ETHNOPHARMACOLOGICAL RESPONSES TO THE CORONAVIRUS DISEASE 2019 (COVID-19) PANDEMIC

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Editorial: Ethnopharmacological Responses to the Coronavirus Disease 2019 Pandemic

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Editorial on the Research Topic

Ethnopharmacological Responses to the Coronavirus Disease 2019 Pandemic

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1 INTRODUCTION

The pandemic of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, has led to severe impact on health globally. There is no effective therapy for SARS-CoV-2 infection except the preventive effects of a vaccine. Several medicines (PF-07321332, EXO-CD24, BRII-196, and BRII-198 etc.) against SARS-CoV-2 infection are under development; but the clinical efficacy of these medicines has not been evaluated rigorously. The outputs and prices make them less accessible, especially in low-and middle-income countries (LMIC). The lack of effective medicines has led to the very high global demand for various forms of traditional medicines (TM) as a potential treatment option for COVID-19. Simultaneously, the use of herbal medicines and supplements (especially those with anti-infective and immunomodulatory effects as well as those used as a supportive therapy) has increased dramatically as a part of adjuvant therapy in economically developed countries, which is often not reported to health care professionals (Bhamra et al. 2021; Smith et al.). In many LMIC regions including in China, India, South Korea, Thailand, the Americas and Africa, TMs are accessible and widely used and numerous TM therapies have started to be investigated as potential treatments. Some of the claims made, however, are very high and seem implausible. We reiterate that these TM treatments should be seen as a part of a broader package of adjuvant and symptomatic treatments and that scientists have a specific responsibility to ascertain a balanced and critical assessment of the evidence as well as the gaps (Heinrich 2010).

For example, traditional African medicine (TAM), traditional Chinese medicine (TCM), traditional Indian medicine (TIM), and traditional Persian medicine (TPM) have shown unique potentials to be used generally as a therapy against COVID-19. This is linked to reported immunomodulatory, anti-inflammatory, antiviral, antioxidant, antihistamine, and bronchodilator effects (Bahramsoltani and Rahimi; Ahmad et al.; Attah et al.; Mosleh et al.; Saggam et al.). This widespread use and the existing preliminary evidence highlight the need of

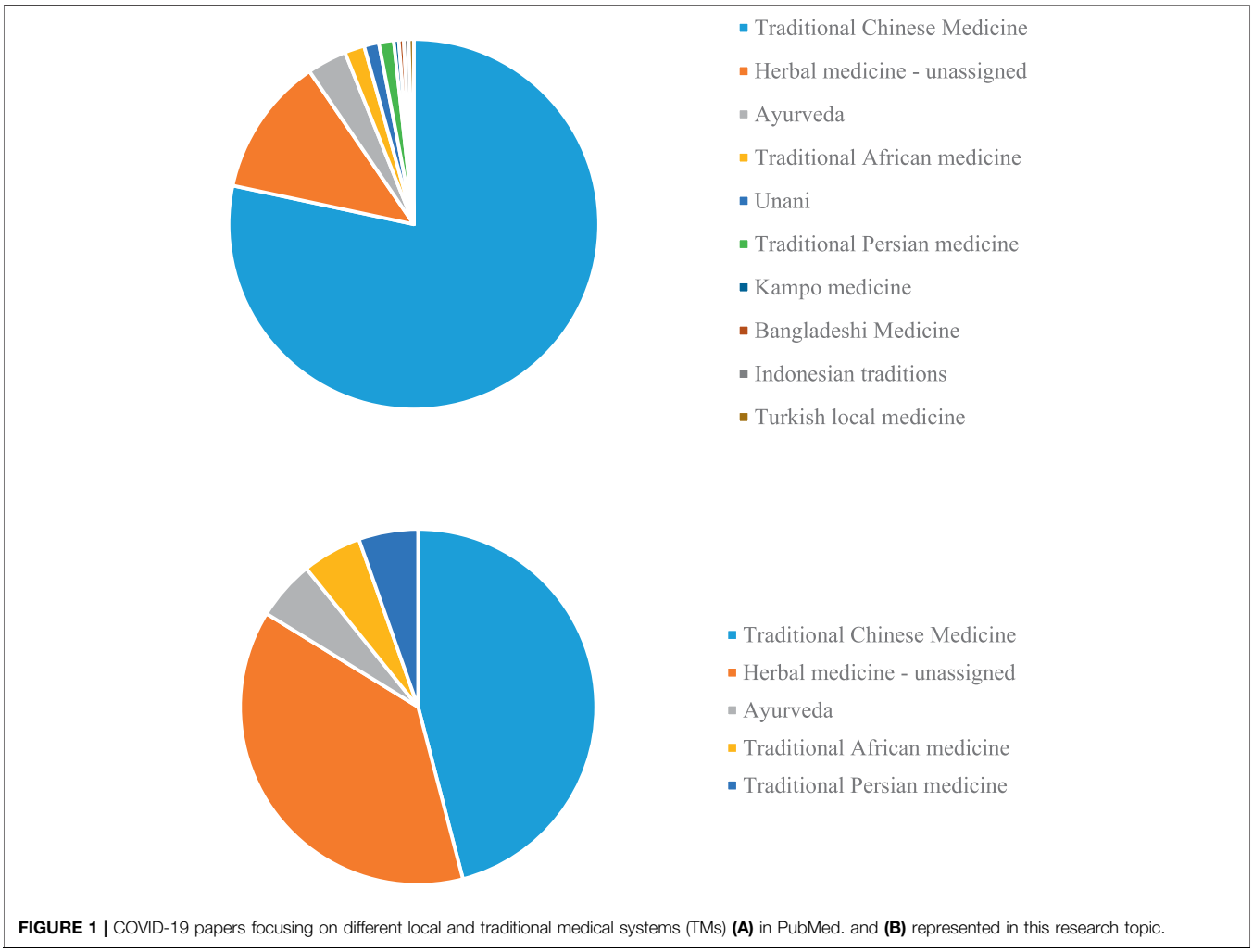

FIGURE 1 | COVID-19 papers focusing on different local and traditional medical systems (TMs) **(A)** in PubMed. and **(B)** represented in this research topic.

clinical evidence from rigorous randomized controlled trials (RCTs) and meta-analysis, as well as *in vitro* and *in vivo* studies to assess the efficacy and the mechanisms of TMs against COVID-19.

This research topic is a collection of 37 articles focusing on the adjuvant effects, pharmacological characteristics, efficacy and safety of TMs, and the potential mechanisms against COVID-19 of TM formulations, medicinal plants, and active components.

2 USAGE OF TMS IN THE TREATMENT OF COVID-19 AND RELATED SYMPTOMS

To get a view of the usage of TMs in the treatment of COVID-19, we searched PubMed to illustrate the proportion of different kinds of TMs with the time range from January 1, 2019 to August 12, 2021. In total, 231 studies of TMs on COVID-19 were retrieved. Among these studies, most abundant were studies on TCM (nearly 80%; 181/231); followed by studies which could not be classified into a specific geographical region or traditional medicine system (28 studies) (cf. also Brendler et al., 2021). The rest of the studies covered traditional medicine systems Ayurveda (Indian medicine), traditional, African, Unani, Kampo, and medicines from Bangladesh, Iran, Indonesia and Turkey (**Figure 1A**) (Ai et al., 2020; Ayatollahi et al., 2021; Benarba and Pandiella, 2020; Chen et al., 2021; Fakhri et al., 2021; Ge and He, 2020; Jha et al., 2021; Kim, 2021; Kalhori et al., 2021; Lem et al., 2021; Li et al., 2020; Lim et al., 2021; Liu et al., 2020; Luo et al., 2020; Majnooni et al., 2020; Ma et al., 2021; Mandal et al., 2021; Peter et al., 2021; Qiu et al., 2020; Remali and Aizat, 2021; Ren et al., 2021; Silveira et al., 2020; Spicer, 2021; Verma et al., 2020; Wu et al., 2020; Wu et al., 2021; Yang et al., 2020; Zhang et al., 2020; Zhou et al., 2020).

This research topic includes research on TCM, herbal medicine generally, Ayurvedic medicine, African, and Iranian medical traditions (**Figure 1B**), with TCM studies accounting for nearly 50% (17/37).

3 EVIDENCE-BASED MEDICINE OF TM STUDIES IN THE TREATMENT OF COVID-19

Rigorous assessment on the level of evidence is of critical importance to guide future research and clinical management

	PubMed	This Research Topic
Systematic reviews and meta-analysis	21	3
Randomized controlled studies	12	0
Cohort studies	8	0
Case control studies	14	1
Case series	5	0
Case reports	4	0
Editorials, opinions	78	18
In vivo pharmacodynamic research	5	3
In vitro ("test tube") research	6	2
In silico approaches	58	4
General reviews based on other respiratory viral diseases and but not focusing on COVID-19	20	6
Total	231	37

FIGURE 2 | Publication on TM associated with COVID-19.

on COVID-19 by TMs. Looking at the top level of evidence regarding the study of TMs on COVID-19 (**Figure 2**), in PubMed there are 21 systematic reviews (SRs) and meta-analyses and 3 SRs in this research topic. In PubMed 12 randomized controlled trials (RCTs) are recorded, and 11 of these RCTs evaluated the efficacy and safety of TCM in the treatment of COVID-19 and the other RCT (published on Frontiers in Pharmacology but not this research topic), tested oral use of a combined curcumin and piperine tablet, which are commonly used in India. We noted that we have no RCT papers but three high-quality SRs in this research topic. Two of these three SRs performed meta-analysis and synthesized the current evidence to evaluate the clinical and safety of TCM intervention in the treatment of COVID-19. Beyond these RCT studies and meta-analysis, however, we can see that the majority of TMs-related COVID-19 studies indicate a relatively low level of evidence. There remains a dearth of original research on this topic, and those opinion papers or descriptive reviews have much overlap of ideas and cited references resulting in the risk of a limited scientific impact. With the limitation of good *in vivo* and *in vitro* SARS-Cov-2 models, we found a very limited body of scientific evidence. However, we found many *in silico* papers using molecular docking or network pharmacology that offer hypothetic leads based on TMs. Such non-validated *in silico* studies on TMs are, without associated *in vivo*, *in vitro* or clinical evidence, of limited relevance for informing clinical practice in COVID-19 and can easily be misleading. Some review papers have been written based on experiences with other respiratory viral diseases but not based on COVID-19, specifically. These review papers were usually published in the early period of the pandemic, with a very limited knowledge regarding to COVID-19 at the time. These reviews may inspire new ideas for future studies on COVID-19, or of future management of a pandemic, considering the potential similarities of corona viruses and common courses of respiratory diseases and inflammatory responses. For instance, TCM treatments were broadly used and played an important role in other respiratory diseases, such as the severe acute respiratory syndrome (SARS) in 2003, the Influenza A (H1N1) in 2009, the Middle East Respiratory Syndrome (MERS) in 2012, and H7N9 avian influenza in 2013 (Xi et al.; Zhuang et al.), which may have some inspirations to understand the treatment of COVID-19. Collectively, although there is a growing body of publications on TMs against COVID-19, we need to be aware that we still lack high-quality clinical evidence, which highlights the importance of future clinical studies.

4 EMPHASIS ON THE CLINICAL EVIDENCES OF TMS IN THE TREATMENT OF COVID-19

Two SRs in this research topic evaluated the clinical efficacy of TCM in the treatment of COVID-19 and concluded that the interventions were safe in COVID-19 patients (Liang et al.; Wang et al.). One of these illustrated that TCM in combination with conventional therapy was better than conventional therapy alone. The purported beneficial effects included increasing the recovery rate of main symptoms of cough and fatigue, shortening the duration of main symptoms of fever, cough and fatigue, but were not suggestive of an increase the recovery rate of main symptoms of fever (Liang et al.). Another SR and meta-analysis focusing on low-risk-of-bias RCTs showed moderate confidence that compare to routine treatment alone, TCM plus routine treatment could reduce the incidence of unfavourable events of clinical deterioration, acute respiratory distress syndrome (ARDS), mechanical ventilation, and death). However, the treatment was not found to reduce the level of positive tests using the SARS-CoV-2 nucleic acid test, and on chest X-ray images could

shows improvements based (Wang et al.). Considering ARDS is the most common complications of COVID-19 (about 7. 4–41.8% of COVID-19 patients developed ARDS), as well as one of the most dangerous (the mortality rate of COVID-19 patients with ARDS was 30.4–52.4%), the potentially favourable effect of specific TCM formula may indicate significant merit in further mechanistic studies (Wang et al.).

5 PERSPECTIVES

TMs have been used extensively in preventing and treating COVID-19 worldwide and in this short period a significant number papers have been published including a considerable share in this RT. These publications attracted considerable attention and have impacted on the fast-evolving discussion about the use of TMs. A wider debate is about the future role of TMs. Some countries including Thailand and the PR China have embraced TM as a potential strategy for ongoing treatment and/or prevention of COVID-19. In some cases, very strong claims about what can be achieved have been made, and these too may warrant further research attention. In many countries, however, the use of TMs as a strategy for COVID-19 is not accepted, resulting in such treatments becoming limited to over-the-counter self-treatment, often in unregulated or informal settings. An example is the dramatic rise of elderberry-based products (*Sambucus nigra* L.) sold in the United States in 2020 (Smith et al., 2021). Here the systematic assessment of opportunities to use such treatments as adjuvants and their appropriate role in the self-management of respiratory conditions more generally needs to be developed further. However, we should keep in mind to the lack of high-quality evidence-based TM-based treatments of COVID-19. More importantly, although many kinds of popular medicines have been used in the management of COVID-19, so far only a few TCM preparations appear to have been tested systematically in RCTs, and even then these studies remain small and of uncertain quality. High quality clinical studies are urgently needed to appropriately guide evidence-informed approaches to incorporation of TMs in public health systems responses to COVID-19. Research using *in silico* or *in vitro* methods may have some values but the results need to be investigated further *in vivo* and clinically in order to allow an assessment of potential therapeutic benefits. Another limitation on the research on TMs against COVID-19 is the limited access to appropriate animal models, for example, transgenic hACE2 mice model (Bao et al., 2020) and cytokine storm syndrome mouse model induced by SARS-CoV-2 Spike protein (Gu et al., 2021). On the other hand, long COVID, the post-acute COVID-19 syndrome, has emerged as a major concern, which deserves further studies by using TMs (Adeloye et al., 2021). Overall, this research topic has been one of the first responses of the scientific communities globally to assess the potential of TM and here specifically TMs in addressing this major global health challenge. It has demonstrated the enormous importance and potential of TMs globally in response to the pandemic and the fast evolving scientific evidence base for some of these treatments. However, it also serves as a calling for a much more systematic study of TMs globally including, obviously, the need for major funding of this research, in order to be appropriately informed on this topic.

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All authors edited MS, contributed to the RT as such and reviewed the MS at various stages.

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Chinese Patent Medicines in the Treatment of Coronavirus Disease 2019 (COVID-19) in China

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Background: Coronavirus Disease 2019 (COVID-19) is an emerging and rapidly evolving disease, with no recommended effective anti-coronavirus drug treatment. Traditional Chinese Patent Medicines (CPMs) have, however, been widely used to treat COVID-19 in China, and a number of clinical practice results have shown them to have a significant role in its treatment. Consequently, numerous guidelines and expert consensus have recommended the use of CPMs to treat COVID-19.

Aim of the Study: The objectives of this review are to provide up-to-date information on the pharmacology and clinical research on CPMs in the treatment of COVID-19, discuss the research findings, and to better guide clinical application and scientific research on CPMs in the treatment of COVID-19.

Methods: The frequencies of CPM recommendations by guidelines and expert consensus for treatment of COVID-19 in China were ranked. This report identifies the top 10 CPMs, which include Huoxiang Zhengqi capsule (HXZQC), Lianhua Qingwen capsule (LHQWC), Jinhua Qinggan granule (JHQGG), Shufeng Jiedu capsule (SFJDC), Tanreqing injection (TRQI), Xiyanping injection (XYPI), Xuebijing injection (XBJI), Shenfu injection (SFI), Shengmai injection (SMI), and Angong Niuhuang pill (AGNHP). Relevant studies from 2000 to 2020 on these top 10 CPMs, covering usage, dosage, mechanism, curative effect, and precautions, were collected from pharmacopoeia, reports, and theses *via* library and digital databases (including PubMed, CNKI, Google Scholar, Web of Science, and Elsevier).

Results: The properties of the top 10 CPMs included antiviral, antibacterial, anti-inflammatory, antipyretic and analgesic, anti-acute lung injury, anti-shock, immune regulation, and enhancement of pulmonary function. In addition, clinical research results and Chinese treatment data showed that the CPMs had good therapeutic efficacy in the treatment of COVID-19, and adverse reactions were minimal.

Conclusions: Knowledge of the characteristics of the top 10 CPMs and precautions that should be taken may help clinicians to rationally improve therapeutic efficacy, and promote the role of Chinese Medicine in the control of the COVID-19 global epidemic.

Keywords: COVID-19, Chinese Patent Medicines, pharmacological action, clinical application, Traditional Chinese Medicine

INTRODUCTION

COVID-19 is an emerging and rapidly evolving epidemic. The cumulative number of confirmed cases globally reached 1,040,772 on April 4, 2020, comprising 149,790 (14.39%) cured cases, and 55,698 (5.35%) deaths. The causative organism has been designated as the 2019 novel coronavirus (2019-nCoV). On January 30, 2020, the epidemic was declared a public health emergency of international concern by the World Health Organization (WHO) (Fisher and Heymann, 2020). On February 11, 2020, the WHO Director-General, Tedros Adhanom Ghebreyesus, announced that the disease caused by this new coronavirus was "COVID-19," which is an acronym for "coronavirus disease 2019." The virus seems to be highly contagious and had quickly spread to 119 countries and regions by March 12, 2020. The clinical spectrum of COVID-19 varies from asymptomatic or paucisymptomatic forms to clinical symptoms characterized by respiratory failure that necessitates mechanical ventilation and support in an intensive care unit (ICU), to multiorgan and systemic manifestations in terms of sepsis, septic shock, and multiple organ dysfunction syndromes (MODS) (Wu and McGoogan, 2020). Currently, there is no effective anti-coronaviral drug that is recommended for treatment of COVID-19, and no vaccine is available. There is no evidence supporting the efficacy of broad-spectrum antibiotics, gamma globulin, interferon, or corticosteroid therapy for COVID-19. Treatment is symptomatic, and oxygen therapy represents the major intervention for patients with severe infection. Mechanical ventilation may be necessary in cases of respiratory failure refractory to oxygen therapy (Huang et al., 2020; Zhang et al., 2020).

Traditional Chinese Medicine (TCM) has a long history and has played an important role in the prevention and treatment of serious epidemic diseases. During the development of the COVID-19 epidemic, more than 3,100 TCM medical staff were deployed to Hubei province, and TCM was included in the guidelines for the diagnosis and treatment of COVID-19. Currently, the total number of confirmed cases treated by TCM has reached 60,107 (Gao, 2020). The decoctions, CPMs, acupuncture, and other TCM treatments have been comprehensively used for treatment, mainly based on syndrome differentiation. Specific CPMs have been widely employed to treat COVID-19 with remarkable therapeutic effects (National Health Commission of the People's Republic of China and National Administration of Traditional Chinese Medicine, 2020).

CPMs are approved by the National Drug Regulatory Authority of China and processed according to prescribed methods using Chinese herbal medicines as raw materials, guided by the theory of TCM. They are available in different dosage forms, such as pill, tablet, capsule, granule, or injection. The use of CPMs is guided by syndrome differentiation and overall analysis of signs and symptoms. Provinces of China have prepared therapeutic schedules for the treatment of COVID-19 based on actual conditions (see **Table 1**). Many guidelines and expert consensus in China have recommended using CPMs to treat COVID-19. In this article, we identify the top 10 recommended CPMs to treat COVID-19 (**Figure 1**). The list of CPMs includes Huoxiang Zhengqi capsule (HXZQC), Lianhua Qingwen capsule (LHQWC), Jinhua Qinggan granule (JHQGG), Shufeng Jiedu capsule (SFJDC), Tanreqing injection (TRQI), Xiyanping injection (XYPI), Xuebijing injection (XBJI), Shenfu injection (SFI), Shengmai injection (SMI), and Angong Niuhuang pill (AGNHP). Information on the drugs include the recommended guidelines, drug ingredients, indications, pharmacological research, clinical research, usage and dosage, adverse reactions, and precautions.

RELEVANT INFORMATION ON THE CLINICAL APPLICATION OF HXZQC

Recommended Therapeutic Regimens

HXZQC has been recommended in 20 therapeutic regimens for COVID-19 in China (see detailed information in **Tables 1** and **2**).

Abbreviations: COVID-19, Coronavirus Disease 2019; CPMs, Chinese Patent Medicines; TCM, Traditional Chinese Medicine; HXZQC, Huoxiang Zhengqi capsule; LHQWC, Lianhua Qingwen capsule; JHQGG, Jinhua Qinggan granule; SFJDC, Shufeng Jiedu capsule; TRQI, Tanreqing injection; XYPI, Xiyanping injection; XBJI, Xuebijing injection; SFI, Shenfu injection; SMI, Shengmai injection; AGNHP, Angong Niuhuang pill; WHO, World Health Organization; ICU, intensive care unit; MODS, multiple organ dysfunction syndromes; AIV, avian influenza virus; LPS, lipopolysaccharide; IFN-γ, interferon-γ; IgG, immunoglobulin G; IBS, irritable bowel syndrome; IL-1, interleukin-1; IL-2, interleukin-2; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-17, interleukin-17; IL-1α, interleukin-1α; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor alpha; MCP-1, macrophage chemotactic factor-1; 5-HT, 5-hydroxytryptamine; LDH, lactate dehydrogenase; CRP, C-reactive protein; PCT, procalcitonin; AST, aspartate aminotransferase; ALT, glutamate transaminase; MDA, malondialdehyde; ATP, adenosine triphosphate; SOD, superoxide dismutase; ROS, reactive oxygen species; CPE, cytopathic effect; WBC, white blood cell; Th, T helper cell; NK, natural killer; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3 kinase; NF-κB, nuclear factor kappa B; HLA-DR, human leukocyte antigen-DR; ICAM-1, intracellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; TXA2, thromboxane A2; ANP, atrial natriuretic peptide; BALF, bronchoalveolar lavage fluid; URI, upper respiratory infection; SARS, severe acute respiratory syndrome; MERS, Middle East respiratory syndrome; RCTs, randomized controlled trials; CAP, community acquired pneumonia; COPD, chronic obstructive pulmonary disease; AECOPD, acute exacerbation of chronic obstructive pulmonary disease; MRSA, methicillin-resistant *Staphylococcus aureus*; SIRS, systemic inflammatory response syndrome; APACHE-II, acute physiology and chronic health evaluation II.

TABLE 1 | Therapeutic regimens for COVID-19 in China.

Serial No.	Therapeutic regimen of COVID-19	Website	Date of issue
1	National Health Commission of the People's Republic of China. Guideline on Diagnosis and Treatment of COVID-19 (Trial 7th edition)	http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml	Mar. 03, 2020
2	Beijing Municipal COVID-19 TCM Preventive and Therapeutic Regime (Trial Version IV)	http://zyj.beijing.gov.cn/sy/tzgg/202003/t20200307_1682382.html	Mar. 06, 2020
3	Tianjin Municipal COVID-19 TCM Preventive and Therapeutic Regime (Trial Version III)	http://wsjk.tj.gov.cn/art/2020/2/21/art_70_71264.html	Feb. 20, 2020
4	Hebei Provincial COVID-19 TCM Therapeutic regime (Trial Version IV)	https://mp.weixin.qq.com/s/9QTqGDw6vkQyWX-VrjulqQ	Feb. 13, 2020
5	Gansu Provincial TCM Preventive and Therapeutic Regime of Novel Coronavirus Infected Pneumonia (Trial Version II)	http://wsjk.gansu.gov.cn/file.jsp?contentId=83488	Feb. 01, 2020
6	Guangdong Provincial COVID-19 TCM Therapeutic Regime (Trial Version II)	http://szyyj.gd.gov.cn/zwgk/gsgg/content/post_2902010.html	Feb. 18, 2020
7	Shaanxi Provincial TCM Therapeutic Regime of Novel Coronavirus Infected Pneumonia (Trial Version II)	http://sxwjw.shaanxi.gov.cn/art/2020/2/2/art_10_67602.html	Feb. 02, 2020
8	Hunan Provincial TCM Diagnosis And Treatment Scheme of Novel Coronavirus Infected Pneumonia (Trial Version III)	http://tcm.hunan.gov.cn/tcm/xxgk/tzgg/202002/t20200203_11168981.html	Feb. 03, 2020
9	Jilin Provincial TCM Therapeutic Regime of Novel Coronavirus Infected Pneumonia (Trial Version I)	http://jltcm.jl.gov.cn/tzgg/xgdt/202001/t20200126_6654768.html	Jan. 26, 2020
10	Technical Guidelines of Sichuan Province on TCM Prevention and Control of COVID-19	http://wsjkw.sc.gov.cn/scwsjkw/zcwj11/2020/2/6/ac6fea21a3ad490aa0a73c9d70004ad6.shtml	Feb. 05, 2020
11	Shanghai Municipal COVID-19 TCM Diagnosis And Treatment Scheme (Trial Version II)	http://wsjkw.sh.gov.cn/zyygz2/20200224/a1f1aab9745e4490867cb4aaf40eaad0.html	Feb. 24, 2020
12	Jiangxi Provincial COVID-19 TCM Preventive and Therapeutic Regime (Trial Version III)	http://www.jxhfpc.gov.cn/doc/2020/02/21/140518.shtml	Feb. 21,2020
13	COVID-19 TCM Therapeutic Regime of Guangxi Zhuang Autonomous Region (Trial Version III)	http://wsjkw.gxzf.gov.cn/zwgk/zfxxgkml/wsjszh/zyzy/2020/0224/1752.html	Feb. 24, 2020
14	TCM Preventive and Therapeutic Regime for Novel Coronavirus Infected Pneumonia of the Xinjiang Uygur Autonomous Region	http://www.xjhfpc.gov.cn/info/2074/17765.htm	Jan. 30, 2020
15	Hainan Provincial COVID-19 TCM Preventive and Therapeutic Regime (Trial Version III)	http://wst.hainan.gov.cn/swjw/xxgk/0200/0202/202003/t20200305_2756534.html	Feb. 14, 2020
16	Heilongjiang Provincial COVID-19 TCM Preventive and Therapeutic Regime (Version III)	http://www.hljdaily.com.cn/article/90/154485.html	Feb. 26, 2020
17	Guizhou Provincial COVID-19 TCM Preventive and Therapeutic Reference Regime (Version II)	http://atcm.guizhou.gov.cn/xwzx/zyyw/202002/t20200219_50116162.html	Feb. 19, 2020
18	Shanxi Provincial of TCM Preventive and Therapeutic Regime of Novel Coronavirus Infected Pneumonia (For Trial Implementation)	http://www.sx.chinanews.com/news/2020/0201/162758.html	Feb. 01, 2020
19	Jiangsu Provincial COVID-19 TCM Diagnosis and Intervention Regime (Trial Version III)	http://www.jstcm.com/article_info.asp?id=10042	Feb. 18, 2020
20	COVID-19 TCM Diagnosis and Treatment Scheme of the Nei Monggol Autonomous Region (Trial Version II)	http://wjw.nmg.gov.cn/doc/2020/02/18/292482.shtml	Feb. 14,2020
21	Liaoning Provincial TCM Diagnosis and Treatment Scheme of Novel Coronavirus Infected Pneumonia (Trial Version II)	http://wsjk.ln.gov.cn/wst_zdzt/xxgzbd/tzgg/202002/t20200203_3733244.html	Feb. 03, 2020
22	Anhui Provincial COVID-19 TCM Therapist Consensus	http://wjw.ah.gov.cn/ahtcm/NewsDetail.aspx?id=987	Feb. 18, 2020
23	Shandong Provincial TCM Diagnosis and Treatment Scheme of Novel Coronavirus Infected Pneumonia	http://wsjkw.shandong.gov.cn/ztzl/rdzt/qlzhfkgz/fkdt/202002/t20200201_2513391.html	Jan. 31, 2020
24	TCM Preventive and Therapeutic Regime for Novel Coronavirus Infected Pneumonia of the Ningxia Hui Autonomous Region (For Trial Implementation)	http://wsjkw.nx.gov.cn/info/1040/13360.htm	Jan. 28, 2020
25	TCM Diagnosis and Treatment Scheme and Preventive Scheme for Novel Coronavirus Infected Pneumonia of Tongji Hospital, Tongji Medical College, Huazhong University of Science & Technology	https://www.tjh.com.cn/html/2020/0208/28991.shtml	Feb. 08,2020

Ingredients of HXZQC

Pogostemon cablin (Blanco) Benth. (Guanghuoxiang), *Atractylodes macrocephala* Koidz. (Baizhu), *Magnolia officinalis* Rehder & E.H.Wilson (Houpo), *Pinellia ternata* (Thunb.) Makino (Banxia), *Perilla frutescens* (L.) Britton (Zisu), *Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav. (Baizhi), *Citrus* × *aurantium* L. (Chenpi), *Poria cocos* (Schw.) Wolf (Fuling), *Platycodon grandiflorus* (Jacq.) A.DC. (Jiegeng), *Glycyrrhiza uralensis* Fisch. ex DC. (Gancao), *Ziziphus jujuba* Mill. (Dazao), *Areca catechu* L. (Binglang), and *Zingiber officinale* Roscoe (Shengjiang). Basic information on HXZQC is provided in the **Supplementary Table**.

Indications for the Treatment of COVID-19 With HXZQC

HXZQC is used for cold outside and inside damp indications during the clinical observation period of COVID-19 and early stage of the disease (mild case). The indicative symptoms include weakness, headache and dizziness, abdominal fullness and distention, vomiting, and diarrhea.

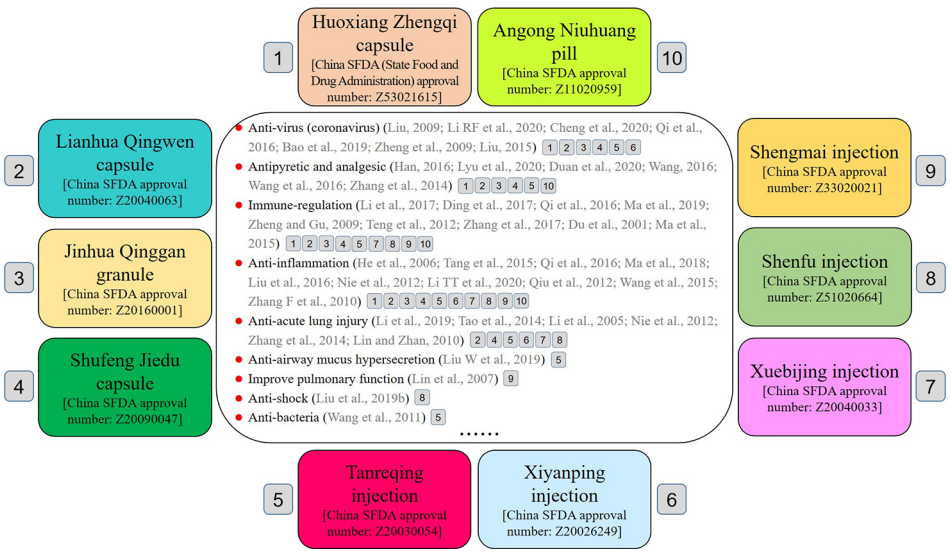

FIGURE 1 | The top 10 CPMs for the treatment of COVID-19: 1) Huoxiang Zhengqi capsule (HXZQC), 2) Lianhua Qingwen capsule (LHQWC), 3) Jinhua Qinggan granule (JHQGG), 4) Shufeng Jiedu capsule (SFJDC), 5) Tanreqing injection (TRQI), 6) Xiyanping injection (XYPI), 7) Xuebijing injection (XBJI), 8) Shenfu injection (SFI), 9) Shengmai injection (SMI), and 10) Angong Niuhuang pill (AGNHP).

TABLE 2 | List of recommended CPMs in therapeutic regimens for COVID-19.

Drug name	Therapeutic regimens of COVID-19	Number of "therapeutic regimens"
AGNHP	2-13, 15-19, 21-23, 25	21
HXZQC	1, 4-8, 10-16, 18, 19, 21-25	20
XBJI	1-4, 6, 7, 10-13, 15, 16, 17-25	20
LHQWC	1, 2, 4-7, 11-16, 18, 19, 21-25	19
SFI	1-4, 6, 10-13, 15, 16, 18-25	19
SMI	1, 3, 6, 7, 10-13, 15, 16, 18-25	18
SFJDC	1, 4-7, 11-13, 16, 18, 19, 21-24	15
XYPI	1, 3, 6, 7, 10-13, 15, 20-25	15
JHQGG	1, 2, 4-7, 11, 12, 18, 21, 22, 24, 25	13
TRQI	1-3, 6, 7, 11-13, 16, 19, 21, 22	12

Progress of Pharmacological Research on HXZQC

Modern pharmacological studies have found that HXZQC has antiviral, anti-inflammatory, and immune regulatory activities, improves gastrointestinal discomfort and other properties (see **Table 3**).

Research by Zhonghua Liu et al. (Liu, 2009) revealed that HXZQC inhibited the lung index of mice infected with avian influenza virus (AIV) H5N1, reduced the development of lung disease, and enhanced the antiviral capacity of mice infected with AIV. The death rate of the infected mice was reduced through regulation of the gastrointestinal tract and strengthening of the stomach Qi. Hongkun Zhang (Zhang, 2013) found that HXZQC inhibited growth of *Vibrio parahaemolyticus*, *Candida albicans*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Research by Wang et al. (2012) found that HXZQC inhibited lipopolysaccharide (LPS)-stimulated expression of proinflammatory cytokines by macrophages and inhibited epithelial barrier disorder induced by interferon-γ (IFN-γ), regulating immunity and improving gastroenteric function. Research by Chunyuan Li et al. (Li et al., 2017) showed that HXZQC significantly improved the thymus coefficient, spleen coefficient and immunoglobulin G (IgG) levels of mice with dampness obstructing spleen-stomach, and enhanced the immune function of the mice. Studies by Yinghui He et al. (He et al., 2006) and Shaobo Zong et al. (Zong et al., 2015) discovered that HXZQC had therapeutic effects in mice with *Bacillus dysenteriae* and *Salmonella typhimurium*-induced diarrhea (BSD mice), mice with bacterial enteritis, and model rats with diarrhea-predominant irritable bowel syndrome (IBS). Clinical symptoms were significantly improved, which might be due to effects on the balance of $CD4^+$ and $CD8^+$ T lymphocytes, and reduction of interleukin-2 (IL-2), interleukin-10 (IL-10), interleukin-1β (IL-1β), and tumor necrosis factor alpha (TNF-α) levels. The results suggested that HXZQC, *via* immune-regulation and anti-inflammatory activity, could have therapeutic effects against many gastrointestinal disorders. Research by Hefei Huang et al. (Huang et al., 2016) showed that HXZQC extracts had a positive regulatory effect on intestinal dysfunction, and had therapeutic efficacy in model rats with diarrhea-predominant IBS. Efficacy was mediated by improving serum NO levels and reducing the concentrations of 5-hydroxytryptamine (5-HT), plasma motilin, and colonic somatostatin.

Clinical Research on HXZQC

Modern clinical studies have shown that HXZQC has therapeutic effects against viral diseases, such as gastrointestinal-type cold, influenza, upper respiratory infection (URI), and viral enteritis

TABLE 3 | Pharmacological functions and clinical research on top 10 CPMs for the treatment of COVID-19.

Drug name	Pharmacological action	Mechanism	Clinical application	Therapeutic efficacy
HXZQC	Regulate the immunity and improve the gastroenteric function	Inhibits LPS and epithelial barrier disorder, stipulate expression of proinflammatory cytokine of macrophage (Wang et al., 2012). Improve thymus coefficient, spleen coefficient, immunoglobulin G (IgG) level of the mice (Li et al., 2017). Regulates effect of the balance of $CD4^+$ and $CD8^+$ T lymphocytes, and reduction of IL-2, IL-10, IL-1β and TNF-α level (He et al., 2006; Zong et al., 2015). Improves serum NO level of rats, reduces concentration of 5-HT, and downregulates the level of plasma motilin and colonic somatostatin (Huang et al., 2016).	Children with rotavirus enteritis	Shortens anti-diarrheal time and total course of time in treating children with rotavirus enteritis (Ma and Wang, 2012).
	Anti-virus	Inhibits Avian Influenza Virus H5N1 regulating the gastrointestinal tract (Liu, 2009).	Influenza	Extends relief time of fever symptom, relieves time of muscle aches and relieves time of fatigue (Han, 2016).
	Inhibition effect of vibrio parahaemolyticus, Candida albicans, staphylococcus aureus and diplococcus pneumonia (Zhang, 2013)	–	Cold	Relieves fever, nasal congestion, running nose, spontaneous sweating, headache, cough and spitting, fatigue and weakness, body ache and other cold symptoms (Wu, 2010; Zhao et al., 2017).
			Acute gastro-enteritis	Improves total response rate and clinical symptoms.
LHQWC	Anti-virus	Inhibits proliferation of influenza virus and regulates immune response to viral infection (Ding et al., 2017; Yao et al., 2020). Inhibits SARS-CoV-2 replication, affects virus morphology and exerts anti-inflammatory activity *in vitro* (Li R. F. et al., 2020).	COVID-19	Improves the fever, weakness, cough, short breath, chest distress, anorexia and other clinical symptoms of COVID-19, reduces the ratio of common to severe (Cheng et al., 2020; Hu et al., 2020; Lyu et al., 2020). Lianhua Qingwen granule combined with Abidole can effectively relieve clinical symptoms of mild COVID-19 patients, regulate expression of related inflammatory factors, improve the curative effect and reduce the rate of severe illness (Liu et al., 2020; Yu P. et al., 2020).
	Anti-acute lung injury	Inhibits expression and secretion level of MCP-1, reduces infiltration of mononuclear macrophages (Li et al., 2019). Reduces LDH and MDA level, increases content of GSH-Px, and relieves the exudation of inflammatory cells in the alveolar cavity (Ping et al., 2016). Downregulates expression of IKK/IκB/NF-κB signaling pathway (Cui et al., 2016). Reduces protein expression and mRNA expression of inflammatory cytokines in lung tissues through reducing content of inflammatory cytokines in mice blood (Tang et al., 2015).	H1N1	Improves cough, sore throat, body ache and other symptoms of the patients infected with H1N1 virus, and reduces the duration of fever (Duan et al., 2011; Zhao P. et al., 2014).
			Influenza	LHQWC has better total response rate, symptom improvement rate and body temperature recovery rate than the control group (Wang et al., 2019).
			URI	Improves the patients' nasal congestion, fever, headache, sore throat, weakness, aches in the limbs, intolerance of cold and other clinical symptoms (Li, 2019).
			Chronic obstructive pulmonary	Improves the condition of the patients with AECOPD, and reduces release of inflammatory mediators (Dong et al., 2014).
JHQGG	Anti-virus	Decreases average level of CRP and IFN-γ in serum of the influenza patients, and decreases inflammatory response (Qi et al., 2016).	COVID-19	Alleviates symptoms of fever, cough, fatigue and sputum (Duan et al., 2020).
			H1N1 Influenza	Shortens antipyretic time (Wang C. et al., 2011). Reduces the serum levels of cytokines and improve their immune function (Li et al., 2013; Qi et al., 2016).
SFJDC	Anti-virus	Improves mice pneumonia symptoms caused by influenza virus, reduce lung index of the mice infected with H1N1, and significantly reduces mortality rate of the infected animals (Liu et al., 2010; Lv et al., 2013; Qiu et al., 2014; Bao et al., 2019).	Acute URI + fever	Improves respiratory symptoms (Wang and Qiu, 2018)
	Anti-inflammation	Reduces the WBC count, and reduces the serum transcription factor NF-κB, chemokine MCP-1, inflammatory mediator BK and COX-2 level (Ma et al., 2018).	CAP	Shortens recover time of multiple symptoms and signs such as fever, reduces levels of PCT, CRP and WBC and other indicators (Wang, 2016).
	Immune-regulation	Reduces levels of B lymphocytes, CD8+ proportion, IL-1α, IL-1β, IL-2, IgM, IgG, etc., reduces quality of thymus, spleen and lung of pneumonia mice, and increase CD4+/CD8+ and NK cell proportion (Ma et al., 2019).	AECOPD	Reduces levels of IL-8, TNF-α, CRP and PCT (Yu H. X. et al., 2020) and increases arterial blood gas PaO_2 (Wang, 2015).
	Anti-acute lung injury	Inhibition of the MAPK/NF-κB signaling pathway, and down-regulation of NF-κB mRNA expression (Tao et al., 2014). The	Bacterial acute	

(Continued)

TABLE 3 | Continued

Drug name	Pharmacological action	Mechanism	Clinical application	Therapeutic efficacy
		action might be closely related with ERK signaling pathway (Li et al., 2017).	bronchitis and Pneumonia	Shortens recovery time of body temperature, duration of cough and the course of treatment, and increases oxygen index (Wang et al., 2014).
TRQI	Anti-virus	Inhibition of intracellular proliferation and enhancement of body immunity of mice infected with influenza virus (Zheng and Gu, 2009). Destroys MRSA biofilm, and induces its death, and when in combined use with vancomycin or linezolid below the MIC concentration (Yang et al., 2018).	Viral pneumonia	A systematic review: TRQI had advantages in response rate of treatment, average length of stay (Pan, 2016).
	Anti-bacteria	Destroys the bacterial biofilm (Wang Y. et al., 2011).	Acute bronchitis	Improves response rate, reduce fever, cough, crackles and X-ray shadow absorption (Wang et al., 2016)
	Anti-inflammation	Inhibits release of inflammatory factors such as TNF-α, IL-6 and NO, and inhibits airway inflammation caused by LPS through MAPK/NF-B pathway (Liu et al., 2016).	Acute attack of chronic bronchitis	Improves clinical symptoms (Gao et al., 2019).
	Anti-airway mucus hypersecretion	Regulates the IL-17 signaling pathway and its downstream protein MUC5AC (Liu W. et al., 2019).	CAP	Improves clinical effect and the symptom of cough with expectoration, shortens the duration of fever and promotes shadow absorption on chest radiography and the hemogram recovery (Jiang et al., 2009).
	Anti-acute lung injury	Improves blood flow status of capillaries in the alveolar walls while repressing LPS-induced inflammatory cascade (Li et al., 2005).	Tuberculosis accompanied by infection	A systematic review: TRQI might have the same overall effect with some antibacterial drugs in treating patients with tuberculosis accompanied by lung infection (Lian et al., 2018).
			AECOPD	Reduction of the patients' serum IL-8 and NE level, and improvement of airway inflammation reaction and mucus hypersecretion (Li et al., 2010). A systematic review: improves clinical effect and lung function of AECOPD patients, reduces pCO_2, and shortens the length of stay (Zhong et al., 2010).
XYPI	Anti-virus	Inhibits proliferation of human rhinovirus in mice (Liu, 2015).	Viral pneumonia	Increases the cure rate, and improve the symptoms and signs (Li, 2015; Qi et al., 2018)
	Anti-acute lung injury	Inhibits release of proinflammatory cytokines such as IL-10, IL4, etc., and could promote the proinflammatory cytokines/ anti-inflammatory cytokines to tend to be balanced, and inhibit excess anti-inflammatory responses during the course of acute lung injury (Nie et al., 2012).	Severe pneumonia	Shortens the course of disease, improves the treatment efficiency, reduces incidence rate of antibiotic resistance, reduces occurrence of double infection, further improves the prognosis and reduces the mortality rate (Zhang and Wang, 2015). Reduces leukocytes, improve oxygen index, lower CPIS score, promotes absorption of pulmonary inflammation, shortens the duration of mechanical ventilation and length of stay in ICU, and improves clinical effect (Yang et al., 2014).
	Inhibition effect of staphylococcus aureus and pneumonia streptococcus (Yu et al., 2009)	–	Upper respiratory infection	Improves symptoms of the patients (Liu and Li, 2015).
XBJI	Anti-inflammation	Downregulates expression of inflammatory cytokines stimulated by Pam3CSK4 and activating MAPK, PI3K/Akt and other pathways (Li T. T. et al., 2020). Reduces TNF-α, IL-6 and IL-10 level of mice with sepsis, prevents the neutrophils from infiltrating the lung and kidney, inhibit Th1/Th2, Th17 and Tregs balance (Zhang et al., 2006; Chen et al., 2018).	COVID-19	Improves the inflammatory markers and prognosis of severe COVID-19 patients (Wen et al., 2020).
			SIRS	Expression of $CD4^+$, $CD4^+/CD8^+$, $CD14^+$/HLA-DR increased, and improves systematic status of the SIRS patients (Zhao W. et al., 2014).
			Severe pneumonia	Reduces the level of inflammatory factors, improves the total treatment efficiency (Qi et al., 2011), reduces infectious indicators and the average length of stays (Zhu et al., 2014).
	Anti-acute lung injury	Reduces TNF-α level, alleviates the degree of pulmonary tissue edema and inflammatory cell infiltration (Zhang et al., 2014).	SCAP	Improves primary endpoint-pneumonia severity index, reduces mortality rate in 28 days, and shortens the duration of mechanical ventilation (Song et al., 2019).
	Immune-regulation	Blocks p-38 MAPK and NF-κB 65 pathways (Liu et al., 2014); reduces IL-1, IL-6 and TNF-α level, improves $CD4^+/CD8^+$ T lymphocyte ratio and NK cell relative activity (Teng et al., 2012).	AECOPD	Lowers the inflammatory indicators, improve cough, expectoration, short breath and other clinical symptoms, and shortens their length of stay (Chen et al., 2011; Zhu et al., 2019).

(Continued)

TABLE 3 | Continued

Drug name	Pharmacological action	Mechanism	Clinical application	Therapeutic efficacy
	Anti-oxidation	Improves activity of SOD, reduce ROS level (Jin et al., 2018). Downregulates MDA (Luo and Zhou, 2017).	Sepsis	Reduces mortality rate of sepsis patients in 28 days, the APACHE-II and body temperature (Li et al., 2018).
SFI	Anti-acute lung injury	Increases the wet/dry weight ratio of lung tissues, neutrophil ratio in BALF, protein content, lung tissue MDA and serum NO (Lin and Zhan, 2010). Reduces activation of NF-κB of lung tissue (Ai et al., 2006). Reduces expression level of p65, P50 mRNA and protein in lung tissues and TNF-α level in serum (Liu et al., 2019a).	Sepsis	Lowers IL-6 level, regulate balance between pro-inflammatory factors and anti-inflammatory factors (Qiu et al., 2012). Increases $CD4^+$ and $CD8^+$T cell counts in peripheral blood and upregulated HLA-DR expression in monocytes (Zhang et al., 2017).
	Anti-shock	Increases content of ATP and taurine, and reduces content of AMP in the heart (Liu et al., 2019b).	Severe pneumonia of elderly	Decreases level of TNF-α, IL-6 and IL-8 (Lv et al., 2017).
			Acute lung injury	Improves respiratory rate, oxygen index, and lowers the ICAM-1, ET-1 and NO level (Ma et al., 2019).
			Respiratory failure	Improves serum prealbumin, oxygen index, shortens the duration of mechanical ventilation (Li, 2013)
SMI	Improve pulmonary function	Raises NO level, dropping oxygen free radical levels and decreases lipid peroxidation (Lin et al., 2007). Lowers expression of NF-κB and activity of iNOS in lung tissues (Liu et al., 2009).	COPD	Improves pulmonary function index, blood gas index, IgG index and disappearance time of lung rale (Huang et al., 2019).
	Anti-inflammation	Inhibits expression of ICAM-1 and VCAM-1 (Liu et al., 2015). Inhibits generation of inflammatory cytokines of ischemia-reperfusion rats, lowers expression level of TNF-α, IL-6, IL-8, etc. (Wang et al., 2015)		
	Immune-regulation	Inhibits monocyte MCP-1 (Liu et al., 2015). Increases the content of serum immunoglobulin IgG and the number of T cells, enhances the phagocytic function of macrophages (Du et al., 2001).	Prevent inflammatory response	Improves microcirculation, protect the organ functions, and prevents further occurrence and development of systemic inflammatory response syndrome (Guo et al., 2004).
	Improve pulmonary function	Raises NO level, dropping oxygen free radical levels and decreases lipid peroxidation (Lin et al., 2007). Lowers expression of NF-κB and activity of iNOS in lung tissues (Liu et al., 2009).		
AGNHP	Anti-inflammation	Inhibits release of superoxide radical; reverses changes in cortical monoamine neurotransmitters (Zhang F. et al., 2010; Zhu and Sun, 2014). Lowers serum LPS level and lung myeloperoxidase (MPO) content (Zhang et al., 2009). Lowers total LDH activity, and changes percentage of isomerase (Tang et al., 2005).	Hyperpyrexia, coma caused by severe infectious diseases	Promotes consciousness, improves the neurologic function (Feng and Yang, 2015), shortens average defervescence time (Long and Wu, 2014) and moderates effect on Th1/Th2 (Ma and Zhou, 2015).
			Viral encephalitis	Reduces body temperature, avoids convulsion, promotes consciousness, and alleviates cerebral edema and brain cell damage (Zhang and Dong, 2014).
	Neuroprotective effect	Regulates Th17/Treg balance, inhibits chronic inflammation, reduces plaque collagen fibers, and reduces inflammatory cells infiltration (Fan et al., 2020)	Pneumonia	Reduces PCT and improves immune function (Zhuo and Wen, 2017).
	Antipyretic and analgesic	–	ACI intracerebral hemorrhage	Neuroprotective effect (Han et al., 2019).

(see **Table 3**). The Diagnosis and Treatment Scheme of Severe Acute Respiratory Syndrome (SARS) (Version 2004) (Chinese Medical Association and China Association of Chinese Medicine, 2004) recommended that HXZQC could be used for advanced stage pulmonary closure.

Research by Shuping Ma et al. showed that, compared with the control group (Ribavirin, interferon), HXZQC + Western medicines (Ribavirin, interferon) had a significant effect on antidiarrheal time and shortened the total time course in the treatment of children with rotavirus enteritis ($p < 0.05$) (Ma and Wang, 2012). Xiaoping Han et al. (Han, 2016) randomized 78 influenza patients into control and observation groups. Patients in the control group were given oral oseltamivir phosphate, while patients in the observation group received HXZQC in addition to oseltamivir phosphate. Compared with those in the control group, patients in the observation group had faster relief of fever symptoms, muscle aches, and fatigue ($p < 0.05$). The total response rate in the observation group was 97.44%, which was higher than the 82.05% of the control group ($p < 0.05$). The results showed that HXZQC enhanced the efficacy of oseltamivir phosphate in the treatment of influenza. Xingzhou Wu (Wu, 2010) randomized 90 cold dampness patients into two groups: the treatment group received HXZQP, while the control group received Ribavirin injection + Compound paracetamol and

amantadine hydrochloride capsules. The total response rate in the treatment group was 88.9% compared to 77.1% in the control group ($p < 0.05$). Patients in the treatment group exhibited greater improvements in aversion to cold, fever, nasal congestion, running nose, spontaneous sweating, headache, cough and spitting, fatigue and weakness, body ache, and other cold symptoms compared to the control group. Hongjie Zhao et al. studied the efficacy and safety of HXZQC in the treatment of gastrointestinal-type cold by systematic evaluation. A total of 680 patients in eight randomized controlled trials (RCTs) were included in the research, and the results showed that the group receiving HXZQC had a significantly better clinical response than the group using Western medicines alone. The effects of HXZQC were superior to Western medicines in improving single symptoms (such as aversion to cold, fever, bowel sound and diarrhea) (Zhao et al., 2017). Dandan Yu et al. conducted a meta-analysis of 44 studies, including a total of 4,153 patients with acute gastroenteritis. The results showed that treatment with HXZQP + conventional therapy or norfloxacin tablets was superior to a single Western medicine in terms of total response rate and improvement of clinical symptoms (Yu et al., 2019).

Usage and Dosage of HXZQC

Oral administration, four capsules, twice a day.

Adverse Reactions of HXZQC

Potential drug eruption, purpura, shock, asthma, intestinal obstruction, upper gastrointestinal hemorrhage, hypoglycemia of childhood, infantile convulsions.

HXZQC Precautions

1) Nourishing traditional Chinese medicines should not be taken during the period of medication. 2) It is advisable that patients are on a light diet during the period of medication.

RELEVANT INFORMATION ON THE CLINICAL APPLICATION OF LHQWC

Recommended Therapeutic Regimens

LHQWC has been recommended in 19 therapeutic regimens for treatment of COVID-19 in China (see detailed information in **Tables 1** and **2**).

Ingredients of LHQWC

Forsythia suspensa (Thunb.) Vahl (Lianqiao), *Lonicera japonica* Thunb. (Jinyinhua), *Ephedra equisetina* Bunge (Zhimahuang), *Prunus armeniaca* L. (Chaoxingren), Gypsum fibrosum (Shigao), *Isatis tinctoria* L. (Banlangen), *Dryopteris crassirhizoma* Nakai (Guanzhong), *Houttuynia cordata* Thunb. (Yuxingcao), *Pogostemon cablin* (Blanco) Benth. (Guanghuoxiang), *Rheum palmatum* L. (Dahuang), *Rhodiola rosea* L. (Hongjingtian), Mentholum (Bohenao), and *Glycyrrhiza uralensis* Fisch. ex DC. (Gancao). Basic information on LHQWC is provided in the **Supplementary Table**.

Indications for the Treatment of COVID-19 With LHQWC

LHQWC is used during the clinical observation period of COVID-19, and wind-heat invading lung in early stage of the disease (mild case). The indicative symptoms are fever, mild aversion to cold, cough, weakness, headache and body pain, sore throat, and constipation.

Progress of Pharmacological Research on LHQWC

Modern pharmacological studies have shown that LHQWC has antiviral, immune-regulatory, anti-inflammatory, and antioxidant properties, efficacy against lung injury, and other effects (see **Table 3**).

LHQWC significantly inhibited SARS-CoV-2 replication in Vero E6 cells and markedly reduced the production of pro-inflammatory cytokines (TNF-α, IL-6, CCL-2/MCP-1, and CXCL-10/IP-10) at the mRNA level. Furthermore, LHQWC treatment resulted in abnormal virion particle morphology in cells. LHQWC significantly inhibits SARS-CoV-2 replication, affects virus morphology and exerts anti-inflammatory activity *in vitro* (Li R. F. et al., 2020).

In vitro experiments have shown significant antiviral activity against SARS-CoV, AIV H7N9, dual H1N1/H3N2, together with inhibition of Middle East Respiratory Syndrome (MERS)-CoV activity to a certain degree (Yao et al., 2020). Yuewen Ding et al. used MTT and plaque reduction assays to show that LHQWC inhibited proliferation of multiple strains of influenza virus, and reduced virus titer and levels of inflammatory cytokines in the lungs of infected mice. The results indicated that LHQWC acted as a broad-spectrum antiviral and, in particular, regulated the immune response to viral infection (Ding et al., 2017). Qi Li et al. discovered that LHQWC not only reversed LPS-stimulated expression of macrophage chemotactic factor-1 (MCP-1) by macrophages, but also significantly improved pulmonary edema in a mouse model of acute lung injury. Inhibition of expression and secretion of MCP-1 in lung tissues of model mice was accompanied by reduced infiltration of mononuclear macrophages and reduction of inflammatory injury (Li et al., 2019). Fen Ping et al. studied the effects of LHQWC on rats with oxidative lung injury caused by fine particulate matters (PM 2.5). The results showed that LHQWC significantly reduced lactate dehydrogenase (LDH) and malondialdehyde (MDA) serum levels in rats with lung injury, increased levels of glutathione peroxidase (GSH-Px), reduced pathological damage of lung tissues, and inhibited exudation of inflammatory cells into the alveolar cavity. Together, the data indicated that LHQWC protected against oxidative stress injury in the lungs of rats (Ping et al., 2016). Wenwen Cui et al. studied the impact of LHQWC in a mouse model of acute lung injury caused by intratracheal infusion of LPS. LHQWC alleviated the inflammatory response in lung tissues by downregulating the IKK/IκB/nuclear factor (NF)-κB signaling pathway, thus, protecting mice from acute lung injury (Cui et al., 2016). Siwen Tang et al. studied the effects of LHQWC intervention on pathological lung tissue injury in mice and expression of

inflammatory cytokines caused by exposure to automobile exhaust. The results showed that LHQWC reduced protein and mRNA expression of inflammatory cytokines in lung tissue by reducing blood levels of inflammatory cytokines, thus, protecting against lung tissue injury caused by automobile exhaust (Tang et al., 2015).

Clinical Research on LHQWC

Modern clinical studies have shown that LHQWC has therapeutic effects against viral diseases, such as COVID-19, SARS, MERS, influenza, and human infection with H7N9 avian influenza. It can also be used to treat URI, chronic obstructive pulmonary disease (COPD) and other conditions (see **Table 3**). LHQWC has been recommended in diagnosis and treatment schemes such as China's SARS Diagnosis and Treatment Scheme (Version 2004) (Chinese Medical Association and China Association of Chinese Medicine, 2004), MERS Diagnosis and Treatment Scheme (Version 2015) (National Health and Family Planning Commission of People's Republic of China, 2015), China's Influenza Diagnosis and Treatment Scheme (Version 2019) (National Health Commission of the People's Republic of China and National Administration of Traditional Chinese Medicine, 2019), and Diagnosis and Treatment Scheme for Human Infection with H7N9 Avian Influenza (Version 1, 2017) (National Health and Family Planning Commission of People's Republic of China, 2017).

Ke Hu et al. conducted a prospective multicenter open-label randomized controlled trial on LHQWC capsule in confirmed cases of COVID-19. Patients (284) were randomized to receive usual treatment alone or in combination with LHQWC capsules (four capsules, thrice daily) for 14 days. The primary endpoint was the rate of symptom (fever, fatigue, coughing) recovery. The recovery rate was significantly higher in the combined treatment group compared with the control group (91.5% vs. 82.4%, $p = 0.022$). The median time to symptom recovery was markedly shorter in the combined treatment group (median: 7 vs. 10 days, $p < 0.001$). Time to recovery of fever (2 vs. 3 days), fatigue (3 vs. 6 days) and coughing (7 vs. 10 days) was also significantly shorter in the combined treatment group (all $p < 0.001$). The rate of improvement in chest computed tomographic manifestations (83.8% vs. 64.1%, $p < 0.001$) and clinical cure (78.9% vs. 66.2%, $p = 0.017$) were also higher in the combined treatment group. However, the two groups did not differ in the rate of conversion to severe cases or viral assay findings ($p > 0.05$). No serious adverse events were reported (Hu et al., 2020).

Ruibing Lyu et al. conducted clinical research on 63 patients receiving conventional therapy in combination with LHQWC (treatment group) and 38 patients receiving only conventional therapy (control group). Clinical data were collected 10 days after the treatment. A comparison between the two groups was performed in terms of disappearance rates of cardinal symptoms (fever, cough, and weakness), duration of fever and disappearance rates of other individual symptoms and signs. The disappearance rates of fever, cough, and weakness in the treatment group were 86.7%, 55.6%, and 82.5%, respectively, which were higher than those in the control group (67.7%, 30.6%, and 58.6%; $p < 0.05$). The median duration of fever was 6 days in patients in the treatment group and 7 days in the control group. There was no statistically significant difference between the groups ($p = 0.171$). The disappearance rates of short breath and moist crackles (68.2% and 56.0%) were higher than those in the control group (20.0% and 20.0%, $p < 0.05$). There were four cases of aggravation in the treatment group (6.4%) and six cases in the control group (15.8%), with no statistically significant difference ($p > 0.05$). There were no obvious adverse reactions in the treatment group (Lyu et al., 2020).

Dezhong Cheng et al. conducted a multi-center retrospective analysis of the therapeutic effect of LHQWC in 51 COVID-19 patients. The control group was treated with simple nutritional support, symptomatic treatment, antiviral therapy and antimicrobial therapy. The treatment group was combined with LHQWC (6 g/bag) on the basis of the control group, one bag each time, 3 times a day. The clinical data of patients treated for 7 days were collected. The results showed that combined application of LHQWC significantly improved fever, weakness, cough, shortness of breath, chest distress, anorexia, and other clinical symptoms of COVID-19. Improvements of the main symptoms and reduced incidence of the severe form suggested that LHQWC could be effective in the treatment of patients with COVID-19 (Cheng et al., 2020).

Ping Yu et al. conducted a study on the therapeutic effect of LHQWC combined with Abidole in the treatment of mild COVID-19. A total of 295 patients were randomly divided into two groups. The control group (n = 148) was treated with Abidole (0.2 g per day) orally, and the observation group (n = 147) was treated with LHQWC (6 g, thrice daily) combined with Abidole. The results showed that the total effective rate of the observation group was significantly higher than that of the control group (80.95% vs 64.86%), and the rate of severe illness was significantly lower than that of the control group (14.29% vs 23.65%). After 7 days of treatment, the scores for the main TCM syndromes (fever, fatigue, cough, dry throat, chest tightness) and the levels of C-reactive protein (CRP) and procalcitonin (PCT) in the observation group were significantly lower than those in the control group ($p < 0.05$), while white blood cells (WBC) and lymphocyte (LYM) were significantly higher than those in the control group. The effective rate of chest computerized tomography (CT) in the observation group was 69.39%, which was higher than that in the control group (62.84%), but the difference was not statistically significant ($p > 0.05$). There were no serious drug-related adverse reactions in either group. The results show that LHQWC combined with Abidole can effectively relieve clinical symptoms in patients with mild COVID-19, regulate the expression of related inflammatory factors, improve the curative effect and reduce the rate of severe illness (Yu P. et al., 2020).

Lili Liu et al. conducted a retrospective analysis of the therapeutic effect of LHQWC in 32 COVID-19 patients. The patients were divided into two groups: Group A + L, in which 18 patients received Abidole (0.2 g, thrice daily) combined with LHQWC; and Group L, in which 14 patients received LHQWC alone. During treatment there was one critical case in each group.

Abnormal liver function was observed in 14 cases (77.78%) in Group A + L and 8 cases (57.14%) in Group L. Antibiotic treatment was applied in 17 cases (94.44%) in Group A + L and 13 cases (92.86%) in Group L. Glucocorticoid use was reported in 10 cases (55.56%) in Group A + L and 9 cases (64.29%) in Group L. Compared with Group L, significantly faster recovery of temperature ($t = -2.471$, $p = 0.019$), recovery of respiratory symptoms ($t = -2.918$, $p = 0.007$), chest CT inflammation absorption ($t = -2.937$, $p = 0.006$), time until two consecutive negative virus nucleic acid tests ($t = -2.930$, $p = 0.006$), and shorter hospital stay ($t = -2.785$, $p = 0.009$) were observed in Group A + L. Abidor combined with LHQWC can be used to treat COVID-19, with good tolerance, to shorten the course of treatment (Liu et al., 2020).

Zhongping Duan et al. conducted a random, double-blind, and positive-drug parallel control clinical trial on the efficiency and safety of LHQWC against H1N1. It was found that LHQWC reduced disease severity and the duration of symptoms. The drug was also well tolerated, indicating that LHQWC might become an alternative therapeutic measure against H1N1 viral infection (Duan et al., 2011). Pan Zhao et al. found by meta-analysis that LHQWC improved cough, sore throat, body ache, and other symptoms of patients infected with H1N1 virus, reduced the duration of fever, and was more effective than oseltamivir (Zhao P. et al., 2014). Shiheng Wang et al. conducted a systematic review of the literature on efficacy and safety of LHQWC in treating viral flu. The results showed that LHQWC gave a better total response rate, symptom improvement rate and body temperature recovery rate than the control group in treating viral flu, but consideration of its safety was important (Wang et al., 2019). Li Tiehui et al. compared the clinical therapeutic effect of LHQWC and vitamin C Yinqiao Tablets in patients with URI, and found that LHQWC significantly improved nasal congestion, fever, headache, sore throat, weakness, aches in the limbs, intolerance of cold, and other clinical symptoms. LHQWC had high efficacy and safety, and was therefore worthy of promotion (Li, 2019). Dong Liang et al. conducted clinical research on 100 patients with COPD, and discovered that LHQWC improved conditions in patients with acute exacerbation of chronic obstructive pulmonary disease (AECOPD), especially those in the high risk subgroup. The mode of action might be related to its ability to reduce release of inflammatory mediators (Dong et al., 2014).

Usage and Dosage of LHQWC:

Oral administration, four capsules, 3 times a day.

Adverse Reactions of LHQWC

Possible nausea, vomiting, diarrhea, stomach discomfort, heartburn, poor appetite, and other gastrointestinal adverse reactions; there might be abnormal liver function, palpitations or rash, and other side effects occasionally.

LHQWC Precautions

1) Pregnant and lactating women should use with caution. 2) It contains ephedrae herba (Mahuang), so should be used with caution by athletes and patients with high blood pressure and heart disease. 3) Those with previous history of liver disease or with abnormal liver function should use with caution. 4) It contains rheum, so the dose should be reduced appropriate in subjects who experience increased stool frequency and shapeless stools after administration. 5) Nourishing traditional Chinese medicine should not be taken at the same time.

RELEVANT INFORMATION ON THE CLINICAL APPLICATION OF JHQGG

Recommended Therapeutic Regimens

JHQGG has been recommended in 13 therapeutic regimens of COVID-19 in China (see detailed information in **Tables 1** and **2**).

Ingredients of JHQGG

Forsythia suspensa (Thunb.) Vahl (Lianqiao), *Lonicera japonica* Thunb. (Jinyinhua), *Ephedra equisetina* Bunge (Zhimahuang), *Prunus armeniaca* L. (Chaoxingren), Gypsum Fibrosum (Shigao), *Scutellaria baicalensis* Georgi (Huangqin), *Fritillaria thunbergii* Miq. (Zhebeimu), *Anemarrhena asphodeloides* Bunge (Zhimu), *Arctium lappa* L. (Niubangzi), *Artemisia annua* L. (Qinghao), *Mentha canadensis* L. (Bohe), and *Glycyrrhiza uralensis* Fisch. ex DC. (Gancao). Basic information on JHQGG is provided in the **Supplementary Table**.

Indications for the Treatment of COVID-19 With JHQGG

JHQGG is used to treat the syndrome of wind-heat invading lung during the clinical observation period of COVID-19 and the early stage of the disease (mild case). Indicative symptoms are fever, mild aversion to cold, weakness, cough, headache and body pain, and sore throat.

Progress of Pharmacological Research on JHQGG

Modern pharmacological studies have found that JHQGG has antiviral, immune-regulatory, and anti-inflammatory effects (see **Table 3**).

Jianping Qi et al. showed that JHQGG significantly decreased the average levels of C-reactive protein (CRP) and IFN-γ in serum of influenza patients. Patients exhibited decreased inflammatory response and improved immune function after treatment, which might be due to the antiviral activity of the main ingredients, such as Lonicerae japonicae flos, Scutellariae radix, Forsythiae fructus, and Artemisiae annuae herba (Qi et al., 2016).

Clinical Research on JHQGG

Modern clinical studies have shown that JHQGG has therapeutic efficacy against viral diseases (see **Table 3**). JHQGG has been recommended in China's Influenza Diagnosis and Treatment Scheme (Version 2019).

COVID-19 outpatients (123) were randomly divided into a treatment group (JHQGG two bags per time, 3 times a day, combined with routine treatment for 5 days, n = 82) and a

control group (only routine treatment, n = 41). The addition of JHQGG significantly alleviated fever, cough, fatigue, sputum and anxiety, and the hospitalization rate tended to be lower than in the control group (Duan et al., 2020). In treatment of H1N1, use of JHQGG alone or in combination with oseltamivir effectively shortened fever duration. The duration of fever in patients treated with oseltamivir in combination with JHQGG was significantly shorter (19%) than in those treated with oseltamivir alone, suggesting that JHQGG could serve as an alternative therapeutic measure against H1N1 (Wang C. et al., 2011). Jianping Qi observed 174 cases of influenza patients and found that JHQGG significantly reduced serum levels of cytokines and improved immune function (Qi et al., 2016). A double-blind, randomized and controlled study on JHQGG in treating influenza syndrome of wind-heat invading lung by Guoqin Li et al. showed that it was effective and safe (Li et al., 2013).

Usage and Dosage of JHQGG

Taken after dissolving in boiled water, one bag, 3 times a day.

Adverse Reactions of JHQGG

Potential for nausea, vomiting, diarrhea, stomach discomfort, heartburn, poor appetite and other gastrointestinal adverse reactions; there might be abnormal liver function, palpitations, or rash occasionally.

JHQGG Precautions

1) Those with deficiency-cold in spleen and stomach should use with caution. 2) It contains ephedrae herba (Mahuang), so should be used with caution by athletes and patients with high blood pressure and heart disease. 3) Those with previous history of liver disease or with abnormal liver function should use with caution. 4) Nourishing traditional Chinese medicine should not be taken at the same time.

RELEVANT INFORMATION ON THE CLINICAL APPLICATION OF SFJDC

Recommended Therapeutic Regimens

SFJDC has been recommended in 15 therapeutic regimens of COVID-19 in China (see detailed information in **Tables 1** and **2**).

Ingredients of SFJDC

Reynoutria japonica Houtt. (Huzhang), Forsythia suspensa (Thunb.) Vahl (Lianqiao), Isatis tinctoria L. (Banlangen), Bupleurum chinense DC. (Chaihu), Patrinia scabiosifolia Link (Baijiangcao), Verbena officinalis L. (Mabiancao), Phragmites australis (Cav.) Trin. ex Steud. (Lugen), and Glycyrrhiza uralensis Fisch. ex DC. (Gancao). Basic information on SFJDC is provided in the **Supplementary Table**.

Indications for the Treatment of COVID-19 With SFJDC

SFJDC is used to treat external wind-heat syndrome during the clinical observation period of COVID-19 and the early stage of the disease (mild case). Indicative symptoms are fever, aversion to cold, cough with yellow phlegm, weakness, and sore throat.

Progress of Pharmacological Research on SFJDC

Modern pharmacological studies have found that SFJDC has antiviral, antibacterial, and anti-inflammatory properties and protects against lung injury (see **Table 3**).

Yanyan Bao et al. evaluated the broad-spectrum antiviral activity of SFJDC by cytopathic effect (CPE) inhibition. A total of eight viruses, including H1N1, herpes simplex (HSV), respiratory syncytial virus, adenovirus (ADV) and Coxsackie virus, were evaluated. SFJDC had significant *in vitro* broad-spectrum antiviral activity and the best inhibitory effect was against parainfluenza virus (PIV). Similar results were obtained from *in vivo* experiments (Qiu et al., 2014; Bao et al., 2019). Ying Liu et al. used H1N1 FM1 and PR8 strains to induce nasal drip infection in an immunocompromised mouse pneumonia model. Therapeutic and preventive effects of SFJDC were observed against H1N1 infection *in vivo*. The results showed that SFJDC influenced the immune function of the mice, improved pneumonia symptoms caused by influenza virus, reduced the lung index of mice infected with H1N1, significantly reduced mortality, and had good therapeutic efficacy (Liu et al., 2010). Research by Weiwei Lv et al. found that SFJDC had inhibitory activity against multiple viruses and bacteria. Its antiviral activity was inferior to that of Ribavirin, but its cytotoxicity was lower. Both antiviral activity and antibacterial action were superior to those of Qingkailing granules (QKLG) (Lv et al., 2013). Li Ma et al. used a mouse pneumonia model induced by *Streptococcus pneumoniae* to study the anti-inflammatory mechanism of SFJDC. They discovered that it reduced white blood cell (WBC) count, reduced serum levels of the transcription factor nuclear factor kappa B (NF-κB), MCP-1, inflammatory mediator BK and COX-2, thus, having a therapeutic effect in the model (Ma et al., 2018). Further studies found that SFJDC had a significant immune regulatory function, reducing levels of B lymphocytes, $CD8^+$ cells, interleukin-1α (IL-1α), IL-1β, IL-2, IgM, and IgG to improve lung function in mice with pneumonia. SFJDC increased the $CD4^+/CD8^+$ ratio and number of natural killer (NK) cells, thus, having a therapeutic effect in the pneumonia model (Ma et al., 2019a; Ma et al., 2019b). Zhengang Tao et al. observed a protective function of SFJDC against endotoxin LPS-induced rat lung injury. Their results showed that SFJDC inhibited the LPS-induced inflammatory response, and reduced LPS-induced lung injury. Its mechanism of action might be inhibition of the MAPK (mitogen-activated protein kinase)/NF-κB signaling pathway and downregulation of NF-κB mRNA expression (Tao et al., 2014). Yanmei Li et al. used a *Pseudomonas aeruginosa* (PAK)-induced KM mouse acute lung injury model to explore the mode of action of SFJDC in treatment of acute lung injury. They found that SFJDC significantly alleviated lung injury in the model and its mode of action might be related to the ERK signaling pathway (Li et al., 2017).

Clinical Research on SFJDC

SFJDC comes from "Detoxification Powder," and is mainly used to treat fever, parotitis, amygdalitis, plague, and other diseases. Recent studies have shown that SFJDC has good clinical efficacy against viral diseases (such as MERS, influenza, human infection with H7N9 avian influenza) and respiratory diseases (such as acute URI, AECOPD, pneumonia, etc.) (see **Table 3**). SFJDC has been recommended in diagnosis and treatment schemes such as MERS Diagnosis and Treatment Scheme (Version 2015), China's Influenza Diagnosis and Treatment Scheme (Version 2019), and Diagnosis and Treatment Scheme for Human Infection with H7N9 Avian Influenza (Version 1, 2017).

Lei Wang et al. conducted a retrospective analysis of 87 patients with acute URI + fever, and found that patients treated with SFJDC had a significantly higher total response rate than those in the control group. SFJDC effectively improved respiratory symptoms in patients with acute URI + fever (Wang and Qiu, 2018). In the treatment of community acquired pneumonia (CAP), application of SFJDC shortened recovery time, reduced the duration of fever and reduced the levels of procalcitonin (PCT), CRP, WBC, and other indicators, effectively shortening the course of treatment (Wang, 2016). Hongxia Yu et al. evaluated the impact of SFJDC on inflammation-associated cytokines in patients with AECOPD. The results showed that SFJDC significantly reduced the levels of interleukin-8 (IL-8), TNF-α, CRP, and PCT, and had significant therapeutic efficacy against AECOPD (Yu H. X. et al., 2020). Tiling Wang et al. added SFJDC treatment to conventional treatment in 60 mild and moderate AECOPD patients and compared with 60 patients receiving conventional treatment as the control group. After 1 week, the treatment group had significantly higher arterial blood gas PaO_2 than the control group, without any adverse reactions (Wang, 2015). Research showed that treatment of bacterial acute bronchitis and pneumonia with a combination of antibacterial drugs and SFJDC significantly shortened recovery of body temperature, duration of cough and the course of treatment compared with antibacterial drug alone. Chunlan Wang et al. observed that combined use of SFJDC and antibiotics significantly improved body temperature, blood sugar, ALT (glutamate transaminase), AST (aspartate aminotransferase) and other indicators compared with the control group, and patients had a higher oxygen index than the control group. The results suggested that SFJDC had a significant protective function against lung injury, and the mechanism might be related to inhibition of inflammatory response by SFJDC (Wang et al., 2014).

Usage and Dosage of SFJDC

Oral administration, four capsules, 3 times a day.

Adverse Reactions of SFJDC

Occasional nausea.

SFJDC Precautions

(1) Use is forbidden in those with allergic constitution or who are allergic to the drug. (2) Use is forbidden in those with deficiency-cold in spleen and stomach.

RELEVANT INFORMATION ON THE CLINICAL APPLICATION OF TRQI

Recommended Therapeutic Regimens

TRQI has been recommended in 12 therapeutic regimens of COVID-19 in China (see detailed information in **Tables 1** and **2**).

Ingredients of TRQI

Scutellaria baicalensis Georgi (Huangqin), Ursi fellis pulvis (Xiongdanfen), *Forsythia suspensa* (Thunb.) Vahl (Lianqiao), and *Lonicera japonica* Thunb. (Jinyinhua). Basic information on TRQI is provided in the **Supplementary Table**.

Indications for the Treatment of COVID-19 With TRQI

TRQI is used for syndromes of epidemic toxin lung closure and phlegm-heat lung obstruction in the progressive stage of COVID-19 (critical case). Indicative symptoms are fever, cough, cough with difficulty in expectoration, chest distress, and shortness of breath.

Progress of Pharmacological Research on TRQI

Modern pharmacological studies have shown that TRQI is effective against influenza virus, destroys bacterial biofilm, inhibits airway inflammation, and improves lung injury (see **Table 3**).

Research by Jinsu Zheng et al. discovered that TRQI improved pathological injury of lung tissues in mice infected with influenza virus, and had significant antiviral activity in influenza virus infected mice. The antiviral activity of TRQI might be due to its inhibition of cellular proliferation and enhancement of immunity (Zheng and Gu, 2009). Weifeng Yang et al. discovered that TRQI could destroy methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm and induce its death. When combined with vancomycin or linezolid below the minimal inhibitory concentration (MIC) concentration, synergistic anti-biofilm activity was observed that was significantly higher than when using TRQI alone (Yang et al., 2018). Research by Yi Wang et al. showed that the efficacy of TRQI in the treatment of acute pneumonia was mediated by destruction of bacterial biofilm, which is different to the mechanism of penicillin (Wang Y. et al., 2011). Wei Liu et al. discovered that TRQI might treat airway mucus hypersecretion by regulating the interleukin-17 (IL-17) signaling pathway and its downstream protein MUC5AC. An *in vivo* experiment showed that TRQI could significantly inhibit excessive secretion of LPS-stimulated MUC5AC and expression of TNF-α, interleukin-6 (IL-6), IL-8, and IL-17A in terms of protein and mRNA levels (Liu W. et al., 2019). Animal experiments conducted by Wei Liu et al. showed that TRQI inhibited airway inflammation caused by LPS through the MAPK/NF-κB pathway, and showed a dose-dependent effect (Liu et al., 2016). Li Wen et al. found that TRQI improved signs and symptoms in AECOPD patients, which might be mediated by reduction of serum IL-8 and neutrophil elastase (NE) levels, and improved airway inflammation and mucus

hypersecretion (Li et al., 2010). Research by Li Pengtao et al. discovered that TRQI improved blood flow in capillaries of the alveolar walls while repressing the LPS-induced inflammatory cascade, which was the pharmacological basis for its effective alleviation of acute lung injury and prevention of decreased arterial partial oxygen pressure (Li et al., 2005).

Clinical Research on TRQI

Modern clinical studies have shown that TRQI has therapeutic efficacy against infectious diseases, such as viral pneumonia, MERS, human infection with H7N9 avian influenza, acute bronchitis, acute attack of chronic bronchitis, CAP, tuberculosis accompanied by infection, and AECOPD (see **Table 3**). TRQI has been recommended in MERS Diagnosis and Treatment Scheme (Version 2015) and Diagnosis and Treatment Scheme for Human Infection with H7N9 Avian Influenza (Version 1, 2017).

A systematic evaluation of eight published randomized and controlled trials that included a total of 590 adult patients with viral pneumonia found that TRQI had advantages in terms of response rate, faster change of chest radiography, average length of stay, and other aspects (Pan, 2016). Research results from Jinzhi Liang et al. showed that there was no statistically significant difference in the clinical effect of combined TRQI and Ribavirin or TRQI alone in the treatment of hand-foot-and-mouth disease. Both treatments were superior to that of Ribavirin alone (Liang et al., 2013). Research by Wang Pei et al. showed that potential benefits of TRQI in the treatment of acute bronchitis included improved response rate, and reduced fever, cough, crackles, and X-ray shadow absorption (Wang et al., 2016). Research results of Lini Gao et al. showed that combined use of TRQI and Western medicines was more effective than Western medicines alone in the treatment of acute bronchitis and gave superior improvement of clinical symptoms (Gao et al., 2019). Hongli Jiang et al. showed by systematic evaluation that administration of TRQI to treat CAP on the basis of antibiotics and symptomatic treatment significantly improved clinical symptoms. Cough with expectoration was improved, the duration of fever was shortened and recovery of chest radiography and hemogram were promoted without significant adverse reactions (Jiang et al., 2009). Lian Xiong et al. showed by systematic evaluation that TRQI might have the same overall effect as some antibacterial drugs in treatment of patients with tuberculosis accompanied by lung infection, but improved efficacy was observed in combination with antibacterial drugs. This might be due to the bacteriostatic effects of TRQI and elimination of inflammatory mediators (Lian et al., 2018). A total of 14 trials and 954 patients were included in a study by Yunqing Zhong, and the results showed that combined use of TRQI and antibacterial drugs improved the clinical effects and lung function in AECOPD patients, reduced pCO_2, and shortened the length of stay without serious adverse reactions (Zhong et al., 2010).

Usage and Dosage of TRQI

20 ml once for adults, 40 ml once for severe patients, with addition of 250–500 ml 5% glucose or 0.9% sodium chloride; intravenous drip at less than 60 drops per min, once a day.

Adverse Reactions of TRQI

1) Some patients may have dizziness, chest distress, nausea, vomiting, and diarrhea. 2) Flushing, rash or itching and other allergic reactions occasionally. 3) Rarely, palpitations, chill and difficulty breathing. 4) Extremely rarely, allergic shock. 5) Other adverse reactions: dry mouth, fever, periorbital facial edema, discomfort at infusion site.

TRQI Precautions

1) Use is forbidden in those with liver and renal failure; 2) Use is forbidden in those with severe lung and heart disease accompanied by heart failure; 3) Use is forbidden in pregnant women and infants less than 24 months; 4) It should be used alone and must not be mixed with other drugs; 5) Dilution ratio of the liquid shall be no lower than 1:10 (liquid: solvent) and the diluted liquid must be used within 4 h.

RELEVANT INFORMATION ON THE CLINICAL APPLICATION OF XYPI

Recommended Therapeutic Regimens

XYPI has been recommended in 15 therapeutic regimens of COVID-19 in China (see detailed information in **Tables 1** and **2**).

Ingredients of XYPI

Andrographolide total sulfonate. Basic information on XYPI is provided in the **Supplementary Table**.

Indications for the Treatment of COVID-19 With XYPI

XYPI is used for syndrome of exuberance of internal heat toxin in progressive stage of COVID-19 (critical case). Indicative symptoms are fever, sore throat, cough with yellow phlegm and chest distress. It could also be used to treat viral infection combined with mild bacterial infection.

Progress of Pharmacological Research on XYPI

The main ingredient of XYPI is andrographolide total sulfonate, which is antipyretic, anti-inflammatory, antiviral, antibacterial and immune-regulatory (see **Table 3**).

Yang Yu et al. conducted *in vivo* experiments with XYPI, and discovered that it could significantly protect mice infected with *Staphylococcus aureus* and *Streptococcus pneumoniae*, and significantly inhibited citric acid-induced cough frequency in guinea pigs (Yu et al., 2009). Using *in vitro* experiments, Lu Wang et al. studied the inhibitory effect of XYPI on inflammatory factors released by LPS-stimulated mouse mononuclear macrophages. The results showed that XYPI significantly inhibited the release of inflammatory factors such as TNF-α and IL-6 (Wang et al., 2008). Yinglan Nie et al. explored the mode of action of XYPI in the treatment of acute lung injury by observing its effect on cytokine content in bronchoalveolar lavage fluid (BALF) following LPS-induced acute lung injury. The results

showed that XYPI could play an anti-inflammatory role by modulating the balance of pro-inflammatory/anti-inflammatory cytokines and prevent excess anti-inflammatory responses during the course of acute lung injury (Nie et al., 2012). Qi Liu et al. observed antiviral activity of XYPI against human rhinovirus-induced mouse infections. XYPI significantly reduced the virus titer in trachea-lung tissue homogenate of infected mice, effectively inhibiting proliferation of human rhinovirus in mice. Respiratory lesions were alleviated in tested animals, survival rate was improved, there were few adverse reactions, and it was an efficient and safe drug against human rhinovirus infection. Its specific mode of action, however, was unclear (Liu, 2015).

Clinical Research on XYPI

XYPI is a broad-spectrum antiviral Chinese patent medicine that is widely used to treat acute URI, viral pneumonia and pulmonary infection in clinical practice with good efficacy. Recent studies have discovered that it can also inhibit some viruses and bacteria, and could be used to treat influenza, human infection with H7N9 avian influenza, capillary bronchitis and other diseases (see **Table 3**). XYPI has been recommended in China's Diagnosis and Treatment Scheme for Human Infection with H7N9 Avian Influenza (Version 2017).

Xiuping Yin et al. used the association rule method to analyze drug combinations, including XYPI in patients with pulmonary infection. The results showed that it could play a role as alternative or as a supplement to antibiotics in the treatment of pulmonary infection, but the safety and rationality of its use in drug combinations required further study in clinical practice (Yin et al., 2015). Guangming Li et al. conducted a retrospective analysis of 92 patients with viral pneumonia and found that XYPI was more effective than Ribavirin, providing significant improvement of symptoms (Li, 2015). Ruihan Qi et al. analyzed the therapeutic effect of XYPI in the treatment of viral pneumonia by systematic evaluation and found that it was more effective than Ribavirin. XYPI increased the cure rate, improved signs and symptoms, and reduced the incidence of adverse reactions (Qi et al., 2018). Lili Zhang et al. used XYPI in combination with Western medicine to treat severe pneumonia of the elderly in clinical practice. The results showed that it significantly shortened the course of disease, improved treatment efficiency, reduced the incidence of antibiotic resistance, reduced occurrence of double infection, improved the prognosis, and reduced mortality (Zhang and Wang, 2015). Zhixu Yang et al. observed the clinical effect of XYPI in treating the syndrome of phlegm-heat obstructing lung of severe pneumonia from the perspective of traditional Chinese medicine. The results showed significant improvements that included reduced fever, reduced numbers of leukocytes, improved oxygen index, lower clinical pulmonary infection score (CPIS), and reduced pulmonary inflammation. It also shortened the duration of mechanical ventilation and length of stay in ICU, and improved the clinical effect (Yang et al., 2014). In addition, XYPI has also shown significant efficacy in the treatment of URI. Xiaowen Liu et al. conducted a retrospective analysis of 660 patients with acute URI and found that the total response rate in the XYPI treatment group was significantly higher than that of the control group. The difference was statistically significant (Liu and Li, 2015).

Usage and Dosage of XYPI

1) Intramuscular injection. Adults: 50-100 mg, 2 or 3 times a day. 2) Intravenous drip. Adults: 250–500 mg a day, diluted with 0.9% sodium chloride or 5% glucose.

Adverse Reactions of XYPI

The main adverse reactions are allergic reaction, damage to the skin, damage to the digestive system, damage to the respiratory system, and general damage to the cardiovascular system. These are manifested by rash, itching, shivering, facial blushing, fever, cyanosis, difficulty breathing, nausea, vomiting, palpitations, chest distress, and allergic shock.

XYPI Precautions

1) Use is forbidden in pregnant women and children under 1 year of age. Use with caution in the elderly above 75 years of age. 2) Use is forbidden in those with a history of allergic or severe adverse reactions to this drug or preparations containing andrographolide total sulfonate. 3) Enhanced monitoring is recommended in patients using XYPI for the first time; pay close attention to reactions during administration, especially if discovering abnormalities within 30 min of administration. Stop administration immediately and take active rescue measures. 4) When used in combination with other injections, XYPI should be administered first. Other injections can be infused after flushing or replacing the infusion tube.

RELEVANT INFORMATION ON THE CLINICAL APPLICATION OF XBJI

Recommended Therapeutic Regimens

XBJI has been recommended in 20 therapeutic regimens of COVID-19 in China (see detailed information in **Tables 1** and **2**).

Ingredients of XBJI

Carthamus tinctorius L. (Honghua), *Paeonia lactiflora* Pall. (Chishao), *Conioselinum anthriscoides* 'Chuanxiong' (Chuanxiong), *Salvia miltiorrhiza* Bunge (Danshen), and *Angelica sinensis* (Oliv.) Diels (Danggui). Basic information on XBJI is provided in the **Supplementary Table**.

Indications for the Treatment of COVID-19 With XBJI

XBJI is used for syndrome of blood-stasis and toxins in the progressive stage of COVID-19 (critical case). Indicative symptoms are fever, dyspnea and tachypnea, palpitations, and dysphoria. It could also be used for treatment of infection-induced systemic inflammatory response syndrome and multiple-organ dysfunction syndrome in the stage of impaired organ function.

Progress of Pharmacological Research on XBJI

Modern pharmacological studies have shown that XBJI is anti-inflammatory, antioxidant, immune-regulatory, and protects against acute lung injury (see **Table 3**).

Tiantian Li et al. found that in mice with MRSA-induced sepsis, XBJI protected the infected mice by downregulating expression of inflammatory cytokines stimulated by Pam3CSK4, MAPK, PI3K (phosphatidylinositol 3 kinase)/Akt and other pathways, thus, inhibiting the inflammatory response (Li T. T. et al., 2020). Shuwen Zhang et al. and Xi Chen et al. found that XBJI significantly reduced TNF-α, IL-6, and IL-10 levels in mice with sepsis, prevented neutrophil infiltration of lung and kidney, modulated T helper cell (Th) 1/Th2, Th17, and Tregs balance, reduced inflammatory response, and improved survival rate in mice with infectious shock (Zhang et al., 2006; Chen et al., 2018). Mingwei Liu et al. studied rats with paraquat-induced acute lung injury and discovered that XBJI could enhance immunity, reduce expression of inflammatory factors, and protect against acute lung injury by blocking p-38 MAPK and NF-κB p65 pathways, (Liu et al., 2014). Research by Yin Teng et al. found that XBJI in combination with conventional treatment significantly reduced interleukin-1 (IL-1), IL-6, and TNF-α levels, improved $CD4^+/CD8^+$ T lymphocyte ratio and NK cell relative activity, reduced inflammatory response, and enhanced cellular immunity in patients with severe pneumonia (Teng et al., 2012). Research by Hui Jin et al. showed that XBJI significantly improved the activity of superoxide dismutase (SOD), reduced reactive oxygen species (ROS) levels and protected against oxidative damage in mice under high-temperature stimulation (Jin et al., 2018). Research by Luo Peng et al. showed that XBJI downregulated MDA levels, upregulated SOD levels, and alleviated LPS-induced acute lung injury in rats (Luo and Zhou, 2017). In a rat model of oleic acid or LPS-induced acute lung injury, XBJI reduced TNF-α levels, alleviated pulmonary tissue edema and inflammatory cell infiltration, and protected against lung injury (Zhang et al., 2014). Research by Yuexia Ma et al. showed that although XBJI had no direct antiviral effect in mice with H1N1 severe pneumonia; it alleviated lung injury and protected against death, which might be due to its regulation of inflammatory cytokine levels in the early stage (Ma et al., 2015).

Clinical Research on XBJI

Modern clinical studies have shown that XBJI in combination with conventional treatment has therapeutic effects in relevant diseases, such as MERS, human infection with H7N9 avian influenza, CAP, severe pneumonia, systemic inflammatory response syndrome, COPD and sepsis (see **Table 3**). XBJI has been recommended in MERS Diagnosis and Treatment Scheme (Version 2015) and Diagnosis and Treatment Scheme for Human Infection with H7N9 Avian Influenza (Version 1, 2017).

Clinical research by Wen Long et al. randomly divided 60 severe COVID-19 patients into routine treatment (n = 20), XBJI 50 ml (n = 20), and XBJI 100 ml (n = 20) groups. On the basis of conventional treatment, XBJI (50 ml) was injected twice a day for 7 days in the XBJI 50 ml group, or 100 ml twice a day for 7 days in the XBJI 100 ml group. After treatment, the white blood cell count (WBC) and lymphocyte count (LYM) of the three groups increased, while CRP and ESR decreased. Compared with the routine treatment group, the WBC count in the XBJI 100 ml group after treatment significantly increased ($\times 10^9$/L: 7.12 ± 0.55 vs. 5.67 ± 0.51, $p < 0.05$), and the levels of CRP and ESR in the XBJI 50 ml and 100 ml groups significantly decreased [CRP (mg/L): 32.3 ± 4.6, 28.0 ± 6.2 vs. 37.3 ± 5.9; ESR (mm/h): 45.9 ± 5.7, 40.5 ± 7.4 vs. 55.3 ± 6.6, all $p < 0.05$]. Compared with the XBJI 50 ml group, the increase of WBC, and the decrease of CRP and ESR were more significant in the XBJI 100 ml group [WBC ($\times 10^9$/L): 7.12 ± 0.55 vs. 5.82 ± 0.49; CRP (mg/L): 28.0 ± 6.2 vs. 32.3 ± 4.6; ESR (mm/h): 40.5 ± 7.4 vs. 45.9 ± 5.7, all $p < 0.05$]. The APACHE II score of three groups decreased. In the XBJI 100 ml group, the APACHE II score after treatment was significantly lower than those in the routine treatment and XBJI 50 ml groups (12.3 ± 1.5 vs. 16.5 ± 1.6, 15.9 ± 1.4, both $p < 0.05$). After treatment, the 2019-nCoV nucleic acid test in the three groups partly turned negative: nine cases in the routine treatment group, eight cases in the XBJI 50 ml group and nine cases in the XBJI 100 ml group, with no significant differences ($p > 0.05$). The conditions of patients in the three groups were improved after treatment. Eight cases in the routine treatment group were transformed into common type and one case into critical type; nine cases and 12 cases in the XBJI 50 ml and 100 ml groups, respectively, were transformed into the common type. Patients in the XBJI 100 ml group improved more obviously than in the XBJI 50 ml and routine treatment groups (both $p < 0.05$). The XBJI injection can effectively improve the inflammatory markers and prognosis of severe COVID-19 patients (Wen et al., 2020).

Clinical research by Qi Fei et al. showed that, of 80 patients with severe pneumonia, those receiving a combination of XBJI and conventional treatment exhibited reduced levels of blood LDH, α1-acid glycoprotein (α1-AG) and α1-antitrypsin (α1-AT). Body temperature was reduced significantly and secretion of TNF-α, IL-6, IL-8, and other cytokines was inhibited. The total treatment efficiency was up to 80%, compared to 67.5% in the control group (Qi et al., 2011). An RCT study comprised of 33 centers and 710 patients conducted by Yuanlin Song et al. showed that XBJI in combination with conventional treatment significantly improved the primary endpoint, pneumonia severity index, in patients with severe CAP (the control group vs XBJI Group, 46.33% vs 60.78%, $p < 0.001$). There was also significantly reduced mortality in 28 days (24.65% vs 15.87%, $p = 0.006$), the duration of mechanical ventilation was shortened (11 vs 16.5 d, $p = 0.012$) and length of stay in ICU was reduced (12 vs 16 d, $p = 0.004$) (Song et al., 2019). Mingjin Zhu et al. conducted a meta-analysis of 12 studies with a total of 860 patients and showed that XBJI in combination with conventional treatment was superior to the treatment group in improving total response rate in patients with severe pneumonia. Infectious indicators (WBC, CRP, CPIS) and inflammatory cytokine (IL-6, IL-8, TNF-α) levels were reduced, and the average length of stay in hospital was reduced (Zhu et al., 2014). Wei Zhao et al. studied 56

patients with systemic inflammatory response syndrome (SIRS) and found that after 7 d treatment with XBJI in combination with conventional treatment, body temperature, WBC, and acute physiology and chronic health evaluation II (APACHE-II) score improved more significantly compared to the control group ($p < 0.05$). Expression of $CD4^+$, $CD4^+/CD8^+$, $CD14^+$/HLA-DR (human leukocyte antigen-DR) increased significantly, and the combination regulated the SIRS immune state and improved systemic status of the patients (Zhao W. et al., 2014). Clinical research found that XBJI in combination with conventional treatment lowered TNF-α, CRP, and other inflammatory indicators in AECOPD patients and had a certain therapeutic effect. In patients with accompanying SIRS, the combination significantly improved cough, expectoration, shortness of breath, and other clinical symptoms, and shortened hospital stay (Chen et al., 2011; Zhu et al., 2019). Meta-analysis by Chengyu Li et al. included sepsis patients from 16 RCTs (total 1,144 cases), and evidence of moderate intensity showed that XBJI in combination with conventional treatment effectively reduced the mortality rate of sepsis patients over 28 d (934/1144, $p < 0.00001$), APACHE-II score (792/1144, $p < 0.00001$) and body temperature (362/1144, $p < 0.00001$) (Li et al., 2018).

Usage and Dosage of XBJI

Intravenous injection. 1) Systemic inflammatory response syndrome: 50 ml plus 100 ml 0.9% sodium chloride injection for intravenous drip, completed in 30–40 min, twice a day. Three times a day for severe patients. 2) Multiple-organ dysfunction syndrome: 100 ml plus 100 ml 0.9% sodium chloride injection for intravenous drip, completed in 30–40 min, twice a day. Three or four times a day for severe patients.

Adverse Reactions of XBJI

Allergic reactions: skin flush, rash, itching, palpitations, cyanosis, laryngeal edema, allergic shock, etc. Cardiovascular system: palpitations, cyanosis, increase, or decrease of blood pressure, arrhythmia. Nervous system: dizziness, headache. Respiratory system: difficulty breathing, chest distress, labored breathing, shortness of breath, and cough. Digestive system: nausea, vomiting, stomach ache, diarrhea, and abnormal liver function. Others: facial edema, conjunctival congestion, abnormal tears, phlebitis, lumbago, backache, and local numbness.

XBJI Precautions

1) Not for use in pregnant women and children under 14 (inclusive) years of age. 2) The product must not be mixed with others, and must be used with caution in combination with others. When used in combination with other drugs, 50 ml 0.9% sodium chloride injection must be used between doses. 3) Allergic history, family allergic history and patient history of medications should be queried before administration. 4) During administration, special attention should be given to the initial 30 min of intravenous drip. In case of abnormality, the drug should be discontinued immediately and symptomatic treatment administered. 5) Monitoring of administration should be enhanced in older patients and in patients receiving TCM injection for the first time.

RELEVANT INFORMATION ON THE CLINICAL APPLICATION OF SFI

Recommended Therapeutic Regimens

SFI has been recommended in 19 therapeutic regimens of COVID-19 in China (see detailed information in **Tables 1** and **2**).

Ingredients of SFI

Panax ginseng C.A.Mey. (Hongshen) and *Aconitum carmichaeli* Debeaux (Fuzi). Basic information on SFI is provided in the **Supplementary Table**.

Indications for the Treatment of COVID-19 With SFI

SFI is used for deliverance due to sudden yang deficiency in the progressive stage of COVID-19 (critical case). Indicative symptoms are dyspnea, pale complexion, and severe symptoms are unconsciousness, drip sweat, and cold limbs.

Progress of Pharmacological Research on SFI

Modern pharmacological studies have shown that SFI has functions, including anti-shock, and protection from lung injury (see **Table 3**).

Yuhang Ai et al. explored the effects and mechanism of SFI in an LPS-induced lung injury model in rats. The results indicated that SFI might protect the lung by reducing activation of NF-κB in lung tissue (Ai et al., 2006). Research by Xia Liu et al. found that SFI improved the inflammatory response of rat lung tissue in an LPS shock model by reducing expression of p65 and p50 mRNA and protein in lung tissue and serum TNF-α (Liu et al., 2019a). Li Lin et al. studied the impact of SFI on LPS acute lung injury in rats, and found that SFI significantly increased the wet/dry weight ratio (W/D) of lung tissue, neutrophil ratio in BALF, protein content, lung tissue MDA, and serum NO. It significantly alleviated injury in lung tissue, indicating that SFI had an important preventive and therapeutic effect on LPS-induced acute lung injury (Lin and Zhan, 2010). Xi Liu et al. used the LPS intravenous injection method to establish a septic shock model in rabbits. Administration of SFI significantly improved mean arterial pressure (MAP), reduced LPS, LDH, and AST serum levels, and significantly improved the morphology of heart, liver, and kidney. In addition, SFI increased levels of adenosine triphosphate (ATP) and taurine in the heart, while reducing the level of adenosine monophosphate (AMP) in the heart. The results showed that SFI had a significant protective effect against LPS-induced septic shock (Liu et al., 2019b).

Clinical Research on SFI

SFI is composed of *Panax ginseng* C.A.Mey. and *Aconitum carmichaeli* Debeaux, and has properties that include enhancing cardiac function, increasing blood pressure, and protecting ischemic myocardium. It is widely used to rescue from shock (infectious or cardiogenic shock) caused by various reasons, cardiac failure, and arrhythmia in clinical practice. Recent studies have shown that SFI significantly protects

against lung injury (see **Table 3**). SFI has been recommended in China's SARS Diagnosis and Treatment Scheme (Version 2004), the MERS Diagnosis and Treatment Scheme (Version 2015), and the Diagnosis and Treatment Scheme for Human Infections with H7N9 Avian Influenza (Version 2017).

Qiu Z.L. et al. observed a therapeutic effect of SFI in patients with severe sepsis and an impact on the expression levels of serum IL-6 and IL-10. They found that SFI significantly lowered IL-6 levels in patients with severe sepsis and regulated the balance between pro- and anti-inflammatory factors, thus, improving the therapeutic effect (Qiu et al., 2012). Ning Zhang et al. randomized 160 patients with sepsis into an SFI treatment group and a conventional treatment group. By collecting post-treatment immunological parameters, they conducted a comparative analysis of the impact on immune function. The results showed that patients in the SFI treatment group had increased $CD4^+$ and $CD8^+$ T cell counts in peripheral blood and upregulated HLA-DR expression in monocytes. In addition, the SFI treatment group had a better response than the control group for duration of vasopressor administration and APACHE-II score. The results showed that SFI enhanced cellular immune function in patients with septic shock and might become an important adjunctive therapy for sepsis patients (Zhang et al., 2017). Another study found that SFI played an active role in the treatment of severe pneumonia in the elderly. Among 89 elderly patients with severe pneumonia, the SFI treatment group had significantly decreased levels of TNF-α, IL-6, and IL-8 7 days after administration, indicating that SFI effectively reduced inflammatory mediators, thus, playing an active therapeutic effect (Lv et al., 2017). Min Ma et al. conducted clinical research on 80 patients with traumatic acute lung injury, and found that SFI significantly improved respiratory rate, improved the oxygen index, and reduced levels of intracellular adhesion molecule 1 (ICAM-1), endothelin-1 (ET-1), and NO, thus, improving prognosis of these patients. This study provided a potential new therapy for traumatic acute lung injury (Ma et al., 2019). Jie Li et al. observed an impact of SFI intervention on duration of mechanical ventilation in patients with respiratory failure. The results showed that the total response rate in the SFI group was higher than that of the control group. SFI significantly improved serum prealbumin and high-sensitivity CRP levels in patients with respiratory failure and improved their oxygen index, thus, shortening the duration of mechanical ventilation (Li, 2013).

Usage and Dosage of SFI

1) Intravenous drip: 20–100 ml, diluted in 250–500 ml of 5%–10% glucose injection. 2) Intravenous injection: 5–20 ml, diluted in 20 ml of 5%–10% glucose injection.

Adverse Reactions of SFI

Dizziness, headache, shivering, fever, palpitations, chest distress, chest pain, difficulty breathing, nausea, retching, abdominal pain, rash, itching, rash or swelling, pain, and other discomfort in local infusion site.

SFI Precautions

1) To be used with caution in pregnant women. 2) Avoid direct mixing with coenzyme A, VitK3, and aminophylline. 3) Prepared drug should be used within 4 h.

RELEVANT INFORMATION ON THE CLINICAL APPLICATION OF SMI

Recommended Therapeutic Regimens

SMI has been recommended in 18 therapeutic regimens of COVID-19 in China (see detailed information in **Tables 1** and **2**).

Ingredients of SMI

Panax ginseng C.A.Mey. (Hongshen) and *Ophiopogon japonicus* (Thunb.) Ker Gawl. (Maidong). Basic information on SMI is provided in the **Supplementary Table**.

Indications for the Treatment of COVID-19 With SMI

SMI is used for the syndrome of deficiency of both qi and yin and deficiency of pulse in the progressive stage of COVID-19 (critical case). Indicative symptoms are weakness and shortness of breath, tachypnea, palpitations, dry mouth, sweating, and even dysphoria and cold limbs.

Progress of Pharmacological Research on SMI

Modern pharmacological studies have shown that SMI has functions that include protection from inflammatory shock, protection of heart and lung function, and immunoregulation (see **Table 3**).

Y. Z. Zhang et al. observed that SMI had strong anti-shock and neuroprotective properties in LPS-induced shock, possibly due to inhibition of brain lipid peroxidation and improvement of SOD activity (Zhang Y. Z. et al., 2010). SMI suppressed apoptosis of lung tissue cells during pulmonary ischemia/reperfusion injury in rabbits, resulting in attenuation of pneumocyte injury by raising NO levels, lowering oxygen free radical levels, and decreasing lipid peroxidation (Lin et al., 2007). It has also been reported that SMI reduced expression of NF-κB and activity of inducible nitric oxide synthase (iNOS) in lung tissues of rats poisoned by paraquat, and significantly alleviated erythrocyte diapedesis in the alveolar space (Liu et al., 2009). Research by Shuhua Xu et al. showed that SMI improved cardiac function and significantly improved hemodynamics in rats with cardiac failure. In addition, it improved the oxygen supply to tissues and the capacity of the tissues to use oxygen, thus, improving oxygen metabolism (Xu and Liu, 2010). SMI also inhibited expression of ICAM-1 and vascular cell adhesion molecule 1 (VCAM-1) to alleviate inflammatory infiltration following ischemia/reperfusion, and alleviated myocardial ischemia/reperfusion injury caused by multiple inflammatory responses (Liu et al., 2015). It also inhibited generation of inflammatory cytokines in rats subjected to ischemia/reperfusion, reduced serum expression of TNF-α, IL-6, IL-8, etc., thus, alleviating

inflammatory factor-induced cardiomyocyte injury and improving immune function (Wang et al., 2015). Research by Xuan Liu et al. discovered that SMI inhibited MCP-1, which indicated that SMI might be important in the inhibition of monocyte and macrophage activation (Liu et al., 2015). Lihua Du discovered that SMI significantly increased the weights of thymus and spleen in mice, raised serum IgG levels and the number of T cells, enhanced the phagocytic function of macrophages and had a significant immunomodulatory effect.

Clinical Research on SMI

SFI has often been used to treat shock caused by various factors, COPD, systematic inflammatory response syndrome and other diseases in clinical practice (see **Table 3**). SMI has been recommended in China's SARS Diagnosis and Treatment Scheme (Version 2004) and MERS Diagnosis and Treatment Scheme (Version 2015).

Biao Deng et al. studied 71 patients with shock and found that SMI in combination with conventional Western medicine had definite therapeutic efficacy, shortened the course of disease, reduced the length of hospital stay, and lowered the fatality rate (Deng et al., 2006). Hefeng Qin observed 68 patients with infectious shock and found that SMI had good clinical efficacy. It significantly improved CRP, PCT and TNF-α serum levels, and shortened the recovery time of vital signs with few adverse reactions (Qin, 2014). Wang Xian'an et al. observed 80 patients treated for infectious shock, and discovered that SMI in combination with ulinastatin had a significant therapeutic effect, enhancing immune function, and alleviating the inflammatory response (Wang et al., 2017). X. Huang et al. evaluated 23 RCTs with a total of 1,804 participants to study the impact of SMI on COPD. The results showed that SMI not only increased the total clinical response rate, but also improved pulmonary function, blood gas, and IgG indexes, and shortened the time for disappearance of lung rales. The results indicated that SMI in combination with Western medicine might have a positive effect in the treatment of COPD (Huang et al., 2019). Zongjun Fang et al. studied 38 patients with COPD. The control group (18 cases) received conventional Western medicine, while 20 cases (the treatment group) received SMI in addition. The results showed that patients in the treatment group had better vital capacity, forced expiratory volume in 1 s (FEV1), maximal breathing capacity (MBC), maximal inspiratory pressure (MIP), load breathing time, arterial blood gas analysis, and Burp dyspnea scores than the control group or the pre-treatment patients. The treatment group also had significantly improved respiratory function and clinical symptoms (Fang et al., 1998). Changxing Guo et al. randomized 33 patients with systemic inflammatory response syndrome into a conventional Western medicine treatment group (15 cases) and SMI + conventional treatment group (18 cases). After treatment, patients in the SMI treatment group had increased prostacyclin PGI2 and PGI2/thromboxane A2 (TXA2) in blood to a certain extent compared to patients in the conventional treatment group. Patients in the SMI group also had decreased levels of TXA2, atrial natriuretic peptide (ANP) and endothelin, and there were significant differences between the two groups. The results indicated that SMI could play an active role in improving microcirculation, protecting organ functions, and preventing further occurrence and development of systemic inflammatory response syndrome (Guo et al., 2004).

Usage and Dosage of SMI

Intravenous drip, 20–60 ml diluted with 250–500 ml of 5% glucose injection.

Adverse Reactions of SMI

The adverse reactions mainly include immediate hypersensitivity, predominantly skin allergy manifested by itching, rash, systematic urticaria, and then allergic shock; there may also be serious abdominal distension, corneal edema, abnormal vision, hypotension, ascending vascular pain, acute hepatic damage, sinus arrest, and drug fever.

SMI Precautions

1) Not to be used in newborns, infants, pregnant women, or those with an allergic constitution. 2) Not for administration by intravenous injection. The administration speed should not be too fast. In those receiving the drug for the first time, the initial administration should be at 15 drips/min for 10 min. If there are no abnormalities, the speed of administration can be increased to normal, which is generally controlled at 40–50 drips/min. 3) This drug has a pressor response, and blood pressure should be monitored in hypertensive patients.

RELEVANT INFORMATION ON THE CLINICAL APPLICATION OF AGNHP

Recommended Therapeutic Regimens

AGNHP has been recommended in 21 therapeutic regimens of COVID-19 in China (see detailed information in **Tables 1** and **2**).

Ingredients of AGNHP

Curcuma kwangsiensis S.G.Lee & C.F.Liang (Yujin), Calculus Bovis (Niuhuang), Cornu Bubali (Shuiniujiao), *Coptis chinensis* Franch. (Huanglian), Cinnabaris (Zhusha), Moschus (Shexiang), Margarita (Zhenzhu), Realgar (Xionghuang), *Scutellaria baicalensis* Georgi (Huangqin), *Gardenia jasminoides* J.Ellis (Zhizi), and *Cinnamomum camphora* (L.) J.Presl (Bingpian). Basic information on AGNHP is provided in the **Supplementary Table**.

Indications for the Treatment of COVID-19 With AGNHP

AGNHP is used for the syndrome of epidemic toxin lung closure and inner blocking causing collapse in the progressive stage of COVID-19 (critical case). Indicative symptoms are hyper-pyretic convulsions, coma and delirium, difficulty breathing, and dysphoria.

Progress of Pharmacological Research on AGNHP

Modern pharmacological studies have shown that AGNHP has antipyretic, analgesic, anti-inflammatory, and neuroprotective effects (see **Table 3**).

Zuguang Ye et al. discovered that AGNHP could significantly reduce the body temperature of hyperpyrexic rabbits in a fever model induced by intravenous injection of typhoid Vi polysaccharide vaccine in rabbit ear (Ye et al., 2003). Feng Zhang, Kunjie Zhu et al. found in an LPS-induced intracerebral inflammation model that AGNHP antagonized the toxic effect of LPS on dopaminergic neurons, inhibited release of superoxide radical, and reverse changes in cortical monoamine neurotransmitters. It was speculated that its impact on cortical monoamine neurotransmitters might be one of the mechanisms by which AGNHP promoted consciousness in LPS brain damage (Zhang F. et al., 2010; Zhu and Sun, 2014). Research by Dan Zhang et al. showed that AGNHP lowered serum LPS and lung myeloperoxidase (MPO) levels in a rat model of sepsis (Zhang et al., 2009). Yishan Tang et al. found that AGNHP lowered total LDH activity in serum and brain tissue, and changed the percentage of isomerase in a rat pertussis-induced infectious cerebral edema model (Tang et al., 2005). Fan Q et al. discovered that AGNHP had anti-atherosclerotic effects in the high fat diet-induced $ApoE^{-/-}$ mouse model at early- and mid-stage *via* regulation of Th17/Treg balance. It inhibited chronic inflammation, reduced plaque collagen fibers, and reduced inflammatory cell infiltration (Fan et al., 2020).

Clinical Research on AGNHP

Modern clinical studies have shown that AGNHP has therapeutic effects against hyperpyrexia, coma caused by severe infectious diseases, and viral encephalitis and severe pneumonia of infants (see **Table 3**).

Yueming Feng et al. conducted a systematic evaluation and found that AGNHP could be used to promote consciousness of coma patients with acute cerebral infarction (ACI) and improve neurologic function. This may be due to the ability of AGNHP to alleviate the inflammatory response, reduce cerebral edema, and promote recovery of neurologic function (Feng and Yang, 2015). Haijun Zhang et al. discovered through clinical observation that AGNHP could be used to treat viral encephalitis in children. AGNHP rapidly reduced body temperature, prevented convulsions, promoted consciousness, and alleviated cerebral edema and brain cell damage (Zhang and Dong, 2014). Research by Zhulin Zhuo et al. found that AGNHP with the adjuvant, Ribavirin was efficacious in acute severe viral pneumonia of children, significantly reduced PCT and improved immune function (Zhuo and Wen, 2017). Yanling Shi discovered through clinical observation that AGNHP in combination with sodium phosphate improved anoxic conditions and myocardial damage in patients with neonatal asphyxia and myocardial damage (Shi, 2019). Xie Long et al. in a study of 70 patients with ACI and central hyperpyrexia found that combined use of conventional Western medicine and AGNHP reduced the duration of fever and significantly improved the prognosis (Long and Wu, 2014). Hanwei Liu et al. conducted a systematic review of relevant literature on AGNHP treatment of ACI and cerebral hemorrhage. The results showed that adjuvant treatment with ANP (AGNHP) appeared to improve the total response rate and neurologic deficit score in patients with ACI and acute intracerebral hemorrhage (AIH) (Han et al., 2019). Research by Ma et al. showed that ANP had a moderating effect on Th1/Th2 in cerebral infarction patients (Ma and Zhou, 2015).

Usage and Dosage of AGNHP

Oral administration. 3 g, once a day.

Adverse Reactions of AGNHP

Overdose administration might cause mercurial nephrosis or allergic reaction and other adverse reactions. Improper use of this product might cause hypothermia.

AGNHP Precautions

1) Nasogastric administration can be used in patients unable to take orally because of high fever and coma. The pills can be dissolved in warm but not hot water. The water or decoction used to dissolve the pills should be controlled at 40–60°C. The use of boiling water is forbidden for two reasons: first, to avoid increased decomposition of realgar and cinnabar by high temperature, and reduce generation of the highly toxic arsenic trioxide, free arsenium, and mercury. Research has shown that arsenic trioxide in realgar preparations is not significantly changed below 60°C but begins to increase at 80°C. The decoction used for dissolving AGNHP should therefore not exceed 60°C secondly, musk, borneol and other aromatic substances are volatile. Boiling water could result in excess volatilization, thus, reducing efficacy. 2) Must not be used with nitrate, nitrite, ferrite or sulfate drugs. 3) Not for use in pregnant women. 4) It contains cinnabar and realgar, and should not be taken at high doses for long periods. Should be used with caution in those with hepatic and renal dysfunction. (5) It contains musk, so athletes should use with caution.

DISCUSSION AND CONCLUSIONS

It is a critical moment in the battle to defeat the current outbreak of novel coronavirus. For this specific indication, rapid performance of TCM can contribute as an alternative measure. TCM can effectively prevent the disease from transforming into severe and critical disease. In severe cases, TCM has won time for recovery by improving symptoms (The State Council Information Office of the People's Republic of China, 2020). Treatment practice for COVID-19 has shown that early intervention with TCM is an important method to improve cure rate, shorten the course of disease, delay disease progression and reduce mortality rate. For example, the total response rate of Qingfei Paidu Decoction was more than 90% in Shanxi and Hebei provinces (He et al., 2020).

CPMs have played an important role in preventing and treating epidemic diseases in China because they are convenient to use,

easily stored and cost-effective. The positive role of CPMs has been emphasized in the "Diagnosis and Treatment of COVID-19 (Trial Version 7)" and other therapeutic regimens. During the medical observation period and early stage of COVID-19, HXZQC, LHQWC, SFJDC, and JHQGG can be selected according to different clinical manifestations. At the same time, they can also promote immunity against the virus. For severe and critical disease, the choice shall be made according to different syndromes during clinical treatment. For viral infections combined with mild bacterial infections, XYPI and TRQI can be used; for high fever with disturbance of consciousness, AGNHP can be used; for systemic inflammatory response syndrome or multiple organ function failure, XBJI is recommended; SMI can be used for immunosuppression; and SFI can be used for shock. Furthermore, the reason that TCM works is not only because it inhibits the virus, but also because it might block infection, regulate the immune response, inhibit the inflammatory storm, and promote repair of the body. Moreover, the prevention and control measures of COVID-19 have fully reflected the ideology of "preventive treatment of disease".

Physicians should pay attention to the reasonable application of CPMs to treat COVID-19. Severe patients are prone to septic shock, and liver and kidney dysfunction. In patients with related underlying diseases, drug metabolism and clearance are reduced. Treatment options: hepato-renal toxic drugs should be avoided to reduce the risk of drug accumulation and poisoning. For example, AGNHP contains cinnabar and realgar, and should not be taken for a long time. People with liver and kidney dysfunction should use with caution. TRQI should be carefully selected because it aggravates liver and kidney function; LHQWC and JHQGG contain ephedrae herba (Mahuang), and doctors need to monitor patients' blood pressure, heart condition and combined use of antihypertensive drugs. In clinical application of XBJI, SFI and other traditional Chinese medicine injections, attention should be paid to the choice of solvent and the interval between infusions with other drugs. As is well known, clinicians use CPMs under the guidance of the theory of TCM. Foreign doctors and patients wishing to use CPMs to treat COVID-19 should exercise caution, especially in countries where they may be used incorrectly without the knowledge of TCM theory.

There are some limitations within this paper. First, as there is little direct clinical evidence for the prevention of COVID-19, the reported studies are from previous reports on the prevention of SARS, MERS, H7N9, and H1N1 influenza by CPMs, which can only be considered as indirect evidence in respect of the current outbreak. Secondly, the programs for prevention of COVID-19 were issued shortly after the outbreak. Chinese medicine experts suggested CPMs to treat COVID-19 based on their previous experience in the prevention and treatment of similar diseases combined with their initial understanding of the disease. The actual effects of these programs need to be verified in clinical application, and updated and improved according to the evidence of new research on COVID-19.

For future studies, we recommend prospective cohort studies, RCTs or registry studies to evaluate the effect of CPMs in prevention of COVID-19. Some clinical trial protocols to treat COVID-19 using the top 10 CPMs are ongoing (see **Table 4**). At present, since COVID-19 has not yet been controlled, a series of prospective population studies with rigorous design and large sample should commence with protocol registration, and

TABLE 4 | Registration information on clinical trial protocols for the top 10 CPMS in the treatment of COVID-19.

CPMS	Registration Number	Registration Date	Clinical research unit	Registration title
XYPJ	ChiCTR2000029756	2020/2/12	Renmin Hospital of Wuhan University (Wuhan, China)	Clinical study of nebulized Xiyanping injection in the treatment of novel coronavirus pneumonia (COVID-19)
	ChiCTR2000030117	2020/2/23	Jiangxi Qingfeng Pharmaceutical Co., Ltd. (Ganzhou, China)	A multicenter, randomized, open, parallel controlled trial for the evaluation of the effectiveness and safety of Xiyanping injection in the treatment of common type novel coronavirus pneumonia (COVID-19)
	ChiCTR2000030218	2020/2/25	Fifth People's Hospital of Ganzhou (Ganzhou, China)	Study of Pinavir/Ritonavir tablets (Trade Name: Kelizhi) Combined with Xiyanping injection for novel coronavirus pneumonia (COVID-19)
LHQWC	ChiCTR2000029433	2020/2/1	Hebei Yiling Hospital (Shijiazhuang, China), Renmin Hospital of Wuhan University (Wuhan, China)	A randomized, open-label, blank-controlled trial for Lian-Hua Qing-Wen Capsule/granule in the treatment of suspected novel coronavirus pneumonia (COVID-19)
	ChiCTR2000029434	2020/2/1	Hebei Yiling Hospital (Shijiazhuang, China), Renmin Hospital of Wuhan University (Wuhan, China)	A randomized, open-label, blank-controlled trial for Lian-Hua Qing-Wen Capsule/granule in the treatment of novel coronavirus pneumonia (COVID-19)
TRQI	ChiCTR2000029432	2020/2/1	The First Affiliated Hospital of Guangzhou University of Chinese Medicine (Guangzhou, China)	A real world study for the efficacy and safety of large dose Tanreqing injection in the treatment of patients with novel coronavirus pneumonia (COVID-19)
	ChiCTR2000029813	2020/2/14	Shanghai Public Health Clinical Center (Shanghai, China)	Clinical trial for Tanreqing capsules in the treatment of novel coronavirus pneumonia (COVID-19)
XBJI	ChiCTR2000029381	2020/1/27	The First Affiliated Hospital of Guangzhou Medical University (Guangzhou, China)	A prospective comparative study for Xue-Bi-Jing injection in the treatment of novel coronavirus pneumonia (COVID-19)
	ChiCTR2000030388	2020/3/1	Jingzhou First People's Hospital (Jingzhou, China)	Efficacy and safety of Xue-Bi-Jing injection in the treatment of severe cases of novel coronavirus pneumonia (COVID-19)
SFJDC	ChiCTR2000030043	2020/2/21	Peking University Third Hospital (Beijing, China)	Shen-Fu injection in the treatment of severe novel coronavirus pneumonia (COVID-19): a multicenter, randomized, open-label, controlled trial

implementation in a timely manner, to produce reliable evidence for CM prevention of COVID-19 or similar emerging respiratory infectious diseases in the future.

AUTHOR CONTRIBUTIONS

WZ and ZF wrote the manuscript. HW, YY, LW, NS, GS and YS searched for related articles. YC, XL, and GG proofread the manuscript. SX guided the writing and critically revised the manuscript. All authors have read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

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REVIEW
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Medicinal Plants as Sources of Active Molecules Against COVID-19

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The Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) or novel coronavirus (COVID-19) infection has been declared world pandemic causing a worrisome number of deaths, especially among vulnerable citizens, in 209 countries around the world. Although several therapeutic molecules are being tested, no effective vaccines or specific treatments have been developed. Since the COVID-19 outbreak, different traditional herbal medicines with promising results have been used alone or in combination with conventional drugs to treat infected patients. Here, we review the recent findings regarding the use of natural products to prevent or treat COVID-19 infection. Furthermore, the mechanisms responsible for this preventive or therapeutic effect are discussed. We conducted literature research using PubMed, Google Scholar, Scopus, and WHO website. Dissertations and theses were not considered. Only the situation reports edited by the WHO were included. The different herbal products (extracts) and purified molecules may exert their anti-SARS-CoV-2 actions by direct inhibition of the virus replication or entry. Interestingly, some products may block the ACE-2 receptor or the serine protease TMPRRS2 required by SARS-CoV-2 to infect human cells. In addition, natural products were shown to inhibit the SARS-CoV-2 life-cycle related proteins such as papain-like or chymotrypsin-like proteases. In conclusion, we suggest that natural products could be used alone or in combination as alternative medicines to treat/prevent COVID-19 infection. Moreover, their structures may offer clues for the development of anti-SARS-CoV-2 drugs.

Keywords: Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2), Coronavirus Disease 2019 (COVID-19), plants, natural products, ACE2

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INTRODUCTION

The Severe Acute Respiratory Syndrome-related Coronavirus 2 or novel coronavirus (COVID-19) infection has been declared world pandemic resulting in thousands of deaths in 216 countries around the world. The disease appeared in late December 2019 in Wuhan (China) as a result of zoonotic transmission (Mackenzie and Smith, 2020). Actually, the Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) was shown to share 96% of the genomic identity with the related bat coronavirus (Zhou et al., 2020). Moreover, the SARS-CoV-2 genome was found to be 91.02% identical to that of the Pangolin-CoV, raising the possibility that the latter

acted as an intermediate zoonotic host between bats and humans (Zhang T. et al., 2020). Till now, no vaccines or specific treatments for SARS-CoV-2 have been developed, although extraordinary efforts are being made (Amanat and Krammer, 2020). Some therapeutic approaches have been suggested such as nucleoside analogs, Remdesivir, anti-inflammatory drugs or Lopinavir/Ritonavir to treat COVID-19. At present, more than 200 clinical trials, some of them analyzing these drugs or others, have been registered in clinicaltrials.gov. Nevertheless, the clinical usefulness of these drugs against COVID-19 infection remains unclear (Li et al., 2020).

Herbal traditional medicines have been used in China since the first days of the COVID-19 outbreak. Indeed, these traditional medicines were shown to result in the recovery of 90% of the 214 patients treated (Hong-Zhi et al., 2020). Furthermore, some traditional herbal medicines prevented SARS-CoV-2 infection of healthy persons and improved the health state of patients with mild or severe symptoms (Hong-Zhi et al., 2020). Similar promising results were reported in Zhejiang Province – China (Xu K. et al., 2020). Chinese traditional medicines known as Shu Feng Jie Du and Lianhuaqingwen have been recommended due to their demonstrated efficacy against previous influenza A (H1N1) or SARS-CoV-1 (Lu, 2020). A group of experts from the Zhongnan Hospital of Wuhan University included the use of traditional medicines in the guidelines for the treatment and prevention of COVID-19. Several methods using medicinal plants were recommended for the prevention of COVID-19. Moreover, to treat the disease, the experts recommended the use of different herbal mixtures according to the disease-stage (Jin et al., 2020).

This review focuses on the possible uses of herbal traditional medicines and natural products in the prevention and treatment of COVID-19 infection (**Table 1**).

SARS-COV-2

SARS-CoV-2 belongs to the β genus, Nidovirales order of the Coronaviridae family. SARS-CoV-2 is an enveloped, single (+) stranded RNA, with symmetric helical nucleocapsid (Khan et al., 2020). The virus encodes twenty different proteins including four main structural proteins (S: spike; E: envelope; M: membrane; N: nucleocapsid), and several nonstructural proteins such as RNA-dependent RNA polymerase (RdRp), coronavirus main protease (3CLpro), and papain-like protease (PLpro) (Chen et al., 2020).

The angiotensin converting enzyme II (ACE2) was found to be a key functional receptor for the SARS-CoV-2 allowing its attachment to human and bat cells and therefore its replication (Walls et al., 2020; Zhou et al., 2020). SARS-CoV-2 binds the host cells through interaction between the receptor binding motif of the spike protein—receptor binding domain (RBD) and the ACE2 receptor. This interaction will trigger conformational changes of the C-terminal S2 subunit (responsible for virus-cell membrane fusion) of the spike protein. The complex S protein-ACE2 is then proteolytically processed by the host cell-type 2II transmembrane serine protease TMPRSS2 leading to the ACE2 cleavage and therefore to viral entry into the host cell (Jiang et al., 2020; Rabi et al., 2020). After entry and uncoating, the genomic RNA is translated into two polyproteins (pp1a and pp1ab) which undergo a proteolytic cleavage generating 15–16 nonstructural proteins. The double-membrane vesicle is then formed from the rearrangement of cellular membrane induced by the nonstructural proteins. On the other hand, the genomic RNA is transcribed into subgenomic RNA which in turn leads to the synthesis of structural (spike, envelope, membrane, and nucleocapsid) and accessory proteins. Finally, virions are assembled in the ER-Golgi intermediate complex, and then released *via* the secretory pathway (Fung and Liu, 2020).

SARS-CoV-2 shares several genetic and clinical similarities with other coronaviruses of the β genus such as SARs-CoV and NL63 (Fani et al., 2020). Indeed, the entry of both viruses needs their interaction with the ACE2 receptor. However, some differences have been reported among these strains such as the length of the S protein and the structure of the receptor binding region (Ceccarelli et al., 2020). On the other hand, a high nucleotides homology has been found between SARS-CoV-2 and SARS-CoV in addition to a high homology (95–100%) that has been demonstrated between the proteins of the two strains. Actually, S2 and N proteins of SARS-CoV-2 and SARS-CoV share 99 and 90% similarities, respectively (Xu J. et al., 2020).

NATURAL PRODUCTS WITH ANTI-SARS-COV-2 EFFECTS

Runfeng et al. (2020) studied the inhibitory effects and anti-inflammatory potential of a Chinese herbal mixture called Lianhuaqingwen (a mixture of 11 medicinal species, a mineral medicine called gypsum and menthol) against SARS-CoV-2 (**Table 2**). Traditionally, Lianhuaqingwen has been widely used to treat fever, cough, fatigue, influenza, bronchitis, pneumonia, and early stage of measles (Ding et al., 2017), and has been included in phase II clinical trial in the USA (Gao et al., 2020). This herbal mixture was recommended by the Chinese National Health Commission to treat or manage COVID-19 (Yang Y. et al., 2020). The anti-SARS-CoV-2 activity was assessed in Vero E6 cells using cytopathic effect inhibition and plaque reduction assays. The herbal mixture inhibited SARS-CoV-2 replication in a dose-dependent manner with an IC_{50} of 411.2 μg/ml. Furthermore, the mixture was able to suppress the release of pro-inflammatory cytokines (TNF-α, IL-6, CCL-2/MCP-1, and CXCL-10/IP-10) in a dose-dependent manner (Runfeng et al., 2020). These results could be interesting since the cytokine storm has been shown to be one of the COVID-19 lethal complications. In a previous study, among the 61 molecules identified in this herbal mixture, seven (arctiin, forsythoside A, gallic acid, isoliquiritigenin, kaempferol, rutin, and secoxyloganin) exhibited important antiviral activities with IC_{50} ranging from 4.9 ± 0.1 (kaempferol) to 47.8 ± 1.5 μM (secoxyloganin) (Wang et al., 2016). Wang et al. (2020) reported results in four COVID-

TABLE 1 | Natural products tested against coronaviruses.

Plant (Family/part)	Product	Model/Strains	Inhibitory assay	Dosage/duration	Control	Effects	Reference
Alnus japonica (Thunb.) Steud. (Betulaceae/bark)	Hirsutenone (Ethanol extract)	*In vitro* SARS-CoV- PLpro	FRET	0–200 μM/60 min	Curcumin (viral protease inhibitor)	A dose-dependent inhibition of the SARS-CoV- PLpro activity (IC_{50} = 4.1 ± 0.3 μM while that of curcumin was 5.7 ± 0.3 μM)	Park et al., 2012b
Angelica keiskei (Miq.) Koidz (Umbelliferae/leaves)	Xanthoangelol E (Ethanol extract)	*In vitro* SARS-CoV- PLpro SARS-CoV- 3CL(pro)	FRET Cell-based cis-cleavage inhibition assay	0, 12.5, 25, 50 μM	NS	A dose-dependent inhibition of SARS-CoV- PLpro activity (IC_{50} = 1.2 ± 0.4 μM) A dose-dependent inhibition of SARS-CoV- 3CL(pro) activity (IC_{50} = 11.4 ± 1.4 μM)	Park et al., 2016
Aglaia perviridis Hiern (Meliaceae/whole)	Myricetin	*In vitro* Angiotensin converting enzyme (ACE) from rabbit lung	FRET	0.01–10 μM	NS	Inhibition of the SARS-CoV helicase by affecting the ATPase activity (IC_{50} = 2.71 ± 0.19 μM)	Yu et al., 2012
Cibotium barometz (L.) J.Sm. (Dicksoniaceae/rhizomes)	Ethanol and methanol extracts	*In vitro* SARS-CoV virus propagated in Vero E6 cells	Cytopathic effect inhibition ELISA FRET	0, 25, 50, 100, and 200 μg/ml	Vero E6 cells without extracts (negative control) SARS-CoV-infected Vero E6 cells (positive control) Valinomycin (reference antiviral standard)	Both extracts inhibited the SARS-CoV-replication at concentrations of 25 and 200 μg/ml. EC_{50} were found to be 8.42 and ≥10 μg/ml, respectively.	Wen et al., 2011
Cullen corylifolium (L.) Medik. (Leguminosae/seeds)	Psoralidin (Ethanol extract)	*In vitro* SARS-CoV- PLpro	Fluorogenic assay	0–100μM	NS	Inhibition of SARS-CoV PLpro in a dose-depenedent manner with IC_{50} = 4.2 ± 1.0 μM	Kim et al., 2014
Ecklonia cava (Laminariaceae/whole)	Dieckol (Ethanol extract)	*In vitro* SARS-CoV- 3CL(pro)	FRET	0–200 μM	Positive controls: hesperetin (60 μM), daidzein (105 μM), aloeemodin (132 μM)	Inhibition of the SARS-CoV- 3CL(pro) activity (IC_{50} = 2.7 ± 0.6 μM)	Park et al., 2013
Paulownia tomentosa (Thunb.) Steud. (Scrophulariaceae/fruits)	Tomentin E (Methanol extract)	*In vitro* SARS-CoV- PLpro	Fluorogenic assay	0, 6.25, 12.5, 25 μM	NS	A dose-dependent inhibition of SARS-CoV-PLpro (IC_{50} = 5.0 ± 0.06 μM)	Cho et al., 2013
Quercus infectoria G. Olivier (Fagaceae/bark)	Ethanol-water extract	*In vitro*	Reverse phase high performance liquid chromatography (RP-HPLC)	330 μg/ml	Methanol solution (negative control)	Inhibition of ACE activity by 93.9 ± 2.5%	Sharifi et al., 2013
Rheum sp. *Polygonum* sp. (Polygonaceae/whole)	Emodin	*In vitro* Vero cells	Luciferase assay	0, 10, 50, 100, 200, and 400 μM	NS	Blockage of the binding SARS-CoV S protein and ACE2 Minimal active concentration: 10 μM IC_{50} = 200 μM	Ho et al., 2007
Salvia miltiorrhiza Bunge (Lamiaceae/roots)	Cryptotanshinone (n-hexane extract)	*In vitro* SARS-CoV- PLpro	FRET	0–200 μM/30 min	NS	A dose- and time-dependent inhibition of the SARS-CoV- PLpro activity in a slow-binding inhibition mechanism (IC_{50} = 0.8 ± 0.2 μM)	Park et al., 2012a
	Dihydrotanshinone I (n-hexane extract)	*In vitro* SARS-CoV- 3CL(pro)	FRET	0–200 μM/60 min	NS	A dose-dependent but not time-dependent inhibition of the SARS-CoV-3CL(pro) activity (IC_{50} = 14.4 ± 0.7 μM)	

(Continued)

TABLE 1 | Continued

Plant (Family/part)	Product	Model/Strains	Inhibitory assay	Dosage/duration	Control	Effects	Reference
Sambucus javanica subsp. chinensis (Lindl.) Fukuoka (Adoxaceae/stem)	95% ethanol extract	*In vitro* HCoV-NL63 in LLC-MK2 cells	Cytopathic effect inhibition	0, 1, 10, and 50 μg/ml/36 h	Virus-infection only with no test extract	Inhibition of: -Viral cytopathicity (IC_{50} = 1.17 ± 0.75 μg/ml) -Virus attachment (IC_{50} = 15.75 ± 6.65 μg/mL) -Plaque formation 4.67 ± 1.21 μg/mL	Weng et al., 2019
	Caffeic acid			0, 10, 50, and 100 μM/36 h		Inhibition of viral cytopathicity (IC_{50} = 3.54 ± 0.77 μM)	
	Chlorogenic acid					Inhibition of viral cytopathicity (IC_{50} = 43.45 ± 6.01 μM)	
	Gallic acid					Inhibition of viral cytopathicity (IC_{50} = 71.48 ± 18.40 μM)	
Scutellaria baicalensis Georgi (Labiatae/whole)	Scutellarein	*In vitro*	FRET	0.01–10 μM	Inhibition of the SARS-CoV helicase by affecting the ATPase activity	Inhibition of the SARS-CoV helicase by affecting the ATPase activity (IC_{50} = 0.86 ± 0.48 μM)	Yu et al., 2012
Torreya nucifera (L.) Siebold & Zucc. (Taxaceae/leaves)	Amentoflavone (Ethanol extract)	*In vitro* SARS-CoV- 3CL(pro)	FRET	0–300 μM	Positive controls: Apigenin (IC_{50} = 280.8 ± 21.4 μM) Luteolin (IC_{50} = 20.0 ± 2.2 μM) Quercetin (IC_{50} = 23.8 ± 1.9 μM)	A dose-dependent inhibition of SARS-CoV- 3CL(pro) activity (IC_{50} = 8.3 ± 1.2 μM)	Ryu et al., 2010
Tribulus terrestris L. (Zygophyllaceae/fruits)	Terrestrimine (Methanol extract)	*In vitro* SARS-CoV PLpro	Fluorogenic assay	1, 10, 100, 1,000 μM	NS	Inhibition of SARS-CoV - PLpro with IC_{50} = 15.8 ± 0.6 μM	Song et al., 2014
———————	Lianhuaqingwen (Herbal mixture) dissolved in DMSO and then in serum-free DMEM	*In vitro* SARS-CoV-2 virus propagated in Vero E6 cells	Cytopathic effect inhibition	0–600 μg/ml/72 h	Remdesivir (5 μM)	Inhibition of the SARS-CoV-2 replication (IC_{50} = 412.2 μg/ml *vs* 0.651 μM for the control) Inhibition of the plaque formation of the SARS-CoV-2 Reduction of TNF-α, IL-6, CCL-2/MCP-1, and CXCL-10/IP-10 expression	Runfeng et al., 2020
———————	Herbacetin	*In vitro* SARS-CoV- 3CL(pro)	FRET	1, 2.5, 20 μM/16 h	NS	A dose-dependent inhibition of SARS-CoV- 3CL(pro) activity (IC_{50} = 33.17 μM)	Jo et al., 2020
———————	Pectolinarin	*In vitro* SARS-CoV- 3CL(pro)	FRET	1, 2.5, 20 μM/16 h	NS	A dose-dependent inhibition of SARS-CoV- 3CL(pro) activity (IC_{50} = 37.78 μM)	Jo et al., 2020
———————	Rhoifolin	*In vitro* SARS-CoV- 3CL(pro)	FRET	1, 2.5, 20 μM/16 h	NS	A dose-dependent inhibition of SARS-CoV- 3CL(pro) activity (IC_{50} = 27.45 μM)	Jo et al., 2020

FRET, Fluorescence resonance energy transfer; GSEA, Microarray and Gene Set Enrichment Analysis; NS, Not specified.

TABLE 2 | Traditional uses of the medicinal species and mixtures with possible anti-SARS-CoV-2 effects.

Plant (family)	Traditional uses	References
Medicinal species		
Alnus japonica (Thunb.) Steud. (Betulaceae)	Cancer, Blood and lymphatic disorders, Gastroenteric disorders, Fever.	Chi et al., 2018
Angelica keiskei (Miq.) Koidz. (Umbelliferae)	Tonic, Galactagogue, Diuretic, Laxative, Analeptic.	Kil et al., 2017 Du et al., 2019
Berberis integerrima Bunge (Berberidaceae)	Hypertension, Abdominal ache, Blood purification, Fever.	Nasab and Khosravi, 2014 Sadat-Hosseini et al., 2017
Cibotium barometz (L.) J. Sm. (Dicksoniaceae)	Osteoporosis, Osteoarthritis, Inflammations, Rheumatism, Lumbago, Dysuria, Age-related leucorrhoea.	Shi et al., 2020 Wu and Yang, 2009
Crataegus laevigata (Poir.) DC. (Rosaceae)	Hyperlipidemia, Arteriosclerosis, Hypertension, Cardiac disorders.	Čopra-Janićijević et al., 2018
Ecklonia cava (Lessoniaceae)	Inflammations, Asthma, Diabetes, Cancer.	Cho et al., 2020 Yun et al., 2018 Park E. Y. et al., 2012
Gentiana scabra Bunge (Gentianaceae)	Inflammatory skin diseases, Gallbladder disorders, Jaundice, Leucorrhea.	Yang et al., 2019
Onopordum acanthium L. (Asteraceae)	Hypertension, Homeostasis, Bacterial infections.	Sharifi et al., 2013
Paulownia tomentosa (Thunb.) Steud. (Scrophulariaceae)	Bacterial and viral infections, Inflammations, Asthma, Hypertension, Hemorrhoids, Gonorrhea, Erysipelas.	Ji et al., 2015 Lee et al., 2018
Cullen corylifolium (L.) Medik. (Leguminosae)	Eczema, Osteoporosis, Colitis, Pollakiuria, Leukoderma, Asthma, Spermatorrhea, Bleeding.	Xu et al., 2020a Zhu et al., 2019
Quercus infectoria G. Olivier (Fagaceae)	Diarrhea, Dysentery, Gonorrhea, Hemorrhages, Infections, Inflammations.	Tayel et al., 2018 Chokpaisarn et al. (2017)
Salvia miltiorrhiza Bunge (Lamiaceae)	Inflammations Cardiovascular and circulatory disorders, Menstrual disorders, Atherosclerosis, Cancer, Hyperglycemia.	Wei et al., 2020 Liang et al., 2020
Sambucus javanica subsp. chinensis (Lindl.) Fukuoka (Adoxaceae)	Bacterial infections, Inflammations, Liver disorders.	Zhang et al., 2010
Senna tora (L.) Roxb. (Fabaceae)	Constipation, Liver disorders, Inflammations, Vision disorders.	Lee et al., 2019
Taxillus chinensis (DC.) Danser (Loranthaceae)	Liver and kidney disorders, Miscarriage, Rheumatic arthralgia, Metrorrhagia, Pregnancy-related Bleeding, Dizziness.	Liu et al., 2019
Torreya nucifera (L.) Siebold & Zucc. (Taxaceae)	Stomachache, Hemorrhoids, Rheumatoid arthritis, Nervous disorders, Viral infections.	Kalpana et al., 2019
Tribulus terrestris L. (Zygophyllaceae)	Inflammation, Oxidative stress, Cancer, Cardiovascular disorders, Hormonal disorders, Muscle aches, Chest pain, Pruritus.	Tian et al., 2020
Dioscorea polystachya Turcz. (Dioscoreaceae)	Inflammations, Liver disorders, Diabetes, Hyperthyroidism, Digestive disorders, Cancer.	Koo et al., 2017 Ma et al., 2018
Herbal mixtures		
Lianhuaqingwen Composition Forsythiae Fructus [*Forsythia suspensa* (Thunb.) Vahl], Lonicerae Japonicae Flos	Respiratory tract infectious diseases, Viral infections,	Runfeng et al., 2020 Ding et al., 2017 Gao et al., 2020

(Continued)

TABLE 2 | Continued

Plant (family)	Traditional uses	References
[*Lonicera japonica* Thunb.], Ephedrae Herba (honey-fried) [*Ephedra sinica Stapf*, *Ephedra intermedia* Schrenk & C. A. Mey. or *Ephedra equisetina* Bunge.], Armeniacae Semen Amarum (stir-baked) [*Prunus armeniaca* L. var. *ansu* Maxim., *Prunus sibirica* L., *Prunus mandshurica* (Maxim.) Koehne or *Prunus armeniaca* L.], Gypsum Fibrosum, Isatidis Radix [*Isatis tinctoria* L.], Dryopteris Crassirhizomatis Rhizoma [*Dryopteris crassirhizoma* Nakai], Houttuyniae Herba [*Houttuynia cordata* Thunb.], Pogostemonis Herba [*Pogostemon cablin* (Blanco) Benth], Rhei Radix & Rhizoma [*Rheum palmatum* L., *Rheum tanguticum* (Maxim. ex Regel) Balf. or *Rheum officinale* Baill.], Rhodiolae Crenulatae Radix et Rhizoma [*Rhodiola crenulata* (Hook.f. & Thomson) H.Ohba], menthol and Glycyrrhizae Radix et Rhizoma [*Glycyrrhiza uralensis* Fisch. ex DC., *Glycyrrhiza inflata* Batalin, or *Glycyrrhiza glabra* L.].	Inflammations, Fever.	
Shu Feng Jie Du Composition Rhizoma Polygoni Cuspidati [*Reynoutria japonica* Houtt.] Fructus Forsythiae [*Forsythia suspensa* (Thunb.) Vahl] Radix Isatidis [*Isatis tinctoria* L.] Radix Bupleuri [*Bupleurum chinense* DC.] Herba Patriniae [*Patrinia scabiosifolia* Link] Herba Verbenae [*Verbena officinalis* L.] Rhizoma Phragmitis [*Phragmites australis* subsp. australis] Radix Glycyrrhizae [*Glycyrrhiza uralensis* Fisch. ex DC.]	Influenza, Viral infections, Immune regulation.	Song et al., 2013 Yang Y. et al. (2020) Xia et al., 2020

19 patients treated using a combination of lopinavir/ritonavir (Kaletra®) and arbidol with capsules of Shufeng Jiedu (a Chinese traditional medicine). After treatment, three patients were found COVID-19 negative and experienced significant improvements of the symptoms. In another study on 132 patients with COVID-19 living in northeast Chongqing (China), the traditional Chinese medicine was applied in almost 92% of them. The study concluded that the best therapeutic approach was a combination of Kaletra and the traditional medicine (Wan et al., 2020). Recently, Lung et al. (2020) demonstrated that theaflavin could be used as an important anti-SARS-CoV-2 drug using *in silico* approaches. Indeed, theaflavin showed promising docking affinities in the catalytic pocket of the SARS-CoV-2 RNA-dependent RNA polymerase. Nevertheless, it is worthy to point out that their bioavailability could limit their use since they are not absorbed in relevant amounts and that the theaflavin skeleton was found to resist to the degradation by the microbiota (Pereira-Caro et al., 2017). By searching single cohort studies undertaken regarding the efficacy of herbal medicines against SARS and H1N1 influenza viruses, it has been concluded that medicinal species, usually used as herbal formula, could be an interesting preventive approach for high-risk populations (medical staff and their families' members, people living in COVID-19 outbreak areas, old populations). Six herbal species were found to be the most frequently used including *Astragalus mongholicus* Bunge, *Glycyrrhiza glabra* L., *Saposhnikovia divaricata* (Turcz. ex Ledeb.) Schischk., *Atractylodes lancea* (Thunb.) DC., *Atractylodes macrocephala* Koidz., *Lonicera japonica* Thunb., and *Forsythia suspensa* (Thunb.) Vahl. These species are the ingredients of the Chinese traditional medicine Yupingfeng powder (Luo et al., 2020). On the other hand, the ethanol extract of *Sambucus javanica* subsp. chinensis (Lindl.) Fukuoka stem exerted promising anti-human coronavirus NL63 effects with IC_{50} ranging from 1.17 (virus yield) to 15.75 μg/ml (virus attachment). The extract significantly decreased virus yield, plaque formation, and virus attachment. Furthermore, three of its major phenolic acids (caffeic, chlorogenic, and gallic acid) were shown to inhibit the NL63 replication and virus attachment. Caffeic acid was the most potent phenolic acid (Weng et al., 2019). Phenolic acids are characterized by their metabolizing ability by the microbiota enhancing their bioavailability. Moreover, their antiviral potential could be increased with the alkyl chain length (Kumar and Goel, 2019). However, their efficacy is still controversial due to their low absorption and instability in alkaline and neutral media, which could limit their use in pure form. Therefore, the clinical utility of phenolic compounds as anti-SARS-CoV-2 agents remains debatable since their bioavailability, delivery mechanisms and efficient doses should be further studied using *in vivo* models.

Wen et al. (2011) evaluated 200 Chinese herbal extracts for their anti-SARS-CoV effect using a cell-based assay. Among them, six extracts [rhizomes of *Gentiana scabra* Bunge; tuber of *Dioscorea polystachya* Turcz.; seed of *Senna tora* (L.) Roxb.; stem and leaves of *Taxillus chinensis* (DC.) Danser; and two extracts of *Cibotium barometz* (L.) J.Sm. rhizome] were found to significantly inhibit SARS-CoV growth and replication. IC_{50} values ranged from 25 to 200 μg/ml. By using FRET assay, the study demonstrated that extracts obtained from tuber of *Dioscorea polystachya* Turcz. and rhizome of *Cibotium barometz* (L.) J.Sm. caused marked inhibition of SARS-CoV- 3CL protease with IC_{50} of 39 and 44 μg/ml, respectively.

Owing to its importance as a key protein for SARS-CoV genome replication, SARS-CoV helicase still remains a target of novel antiviral drugs. Sixty-four natural molecules originated from 15 medicinal species were evaluated regarding their inhibitory activity of SARS-CoV helicase. Myricetin and scutellarein (**Figure 1**) significantly inhibited the SARS-CoV helicase activity. At 10 μM, myricetin (IC_{50} = 2.71 ± 0.19 μM) and scutellarein (IC_{50} = 0.86 ± 0.48 μM) were able to inhibit 90% of the ATPase activity of the SARS-CoV helicase. Accordingly, Myricetin and scutellarein were suggested to be promising future anti-SARS drugs (Yu et al., 2012).

SARS-CoV-2 life-cycle related proteins are considered promising targets of antiviral drugs. Therefore, molecules and/or products able to inhibit these proteins may be used to treat or even prevent the SARS-CoV-2 infections (**Table 1**).

NATURAL PRODUCTS AS ACE2-BLOCKERS

The penetration of the SARS-CoV-2 genome into the host cells occurs as a result of the SARS-CoV-2 spike protein binding to host receptors (Sigrist et al., 2020). By using phylogenetic analysis and critical site of ACE2 structure, different animals such as cat, pigeon, and sheep were predicted to be important intermediate hosts for SARS-CoV-2 (Qiu et al., 2020). Hoffmann et al. (2020) demonstrated that the ACE2 receptor is used by SARS-CoV-2 to enter human cells. Moreover, they reported that the use of TMPRSS2 inhibitors may be a promising therapeutic approach against SARS-CoV-2. TMPRSS2 is a transmembrane serine protease that cleaves both ACE2 and the S protein. Recently, Ortega et al. (2020) used *in silico* approaches to understanding the relationship between changes in SARS-CoV-2 Spike protein and ACE2 receptor. They demonstrated superior affinity of SARS-CoV-2 spike protein towards human ACE2 as compared to that of the Bat-CoV spike and ACE2. This study supported the idea that the ACE2 receptor may be the key "bridge" used by SARS-CoV-2 to transmit among humans. Chen et al. (2020) confirmed that although SARS-CoV and SARS-CoV-2 RBD of spike glycoprotein had 72% of structural similarities, SARS-CoV-2 RBD exhibited higher interaction with ACE2. ACE2 inhibitors are thought to indirectly alter the RBD binding site and therefore block SARS-CoV-2 infection. Likewise, Wrapp et al. (2020) found that the SARS-CoV-2 spike exhibited a higher affinity to ACE2 than SARS-CoV. Adedeji et al. (2013) demonstrated that early blocking of SARS-CoV with ACE2 inhibitors was one of the mechanisms used by novel efficient anti-SARS drugs. Nonetheless, it has been shown in three recent studies on COVID-19 that hypertension and diabetes mellitus significantly enhanced the risk of COVID-19 infection, in spite of using ACE2 inhibitors (Guan et al., 2019; Yang X. et al., 2020; Zhang J. J. et al., 2020). ACE2 inhibitors, angiotensin II type-I receptor blockers, and ibuprofen lead to ACE2 upregulation which justifies the urgent need to use and/or identify alternative ACE2 blockers (Fang et al., 2020). Hence, medicinal plants-derived products or natural products able to selectively block the ACE2 receptor without inhibiting the enzyme activity may be useful to prevent and/or treat SARS-CoV-2 spread in humans without increasing ACE2 expression in patients and therefore increased risk for COVID-19.

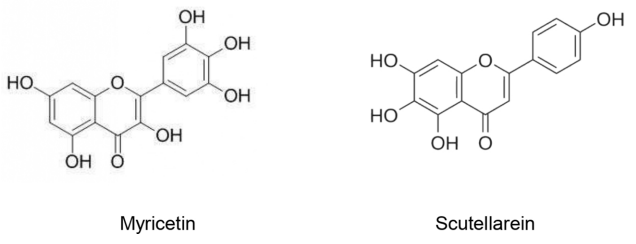

FIGURE 1 | Natural products that act as SARS-CoV helicase activity.

Since important similarities exist between the sequences of ACE and ACE2 (Guy et al., 2005), molecules with inhibitory effects toward ACE may exert the same effect toward ACE2 and thus lead to reduce the viral entry. However, further studies should be undertaken to evaluate this hypothesis. Patten et al. (2016) reviewed the medicinal plants for their inhibitory effects on ACE2. They reported 141 medicinal species belonging to 73 families and 49 purified natural compounds with documented ACE inhibitory potential. Moreover, 16 medicinal species (16 families) were found to be able to block the angiotensin type 1A receptor *in vitro*. Sharifi et al. (2013) identified four Iranian medicinal species able to inhibit more than 80% of ACE activity *in vitro*. These active species were: *Berberis integerrima* Bunge., *Crataegus laevigata* (Poir.) DC., *Onopordum acanthium* L., and *Quercus infectoria* G. Olivier. At 330 μg/ml, *Quercus infectoria* G. Olivier. was found to be the most active and caused 94% ACE inhibition. This important inhibitory activity might be attributed to its higher phenolic content and enhanced antioxidant potential. In spite of the important ACE inhibition and antioxidant activities exhibited by *Q. Infectoria* extract, the presence of condensed tannins compromised its usefulness due to their interference in the functions of ACE. On the other hand, *B. integerrima, C. microphylla* and *O. acanthium* were found to have both important ACE inhibitory activities and enhanced antioxidant potential without the presence of tannins (Sharifi et al., 2013). These species could be promising sources of antiviral molecules. Indeed, viral infections are accompanied by oxidative stress which in turn promotes virus replication. Antioxidant species decrease the ROS production in infected cells and target different oxidative stress-related signalling pathways resulting in reduction of viral spread (Fedoreyev et al., 2018). Walls et al. (2020) demonstrated that SARS-CoV-2 and SARS-CoV bind with similar affinities to ACE2. In another study, 25 Chinese herbal families were found to significantly inhibit the interaction SARS-CoV – ACE2. Among them, species belonging to Polygonaceae, Labiatae, Oleaceae, Magnoliaceae, Lauraceae, and Nelumbonaceae exhibited the most important inhibitory effects. These inhibitory effects were attributed to

emodin (1,3,8-trihydroxy-6-methylanthraquinone) (**Figure 2**) produced in high levels in in genus Rheum and Polygonum. Emodin blocked the interaction SARS-CoV S protein and ACE2 in a dose-dependent manner with an IC_{50} of 200 μM (Ho et al., 2007).

NATURAL PRODUCTS TARGETING THE TMPRSS2

The type II transmembrane serine-proteinase serine type 2 RSS2 type II transmembrane serine protease that cleaves the S spike proteins of SARS-CoV and MERS and ACE2 (Iwata-Yoshikawa et al., 2019). Recently, Hoffmann et al. (2020) demonstrated that besides using ACE2 receptor to enter the host cells, SARS-CoV-2 uses also TMPRSS2 for S protein priming. After the interaction between the S spike protein (SARS-CoV-2) and the ACE2 (host cell), the complex is cleaved by the TMPRSS2 to facilitate viral entry (Rabi et al., 2020). Matsuyama et al. (2020) found that an important TMPRSS2 expression in cells makes them highly susceptible to SARS-CoV-2. Since SARS-CoV-2 viral entry is conditioned by its binding to the ACE2 receptor, and the latter should be cleaved by the TMPRSS, finding agents able to suppress or downregulate the TMPRSS2 expression in human cells could represent a promising therapeutic or preventive approach (Schlagenhauf et al., 2020). Several studies have demonstrated that natural products could downregulate or suppress TMPRSS2. It has been shown that kaempferol was able to inactivate TMPRSS2 expression by 49.14 and 79.48% at 5 and 15 μM, respectively (Da et al., 2019). Likewise, sulforaphane (an isothiocyanate) was found to downregulate the TMPRSS2 expression through the release and translocation of the Nrf2 (nuclear factor (erythroid-derived 2)-like 2) (Gibbs et al., 2009; Meyer and Jaspers, 2015). Mamouni et al. (2018) found that a standardized flavonoids formulation including luteolin, quercetin, and kaempferol significantly suppressed TMPRSS2 expression. In spite of the diverse biological effects attributed to the three flavonoids, this study has demonstrated that at low concentrations, they exhibited an important synergic effect. Nonetheless, the efficacy and safety of these compounds in COVID-19 patients is still unclear. Moreover, modes of administration, the health of the patients' digestive system, and disease stage may limit the clinical usefulness of such formulations and compounds (Fuzimoto and Isidoro, 2020).

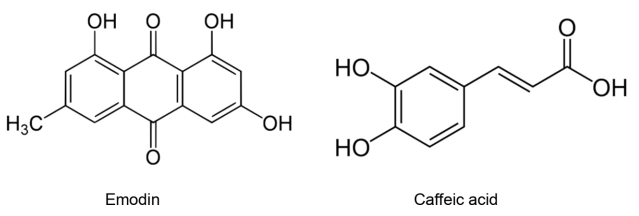

FIGURE 2 | Natural products that act as inhibitors of ACE2.

Xu et al. (2012) demonstrated that cryptotanshinone at 0.5 μM effectively exhibited anti-TMPRSS2 activity (**Figure 3**).

NATURAL PRODUCTS TARGETING THE PAPAIN-LIKE PROTEINASE (PLPRO)

PLpro is one of the nonstructural proteins encoded by the SARS-CoV-2 genome. This protease is vital for virus replication since it contributes to the cleavage of viral polyproteins (PP1A and PP1AB) into effector proteins (Jiang et al., 2020). Moreover, PLpro has been found to be an antagonist of the host's innate immunity (Yuan et al., 2015; Li et al., 2016). Actually, PLpro was shown to target the interferon production by blocking the IRF3 phosphorylation, dimerization, and nuclear translocation and NF-κB signaling pathways (by preventing IκBα degradation) (Wong et al., 2016). These effects were shown to occur in Toll-like receptor 3 and retinoic acid-inducible gene 1 pathways. Moreover, it has been demonstrated that SARS-CoV PLpro inhibits the TLR7 pathway *via* inactivation of TRAF3/6-TBK1-IRF3/NF-κB/AP1 signalling pathways (Yuan et al., 2015).

Recently, Arya et al. (2020) screened FDA-approved drugs for their *in silico* inhibitory potential of PLpro. They demonstrated that sixteen FDA-approved drugs (Biltricide, Cinacalcet, Procainamide, Terbinafine, Pethidine, Labetalol, Tetrahydrozoline, Ticlopidine, Ethoheptazine, Levamisole, Amitriptyline, Naphazoline, Formoterol, Benzylpenicillin, Chloroquine, and Chlorothiazide) exhibited important binding affinity to SARS-CoV-2 PLpro suggesting their possible effectiveness as anti-SARS-CoV-2 agents. Likewise, Disulfiram (an alcohol-aversive drug) was found to be a competitive inhibitor of SARS-CoV PLpro (Lin et al., 2018).

Several compounds have been shown to target the SARS-CoV PLpro (**Figure 4**).

Cinnamic Amides From *Tribulus terrestris*

Several natural compounds were found to possess promising PLpro inhibitory effects. Indeed, Song et al. (2014) demonstrated that six cinnamic amides (N-trans-Feruloyloctopamine, N-trans-Coumaroyltyramine, N-trans-Caffeoyltryamine, Terrestrimine, N-trans-Feruloyltryamine, and Terrestriamide) extracted from *Tribulus terrestris* L. fruits were able to inhibit SARS-CoV PLpro in a dose-dependent manner. PLpro inhibitory IC_{50} of these compounds were found to be 15.8–70.1 μM. Terrestrimine [(E)-N-(1-hydroxy-2-(4-hydroxyphenyl)-2- oxoethyl)-3-(4-hydroxy-3-methoxypheny) acrylamide] showed the best inhibitory activity of SARS-CoV PLpro with an IC_{50} of 15.8 ± 0.6 μM. The presence of a polar substituent (ketone or alcohol) on the methylene groups (C8'and C7') was associated with enhanced inhibitory activity.

Flavonoids From *Cullen corylifolium* (L.) Medik.

Ethanolic extract of *Cullen corylifolium* (L.) Medik. seeds showed an important inhibitory effect of SARS-CoV PLpro

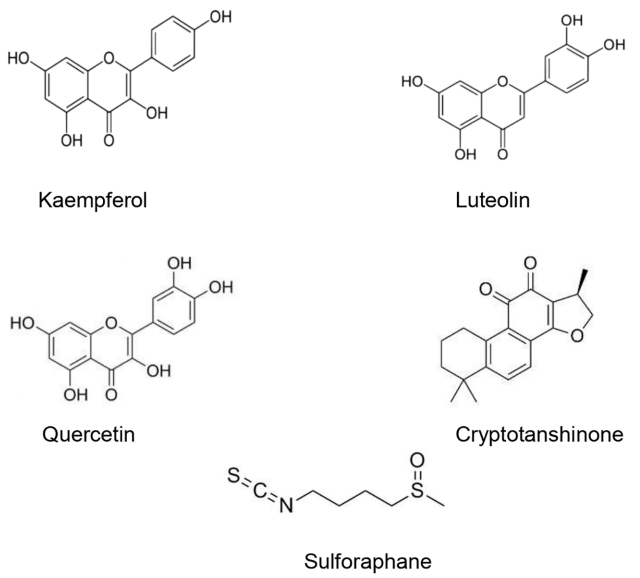

FIGURE 3 | Natural products that act as inhibitors of TMPRSS2.

with an IC_{50} of 15 μg/ml. Furthermore, six flavonoids present in the extract (Bavachinin, neobavaisoflavone, isobavachalcone,40 –O-methylbavachalcone, psoralidin, and corylifol A) inhibited SARS-CoV PLpro activity in a dose-dependent manner with IC_{50} estimated to be 4.2–38.4 μM. The highest inhibitory effect was exerted by psoralidin (IC_{50} = 4.2 ± 1.0 μM) and isobavachalcone (IC_{50} = 7.3 ± 0.8 μM) (Kim et al., 2014).

Flavonoids From *Paulownia tomentosa* (Thunb.) Steud.

Cho et al. (2013) identified five new geranylated flavonones, tomentin A, tomentin B, tomentin C, tomentin D, tomentin E from the ethanolic extract of *Paulownia tomentosa* (Thunb.) Steud. fruits. These flavonoids besides seven other knowns resulted in significant inhibition of SARS-CoV PLpro in a dose-dependent manner with IC_{50} of 5.0 and 14.4 μM. Tomentin E exhibited the highest inhibitory effect with an IC_{50} of 5.0 ± 0.06 μM. It has been found that molecules with 3,4-dihydro-2H-pyran moiety possessed higher inhibition. The *P. tomentosa* flavonoids were found to be reversible, mixed inhibitors.

Chalcones From *Angelica keiskei* (Miq.) Koidz

Park et al. (2016) investigated the inhibitory potential of nine alkylated chalcones (isobavachalcone, 4-hydroxyderricin, xanthoangelol, xanthoangelol F, xanthoangelol D, xanthoangelol E, xanthoangelol B, xanthoangelol G, xanthokeistal A) and four coumarins extracted from the ethanolic extract of *Angelica keiskei* (Miq.) Koidz. The alkylated chalcones inhibited SARS-CoV PLpro in a significant dose-dependent manner with IC_{50} ranging from 1.2 ± 0.4 to 46.4 ± 7.8 μM. On the other hand, the analyzed coumarins did not show a significant inhibitory effect toward SARS-CoV PLpro. Kinetic studies revealed that isobavachalcone was a mixed inhibitor whereas the other chalcones were

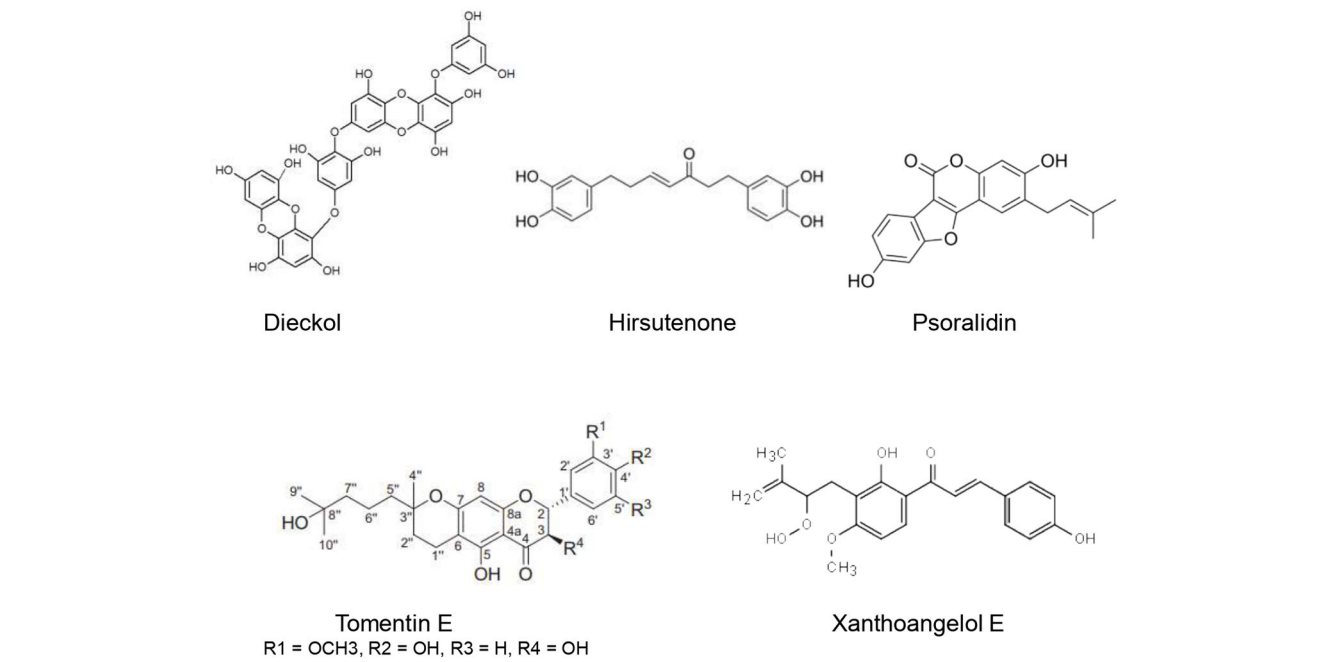

FIGURE 4 | Natural products that act as inhibitors of PLpro.

noncompetitive. Interestingly, xanthoangelol E, an –OOH substituted analogue, exhibited the most enhanced inhibitory effect (IC_{50} = 1.2 ± 0.4 μM), which was 40-fold higher when compared to other tested chalcones. This important inhibitory activity of xanthoangelol E was confirmed using *in silico studies.*

Tanshinones From *Salvia miltiorrhiza* Bunge

Salvia miltiorrhiza Bunge ethanolic extract (30 μg/ml) caused 88% inhibition of SARS-CoV PLpro. Moreover, seven bioactive tanshinones (tanshinone IIA, tanshinone IIB, methyl tanshinonate, cryptotanshinone, tanshinone I, dihydrotanshinone I, and rosmariquinone) were identified from the n-hexane fraction. These tanshinones were evaluated with regard to inhibition of SARS-CoV PLpro activity using a fluorometric assay. Both molecules exhibited important inhibitory time-dependent activities with IC_{50} of 0.8 to 30 μM. The dimethyl tetrahydronaphthalen structure was associated with enhanced inhibitory potential. The results obtained showed that cryptotanshinone was the most potent inhibitor of SARS-CoV PLpro with an IC_{50} of 0.8 ± 0.2 μM. Kinetic investigations demonstrated that rosmariquinone was a mixed-type inhibitor of SARS-CoV PLpro, whereas the other tanshinones were noncompetitive inhibitors (Park et al., 2012a).

Diarylheptanoids From *Alnus japonica* (Thunb.) Steud.

Park et al. (2012b) used activity-guided fractionation to identify nine diarylheptanoids (platyphyllenone, hirsutenone, platyphyllone, platyphyllonol-5-xylopyranoside, hirsutanonol, oregonin, rubranol, rubranoside B, and rubranoside A) from the ethanol extract of *Alnus japonica (Thunb.) Steud.* They evaluated their SARS-CoV PLpro inhibitory effect using a continuous fluorometric assay. The results showed that hirsutenone, hirsutanonol, oregonin, rubranol, rubranoside B, and rubranoside A exerted a significant dose-dependent inhibitory activity towards SARS-CoV PLpro with IC_{50} ranging from 3 to 44.5 μM. Hirsutenone possessed the most potent inhibitory effect with IC_{50} of 4.1 ± 0.3 μM which was less important than that of the reference inhibitor curcumin (5.7 μM). The enhanced inhibitory potential of diarylheptanoids seems to be related to the presence of α,β-unsaturated carbonyl, and catechol groups.

NATURAL PRODUCTS TARGETING THE CHYMOTRYPSIN-LIKE PROTEASE [3CL (PRO)]

3CL(pro) belongs to the 16 nonstructural proteins of the SARS-CoV-2. Since it plays an important role in the SARS-CoV-2 replication process polyproteins, 3CL(pro) is considered a potential therapeutic target for anti-COVID-19 drugs (Zhang L. et al., 2020). Different natural compounds exhibited promising anti3CL(pro) activities (**Figure 5**).

Alkylated Chalcones From *Angelica keiskei* (Miq.) Koidz

Inhibitory potential toward SARS-CoV- 3CL(pro) of alkylated chalcones and coumarins extracted from *Angelica keiskei* (Miq.) Koidz was investigated using a fluorescence resonance energy

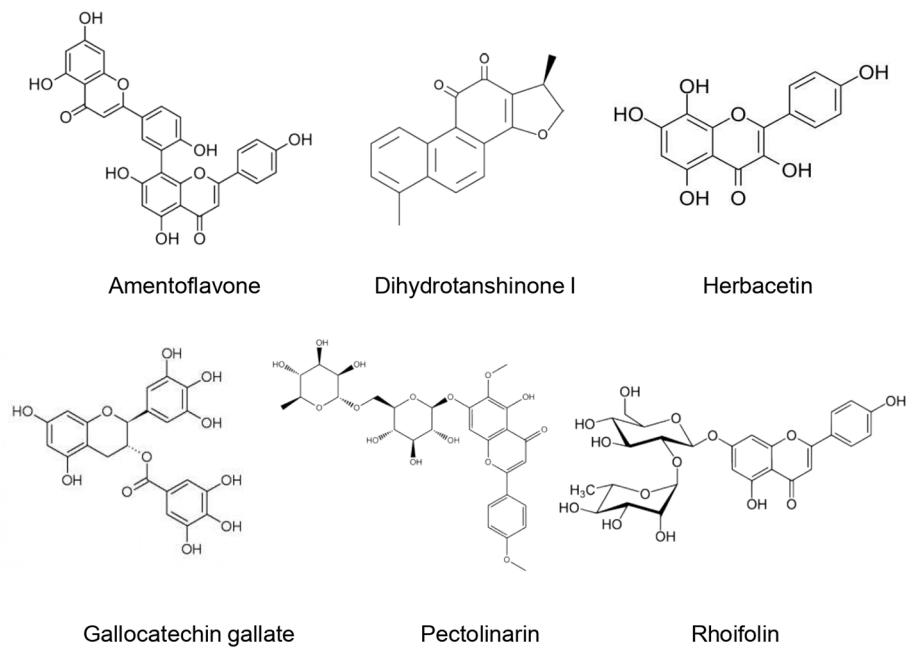

FIGURE 5 | Natural products that act as inhibitors of 3CL(pro).

transfer (FRET) method. Except for coumarins, alkylated chalcones exhibited marked inhibitory effects in a dose-dependent fashion. IC_{50} ranged from 11.4 ± 1.4 to 129.8 ± 10.3 μM. Moreover, xanthoangelol E (**Figure 5**) was found to be the most potent SARS-CoV- 3CL(pro) inhibitor. Kinetic studies showed that both alkylated chalcones were competitive inhibitors (Park O. K. et al., 2016). Since xanthoangelol E was also found to inhibit SARS-CoV- PLpro (Park O. K. et al., 2016) it could be a promising candidate in the therapeutic approach against COVID-19.

Phlorotannins From *Ecklonia cava* (Algae)

Park et al. (2013) isolated nine phlorotannins from the ethanolic extract of brown Alga *Ecklonia cava*. These phlorotannins were assessed regarding their inhibitory effects towards SARS-CoV-3CL(pro) using a cell-free based assay. Eight phlorotannins (triphloretol A, eckol, dioxinodehydroeckol, 2-phloroeckol, 7-phloroeckol, fucodiphloroethol G, dieckol, and phlorofucofuroeckol A) were shown to be competitive inhibitors of SARS-CoV- 3CL (pro) in a dose dependent manner. IC_{50} ranged from 2.7 ± 0.6 (dieckol) to 164.7 ± 10.8 μM (triphloretol A). Moreover, six phlorotannins (dioxinodehydroeckol, 2-phloroeckol, 7-phloroeckol, fucodiphloroethol G, dieckol, and phlorofucofuroeckol A) resulted in a significant micromolar dose-dependent inhibition of SARS-CoV-3CL(pro) cis-cleavage activity. Of the tested molecules, diekcol (possessing two eckol groups linked by a diphenyl ether) exhibited the best inhibitory effect of SARS-CoV-3CL(pro). Molecular docking studies corroborated this result since diekcol possessed the lowest binding energy (11.51 kcal/mol) towards SARS-CoV-3CL(pro). Diekcol was shown to form strong H bonds to the catalytic dyad (Cys145 and His41). Nevertheless, the bioavailability of phlorotannins and the inter-individual differences regarding their metabolization is still a substantial limitation to validate their usefulness. Gut microbiota composition seems to play a critical role in determining their health benefits (Corona et al., 2016). Moreover, the complexity of their structures due to the diversity of structural linkages and the different structural and conformational isomers for the same molecular weight, in addition to the absence of analytical standards and the lack of clear relationship between their structure and bioactivity may be another limitation to their clinical use (Li et al., 2017).

Tanshinones From *Salvia miltiorrhiza* Bunge

Park O. K. et al. (2012) investigated the inhibitory potential of *Salvia miltiorrhiza* Bunge towards SARS-CoV-3CL(pro). They found that *Salvia miltiorrhiza* Bunge ethanolic extract (30 μg/ml) resulted in 60% inhibition of SARS-CoV-3CL(pro). Furthermore, they demonstrated that six tanshinones of the plant (lipophilic fraction) exerted marked inhibition of SARS-CoV-3CL(pro) in a dose- but not–time- dependent manner. IC_{50} was estimated at 14.4–89.1 μM. Dihydrotanshinone I exhibited the most important inhibitory effect with an IC_{50} of 14.4 ± 0.7 μM. With regard to the kinetic mechanism of SARS-CoV-3CL(pro) inhibition, *Salvia miltiorrhiza* Bunge tanshinones were found to be noncompetitive inhibitors.

Biflavonoids From *Torreya nucifera* (L.) Siebold & Zucc.

Four biflavonoids (amentoflavone, bilobetin, ginkgetin, and sciadopitysin) were isolated from the ethanol extract of *Torreya nucifera* (L.) Siebold & Zucc. leaves and evaluated for their SARS-CoV-3CL(pro) inhibitory effect by using a FRET method. All biflavonoids exhibited a marked inhibitory effect of SARS-CoV-3CL(pro) with IC_{50} of 8.3–72.3 μM. This inhibitory effect was stronger than that of eight diterpenoids isolated from the *T. nucifera* extract (IC_{50}: 49.6–283.5 μM). Amentoflavone exerted the most important inhibitory activity since it possessed the lowest IC_{50} (8.3 ± 1.2 μM). Moreover, its inhibitory potential was more important than that of apigenin (IC_{50} = 280.8 ± 21.4 μM), quercetin (IC_{50} = 23.8 ± 1.9 μM) and luteolin (IC_{50} = 20.0 ± 2.2 μM). Molecular docking demonstrated that amentoflavone showed a good affinity with SARS-CoV-3CL(pro) and formed strong hydrogen bonds. An apigenin moiety at position C-30 of flavones was suggested to be responsible for a better inhibitory effect (Ryu et al., 2010).

Flavonoids

Seven flavonoids (Quercetin, Puerarin, Daidzein, gallocatechin gallate, epigallocatechin gallate, epigallocatechin, ampelopsi) were evaluated for their inhibitory effects of SARS-CoV-3CL(pro) expressed in *Pichia pastoris* GS115. At 200 μM, gallocatechin gallate, epigallocatechin gallate, and quercetin were able to inhibit the SARS-CoV-3CL(pro) activity by 91, 85, and 82%, respectively. Gallocatechin gallate was found to be a competitive inhibitor of SARS-CoV-3CL(pro) with IC_{50} of almost 47μM. Molecular docking studies confirmed the important inhibitory potential of gallocatechin gallate owing to the hydrophobic and H-bonds interaction formed with the active site of SARS-CoV-3CL(pro) (Nguyen et al., 2012). Nonetheless, it is difficult to predict how these H-bonds may contribute to the biological functions of the supposed active molecules (Chen et al., 2016). Furthermore, the strength of the H-bonds is not evaluated in this study which could limit the selectivity of the tested molecules since an important number of weak H-bonds often increase their affinity and therefore their interaction with off-target proteins.

Jo et al. (2020) evaluated the inhibitory effect of 64 flavonoids towards SARS-CoV-3CL(pro) using the FRET method. They found that at 20 μM, rhoifolin, herbacetin, and pectolinarin possessed the higher inhibitory effect, with IC50 values of 27.45, 33.17, and 37.78 μM, respectively. In addition, molecular docking revealed that flavonoids possessed an important binding affinity for SARS-CoV-3CL(pro) owing to their hydrophobic aromatic rings and hydrophilic hydroxyl groups.

Figure 6 summarizes the possible anti-SARS-CoV 2 actions of natural products.

CRITICAL CONSIDERATIONS

In the present review, 15 *in vitro* studies were included. They were from Korea (9/15), Taiwan (3/15), China (2/15), and Iran

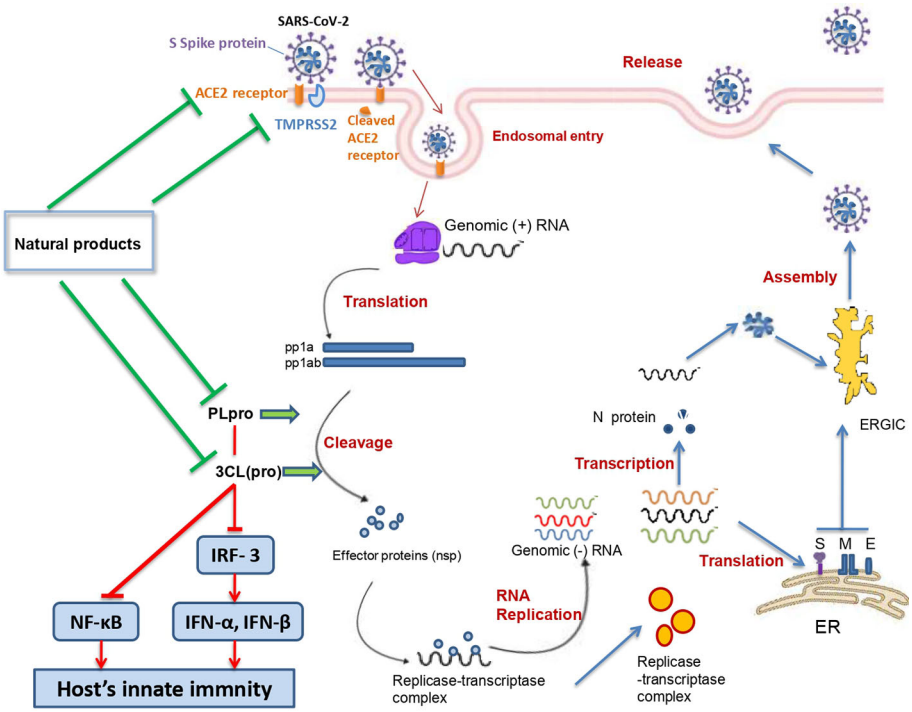

FIGURE 6 | Summary of possible anti SARS-CoV 2 actions of natural products.

(1/15). Most of the studies (10/15) were published between 2011 and 2018 whereas only two studies (Runfeng et al., 2020; Jo et al., 2020) were published in 2020 and one study (Weng et al., 2019) in 2019. Of them, only one study (Runfeng et al., 2020) investigated the antiviral activity against SARS-CoV-2, and another (Weng et al., 2019) on the human coronavirus NL63, whereas the other studies were not cell-based. Three studies used plant extracts and one study (Runfeng et al., 2020), a herbal mixture of 11 Chinese plants. Ethanol, methanol, and water were the solvents used to prepare the plant extracts included in these studies. Most of the studies were conducted with isolated natural compounds belonging to different phytochemical classes. Phenolic compounds were the most frequently reported.

When studies were analyzed using the best practice recommendations in phytopharmacological research (Heinrich et al., 2020), several concerns were detected especially regarding reporting data and outcomes. A large number of natural products reported in this review such as phenolic acids, quercetin, or kaempferol belong to phytochemical classes known to possess a broad spectrum of biological activities both *in vitro* and *in vivo*. Therefore, most of the studies failed to demonstrate the specificity of such products. In addition, all the included studies did not consider the drugability of the "active" compounds or extracts and presented an insufficient interpretation of the obtained data. Likewise, all the studies presented limitations regarding concepts and methods and also in the development of the project. In fact, all the studies investigating herbal extracts and isolated compounds did not consider the sustainable sourcing of the species nor the registration standards of both compounds or accepted plants' names. Likewise, the included studies presented serious bias concerning the dose range and the toxic doses. On the other hand, eight studies did not report the use of control, whereas the other studies did not justify the choice of the positive controls used for comparison. This could be considered as a risk of bias resulting in possible limitations in the methodology of the studies. Besides, the lack of full taxonomic validity has been detected in all these studies.

It has been reported that IC_{50} of chloroquine inhibition of SARS-CoV-2 replication was found to be 1.13 to 5.47 μM (Smit et al., 2020) whereas that of SARS-CoV was 8.8 μM (Keyaerts et al., 2004). On the other hand, IC_{50} of ivermectin was found to be ~2 μM (Caly et al., 2020). Therefore, molecules with IC_{50} ranging from 0 to 10 μM have been considered as possible active molecules against SARS-CoV-2. Accordingly, few natural molecules reported in the included studies could be considered active against SARS-CoV-2 including amentoflavone, dieckol, hirsutenone, cryptotanshinone, xanthoangelol E, tomentin E, psoralidin, scutellarein, myricetin, and caffeic acid. Nevertheless, most of these active molecules are phenolic compounds characterized by a low bioavailability and rapid elimination (Górniak et al., 2019) which could compromise their clinical usefulness in the context of COVID-19.

Despite the promising possible anti-SARS-CoV-2 effects exhibited by both plant extracts and natural molecules, several limitations should be considered. Overall, due to the recent outbreak, the clinical usefulness of these products needs to be demonstrated since the current data are still immature, and no final conclusions have been validated. As shown in **Table 2**, for some species, there was no relationship between the traditional ethnopharmacological uses and the anti-SARS-CoV-2 effects. In

spite of that, these plants are currently used to treat or manage symptoms reported in SARS-CoV-2 disease such as fever, inflammations, or cardiovascular and circulatory disorders. Moreover, efficacy and safety of the active natural products should be further studied *in vivo* and clinically validated in COVID-19 patients. Importantly, bioavailability, modes of administration, safe doses, time of exposure, pharmacokinetic profile, the health of the patients' digestive system, and disease stage are to be considered in the evaluation of the beneficial effects of natural products against SARS-CoV-2. On the other hand, further studies are needed to clarify the mechanisms and pathways targeted by such products, which will help to improve their clinical usefulness. Assessing the effects of combinations of active natural products with validated antiviral drugs could be a promising alternative to explore.

CONCLUSION

Medicinal plants and natural products are still considered promising alternatives to prevent or treat several diseases. Since the outbreak of the COVID-19 pandemic in December 2019, various traditional herbal medicines have been used and resulted in positive health effects among COVID-19 patients, mainly in China. In the present review, we have discussed the possible potential uses of medicinal plants and/or natural products to prevent or even treat COVID-19. Although the studies evaluating the anti-SARS-CoV-2 effects of medicinal plants are still insufficient and relatively immature, some natural products with IC_{50} below 10 μM could be considered as promising anti-SARS-CoV-2 agents since they were able to block its life-cycle related proteins such as the cellular receptor ACE2, papain-like or chymotrypsin-like proteinases. Nevertheless, several limitations have been detected in relation to the specificity of the action exerted by such products, sustainable sourcing of the species, doses range used, or the use of appropriate controls.

While available studies offer several indications that these plant-derived products may help in fighting COVID-19, further studies should be carried out to evaluate the clinical usefulness of such products against COVID-19 infection. Furthermore, the bioavailability of natural products with possible anti-SARS-CoV-2 effects such as tannins should be considered besides the need for clinical validation of their usefulness and safety. The herbal mixtures, medicinal plants, or natural products with possible anti-SARS-CoV-2 effects must be evaluated through prospective and interventional studies. A combination of natural products or herbal mixtures with validated anti-COVID-19 drugs may constitute a promising preventive and therapeutic alternative to be assessed.

AUTHOR CONTRIBUTIONS

BB and AP contributed equally to the study. All authors contributed to the article and approved the submitted version.

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REVIEW
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Clinical Studies on the Treatment of Novel Coronavirus Pneumonia With Traditional Chinese Medicine—A Literature Analysis

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Objective: This study aims to analyze the current situation and characteristics of traditional Chinese medicine for treatment of novel coronavirus pneumonia, clarify its clinical advantages and provide a reference for clinical treatment.

Methods: Clinical randomized controlled trials, clinical control trials and case series research involving the use of Chinese medicine for novel coronavirus pneumonia treatment were selected from PubMed, Chinese Journal Service Platform of CNKI, VIP, and WanFang Data Knowledge Service Platform from the establishment of the library to 11:00 AM on April 15, 2020. The published information, research design, intervention measures and research observation index were statistically analyzed.

Results: Twenty studies were included. The research methods were mainly clinical controlled trials. The observation indicators were mostly fever improvement time, cough improvement time, shortness of breath improvement time, chest CT and CRP examination. Maxing Ganshi (*Ephedrae Herba, Armeniacae Semen Amarum, Glycyrrhizae Radix Et Rhizoma*, and *Gypsum Fibrosum*) decoction was the core prescription. The most frequently used drugs were *Glycyrrhizae Radix Et Rhizoma* (Gancao), *Ephedrae Herba* (Mahuang), *Armeniacae Semen Amarum* (Kuxingren), *Atractylodis Rhizoma* (Cangzhu), and *Scutellariae Radix* (Huangqin). The most frequently used drug combination was *Ephedrae Herba* (Mahuang)–*Armeniacae Semen Amarum* (Kuxingren). The most frequently used Chinese patent medicine was Lianhua Qingwen capsule/granule.

Conclusions: Traditional Chinese medicine has widely used for novel coronavirus pneumonia in China. It is worthy of global attention. Also, high-quality randomized controlled clinical trials on the effectiveness and safety of traditional Chinese medicine in the treatment of novel coronavirus pneumonia need to carry out.

Keywords: novel coronavirus pneumonia, traditional Chinese medicine, clinical research, Drug application rule, literature analysis

INTRODUCTION

Recently, new coronary pneumonia (NCP) outbreaks worldwide, according to the daily information released by the Chinese State and Regional Health Committees' daily information as of 21:31 on April 16, 2020, China has confirmed a total of 83,798 cases and 3,352 cumulative deaths; among the cumulative confirmed cases of 2,019,857 worldwide, 135,165 died and 1,422,853 remained infected (Dingxiangyuan, 2020). The epidemic trend in regions outside of China has greatly erupted, overseas outbreaks have escalated, and more than 20 countries and regions have been infected. Except for Antarctica, all continents have confirmed cases. How to effectively treat NCP remains a key problem. The Office of the State Administration of Traditional Chinese Medicine and the General Office of the National Health And Health Commission have issued seven editions of the "Diagnosis and Treatment Plan of Novel Coronavirus Infection Pneumonia"; each version of the diagnosis and treatment plan has always emphasized the active role of Chinese medicine in the treatment and the strengthening of its combination with Western medicine to promote medical treatment and achieve good results (National Health Commission of the People's Republic of China, 2020). In an interview, Zhong Nanshan affirmed the role of Chinese medicine in treatment of NCP; Chinese medicine can effectively suppress inflammatory damages and can also be popularized in foreign countries (Tencent News, 2020a). The article aimed to systematically organize clinical research by literature metrology and data mining methods, analyze the current situation of clinical treatment research in Chinese medicine, explore the clinical treatment characteristics of Chinese medicine and provide a reference for global clinical treatment of NCP.

MATERIALS AND METHODS

Search Strategy

Two reviewers (ZZ and NG) independently isolated the useful information from the database. Studies that used Chinese medicine to treat NCP were selected from PubMed, Chinese Journal Service Platform of CNKI, VIP, and WanFang Data Knowledge Service Platform. Advanced search was conducted using the following terms: "NCP" or "Novel Coronavirus Infection" or "New Coronavirus" "2019-nCoV" "COVID-19" "SARS-CoV-2" containing "Chinese and Western medicine" or "Chinese medicine" or "Traditional Chinese medicine" or "prescription." The search time was from the establishment of the library to 11:00 on 15 April 2020.

Inclusion and Exclusion Criteria

Inclusion criteria: All studies on clinical treatment of NCP in Chinese medicine that state complete treatment options and processes and are classified as clinical control trials (CCT), randomized controlled trials (RCT), and case series studies (CS) were included.

Exclusion criteria: Studies categorized as review, basic research, regional epidemiological research, experience summary, and syndrome analysis were excluded.

Data Extraction and Analysis

Noteexpress, a document management software program, was used to manage the studies obtained from different databases. An access database was established to extract information on the publication of the literature (author, time of issue, issue journal, type of fund), research design (number of cases, subject gender and age), intervention measures (prescription, traditional Chinese medicine), research observation indicators and other information for statistical analysis. For eligible studies, two review authors (ZZ, and GN) extracted the data independently. Disagreements were resolved through consultation with a third party (FS).The law of the prescription use of Chinese medicine was analyzed statistically through the "Traditional Chinese medicine inheritance auxiliary system."

RESULTS

Description of Studies

We identified 757 potentially relevant articles. After removal of duplicates, 625 records remained. After going through the titles and abstracts, we exclude 605 papers. By reading the full text of the remaining 46 articles, 26 were exclude because they were case reports. Ultimately, 20 studies were included in present study (Bin et al., 2020; Cheng and Li, 2020; Cheng et al., 2020; Ding et al., 2020; Duan et al., 2020; Fang et al., 2020; Fu et al., 2020; Gong et al., 2020; Hu et al., 2020; Lv et al., 2020; Qv et al., 2020; Shi et al., 2020; Wang Y. et al., 2020; Wang T. et al., 2020; Xia et al., 2020; Xiao et al., 2020; Yang Q. et al., 2020; Yang Z. et al., 2020; Yao et al., 2020; Zhu et al., 2020). Among these studies, 2 RCTs, 10 CCTs and 8 CSs were included, which accounted for 10.00%, 50.00% and 40.00% of the total number of studies, respectively. The specific screening process is shown in **Figure 1**.

Basic Characteristics of the Literature

The basic characteristics of the 20 trials are summarized in **Tables 1** and **2**. The first study on clinical treatment involving Chinese medicine for treatment of NCP was published on February 6, 2020 (Gong et al., 2020). After February 15, the volume of studies published began to increase. By March 25, 19 articles were published. By April 4, the volume of literature published showed a downward trend. The total number of observations was 1,810, of which 1,021 and 789 were males and females, respectively. The age ranged from 0.6 to 95 y. The largest number of subjects in the study was 308 (Wang T. et al., 2020), and the minimum number of study cases was 13 (Cheng and Li, 2020). About the research areas, the worst-affected area, Hubei region, had the largest volume of studies, accounting for more than 50%, followed by Henan and Anhui regions. 12 trails were funded by research projects. All trials adopted decoction or patent medicine of traditional Chinese medicine (TCM) therapy

Abbreviations: NCP, new coronary pneumonia; CCT, clinical control trials; RCT, randomized controlled trials; CS, case series studies; TCM, Traditional Chinese medicine.

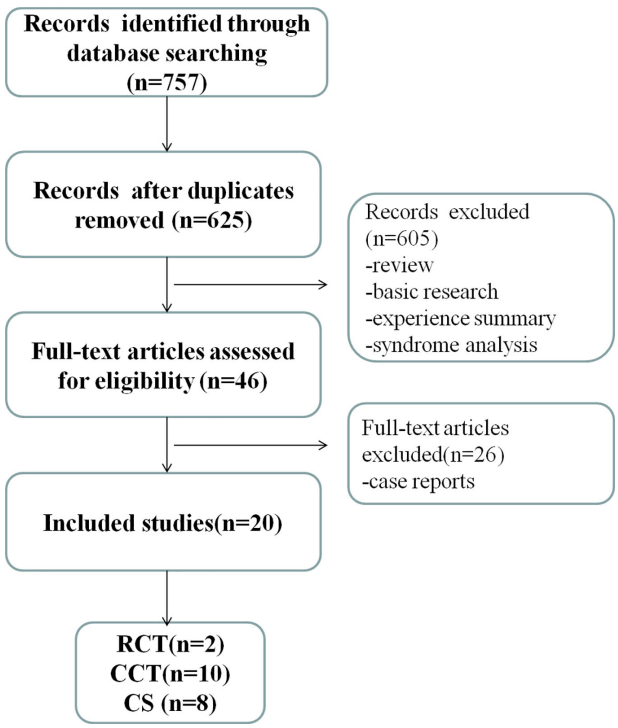

FIGURE 1 | Literature screening process and results.

combination with western treatment in the trial group for NCP. While the control group only adopted western treatment. For the severity of included subjects, most RCTs and CCTs included subjects who were the mild or common type, while the subjects in CS were common type and serious type. Three studies mentioned death cases (Bin et al., 2020; Xia et al., 2020; Yang Q. et al., 2020). One study mentioned there were no death cases (Wang T. et al., 2020). The other 16 studies did not mention the death condition. Adverse reactions were reported in eight studies, while no mention in the other studies. Specific research characteristics of RCTs and CCTs are shown in **Table 1** and CSs are shown in **Table 2**.

Analysis of the Law of Prescription Use in TCM

Frequency Analysis of Single Chinese Herbal Medicine

The statistical analysis showed that 34 traditional Chinese medicine prescriptions, involving 106 traditional Chinese medicines, were used in 20 clinical studies. The frequency of traditional Chinese medicine use was sorted. The top three drugs were *Glycyrrhizae Radix Et Rhizoma* (Gancao), *Ephedrae Herba* (Mahuang), and *Armeniacae Semen Amarum* (Kuxingren). *Ephedrae Herba* (Mahuang) aids in freeing lung, relieving cough and asthma and releasing exterior syndrome; *Armeniacae Semen Amarum* (Kuxingren) helps to depress qi and relieve cough and asthma; and *Glycyrrhizae Radix Et Rhizoma* (Gancao) facilitates in relieving cough and reducing sputum and coordinating of drugs. The three drugs are commonly used for cough and sputum and are also the basic components of Maxing Ganshi decoction in traditional Chinese medicine to treat cough and asthma. In the included prescriptions, 24 drugs were found with a frequency of ≥5 (**Table 3**). According to the traditional Chinese medicine category to sort out the 106 traditional Chinese medicines, the top 3 most frequently used are heat-clearing medicines, exterior syndrome-relieving medicines and phlegm-resolving and cough and asthma-relieving medicines, followed by damp-resolving medicines, tonify medicines, and damp-draining diuretic medicines. The details are presented in **Table 4**.

Analysis of the Association Rules of Traditional Chinese Herbal Medicine

The association rules of traditional Chinese medicine for the included prescriptions were analyzed. The support was set to 20%. The results showed 10 associations of traditional Chinese medicine with a confidence of above 0.8. The association of traditional Chinese medicine with a confidence of 1 was *Gypsum Fibrosum* (Shigao)->*Armeniacae Semen Amarum* (Kuxingren), *Tsaoko Fructus* (Caoguo) -> *Arecae Semen* (Binglang). The association of traditional Chinese medicine with a confidence level of above 0.86 was *Gypsum Fibrosum* (Shigao) -> *Ephedrae Herba* (Mahuang), *Gypsum Fibrosum* (Shigao), *Armeniacae Semen Amarum* (Kuxingren) -> *Ephedrae Herba* (Mahuang), *Ephedrae Herba* (Mahuang), *Arecae Semen* (Binglang) -> *Atractylodis Rhizoma* (Cangzhu), *Ephedrae Herba* (Mahuang), *Arecae Semen* (Binglang) -> *Armeniacae Semen Amarum* (Kuxingren), *Atractylodis Rhizoma* (Cangzhu), *Arecae Semen* (Binglang) -> *Ephedrae Herba* (Mahuang). **Table 5** presents the analysis of specific association rules.

Analysis of Chinese Herbal Medicine Combinations Network

The relationship among different drug combinations was visualized using the network display function of the traditional Chinese medicine inheritance auxiliary system. The results showed that *Ephedrae Herba* (Mahuang)–*Armeniacae Semen Amarum* (Kuxingren) had the highest support, as the most common core combination, followed by *Pinelliae Rhizoma* (Banxia)–*Poria* (Fuling), *Ephedrae Herba* (Mahuang)–*Glycyrrhizae Radix Et Rhizoma* (Gancao) and *Ephedrae Herba* (Mahuang)–*Atractylodis Rhizoma* (Cangzhu). This result indicates that commonly used clinical treatments for NCP involve depressing qi, relieving cough, eliminating dampness and eliminating phlegm. The Chinese herbal medicine combinations network is presented in **Figure 2**.

Analysis of Application of Classical Prescriptions of TCM

Studies involving the application of classical prescriptions of TCM were collected and summarized. Six studies were obtained. Among these classical prescriptions, Da Yuan decoction and Ganlu Xiaodu pill were created by doctors Wu Youke (Ming Dynasty and Ye Tianshi (Qing Dynasty) and who studied in epidemic exogenous febrile diseases, while Maxing Ganshi

TABLE 1 | Basic characteristics of the included studies (RCT and CCT).

Included trials	Funding	Study designs	Study region	Sample characteristics type; male/female; age(y)		Interventions		Duration	Fever improvement time(d)	Outcome index	Intergroup differences	Adverse reactions
				Trial	Control	Trial	Control					
YAO 0206 (Yao et al., 2020)		CCT	Hubei	CT:21 M: 16, F: 5 57.1 ± 14.0	CT: 21 M: 12, F: 9 62.4 ± 12.3	Chinese patent drug +WT1.2.6.7	WT1.2.6.7		T: 4.6 ± 3.2 C: 6.1 ± 3.1	1. Disappearance rate of fever and cough 2. Disappearance rate of fatigue 3. Fever improvement time 4. Disappearance rate of anhelation, expectoration 5. Disappearance rate of sore throat, choking sensation in chest, dyspnea, headache, nausea, anorexia, diarrhea, muscle pain 6. Death rate	1.P<0.05 2.P>0.05 3.P>0.05 4.P<0.05 5.P>0.05 6.Not mentioned	
LV 0217 (Lv et al., 2020)		CCT	Hubei	MT, CT: 63 M: 28, F: 35 59.1 ± 15.61	MT, CT: 38 M: 18, F: 20 60.2 ± 17.01	Chinese patent drug +WT1.2.3.5.7.8	WT1.2.3.5.7.8	10 d	T: 6 (median) C: 7 (median)	1. Disappearance rate of fever, fatigue, cough 2. Disappearance rate of anhelation, moist rale 3. Fever improvement time 4. Disappearance rate of muscle pain, expectoration, nasal obstruction, nasal discharge, sore throat, choking sensation in chest, dyspnea, headache, nausea, vomiting, anorexia, diarrhea 5. Aggravation rate 6. Death rate	1.P<0.05 2.P<0.05 3.P>0.05 4.P>0.05 5.P>0.05 6. Not mentioned	No adverse response
XIA 0218 (Xia et al., 2020)	√	CCT	Hubei	CT: 27 ST: 7 M: 17, F: 17 54.18 ± 13.08	CT: 13 ST: 5 M: 6, F: 12 53.67 ± 12.70	decoction +WT1.2.7.8	WT1.2.7.8	7–10 d	T: 2.64 ± 1.31 C: 4.38 ± 1.90	1. Fever improvement time 2. Recovery time of cough, fatigue, dyspnea, diarrhea) 3. Score of TCM syndrome scale 4. Incidence of mild type to severe type 5. Improvement rate of lung	1.P<0.01 2.P<0.01 3.P<0.05 4.<0.05 5.P>0.05	No adverse response

(Continued)

TABLE 1 | Continued

Included trials	Funding	Study designs	Study region	Sample characteristics type; male/female; age(y)		Interventions		Duration	Fever improvement time(d)	Outcome index	Intergroup differences	Adverse reactions
				Trial	Control	Trial	Control					
										CT 6. Death rate	6. Trial 0%; Control 5.6%	
QU 0226 (Qv et al., 2020)	√	CCT	Anhui	MT, CT: 40 M: 25, F: 15 40.65 ± 8.23	MT,CT:30 M: 16, F: 14 39.82 ± 6.40	Chinese patent drug+WT1.2	+WT1.2	10 d	T:3.24 ± 0.89 C:5.10 ± 1.40	1. Improvement time of temperature, dry cough, nasal obstruction, Fever improvement time, sore throat, fatigue, diarrhea 2. Dime of nucleic acid test turning negative 3. Death rate	1.P<0.05 2.P<0.05 3. Not mentioned	Trail: 1 case of nausea; Control: 2 cases of nausea
DING 0303 (Ding et al., 2020)		RCT	Hubei	MT: 10 CT: 36 ST: 5 M: 39, F: 12 54.7 ± 21.3	MT: 11 CT: 34 ST: 4 M: 39, F: 10 50.8 ± 23.5	decoction +WT1.2.6	WT1.2.6	10 d		1. Disappearance rate of fever, cough, choking sensation in chest and anhelation 2. Disappearance rate of nasal obstruction, abdominal pain, and diarrhea 3. Improvement rate of ESR 4. Improvement rate of CRP, IL-6 5. Improvement rate of TNF-γ, TNF-α 6. Improvement rate of lung CT 7. Liver function 8. Death rate	1.P<0.05 2.P>0.05 3.P<0.01 4.P<0.05 5.P>0.05 6.P<0.05 7.P>0.05 8. Not mentioned	
SHI 0305 (Shi et al., 2020)	√	CCT	Shanghai	MT: 1 CT: 40 ST: 8 M: 26, F: 23 47.94 ± 14.46	MT: 1 CT: 14 ST: 3 M: 10, F: 8 46.72 ± 17.40	Chinese patent drug +decoction +WT1.2.3.8	WT1.2.3.8	6 d	T: 16 (4,42) C: 17.5 (8,42)	1. Clinical syndrome integral 2. Hospitalization time 3. Course of disease, fever improvement time 4. Improvement rate of lung CT 5. Death rate	1.P<0.05 2.P<0.05 3.P>0.05 4.P>0.05 5. Not mentioned	

(Continued)

TABLE 1 | Continued

Included trials	Funding	Study designs	Study region	Sample characteristics type; male/female; age(y)		Interventions		Duration	Fever improvement time(d)	Outcome index	Intergroup differences	Adverse reactions
				Trial	Control	Trial	Control					
XIAO 0310 (Xiao et al., 2020)		CCT	Hubei	MT: 100 M: 64, F: 36 60.90 ± 8.70	MT: 100 M: 66, F: 34 62.20 ± 7.50	Chinese patent drug+WT1	WT1	2 w	T: 2.25 ± 1.12 C: 3.08 ± 1.64	1. Total effective rate 2. Lung CT 3. Fever improvement time 4. Disappearance time of cough, fatigue, dizziness, nasal discharge 5. WBC, Lymph% 6. Death rate	1.P<0.05 2.P<0.05 3.P<0.05 4.P>0.05 5.P<0.05 6. Not mentioned	Trail: 1 case of drug allergy: 2 cases of abdominal pain and diarrhea; Control: 2 cases of drug allergy, 1 case of abdominal pain and diarrhea
CHENG 0311 (Cheng et al., 2020)		CCT	Hubei	CT: 51 M: 26, F: 25 55.5 ± 12.3	CT: 51 M: 27, F: 24 55.8 ± 11.6	Chinese patent drug+WT1.2.8	WT1.2.8	7 d	T:2.9 ± 1.7 C:3.9 ± 1.3	1. Disappearance rate and time of fever, fatigue, cough 2. Effective rate of main symptoms 3. Disappearance rate of expectoration, anhelation, choking sensation in chest, anorexia 4. Disappearance rate of muscle pain, dyspnea, nausea 5. Improvement rate of lung CT 6. Rate of turn to severe type 7. Death rate	1.P<0.05 2.P<0.05 3.P<0.05 4.P>0.05 5.P>0.05 6.P<0.05 7. Not mentioned	
FU 0320 (Fu et al., 2020)	√	CCT	Hubei	CT: 37 M: 19, F: 18 45.26 ± 7.25	CT: 36 M: 19, F: 17 44.68 ± 7.45	Chinese patent drug+WT1.7	WT1.7	10–15 d		1. Accumulated points of fever, cough, dry throat and sore throat, choking sensation in chest and anhelation, fatigue 2. Effective rate, hospital discharge rate 3. Absolute value of LYM, CRP 4. WBC, LYM ratio 5. Death rate	1.P<0.05 2.P<0.05 3.P<0.05 4.P>0.05 5. Not mentioned	No adverse response
WANG 0323 (Wang Y. et al., 2020)	√	RCT	Hubei	MT, CT: 10 M: 5, F: 5 54.90 ± 3.71	MT,CT:10 M:5.F:5 55.90 ± 3.71	decoction, incense+WT1.	WT 1.2.8.	7 d		1. Clinical symptoms improved conditions (fatigue, cough, dry throat, short of breath)	1.P<0.05	

(Continued)

TABLE 1 | Continued

Included trials	Funding	Study designs	Study region	Sample characteristics type; male/female; age(y)		Interventions		Duration	Fever improvement time(d)	Outcome index	Intergroup differences	Adverse reactions
				Trial	Control	Trial	Control					
										2. Lung CT 3. Nucleic acid test turning negative 4. Death rate	2.P>0.05 3.P>0.05 4. Not mentioned	
DUAN 0324 (Duan et al., 2020)	√	CCT	Hubei	MT: 82 M: 39, F: 43 51.99 ± 13.88	MT:41 M:23.F:18 50.29 ± 13.17	Chinese patent drug +WT1.2.6.7	WT1.2.6.7	5 d		1. Disappearance condition of fever 2. Disappearance time of fatigue, cough, expectoration, diarrhea 3. Disappearance time of aversion to cold, bodily pain, sore throat, pharyngalgia, dry throat 4. Score of TCM syndrome scale 5. Hamilton Anxiety Scale 6. Death rate	1.P<0.01 2.P<0.05 3.P>0.05 4.P<0.01 5.P<0.01 6. Not mentioned	Trail: 27 cases of diarrhea Control: no adverse response
YANG 0414 (Yang Z. et al., 2020)	√	CCT	Hubei	ST: 51 M: 28, F: 23 61.57 ± 1.84	ST: 52 M: 24, F: 28 66.35 ± 1.82	decoction +Chinese patent drug +WT1.2.6.7	WT1.2.6.7			1. CRP 2. Albumin 3. Cases number of absorption and improvement by lung CT 4. Cure rate 5. Death rate	1.P<0.01 2.P<0.05 3.P<0.05 4.P>0.05 5. Trial 21.6%; Control 30.77%	Trail: 2 cases of mild gastrointestinal reactions

MT, mild type; CT, common type; ST, serious type; WT, western treatment.

WT: 1. antiviral; 2. anti-infection/anti-inflammatory/antibiotics; 3. immunoregulation; 4. gastrointestinal regulation; 5. relieving cough and asthma; 6. oxygen therapy; 7. glucocorticoid; 8. nutritional support; 9. nlgesics; 10. liver protection; 11. anti-anxiety.

TABLE 2 | Basic characteristics of the included studies (CS).

Included trials	Funding	Study region	Sample characteristics type; male/female; age (y)	Interventions	Duration	Fever improvement time(d)	Outcome index	Self before and after comparison	Adverse reactions
CHENG 0219 (Cheng and Li, 2020)		Hubei	CT:54 M:29.F:25 60.1 ± 16.98	Chinese patent drug +WT1.3.2.7	7 d	3.6 ± 2.14	1. Disappearance rate of fever 2. Disappearance rate of fatigue, disappearance days of fatigue 3. Disappearance rate of cough, disappearance days of cough 4. Disappearance rate of choking sensation in chest 5. Disappearance rate of anhelation 6. Disappearance rate of anorexia 7. Disappearance rate of moist rale 8. Effective rate 9. Death rate	1.80%, 2.75.7%, 4.1 ± 2.58 3.76.7%, 5.3 ± 2.63 4.84.6% 5.100% 6.40.0% 7.89.5% 8.81.6% 9. Not mentioned	No adverse response
WANG 0228 (Wang T. et al., 2020)		Jilin	MT,CT,ST:50 M:30.F:20 44.52 ± 16.12	decoction +WT1.2.6.7	7 d		1. Total effective rate 2. Disappearance rate of aversion to cold 3. Disappearance rate of thirsty 4. Disappearance rate of fever 5. Disappearance rate of sweating 6. Disappearance rate of nasal obstruction 7. Disappearance rate of headache body ache 8. Disappearance rate of short of breath 9. Disappearance rate of nausea 10. Disappearance rate of choking sensation in chest 11. Disappearance rate of diarrhea 12. Disappearance rate of anorexia 13. Disappearance rate of expectoration 14. Disappearance rate of fatigue 15. Disappearance rate of cough 16. Death rate	1.98.00% 2.100% 3.100% 4.96.96% 5.90.91% 6.73.33% 7.73.33% 8.72% 9.64.54% 10.64% 11.63.64% 12.55.56% 13.30.30% 14.25.93% 15.10.53% 16.0%	
BIN 0229 (Bin et al., 2020)	√	Hubei	MT:45 ST:10 M:31.F:24 53.9 ± 17.1	Chinese patent drug +WT1.2.6.7			1. Effective rate of mild patients 2. Effective rate of severe patients 3. Death rate	1.95.6% 2.90.0% 3.9.1%	
GONG 0309 (Gong et al., 2020)	√	Chongqing	CT:188 ST:37 M:125.F:100 0.6-82	decoction+WT1.2			1. Lymphocyte of severe patients 2. Albumin of severe patients 3. CRP of severe patients 4. CD4+,CD8+ of severe patients 5. Death rate	1.Gradually increase 2.Gradually increase 3.Drop to normal 4.Increase 5. Not mentioned	
FANG 0312 (Fang et al., 2020)	√	Hubei	MT:90 CT:98 ST:120 M:156.F:152 30-86	decoction, Chinese patent drug +WT1.2.7		5.0 ± 3.8	1. Remaining proportion of fever 2. Improvement time and remaining proportion of diarrhea 3. Improvement time and remaining proportion of choking sensation in chest 4. Improvement time and remaining proportion of fatigue	1.0% 2.6.3 ± 3.8, 0% 3.8.5 ± 4.4,2.4% 4.7.1 ± 3.6,3.6%	

(Continued)

TABLE 2 | Continued

Included trials	Funding	Study region	Sample characteristics type; male/female; age (y)	Interventions	Duration	Fever improvement time(d)	Outcome index	Self before and after comparison	Adverse reactions
							5. Improvement time and remaining proportion of cough 6. Death rate	5.10.4 ± 4.8,35.7% 6. Not mentioned	
ZHU 0319 (Zhu et al., 2020)		Jiangsu	CT:22 ST:1 M:10.F:13 50.0 ± 13.0	Chinese patent drug +decoction +WT1.2.6.7			1. Absolute value of LY 2. CRP 3. Improvement rate of inflammatory change absorption of lung CT 4. Time of nucleic acid test turning negative 5. Death rate	1.Obviously increase 2.Obviously decline 3.65.2% 4.11.6 ± 0.8 5. Not mentioned	
HU 0320 (Hu et al., 2020)	√	Henan	CT:19 M:8.F:11 40.55 ± 10.59	decoction+WT1.6			1. Effective rate 2. Hospitalization average time 3. Fever, cough 4. Shortness of breath, fatigue, sweating, painful abdominal mass, nausea, anorexia, diarrhea 5. Lung CT 6. Rate of turning to severe type 7. Death rate	1.100% 2.(16.36 ± 4.95)d 3.Disappear 4.Relief 5.Obvious improvement 6.0% 7. Not mentioned	
YANG 0324 (Yang Z. et al., 2020)	√	Henan	MT,CT:13 M:10,F:3 41.31 ± 13.51	decoction +WT1.2.3.4.5		3 ± 0.71	1. Improvement time of cough 2. Improvement time of fatigue 3. Improvement time of diarrhea 4. Improvement time of choking sensation in chest 5. Lung CT 6. NEUT, LY, LY/%, SCR 7. PLT, CRP, ALT, AST, TBIL, ALP, GGT, BUN, LDH 8. Death rate	1.(6 ± 2)d 2.(5 ± 1.10)d 3.(6 ± 2.12)d 4.(4 ± 1.54)d 5.Most of them still had lesions, and only 1 mild case was cured 6.P<0.05 7.P>0.05 8. Not mentioned	

MT, mild type; CT, common type; ST, serious type; WT, western treatment, WT: 1. antiviral; 2. anti-infection/anti-inflammatory/antibiotics; 3. immunoregulation; 4. gastrointestinal regulation; 5. relieving cough and asthma; 6. oxygen therapy; 7. glucocorticoid; 8. nutritional support; 9. nlgesics; 10. liver protection; 11. anti-anxiety.

TABLE 3 | Frequency of traditional Chinese herbal medicine (frequency≥5).

No.	Chinese name	Latin name	Freq.	No.	Chinese name	Latin name	Freq.
1	Gancao	*Glycyrrhizae Radix Et Rhizoma*	18	13	Renshen	*Ginseng Radix Et Rhizoma*	8
2	Mahuang	*Ephedrae Herba*	16	14	Shigao	*Gypsum Fibrosum*	8
3	Kuxingren	*Armeniacae Semen Amarum*	14	15	Taoren	*Persicae Semen*	7
4	Huangqin	*Scutellariae Radix*	12	16	Chaihu	*Bupleuri Radix*	7
5	Cangzhu	*Atractylodis Rhizoma*	12	17	Lianqiao	*Forsythiae Fructus*	7
6	Fuling	*Poria*	11	18	Huangqi	*Astragali Radix*	6
7	Banxia	*Pinelliae Rhizoma*	11	19	Yiyiren	*Coicis Semen*	6
8	Binglang	*Arecae Semen*	10	20	Dahuang	*Rhei Radix Et Rhizoma*	5
9	Chenpi	*Citri Reticulatae Pericarpium*	9	21	Baizhu	*Atractylodis Macrocephalae Rhizoma*	5
10	Houpo	*Magnoliae Officinalis Cortex*	9	22	Baishao	*Paeoniae Radix Alba*	5
11	Caoguo	*Tsaoko Fructus*	8	23	Zhimu	*Anemarrhenae Rhizoma*	5
12	Guanghuoxiang	*Pogostemonis Herba*	8	24	Chantui	*Cicadae Periostracum (Periostracum Cicadae Cryptotympana atrata Fabricius)*	5

TABLE 4 | Frequency of types of traditional Chinese herbal medicine.

No	Types	Freq.	Types of Medicines
1	Heat-clearing medicines	65	23
2	Exterior syndrome-relieving medicines	55	16
3	Phlegm-resolving and cough and asthma-relieving medicines	48	15
4	Damp-resolving medicines	43	7
5	Tonify medicines	42	15
6	Damp-draining diuretic medicines	30	9
7	Qi-regulating medicines	23	4
8	Blood-activating and stasis-resolving medicines	11	4
9	Interior-warming medicines	8	3
10	Resolving wind-damp medicines	6	4
11	Astringent medicines	5	3
12	Purgative medicines	5	1
13	Clearing away toxin and killing parasites medicines	2	2
14	Liver-calming and wind-extinguishing medicines	1	1

The frequency of application of Glycyrrhizae Radix Et Rhizoma (gancao) has not been counted in the statistics, because of Glycyrrhizae Radix Et Rhizoma (gancao) commonly used as harmonizing herb in TCM decoctions.

decoction was created by doctor Zhang Zhongjing (Han Dynasty) who researched on exogenous cold induced febrile diseases. Modern prescriptions are mostly added and subtracted by classical prescriptions. For example, the Qingfei Paidu decoction recommended by the State Administration of Traditional Chinese Medicine is based on Maxing Ganshi decoction, Shegan Mahuang decoction, Wuling powder and Xiao Chaihu decoction. The classical prescriptions with a literature frequency of ≥ 2 are presented in **Table 6**.

Analysis of Application of Chinese Patent Medicine

Given its convenient application, Chinese patent medicine has gained increasing research attention. An analysis of the use of Chinese patent medicine in 20 clinical studies showed that Lianhua Qingwen capsules/granules are the most widely used. These capsules have been widely studied to verify their clinical efficacy. Lianhua Qingwen can act on coronavirus through multiple components, targets and pathways *via* their broad-spectrum antiviral, antibacterial and antipyretic; cough relief; sputum reduction and immune regulation effects (Ling et al., 2020). In the treatment of NCP, Xuebijing and other traditional Chinese medicine injections have been used several times. Xuebijing can antagonize endotoxins (Zhang, 2018; Wang, 2019) and inhibit the excessive release of inflammatory mediators, such as interferon and interleukin (Tian et al., 2019), thereby inhibiting inflammation and enhancing immunity (Diao et al., 2015). The academician Zhang Boli emphasized that the early application of traditional Chinese medicine injection can play a vital role in treating critical patients (Tencent news, 2020b). **Table 7** presents The commonly used Chinese patent medicine for NCP.

Investigation of the Observation Indicators

In 20 studies on the treatment of NCP, the most commonly used clinical observation and evaluation indices was fever improvement time, followed by cough improvement time, shortness of breath improvement time, chest CT, and TCM syndrome scale score. Some articles also used the disappearance rate of other accompanying

TABLE 5 | Analysis of the association rules of traditional Chinese herbal medicine.

No.	Chinese name	Latin name	Confidence coefficient
1	Shigao -> Kuxingren	*Gypsum Fibrosum -> Armeniacae Semen Amarum*	1
2	Caoguo-> Binglang	*Tsaoko Fructus -> Arecae Semen*	1
3	Shigao -> Mahuang	*Gypsum Fibrosum -> Ephedrae Herba*	0.875
4	Shigao, Kuxingren -> Mahuang	*Gypsum Fibrosum*, *Armeniacae Semen Amarum -> Ephedrae Herba*	0.875
5	Mahuang, Binglang -> Cangzhu	*Ephedrae Herba*, *Arecae Semen -> Atractylodis Rhizoma*	0.875
6	Mahuang, Binglang -> Kuxingren	*Ephedrae Herba*, *Arecae Semen-> Armeniacae Semen Amarum*	0.875
7	Cangzhu, Binglang ->Mahuang	*Atractylodis Rhizoma*, *Arecae Semen -> Ephedrae Herba*	0.875
8	Kuxingren -> Mahuang	*Armeniacae Semen Amarum-> Ephedrae Herba*	0.857
9	Banxia -> Fuling	*Pinelliae Rhizoma -> Poria*	0.82
10	Fuling -> Banxia	*Poria -> Pinelliae Rhizoma*	0.82

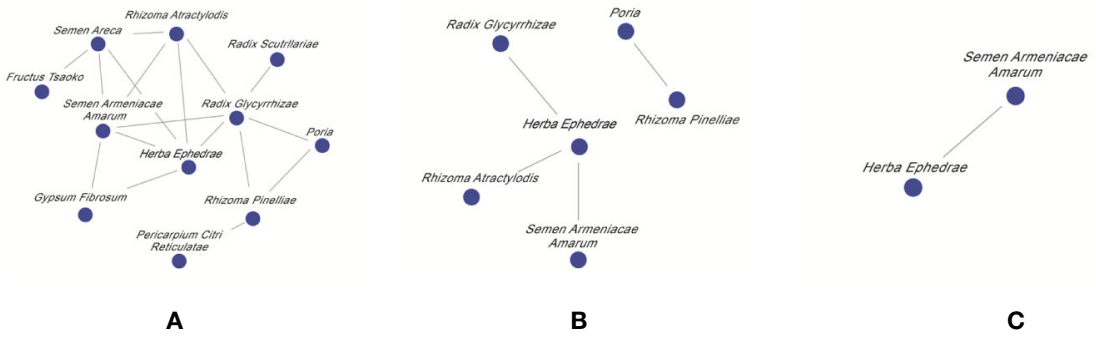

FIGURE 2 | Commonly used Chinese herbal medicine combinations network diagram for NCP with different support rate. Support rate was **(A)** ≥20%, **(B)** ≥25%, and **(C)** ≥30%.

TABLE 6 | The commonly used classical prescriptions of TCM for NCP.

No.	Classical Prescriptions of TCM	Components Latin name(Chinese name)	Source (year of completion)	Freq.	Application of cases
1	Ganlu Xiaodu Pill	*Amomi Fructus Rotundus*(Doukou), *Pogostemonis Herba*(Guanghuoxiang), *Acori Tatarinowii Rhizoma*(Shichangpu), *Menthae Haplocalycis Herba*(Bohe), *Forsythiae Fructus*(Lianqiao), *Belamcandae Rhizoma*(Shegan), *Fritillariae Cirrhosae Bulbus* (Chuanbeimu), *Scutellariae Radix* (Huangqin), *Artemisiae Scopariae Herba* (Yinchen), *Talcum*(Huashi), *Akebiae Caulis*(Mutong)	Secret of Medical Efficacy AD 1831	3	40
2	Maxing Ganshi Decoction	*Ephedrae Herba*(Mahuang), *Armeniacae Semen Amarum*(Kuxingren), *Gypsum Fibrosum*(Shigao), *Glycyrrhizae Radix Et Rhizoma* (Gancao)	Treatise on Febrile Diseases AD 200	2	80
3	Huopo Xialing Decoction	*Pogostemonis Herba* (Guanghuoxiang), *Sojae Semen Praeparatum (Dandouchi)*, *Amomi Fructus Rotundus* (Doukou), *Magnoliae Officinalis Cortex*(Houpo), *Pinelliae Rhizoma* (Banxia), *Armeniacae Semen Amarum*(Kuxingren), *Poria* (Fuling), *Polyporus*(Zhuling), *Alismatis Rhizoma*(Zexie), *Coicis Semen* (Yiyiren)	Original Medical Theory AD 1861	2	45
4	Da Yuan Decoction	*Arecae Semen* (Binglang), *Magnoliae Officinalis Cortex* (Houpo), *Tsaoko Fructus* (Caoguo), *Anemarrhenae Rhizoma*(Zhimu), *Paeoniae Radix Alba*(Baishao), *Scutellariae Radix* (Huangqin), *Glycyrrhizae Radix Et Rhizoma*(Gancao)	Treatise on Acute Epidemic Febrile Diseases AD 1642	2	42
5	Haoqin Qingdan Decoction	*Artemisiae Annuae Herba*(Qinghao), *Bambusae Caulis In Taenias*(Zhuru), *Pinelliae Rhizoma*(Banxia), *Poria* (Fuling), *Scutellariae Radix* (Huangqin), *Aurantii Fructus* (Zhiqiao), *Citri Reticulatae Pericarpium* (Chenpi), *Talcum*(Huashi), Indigo Naturalis (Qingdai), *Glycyrrhizae Radix Et Rhizoma*(Gancao)	Revisiting of Treatise on Acute Epidemic Febrile Diseases AD 1956	2	25
6	Xuanbai Chengqi Decoction	*Gypsum Fibrosum* (Shigao), *Rhei Radix Et Rhizoma* (Dahuang), *Armeniacae Semen Amarum*(Kuxingren), *Trichosanthis Fructus* (Gualou)	Item Differentiation of Warm Febrile Diseases AD 1798	2	18
7	Tingli Dazao Xiefei Decoction	*Descurainiae Semen Lepidii Semen* (Tinglizi), *Jujubae Fructus*(Dazao)	Synopsis of Golden Chamber AD 200	2	18

TABLE 7 | The commonly used Chinese patent medicine for NCP.

No.	Chinese patent medicine	Components Latin name(Chinese name)	Freq.	Prop.
1	Lianhua Qingwen capsule/ granule	*Forsythiae Fructus*(Lianqiao), *Lonicerae Japonicae Flos* (Jinyinhua), *Ephedrae Herba*(Mahuang), *Armeniacae Semen Amarum*(Kuxingren), *Gypsum Fibrosum* (Shigao), *Isatidis Radix*(Banlangen), *Dryopteridis Crassirhizomatis Rhizoma* (Mianma Guanzhong), *Houttuyniae Herba*(Yuxingcao), *Pogostemonis Herba* (Guanghuoxiang), *Rhei Radix Et Rhizoma*(Dahuang), *Rhodiolae Crenulatae Radix Et Rhizoma*(Hongjingtian)	7	35.00%
2	Xue Bi Jing Injection	*Carthami Flos*(Honghua), *Paeoniae Radix Rubra* (Chishao), *Chuanxiong Rhizoma* (Chuanxiong), *Salviae Miltiorrhizae Radix Et Rhizoma* (Danshen), *Angelicae Sinensis Radix*(Danggui)	3	15.00%
3	Shufeng Jiedu Capsule	*Polygoni Cuspidati Rhizoma Et Radix* (Huzhang), *Forsythiae Fructus* (Lianqiao), *Isatidis Radix* (Banlangen), *Bupleuri Radix* (Chaihu), *Herba Patriniae*(Baijiangcao), *Verbenae Herba* (Mabiancao), *Phragmitis Rhizoma* (Lugen), *Glycyrrhizae Radix Et Rhizoma* (Gancao)	3	15.00%

symptoms and CRP examination as observation indices. From **Table 1**, we can see the fever improvement time in the trial group was significantly shorter than that in the control group. In **Table 8**, we listed the Chinese name, Latin name in Chinese pharmacopeia, and Name in Medicinal Plant Names Services.

DISCUSSION

On the discussion of epidemic, the ancient Chinese doctor Wu Youke from the Ming Dynasty pointed out it was caused by epidemic pathogenic evils. Given its strong infectivity,

TABLE 8 | Drug name comparison table.

No.	Chinese name	Latin name in Chinese pharmacopeia	Name in Medicinal Plant Names Services(MPNS)
1	Baijiangcao	*Herba Patriniae*	Patrinia scabiosifolia Link
2	Baishao	*Paeoniae Radix Alba*	Paeonia lactiflora Pall.
3	Baizhi	*Angelicae Dahuricae Radix*	Angelica dahurica (Hoffm.) Benth. & Hook.f. ex Franch. & Sav.
4	Baizhu	*Atractylodis Macrocephalae Rhizoma*	Atractylodes macrocephala Koidz.
5	Banlangen	*Isatidis Radix*	Isatis tinctoria L.
6	Banxia	*Pinelliae Rhizoma*	Pinellia ternata (Thunb.) Makino
7	Binglang	*Arecae Semen*	Areca catechu L.
8	Bohe	*Menthae Haplocalycis Herba*	Mentha canadensis L.
9	Cangzhu	*Atractylodis Rhizoma*	Atractylodes lancea (Thunb.) DC.
10	Caoguo	*Tsaoko Fructus*	Lanxangia tsao-ko (Crevost & Lemarié) M.F.Newman & Skornick.
11	Chaihu	*Bupleuri Radix*	Bupleurum chinense DC.
12	Chantui	*Cicadae Periostracum (Periostracum Cicadae Cryptotympana atrata Fab- ricius)*	——
13	Chenpi	*Citri Reticulatae Pericarpium*	Citrus × aurantium L.
14	Chishao	*Paeoniae Radix Rubra*	Paeonia anomala subsp. veitchii (Lynch) D.Y.Hong & K.Y.Pan
15	Chuanbeimu	*Fritillariae Cirrhosae Bulbus*	Fritillaria cirrhosa D.Don
16	Chuanxiong	*Chuanxiong Rhizoma*	Conioselinum anthriscoides 'Chuanxiong'
17	Dahuang	*Rhei Radix Et Rhizoma*	Rheum palmatum L.
18	Dandouchi	*Sojae Semen Praeparatum*	Glycine max (L.) Merr.
19	Danggui	*Angelicae Sinensis Radix*	Angelica sinensis (Oliv.) Diels
20	Danshen	*Salviae Miltiorrhizae Radix Et Rhizoma*	Salvia miltiorrhiza Bunge
21	Daqingye	*Isatidis Folium*	Isatis tinctoria L.(Folium Isatidis)
22	Dazao	*Jujubae Fructus*	Ziziphus jujuba Mill.
23	Dihuang	*Rehmanniae Radix*	Rehmannia glutinosa (Gaertn.) DC.
24	Doukou	*Amomi Fructus Rotundus*	Alpinia hainanensis K.Schum.
25	Fangfeng	*Saposhnikoviae Radix*	Saposhnikovia divaricata (Turcz. ex Ledeb.) Schischk.
26	Fengfang	*Vespae Nidus*	——
27	Fuling	*Poria*	Smilax glabra Roxb. (Poria cocos (Schw.) Wolf.)
28	Fuzi	*Aconiti Lateralis Radix Praeparata*	Aconitum carmichaeli Debeaux (Radix Aconiti Lateralis Preparata)
29	Gancao	*Glycyrrhizae Radix Et Rhizoma*	Glycyrrhiza uralensis Fisch. ex DC.
30	Ganjiang	*Zingibneris Rhizoma*	Zingiber officinale Roscoe (Rhizoma Zingiberis)
31	Gegen	*Puerariae Lobatae Radix*	Pueraria montana var. lobata (Willd.) Maesen & S.M.Almeida ex Sanjappa & Predeep
32	Gualou	*Trichosanthis Fructus*	Trichosanthes kirilowii Maxim.
33	Guanghuoxiang	*Pogostemonis Herba*	Pogostemon cablin (Blanco) Benth.
34	Guizhi	*Cinnamomi Ramulus*	Cinnamomum cassia (L.) J.Presl
35	Honghua	*Carthami Flos*	Carthamus tinctorius L.
36	Hongjingtian	*Rhodiolae Crenulatae Radix Et Rhizoma*	Rhodiola crenulata (Hook.f. & Thomson) H.Ohba
37	Hongshen	*Ginseng Radix Et Rhizoma Rubra*	Panax ginseng C.A.Mey.
38	Houpo	*Magnoliae Officinalis Cortex*	Magnolia officinalis Rehder & E.H.Wilson
39	Huanglian	*Coptidis Rhizoma*	Coptis chinensis Franch.
40	Huangqi	*Astragali Radix*	Astragalus mongholicus Bunge
41	Huangqin	*Scutellariae Radix*	Scutellaria baicalensis Georgi
42	Huashi	*Talcum*	——
43	Huzhang	*Polygoni Cuspidati Rhizoma Et Radix*	Reynoutria japonica Houtt.
44	Jiangcan	*Bombyx Batryticatus*	——
45	Jianghuang	*Curcumae Longae Rhizoma*	Curcuma longa L.
46	Jinyinhua	*Lonicerae Japonicae Flos*	Lonicera japonica Thunb.
47	Kuxingren	*Armeniacae Semen Amarum*	Prunus armeniaca L.
48	Lianqiao	*Forsythiae Fructus*	Forsythia suspensa (Thunb.) Vahl
49	Lugen	*Phragmitis Rhizoma*	Phragmites australis subsp. australis
50	Mabiancao	*Verbenae Herba*	Verbena officinalis L.
51	Mahuang	*Ephedrae Herba*	Ephedra sinica Stapf
52	Maidong	*Ophiopogonis Radix*	Ophiopogon japonicus (Thunb.) Ker Gawl.

(Continued)

TABLE 8 | Continued

No.	Chinese name	Latin name in Chinese pharmacopeia	Name in Medicinal Plant Names Services(MPNS)
53	Mianma Guanzhong	*Dryopteridis Crassirhizomatis Rhizoma*	Dryopteris crassirhizoma Nakai
54	Moyao	*Myrrha*	Commiphora myrrha (T.Nees) Engl.
55	Mudanpi	*Moutan Cortex*	Paeonia × suffruticosa Andrews
56	Mutong	*Akebiae Caulis*	Akebia quinata (Thunb. ex Houtt.) Decne.
57	Niubangzi	*Arctii Fructus*	Arctium lappa L.
58	Pugongying	*Taraxaci Herba*	Taraxacum mongolicum Hand.-Mazz.
59	Qianhu	*Peucedani Radix*	Kitagawia praeruptora (Dunn) Pimenov
60	Qingdai	*Indigo Naturalis*	Persicaria tinctoria (Aiton) Spach
61	Qinghao	*Artemisiae Annuae Herba*	Artemisia annua L.
62	Renshen	*Ginseng Radix Et Rhizoma*	Panax ginseng C.A.Mey.
63	Sangbaipi	*Mori Cortex*	Morus alba L.
64	Shancigu	*Cremastrae Pseudobulbus Pleiones Pseudobulbus*	Pleione yunnanensis (Rolfe) Rolfe
65	Shegan	*Belamcandae Rhizoma*	Iris domestica (L.) Goldblatt & Mabb.
66	Shengjiang	*Zingiberis Rhizoma Recens*	Zingiber officinale Roscoe
67	Shengma	*Cimicifugae Rhizoma*	Actaea cimicifuga L.
68	Shichangpu	*Acori Tatarinowii Rhizoma*	Acorus calamus var. angustatus Besser
69	Shigao	*Gypsum Fibrosum*	——
70	Taizishen	*Pseudostellariae Radix*	Pseudostellaria heterophylla (Miq.) Pax
71	Taoren	*Persicae Semen*	Prunus persica (L.) Batsch
72	Tinglizi	*Descurainiae Semen Lepidii Semen*	Descurainia sophia (L.) Webb ex Prantl
73	Weilingxian	*Clematidis Radix Et Rhizoma*	Clematis chinensis Osbeck
74	Wumei	*Mume Fructus*	Prunus mume (Siebold) Siebold & Zucc.
75	Wuweizi	*Schisandrae Chinensis Fructus*	Schisandra chinensis (Turcz.) Baill.
76	Xinyi	*Magnoliae Flos*	Magnolia biondii Pamp.
77	Xixiancao	*Siegesbeckiae Herba*	Sigesbeckia orientalis L.
78	Xixin	*Asari Radix Et Rhizoma*	Asarum sieboldii Miq.
79	Xuanshen	*Scrophulariae Radix*	Scrophularia ningpoensis Hemsl.
80	Yinchen	*Artemisiae Scopariae Herba*	Artemisia capillaris Thunb.
81	Yiyiren	*Coicis Semen*	Coix lacryma-jobi var. ma-yuen (Rom.Caill.) Stapf
82	Yuxingcao	*Houttuyniae Herba*	Houttuynia cordata Thunb.
83	Zexie	*Alismatis Rhizoma*	Alisma plantago-aquatica subsp. orientale (Sam.) Sam.
84	Zhebeimu	*Fritiliariae Thunbergil Bulbus*	Fritillaria thunbergii Miq.
85	Zhimu	*Anemarrhenae Rhizoma*	Anemarrhena asphodeloides Bunge
86	Zhiqiao	*Aurantii Fructus*	Citrus trifoliata L.
87	Zhuling	*Polyporus (Polyporus umbellatus(Pers.) Fr.)*	——
88	Zhuru	*Bambusae Caulis In Taenias*	Bambusa beecheyana Munro
89	Ziwan	*Asteris Radix Et Rhizoma*	Aster tataricus L.f.

The drugs were listed in the order of their Chinese name.
The top frequency that search term appeared in medicinal plant literature was chosen.
—— MPNS could not match the search term.

disease location and clinical characteristics, NCP can be named "pulmonary epidemic disease" (Guo and Wan, 2020). The main consensus regarding its pathogenesis is that the virus invades the lungs and causes vital qi deficiency. The pathological nature is dampness, heat, toxin, deficiency and stasis.

This study mainly uses bibliometrics and data mining methods to obtain a systematic summary of clinical studies published at this stage and systematically analyses the published information, research design, intervention measures and observation indicators. A summary of the research methods indicates that only 2 RCTs were conducted. Most of the studies were CCTs and CSs. Considering the large number of patients and the rapid spread of the epidemic, the shortage of medical resources has led to the unconditional implementation of RCT research. The treatment of patients is the first priority at this time.

Regarding the time distribution of publications, the time that research on traditional Chinese medicine treatment of NCP was conducted synchronized with the epidemic. Furthermore, the symptom improvement rate and symptom scores in the observation and evaluation indicators fully reflect the characteristics of the judgment standard of clinical efficacy of traditional Chinese medicine. The total number of observation cases also reflects the high participation of traditional Chinese medicine in this anti-epidemic treatment. A clear understanding of Chinese herbal medicines use has been achieved through the data mining and analysis of prescriptions for treatment of NCP. In addition to *Glycyrrhizae Radix Et Rhizoma* (Gancao), *Ephedrae Herba* (Mahuang), *Armeniacae Semen Amarum* (Kuxingren) *Atractylodis Rhizoma* (Cangzhu) and *Scutellariae Radix* (Huangqin) are frequently used. An analysis of drug categories showed that heat-clearing medicine, exterior syndrome-relieving medicines, phlegm-resolving and cough and asthma-relieving medicines, and humidifying drugs are frequently used. This finding suggests that dampness and toxin accumulating in the lung are the main pathogenesis of NCP. *Ephedrae Herba* (Mahuang)-*Armeniacae Semen Amarum* (Kuxingren) had the highest support and high confidence in the association rules, which reflects the classic compatibility of Maxing Shigan

decoction. About the high frequency Chinese herbal medicines, most of it enters the lung meridian or spleen meridian. Chinese medicine recognizes that NCP mainly involves the lung. The spleen is the source of phlegm, and the lung is the sputum storage position, phlegm and dampness caused by lung and spleen disease. The results of clinical application analysis of Chinese patent medicines reflect the participation in clinical treatment. Given their wide range of applications and convenient application, Chinese patent medicines play an important role in clinical treatment of the epidemic in China. Traditional Chinese medicine for treatment of NCP is worthy of global attention.

Our study has several limitations. Randomized controlled trials are the most commonly used to judge the effectiveness of interventions. This review only included two RCTs. And they did not mention blinding method. In addition, the interventions, treatment courses, and observation indicators of each study were quite different, so meta-analysis cannot be done. High-quality RCTs on the effectiveness and safety of traditional Chinese medicine in the treatment of new coronary pneumonia need further study.

AUTHOR CONTRIBUTIONS

ZZ conceived and wrote the manuscript draft. SF designed the study and revised the manuscript. NG drafted the manuscript. YW was responsible for data collection. PC helped data management. YT was in charge of statistical analysis of data. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.560448/full#supplementary-material

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REVIEW
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Research Progress on Main Symptoms of Novel Coronavirus Pneumonia Improved by Traditional Chinese Medicine

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Novel coronavirus (COVID-19) pneumonia has become a major threat to worldwide public health, having rapidly spread to more than 180 countries and infecting over 1.6 billion people. Fever, cough, and fatigue are the most common initial symptoms of COVID-19, while some patients experience diarrhea rather than fever in the early stage. Many herbal medicine and Chinese patent medicine can significantly improve these symptoms, cure the patients experiencing a mild 22form of the illness, reduce the rate of transition from mild to severe disease, and reduce mortality. Therefore, this paper summarizes the physiopathological mechanisms of fever, cough, fatigue and diarrhea, and introduces Chinese herbal medicines (Ephedrae Herba, Gypsum Fibrosum, Glycyrrhizae Radix et Rhizoma, Asteris Radix et Rhizoma, Ginseng Radix et Rhizoma, Codonopsis Radix, Atractylodis Rhizoma, *etc.*) and Chinese patent medicines (Shuang-huang-lian, Ma-xing-gan-shi-tang, *etc.*) with their corresponding therapeutic effects. Emphasis was placed on their material basis, mechanism of action, and clinical research. Most of these medicines possess the pharmacological activities of anti-inflammatory, antioxidant, antiviral, and immunity-enhancement, and may be promising medicines for the treatment or adjuvant treatment of COVID-19 patients.

Keywords: COVID-19, traditional Chinese medicine, fever, cough, fatigue, diarrhea

INTRODUCTION

Novel coronavirus (Corona Virus disease 2019, COVID-19) pneumonia has become a massive threat to global public health. It is highly infectious, with a relatively high mortality rate, causing a sharp increase in the number of infections in a short period. Of even greater concern, is that some people infected with COVID-19 do not have obvious symptoms in the initial stage, and become potential super-communicators. More than 80,000 COVID-19 cases have been confirmed in China so far. Surprisingly, by the end of April, 2020, novel infections were almost zero, the country was recovering, and the epidemic in China was nearing completion. However, the virus is still causing panic around the world. The Director-General of the World Health Organization (WHO) declared

that COVID-19 can be characterized as a pandemic on March 11, 2020 (World Health Organization, 2020). The epidemic is spreading rapidly in Italy, the United States, Spain, Germany, Iran, France, South Korea, Japan, and other countries. More than 1.6 billion confirmed cases and 650,000 cumulative deaths have been reported worldwide as of July 28, 2020. Although most of these countries have a well-developed medical and health service system, they are caught in the dilemma of shortage and exhaustion of public medical resources. At the same time, they are facing a sharp increase in the number of patients, which will lead to a series of serious consequences, such as a severe shortage of timely medical treatment, a high incidence of transition from mild to severe disease, an increasing mortality of the severely-affected patients, and a large-scale infection of medical staff. Therefore, it is of great value and significance to share the treatment experience of China in anti-epidemic to all countries in the world, so that more infected people can get treatment, and a greater public health crisis can be avoided.

At present, the transmission characteristics and clinical symptoms of COVID-19 have been relatively fully recognized. It is infectious and can be transmitted through respiratory droplets, digestive tracts, and contact; and the population is generally susceptible (Guan and Xian, 2020; Medical Administration Bureau, 2020). The incubation period is about 7 days on average and up to 14 days. Fever, dry cough, and fatigue are the main clinical manifestations. Half of the patients developed dyspnea after 8 days. Severe patients rapidly progressed to acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis, and coagulation dysfunction that are difficult to correct (Huang et al., 2020). However, there is still a lack of effective means of treatment. The research and development cycle of new drugs and vaccines is too long, so it is the first choice to seek effective treatment strategies in the existing treatment methods.

The COVID-19 belongs to the scope of "Wen Yi" in traditional Chinese medicine (TCM). And TCM has unique cognition and rich experience in diagnosis and treatment of "Wen Yi". The integration of traditional Chinese and western medical treatments played a unique role in the prevention and treatment of SARS in 2003 (Zhang et al., 2004). It may be one of the reasons that the mortality rate in Mainland China (7%, 349/5327) was lower than that in Hong Kong, (17%, 299/1755), Taiwan (11%, 37/346), or even in the world (9.6%, 774/8096) (Wu et al., 2008). Dr. J. Kenneth Baillie, a member of the WHO panel on clinical management for COVID-19, suggested that corticosteroid treatment should be avoided, and argued that steroids have little benefit to patients, with harm outweighing the benefit. He proposed that clinicians may give priority to symptomatic and supportive treatment (Russell et al., 2020), which is highly consistent with the concept of syndrome differentiation and treatment in TCM. According to the clinical observation of 34 cases carried out by Professor Zhang Boli and others in the Wuhan Jiangxia makeshift hospital, the disappearance rate of other concomitant symptoms, the clinical cure rate, and the incidence of common type patient to severe type in the integrated group were respectively 85.3%, 91.2%, and 5.9%. Compared with conventional western medical therapy, treatment with TCM was significantly better than those in the western medicine group (38.9%, 61.1%, and 33.3%). It was found that the treatment of COVID-19 using an integration of TCM and western medicine may significantly relieve the clinical symptoms, shorten the course of the disease, and improve the clinical cure rate, which is superior to the results using western medicine alone (Xia et al., 2020). Moreover, the participation of TCM in all provinces of China is as high as 90%, which has demonstrated that TCM has made an important contribution to the prevention and control of this epidemic.

Therefore, this review aimed to summarize various Chinese herbal medicines (**Table 1**, **Figure 1**) and Chinese patent medicines that have properties which would be beneficial in treating symptoms associated with coronavirus infection (fever, cough, fatigue and diarrhea). Additionally, evidence quality evaluation criteria were established to select references suitable for this study, as shown in **Table 2**. Based on the existing literature, we sought drugs with scientific evidence that improve the clinical manifestations of patients with COVID-19, which may provide supplementary and alternative treatments to underdeveloped or medically under-resourced areas. More importantly, we hope to explore the potential drugs for COVID-19 and provide novel ways and ideas for the prevention and treatment of COVID-19.

FEVER

The Mechanism of Fever

Fever is known as a characteristic defensive host mechanism, consisting of an increase in body temperature, occurring in response to various types of infectious or non-infectious stimuli(Aryal et al., 2019). Based on guidelines for the management of febrile illnesses provided by authorities such as the WHO and the Society of Critical Care Medicine and the Infectious Disease Society of America, among others, equivalent rectal temperature of ≥ 38°C or axillary temperatures of ≥ 37.5°C are indicative of fever in both adults and children (Ogoina, 2011). Fever is not only a disease, but also an important clinical manifestation of many diseases (Dewitt et al., 2017). One of the important clinical manifestations of COVID-19 is fever. From January 1 to January 28, 2020, 136 (98.6%) of 138 consecutive confirmed COVID-19 patients in the Central South Hospital of Wuhan University in China had clinical manifestations of fever. Early fever generally lasts for 5 to 7 days, during which the virus is strong, but the patient's vital energy is not declining, approaching the turning point. Early control can directly lead to the recovery. Therefore, understanding the mechanism of fever is crucial for the diagnosis, treatment, and prognosis of COVID-19 patients.

Material Basis of Fever

Fever is usually caused by the interaction of immune cells with exogenous pyrogen and endogenous pyrogenic cytokines. The peripheral fever signal is transmitted to the central temperature

TABLE 1 | Descriptive table of the Chinese herbal medicines mentioned in this paper.

Number	Scientific name	Latin name	Common name	Local Chinese name	Parts used
1	*Acorus tatarinowii* Schott	Acori Tatarinowii Rhizoma	Grassleaf sweetflag rhizome	Shi-chang-pu	Rhizome
2	*Alisma orientale* (Sam.) Juz.	Alismatis Rhizoma	—	Ze-xie	Rhizome
3	*Amomum villosum* Lour., *Amomum villosum* Lour. var. *xanthioides* (Wall. ex Baker) T.L.Wu & S.J.Chen, *Amomum longiligulare* T.L.Wu	Amomi Fructus	Villous amomum fruit	Sha-ren	Fruit
4	*Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav., *Angelica dahurica* var. *formosana* (Boissieu) Yen	Angelicae Dahuricae Radix	Dahurian angelica root	Bai-zhi	Root
5	*Angelica sinensis* (Oliv.) Diels	Angelicae Sinensis Radix	Chinese angelica root	Dang-gui	Root
6	*Arctium lappa* L.	Arctii Fructus	Great burdock achene	Niu-bang-zi	Fruit
7	*Areca catechu* L.	Arecaesemen	Areca seed	Bing-lang	Seed
8	*Areca catechu* L.	Arecae Pericarpium	Areca peel	Da-fu-pi	Pericarpium
9	*Aster tataricus* L. f.	Asteris Radix	Aster root	Zi-wan	Root
10	*Atractylodes lancea* (Thunb.) DC., *Atractylodes chinensis* (DC.) Koidz.	Atractylodis Rhizoma	Atractylodes	Cang-zhu	Rhizome
11	*Atractylodes macrocephala* Koidz.	Atractylodis Macrocephalae Rhizoma	Largehead atractylodes rhizome	Bai-zhu	Rhizome
12	*Aucklandia lappa* DC.	Aucklandiae Radix	Common aucklandia root	Mu-xiang	Root
13	*Bubalus bubalis* Linnaeus	Bubali Cornu	Buffalo horn	Shui-niu-jiao	Horn
14	*Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd.	Bupleuri Radix	Chinese thorowax root	Chai-hu	Root
15	*Chaenomeles speciosa* (Sweet) Nakai	Chaenomelis Fructus	Common floweringqince fruit	Mu-gua	Fruit
16	*Cinnamomum cassia* (L.) J.Presl	Cinnamomi Ramulus	Cassia twig	Gui-zhi	Branch
17	*Cinnamomum cassia* (L.) J.Presl	Cinnamomi Cortex	Cassia bark	Rou-gui	Bark
18	*Citrus* × *aurantium* L., *Citrus sinensis* (L.) Osbeck	Aurantii Fructus Immaturus	—	Zhi-shi	Fruit
19	*Citrus grandis* 'Tomentosa', *Citrus grandis* (L.) Osbeck	Citri Grandis Exocarpium	Pummelo peel	Hua-ju-hong	Pericarpium
20	*Citrus medica* L.	Citri Sarcodactylis Fructus	Finger citron	Fo-shou	Fruit
21	*Citrus reticulata* Blanco	Citri Reticulatae Pericarpium	Dried tangerine peel pericarpium	Chen-pi	Pericarpium
22	*Codonopsis pilosula* (Franch.) Nannf., *Codonopsis pilosula* Nannf.var.modesta (Nannf.) L.T.Shen, *Codonopsis tangshen* Oliv.	Codonopsis Radix	Tangshen	Dang-shen	Root
23	*Coix lacryma-jobi* var. *ma-yuen* (Rom.Caill.) Stapf	Coicis Semen	—	Yi-yi-ren	Seed
24	*Cryptotympana pustulata* Fabricius	Cicadae Periostracum	Cicada slough	Chan-tui	Slough
25	*Cuscuta australis* R.Br., *Cuscuta chinensis* Lam.	Cuscutae Semen	Dodder seed	Tu-si-zi	Seed
26	*Cynanchum stauntonii* (Decne.) Schltr. ex H.Lév., *Cynanchum glaucescens* (Decne.) Hand.-Mazz.	Cynanchi Stauntonii Rhizoma et Radix	Willowleaf	Bai-qian	Root and rhizome
27	*Descurainia Sophia* (L.) Webb. ex Prantl	Descurain Semen	Pepperweed seed	Ting-li-zi	Seed
28	*Dimocarpus longan* Lour.	Longan Arillus	Langan aril	Long-yan-rou	Aril
29	*Dryopteris crassirhizoma* Nakai	Dryopteridis Crassirhizomatis Rhizoma	Male fern rhizome	Mian-ma-guan-zhong	Rhizome
30	*Ephedra sinica* Stapf, *Ephedra intermedia* Schrenk et C. A. Mey and *Ephedra equisetina* Bunge	Ephedrae Herba	Ephedra aerial parts	Ma-huang	Aerial parts
31	*Epimedium brevicomu* Maxim., *Epimedium sagittatum* (Siebold & Zucc.) Maxim., *Epimedium pubescens* Maxim., *Epimedium koreanum* Nakai	Epimedii Folium	—	Yin-yang-huo	Leaf
32	*Eriobotrya japonica* (Thunb.) Lindl.	Eriobotryae Folium	Loquat leaf	Pi-pa-ye	Leaf
33	*Eucommia ulmoides* Oliv.	Eucommiae Cortex	Eucommia bark	Du-zhong	Bark
34	*Euodia rutaecarpa* (Juss.) Benth., *Euodia rutaecarpa* (Juss.) Benth. var. *officinalis* (Dode) Huang, *Euodia rutaecarpa* (Juss.) Benth. var. *bodinieri* (Dode) Huang	Euodiae Fructus	Medicinal evodia fruit	Wuzhuyu	Fruit
35	*Fritillaria cirrhosa* D.Don, *Fritillaria unibracteata* P.K.Hsiao & K.C.Hsia, *Fritillaria przewalskii* Maxim. ex Batalin., *Fritillaria delavayi* Franch., *Fritillaria taipaiensis* P.Y.Li, *Fritillaria unibracteata* var. wabuensis (S.Y.Tang & S.C.Yueh) Z.D.Liu, Shu Wang & S.C.Chen	Fritillariae Cirrhosae Bulbus	Fritillaria bulb	Chuan-bei-mu	Bulb
36	*Fritillaria usuriensis* Maxim.	Fritillariae Ussuriensis Bulbus	—	Ping-bei-mu	*Bulbus*
37	*Gardenia jasminoides* J.Ellis	Gardeniae Fructus	—	Zhi-zi	Fruit

(Continued)

TABLE 1 | Continued

Number	Scientific name	Latin name	Common name	Local Chinese name	Parts used
38	*Glycine max* (L.) Merr.	Sojae Semen Praeparatum	Fermented soybean	Dan-dou-chi	Seed
39	*Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Batalin, *Glycyrrhiza glabra* L.	Glycyrrhizae Radix et Rhizoma	Licorice root	Gan-cao	Root and rhizome
40	Gypsum Fibrosum	Gypsum Fibrosum	Gypsum	Shi-gao	
41	*Houttuynia cordata* Thunb.	Houttuyniae Herba	—	Yu-xing-cao	Herb
42	*Hyriopsis cumingii* (Lea), *Cristaria plicata* (Leach), *Pteria martensii* (Dunker)	Margaritifera Concha	Margaritifera	Zhen-zhu-mu	Shell
43	*Isatis indigotica* Fortune ex Lindl.	Isatidis Radix	Isatis root	Ban-lan-gen	Root
44	*Ligusticum chuanxiong* Hort.	Chuanxiong Rhizoma	Szechwan lovage rhizome	Chuan-xiong	Rhizome
45	*Lonicera japonica* Thunb.	Lonicera Japonica Flos	Honeysuckle flower	Jin-yin-hua	Flower
46	*Lophatherum gracile* Brongn.	Lophatheri Herba	Lophatherum herb	Dan-zhu-ye	Stem and leaf
47	*Lycium barbarum* L.	Lycii Fructus	Arbary wolfberry fruit	Gou-qi-zi	Fruit
48	*Magnolia officinalis* Rehder & E.H.Wilson, *Magnolia officinalis* var. *biloba* Rehder & E.H.Wilson	Magnoliae Officinalis Cortex	Officinal magnolia bark	Hou-po	Bark
49	*Mentha haplocalyx* Briq.	Menthae Haplocalycis Herba	Peppermint	Bo-he	Aerial parts
50	Mongolian *Astragalus membranaceus* (Fisch.) Bge.var.mongholicus (Bge.) Hsiao, *Apodium Astragalus membranaceus* (Fisch.) Bge.	Astragali Radix	Milkvetch root	Huang-qi	Root
51	*Morus alba* L.	Mori Cortex	White mulberry root-bark	Sang-bai-pi	Root-bark
52	*Myristica fragrans* Houtt.	Myristicae Semen	Nutmeg seed	Rou-dou-kou	Seed
53	*Paeonia lactiflora* Pall.	Paeoniae Radix Alba	White peony root	Bai-shao	Root
54	*Panax ginseng* C.A.Mey.	Ginseng Radix Et Rhizoma	Ginseng root	Ren-shen	Root and rhizome
55	*Perilla frutescens* (L.) Britton	Perillae Folium	—	Zi-su	Leaf
56	*Perilla frutescens* (L.) Britton	Perillae Fructus	—	Zi-su-zi	Fruit
57	*Peucedanum praeruptorum* Dunn	Peucedani Radix	—	Qian-hu	Root
58	*Pheretima aspergillum* (E.Perrier), *Pheretima vu1garis* Chen, *Pheretima guillelmi* (Michaelsen), *Pheretima pectinifera* Michaelsen	Pheretima	Earthworm	Di-long	Body
59	*Phragmites communis* Trin.	Phragmitis Rhizoma	Reed rhizome	Lu-gen	Rhizome
60	*Pinellia ternate* (Thunb.) Makino	Pinelliae Rhizoma	Pinellia tuber	Ban-xia	Tuber
61	*Platycodon grandiflorus* (Jacq.) A. DC.	Platycodonis Radix	Platycodon root	Jie-geng	Root
62	*Pogostemon Cablin* (Blanco) Benth.	Pogostemonis Herba	Cablin patchouli herb	Guang-huo-xiang	Aerial parts
63	*Polygala tenuifolia* Willd.	Polygalae Radix	—	Yuan-zhi	Root
64	*Polygonum cuspidatum* Siebold & Zucc.	Polygoni Cuspidati, Rhizoma Et Radix	Giant knotweed rhizome	Hu-zhang	Root and rhizome
65	*Polyporus umbellatus* (Pers.) Fries	Polyporus	—	Zhu-ling	Sclerotium
66	*Poria cocos* (Schw.) Wolf	Poria	Indian bread	Fu-ling	Sclerotium
67	*Prunus armeniaca* L., *Prunus sibirica* L., *Prunus mandshurica* (Maxim.) Koehne	Armeniacae Semen Amarum	Apricot kernel	Ku-xing-ren	Seed
68	*Psoralea corylifolia* L.	Psoraleae Fructus	Malaytea scurfpea fruit	Bu-gu-zhi	Fruit
69	*Rehmannia glutinosa* (Gaertn.) DC.	Rehmanniae Radix	Rehmannia root	Di-huang	Root
70	*Rheum palmatum* L., *Rheum tanguticum* Maxim.ex Balf., *Rheum officinale* Baill.	Rhei Radix Et Rhizoma	Rhubarb	Da-huang	Root and rhizome
71	*Rhodiola crenulata* (Hook. f. & Thoms.) H. Ohba	Rhodiolae Crenulatae Radix et Rhizoma	Rhodiolae root	Hong-jing-tian	Root and rhizome
72	*Schisandra Chinensis* (Turcz.) Baill.	Schisandrae Chinensis Fructus	Schisandra fruit	Wu-wei-zi	Fruit
73	*Schizonepeta tenuifolia* (Benth) Briq.	Schizonepetae Herba	Fineleaf schizonepeta herb	Jing-jie	Aerial parts
74	*Scutellaria baicalensis* Georgi	ScutellariaeRadix	Baical skullcap root	Huang-qin	Root
75	*Stemona sessilifolia* (Miq.) Miq., *Stemona japonica* (Blume) Miq, *Stemona tuberosa* Lour.	Stemonae Radix	Stemona root	Bai-bu	Root
76	*Tussilago farfara* L	Farfarae Flos	Coltsfoot flower	Kuan-dong-hua	Flower
77	*Ziziphus jujuba* Mill.	Ziziphus Jujuba	Chinese date	Da-zao	Fruit
78	*Ziziphus jujube* Mill. var. *spinosa* (Bunge) HuexH.F.Chou	Ziziphi Spinosae Semen	Spine date seed	Suan-Zao-ren	Seed

regulating center through humoral and neural pathways, thus producing fever (Zeisberger, 1999). Exogenous pyrogen refers to microorganisms and their metabolites from outside, and also the most common fever activators, mainly including bacteria, viruses, fungi, parasites, mycobacteria, *etc.* (Laupland, 2009). The currently recognized major pyrogenic cytokines are interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) (Prajitha et al., 2018).

IL-1 represents a family of two agonists (IL-1α and IL-1β) (Conti et al., 2004). Numerous studies have demonstrated the

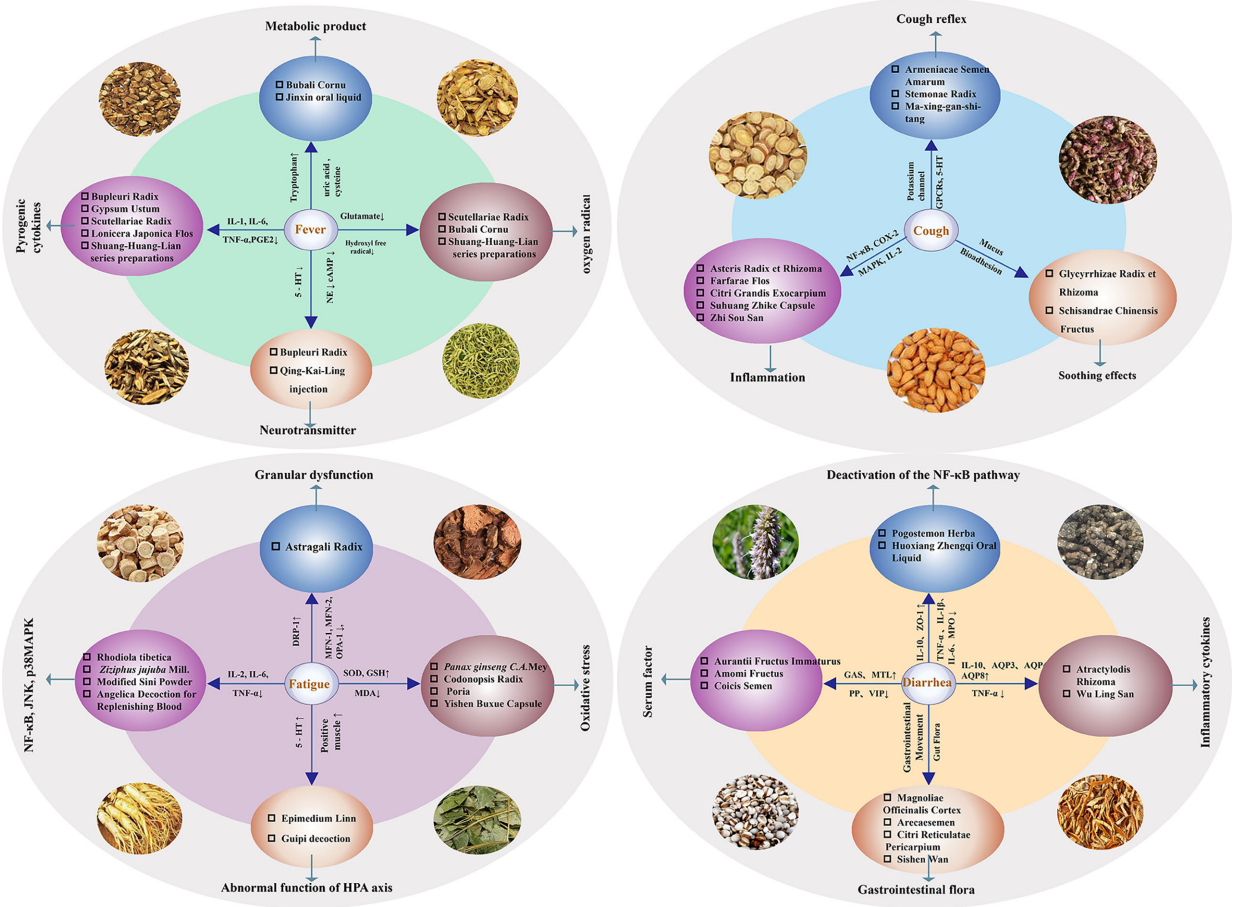

FIGURE 1 | Therapeutic effect of Chinese Herbal Medicine on the main symptoms (fever, cough, fatigue and diarrhea) of COVID-19 in the early stage.

capacity of peripherally administered IL-1α and IL-1β to evoke fever in a variety of species (Kluger, 1991; Dinarello, 1996). The current explanation for this is that IL-1 induces intermediates, prostaglandin E2 (PGE2), and cyclooxygenase 2 (COX-2), which are considered necessary downstream events which mediate peripheral IL-1-induced fever (Li et al., 2001; Ching et al., 2007). Receptors for IL-6 exist in two forms, a soluble receptor, sIL-6R, and a membrane bound receptor, IL-6R (Vallières and Rivest, 1997). Injection of IL-6 into lateral ventricle can upregulate COX-2 (Cao et al., 2001), increase the level of PGE2 in CSF, and produce fever (Dinarello et al., 1991). Recent research further confirms this view that the pyrogenic effect of IL-6 is exerted by its binding to IL-6 receptors on brain endothelial cells, and that the ligand binding in turn leads to induced expression of the prostaglandin synthesizing enzyme COX-2 *via* intracellular signaling involving the STAT3 pathway (Eskilsson et al., 2014). Intravenous administration of recombinant human TNF (rh TNF) into rabbits can cause fever, and also reveals that the pyrogenic potential of rh TNF is correlated with increased production of PGE2 (Nakamura et al., 1988). TNF-α is the first member of the LPS-induced cytokine cascade to appear following the injection of this exogenous pyrogen (Roth et al., 1998). Again, the mechanism is related to glutathione. It has been shown that the regulation of TNF-α biosynthesis induced by LPS is redox sensitive and requires the participation of the glutathione mediated signaling pathway. In the presence of glutathione, it can activate the activity of PGE synthase-1 (mPGES-1) to produce PGE2 (Wrotek et al., 2015).

The Humoral Transmission Pathway of Fever Signal

As mentioned above, the production of PGE2, a common connection has been found in the three kinds of important pyrogenic cytokines. Therefore, PGE2 is considered as the final medium of fever (Roth and Blatteis, 2014). It has been shown that PGE2 from peripheral or central all cause fever (Romanovsky et al., 1999; Blatteis et al., 2000). The pyrogenic cytokines released in the blood by exogenous pyrogen stimulation may play a role outside the brain by binding and activating the cytokine receptor on the capillaries located in the periventricular organs, thus leading to the release of PGE2 (Blatteis, 2006). In addition, in this pathway, the fever signal can also be carried by the PAMPS. The

TABLE 2 | Evidence quality evaluation criteria.

Type	Evidence degree	Treatment
Clinical trials	Ia	Meta-analyses of randomized controlled trials
	Ib	Evidence from randomized controlled trial (n ≥ 50)
	Ic	Evidence from randomized controlled trial (n ≥ 20)
	IIa	Evidence from well-performed nonexperimental descriptive studies as well as comparative studies, correlation review studies, network pharmacology studies and case- studies
Animal trials	I	Evidence from *in vivo* experiments with reasonable groups (multi-dose, positive and negative control group, n ≥ 8) and credible results
	II	Evidence from *in vivo* experiments with reasonable groups (a single dose, positive and negative control group, n ≥ 5) and credible results
	III	Evidence from *in vivo* experiments with relatively reasonable groups (a single dose, n ≥ 5) and credible results
	IV	Evidence from *in vitro* experiments with credible results

Evidence below this criterion will be excluded.

circulating PAMPs represented by LPS release PGE2 through arachidonic acid pathway by binding and activating TLR-4 on the capillaries of the organs around the central chamber of the BBB, and then activate the thermal neurons in the front of the hypothalamus, causing fever (Steiner et al., 2006; Turrin and Rivest, 2004). The synthesis of PGE2 is related to the activation of NF-κB or STAT3 in brain endothelial cells (Nadjar et al., 2005; Rummel et al., 2006).

The Neural Transmission Pathway of Fever Signal

The characteristics of febrile reactions are early rapid reaction and late delayed reaction. The activation of the neural pathway is believed to be another mechanism by which fever is rapidly initiated (Roth and De Souza, 2001). It has been shown that the activation of complement component 5A (C5a) immediately triggered the release of PGE2 from Kupffer cells (KC) after LPS injection (Perlik et al., 2005). PGE2 stimulates homologous receptors on the afferent vagus of the liver and binds to EP3 receptors in the OVLT/POA region, resulting in fever (Oka, 2004). PGE2 can also enhance the release of neurotransmitters, especially cAMP released by hypothalamic cells that can change the temperature setting (Dinarello, 2004). Therefore, we believe that PGE2, a rapid fever signal, plays a triggering role in the initial stage of fever through the neural pathway, while the fever signal in the humoral pathway plays a more important role in maintaining fever (**Figure 2**).

Treatments

Fever is caused by the interaction of immune cells with exogenous pyrogen and endogenous pyrogenic cytokines. The most widely studied pyrogenic cytokines include 1L-1, IL-6, TNF-α, IFNs, and CNTF. After the virus and other pathogens infect the body, they can activate NF-κB to cause the release of TNF-α, IL-1, IFNs, chemokine, etc., which can mediate the aggregation and infiltration of a large number of immune cells into the lung tissue, activate the signal transduction pathway in the cells, start the cascade reaction of waterfall inflammation, release the amount of cytokines, and continuously activate more inflammatory cells to form a vicious cycle. It eventually leads to a cytokine storm. Chen et al. (2020) analyzed 99 confirmed cases of COVID-19, and proposed that the virus spreads through respiratory mucosa to infect other cells, induce a cytokine storm *in vivo*, and produce a series of immune responses. Therefore, it is essential for in the treatment of COVID-19 to inhibit excessive immune cell activation and cytokine production. TCM and its preparations can achieve an antipyretic effect by inhibiting 1L-1, IL-6, TNF-α, and other pyrogenic cytokines, and also can indirectly achieve the effect of initial treatment of COVID-19 by inhibiting the cytokine storm.

Single Chinese Herbal Medicine

Gypsum Fibrosum

Gypsum Fibrosum is a variety of fibrous crystalline aggregate of hydrous calcium sulfate. Although its main component is hydrous calcium sulfate, it also contains inorganic elements such as sodium, magnesium and iron. Wang et al. (2009) studied the antipyretic activity of Gypsum Fibrosum by intraperitoneal injection of LPS in rats. The body temperature of rats with fever decreased significantly after the intragastric administration of Gypsum Fibrosum extract (0.8 g/ml), indicating that it exerts an antipyretic effect; furthermore, it was speculated that its active components may be the inorganic elements. Calcium is the main ion component of Gypsum Fibrosum. Following the action of gastric acid, part of the gypsum decoction can be transformed into soluble calcium, which can then be absorbed into the bloodstream through the intestines, increasing the calcium ion concentration in the blood, so as to regulate the temperature center and relieve the fever. Zhou et al. (2012) injected a 15% yeast suspension subcutaneously into rat backs, resulting in fever. The rats were infused with gypsum suspension for 7 days (10 g/kg). The results showed that gypsum could play an antipyretic role by reducing the synthesis of PGE2. Gypsum Fibrosum is usually used with Anemarrhena Rhizoma in clinic as antipyretic. One of the most classical prescriptions is Baihu Decoction. When they are used together, it can enhance the dissolution rate of calcium ion and the antipyretic effect of Gypsum Fibrosum (Jia et al., 2013).

Bupleuri Radix

Bupleuri Radix is the dry root of *Bupleurum chinense* DC and *Bupleurum scorzonerifolium* Willd. Phytochemical studies reveal that this plant contains essential oils, triterpenoid saponins, polyacetylenes, flavonoids, lignans, fatty acids, and sterols (Yang et al., 2017). In the fight against SARS, it once appeared on the treatment list and attracted scientific attention (Zhao et al., 2007). Bupleuri Radix has good antipyretic effect and has been widely used in clinic. The main components of essential oil and saikosaponin play the role of antipyretic. Chen et al. (2010) injected ET into the normal rabbit to induce fever. The essential oil was extracted from Bupleuri Radix as raw material to prepare the gel, which was sprayed into the nasal cavity of the febrile rabbits. Once 0.2 ml was given, the temperature dropped 0.5°C after 5 h, and the temperature

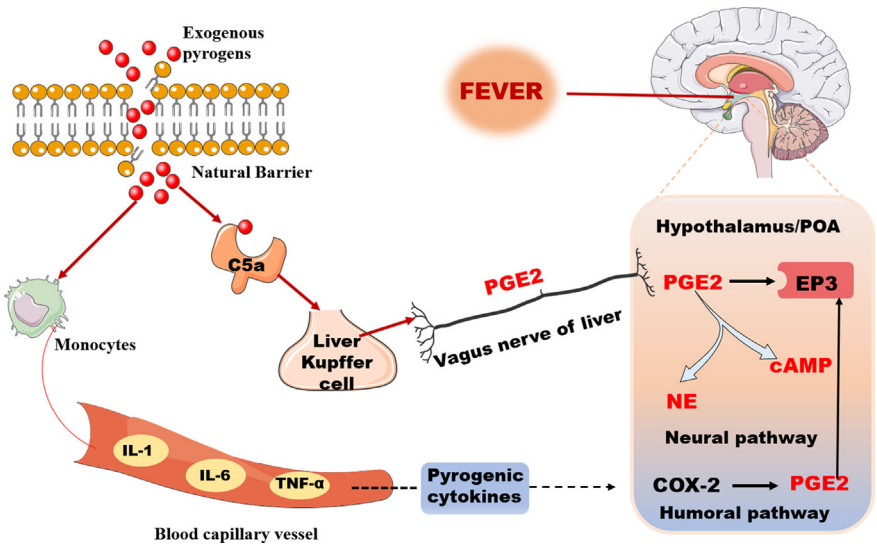

FIGURE 2 | The transmission pathway of fever signal: Humoral pathway and Neural pathway.

decreased by 0.8°C after 24 h. The results showed that the essential oil of Bupleuri Radix had an antipyretic effect which could play an antipyretic role by reducing the concentration of cAMP in the cerebrospinal fluid of febrile rabbits. Jin et al. (2014) found the antipyretic effect of essential oil and saikosaponin of Bupleuri Radix. The results demonstrated that the antipyretic effect of the treatment group was significant as compared with the control group. Some studies have shown that saikosaponin can significantly reduce the expression of TNF-α, IL-1β, IL-6, and other cytokines. It can also inhibit the NF-κB signaling pathway by inhibiting the phosphorylation of extracellular signal regulated kinase (downstream of TNF-α) (Kim et al., 2015). In conclusion, Bupleuri Radix may play an antipyretic role by reducing cAMP concentration and inhibiting the expression of TNF-α, IL-1β, IL-6, and the NF-κB inflammatory signaling pathway. However, Bupleuri Radix could lead to hepatotoxicity in high doses and with long-term use (Yang et al., 2017).

The commonly used Chinese herbal medicines as antipyretics are listed in **Table 3** in addition to the above.

Chinese Patent Medicine

Shuang-huang-lian

Shuang-huang-lian series preparations are made from Lonicera Japonica Flos (Jin-yin-hua), Scutellariae Radix (Huang-qin), and Forsythiae Fructus (Lian-qiao). The existing clinical randomized controlled trials demonstrate that Shuang-huang-lian preparations exhibit a certain antipyretic effect. Although it contains a large number of active ingredients, only chlorogenic acid, baicalin, and forsythin have been officially included in the quality control standard (Gao et al., 2014). Baicalin is a type of flavonoid extracted from Scutellariae Radix, which has an obvious antipyretic effect. *In vivo* studies demonstrated that the antipyretic effect of baicalin was related to a decrease in TNF-α, IL-1 β, IL-6, and other cytokines in serum, hypothalamus, and CSF (Li and Ge, 2010).In addition, baicalin inhibited the LPS-modulated upregulation of TLR4 mRNA and protein expression and TNF-α and IL-1β mRNA expression in rats, and downregulated NF-κB activation with simultaneous decreases in TNF-α and IL-1β protein expression (Ye et al., 2015). Forsythoside A (FTA), a monomer of phenethyl alcohol glycosides extracted from Forsythiae Fructus. A previous study suggested that FT-A significantly downregulated TRPV1 expression in the hypothalamus and DRG of yeast-induced pyrexia mice. TRPV1 is a non-selective cation channel gated by noxious heat, playing major roles in thermoregulation. FT-A alleviated fever of yeast-induced pyrexia mice *via* suppression of TRPV1 expression and activation, inhibition of MAPKs, activation of the hypothalamus and DRG, and subsequently decreased secretion of pyretic cytokine as PGE2 and IL-8 (Liu et al., 2017).In addition, FT-A can significantly enhance the phagocytic function of macrophages in LPS-stimulated ra-w264.7 cells and reduce the secretion of TNF-α (Guan et al., 2013). FT-A can also inhibit TNF-α and NF-κB by blocking the LPS/TLR4 signaling pathway (Zeng et al., 2017). It is suggested that FT-A can also inhibit the LPS/TLR4 signaling pathway and reduce TNF-α secretion in order to achieve an antipyretic effect. In a randomized controlled trial to systematically evaluate Shuang-huang-lian injection in the treatment of acute upper respiratory tract infection, it was found that Shuan-ghuang-lian can significantly reduce the fever caused by acute upper respiratory tract infection (Zhang et al., 2013).In addition, Shuang-huang-lian is widely used in the clinical treatment of infectious diseases such as pneumonia, influenza, acute tonsillitis and acute pharyngitis (Song et al., 2000; Chen et al., 2002).

The commonly used Chinese patent medicine as antipyretics are shown in **Table 4**.

TABLE 3 | The commonly used Chinese herbal medicines as antipyretics.

Chinese herbal medicine	Bioactive components	Model	Treatment	Mechanisms	The species investigated	Result	References	Qualityof evidence
Cinnamomi Ramulus	Essential oil, organic acids, triterpenoid saponins, coumarins, tannins, flavonoid glycosides and polysaccharides	The dorsal root ganglion (DRG) of newborn rats was stimulated at different experimental temperatures	Cultured cells of DRG neurons were incubated with cinnamaldehyde of different concentrations for 12 h	Cinnamaldehyde upregulates the expression and function of Transient receptor potential vanilloid 1 (TRPV1) in DRG neurons through non TRPA1 pathway	New-born SD rats	Cinnamaldehyde, the extract of Cinnamomi Ramulus, has a significant antipyretic effect	(Sui et al., 2010)	IV
Lonicera Japonica Flos	Organic acids, essential oil, flavonoids, triterpenoid saponins	Fever caused by intravenous injection of IL-1β (100 ng)	Intravenous Jin-Yin-Hua injection 1 ml	Inhibition of EP3 expression in preoptic anterior hypothalamic neurons, thus inhibiting the production of PGE2	Healthy New Zealand rabbits	Lonicera Japonica Flos has a significant antipyretic effect	(Xie et al., 2009)	III
Scutellariae Radix	Flavonoids, essential oil, terpenes	Fever caused by intravenous injection of LPS (2 mg/kg)	Baicalin (2 mg/kg, 10 mg/kg, 20 mg/kg) was injected randomly into rabbits	Reduce the excessive production of TNF-α and glutamate; Inhibition of NMDA receptor dependent hydroxyl radicals and PGE2 pathway	Healthy rabbits	Scutellariae Radix has a significant antipyretic effect	(Tsai et al., 2006)	II
Bubali Cornu	Protein, polypeptide and amino acid	Fever caused by subcutaneous injection of 20% yeast (10 ml/kg)	400 mg/kg Bubali Cornu powder extract was administrated orally with a dosage of 10 ml/kg	Change the metabolism of uric acid and cysteine; enhance the activity of antioxidant enzymes; reduce the level of TNF-α; reduce the ROS production and PGE2 synthesis	Aged SD rats (200 ± 20 g)	Bubali Cornu has a significant effect of fever induced by yeast.	(Liu et al., 2016)	II

TABLE 4 | The commonly used Chinese patent medicines as antipyretics.

Chinese patent medicine	Formation	Model	Treatment	The species investigated	Mechanisms	Result	References	Quality of evidence
Qingkailing injection (QKLI)	Gardeniae Fructus, Bubali Cornu, Margaritifera Concha, Isatidis Radix, Lonicera Japonica Flos, Baicalin, Cholic acid	Fever caused by subcutaneous injection of 20% yeast (15 ml/kg)	4.2 ml/kg QKLI into tail vein of rats	Aged SD rats	Decrease the expression of 5-HT and the concentration of 4-aminobutyric acid; improve the metabolism of amino acids and the urea cycle	QKLI has an antipyretic effect	(Gao et al., 2013; Zhang et al., 2017)	II
Jinxin oral liquid (JXOL)	Ephedrae Herba, Descurain Semen, Mori Cortex, Armeniacae Semen Amarum, Gypsum Fibrosum, Peucedani Radix, Scutellariae Radix, Polygoni Cuspidati, Rhizoma et Radix	Fever caused by subcutaneous injection of 20% yeast (15 ml/kg)	Subcutaneous injection of 7.02 g/kg JXOL	Aged SD rats (80 ± 20 g)	Reduce the production of IL-1β, PGE2 and the level of quinolinic acid and pantothenic acid, regulate the metabolism level of 3-phosphoglycerate, pyruvate and other metabolites	JXOL has an antipyretic effect on fever rats	(Qian et al., 2019)	II
Yin Qiao San (YQS)	Lonicera Japonica Flos, Forsythiae Fructus, Platycodonis Radix, Menthae Haplocalycis, Herba, Sojae Semen Praeparatum, Lophatheri Herba, Arctii Fructus, Schizonepetae Herba, Phragmitis Rhizoma, Glycyrrhizae Radix et Rhizoma		weight <20 kg, 1 g/8 h; 20 kg < weight <40 kg,1.5 g/8 h; weight >40 kg,3 g/8 h	21 fever patients		YQS can effectively treat upper respiratory tract infection and fever without serious adverse reactions.	(Liew et al., 2015)	Ic

COUGH

The Pathophysiological Mechanism of Cough

Cough is a common respiratory disease and one of the early symptoms of bronchitis, pneumonia, asthma, and pertussis (Swarnkar et al., 2013). It is a natural protective mechanism, which helps to clear the secretion of the respiratory tract and prevent harmful particles from entering the respiratory system (Song et al., 2015). From another perspective, cough is one of the ways to enhance the transmission of the virus to the next victim, so inhibiting cough can help reduce the transmission between people (Morice et al., 2015). Cough is usually divided into three types: acute cough (lasting for less than three weeks), subacute cough (lasting for three to eight weeks), and chronic cough (persistent greater than eight weeks) (Kim et al., 2016). Acute cough is commonly associated with viral upper respiratory infection (O'Connell, 1998). Acute infection and inflammation (such as bronchitis or pneumonia) may cause dry cough in some cases (Urso and Michaels, 2016). The cough caused by the COVID-19 lasts for less than 3 weeks, and most of them do not produce sputum, which is actually an acute dry cough.

In general, cough is characterized by changes in the normal respiratory pattern caused by reflex, which is mediated by stimulation of extrapulmonary vagal afferent nerves and the brainstem (Mahashur, 2015). The pathophysiological mechanism of a cough may be related to the following two aspects: sensitizing cough receptors by increasing inflammatory mediators, such as bradykinin, tachykinin or prostaglandin, these sensitive cough receptors will cause an increase in the cough reflex; inducing or enhancing cough sensitivity by contraction of bronchial smooth muscle. The molecular mechanism involved in the former may be regulated by a series of G-protein coupled receptors (GPCRs). The activation of GPCRs has both inhibitory effects (e.g. β2-adrenoceptor and cannabinoid receptors), and excitatory effects (e.g. EP3 and bradykinin B2 receptors) on sensory nerves and the cough reflex (Maher et al., 2011). Prostaglandin E2 and bradykinin can activate airway sensory nerve through EP3 and B2 receptors, and mediate its action through TRPV1 and TRPA1 receptors. The activation of β2-adrenergic and cannabinoid CB2 receptors can inhibit sensory nerves and cough. The main receptors associated with cough reflex are shown in **Table 5**.

Postinfectious cough may occur in the following three ways: 1) The viral infection causes dripping of nasal secretions or produces inflammatory mediators, which lead to an inflammatory reaction of the bronchial mucosa. The inflammatory mediators act on the sensory nerve endings of the airway, increasing the sensitivity of the cough receptors. 2) The virus increases the activity of neuraminidase, destroys the cholinergic M receptor, reduces the affinity with the M receptor, and finally leads to the hyperfunction of cholinergic nerve, which increases the airway responsiveness. 3) By upregulating the expression of neuropeptides, the virus induces neurogenic inflammation, which affects the excitability of afferent nerves and indirectly stimulates cough receptors (McGarvey et al., 2014; Schnoeller et al., 2014). Therefore, the treatment of a cough after infection requires controlling airway inflammation, and reducing airway hyperresponsiveness and cough sensitivity.

TABLE 5 | The main receptors associated with cough reflex (Dicpinigaitis, 2004; Maher et al., 2011).

Receptors that excite the cough reflex	
TRPV1	Peripheral pain-sensing neurones and throughout the central nervous system A member of transient receiver potential (TRP). It can respond to various harmful stimuli such as capsaicin, PGE2 and LTB4, which may lead to airway hyperresponsiveness and increase cough sensitivity
Endogenous cannabinoids	
Tachykinin receptor	The tachykinins include substance P, neurokinin A, neurokinin B, and calcitonin gene-related peptides and other neuropeptide transmitters. Neurokinin may induce bronchial hyperresponsiveness, neurogenic inflammation and cough
Bradykinin receptor	
5-HT receptor	Central nervous system
Eosinophil	Eosinophilic airway inflammation is an important cause of chronic non-asthmatic cough
Receptors that inhibit the cough reflex	
Opioid and opioid-like receptor	Central nervous system Antitussive effects mainly mediated by μ- and κ-opioid receptors
Gamma-aminobutyric acid (GABA)-B receptor	
β_2-adrenoceptor	
Potassium channel	Peripheral nervous system The activation of potassium channels can inhibit the activity of airway sensory nerve, and the regulation of these channels can alleviate cough

Treatments

Cough is not only one of the main symptoms of COVID-19, but also one of the main routes of transmission of SARS-CoV-2. The virus causes upper respiratory tract infections and pulmonary inflammation, which results in coughing. Many Chinese herbal medicines like Glycyrrhizae Radix et Rhizoma, Asteris Radix et Rhizoma, Farfarae Flos not only have excellent antitussive effect, but also have anti-inflammatory activity. They may reduce airway or pulmonary inflammation by mediating inflammation-related pathways such as NF-κB and reducing airway inflammatory factors. There is a strong association in hyperinflammatory responses in patients with severe COVID-19 infection, so the intervention of these the TCM substance in the early stage of COVID-19 may prevent the disease from mild to severe. TCM possesses the potential effect of synergistic treatment of COVID-19 through multi-components, multi-targets, and multi-ways, which is also in line with the concept of holistic treatment of TCM.

The treatment of a cough includes: blocking the level of corresponding receptor of the cough reflex; covering the irritated mucosa in the mouth and throat with mucilaginous herbs, and protecting cells from local stimulation of the mouth or throat, so as to relieve the cough reflex (Fink et al., 2018);

controlling airway inflammation and reducing airway hyperresponsiveness (**Figure 3**).

Single Chinese Herbal Medicine

Glycyrrhizae Radix et Rhizoma

Glycyrrhizae Radix et Rhizoma, the dried root and rhizome of the legume *glycorrhiza urensis* Fisch., *glycorrhiza inflata* Bat., *glycorrhiza glabra* L., is one of the oldest and most popular herbs in the world, exhibiting anti-inflammatory, antiviral, antibacterial, antioxidant, and anticancer activities, immunomodulatory pharmacological activity. It is commonly used in the treatment of cough and lung disease, bronchitis, and gastric ulcer (Pastorino et al., 2018). It contains mainly flavonoids, triterpenoid saponins and glycosides (Hosseinzadeh and Nassiri-Asl, 2015). Among them, the active components with an antitussive effect may be liquiritin apioside, liquiritin, liquiritigenin, 18β-glycyrrhetinic acid and its derivatives, and polysaccharides (Anderson and Smith, 1961; Kamei et al., 2005; Kuang et al., 2018). It has been reported that 50% methanol extract (100 mg/kg) and 70% ethanol extract (800 mg/kg) of Glycyrrhizae Radix et Rhizoma have significant inhibitory effects on the cough reflex in a capsaicin-induced guinea pig cough model and sulfur dioxide gas-induced mouse cough model respectively (Kamei et al., 2003; Jahan and Siddiqui, 2012). In addition, the antitussive effect of the water-extracted polysaccharide fraction (arabinose (52%), galactose (22%), rhamnose (6%) and fucose (2%)) in the citric acid-induced cough model of guinea pigs was even stronger (81%) than that of codeine (62%) at a dose of 50 mg/kg (Saha et al., 2011; Nosalova et al., 2013). Mucus and bioadhesion may be two of the reasons behind its pharmacodynamic effect. The water-extracted polymer fraction of Glycyrrhizae Radix et Rhizoma exhibits bioadhesion to the epithelial mucosa, forming the polysaccharide layer in airway mucus, protecting cells from local oral or pharyngeal stimulation, indirectly affecting the sensitivity of cough receptors and inhibiting cough. Polysaccharides also have the ability to replenish water to epithelial cells, reducing dry cough and supporting phlegm, and increasing mucus secretion through vagal reflex (Nosalova et al., 2013). In addition, it was shown that the cough suppressant effect of liquiritin apioside may depend on the peripheral (regulation of the ATP sensitivity K^+ channel) and the central mechanism (regulation of the 5-HT system), a possible additional pathway for the cough suppressant effect of Glycyrrhizae Radix et Rhizoma, which may be another way for Glycyrrhizae Radix et Rhizoma to play an antitussive role (Kamei et al., 2003).

Asteris Radix et Rhizoma

Asteris Radix et Rhizoma consists of the dried roots and rhizomes of *Aster tataricus* L. f. In the clinical application of TCM, it has been proved to be an effective medicine for the treatment of phlegm cough disease, which has a history of thousands of years. Aster extract exhibits anti-inflammatory, anti-oxidation, anti-tumor and other biological activities. Asteris Radix et Rhizoma is rich in chemical components, including terpenes, flavonoids, sterols, cyclopeptides, *etc.* (Xu et al., 2013). Among them, the main active components of antitussive effect may be asterone, episorbitol, caffeoylquinic acids, astersaponins, aster peptides (Yu et al., 2015), luteolin, and quercetin (Yang et al., 2016). The antitussive effect of Asteris Radix et Rhizoma has been reported many times. Ren et al. observed the antitussive effect of different polar segments of Asteris Radix et Rhizoma through ammonia liquor-induced mice cough model, and the results showed that petroleum ether group, final mother liquor group and 75% ethanol group (5 g/kg) could prolong the latent period of mice cough, inhibit the frequency of cough within 2 min, and n-butanol group had significant

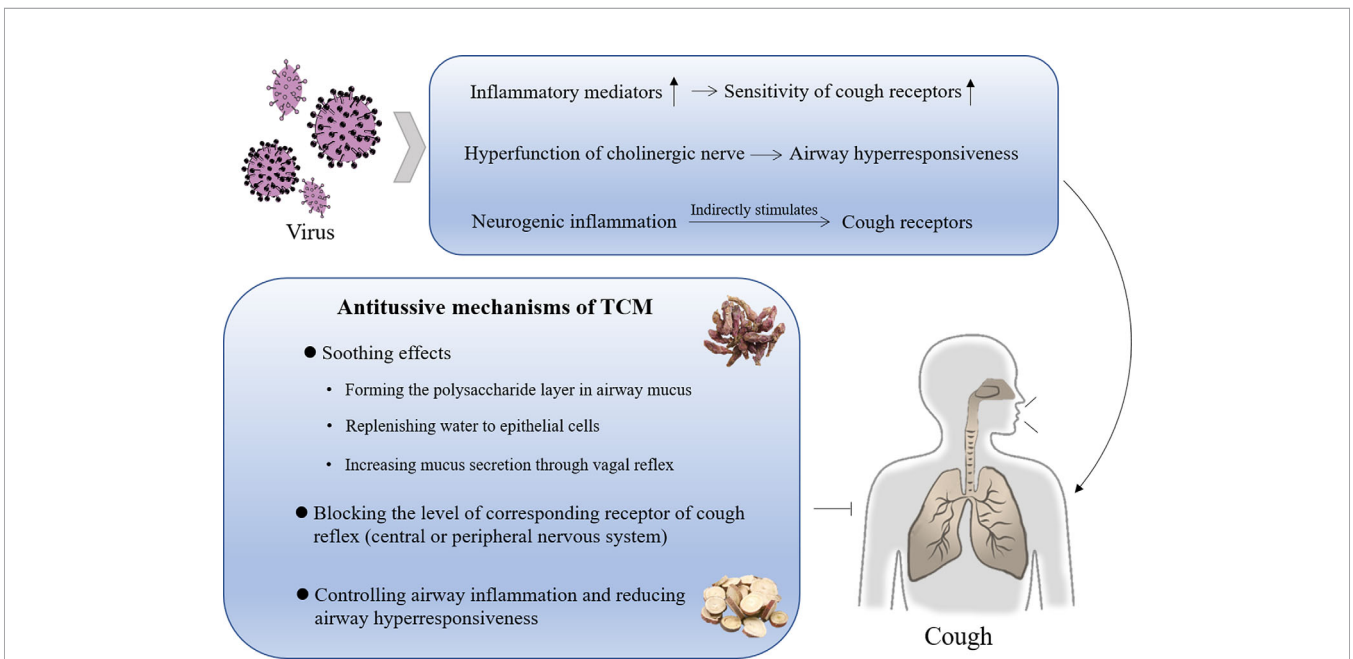

FIGURE 3 | Possible pathogenesis of postinfectious cough and antitussive mechanism of TCM.

antitussive effect, which indicated that aster Asteris Radix et Rhizoma had antitussive effect (Ren et al., 2015). Recently, it was found that in an ammonia-induced mouse cough model, the cough frequency of mice treated with 50% ethanol fraction (40 and 80 mg/kg) eluted from 70% ethanol extract was significantly reduced by 42.9% and 56.5% (both ($p < 0.001$), cough latency increased by 50.5%, 70.9% (both $p < 0.01$). Through further analysis, it is speculated that eliminating or reducing tracheal inflammation (a major source of cough and sputum) through the TLR4-mediated NF-κB pathway may be the mechanism behind its antitussive effect (Yu et al., 2015).

Farfarae Flos

Farfarae Flos, flower bud of *Tussilago farfara* L., is widely used in the treatment of cough, tuberculosis and other diseases (Zhao et al., 2014). Farfarae Flos possesses a variety of pharmacological activities, such as anti-inflammatory, antioxidant, anticancer, neuroprotective activities (Lee et al., 2014; Lee et al., 2018). Extensive phytochemical studies have shown that Farfarae Flos contains a large number of components, including volatile oils, phenolic acids, sterols, alkaloids, terpenes, *etc.* Besides, alkaloids, flavonoids, terpenes and saponins are considered to exert antitussive effects (Han et al., 2016). A series of studies have found that the monomeric components with antitussive bioactivity may be 4,5-O-dicaffeoylquinic acid, caffeic acid, chlorogenic acid, 3,5-O-dicaffeoylquinic acid, 3,4-O-dicaffeoylquinic acid, rutin, kampferol analogues, 2,2-dimethyl-6-acetylchromanone, tussilagone, Bauer-7-ene-3β,16α-diol, β-sitosterol, and sitosterone (Li et al., 2012; Wu et al., 2016; Li et al., 2018a; Yang et al., 2020). The antitussive effect of Farfarae Flos has been reported in an animal cough model induced by ammonia. Its aqueous extract at the dose of 2.8 g/kg significantly prolonged the latent period of expectoration and reduced the cough frequency in mice (Li et al., 2012). Further study found that the antitussive effect of Farfarae Flos may be related to four pathways, including the alterations of valine, leucine and isoleucine biosynthesis, pyruvate metabolism, glycerolipid metabolism, phenylalnine, tyrosine and tryptophan biosynthesis, and the imbalance of these pathways is related to a variety of neurological and inflammatory diseases (asthma, emphysema, chronic obstructive pulmonary disease (COPD)) (Li et al., 2018a). In addition, caffeoylquinic acid in Farfarae Flos may inhibit the release of PGE2 in raw 264.7 cells and mediate the cough response by inhibiting leukocytosis or decreasing LPS induced up regulation of COX-2 protein and mRNA levels (Wu et al., 2016). What is more, a network pharmacology study has found that the active components of Farfarae Flos involve 18 targets such as interleukin-2 (IL-2), COX-2, human ribonuclease A3 (RNase3), and biological processes and metabolic pathways related to signal transduction, inflammation and energy metabolism (Li et al., 2018b). These researches have provided a scientific basis for further elaboration of the mechanism of cough and phlegm elimination of Farfarae Flos. Farfarae Flos is safe and effective in the traditional dose range, but the potential toxicity due to the emergence of pyrrolidine alkaloids also needs to be paid attention (Liu et al., 2020).

The commonly used Chinese herbal medicines for cough are listed in **Table 6** in addition to the above.

Chinese Patent Medicine

Ma-xing-gan-shi-tang (MXGST) is composed of four Chinese herbal medicines, Ephedrae Herba, Armeniacae Semen Amarum, Glycyrrhizae Radix et Rhizoma, and Gypsum Fibrosum. It is a commonly used antitussive prescription in China. It has been made into a variety of prescription preparations, including Maxingzhike tablets, Maxingganshi soft capsules, Maxingganshi concentrated granules, Maxingzhike syrup, Maxingganshi mixture, *etc.* Ephedrine, amygdalin, ephedrine, glycyrrhizic acid, and amygdalin are considered as the main active components (Wang et al., 2016). A series of clinical studies have shown that MXGST is widely used in the treatment of cough, asthma, pneumonia, COPD, and other diseases (Chen et al., 2013; Wang et al., 2014; Lin S. K. et al., 2016; Liao et al., 2017). In an animal experimental study, Lin et al. adopted citric acid-induced cough in guinea pigs cough model to study the pharmacological effect of MXGST water extract in clinical application, and evaluated its subacute toxicity, and found that MXGST water extract (0.4, 1.0 g/kg) has a significant dose-dependent antitussive effect on guinea pigs, which is a safe and effective traditional Chinese medicine prescription (Lin Y. C. et al., 2016). In addition, this study also proved that MXGST water extract has an antipyretic effect on LPS-induced fever rats, which suggests that MXGST may be a promising drug for the treatment of COVID-19. Further studies have found that the antitussive mechanism of MXGST is related to the partial relaxation of bronchial smooth muscle by blocking the acetylcholinergic receptor and histaminergic receptor (Lin Y. C. et al., 2016). Another study showed that MXGST may stimulate the β2-adrenoceptor of bronchial smooth muscle and has an anti-inflammatory effect that inhibits neutrophils from entering the airway (Kao et al., 2001). In terms of composition, the antitussive mechanism of MXGST may be related to the sympathetic α- and β-adrenoceptors activated by ephedrine alkaloids and amygdalin inhibit the central cough center (Miyagoshi et al., 1986).

The commonly used Chinese patent medicines with antitussive effects are shown in **Table 7**.

FATIGUE

Pathological Process and Possible Pathogenesis

Pathological fatigue refers to fatigue caused by a certain disease, and it is also a symptom of the onset of the disease (Matura et al., 2018). It will cause a sub-healthy state of the decline of the function of body, which should be paid great attention (Wang et al., 2018). This kind of fatigue is common in chronic fatigue syndrome (CFS). Some scholars have proposed several hypotheses about the causes and mechanisms of CFS, such as viral infection, immune dysfunction (Yang et al., 2010), and neuroendocrine system disorders (Norheim et al., 2011; Cho et al., 2013). Among them, virus infection is an important factor causing CFS.

TABLE 6 | Frequently used Chinese herbal medicines for cough.

Chinese herbal medicine	Bioactive components	Model	Treatment	The species investigated	Mechanisms	Result	References	Quality of evidence
Armeniacae Semen Amarum(its therapeutic applications may be limited by reported toxicity and the presence of cyanogenic glycosides)	Amygdalin	Ammonia liquor induced mice cough	Water extract 2, 4 g/kg for 5 days	ICR mice (18–20 g)	Inhibition of proliferation of tracheal smooth muscle cells; stimulation of β2-adrenergic receptor; amygdalin can decompose under the action of β-glucosidase to produce hydrocyanic acid, which has a certain inhibitory effect on respiratory center, making respiratory movement tend to be quiet, thus relieving cough and asthma	Effective in decreasing cough frequency, prolonging cough latency	(Zhang et al., 2010; Xia et al., 2013)	I, IIa
Stemonae Radix	Croomine, neotuberostemonine, stemoninine, tuberostemonine, protostemonine, stemospironine, maistemonine, tuberostemonine H, stemoninoamide, bisdehydrostemoninine	Ammonia liquor induced mice cough	Total alkaloid extract 0.03 g/ml, 0.6 g/kg	Kunming mice of either sex (18–22 g)	Exerting antitussive effect through central and peripheral pathways	Decreasing cough frequency significantly	(Lin et al., 2006; Lin et al., 2008; Yang et al., 2009; Zhou et al., 2009; Xu et al., 2010)	III, III, III, III, III
Citri Grandis Exocarpium	Naringin	Ammonia liquor induced mice cough	Water extract and 70% ethanolic extract (247, 493, and 986 mg/kg)	NIH mice of either sex (18–22 g)	Anti-inflammation effects (blocking the NF-κB pathway); Peripheral antitussive action, but neither through the sensory neuropeptide system nor through the regulation of ATP sensitive K^+ channels	Obvious antitussive, expectorant and anti-inflammatory effects. And the activity of 70% ethanol extract is much better than that of water extract, even better than that of positive drugs	(Gao et al., 2011; Luo et al., 2012; Jiang et al., 2014)	I, II
Fritillariae Cirrhosae Bulbus	Imperialine, imperialine–N-oxide, isoverticine, and isoverticine–N-oxid, chuanbeinone, verticinone	Ammonia liquor induced cough	80% ethanol extract 1.2, 3.6, 10.8, and 18.0 g/kg for 3 days	Kunming mice of either sex (18–22 g)	Relaxing the bronchi; increasing respiratory secretions; anti-inflammatory effects	Dose dependence significantly increasing cough latency and suppressed cough frequency in mice Low toxicity	(Wang et al., 2011; Wang et al., 2012; Xu et al., 2019)	I, I, I

(Continued)

TABLE 6 | Continued

Chinese herbal medicine	Bioactive components	Model	Treatment	The species investigated	Mechanisms	Result	References	Quality of evidence
Schisandrae Chinensis Fructus	Polysaccharides, lignans (schizandrin, schisantherin A, deoxyschizandrin and γ-schisandrin)	Cough models in guinea pigs induced by cigarette smoke (Chronic cough model) and citric acid (Acute cough model),	Polysaccharide extract SCFP-1 (66.5% glucose and 29.4% arabinose) 250, 500, and 1000 mg/kg for 5 days (acute cough model), 14 days (chronic cough model)	Male Hartley guinea pigs (250–350 g)	Reducing the sensitivity of cough receptors; rehydrating epithelial cells and thereby reducing dry cough; increasing mucus secretion through vagus nerve reflex	Remarkable suppressive effects on cough both in chronic cough model and acute cough model	(Zhong et al., 2016)	I
		A guinea pig model of cough hypersensitivity induced by 14 days	Ethanol extract and ethanol-water extract 1 g/kg for 14 days	Male Hartley guinea pigs (250–350 g)	Reducing the infiltration of neutrophils and inflammatory cells, the content of MDA, TNF - α and IL-8 in lung tissue; inhibiting the proliferation of airway epithelium, smooth muscle thickening, inflammatory cell infiltration, TRPV1 and TRPA1 expression	Reducing the frequency of cough and lung inflammation in guinea pigs with cigarette smoke induced cough hypersensitivity	(Zhong et al., 2015)	II

TABLE 7 | Chinese traditional patent medicines with antitussive effect.

Chinese patent medicine	Formation	Bioactive components	Model	Treatment	The species investigated	Mechanisms	Result	References	Quality of evidence
Suhuang Zhike Capsule	Ephedrae Herba, Perillae Folium, Pheretima, Eriobotryae Folium, Perillae Fructus, Cicadaeperiostracum, Peucedani Radix, Arctii Fructus, Schisandrae Chinensis Fructus	Arctiin, ephedrine, schisandrin, pseudoephedrine, schisandrin B, and 1-caffeoylquinic acid	Postinfectious cough	7–14 days	7 randomized controlled trials involving 573 patients	Reducing airway inflammatory factors; alleviating airway hyperresponsiveness and cough sensitivity; relieving airway inflammation	Effective in the treatment of postinfectious cough in adults No serious adverse events	(Ding et al., 2016)	Ia,
Zhi Sou San	Platycodonis Radix, Schizonepetae Herba, Asteris Radix et Rhizoma, Stemonae Radix, Cynanchi Stauntonii Rhizoma et Radix, Glycyrrhizae Radix et Rhizoma, Citri Reticulatae Pericarpium	Total flavonoids	Cough	3–28 days	46 randomized controlled trials with a total of 4007 participants	Relieving pneumonia and airway mucus obstruction; relaxing bronchial smooth muscle;inhibiting the release of eosinophil	Significantly improving the total effective rate and the pulmonary function No serious adverse events	(Xu et al., 2004; Cheng et al., 2017; Zhen et al., 2018)	Ia, III
Eriobotrya japonica-Fritillaria usuriensis dropping pills	Eriobotryae Folium, Fritillariae Ussuriensis Bulbus, Platycodonis Radix, Pinelliae Rhizoma, volatile oil extracts from Mentha haplocalyx Briq.	Ursolic acid, oleanolic acid, peiminine, platycodigenin, polygalacic acid, guanosine	A network pharmacology approach			Acting on the mitogen activated protein kinase (MAPK) pathway, transforming growth factor (TGF)-beta pathway, focal adhesion, tight junctions and the action cytoskeleton		(Tao et al., 2016)	IIa, II

large number of clinical observations have found that the clinical symptoms of CFS are very similar to the symptoms of viral infections, such as fever, sore throat, and muscle swelling when some CFS patients develop symptoms (Collin et al., 2018). At present, there is no firm evidence that CFS is necessarily related to viral infections. The theoretical basis for CFS caused by viral infections is not sufficient, and experts and scholars have not reached a consensus. However, experts agree on this point that virus infection will further cause an imbalance in the immune system of the body, resulting in damage to the central nervous system and muscle structure (Nair and Diamond, 2015).

Pathological Fatigue and Immune Function

Brenu et al. (2010) used flow cytometry and found that compared with normal healthy people, granulocyte respiration broke out in patients with CFS, and natural killer (NK) cell expression of CD56 decreased significantly. Nakamura et al. (Nakamura et al., 2010) found that the anti-inflammatory factor interleukin 10 (IL-10) in patients with CFS is higher than that in normal people and the levels of immunoglobulins IgA, IgG, and IgM are disordered. This suggests that immune dysfunction may be one of the mechanisms of CFS. In addition, FIL-1 is a pro-inflammatory cytokine that contains two receptor agonists that induce the expression of other pro-inflammatory factors: IL-1α and IL-1β (Lampa et al., 2012). Cyclooxygenase-2 (Coxoxy-2) inhibitors and inducible nitric oxide synthase (iNOS) inhibitors are targets for the treatment of pain, depression, and fatigue (Von Ah et al., 2008). IL-1 causes expressions of COX-2 and iNOS (Maes, 2009). Therefore, elevated IL-1 levels may be related to fatigue (Miaskowski et al., 2012). At the same time, it was found that in human and animal models affected by CFS, levels of IL-1, IL-6, and TNF-α were also increased (Reyes-Gibby et al., 2013). These pro-inflammatory cytokines can signal the central nervous system and produce behavioral symptoms such as fatigue.

IL-2 is a multifunctional small molecule protein with high activation. It is an immunoregulatory lymphokine produced by activated $CD4^+$ T cells and a small amount of $CD8^+$ T cells. It can enhance the activity of NK cells and CD^+ T cells, thereby inducing IL-1B (Almeida et al., 2002). The production of receptors produces λ-INF, which maintains the growth of T cells *in vitro* and activates a variety of immune cells. The level of IL-2 reflects the functional status of T cells, and its ability to produce is an important indicator of the immune function of the body's cells (Hilgers and Frank, 1994). Therefore, a decrease in the level of IL-2 may produce a fatigue state.

Pathological Fatigue and Neuroendocrine System Disorders

The occurrence of CFS is also closely related to changes in the neuroendocrine system (You et al., 2011). The clinical manifestations of fatigue, depression, bone, and muscle pain in patients with CFS are similar to those in patients with decreased adrenal function (Klimas and Koneru, 2007). The hypothalamus-pituitary-adrenal (HPA) axis contains neurons that synthesize corticotropin releases hormones (CRH) (Tak et al., 2011). CRH regulates adrenocorticotropic hormone (ACTH) through the pituitary. ACTH stimulates the synthesis of corticosteroids such as cortisol or corticosterone through the adrenal cortex. HPA axis disorders often occur in CFS. Inflammatory mediators cause excessive release of corticosterone, which can cause chronic pain, immunosuppression and chronic fatigue. According to Zhao's research (Zhao, 2010), chronic compound stimulation can lead to a decrease in 5-HT levels in the hippocampus and occipital cortex, disrupt the balance of the hypothalamus-pituitary-adrenal axis, and disrupt the internal environment and cause CFS (Katafuchi, 2006). In addition, cortisol is one of the main effective hormones of the adrenal system acting on peripheral tissues. It was found that cortisol levels in CFS patients were significantly elevated, and the pathogenesis of CFS was related to abnormal HPA negative feedback regulation or excessive activation (Luo et al., 2019). CFS patients have a low level of serum cortisol during steady state, and often experience physical or emotional stress prior to the onset of the disease, which in turn activates the hypothalamic-pituitary-adrenal axis system, leading to increased release of cortisol and adrenocorticotropic hormone. It affects the immune, nervous and other systems, and then produce fatigue symptoms. Therefore, reducing the release of glucocorticoids such as cortisol by adjusting the HPA axis may alleviate the development of pathological fatigue.

Pathological Fatigue and Oxidative Stress

The body will produce a large amount of oxygen free radicals during metabolism, which can attack polyunsaturated fatty acids in biofilms, trigger lipid peroxidation reactions, and thus form lipid peroxides, such as malondialdehyde (MDA), resulting in damage to cells and tissues. The level of superoxide dismutase (SOD) activity indirectly reflects the body's ability to scavenge oxygen free radicals, while the level of MDA indirectly reflects the severity of the body's cells attacked by free radicals (Hui et al., 2014). MDA is a lipid peroxide formed by free radical attack on polyunsaturated fatty acids in the biofilm to trigger lipid peroxidation. The increase of free radicals will lead to damage to the integrity of the biofilm, increase permeability of the biofilm, release extracellular of enzymes, make electrolyte imbalance, decrease enzyme activity and cell function, resulting in fatigue (Chen and Yan, 2005). At the same time, the body also has antioxidant systems including: SOD, glutathione (GSH), CAT, *etc.* At present, studies have shown that the mental fatigue of CFS is related to the large amount of oxygen free radicals generated in the brain and the antioxidant system is inhibited (Logan and Wong, 2001). Therefore, it is of great significance to seek ways to improve the antioxidant capacity of the body for the treatment of CFS.

Treatments

It is known that the early pathogenesis of diseases such as COVID-19, SARS, and MERS are immunodeficiency and excessive oxidative stress. These two factors are the common pathological basis for death. For example, peripheral blood flow cytometry was performed on the lung tissue of COVID-19 dead patients. The results showed that $CD4^+$ and $CD8^+$ T cells were significantly reduced, T cells were overactivated, and $CCR4^+$, $CCR6^+$, Th17 in $CD4^+$ T cells increased, and $CD8^+$T cells are rich in cytotoxic particles, which activate the immune system and

induce a large number of immune cells to infiltrate into the lung tissue. Other studies have shown that viral infections can directly lead to increased ROS production in alveolar epithelial cells, GSH, SOD, and glutathione peroxidase (GSH-Px) activity is reduced, causing severe oxidative stress in cells, which further aggravating acute lung injury.

These two factors happen to be the same as the causes of fatigue. We hope that the drugs can also treat coronavirus while relieving fatigue symptoms. Therefore, based on the basic pathophysiological mechanism of COVID-19, this section focuses on summarizing the anti-inflammatory and antioxidant intervention strategies of Chinese herbal medicines to specifically block or reverse its pathological development process. This is of great significance for improving the clinical cure rate and reducing the case fatality rate.

Single Chinese Herbal Medicine

Astragali Radix

Astragali Radix is the dried root of the legume Mongolian *Astragalus membranaceus* (Fisch.) Bge.var.mongholicus (Bge.) Hsiao or Apodium *Astragalus membranaceus* (Fisch.) Bge. Its main ingredients are saponin, flavonoids, polysaccharides, and amino acids (Zhang H. et al., 2014). Huang et al. (2017) administered 40 male pathologically-fatigued BALB/c mice with astragalus polysaccharides (APS) *via* intragastric administration every morning at 8:00 am for 28 days. The required APS was dissolved in 2.0 mL of normal saline. Chronic fatigue can significantly reduce mRNA levels of mitochondrial fusion-related proteins Mfn-1, Mfn-2, and Opa-1 in mice, while mRNA levels of mitochondrial division-related protein Drp-1 significantly increase, indicating that chronic fatigue can make mice mitochondrial fusion-split imbalance in skeletal muscle, eventually causing mitochondrial dysfunction. APS can improve mitochondrial autophagy in skeletal muscle cells by reducing the level of oxidative stress in tissues. In addition, APS can stimulate the origin of mitochondria, maintain the mitochondrial fusion-split balance, improve mitochondrial dysfunction, and ultimately improve cell energy metabolism, thereby increasing the ability of mice to resist chronic fatigue.

Ginseng Radix et Rhizoma

Ginseng Radix et Rhizoma consists of the dried roots and rhizomes of the genus *Panax ginseng* C.A.Mey. The main components of ginseng are saponins, polysaccharides, proteins, volatile oils, amino acids, and flavonoids (Lee et al., 2002). Song (2014) Treated 30 male SD rats after successful CFS modeling with ginsenoside aqueous solution 60 mg/kg/d for 6 consecutive weeks. The results showed that compared with the model group, the SOD and GSH activities in the ginsenoside group were significantly increased, and the MDA content was significantly reduced, with a very significant difference ($p < 0.01$). This shows that the increase of free radicals will lead to the damage of the integrity of the biofilm, the increase of the permeability of the biofilm, the release of enzymes inside and outside the cell, leading to abnormal conditions inside and outside the cell, electrolyte imbalance, and decline in cell function, which will cause fatigue. Therefore, ginsenoside Rg1 is thought to reduce the production of the peroxidation product MDA, increase the activity of antioxidant enzymes, improve the antioxidant capacity of nerve cells, reduce the generation of free radicals, and thus increase the ability to resist CFS (Soares et al., 2007).

The commonly used Chinese herbal medicines for fatigue are listed in **Table 8** in addition to the above.

Chinese Patent Medicine

Angelica Decoction for Replenishing Blood, is composed of Astragali Radix and Angelicae Sinensis Radix in a 5:1 ratio. The primary chemical components of Astragalus are saponins, flavonoids, and polysaccharides; the main chemical components of angelica are volatile oils, organic acids, and polysaccharides. Liu et al. modeled 56 SD male rats (180–220 g) by tying an iron block weighing approximately 10% of the rat's own weight to its tail, and then placing it into a transparent water tank with a depth of 30 cm, at a constant temperature of 25 degrees Celsius. The rats were forced to swim exhaustively. When the rats' swimming movements were uncoordinated or their heads sank into the water surface within 10 s, they could not return to the water surface, and the 15.00 g/kg drug was administered to the stomach for 29 consecutive days after modeling successfully. The results showed that compared with the blank group, the Angelica Decoction for Replenishing Blood could significantly reduce the levels of TNF-α and IL-6 in the serum ($p < 0.01$). This demonstrates that CFS can produce inflammation in the body, and Angelica Decoction for Replenishing Blood can effectively alleviate this situation. At the same time, the activity of SOD in the serum of angelica buxue decoction group increased significantly ($p < 0.01$), suggesting that a large number of free radicals in CFS rats may lead to oxidative stress. Angelica buxue decoction can effectively remove free radicals in the body and alleviate the oxidative stress reaction of the body. Threonine is an essential amino acid. When it is lacking, the synthesis of immunoglobulins and the production of T-lymphocytes and B-lymphocytes will be affected, thereby upsetting the body's immune functioning. Serine is involved in the production of immune hemoglobin and antibodies, and plays an important role in the maintenance of the immune system. Metabolomics results show that Angelica Decoction for Replenishing Blood can improve the thymic degenerative changes by increasing the levels of threonine and serine, promote the differentiation and maturation of white blood cells, and block the NF-κB, JNK, and p38MAPK signaling pathways to regulate the immune system and improve chronic fatigue syndrome (Liu et al., 2011).

The commonly used Chinese patent medicines for fatigue are shown in **Table 9**.

DIARRHEA

The Mechanism of Diarrhea

Diarrhea, a common digestive disease, is caused by a variety of pathogens and other factors. Diarrhea results from the abnormal absorption or transport of water and electrolytes in the intestine. Any substance, whether infectious biological factor, noninfectious humoral factor, or some drugs, which blocks the active absorption

TABLE 8 | Chinese herbal medicines for fatigue.

Chinese herbal medicine	Bioactive components	Model	Treatment	The species investigated	Mechanisms	Result	References	Qualityof evidence
Codonopsis Radix	Codonopsis flavonoids	The mice were placed in a glass tank with a water depth of 30 cm, a diameter of 15 cm, and a water temperature of 27–30°C for 30 minutes for 25 consecutive days	Xinjiang wild Codonopsis flavonoid solution (1 mg/kg), continuous gavage for 25 days	160 Kunming male mice (18–22 g)	Improve SOD vitality and reduce the accumulation of free radicals, which helps to eliminate lipid peroxides in the body, thereby delaying fatigue	Compared with the control group, the serum MDA value of the drug group decreased by 55.65%, and the serum SOD activity increased by 186.91%	(Wang and Yuan, 2012)	I
Ziziphus jujuba	Jujubepolysaccharide(JP)	(1) Electric shock method. (2) Restriction method (2 h each time). (3) Cold water swimming 21° C once a day for 30 min each time. Modeling time is 4 weeks	Intragastric administration (400 mg/kg/d) for 28 consecutive days	40 male SD rats (180–220 g)	Related to regulating the body's immune function status and reducing the content of related inflammatory factors	Compared with the control group, the drug group significantly increased the spleen index, lowered the serum MDA content, improved the T and B lymphocyte transformation ability, and thereby adjusted the body's immune ability	(Wang et al., 2015)	I
Epimedii Folium	herbaepimediipolysaccharide (HEP)	(1) Swimming for 4 h each time. (2) Noise: Noisy music is played every day from 8 pm to 8 am the next day. (3) Treadmill exercise for 1 hour (20 m/min) every day. (4) Crowding: Each group of 10 rat lives in a standard feeding cage. The time is 28 days	Inject HEP 100 mg/kg daily for 14 days	50 female SD rats (180–220 g)	HEP indirectly regulates HPA axis function in CFS patients by increasing norepinephrine levels	The weight of rats, the number of crossing the adjacent lattice, and the number of standing (open field test) in the drug group all increased significantly ($P < 0.01$). Both the time to find the platform (Morris water maze) and the time to rest (suspended tail experiment) decreased significantly ($P < 0.01$)	(Chi et al., 2017)	I
Rhodiolae Crenulatae Radix et Rhizoma	SHR-5	(1) Cold water swimming (16 ± 1°C) for 7 min each time (2) Restraint: After the restraint is placed in the rat's head to the vent for 30 minutes The modeling time is 21 days	Intragastric administration 168 mg/kg daily for 21 consecutive days	40 male SD rats (180–220 g)	IL-2 and TNF-α levels in serum were significantly increased	The differences in the levels of IL-2 and TNF-α in the serum of the model group and the normal group were statistically significant ($P < 0.05$)	(Wang, 2014)	II

TABLE 9 | Chinese patent medicines for fatigue.

Chinese patent medicine	Formation	Bioactive components	Model	Treatment	The species investigated	Mechanisms	Result	References	Qualityof evidence
Jiawei Sini Powder granules	Radix Bupleuri 10 g, Aurantii Fructus Immaturus 10 g, Radix Paeoniae Rubra 10 g, Glycyrrhizae Radix et Rhizoma Praeparata cumMelle 10 g, Cinnamomi Ramulus 10 g, Acori Tatarinowii Rhizoma 6 g	—	Received various stresses within 49 days, including electroacupuncture (sparse wave, 10 s each time, 5 times each), exhausted swimming, dark box roller (60 r·min^{-1}, 10 min), tail suspension (10 min, and gradually extended), sleep deprivation for 24 h, an average of 6 times per stimulation	Dosing was started on the 49 th day after successful modeling, continuous administration for 7 days, each dose was 8.64 g/kg	70 Kunming mice (17–20 g)	Related to regulating the body's immune function and reducing IL-2 content	Compared with the model group, the quality and behavior changes of the drug group model were statistically significant ($P < 0.05$)	(Zhang T. et al., 2014)	II
Addition and subtraction of Guipi Decoction	Astragali Radix 30 g, Ziziphi spinosae semen 25 g, Codonopsis Radix 15 g, Longan Arillus 15 g, Atractylodis Macrocephalae Rhizoma 15 g, Polygalae Radix 15 g, Angelicae Sinensis Radix 15 g, Glycyrrhizae Radix et Rhizoma 10 g, Aucklandiae Radix 7 g	Astragalus saponin I.V.	—	1 dose daily, 300 ml each morning and evening, 30 days as a course of treatment, a total of 3 courses of treatment	80 patients with CFS	Astragalus saponin IV is the main active ingredient that exerts a positive inotropic effect, which can effectively improve cardiac contraction and diastolic function, and enhance myocardial contractility by inhibiting Na^+ - K^+ - ATP	The total effective rate in the treatment group was 85.0% (34/40); the control group was only 67.5% (27/40) There was a significant difference between the two groups ($P < 0.05$)	(Ouyang et al., 2018)	Ib
Yishen Buxue Ointment	Angelicae Sinensis Radix 10 g, Rehmanniae Radix 15 g, Radix Paeoniae Alba 10 g, Chuanxiong Rhizoma 10 g, Cuscutae Semen 15 g, Epimedii Folium 12 g, Psoraleae Fructus 10 g, Lycii Fructus 10 g	Tetramethylpyrazine	—	Decoction 300–400 ml, take 2 times in the morning and evening, continuous treatment for 6 weeks	104 patients with CFS	Enhance the immune function of patients, IgG, IgM, IgA levels are significantly increased, and ligustrazine has the effects of scavenging oxygen free radicals, improving blood rheology, regulating lipid metabolism	The total effective rate of treatment in the drug group was significantly higher than that in the control group ($P < 0.05$)	(Yuan, 2018)	Ib

TABLE 10 | The single Chinese herbal medicines of antidiarrheal.

Chinese herbal medicine	Bioactive components	Model	treatment	The species investigated	Mechanisms	Result	References	Quality of evidence
Coicis Semen	Fatty acids and esters, coixol, coixan, flavonoids, glycoproteins, sterols, lactams	Rhei Radix et Rhizoma : Magnoliae officinalis Cortex : Aurantii Fructus Immaturus (4:5:3) 1.5 mL/100 g, administered once every other day, fasted on the same day, fed enough and swam to endurance limit on the next day, lasting for 15 days	Coicis Semen decoction high and low dose groups were given 200 $g \cdot kg^{-1} \cdot d^{-1}$ and 10 $g \cdot kg^{-1} \cdot d^{-1}$ by gavage, respectively, for 10 consecutive days	50 SD rats (200 ± 10 g)	Increasing the levels of serum SP, MTL, GAS, CCK, and SS, reducing the content of serum PP, and then regulating gastrointestinal motility	Rat serum hormones have been improved to varying degrees, the number of stools has decreased, and the stools have changed	(Li and Liu, 2019)	II
Aurantii Fructus Immaturus	Flavonoids, essential oil, alkaloids	Ig Rhei Radix et Rhizoma decoction once a day (8.9 g/kg, calculated by crude drugs), 10 ml/kg, for 14 consecutive days	Ig Aurantii Fructus Immaturus solution 10 mL/kg, once a day for 7 consecutive days	170 SD rats (180–200 g)	Promoting the secretion of serum gastrin, acetylcholine, motilin and inhibiting the secretion of vasoactive intestinal peptide	Promote Gastrointestinal motility of spleen deficiency model rats	(Hu et al., 2017)	II
Arecaesemen	Alkaloids, flavonoids, tannins, fatty acids, terpenes, steroids	Gastrointestinal *in vitro* experiment	The Arecaesemen decoction with 12.5% concentration was added 0.025 ml, 0.05, 0.1, and 0.2 ml in sequence, and the interval was 5 minutes	Isolated stomach from rats	Obviously promoting the contraction of the fundus muscle strips in rats, which is manifested by a marked increase in the baseline tension and a significantly increased amplitude	Promote gastrointestinal movement	(Ni et al., 2003)	IV
Magnoliae Officinalis Cortex	Magnolol, honokiol	Each one was given 0.4 ml castor oil by gavage	Water extracts (100, 200, 400 mg/kg) were perfused into stomach respectively	30 sterile Kunming mice	Magnolol and honokiol may exert anti-diarrheal effects by regulating gastrointestinal motility and inhibiting inflammation in the form of Ca^{2+} antagonists	Significantly reducing the diarrhea rate and diarrhea index of mice, and also inhibiting the frequency of loose stools in mice	(Xie et al., 2017)	II
Amomi Fructus	Essential oil, polysaccharides, flavonoids, organic acids, phenols, inorganic compounds	Intragastric administration of 8% Sennae Folium powder suspension once (0.25 ml/10 g)	The essential oil (2, 1, 0.5 ml/kg) were given by gavage once a day, lasting for 3 days	SPF Kunming mice (20 ± 2 g)	Regulating gastrin and prostaglandin E2(PGE2) secretion and VIP expression	Inhibiting diarrhea in mice caused by Sennae Folium	(Zhao et al., 2009)	I

TABLE 11 | Chinese traditional patent medicines of antidiarrheal.

Chinese traditional patent medicine	Components	Model	Drug delivery cycle	The species investigated	Mechanisms	Result	References	Quality of evidence
Wu Ling San	Poria, Alismatis Rhizoma, Polyporus, Cinnamomi Cortex, Atractylodis Macrocephalae Rhizoma	Daily morning gavage: 20 ml/kg Senna solutionfor 6 day	Wu Ling San (1.35, 2.7, 5.4 g/kg) was administered once a day for 7 days	216 SPF grade SD rats, (160 ± 20 g)	Up regulated expression of AQP4 and AQP4mRNA in colon mucosa of diarrhea rat	Compared with the control group, the stool in the model group was still soft	(Liu et al., 2012)	I
Sishen Wan	Myristicae Semen, Psoraleae Fructus, Schisandrae Chinensis Fructus, Euodiae Fructus, Jujubae Fructus	0.2 g/kg of adenine was administered to each stomach for 4 weeks, then 10 mL/kg of ice Sennae Folium water from the third week for 2 weeks	Intragastric administration at 4.2, 3.23, 0.97 g/kg, respectively, once a day for 2 weeks	45 SPF grade SD rats, (140–160 g)	Sishen Wan has the potential as a therapeutic regiment for treatment of diarrhea-predominant irritable bowel syndrome (IBS-D) due to partial regulation of the intestinal flora	Compared with the model group, the diarrhea index decreased and the intestinal sensitivity decreased	(Liu et al., 2019)	III

or activation of active secretion of the intestine, will cause diarrhea. Diarrhea is also caused by the increase of osmotic gradient or hydrostatic pressure in intestinal tissue. Infection with bacteria, viruses, or parasites is the main cause of diarrhea, which is also known as infectious diarrhea or gastroenteritis (Thapar and Sanderson, 2004; Schlossberg, 2015). The occurrence and spread of infectious diarrhea are considered to be the results of poor sanitation. Other causes of diarrhea include hyperthyroidism, lactose intolerance, inflammatory bowel disease, drug effects, and irritable bowel syndrome.

Clinically, quite a few patients infected with COVID-19 experienced diarrhea in the early stage or in the course of the disease, which is mostly self-limiting and varies in severity. In the course of treatment, it was also found that diarrhea caused by COVID-19 mainly occurred after antiviral treatment. Infectious virus particles were isolated from feces of some patients, which increased the possibility of feces-oral transmission. The main causes of diarrhea were considered to be gastrointestinal mucosal injury or gastrointestinal dysfunction caused by COVID-19 and adverse reactions caused by the use of antiviral drugs. The mechanism may be related to the rapid and massive production of various cytokines such as TNF-, IL-6, IL-1, and IL-8 in body fluids when patients were infected with COVID-19.

Treatments

TCM has thousands of years of valuable experience in the treatment of diarrhea. Many antidiarrheal TCM substances, such as Pogostemon Herba, Atractylodis Rhizoma, and Citri Reticulatae Pericarpium, may prevent and treat diarrhea by TNF, IL-6, VIP, and NF-κB. Some of them can also enhance the anti-virus ability of the body by enhancing the immunity, and protect various organs at the same time. Patients with mild conditions can recover quickly by strengthening their own immunity and eliminating the virus by the immune mechanism.

Single Chinese Herbal Medicine

Pogostemon Herba

Pogostemon Herba is a dry aboveground part of the *Pogostemon cablin* (Blanco) Benth. The chemical constituents of Pogostemon Herba can be divided into two categories: volatile components (patchouli oil) and non-volatile components, including monoterpenes, and sesquiterpenes, flavonoids, organic acids, and alkaloids. A large number of studies showed that the main components of patchouli oil include patchouli alcohol and patchouli ketone. Chen et al. (1998) found that the water extraction and oil-free extraction can inhibit the gastrointestinal propulsion of the normal mice and treat diarrhea in mice caused by Sennae Folium, suggesting that two extractions can inhibit diarrhea by inhibiting the excessive peristalsis of the small intestine. Therefore, the effective component of Pogostemon Herba to improve intestinal function may be water-soluble. Wu et al. (2020) established a rat model of intestinal mucositis *via* intraperitoneal injection of 5-fluorouracil, and intragastrically administrated Patchouli alcohol (PA) (10, 20, and 40 mg/kg) to evaluate the effect of PA on intestinal mucositis. The results showed that PA could effectively improve diarrhea in intestinal mucositis rats,

preliminary confirming PA efficacy. Further experiments revealed that PA not only decreased the levels of TNF-α, IL-1β, IL-6, and MPO but also increased the level of IL-10 significantly. In addition, the expression of mucosal barrier proteins and the microbiota community were also improved after PA treatment in diseased rats. Hence, PA may prevent the development and progression of intestinal mucositis by improving inflammation, protecting the mucosal barrier, and regulating intestinal microbiota.

Citri Reticulatae Pericarpium

Citri Reticulatae Pericarpium, commonly referred to as "Chen-pi" in Chinese, is an orange-colored *Citrus reticulata* Blanco fruit peel. Up to now, approximately 140 chemical components have been isolated and identified from Citri Reticulatae Pericarpium, including alkaloids, flavonoids, and essential oils. And among them, flavonoids were considered to be the primary bioactive constituents of herbal medicine, mainly including hesperidin and nobiletin. The Citri Reticulatae Pericarpium decoction can alleviate diarrhea of rats caused by Sennae Folium. Guan (Guan et al., 2002) found that the Citri Reticulatae Pericarpium decoction (6.25%, 12.5%, 25%, 50%, 75%, 100%) can significantly inhibit the spontaneous activity of the isolated duodenum of rabbits, reduce the contractility and tension, and show a dose-response relationship. It has an antagonistic effect on the enhancement of ileal contraction induced by acetylcholine, $BaCl_2$, and 5-HT. Moreover, it may further relax the isolated rabbit intestines which first used atropine, epinephrine and dopamine, but the tension decreased. It is suggested that the inhibitory effect of hesperidin on intestinal motility may not be the main component of Citri Reticulatae Pericarpium. The inhibitory effect of Citri Reticulatae Pericarpium is mediated by the cholinergic receptor, 5-HT receptor, or directly on smooth muscle.

Atractylodis Rhizoma

Atractylodis Rhizoma is derived from the dried roots of *Atractylodes lancea* (Thunb.) DC. and *Atractylodes chinensis* (DC.) Koidz. The main components in its essential oil are atractylol (a mixture of β-cineole and atractylol), atractylone, atractylon, *etc*. Ancient Chinese doctors thought that Atractylodis Rhizoma could be used for dampness blocking, abdominal distention, diarrhea and so on. Modern research has found that Atractylodis Rhizoma has anti-diarrhea and anti-inflammatory effects. Wang et al. (2002) found that β-cineole can significantly improve the physical signs and inhibit the gastrointestinal movement of spleen deficient mice. β-eucalyptol has an obvious antagonistic effect on the acceleration of gastrointestinal motility induced by neostigmine loaded mice, and also on the gastrointestinal motility induced by Rhei Radix et Rhizoma. Research has shown that (Chen et al., 2018) the N-butanol portion of Atractylodis Rhizoma can significantly improve the level of serum anti-inflammatory factor IL-10 and AQP3 of colon mucosa, reduce the level of TNF-α and diarrhea index, relieve the inflammation of the digestive tract, promote the absorption of water by the colon, and play a role in strengthening the intestine and stopping diarrhea. The results showed that the antidiarrheal effect of Atractylodis Rhizoma was enhanced after frying coke and the N-butanol extract was one of the effective parts of Atractylodis Rhizoma. Shi (Shi et al., 2020) found that the ethanol extract of deep-fried Atractylodis Rhizoma can significantly reduce the level of intestinal inflammatory cytokines, increase the expression of AQP3 and AQP8, and restore the abnormal water metabolism. In addition, it can regulate intestinal flora and improve intestinal structure. The commonly used Chinese herbal antidiarrheal medicines are listed in **Table 10** in addition to the above.

Chinese Traditional Patent Medicine

Huoxiang Zhengqi Oral Liquid is composed of Atractylodis Rhizoma, Citri Reticulatae Pericarpium, Magnoliae officinalis Cortex, Angelicae dahuricae Radix, Poria, Arecae Pericarpium, Pinelliae Rhizoma, licorice extract, patchouli oil, and perilla leaf oil. The active ingredients of Huoxiang Zhengqi Oral Liquid mainly include liquiritin, narirutin, hesperidin, ammonium glycyrrhetate, honokiol, magnolol, thymol, guanosine, adenosine, imperatorin, isoimperatorin. Different Huoxiang-Zhengqi preparations have certain anti-diarrhea effects. Taking Huoxiang Zhengqi Oral Liquid as an example, long-term clinical experience shows that it has a significant effect on improving gastrointestinal symptoms. It was found that Huoxiang Zhengqi Oral Liquid could significantly improve the symptoms of diarrhea in spleen deficient rats with dampness syndrome (Xue et al., 2011). Its mechanism might be related to the increased of the expression of ZO-1 in the ileal mucosa, the regulation of CD4 and CD8 T cells in Peyer's patch, and the inhibition of TNF-α level in intestinal homogenate (He et al., 2007).

The commonly used Chinese traditional patent medicines of antidiarrheal are shown in **Table 11**.

CONCLUSION AND PERSPECTIVES

The anti-epidemic experience of China shows that it is of great value in the clinical treatment for COVID-19 to intervene early, improve clinical symptoms of patients, block the transition of mild cases to severe cases, shorten the course of the disease and promoting self-recovery, which can minimize the incidence and mortality of severe illness, and make full use of tight and limited medical resources. At present, the focus is on the research of antiviral drugs and vaccines, but there are few reports on treating early mild symptoms. Fever, cough, and fatigue are the most common symptoms of COVID-19, while some special patients experience diarrhea rather than fever in the early stage. Therefore, this paper summarizes the physiological and pathological processes of fever, cough, fatigue, and diarrhea, and explores the material basis, action mechanism and clinical research of Chinese herbal medicines and Chinese patent medicines with corresponding therapeutic effects, in order to provide reference for the efficient use of existing drugs. TCM has the unique properties of multi-components and multi-targets. The majority of these mentioned drugs may not only

exert the effects of antipyretic, antitussive, anti-fatigue, and antidiarrheal, but also have the properties of anti-inflammation, antioxidation and immunity enhancement; and some of them are antiviral.

Such rich pharmacological activities are of great benefit in the initial treatment of COVID-19. On the one hand, these medicines may have the ability to relieve symptoms, reduce the rate of infection and prevent the transition from mild to severe in the early stages of infected patients. On the other hand, it is possible to cut down the dosage or use of hormones, decrease the dosage of first-line antiviral drugs or shorten their usage time, so as to minimize the potential damage to the liver by these drugs, and reduce the mortality of critically ill patients through the treatment of integrated traditional Chinese medicine and western medicine. However, these medicines also have shortcomings, mainly manifested in the lack of in-depth research on the material basis and mechanism of action, as well as the imperfection of clinical trials. Therefore, it is urgent to design more stringent controlled clinical trials in order to provide more scientific and reliable evidence for fighting COVID-19 all over the world.

AUTHOR CONTRIBUTIONS

D-KZ and LH put forward the idea. C-HL, L-LM, H-ML, and WL gather the materials, and wrote the paper. R-CX, Z-MC, and J-ZL contributed to the revisions. All authors contributed to the article and approved the submitted version.

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Systems Pharmacology and Verification of ShenFuHuang Formula in Zebrafish Model Reveal Multi-Scale Treatment Strategy for Septic Syndrome in COVID-19

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The outbreak of coronavirus disease 2019 (COVID-19) has affected millions of people worldwide. Critically ill COVID-19 patients develop viral septic syndrome, including inflammatory damage, immune dysfunction, and coagulation disorder. In this study, we investigated ShenFuHuang formula (SFH), a traditional Chinese medicine, which has been widely used as complementary therapy for clinical treatment of COVID-19 in Wuhan, to understand its pharmacological properties. Results of systems pharmacology identified 49 active compounds of SFH and their 69 potential targets, including GSK3β, ESR1, PPARG, PTGS2, AKR1B10, and MAPK14. Network analysis illustrated that the targets of SFH may be involved in viral disease, bacterial infection/mycosis, and metabolic disease. Moreover, signaling pathway analysis showed that Toll-like receptors, MAPK, PPAR, VEGF, NOD-like receptor, and NF-kappa B signaling pathways are highly connected with the potential targets of SFH. We further employed multiple zebrafish models to confirm the pharmacological effects of SFH. Results showed that SFH treatment significantly inhibited the inflammatory damage by reducing the generation of neutrophils in Poly (I:C)-induced viral infection model. Moreover, SFH treatment could improve the phagocytosis of macrophages and enhance the expression of immune genes in an immune deficiency model. Furthermore, SFH treatment exhibited promising anti-thrombosis effect in a thrombus model. This study provided additional evidence of SFH formula for treating COVID-19 patients with septic syndrome using multiple-scale estimation.

Keywords: COVID-19, sepsis, traditional Chinese medicine, systems pharmacology, zebrafish

INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19) pandemic caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, 2019-nCoV) has caused an enormous impact worldwide since the end of 2019, resulting in great loss to global health and economy (Wu et al., 2020). It has been confirmed that bats and birds are the hosts of typical coronavirus, with zoonotic spread and animal-animal-human transmission (Connors and Levy, 2020). By July 8, 2020, data from the World Health organization (WHO) showed that the number of COVID-19 confirmed cases worldwide had increased to over 11 000 000. Although most COVID-19 patients suffered from a mild illness, 5% of the patients developed severe lung injury or even systemic organ failure. The number of confirmed deaths worldwide has reached 500 000. Hence, it is critical to urgently improve the therapeutic schedule and develop more effective drugs against SARS-CoV-2. The efforts of clinicians worldwide have led to considerable experience and understanding of this infectious disease. Recent studies reported that many critically ill COVID-19 patients developed typical septic syndrome, including inflammatory injury, immune dysfunction, coagulation disorder, and multiple organ failure (Bellinvia et al., 2020; Coronado et al., 2020; Li et al., 2020). Diagnosis and clinical symptoms of these patients met the criteria of the Sepsis-3 International Consensus. Guidelines on the management of critically ill adults with COVID-19 established by Surviving Sepsis Campaign (SSC) also emphasized the treatment and supportive care of patients with septic syndrome (Alhazzani et al., 2020).

Infectious complications in critically ill patients are known to activate multiple systemic coagulation and inflammatory responses that are vital for host defense. Early inflammatory response and activation of coagulation system are critical for the elimination of SARS-CoV-2 *in vivo* (Giannis et al., 2020; Merad and Martin, 2020; Tay et al., 2020). However, when the inflammatory reaction is not controlled in time, the exogenous coagulation pathway will promote coagulation reaction. The uncontrolled coagulation dysfunction may lead to vascular damage and eventually disseminated intravascular coagulation (DIC) (Jose and Manuel, 2020). Given the important role of the inflammation and coagulation system in the development of sepsis, it is critical to identify drugs that can effectively modulate these pathological processes. Early use of anti-infective drugs combined with hemodynamic support achieves better therapeutic effect, but when the immune balance of the host is disrupted, the interaction between inflammatory response and coagulation function complicates the treatment.

ShenFuHuang (SFH) formula, which is composed of *Panax ginseng C.A.Mey*, *Aconitum carmichaeli Debeaux*, and *Rheum palmatum L.*, has been extensively used for clinical treatment of critical illnesses, including sepsis and septic shock. According to our statistics, SFH formula was empirically applicated on more than 500 critically ill patients with sepsis or septic shock in three hospitals during the COVID-19 outbreak in Wuhan. However, as a traditional Chinese medicine (TCM) with complex composition, it is necessary to understand its pharmacological effects and mechanisms *via* multi-scale investigations. Systems pharmacology has been used to identify the active compounds and potential targets of herbal medicines, combining pharmaco-chemistry information and multi-target prediction with network analyses. In this study, we employed systems pharmacology to reveal the underlying mechanisms of SFH. Zebrafish (*Danio rerio*) have become powerful tools in drug development research due to their molecular conservation, genetic accountability, ease of experimental use, and diverse behavioral properties. Zebrafish models and tests are particularly useful in genetics research, drug screening, global inflammatory research, as well as coagulation system disorders.

In this study, we used multiple tools to understand the effects and molecular mechanisms of SFH, in order to provide new insights into the clinical application of TCM for treating COVID-19 patients with septic syndrome.

MATERIALS AND METHODS

Preparation of SFH Formula

SFH formula, consists of Hong Shen (*Panax ginseng C.A.Mey*) (ratio 1/4), Fu Zi (*Aconitum carmichaeli Debeaux*) (ratio 2/4), and Da Huang (*Rheum palmatum L.*) (ratio 1/4), was decocted and provided by TCM Pharmacy of Beijing hospital of traditional Chinese Medicine. Briefly, 120 g crude drugs of SFH formula were soaked and decocted in 400 ml pure water for 30 min. Then the water decoction was concentrated to 120 ml, and the final dosage of crude drugs was 1 g/ml. The dosages of SFH formula used for this study were 1.11, 3.33, and 10 μl/ml water (autoclaved and sterilized), respectively.

Reagents and Herbs

Indomethacin (No. 13931) and arachidonic acid (No. A1831030) were purchased from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). LPS (No. 017M4112V) and aspirin (No. MKCD0957) were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). FastQuant RT Kit (No. KR106) was purchased from TIANGEN Biotech Co., Ltd (Beijing, China). TRIzol® reagent was purchased from Gibco (Grand Island, NY, USA). *Panax ginseng C.A.Mey* was purchased from Beijing TaiYangShuKang herbs company (Beijing, China, Voucher number 1805060). *Aconitum carmichaeli Debeaux* was purchased from China SinoPharm (Beijing, China, Voucher number xf5271). *Rheum palmatum L.* was purchased from Beijing Xinglin pharmaceutical (Beijing, China, Voucher number 20012201)

Data Set Construction

The current data were obtained from literature mining and the TCM pharmacology analysis platforms, including TCMSP (http://lsp.nwsuaf.edu.cn/tcmsp.php) (Ru et al., 2014) and ETCM (http://www.tcmip.cn/ETCM/) (Xu et al., 2019). We collected the physical and chemical information of 231 compounds from SFH formula, including 92 compounds of *Rheum palmatum L.*, 74 compounds of *Panax ginseng C.A.Mey*, and 65 compounds of *Aconitum carmichaeli Debeaux*.

Active Compound Screening Model

Oral Bioavailability

Oral Bioavailability (OB) is an important pharmacokinetic parameter in the drug's ADME (absorption, distribution, metabolism, excretion) curve. In this study, OB was evaluated based on OBioavail 1.1 (Xu et al., 2012) and IntegrOB. Suitable molecules with OB ≥ 30% are used as candidate compounds for further study.

Caco-2 Permeability

The oral absorption of drugs is mainly done by intestinal epithelial cells (IEC). In this study, the computer Caco-2 permeability prediction model was used to predict the intestinal permeability of all TCM components in TCMSP. Molecules with Caco-2 > −0.4 are considered to exhibit sufficient intestinal epithelial permeability.

Drug-Likeness

Drug similarity is a qualitative concept used in drug design to illustrate the "drug -Likeness (DL)" of a substance in terms of bioavailability and other factors. In this work, Tanimoto coefficient is used for estimating DL. Compounds with DL ≥ 0.18 are considered to have high drug-like properties and are selected as candidates for further study.

Half-Life

The half-life (HL) of a drug reflects the rate of elimination (excretion, biotransformation, storage, etc.) of the drug in the body, and represents the relationship between the time and drug in the body. The compounds with HL ≥ 4 in SFH were screened as candidate active molecules for study.

Drug Targeting

To obtain targets we used weighted ensemble similarity (WES) model, a comprehensive drug targeting method that integrates chemical, genomics, and pharmacological information to predict direct target data of SFH. Full data mining is performed in the TCMSP analysis platform to obtain the three-dimensional structure information corresponding to the molecule. Run the WES model with the obtained molecular three-dimensional structure as an index to obtain all species targets. Finally, put the targets from different sources in the UniProt (http://www.uniprot.org) database with a unified name, and then submit them to the PharmGKB database, Therapeutic Targets Database (TTD) and the Comparative Toxicogenomics Database (CTD) to delete redundant and wrong targets to ensure the accuracy of the targets (Zheng et al., 2015).

Network Construction

We performed gene ontology functional enrichment analysis by using BatchQuery CTD database (http://ctdbase.org/tools/batchQuery.go) to obtain all disease information related to the targets. The drug-target network is constructed using the software Cytoscape 2.8.1. In the generated network, nodes represent compounds, proteins, or diseases, and edges represent compound-target, target-disease, and target-pathway interactions (Smoot et al., 2011).

Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC–MS/MS) Analysis

A Waters UPLC-MS/MS spectrometer equipped with a HESI-II probe was used to identify the main composition of SFH formula. The details of the protocol were as previously described (Xu et al., 2018). Data were collected and analyzed by using the Waters Masslynx 4.1 system.

Zebrafish Models

Zebrafish Embryo and Larvae Maintenance

Wild type AB zebrafish, transgenic neutrophil fluorescent AB zebrafish, melanin allele mutated translucent Albino strain zebrafish were purchased from Hunter Biotech (Hangzhou, China). Zebrafish were maintained and raised according to the protocol described before (Yang et al., 2014; Mehrdana et al., 2017).

Poly I:C-Induced Viral Infection Model

Randomly select 180 transgenic neutrophil fluorescent AB zebrafish (3 days post fertilization, 3 dpf) into six-well plates. Each well (3 ml) contains 30 tails of zebrafish. Poly (I:C) (100 ng/fish) was injected into the swim bladder to establish a zebrafish infection model. The model zebrafish were administered with or without SFH formula at different dosages for 3 h. The concentration of the positive control indomethacin was 60 μM. After treatment with Poly (I:C) for 3 h, the zebrafish were collected for research.

Macrophage Activation Model

Randomly select 180 wild-type AB zebrafish (3 dpf) into a six-well plate. Each well contains 30 tails. Fish were given intravenous injection of Indian ink (10 nl/fish) to establish a model of phagocytosis of zebrafish macrophages. Neutral red was used to stain macrophage. The model zebrafish were administered with or without SFH formula at different dosages for 3 h for research.

AA-Induced Thrombosis Model

Randomly select 180 melanin allele mutated translucent Albino strain zebrafish (3 dpf) into a six-well plate. Zebrafish were given arachidonic acid to establish thrombosis model and then were administered with or without SFH formula at different dosages for 3 h for research. To evaluate the antithrombosis capacity, zebrafish was stained with o-anisidine staining solution, and the antithrombotic activity of medicines were quantitatively analyzed by calculating the staining intensity of zebrafish heart erythrocytes (Zhu et al., 2016).

$$\text{Efficacy}(\%) = [\text{S(drug)} - \text{S(model)}]/[\text{S(vehicle)} - \text{S(model)}] \times 100\%$$

Staining

The zebrafish was fixed with 4% paraformaldehyde. After fixation, the zebrafish was transferred to 70% ethanol for

dehydration, embedding, sectioning, staining, and mounting. The stained zebrafish sections were analyzed for pathology.

Real-Time PCR

After extracting total zebrafish RNA from each experimental group using the classic Trizol method, the concentration and purity of total RNA were determined using Thermo ultra-micro spectrophotometer. The RNA was then quantified by UV spectrophotometry (Thermo, NanoDrop 2000). Then use the PrimeScript® RT kit to amplify the transcribed cDNA according to the manufacturer's instructions. Perform real-time quantitative PCR according to the manufacturer's instructions (SYBR Green PCR Reagent kit). The primer sequences used are listed in **Supplementary Table S1**.

Statistical Analysis

SPSS15.0 software was used to analyze the data. The analysis of variance was combined with Dunnett's T-test for statistical analysis. $p < 0.05$ indicated a significant difference.

RESULTS

In this study, parameters including OB (≥ 30%), DL (≥ 0.18), Caco-2 (> −0.4), and HL (≥ 4) were utilized to identify the active compounds of SFH. Compounds with properties OB < 30%, DL < 0.18, Caco-2 ≤ −0.4, and HL< 4 were considered as candidate compounds, since there are some typical pharmacodynamic molecules that possess well-documented biological activities established in *in-vivo* and *in-vitro* studies. Finally, 49 potential ingredients were screened as candidate compounds of SFH (**Table 1**).

Target Prediction of Potential Compounds

In order to reveal the interactions between all candidate compounds and the target proteins on a large scale, the WES model was employed to calculate and forecast the targets. Based on the results of simulation, 64 potential targets were identified for the 49 candidates (**Supplementary Table S2**). The full names of these symbols of targets were listed in **Supplementary Table S3**.

Network Analysis

Compound-Target Network

The compounds of SFH acted on multiple targets, and each target was involved in various physiological and pathological processes. The compound-target network includes 113 nodes and 291 interactions. A total of 64 target proteins were screened out, which may be related to inflammatory response, coagulation disorder, and tissue damages. Compounds such as sitosterol, emodin, chrysophanol, and deltoin had the highest weight and number of targets. Additionally, retrieval of multiple relationships between the compounds and targets showed that proteins including GSK3β, ESR1, PPARG, PTGS2, AKR1B10, and MAPK14 may act as potential targets of SFH and impact downstream signaling pathways (**Figure 1A**).

Target-Diseases Network

To understand the role of targets of SFH on diseases, we established a target-disease network using PharmGKB, Drugbank, and TTD databases. Total of 46 targets were directly involved in immune system disease, while nine targets were related to inflammation. Moreover, viral disease, bacterial infection/mycosis, and metabolic disease were also highly linked to the potential targets (**Figure 1B**).

Target-Pathway Network

To further investigate the intervention of SFH on various diseases, KEGG and DAVID databases were used to identify the relationship between the potential targets and related signaling pathways. The target-pathway interaction network comprised of 45 targets and 46 pathways, which included 91 nodes and 293 edges. Most of the predicted targets were involved in pathways that contributed to diseases. These high-weight signaling pathways included Toll-like receptor, MAPK, JAK/STAT, PPAR, VEGF, NOD-like receptor and NF-kappa B signaling pathways, which are closely related to sepsis, infection immunity, inflammatory response, coagulation function, organ damage, immune disorders and other diseases (**Figure 1C**).

Details of the topological properties of the networks in **Supplementary Table S4**.

Integration of Networks

To systematically estimate the synergistic effects of these three herbal ingredients of SFH on sepsis, an integrated "sepsis-related pathway" approach was structured based on the current data on sepsis. The targets of SFH were associated with key pathological processes of sepsis, including the calcium signaling pathway, MAPK signaling pathway, T cell receptor signaling pathway, and PI3K-AKT signaling pathway (**Figure 2**).

Identification of Major Components of SFH Formula

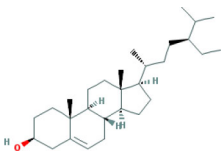
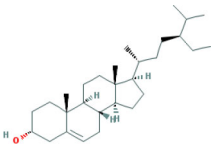
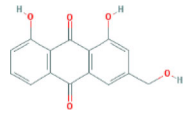
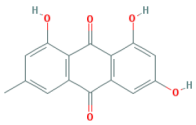
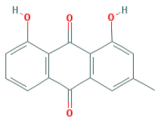
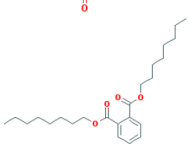
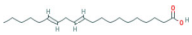
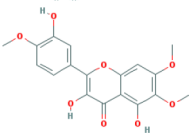
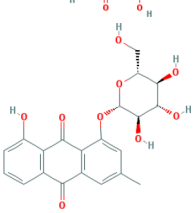
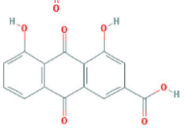
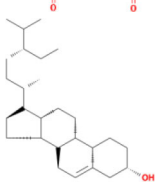
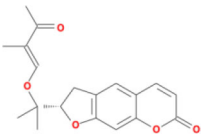
This study employed UPLC–MS/MS detection to investigate the ingredients and evaluate the repeatability and stability of SFH formula. As shown in **Figure 3**, the total ion chromatogram of three parallel samples illustrated the composition and percentage of ingredients in the SFH formula. Moreover, data based on the characteristic peaks found that the SFH formula showed high repeatability and stability according to coefficient correlation. The contents of major compounds were further examined by using UPLC–MS/MS. Data showed that main compounds including aloe-emodin (48.13 μg/ml), rhein (110.69 μg/ml), fuziline (68.78 μg/ml), deoxyaconitine (12.08 μg/ml), ginsenoside rh2 (1.74 μg/ml), quercetin (2.01 μg/ml), gallic acid (157.47 μg/ml) were detected in SFH formula, details in **Supplementary Table S5**.

Effect of SFH on Zebrafish Model

Effect of SFH on Poly (I:C)-Induced Infection

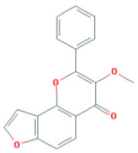
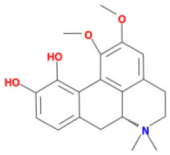
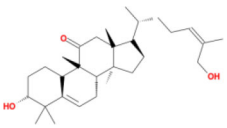
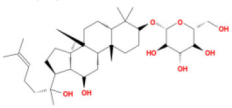
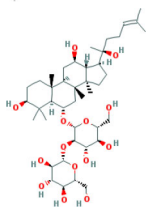
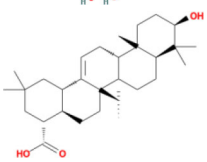
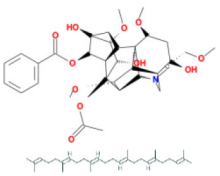
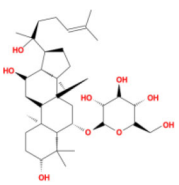
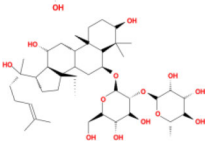
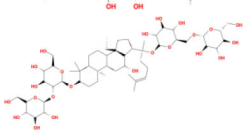
Neutrophils are important immunocytes in the innate immunity system. We investigated the effect of SFH on neutrophil activation using a Poly (I:C)-induced zebrafish infection model.

TABLE 1 | Candidate Information.

Molecular ID	Compound	Herb	OB	Caco2	DL	HL	Structure
M1	beta-sitosterol	*Rheum palmatum* L., Panax ginseng C.A.Mey	36.91	1.32	0.75	5.35	
M2	sitosterol	*Aconitum carmichaeli* Debeaux	36.91	1.32	0.75	5.37	
M3	aloe-emodin	*Rheum palmatum* L.	83.38	-0.12	0.24	31.5	
M4	emodin	*Rheum palmatum* L.	24.40	0.22	0.24	0	
M5	chrysophanol	*Rheum palmatum* L.	18.64	0.62	0.21	0	
M6	dioctyl phthalate	*Panax ginseng* C.A.Mey	40.58	0.95	0.40	9.73	
M7	11,14-eicosadienoic acid	*Aconitum carmichaeli* Debeaux	39.99	1.22	0.20	5.60	
M8	eupatin	*Rheum palmatum* L.	50.80	0.53	0.41	13.9	
M9	chrysophanol glucoside	*Rheum palmatum* L.	20.06	-1.17	0.76	0	
M10	rhein	*Rheum palmatum* L.	47.07	-0.20	0.28	32.1	
M11	daucosterol_qt	*Rheum palmatum* L.	35.89	1.35	0.70	6.11	
M12	Deltoin	*Aconitum carmichaeli* Debeaux	46.69	0.55	0.37	7.69	

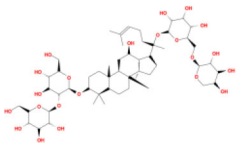
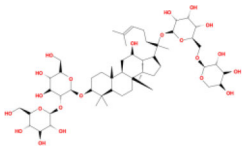
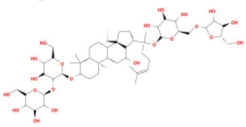
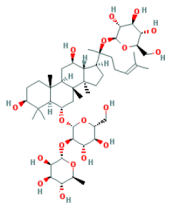
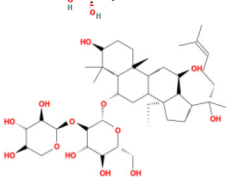
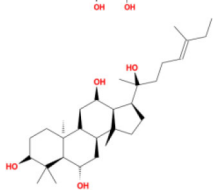
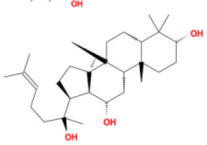
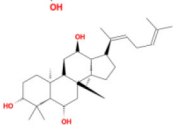
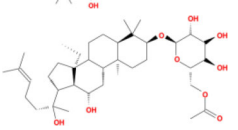
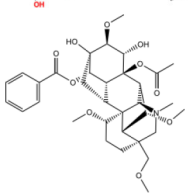
(Continued)

TABLE 1 | Continued

Molecular ID	Compound	Herb	OB	Caco2	DL	HL	Structure
M13	karanjin	*Aconitum carmichaeli* Debeaux	69.56	1.22	0.34	13.1	
M14	fuzitine	*Aconitum carmichaeli* Debeaux	25.78	1.02	0.54	0	
M15	carnosifloside I_qt	*Aconitum carmichaeli* Debeaux	38.15	0.28	0.79	6.99	
M16	ginsenoside rh2	*Panax ginseng* C.A.Mey	36.32	-0.50	0.56	11.07	
M17	ginsenoside rf	*Panax ginseng* C.A.Mey	17.74	-2.23	0.24	0	
M18	ginsenoside R0_qt	*Panax ginseng* C.A.Mey	17.41	0.43	0.76	0	
M19	mesaconitine	*Aconitum carmichaeli* Debeaux	8.70	-0.35	0.25	0	
M20	(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	*Panax ginseng* C.A.Mey	33.55	2.07	0.42	3.14	
M21	ginsenoside-Rh1	*Panax ginseng* C.A.Mey	3.86	-1.17	0.57	0	
M22	20(R)-ginsenoside Rg2	*Panax ginseng* C.A.Mey	10.09	-1.96	0.26	0	
M23	ginsenoside Rb1	*Panax ginseng* C.A.Mey	6.24	-3.99	0.04	0	

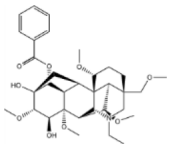
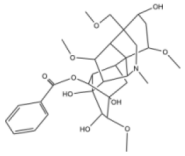
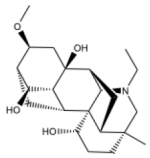
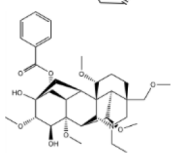
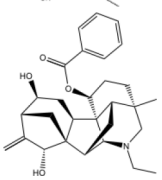
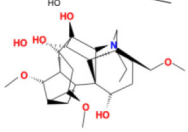
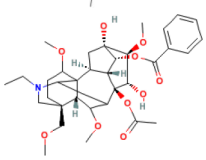
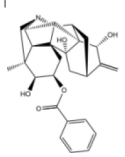
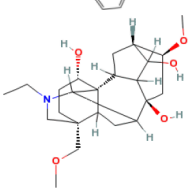
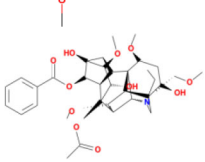
(Continued)

TABLE 1 | Continued

Molecular ID	Compound	Herb	OB	Caco2	DL	HL	Structure
M24	ginsenoside-Rb2	*Panax ginseng* C.A.Mey	6.02	-3.92	0.04	0	
M25	ginsenoside-Rc	*Panax ginseng* C.A.Mey	8.16	-3.97	0.04	0	
M26	ginsenoside Re	*Panax ginseng* C.A.Mey	4.27	-3.20	0.12	0	
M27	notoginsenoside R2	*Panax ginseng* C.A.Mey	17.74	-2.22	0.28	0	
M28	ginsenoside Rg2_qt	*Panax ginseng* C.A.Mey	20.12	0.05	0.82	0	
M29	ginsenoside Rg2_qt	*Panax ginseng* C.A.Mey	20.12	0.05	0.82	0	
M30	ginsenoside Rg3_qt	*Panax ginseng* C.A.Mey	29.70	0.34	0.77	0	
M31	ginsenosideRh4_qt	*Panax ginseng* C.A.Mey	9.84	0.36	0.78	0	
M32	ginsenoside Rs1_qt	*Panax ginseng* C.A.Mey	11.87	-0.86	0.46	0	
M33	hypaconitine	*Aconitum carmichaeli* Debeaux	31.39	-0.34	0.26	19.87	

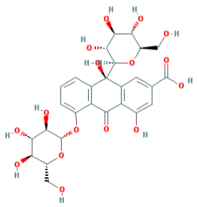
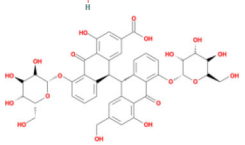
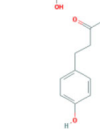
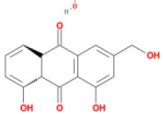
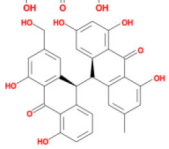
(Continued)

TABLE 1 | Continued

Molecular ID	Compound	Herb	OB	Caco2	DL	HL	Structure
M34	Benzoylmesaconine	*Aconitum carmichaeli* Debeaux	8.55	-0.52	0.27	0	
M35	karakoline	*Aconitum carmichaeli* Debeaux	51.73	0.32	0.73	11.10	
M36	neojiangyouaconitine	*Aconitum carmichaeli* Debeaux	9.83	0.01	0.26	0	
M37	benzoylhypaconine	*Aconitum carmichaeli* Debeaux	8.70	-0.29	0.29	0	
M38	benzoylnapelline	*Aconitum carmichaeli* Debeaux	34.05	0.19	0.52	15.7	
M39	6-demethyldesoline	*Aconitum carmichaeli* Debeaux	51.87	-0.26	65	13.1	
M40	deoxyaconitine	*Aconitum carmichaeli* Debeaux	30.95	-0.23	0.24	22.6	
M41	ignavine	*Aconitum carmichaeli* Debeaux	84.07	-0.07	0.24	28.9	
M42	isotalatizidine	*Aconitum carmichaeli* Debeaux	50.82	-0.11	0.73	11.5	
M43	aconitine	*Aconitum carmichaeli* Debeaux	7.87	-0.58	0.23	0	

(Continued)

TABLE 1 | Continued

Molecular ID	Compound	Herb	OB	Caco2	DL	HL	Structure
M44	mutatochrome	*Rheum palmatum* L.	48.64	1.97	0.61	15.7	
M45	rheinoside A	*Rheum palmatum* L.	0.82	-3.17	0.68	0	
M46	sennoside C	*Rheum palmatum* L.	3.99	-3.53	0.08	0	
M47	rheosmin	*Rheum palmatum* L.	26.79	0.97	0.04	0	
M48	aloeemodin	*Rheum palmatum* L.	20.65	-0.22	0.24	0	
M49	palmidin A	*Rheum palmatum* L.	32.45	-0.36	0.65	32.1	

Obvious inflammatory infiltration and cell shedding were observed in the air bladder of Poly (I:C) stimulated group. However, SFH treatment at doses of 3.33 and 10 mg/ml notably improved the symptoms, with no difference with indomethacin group (**Figure 4A**). Moreover, data showed that the number of neutrophils were markedly increased when challenged by Poly (I:C), compared with the control group. Treatment with indomethacin as a positive control notably suppressed the generation of neutrophils induced by Poly (I:C) ($p < 0.05$). SFH treatment at doses of 3.33 and 10 mg/ml could significantly inhibit the production of neutrophils, compared with the Poly (I:C) group ($p < 0.001$). Moreover, SFH at these two doses exhibited a better treatment effect than indomethacin ($p < 0.05$) (**Figures 4B, C**).

Effect of SFH on Macrophage Activation

Macrophages play a key role in sepsis by phagocytosis of pathogens and inflammatory response. We used ink to establish a macrophage phagocytosis model. Data showed that SFH treatment significantly enhanced the phagocytic capacity of macrophages by increasing the number of macrophages that engulfed ink (**Figures 5A, B**). The expression of several key phenotypic indexes of macrophages represents their functional activation. Hence, we detected the mRNA expression levels of polarization biomarkers such as TNF-α, iNOS, IL-1β, IL-10, and Arg-1 in macrophages of zebrafish treated by SFH. SFH treatment significantly enhanced the mRNA expression of TNF-α at doses of 3.33 mg/ml ($p < 0.05$) and 10 mg/ml ($p < 0.001$), compared with the model group. The expression levels of IL-1β and IL-10 were also increased after treatment with SFH at the dose of 10 mg/ml ($p < 0.05$). However, the expression of AGR-1 was suppressed by SFH treatment at the dose of 10 mg/ml ($p < 0.05$). SFH had limited regulatory effect on iNOS expression (**Figure 5C**).

Effect of SFH on Coagulation Function

To investigate the role of SFH in coagulation function, we evaluated the staining intensity of red blood cells (RBC) in the heart and trunk of zebrafish. Compared with the control group, treatment with arachidonic acid (AA) significantly decreased the number of red blood cells in the heart but increased their number in the trunk ($p < 0.001$). However, SFH treatment at doses of 3.33 and 10 mg/ml effectively enhanced the generation of red blood cells in the heart ($p < 0.001$). Treatment with aspirin as a positive control showed a similar effect to SFH ($p > 0.05$) (**Figures 6A, B**). By using the model of zebrafish thrombosis assay technology, we found that SFH treatment at doses of 3.33 and 10 mg/ml had promising antithrombosis properties, compared with the aspirin treatment ($p > 0.05$) (**Figure 6C**).

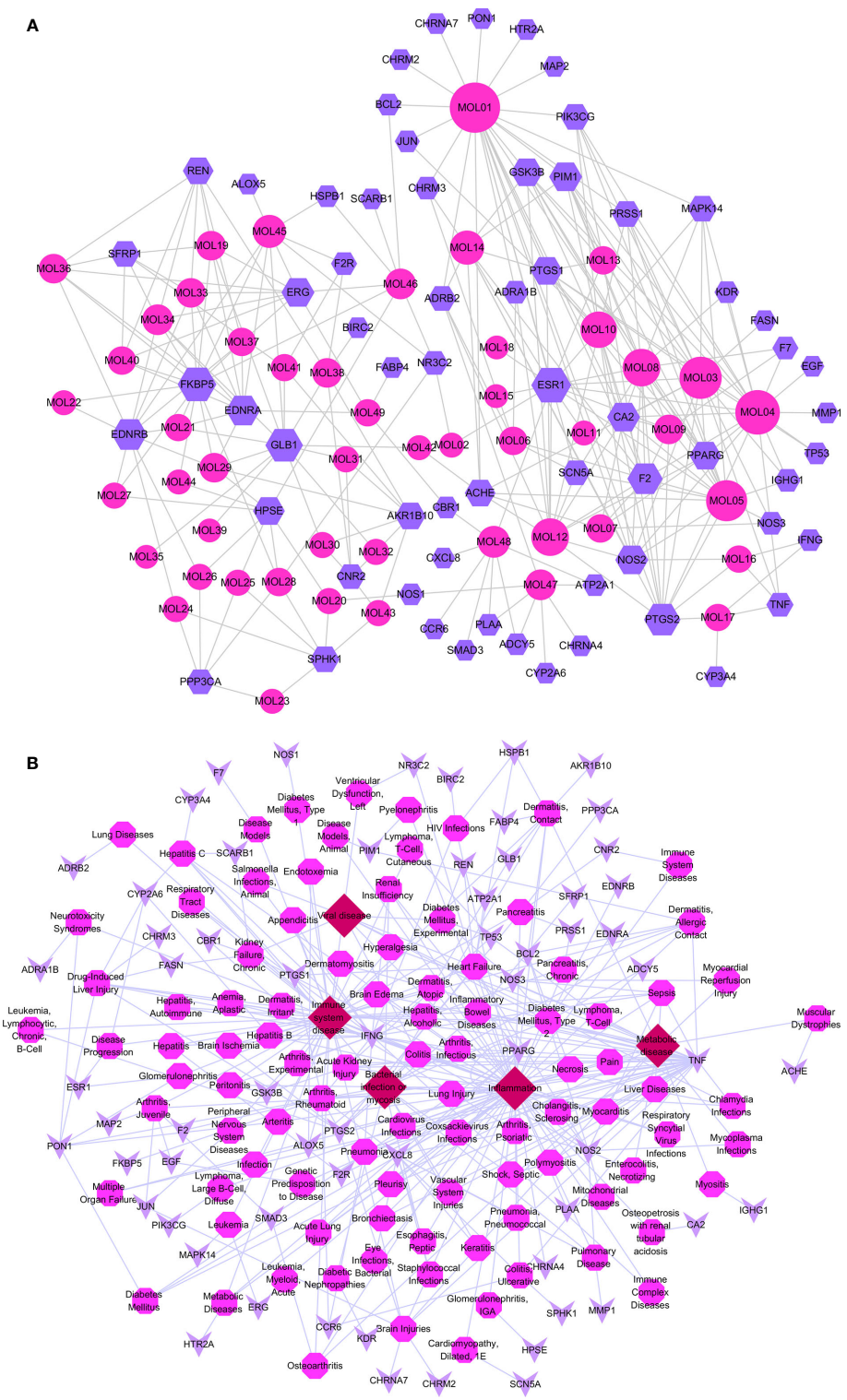


FIGURE 1 | Continued

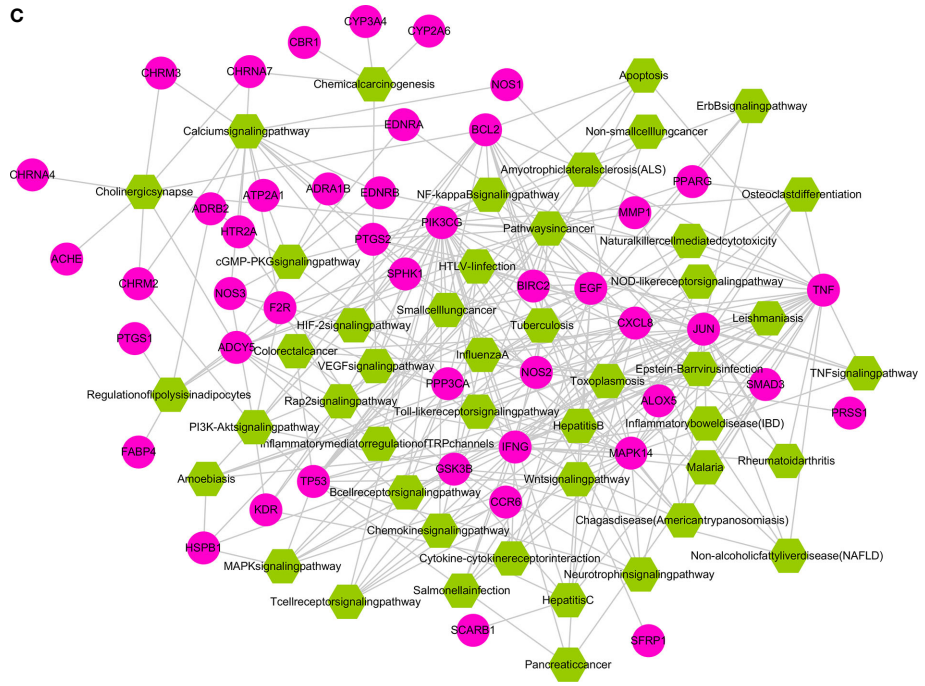


FIGURE 1 | Compound-target-disease-pathway networks. **(A)** Compound-target network of SFH consisting of 113 nodes and 291 interactions. **(B)** Target-disease network including 46 candidate targets and 5 important diseases. **(C)** Target-pathway network including 45 candidate targets and 46 KEGG pathways.

DISCUSSION

The outbreak of COVID-19 caused by SARS-CoV-2 has resulted in an acute respiratory illness pandemic worldwide. A global health emergency was announced by the WHO Emergency Committee on January 30, 2020, based on the growing number of infected and dead patients in China and several other countries. The lack of knowledge of this virus in the initial stage resulted in large numbers of infected patients, with approximately 5% mortality rate. Extensive efforts of clinicians and scientists worldwide have gradually uncovered several critical aspects of this disease. It has been well-documented that critically ill COVID-19 patients accord with the Sepsis-3.0 guideline. Moreover, negative results for bacteria and fungus based on specimen cultures were observed in the lower respiratory tract and blood samples of 76% of sepsis patients, indicating that severe patients are more likely to suffer from viral sepsis (Lin et al., 2018; Zhou et al., 2020).

Sepsis is one of the complications in critical patients. The pathophysiological process of sepsis is complex. Excessive activation of inflammation in the early stage of sepsis contributes to unexpected immune response and tissue damages. However, with the increase of inflammatory mediators and anti-inflammatory reaction *in vivo*, the host gradually reaches a state of immunosuppression, which leads to immune function disorder, apoptosis, necrosis and coagulation dysfunction. The progression of COVID-19 infection is also characterized by these features. Despite the pulmonary injury, the production of pro-inflammatory cytokines including IL-1β, IL-6, TNF-α, macrophage inflammatory protein 1-α, interferon gamma-induced protein-10, and monocyte chemoattractant protein-1 were significantly enhanced in COVID-19 patients (Huang et al., 2020; Liu et al., 2020). Besides, 71.4% of dead patients matched the grade of disseminated intravascular coagulation (DIC) according to the International Society on Thrombosis and Hemostasis criteria, and showed abnormal coagulation in later stages of the disease (Tang et al., 2020; Zhou et al., 2020).

SFH formula, which is composed of *Panax ginseng C.A.Mey*, *Aconitum carmichaeli Debeaux*, and *Rheum palmatum L.*, was designed for treating septic syndrome and used for treating COVID-19 patients. In addition to the well-published LianHuaQingWen capsule (Hu et al., 2020), many unreported TCMs are effective for COVID-19 and its complications, including SFH formula. Chinese herbs have complex mechanism and pharmacology, with multi-component, multi-target, and multi-channel systems. Systems pharmacology has been used to study TCMs by screening the active compounds, predicting the targets, and analyzing the potential compound-target-disease relationships. In this study, we screened 49 active candidates from SFH formula using indicators such as OB, DL, Caco-2, and HL. There are some typical pharmacodynamic molecules who are not up to standard, possessing well-documented biological activities established in *in-vivo* and *in-vitro* studies. For example, emodin (M4) from *Rheum palmatum L.* has been reported to exhibit promising anti-inflammatory, anti-oxidative, hepatoprotective, and anticancer effects (Dong et al., 2016). Again as an example, chrysophanol (M5) from *Rheum palmatum L.* has been established to have anticancer, antioxidation, neuroprotection, antibacterial and antiviral

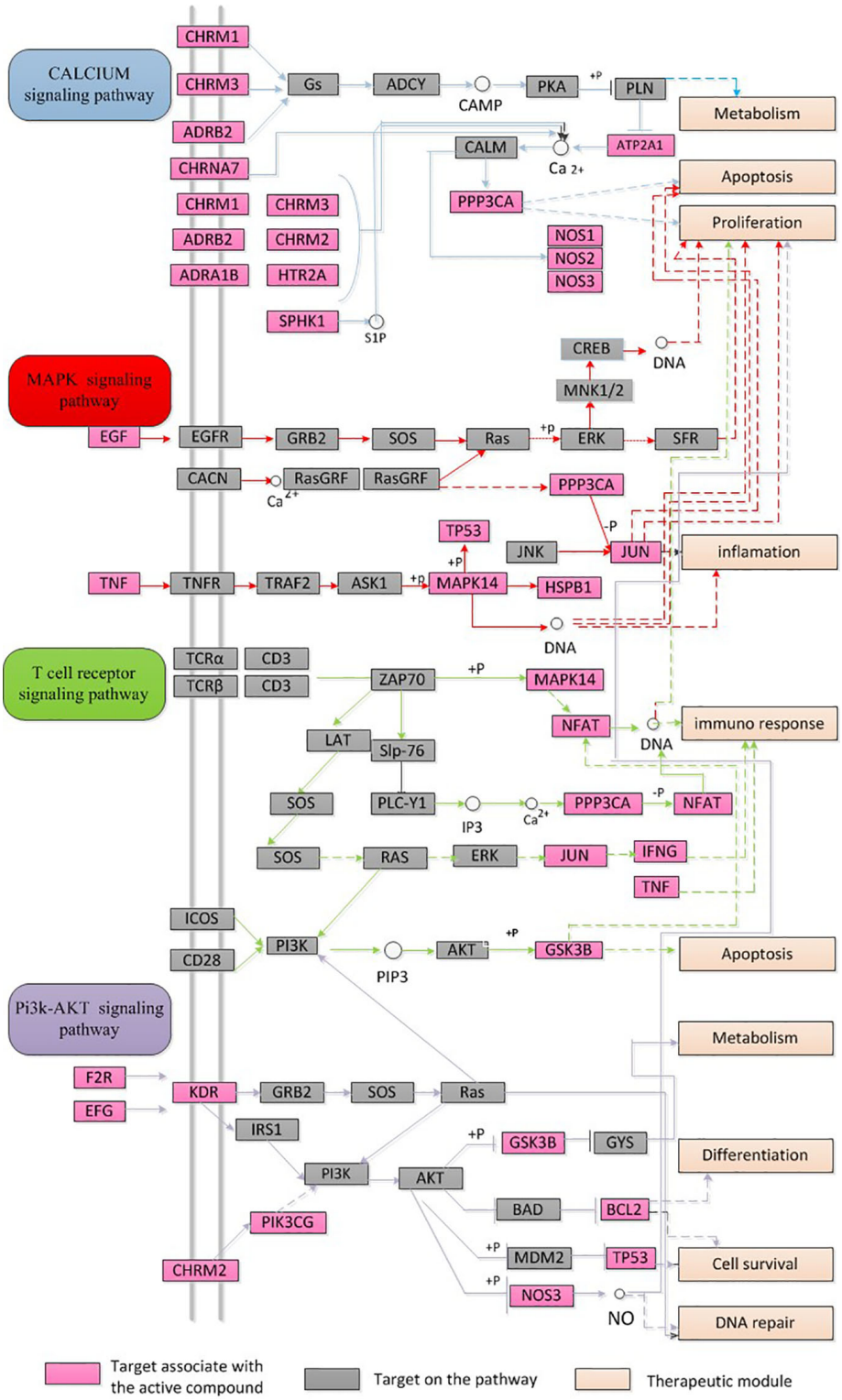


FIGURE 2 | Integration of networks of SFH targets. Sepsis-related pathway including calcium signaling pathway, MAPK signaling pathway, T cell receptor signaling pathway, and PI3K-AKT signaling pathway.

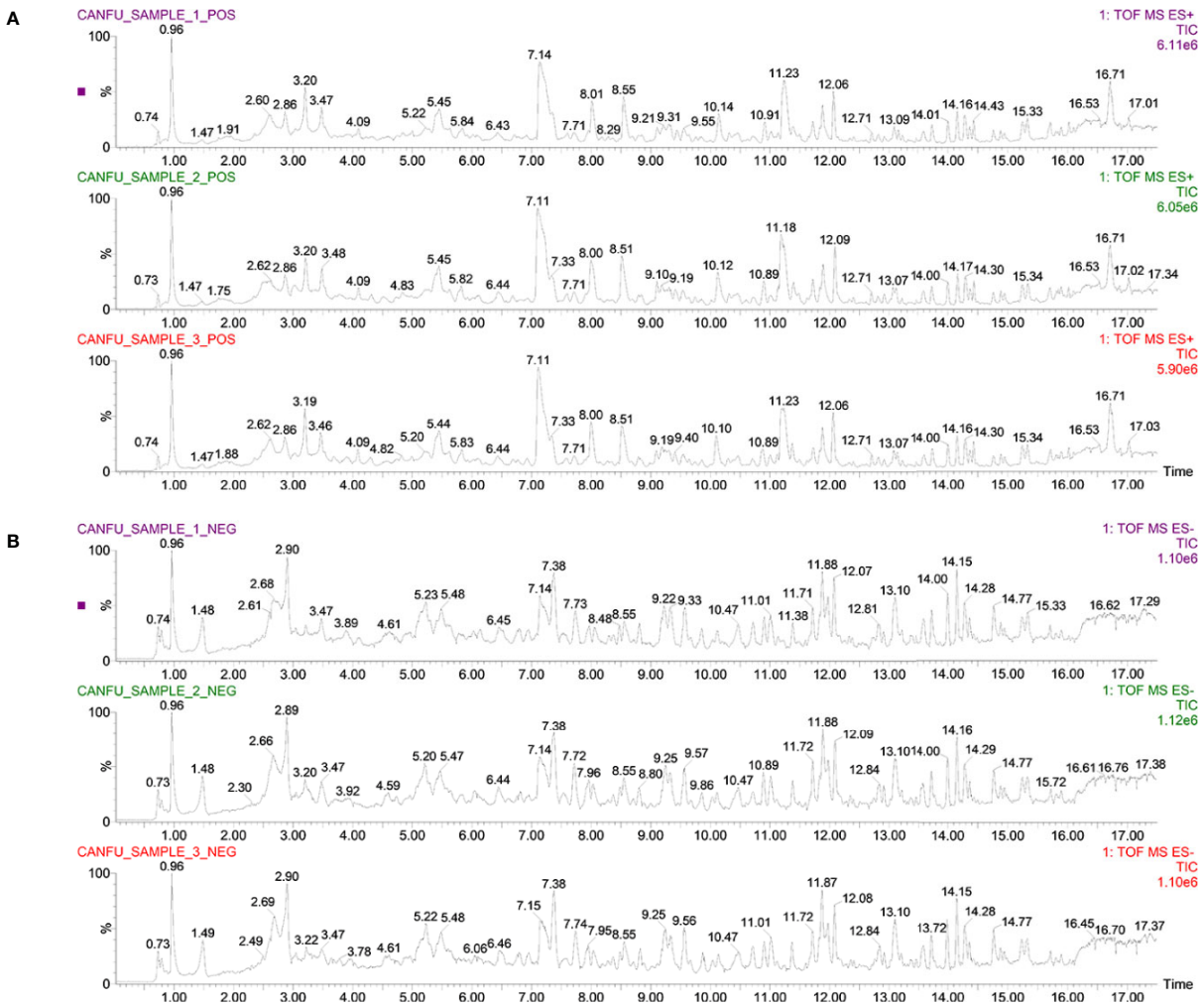


FIGURE 3 | Identification of major components of SFH formula. Three parallel samples of SFH formula were detected by employing the UPLC–MS/MS system. Data were collected and proceeded by software Masslynx 4.1. The positive **(A)** and negative **(B)** ion chromatograms of SFH formula were shown as indicated.

properties (Xie et al., 2019). Therefore, by retrieving in DrugBank and NCBI database, we listed these compounds as typical pharmacodynamic candidates of SFH formula.

In-depth analysis revealed many compounds with well-established pharmacological effects. For example, emodin from *Rheum palmatum L.* has been proven to possess hepatoprotective, anti-inflammatory, antioxidant, anticoagulant, and anti-microbial activities (Nemmar et al., 2015; Dong et al., 2016). Ginsenoside rh2 from *Panax ginseng C.A.Mey* exhibits antimicrobial resistance and cure for pneumonia (Hsieh et al., 2018; Liu et al., 2018). Benzoylmesaconine from *Aconitum carmichaeli Debeaux* has antiviral and anti-nociceptive activities (Suzuki et al., 1994; Kobayashi et al., 2003). To further investigate the role of these 49 candidates, we predicted their potential targets using the WES model. A total of 64 targets were identified, including GSK3β, ESR1, PPARG, PTGS2, AKR1B10, and MAPK14. Evaluation of the roles of these targets suggested that they may be involved in the therapeutic effect of SFH on sepsis. PPARG has been proven to regulate macrophage activity in inflammatory response (Majithia et al., 2016). GSK3β is active in many important signaling pathways, including cell proliferation, migration, inflammation, immune response, and apoptosis (Patel and Woodgett, 2017). The MAPK superfamily members, such as MAPK14, are key regulators of macrophage inflammation, autophagy and cell proliferation (He et al., 2018; Wu et al., 2019). The target-disease association network further illustrated that the targets of SFH may be involved in inflammatory disease, viral disease, bacterial infection/mycosis, and metabolic disease. These pathological processes have been reported to have a strong relationship with sepsis (Van der Poll et al., 2017; Cecconi et al., 2018; Lin et al., 2018; Van Wyngene et al., 2018). To systematically understand how SFH formula exhibits curative effect on sepsis, we summarized an integrated "sepsis-related pathway" approach. The pathways regulated by SFH, including calcium signaling pathway, MAPK signaling

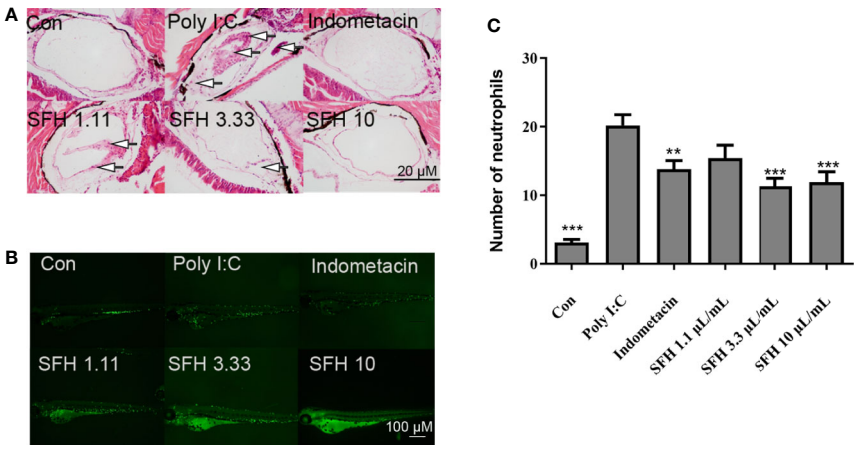


FIGURE 4 | Effect of SFH on Poly (I:C)-induced pneumonia. **(A)** HE staining to observe the pathological features. Arrows mark the inflammatory infiltration and cell shedding. **(B)** Fluorescence detection on neutrophils. **(C)** The numbers of neutrophils in air bladder tissue are counted. For all experiments, at least 30 larvae were used for each condition. ** indicated significant difference at $p < 0.01$, *** indicated significant difference at $p < 0.001$, compared with Poly (I:C) group.

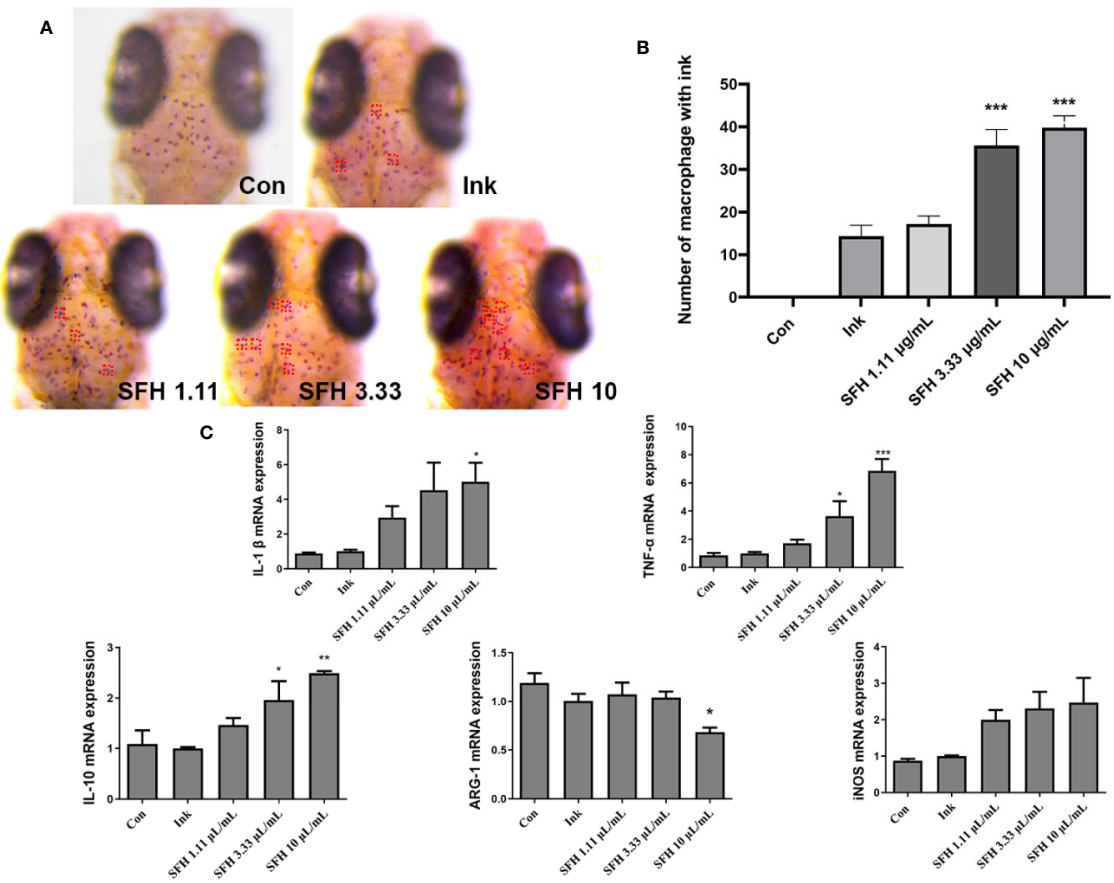


FIGURE 5 | Effect of SFH on macrophage activation. **(A)** Observation of macrophages with or without ink under dissecting microscope. **(B)** The numbers of macrophages with ink are counted. For all experiments, at least 30 larvae were used for each condition. *** indicated significant difference at $p < 0.001$, compared with ink group. **(C)** RT-PCR detection on the mRNA expression of M1/M2 markers in macrophage. For all experiments, at least 30 larvae were used for each condition. * indicated significant difference at $p < 0.05$, ** indicated significant difference at $p < 0.01$, *** indicated significant difference at $p < 0.001$, compared with ink group.

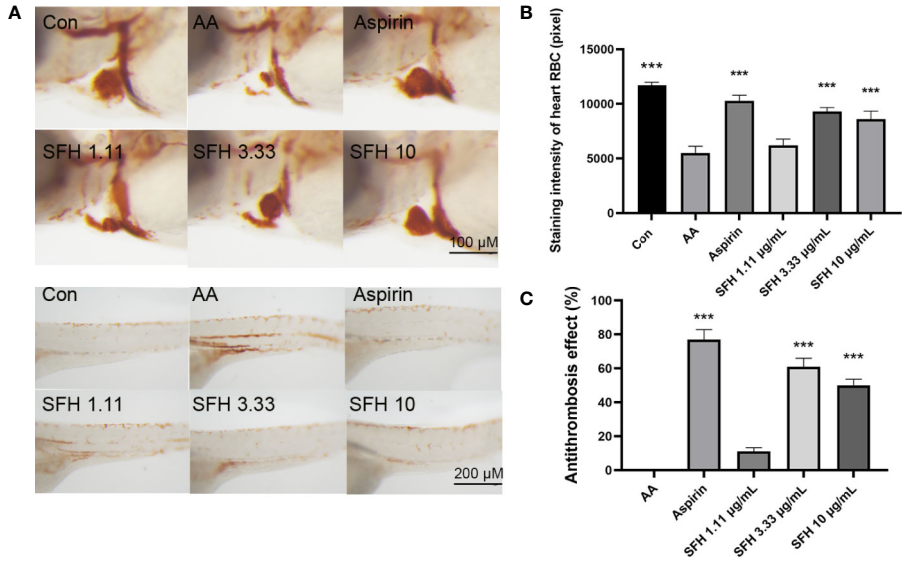


FIGURE 6 | Effect of SFH on coagulation function. **(A)** Observation of RBC in the heart and trunk of zebrafish under dissecting microscope. **(B)** Detection of staining intensity of RBC in the heart of zebrafish using dissecting microscope. For all experiments, at least 30 larvae were used for each condition. *** indicated significant difference at $p < 0.001$, compared with AA group. **(C)** Evaluation of the antithrombosis effect of SFH. For all experiments, at least 30 larvae were used for each condition. *** indicated significant difference at $p < 0.001$, compared with AA group.

pathway, T cell receptor signaling pathway, and PI3K-AKT signaling pathway, contribute to the development of sepsis.

Current studies reported that severe COVID-19 patients with septic syndrome mainly showed abnormal pathological features, including virus infection and tissue damage, excessive inflammation in early stage but immune suppression in late stage, and coagulation dysfunction (Li et al., 2020). Since the data of systems pharmacology illustrated that SFH may regulate several key targets and biological processes of sepsis, such as PPARG in inflammatory response, GSK3β and MAPK14 in cell proliferation, and PTGS2 in coagulation, we hypothesized that SFH improves the condition of critically ill COVID-19 patients with septic syndrome by ameliorating lung injury, suppressing excessive inflammation but enhancing the capacity of pathogen phagocytosis and killing, and improving the function of blood coagulation. Therefore, we employed various zebrafish models to test our hypothesis. We first simulated SARS-CoV-2 pulmonary infection using polyinosinic-polycytidylic acid (poly I:C), a synthetic double-stranded RNA immune-stimulant for study of SARS-CoV-2 infection (Kumaki et al., 2017; Gao et al., 2020). It is documented that hyper-inflammation due to neutrophils occurs in viral infections of the upper respiratory tract (Drescher and Bai, 2013). Recent studies also reported that COVID-19 lung injury in some patients might involve dysregulated neutrophil activity (Didangelos, 2020). Thus, inhibition of excessive neutrophil activation may help to control the lung damage. Our data showed that Poly I:C stimulation significantly increased the number of neutrophils in the air bladder of zebrafish. However, SFH treatment ameliorated the hyper-inflammation in air bladder tissue by suppressing the neutrophil infiltration. Macrophages, as innate immune cells, are the principal players in viral infections (Jakubzick et al., 2017). Several observational studies have characterized over-activation of monocytes in the early stage of SARS-CoV-2 infection (Pence, 2020). However, studies involving severe and critically ill COVID-19 patients demonstrated a substantial decrease in circulating monocytes and a sudden decrease in their expression of antigen (HLA)-DR (Giamarellos-Bourboulis et al., 2020; Sanchez-Cerrillo et al., 2020). These data further support the macrophage dysfunction and adaptive immune system impairments, which may lead to worsened medical condition or death. According to our data, SFH treatment promoted M1 macrophage activation by enhancing the expression of TNF-α, iNOS, IL-1β, and IL-10, while suppressing ARG-1 expression. This finding offers additional methods for treating viral infection patients with immune dysfunction. Coagulation dysfunction is another important pathogenesis of COVID-19 patients with septic syndrome. Coagulation function in SARS-CoV-2 infected patients is notably impaired compared with normal patients (Guevara-Noriega et al., 2020). Disseminated intravascular coagulation (DIC) with excessive thrombin generation poses a challenge to clinical therapeutics of COVID-19. We used a zebrafish thrombus model to evaluate the anti-thrombosis property of SFH. Results showed that SFH treatment significantly suppressed AA-induced generation of thrombus. Thus, SFH treatment may help to improve the coagulopathy of COVID-19 patients.

COVID-19 is an emerging and rapidly evolving pandemic. Traditional Chinese herbs have played important roles in treating

SARS-CoV-2 infection in China. SFH treatment showed promising therapeutic effects in critically ill COVID-19 patients. This study delineated the molecular mechanism of SFH using systems pharmacology tools and *in-vivo* zebrafish models. The results provide additional evidence of TCMs as complementary and alternative therapies for treating COVID-19.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The animal study was reviewed and approved by Committee for the Care and Use of Experimental Animals at Beijing Institute of Traditional Chinese Medicine.

AUTHOR CONTRIBUTIONS

TL: substantial contributions to the conception and design of the work, drafting the work, and revising it critically for important intellectual content. YG: substantial contributions to the design of the work, interpretation of data for the work. SH, JZ, YB, and NW: acquisition, analysis, and interpretation of data for the work. YL: drafting the work and revising it critically for important intellectual content. QL: language polishing and adjustment of structure of manuscript. XX: substantial contributions to the design of the work, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.584057/full#supplementary-material

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In Silico Prediction of Molecular Targets of Astragaloside IV for Alleviation of COVID-19 Hyperinflammation by Systems Network Pharmacology and Bioinformatic Gene Expression Analysis

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Introduction: The overproduction of cytokines and chemokines caused by excessive and uncontrolled inflammation contributes to the development of COVID-19. Astragaloside IV is considered as an anti-inflammatory and antioxidant agent. This study aimed at undertaking a network pharmacology approach and bioinformatics analysis to uncover the pharmacological mechanisms of Astragaloside IV on COVID-19.

Methods: Potential targets of Astragaloside IV were screened from public databases. Differentially expressed genes (DEGs) in SARS-CoV-2 were screened using bioinformatics analysis on the Gene Expression Omnibus (GEO) datasets GSE147507. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were subsequently performed. The overlapping genes, GO terms and KEGG pathways between Astragaloside IV targets and SARS-CoV-2 DEGs were confirmed, and the location of overlapping targets in the key pathways was queried using KEGG Mapper.

Results: A total of 425 potential targets of Astragaloside IV were screened. Besides, a total of 546 DEGs were identified between SARS-CoV-2 infected samples and control samples, including 380 up-regulated and 166 down-regulated genes. There was a significant overlap in GO terms and KEGG pathways between Astragaloside IV targets and SARS-CoV-2 DEGs. The shared genes included MMP13, NLRP3, TRIM21, GBP1, ADORA2A, PTAFR, TNF, MLNR, IL1B, NFKBIA, ADRB2, and IL6.

Conclusions: This study is the first to propose Astragaloside IV as a new drug candidate for alleviating hyper-inflammation in COVID-19 patients. Besides, the key targets and pathways may reveal the main pharmacological mechanism of Astragaloside IV in the treatment of COVID-19.

Keywords: COVID-19, Astragaloside IV, hyperinflammation, network pharmacological, cytokine storms

INTRODUCTION

COVID-19 has reached pandemic proportions around the world. Severe acute respiratory distress syndrome (ARDS) represents an important clinical feature of COVID-19, and a primary cause of death in COVID-19 patients (McGonagle et al., 2020; Ramanathan et al., 2020; Wu et al., 2020). In response to SARS-CoV-2 infection, immune cells and nonimmune cells release large amounts of proinflammatory cytokines, which lead to "cytokine storms". Clinical studies have revealed that COVID-19 patients admitted to intensive care have increased expression of inflammatory cytokines (IL-6, IL-10, IL-2, and IFN-γ). Other studies confirm that a large number of patients with severe COVID-19 are likely to suffer a cytokine storm syndrome (Huang et al., 2020; Shen et al., 2020; Ye et al., 2020). ARDS and cytokine storms occur very often in patients with COVID-19 since excess production of pro-inflammatory cytokines results in ARDS aggravation (Howell and Davis, 2018). Therefore, there a need to search for therapies to reduce hyper-inflammation and improve prognosis in severe COVID-19 patients.

Huangqi (Radix Astragali Mongolici) is a Well-Known Chinese Tonic. According to the Chinese Pharmacopeia, it is the dried root of the leguminous plants Astragalus mongholicus. It has been widely used in ischemic cardio-cerebrovascular disease, viral hepatitis, kidney disease, and skin diseases for the nourishment of Qi and blood (Li et al., 2017; Gong et al., 2018).

More than 40 active constituents of astragalus root have been identified, with Astragaloside IV (PubChem, CID13943297) being the major active compound.

The pharmacopeia of the People's Republic of China has regarded Astragaloside IV as the quality standard for Astragalus membranaceus injection (Cao et al., 2019; Wang C. J. et al., 2020). Astragaloside IV has been widely used due to its anti-inflammatory effects associated with various molecular mechanisms (Ren et al., 2013; Li et al., 2017). Astragaloside IV has been reported to decrease *TNF-α, IL-1β* release, and the expression of other inflammatory cytokines by inhibiting the phosphorylation of *IκB* and decrease nuclear translocation of *NF-κB* (Huang et al., 2017). Astragaloside IV also suppresses neutrophils adhesion-related molecules (Shen et al., 1998; Jin et al., 2010).

Currently, no studies have reported the use of Astragaloside IV in the treatment of COVID-19. In this study, the network pharmacology approach and bioinformatics analysis were used to investigate the possible mechanism of action underlying the effectiveness of Astragaloside IV in the treatment of COVID-19. The analysis workflow of network pharmacology is shown in **Figure 1**.

MATERIALS AND METHODS

Potential Astragaloside IV-Related Targets

The 2D molecular structure and PubChem CID of Astragaloside IV were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/), which is the world's largest database of free access to chemical information (Wang et al., 2017). To predict the potential targets for Astragaloside IV with the AUC >0.7, SwissTargetPrediction(http://www.swisstargetprediction.ch/), (Daina et al., 2019), Comparative Toxicogenomics Database (CTD, http://ctdbase.org/about/), (Davis et al., 2019) and TargetNet (http://targetnet.scbdd.com) (Yao et al., 2016) were used. Generate Conformers was confirmed, maximum generated conformations are 300, select targets set of Pharmacophore Mapping was choosed as Druggable Pharmacophore Models, then Astragaloside IV related targets were predicted using PharmMapper (http://www.lilab-ecust.cn/pharmmapper/) (Wang et al., 2017). However, due to the nonstandard naming, the names of the targets were converted to official symbols using the UniProt Knowledgebase (UniProtKB, http://www.uniprot.org/) and species was restricted to "Homo sapiens".

SARS-CoV-2 Related Genes

GSE147507 was downloaded from the GEO (http://www.ncbi.nlm.nih.gov/geo/)(Barrett et al., 2013) database. In the publisher's study, the transcriptional responses of hosts to SARS-CoV-2 and other respiratory infections were systematically described. These data suggest that the unique transcriptional characteristics may be related to the pathogenesis of COVID-19 (Blanco-Melo et al., 2020). The transcriptional results of A549 cells (Series7) were selected for analysis. Impute and limma packages in R provided by the Bioconductor project (http://www.bioconduct-or.org/packages/release/bioc/html/affy.html) (Chen et al., 2007) were used to assess the transcriptional results. Quantile normalization and log2-transformation were performed before analyzing the matrix data. Original p-values were adjusted using the Benjamini-Hochberg method, and fold-changes (FC) were calculated using the false discovery rate (FDR). The $|\log2\ FC|>2$ and adj.P.Val <0.05 were used to filter the differentially expressed genes (DEGs).

KEGG Pathway and GO Enrichment Analysis

A list of SARS-CoV-2 DEGs and Astragaloside IV related targets were submitted to Metascape (http://metascape.org) (Zhou et al., 2019), with the species limited to "Homo sapiens". Functional enrichment analysis was performed based on the three categories of GO terms; Biological Processes, Cellular Components, and Molecular Functions. All genes present in the GSE147507 dataset were used as the enrichment background. Terms with a p-value < 0.01, a minimum count of 3, and an enrichment factor >1.5 were collected and grouped into clusters based on their membership similarities. The intersection of KEGG Pathways and GO terms were identified between Astragaloside IV-related targets and SARS-CoV-2 DEGs.

PPI Network Construction and Key Pathways

SARS-CoV-2 DEGs utilized as hub proteins and submitted to String (https://string-db.org/) (Szklarczyk et al., 2019), with species limited to "Homo sapiens". A confidence score>0.9 was used to obtain the PPI networks, which were visualized using Cytoscape 3.7.2 (http://www.cytoscape.org/)

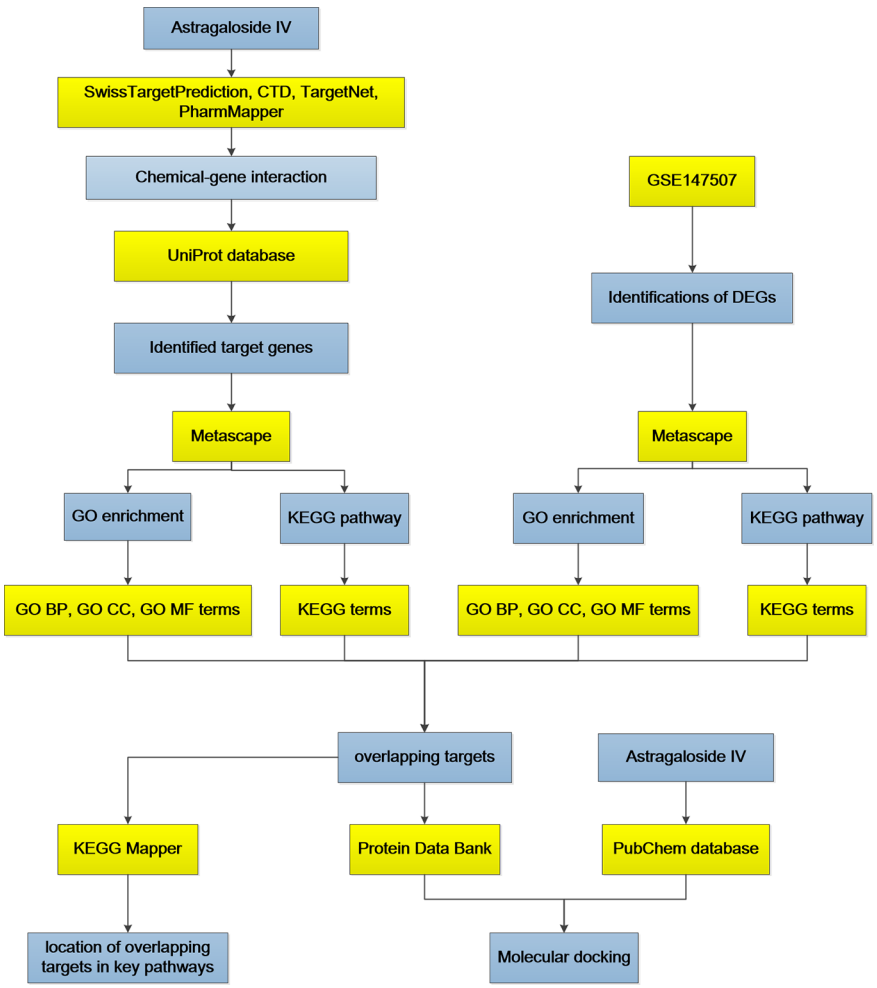

FIGURE 1 | The schematic diagram based on pharmacology analysis.

(Otasek et al., 2019). Overlapping targets were identified by considering the intersection of Astragaloside IV-related targets and SARS-CoV-2 DEGs. The location of overlapping targets in critical pathways was queried using KEGG Mapper.

In Silico Molecular Docking Study of Astragaloside IV Key Targets

Autodock Vina was used to carrying out molecular docking of Astragaloside IV key targets (Trott and Olson, 2010). The PDB format molecular structure of Astragaloside IV was obtained from the PubChem database (https://www.ncbi.nlm.nih.gov/). The structures of the key targets were downloaded from the Protein Data Bank (http://www.rcsb.org/). The water molecules were deleted from the protein crystal structure, hydrogen atoms added, and charge calculated. The molecular docking mode of Astragaloside IV to the protein targets was selected as Local Search Parameters. The docking score was used to evaluate the theoretical binding affinities of Astragaloside IV to the key targets. In order to compare the binding affinities of standard pharmacological immunosuppressive drugs with Astragaloside IV, the same methods were used to evaluate binding affinities of Rapamycin to the key targets.

RESULTS

Potential Targets of Astragaloside IV

The molecular structure of Astragaloside IV was download from PubChem (**Figure 2**), and PubChem CID of Astragaloside IV is 13943297. A total of five corresponding potential targets of Astragaloside IV were identified from TargetNet.19, while the corresponding potential targets of Astragaloside IV were identified from CTD. Besides, 154 corresponding potential targets of Astragaloside IV were identified using PharmMapper and 104 corresponding potential targets of Astragaloside IV were identified from SwissTargetPrediction. After data de-duplication,

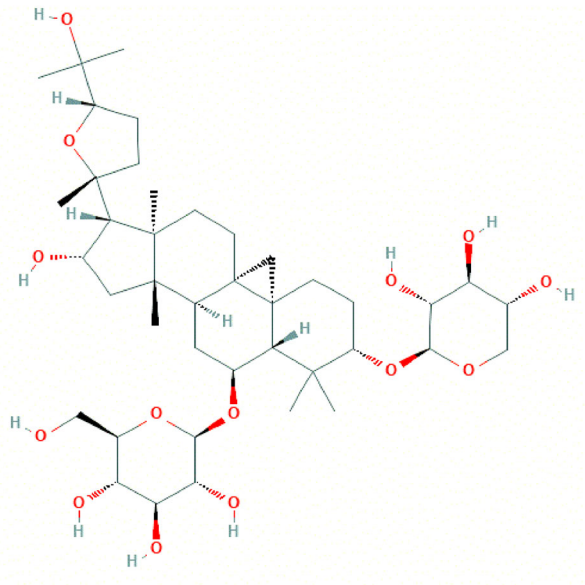

FIGURE 2 | 2D molecular structure of Astragaloside IV.

282 potential targets were retained (**Figure 3**, **Supplemental file Table S1**).

Identification of SARS-CoV-2 DEGs

A total of 23,710 genes and 546 DEGs were identified from SARS-CoV-2 infected samples compared with the control samples, including 380 up-regulated and 166 down-regulated genes. The identified DEGs between the control and SARS-CoV-2 groups were presented in volcano plots. **Figure 4** shows the heatmaps showing genes expression of the DEGs (**Supplemental file Table S2**).

Gene Ontology and KEGG Enrichment Analysis of Astragaloside IV-Related Targets and SARS-CoV-2 DEGs

Based on GO enrichment, the SARS-CoV-2 DEGs were enriched in 150 terms, 127 in the category Biological Processes, and 23 in Molecular Functions. GO enrichment revealed that the DEGs were mainly involved in various biological processes (BP), including type I interferon signaling pathway, cellular response to type I interferon, response to type I interferon, defense response to a virus, mRNA binding involved in posttranscriptional gene silencing, response to a virus, defense response to other organism and negative regulation of viral genome replication. KEGG analysis revealed that the DEGs were enriched in nine pathways including, Influenza A, Measles, NOD-like receptor signaling pathway, Hepatitis C, Herpes simplex infection, Cytokine-cytokine receptor interaction, Chemokine signaling pathway. Top 20 terms in which the DEGs were enriched and ordered by *p*-value are listed in **Figure 5A**.

The Astragaloside IV related targets were enriched in 385 terms, including 341 in Biological Processes, 13 in Molecular Functions, and 31 in Cellular Components. The targets were involved in biological processes (BP) including, blood circulation, circulatory system process, G protein-coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger, response to toxic substances, the vascular process in the circulatory system, regulation of system process, positive regulation of cell death, and regulation of tube diameter. KEGG analysis revealed that the targets were enriched in 74 pathways, all targets were primarily enriched in neuroactive ligand-receptor interaction, calcium signaling pathway, cAMP signaling pathway, pathways in cancer, and neurotrophin signaling pathway. The top 20 terms in which the targets were enriched and ordered by *p*-value are listed in **Figure 5B**.

The results revealed that there was a significant overlap in GO terms between Astragaloside IV targets and SARS-CoV-2 DEGs. The intersection of Go terms between Astragaloside IV related targets and SARS-CoV-2 DEGs included 34 terms (**Figure 7**). The top 10 overlapping GO terms ordered by *p*-value are listed in **Table 1**.

PPI Network Analysis

Overlapping targets of Astragaloside IV and SARS-CoV-2 DEGs included MMP13, NLRP3, TRIM21, GBP1, ADORA2A, PTAFR, TNF, MLNR, IL1B, NFKBIA, ADRB, and IL6. PPI network can visualize and quantify the function of specific proteins in cells at the systematic level (Jordan et al., 2012). Therefore, a PPI network of overlapping targets and SARS-CoV-2 DEGs was constructed and the common targets marked (**Figure 6**). The location of overlapping genes and SARS-CoV-2 DEGs in the key pathways are listed in **Figure 7**.

Molecular Docking Analysis

Molecular docking analysis was conducted to evaluate the binding affinity of Astragaloside IV with key target receptors. The results showed that docking scores of Astragaloside IV with MMP13, NLRP3, GBP1, ADORA2A, PTAFR, TNF, MLNR, IL1B, NFKBIA, ADRB2, and IL6 ranged from −6.72 to −9.05. Particularly, Astragaloside IV presented the highest docking score with MMP13 and IL6 (docking score: −9.05, −9.04), demonstrating that Astragaloside IV is perfectly located inside the binding site together with MMP13 and IL6. Other key targets also showed an affinity with Astragaloside IV. Compared to Rapamycin, Astragaloside IV showed weaker binding affinities to these targets (**Figure 8**; **Table 2**).

DISCUSSION

Astragaloside IV has been widely used in clinical practice (Qi et al., 2006; Xie et al., 2012; Yang et al., 2012). Pharmacokinetic properties of Astragaloside IV have been based on linear pharmacokinetics on doses ranging from 0.3mg/kg to 0.75mg/kg of Astragaloside injection. Astragaloside IV is safe and well-tolerated after intravenous infusion in clinical practice (Xu et al., 2013).

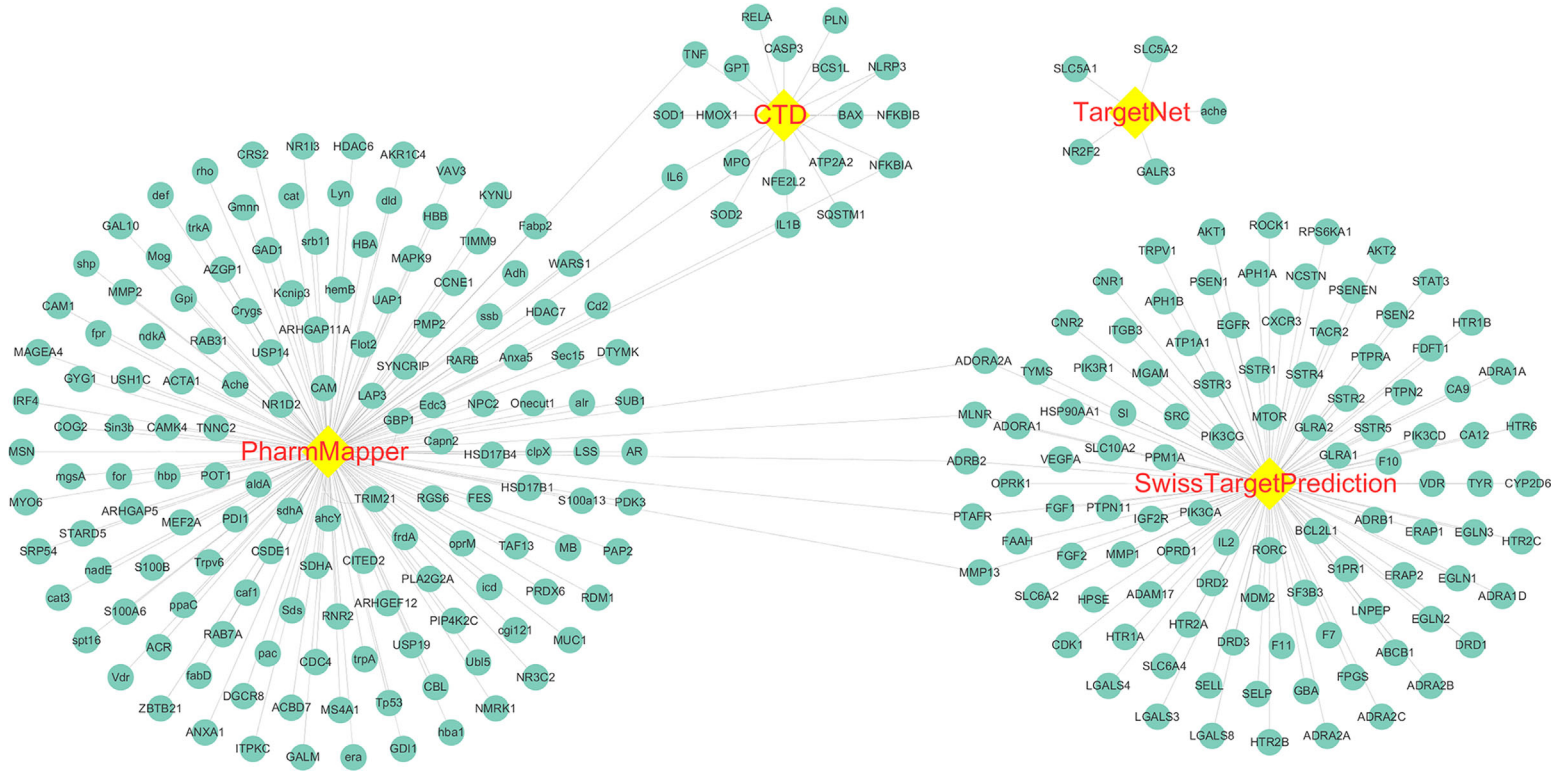

FIGURE 3 | Astragaloside IV related targets. The targets were identified from PharmMapper, Comparative Toxicogenomics Database (CTD), TargetNet, and SwissTargetPrediction.

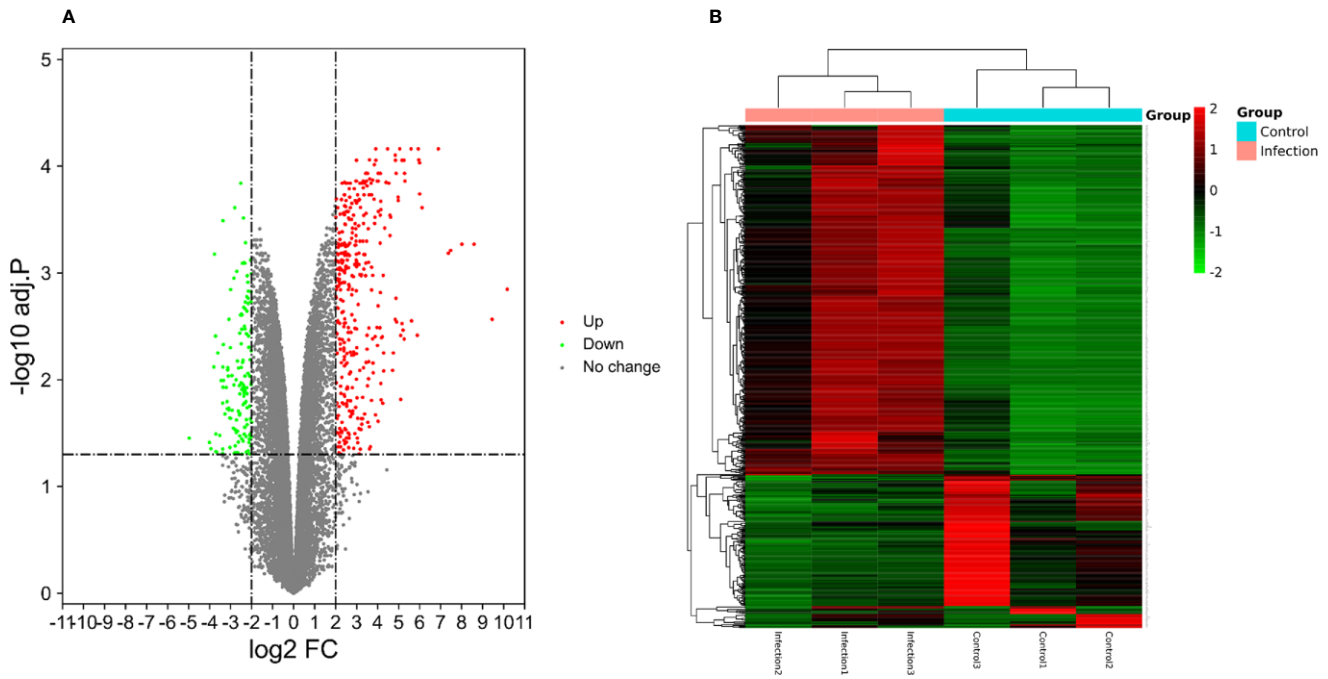

FIGURE 4 | **(A)** The volcano plots displaying significantly expressed (|log2 FC|>2, adj.P.Val <0.05) mRNAs between the control and SARS-CoV-2 groups. Red dots represent up-regulated genes, green dots represent down-regulated genes. **(B)** Heatmaps depicting the expression levels of SARS-CoV-2 differentially expressed genes (DEGs). The upregulated genes are marked in red, the downregulated genes are marked in green.

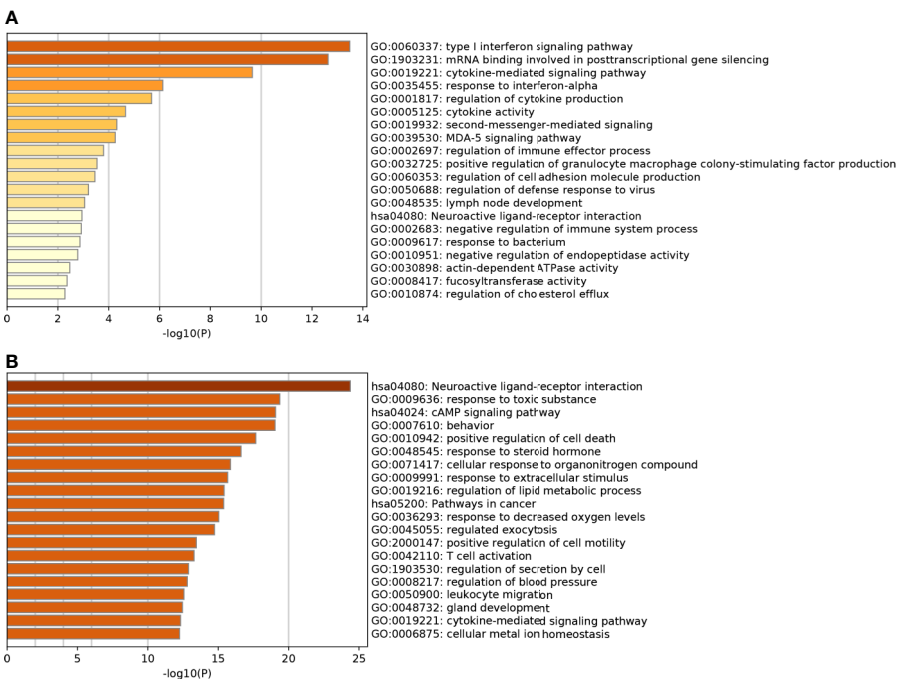

FIGURE 5 | **(A)** Top 20 most significant gene ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis for SARS-CoV-2 DEGs. **(B)** Top 20 most significantly GO terms and KEGG enrichment analysis for Astragaloside IV related targets.

Studies have demonstrated that Astragaloside IV exerts anti-inflammatory effects *via* regulation of the NF-κB and JNK signaling pathway and inhibiting the release of inflammatory cytokines. Astragaloside IV inhibits the activation of NF-κB, by decreasing the phosphorylation of IκB and the nuclear translocation of NF-κB, thus downregulating the expression of TNF-α and IL-1β (Leng et al., 2018; Song et al., 2018; Wang et al., 2018; Leng et al., 2019). Astragaloside IV also inhibits adhesion-related molecules which are important in exerting protective anti-inflammatory effects. CD11b/CD18 is the key integrin on the surface of neutrophils. Astragaloside IV decreases the proportion of CD11b/CD18-positive neutrophils and reduces the expression of intercellular adhesion molecule-1 (ICAM-1), which is achieved by inhibiting the level of NF-κB and attenuating the expression of TNF-α and IL-1β (Li et al., 2012; Li et al., 2013).

Astragaloside IV has been widely in treating various inflammatory diseases caused by viruses. Astragaloside IV inhibits the replication of human adenovirus type 3 and apoptosis of A549 cells *in vitro*. The anti-virus properties are correlated with the concentration of astragaloside IV (Shang et al., 2011). Astragaloside IV exerts potential cardioprotective properties in viral myocarditis (Chen et al., 2011; Liu et al., 2019; Zhuang et al., 2019). Coxsackievirus B3 (CVB3) is an important pathogen for viral myocarditis. Astragaloside IV inhibits the proliferation of CVB3 by enhancing the expression of IFN-gamma (Zhang et al., 2006). In H1N1 Infection, Astragaloside IV inhibits IL-1β secretion by up-regulating Autophagy (Zhang J. et al., 2020).

Significantly up-regulated levels of cytokines and chemokines including IL1-β, IL1RA, IL10, IL9, IL8, IL7, basic FGF2, GCSF, GMCSF, IFNγ, IP10, MCP1, MIP1α, MIP1β, PDGFB, TNFα, and VEGFA in blood, have been confirmed in COVID-19 patients. The hyper-inflammation triggers the immune system to attack the body, and cause ARDS and multiple organ failure, and finally lead to death in severe cases of COVID-19 (Huang et al., 2020; Li et al., 2020; Rothan and Byrareddy, 2020). Therefore, there is an urgent need to find specific drugs or therapy for treating hyperinflammation in COVID-19.

In this study, a total of 425 potential Astragaloside IV related targets were screened from an online database. GO terms and KEGG pathways in which these targets enrich are consistent with

TABLE 1 | Top 10 overlapping gene ontology (GO) terms between Astragaloside IV targets and SARS-CoV-2 differentially expressed genes (DEGs).

Category term	Description	Count	P value
GO:0002526	cytokine-mediated signaling pathway	53	2.22498E-10
GO:0032755	regulation of cytokine production	41	2.05545E-06
GO:0010874	second-messenger-mediated signaling	26	4.79803E-05
GO:0002438	positive regulation of cytokine production	27	8.37368E-05
GO:0002526	cytokine secretion	17	0.000161558
GO:0032755	regulation of cytokine secretion	15	0.000335339
GO:0002438	negative regulation of protein secretion	11	0.000653223
GO:0002438	negative regulation of peptide secretion	11	0.00095377
GO:0001730	cellular calcium ion homeostasis	24	0.001033831
GO:0002438	negative regulation of immune system process	24	0.001200594

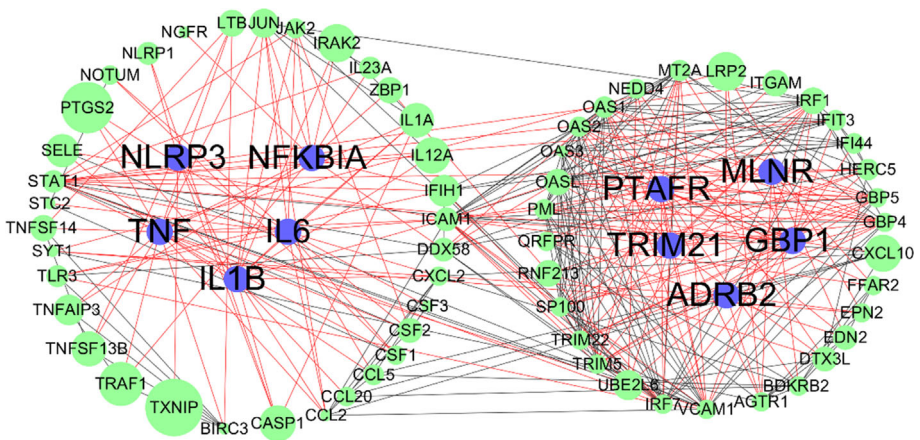

FIGURE 6 | Protein-protein interaction (PPI) networks of SARS-CoV-2 differentially expressed genes (DEGs); blue nodes represent overlapping targets of Astragaloside IV and SARS-CoV-2; green nodes represent SARS-CoV-2 DEGs; edges represent correlations between targets, red edges represent overlapping targets related correlations, black edges represent SARS-CoV-2 DEGs related correlations; the size of the nodes indicates the value of combined score in STRING.

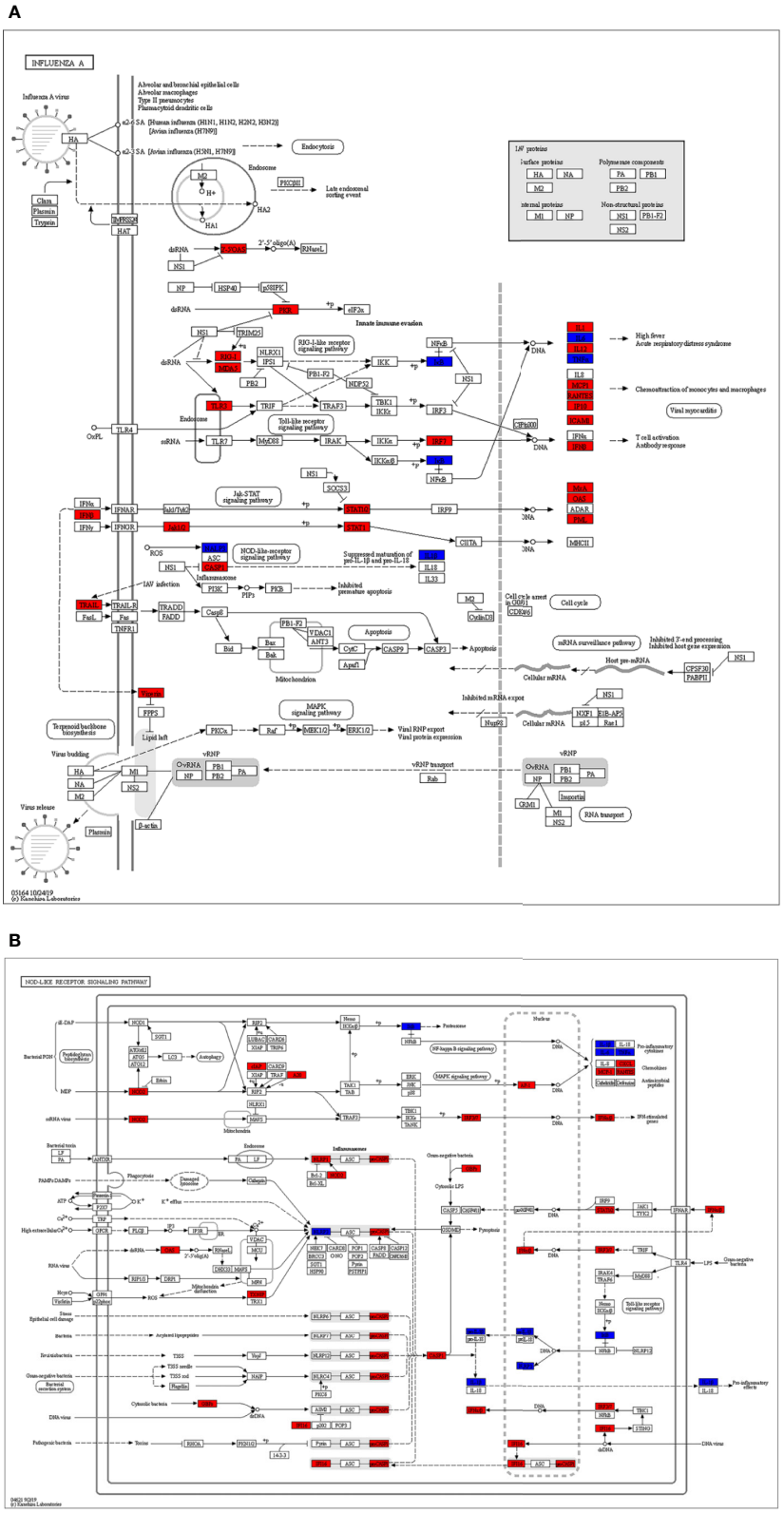

FIGURE 7 | The identified key pathways. **(A)** Influenza A pathway. **(B)** NOD-like receptor signaling pathway. The red marked nodes represent up-regulated SARS-CoV-2 differentially expressed genes (DEGs), while the blue marked nodes represent overlapping targets of Astragaloside IV and SARS-CoV-2.

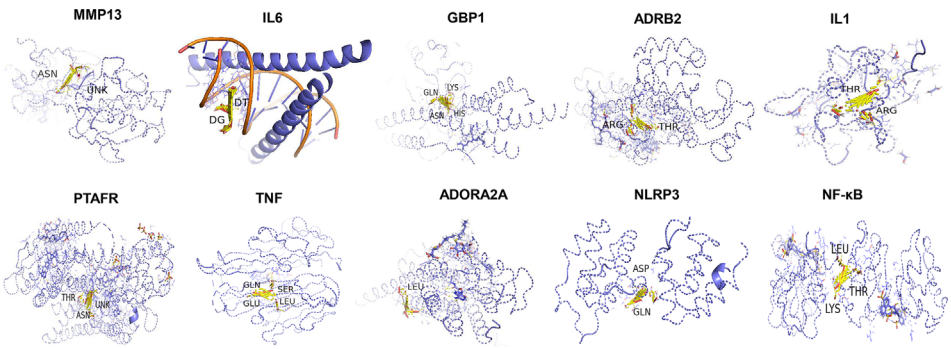

FIGURE 8 | Structural interactions of Astragaloside IV and key target receptors.

TABLE 2 | The docking scores of Astragaloside IV and Rapamycin with key proteins.

Target	PDB ID	Binding energy (kcal/mol)	
		Astragaloside IV	Rapamycin
MMP13	3I7I	−9.05	−16.27
IL6	4CNI	−9.04	−20.19
GBP1	6K1Z	−8.91	−13.8
ADRB2	3D4S	−8.18	−14.08
IL1	5BOW	−8.16	−17.84
PTAFR	5ZKQ	−7.84	−16.66
TNF	2ZJC	−7.78	−14.24
ADORA2A	2YDV	−7.13	−12.99
NLRP3	3QF2	−6.97	−12.11
NF-κB	6GJW	−6.72	−12.63

the current understanding of Astragaloside IV(Ren et al., 2013; Li et al., 2017). Some of the upregulated SARS-CoV-2 DEGs included TNF, IL6, IL1A, IL1B, NLRP3, IL17C, IL12A. These results are consistent with current COVID-19 research, that there are elevated levels of cytokines in plasma of patients with COVID-19 (Huang et al., 2020).

The GO analysis results of SARS-CoV-2 DEGs revealed that type I interferon signaling pathway, cellular response to type I interferon, response to type I interferon, defense response to the virus, mRNA binding involved in posttranscriptional gene silencing were the most significant terms. Influenza A, Measles, NOD-like receptor signaling pathway, Hepatitis C, Herpes simplex infection, Cytokine-cytokine receptor interaction were the most enriched pathways in KEGG enrichment analysis. Hyper-activation of these pathways is involved in the SARS-CoV-2 infection, leading to the induction of a variety of pro-inflammatory cytokines, including IL-6, TNFα, and chemokines (Miossec and Kolls, 2012; Murakami et al., 2019; Xu et al., 2020). Inhibiting these signaling pathways to reduce the release of inflammatory factors may be key in the treatment of hyperinflammation in COVID-19. *NF-κB, IL-6, TNF* are considered to be therapeutic targets for COVID-19 (Mehta et al., 2020; Wang L. et al., 2020).

The overlapping targets of Astragaloside IV-related targets and SARS-CoV-2 DEGs can be considered as potential drug targets in the treatment of COVID-19. Astragaloside IV attenuates upstream nuclear translocation and phosphorylation of NF-κB-p65, which is a key component of the NF-κB pathway, and the activation of NF-κB leads to up-regulated expression of TNF-α and IL-1β (Liu et al., 2014).

Immunosuppressive drugs are likely to be beneficial in the treatment of COVID-19 infections. A study on IL-1 blockade (anakinra) in sepsis, showed a significant survival benefit in patients with hyperinflammation, without any apparent increased adverse events (Shakoory et al., 2016). Administration of IL-6 receptor blockade, licensed for cytokine release syndrome, has been approved in patients with COVID-19 pneumonia and elevated IL-6 in China (Liu et al., 2020; Zhang C. et al., 2020). Therefore, this study shows that the anti-inflammatory effects of astragaloside IV are sufficient to ameliorate a cytokine storm in the lungs, caused by COVID-19. However, these results need further experimental validation.

According to the molecular docking analysis results in our study, Astragaloside IV showed milder anti-inflammatory effects compared to standard pharmacological immunosuppressive drugs such as Rapamycin. Probably, it is less likely to affect the immune function and reduce the ability of the host to eliminate virus or bacteria. A large number of studies have confirmed that the pharmacological effects of Astragaloside IV are multi-level and multi-target (Ren et al., 2013; Li et al., 2017; Liu et al., 2019), which is beneficial to be a potential clinical therapeutic drug for COVID-19 considering the complex pathogenesis and complications.

In this study, the pharmacological mechanism of Astragaloside IV on COVID-19 is investigated using network pharmacology and bioinformatics analysis. Astragaloside IV is shown to be a potential drug for alleviating hyperinflammation in COVID-19 by inhibiting the Influenza A pathway, NOD-like receptor signaling pathway. Although direct evidence of Astragaloside IV application in COVID-19 is unclear, the anti-inflammatory effects of Astragaloside IV have been proven in numerous studies. Therefore, we believe that Astragaloside IV is likely to be beneficial in treating severe COVID-19 patients.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

CG and YH contributed equally to this study. CG and YH participated in the design of this study and performed the statistical analysis. CG drafted the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.556984/full#supplementary-material

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REVIEW
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COVID-19: Is There Evidence for the Use of Herbal Medicines as Adjuvant Symptomatic Therapy?

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Background: Current recommendations for the self-management of SARS-Cov-2 disease (COVID-19) include self-isolation, rest, hydration, and the use of NSAID in case of high fever only. It is expected that many patients will add other symptomatic/adjuvant treatments, such as herbal medicines.

Aims: To provide a benefits/risks assessment of selected herbal medicines traditionally indicated for "respiratory diseases" within the current frame of the COVID-19 pandemic as an adjuvant treatment.

Method: The plant selection was primarily based on species listed by the WHO and EMA, but some other herbal remedies were considered due to their widespread use in respiratory conditions. Preclinical and clinical data on their efficacy and safety were collected from authoritative sources. The target population were adults with early and mild flu symptoms without underlying conditions. These were evaluated according to a modified PrOACT-URL method with paracetamol, ibuprofen, and codeine as reference drugs. The benefits/risks balance of the treatments was classified as *positive*, *promising*, *negative*, and *unknown*.

Results: A total of 39 herbal medicines were identified as very likely to appeal to the COVID-19 patient. According to our method, the benefits/risks assessment of the herbal medicines was found to be positive in 5 cases (*Althaea officinalis, Commiphora molmol, Glycyrrhiza glabra, Hedera helix*, and *Sambucus nigra*), promising in 12 cases (*Allium sativum*, *Andrographis paniculata*, *Echinacea angustifolia, Echinacea purpurea, Eucalyptus globulus* essential oil, *Justicia pectoralis, Magnolia officinalis*, *Mikania glomerata*, *Pelargonium sidoides*, *Pimpinella anisum*, *Salix* sp, *Zingiber officinale*), and unknown for the rest. On the same grounds, only ibuprofen resulted promising, but we could not find compelling evidence to endorse the use of paracetamol and/or codeine.

Conclusions: Our work suggests that several herbal medicines have safety margins superior to those of reference drugs and enough levels of evidence to start a clinical

discussion about their potential use as adjuvants in the treatment of early/mild common flu in otherwise healthy adults within the context of COVID-19. While these herbal medicines will not cure or prevent the flu, they may both improve general patient well-being and offer them an opportunity to personalize the therapeutic approaches.

Keywords: herbal medicine, coronavirus (2019-nCoV), COVID-19, benefit/risk assessment, respiratory diseases

INTRODUCTION

The outbreak of Coronavirus SARS-Cov-2 disease (COVID-19) in Wuhan (China) in late 2019 and its Worldwide spread has caused hundreds of thousands of deaths so far. As of July 2020, the disease seems to be mostly affecting Europe and the Americas. Most people infected with the COVID-19 virus will experience mild to moderate respiratory illness and recover without requiring special treatment. Older people and those with underlying medical problems such as cardiovascular disease, diabetes, chronic respiratory disease, and cancer are more likely to develop serious illness (WHO, 2020a). Teens and adults without underlying medical conditions are asked to self-manage their symptoms in isolation with a minimum of drugs (paracetamol, if fever is high) and lifestyle adjustments (increased rest and hydration). However, most of the current guidelines do not specifically advise on how to treat cough, one of the main symptoms, which, apart from being very debilitating, contributes to the spread of the virus.

There is not yet any evidence-based specific therapy for COVID-19, and the real efficacy and safety of current therapeutic approaches will need further scrutiny when enough multi-site clinical data become available. The examples of ibuprofen and hydroxychloroquine illustrate how clinical protocols may include and/or exclude drugs in their therapeutic approaches based on limited evidence (Kim et al., 2020; Sodhi and Etminan, 2020; Taccone et al., 2020; Torjesen, 2020). Predictably, patients will largely try to increase their well-being at least by self-administering cough suppressing medication (natural or not) plus natural medication or supplements to combat cold/flu symptoms. These are readily accessible both in retail commerce and community pharmacies. In Europe, there are several herbal medicines registered under the European Directive 24/2004 for self-prescription (EU, 2004). Their labeling establishes that these medicines are indicated for the treatment of common cold and flu symptoms based on traditional use only. We agree in that COVID-19 is not the common flu, but the WHO definition is clear in that it is a mild, self-limiting condition and, therefore, fitting the boundaries of self-prescription, moreover if the patients have not been tested for the virus (WHO, 2020a). In that sense, there is a need to clarify the real potential and safety profile of herbal medicines to scientifically substantiate future recommendations on their benefits and risks of use them. Therefore, the impetus of this work is twofold. First, it intends to highlight which species may provide a more rational phytotherapeutic choice to the disease and second to showcase which plants can be a clinically compatible option as adjuvant therapy for the self-management of common cold/flu symptoms by otherwise healthy adults within the context of the COVID-19 pandemic.

Clinical Background

Early COVID-19 symptoms include fever, dry cough, and dyspnea, among other similar ones to other viral respiratory diseases such as common flu (Rothan and Byrareddy, 2020). Therefore, the diagnosis of COVID-19 based on anamnesis remains problematic. In general, the incubation period is around 15 days, but the reported range is 0 to 24 days (Bai et al., 2020). SARS-Cov-2 presents a strong transmission capacity (Zheng et al., 2020). To complicate matters further, there is a significant number of asymptomatic patients (up to 60%) who unknowingly contribute to the spread of the disease (Gao et al., 2020; Kronbichler et al., 2020).

The clinical spectrum of the infection goes from mild upper respiratory tract illness to the so-called 'severe acute respiratory syndrome': respiratory failure, shock, and multiple organ failure (Bai et al., 2020; Zhou et al., 2020); and may be accompanied by fatigue, headache, diarrhea, and lymphopenia (Rothan and Byrareddy, 2020), and high incidence of cardiovascular symptoms (Zheng et al., 2020). Older people and those with underlying medical problems such as cardiovascular disease, diabetes, chronic respiratory disease, and cancer are more likely to develop severe illness (WHO, 2020a).

The precise pathology of the disease is not yet clear, but seems to include a systemic pro-inflammatory response, inducing hemodynamic changes and, consequently, a predisposition to ischemia and thrombosis (Tang et al., 2020; Zheng et al., 2020; Zhou et al., 2020). A hallmark of the disease is the "cytokine storm", a massive cytokine and chemokine release due to an uncontrolled dysregulation of the host immune defense that causes loss of function of multiple organs (Catanzaro et al., 2020).

Preventative and Therapeutic Approaches

Due to the emergence of the propagation of the disease, Health Systems have become overloaded, even having sufficient diagnostic capacity and hospital facilities to handle such an outbreak. In the most vulnerable regions, the COVID-19 epidemic effectively paralyzes health systems at the expense of primary health care (Velavan and Meyer, 2020). Some measures, such as lock-down of communities, social distancing, and quarantine-type for those suspected to be infected can, at least in part, slow the COVID-19 spread (Heymann and Shindo, 2020) and, so, enable the health systems to cope. However, these measures are palliative, and people tend to ignore them after a few days of isolation, mainly those in disadvantaged and vulnerable communities.

Importantly, at this stage, we are starting to build up an evidence-base for the best strategy to treat, mitigate, and prevent the diseases. Currently, none of the approaches used is evidence-based.

In the worldwide search for a response to the COVID-19 pandemic, news about "alternative remedies against COVID" have been disseminated (ANSES, 2020; Nordling, 2020). As of July 2020, the evidence-base for such treatments is often limited if not non-existent. However, often strong, unsubstantiated claims are made about the pros and cons of herbal medicines, which will also result both in false hopes or strong fears of those at risk or ill with COVID-19 (Brennen et al., 2020; Guastalegname and Vallone, 2020; Thorp, 2020).

While some preparations have been claimed to be specifically active, some commonly used medicinal plants are assessed here, especially those on the WHO list of selected medicinal plants, as adjuvant treatments. For example, Hensel et al. (2020), in their recent work, state that extracts prepared with *Echinacea* species (Asteraceae) have an important role with the immune system due to their alkylamide interacting with the cannabinoid receptors (Hensel et al., 2020). Additionally, curcumin, the main constituent in *Curcuma longa* L. (Zingiberaceae), is suggested as a potential clinical option for the treatment of SARS-CoV-2 infection, due to its action in several steps of a viral infection such as protease inhibition, cellular signalling pathways modulation, among others (Zahedipour et al., 2020)

In another paper (Panyod et al., 2020), 11 medicinal plants were discussed concerning their *in vitro* antiviral activity using different models. Two species (*Allium sativum* L. and *Zingiber officinale* Roscoe) mentioned in this study that had their *in vitro* potential and immune capacity assessed, were also included in our study. Moreover, in an elegant evidence-based analysis, eighteen phytotherapeutic preparations were mentioned as having some role in the clinical management of viral respiratory diseases, showing different levels of immunological response (Portella et al., 2020). Four plants mentioned in that report [*Echinacea purpurea* (L.) Moench, *Glycyrrhiza glabra* L., *Sambucus nigra* L., and *Scutellaria baicalensis* Georgi] were also included in our study.

The rationale used in our study was to include, mostly, species known in the Americas and Europe and those already more widely available for the management of respiratory conditions (Blumenthal, 2003; Edwards et al., 2015; Anheyer et al., 2018; Langeder et al., 2020), mainly regarding the symptoms (cough, pain, fever). We recognize that patients suffering from COVID-19 are likely to seek such herbal medications. Complementing other papers published on medicinal plants and their potential to be used for COVID-19, we focused on the therapeutic potential of 39 species, the limitations for their use, and their possible risks. It must be strongly emphasized that this is not an assessment of any mainline treatment for COVID-19 with such herbal medicines. The focus is on assessing their potential as adjuvant therapies to COVID-19. Although the listed herbal drugs included herein have been used for a long time, the evidence level of their action in the relief of mild respiratory symptoms varies, and they are pointed out here.

The Frame of the Problem

- Apart from a handful of antiviral drugs with limited efficacy, there is only symptomatic therapy for influenza.
- There is no specific therapy for COVID-19.
- There are several herbal medicines recognized by various Health Authorities for the treatment of flu, its symptoms, and other respiratory disorders.
- Health authorities in Europe and the Americas have warned the population against taking any natural/herbal medicines/supplements. They sustain this advice on theoretical potential effects on the immune system due to unspecific anti-inflammatory effects in early stages, facilitating the infection as well as potential immunostimulation, contributing to the aggravation of the "cytokines storm". As of today, there is no clinical backup for such strong recommendations other than trying to prevent unspecific herb-drug interactions should the patient need emergency care.
- There is a significant OTC use for these herbal medicines, and a realistic prospect is that patients will self-administer them to increase their well-being.

Aims

To apply a decision-making framework to provide a benefits/risks assessment for selected herbal medicines traditionally indicated for "respiratory diseases" within the current frame of the COVID-19 pandemic.

METHODS

For a treatment to be recommended as adjuvant therapy for respiratory diseases in the context of COVID-19, we here determine that the treatment is effective and that its expected benefits outweigh its potential risks to patients. Briefly, this assessment is informed by the body of evidence about each treatment's safety and efficacy retrieved in a literature search. This assessment is also informed by a number of other factors, including the severity of the underlying condition and how well patients' medical needs are addressed by currently available therapies ("reference drugs"). The decision also reflects current applicable laws, regulations, and healthcare recommendations, taking into consideration the uncertainty associated with COVID-19.

Selection of Herbal Medicines

A search was conducted, considering herbal medicines traditionally used to relieve cold and flu symptoms. The criterion used to limit the investigation, and to grant minimal evidence of efficacy was that the species, linked to the chosen indications, must be listed at least in one of the following organizations: World Health Organization Monographs (WHO); European Medicines Agency (EMA); European Scientific Cooperative on Phytotherapy; ANVISA (Brazilian Pharmacopoea, Brazilian Pharmacopoea Herbal Medicines Formulary, Brazilian Pharmacopoea Herbal Medicines Mementum), Ministry of Health of Chile, Ministry of Health of

Cuba, Ministry of Health of Colombia, and Government of Canada. Several South American Countries use the Brazilian Pharmacopeia documents as a reference, so they are also covered within this search. Based on these documents, a list of target species was prepared to establish a search of clinical evidence for the given indication. We refer to these as "herbal medicines" as they are endorsed by Scientific and/or Regulatory committees.

We also included some species which are not listed in monographs based on their significant widespread use in the self-management of respiratory diseases. Some of them are also linked to food uses. We refer to these as "herbal remedies".

We did not consider species that have given rise to major safety concerns (such as *Ephedra* sp.) and species which are only indicated for relieving mucous phlegm. Furthermore, we do not assess multi-herbal preparations.

Decision-Making Framework

To assist our decision-making and/or the benefits/risks assessment of herbal medicines, we adopted some of the procedures described in many qualitative or semi-quantitative guidelines to conduct a benefit-risk assessment (PROTECT, 2020). They consist of a step-by-step approach to follow for good decision-making practice and to increase transparency. Descriptive frameworks are usually general, and most of the time, reiterate common sense. Our framework is inspired by the PrOACT-URL method (EMA, 2011c).

The main decision-making elements that we considered for this work are:

A. Frame the problem. The appearance of early/mild respiratory symptoms during the pandemic including fever, cough, catarrh, aches, and pains, nasal congestion, runny nose, sore throat, cough, sneezing (= *Condition*) in adults otherwise healthy (= *Target Population*). The patient did not have a test or was negative for COVID-19, but continues at risk of infection (= *Uncertainty*). The patient uses herbal medicines alone or with drugs (= *Treatment*, main or adjuvant). The patient experiences relief of upper respiratory symptoms within 1-2 weeks (= *Favorable effect*). The treatment interferes with hospital/emergency treatment in case of severe acute respiratory syndrome (*Unfavorable effects*).
B. Set criteria for Favorable/Unfavorable effects. We followed the "General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine-World Health Organization" for clinical evidence (WHO, 2000) (**Table 1**). We agreed on six key criteria for safety (**Table 2**).
C. Consider options to be evaluated against the treatment. Currently available over-the-counter (OTC) medications

TABLE 1 | Grading criteria for clinical evidence of the treatments.

Grade	Evidence levels quality/Type of evidence	Requirements
High	**Ia** Evidence obtained from meta-analysis of randomized controlled trials **Ib** Evidence obtained from at least one randomized controlled trial	Requires at least one randomized controlled trial as part of the body of literature of overall good consistency addressing the specific recommendation.
Medium	**IIa** Evidence obtained from at least one well-designed controlled study without randomization **IIb** Evidence obtained from at least one other type of well-designed quasi-experimental study **III** Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies, and case-control studies	Requires availability of well-conducted clinical studies with no randomization clinical trials on the topic of recommendation
Low	**IV** Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities	Requires evidence from expert committee reports or opinions and/or clinical experience of respected authorities. Indicates the absence of directly applicable studies of good quality

Adapted from General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine World Health Organization (WHO, 2000).

TABLE 2 | Grading criteria for safety level of the treatments and its evidence.

Grade	Type of adverse effects	Evidence levels quality/Type of evidence*
High	A. Other/s than those listed below (B-F). May include allergies, contraindication in pregnancy/lactation, children/elderly, GI disturbances, etc.	**Ia** Evidence obtained from pharmacovigilance data **Ib** Evidence obtained from medically reviewed drug monographs/Patients information leaflets **Ic** Evidence obtained from clinical trials
Medium	B. Reported interactions which may affect cardiovascular and/or platelet function C. Presence of compounds potentially able to theoretically produce any in the COVID-19 context (such as coumarins, salicin, ephedrine, etc.) D. No adverse effects reported but known immunostimulant activities	**II** Evidence obtained from case reports or clinical practice/ well stablished use. **III** Preclinical evidence in relevant experimental models.
Low	E. Reported adverse effects which may affect the respiratory function F. Reported interactions which may affect emergency treatments (anesthesia, mechanical ventilation, etc.)	**IV** Recommendations from expert committee reports or opinions of respected authorities.

**Adapted from General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine World Health Organization (WHO, 2000).*

endorsed by health authorities are non-steroidal anti-inflammatory drugs (NSAIDs) (ibuprofen, naproxen, etc.), antipyretics (acetaminophen/paracetamol), and cough medicines (dextromethorphan, codeine, etc.). They may be taken as monotherapy or alternating therapies. Therefore, one of each category (ibuprofen, paracetamol, and codeine) was chosen to be evaluated based on the same criterion.

D. Assess the balance between favorable and unfavorable effects and the associated uncertainty. All possible combinations of clinical and safety grades lead to four possible results: "positive", "promising", "negative", and "unknown". The last two categories allowed for the inclusion of two different degrees of uncertainty (**Table 3**).
E. Recommendation. See conclusions.

Retrieval of Evidence and Grading

In the second step, we assessed the existing clinical evidence and safety data of all shortlisted herbal medicines and remedies through a bibliographic search, including PubMed, Web Of Science, and other available sources, using the terms "<plant name>" AND cough OR flu OR cold. Also, Cochrane, Drug.com, governmental agencies (EMA, ANSES, and others) were used.

All possible combinations of clinical and safety grades lead to four possible results: "positive", "promising", "negative", and "unknown". The last two categories allowed for the inclusion of two different degrees of uncertainty. **Table 3** shows how the consensus criteria were translated into a preliminary benefits/risks assessment.

We are aware that for many herbal drug preparations, preclinical evidence exists, and this is not considered in this assessment since it cannot be translated directly into clinical practice. However, such estimation might be of relevance to unveil potential mechanisms of action only so in that case this information was included.

RESULTS

Options to Be Evaluated Against the Criteria

Based on the defined parameters, three recommended drugs for early symptoms of COVID-19 - codeine, ibuprofen, and paracetamol - were evaluated.

Ibuprofen

Indications in the context of respiratory conditions. Fever, pain, inflammation.

TABLE 3 | Benefits/Risks Decision Consensus Criteria.

Clinical evidence (Table 1)	Safety evidence (Table 2)	Benefits/risks Balance
High	High	Positive
High/Medium	Medium	Promising
Low	High/Medium	Unknown
High/Medium/Low	Low	Negative

Posology. Up to 2,400 mg a day in doses not bigger than 400 mg every 6 h.

Preclinical evidence. *In vitro* and *in vivo* studies showed evidence of antipyretic and mild analgesic activities (Rainsford, 2015).

Clinical evidence. In a review on the effects of non-steroidal anti-inflammatory drugs (NSAIDs) for treating pain or respiratory symptoms (e.g., cough associated with the common cold), the conclusion was NSAIDs are somewhat effective in relieving the discomfort caused by cold. However, there is no clear evidence of their effect in easing respiratory symptoms. Therefore, the balance of benefits and risks needs to be considered when using NSAIDs for colds (Kim et al., 2013). There is an ongoing clinical trial in the UK with COVID-19 patients receiving a liquid ibuprofen formulation on top of standard care (ClinicalTrials.gov, 2020). Overall, the clinical evidence is High.

Safety. Side effects of ibuprofen include anemia, decreased hemoglobin, eosinophilia, hemorrhage, vomiting, and hypertension. Other side effects include upper gastrointestinal hemorrhage, upper gastrointestinal tract ulcers, dizziness, and dyspepsia. A comprehensive list of very common (10% or more) to common (1% to 10%) adverse effects include nausea (up to 57%), vomiting (up to 22%), flatulence (up to 16%), diarrhea (up to 10%); epigastric pain, heartburn, abdominal distress, indigestion, dyspepsia, abdominal discomfort, constipation, abdominal cramps/pain, fullness of GI tract, bloating, GI hemorrhage, melena (1% to 10%) (Drugs.Com, 2020a). However, regarding COVID-19 patients, there is not enough evidence supporting the potential harmful effects (Sodhi and Etminan, 2020). Overall, safety is Medium.

Specific warnings and precautions of use. Ibuprofen may cause severe cardiovascular thrombotic events, myocardial infarction, and stroke, which can be fatal. This risk may be increased in patients with cardiovascular disease or risk factors for cardiovascular disease. Ibuprofen is contraindicated for the treatment of peri-operative pain in the setting of coronary artery bypass graft (CABG) surgery. NSAIDs can also cause an increased risk of serious gastrointestinal adverse events, especially in the elderly, including bleeding, ulceration, and perforation of the stomach or intestines, which can also be fatal (McGettigan and Henry, 2011).

Overall assessment. According to well-established use, ibuprofen may be useful in the symptomatic relief of respiratory conditions by reducing fever and aches. However, there is only a relatively low number of clinical studies, and meta-analyses do not provide consistent evidence that ibuprofen is effective in reducing symptoms and duration and prevention of the common cold. Overall, the clinical evidence is High. Ibuprofen may have antiplatelet activities; its safety may be considered Medium.

Codeine

Indications in the context of respiratory conditions. Cough.

Posology. Up to 360 mg a day in doses not bigger than 60 mg every 4 h.

Preclinical evidence. Some *in vitro, ex vivo*, and *in vivo* studies show evidence of anti-cough activity (Ohi et al., 2007; Cui et al., 2019).

Clinical evidence. Although codeine is widely used as antitussive, the clinical evidence supporting this action is controversial. A study, involving 91 adults presenting cough associated with acute upper respiratory tract infection, showed codeine statistically had the same effect than vehicle (syrup) in controlling cough (Eccles et al., 1992). Overall, the clinical evidence is Low.

Safety. Commonly reported side effects of codeine include drowsiness, lightheadedness, dizziness, sedation, shortness of breath, nausea, vomiting, sweating, and constipation. Other possible effects include bronchospasm, laryngospasm, respiratory depression; heartbeat irregularities, blood pressure changes, syncope, itching, facial swelling, pruritus, urticaria, histamine release; dry mouth, loss of appetite, nausea, vomiting, paralytic ileus, toxic megacolon, anorexia, stomach cramps; miosis, blurred or double vision; euphoria, dysphoria, unusual dreams, hallucinations, insomnia, anxiety (0.1% to 1%); respiratory arrest, dyspnea; flushing, hypotension, palpitations, circulatory depression, shock, cardiac arrest, circulatory depression, bradycardia, tachycardia, edema (Drugs.com, 2019). Overall, safety is Low.

Specific warnings and precautions of use. Death due to respiratory depression has been reported in children (Friedrichsdorf et al., 2013; Tobias et al., 2016). Moreover, codeine can lead to opioid misuse, abuse, and addiction (Casati et al., 2012).

Overall assessment. According to established use, codeine may be useful in the symptomatic relief of cough. However, clinical studies and meta-analyses do not provide consistent evidence that codeine is effective in treating cough. Overall, the clinical evidence is Low. Due to the severe side effects, codeine safety may be considered Low.

Paracetamol

Indications in the context of respiratory conditions. Fever, pain.

Posology. Up to 4 g a day in dose not bigger than 1 g every 6 h.

Preclinical evidence. Numerous *in vitro* and *in vivo* studies show evidence of antipyretic and mild analgesic activities (Graham et al., 2013).

Clinical evidence. Paracetamol (acetaminophen) did not show any efficacy in flu, according to a clinical trial involving 80 patients (Jefferies et al., 2016). In a systematic review, the authors concluded that the data did not provide sufficient evidence to inform practice regarding the use of acetaminophen for common cold in adults (Li S. et al., 2013). Overall, the clinical evidence is Low.

Safety. Paracetamol (acetaminophen) is hepatotoxic (Athersuch et al., 2018), and several side effects have been reported (Ishitsuka et al., 2020), such as nausea (up to 34%), vomiting (up to 15%); abdominal pain, diarrhea, constipation, dyspepsia and enlarged abdomen (1% to 10%); anemia, postoperative hemorrhage (1% to 10%); rash, pruritus (1% to 10%); dyspnea, abnormal breath sounds, pulmonary edema, hypoxia, pleural effusion, stridor, wheezing, coughing (1% to 10%); cardiovascular effects (1% to 10%): peripheral edema, hypertension, hypotension, tachycardia, chest pain; metabolic alterations (1% to 10%): hypokalemia, hyperglycemia; headache, dizziness (1% to 10%). Other side effects: dystonia; muscle spasms, trismus (1% to 10%); insomnia, anxiety (1% to 10%); oliguria (1% to 10%); pyrexia, fatigue (1% to 10%) (Drugs.com, 2020b). There are reported cases of deaths in flu patients taking paracetamol (Stevenson et al., 2001). Overall, the safety evidence is Low.

Specific warnings and precautions of use. Not indicated in cases of liver disease and alcoholism (Drugs.com, 2020b).

Overall assessment. According to a well-established use, paracetamol may be useful in the symptomatic relief of respiratory conditions by reducing fever and aches (although a relatively low number of clinical studies and meta-analyses do not provide consistent evidence that paracetamol can reduce symptoms and duration), and prevention of the common cold. The clinical evidence may be considered Low or at best Medium. It is known to be hepatotoxic, and a frequent incidence of respiratory adverse effects may justify serious concerns and a safety rating of Low.

Assess the Relative Importance of the Decision Maker's Risk Attitude Towards Herbal Substance

Risk is inherent to any therapeutic intervention (herbal or not). The level of risk of herbal interventions in adults experiencing common flu symptoms without underlying conditions is very low. According to WHO, COVID-19 is self-limiting and mild in this segment of the population (WHO, 2020a). However, we took extra care in integrating current health authorities' advice with current clinical evidence to emit our assessment.

Scientific and Clinical Data of Current Herbal Therapy Referred to as Useful to Relieve Symptoms Related to Respiratory Conditions (Cold/Flu)

General warning: allergic reactions and gastrointestinal (GI) disturbances are common adverse effects in all medicines and apply to herbal ones. Their use in pregnancy and lactation, babies, children, and the elderly, as well as patients with known severe conditions, is to be individually assessed by a registered healthcare professional.

The recommendations made here are for medicinal products regulated by national authorities to ensure their quality and safety. Other products may be unsafe due to contamination, adulteration, and the presence of naturally occurring toxins in levels above those permitted.

Allium sativum L. - Amaryllidaceae (Bulbs, Powder)

Indications in the context of respiratory conditions. *Allium sativum* is indicated for respiratory disease, namely cold and cough (CUBA, 2014; EMA, 2017b), and other symptoms related to influenza (BRASIL, 2011). Other related indications of garlic preparations (fresh, garlic powder) included diaphoretic, antiseptic, bacteriostatic, and antiviral effects. It is also used to treat chronic bronchitis and recurrent upper respiratory tract infections (EMA, 2017b; El-Saber Batiha et al., 2020b).

Traditional indications. *Allium sativum* has been traditionally used for alleviation of symptoms of the common cold in adults and children over 12 years. Indeed, garlic is considered as a

traditional herbal medicinal product used for the relief of cold symptoms. Moreover, the British Herbal Pharmacopoeia considers that garlic products are indicated for recurrent colds and whooping cough (BHMA, 1983).

Chemical composition. Sulfur compounds (allicin, mercaptan, allyl methyl thiosulphinate, allyl methyl trisulphide, diallyl disulfide, diallyl trisulfide, S-allyl cysteine sulphoxide, and others), glucosides (sativoside B1, proto-degalactotigonin), amino acids (alanine, arginine, aspartic acid, asparagine, histidine, proline, alanine, valine), monoterpenoids (citral, geraniol, alfa and beta-phellandrene and other), peptides, minerals, flavonoids, and vitamins (Lanzotti, 2006; Omar and Al-Wabel, 2010). In the presence of the enzyme alliinase, alliin will be converted to allicin (1 mg alliin to be equivalent to 0.45 mg of allicin). Allicin is also the precursor of other non-volatile products such as ajoenes or oligo- and polysulphides (ESCOP, 2003a; Kovarovič et al., 2019).

Posology (based on traditional uses). Fresh garlic: 2.0–4.0 g average daily dosage (EMA, 2017b). However, it is preferable to use a commercial preparation with defined composition and an adequate dose.

Preclinical evidence. This herbal medicine has been experimentally proven to have antiviral activity. Among the viruses which are sensitive to garlic extracts, are the Human *Cytomegalovirus* (HCMV), *Influenz*a B virus, *Herpes simplex virus* type 1, *Herpes simplex virus* type 2, vesicular stomatitis virus, *Parainfluenza virus* type 3, *Vaccinia virus*, and human *Rhinovirus* type 2 (Tsai et al., 1985; Mikaili et al., 2013). Allicin-containing supplements can prevent attacks by the common cold virus.

Clinical evidence. There is no clinical data to support garlic in the treatment of upper respiratory infections, only in the prevention and treatment of symptoms of the common cold (Josling, 2001). Regarding the prevention or treatment of the common cold, a Cochrane meta-analysis concludes that there is insufficient clinical evidence (Lissiman et al., 2014); the sole study retained for the analysis showed fewer days of illness in the garlic group compared with the placebo group (Josling, 2001). Another trial suggested that consuming the aged garlic extract could reduce the severity of cold symptoms reported (Nantz et al., 2012). Overall, the clinical evidence is High for cold.

Safety. Garlic preparations are generally considered to be safe (EMA, 2016a). However, patients taking anticoagulation and/or antiplatelet therapy should use garlic preparations with caution because they may increase bleeding times (EMA, 2017b). Overall, safety is Medium.

Specific warnings and precautions of use. Patients should avoid concomitant use with anti-platelet drugs.

Overall assessment. Although *Allium sativum* preparations have been used to relieve cold symptoms since ancient times, there is no evidence this herbal medicine can relieve flu symptoms. Whether it may indirectly provide anti-inflammatory and soothing effects on the upper respiratory tract remains to be seen. The clinical evidence may be considered High. Even though garlic is known to have potential antiplatelet effects, overall, it can be considered presenting Medium safety, due to products with less than 0.6% allicin content do not appear to have any such effects (Scharbert et al., 2007).

Althaea officinalis L. - Malvaceae (Roots, Leaves)

Indications in the context of respiratory conditions. *Althaea officinalis* is indicated for respiratory disease symptoms, namely dry, irritable coughs, and irritations of oral and pharyngeal mucosa (EMA, 2016c).

Chemical composition. Mucilage polysaccharides, such as galacturonorhamnans (rhamnogalacturonan), arabinans, glucans, arabinoglucans, mainly of acidic polysaccharides; flavonoids (e.g., isoscutellarein, hypolaetin, kaempferol and luteolin derivatives); phenolic acids; coumarin (scopoletin); tannins (ESCOP, 2019; Kianitalaei et al., 2019).

Posology (based on traditional uses). 0.5–5.0 g in 150 ml of water as a macerate, three times daily (EMA, 2016c). Marshmallow root's syrup is also a commonly used preparation in a daily dose of 2.0-8.0 ml (ESCOP, 2019; Kianitalaei et al., 2019).

Preclinical evidence. This herbal medicine has been experimentally proven for respiratory disease symptom, namely, cough. The aqueous extract of marshmallow roots inhibited the tracheobronchial smooth muscle contractions in rats in a dose-dependent manner (Alani et al., 2015). The antitussive effects of oral rhamnogalacturonan (50 mg/kg) were evaluated against mechanically induced cough reflux in both sexes of non-anesthetized cats. The polysaccharide significantly reduced the number of efforts, cough frequency, and intensity of cough attacks from laryngopharyngeal and tracheobronchial areas (Nosalova et al., 2005). The antitussive effects of oral syrup and the polysaccharide were tested against mechanically induced cough of non-anesthetized cats in comparison with non-narcotic antitussives (Nosal'ova et al., 1992).

Clinical evidence. *Althaea officinalis* preparations have been trialed clinically for respiratory disease, and the following symptom was evaluated: cough. In a randomized clinical study, 63 adults suffering from dry cough associated with angiotensin-converting enzyme inhibitors ingested 20 drops, three times per day, of either a marshmallow root preparations or a placebo, for four weeks. The severity of the cough in the marshmallow group was significantly reduced (Rouhi and Ganji, 2007). In one clinical trial on 822 patients with dry cough associated with pharyngeal irritation, the efficacy, tolerability, and satisfaction of *A. officinalis* root aqueous extract in the form of lozenges and syrup was evaluated. *Althaea officinalis* root aqueous extract improved the symptoms of dry cough within 10 min with very good tolerability. There were only three minor adverse events in the syrup group (Fink et al., 2017). Overall, the clinical evidence is High for cough.

Safety. No toxicity was reported at the indicated doses (EMA, 2016b). Overall, safety is High.

Specific warnings and precautions of use. The absorption of other drugs taken simultaneously may be retarded due to the presence of mucilage. As a precautionary measure, all preparations with *A. officinalis* should not be taken 30 min to 1 h before or after intake of other drugs/minerals/vitamins. The macerate should be used immediately after preparation (EMA, 2016b).

Overall assessment. *Althaea officinalis* preparations can suppress cough and diminish irritation through anti-

inflammatory and soothing effects on the respiratory tract. Its traditional use as cold therapy in the context of upper respiratory conditions is not backed up by robust clinical data, but the evidence allows us to infer a potential use in the relief of early symptoms of COVID-19. The clinical evidence may be considered High, and as no severe concerns are reported for this herbal medicine, it can be rated as High safety.

*Andrographis paniculata (*Burm.f.) Nees - Acanthaceae (Leaves, Aerial Parts)

Indications in the context of respiratory conditions: *Andrographis paniculata* is indicated for respiratory disease, namely common cold, influenza type, and other upper respiratory tract infections, cough, and fever (WHO, 2002). It is often included in multi-herbal preparations, which are not assessed here [aside from the combination with *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim.].

Chemical composition. Relevant secondary metabolites are diterpenes like andrographic acid, their glucosides (deoxyandrographolide-19β-D-glucoside) and dimers (bis-andrographolides A, B, C, and D) and labdane diterpenoids like andrographolide, neoandrographolide; flavonoids (methoxylated flavones, flavanones, chalcones); steroids (β-sitosterol (Koteswara Rao et al., 2004; Akbar, 2011; Dai et al., 2019; Hanh et al., 2020). Andrographolides are considered the active metabolites and chemical markers of this species.

Posology (based on traditional uses). 1–3 g as a decoction, three times daily (WHO, 2002). However, it is preferable to use a commercial preparation with defined composition and an adequate dose.

Preclinical evidence. This herbal medicine has been experimentally proven for respiratory disease, based on a mouse-model for influenza (an adapted H1N1 strain PR8A/PR/8/34). Treatment with andrographolide decreased the virus loads and the expression of the inflammatory cytokines. Also, diminished lung pathology and overall survival rate (Ding et al., 2017). Anti-inflammatory and immunomodulatory properties of the extract of *A. paniculata* and andrographolide have been linked to the increasing proliferation of lymphocytes and the production of IL-2 and inhibition of the tumor cell proliferation immune system (Rajagopal et al., 2003; Kumar et al., 2004). Also, diminished lung pathology and overall survival rate (Ding et al., 2017). Neoandrographolide has *in vivo* anti-inflammatory effects (Panossian et al., 2002). Andrographolide and a standardized registered fixed combination of *A. paniculata* extract SHA-10 and *E. senticosus* extract SHE-3 showed an *in vitro* effect on the activation and proliferation of immune-competent cells as well on the production of key cytokines and immune activation markers (Dai et al., 2019; Kim et al., 2019). A range of anti-inflammatory effects has been reported on diverse disease targets for *A. paniculata* and key constituents (Dai et al., 2019; Kim et al., 2019).

Clinical evidence. This herbal medicine has been trialed clinically for cold symptoms. Three systematic reviews pointed to the beneficial effects and safety of Andrographis for relieving symptoms of acute respiratory tract infections and shortening time to the symptom (Kligler et al., 2006; Akbar, 2011). The most recent study included 33 randomized controlled trials (RCT) with 7175 patients. The results indicated that compared to usual care, a shortening of the duration of symptoms including cough, sore throat, and sick leave/time to resolution was observed. Concerns were raised due to the low quality of many studies and their heterogeneity (Hu et al., 2017; Hu et al., 2018). Overall, the clinical evidence is High for cold and cough.

Safety. An assessment report of EMA concludes that while 'there is a clear effect on some CYP isoenzymes, the available acute, and reproductive toxicity and genotoxicity data support the safety of Andrographis' (EMA, 2014a). On the other hand, the Australian Therapeutic Goods Administration (TGA) highlighted the potential risks of severe allergic reactions (TGA, 2015), which, however, seems to be of very limited clinical concern. There are some preclinical indications of immunomodulatory activities of unknown implications for the COVID-19 cytokines storm. Overall, safety is Medium.

Specific warnings and precautions of use. Allergic reactions may be of concern (TGA, 2015).

Overall assessment. Andrographis may be useful in the symptomatic relief of respiratory symptoms, especially in terms of alleviating the symptoms of uncomplicated upper respiratory tract infections. Its traditional use as cold therapy in the context of upper respiratory conditions is not backed up by robust clinical data, but the evidences allow to infer a potential use in the relief of early symptoms of COVID-19. The clinical evidence may be considered High, and as although no severe concerns are reported, this herbal medicine may exert immunomodulatory activities so can be cautiously rated as Medium safety.

Commiphora molmol Engle [syn. *Commiphora myrrha* (T.Nees) Engl.] and Other *Commiphora* sp. - Burseraceae (Air-Dried Oleo-Gum Resin Exudate)

Indication in the context of respiratory conditions. *Commiphora molmol* is indicated symptoms of respiratory disease, namely mild inflammation of pharyngeal mucosa (WHO, 2007). Other related symptoms are cough, anti-inflammatory (Akbar, 2020); supportive treatment for tonsillitis (WHO, 2007; Barnes et al., 2012; ESCOP, 2014).

Chemical composition. Sesquiterpenes (furanoeudesma1,3-diene and lindestrene as the major components) (Marongiu et al., 2005).

Posology (based on traditional uses). It is preferable to use a commercial preparation with a defined composition, such as a tincture (0.5–5 ml in 150 ml of water for rinsing or gargling three times daily) (WHO, 2007).

Preclinical evidence. This herbal medicine has not been experimentally proven for symptoms of respiratory disease. Although *C. molmol* has been used as a remedy since ancient times (Akbar, 2020), so far, only a few studies on anti-inflammatory or antinociceptive action can be found. An ethanol extract from the resin (at the doses of 100 mg/kg and 200 mg/kg, p.o.) presented analgesic and anti-inflammatory activities in mice. In the swelling paw test, the effect of the extract (100 mg/kg, p.o.) was similar to indomethacin (10 mg/kg, i.p.) (Su et al., 2011).

Clinical evidence. *Commiphora molmol* preparations have not been trialed clinically for respiratory disease, only for inflammation. A standardized extract of *C. molmol* (curzerene 17.93%, furanoeudesma-1,3-diene 27.44%, lindestrene 9.08%) was evaluated about the anti-nociceptive effect. The volunteers (89 men and 95 women), presenting headache, fever-dependent pain, joint pain, muscle aches, lower back pain, or menstrual cramps, received extract (200 mg or 400 mg), or placebo, for 20 days. According to this RCT, the extract presented a similar effect to some frequently used drugs, such as diclofenac, ibuprofen, and paracetamol, although requiring a longer time of use (20 days). In the male group, the extract, at the dose of 400 mg, the effect was significant against all the symptoms. For the female group, the extract was effective against low back pain and fever-dependent pain at the doses of 200 mg/day. Furthermore, no side effect was reported by any of the volunteers (Germano et al., 2017). Overall the clinical evidence is High for pain associated with fever.

Safety. The safety of *C. molmol* is well established. There are no known safety concerns from non-clinical or clinical data (EMA, 2011d; EMA, 2018b). Overall, safety is High.

Specific warnings and precautions of use. Due to a uterine stimulant effect (Vafaei et al., 2020), products containing *C. momol* resin should be avoided in pregnancy and lactation (EMA, 2011d). The concomitant use of *C. molmol* with theophylline or cyclosporine A should be avoided (Al-Jenoobi et al., 2015a; Al-Jenoobi et al., 2015b; EMA, 2018a).

Overall assessment. *Commiphora molmol* preparations seem to have a supportive effect as antinociceptive and thus be useful in the relief of respiratory symptoms. However, its effect is evident only after a few weeks, thus exceeding the normal, uncomplicated evolution of COVID-19. The clinical evidence may be considered High. As no severe concerns are reported, this herbal medicine safety can be rated as High.

Cymbopogon citratus (DC.) Stapf - Poaceae (Leaves)

Indications in the context of respiratory conditions. *Cymbopogon citratus* is indicated for respiratory infections (CUBA, 2014).

Chemical composition. Essential oil (with geranial, neral and myrcene as the major constituents) (Leclercq et al., 2000; Menut et al., 2000; Pino and Rosado, 2000; Sidibé et al., 2001; Ali et al., 2004; Kanko et al., 2004; Pérez et al., 2006; Rodriguez-Pérez et al., 2006; Brito et al., 2011; Silva et al., 2014; Diop et al., 2017; Soliman et al., 2017; Alam et al., 2018; Silva et al., 2018; BRASIL, 2019a); triterpenes (e.g., cymbopogonol; cymbopogone) (Hanson et al., 1976); flavonoids (e.g., luteolin, apigenin, kaempferol) (Cheel et al., 2005; Orrego et al., 2009; Costa et al., 2015a; Costa et al., 2015b); phenolic acids (e.g., caffeic, chlorogenic, ferulic acids and derivatives) (Tapia et al., 2007).

Posology (based on traditional uses). 1–2 g of dried leaves (or 4–5 g of fresh leaves) in 150 ml, up to three times daily (Carballo, 1995; Matos et al., 2001).

Preclinical evidence. *Cymbopogon citratus* has been experimentally evaluated for fever. However, the evaluation of the antipyretic activity of *C. citratus* herbal tea in rats (p.o. or i.p.) did not result in body temperature reduction (Carlini et al., 1986). On the other hand, other related experimental effects include the anti-inflammatory potential of this species, mainly the essential oil. The essential oil from *C. citratus* has been reported to suppress inhibition of TNF-α-induced neutrophil adherence, inducible nitric oxide synthase (iNOS), and other lipopolysaccharides (LPS)-induced pathways, suppression of COX-2 and peroxisome proliferator-activated receptor alpha (PPAR-α) (Katsukawa et al., 2010; Francisco et al., 2013). Another experiment (murine model of allergic asthma) showed a standardized hexane extract of *C. citratus* led to the reduction of inflammatory cells and eosinophils, as well as the expression of NF-kB/p65, in mice sensitized by Bt-antigen (Machado et al., 2015). Citral, the main substance in *C. citratus* essential oil, presented antinociceptive and anti-inflammatory activity in mice (Quintans-Júnior et al., 2011).

Clinical evidence. This herbal medicine has been not trialed clinically for respiratory disease. Overall, the clinical evidence is Low.

Safety. Some reports considered *C. citratus* safe with no health issues from its usage due to the acceptable concentration limit of the essential oil compounds (Carlini et al., 1986; Ekpenyong et al., 2015). Overall, safety is High.

Specific warnings and precautions of use. None

Overall assessment. This herbal medicine is widely used, and although its profile fit as safety relief therapy for flu, the anti-inflammatory effect on the respiratory tract of *C. citratus* preparations could be useful in the symptomatic relief of respiratory disease. The clinical evidence may be considered Low. As no severe concerns are reported, this herbal medicine safety can be rated as High.

Echinacea sp. (*E. angustifolia* DC., *E. purpurea* (L.) Moench and *E. pallida* (Nutt.) Nutt. - Asteraceae (Aerial Parts, Rootstock)

Indications in the context of respiratory conditions. This herbal medicine is indicated for symptoms of respiratory disease, namely, those associated with common cold (EMA, 2017c). *Echinacea* preparations are widely used to 'prevent colds and other respiratory infections, as immunostimulants and in conditions associated with respiratory discomfort. The European Medicine Agency (Herbal Medicinal Product Committee, HMPC) granted registrations for *Echinacea purpurea* (L.) Moench (purple coneflower) for a preventive continuous use for maximal ten days, specifically excluding pediatric populations and with autoimmune illnesses as a contraindication (EMA, 2017a).

Chemical composition. Alkylamides, polysaccharides. Caffeic acid derivatives serve as marker substances (e.g., echinacoside for *E. pallida* root; cichoric acid for *E. purpurea* aerial parts) but are not considered to be of therapeutic relevance (Edwards et al., 2015).

Posology. It is essential to use a commercial preparation with defined composition and an adequate dose. Preparations that administer the extract to the upper respiratory tract (like lozenges) may well be preferable over solid formulations.

Preclinical evidence. This herbal medicine has been not experimentally proven for respiratory disease, namely cough, fever, flu, cold (EMA, 2017a). *In vitro* studies were reported against a range of respiratory viruses but for some preparations

only at higher concentrations (Pleschka et al., 2009; Hudson and Vimalanathan, 2011). The immunomodulatory effects of a standardized extract of *Echinacea purpurea*, as well as fractions rich in chicoric acid, polysaccharide, and alkylamide, were evaluated in rats. Phagocytic activity of alveolar macrophage was increased with increasing concentrations of the Echinacea components. Also, a trend of increase in TNF-α and NO release, after *in vivo* LPS stimulation, by the alveolar macrophages was observed. High concentration led to a release of cytokines (such as TNF-α and IFN-γ) in rat's spleen macrophage (Goel et al., 2002).

Clinical evidence. Echinacea preparations have been trialed clinically for flu and cold. However, the overall problem with the assessment of the evidence refers to the variability of the pharmaceutical preparations investigated in clinical studies (Linde et al., 2006; Karsch-Völk et al., 2014; David and Cunningham, 2019). Therefore, a large number of clinical studies, as well as a range of pharmacodynamics studies, have been conducted, overall indicating at best a weak evidence for benefits for treating colds. Several metanalyses failed to find any evidence of clear benefits. Still, two RCT reported statistically significant benefits for patients in terms treated with *E. purpurea* and *E. angustifolia* preparations of both symptoms and reduced duration of flu or cold (Linde et al., 2006; Karsch-Völk et al., 2014; David and Cunningham, 2019), thus qualifying for A (Ib). Of note, one study showed no difference between an *Echinacea pallida* preparation and oseltamivir (Rauš et al., 2015). Overall, the clinical evidence is High for cold and flu.

Safety. Echinacea preparations are generally considered to be safe, although some allergic reactions have been recorded (EMA, 2017a). Interactions risks seem to be of no therapeutic concern (Modarai et al., 2010). Although French Agency (ANSES) postulated that echinacea, among other herbal medicines, could interfere with the immune response in the context of COVID-19 pandemic (ANSES, 2020), at this stage, there is no evidence to support this and, more generally, herbal treatments are not known to rigorously block inflammatory processes and to negatively affect immune responses. Therefore, these concerns seem to be a theoretical postulate which would require further evaluation. While there are no clinical signals for interaction with other medications, ANSES's concerns might be linked to theoretical interaction with immunosuppressants (such as ciclosporin and methotrexate) due to an antagonistic effect (Williamson et al., 2013). Overall, safety is Medium.

Specific warnings and precautions of use. Like all other products discussed here, there is no evidence for specific therapeutic benefits, and it is important to communicate this to potential users. The safety concerns listed above need to be kept in mind.

Overall assessment: *Echinacea* sp. may be useful in the relief of respiratory symptoms by exerting a soothing effect on the respiratory tract. Overall, a relatively large number of clinical studies and a series of meta-analyses provide evidence that *Echinacea* preparations seem to be efficacious both in the treatment (reducing symptoms and duration) and prevention of the common cold. The clinical evidence may be considered High except for *E. pallida*. Although in the COVID-19 context, caution needs to be taken in order to avoid immunostimulation in complications in later phases of the disease, this herbal medicine safety can be rated as Medium.

Eucalyptus globulus Labill. - Myrtaceae (Leaves, Essential Oil)

Indication in the context of respiratory conditions. *Eucalyptus globulus* is indicated for symptoms of respiratory disease, namely cough (WHO, 2002; BRASIL, 2011; EMA, 2013a). Other related indications include respiratory antiseptic (CHILE, 2010) and expectorant (COLOMBIA, 2008), due to the presence of 1-8-cineol (Fischer and Dethlefsen, 2013; Salehi et al., 2019b).

Chemical composition. Essential oil (1,8-cineol as the major component); phenolic acids (cafeic, ferulic acid, and derivatives), tannins (gallic and protocatechuic acids), flavonoids (quercetin derivatives) (Dixit et al., 2012; Sonker et al., 2017).

Posology (based on traditional uses). 1.5–3 g of dried leaves in 150 ml, up to four times daily (EMA, 2013a).

Preclinical evidence. This herbal medicine has not been experimentally proven for symptoms of respiratory disease. *In vitro* and *in vivo* studies with leaves extracts, essential oil, and 1,8-cineol are supportive for some ethnomedicinal use (Ross, 2001; Jun et al., 2013; Brezáni et al., 2018; Dhakad et al., 2018; Galan et al., 2020); for example, the essential oil (300 mg/kg) exerted an anti-inflammatory effect on LPS-induced bronchitis in rats, inhibiting the airway mucin hypersecretion (Lu et al., 2004).

Clinical evidence. *Eucalyptus globulus* essential oil preparations have been trialed clinically for respiratory disease (bronchitis, rhinitis), and the following symptoms were evaluated: cough and throat irritation. In an aromatherapy experiment with 48 students diagnosed with allergic rhinitis, eucalyptus reduced coughing, itching sensation in the throat and oral cavity, as well as other symptoms. After four weeks of treatment, the volunteers related a reduction of the discomfort provoked by rhinitis (Song and Kim, 2014). An RCT, involving 152 volunteers with acute non-purulent rhinosinusitis, was carried out with 1,8-cineol, the main compound of eucalyptus essential oil (capsules of 200 mg of oil or placebo, three times daily). After four and seven days, the cineol group presented better symptoms scores than the control group. Moreover, inflammatory processes, such as bronchitis, pharyngitis, tracheitis, conjunctivitis, were less frequent among the verum group (Kehrl et al., 2004). Overall, the clinical evidence is Medium for bronchitis and cough for the essential oil, while for the herbal drug is Low.

Safety. In traditional doses, there is no report on the toxicity of *E. globulus*. However, high doses can cause nausea, vomiting, and diarrhea (WHO, 2002). In a preclinical assay of 1,8-cineol in mice, this compound was classified as presenting low toxicity (Xu et al., 2014). However, high doses can cause nausea, vomiting, and diarrhea (WHO, 2002). However, in the cited clinical trial, the essential oil and 1,8-cineol did not present a significant side effect. Overall, safety is High.

Specific warnings and precautions of use. The use is contraindicated for patients with hypersensitivity to the active

substance. Moreover, this plant should not be used by children under 30 months of age due to 1,8-cineole containing preparations, which can induce laryngospasm (EMA, 2013a). In rats, 1,8-cineol induced CYP-450 activity and reduced the levels of amphetamine, pentobarbital, and aminopyrine in plasma or brain (Jori et al., 1970). Moreover, the usual precautions relevant to essential oils should be taken into account.

Overall assessment. *Eucalyptus globulus* may be useful in the relief of symptoms associated with upper respiratory infection by exerting a soothing effect on the respiratory tract. However, even though the extensive use of products containing eucalyptus derivatives, more evidence is need on the impact in the respiratory tract. The clinical evidence may be considered Medium. Although there are concerns about the eucalyptus use by babies, this herbal medicine safety can be rated as High.

Foeniculum vulgare Mill. – Apiaceae (Fruits)

Indications in the context of respiratory conditions. *Foeniculum vulgare* is indicated for respiratory disease, namely cough associated with cold and fever (EMA, 2007; WHO, 2007).

Chemical composition. Essential oil (trans-anethole, estragole, and limonene as major components) (Singh et al., 2006; Badgujar et al., 2014); stilbenes (e.g., foeniculosides X and XI, cis- and trans-miyabenol) (De Marino et al., 2007); flavonoids (e.g., eriodictyol, quercetin, and derivatives) (Parejo et al., 2004; Faudale et al., 2008); and triterpenes and steroids (e.g., oleanolic acid, 7α-hydroxycampsterol) (Parejo et al., 2004; De Marino et al., 2007; Rather et al., 2016).

Posology. 1.5 to 2.5 g in 200 ml of boiling water (brew for 15 min) three times daily (EMA, 2007; WHO, 2007).

Preclinical evidence. This herbal medicine has been experimentally proven for cough. The ethanol extract, as well as the essential oil of *F. vulgare* presented analgesic and anti-inflammatory activity in rats (Tanira et al., 1996; Özbek, 2005; Him et al., 2008; Araujo et al., 2013; Elizabeth et al., 2014). The aqueous and ethanolic extract and essential oil of *F. vulgare* were evaluated about the myorelaxant activity using isolated guinea-pig trachea, as a model to bronchodilatory effect. The ethanolic extract and essential oil exert relaxant effects similar to those presented by theophylline. The aqueous extract, on the other hand, presented a contraction effect. The myorelaxant effect was not due to inhibition of muscarinic and histamine H1 and/or stimulation on β2-adrenergic receptors (Boskabady and Khatami, 2003).

Clinical evidence. *Foeniculum vulgare* has not been trialed clinically for respiratory diseases. Overall, the clinical evidence is Low.

Safety. Although essential oil of *F. vulgare* presents estragole as one of the main components, and due to its genotoxic carcinogenicity, the exposure to this compound should be kept as low as possible (EMA, 2019), in the recommended herbal preparation and posology, *F. vulgare* is considered safe. Overall, safety is High.

Specific warnings and precautions of use. The safety of fennel was long considered to be of no concern and importantly linked to the long history of use as a medicine and food. The German Commission E lists no risks. In recent years, concerns were raised related to the content of estragole (methyl chavicol), which is known as a potential carcinogen. No clinical reports of fennel's toxicity are known. The dose and the relevance of some studies with pure estragole at high doses have been disputed. Overall, the evidence is very limited, and there is no known reason for concern (Edwards et al., 2015). Administration of different doses of fennel essential oil reduced the intensity of oxytocin and PGE2 induced contractions significantly (25 and 50 μg/ml for oxytocin and 10 and 20 μg/ml for PGE2, respectively) (Ostad et al., 2001). Fennel is a CYP3A4 inhibitor and can interfere in the metabolism of several drugs (Subehan et al., 2006).

Overall assessment. Even though its profile fits as safety relief therapy for cough in the context of upper respiratory affections, therapeutic benefits are likely to be limited. The clinical evidence is Low. This herbal medicine safety can be rated as High, although the recommended dosage must be observed.

Glycyrrhiza glabra L. - Fabaceae (Roots)

Indication in the context of respiratory conditions. *Glycyrrhiza glabra* is indicated for symptoms of respiratory disease, namely, cough, sore throat (WHO, 1999; EMA, 2012a).

Chemical composition. Saponins (e.g., glycyrrhizin); triterpenes (glycyrrhetinic acid); flavonoids (liquiritin, rhamnoliquirilin, liquiritigenin, besides others); coumarins (e.g., licoarylcoumarin); essential oil (Saxena, 2005; Öztürk et al., 2017; Frattaruolo et al., 2019; El-Saber Batiha et al., 2020a).

Posology (based on traditional uses). 1.5 g of roots in 150 ml, as herbal decoction two times daily (EMA, 2012a).

Preclinical evidence. This herbal medicine has not been experimentally proven for cold symptoms. Other related experimental effects are anti-asthma and antiviral. The anti-asthma activity of licorice was proven in sensitized rats. A crude hydroethanolic extract (100 mg/kg, p.o) exerted a similar effect to prednisolone (10 mg/kg, p.o.) in mast cells degranulation (Patel et al., 2017). Glycyrrhizin improved the survival time of mice infected with the *Influenza* virus. Also, inhibited the SARS-related coronavirus proliferation *in vitro*. Glycyrrhizic acid inhibited the growth of the *Influenza* virus, inflammatory cytokines, as well as the cytopathic effect of the Respiratory Syncytial Virus (RSV) (Fiore et al., 2008).

Clinical evidence. *Glycyrrhiza glabra* preparations have been trialed clinically for respiratory disease (asthma). An RTC, involving 36 patients presenting chronic asthma, showed licorice in a dose of 3.5 mg/kg in 200 ml water, three times daily, was able to improve the pulmonary function's parameter similarly to prednisolone (0.15 mg/kg) as a single daily dose (Al-Jawad et al., 2012). Overall, the clinical evidence is High for asthma.

Safety. Patients affected by hypertension, kidney diseases, liver or cardiovascular disorders, or hypokalemia, should avoid *G. glabra* (Nazari et al., 2017). On the other hand, licorice is widely used in food preparation and, therefore, short term uses seem of little concern in otherwise patients with no history of major diseases. Overall, safety is High.

Specific warnings and precautions of use. In an *in vitro* experiment, licorice ethanol extract inhibits CYP3A4 and CYP2D6 (Budzinski et al., 2000; Pandit et al., 2011).

Overall assessment. *Glycyrrhiza glabra* is used for a long time and can be useful in the relief of respiratory symptoms by

exerting a soothing effect on the respiratory tract. The clinical evidence can be considered as High. This herbal medicine safety can be rated as High, although it should be avoided for some risk groups.

Hedera helix L. - Araliaceae (Leaves)

Indication in the context of respiratory conditions. *Hedera helix* is indicated for some symptoms of respiratory disease, namely expectorant (a medicine that helps to bring up phlegm) for productive (chesty) coughs (EMA, 2015a). Other related indications include its actions as antispasmodic and in the treatment of flu and fever (Bisset, 1994; Blumenthal et al., 1998; Hong et al., 2015; Kruttschnitt et al., 2019).

Chemical composition. Flavonoids and other phenolics (Urban, 1958; Trute and Nahrstedt, 1997; Al-Snafi, 2018); polyacetylenes (falcarinol, dehydrofalcarinol) (Bohlmann et al., 1961; Gafner et al., 1989); saponins (Elias et al., 1991; Crespin et al., 1995; Yakovishin and Grishkovets, 2018); and essential oils (β-caryophyllene, germacrene D, limonene, α- and β-pinene, and sabinene as the main components) (Tucker and Maciarello, 1994).

Posology. Pharmaceutical preparation with a defined chemical profile and an adequate dose need to be used (EMA, 2015a).

Preclinical evidence. This herbal medicine has been experimentally proven for symptoms of respiratory disease, namely productive cough (Hong et al., 2015; Pizzorno et al., 2016). Other related experimental effects include acute and chronic bronchitis, asthma, and pneumonia (Hong et al., 2015; Pizzorno et al., 2016). The *H. helix* extract and isolated compounds exerted a spasmolytic effect in isolated guinea-pig ileum (Trute et al., 1997).

Clinical evidence. *Hedera helix* preparations have been trialed clinically for respiratory diseases such as bronchial asthma, and improvement of airway resistance, intrathoracic gas volume, and forced expiratory volume were evaluated (Hofmann et al., 2003; Guo et al., 2006; Holzinger and Chenot, 2011). However, despite its established traditional use, very few controlled clinical studies have been published. A randomized, double-blind trial was carried was conducted with an *H. helix* standardized extract. A total of 181 patients presenting acute cough was treated with 35 mg of the extract, three times daily, for 7 days. The group treated with ivy extract showed a clinically relevant reduction in cough score, the severity of symptoms associated with cough, and bronchitis, in comparison with the control group. The reduction of symptoms occurred in the first 48 h. of treatment. The observed adverse effects were non-serious, mild, or moderate severity and not related to the treatment (Schaefer et al., 2016). A study involving 139 patients having acute bronchitis and productive cough for at least three days, compared the treatment using a standardized extract of *H. helix* and acetylcysteine. No statistically differences were observed between the treatments. Moreover, both treatments showed to be safe and effective in children and adults (Kruttschnitt et al., 2019; Kruttschnitt et al., 2020). Moreover, a review of the treatment of upper respiratory tract infections identified 10 clinical trials, including three controlled trials (one of which was placebo-controlled) and 7 non-randomized observational studies (Holzinger and Chenot, 2011). Overall, the clinical evidence is High for bronchitis and the common cold.

Safety. Although gastrointestinal reactions (EMA, 2015a) and nausea, vomiting, and diarrhea have been listed among the symptoms of overdose (Bisset, 1994), and although there might be allergic reactions to the saponins, *H. helix* preparations are generally considered to be safe. There was a minimal report of adverse effects in 10 clinical trials, including three controlled trials (one of which was placebo-controlled) and 7 non-randomized observational studies (Holzinger and Chenot, 2011). Overall, the safety is High, and there are no reports to infer that the preparations from the plant may interfere negatively with the disease or NSAIDs.

Specific warnings and precautions of use. *Hedera helix* preparations are contraindicated if the patient presents dyspnea, fever, or purulent sputum. They should be used with care in patients suffering from gastritis or gastric ulcers (EMA, 2015a).

Overall assessment. *Hedera helix* preparations could be useful in the symptomatic relief of respiratory diseases by exerting an expectorant and anti-inflammatory effect on the respiratory tract. In many countries, licensed preparations are regulated for use in children. There is a robust body of clinical evidence that can be considered as High. This herbal medicine safety can be rated as High, due to the side effects that had been related only in an overdose situation.

Justicia pectoralis Jacq. [syn. *Dianthera pectoralis* (Jacq.) J.F.Gmel.] – Acanthaceae (Leaves)

Indications in the context of respiratory conditions. *Justicia pectoralis* (chambá) is indicated for symptoms of respiratory disease, as expectorant (BRASIL, 2011) and immunostimulant (CUBA, 2014).

Chemical composition. Coumarins (mainly coumarin and umbelliferone); flavonoids (e.g., quercetin, kaempferol, swertisin, and derivatives) (Lino et al., 1997; Oliveira et al., 2000); lignans (e.g., justicidin B) (Joseph et al., 1988).

Posology (based on traditional uses). 5 g in 150 ml of water as an infusion (BRASIL, 2011).

Preclinical evidence. This herbal medicine has been experimentally proven for symptoms of respiratory disease, namely asthma. A standardized hydroalcoholic extract of *J. pectoralis* presented inhibitory effects on the tracheal smooth muscle of rats subjected to challenge with ovalbumin (OVA) in an allergen model that reproduces many features of clinical asthma such as bronchial hyper-reactivity. Administered by gavage to sensitized rats after challenge with saline or OVA, the extract of *J. pectoralis* decreased the exacerbated responsiveness of rat trachea caused by the challenge with the sensitizing antigen. The oral administration of the standardized extract reduced the hyperresponsiveness in OVA-challenged trachea in preparations stimulated with KCl (potassium chloride) or ACh (acetylcholine). These effects on rat airways are possibly related to its anti-inflammatory activity, as observed by its ability to significantly inhibit the increase of the levels of TNF-α and IL-1β, pro-inflammatory cytokines, in bronchoalveolar lavage of OVA-

challenged rats. These work findings reinforce the notion that the extract of *J. pectoralis* possesses potential anti-asthmatic properties (Moura et al., 2017). In another study, the aqueous extract of *J. pectoralis* was evaluated in sensitized guinea-pig. The crude extract reduced the formation of histamine-induced wheals. Moreover, the extract was tested on guinea-pig tracheal contraction caused by the cumulative dosing of histamine. *Justicia pectoralis* reduced histamine-induced tracheal smooth muscle contractions (Cameron et al., 2015).

Clinical evidence. *Justicia pectoralis* preparations have been trialed clinically for cough in children only. The efficacy of chambá syrup at 5% was evaluated in a randomized, double-blind clinical trial, in 114 children presenting cough and other respiratory symptoms. Chambá syrup was effective in relieving symptoms of cough, nasal congestion, and rhinorrhea, as well as improving the sleeping capacity of children when compared to placebo (Nascimento, 2018). Overall, the clinical evidence is High for cough.

Safety. In traditional doses, there is no report about the toxicity of *J. pectoralis*, including children.

Specific warnings and precautions of use. Due to the presence of coumarin and derivatives, *J. pectoralis* use should be avoided together non-steroidal anti-inflammatory or anti-coagulant drugs (Wittkowsky, 2003). Overall, safety is High.

Overall assessment. *Justicia pectoralis* preparations might be useful in the symptomatic relief of respiratory disease through exerting an anti-inflammatory effect on the respiratory tract. The clinical evidence may be considered High. Even though chambá is known to have potential antiplatelet effects, overall, it can be considered presenting Medium safety.

Magnolia officinalis Rehder & E.H. Wilson - Magnoliaceae (Bark)

Indications in the context of respiratory conditions. *Magnolia officinalis* is indicated for symptoms of respiratory disease, namely cough, fever, and shortness of breath (WHO, 2009).

Chemical composition. Biphenyl neolignan derivatives: magnolol (5,5'-diallyl-2,2'-dihydroxy biphenyl) and honokiol (5,3'-diallyl-2,4'-dihydroxy biphenyl); isoquinoline-type alkaloids, the majority of which are aporphine (*N*-methylcoxylonine, (*S*)-magnoflorine, magnofficine, (*R*)-asimilobine, corytuberine, anonaine, liriodenine) and benzylisoquinoline derivatives (magnocurarine, (*S*)-tembetarine, lotusine, (*R*)-oblongine, reticuline); essential oil (the major constituents are α-, β-, and γ-eudesmol) (Yan et al., 2013; Poivre and Duez, 2017).

Posology (based on traditional uses). 3-9 g of crude drug (decoction) daily in divided doses (WHO, 2009).

Preclinical evidence. This herbal medicine has been experimentally proven for asthma. Studies in animals and *in vitro* models have demonstrated multiple biological properties of honokiol, including anti-asthma (through IL4 and IFN-NF-kB) (Hong et al., 2018). Other related symptom studies are anti-histamine (Shin et al., 2001) and anti-HIV (human immunodeficiency viruses) activities (Amblard et al., 2006).

Clinical evidence. Magnolia preparations have been trialed clinically for asthma. Preliminary clinical studies showed the benefits of magnolia for oral health (Campus et al., 2011). In non-comparative research, as add-on therapy in 148 patients with mild to moderate asthma using inhaled corticosteroids, an extract of Magnoliae flos had a beneficial effect on asthma control (Park et al., 2012). Overall, the clinical evidence is Medium for asthma.

Safety. Magnolol and honokiol are the main ingredients of magnolia bark and, therefore, were considered directly relevant to evaluate the safety of the product. Full clinical monitoring, including biochemical and hematological analysis, showed no evidence of toxicity reported in subjects consuming magnolia bark containing supplements at a dose of 750 mg/person/day (approximately 15 and 60 mg/person per day of honokiol and magnolol respectively) for 42 days (Garrison and Chambliss, 2006). According to other studies, safety and toxicity of magnolia bark do not differ notably but may reflect insufficiencies of these toxicity studies. Therefore, currently, studies of the mechanisms of toxicity of magnolia bark are insufficient (Luo et al., 2019). However, in the context of COVID-19, magnolol has been identified as a potential enhancer of ACE2 expression (ANSES, 2020). Overall, the safety of *M. oficinalis* is Medium.

Specific warnings and precautions of use. None known.

Overall assessment. Magnolia bark has been used in Chinese and Japanese traditional medicines for the treatment of asthma, allergic disease, as well as for the alleviation of headaches, muscular pain, and fever (Forrest, 1995; Lee et al., 2011). As *M. officinalis* preparations are not clinically proven to provide symptomatic relief of flu symptoms and its active principle (magnolol) may enhance the entry SARS-CoV-2 virus into the cell, the clinical evidence may be considered Medium and the safety, in the COVID-19 context, Medium.

Malva sylvestris L. – Malvaceae (Leaves)

Indication in the context of respiratory conditions. *Malva sylvestris* is indicated for respiratory disease, namely oral or pharyngeal irritations and dry cough (BRASIL, 2011; EMA, 2018d).

Chemical composition. Mucilages (mainly glucuronic and galacturonic acids, rhamnose, galactose, fructose, glucose, trehalose); flavonoids (e.g., malvidin, delphinidin, myricetin, apigenin, kaempferol, genistein, and derivatives); tannins (Farina et al., 1995; Paul, 2016); hydroxycinnamic acid and derivatives; benzoic acid and derivatives; monoterpenes (Cutillo et al., 2006).

Posology (based on traditional uses). 1.8 g in 150 ml as infusion or decoction three times daily (EMA, 2018d).

Preclinical evidence. *Malva sylvestris* preparations have been experimentally studied for cough. The anti-tussive activity of its mucilage and isolated rhamnogalacturonan was evaluated in cats. Both substances suppressed the cough reflex and decreased the frequency of cough, especially in the laryngopharynx area, although the polysaccharide was more active than mucilage (Nosalova et al., 2005). Another study showed anti-inflammatory and analgesic action through traditional pharmacological *in vivo* models (Seddighfar et al., 2020). Mucilage seems to be responsible for the cough suppressive activity of this species (Čapek et al., 1999). Another experimental related activity is anti-inflammatory. *Malva sylvestris* ethanolic extract presented anti-inflammatory activity in

mice through the ear edema model. The extract reduced the levels of IL-1β in tissue challenged by TPA. Also, the extract and isolated compounds inhibited myeloperoxidase activity. Malvidin-3-glucoside presented inhibitory activity similar to dexamethasone (Prudente et al., 2013). The aqueous extract can suppress the expression of several pro-cytokine genes such as TNF-α, IL-1β, COX-2, iNOS (Mirghiasi et al., 2015).

Clinical evidence. *Malva sylvestris* has not been trialed clinically for respiratory disease. Overall, the clinical evidence is Low.

Safety. In traditional doses, there is no report about the toxicity of *M. sylvestris*. However, high doses may cause nausea, vomiting and diarrhea, nervous excitement, and insomnia (BRASIL, 2015a; BRASIL, 2019b). Overall, safety is High.

Specific Warnings and precautions of use. In general, mucilage can alter the absorption of some drugs (BRASIL, 2015a).

Overall assessment. *Malva sylvestris* has been traditionally used as cough therapy and may be useful in the relief of COVID-19 symptoms through exerting a soothing effect on the respiratory tract. The clinical evidence may be considered Low, but this herbal medicine is considered presenting High safety.

Mikania glomerata Spreng. and *M. laevigata* Sch.Bip. ex Baker Asteraceae (Leaves)

Indications in the context of respiratory conditions. *Mikania glomerata* is indicated for symptoms of respiratory disease, namely cough, and as expectorant (BRASIL, 2011; BRASIL, 2017; BRASIL, 2018).

Chemical composition. Essential oil (germacrene D and β-caryophyllene as the major components) (Ueno and Sawaya, 2019); coumarins (e.g., coumarin, trans-*o*-coumaric acid),; phenolic acids (e.g., chlorogenic and caffeoylquinic acids) (Lazzari Almeida et al., 2017); terpenoids and steroids (e.g., friedelin, ent-kaurenoic acid, ent-kaur-16(17)-en-19-oic acid, ent-beyer-15(16)-en-19-oic acid, ent-15β-benzoyloxykaur16(17)-en-19-oic acid, grandifloric acid, ent-cinnamoylgrandifloric acid, ent-benzoylgrandifloric acid 17-hydroxy-ent-kaur-15(16)-en-19-oic acid, stigmasterol, β-sitosterol) (Veneziani and Oliveira, 1999; Bertolucci et al., 2013).

Posology (based on traditional uses). 3 g of dried leaves in 150 ml, as an infusion, 2 times daily. However, the use of dosage forms, with defined composition, is recommended.

Preclinical evidence. This herbal medicine has been experimentally proven for cough. The aqueous extract reduced the contractile effect of histamine on the isolated guinea-pig trachea and human bronchi (Moura et al., 2002). A fraction of *M. glomerata* ethanolic extract was evaluated through an allergic pleurisy model in rats and resulted in the inhibition of leukocyte infiltration (Fierro et al., 1999).

Clinical evidence. *Mikania glomerata* preparations have been trialed clinically for one of the related diseases, namely cough. This herbal medicine has been used in Brazil in respiratory diseases, such as asthma, cough, and throat inflammation (Agra et al., 2008; Brandao et al., 2009). A clinical trial sponsored by the Brazilian government showed that the infusion of *M. glomerata* (5, 10, and 15 g in 200 ml of water) had an unequivocal bronchodilator action and evident dose-dependent antitussive effect (Amaral et al., 2006). A randomized study involving 62 patients compared the bronchodilator effect of *M. glomerata* syrup and salbutamol inhaler and found *M. glomerata* syrup did not present effect (Garcia et al., 2020). However, the overall problem with this study refers to the differences between the investigated pharmaceutical preparations. Overall, the clinical evidence is High for asthma, but Low for other types of cough.

Safety. Some non-clinical studies report its safety. *Mikania glomerata* hydroalcoholic (70%) extract presented LD_{50} ~ 3000 mg/kg and did not produce any biochemical, hematological, and morphological changes in mice (Santana et al., 2013). Moreover, *M. glomerata* did not present genotoxicity, mutagenicity, teratogenicity or antifertility activity (Sa et al., 2003; Sá et al., 2006; Barbosa et al., 2012; Fulanetti et al., 2016). Doses above those recommended may cause vomiting and diarrhea (Matos, 2000; BRASIL, 2011). *Mikania glomerata* syrup is included in the Brazilian List of Essential Medicines, and so far, there are no reported concerns relating to safety from the point of view of pharmacovigilance.

Specific Warnings and precautions of use. Not to be used while under treatment with NSAIDs. The use may interfere with coagulation due to the presence of coumarin derivatives (Matos, 2000; Wittkowsky, 2003; BRASIL, 2011). Overall, safety is Medium.

Overall assessment. *Mikania glomerata* is in the list of essential medicine in Brazil for the symptomatic relief of cough and asthma and has been prescribed for children for several years without a pharmacovigilance report. Preclinical and clinical data back up this indication, although guaco is not clinically proven to provide relief of flu symptoms. The clinical evidence may be considered High. Even though guaco is known to have potential antiplatelet effects, overall, it can be considered presenting Medium safety.

Ocimum gratissimum L. -Lamiaceae (Leaves)

Indication in the context of respiratory conditions. *Ocimum gratisssimum* is indicated for symptoms of cold, influenza, fever, asthma, bronchitis (WHO, 2002).

Chemical composition. Essential oil (eugenol, thymol, and 1,8-cineol as the major components) (Prabhu et al., 2009; Monga et al., 2017); phenolics (quercetin and derivatives, luteolin and derivatives, kaempferol and derivatives, catechin, epi-catechin, cafeic acid); triterpenes (ursolic acid) (Prabhu et al., 2009; BRASIL, 2015b; Siva et al., 2016; Monga et al., 2017).

Posology (based on traditional uses). 1-3 g in 150 ml of boiling water, as an infusion, 3-4 times daily (BRASIL, 2019b).

Preclinical evidence. This herbal medicine has been experimentally proven for cough. An aqueous extract of *O. gratissimum* was evaluated in OVA-sensitized guinea-pig about anti-tussive and anti-asthma activities. The aqueous extract did not exert effect in pre-convulsive dyspnea; however, reduced in a significant manner, the volume of tracheal liquid, although it did not interfere in the fluid viscosity, and reduced in more than 80% the number of cough episodes (Ozolua et al., 2016). Another experimental related activity is anti-inflammatory. The essential oil of *O. gratissimum* presented an antinociceptive effect in mice,

in evaluation using classical methods (Rabelo et al., 2003; Paula-Freire et al., 2013). A flavonoid-rich fraction of *O. gratissimum*, in mice, presented anti-inflammatory activity, reducing the number of the leucocytes in the peritoneum, and inhibited iNOS, COX-2 (in the same degree than indomethacin). Also, inhibited LPS-induced NO, IL-1β, and TNF-a production in RAW 264.7 cells (Ajayi et al., 2017).

Clinical evidence. *Ocimum gratissimum* has not been trialed clinically for respiratory diseases. Overall, the clinical evidence is Low.

Safety. In traditional doses, there is no report about the toxicity of *O. gratissimum*.

Specific Warnings and precautions of use. Due to the presence of estragole, a naturally occurring genotoxic carcinogen, *O. gratissimum* should be used for a short time, in the recommended posology (EMA, 2005) (see also *Foeniculum vulgare*).

Overall assessment. Its traditional use as cough therapy in the context of upper respiratory conditions is not backed up by clinical data profile, but its preclinical evidence and safety profile may allow the potential use in the relief of early symptoms of COVID-19. The clinical evidence is Low, and although there are some concerns about the presence of estragole, in the traditional doses, this herbal medicine is considered presenting High safety.

Pelargonium sidoides DC and/or *Pelargonium reniforme* Curt. -Geraniaceae (Root)

Indications in the context of respiratory conditions. *Pelargonium sidoides* is indicated for the common cold (EMA, 2018c), cough, and bronchitis (COLOMBIA, 2008). Umkaloabo preparations are available in some European Countries with a full marketing authorization (e.g., Bulgaria, Czech Republic, Germany, Latvia) or register as a traditional herbal medicinal product (e.g., Austria, Hungary, Italy, The Netherlands, Poland, Spain, Sweden), and are widely used for 'acute bronchitis' and other respiratory infections' (EMA, 2018c). Practically all research has been conducted with the proprietary extract EPs 7630®. The species originate from Southern Africa, and the term Umkaloabo is claimed to be a fusion of two terms from Zulu ("Umkuhlune" - coughing and fever; and "Uhlabo" = pain in the chest) (Heinrich et al., 2018).

Chemical composition. Relevant key metabolites assumed to be active are hydrolyzable tannins, (+)-catechin, gallic acid, and methyl gallate, including some unusual *O*-galloyl-C-glucosyl flavones., scopoletin, 6,8-dihydroxy-5,7-dimethoxycoumarin, 5,6,7-trimethoxycoumarin. Other coumarins, as well as, quercetin 3-*O*-β-D-glucoside, myricetin, and other flavonoids have been isolated from both species. *Pelargonium sidoides* yielded some benzopyranones. Umckalin is only known from *P. sidoides* (Maree and Viljoen, 2012); pelargoniins (an ellagitannin) and the diterpene reniformin have been identified in *P. reniforme* (Edwards et al., 2015).

Posology. Oral drops, tablets, and syrups are most commonly used (EMA, 2018c). Commercial preparations with regulated quality, a defined composition, and an adequate dose are available on some markets globally.

Preclinical evidence. This herbal medicine has been experimentally proven for anti-viral activity. The commercial extract EPs 7630® interfered *in vitro* with the replication of different respiratory viruses, including human coronavirus (Michaelis et al., 2011), *Influenza* virus (*in vitro* and *in vivo*) (Theisen and Muller, 2012), and *Rhinovirus* isolated from patients with severe asthma (Roth et al., 2019). EPs 7630 was able to stimulate IFN-β *in vitro*, and gallic acid enhanced the expression of iNOS and TNF-α (Kolodziej et al., 2003).

Clinical evidence. *Pelargonium sidoides* preparations have been trialed clinically for cough. In a randomized, controlled trial, involving 124 adults presenting acute bronchitis, were treated with 30 drops of EPs 7630®, for seven days. The treated group showed a significant improvement regarding the Bronchitis Severity Score (BSS) compared with the placebo group (Chuchalin et al., 2005). In a Cochrane review, the efficacy of *P. sidoides* preparations in acute respiratory infections in RCT was assessed and indicated that it might be useful in the alleviation of symptoms of acute rhinosinusitis and the common cold in adults. However, the authors also raised concerns regarding the studies' quality (Timmer et al., 2013). Similarly, Schapowals' meta-analyses of randomized, double-blind, placebo-controlled trials concluded that it is useful in the management of the common cold (Schapowal et al., 2019). Overall, the clinical evidence is High for bronchitis and the common cold.

Safety. Umkaloabo preparations are generally considered to be safe, although gastrointestinal discomfort (stomach pain, heartburn, nausea, or diarrhea) might occur with no major concerns about interaction risks (Edwards et al., 2015). However, due to the potential immunomodulatory activity, overall, safety is Medium.

Specific warnings and precautions of use. As with all other products discussed here, there is no evidence for specific therapeutic benefits, and it is important to communicate this to potential users. The safety concerns listed above need to be kept in mind.

Overall assessment. Umkaloabo may be useful in the symptomatic relief of respiratory symptoms through exerting a soothing effect on the respiratory tract. A relatively large number of clinical studies and a series of meta-analyses provide evidence that Umkaloabo preparations seem to be efficacious both in the treatment (reducing symptoms and duration) and prevention of the common cold. However, it must be discontinued to avoid immunostimulation in complications in later phases of the disease. The clinical evidence is High, and this herbal medicine is considered presenting Medium safety.

Pimpinella anisum L. – Apiaceae (Fruits)

Indications in the context of respiratory conditions. *Pimpinella anisum* is indicated for cough and fever (WHO, 2009; CHILE, 2010) and as antispasmodic (WHO, 2009; CHILE, 2010; BRASIL, 2011).

Chemical composition. Essential oil (*trans*-anethole as the main compound) (Orav et al., 2008); chlorogenic acid derivatives; flavonoids (orientin, vitexin, and others, coumarin

derivatives; triterpenes and steroidal compounds (Zobel et al., 1991; Reichling and Galati, 2004; Abdollahi Fard, 2012).

Posology (based on traditional uses). 1,5 g of the dried fruits in 150 ml, as an infusion, three times daily (BRASIL, 2011; Barnes et al., 2012; EMA, 2013b).

Preclinical evidence. This herbal medicine has been experimentally proven for cough. Aqueous and ethanol extracts of *P. anisum* presented myorelaxant effect in isolated tracheal chains of guinea-pig. The relaxant effect was similar to promoted by theophylline. Interesting to note that the essential oil did not present a significant effect (Boskabady and Ramazani-Assari, 2001).

Clinical evidence. *Pimpinella anisum* preparations have been trialed clinically for asthma. The bronchodilator activity of *P. anisum* was evaluated in a study involving 50 patients presenting bronchial asthma. The patients ingested tea (2 g in 200 ml of water), twice a day for 40 days. All patients presented a reduction of cough episodes after 21 days of treatment (from more than 6 episodes/day to none), as well as dyspnea and wheezing. Also, an improvement in the breath-holding time and respiratory rate was observed (Paheerathan, 2019). Overall, the clinical evidence is High for asthma-related cough and Low for cough and fever.

Safety. In the traditional doses, there is no report about the toxicity of *P. anisum*. However, allergic reactions can occur, for example, rhinoconjunctivitis (García-González et al., 2002; EMA, 2013b). Overall, safety is High.

Specific warnings and precautions of use. The concomitant use with anti-coagulant drugs should be avoided due to the presence of coumarins (Dinehart and Henry, 2005).

Overall assessment. Its traditional use as cough therapy in the context of upper respiratory conditions is not backed up by robust clinical data profile, but its safety profile allows for potential use in the relief of early symptoms of COVID-19. The clinical evidence may be considered High. Even though *P. anisum* has potential antiplatelet effects, overall, it can be considered presenting Medium safety.

Plantago lanceolata L. – Plantaginaceae (Leaves)

Indication in the context of respiratory conditions. *Plantago lanceolata* is indicated for symptoms of respiratory disease, namely cough, pharyngitis, and fever (CHILE, 2010). Other indications include as demulcent, to reduce bronchial catarrh and mild inflammation of the pharynx (Blumenthal et al., 1998; ESCOP, 2003b; Wichtl, 2004; Boskabady et al., 2006a).

Chemical composition. Carbohydrates (Jiang et al., 2019); flavonoids (apigenin, luteolin and their derivatives) (Haddadian and Zahmatkash, 2014; Ferrazzano et al., 2015); iridoid glycosides (aucubin and catalpol as the main ones) (EMA, 2014b; Lotter et al., 2017; Hasan et al., 2018); phenylethanoids (Bahadori et al., 2020); saponins (Wichtl, 2004); tannins (Wichtl, 2004; Grigore et al., 2015); and essential oil (amyl vinyl carbinol (E),4(3-oxo-2,6,6-trimethylcyclo-hex-2-en-1-yl)-3-buten-2-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-7-oxabicyclo[4,1,0] heptan-3-ol, and benzoic acid as major components) (Fons et al., 1998; Bajer et al., 2016).

Posology (based on traditional uses). 2 g of leaves in 150 ml boiling water, two to three times daily; 160-190 mg of powdered plant coated tablet or lozenges, maximum dose of 1.28 g daily (EMA, 2014b). It is preferable to use a commercial preparation with a defined composition and an adequate dose (EMA, 2014b).

Preclinical evidence. This herbal medicine has been experimentally proven for cough. An ethanol extract of *P. lanceolata* reduces the number of coughs provoked by citric acid, in guinea-pig, in a similar way to codeine (Boskabady et al., 2006a). Other experiments showed that an ethanol extract of this species inhibited the barium-induced contraction of guinea-pig isolated trachea (Fleer and Verspohl, 2007). The pharmacological effects described in literature support both the oral and oromucosal traditional use of herbal preparations of *P. lanceolata* as a demulcent for the symptomatic treatment of oral and pharyngeal mucosa irritation, associated to dry cough (Ortiz de Urbina et al., 1994; Herold et al., 2003; Vigo et al., 2005; Hausmann et al., 2007).

Clinical evidence. *Plantago lanceolata* has not been trialed clinically for respiratory tract disorders. However, there is some evidence in the literature for the traditional internal use of *P. lanceolata* as a mucilage in the treatment of irritations of oral and pharyngeal mucosa and associated dry cough (Wegener and Kraft, 1999). However, data on this clinical assay are scarce. Overall, the clinical evidence is Low.

Safety. Currently, there is a lack of toxicity and clinical safety data for *P. lanceolata*, which warrants further investigation for this plant. However, popular use points to hypersensitivity reactions as the main side effects reported (Ozkol et al., 2012; EMA, 2014b). Overall, the safety is High as there are no reports to infer that the preparations may interfere with the disease or NSAIDs.

Specific warnings and precautions of use. The topical use of this plant is not recommended (EMA, 2011a; Ozkol et al., 2012; EMA, 2014b). *Plantago lanceolata* pollen may cause hayfever, especially if the plant is abundant in a region like in many subtropical regions (Calabozo et al., 2001). Pla 11 has been named the main allergen with IgE-binding capacity present in this plant, although other IgE-binding proteins have also been identified (Sousa et al., 2014). Treatment should not exceed one week (EMA, 2014b). Minor diarrhea has also been reported (EMA, 2011a).

Overall Assessment. Although *P. lanceolata* is not clinically proven to provide symptomatic relief of flu symptoms, there are enough preclinical evidences to allow the use of this herbal medicine to relieve respiratory symptoms through exerting an expectorant and anti-inflammatory effect. The clinical evidence is Low, but this herbal medicine is considered presenting High safety.

Platycodon chinensis (Jacq). A.DC. [syn. *Platycodon grandiflorus* (Jacq.) A.DC.] – Campanulaceae (Roots)

Indications in the context of respiratory conditions. *Platycodon chinensis* is indicated for cough (WHO, 1999). Other related indications include expectorant (WHO, 1999); upper respiratory infections; sore throat (PRC, 1992; Li Y. H. et al., 2013).

Chemical composition. Triterpene saponins (2%) (e.g., platycodigenin; polygalacic acid) ((Fu et al., 2011); steroids (δ-stigmasterol, α-spinasterin, betulin) (Wagner H. et al., 2015); essential oil (He et al., 2013).

Posology (based on traditional uses). 2–9 g in 150 ml as a decoction, daily (Chang and But, 1987).

Preclinical evidence. *Platycodon chinensis* has been experimentally proven for cough and fever in mice (Oh et al., 2010; Zhang et al., 2015). An aqueous extract from *P. chinensis* presented anti-inflammatory activity in rats, in the carrageenan model. The extract inhibited the production of cytokines and the expression of COX-2 (Kim et al., 2006). Platycosides are inhibitors of the production of IL-6, PGD2, LTC4, and β-Hex, therefore presenting potential for allergy symptoms treatment (Oh et al., 2010). Crude platycodin (80 mg/kg, p.o.) presented expectorant activity, higher than ammonium chloride, in guinea-pig. The author showed that crude platycodin also presented analgesic and antipyretic activities in mice (Lee, 1975). Crude platycodin presented anti-histaminic and anticholinergic effects in guinea-pig, but not presented anti-serotonin or anti-bradykinin effect (Takagi and Lee, 1972). Isolated platycodin D and D3 presented anti-inflammatory and potential expectorant activities. In rat and hamster tracheal surface epithelial cells, both compounds increased mucin release. Platycodin D3 was most active than ATP (mucin secretagogue) and ambroxol (mucolytic) (Shin et al., 2002). A decoction of *P. chinensis* inhibited cough in mice (Zhang et al., 2015).

Clinical evidence. This herbal medicine has been trialed clinically for pneumonia. Two patients presenting pneumoniae were treated with aqueous extract of *P. chinensis* and acupuncture. Patients presented improvement in cough, phlegm, and fever. Chest X-ray showed a decrease in lung infiltration (Kim et al., 2001). Overall, the clinical evidence is Low.

Safety. *Platycodon chinensis* was considered safe in preclinical evaluation. Platycodins present hemolytic activity; however, as they are poorly absorbed, the oral ingestion in the suggested doses seems to be safe (WHO, 1999). In the context of COVID-19, overall, the safety is High, as there are no reports to infer that such herbal preparations may interfere with the disease or NSAIDs.

Specific warnings and precautions of use. Radix *Platycodi* reportedly depresses the central nervous system (CNS) (Lee, 1975; WHO, 1989). Therefore, the concomitant use of this herbal medicine with alcohol or hypnotic and sedative drugs should be avoided. Overall, the safety is Medium, but there are no reports to infer that such herbal preparations may interfere with the disease or NSAIDs.

Overall assessment. Based on traditional use, preparations could be useful in respiratory symptoms relief, through exerting an antitussive effect, although it is not backed up by robust clinical data. The clinical evidence is Low, but this herbal medicine is considered presenting High safety.

Polygala senega L. – Polygalaceae (Roots)

Indication in the context of respiratory conditions. *Polygala senega* is indicated for cough (WHO, 2002; ESCOP, 2003b). Other related indications include its actions as expectorant bronchitis, emphysema, and catarrh of the upper respiratory tract (Briggs, 1988; WHO, 2002; ESCOP, 2003b; Lacaille-Dubois et al., 2020).

Chemical composition. Organic acids (salicylic acid and its methyl ester) (Bradley, 1992; COE, 2008); monosaccharides (Takiura et al., 1974; Takiura et al., 1975); oligosaccharides (Ikeya et al., 1991; Saitoh et al., 1994); acylated glucosides (Ikeya et al., 1991; Ikeya et al., 1994); triterpene saponins (Tsukitani et al., 1973; Tsukitani and Shoji, 1973; Sakuma and Shoji, 1981; Sakuma and Shoji, 1982; Bradley, 1992; Yoshikawa et al., 1995b; Yoshikawa et al., 1995a; Yoshikawa et al., 1996; Wichtl, 2004; COE, 2008; Evans, 2009); essential oils (Sakuma and Shoji, 1982; COE, 2008), flavonoids; coumarins (Lacaille-Dubois et al., 2020); and xanthones (Ikeya et al., 1994).

Posology (based on traditional uses). Herbal tea (dried root): 0.5–1.0 g three times a day (BHMA, 1983; Bradley, 1992). It is preferable to use a commercial preparation with a defined composition and an adequate dose.

Preclinical evidence. This herbal medicine has not been experimentally proven for symptoms of respiratory disease. However, the expectorant activity of the crude drug is due to the constituent saponins, which produce local irritation of the mucous membranes of the throat and respiratory tract. This irritation stimulates an increase in bronchial secretions, thereby diluting the mucus, reducing its viscosity and facilitating expectoration (Boyd and Palmer, 1946; Misawa and Yanaura, 1980; Reynolds and Parfilt, 1996). Isolated saponins from roots of this species were able to enhance anti-OVA specific IgG and IgG2a in mice (Katselis et al., 2007).

Clinical evidence. *Polygala senega* preparations have not been trialed clinically for respiratory diseases. French patent mentioned that a triterpene acid isolated from *P. senega* possesses anti-inflammatory action (Mitchell and Rook, 1979). Another French Patent (Tubery P., Fr Demande Patent 2,202,683) claimed the fluid extract prepared with *P. senega* could reduce the viscosity of phlegm in patients with bronchiectasis (Barnes et al., 2012). However, data on this clinical trial are limited. Overall, the clinical evidence is Low.

Safety. Generally, there is a lack of toxicity data and clinical safety for *Polygala* species. Saponins of this species are usually irritant to the gastrointestinal mucosa (Mitchell and Rook, 1979), and large doses of the plant are reported to cause nausea, diarrhea, and vomiting (Foster and Tyler, 1999; WHO, 2002). In the context of COVID-19, overall safety is High, as there are no reports to infer that the preparations may interfere with the disease or NSAIDs.

Specific warnings and precautions of use. Due to the lack of toxicity data and potential risks associated with chronic ingestion of this herbal medicine, the use by patients with gastric ulcer or gastric inflammation should be avoided. Chronic exposure of the intestinal mucosa to saponins may also inhibit active nutrient absorption (Capasso et al., 2003).

Overall assessment. Senega preparations might be useful in the symptomatic relief of respiratory symptoms through exerting an antitussive effect. Although the clinical evidence is Low, this herbal medicine is considered presenting High safety.

Polypodium vulgare L. – Polypodiaceae (Rhizomes)

Indication in the context of respiratory conditions. *Polypodium vulgare* is indicated for symptoms of respiratory disease, namely cough (EMA, 2008c).

Chemical composition. Flavonoids (Grzybek, 1976; Karl et al., 1982); hydroxycinnamic acids (caffeic acid 4'-glucoside, 0.6% in

the rhizome) (Jizba and Herout, 1967; Grzybek, 1976); phytoecdysteroids (Arai et al., 1991; Yamada et al., 1992; Marco et al., 1993; Coll et al., 1994; Reixach et al., 1996; Messeguer et al., 1998; Simon et al., 2011); triterpenes (Devys et al., 1969; Robinson et al., 1973; EMA, 2008b).

Posology (based on traditional uses). 4–5 g, as a decoction, 3–4 times a day. Not to be taken for more than a week (EMA, 2008c).

Preclinical evidence. This herbal medicine has been experimentally proven for respiratory disease. A hydroalcoholic extract presented a myorelaxant dose-dependent effect in rabbit isolated tracheal preparation. The effect was cholinergic-like (Naz et al., 2016). However, the expectorant and antitussive effects of saponins are known (Hostettmann and Marston, 2005), and the saponin in the fresh plant of polypody acts as a cough suppressant (Høeg, 1984).

Clinical evidence. *Polypodium vulgare* has not been trialed clinically for respiratory diseases. Overall, the clinical evidence is Low.

Safety. Overall, the safety is High as there are no reports to infer that the preparation from this plant may interfere with the disease or NSAIDs.

Specific warnings and precautions of use. A mild laxative effect may happen when this plant is used as expectorant (EMA, 2008c). Moreover, due to possible hypotensive and hypnotic effects, patients should be aware of potential interactions (Ulbricht et al., 2006).

Overall assessment. *Polypodium vulgare* preparations could be useful in the relief of the respiratory symptoms, through exerting an antitussive effect. Although the clinical evidence is Low, this herbal medicine is considered presenting High safety.

Potentilla erecta (L.) Raeusch. – Rosaceae (Rhizomes, Roots)

Indications in the context of respiratory conditions. *Potentilla erecta* is indicated for cough and symptomatic treatment of minor inflammations of the oral mucosa (EMA, 2010; ESCOP, 2013).

Chemical composition. Hydrolysable tannins (pedunculagin, pentadigalloylglucose, agrimoniin, laevigatin B, tormentillin); condensed tannins/proanthocyanidins ([4,6]-all-trans-bi-(+)-catechin = procyanidin B6, [4,8]-2,3-trans-3,4-cis-bi-(+)-catechin, [4,8]-all-trans-bi-(+)-catechin = procyanidin B3), [6′,6]-all-trans-bi-(+)-catechin, procyanidin B1, procyanidin B2, procyanidin B5, (+)-catechin-[4,8]-(+)-catechin-[4,8]-(+)-catechin, (+)-catechin-[6′,8]-(+)-catechin-[4,8]-(+)-catechin; triterpenoids: 2α,3β-dihydroxyurs-12 -en-28-oic acid β-D-glucopyranosyl ester, 3-epi-pomolic acid 28-*O*-β-D-glucoside, 3β,19α-dihydroxyolean-12-en-28-oic acid β-D-glucopyranosyl ester, 3β,19α-dihydroxyurs-12-en-28-oic acid β-D-glucopyranosyl ester, arjunetin, chinovic acid, kaji-ichogoside F1, tormentic acid, tormentoside (Tomczyk and Latté, 2009; Melzig and Böttger, 2020).

Posology (based on traditional uses). 1.5–2.0 g per 100 ml of water, as an infusion, or 0.8–3.0 g per 100 ml of water, as a decoction. The preparation should be used to rinse the mouth several times daily (EMA, 2010). It is preferable to use a commercial preparation (e.g., tincture) with a defined composition and an adequate dose.

Preclinical evidence. This herbal medicine has not been experimentally proven for symptoms of respiratory disease. However, a moderate antiviral effect against *Herpes viridae* has been demonstrated for extracts of *P. erecta* and hydrolyzable and condensed tannins isolated from this plant. In particular, the antiviral activity against *Herpes virus* types I and II was reported, as well as the cytotoxic activity against *Influenza* virus type A2 and Cowpox (May, 1978). An agrimoniin-rich ethanolic extract of *P. erecta* inhibited the NF-kB and formation of IL-6, and PGE2 in TNF−α stimulated HaCaT cells (Wölfle et al., 2017). An animal test system with mice supported the demonstrated antiviral effects against the *Vaccine* virus together with an induction of interferon synthesis (May, 1978; Lund and Rimpler, 1985).

Clinical evidence. *Potentilla erecta* has not been trialed clinically for respiratory disease. Overall, the clinical evidence is Low.

Safety. Due to the limited studies in humans, animal models are used as alternatives to evaluate the safety of *P. erecta*. Acute toxicity of an aqueous extract of tormentil was assessed in animals using a single oral dose of 2.5 and 6.8 g/kg. No apparent toxic effect was observed. *Potentilla erecta* extracts should be considered safe for acute toxicity when also applied to humans (Shushunov et al., 2009).

Special warnings and precautions of use. Due to thrombolytic activity (Melzig and Böttger, 2020), caution should be taken to avoid interaction with thrombolytic drugs. Therefore, safety is Medium.

Overall assessment. *Potentilla erecta* preparations reportedly have relevant anti-viral effects, but the main uses are for buccal sores and inflammation. It is not established that it has a specific effect on the respiratory tract. The clinical evidence is Low, but this herbal medicine is considered presenting Medium safety.

Primula veris L. - Primulaceae (Roots)

Indications in the context of respiratory conditions. *Primula veris* is indicated for some symptoms of respiratory diseases, namely cough (EMA, 2012b).

Chemical composition. Saponins (e.g., primulasaponin I, primulasaponin II) (Müller et al., 2006); flavonoids (e.g., quercetin, luteolin, kaempferol, and isorhamnetin derivatives); phenolic acids (e.g., chlorogenic acid) (Bączek et al., 2017).

Posology. 0.2–0.5 g in 150 ml of boiling water, as an infusion, 3 times daily (EMA, 2012b). It is preferable to use a commercial preparation with a defined composition and an adequate dose.

Preclinical evidence. This herbal medicine has not been experimentally proven for symptoms of respiratory diseases. However, a *P. veris* dry extract presented potential anti-inflammatory activity *in vitro*, reducing IFN-γ, and inhibited prostaglandin and leukotriene synthesis. Also, inhibited the replication of human rhinovirus (HRV) and respiratory syncytial virus (RSV) (Seifert et al., 2012). A hydroalcoholic extract of *P. veris* presented anti-inflammatory activity in rats in the carrageenan-induced paw edema test (Marchyshyn et al., 2017).

Clinical evidence. *Primula veris* has not been trialed clinically for respiratory diseases. Overall, the clinical evidence is Low.

Safety. Cases of nausea, vomiting, gastric disturbances, and allergies may occur in doses higher than recommended (EMA, 2012b; Ghédira and Goetz, 2017). Overall, safety is High.

Special warnings and precautions of use. Concerns were raised over the concomitant use of *P. veris* and warfarin (Řehulková, 2001).

Overall assessment. Although *Primula veris* is not clinically proven to provide symptomatic relief of flu symptoms, it could be useful in the relief of the respiratory symptoms through an anti-inflammatory effect. The clinical evidence is Low, but this herbal medicine is considered presenting Medium safety.

Salix alba L., *Salix* sp. – Salicaceae (Cortex)

Indications in the context of respiratory conditions. *Salix alba* is indicated as antipyretic (BRASIL, 2011; EMA, 2017d); Other indications are as anti-inflammatory, and in the treatment of the flu and common cold (WHO, 2009).

Chemical composition. It is generally standardized to salicin. However, other salicylates, polyphenols, and flavonoids may also play important roles in therapeutic actions (Shara and Stohs, 2015). Some components: (+)-catechin, syringin, triandrin, ampelopsin, ethyl-1-hydroxy-6-oxocyclohex-2-enecarboxylate taxifolin, 3-*O*-methyltaxifolin, 7-*O*-methyltaxifolin-3-*O*-glucoside; *p*-coumaric, benzoic, *p*-hydroxybenzoic, *p*-methoxy benzoic, cinnamic, trans-p-methoxycinnamic, and cis-p-methoxy cinnamic acids (Agnolet et al., 2012).

Posology (based on traditional uses). 3 g dry stem barks in 150 ml as a decoction, 2–3 times a day (BRASIL, 2011). 1–3 g of the stem bark in 150 ml of boiling water three times daily (EMA, 2017d).

Preclinical evidence. This herbal medicine has been experimentally proven for fever. Its efficacy and safety have been reported in several studies. *Salix alba* extract showed significant analgesic as well as anti-inflammatory properties in mice and showed more potency than the standard drug aspirin in all the doses tested (Gyawali et al., 2013).

Clinical evidence. *Salix alba* preparations have been trialed clinically for fever and inflammation. A randomized study with 210 patients was conducted to evaluate the effectiveness of *Salix* bark extract for the treatment of low back pain. Patients received bark extract orally with 120 mg or 240 mg of salicin or placebo in 4-week. In the last week of treatment, 39% of patients were pain-free for the high dose, 21% for the low dose, and 6% for the placebo (Chrubasik et al., 2000). Another randomized placebo-controlled, double-blind study was conducted involving 78 patients with osteoarthritis. Patients received placebo or willow bark extract containing 240 mg salicin per day for 2 weeks. In the willow bark extract group, the WOMAC pain score was reduced by 14% after 2 weeks, compared with an increase of 2% in the placebo group. Therefore, *Salix* extract was well tolerated, with no adverse effects reported (Schmid et al., 2001). Patients (436) with osteoarthritis and rheumatic pain or back pain or both conditions received by 6-month mono or combination therapy with aqueous *Salix* extract, opioids, and NSAIDs. The results showed that in conjunction with the use of the *Salix* extract, the pain scores were between 33% and 44% of baseline. Drug interactions were not reported (Uehleke et al., 2013). Overall, the level of evidence is High for pain.

Safety. Oral administration of a *Salix alba* ethanol extract when administered to female rabbits and rats did not disrupt the estrous cycle, or inhibit ovulation or fertility and was not teratogenic or embryotoxic at a dose of 1.6 ml/kg (WHO, 2009). *Salix alba* ethanol extract (1.6 ml/kg, intragastric administration, in rats for 13 weeks) had no effect on kidney function, hematological parameters, cholesterol levels, or liver function. The results of the histological evaluation showed no pathological changes in the heart, brain, lungs, kidneys, bone, liver, mammary tissue, reproductive organs, intestines, or stomach (WHO, 2009). In mice (both sexes), *S. alba* ethanol extract presented a median lethal dose range of 28.0–42.0 ml/kg (WHO, 2009). In children can cause a risk of Reye's syndrome, then it is not recommended (El-Radhi, 2018). Also, the use is not recommended in patients with hypersensitivity to other nonsteroidal anti-inflammatory drugs. People with asthma (sensibility to salicylates) due to severe reactions (acute bronchospasms) should avoid the use (WHO, 2009).

Specific warnings and precautions of use. In the case of treatment with anticoagulants, antacids, corticosteroids, or NSAIDs, the use of salix should be avoided (EMA, 2017d). Do not use it in people with gastrointestinal disorders and sensitivity to salicylic acid (BRASIL, 2011). Overall, due to the antiplatelet effect, in the context of COVID-19, the safety should be considered Medium.

Overall assessment. *Salix alba* profile and chemistry fit as an anti-inflammatory and antipyretic therapy in the context of upper respiratory affections. Therefore, this herbal medicine may be useful in the symptomatic relief of respiratory symptoms through exerting an anti-inflammatory and antipyretic effect. The clinical evidence is High. Due to its antiplatelet effects, this herbal medicine is considered presenting Medium safety in the COVID-19 context.

Sambucus nigra L. – Adoxaceae (Dried Flowers)

Indications in the context of respiratory conditions. *Sambucus nigra* is indicated for fever and inflammation of the respiratory tract (WHO, 2002; BRASIL, 2011). Other indications are to relieve cold and flu symptoms, to alleviate headaches, and as expectorant (EMA, 2018e; Torabian et al., 2019).

Chemical composition. Flavonoids, such as kaempferol, astragalin, quercetin, rutin, isoquercitrin, hyperoside; triterpenes (α- and β-amyrin, ursolic acid, oleanolic acid); sterols (β-sitosterol, campesterol, stigmasterol); phenolic acids and their corresponding glycosides (chlorogenic, ferulic, caffeic and *p*-coumaric acids); and essential oil (Sidor and Gramza-Michałowska, 2015).

Posology (based on traditional uses). 3–5 g of dried flowers in 25 ml as an infusion three times daily. Store in a well-closed container, protected from light (Bradley, 1992). 2–5 g dried flowers in 150 ml as an infusion, 2–3 times a day; 3–6 g dried flowers in 150 ml as a decoction, 2–3 times a day (EMA, 2018e).

Preclinical evidence. This herbal medicine has been experimentally proven for fever. The active constituents inhibit the biosynthesis of the inflammatory cytokines IL-1a, IL-1b, and tumor necrosis factor-α in human peripheral mononuclear cells

in vitro (Yeşilada et al., 1997; Torabian et al., 2019). Several *in vitro* and *in vivo* experiments have shown the antiviral effects of extracts from elderberries, against *Influenza* virus (Serkedjieva et al., 1990; Zakay-Rones et al., 1995; Krawitz et al., 2011; Kinoshita et al., 2012; Porter and Bode, 2017).

Clinical evidence. *Sambucus nigra* preparations have been trialed clinically for common cold and flu. Two randomized studies with a standardized extract, involving patients presenting influenza symptoms and positive reaction for *Influenza* virus type A or B, showed the reduction of the symptoms after the second day of treatment, without significant adverse effects (Zakay-Rones et al., 1995; Zakay-Rones et al., 2004). In another randomized study with air travelers, the group using *S. nigra* presented less cold episodes than the placebo group (Tiralongo et al., 2016). A meta-analysis of randomized clinical studies led to the conclusion that *S. nigra* supplementation reduces upper respiratory symptoms of common cold and influenza (Hawkins et al., 2019). Overall, the level of evidence is High for cold and flu.

Safety. No information available on general precautions or concerning drug interactions; non-teratogenic effects in pregnancy or pediatric use (EMA, 2017c). Overall, safety is High.

Specific warnings and precautions of use. None.

Overall assessment. *Sambucus nigra* is traditionally used as cold therapy in the context of upper respiratory conditions and could be useful in the relief of respiratory symptoms through exerting anti-inflammatory and antipyretic effects. The clinical evidence is High, and this herbal medicine is considered presenting High safety.

Scutellaria baicalensis Georgi – Lamiaceae (Roots)

Indications in the context of respiratory conditions. *Scutellaria baicalensis* is indicated for fever (WHO, 2007). Other related indications are as antiviral and to relieve bronchitis symptoms (WHO, 2007; Zhao et al., 2019).

Chemical composition. Flavonoids such as wagonin, baicalcin, and baicalin are the main components (Zobel et al., 1991; Kovács et al., 2004; Reichling and Galati, 2004; Abdollahi Fard, 2012); essential oil (Fukuhara et al., 1987); terpenoids and steroids (e.g., β-caryophyllene, scutebaicalin, β-sitosterol) (Hussein et al., 1996).

Posology (based on traditional uses). 1-2 g of dried roots in 150 ml of boiling water, as an infusion, three times daily (Barnes et al., 2012). 3-9 g of dried roots as infusion or decoction (WHO, 2007).

Preclinical evidence. *Scutellaria baicalensis* has been not experimentally proven for symptoms of respiratory disease. Other related experimental effects include anti-inflammatory. The anti-inflammatory activity was evaluated by *in vitro* and *in vivo* experiments. Extracts inhibited the production of NO, interleukin (IL)-3, IL-6, IL-10, IL-12p40, IL-17, interferon-inducible protein (IP)-10, keratinocyte-derived chemokine, and vascular endothelial growth factor (VEGF) in LPS-induced RAW 264.7 cells (Yoon et al., 2009). Moreover, the aqueous extract of *S. baicalensis* suppressed LP-induced COX-2 protein expression *in vitro*, as well as presented analgesic and anti-inflammatory activities *in vivo* (Lee et al., 2007).

Clinical evidence. This herbal medicine has been not trialed clinically for the following symptom: bronchitis, cough, fever, flu, cold. Overall, the clinical evidence is Low.

Safety. Some stomach discomfort and diarrhea were observed in patients taking preparation of *S. baicalensis* (WHO, 2007). In a randomized, double-blind trial, involving 72 healthy subjects, baicalein (100–2,800 mg) was safe and did not present severe adverse effects (Li et al., 2014). There are reports of baicalein being able to stimulate the ACE2 activity, though (ANSES, 2020). Therefore, safety is Medium.

Special warnings and precautions of use. *Scutellaria baicalensis* should not be used in people with spleen and stomach deficiency (Dinehart and Henry, 2005).

Overall assessment. *Scutellaria baicalensis* may be useful in the relief of respiratory symptoms through the anti-inflammatory activity. The clinical evidence is Low. This herbal medicine is considered presenting Medium safety.

Silybum marianum L. Gaertn. - Asteraceae (Fruits)

Indications in the context of respiratory conditions. *Silybum marianum* is indicated for symptoms of respiratory disease, namely fever, and catarrh (WHO, 2002).

Chemical composition. Flavonolignans such as silymarin, silybin, isosilybin and taxifolin (1.5–3.0%) are the main components reported for *S. marianum* (Wynn, 1999; Porwal et al., 2019); flavonoids (quercetin derivatives); phenolic acids (chlorogenic, caffeic and ferulic acids) (Lucini et al., 2016; Valková et al., 2020).

Posology (based on traditional uses). It is preferable to use a commercial preparation with a defined composition and an adequate dose.

Preclinical evidence. *Silybum marianum* has not been experimentally proven for symptoms of respiratory disease. Silybin, the main flavonolignan from the seed of *S. marianum*, has demonstrated an anti-inflammatory activity by inhibiting the spontaneous and LPS-stimulated NF-kB activation as well as the production of inflammatory cytokines (Corchete, 2008; Giorgi et al., 2012).

Clinical evidence. *Silybum marianum* has not been trialed clinically for respiratory diseases. Overall, the clinical evidence is Low.

Safety. At high doses, mild laxative effect, as well as mild allergic reactions, have been reported (Blumenthal, 2003; Lucini et al., 2016), besides dry mouth, nausea, upset stomach, gastric irritation or headache (EMA, 2016d). A preparation containing 7%–8% silymarin appeared to be safe for up to 41 months of use (Corchete, 2008). Overall, safety is High.

Specific warnings and precautions of use. None.

Overall assessment. Although *S. marianum* is not clinically proven to provide symptomatic relief of flu symptoms, it could be useful in the relief of respiratory symptoms by exerting a systemic anti-inflammatory effect. The clinical evidence is Low. This herbal medicine is considered presenting High safety.

Thymus vulgaris L. - Lamiaceae (Aerial Parts, Leaves)

Indications in the context of respiratory conditions. *Thymus vulgaris* is indicated for productive cough associated with cold,

laryngitis, and tonsilitis (WHO, 1999; CHILE, 2010; EMA, 2013c).

Chemical composition. Essential oil (thymol as the major component); phenolic acids (rosmarinic, ferulic, syringic, coumaric acids), tannins (gallic and protocatechuic acids), flavonoids (luteolin, apigenin, cirsilenol, thymonin, and derivatives, besides others) (Van Den Broucke and Lemli, 1983; Kuete, 2017; Salehi et al., 2019a).

Posology (based on traditional uses). 1–2 g of dried leaves in 150 ml, as an infusion, 3–4 times daily (EMA, 2013c).

Preclinical evidence. *Thymus vulgaris* has not been experimentally proven for symptoms of respiratory disease. However, the myorelaxant effect of ethanolic extract and flavonoids from *T. vulgaris* was evaluated in isolated guinea-pig trachea contracted by acetylcholine. The extract promoted relaxation, similar to DL-isoprenaline (Van Den Broucke and Lemli, 1981). The most active flavonoids were cirsilineol, thymonin, and 8-methylcirsilineol, acting as non-competitive and non-specific Ca^{2+} antagonists (Van Den Broucke and Lemli, 1983). An evaluation of *T. vulgaris* essential oil in rats, through classic methods, showed that the essential oil presents anti-inflammatory activity. Additional experiments showed that carvacrol plays an important role in the activity, with anti-edematogenic and anti-chemotactic action. The inhibition of chemotaxis was also proven by *in vitro* experiments. On the other hand, thymol promoted the release of histamine acting as an irritative agent (Fachini-Queiroz et al., 2012). The hydroethanolic extract of *T. vulgaris* also presents myorelaxant activity *ex vivo* (Meister et al., 1999; Boskabady et al., 2006b). An aqueous extract of *T. vulgaris* presented *in vitro* activity against *Herpes simplex* virus type 1 (HSV-1), type 2 (HSV-2), and HSV-1 acyclovir-resistant (Nolkemper et al., 2006).

Clinical evidence. This herbal medicine has been not trialed clinically, as monodrug, for respiratory disease. Clinical evaluation of thyme has been carried out as an association with another herbal drug, such as *P. vulgaris*. Overall, the clinical evidence is Low.

Safety. In traditional doses, there is no report about the toxicity of *T. vulgaris*. However, high doses (around 10 g) are considered unsafe due to thymol (Basch et al., 2004). Overall, safety is High.

Specific warnings and precautions of use. The use is contraindicated for hypersensitivity to the active substance. Children under 30 months of age should not use this plant due to thymol containing preparations, which can induce laryngospasm (WHO, 1999; EMA, 2013c).

Overall assessment. Although *T. vulgaris* is not clinically proven to provide symptomatic relief of flu symptoms, it may be useful in the relief of respiratory symptoms through spasmolytic and anti-inflammatory effects. The clinical evidence is Low. This herbal medicine is considered presenting High safety.

Tilia cordata Mill. – Malvaceae (Flowers)

Indications in the context of respiratory conditions. *Tilia cordata* is indicated for symptoms of the common cold (CHILE, 2010; EMA, 2012c).

Chemical composition. Essential oil (main components are alkanes, such as 6,10,14-trimethyl-2-pentadecanone and tricosane, besides linalool, E-anethole) (Fitsiou et al., 2007; Kowalski et al., 2017); flavonoids (e.g., quercetin, kaempferol, and derivatives; tiliroside), anthocyanins (Negri et al., 2013; Fawzy et al., 2018).

Posology. 1.5 g in 150 ml of boiling water, as an infusion, 2–4 times daily (CHILE, 2010; EMA, 2012c)

Preclinical evidence. This herbal medicine has not been experimentally proven for the symptoms of respiratory disease. However, procyanidins present in small flowers were evaluated about an inflammatory response in human neutrophils in the concentration ranging from 5 to 20 μM. All compounds were able to decrease ROS production from f-MLP-stimulated neutrophils. Most compounds were able to inhibit LPS-induced IL-8 release. Some trimeric and tetrameric derivatives were also able to decrease the production of MIP-1β. A hydromethanolic extract presented anti-inflammatory and antinociceptive activities in rats, in a similar way than indomethacin and acetylsalicylic acid, respectively (Fawzy et al., 2018).

Clinical evidence. This herbal medicine has been not trialed clinically for respiratory diseases. Overall, the level of evidence is Low.

Safety. Overall safety is High, as there are not reports to infer that the preparations from the plant may interfere with the disease.

Specific warnings and precautions of use. Flavonoids present in *T. cordata* exert an anxiolytic effect and can, potentially, interact with serotonergic drugs (Noguerón-Merino et al., 2015).

Overall assessment. *Tilia cordata* is not clinically proven to provide symptomatic relief of flu symptoms. However, this herbal medicine may be useful in the relief of respiratory symptoms through an anti-inflammatory effect. The clinical evidence is Low. This herbal medicine is considered presenting High safety.

Zingiber officinale Roscoe - Zingiberaceae (Rhizome)

Indications in the context of respiratory conditions. *Zingiber officinale* is indicated for common cold and cough (COLOMBIA, 2008). Its use as anti-asthma (WHO, 1999) and expectorant (BRASIL, 2011) have also been reported.

Chemical composition. Zingerone, shogaols, gingerols, paradols, wikstromol, and carinol (Idris et al., 2019). Total phenolic of 1.02 ± 0.03 mg gallic acid equivalent/L of infusion (Mesquita et al., 2018).

Posology (based on traditional uses). 1 g of dried rhizoma in 150 ml, 3–4 times daily (BRASIL, 2011; EMA, 2011b).

Preclinical evidence. This herbal medicine has been experimentally proven for fever (Ueki et al., 2008; Akbar, 2020). It is described in several preclinical studies report its anti-inflammatory, antipyretic and analgesic properties (Mascolo et al., 1989; El-Abhar et al., 2008; Sepahvand et al., 2010; Ahmed et al., 2011; Darvishzadeh-Mahani et al., 2012; Hsiang et al., 2013; Rashidian et al., 2014). The anti-inflammatory effect of ginger is well established as *in vivo* as *in vitro* models (Thomson et al., 2002; Ali et al., 2008). 6-Shogaol interferes with the inflammatory cascade, inhibits COX, and the prostaglandin release (Rehman et al., 2011). Moreover, 6-gingerol and 6-shogaol present anti-platelet aggregation activity *in vitro* (Liao et al., 2012).

Clinical evidence. This herbal medicine has been trialed clinically for respiratory diseases. In a randomized study, 32 patients with acute respiratory distress syndrome (ARDS) received an enteral diet enriched with ginger or placebo, through a nasogastric tube, during 21 days. On day 5, the patients that received ginger presented lower serum levels of IL-1, IL6, and TNFa, while the level of RBC glutathione presented higher in comparison with the placebo group. Moreover, a significant improvement in oxygenation was observed in the ginger group. The same results were observed on day 10. The authors described that there was a significant difference in the duration of mechanical ventilation and in the time expended in the intensive care unit. However, barotrauma, organ failure, and mortality between ginger and placebo groups were similar (Vahdat Shariatpanahi et al., 2013). Overall, the level of clinical evidence is Medium.

Safety. Although regulatory authorities recommend avoiding the use in pregnancy and lactation (EMA, 2011b), ginger use during pregnancy was reported as safe in Canadian, Australian and Norwegian women (Portnoi et al., 2003; Willetts et al., 2003; Westfall, 2004; Heitmann et al., 2013). Clinical studies found ginger as effective and safe to prevent pregnancy-associated nausea and vomiting (Borrelli et al., 2005; Ding et al., 2013). In human liver microsomes, the hydroethanolic extract inhibited CYP2C19 and CYP2D6 (Kim et al., 2012; Gorman et al., 2013).

Specific warnings and precautions of use. Stomach upset, eructation, dyspepsia, and nausea have been reported (EMA, 2011b). High doses (12–14 g) of ginger may increase the effects of anticoagulant therapy (Thomson et al., 2002; BRASIL, 2011; BRASIL, 2016). Studies showed the effect of ginger on cytochrome P450 enzyme-mediated drug metabolism, which can cause interference on the outcome of the treatment of some conventional drugs. Ginger stimulated levels of CYP450 and cytochrome b5 in rats (Sambaiah and Srinivasan, 1989). The use is contraindicated for people with gallstones, gastric irritation, and high blood pressure (BRASIL, 2011). Overall, safety is Medium due to the potential antiplatelet activity.

Overall assessment. *Zingiber officinale* profile and chemistry fit as an anti-inflammatory therapy in the context of upper respiratory affections. Therefore, this herbal medicine may be useful in the relief of respiratory symptoms through exerting an anti-inflammatory effect. The clinical evidence is Medium. This herbal medicine is considered presenting Medium safety.

Herbal Remedies Used to Relieve Symptoms Related to Respiratory Conditions Not Listed By International Monographs

Some species, although without the support of official documents, have been widely used to relieve common cold symptoms, usually as homemade preparations. Some of them are analyzed here, in the COVID-19 context.

Citrus limon (L.) Burm. f. – Rutaceae (Fruit)

Indications in the context of respiratory conditions. *Citrus limon* has been used to relieve cough, and as expectorant in bronchitis (Papp et al., 2011; Klimek-Szczykutowicz et al., 2020); and as anti-inflammatory (Parhiz et al., 2015), sore throat (Balogun and Ashafa, 2019).

Chemical composition. Essential oil (limonene and α-terpineol as major components) (Mahalwal and Ali, 2003); flavonoids (e.g., hesperidin, hesperetin, naringenin) (Abad-García et al., 2012; Parhiz et al., 2015); phenolic acids (e.g., *p*-cumaric, ferulic, quinic acids and derivatives) (Abad-García et al., 2012), coumarins and furanocoumarins; vitamin C (Klimek-Szczykutowicz et al., 2020).

Posology. Slices of whole fruit or fresh pericarp, as a decoction, 2–3 times daily; expressed juice (Panizza et al., 2012).

Preclinical evidence. This herbal medicine has not been experimentally proven for respiratory disease.

Clinical evidence. This species has not been trialed clinically for respiratory disease. Overall the clinical evidence is Low.

Safety. *Citrus limon* is an edible fruit, therefore, considered safe, although there may be a risk of minor burns on the skin if in contact with sun exposure, due to the presence of furanocoumarins (Palazzolo et al., 2013). Overall safety is High.

Specific warnings and precautions of use. *Citrus* flavonoids can exert severe interaction with drugs metabolized by inhibition of CYP3A4, CYP2C9 (Bailey et al., 1998; Mallhi et al., 2015). *Citrus limon* presents anti-platelet activity. Therefore, the concomitant use with anti-platelet drugs should be avoided.

Overall assessment. *Citrus limon* can be useful in the relief of respiratory symptoms, especially cough and sore throat. The clinical evidence is Low. Due to its antiplatelet effects, this herbal medicine is considered presenting Medium safety in the COVID-19 context.

Culcitium canescens H&B. – Asteraceae (Leaves and Roots)

Indications in the context of respiratory conditions. In Peru and adjoining countries, *Culcitium canescens* is used to relieve cough and fever (Okuyama et al., 1994). Another related indication includes asthma (D'Agostino et al., 1995; Ramirez et al., 2020)

Chemical composition. Furanoeremophilanes (e.g., cacalonol, cacalohatine, dehydrocacalohastine) (Abdo et al., 1992; Okuyama et al., 1994); phenolic compounds (e.g., kaempferol and derivatives, quercetin and derivatives, caffeic acid) (D'Agostino et al., 1995); terpenes and steroids (spatulenol, lupeol, damaradienone) (Ramirez et al., 2020).

Posology (based on traditional uses). 1–2 g of leaves in 150 ml, as an infusion, up to three times daily (Ramirez et al., 2020).

Preclinical evidence. *Culcitium canescens* has not been experimentally proven for respiratory disease.

Clinical evidence. This species has not been trialed clinically for respiratory disease. Overall the clinical evidence is Low.

Safety. Little or anything is known about the toxicity of this plant (Ramirez et al., 2020).

Specific warnings and precautions of use. None.

Overall assessment. Although *Culcitium canescens* profile and chemistry fit as an anti-inflammatory therapy in the context of upper respiratory affections, and preparations are traditionally used, the lack of knowledge about this species does not allow a clear recommendation for the relief of early symptoms of COVID-19. The clinical evidence is Low.

Laurus nobilis L. – Lauraceae (Berries/Leaves)

Indications in the context of respiratory conditions. *Laurus nobilis* is used to treat respiratory infections (Chevallier, 1996; Ross, 2001).

Chemical composition. Berries: essential oil (β-ocimene and 1,8-cineole as the main components); sterols (e.g., β-sitosterol); flavonoids (cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside, as the major anthocyanins) (Kilic et al., 2004; Beis and Dunford, 2006; Abu-Dahab et al., 2014). Leaves: essential oil (1,8-cineole and *a*-terpinyl acetate as the major compounds) (Fidan et al., 2019). Further phytochemical investigations of laurel leaves led to the isolation of sesquiterpene lactones, alkaloids, glycosylated flavonoids, and monoterpene and germacrane alcohols (Dall'Acqua et al., 2009).

Posology (based on traditional uses). The only dosage information on *L. nobilis* is given by the American Pharmaceutical Association: 1–2 tablespoons leaf/cup water and 3 times/day or 1–2 drops of essential oil added to honey, or tea (Duke et al., 2002).

Preclinical evidence. This species has not been experimentally proven for respiratory disease. The closest experiment is an *in vitro* study examining SARS-CoV and the effect of several essential oils. The authors reported that a distilled oil extracted from *Laurus nobilis* berries was an effective virucidal against SARS-CoV. This essential oil also contained eremanthin and dehydrocostus lactone as minor constituents at 3.65% and 7.57%, respectively. These compounds are somewhat unusual in essential oils, but at least one *in vitro* study found that dehydrocostus lactone had activity against the hepatitis B virus, an enveloped DNA virus (Loizzo et al., 2008). The essential oil of *L. nobilis* has also been evaluated for its antinociceptive and anti-inflammatory activities in mice and rats. The essential oil exhibited a significant analgesic effect in tail-flick and formalin tests, a dose-dependent anti-inflammatory effect in formalin-induced edema, and a moderate sedative effect at the anti-inflammatory doses (Sayyah et al., 2003).

Clinical evidence. This species has not been trialed clinically for respiratory disease. Overall, the clinical evidence is Low.

Safety. There is not enough reliable information about the safety of taking *L. nobilis* leaves and berries. As *L. nobilis* has been used as food since ancient times, overall, it can be considered safe in the recommended doses. Therefore, safety is High.

Specific warnings and precautions of use. None.

Overall assessment. *Laurus nobilis* profile and chemistry fit as an anti-inflammatory therapy in the context of upper respiratory affections. Therefore, this herbal medicine could be useful in the relief of respiratory symptoms through exerting anti-viral and anti-inflammatory effects on the respiratory tract. The clinical evidence is Low. This herbal medicine is considered presenting High safety.

Lippia graveolens Kunth. – Verbenaceae (Leaves, Aerial Parts)

Indications in the context of respiratory conditions. *Lippia graveolens* is used to treat symptoms of respiratory diseases, and as anti-inflammatory (Rastrelli et al., 1998).

Chemical composition. Iridoid and secoiridoid glucosides and ester derivatives (Rastrelli et al., 1998); essential oil (thymol, limonene, carvacrol, as the major components) (Compadre et al., 1987; Uribe-Hernández et al., 1992); lipids (esterols) and flavonoids (e.g., pinocembrin, luteolin, apigenin, quercetin and derivatives) (Lin et al., 2007; Arias et al., 2020); rosmarinic acid (Compadre et al., 1987).

Posology (based on traditional uses). 1–2 g of dried leaves or stem in 150 ml of water (Dominguez et al., 1989).

Preclinical evidence. This species has not been experimentally proven for respiratory disease. Flavonoid and terpenoid fractions from *Lippia* genus have demonstrated a significant inhibitory effect on ROS and NO production and mitochondrial activity in LPS-induced inflammation in RAW 264.7 macrophage cells. Additionally, these fractions exhibited non-selective inhibitions against the activity of the cyclooxygenases COX-1 and COX-2 (Leyva-López et al., 2016).

Clinical evidence. This species has not been trialed clinically for respiratory disease. Overall, the clinical evidence is Low.

Safety. In the United States, the regulatory status "generally recognized as safe" has been accorded to Mexican oregano (GRAS 2827). Therefore, safety is High.

Specific warnings and precautions of use. None.

Overall assessment. *Lippia graveolens* profile and chemistry fit as an anti-inflammatory therapy in the context of upper respiratory affections. Therefore, this herbal medicine could be useful in the relief of respiratory symptoms through exerting an anti-inflammatory effect. The clinical evidence is Low. This herbal medicine is considered presenting High safety.

Nigella sativa L. - Ranunculaceae (Seeds)

Indications in the context of respiratory conditions. Black seeds are globally known as a spice and as such as a food item. In Arabic medicine, they have very high status as an herbal medicine used for a wide range of diseases. In the context of COVID-19, the relevant uses are for asthma and in the more general management of inflammatory conditions. In many Arabic countries, black seed is used for asthma and cough, among many other uses, especially for gastrointestinal diseases (like abdominal pain, stomach ache, colic), rheumatism, skin diseases (Lebling and Pepperdine, 2006).

Chemical composition. Relevant secondary metabolites in the seed include large quantities of fixed oil (30%–45%; especially linoleic and oleic acids), various triterpenoids, including saponins; essential oil, with a relatively high concentration of thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone), which is also a key therapeutically relevant of the fixed oil, where it can be found in concentrations varying from 3.5 to 8.7 mg/g of fixed oil (Lutterodt et al., 2010). Thymoquinone is not found in relevant concentrations in aqueous preparations.

Posology. Capsules containing fatty oil with a defined amount of thymoquinone are likely to be the best option. Herbal teas and other hydrophilic preparations seem less likely to have benefits, and potentially the essential oil may offer some relief.

Preclinical evidence. In rats, a chemically not well characterized 50% ethanol extract (1:4) of chopped *N. sativa* seeds (extraction at room temperature for 72 h) showed ameliorating effects on lung inflammation and oxidative stress induced by lipopolysaccharide. In general, many study focus on

the effects of *N. sativa* preparations (Yimer et al., 2019), but mostly these are of highly inadequate scientific quality.

Clinical evidence. Some small studies demonstrate clinical benefits in asthma patients. In a randomized, double-blind, placebo-controlled trial supplementation of standard therapy with soft gel capsules of cold-pressed *N. sativa* oil (0.7% thymoquinone) improved asthma control with a trend in pulmonary function improvement. Two of the five subscores (impact of asthma on work, school, or at home and shortness of breath and the overall score) showed a significant improvement. The other three were not significantly improved. At the same time, a remarkable normalization of blood eosinophilia count was observed (Koshak et al., 2017). The release of IL-2, IL-6, and PGE2 in T-lymphocytes, as well as IL-6 and PGE2 in monocytes, were suppressed. Overall, the clinical evidence is Medium for asthma.

Safety. As a commonly used food item, it can be considered to be intrinsically safe. However, this does not apply to the lipophilic extracts discussed above. Here further evidence is essential. Insufficient evidence is available in the context of uses as an adjuvant medication (Nguyen et al., 2019). Overall, safety is High.

Specific warnings and precautions of use. Again, there is no evidence for specific therapeutic benefits, and it is important to communicate this to potential users. There is insufficient evidence for use during pregnancy and while breastfeeding.

Overall assessment. Black seed may be useful in the relief of respiratory symptoms mainly associated with the severe cough experienced, and it may reduce inflammatory parameters. The clinical evidence is very limited, focusing on asthma. A particular concern, in this case, are the many well-intended but very low-quality studies and the broad range of claims they try to support, making any assessment problematic. The clinical evidence is Medium. This herbal medicine is considered presenting High safety.

Plectranthus amboinicus Lour. – Lamiaceae (Leaves)

Indications in the context of respiratory conditions. *Plectranthus amboinicus* has been used in asthma and to relieve cold, headache, and fever (Menéndez Castillo and Pavón González, 1999; Duke et al., 2002).

Chemical composition. Essential oil (thymol and carvacrol as the major components) (Pino et al., 1990; Arumugam et al., 2016); phenolic compounds (e.g., rosmarinic, caffeic, gallic and *p*-coumaric acids; quercetin derivatives; luteolin) (Brieskorn and Riedel, 1977b; Arumugam et al., 2016), terpenoids and steroids (e.g., β-sitosterol, α-amyrin, stigmasterol) (Brieskorn and Riedel, 1977a; Risch et al., 2012).

Posology (based on traditional uses). 3–5 g of fresh leaves in 150 ml, as an infusion, up to three times daily (Albornoz, 1993; Matos et al., 2001).

Preclinical evidence. This herbal medicine has not been experimentally proven for respiratory disease. Aqueous and ethanolic extracts of *P. amboinicus* present *in vitro* anti-inflammatory activity (Devi and Periyanayagam, 2009; Ravikumar et al., 2009; Janakiraman and Somasundaram, 2014). The active constituents of *P. amboinicus* present inhibitory effects on pro-inflammatory cytokines TNF-α, IL-6, and IL-1β (Yashaswini and Vasundhara, 2011).

Clinical evidence. This species has not been trialed clinically for respiratory disease. Overall, the clinical evidence is Low.

Safety. No toxicity reported at the indicated dose (Albornoz, 1993; Matos et al., 2001). Overall, safety is High.

Specific warnings and precautions of use. The use is contraindicated for hypersensitivity to the active substance. Children, under 30 months of age, should not use this plant due to thymol containing preparations, can induce laryngospasm (see *Thymus vulgaris*).

Overall assessment. *Plectranthus amboinicus* profile and chemistry fit as an anti-inflammatory therapy in the context of upper respiratory affections and could be useful in the relief of respiratory disease through exerting an anti-inflammatory effect on the respiratory tract and in the treatment of fever. The clinical evidence is Low, but this herbal medicine is considered presenting High safety.

Discussion

General Discussion About the Position of International Health Organizations, National Authorities, and Professional Bodies on the Use of Herbal Medicines Within the Context of COVID-19 Disease

The use of herbal medicines/food supplements to prevent, treat, mitigate, diagnose, or cure coronavirus disease 2019 has not been consistently addressed at a global level. China has been actively exploring how to integrate traditional Chinese medicine (TCM) into western therapy since the SARS outbreak in 2003. Based on clinical results, the General Office of the National Health, and the Office of the State Administration of Traditional Chinese Medicine encouraged the integration of herbal TCM and Western medicine in the treatment of respiratory complications in Coronavirus infections with different prescriptions recommended in different stages of disease (Leung, 2007). After SARS-CoV-2 disease outbreak, a number of clinical trials with Traditional Herbal Medicines (THM) have been added to the body of evidence with some favorable findings when compared to standard antiviral therapy (Langeder et al., 2020; Li et al., 2020; Luo et al., 2020; Rastogi et al., 2020).

Therefore, it is not surprising that thousands of TCM practitioners across China have been working along with regular medical doctors to control the COVID-19 disease. The clinical data generated from tens of thousands of confirmed cases are being subjected to meta-analyses to ascertain their actual effectiveness (Li et al., 2020). In the meantime, the Diagnosis and Treatment Protocol of COVID-19 by the National Health Commission has already advised to integrate TCM in the treatment of COVID-19 patients to effectively relieve symptoms such as fever, cough, sore throat, myalgia, and fatigue, shorten the course of the disease, reduce the probability of life-threatening complications (Xu et al., 2020).

In India, the Ayurveda Practitioners are following suit by working on setting clear protocols with a rationale consistent with their millenary system. The first line of defense recommended to early-stage patients consists of the following herbal medicines: *Tinospora cordifolia* (Willd.) Myers (Menispermaceae), *Zingiber officinale* Roscoe (Zingiberaceae), *Curcuma longa* L. (Zingiberaceae), *Ocimum sanctum* L. (Lamiaceae), *Glycyrrhiza*

glabra L. (Fabaceae), *Adhatoda vasica* Ness (Acanthaceae), (Acanthaceae), *Andrographis paniculata* (Burm.f.) (Acanthaceae), *Swertia chirata* Buch.-Ham. ex Wall. (Gentianaceae), *Moringa oleifera* Lam (Moringaceae), Triphala [a mixture of the dried fruits of *Emblica officinalis* Gaertn. (Phyllanthaceae), *Terminalia bellirica* (Gaertn.) Roxb. (Combretaceae), and *Terminalia chebula* Retz.(Combretaceae)] and Trikatu [a complementary formula to Triphala, including *Piper nigrum* L. (Piperaceae), *Piper longum* L. (Piperaceae), and *Z. officinale* Roscoe] (Rastogi et al., 2020). Clinical settings and regulations of research on COVID-19 through Ayurveda, Unani, Siddha, and Homeopathy systems have already been published by the Indian Ministry of Ayush (INDIA, 2020).

However, in Western medicine, we are witnessing quite the opposite. Not only integration of herbal medicine is "out of the question" from an emergency clinical point of view, but authorities are also reluctant - if not discouraging - the self-prescription of any products other than paracetamol in early stages of the disease. The media trying to get answers about the benefits of herbal medicines turn to medical experts who usually caution against the use of herbal medicines in order to avoid any liabilities. Oftentimes, dismissing the potential of herbal medicines to relieve early COVID-19 symptoms is just an uncritical and legalist approach. An international initiative from Brazilian Academic Consortium for Integrative Health - CABSIn, TCIM Americas Network, and the Latin-American and Caribbean Center on Health Sciences Information (BIREME/PAHO/WHO) is systematizing the available evidence about Traditional, Complementary and Integrative Medicine (including herbal medicines) potentially useful in the COVID-19 pandemic (Portella et al., 2020).

Traditional herbal medicines are poorly or not regulated as licensed/registered medicines in quite a few countries, including the USA, and therefore are considered "food," precluding any high-level discussion on their potential contributions to human health. Consequently, the U.S. Food and Drug Administration (FDA) considers fraudulent to sell any herbal products with attached claims and has sent warning letters to companies offering herbal products such as teas, essential oils, and tinctures claiming to prevent, treat or cure the symptoms of COVID-19. It is also encouraging the public to report the unlawful sales of medicinal products on the Internet (FDA, 2020). The North American public is receiving advice on the use of herbal products from dedicated academic groups. One of them is the Andrew Weil Center for Integrative Medicine which recommends polyphenol-rich plants (chamomille, Chinese skullcap, licorice, onions, apples, tomatoes, oranges, nuts, berries, parsley, celery, turmeric root, and green tea) to reduce the risk of infection (Alschuler et al., 2020), but no detailed assessment is available.

In Europe, Traditional Herbal Medicine Products (THMP) are regulated by the EMA as OTC medicinal products for the relief of mild, self-limiting conditions, including flu symptoms and cough. Surprisingly, there is not any overarching official guidance with respect to the use of THMP in the context of the pandemic. In principle, THMP would not qualify as therapy for COVID-19 symptoms as it is considered a severe life-threatening condition. However, this disease is self-limiting in most of the patients and - in the absence of a positive result in a test – COVID-19 patients are just 'flu' patients and, as such, could self-medicate with THMP. Several herbal drugs here reviewed have been registered as THMP. EMA stresses in its guidelines to healthcare professionals to report all "*suspected side effects your patients experience while infected, regardless of the medicines intended to treat the disease or pre-existing conditions. Suspected side effects should be reported even if the medicine is not authorized for use in COVID-19. When reporting a suspected side effect in a patient, you should tell us of any other medicines being taken around the same time, including non-prescription medicines, herbal remedies, or contraceptives*" (EMA, 2020).

We have witnessed how, in different countries, the authorities have developed an array of tools both to inform and enforce guidelines: Italian police are acting at retail commerce level by seizing on the spot any products sold under fraudulent claims; the Brazilian Ministry of Health is fighting back any misleading claims with a dedicated channel in WhatsApp (BRASIL, 2020). The case of France is unique in that its health authorities have provided an extensive and detailed analysis of the potential interactions of natural products with either the disease or its clinical treatment (ANSES, 2020). Finally, many other countries lack the resources to either reach out to the public in this specific matter or enforce any significant actions regarding to the sale of such products.

The WHO regional office for Africa has issued a statement supporting scientifically-driven traditional medicine as "a valid approach towards the treatment of the virus, as long as their efficacy and safety are proven through rigorous clinical trials" (WHO, 2020b), in reaction to a self-proclaimed "COVID-19 herbal cure" promoted at high governmental levels in Madagascar under the name COVID Organics (CVO) (BBC, 2020; WHO, 2020b).

The potential risks and public impact associated with any advice on this disease are huge, and understandably; so, Herbalists associations are more cautious than usual in promoting herbal medicines in this context. In the UK, the NMHI just encourages its members to "integrate the herbal advice they provide with any conventional treatment or management strategies that are appropriate for their patients, and to observe the current infection control and management guidelines formulated by the Department of Health" (NMHI, 2020); and the College of Medicine actively recommends dietary interventions such as boosting immunity against coronavirus by 'turning' to antioxidants and polyphenol-rich food (Dixon, 2020).

General Discussion About the Efficacy of Reference Drugs and Herbal Medicines in the Relief of Flu Symptoms in the Context of COVID-19

Despite the massive use of codeine, paracetamol, and ibuprofen for the relief of respiratory diseases, the clinical body of evidence supporting their use in flu is very low. These drugs are mostly used based on medical experience and clinicians are familiar with their benefits/risks profiles. These drugs have very common toxicities and their adverse effects are potentially dangerous in the context of COVID-19 (Jóźwiak-Bebenista and Nowak, 2014; Drugs.com, 2020a; Drugs.com, 2020b).

It is surprising the lack of clinical trials to substantiate the use of both paracetamol and ibuprofen in the treatment of cold/flu symptoms. As noted in previous sections, clinical evidence is somewhat higher for ibuprofen than for paracetamol.

General Discussion About the Safety of Reference Drugs and Herbal Medicines in the Relief of Flu Symptoms in the Context of COVID-19

There is quite a consensus in that the patient may safely choose to use herbal medicines and food supplements in the prevention of COVID-19. In line with EMA, we advise the patients to keep a diary of the medication (herbal or not) to inform medical doctors and other health care professionals in case of hospitalization worsening of the disease.

Two types of interactions are important in COVID-19: disease-herbal medicine and herbal medicine-drug interactions. There is a complicated relationship between the immune system and viral infections: infection is favored by a weak immune response, whilst complications arise from an 'overreaction' of the immune system known as "cytokines storm". As a result, health authorities discourage any therapies modulating the function of immune cells in any of both directions -including anti-inflammatory drugs- and reserve those to clinical management of advanced stages of COVID-19.

To complicate matters further, a significant fraction of COVID-19 patients in advance stages may require emergency treatment consisting of mechanical ventilation under general anesthesia. It is standard protocol for anesthetists to advise against taking herbal supplements at least 2 to 3 weeks before surgery or other procedures requiring anesthesia, due to potential pharmacodynamic and pharmacokinetic interactions with these procedures (Levy et al., 2017).

As a result of all the above, the use of any herbal-based products has been strongly discouraged by medical authorities, even for typical symptoms of the early stages of COVID-19. Patients are asked to rest, take paracetamol - if fever is dangerous only - and plenty of water. Such "Keep calm and carry on" clinical advice is not realistic and causes dissatisfaction in patients that are not ready to just 'sit and suffer' with the added distress of the potentially lethal complications. It is all human that they will try whatever is offered from alternative medicinal systems to minimize both symptoms and viral load.

Interestingly, there is seldom any mention to anti-tussive medication, one of the most characteristic symptoms of the COVID-19 disease, which chiefly contributes to its spread. Available cough medicines range from OTC products such as simple linctus (usually containing citric acid in a water-glycerol base) and THMP (in Europe) to pharmacy/prescription-only opioids such as dextromethorphan or codeine.

Potential Drug-COVID-19 Interactions

Early clinical data on the evolution of COVID-19 patients pointed out to a deleterious influence of ibuprofen and ketoprofen even taken as short as 2–4 days during the onset of the infection (ANSES, 2020), and prompted French authorities to discourage public to use NSAIDs other than paracetamol. Later data did not clearly substantiate the link between NSAIDs and worsening of the symptoms, so EMA released a communication clarifying this point and calling for rational use of NSAIDs 'at the lowest effective dose for the shortest possible period' (EMA, 2020).

The basis for lower use of NSAIDs has been substantiated on two lines of thinking: (a) they non-specifically impair the immune response thus potentially facilitating the viral infection; (b) some NSAIDs such as ibuprofen, potentially increase the expression of proteins necessary for the entry of the virus into the cell. The later includes proteins vimentin (streptococcus) and ACE-2 (coronavirus).

Surprisingly, paracetamol counts among its many potential adverse effects "very common incidence" (1% to 10%) of dyspnea, abnormal breath sounds, pulmonary edema, hypoxia, pleural effusion, stridor, wheezing, and coughing.

Potential Herbal-COVID-19 Interactions

Herbal Medicines Acting as NSAIDs and/or Immunomodulators

The French authorities have issued a detailed document warning against all herbal medicines with anti-inflammatory and immunomodulatory activities and discouraging their liberal use if flu symptoms appear, whilst the COVID-19 pandemic is going on - unless the patient is tested negative (ANSES, 2020).

COVID-19 Interactions With Herbs Modulating Host Cell Proteins

Coronaviruses have spike or surface (S) glycoproteins giving them their characteristic shape and name. These help the virus to enter the host cell by a receptor-mediated process (Lu et al., 2020). Although they show a complex pattern for receptor recognition, Angiotensin-converting enzyme 2 (ACE2) is considered the main host cell receptor of human pathogenic coronaviruses. In principle, herbal products down-regulating ACE2 may provide a baseline defense against infection. In the wake of SARS, Taiwanese researchers described emodin as an inhibitor of both S and ACE2 proteins. Emodin is present in medicinal plants such as *Rheum officinale* Baill. and *Polygonum multiflorum* Thunb (Ho et al., 2007).

However, some other plant metabolites have been identified as potential enhancers of ACE2 expression, including baicalin, tanshinones, magnolol, curcumin, and rosmarinic acid (ANSES, 2020). Therefore, French authorities have warned that the use of *Scutellaria* sp., *Magnolia* sp., *Salvia* sp., *Curcuma* sp., and *Rosmarinus officinalis* L., by COVID-19 patients, needs further evaluation (ANSES, 2020).

Immunomodulators may both stimulate or suppress the immune system by inhibiting and/or increasing the synthesis and release of pro-inflammatory mediators, including cytokines and eicosanoids. There is preclinical evidence of increased IL-1B and/or IL-18 production in infected immune cells by the use of *Sambucus nigra* L., polysaccharide-rich extracts from medicinal mushrooms, *Echinacea angustifolia* DC. and *Echinacea purpurea* L. (Moench.), Larch (*Larix* sp.) arabinogalactans-rich extracts,

and plant extracts or food supplements rich in Vitamin D (Alschuler et al., 2020).

COVID-19 Interactions With Herbal Medicines Acting as Antithrombotic

The incidence of venous thromboembolism (VTE) in hospitalized patients with COVID-19 has been a controversial matter during the pandemic. Initial studies seemed to point towards patients the benefits of VTE prophylaxis in emergency care. Currently, there is insufficient data to recommend for or against the use of thrombolytics or increasing anti-coagulant doses in hospitalized COVID-19 patients. More epidemiologic studies with better control for underlying comorbidities, prophylactic anticoagulation, and COVID-19-related therapies are needed (NIH, 2020).

The current recommendation is that anticoagulants/antiplatelet therapy should not be initiated for the prevention of VTE in non-hospitalized patients with COVID-19 unless there are other indications. Similarly, modern phytotherapy should avoid recommending the use of plants with anticoagulant effects as prophylaxis of thromboembolic events triggered by COVID-19 disease. Some of the herbal medicines here reviewed are known to have anti-coagulant activity on top of their beneficial effects upon cold/flu symptoms such as garlic (*A. sativum* L.) and guaco (*M. glomerata* Spreng.). Although caution needs to prevail in assessing whether they are providing a benefit that outweighs the potential risks in case the disease gets complicated. This assessment needs to take into consideration quantitative scientific criteria that, in many times, are lacking. A case in point is the 'zero tolerance' to garlic, despite experimental evidence showing that an equivalent of 0.6% allicin does not impair platelet function and does no potentiate platelet-inhibiting drugs (Scharbert et al., 2007).

Herb-Drug Interactions in the Context of COVID-19 Emergency Treatment

Standard protocols in case of pneumonia contemplate assisted respiration for long periods, which in turn requires general anesthesia. Concerns about herb-drug interaction in anaesthesiology have been historically a major driver for objections to herbal medicine from clinicians. There is a substantial body of knowledge of how herbal medicines interact with anesthesia (Levy et al., 2017; AANA, 2020). There is a consensus among anesthesiologists in advising against any of the following herbal medicines that may be taken for flu symptoms:

- Echinacea (*Echinacea* sp.), because they may (theoretically) increase the risk of liver damage in patients under anesthesia.
- Ginkgo (*Ginkgo biloba* L., Ginkgoaceae), St. John's wort (*Hypericum perforatum* L., Hypericaceae), and valerian (*Valeriana officinalis* L., Caprifoliaceae) because they may increase the effects of anesthesia and make it harder to wake up. They may also cause irregular heart rhythms.
- Ginseng (*Ginseng* sp., Araliaceae), licorice (*Glycyrrhiza glabra* L.), and milk thistle [*Silybum marianum* (L.) Gaertn.], because they may cause high blood pressure and a rapid heart rate.
- Garlic (*Allium sativum* L., Amaryllidaceae), ginkgo, green tea [*Camellia sinensis* (L.) Kuntz., Theaceae], feverfew (*Tanacetum parthenium* L., Asteraceae), ginger (*Zingiber officinale* L.) and Saw palmetto [*Serenoa repens* (W. Bartram) Small, Arecaceae], because they may cause prolonged bleeding.
- Garlic, in addition, can increase the effects of some OTC pain relievers.
- Ephedra: Several studies and clinical trials have been carried out to identify drugs that can effectively treat the disease, but, at the moment, the strategies to deal with the infection are only supportive (Cascella et al., 2020). Two recent reviews to on Chinese Herbal Medicine (CHM) can illustrate this. In the first one, the authors presented some CHM formulae used in the H1N1 outbreak that can be useful to prevent COVID-19 (Luo et al., 2020). The other offers guidelines for the treatment of COVID-19, at different stages, using CHM (Ang et al., 2020a). Among the most cited plant drug, *Ephedra* plays an important role. Both indicate a lack of evidence for the efficacy of those CHM preparations. The presence of a few medicinal plants such as *Ephedra* in the suggested guidance for COVID-19 is a subject of major concerns. Although *Ephedra*, listed in the WHO monographs (WHO, 1999), has been used for the treatment of asthma, cough, cold as well as in the management of weight loss (its primary use as a supplement in the USA), globally regulatory agencies have banned food supplements and medicines containing the ephedra alkaloid ephedrine, due to the serious adverse effects especially on the cardiovascular and nervous system and reported deaths (FDA, 2008; EFSA, 2013; EMA, 2015b).

Benefits/Risks Assessment of Herbal Medicines Officially Referred as to as Useful to Relieve Symptoms Related to Respiratory Conditions

There have been a few metanalyses of the impact of herbal medicines in the treatment of common flu (Wagner L. et al., 2015; Ang et al., 2020b). Here, the analysis of the level of evidence was done considering COVID-19 and the current knowledge about symptoms and mechanisms of action involved in the coronavirus infection. In order to support the decision, the same criteria used for three drugs currently used to mitigate the same symptoms defined here: fever (paracetamol), inflammation (ibuprofen), and cough (codeine) were applied to the selected herbal medicines.

A modified PrOACT-URL approach has been used here as a tool for the evaluation of herbal medicines. Based on the defined criterion and the benefits/risks assessment, thirty-six herbal medicines, and three conventional drugs were evaluated (**Table 4**).

The potential usefulness of the herbal medicines in the management of early symptoms of flu in the context of the current COVID-19 pandemic was compared with the baseline benefits/risks assessment performed on the reference drugs paracetamol (acetaminophen), ibuprofen and codeine. These drugs resulted not to be backed up by robust clinical evidence,

TABLE 4 | Benefits/risks assessment in adult patients without any other conditions suffering early/mild flu symptoms in the context of COVID-19.

Treatment		Evidence Levels in Respiratory conditions[1]							Overall Benefit	Adverse Effects[2]	Overall Safety	Benefits/Risks
Plant Species/Drug	**Pharmacopoeial name**	**Cold**	**Flu**	**Bronchitis**	**Asthma**	**Cough**	**Pain**	**Fever**				
Allium sativum/bulbs, powder	Allii sativi bulbus (EMA)	Ib							High	B-II	Medium[a]	Promising
Althaea officinalis/roots, leaves	Althaeae radix					Ib			High	A-IV	High	Positive
Andrographis paniculata/leaves	Andrographidis paniculatae folium	Ib				Ib	Ib		High	D-III	Medium	Promising
Commiphora molmol/gum	Myrrha gummi-resina						1b		High	A-Ic	High	Positive
Cymbopogon citratus/leaves	Cymbopogonis folium	IV	IV						Low	A-IV	High	Unknown
Echinacea angustifolia/roots	Echinaceae angustifoliae radix	Ib	Ib						High	D-IV	Medium	Promising
Echinacea pallida/roots	Echinaceae pallidae radix	IV	IV						Low	D-IV	Medium	Unknown
Echinacea purpurea/herb	Echinaceae purpureae herba	Ib	Ib						High	D-IV	Medium	Promising
Eucalyptus globulus/essential oil	Eucalypti aetheroleum			III		III			Medium	A-IV	High	Promising
Eucalyptus globulus/leaves	Eucalypti folium			IV		IV			Low	A-IV	High	Unknown
Foeniculum vulgare/fruits	Foeniculi amari/dulcis fructus	IV				IV		IV	Low	A-IV	High	Unknown
Glycyrrhiza glabra/roots	Liquiritiae radix				Ib				High	A-II	High	Positive
Hedera helix/leaves	Hederae helicis folium			Ib	Ib	Ib			High	A-Ic	High	Positive
Justicia pectoralis/leaves	*Justicia pectoralis* folium					Ib			High	B-IV	Medium	Promising
Magnolia officinalis/bark	Magnoliae cortex				III				Medium	C-III	Medium	Promising
Malva sylvestris/leaves	Malvae sylvestris folium					IV			Low	A-IV	High	Unknown
Mikania glomerata/leaves	*Mikania glomerata* folium				Ib	IV			High	C-IV	Medium	Promising
Ocimum gratissimum/leaves	Ocimi Sancti folium	IV	IV	IV	IV			IV	Low	A-IV	High	Unknown
Pelargonium sidoides/roots	Pelargonii radix	Ia		Ib		Ib			High	D-IV	Medium	Promising
Pimpinella anisum/fruits essential oil	Anisi aetheroleum/fructus				Ib	IV		IV	High	C-IV	Medium	Promising
Plantago lanceolata/leaves	Plantaginis lanceolatae folium			IV		IV		IV	Low	A-IV	High	Unknown
Platycodon chinensis/roots	Platicodi radix					IV			Low	A-III	High	Unknown
Polygala senega/roots	Polygalae radix			IV		IV			Low	A-IV	High	Unknown
Polypodium vulgare/rhizome	Polypodii rhizoma					IV			Low	A-IV	High	Unknown
Potentilla erecta/rhizome	Tormentillae rhizoma					IV			Low	A-III	Medium	Unknown
Primula veris/roots	Primulae radix					IV			Low	B-III	High	Unknown
Salix sp./bark	Salicis cortex	IV	IV				Ib	IV	High	C-IV	Medium	Promising
Sambucus nigra/fruits	Sambuci fructus	Ia	Ia						High	A-Ic	High	Positive
Scutellaria baicalensis/roots	Radix Scutellariae			IV				IV	Low	C-III	Medium	Unknown
Silybum marianum/fruits	Silybi mariani fructus							IV	Low	A-Ic[b]	High	Unknown
Thymus vulgaris/herb essential oil	Thymi herba/aetheroleum					IV			Low	A-IV	High	Unknown
Tilia cordata/flowers	Tiliae flos	IV							Low	A-IV	High	Unknown
Zingiber officinale/rhizome	Zingiberis rhizoma	IV	IIa*		IV	IV			Medium	B-II	Medium	Promising
Ibuprofen	–	Ia	IV				Ia		Medium	B-II	Medium	Promising
Codeine	–					IV			Low	E-Ib	Low	Negative
Paracetamol	–	IV	IV						Low	E-Ib	Low	Negative

(1) Grading as per ***Table 1****;(2) Codes and Grading as per* ***Table 2****;(3) As per* ***Table 3****; (a) Products with less than 0.6% allicin content are safe (Scharbert et al., 2007); (b) Chronic administration of products with 7%–8% silymarin content are safe up to 40 months (Corchete, 2008); (*) Clinical experiment in SARS patients (Vahdat Shariatpanahi et al., 2013).*

and their safety profile was concerning in some cases. According to this 'baseline', five herbal medicines were found as potentially valid candidates in managing early or mild symptoms of cold, flu and bronchitis in the context of COVID-19 as they provide with ample safety margins and good evidence for efficacy: *Althaea officinalis*, *Commiphora molmol, Glycyrrhiza glabra, Hedera helix*, and *Sambucus nigra*. The authors recommend starting their integration into clinical advice as adjuvant therapies for respiratory diseases, even in the context of COVID-19.

A second group of twelve herbal medicines is to be considered as promising candidates, due to their reasonable safety margins and emerging evidence for efficacy: *Allium sativum*, *Andrographis paniculata*, *Echinacea angustifolia, Echinacea purpurea, Eucalyptus globulus* essential oil, *Justicia pectoralis, Magnolia officinalis, Mikania* glomerata *Pelargonium sidoides*, *Pimpinella anisum*, *Salix* sp, and *Zingiber officinale*. The authors recommend the scientific community to prioritize working on these herbals towards their full integration into clinical use. These two groups are, in the opinion of the authors, comparable in terms of safety -and sometimes efficacy- with the three reference drugs currently in clinical use for the target symptoms, so these herbal medicines may help to mitigate the discomfort in the early stages of the disease, based on their anti-inflammatory, immunomodulatory, and antitussive properties.

The following plants have good evidence for the treatment of asthma: *Glycyrrhiza glabra*, *Magnolia officinalis*, *Mikania glomerata*, and *Pimpinella anisum*. These plants cannot be candidates to treat respiratory infections but may be safe to use if the asthmatic patient suffers flu symptoms.

The rest of herbal medicines (approximately half of the analyzed herbal medicines) appear to have a good safety profile, but simply there is not enough evidence – clinical or pre-clinical – about their action on the target symptoms (cough, fever) or conditions (cold, flu). Therefore, they must be categorized as of 'unknown' potential and would need much more research to build up the necessary evidence to justify their use in the management of flu, let alone in the context of COVID-19.

Benefits/Risks Assessment of Herbal Remedies Not Covered by International Monographs

Several food/herbal-based remedies are associated with the treatment of common cold and flu because they include vitamins (such as lemon juice), aromatic principles with perceived benefits for the upper respiratory system (such as mint, bayleaf), or because they are culturally considered as a "cure-all" (such as Nigella). Some of them are presented to the public in a "pharmaceutical forms" (capsules, tablets, syrups), thus looking like a "safe and effective medicinal approach". The regulatory status of these products varies from country to country, and we advise here that only products that are regulated as (herbal) medicines should be used since this is an essential requirement for a product's safety.

The following herbals and foods were identified by the authors as falling into this category: *Citrus limon, Culcitium canescens, Laurus nobilis, Lippia graveolens*, and *Plectranthus amboinicus*. We subjected the evidence for the efficacy and safety of these plants to the same Benefit/Risks assessment applied to the "officially recognized" herbal medicines, and the collected preclinical, clinical, and safety data are provided.

Nigella may be considered as a borderline "promising" treatment example as there is some preliminary clinical evidence for its relief of cough but it seems to be restricted to asthma patients, so from a physiopathological point of view is not clear how useful it would be in cold/flu. As for the others, although considered safe, there is simply not enough evidence – clinical or pre-clinical – in relation to their benefits on the target symptoms (cough, fever) or conditions (cold, flu).

Despite their lack of evidence in the treatment of flu, lemon, bay leaves, mint, and Nigella are deeply ingrained 'polyvalent' remedies in many cultures all over the World. Therefore, a succinct discussion on their efficacy and safety in the treatment of respiratory conditions is essential:

- The juice or tea of *Citrus limon* (L.) Burm.f fruits, alone or associated with salt, honey or ginger has been used to relieve cough and fever related to flu and cold in several traditional systems (Papp et al., 2011; Panizza et al., 2012; Sultana et al., 2016; Klimek-Szczykutowicz et al., 2020). However, there is no clinical evidence of such action in the respiratory tract, although this species is well-known as a rich source of vitamin C (Ye, 2017).
- Besides the use as food, the decoction of *Laurus nobilis* L. dried leaves is taken orally to treat respiratory distress (Chevallier, 1996; Ross, 2001), and the only information on *L. nobilis* posology is given by the American Pharmaceutical Association (Duke et al., 2002). The closest to an applicable study is an *in vitro* study examining SARS-CoV and the effect of several essential oils (Chen et al., 1995). The essential oil of *L. nobilis* leaves was also evaluated for its antinociceptive and anti-inflammatory activities in mice and rats (Sayyah et al., 2003). However, the lack of clinical evidence led to concerns about the use of this species.
- *Mentha* x *piperita* L. (peppermint) is widely used to treat bronchitis, fever and other respiratory disorders (WHO, 2002; Blumenthal, 2003), although no evidence can be found to corroborate these actions. The essential oil contains, among other compounds, menthol, menthone, menthofuran, and pulegone, a well-known hepatotoxin (Engel, 2003), leading to a deep concern about the use of essential oil (EMA, 2016e). However, peppermint tea usually contains a small amount of these compounds, and the normal use rarely led to serious adverse effects (EMA, 2008a).
- *Nigella sativa* L. (black seeds) is globally known as a spice and as a food item. In Arabic medicine, this species has very high status as a herbal medicine used for a wide range of diseases. In the context of COVID-19, the relevant uses are for asthma and in the more general management of inflammatory conditions. With regards to respiratory diseases its use can be traced back at least to Avicenna (ca. 980 – 1037, C.E.), who indicated it for shortness of breath and for stopping phlegm and Imam Ibn Qayyim Al-Jawziyya (1292–1350 C.E.), who

recommended it for gasping and hard breathing (Koshak et al., 2018). In many Arabic Countries, "black seed" is used for asthma and cough, among many other uses, especially of gastrointestinal diseases (like abdominal pain, stomach ache, colic), rheumatism, skin diseases and recently use to manage diabetes have become popular (Aisa et al., 2019). Some small studies demonstrate clinical benefits in asthma patients (Koshak et al., 2017). Although black seed may be useful in the symptomatic relief of respiratory symptoms, especially associated with the severe asthmatic cough, the clinical evidence is very limited. A particular concern, in this case, are the many well-intended but very low-quality studies and the broad range of claims they try to support, making any assessment problematic.

Limitations of the Present Work

The authors are aware of the limitations of this assessment: first, the body of studies is not large, even for reference drugs, and this is further complicated by the uncharted territory, which is the current pandemic. Second, the decision-making framework is here applied to herbal medicines for the first time and would need external validation.

The PrOACT-URL method is a valid approach to inform decision-making at high regulatory levels. The way we apply it here may well attract the critique of experts in the field of evaluation of the benefits/risks balance for medicines. We welcome constructive comments since this is exactly what we pursue: to start a high-level discussion on the potential of herbal medicines for the management of flu and their safety implications during this pandemic. For this, we need to work towards further adapting decision-making frameworks to bring about advances in 'clinical phytotherapy'.

We acknowledge that the selection of herbal medicines is geographically limited and that many other species could have been considered. We took a "legalist" approach by only accepting entries provided by EMA or WHO, and we recognize we hold a collective expertise, especially in European and American herbal medicines. Nevertheless, the list includes globally-recognized, therapeutically relevant herbs. At the same time, there is, clearly, an emerging body of evidence on specific Asian (often multiherbal) preparations (especially TCM), which also is included in this Research Topic of Frontiers in Pharmacology, Sect. Ethnopharmacology, and thus these studies mutually complement each other.

CONCLUSIONS

A total of 39 herbal medicines were identified as very likely to appeal to COVID-19 patients. According to our method, the benefits/risks assessment of the herbal medicines was found *positive* in 5 cases, *promising* in 10 cases, and *unknown* for the rest. On the same basis, only ibuprofen resulted promising, but we could not find compelling clinical evidence for a positive assessment to endorse the adjuvant use of paracetamol and/or codeine due to their common adverse effects in the respiratory function.

Our work suggests that *Althaea officinalis*, *Commiphora molmol, Glycyrrhiza glabra, Hedera helix*, and *Sambucus nigra* have safety margins superior to those of reference drugs and enough levels of evidence to merit their potential clinical use as adjuvants in the treatment of early/mild cases of COVID-19.

Herbal medicines are not a "magic bullet" to solve the problems related to flu - let alone COVID-19 or any other coronavirus - neither can avoid the virus infection but may alleviate symptoms and potentially improve the general wellbeing of patients. It requires a careful assessment of whether such adjuvant therapies are justified or not. Then again, it can only be a suitable therapy at a stage when the severity of the disease is minor. Overall, we highlighted the potential of some medicinal plants for the adjuvant management of early symptoms of flu within the context of COVID-19 in otherwise healthy adults. For an herbal medicine to be a medicine high quality, chemically well-characterized, and pharmacologically well-studied preparations are acceptable only. Equally, it will be essential that such well-characterized preparations are used in all future pharmacological and clinical studies (Heinrich et al., 2020).

In a broader context, this study also offers a novel approach for assessing the risks and benefits of using herbal medicines. Since the PrOACT-URL strategy is used here for the first time with a set of herbal medicines, we want to highlight the opportunities for a rigorous and evidence-based approach will have in a balanced assessment of such medicines.

AUTHOR CONTRIBUTIONS

DS Conceptualization of overarching research goals and aims, data/evidence collection, data curation, coordination of the overall work, formal analysis of study data, preparation, creation and/or presentation of the published work, critical review and revision. JP-G: Conceptualization of overarching research goals and aims, development and design of the methodology, visualization/data, formal analysis of study data, preparation, creation and/or presentation of the published work, critical review and revision. FB: Data/evidence collection, formal analysis of study data, critical review and revision. OE, YF-B, CJ, PM, EP, and MT: Data/evidence collection, formal analysis of study data. MH: Conceptualization of overarching research goals and aims, data/evidence collection, formal analysis of study data, critical review and revision.

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REVIEW
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Anti-SARS-CoV Natural Products With the Potential to Inhibit SARS-CoV-2 (COVID-19)

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The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), known to cause the disease COVID-19, was declared a pandemic in early 2020. The objective of this review was to collate information regarding the potential of plants and natural products to inhibit coronavirus and targets associated with infection in humans and to highlight known drugs, which may have potential activity against SARS-CoV-2. Due to the similarity in the RNA genome, main proteases, and primary host receptor between SARS-CoV and SARS-CoV-2, a review was conducted on plants and secondary metabolites, which have shown activity against SARS-CoV. Numerous scientific reports on the potential of plants and secondary metabolites against SARS-CoV infection were found, providing important information on their possible activity against SARS-CoV-2. Based on current literature, 83 compounds have been identified with the potential to inhibit COVID-19. The most prominent selectivity was found for the alkaloid, lycorine, the lignan, savinin, and the abietane terpenoid, 8-beta-hydroxyabieta-9(11),13-dien-12-one with selectivity index values greater than 945, 667, and 510, respectively. Plants and their secondary metabolites, with activity against targets associated with the SARS-CoV infection, could provide valuable leads for the development into drugs for the novel SARS-CoV-2. The prospects of using computational methods to screen secondary metabolites against SARS-CoV targets are briefly discussed, and the drawbacks have been highlighted. Finally, we discuss plants traditionally used in Southern Africa for symptoms associated with respiratory viral infections and influenza, such as coughs, fever, and colds. However, only a few of these plants have been screened against SARS-CoV. Natural products hold a prominent role in discovering novel therapeutics to mitigate the current COVID-19 pandemic; however, further investigations regarding *in vitro*, *in vivo*, pre-clinical, and clinical phases are still required.

Keywords: coronavirus, COVID-19, ethnomedicine, HCoV, natural products, novel drug candidates, SARS-CoV, viral infections

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INTRODUCTION

Severe acute respiratory syndrome coronavirus (SARS-CoV) is a highly contagious viral infection that causes considerable morbidity and mortality (Simmons et al., 2004). The SARS-CoV is part of the family Coronaviridae, which are enveloped viruses with single and positively stranded RNA (Du et al., 2009). This virus is known to cause respiratory, enteric, and neurological diseases in humans (Simmons et al., 2004). It is one of seven coronaviruses that have been shown to cause human infection. This includes the novel SARS-CoV-2, which is responsible for causing the coronavirus disease of 2019 (COVID-19). Other coronaviruses include the alpha coronaviruses (HCoVs-NL-63 and HCoVs-229E) and the beta coronaviruses [HCoVs-OC43, HCoVs-HKu1, Middle East respiratory syndrome-CoV (MERS-CoV), and SARS-CoV]. The COVID-19 outbreak originated from the Wuhan province in China during December 2019. It has developed into a global pandemic in a matter of months, spreading to 214 countries, areas, or territories.

Currently, there is no antiviral treatment for COVID-19; therefore, the control of this disease has become a global health emergency. Given the rapid transmission of the virus, researchers and public health agencies are investigating the possibility of repurposing existing drugs for the potential treatment of COVID-19 (**Figure 1**). The WHO is focusing on four promising therapies; an experimental antiviral drug remdesivir (used for the treatment of Ebola), the antimalarial drugs chloroquine and hydroxychloroquine, a combination of two HIV drugs (lopinavir and ritonavir), and the latter combined with interferon-β, an antiviral cytokine and modulator of the immune system (Kupferschmidt and Cohen, 2020).

In a study by Wang et al. (2020), patients admitted to hospital with severe COVID-19, displayed a faster improvement when using remdesivir to those receiving the placebo; however, this was not statistically significant, and it was concluded that larger-scale studies were required to adequately assess the potential therapeutic efficacy of remdesivir. Additionally, remdesivir did not significantly improve mortality or clearance time of the virus (Wang et al., 2020). In a study by Beigel et al. (2020), which was a larger-scale study than conducted by Wang et al. (2020), remdesivir shortened the recovery time in patients that were hospitalized with COVID-19 and showed signs of lower respiratory tract infection compared to recovery times of patients receiving the placebo, from an average of 15 to 11 days; however, it was found that there was no significant difference in mortality rate compared to the placebo control, and therefore, this study concluded that treatment with an antiviral drug alone might not be sufficient in treating COVID-19 (Beigel et al., 2020).

The US food and drug administration (FDA) recently approved the antimalarial drug, hydroxychloroquine, for the experimental treatment of COVID-19 (Cortegiani et al., 2020). Hydroxychloroquine has also been recommended by the Indian Council of Medical Research (ICMR) for the treatment of COVID-19 (Indian Council of Medical Research, 2020). A study reported that the treatment of COVID-19 patients with hydroxychloroquine significantly reduced the viral load, while combining the treatment with azithromycin enhanced the reduction of the viral load when compared to the controls (Gautret et al., 2020). However, due to the urgency of finding a cure for COVID-19, Gautret et al. (2020) published these results using only a small sample size; therefore, results should be confirmed in a larger study (Gautret et al., 2020). Additionally, *in vitro* results regarding the potential of hydroxychloroquine and chloroquine to inhibit SARS-CoV-2 showed that hydroxychloroquine was a more potent inhibitor of SARS-CoV-2 than chloroquine, with 50% effective concentrations (EC_{50}) of 0.72 and 5.47 μM, respectively (Yao et al., 2020). However, on the 5th of June 2020, a statement was released by the Chief Investigators of the Randomized Evaluation of COVID-19 therapy (RECOVERY) trial on the use of hydroxychloroquine for COVID-19. The Independent Data Monitoring Committee reviewed clinical trial data that used hydroxychloroquine and concluded that there was no beneficial effect when COVID-19 hospitalized patients were treated with hydroxychloroquine compared to patients which received standard COVID-19 care and, therefore, RECOVERY has stopped enrolling participants for the hydroxychloroquine trials (Horby and Landray, 2020).

A study by Cao et al. (2020), found that patients hospitalized with severe COVID-19 that were treated with lopinavir-ritonavir showed no significant difference compared to patients who received the standard care for COVID-19 (Cao et al., 2020). However, a study by Hung et al. (2020), which investigated the efficacy of a triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of hospitalized COVID-19 patients, found that the triple combination treatment was effective in shortening virus shedding and alleviated symptoms in patients with mild to moderate COVID-19 compared to the group treated with lopinavir-ritonavir alone. However, the study lacked the required placebo control and did not include an interferon beta-1b control group in which to compare efficacy (Hung et al., 2020). Therefore, further studies are required to compare the triple combination treatment to that of a placebo group and to establish the role of interferon beta-1b in the efficacy of the triple combination treatment.

A natural product derivative, ivermectin, which is a mixture of two major homologues, ivermectin B1a (>80%) and ivermectin B1b (<20%), is an anti-parasitic natural product that was isolated from a microorganism found in Japanese soil (Crump and Omura, 2011). It is used for the treatment of parasitic infections such as head lice, scabies, river blindness (onchocerciasis), strongyloidiasis, trichuriasis, ascariasis, and lymphatic filariasis (Ottesen and

Abbreviations: $3CL^{pro}$: Coronavirus main protease $3CL^{pro}$; ACE2: Angiotensin-converting enzyme 2; COVID-19: Coronavirus disease of 2019; EC_{50}: Fifty percent effective concentration; FDA: Food and drug administration; HCoV: Human coronavirus; IC_{50}: Fifty percent inhibitory concentration; ICMR: Indian Council of Medical Research; IFN: Interferons; IFNAR: Interferon alpha-receptor; MERS-CoV: Middle East respiratory syndrome-related coronavirus; NF-κβ: nuclear transcription factor; Nsp1: Nonstructural protein 1; NSP13: Non-structural protein 13; ORF7a: Open reading frame 7a; PAINS: Pan assay interference compounds; PL^{pro}: Papain-like protease; RBD: Receptor binding domain; RdRp: RNA-dependent RNA polymerase; RNA: Ribonucleic acid; S protein: Viral spike glycoprotein; SARS-CoV: Severe acute respiratory syndrome-related coronavirus; SARS-CoV-2: Severe acute respiratory syndrome-related coronavirus-2; SI: Selective index; TLR: Toll-like receptors; TMPRSS2: Transmembrane protease, serine 2; TNF-α: Tumor necrosis factor-alpha; WHO: World Health Organization.

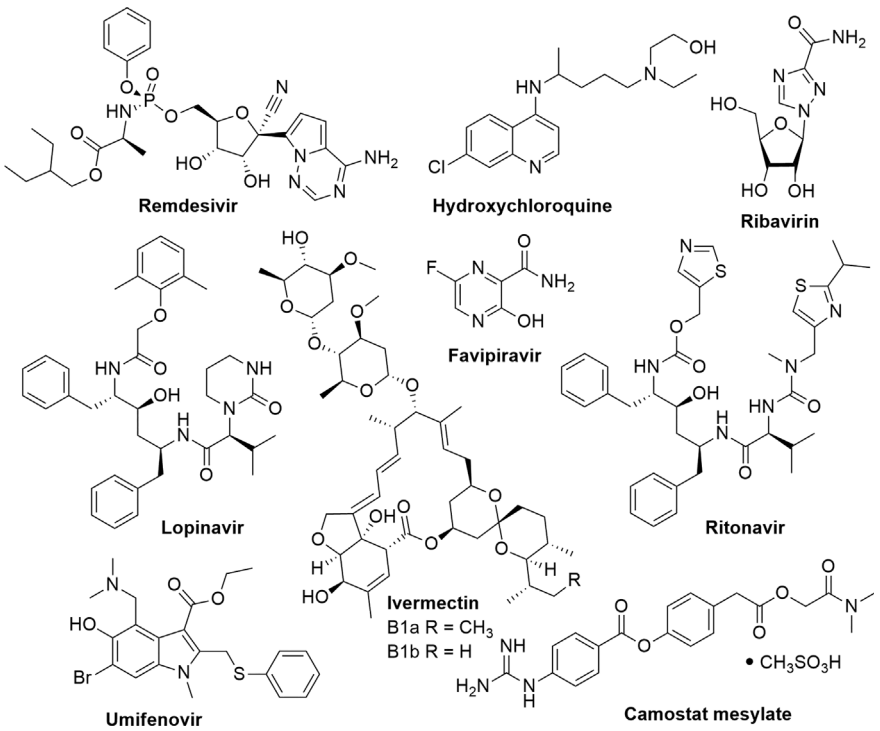

FIGURE 1 | Existing drugs which are being repurposed for the experimental treatment of COVID-19.

Campbell, 1994). Ivermectin has shown potent *in vitro* activity against SARS-CoV-2. It reduced viral replication by 99.98% within 48 h after treatment with a single dose of 5 μM. The 50% inhibitory concentration (IC_{50}) was determined to be ~2 μM (Caly et al., 2020); however, further studies are required to determine the therapeutic potential against COVID-19. **Table 1** and **Figure 1** summarize existing drugs and chemical structures that are being used for the experimental treatment of COVID-19 based on their efficacy in targeting key proteins found on the COVID-19 virus.

This review focuses on natural products, which have shown activity against SARS-CoV, as a selection criterion for potential inhibition of SARS-CoV-2, due to the genome similarity and the similarity in the main protease structure and the primary host receptor between SARS-CoV and SARS-CoV-2 (Chan et al., 2020; Chen and Du, 2020; Zhang et al., 2020b). In addition, the current state of this research topic is briefly discussed, and gaps in the research are identified. Finally, this review discusses the potential use of Southern African medicinal plants, which have traditionally been used for the treatment of symptoms related to respiratory viral infections, and influenza, to inhibit SARS-CoV-2.

SIMILARITIES BETWEEN SARS-COV AND SARS-COV-2

Both the SARS-CoV and the SARS-CoV-2 are considered zoonotic coronaviruses within the genus Betacoronavirus. Coronaviruses are spherical enveloped viruses which range between 100 and 160 nm in diameter. The positive-sense single-stranded RNA genome (27–32 kb), contained in each particle, forms a complex with the nucleocapsid protein (Salata et al., 2019; Kannan et al., 2020). The genome of the novel SARS-CoV-2 was determined to have an 82% nucleotide identity with SARS-CoV. Through phylogenetic analysis, it was found that the membrane, envelope, spike, nucleoprotein, and the orf1a/b polyproteins clustered closely together; however, the orf3b protein encoded a novel short protein (Chan et al., 2020). It was further confirmed that the primary host receptor for SARS-CoV-2 is the human angiotensin-converting enzyme 2 (ACE2), similar as in the case of SARS-CoV (Ou et al., 2020; Rothan and Byrareddy, 2020; Yan et al., 2020). Furthermore, the homology of the spike-receptor binding domain (RBD) sequence between SARS-CoV-2 and SARS-CoV was found to be 76% similar, and the main proteases between the two viruses were closely related (96% identity) (Chen et al., 2020; Lung et al., 2020). Other similarities between SARS-CoV and SARS-CoV-2 include symptom progression and mode of infection. The initial symptoms observed in infected patients are fever, fatigue, and respiratory problems (Wu et al., 2020). Within 8 to 20 days after the initial onset of symptoms, patients suffer from acute respiratory distress syndrome. After 10 days from the onset of symptoms, patients suffer from lung abnormalities (Prompetchara et al., 2020).

During viral infections, the innate immune cells recognise viral RNA through endosomal RNA receptors, cytosolic RNA sensors, and toll-like receptors (TLR) 3 and 7 (Xagorari and

TABLE 1 | Drug candidates for key proteins during the coronavirus infection process [adapted from Liu et al. (2020)].

Target protein	Drug name	References
Coronavirus main protease $3CL^{pro}$ ($3CL^{pro}$)	Lopinavir	(Dayer et al., 2017)
Papain-like protease PL^{pro} (PL^{pro})	Lopinavir	(Dayer et al., 2017)
RNA-dependent RNA polymerase (RdRp)	Remdesivir, ribavirin	(Contreras et al., 2002; Gordon et al., 2020)
Viral spike glycoprotein (S protein)	Arbidol (umifenovir)	(Boriskin et al., 2008)
Transmembrane protease, serine 2 (TMPRSS2)	Camostat mesylate	(Hoffmann et al., 2020)
Angiotensin-converting enzyme 2 (ACE2)	Arbidol (umifenovir)	(Boriskin et al., 2008)

Chlichlia, 2008; Ahmadpoor and Rostaing, 2020). Once the virus has been recognized, a cascade occurs, which activates transcription factors such as nuclear transcription factor (NF-κβ). These transcription factors induce the expression of type I interferons (IFN), which binds to an interferon alpha-receptor (IFNAR) (Ivashkiv and Donlin, 2014). This process activates the JAK-STAT pathway, which suppresses viral replication and removes the virus within the body (Fleming, 2016). During SARS-CoV and SARS-CoV-2 viral infection, the RNA enters into a patient's tissue by binding to the ACE2 receptor, expressed on host cells using the spike glycoprotein (S protein), which contains the receptor-binding domain (RBD) (Hoffmann et al., 2020).

Patients infected with the human coronavirus SARS-CoV-2 undergo what is denoted a "cytokine storm," where pro-inflammatory cytokines are generated as a result of SARS-CoV-2 infection (Zhang et al., 2020c). Patients who tested positive for the SARS-CoV-2 coronavirus showed an increased level of interleukin-2 (IL-2), IL-7, IL-10, IL-1β, IL-1 receptor agonist (IL-1RA), IL-8, IL-9, basic fibroblast growth factor (b-FGF), granulocyte-colony stimulating factor (GCSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-gamma (IFNγ), inducible protein 10 (IP10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1A (MIP1A) and MIP1B, platelet-derived growth factor (PDGF), tumor necrosis factor-alpha (TNFα), and vascular endothelial growth factor (VEGF) in their serum levels. When serum levels of ICU-patients were compared to non-ICU patients, IL-2, IL-7, IL-10, GCSF, IP10, MCP1, MIP1A, and TNFα were elevated in ICU patients (Channappanavar and Perlman, 2017). Furthermore, patients who develop mild or high acute respiratory syndrome due to SARS-CoV-2 infection show an increased level of IL-1β and IL-6, which mediate lung inflammation, fever, and fibrosis (Gallagher and Buchmeier, 2001). It has been reported that IL-6 is one of the main cytokines involved in pulmonary complications associated with SARS-CoV-2 infection (Simmons et al., 2005). Therefore, the inhibition of these overexpressed cytokines could be a potential therapeutic target for COVID-19. Numerous potential therapeutic targets associated with coronavirus infections in humans have been identified (**Table 2**).

COVID-19 infections in humans are not only associated with various pulmonary complications or respiratory illnesses but also several other organs, such as the kidney and liver, are also affected, which could contribute toward impaired metabolism and excretion of potential drugs used to treat the disease (Rismanbaf and Zarei, 2020). A study by Zhang et al. (2020a), reported that 2–11% of patients infected with COVID-19 showed signs of liver dysfunction with 14–53% of cases displaying elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). This was confirmed in a study by Huang et al. (2020), where increased levels of AST were detected in 37% of COVID-19 patients (Huang et al., 2020). Guan et al. (2020) found that elevated AST and ALT levels were more prominent in patients with severe COVID-19 compared to non-severe cases. Additionally, this study reported that, on admission, 83.2% of patients suffered from lymphocytopenia (low levels of lymphocytes in the blood), 36.2% had thrombocytopenia (low blood platelet count), and 33.7% had leukopenia (low white blood cell count) (Guan et al., 2020). In a study by Xu et al. (2020), biopsies were taken from the lung, liver, and heart tissue of a patient who died from a cardiac arrest associated with COVID-19. Histological examination of the liver tissue revealed that the patient showed moderate microvesicular steatosis and mild lobular and portal activity, which could have been due to the COVID-19 infection or drug-induced damage, whereas a few interstitial mononuclear inflammatory infiltrates were found in the heart tissue (Xu et al., 2020). Additionally, several features characteristic of COVID-19 infection were found within the lung tissue, such as pulmonary oedema and alveolar damage (Xu et al., 2020). Acute kidney injury has also been reported as a severe symptom in patients hospitalized with COVID-19. Hirsch et al. (2020) reported that 36.6% of patients admitted with COVID-19 developed acute kidney injury, which was most prominent in patients with respiratory failure (89.7% of patients on ventilators compared to 21.7% not using ventilators) (Hirsch et al., 2020). The expression of ACE2 receptors are not only prevalent in lung cells but are also expressed in kidney cells; however, it has been reported that the incidence of acute kidney injury (29%) is lower than incidence of lung damage (71%) associated with COVID-19 infection (Malha et al., 2020).

A study by Zou et al. (2020) aimed at identifying high-risk organs vulnerable to COVID-19 infection through single-cell RNA sequencing techniques. This study identified the lungs, heart, bladder, kidneys, oesophagus, and ileum as high-risk organs for COVID-19 infection, specifically identifying type II alveolar lung cells, myocardial cells, bladder urothelial, ileum, oesophagus epithelial, and kidney proximal tubule cells, which express ACE2 (Zou et al., 2020). Xiao et al. (2020) found that 53.42% of COVID-19 hospitalized patients tested positive for SARS-COV-2 RNA in stool samples, of which 23.29% tested negative for SARS-CoV-2 when respiratory samples were tested, which confirms that SARS-CoV-2 is able to infect the

TABLE 2 | Potential therapeutic targets associated with coronavirus infections in humans.

Target	Function	Coronavirus type	Reference
Angiotensin-converting enzyme 2 (ACE2)	Functional cellular receptor for SARS-CoV and SARS-CoV-2 (COVID-19)*	SARS-CoV SARS-CoV-2	(Yan et al., 2020)
Spike glycoprotein (S protein)—during viral infection in cleaved into S1 and S2 subunits	Mediates receptor recognition and membrane fusion for viral entry. S1 subunit: contains receptor-binding domain (RBD)** which binds to the peptidase domain (PD) of ACE2 S2 subunit: responsible for membrane fusion; cleaved by host proteases once S1 binds to ACE2 which is needed for a viral infection to occur	SARS-CoV	(Gallagher and Buchmeier, 2001)
Cathepsin L–cysteine peptidase	Facilitates the cleavage of the S protein of SARS-CoV, therefore aids in the activation of membrane fusion	SARS-CoV	(Simmons et al., 2005)
Transmembrane protease serine 2 (TMPRSS2)	Cleaves C-terminal segment of ACE2, enhancing S-protein viral infection	SARS-CoV	(Shulla et al., 2011)
Nonstructural protein 1 (Nsp1) coronavirus virulence factor	Induces host mRNA degradation by interacting with the hosts 40S ribosomal subunit and inhibits type-I interferon production	SARS-CoV	(Kamitani et al., 2006; Narayanan et al., 2008)
Open reading frame 7a (ORF7a) coronavirus virulence factor	ORF7a binds directly to bone marrow stromal antigen 2 (BST-2), blocking the activity of BST-2 by disrupting the glycosylation of BST-2. BST-2 mediates the restriction of virus-like particle release	SARS-CoV	(Taylor et al., 2015)
Replicase polyproteins	Involved in the transcription and replication of viral RNAs. Encoded by open reading frames (ORF) 1a and 1b.	SARS-CoV	(Wu et al., 2020)
Papain-like proteinase (PL^{pro})	Essential in the replication and infection for coronaviruses. Cleaves the N-terminal of the replicase polyprotein causing the release of Nsp1, Nsp2 and Nsp3, which are in turn involved in viral replication	SARS-CoV	(Harcourt et al., 2004)
Viral main protease ($3CL^{pro}$, also called Mpro) –cysteine protease	Controls the activities of the coronavirus replication complex and is therefore essential for viral replication***	SARS-CoV SARS-CoV-2	(Anand et al., 2003)
RNA dependent RNA polymerase (RdRp) (nsp12)	Essential protease enzyme that catalyzes the replication of RNA from the RNA template	SARS-CoV SARS CoV-2	(Lung et al., 2020)
Non-structural protein 13 (NSP13)/ helicase	Enhances the efficiency of viral replication and proliferation through its NTPase, duplex RNA/DNA-unwinding and RNA-capping activities	SARS CoV	(Shum and Tanner, 2008)

**Functional cellular receptor (ACE2) are identical for SARS-CoV and SARS-CoV-2 (Ou et al., 2020); **Homology of the spike-receptor binding domain (RBD) sequence between SARS-CoV-2 and SARS-CoV is 76% (Wu et al., 2020); ***The SARS-CoV-2 main protease is closely related (96% identity) to the SARS-CoV protease (Chen and Du, 2020).*

gastrointestinal system, which also suggests that the spread of COVID-19 could be through fecal-oral transmission (Xiao et al., 2020). The infection of the gastrointestinal tract could furthermore explain the prevalence of diarrhea in COVID-19 patients, which highlights the need to monitor individuals with diarrhea as a potential initial symptom of COVID-19 infection (Zhang et al., 2020d).

A study by Varga et al. (2020) described the involvement of vascular endothelial cells, which express ACE2 receptors, in multi-organ toxicity related to COVID-19 infected patients. Histological analysis of a patients' tissue, who suffered from pre-existing heart conditions, showed that there was an increase in inflammatory cells associated with the endothelium and an increase of mononuclear cells in the lung, as well as the presence of apoptotic bodies in the heart, lung, and small bowel. Histological analysis of a second patients' tissue, who suffered from heart comorbidities and obesity, showed the presence of lymphocytic endotheliitis in the lung, heart, kidney and liver; necrosis of liver cells; and endotheliitis of the submucosal vessels in the small intestine. In a third patient who suffered from high blood pressure, endotheliitis of the submucosal vessels in the small intestine was also observed and the presence of apoptotic bodies. Varga and colleagues were able to conclude that SARS-CoV-2 is able to directly infect endothelial cells, thereby causing endotheliitis in several organs and increased inflammatory response (Varga et al., 2020).

Although SARS-CoV and SARS-CoV-2 share several similarities, there are many differences. SARS-CoV-2 is considered the most contagious, as asymptomatic hosts can spread the virus *via* respiratory droplets and contaminated fomites (Chen et al., 2020; Lai et al., 2020; Prompetchara et al., 2020; Yuen et al., 2020), whereas SARS-CoV can only be spread by those that have severe respiratory illnesses (Lung et al., 2020; Wilder-Smith et al., 2020). This has allowed SARS-CoV-2 to infect more countries and have higher case numbers than SARS-CoV and MERS-CoV (Arabi et al., 2020; Wu et al., 2020). Numerous studies have been conducted on the use of medicinal plants and their isolated secondary metabolites to target and inhibit proteins related to coronavirus infections in humans. Following the outbreak of SARS in China during 2002, the State Administration of Traditional Chinese Medicine of the People's Republic of China initiated clinical research projects regarding the combined use of Traditional Chinese medicine (TCM) and Western medicine for treating SARS. A total of 21 research projects were initiated to cover three aspects of SARS, namely, prevention, treatment, and rehabilitation (World Health Organization, 2004).

Of the 5327 patients diagnosed with SARS across the country, 3104 cases received TCM treatment. The WHO reviewed clinical and research reports on patients treated with a combination of Traditional Chinese Medicine and Western Medicine to better understand the potential of these treatments for SARS. They

concluded that the integrated use of TCM and Western medicine for SARS patients was safe and that there could be potential benefits to SARS patients using this combined treatment method. A reduction in case fatality rate, when treated with the combination therapy as opposed to treatment with Western medicine alone, was also observed. In addition to these benefits, the combination treatment regime lowered the overall cost of effective treatment. This highlights the importance of introducing complementary medicine, such as through the use of medicinal plants, for the treatment of SARS (World Health Organization, 2004).

LITERATURE STUDY ON THE USE OF NATURAL PRODUCTS AGAINST CORONAVIRUSES

To assess the current literature on the potential use of natural products against coronaviruses, a detailed literature study was conducted using published research articles ranging from the year 2010–2020. This analysis was conducted to indicate the current state of the art and identify potential gaps and areas in the field that can be explored in future research studies. Four databases were used to conduct the literature search, namely, ScienceDirect, SciFinder^{n}, Scopus, and Web of Science. The search terms included "coronavirus" and "natural product*." VOSviewer was used to analyse the co-occurrence of related keywords. Similar trends and keywords were identified in each of the databases. ScienceDirect, followed by Scifinder^{n}, identified the largest hit ratio with 120 and 124 papers, respectively. The most recent, prevailing, and obvious co-occurrence of keywords were "SARS-CoV-2" and "COVID-19" (**Figure 2**). This was followed by the identification of the keywords "medicinal plants," "natural products," "natural compounds," and "phytochemicals." The only potential drug target or mechanism that was identified, and associated with natural products, was the cysteine protease, 3CL^{pro}. Similarly, flavonoids were identified as the largest class of compounds with potential activity; however, this group does not have any link to the 3CL^{pro} group, indicating that the mechanism is poorly understood and has not yet been identified. Research articles which include computational approaches, such as molecular docking, have increased over the past few months, which is expected due to the rapid outcome of results using these approaches. Molecular docking and its applicability to identifying potentially biologically active compounds are later discussed in this review. Due to the outbreak of SARS-CoV-2 as a new and emerging disease, it is expected that the body of published research is still fairly limited, and it is of utmost importance to structure future research projects with a clear hypothesis, research justification, and relevant and appropriate methods.

Analysis of the test systems used, as well as the proposed mechanisms associated with natural products activity, revealed interesting trends (**Figure 3**). The data has been compiled from Islam et al. (2020). The most prevalent test system used to date is the SARS-CoV-1, regardless of the strain. Moreover, there are some reports on other coronavirus strains, including MERS-CoV and coronaviruses associated with other animal diseases. Many of the proposed mechanisms were 'undefined,' indicating one of the major concerns and obstacles in drug discovery and natural product pharmacology. The proteases, 3CL^{pro} and PL^{pro}, were identified as the second and third most investigated proposed mechanisms associated with natural product activity, respectively. The group, 'viral infection and replication,' was identified as the fourth-highest proposed mechanism. The exact molecular targets have not been identified in these reports; however, it can be hypothesized that inhibition of viral infection can be associated with the ACE2 receptor. The success of natural product research as anti-coronavirus compounds does not only lie in the rapid identification of active compounds but also the identification of a targeted mechanism of action (Islam et al., 2020).

PLANTS AND ISOLATED COMPOUNDS WITH ACTIVITY AGAINST SARS-COV TARGETS AND OTHER HUMAN CORONAVIRUSES

Several natural products have shown activity and their structures are represented in **Figure 4**. A study conducted by Wen et al. (2007) investigated whether 22 terpenoids and lignoids were able to inhibit viral replication of SARS-CoV in African green monkey kidney (Vero) E6 cells. The cytotoxic effect of the compounds against Vero E6 cells and the ability to inhibit viral replication were measured. The most potent compounds were found to be ferruginol (**1**), 8β-hydroxyabieta-9(11),13-dien-12-one (**2**), 7β-hydroxydeoxycryptojaponol (**3**), 3β,12-diacetoxyabieta-6,8,11,13-tetraene (**4**), betulonic acid (**5**), and savinin (**6**). Compounds **1**–**6** were found to be potent inhibitors of viral replication with effective concentrations (EC_{50}), concentration where 50% of viral replication was inhibited, of 1.39, 1.47, 1.15, 1.57, 0.63, and 1.13 μM, respectively (Wen et al., 2007).

The selective index (SI) values of compounds **1**–**6** were found to be 58, >510, 111, 193, 180, and >667, respectively, indicating that these plants were able to inhibit viral replication without having a cytotoxic effect on the host cells. Compounds **1**, **2**, and **6** were purified from the ethyl acetate extracts of the heartwood of *Chamaecyparis obtuse* var. *formosana* Hayata, whereas compounds **4** and **5** were isolated from the heartwood of *Juniperus formosana* Hayata and compound **3** from *Cryptomeria japonica* (Thunb. ex L.f.) D.Do. Furthermore, betulinic acid (**7**) and savinin (**6**) were able to inhibit SARS-CoV 3CL protease activity (3CL^{pro}) with IC_{50} of 10 and 25 μM. The inhibitory mechanism of betulinic acid (**7**) and savinin (**6**) was also calculated, showing Ki values of 8.2 ± 0.7 and 9.1 ± 2.4 μM, respectively, with a competitive mode of inhibition (Wen et al., 2007).

A chalcone, xanthoangelol E (**8**), isolated from the ethanolic leaf extract of *Angelica keiskei* (Miq.) Koidz., showed inhibitory activity against SARS-CoV 3CL^{pro} and a papain-like protease (PL^{pro}) with IC_{50} values of 11.4 and 1.2 μM, respectively, using cell-free assays. The chalcone was shown to be a competitive inhibitor of the SARS-CoV 3CL^{pro}, whereas noncompetitive inhibition was observed with

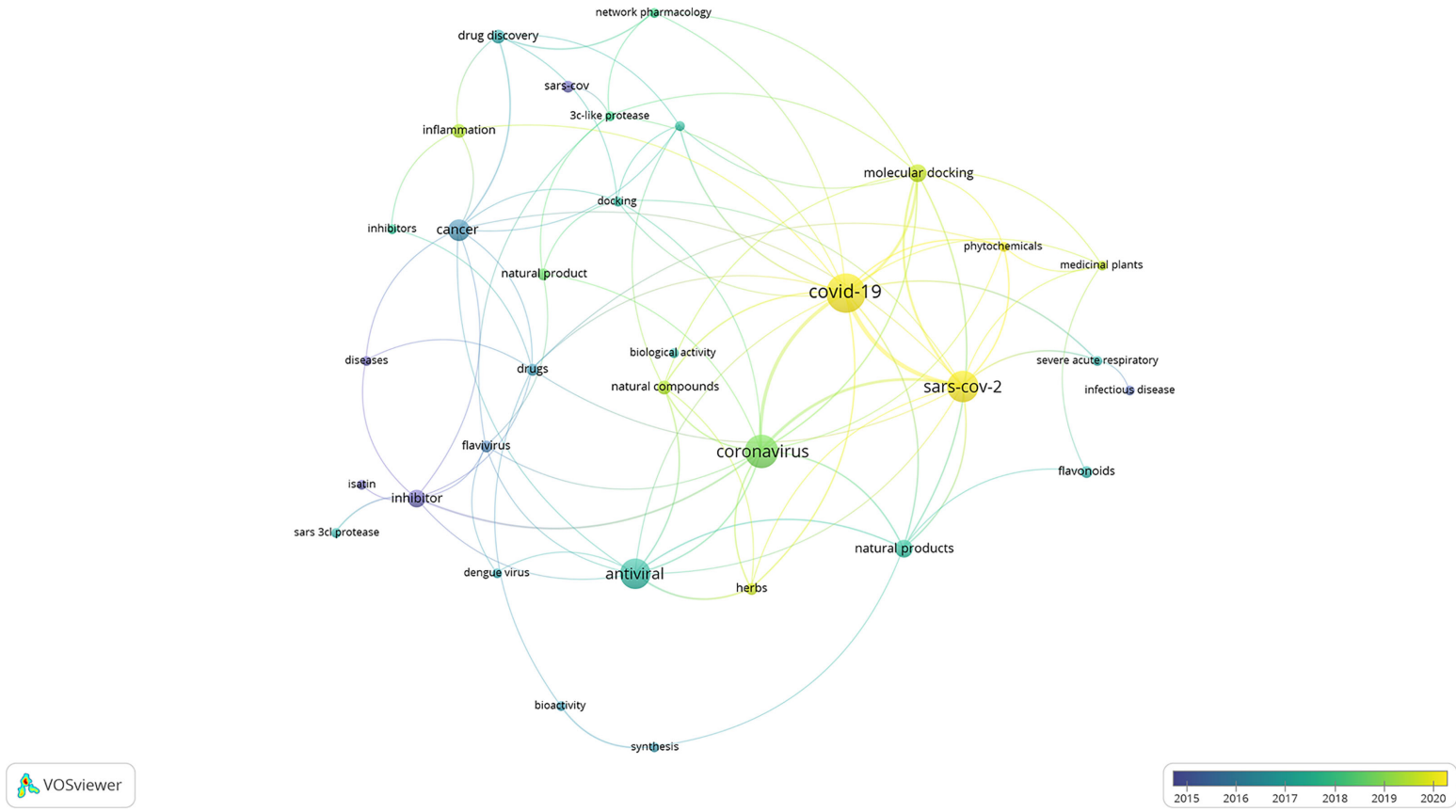

FIGURE 2 | Network map of the literature data analysis (2010 to 2020). Circles represent identified keywords and the size correspond to the occurrence count of the keyword. Curved lines represent the connectivity between different keywords. The color corresponds to the year associated with the specific keyword.

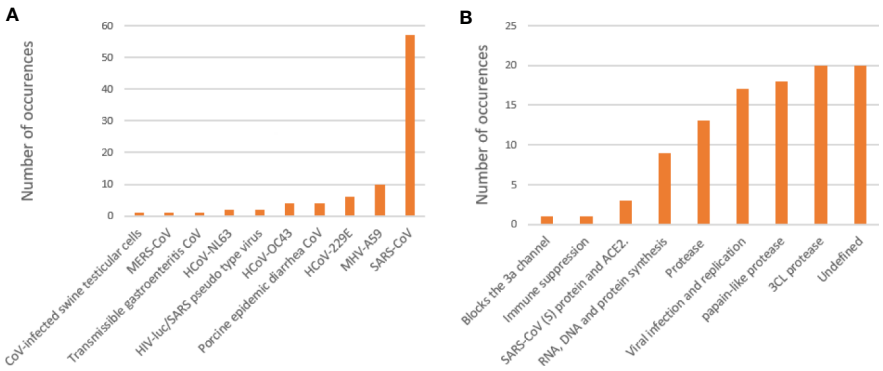

FIGURE 3 | Test systems used in the assessment of natural products against coronaviruses **(A)**. Proposed mechanisms associated with natural products **(B)**.

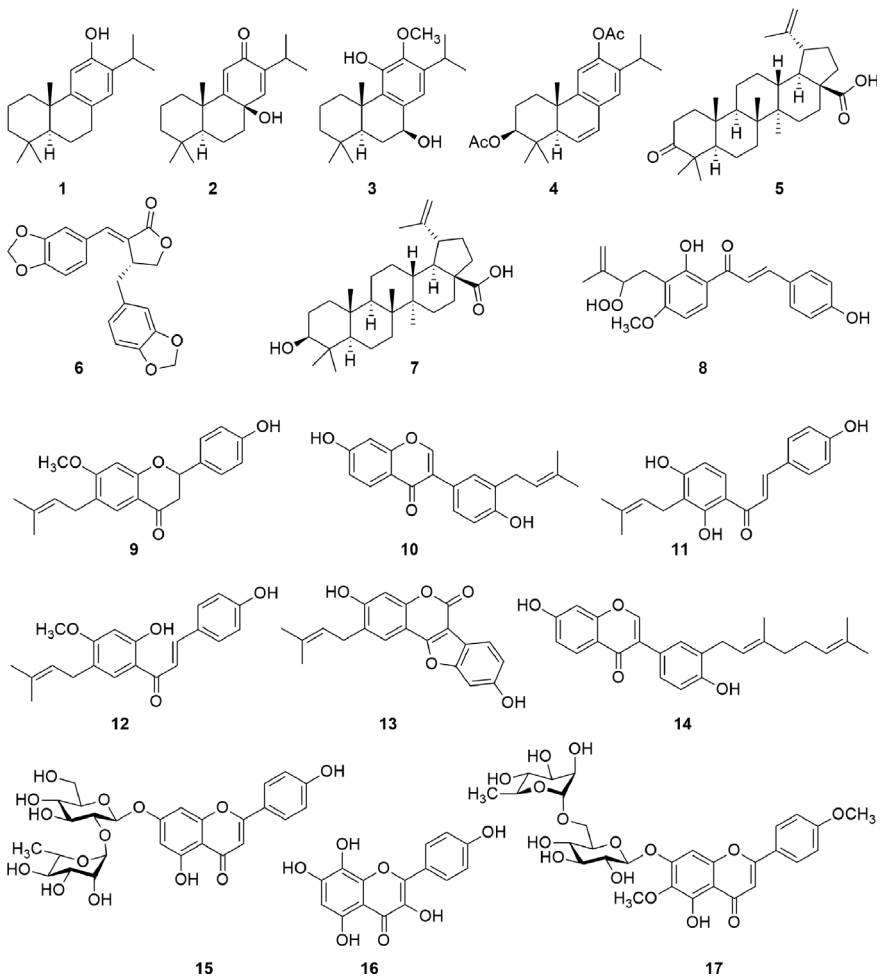

FIGURE 4 | Chemical structures, which target proteins associated with SARS-CoV (1–17).

the SARS-CoV PL^{pro}. In a cell-based assay, xanthoangelol E (**8**) showed an IC_{50} value of 7.1 μM against the SARS-CoV $3CL^{pro}$ and a 50% cytotoxic concentration (CC_{50}) of 65.6 μM against Vero cells (SI = 9.2) (Park et al., 2016). In a study by Kim et al. (2014), six flavonoid compounds bavachinin (**9**), neobavaisoflavone (**10**), isobavachalcone (**11**), 4'-*O*-methylbavachalcone (**12**), psoralidin

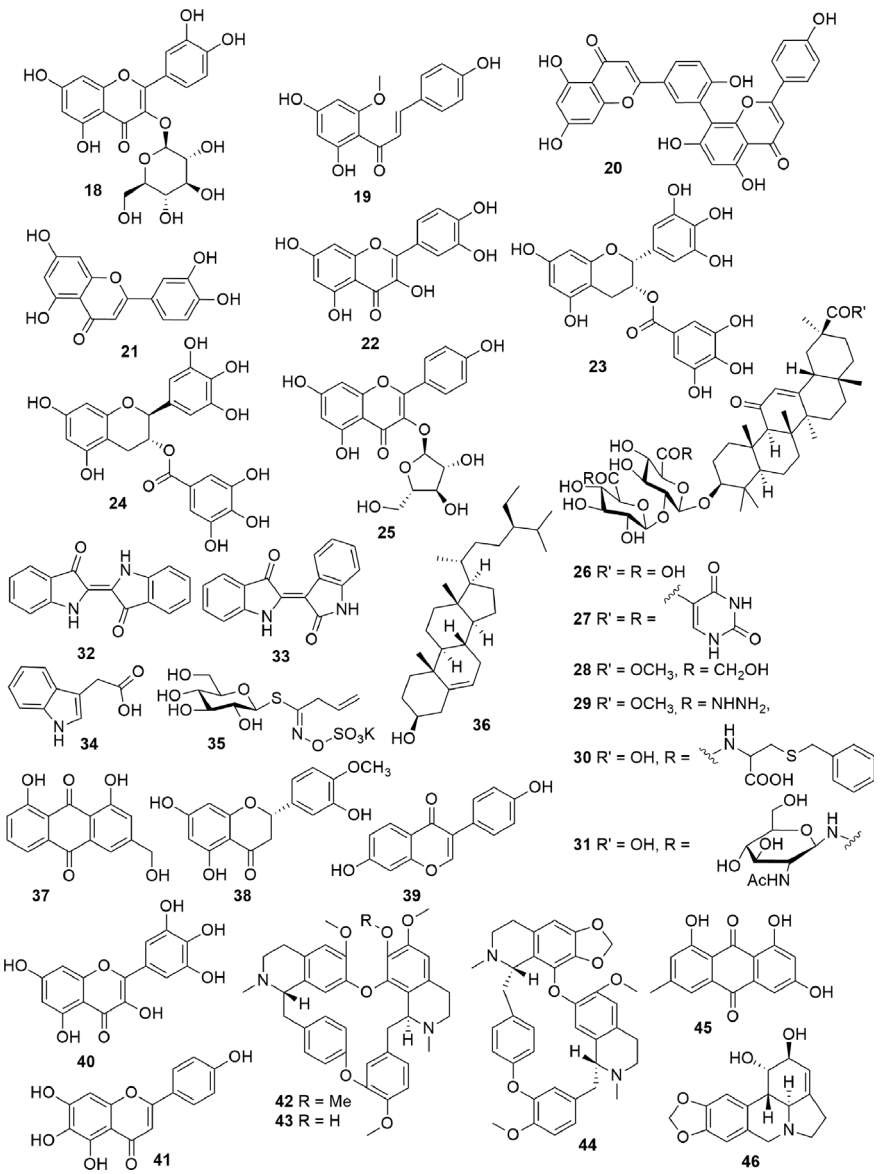

FIGURE 4 | (Continued) Chemical structures which target proteins associated with SARS-CoV (18–46).

(**13**), and corylifol A (**14**) were isolated from the ethanolic extract of the seeds of *Psoralea corylifolia* L. Each of these compounds were able to inhibit PL^{pro} in a dose-dependent manner. Compounds **11** and **13** showed the highest inhibition with IC_{50} values of 7.3 and 4.2 μM, respectively, whereas the other compounds showed lower inhibition with IC_{50} values ranging between 10.1 and 38.4 μM (Kim et al., 2014).

In a recent study by Jo et al. (2020), a flavonoid library was used to examine whether these compounds displayed inhibitory activity against SARS-CoV $3CL^{pro}$. The compounds rhoifolin (**15**), herbacetin (**16**), and pectolinarin (**17**) were found to have noteworthy inhibitory activity against $3CL^{pro}$ with IC_{50} values of 27.45, 33.17, and 37.78 μM, respectively (Jo et al., 2020). The authors, furthermore reported that the compounds herbacetin (**16**), isobavachalcone (**11**), quercetin-3-β-D-glucoside (**18**), and helichrysetin (**19**) were able to inhibit MERS-CoV $3CL^{pro}$ with IC_{50} values of 40.59, 35.85, 37.03, and 67.04 μM, respectively (Jo et al., 2019). In another study, it has been estimated, through a bioinformatic meta-analysis, that the leaves of the Barley varieties, Stratus, and Morex, as well as the leaves of *Ficus deltoidea* Jack, contain high percentages of rhoifolin (**15**), while the leaves of *Cirsium chlorolepis* Petr. ex Hand.-Mazz. contain a high quantity of pectolinarin (**17**). The authors, therefore, hypothesize that these plants may be effective in inhibiting coronaviruses; however, the plant extracts have not been tested (Sawikowska, 2020).

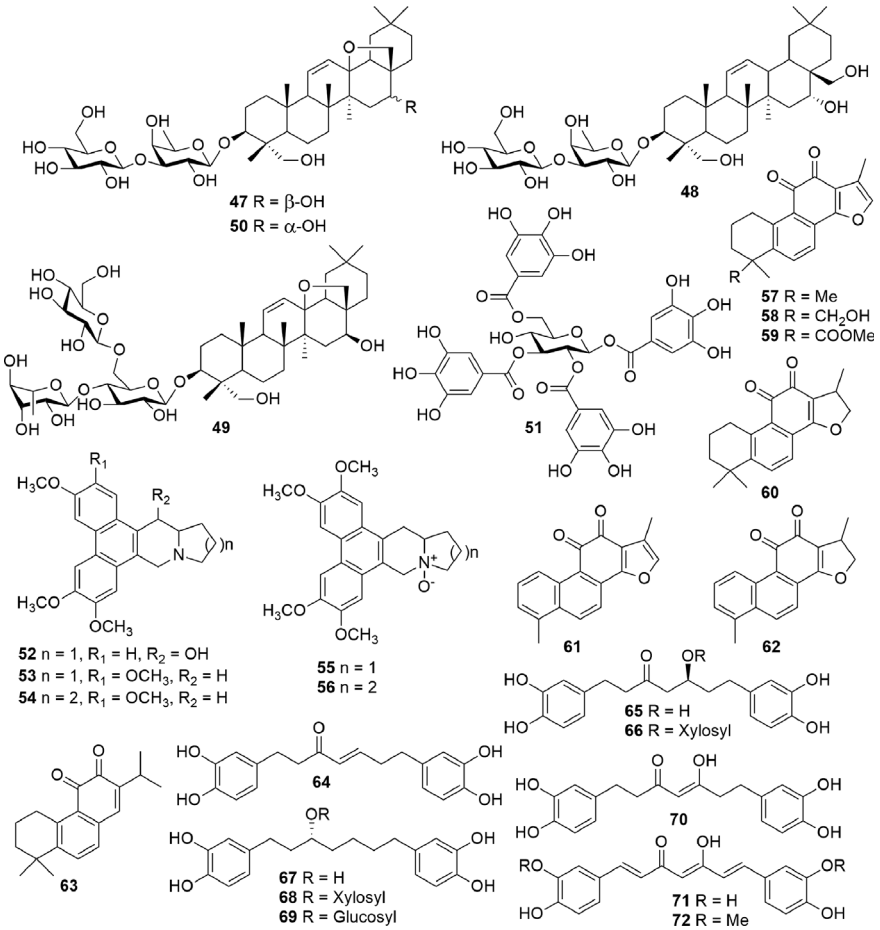

FIGURE 4 | (Continued) Chemical structures which target proteins associated with SARS-CoV (47–72).

A study by Wen et al. (2011) reported that an ethanolic rhizome extract of *Cibotium barometz* (L.) J.Sm., a hexane rhizome extract of *Gentiana scabra* Bunge, a methanolic tuber extract of *Dioscorea batatas* Decne., a hexane seed extract of *Cassia tora* L. and a hexane stem and leaf extract of *Taxillus chinensis* (DC.) Danser showed effective inhibition of SARS-CoV replication in Vero E6 cells with EC_{50} values of 8.42, 8.70, 8.06, 8.43, and 5.39 μg/mL, respectively. The SI values were found to be >59.4, >57.5, >62.0, >59.3, and >92.8, respectively, with each of the extracts having a CC_{50} value of >500 μg/mL. Additionally, the methanolic extracts of *C. barometz* and *D. batatas* showed inhibition of SARS-CoV $3CL^{pro}$ with IC_{50} values of 39 and 44 μg/mL (Wen et al., 2011).

The essential oil from *Laurus nobilis* L. exhibited inhibition against SARS-CoV with an EC_{50} value of 120 μg/mL and an SI value of 4.16 (Loizzo et al., 2008). At a concentration of 100 μg/mL, an ethanolic leaf extract of *Torreya nucifera* (L.) Siebold and Zucc. exhibited 62% inhibition of SARS-CoV $3CL^{pro}$ compared to the untreated enzyme control. Through bioassay-guided fractionation, the compound amemtoflavone (**20**), a biflavone, was isolated, which showed the most potent $3CL^{pro}$ activity with a non-competitive IC_{50} value of 8.3 μM. In this study, luteolin (**21**) and quercetin (**22**) were also tested, which showed IC_{50} values of 20.2 and 23.8 μM, respectively. The type of inhibition for these two compounds could not be determined, which might be indicative of a false positive (Ryu et al., 2010). In a study by Nguyen et al. (2012), quercetin (**22**) was reported to have an IC_{50} value of 73 μM against SARS-CoV $3CL^{pro}$; however, no mention is made to the type of enzymatic inhibition. Luteolin (**21**) and quercetin (**22**) are known as pan-assay interference compounds due to the catechol moiety. Additional assays are required if a compound is classified as a pan assay interference compound (PAINS) (Nguyen et al., 2012). To determine specific enzyme activity, the assays should include counter-screening on unrelated targets, kinetic investigation to determine if the compound is a competitive or non-competitive inhibitor, and clearly identifying and carefully describing the concentration-response curves (Aldrich et al., 2017). Both epigallocatechin gallate (**23**) and gallocatechin gallate (**24**) also inhibited SARS-CoV $3CL^{pro}$ with IC_{50} values of 73 and 47 μM, respectively. Furthermore, gallocatechin gallate (**24**) was found to be a competitive inhibitor of $3CL^{pro}$ with a Ki value of 25 μM

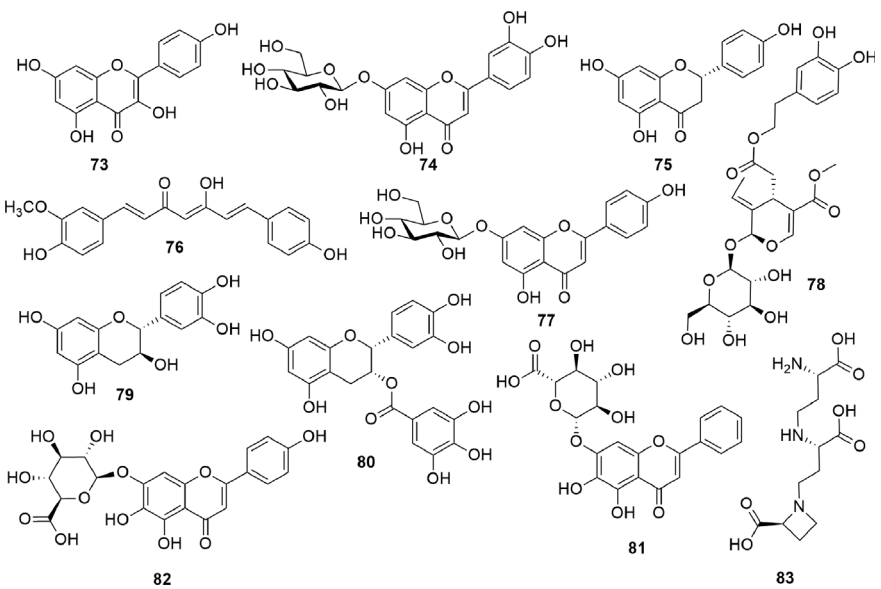

FIGURE 4 | (Continued) Chemical structures which target proteins associated with SARS-CoV (73–83).

(Nguyen et al., 2012). Similar to luteolin (**21**) and quercetin (**22**), both epigallocatechin gallate (**23**) and gallocetechin gallate (**24**) contain substructures classified as PAINS. To further assess the antiviral activity, PAINS need to undergo cellular-based inhibitory assays in order to eliminate false positives. The different cell-based assays are briefly summarized in the discussion section. The compound juglanin (**25**), a glycoside of kaempferol, was shown to effectively inhibit the 3a-mediated current with an IC_{50} of 2.3 μM. The protein which is encoded by the open-reading frame 3a (ORF3a) of SARS is involved in virus release and production (Schwarz et al., 2014). Glycyrrhizin (**26**), a triterpenoid glycoside isolated from *Glycyrrhiza glabra* L., was one of the first compounds found to inhibit SARS-CoV replication *in vitro*. Several derivatives (**27**–**31**) of glycyrrhizin (**26**) have also been synthesized which showed up to 70-fold increased activity. Glycyrrhizin (**26**) was found to inhibit viral replication with an EC_{50} value of 365 μM and an SI value of >65. The EC_{50} values for derivatives **27**–**31** were 5.0, 8.0, 16.0, 35.0, and 40.0 μM, respectively, while the SI values were 3, 6, 4, 41, and >75, respectively (Hoever et al., 2005). In a similar study, **26** was found to inhibit the cytopathic effect of SARS-CoV with an EC_{50} value of 300 μg/mL and an SI value of >33. (Cinatl et al., 2003).

A root extract of *Isatis indigotica* Fortune ex Lindl., as well as compounds isolated from the plant; indigo (**32**), indirubin (**33**), indican (**34**), sinigrin (**35**), *β*-sitosterol (**36**), aloeemodin (**37**), hesperetin (**38**), and daidzein (**39**) were able to inhibit the cleavage of $3CL^{pro}$ in a cell-free assay with IC_{50} values of 53.8, 37.3, 81.3, 33.1, 50.3, 47.8, 35.7, 18.1, and 26.8 μg/mL, respectively (Lin et al., 2005). However, when tested in a cell-based assay, only the extract and indigo (**32**), sinigrin (**35**), *β*-sitosterol (**36**), aloeemodin (**37**), and hesperetin (**38**) showed inhibition, with IC_{50} values of 191.6, 190, 90.1, 502.1, 99.1, and 2.5 μg/mL, respectively (Lin et al., 2005). An aqueous extract prepared from the whole plant of *Houttuynia cordata* Thunb. showed low inhibition of both SARS $3CL^{pro}$ and RdRp activity in a dose-dependent manner with 50% inhibition of $3CL^{pro}$ at a concentration >1,000 μg/mL and 50% inhibition of RdRp activity at >200 μg/mL. Although a concentration-response curve was present in the reported activity, the inhibitory activity, on both $3CL^{pro}$ and RdRp, is lower when compared to other plants. The study does not report on the potential active compound/s, and therefore, bioassay-guided fractionation is needed to identify bioactive compounds. However, this is unlikely due to the low activity observed. Moreover, the study reported acute oral toxicity conducted in mice, which found that the extract was non-toxic when administered at 16 g/kg (16,000 mg/kg). However, there was a mortality rate of 10% among the female mice (Lau et al., 2008). In addition, this dosage is considered extremely high, exceeding the recommended upper limit of 2000 mg/kg (5,000 mg/kg in extreme cases), set out by the OECD guidelines (Erhirhie et al., 2018). In addition, the study by Lau et al. (2008) identified that the extract was able to induce T cell proliferation, specifically $CD4^+$ and $CD8^+$ T cells in an *in vitro* splenic lymphocyte assay at concentrations ranging from 50–400 μg/mL (Lau et al., 2008). Woranam et al. (2020) reported that aqueous and methanolic extracts prepared from the aerial parts of *H. cordata*, at concentrations ranging between 5–750 and 4–12 μg/mL, respectively, were able to reduce nitric oxide production in murine macrophages (RAW 264.7) and decreased the expression of PGE2, iNOS, IL-1β, TNF-α, and IL-6 in LPS-stimulated RAW 264.7 cells (Woranam et al., 2020). Therefore, this plant should rather be considered as a potential immune modulator, as opposed to an antiviral, but further investigation is needed.

In a study by Yu et al. (2012), 64 compounds were tested for their inhibitory activity against the SARS helicase enzyme (nsP13). Myricetin (**40**) and scutellarein (**41**) were able to significantly inhibit nsp13 ATPase activity with IC_{50} values of 2.71 and 0.83 μM, respectively. Furthermore, cytotoxicity studies revealed that these compounds, at a concentration of 2 μM, did not affect the growth of normal epithelial breast cells (MCF10A) (Yu et al., 2012).

The alkaloids tetrandrine (**42**), fangchinoline (**43**), and cepharanthine (**44**) were able to inhibit the cytopathic effect of HCoV-OC43 in human lung cells (MRC-5) with EC_{50} values of 295.6, 919.2, and 729.7 nM, respectively. The cytotoxic effect of the compounds in the MRC-5 cells was determined and showed CC_{50} values of 15.51, 12.40, and 10.54 μM and SI values of >40, 11, and 13, respectively (Kim et al., 2019). These compounds additionally were able to inhibit the expression levels of the N and S proteins and the inflammatory cytokines interleukin 1β (IL-1β), IL-6, and IL-8. Furthermore, Zou et al. (2019) reported that tetrandrine (**42**) was able to inhibit pro-inflammatory Th1, Th2, and Th17 cells (Zou et al., 2019).

A chloroform fraction, from an ethyl acetate partition, from a 75% ethanolic extract of the whole plant of *Rheum palmatum* L., showed a high inhibition of SARS-CoV $3CL^{pro}$ with an IC_{50} value of 13.76 μg/mL. The inhibitory activity of the crude extract was 38.09 μg/mL, while the fractions and partitions showed inhibition ranging between 13.76 and 59.33 μg/mL (Luo et al., 2009). The study does not report the identification of an active compound or the mechanism of action by binding to a specific target. In addition, the activity does not appear to be specific to polarity, which may be indicative of a false positive. An in-depth investigation is needed to confirm the suitability of *R. palmatum* and its constituents as potential candidates for further investigation. A water extract prepared by boiling (decoction) the leaves of *Toona sinensis* (Juss.) M. Roem. inhibited HCoV 229E viral replication in Vero cells with an EC_{50} of 30 μg/mL. This plant is consumed as a cooked vegetable and the extract was, therefore, prepared from the cooked/boiled menstruum. When the extract preparation did not include boiling, an EC_{50} value of 43 μg/mL was obtained. Both the boiled and non-boiled extract did not show cytotoxic effects against the Vero cells, with CC_{50} values of >500 μg/mL and SI values of 17 and >12, respectively. The proposed active compound/s, mechanism of action, and the effect of boiling on the chemical profile have not been identified or discussed (Chen et al., 2008). Although the article identified a difference in activity between boiled and non-boiled extracts, the difference appears insignificant, however, the difference noted in the activity might be due to the breakdown and release of glucose and an aglycon from glycosides during the heating process (Fabbri and Chiavari, 2001). The compound emodin (**45**), found within the genus *Rheum* and *Polygonum*, was able to block the binding of the SARS-CoV S protein to the ACE2 receptor with an IC_{50} value of 200 μM (Ho et al., 2007). Emodin (**45**) was furthermore able to inhibit the SARS-CoV and HCOV-OC43 3a ion channel with a $K_{1/2}$ value of 20 μM (Schwarz et al., 2011). An ethanolic stem cortex extract of *Lycoris radiata* (L'Hér.) Herb. exhibited anti-SARS-CoV activity against viral strains BJ-001 and BJ-006 with EC_{50} values of 2.4 and 2.1 μg/mL and SI values of 370 and 422, respectively. The EC_{50} and SI values for the total alkaloid fraction from *L. radiata* was found to be 1.0 μg/mL and 94, respectively. This led to the isolation of lycorine (**46**) from *L. radiate*, which showed significant inhibition with an EC_{50} and SI value of 15.7 μg/mL and 954, respectively (Li et al., 2005).

Saikosaponins, which are oleanane derivatives, were tested for antiviral activity against the coronavirus 229E. Saikosaponin A (**47**), B_2 (**48**), C (**49**), and D (**50**) showed inhibition of HCoV-229E viral infection in MRC-5 cells with EC_{50} values of 8.6, 1.7, 19.9, and 13.2 μmol/L. These saikosapnins furthermore were not cytotoxic to the MRC-5 cells with CC_{50} values of 228.1, 383.3, 151.5, and 176.2 μmol/L with SI values of 26.6, 221.9, 19.2, and 13.3, respectively. In addition, saikosaponin B_2, which showed the highest activity, was able to inhibit viral attachment and penetration (Cheng et al., 2006). In a study by Yi et al. (2004), tetra-*O*-galloyl-*β*-D-glucose (**51**) and luteolin (**21**) were tested for their activity against SARS-CoV. Both compounds were able to dose-dependently inhibited SARS-CoV infection in Vero E6 cells with EC_{50} values of 4.5 and 10.6 μM, respectively. The cytotoxic effect was also determined, and both were found to be non-toxic with CC_{50} values of 1.08 and 0.115 mM and SI values of 240 and 14.62, respectively (Yi et al., 2004).

Tylophorinine (**52**), isolated from *Tylophora indica* (Burm. f.) Merr., and four synthetic an tylophorine (**53**), 7-methoxycryptopleurine (**54**), tylophorine N-oxide (**55**) (a naturally occurring compound), and 7-methoxycryptopleurine N-oxide (**56**) showed significant activity against SARS-CoV with EC_{50} values ranging from <5 to 340 nM and SI values ranging from 1.7 to >100 (Yang et al., 2010). Tanshinones (**57**–**63**), isolated from *Salvia miltiorrhiza* Bunge, were found to be time-dependent selective inhibitors against the cysteine protease SARS-CoV PL^{pro}. Tanshinone IIA (**57**), tanshinone IIB (**58**), methyl tanshinonate (**59**), cryptotanshinone (**60**), tanshinone I (**61**), and dihydrotanshinone I (**62**) were identified as non-competitive enzyme isomerization inhibitors, whereas rosmariquinone (**63**) showed a mixed-type simple reversible slow-binding inhibition. The IC_{50} values of compounds **57**–**63** were found to range between 0.8 and 30.0 μM against SARS-CoV PL^{pro} and between 14.4 and 226.7 μM against SARS-CoV CL^{pro} (Park et al., 2012b). Six diarylheptanoids (**64**–**69**), isolated from *Alnus japonica* (Thunb.) Steud., as well as two synthetic derivatives (**70**–**71**), showed inhibitory activity against SARS-CoV PL^{pro} with IC_{50} values ranging between 4.1 and 59.8 μM. Curcumin (**72**) was used as a positive control in this study, which showed an IC_{50} value of 5.7 μM (Park et al., 2012a).

PROSPECTS OF USING COMPUTATIONAL TECHNIQUES TO SCREEN POSSIBLE ANTI-COVID-19 AGENTS FROM PLANTS

Zhang et al. (2020b), recently reported the crystal structure of the SARS-CoV-2 main protease (Mpro also called $3CL^{pro}$), which is essential for viral replication. The availability of the crystal structure

allows compounds, which have shown activity against SARS-CoV proteases, and other similar compounds to be screened through computational studies to identify possible lead molecules active against COVID-19. Based on a molecular docking study reported by Khaerunnisa et al. (2020), kaempferol (**73**), quercetin (**22**), luteolin-7-*O*-glucoside (**74**), naringenin (**75**), desmetho-xycurcumin (**76**), curcumin (**72**), apigenin-7-*O*-glucoside (**77**), oleuropein (**78**), catechin (**79**), and epicatechin-gallate (**80**) could potentially inhibit SARS-CoV-2 $3CL^{Pro}$ and therefore act as anti-COVID-19 agents (**Figure 4**); however, *in vitro* studies are required to assess these results further (Khaerunnisa et al., 2020). According to another report, the host receptor for SARS-CoV-2, ACE2, is the same as the host receptor of SARS-CoV; therefore, the inhibitors of SARS-CoV ACE2 might be able to inhibit the same receptor in SARS-CoV-2 (Salata et al., 2019). Based on the molecular docking study performed by Chen and Du (2020), baicalin (**81**), scutellarin (**82**), hesperetin (**38**), nicotianamine (**83**), and glycyrrhizin (**26**) have been identified as potential ACE2 inhibitors and could be used as possible anti-2019-nCoV agents (Chen and Du, 2020). Molecular docking can be a useful tool to describe binding affinities and molecular interactions and is a rapid technique in which to identify potentially active compounds during drug discovery. However, *in vitro* or *in vivo* antiviral tests are crucial in order to support molecular docking data, which describes a compound with potent activity. Studies have shown that a positive correlation between docking scores and pharmacological activity are relatively low and docking is not very effective in ranking active compounds (Vilar and Costanzi, 2012). This emphasizes the need to include wet-lab experimentation to substantiate the activities of natural products, especially in the context of a global pandemic.

POTENTIAL LEADS FROM SOUTHERN AFRICAN PLANTS

In Southern Africa, a major portion of the population relies primarily on traditional medicine as a source of health care. In traditional knowledge systems, the use of a plant for the treatment of a specific symptom, rather than a specific disease or infectious organism is recorded. In this section, Southern African plants that are traditionally used in the treatment of coughs, fevers, colds, and influenza have been listed as potential candidates for testing against SARS-CoV-2 and related targets (**Table 3**) (Van Wyk et al., 2009). This aids in identifying a large number of potential plant species, especially Southern African plants, which can be considered for investigating the potential inhibition against coronaviruses. Only a few of the plant species listed in **Table 3** have been tested for their antiviral potential, indicating the major gap in scientifically assessing the medicinal potential of traditionally used plants, thereby emphasizing the importance for African-based researchers to include these types of studies within their research focus. Furthermore, extensive toxicity and *in vivo* testing is necessary to investigate the pharmacological use of these plants and compounds.

Artemisia afra, has not been tested for its inhibitory potential against coronaviruses, however, a closely related species, *A. annua*, was able to inhibit SARS-CoV BJ-001 viral replication in Vero cells, with an EC_{50} value of 34.5 ± 2.6 μg/mL. Although these are two different species, it has been shown that within the *Artemisia* genus, many compounds are conserved; however, it is the small chemical nuances and profile that have a large effect on the biological activity (Abad et al., 2012). Medicinal plants species that are closely related may also produce similar or chemically similar compounds responsible for their biological activity (Nigam et al., 2019). This forms the basic definition for chemotaxonomy, which is the "closely related plants contain the same or similar chemical profiles" (Hao and Xiao, 2020). As an example, in a review article published by da Silva Mendes et al. (2020), many aspects of the *Cissampelos* genus were investigated, including the ethnobotanical aspects, isolated phytochemicals, and biological activity of the different species. Most of the biological activity described to species within the *Cissampelos* genus is attributed to the presence of alkaloids. The review, furthermore, describes the presence of similar compounds within different *Cissampelos* species. Many biological activities are attributed to warifteine, including results from clinical studies, and this compound was isolated from both *Cissampelos ovalifolia* and *Cissampelos sympodialis* (da Silva Mendes et al., 2020). Another example is the Southern African species, *Ziziphus mucronata*, which has not been investigated for its antiviral activity; however, cyclopeptide alkaloids isolated from *Z. jujuba* showed inhibition of a porcine-related coronavirus (porcine epidemic diarrhea virus (PEDV)), with SI values ranging from 7.98 to 47.11 on Vero cells (Kang et al., 2015).

Helichrysetin, a compound found within numerous *Helichrysum* species was able to inhibit MERS-CoV $3CL^{pro}$ (Jo et al., 2019). A commercial product from *Pelargonium sidoides*, EPs® 7630, showed a low selectivity index of 2.3 when tested against the human coronavirus strain 229E in Caco-2 cells. Two significant compounds identified within the traditionally used plants have been investigated for their potential against coronaviruses. *β*-Sitosterol, present in *Dodonaea viscosa* and *Prunus africana*, showed an EC_{50} value of 1210 μM against human coronavirus (HCoV-NL63) (Lin et al., 2005). Reserpine, a major constituent of *Rauvolfia caffra*, inhibited SARS-CoV viral replication with an EC_{50} value of 3.4 μM, CC_{50} value of 2.5 μM, and SI value of 7.3 (Wu et al., 2004). The further testing of the listed plant species could potentially identify a lead candidate for the treatment of COVID-19.

DISCUSSION

The COVID-19 pandemic has resulted in numerous clinical trials to evaluate whether existing drugs can be repurposed for the potential treatment of COVID-19. Studies have led to the following conclusions; treatment of COVID-19 might not be efficient if an antiviral drug alone is used, although small scale studies have shown some promise, larger-scale *in vivo* clinical studies are required to effectively evaluate the efficacy and safety of drugs. Furthermore, it is crucial to include placebo controls to adequately evaluate the potential benefit of a drug. Despite the publicity surrounding the drug, hydroxychloroquine as a potential treatment for COVID-19, RECOVERY has recently concluded that it has no beneficial effect toward severe cases of COVID-19 patients and therefore have stopped recruiting patients for clinical trials using

TABLE 3 | Potential Southern African medicinal plants (traditionally used for coughs, fevers, colds and influenza) that showed activity against coronaviruses or against similar viruses [the list have been compiled from Medicinal Plant of South Africa (Van Wyk et al., 2009)].

Name	Vernacular name	Reported activity against human coronaviruses
Adansonia digitata L.	Kremetart, Baobab, Shimuwu, Movana, Muvhuyu	NT[#]
Agathosma betulina (P.J.Bergius) Pillans	Boegoe, Buchu, Ibuchu	NT
Alepidea amatymbica Eckl. & Zeyh.	Kalmoes, Lesoko, Iqwili, Ikhathazo	NT
Aloe excelsa A.Berger	Noble aloe, Zimbabwe aloe	NT
Artemisia afra Jacq. ex Willd.	Als, Wildeals, African wormwood, Lengana, Umhlonyane	*Artemisia annua*, closely related species to *A. afra*: EC_{50}^{+} = 34.5 ± 2.6 μg/mL (SARS-CoV BJ-001); CC_{50}^{++} = 1053 ± 92.8 μg/mL (Vero cells); SI[##] = 27 (Li et al., 2005)
Aspalathus linearis (Burm.f.) R.Dahlgren	Rooibostee, Rooibos tea	Quercetin: IC_{50}^{+++} = 73 μM (Recombinant $3CL^{pro}$) (Nguyen et al., 2012) Luteolin: EC_{50} = 10.6 μM (wild-type SARS-CoV); CC_{50} = 0.16 mM (Vero cells); SI = 14.62 (Yi et al., 2004)
Ballota africana (L.) Benth.	Kattekruid	NT
Camellia sinensis (L.) Kuntze	White tea, green tea, mchai (Kiswahili)	Epigallocatechin gallate: IC_{50} = 73 μM (Recombinant $3CL^{pro}$) (Nguyen et al., 2012)
Cannabis sativa L.	Dagga, Marijuana, Matokwane, Umya, Nsangu	NT
Catha edulis (Vahl) Endl.	Boesmanstee, Khat, Bushman's tea	NT
Chondropetalum mucronatum (Nees) Pillans	Mountain Restio	Myricetin: IC_{50} = 2.71 ± 0.19 μM (nsP13, SARS helicase protein); Cytotoxicity: No toxicity at 2 μM against MCF10A cells (Yu et al., 2012) Quercetin: IC_{50} = 73 μM (Recombinant $3CL^{pro}$) (Nguyen et al., 2012)
Cinnamomum camphora (L.) J.Presl	Kamferboom, Camphor tree, Uroselina	NT
Croton gratissimus Burch.	Bergboegoe, Lavender croton, Maquassie, Umahlabekufeni	NT
Cyclopia latifolia DC.	Heuningbos, Honeybush	Epigallocatechin gallate: IC_{50} = 73 μM (Recombinant $3CL^{pro}$) (Nguyen et al., 2012) Luteolin: EC_{50} = 10.6 μM (wild-type SARS-CoV); CC_{50} = 0.16 mM (Vero cells); SI = 14.62 (Yi et al., 2004)
Datura stramonium L.	Stinkblaar, Thornapple, Lethsowe, Zaba-zaba, Iloyi, Ijoyi	NT
Dicoma capensis Less.	Wilde karmedik, Koorsbossie	NT
Dodonaea viscosa (L.) Jacq.	Sandolien, Sand olive, Mutepipuma, Mutata-vhana	β-sitosterol: EC_{50} = 1210 μM (HCoV-NL63) (Lin et al., 2005)
Drimia elata Jacq.	Brandui, Indongana-zibomvana	NT
Glycyrrhiza glabra L.	Soethoutwortel, Liqourice root, Mlomo-mnandi	Glycyrrhizin: EC_{50} = 300 mg/L (SARS-CoV); CC_{50} >20 000 mg/L (Vero cells); SI >67 (Cinatl et al., 2003)
Halleria lucida L.	Tree fuschia, white olive	NT
Helichrysum spp.	Kooigoed, Everlastings, Isicwe, Imphepho	Helichrysetin: IC_{50} = 67.04 μM (MERS-CoV 3C like-protease) (Jo et al., 2019)
Heteropyxis natalensis Harv.	Laventelboom, Lavender tree, Inkunzi	NT
Leonotis leonurus (L.) R. Br.	Wilde dagga, Wild dagga, Umhlahlampetu, Lebake, Umunyane	NT
Lippia javanica (Burm.f.) Spreng	Koorsbossie, Fever tea, Mumara, Musukudu, Inzinziniba, Umsuzwane	NT
Mentha longifolia (L.) L.	Kruisement, Wild mint, Koena-ya-thaba, Inixina, Ufuthanen lomhlange	NT
Myrothamnus flabellifolia Welw.	Bergboegoe, Resurrection plant, Uvukwabafile	NT
Myrsine melanophloeos (L) R. Br.	Kaapse boekenhout, Cape beech, Isiqwane-sehlati, Umaphipha	NT
Osmitopsis asteriscoides Less.	Bels, Belskruie	NT
Pelargonium sidoides DC.	Rabas, Khoaara e nyenyane, Ikhubalo	EPs® 7630 (commercial product prepared from *P. sidoides*): EC_{50} = 44.50 ± 15.84 μg/mL (HCoV 229E); CC_{50} >100 μg/mL (Caco-2 cells); SI > 2.3 (Michaelis et al., 2011)
Pellaea calomelanos (Sw.) Link	Hard fern, Lehorometso, Inkomankomo	NT
Protea repens L.	Suikerbos, Sugarbush	NT
Prunus africana (Hook.f) Kalkman	Rooistinkhout, Red stinkwood, Umkakase, Inyazongoma-elimnyana	β-sitosterol: EC_{50} = 1210 μM (HCoV-NL63) (Lin et al., 2005)
Rauvolfia caffra Sond.	Kinaboom, Quinine tree, Umhlambamase, Umhlambamanzi	Reserpine: EC_{50} = 3.4 μM (SARS-CoV); CC_{50} = 2.5 μM (Vero-cells); SI: 7.3 (Wu et al., 2004)
Salix mucronata (Thunb.)	Wilde wilger, Wild wilow	NT
Scadoxus puniceus (L.) Friis & Nordal	Rooikwas, Red paintbrush, Umphompo	NT

(Continued)

TABLE 3 | Continued

Name	Vernacular name	Reported activity against human coronaviruses
Searsia undulata (Jacq.) T. S. Yi, A.J.Mill. & J. Wen	Koeniebos, Kuni-bush, T'kuni	NT
Securidaca longipedunculata Fresen.	Krinkhout, Violet tree, Mpesu	NT
Siphonochilus aethiopicus (Schweinf.) B.L.Burtt.	African ginger, Isiphephetho, Indungulo	NT
Tarchonanthus camphoratus L.	Wildekanferbos, Wild camphor bush, Sefehla, Umgebe, Mofahlana, Mohata, Mathola	NT
Tetradenia riparia (Hochst.) Codd.	Watersalie, Ginger bush, Iboza	NT
Thesium hystrix A.W. Hill	Kleinswartstorm	NT
Tulbaghia violacea Harv.	Wilde knoffel, Wild garlic, Isihaqa	NT
Viscum capense L. f.	Lidjiestee, Cape mistletoe	NT
Withania somnifera (L.) Dunal	Geneesblaarbossie, Winter cherry, Bofepha, Ubuvuma, Ubuvimbha	NT
Xerophyta retinervis Baker	Bobbejaanstert, Monkey's tail, Isiphemba, Isiqumama	NT
Zanthoxylum capense (Thunb.) Harv.	Kleinperdepram, Small knobwood, Monokwane, Umlungumabele, Umnungamabele	NT
Zingiber officinale Roscoe	Gemmer, Ginger	NT
Ziziphus mucronata Willd.	Blinkblaar-wag-'n-bietjie, Buffalo thorn, Mokgalo, Umphafa, Umlahlankosi	*Z. jujuba* cyclopeptide alkaloids Jubanine H: EC_{50} = 4.49 ± 0.67 μM (PEDV, CoV); CC_{50} = 211.26 ± 29.64 μM (Vero cells); SI = 47.11 ± 0.49 Nummularine B: EC_{50} = 6.17 ± 0.50 μM (PEDV, CoV); CC_{50} = 165.30 ± 16.49 μM (Vero cells); SI = 26.75 ± 0.54 (Kang et al., 2015)

The plants have been selected from Medicinal Plants of South Africa (Van Wyk et al., 2009) and other resources, #Not tested (selected based on traditional usage), +50% effective concentration, ++50% cytotoxic concentration; ##Selective index (CC_{50}/EC_{50}), +++50% inhibitory concentration.

hydroxychloroquine. However, treatment of patients with a combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin was shown to be effective in alleviating COVID-19 symptoms and shortening virus shedding; however, this study lacked the required placebo control, and therefore, no conclusion can be made with regard to the therapeutic effect against COVID-19. Additional studies are required to substantiate these findings. *In vitro* studies showed that ivermectin was able to significantly reduce viral replication; however, no clinical trials have been completed to substantiate these results.

Studies have recently started to focus on the infection of SARS-CoV-2 in several other organs such as the heart, kidney, and liver, which widely express the ACE2 receptor, thereby leading to multiple organ toxicity and not only the severe infection of the lungs. Multiple studies have reported an increased inflammatory response in the endothelium, apoptotic bodies present within the heart, lungs, and small bowel; endotheliitis in the lungs heart, kidney, and liver; and necrosis of liver cells, which further suggests that antiviral treatment alone will not be sufficient and that a combination of drugs, including anti-inflammatories might be more effective, as shown in the preliminary study by Hung et al. (2020) (Hung et al., 2020). Additionally, the use of hepatoprotective drugs might be beneficial as Xu et al. (2020) reported that a COVID-19 patients' liver tissue showed moderate microvesicular steatosis, which may have been due to COVID-19 infection or was due to drug-induced damage (Xu et al., 2020).

There have been countless studies where plant extracts and isolated compounds have been tested for activity against several strains of human coronaviruses. In this review, it was noted that extracts and compounds have been tested mainly against various target proteins of the coronaviruses such as protease activity ($3CL^{pro}$), RNA-dependent RNA polymerase (RdRp), and papain-like proteinase (PL^{pro}). These target proteins are critical for viral replication and infection in the host cell, thereby providing valuable targets for potentially inhibiting these processes. Cell culture–based techniques for testing the potential antiviral activity have been developed, which focus on screening of samples as potential viral inhibitors in an intracellular assay rather than testing activity using biochemical assays involving specific viral enzymes as mentioned above. There are several techniques that can be used to determine the antiviral activity of a sample. As an initial assay, the cytopathic effect (CPE) assay is most often used, which determines the ability of samples to prevent the virus from causing a cytopathic effect in the host cell. This also involves determining the potential toxicity of the sample against the host cell line used to perform the assay, which is most often depicted as the concentration required to cause toxicity to 50% of the host cells (CC_{50}). The CPE assay is frequently followed by the viral reduction assay, which determines whether a sample is able to inhibit viral production in the host cell, post-infection. Additionally, the virucidal assay is used to determine the ability of a sample to kill the virus extracellularly before it infects the mammalian host cell line. This can be performed in a time-dependent manner in order to establish the shortest time necessary for the sample to display inhibition of viral infectivity. This can also include determining whether a sample is able to inhibit viral attachment and inhibit viral entry into the host cells (Lalani et al., 2020). The plaque assay was adopted for reliable determination of the titers of a wide variety of viruses. Each infectious particle produces a circular zone of infected cells, known as a plaque, which can be visually observed. This assay

can only be performed using viruses that cause visible damage to the host cell (Flint et al., 2009). In a recent publication by Harcourt et al. (2020), it was shown that SARS-CoV-2 was not compatible with human lung adenocarcinoma (A549) cells and was able to moderately replicate in human liver (HUH7.0) and human embryonic kidney (HEK-293T) cells and was not able to replicate in big brown bat kidney (EFK3B) cells. However, results suggested that the best candidate for viral amplification and quantification was the VeroE6 cell line, which is widely used as a host cell line for antiviral studies (Harcourt et al., 2020).

When identifying a potential lead candidate with antiviral activity, pre-clinical toxicity studies are important to establish the margin of safety and to efficiently consider the risk-benefit of a proposed drug. The antiviral activity of a sample is determined by the 50% effective concentration (EC_{50}), which is the concentration required to inhibit 50% viral replication/production using cellular-based assays or by the IC_{50} in assays where viral enzymes are targeted, such as proteases and polymerases. The overall therapeutic activity can be determined by calculating the selectivity index, which is defined as the ratio of the CC_{50} to the 50% concentration needed to inhibit viral replication (EC_{50}). This provides valuable information on whether a sample is inhibiting viral replication without killing the host cell. Therefore, SI values that are >1 indicate that the inhibition is targeted toward viral replication and are less cytotoxic toward the host cell; therefore, the higher the SI value, the better the sample. There are no guidelines or cut-off values for an acceptable or appropriate SI value. It has been recommended that an SI value greater than 10 should be considered a good candidate. However, other factors, including the pharmacokinetic profile and drug delivery systems, can be used to mitigate associated toxicities. Extracts with a selectivity index of <10 should either undergo fractionation or purification to identify if a bioactive compound has increased therapeutic activity. When referring to *in vivo* animal studies it is denoted as the therapeutic index, where 50% lethal dose (LD_{50}), which is determined from toxicity studies in animal models, is used instead of CC_{50} values obtained from *in vitro* toxicity studies. The therapeutic efficacy, in other words, described the margin of safety of a sample, compound, or drug (Abughazaleh and Tracy, 2014). However, there is no set guideline on defining whether a calculated selectivity index depicts significant therapeutic activity or not. Feng (2018) discussed that safety margins differ depending on the severity of a viral disease, where drugs used to treat acute diseases, such as Ebola, will differ in safety criteria compared to chronic viral infections, such as HIV (Feng, 2018). Muller and Milton (2012) further describe that when assessing the therapeutic efficacy of a drug, the risk-benefit analyses should be used, taking into account toxic effects that appeared frequently in clinical trials and not placing too much emphasis on rare toxic effects, which were only reported in large scale studies (Muller and Milton, 2012).

As mentioned, there are various reports of activity on the proteases and other molecular targets, although many lack the proper hypothesis, experimental design, and justification for the conclusion. The criteria for the effective identification of enzymatic inhibitors should be three-fold, namely, specificity, concentration-response, and kinetic characteristics. Firstly, a study needs to provide sufficient evidence to indicate that the enzyme activity is specific to the selected target. This can be achieved by testing on non-related targets and by screening the compounds for Pan-assay interference (PAINS) to rule out false positives. Secondly, the concentration range and response need to be appropriate, relevant, and realistic for the test system. A single test concentration or exorbitantly high concentration is not sufficient and appropriate to confirm enzymatic inhibition. Lastly, an attempt should be made to identify the kinetic properties and mode of inhibition (competitive, non-competitive or un-competitive) through appropriate kinetic assessment.

The most prominent compounds identified in this review, are the abietane diterpenoids, triterpene glycosides and chalcones. Since these compounds possess medium polarity, these can be easily extracted with organic extracts such as dichloromethane, chloroform, ethyl acetate and alcohols. These are common classes of natural products occurring abundantly in several plant species including South African plants. However, clinical toxicity and efficacy trials are still necessary for each of the identified natural products. In this review, we also attempted to identify potential leads from a Southern African perspective. Two propositions were evident. Firstly, these plants are highly under-investigated. Secondly, the "related-species" approach can be useful in selecting the initial candidates for further testing. This approach might be somewhat speculative but should not be overlooked. Related species may well have similar chemical profiles or slightly varying constituents that can have a beneficial effect on the biological activity. Lastly, bioprospecting, access, and benefit-sharing related to traditional knowledge on the usage of medicinal plants for COVID-19 pathogenesis and/or related symptoms should beincluded in the study design. Should any plant samples or related natural products show potential for commercialization (pharmaceutical or nutritional supplement development), a bioprospecting permit should be obtained in the respective countries. Although COVID-19 is considered a novel viral disease many plant species mentioned in this review article, have a direct link to the traditional usage of the plants for COVID-19 related symptoms. The Nagoya protocol guidelines, as well as national and international regulations, should be followed for commercialization purposes to ensure the knowledge holders and communities benefit.

CONCLUSION

The current COVID-19 pandemic, caused by SARS-CoV-2, is a major global health concern and there is a social and ethical responsibility for communities and scientists around the world to work together to effectively combat the disease. In this review, we investigated the current state of natural products research to identify potential anti-coronaviral compounds, current drugs being used and potential lead candidates for the treatment of COVID-19, specifically from plants. Lycorine, savinin, and 8-hydroxe were the most prominent compounds identified that showed high selectivity. Southern Africa boasts a large biodiversity and subsequent natural products diversity, providing a substantial source of candidates to be screened

against SARS-CoV-2 and its protein targets. Combining this with an ethnobotanical approach, it is evident that there exists a vast potential to discover new antiviral compounds. Several techniques have been used to identify potential lead from natural sources; these include the ethnopharmacological approach, similarities in previously identified active compounds, and computational models such as molecular docking. However, selecting compounds for further clinical assessment should be carefully considered, and the necessary *in vitro* and *in silico* experimental evidence needs to be conclusive. Finally, matters relating to bioprospecting and the fair and equitable sharing of benefits should be included in projects that are related to traditional knowledge systems.

AUTHOR CONTRIBUTIONS

All listed authors contributed equally to this review.

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REVIEW
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The Important Role of Volatile Components From a Traditional Chinese Medicine Dayuan-Yin Against the COVID-19 Pandemic

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Aromatic Chinese herbs have been used to prevent plagues since ancient times. Traditional Chinese medicine has unique advantages in the prevention and treatment of epidemic diseases. According to the traditional Chinese medicine treatment plan in the National COVID-19 Diagnosis and Treatment Plan (Trial Seventh Edition) of the National Health Commission, Chinese patent medicines or prescriptions rich in aromatic Chinese herbs are selected for prevention and treatment during the period of medical observation, clinical treatment, and recovery of confirmed COVID-19 patients. Some local health committees or traditional Chinese medicine administrations recommend a variety of other ways of using traditional aromatic Chinese herbs to prevent and cure COVID-19. These involve external fumigation, use of moxibustion, and wearing of sachet. The efficacy of aromatic Chinese herbs plays a decisive role in the prevention and treatment of COVID-19. The unique properties, chemical composition, and mechanism of action of aromatic Chinese herbs are worthy of extensive and in-depth experimental and clinical research. The findings are expected to provide a reference for follow-up treatment of novel coronavirus and the development of corresponding drugs. In 2003, Dayuan-Yin produced excellent results in the treatment of the SARS virus. Individually, 112 confirmed cases were administered this drug between January and April 2003, and more than 93.7% of the patients showed noticeable mitigation of the symptoms, as well as recovery. Dayuan-Yin also was selected as one of the nationally recommended prescriptions for the COVID-19. Based on the national recommendation of Dayuan-Yin prescription, this review discusses the role of volatile components in the prevention and treatment of COVID-19, and speculates the possible mechanism of action, so as to provide a basis for the prevention and treatment of COVID-19.

Keywords: COVID-19, coronavirus, volatile components, aromatic Chinese herbs, Dayuan-Yin, traditional Chinese medicine

INTRODUCTION

When the new coronavirus infection broke out in Wuhan, China, in December 2019, WHO announced that it was PHEIC, which is named "COVID-19" (Wu et al., 2020). By mid-August 2020, more than 21,815,000 patients had been diagnosed with the disease worldwide, while 772,856 infected persons died. At present, the coronavirus has spread to 188 countries, with the US, Brazil, and India having a total of about 11,444,806 infected cases as of August 18, 2020 (Johns Hopkins University & Medicine, J.H.U, 2020). The situation is deteriorating every day, although the number of new cases in China has declined significantly since mid-March 2020. It is known that COVID-19 is harmful to different organs of the human body. Many governments have launched a joint prevention and control plan to prevent the spread of the COVID-19 pandemic.

Despite extensive and global scientific efforts, there is little drug has had a significant clinical effect on COVID-19 (Cao B. et al., 2020). Interestingly, Traditional Chinese medicine (TCM) plays an important role in the prevention, treatment, and rehabilitation of COVID-19 (Ren et al., 2020). According to the latest data from the State Administration of traditional Chinese medicine, Dayuan-Yin, a cocktail of aromatic Chinese herbs, has a significant therapeutic effect on COVID-19 (Ruan et al., 2020). In 2003, Dayuan-Yin produced excellent results in the treatment of the SARS virus. A total of 112 confirmed cases were individually administered Dayuan-Yin between January and April 2003, with more than 93.7% of the patients showing noticeable reduction in symptoms, as well as recovery (Li H. et al., 2020). As a result of this excellent therapeutic outcome, the TCM treatment plan in the National COVID-19 Diagnosis and Treatment Plan (Trial Seventh Edition) of the National Health Commission issued by the People's Republic of China, has recommended Dayuan-Yin for normal COVID-19 patients (General Office of the National., Health Commission of the people's Republic of China. et al., 2020). It has been used clinically in improving symptoms of lung condition for a long time, with the results showing that the prescription shortened the course of the disease by reducing the clinical symptoms and improving prognosis of patients. Thus, it is worthy of clinical application (Ruan et al., 2020; Wang W. et al., 2020). At present, the TCM has adopted Dayuan-Yin for the treatment of COVID-19, and it has achieved good curative effect (Wang B. et al., 2020; Li D. et al., 2020). The bioactive components of Dayuan-Yin remain unknown. This is probably due to the fact that the TCM decoction has nine herbal components derived from several prescriptions in a classic TCM fashion. The complex constituents of Dayuan-Yin make it hard to carry out a detailed study on its bioactive components in a short time.

These are Atractylodes lancea (Thunb.) DC., Citrus × aurantium L., Magnolia officinalis Rehder & E.H.Wilson, Pogostemon cablin (Blanco) Benth., Lanxangia tsao-ko (Crevost & Lemarié) M.F.Newman & Skornick., Ephedra sinica Stapf, Hansenia weberbaueriana (Fedde ex H.Wolff) Pimenov & Kljuykov, Zingiber officinale Roscoe, and Areca catechu L. in Dayuan-Yin. These plants are present in Dayuan-Yin in the ratio of 15: 10: 10: 10: 6: 6: 10: 10: 10. (General Office of the National., Health Commission of the people's Republic of China. et al., 2020) (**Figure 1**). Eight of them are aromatic Chinese herbs.

Air pollution is a major environmental problem affecting global respiratory health (Guan et al., 2016). Moreover, aromatic Chinese herbs can be used for air disinfection (Sun and Liang, 2015). It is one of the reasons why aromatic Chinese medicine have been successfully used as key TCM for prevention of epidemics since ancient times (Luo et al., 2020). Volatile components are the main active components of aromatic Chinese herbs (Chen and Wang, 1994). It is supposed that the volatile components which are essential active ingredients in Dayuan-Yin may play a vital role in treating COVID-19 patients.

Abbreviations: ALI, acute lung injury; CDK1, cyclin-dependent kinases 1; CDK1, cyclin-dependent kinases 1; COPD, chronic obstructive pulmonary disease; COX, cyclooxygenase; COX-2, cyclooxygenase; DPPH, 1,1-diphenyl-2-picrylhydrazyl; fMLP, N-formyl methionyl leucyl phenylalanine; FRAP, ferric reducing antioxidant power; FRAP, ferric reducing antioxidant power; IAV, influenza A virus; IC50, 50%inhibiting concentration; ICAM, intercellular adhesion molecule; ICAM-1, intercellular adhesion molecule; IFN, Interferon; IFN-gamma, Interferon-gamma; IL, interleukin; IL-10, interleukin-10; IL-1β, interleukin 1 beta; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; iNOS, Nitric oxide synthase; IRF3, Interferon regulatory Factor 3; LDL, low-density lipoprotein; LTB4, Leukotriene B4; MAPKs, mitogen activated protein kinases; MCP, monocyte chemotactic protein; MIC, minimum inhibitory concentration; MUC, mucoprotein; NF-κB, nuclear factor-kappa B; NLRP3, Nucleotide-binding domain-(NOD-) like receptor protein 3; NO, nitric oxide; NP-SH, non-protein-sulthydry; Nrf2, nuclear factor erythroid 2-related factor 2; NSAIDs, nonsteroidal anti-inflammatory drugs; oxLDL, lipid peroxidation because oxidized LDLs; PGE2, prostaglandin E2; PGE2, prostaglandin E2; PHEIC, a public health emergency of international concern; RANTES, regulated upon activation normal T-cell expressed and secreted; RPMCs, Rat peritoneal mast cells; ROS, reactive oxygen species; SARS, Severe Acute Respiratory Syndrome; SFJD, Shufengjiedu Capsule; SHL, Shuanghuanglian; T3SS, type III secretion system; TBA, thiobarbituric acid; TCM, traditional Chinese medicine; TI, therapeutic index; TLR4, toll like receptor 4; TLR4, toll like receptor 4; TLR7, toll like receptor 7; TNF-alpha, tumor necrosis factor-alpha; TNF-α, tumor necrosis factor alpha; VCAM-1, vascular cell adhesion molecule; WHO, Worldwide Health Organization.

BIOACTIVE VOLATILE COMPONENTS OF DAYUAN-YIN

Most of the volatile components of Dayuan-Yin have been elucidated, and their structures are well established (**Table 1**). However, there are no data on volatile components extracted from Areca catechu L. Based on clinical evidence of therapeutic results with Dayuan-Yin, we summarized its potential bioactive volatile components in the treatment of COVID-19. The biological benefits of Dayuan-Yin seem to involve anti-inflammatory, anti-viral, antibacterial, and immunomodulatory effects (**Table 2**).

Anti-Viral Effect

In autopsy studies and animal models, COVID-19 manifests mainly as acute viral pneumonia leading to respiratory failure (Chan et al., 2020; Yao et al., 2020). Antiviral drugs have been used to treat common cold, fever and influenza viruses by destroying the viral surface structure and inhibiting its entry (Hsieh et al., 2012), suggesting that antiviral drugs can be used for COVID-19. Unfortunately, no specific antiviral treatment has

FIGURE 1 | The processed raw materials of Dayuan-Yin in treating COVID-19.

been recommended for COVID-19 treatment because of insufficient evidence from randomized trials (Hung et al., 2020). It has been shown that many re-purposed drugs have effects against close relatives of SARS-COV-2, such as β-coronavirus, in vitro. Furthermore, lopinavir and many interferons, especially interferon beta, have moderate effects against SARS-COV in vitro and can be used in combination with ribavirin (Chen et al., 2004; Chan et al., 2013). Administration of antiviral drugs soon after symptoms appear reduces the release of virus in respiratory secretions of patients with COVID-19, thereby decreasing their infectivity to others. Targeted preventive treatment for contacts reduces their risk of infection (Welliver et al., 2001; Oriol and Bonaventura, 2020).

Patchouli oil is extracted from *Pogostemon cablin* (Blanco) Benth. Some studies in vitro have shown that patchouli oil exerted anti-viral effects against Coxsackie virus (IC_{50} = 0.081 mg/ml, TI 1.25), adenovirus(IC_{50} = 0.084 mg/ml, TI 1.20), influenza A virus (IC_{50} = 0.088 mg/ml, TI 1.15), and respiratory syncytial virus (IC_{50} = 0.092 mg/ml, TI 1.10) (Wei et al., 2012). Evaluation of the antiviral properties of six chemical compositions of *Atractylodes lancea* (Thunb.) D DPPH.C. revealed that atractylodin produced the most significant effect at doses of 10–40 mg/kg for five days, and attenuated IAV–induced pulmonary injury via regulation of the TLR7 signaling pathway (Cheng et al., 2016). Moreover, 1,8-cineole, the major constituent of the essential oil of *Lanxangia tsao-ko* (Crevost & Lemarié) M.F.Newman & Skornick., is commonly applied for treating inflammatory diseases of the respiratory tract caused by viruses since it potentiates the antiviral effect of IRF3, in addition to its inhibitory effect on proinflammatory NF-κB signaling (Müller et al., 2016).

Anti-Inflammatory Effect

The levels of proinflammatory factors i.e. IL-2, IL-7, IL-10, GCSF, IP10, MCP1, mip1a, and TNF – α in the plasma of critically-ill patients were higher than those in plasma of patients who were not in intensive care, suggesting that "cytokine storm" is closely related to the severity of COVID-19 (Huang et al., 2020). *Cytokine storm* is a very prominent pathophysiological feature of COVID-19 infection (Lin et al., 2020). Extensive endothelial barrier disruption and uncontrolled *cytokine storm* promote uncontrolled inflammatory response which is the basis of the core mechanism underlying acute respiratory distress syndrome (ARDS) (Huang et al., 2017), although this phenotype varies among individuals. Experimental models of acute lung injury (ALI) and human genome-wide association studies of ARDS indicate that *cytokine storm* plays an essential role in the pathophysiology of ARDS (Biondi et al., 1986; Huang et al., 2017). Moreover, the most common and severe complication of COVID-19 is ARDS (Huang et al., 2017). Therefore, an understanding of the *cytokine storm* that aggravates ARDS in COVID-19 may lead to early and effective intervention in

TABLE 1 | Name of volatile oil in Dayuanyin prescription.

Volatile oils of herbs	Compounds	PubChem CID	References
Pogostemon cablin (Blanco) Benth. volatile oils	Patchoulol	10955174	(Lin et al., 2016; Tang et al., 2019)
	α-bulnesene	94275	
	α-guaiene	5317844	
	Seychellene	519743	
	α-patchoulene	521710	
	Carvacrol	10364	
	p-cymene	7463	
	γ-Terpinene	7461	
	β-guaiene	15560252	
	Eicosene	18936	
	Caryophyllene	5281515	
	Pogostone	54695756	
Magnolia officinalis Rehder & E.H.Wilson Cortex volatile oils	γ- eudesmol	6432005	(Tang et al., 2019)
	β- eudesmol	91457	
	α-pinene	6654	
	β-pinene	14896	
	Camphene	6616	
	Limonene	22311	
	Bornyl acetate	6448	
	Caryophyllene	5281515	
	Caryophllene Epoxide	14350	
	α-eudesmol	92762	
	Cryptomeridiol	165258	
	β-Caryophyllene	5281515	
	β-selinene	442393	
	Cardene	41114	
	β- cadinene	10657	
	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	605019	
	Eudesm-4-en-11-ol(8CI)	6432005	
volatile oils of *Pogostemon cablin* (Blanco) Benth. & *Magnolia officinalis* Rehder & E.H.Wilson Cortex	2-Cyclohexen-1-ol	13198	(Tang et al., 2019)
	1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl-ester	549130	
	Estragole	8815	
	2-Cyclohexen-1-ol,3-methyl-6-(1-methylethyl)-,cis;p-Menth-1-en-3-ol,cis-(8CI)	85567	
	Cadina-1,3,5,9-tetraene(8CI)	12302243	
	Cyclohexanemethanol,4-ethenyl-a,a,4-trimethyl-3-(1-methylethenyl)-,[1R-(1a,3a,4b)]-	92138	
	Hexamethylbenzene	6908	
Atractylodes lancea (Thunb.) DC. volatile oils	atractylon	3080635	(Yao et al., 2001; Guo et al., 2008; Chu et al., 2011)
	hinesol	10878761	
	α-phellandrene	7460	
	3- carene	26049	
	4-Biphenylcarbaldehyde	76689	
	Furanodiene	9601230	
	β- selinene	442393	
	β-eudesmol	91457	
	γ-elemene	6432312	
	α-elemol	6429032	
	Eudesma-4(14),11-diene	442393	
	aromadendrene	91354	
	β-sesquiphellandrene	12315492	
	patchoulene	91746471	
	atractylodin	5321047	
	α-pinene	6654	
	β-selinene	442393	
	Methyl palmitate	8181	
	Methyl linoleate	5284421	
	Methyl -9-octadecenoate	8202	
	Ethyl linoleate	5282184	
	4-hydroxyphthalic acid	11881	
	β-selinene	442393	
Citrus × *aurantium* L. eticulatae Pericarpium volatile oils	limonene	22311	(Jing et al., 2015; Castro et al., 2018; Vieira et al., 2018; Pan et al., 2020)
	sesquiterpene	6473767	
	α-pinene	6654	
	β-pinene	14896	
	sabinene	18818	
	γ-Terpinene	7461	
	β-myrcene	31253	
Lanxangia tsao-ko (Crevost & Lemarié) M.F.Newman & Skornick. volatile oils	1,8-Cineole	2758	(Xiong and Hua, 2012)
	4- Propylbenzaldehyde	120047	
	Geraniol	637566	
	Geranial	638011	
	α-Terpineol	17100	
	α-Phellandrene	7460	
	β-Pinene	14896	
	α-Pinene	6654	
	α-phellandrene	7460	
	2-Propenal,3-methyl-3-phenyl	5372857	
	neral	643779	
	Cineole	2758	
	γ-terpineol	11467	
	β-citral	643779	
	α-citral	638011	
	2-decenal	147309	
	6-methyl-1,2,3,5,8,8a-hexahydronaphthalene	562093	
	2-oxaadamantane	520365	
	2-phenyl-2-butenal	6429333	
	geraniol acetate	1549026	
	trans-2-undecen-1-ol	5365004	
	2-dodecenal	5283361	
	trans-nerolidol	5284507	
	mint furanone	91753282	
	hedycaryol	6432240	
	cyclohexanol,2-methylene-3-(1-methylethenyl)-,acetate,cis-	22213002	
	Citral	638011	
Hansenia weberbaueriana (Fedde ex H.Wolff) Pimenov & Kljuykov volatile oils	α-Pinene	6654	(Wu et al., 2008)
	β-Pinene	14896	
	D-Limonene	440917	
	α-Bisabolol	1549992	
	β-Ocimene	18756	
	β-Bisabolene	10104370	
	γ-Terpinene	7461	
	β-Thujene	520384	
	α-Terpinolene	11463	
	Terpinen-4-ol	11230	
	1-Bornyl acetate	93009	
	α-Copaene	70678558	
	Trans-β-farnesene	5281517	
	Apiol	10659	
	Guaiol	227829	
	Benzyl benzoate	2345	
	5-Allyl-2,3-(methylendioxy)anisole	4276	
	β-Cedarene	11106485	
	Spathulenol	97032059	
	9,12-Octadecadienoic acid(Z,Z)-, ethyl ester	5282184	
	Palmitoleic acid	445638	
	α- phellandrene	7460	
	3-carene	26049	
	1-Methyl-2-(1-Methylethyl)Benzene	10703	
	3,7-dimethyl-1,3,6-octatriene	5281553	
	1-Isopropyl-2-methoxy-4-methyl-benzene	14104	
	Z-3-decen-1-ylacetate	5363204	
	Agarospirol	21675005	
	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	24848167	
	Benzene, 1-methyl-3-(1-methylethyl)-	10812	
	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,	90805	
	o-Cymene	10703	
	β-Phellandrene	11142	
Ginger essential oil	camphene	6616	(El-Ghorab et al., 2010)
	p-cineole	2758	
	α-terpineol	17100	
	zingiberene	92776	
	pentadecanoic acid	13849	
Ephedra sinica Stapf volatile oil	2,3,5,6-tetramethylpyrazine	14296	(Kun et al., 2000; Xue et al., 2020)
	linalool	6549	
	4-methoxystyrene	12507	
	α-terpineol	17100	
	myrtenol	10582	
	geraniol	637566	
	(4-Isopropyl-2-cyclohexen-1-yl) methanol, trans-	22215197	
	phytol	5280435	

(Continued)

critically-ill COVID-19 patients. It seems necessary to directly inhibit inflammatory response in the lungs because *cytokine storm* can be alleviated with inflammatory therapy (Cao P. et al., 2020). Previous studies have shown the benefits of anti-inflammatory drugs in lung disease: they slow down impairment of lung function, reverse the inflammatory parameters nearly back to normal values, and improve patients' survival (Konstan et al., 2011; Lubamba et al., 2011). Ibuprofen, a popular anti-inflammatory drug, is recommended for airway inflammation in cystic fibrotic lung disease (Flume et al., 2007). Studies have shown

TABLE 2 | The mechanism of action of volatile components in Dayuanyin prescription.

Bioactivities	Volatile oils of herbs	Mechanisms	References
Anti-inflammatory activity	*Pogostemon cablin* (Blanco) Benth. volatile oils	Patchoulene: cyclin E↓, cyclin B↓, CDK1↓; the subsequent S-phase arrest, IFN-gamma↓, IL-10↓	(Su et al., 2015)
		Pogostemon cablin (Blanco) Benth. volatile oils: TNF-α↓, IL-6↓, IL-10↑, NP-SH↑	(Chen et al., 2015)
		Patchoulene: regulates on the balance between Nrf2 and NF-κB p65 signaling pathways	(Yang et al., 2018)
		Patchoulene: vitro neutrophil fMLP chemotaxis↓, phagocytic activity↑; ear edema↑, myeloperoxidase (MPO) activity↑	(Silva-Filho et al., 2016)
		Patchoulene: NF-κB↓, Nrf2↑, miR-146a expression↑	(Chen et al., 2017)
		Patchoulene: NF-κB↓, p38 MAPK phosphorylation↓	(Li et al., 2014)
		Patchoulene: regulates on the balance between Keap1-Nrf2 and NF-κB signaling pathways	(Sun et al., 2016)
	Magnolia officinalis Rehder & E.H.Wilson Cortex volatile oils	*Magnolia officinalis* Rehder & E.H.Wilson Cortex volatile oils: PGE2/TNF-α↓, IL-1β↓	(Cao et al., 2015)
	Atractylodes lancea (Thunb.) DC. volatile oils	Atractylone: caspase-1/NF-κB/MAPKs activations↓, reduce IL-1↓, IL-4↓, IL-5↓, IL-6↓, IL-13↓, COX-2↓, intercellular protein-2 expression↓	(Kim et al., 2016)
		Atractylone: RPMCs degranulation intracellular Ca2+ level ([Ca2+])↓, tryptase↓, histamine↓ p56lck tyrosine kinase activity↓; histidine decarboxylase activity and expression↓, tryptase and histamine releases↓in PMACI-induced HMC-1 cells; morphological alteration and flamentous actin formation in stem cell factor-stimulated RPMCs animal model	(Han et al., 2016)
		Atractylone: NLRP3 inflammasome↓, TLR4 activation↓	(Tang et al., 2018)
		Hinesol: H^+, K^+ -ATPase activity↓	(Kanako et al., 2000)
	Citrus × *aurantium* L. Pericarpium volatile oils	Limonene: TNF-α↓, neutrophils chemotaxis↓, leukocytes chemotaxis↓	(Vieira et al., 2018)
		Limonene: iNOS↓, COX↓, PGE2↓; TNF-α↓, IL-1β↓, and IL-6↓;	(Yoon et al., 2010)
	Lanxangia tsao-ko (Crevost & Lemarié) M.F.Newman & Skornick. volatile oils	*Citrus* × *aurantium* L. Pericarpium volatile oils: Nitric oxide↓, iNOS↓, COX-2↓	(Dang et al., 2020)
		1,8-cineole: IL-4↓, IL-5↓, IL-10↓, MCP-1↓, IL-1 beta↓, IL-6↓, TNF-alpha↓, IFN-gamma↓, NF-kB p65↓, ICAM-1↓; VCAM-1↓ in lung tissues of mice infected with influenza A virus.	(Li et al., 2016)
		1,8-cineole: mucin-filled goblet cells↓, MUC2↓, MUC19↓, NF-kappa B-activity↓	(Sudhoff et al., 2015)
		1,8-cineole: IL-10↑, TNF-α↓, IL-1β↓, NF-κB's subunit p65↓ and TLR4↓	(Zhao et al., 2014)
		1.8-cineole: LTB4↓ and PGE2↓in human blood monocytes ex vivo in the treatment of bronchial asthma.	(Juergens et al., 1998b)
		1.8-cineole: TNFαC, IL-1β↓, leukotriene B4↓, thromboxane B2↓ in human blood monocytes *in vitro*	(Juergens et al., 1998b)
	Hansenia weberbaueriana (Fedde ex H.Wolff) Pimenov & Kljuykov volatile oils	*Hansenia weberbaueriana* (Fedde ex H.Wolff) Pimenov & Kljuykov volatile oils: NO↓ in RAW 264.7 cells.	(Bi et al., 2018)
		α-pinene: MAPKs↓, NF-κB↓ in mouse peritoneal macrophages	(Kim et al., 2015)
		α-pinene: LPS-induced nuclear translocation of NF-κB↓ in TPS-1 cells by κBα expression↑ in a dose-dependent manner	(Zhou et al., 2004)
	Ginger volatile oil	PLS-induced IL-8 secretion↓, RANTES↓ in human bronchial epithelial cells (BEAS-2B)	(Podlogar and Verspohl, 2012)
Antiviral activity	*Pogostemon cablin* (Blanco) Benth. volatile oils	anti-Coxsackie virus (IC50 0.081 mg/ml, TI 1.25), anti-adenovirus (IC50 0.084 mg/ml, TI 1.20), anti-influenza A virus (IC50 0.088 mg/ml, TI 1.15), and anti-respiratory syncytial virus (IC50 0.092 mg/ml, TI 1.10)	(Wei et al., 2012)
	Atractylodes lancea (Thunb.) DC. volatile oils	Regulate the TLR7 signaling pathway.	(Cheng et al., 2016)
	Lanxangia tsao-ko (Crevost & Lemarié) M.F.Newman & Skornick. volatile oils	1,8-cineole: IRF3 antiviral activity↑, proinflammatory NF-κ B signalling↓	(Müller et al., 2016)
Anti-oxidative activity	*Magnolia officinalis* Rehder & E.H.Wilson Cortex volatile oils	Scavenging DPPH free radicals, providing hydrogen atoms, scavenging superoxide free radicals	(Guo, 2012)
	Atractylodes lancea (Thunb.) DC. volatile oils	Scavenge DPPH- radical activity with an IC50 of 288.7 μg/mL, lipid peroxidation↓, and effects on T-AOC in the serum and organ tissues of mice	(He et al., 2020)
	Citrus × *aurantium* L. Pericarpium volatile oils	Citrus reticulata peel oil prevented LDL lipid peroxidation because oxLDL are absorbed by the macrophages' scavenger molecules, forming foam cells	(Yoon et al., 2010; Castro et al., 2020)
		Certain monoterpenes and essential oils: LDL oxidation↓	(Barter, 2005) (Naderi. et al., 2004)

(Continued)

TABLE 2 | Continued

Bioactivities	Volatile oils of herbs	Mechanisms	References
		Limonene: protect the lens epithelial cells from oxidative stress through antioxidant and anti-apoptotic pathways.	
		Limonene: be able to attenuate the oxidative stress impairment on *in vitro* and *in vivo* models	(Ahmad and Beg, 2013)
	Lanxangia tsao-ko (Crevost & Lemarié) M.F.Newman & Skornick. volatile oils	Weak in scavenge DPPH- radical activity, TBA, and FRAP	(Yang et al., 2010)
		1,8-cineole: reactive oxygen species↓, superoxide dismutase↓, catalase↓, malondialdehyde↓	(Kennedy-Feitosa et al., 2016)
Anti-bacterial activity	*Pogostemon cablin* (Blanco) Benth. volatile oils	Inhibit Candida albicans (MIC 0.9 ml/L), Crytococus neoformans (MIC 0.15 ml/L), Sporothrix schemckii (MIC 0.6 ml/L), Microporum lanosum (MIC0.7 ml/L), M.gypseum (MIC 0.6 ml/L), Aspergillus flavus,AS3.3950 (>1.0 ml/L), A.niger,AS3.3928) (MIC>1.0 ml/L, Mucor globosum,AS3.963 (MIC 1.0 ml/L), Chaetomium globosum,AS3.963 (MIC 0.45 ml/L), Rhizopus nigricans, AS3.31 (MIC 0.8 ml/L), Scopulariopsis brevicaulix (MIC0.5 ml/L), Escherichia coli, 8099 (MIC>1.0 ml/L), Bacillus subtilis, ATCC 9379 (MIC 0.7 ml/L), Staphylococcus albus, AS1.184 (MIC 0.8 ml/L),Micrococcus tetrgenus (MIC 0.8 ml/L),Staphylococcus aureus (MIC 0.7 ml/L),methicillin-resistant Staphylococcus aureus, Staphylococcus epidermidis (MIC 0125 mg/ml), Shigella sonnei standard strain (MIC 0125 mg/ml), Hemolytic Streptococcus A, Pseudomonas aeruginosa, Bacillus subtilis, yeast, penicillium.	(Zhou et al., 2014; Lin et al., 2016; Wang et al., 2018)
	Magnolia officinalis Rehder & E.H.Wilson Cortex volatile oils	Inhibit Staphylococcus aureus, Candida albicans, methicillin-resistant Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Shigella sonnei, Escherichia coli, Listeria monocytogenes, Salmonella, Bacillus cereus, Pseudomonas aeruginosa.	(Guo, 2012; Tang et al., 2019)
	Atractylodes lancea (Thunb.) DC. volatile oils	Inhibit Gram-positive and Gram-negative bacteria due to disruption of the cell membrane.	(He et al., 2020)
		β-eudesmol has two-way regulation of gastrointestinal motility, which may be anticholinergic or directly acting on gastrointestinal smooth muscle.	(Wang et al., 2002)
		Hinesol: H^+, K^+-ATPase activity↓	(Kanako et al., 2000)
	Citrus × *aurantium* L. Pericarpium volatile oils	Inhibit the phytopathogenic fungus *Sclerotinia sclerotiorum*.	(Dias et al., 2020)
	Lanxangia tsao-ko (Crevost & Lemarié) M.F.Newman & Skornick. volatile oils	Protect the mice from Staphylococcus aureus or Escherichia coli infection.	(Dai et al., 2016)
		1,8-cineole: inhibits *S. aureus, Escherichia coli, Moraxella catarrhalis*.	(Schürmann et al., 2019)
	Ginger volatile oil	Inhibit Pseudomonas aeroginosa bacteria, S. typhimurium and S. flexneri, Gram-negative bacteria, slightly.	(Mesomo et al., 2013)
Other aspects	*Lanxangia tsao-ko* (Crevost & Lemarié) M.F.Newman & Skornick. extract	Epicatechin has anti-inflammatory properties, quercetin has the strongest neuroprotective effect of PC-12 cells induced by H_2O_2, and DPPH radical-scavenging activity.	(Zhang et al., 2014)
	Hansenia weberbaueriana (Fedde ex H.Wolff) Pimenov & Kljuykov extract	Inactivate the influenza virus A/FM/1/47 directly and reduce the titer.	(Guo et al., 2005)
		Falcarindiol inhibited DC maturation by blocking the canonical pathway of nuclear factor-kappaB and phosphorylated p38.	(Mitsui et al., 2010)
		Falcarindiol inhibit Pseudomonas aeruginosa by repressing virulence-related genes, including the T3SS; quorum sensing synthase genes lasIR and rhlIR; lasB; motility-related genes fliC and fliG; and phenazine synthesis genes phzA1 and phzA2.	(Zhang et al., 2020)

that long-term prediagnostic use of nonaspirin NSAIDs (e.g., ibuprofen) is associated with a significant reduction in lung cancer survival (Brasky et al., 2012). In addition, baricitinib, fedratinib, and ruxolitinib are active and selective JAK inhibitors which have been approved for rheumatoid arthritis and myelofibrosis. All three drugs are effective anti-inflammatory agents, and as JAK-STAT signaling inhibitors, they may be effective against the consequences of elevated levels of cytokines (including interferon-γ) usually observed in patients with COVID-19 (Stebbing et al., 2020). The UK is currently conducting a randomized evaluation of COVID-19 treatment (recovery) trial, based on the announcement on June 16, 2020, that dexamethasone had been shown to significantly improve the prognosis of COVID-19 patients receiving respiratory support (Recovery, Randomised Evaluation of COVid-19 therapy trial, 2020). Dexamethasone is a glucocorticoid which can be used as a synthetic form of the natural hormone cortisol (Cain and Cidlowski, 2017). It has the same anti-inflammatory effect as cortisol. It inhibits the release of inflammatory chemokines by immune cells, thereby improving the prognosis of patients by reducing the severity of ARDS (Lester et al., 2020). In European patients, low-dose dexamethasone reduces mortality by 33% in critically patients requiring invasive ventilation (Lim et al., 2020). However, the implementation of appropriate dexamethasone use in low-and-middle-income countries has been a challenge. For example, corticosteroids may cause sepsis in some prevalent parasitic infections in Africa (Nutman, 2020). Therefore, the use of dexamethasone in African patients who have not been

diagnosed with COVID-19 may lead to unexpected consequences (Brotherton et al., 2020). During the treatment of COVID-19, Dayuan-Yin also reduces the severity of ARDS by inhibiting the release of inflammatory chemokines from immune cells. As a classic prescription in ancient China, Dayuan-Yin can play a safe and effective role in the treatment of respiratory infections in a more adverse environment. Thus, it can avoid such problems in a large extent.

Anti-inflammatory property is widespread in various sources of volatile components. Several data have found that 1,8-cineole significantly improved lung function and health conditions, and reduced dyspnea in patients with asthma, acute bronchus, and chronic obstructive pulmonary disease (COPD). Moreover, it significantly reduced the frequency of cough in patients with acute bronchitis, and alleviated frequent exacerbations in patients with COPD and frequent exacerbations, notably (Worth et al., 2009; Worth and Dethlefsen, 2012; Fischer and Dethlefsen, 2013; Vogelmeier et al., 2018). In a mouse model of LPS-induced acute pulmonary inflammation, 1,8-cineole upregulated IL-10 in lung tissues, and decreased the expressions of TNF-α, IL-1β, NF-κB's subunit p65 and TLR4 (Zhao et al., 2014). Moreover, 1,8-cineole was shown to inhibit LTB4 and PGE2 (pathways of AA-metabolism in human blood monocytes) in bronchial asthma in vitro (Juergens et al., 1998b). In addition, 1,8-cineole decreased levels of TNFα, IL-1β, leukotriene B4, and thromboxane B2 in human blood monocytes in vitro (Juergens et al., 1998a).

Pogostone, a bioactive component extracted from *Pogostemon cablin* (Blanco) Benth., reduced the total population of T cells under ConA stimulation by blocking T cell proliferation via down-regulation of cyclin E, cyclin B, and CDK1. Subsequent S-phase arrest inhibited the production of IFN-gamma and IL-10 (Su et al., 2015). Simultaneously, pogostone pretreatment mitigated ethanol-induced gastric ulcer in rats by downregulation of IL-6 and TNF-alpha, and upregulation of IL-10 and non-protein-sulfhydryl (NP-SH) groups in the gastric mucosa (Chen et al., 2015). In lung disease, pogostone exerted potent protective effects against lipopolysaccharide-induced acute lung injury in mice by decreasing TNF-α-induced cell injury in A549 cells through modulation of the balance between Nrf2 and NF-κB-p65 signaling pathways (Yang et al., 2018). Pogostone significantly inhibited the protein and mRNA expressions of proinflammatory mediators such as TNF-α, IL-6, IL-1β, NO, and PGE2. Pogostone also significantly reduced LPS-induced mortality in mice, suppressed the production of proinflammatory mediators in serum. And it attenuated liver and lung injury via downregulation of the mRNA expressions of inflammatory mediators in multiple organs due to inhibition of activation of NF-κB and phosphorylation of p38 MAPK (Li et al., 2014). Pre-treatment with pogostone markedly mitigated LPS-induced acute lung injury in mice, improved survival, attenuated histological alterations in the lungs, reduced MPO and MDA levels, decreased the wet/dry weight ratio of lungs, and down-regulated proinflammatory mediators, such as TNF-a, IL-1 beta and IL-6. Furthermore, pretreatment with pogostone enhanced the Nrf2-dependent genes NQO-1, GCLC, and HO-1, but suppressed the NF-kappa B regulated genes TNF-alpha, IL-1 beta, and IL-6. The mechanism involved in the protective effect of pogostone was correlated with its regulation of the balance between Keap1-Nrf2 and NF-kappa B signaling pathways (Sun et al., 2016). Moreover, volatile oils from *Pogostemon cablin* contain a bioactive component named β-patchoulene which has been shown to significantly decrease mortality and lung wet/dry weight ratio of mice, and mitigate pathological changes in lungs, when compared to model group. It suppressed LPS-induced activation of NF-kappa B, and markedly upregulated Nrf2 and miR-146a (Chen et al., 2017).

Anti-Oxidative Properties

Oxidative stress and inflammation form a positive feedback cycle (Mittal et al., 2014). In lung disease, excessive inflammation and oxidative stress lead to adverse outcomes. For instance, patients with COPD are usually affected by other diseases (Rabe and Watz, 2017). Several mechanisms in lung inflammation and oxidative stress destroy DNA and lead to an imbalance between tissue repair and cell proliferation, which seems to promote the link between COPD and lung cancer (Wilson et al., 2008; Houghton, 2013; Durham and Adcock, 2015). Under normal conditions, the production and elimination of ROS maintain a crucial balance between oxidation and antioxidation (Cao et al., 2019). In such a balance, the signal pathways are regulated, and cell proliferation can be guaranteed. When inflammatory factors destroy this balance, oxidative stress enhances the maturation of proinflammatory factors, leading to oxidative damage to cells and multisystem diseases (Sies, 2015; Kruk et al., 2019). Antioxidant drugs have been used in lung diseases. For example, antioxidants have been recommended for reduction of mortality or prevention of organ damage in animal models of acute lung injury induced by lipophilic acids (Hsu et al., 2006; Zhu et al., 2020). Vitamin C has also been shown to reduce the incidence of pneumonia in several controlled trials for human subjects (Hemila, 2017).

The essential oil of *Magnolia officinalis* Rehder & E.H. Wilson exerts antioxidant effect by scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and superoxide anion radical. The essential oil contains β–eucalyptol with a hydroxyl group which can provide hydrogen atom for scavenging DPPH radical. Moreover, with increase in volatile oil concentration, the antioxidant capacity gradually increased (Guo, 2012). The essential oil from *Atractylodes lancea* (Thunb.) DC. showed a strong antioxidant effect in vitro and indicated by DPPH-radical scavenging property, with an IC_{50} of 288.7 μg/ml. Moreover, it inhibited lipid peroxidation, and affected total antioxidant capacity (T-AOC) in the serum and organ tissues of mice (He et al., 2020). In short-term cigarette smoke (CS)-induced acute lung inflammation, 1,8-cineole decreased oxidative stress involving reactive oxygen species, by increasing superoxide dismutase and catalase, while reducing levels of malondialdehyde, inflammation, and the NF-kappa B p65 subunit (Kennedy-Feitosa et al., 2016).

Antibacterial Properties

The major components of *Pogostemon cablin* (Blanco) Benth. are carvacrol (47.5%) and p-cymene (15.2%). It completely inhibits the growth of *E. coli* at a level of 0.05% (Lin et al., 2016). The essential oil of *Atractylodes lancea* (Thunb.) DC. exhibited antibacterial effects against Gram-positive and Gram-negative bacteria due to the cell membrane (He et al., 2020). In chronic

rhinosinusitis, 1,8-cineole suppressed the growth of *S. aureus*, *Escherichia coli*, *Moraxella catarrhalis* due to downregulation of significant and critical players in biofilm generation (Agra, Sara, and σ^B) (Schürmann et al., 2019). On the other hand, the major constituent of the essential oil of *Atractylodes lancea* (Thunb.) DC. is β-eudesmol. In terms of intestinal flora, β-eudesmol has two-way regulation for gastrointestinal motility: anticholinergic pathway and direct effect on gastrointestinal smooth muscle.

Antibacterial effect is an essential pharmacological property of volatile compounds (Houdkova et al., 2017; Riad et al., 2020). Secondary bacterial co-infection is common in patients with COVID-19 infection, and it leads to adverse prognosis (Macintyre et al., 2018). At present, many antibiotics have been used in the treatment of COVID-19. For example, Shufeng Jiedu Capsule (SFJD) prevents acute upper respiratory tract infection and positively affects fever, cough, and headache. Studies have shown that SFJD significantly reduced the levels of serum PGE2, IL-1 β, and TNF-α in rats with acute pharyngitis (Qian, 2019). Being a popular antiviral and antibacterial drug, SFJD is one of the drugs for COVID-19 treatment in China (Pan X. et al., 2020). In addition, Shuanghuanglian (SHL) is a popular anti-bacterial drug. It has various pharmacological potential such as antibacterial, antiviral, and immune-enhancing properties which can be exploited in the treatment of acute upper respiratory tract infection (Zhang et al., 2013). Preliminary studies in vitro showed that SHL oral liquid inhibited SARS-COV-2. Indeed, SHL has been used to carry out clinical research on COVID-19 in Shanghai Public Health Clinical Center and Tongji Hospital Affiliated to Huazhong University of Science and Technology (Pan X. et al., 2020).

The antibacterial effects of volatile components of Dayuan-Yin are not limited to upper respiratory tract infections: these volatile components also regulate intestinal flora, treat gastric ulcers, and improve gastrointestinal symptoms. Human gut microbes are the "second genome" of the human body (Backhed et al., 2005; Gill et al., 2006; Cani and Delzenne, 2007). The composition of intestinal flora is closely related to human health status, and it plays an essential role in maintaining physiological balance (Schuijt et al., 2016). It has been confirmed that intestinal flora reduces ventilator-associated pneumonia and enteritis by enhancing the function of primary alveolar macrophages (Bradley et al., 2019). Patients with COVID-19 showed intestinal microbial malnutrition and decreased microbial flora levels of some probiotics such as *Lactobacillus* and *Bifidobacterium*. The latest version of novel coronavirus pneumonia diagnosis and treatment plan released by the People's Republic of China National Health Council suggests that intestinal microbiota should be used in severe and critical cases to maintain intestinal micro ecological balance (General Office of the National., Health Commission of the people's Republic of China. et al., 2020).

EFFECTS OF NON-VOLATILE COMPONENTS

Apart from the biological effects of the volatile components mentioned above, other non-volatile components of Dayuan-Yin also have abundant pharmacological properties.

Epicatechin, one of the chemical components of *Lanxangia tsao-ko* (Crevost & Lemarié) M.F.Newman & Skornick., exhibited excellent anti-inflammatory properties in LPS-stimulated macrophage RAW 264.7 cells. Quercetin, one of the chemical components of *Lanxangia tsao-ko* (Crevost & Lemarié) M.F. Newman & Skornick., produced the most potent neuroprotective effect on PC-12 cells induced via H_2O_2 and DPPH radical-scavenging properties (Zhang T. T. et al., 2014). It was shown that SFE-CO_2 extract of *Hansenia weberbaueriana* (Fedde ex H.Wolff) Pimenov & Kljuykov significantly prolonged average survival time of mice with influenza virus pneumonia, directly killed the influenza virus, and reduced the hemagglutination titer. *Hansenia weberbaueriana* (Fedde ex H.Wolff) Pimenov & Kljuykov induced immunosuppressive effects in vitro. Falcarindiol, the main bioactive compound in *Hansenia weberbaueriana* (Fedde ex H.Wolff) Pimenov & Kljuykov, inhibited DC maturation by blocking the canonical pathway of nuclear factor-kappaB and phosphorylated p38 (Mitsui et al., 2010). Falcarindiol inhibited the growth of *Pseudomonas aeruginosa* by repressing virulence-related genes, including the T3SS; quorum sensing synthase genes lasIR and rhlIR; lasB; motility-related genes fliC and fliG; and phenazine synthesis genes phzA1 and phzA2 (Zhang et al., 2020).

It is obvious that Dayuan-Yin exerts extensive biological properties such as antiviral, anti-inflammatory, antioxidative, and antibacterial effects. It can be inferred that Dayuan-Yin may play an essential role in preventing COVID-19 pandemic.

SUMMARY AND FUTURE PROSPECTS

At present, there are no conventional drugs that can cure COVID-19 (Cao B. et al., 2020). However, according to data collected by the National Health Commission of the people's Republic of China, clinical practice in Chinese hospitals have reported that traditional Chinese medicine has a definite therapeutic effect in the early stages of COVID-19 infection (Liu C.-X. et al., 2020). As a significant part of medical practice, Chinese medicine has been used to treat human diseases for more than 5,000 years (Li and Kan, 2017). In recent decades, volatile compounds extracted from medicinal plants have attracted more and more attention due to their important biological effects such as antiviral, anti-inflammatory, and antibacterial properties. Besides, they are non-toxic and have few side effects, making them suitable for use as drugs. This review discussed the potential role of traditional Chinese medicine in terms of volatile components. The anti-inflammatory, antiviral, antibacterial and immunomodulatory effects of these volatiles seem to play the most critical roles in treating patients infected with COVID-19. However, there are still lack of clinical trials on Dayuan-Yin. These need to be done in future.

In China, the situation of COVID-19 pandemic prevention and control has improved. The national pandemic situation has been controlled. However, with the resumption of factory work, re-opening of shopping malls, and resumption of transportation, the cross-flow of personnel has increased significantly, and the

probability of close contact between people has increased tremendously too. In particular, with likelihood of increase in imported cases from abroad, the epidemic prevention and control should not be relaxed. It is essential to improve the ability of the human body to withstand infection. In addition to frequent washing of hands, wearing masks, social distancing and other measures, the "Chinese medicine sachet" can be used as an essential means of prevention. This stems from a very important theory of traditional Chinese medicine, namely "treating pre-disease". This idea in traditional Chinese medicine originated in the Yin and Shang Dynasties, took shape in Zhouyi, and formed in Huangdi Neijing (Xu et al., 2016). Chinese doctors in the past dynasties attached great importance to the prevention and treatment of diseases. They emphasized the prevention of diseases first, especially infectious diseases (Lian et al., 2020). Wearing *Chinese medicine sachet* is another special treatment of "treating pre-disease" (Chen et al., 2020). *Chinese medicine sachet* has been used to prevent disease since ancient times. In this method, aromatic Chinese medicine is put into a unique bag and worn on the chest to prevent respiratory diseases. This is known as "Xiangpei therapy" (Zhang Q. et al., 2014). From the perspective of modern medicine, the medicinal fragrance (i.e., volatile oil components) of Chinese medicine sachet stimulates the nasal mucosa, promotes the secretion of immunoglobulins, and kills all kinds of viruses at the same time, thereby playing multiple roles in regulating immune function, and exerting antibacterial and anti-viral effects (Lvy and Bai, 2017). Interestingly, early intervention with aromatic Chinese medicine blocks the course of diseases and relieves symptoms in clinical practice through oral administration, external fumigation, and moxibustion (Lun and Chen, 1987; Chen et al., 2013). Aromatic Chinese medicine dispels exterior pathogenic factors, regulates *qi*, activates blood circulation, *breaks blood stasis*, and *disperses nodules*. The application of aromatic Chinese medicine embodies the theory of "internal disease and external treatment" of traditional Chinese medicine (Hu et al., 2010). Since the outbreak of COVID-19, fumigation has been used for air disinfection to prevent the spread of the virus. In the clinical treatment period, the application of moxibustion plays the role of anti-inflammatory agent, regulates immune function, and prevents deterioration of the patients (Zhang, 2012; Liu K. et al., 2020). Some local health committees or Chinese medicine administration bureaus are actively involved in promoting aromatic traditional Chinese medicine as an anti-epidemic, as well as the use of fumigation or *Chinese medicine sachet* to prevent and control COVID-19 (Chen et al., 2020).

There is no doubt that the pharmacological effects of volatile components of traditional Chinese medicine are beneficial in the global fight against COVID-19. However, each TCM prescription has multiple goals and links in the treatment of diseases, making it difficult to clearly and thoroughly explain its mechanism in a short period. More research should be carried out on volatile components of traditional Chinese medicine to elucidate the associated regulatory mechanism, evaluate possible side effects, and conduct standard clinical trials. The insights provided in this review may help ease the COVID-19 pandemic worldwide.

AUTHOR CONTRIBUTIONS

Q-wH and JW are the corresponding authors on the study. X-rZ and T-nL are first authors and responsible for collecting materials and writing the paper. Y-yR, Y-jZ, and H-yL helped in organizing the information and edited the article pictures. All authors contributed to the article and approved the submitted version.

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"Fei Yan No. 1" as a Combined Treatment for COVID-19: An Efficacy and Potential Mechanistic Study

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There has been a large global outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), representing a major public health issue. In China, combination therapy, including traditional Chinese medicine (TCM) as a treatment for COVID-19 has been used widely. "Fei Yan No. 1" (QFDYG) is a formula recommended by the Hubei Government to treat COVID-19. A retrospective study of 84 COVID-19 patients from Hubei Provincial Hospital of TCM and Renmin Hospital of Hanchuan was conducted to explore the clinical efficacy of QFDYG combination therapy. TCMSP and YaTCM databases were used to determine the components of all Chinese herbs in QFDYG. Oral bioavailability (OB) ≥ 30% and drug-like (DL) quality ≥ 0.18 were selected as criteria for screening the active compounds identified within the TCMSP database. The targets of active components in QFDYG were determined using the Swiss TargetPrediction (SIB) and Targetnet databases. The STRING database and the Network Analyzer plugin in Cytoscape were used to obtain protein-protein interaction (PPI) network topology parameters and to identify hub targets. Gene Ontology (GO) enrichment was conducted using FunRich version 3.1.3, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment using ClueGO version 2.5.6 software. PPI and compound-pathway (C-T) networks were constructed using Cytoscape 3.6.0. Compared with the control group, combined treatment with QFDYG resulted in a significantly higher rate of patients recovering from symptoms and shorter the time. After 14 days of treatment, QFDYG combined treatment increased the proportion of patients testing negative for SARS-CoV-2 nucleic acid by RT-PCR. Compared with the control group, promoting focal absorption and inflammation as viewed on CT images. GO and KEGG pathway enrichment indicated that QFDYG principally regulated biological processes, such as inflammation, an immune response, and apoptosis. The present study revealed that QFDYG combination therapy offered particular therapeutic advantages, indicating that the theoretical basis for the treatment of

COVID-19 by QFDYG may play an antiviral and immune response regulation through multiple components, targets, and pathways, providing reference for the clinical treatment of COVID-19.

Keywords: Fei Yan NO1, COVID-19, SARS-CoV-2, clinical efficacy, network pharmacology

INTRODUCTION

Since December 2019, coronavirus disease 2019 (COVID-19), caused by infection with the SARS-CoV-2 virus, has spread to most countries and regions around the world (Zhu et al., 2020). In common with two other highly pathogenic coronaviruses, SARS-CoV and MERS-CoV, SARS-CoV-2 is also a β-coronavirus (Xu et al., 2020), principally transmitted by close contact and through the respiratory tract, resulting in severe respiratory illness and even death. The main clinical manifestations of COVID-19 are fever, cough. A small number of patients also display symptoms that include sore throat, stuffy or runny nose, muscle pain, and diarrhea. A number of patients rapidly progress to acute respiratory failure or acute respiratory distress syndrome (ARDS) (Chen N. et al., 2020). Because of the scale of the outbreak, the World Health Organization (WHO) announced a public health emergency of international concern (PHEIC) on 30th January 2020 (Sohrabi et al., 2020). According to the recent data from the WHO, the number of global infections has passed 27 million, with a death toll greater than 880,000, threatening 214 countries. Therefore, there is a pressing need to establish a specific and effective therapeutic schedule for the treatment of COVID-19.

Traditional Chinese medicine (TCM), which has a history of successful therapy over thousands of years, has made a substantial contribution to the health and prosperity of the entire nation. TCM has accumulated the rich experience of fighting emerging infectious diseases over a considerable period of time with a solid theoretical foundation for their treatment (Luo et al., 2020). Although COVID-19 is a novel infectious disease caused by SARS-CoV-2, TCM has been used to successfully treat similar syndromes caused by coronaviruses. In addition, TCM has played an important role in combating coronavirus pneumonia, such as that caused by SARS and MERS, in addition to H7N9 avian influenza, over the last two decades (Ren et al., 2020). In 2003, SARS broke out in China. Studies found that the earlier an intervention with TCM was initiated, the lower the rate of mortality and incidence of complications was observed (Du et al., 2020).

Following the outbreak of COVID-19, China's National Health Commission proposed a diagnosis and treatment program through the integration of TCM with modern medicine, as no antiviral drug around the world had at that time been shown to be effective. The combination treatment of COVID-19 using traditional Chinese medicine is widely used in China. TCM marked improvement of symptoms and shortened the disease course, and also better control of fever, quicker clearance of chest infection and other symptoms. The symptoms of the majority of COVID-19 patients improving markedly, demonstrating its capacity to promote recovery (Yang et al., 2020a). Of the proposed diagnoses and treatments, a TCM formula termed "Fei Yan No. 1" (QFDYG) was recommended by the Hubei Headquarters for the Prevention and Control of Novel Coronavirus Pneumonia Epidemic and approved by the Hubei Provincial Drug Administration (Z20200003) for the treatment of COVID-19. It consists of 13 traditional Chinese medicines, including *Bupleurum chinense* DC. (Chaihu), *Scutellaria baicalensis* Georgi (Huangqin), *Pinellia ternata* (Thunb.) Breit. (Banxia), *Codonopsis pilosula* (Franch.) Nannf. (Dangshen), *Trichosanthes kirilowii* Maxim. (Gualou), *Areca catechu* L. (Binglang), *Amomum tsao-ko* Crevost et Lemaire (Caoguo), *Magnolia officinalis* Rehd. et Wils. (Houpo), *Anemarrhena asphodeloides* Bge. (Zhimu), *Paeonia lactiflora* Pall. (Chishao), *Glycyrrhiza uralensis* Fisch. (Gancao), *Citrus reticulata* Blanco (Chenpi), and *Polygonum cuspidatum* Sieb. et Zucc. (Huzhang). Production quality control standards for QFDYG have been established and it has been widely used for the clinical treatment of COVID-19 patients in Hubei Province. The process of preparation of QFDYG can be described as follows: prescribed quantities of the Chinese Traditional Medication were weighed out for decoction, extracted twice in water using an automatic digital instrument, concentrated under reduced pressure, spray-dried to obtain a powder, to which auxiliary materials were added. It was granulated using a dry process and then packaged. High performance liquid chromatography (HPLC) was used to measure its 6 principal components and thin-layer chromatography (TLC) used to detect the three components of greatest concentration in QFDYG, from which quality control standards were established. All materials and operations met the requirements of the Chinese Pharmacopoeia (*2015 edition*). Although QFDYG was used for the treatment of COVID-19, its clinic effectiveness remains uncertain, and the bioactive components and their mechanism of antiviral action remain unclear.

Network pharmacology is based upon the theories of system biology and multi-directional pharmacology (Zhou Y. et al., 2020). It allows the construction of biological networks and network visualization analysis of bioactive components, hub targets, and signaling pathways to elucidate the complex relationships between drugs and diseases (Zhou S. et al., 2020). Network pharmacology can reveal the mechanisms of action of a TCM formula with multiple components and target characteristics (Li and Zhang, 2013).

Therefore, as a first step, studies that used combination therapy of COVID-19 patients with QFDYG were systematically reviewed in order to explore the evidence for its clinical efficacy. Based on definite curative outcomes, network pharmacology was used to

explore the potential bioactive components and therapeutic mechanisms of QFDYG in treating COVID-19. In the present study, we aimed to establish the evidence as a reference for the optimal clinical practice of treatment of COVID-19 using QFDYG and determine the therapeutic mechanisms. A graphical abstract of the study is displayed in **Figure 1**.

MATERIALS AND METHODS

Clinical Efficacy Study

Study Design and Participants

The design was a retrospective study and was approved by the Institutional Ethics Board of Hubei Province Hospital of TCM (HBZY2020-C02-01), for which the requirement for written informed consent was waived. Patients included all those aged 18 years old or greater of either sex. The diagnosis of COVID-19 was confirmed by a positive nucleic acid RT-PCR test for SARS-CoV-2. If an individual's sample were tested positive, it will be included in further study. In general, adults with COVID-19 can be grouped into the following categories of illness severity **(Table 1)**, in accordance with the "*Protocol for Diagnosis and Treatment of COVID-19 guidelines (4th edition)*," issued by the National Health Commission of China (General Office Of The National Health And Health Commission, 2020).

Key exclusion criteria included: (1) Severe systemic disease; (2) Women that were pregnant or lactating; (3) Known allergies to the investigational medication; (4) Respiratory disease caused by bacterial infection; (5) Transfer to other hospitals due to policy reasons; (6) Self withdrawal during the course of treatment; (7) Incomplete clinical data records.

Patients with confirmed COVID-19 admitted to the Hubei Province Hospital of TCM and Renmin Hospital of Hanchuan between January 27 and March 12, 2020, were included in the study.

TABLE 1 | The clinical classification of COVID-19.

Clinical classification	Protocol for Diagnosis
Mild	1. Individuals who test positive for SARS-CoV-2 with RT-PCR. 2. The clinical symptoms were mild. 3. With or without fever or respiratory diseases. 4. With or without showed obvious lung infiltrates on chest computed tomography (CT).
Severe	1. shortness of breath and respiratory rate (RR) ≥30 times/min. 2. A saturation of oxygen (SpO2) ≤93% at rest. 3. Ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300 mmHg (1mmHg =0.133kPa) on room air at sea level. *In high-altitude areas (at an altitude of over 1,000 meters above the sea level), PaO_2/FiO_2 shall be corrected by the following formula: PaO_2/FiO_2 x [Atmospheric pressure (mmHg)/760]. 4. Cases with chest imaging that showed obvious lung infiltrates >50% within 48 h. 5. Individuals who have respiratory failure, septic shock, and/or multiple organ failure.

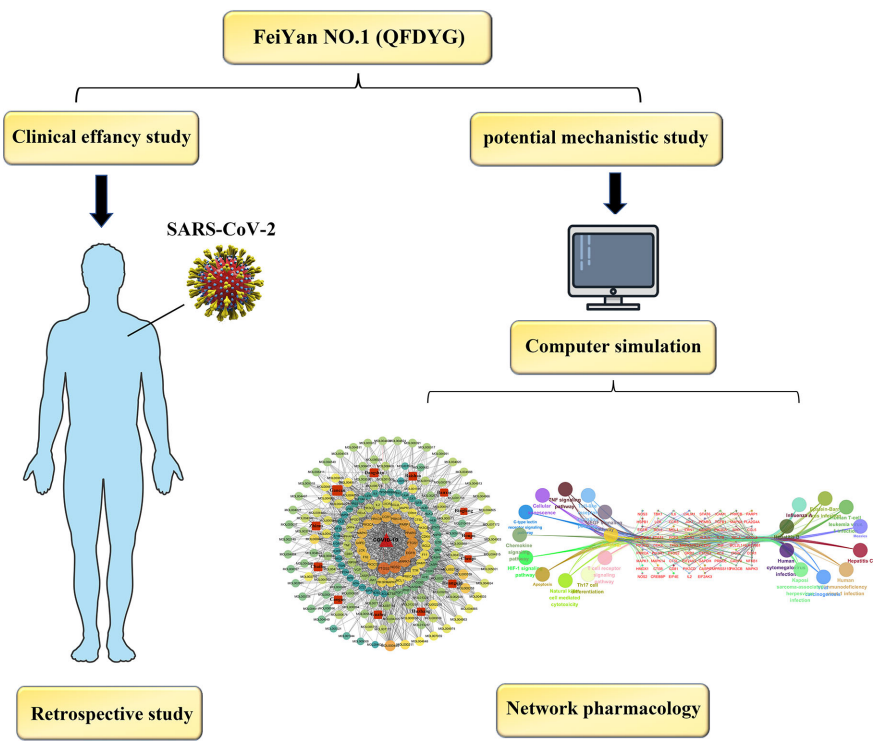

FIGURE 1 | Clinical efficacy.

Clinical Treatments and Data Collection

Patients were divided into two groups depending on whether they were prescribed QFDYG as a clinical treatment. In accordance with the "*Protocol for Diagnosis and Treatment of COVID-19 guidelines (4th edition, 5th edition and 6th edition)*" (General Office Of The National Health And Health Commission, 2020) **(Figure 2)**, symptomatic treatment (ST) included the antivirals oral alpha interferon inhalation (50 μg twice daily), oseltamivir (500 mg twice daily), arbidol (200mg three times daily),and other treatments or interventions based on disease progression. There are no obvious differences with respect to the use of other drugs and treatments between the two groups. For ST+QFDYG group, each patient was given the same treatments as in the basic therapy plus QFDYG (18-g granules, three times daily). symptom recovery was represented by complete remission of both symptoms. Clinical cure was defined as having met all of the following criteria: recovery of body temperature for more than 3 days, symptom recovery, marked improvement in chest CT imaging, and two consecutive negative SARS-CoV-2 RNA tests (at least 1 day apart).

The clinical symptoms, treatment, and outcomes were collected from electronic medical records. Primary outcomes consisted of the rate of recovery from symptoms, the time required and rate for SARS-CoV-2 nucleic acid testing to be negative by RT-PCR and chest CT imaging. Secondary outcomes consisted of leukocyte, neutrophil, lymphocyte counts, hs-CRP. Data prior to admission was compared with that prior to discharge of the two groups, including age, sex, clinical classification, signs and symptoms, underlying disease, laboratory biochemical test results, and nucleic acid RT-PCR becoming negative.

Safety Evaluation of Two Groups

Previous reports of QFDYG showed that no obvious adverse reactions were found during the treatment, and the safety of clinical application was good (Ba et al., 2020). In our study, we recorded the timing, severity, duration, measures and consequence of adverse events, and the association with QFDYG.

Analysis of Network Pharmacology

Screening of Bioactive Ingredients

The compounds within QFDYG were identified in the TCMSP database (https://tcmspw.com/tcmsp.php), compensating for insufficient information within the YaTCM database (http://cadd.pharmacy.nankai.edu.cn/yatcm/home). Oral bioavailability (OB) ≥ 30% and drug-like quality (DL) ≥ 0.18 were selected as criteria for screening active compounds. OB denotes the relative quantity absorbed into the bloodstream after administration

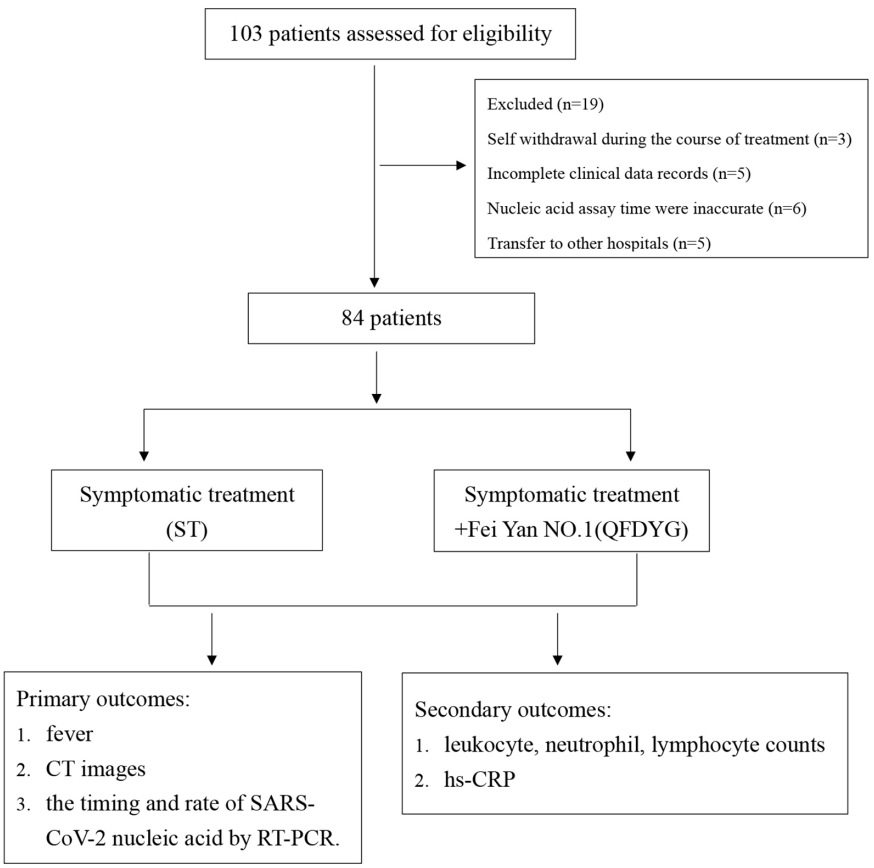

FIGURE 2 | Data collection for the treatment of COVID-19 patients.

through an extravascular route. DL refers to the "drug-like" qualities of a compound, used to optimize pharmacokinetics and the properties of a drug. The OB values and DL indices of the components in QFDYG were retrieved from the TCMSP database.

Potential Targets Intersection of QFDYG With COVID-19

Targets of the active components in QFDYG were explored using Swiss TargetPrediction (SIB) (http://www.swisstargetprediction.chand) and Targetnet (http://targetnet.scbdd.com/). The Uniprot protein sequence resource (http://www.Uniprot.org/) was used to confirm and standardize protein names. Duplicates were deleted to obtain target genes. Genes associated with COVID-19 were identified using GeneCards (https://www.genecards.org/). Intersection targets for COVID-19 and their compounds were considered potential targets. Potential targets of the active components were then imported into the STRING (http://string-db.org/) database to identify protein-protein interactions (PPIs).

GO and KEGG Pathway Enrichment Analysis of Candidate Targets

Gene ontology (GO) functional enrichment analysis, including molecular function (MF), biological processes (BP) and cytological components (CC), was performed by inputting all potential targets into the Functional Enrichment Analysis Tool (FunRich version 3.1.3) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis conducted using the ClueGO plugin v2.5.6 in Cytoscape.

Network Construction and Analysis

Cytoscape 3.6.0 software was used to construct PPI and drug-compound-target (D-C-T) networks. The Cytoscape Network Analyzer plugin was used to analyze network topology, in which nodes represent targets or compounds and the size and color of the nodes indicate the degree of the node.

Statistical Analysis

Categorical variables are described as frequency rates and percentages, and compared using χ^2 tests between two groups. Continuous variables are described using median values (interquartile range (IQR)) or means ± SD, and compared using an independent sample t-test between two groups. All statistical analyses were performed using SPSS 25.0 software. P <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics of the Patients in the Study

Of the 103 patients assessed for eligibility, 19 were excluded, including 3 that were asymptomatic infection who stopped taking the medication by themselves, 5 cases did not undergo CT image scanning at discharge, 6 where nucleic acid assay time were inaccurate (2 of patients did not cooperate with RT-PCR test for SARS-CoV-2 testing. 4 cases were due to insufficient nucleic acid detection capabilities at that time), and 5 that had transferred to other hospitals. Therefore, a total of 84 patients confirmed to have COVID-19 participated in the study. Of these, symptomatic treatment of 49 was supplemented with QFDYG (ST+QFDYG group) while the remaining 35 patients were administered symptomatic treatment only (ST group). From the baseline characteristics displayed in **Table 2**, both groups were comparable in terms of demographic characteristics, vital signs, symptoms, and concomitant treatment. The median age of patients in the two groups was 48 years in the ST group (range: 39 - 53 years) and 57 years in the ST + QFDYG group (range: 49.5 - 65.5 years). There was no significant difference in the distribution of sex or clinical classification ($P > 0.05$). The most common underlying diseases were diabetes, hypertension, hyperlipemia, coronary disease and chronic hepatitis B. Of the recorded symptoms, cough (91.42% *vs.* 91.83%), fever (85.71% *vs.* 83.67%) and chest CT (88.57% *vs.* 91.84%) were the most common in the two groups, respectively.

Comparison of Treatment Responses Between the Groups With or Without QFDYG

In the primary outcomes, the rate of symptom recovery was greater in the ST + QFDYG group compared with the ST group. Furthermore, the mean duration of recovery from fever (7.6 days *vs.*3.8 days, $P < 0.05$) was significantly shorter in the ST + QFDYG group. A nasopharyngeal swab test for SARS-CoV-2 nucleic acid by RT-PCR is considered the most important indicator of a treatment effect. The time for a negative RT-PCR result to be recorded was shorter in the ST+QFDYG group than in the ST group (7.0 days *vs.* 14.0 days, $P < 0.05$). After 14 days, coronavirus was not detected in 47 out of 49 (95.92%) and 28 out of 35 (80.00%) patients in the ST+QFDYG and ST groups, respectively ($P < 0.05$) (**Figure 3**). Chest CTs indicated that the majority of patients had apparent lung infiltrates prior to

TABLE 2 | Baseline characteristics and symptoms of the groups with or without QFDYG.

	ST(n = 35)	ST+QFDYG (n = 49)	*P*
Age, years	48 (39, 53)	57 (49.5, 65.5)	0.137
Sex (male/female)	22/13	28/21	0.599
Clinical classification (mild/severe)	31/4	37/12	0.133
Signs and symptoms			
Fever (> 37.2°C)	30 (85.71%)	41 (83.67%)	0.799
Cough	32 (91.42%)	45 (91.83%)	0.947
Mild insomnia	29 (82.86%)	43 (87.76%)	0.527
Chest CT	31(88.57%)	44 (91.84%)	0.858
Laboratory indices			
Leukocyte count ($\times10^9$/L)	4.23 (3.86, 4.79)	6.23 (4.93, 9.26)	0.058
Neutrophil count ($\times10^9$/L)	2.57 (2.45, 3.35)	4.69 (3.40, 7.36)	0.024
Lymphocyte count ($\times10^9$/L)	1.38 (0.71, 1.38)	1.19 (1.06, 1.21)	0.79
Hs-CRP (mg/L)	9.00 (9.00, 12.29)	9.70 (2.40, 9.80)	0.886
Underlying diseases			
Diabetes	8 (22.86%)	13 (26.53%)	0.701
Hypertension	9 (25.71%)	12 (24.49%)	0.898
Hyperlipemia	5 (14.29%)	8 (16.33%)	0.799
Coronary disease	3 (8.57%)	5 (10.20%)	0.802
Chronic hepatitis B	2 (5.71%)	4 (8.16%)	0.693

admission (88.57% *vs.* 91.84%), with ground glass or flocculent shadows observed in the lungs of patients with both mild and severe disease. Manifestations in the chest as observed by computed tomography in mild and severe patients are displayed in **Figure 4**.

In 84 cases, the leukocyte count of 18 patients and the neutrophil count of 16 patients were not in the normal range and returned to normal after treatment. The lymphocyte count of 33 patients were lower than the normal value. After treatment, the lymphocytes increased, which was significantly different ($P < 0.05$) (**Table 3**). Median hs-CRP (9.00 *vs.* 9.70) was significantly higher than the normal reference range prior to hospitalization, which decreased significantly ($P < 0.05$), close to normal, following ST+QFDYG treatment.

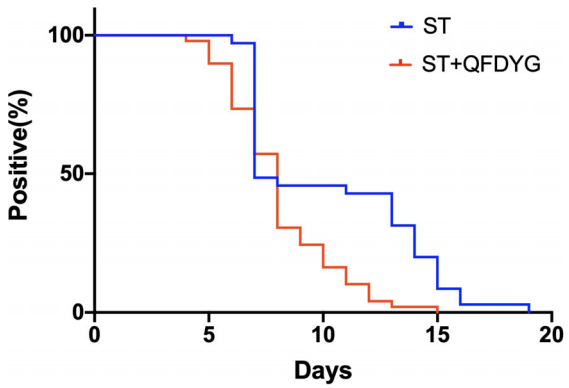

FIGURE 3 | SARS-CoV-2 nucleic acid by RT-PCR between two groups ($P < 0.05$).

Safety of Two Groups

No significant difference in the incidence of adverse events was observed between the two groups. No serious adverse events were reported and there were few reports of abnormal laboratory blood tests (**Table 4**).

Active Components in QFDYG

The TCMSP database was used to search for active components in QFDYG. A total of 122 active ingredients fulfilling the specified criteria, OB ≥ 30% and DL index ≥ 0.18, were identified (**Table 5**).

Candidate Targets Screening and PPI Network Analysis

The SIB and TargetNet databases were used to screen 1116 targets corresponding to the 122 active components identified within QFDYG. A total of 259 targets relating to COVID-19 were obtained from GeneCards. In total, 85 common targets were identified as potential therapeutic targets for QFDYG against COVID-19. These targets were then uploaded into the STRING database to obtain information about the PPI, and then finally imported into Cytoscape for network analysis. Targets in the PPI network (**Figure 5**) with a high degree represented those with an important role in the central correlation. For example, the top 16 targets, ranked in order of degree, namely TNF, IL6, GAPDH, TP53, MAPK1, MAPK3, ALB, EGFR, CASP3, MAPK8, CXCL8, L2, ICAM1, IL1B, MAPK14, STAT1 tended to have more critical roles in the target network of QFDYG.

GO Enrichment Analysis

GO enrichment analysis of the 85 potential therapeutic targets was performed in order to identify relevant biological functions of QFDYG. The 10 most significantly enriched terms of a high

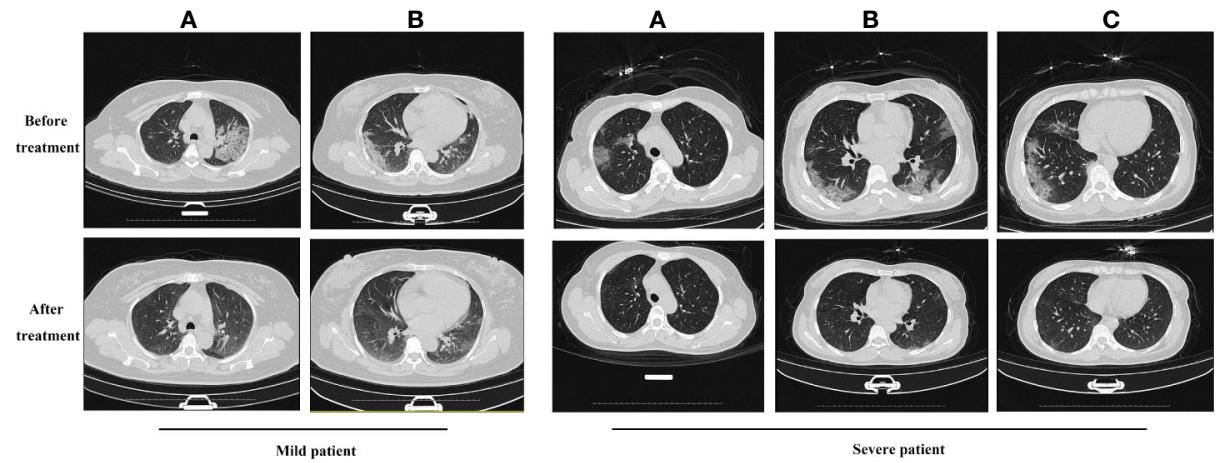

FIGURE 4 | The results of chest CT scan with QFDYG combined therapy. A, B and C represent different slices. Mild patient: A 55-year-old female with bilateral pulmonary infiltrate prior to treatment with ST+QFDYG observed in A and B slices. Clear absorption of bilateral pulmonary infiltrate was observed by day 9. Severe patient: A 50-year-old female with shortness of breath and hypertension at the initiation of treatment with ST+QFDYG. Images display bilateral pulmonary infiltrate in A, B and C slices over an area larger than that of a mild patient. Marked absorption of the bilateral pulmonary infiltrate was observed by day 11, with only a few small patches of faint shadow remaining.

TABLE 3 | Comparison of treatment responses between the groups with or without QFDYG.

	Reference range	ST (n=35)	ST+QFDYG (n=49)	*P*
Leukocyte count ($\times10^9$/L)	3.5-9.5	5.20 (4.10, 5.20)	3.97 (3.97, 7.65)	0.01
Neutrophil count ($\times10^9$/L)	1.8-6.3	3.48 (3.37, 3.48)	2.60 (2.20, 3.66)	0.436
Lymphocyte count ($\times10^9$/L)	1.1-3.2	1.24 (0.49, 1.24)*	1.20 (0.96, 3.01)*	0.510
Hs-CRP (mg/L)	0-3	4.50 (4.50, 9.64)*	2.40 (1.20, 2.50)*	0.002
Nucleic acid RT-PCR negative, days	–	14.0 (7.0, 16.0)	7.0 (5.0, 8.0)	0.04
Fever, days	–	7.6 ± 1.7	3.8 ± 1.3	0.001

Compared with before admission data in ***Table 2*** **P < 0.05*

TABLE 4 | Comparison of the adverse events between the groups with or without QFDYG.

Adverse events	ST (n = 35)	ST+QFDYG (n = 49)	*P*
Total	19 (54.29%)	23 (46.94%)	0.507
Nausea	8 (22.86%)	11(22.45)	0.965
Abnormal liver function	5 (14.29%)	4(8.16%)	0.371
Vomiting	1 (2.86%)	1 (2.04)	0.809
Headache	3 (8.57%)	4 (8.16)	0.947
Abnormal renal function	2 (5.71%)	3 (6.12%)	0.938

percentage of genes in the BP, CC, and MF categories are displayed in **Figure 6**. BP analysis indicated that the targets were principally related to signaling transduction, cell communication, protein metabolism, energy pathway, metabolism, apoptosis and immune response. Analysis of the CC category indicated that the targets were mostly within the cytoplasm, nucleus, plasma membrane, cytosol, lysosomes and also extracellular. Analysis of MF indicated that the targets were mainly involved in activities related to serine/threonine kinases, catalysts, transcription factors, cysteine-type peptidases, lipid kinases and cytokines.

KEGG Pathway Enrichment Analysis

To reveal the potential therapeutic mechanism of QFDYG on COVID-19, KEGG pathway enrichment analysis was conducted on 85 potential targets with 35 signaling pathways screened for those with *P*-values < 0.01, a kappa score ≥ 0.8, and numbers of genes ≥ 15. An overview map of the 35 KEGG signaling pathways is displayed in **Figure 7A**. KEGG pathway enrichment suggested that the targets were highly enriched in signaling pathways regulating virus infection and immune response, such as influenza A, hepatitis B, hepatitis C, Kaposi sarcoma-associated herpesvirus infection, human cytomegalovirus infection, viral carcinogenesis, human immunodeficiency virus 1 infection, Epstein-Barr virus infection, human T-cell leukemia virus 1 infection, the TNF signaling pathway, T cell receptor signaling pathway and Natural killer cell-mediated signaling pathway. The 11 most enriched signaling pathways were then selected to identify the relationship with their associated genes. As shown in **Figure 7B**, TNF, NFκB, IL-6, IL-1β, TP53, MAPK1, MAPK3 and MAPK14 were highly enriched.

Drug-Compound-Target Network Construction

The 85 potential therapeutic targets and components and Chinese herbs within QFDYG were imported into Cytoscape 3.6.0 to construct a D-C-T network. The network (**Figure 8**) indicates that one Chinese herb contained multiple compounds, one compound acted on multiple targets, and one target was the target of multiple compounds, suggesting that the therapeutic

TABLE 5 | Information of the potential components with higher OB and DL values in QFDYG.

NO.	Mol ID	Molecule Name	MW	OB (%)	DL	Classification
1	MOL001918	paeoniflorgenone	318.35	87.59	0.37	Baishao
2	MOL000449	Stigmasterol	412.77	43.83	0.76	Banxia
3	MOL002776	Baicalin	446.39	40.12	0.75	Banxia
4	MOL000004	Procyanidin B1	578.56	67.87	0.66	Binglang
5	MOL000098	Quercetin	302.25	46.43	0.28	Caoguo
6	MOL000422	Kaempferol	286.25	41.88	0.28	Chaihu
7	MOL004644	Sainfuran	286.3	79.91	0.23	Chaihu
8	MOL005815	Citromitin	404.45	86.9	0.51	Chenpi
9	MOL000006	Luteolin	286.25	36.13	0.25	Dangshen
10	MOL006554	Taraxerol	426.80	38.40	0.77	Dangshen
11	MOL002311	Glycyrol	366.39	90.78	0.67	Gancao
12	MOL004904	licopyranocoumarin	384.41	80.36	0.65	Gancao
13	MOL005017	Phaseol	336.36	78.77	0.58	Gancao
14	MOL005970	Eucalyptol	266.36	60.62	0.32	Houpo
15	MOL013288	Picralinal	366.45	58.01	0.75	Huzhang
16	MOL000173	Wogonin	284.28	30.68	0.23	Huangqin
17	MOL001689	Acacetin	284.28	34.97	0.24	Huangqin
18	MOL007165	10α-cucurbita-5,24-diene-3β-ol	426.8	44.02	0.74	Gualou
19	MOL001924	Paeoniflorin	480.51	53.87	0.79	Zhimu

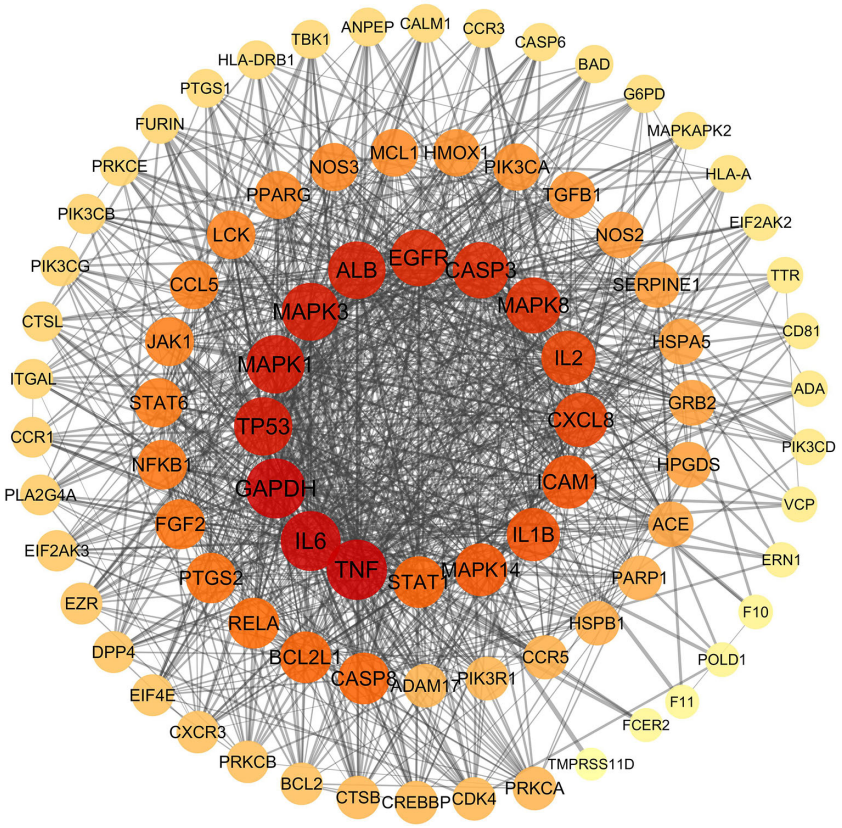

FIGURE 5 | PPI network of candidate target. The colors of the nodes are illustrated from red to orange to yellow in descending order of degree values.

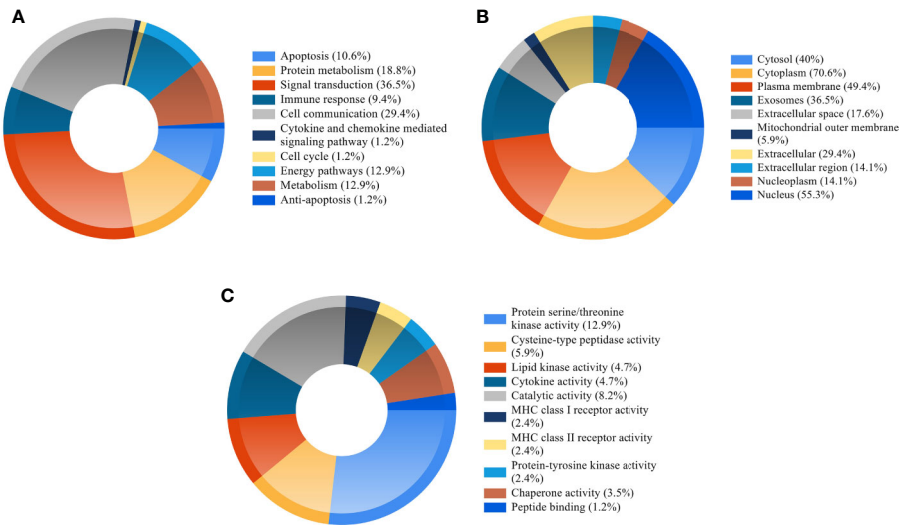

FIGURE 6 | GO enrichment of the candidate targets. **(A)** Biological process of the candidate targets. **(B)** Cellular component of the candidate targets. **(C)** Molecular function of the candidate targets.

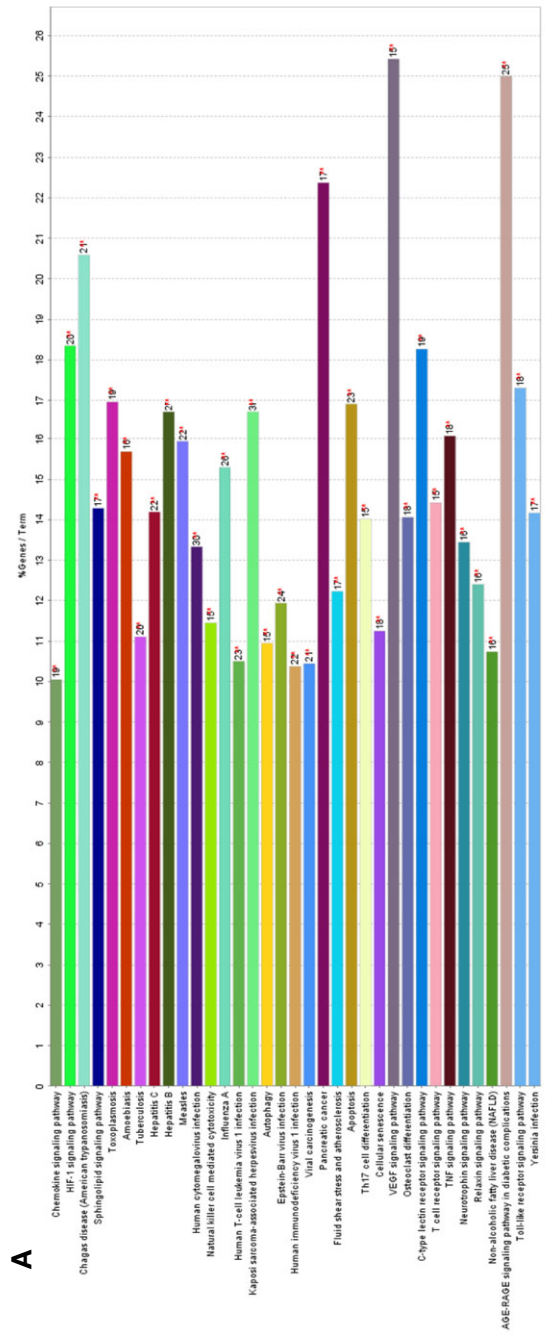

FIGURE 7 | KEGG pathway enrichment of the potential targets **(A)**. Pathways and associated genes relationships network **(B)**.

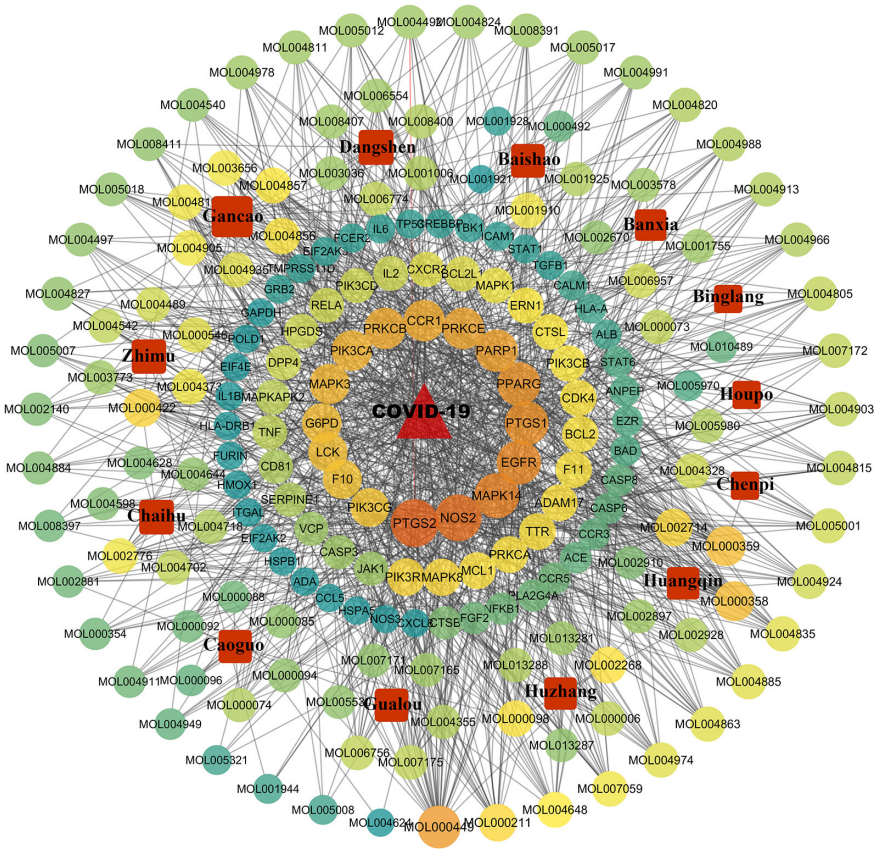

FIGURE 8 | Drug-compound-target network of QFDYG. The red square nodes represent Chinese herbs. and the circular nodes represent active compounds and targets. Nodes size and color are illustrated from dark orange to yellow to green in descending order of degree values.

characteristics of QFDYG were that of multiple components and multiple targets.

DISCUSSION

With the outbreak of COVID-19, there is an urgent need for specific and clinical antiviral regimens (Wu and McGoogan, 2020). However, there are no effective antiviral drugs for COVID-19, and the main strategy involves individualized treatment for symptomatic patients (Lai et al., 2020). Fortunately, in China, the combination of TCM treatment and effective life support was widely used in the early stage of the outbreak. The available data showed that the combination of TCM treatment with symptomatic treatment could reduce the transition of COVID-19 patients from mild to severe and promote the recovery from COVID-19 (Wan et al., 2020). QFDYG is a recommended TCM formula, which has been used clinically since the COVID-19 epidemic in Hubei Province. It has been reported that its clinical effect is better. We found that COVID-19 patients have the most common symptoms of fever and cough. It has been reported that fever is more than 80% and cough is more than 60%. Therefore, improving the symptoms of fever and cough is of great significance for the treatment of COVID-19 (Guan et al., 2020).

To clarify the clinical efficacy of QFDYG in treating COVID-19, a retrospective study was conducted, in which 103 patients were selected. Following the exclusion of 19 patients due to the clinical requirements of the study, data from 84 patients were analyzed in detail. Compared with the control group, combination treatment with QFDYG resulted in a significantly higher rate of recovery from symptoms over a shorter time. Nasopharyngeal swab tests for SARS-CoV-2 nucleic acid by RT-PCR is the principal text of effectiveness of COVID-19 treatment. The shorter the duration for a test to be negative, the better the drug treatment (Tahamtan and Ardebili, 2020). After 14 days of treatment, the proportion of negative nucleic acid tests in the combined treatment group was higher than that in the control group and the time was lower than the control group. QFDYG combined treatment, therefore, enhanced the negative rate and short recovery time of SARS-CoV-2 nucleic acid tests and shortened the duration of recovery. Chest CT is one of the vital evidences in the diagnosis for patients suspected of having COVID-19 infection, which improves the diagnosis rate and is also used to observe the patient's treatment together with RT-PCR (Bernheim et al., 2020). Combination

therapy with QFDYG shows better absorption of pulmonary inflammatory infiltration. Due to the complexity of COVID-19, other laboratory parameters such as hs-CRP, leukocyte count, neutrophil count and lymphocyte count are meaningful for clinical diagnosis as well (Soraya and Ulhaq, 2020). As a result, hs-CRP decreased significantly through the combination therapy with QFDYG compared with the data before admission, and what is more, the decrease rate was greater compared with the control group. Moreover, the lymphocyte count increased remarkably, indicating the obvious effect of the combination therapy with QFDYG on the recovery of the disease. No serious adverse events were reported in the clinical records, and there was no significant difference in the number of adverse reactions between the two groups, indicating that QFDYG treatment of COVID-19 was safe.

To further explore the foundation for the efficacy of QFDYG, network pharmacological approaches were integrated to investigate the bioactive components of QFDYG and their latent mechanisms for treating COVID-19. A total of 1643 components were obtained. Since antiviral components are absorbed *via* oral administration, the screening process for bioactive ingredients should include an assessment of drug-likeness (DL) and oral bioavailability (OB) (Yang et al., 2020b). The OB and DL of all compounds within QFDYG were extracted from the TCMSP database, finding that 122 compounds met the specified requirements. Targets are the key locations of disease treatment. Drugs can act both directly or indirectly on targets to fight a disease (Vinayagam et al., 2016). Therefore, all identified targets of compounds were compared with COVID-19 disease targets to obtain common cross-targets, of which 85 were finally identified. These common targets are considered potential therapeutic targets for drugs to treat COVID-19 disease and were used for further network pharmacological analysis.

Network pharmacological analysis indicated that the potential therapeutic targets of QFDYG were widely involved in mediating biological processes, such as inflammation, the immune response, both related to the body's immune response after a viral infection. PPI network analysis indicated that TNF, IL-6, GAPDH, TP53, MAPK1, MAPK3, ALB, EGFR, CASP3, MAPK8, CXCL8, L2, ICAM1, IL1B, MAPK14 and STAT1 were located in the center of the network, and may represent hub targets of QFDYG. GO enrichment analysis suggested that the potential therapeutic targets in QFDYG may exert their therapeutic effect by regulation of signaling transduction, apoptosis, the immune response, and other biological processes *via* modulation of transcription factors and functional protease activity in the plasma membrane, cytosol, and nucleus. KEGG pathway enrichment analysis indicated that the 85 therapeutic targets were highly enriched in viral infection signaling pathways, such as influenza A and HIV-1 virus infection. They were also involved in immune respone, such as human T-cell, and activcation of the TNF signaling pathway, Tcell receptor signaling pathway and Natural killer cell-mediated signaling pathway. In the signaling pathway network, TNF, NF-κB, IL-6, IL-1β, TP53, MAPK1, MAPK3 and MAPK14 were the most enriched targets.

From the enrichment results of the KEGG pathway, 35 pathways were obtained, of which 10 were related to anti-viral action and 14 were related to the immune response. It can be inferred that the potential mechanism of action of QFDYG is mostly through anti-viral mechanisms and an immune response. SARS-CoV-2 is a type of β-coronavirus, a single-stranded positive-sense RNA virus that causes respiratory and enteric disease in humans and other animals (Wei et al., 2020). According to the results of this research, 6 Chinese medicines in QFDYG have antiviral effects, namely Chaihu, Huangqin, Houpo, Chishao, Gancao and Huzhang (Iore et al., 2007; Zhang et al., 2007; Shang et al., 2010; Xue et al., 2016; Yang et al., 2017). Components isolated from the Chinese medicine Chaihu, such as saikosaponins, have been reported to inhibit coronavirus and influenza virus activity, *via* an inhibitory effect of viral attachment, penetration and replication (Li et al., 2018). Baicalein, isolated from the Chinese medicine Huangqin can inhibit H1N1 virus activity by interfering with mRNA synthesis during the middle to late stages (Su et al., 2012). Similarly, glycyrrhizic acid isolated from the Chinese medicine Gancao can inhibit HIV virus activity. The mechanism of action may be to reduce membrane fluidity, reduce HIV-1-induced cell fusion and protein kinase C activity (Sun et al., 2019). Recent studies have demonstrated that SARS-CoV-2 displays a genomic organization similar to that of other β-coronaviruses, including the genes for three glycoproteins on the surface of the membrane, a spike protein (S), an envelope protein (E) and a membrane protein (M) (Li H. et al., 2020). The coronavirus enters cells by binding surface proteins *via* a receptor, causing infection and simultaneously inducing an immune response (Columbus et al., 2020). For example, MERS-CoV uses its S protein to bind to a specific dipeptidyl peptidase-4 receptor, which is considered a key factor in signal transduction and causes the activation of both the innate and specific immune response (Al-Tawfiq, 2020). The spike (S) glycoproteins of SARS-CoV-2 are no exception and mediate binding to host cells followed by membrane fusion (Chen W. et al., 2020). In the present study, molecular docking was used to establish that saikosaponins bind strongly to the S protein of the new coronavirus, saikosaponins U and V having the strongest binding, which is the subject of a potential future research study. From binding energy and interaction studies, Saikosaponins U and V displayed the strongest affinity towards the S protein (Sinha et al., 2020a). Similarly, a molecular docking simulation study of 20 compounds and an additional simulation study using molecular dynamics (MD) found that components of glycyrrhizic acid and glyasperin A displayed high binding affinity towards the S protein (Sinha et al., 2020b). COVID-19 principally spreads through the respiratory tract, and so the pharynx is a location that the virus can be found in high concentration. Samples were collected using nasopharyngeal swabs for an RT-PCR SARS-CoV-2 nucleic acid assay to determine whether the patients were infected with the virus, it is simple and reliable (Yang and Yan, 2020). The results of the present study indicate that combined treatment with QFDYG increased the proportion of positive to negative nucleic acid tests and reduced the time required for the nucleic acid test to become negative, indicating that QFDYG combination therapy may have a direct or indirect antiviral effect. From the results of these

studies, we hypothesize that when treating patients positive for COVID-19, the active components of QFDYG, such as saikosaponins and glycyrrhizic acid, may enter cells infected with SARS-CoV-2, bind to membrane proteins, such as the S protein, regulate various pathways resulting in an anti-viral effect.

To date, seven types of human coronavirus (HCoV) have been found (Hasoksuz et al., 2020). Among them, Hcov-HKU1, HCoV-OC43, HCoV-NL63, and HCoV-229E mainly just cause common colds in adults and upper respiratory tract infections in children (Kong et al., 2020). The last three are SARS-CoV, MERS-CoV, and SARS-CoV-2. Although the pathogenesis of SARS-CoV-2 is not clear, the clinical manifestations similar to SARS-CoV and MERS-CoV suggest that the immune response plays an important role in the pathogenic mechanism (Pearson et al., 2019). The cytokine storm is an immune system in producing an uncontrolled and generalized inflammatory response (Mehta et al., 2020). SARS-CoV and SARS-CoV-2 infection can cause cytokine storm, accelerate the progression of systemic diseases, and cause multiple organ failure (Coperchini et al., 2020). The cytokine storm induced by abnormal immune activation was shown to be related to ARDS and respiratory failure in patients with COVID-19 (Conti et al., 2020). As showed in the KEGG pathway, chemokines, interleukins and TNF are associated with cytokine storm. Chemokines are a large family of cytokines characterized by a powerful chemotactic effect. Chemokines act as powerful chemoattractants (Rotondi et al., 2007). According to the gradient of chemokines, chemokines absorb inflammatory cells and make them migrate from the intravascular space through the endothelium and epithelium to the inflammation site (Zemans et al., 2009). Recently, several studies have investigated the role of chemokines in coronavirus associated infectious diseases. The results suggest that specific chemokines (such as CXCL8 and CXCL10) may play an important role in the development of COVID-19 related symptoms (Coperchini et al., 2020). Interleukins are related to the cytokine family of immune cell differentiation and activation (Scheller and Rose-John, 2006). They are widely involved in the expression and regulation of immune response, mediate the transport of immune cells to the site of infection, induce signal transmission in the acute phase, activate epithelial cells to induce the production of secondary cytokines, and play a key role in immune response (Hunter and Jones, 2015). Previous studies showed that the serum IL-6 level in patients with COVID-19 is increased, and its circulating level is positively correlated with the severity of the disease (Liu B. et al., 2020). Interleukin-6 (IL-6) is involved in the cytokine storm caused by coronavirus. It can be produced by B cells and T cells, and is closely related to the acute inflammatory phase. IL-1 β and tumor necrosis factor (TNF-α) can increase the production of this cytokine (Hunter and Jones, 2015). IL-6 may also be responsible for activating T helper cell 17 (Th17) in the interaction between dendritic cells and T cells. In patients with covid-19 infection, the high activation of Th17 cells may be caused by the increase of IL-6 production driven by the virus caused by the immune system (Kimura and Kishimoto, 2010). Due to its pleiotropy, IL-6 plays a key role in the pathogenesis of cytokine storm. TNF is mainly secreted by activated macrophages, NK cells, and T lymphocytes. TNF-α is a well-known typical proinflammatory cytokine and a key effector of a lethal cytokine storm. IL-1β and IL-6 are also proinflammatory cytokines. Diffuse alveolar injury is the main cause of respiratory dysfunction caused by a viral infection (Pirozyan et al., 2020). Continued disruption of the alveolar endothelial and epithelial barriers will finally lead to pulmonary alveolar-capillary barrier dysfunction. IL-1β and TNF can break the alveolar endothelial and epithelial barriers.

The innate immune system is deemed essential to preventing or inhibiting initial viral infections (Jawhara, 2020). The innate immune cells with antiviral activities are mainly phagocytes, dendritic cells, and natural killer cells. Innate immune cells usually use pattern recognition receptors (PRRs) to recognize pathogen-associated molecular patterns (PAMP) (Almeida et al., 2020). After the virus enters the infected cells through interaction with the receptor, it can further promote the innate immune cells to secrete cytokines and chemokines, such as IL-1β, IL-6, TNF-α, and IFN-β, which lead to the activation of inflammasomes (Yusuf et al., 2019). The phagocytes can phagocytize and degrade the viral particles. NK cells can secret granzymes and perforins, which induce autophagy and apoptosis of the infected cells (Zambello et al., 2020). Autopsies of COVID-19 patients showed apoptosis in the lung, spleen, and thyroid tissues (Hanley et al., 2020). In addition, SARS-CoV-2 infection can activate NF-κB, leading to multiple inflammatory and autoimmune diseases, which induces pro-inflammatory cytokines and chemokines, including IL-6, and recruits lymphoid cells and myeloid cells, such as activated T cells and macrophages (Hirano and Murakami, 2020). Studies have shown that nearly 20% of the active components in QFDYG have a therapeutic effect on pneumonia model, such as Baicalin, Emodin, Acacetin, Luteolin and Taraxerol et al. They can regulate the levels of IL-1β, IL-6, TNF-α inflammatory cytokines and chemokines, most of them can inhibit the expression of NF-κB signaling pathway to play an anti-inflammatory effect (Su et al., 2012; Dai et al., 2017; Khanra et al., 2017; Kong et al., 2019; Li S. et al., 2020). CT images show that most patients with COVID-19 have different degrees of infiltration in the lungs. The combined treatment of QFDYG can speed up the absorption of inflammatory infiltration in both lungs and promote the recovery of lungs to normal. It indicates that QFDYG may play a role in the treatment of pneumonia and reduced the mean time to symptoms recovery (cough and fever), by regulating the expression of inflammatory cytokines and the NF-κB signal pathway. IL-6 and TNF-α are inflammatory cytokines and the main inducers of C-reactive protein (CRP) secretion in liver (Liu F. et al., 2020). CRP is a sensitive biomarker associated with inflammation, infection, and tissue damage. In normal organisms, the protein expression level of CRP is low. During acute inflammation such as viral infections, the protein level rises rapidly, which is a non-specific acute-phase protein (Wang, 2020). Clinical results showed that combined treatment QFDYG with can effectively reduce the level of hs-CRP, which may reduce IL-6, TNF-α and other cytokines to play an anti-inflammatory or reduce cytokine storm effect. we speculate that

QFDYG could treat COVID-19 by regulating the immune response and reducing the cytokine storm.

CONCLUSION

In summary, QFDYG combination therapy confers a therapeutic effect on COVID-19 by both increasing the proportion of patients that recovered and accelerating the recovery from symptoms. QFDYG combined treatment increased the proportion of patients with a SARS-CoV-2 negative nucleic acid RT-PCR test and promoted focal absorption and inflammation on CT images. The mechanism by which QFDYG treats COVID-19 may be related to anti-viral action and regulation of an immune response and reduction of a cytokine storm. As an adjuvant therapy, QFDYG was beneficial for the treatment of COVID-19. However, due to the small sample size of this retrospective study, further double-blind, prospective, multi-center and randomized controlled trials are required to fully evaluate the clinical efficacy of QFDYG in a larger patient population. Additional investigation and experimental research are required to reveal the specific mechanisms and confirm its clinical efficacy.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study will be available from the corresponding authors upon request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Ethics Board of Hubei Province Hospital of TCM. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

YB, HW, YY, ZA and SZ: Clinical research program design and management. ZA, SZ and MW: Visualization and Software. WL, LW, GH, RT, XW, YS, LX: Clinical research plan execution and clinical data collection. ZA, WL and LW: Key revisions to the important knowledge content of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Role of Traditional Chinese Medicine in the Management of Viral Pneumonia

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Viral pneumonia is one kind of acute respiratory tract infection caused by the virus. There have been many outbreaks of viral pneumonia with high contagiousness and mortality both in China and abroad, such as the great influenza in 1918, the severe acute respiratory syndrome (SARS) coronavirus in 2003, the Influenza A (H1N1) virus in 2009, and the Middle East Respiratory Syndrome coronavirus (MERS-CoV) in 2012 and the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019. These outbreaks and/or pandemic have significant impact on human life, social behaviors, and economic development. Moreover, no specific drug has been developed for these viruses. Traditional Chinese medicine (TCM) plays an important role in the treatment of viral pneumonia during these outbreaks especially in SARS and SARS-CoV-2 because studies suggest that TCM formulations may target several aspects of the disease and may have lesser side effects than manufactured pharmaceuticals. In recent years, a lot of clinicians and researchers have made a series of in-depth explorations and investigations on the treatment of viral pneumonia with TCM, which have understood TCM therapeutic mechanisms more specifically and clearly. But critical analysis of this research in addition to further studies are needed to assess the potential of TCM in the treatment of viral pneumonia.

Keywords: traditional Chinese medicine, viral pneumonia, severe acute respiratory syndrome coronavirus, influenza virus, coronavirus induced disease 2019

INTRODUCTION

Viral pneumonia is an acute respiratory infectious disease caused by viruses with different degrees of contagiousness. The main clinical manifestation is fever, which may be accompanied by symptoms such as anhidrosis or sweating, nasal congestion, runny nose, sore throat and cough (Figueiredo, 2009). Common viruses that cause pneumonia include adenovirus, coronavirus, human metapneumovirus, rhinovirus, respiratory syncytial virus, influenza virus and parainfluenza virus (Jain, 2017). Among them, severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) in

2003, Influenza A (H1N1) virus in 2009, and middle east respiratory syndrome coronavirus (MERS-CoV) in 2012 and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or called novel coronavirus in 2019 are highly contagious and fatal. As of 30 May 2020, there were 5,817,385 confirmed cases and 362,705 deaths in the coronavirus induced disease 2019 (COVID-19) outbreak since December 2019, and the trend is still on the rise (World Health Organization, 2020). At present, the commonly-used antiviral drugs in western medicine are probavirin, acyclovir, interferon, adenosine arabine, etc., which are easy to produce drug resistance, have many side effects and poor efficacy as well as other disadvantages (Amarelle et al., 2017). Because no specific and effective antiviral drugs have been developed in western medicine and Chinese herbal medicine possess clinical features of targeting multiple components and having multiple approaches, traditional Chinese medicine (TCM) has unique advantages in relieving symptoms, shortening treatment time and reducing the development of severe pneumonia. In the fight against COVID-19, the State Administration of Traditional Chinese Medicine of China has actively promoted the therapeutic role of TCM. As the member of the Leading Group of the National Health Commission and Secretary of the Leading Group of the National Administration of Traditional Chinese Medicine of China, Dr. Yanhong Yu pointed out that among the confirmed COVID-19 cases in China, a total of 74,187 people have used Chinese medicine, which accounts to 91.5% of patients (National Administration of Traditional Chinese Medicine, 2020). Academician of Chinese Academy of Engineering Dr. Boli Zhang analyzed 52 patients with COVID-19 retrospectively and found the clinical effective rate of 91.2% in patients treated with integrated traditional Chinese and western medicine as compared to effective rate of 61.1% in patients treated with western medicine alone (Xia et al., 2020).

Although there is no name of "viral pneumonia" in TCM, it is mainly attributed to "exogenous diseases" or "exterior syndrome". Traditional Chinese medical physicians usually classified them as "cough" or "lung distention" according to its clinical manifestations. Moreover, viral pneumonia with strong infectivity and high fatality rate is usually classified as "epidemic disease" in TCM. There has been a long history in China that TCM has been used to treat "epidemic disease" and there are a lot of clinical experiences and excellent efficacy. Therefore, different health organizations in China focus on TCM prevention and treatment of viral pneumonia and have formulated a series of diagnosis and treatment guidelines (China Association of Chinese Medicine, 2003; National Health and Family Planning Commission of People's Republic of China, 2015; National Health and Family Planning Commission of People's Republic of China, 2017; National Health Commission of the People's Republic of China and National Administration of Traditional Chinese Medicine, 2019; National Health Commission of the People's Republic of China, 2020). Among them, dozens of Chinese herbal medicines and formulae have been proposed (See **Figures 1** and **2**). Single traditional Chinese herbal medicine commonly-used in these diagnosis and treatment guidelines includes *Gypsum Fibrosum* (Shengshigao), *Glycyrrhiza uralensis* Fisch. ex DC. (Gancao), *Prunus armeniaca* L. (Xingren), *Ephedra sinica* Stapf (Mahuang), *Scutellaria baicalensis* Georgi (Huangqin), *Artemisia annua* L. (Qinghao), *Lonicera japonica* Thunb. (Jinyinhua), *Forsythia suspensa* (Thunb.) Vahl (Lianqiao), *Lepidium apetalum* Willd. (Tinglizi), *Anemarrhena asphodeloides* Bunge (Zhimu), *Fritillaria thunbergii* Miq. (Zhebeimu), *Pogostemon cablin* (Blanco) Benth. (Huoxiang) and *Ophiopogon japonicus* (Thunb.) Ker Gawl. (Maidong), etc. The recommended basic medical formulae for the treatment of viral pneumonia include the Ephedra, Apricot Kernel, Gypsum and Licorice Decoction (Maxingshigan Tang), which is used in the highest frequency (China Association of Chinese Medicine, 2003; Wang et al., 2011; National Health Commission of the People's Republic of China, 2020; Xi et al., 2020). For the main clinical manifestations of viral pneumonia such as fever, cough and panting, *Gypsum Fibrosum* (Shengshigao) can clear and discharge lung-heat, and vent pathogen with acrid-cool (medicinals). *Prunus armeniaca* L. (Xingren) and *Ephedra sinica* Stapf (Mahuang) can diffuse the lung, relieve cough and calm panting. *Glycyrrhiza uralensis* Fisch. ex DC. (Gancao) and *Ephedra sinica* Stapf (Mahuang) have antiviral and immune regulating effect (Mantani et al., 2001; Cinatl et al., 2003). *Lonicera japonica* Thunb. (Jinyinhua), *Forsythia suspensa* (Thunb.) Vahl (Lianqiao), *Artemisia annua* L. (Qinghao), *Pogostemon cablin* (Blanco) Benth. (Huoxiang), and *Scutellaria baicalensis* Georgi (Huangqin) are also commonly used in the recommended prescription and have been reported to possess immunoregulatory and antiviral activities (Efferth et al., 2008; Duan et al., 2012; Shen et al., 2012; Liu F. et al., 2016; Xu et al., 2019).

The role of Chinese herbal medicine in antivirus is usually considered interfering the procession of virus pathogenesis to achieve anti-virus effects, such as suppressing the virus proliferation, preventing the adhesion of virus into susceptible host cells, promoting the immune response, suppressing the excessive abnormal inflammatory response and regulating the immune function of the body. These antiviral effects are often referred to as "detoxification" or "resolving toxins" in the theory of traditional Chinese medicine (Xi and Gong, 2017). In recent years, many researches and progresses have been made to understand the action and mechanism of TCM in the treatment of viral pneumonia for clinical purpose. Through the collection and analysis of nearly 20 years of literature, these actions and mechanisms were discussed from the perspective of direct and indirect antiviral effects as well as immunomodulatory effects in this report.

METHODS

All data were retrieved from the PubMed, Web of Knowledge, China National Knowledge Infrastructure (CNKI), Wanfang Database, VIP Database, China Biology Medicine disc (CBMdisc) and official websites from January 1, 2000 to August 8, 2020 (including), and collected from the TCM

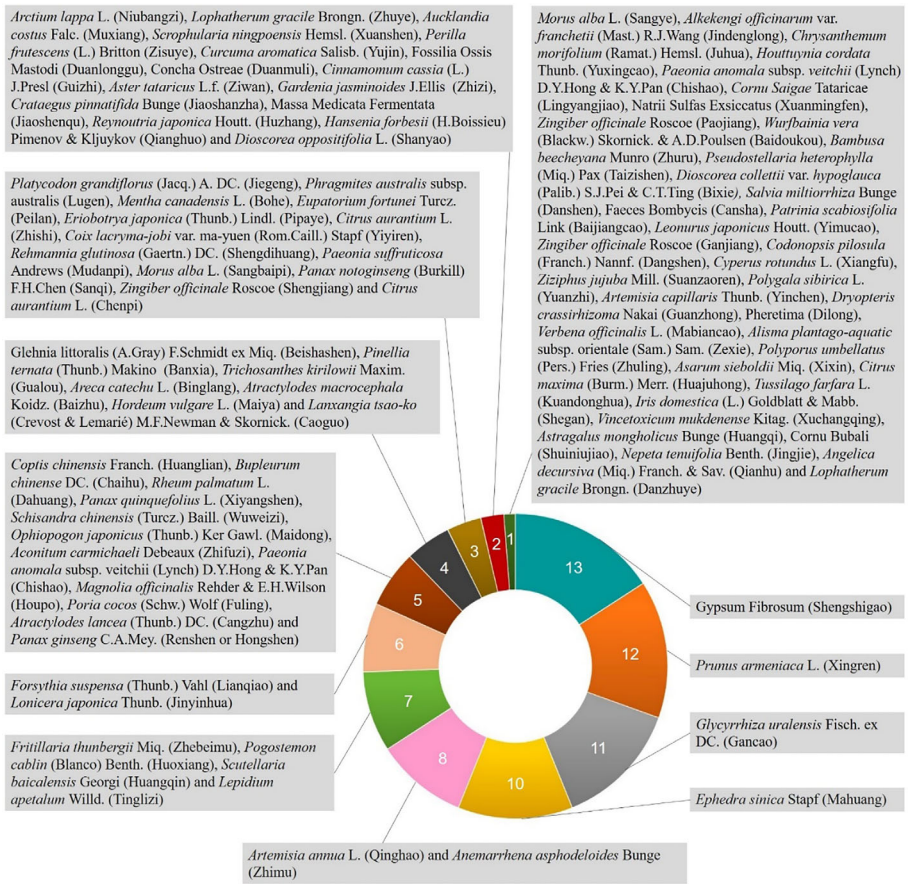

FIGURE 1 | Usage frequency of single herbal medicine in traditional Chinese medicine (TCM) part of the China National Guidelines of Diagnosis and Treatment for SARS-CoV, MERS-CoV, SARS-CoV-2, and influenza virus (The statistical data were based on the analysis of each traditional Chinese herbal medicine involved in different syndromes of viral pneumonia in China's National Guidelines for TCM treatment of SARS-CoV, MERS-CoV, SARS-CoV-2, and influenza virus).

diagnosis and treatment literatures and data related to viral pneumonia issued by the National Health Commission of China, the Health Commissions of provinces, autonomous regions and municipalities directly under the central government, the State Administration of Traditional Chinese Medicine of China and the Administration of Traditional Chinese Medicine of all provinces, as well as the opinions expressed from TCM masters, academicians and famous TCM clinical experts through open channels. These date were extracted into two tables by two independent researchers according to the inclusion and exclusion criteria after reaching a consensus. When there are differences in the process of screening and data extraction, it was submitted to the third party for joint decision. The inclusion criteria included the following: *a*. Clear literatures on the experimental and clinical research on the treatment of viral pneumonia with herbs, herbal extracts, or Chinese medical formulae. *b*. Literatures written in English and Chinese. The exclusion criteria were as follows: *a*. Literatures without control medicinals in experimental research; *b*. Literature review, individual case report, expert experience introduction and other types of literature; *c*. For the content of the repeatedly published literature or the repeatedly quoted literature, only one article is included.

RESULTS

Experimental Research

Animal or cell studies have found that some traditional Chinese herbal medicines and medical formulas have a variety of pharmacological effects in the treatment of viral pneumonia. In addition to the direct or indirect antiviral effect (See **Tables 1** and **2**), the best advantage of TCM is the regulation of immune function and low adverse effects (Ma et al., 2013).

Inhibition/Inactivation of Virus by TCM

The antiviral activity of TCM first manifests as the inhibition or inactivation of the virus. Studies have shown that a variety of TCM can directly inactivate or prevent the virus from adsorbing or penetrating into the cells, or induce the body to produce substances such as interferon, thereby inhibiting the replication of the virus.

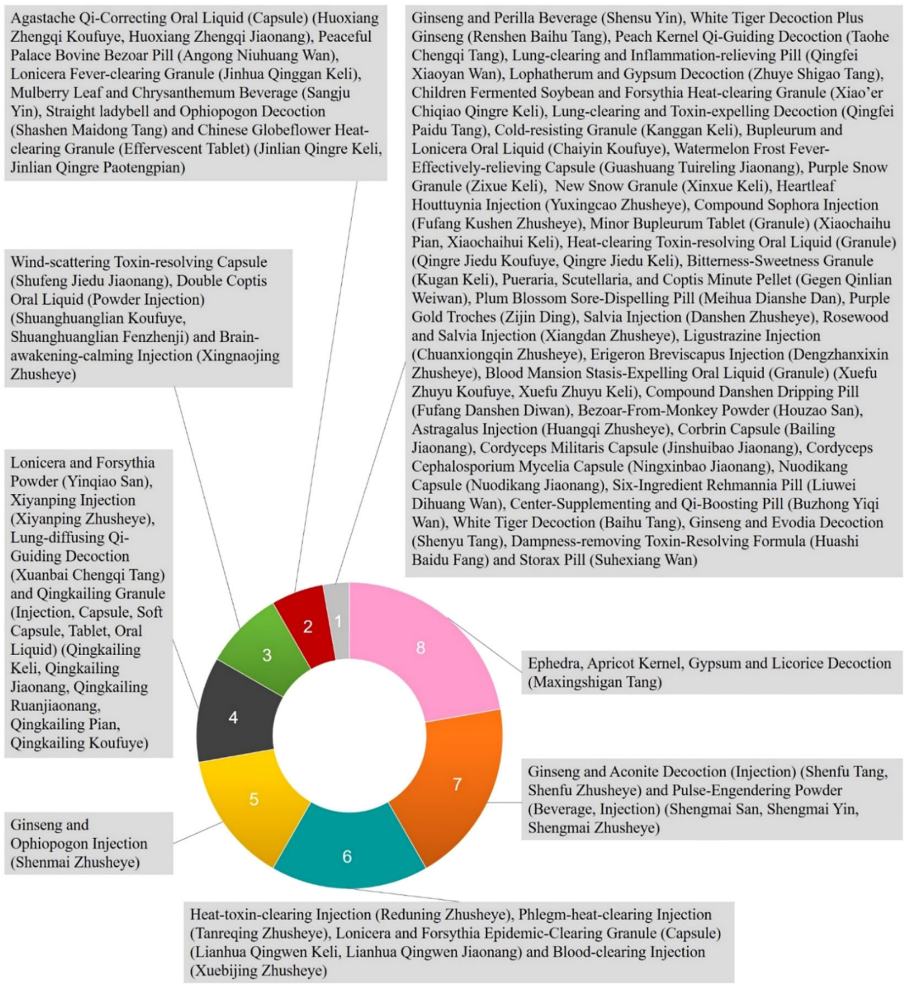

FIGURE 2 | Usage frequency of Chinese medical formulas and proprietary traditional Chinese medicine products in traditional Chinese medicine (TCM) part of the China National Guidelines of Diagnosis and Treatment for SARS-CoV, MERS-CoV, SARS-CoV-2, and influenza virus (The statistical data were based on the analysis of Chinese medical formulas and proprietary traditional Chinese medicine products involved in different syndromes of viral pneumonia in China's National Guidelines for TCM treatment of SARS-CoV, MERS-CoV, SARS-CoV-2, and influenza virus).

Direct Inhibition of Viruses

Virus first attaches to membrane of host cells and then enters cells. After dissociation of virus particle, virus will employ host cells to replicate its genes and process proteins for viral assembly and release. In view of this series of processes, the use of drugs in the pre-infection stages of the virus can play a direct inhibitory effect on the virus. Studies showed by MTT method that the volatile oil from *Cinnamomum cassia* (L.) J. Presl and Cinnamic aldehyde could significantly inhibit the proliferation of influenza A virus (H1N1) in MDCK (Madin-Darby canine kidney) cells (p<0.05) (Liu et al., 2012). Ling Gou et al. confirmed that the medicated serum containing volatile oil from *Nepeta tenuifolia* Benth. and *Cinnamomum cassia* (L.) J. Presl also could significantly inhibit the proliferation of influenza A virus in MDCK cells (p<0.05) and show a certain degree of direct killing of virus (Gou et al., 2013). Studies have shown that Glycyrrhizic acid from *Glycyrrhiza uralensis* Fisch. ex DC. not only directly inhibits the replication of coronavirus, but also acts on the early stage of virus adsorption and membrane penetration, which may be related to its activation of protein kinase C, casein kinase II and nuclear transcription factor B (Cinatl et al., 2003). Arctiin and its aglucone, arctigenin from the fruits of *Arctium lappa* L. showed potent *in vitro* antiviral activities against influenza A virus (A/NWS/33, H1N1) (IFV). Based on the data from time-of-addition experiments and on release tests of progeny viruses, arctigenin was assumed to interfere the early event(s) of viral replication after viral penetration into cells, and to suppress the release of progeny viruses from the host cells (p<0.01 or p<0.001) (Hayashi et al., 2010). The classical Chinese medical formula, Pueraria Decoction (Gegen Tang) can play an antiviral role in the adsorption stage of virus (p<0.01), and Pueraria decoction and its antiviral activity are positively correlated with dose (Geng et al., 2019). Neuraminidase (NA) can be another target molecule

TABLE 1 | Effects and mechanisms of single Chinese medicine and its components on antiviral pneumonia.

Name of Herb	Components contained/ product	Model/Strains	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_{50}, CD_{50} *	Control	Actions and mechanisms	References
Rhus chinensis Mill. (Wubeizi)	Ethyl acetate extract: acyl pentagalic acid glucose, ellagic acid and gallic acid	*In vitro*: influenza virus neuraminidase (NA)	10, 25, 50, 75, 100 mg/L	62.81 mg/L (IC_{50})	Oseltamivir	Inhibits neuraminidase activity, restrain influenza virus A/PR/8/34 (H1N1), inhibits the activation of TLR4 and the downstream MyD88 dependent transduction pathway, reduces the transcription of inflammatory factors, and alleviates lung inflammation and slow down the process of acute lung injury.	Yang et al., 2017; Zhang et al., 2018
	Ethanol extract: acyl pentagalic acid glucose, ellagic acid and gallic acid			58.31 mg/L (IC_{50})			
	Gallic acid	*In vitro*: A/California/07/ 2009 (H1N1) in MDCK	2.5 μg/ml	2.6 ± 0.07 μg/ml (EC_{50})	Amantadine		
Osmunda japonica Thunb. (Ziqiguanzhong)	Isoginkgetin	*In vitro*: A/Califomia/04/ 2009 (H1N1)	50 mg/L	42.54 ± 2.85 μmol/L (IC_{50})	Oseltamivir	Inhibits neuraminidase activity, and plays an anti-inflammatory role through IκB and c-JUN pathway.	Lang et al., 2019; Li et al., 2019
Nepeta tenuifolia Benth. (Jingjie)	Volatile oil of Nepeta tenuifolia	In vitro: A/PR/8/34 (H1N1) in MDCK	3.1×10^{-3}-0.10 mg/ml	3.1×10^{-3} mg/ml (IC_{50}); 0.20 mg/ml (TC_{50}); 0.10 mg/ml (TC_0)	Ribavirin	Inhibits and kills influenza virus, increases the content of IFN-α, TLR7, IFN-β and IL-2 and inhibits the secretion of IL-6 and TNF-α in the serum of mice, and restrains the protein expression of Myd88 and TRAF6.	Xie X. H. et al., 2007; He et al., 2012; Gou et al., 2013; He et al., 2013
	Pulegone		1.3×10^{-2}-0.10 mg/ml	7.2×10^{-3} mg/ml (IC_{50}); 0.36 mg/ml (TC_{50}); 0.10 mg/ml (TC_0)			
	Menthone		7.8×10^{-3}-0.10 mg/ml	1.9×10^{-3} mg/ml (IC_{50}); 0.43 mg/ml (TC_{50}); 0.25 mg/ml (TC_0)			
Arctium lappa L. (Niubangzi)	Arctigenin	*In vitro*: A/FM1/1/47 (H1N1) in MDCK	500, 125, 31.25, 7.81 mg/ml	31.25 mg/ml (IC_{50})	Ribavirin	Inhibits the release of progeny viruses from the host cells and induces interferon *in vivo*; and improves the protein expression of IFN to regulate human immune function.	Tsou, 2007; Fu et al., 2008; Hayashi et al., 2010
Ilex asprella (Hook. et Arn.) Champ. ex Benth. (Gangmei)	Water extraction of Ilex asprella root	*In vitro*: A/FM1/1/47 (H1N1) in MDCK	50, 12.5, 3.12, 0.78 mg/ml	23.04 mg/ml (TC_{50})	Ribavirin	Inhibits influenza virus type A FM1 strain, RSV and parainfluenza virus type 3, increases the percentage of CD3+ in peripheral blood T lymphocyte subsets, and regulates the ratio of CD4/CD8 to enhance the immune function of mice.	Chen et al., 2016
	Water extraction of Ilex asprella stem			10.82 mg/ml (TC_{50})			
Reynoutria japonica Houtt. (Huzhang)	Resveratrol	*In vitro*: A/PR/8/34 (H1N1) in MDCK In vitro: A/Guangdong/ 243/72 (H3N2) in MDCK	31.25–1,000 mg/L	129.8 μmol/L (IC_{50}); 5.9 μmol/L (EC_{50}) 144.7 μmol/L (IC_{50})	Zanamivir	Inhibits neuraminidase activity, reduces lung index, alleviates pulmonary edema, reduces inflammatory factors TNF-α, IL-1β, increases PI3K protein expression, and reduces the protein expression of p-Akt, caspase-3 and NF-κB.	Chen et al., 2012; Li M. et al., 2020
	Catechin-3-O-gallate	*In vitro*: A/PR/8/34 (H1N1) in MDCK *In vitro*: A/Guangdong/ 243/72 (H3N2) in MDCK		21.3 μmol/L (IC_{50}); 0.9 μmol/L (EC_{50}) 21.5 μmol/L (IC_{50})			
Isatis tinctoria L. (Banlangen)	Isatis root polysaccharide (IRP) Acid Isatis root polysaccharide (IRPA) Neutral Isatis root polysaccharide (IRPN)	*In vitro*: A/Califomia/04/ 2009 (H1N1), A/Anhui/1/ 2005 (H5N1) in Fluorescein-n-acetylneuraminic acid solution	0.5-35 mg/ml	H1N1: 24.13 mg/ml (IC_{50}); H5N1: 22.91 mg/ml (IC_{50}) H1N1: 5.88 mg/ml (IC_{50}); H5N1: 4.12 mg/ml (IC_{50}) H1N1: 52.34 mg/ml (IC_{50}); H5N1: 49.39 mg/ml (IC_{50})	Oseltamivir, apigenin	Inhibits neuraminidase activity in influenza virus H1N1 and H5N1, and decrease the protein expression of IFN-β by downregulating the expression of TLR3, TBK1 and p-IRF3 in RAW264.7 cells infected by respiratory syncytial virus (RSV), attenuates inflammation, and decreases H1N1 viral replications in lungs, reduces the protein expression of mitofusin-2	Li et al., 2009; Li J, 2013; Hou et al., 2017; Luo et al., 2019
	Isatis root polysaccharide (IRP)	*In vitro*: A/Califomia/04/ 2009 (H1N1), A/Anhui/1/ 2005 (H5N1) in		H1N1: 25.66 mg/ml (IC_{50}); H5N1: 23.10 mg/ml (IC_{50})			

(Continued)

TABLE 1 | Continued

Name of Herb	Components contained/ product	Model/Strains	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_{50}, CD_{50} *	Control	Actions and mechanisms	References
	Acid Isatis root polysaccharide (IRPA) Neutral Isatis root polysaccharide (IRPN)	Fluorescein-4,7-dimethoxy-n-acetylneuraminic acid solution		H1N1: 6.09 mg/ml (IC_{50}); H5N1: 5.08 mg/ml (IC_{50}) H1N1: 55.19 mg/ml (IC_{50}); H5N1: 52.16 mg/ml (IC_{50})		(MFN2) to reduce the susceptibility to influenza virus *via* mitochondrial antiviral signaling.	
Gardenia jasminoides J. Ellis (Zhizi)	Geniposide, Gardenia extract ZG	*In vitro*: A3/Guifang/81/23 (H3N2) in A549	6.25–1,600 μg/ml	50-60 μg/ml (IC_{50})	Ribavirin	Decreases the protein expression of IL-6, TNFα, TLR3 and NF-κB and mRNA expression of TLR7/MyD88 and TRIF in lung tissue of H3N2-infected mice, and improve cell membrane fluidity.	Guo et al., 2007; Zhang and Yu, 2010; Wang Y. F. et al., 2020
Hypericum perforatum L. (Guanyelianqiao)	Hypericin, hyperoside	In vitro: 2337/A/Gansuchengguan/1771/2006 (H1N1) in MDCK	100, 50, 25, 12.5 μg/ml	$10^{-4.90}$/0.1 ml ($TCID_{50}$); 200 μg/ml (TC_0)	Oseltamivir	Increases the function of T and B lymphocyte conversion, phagocytic function of macrophages and NK killing activity of influenza virus-infected mice, decreases the content of IL-6 and TNF-α and increases the protein expression of IFN-γ and IL-10 in lung tissue and serum of mice.	Wang et al., 2009; Xu et al., 2016
Andrographis paniculata (Burm.f.) Nees (Chuanxinlian)	Water extraction of Andrographis paniculata	*In vitro*: A/FM1/1/47 (H1N1) in MDCK	250, 62.5, 15.63, 3.9, 0.98, 0.24 mg/ml	$10^{-3.50}$/0.1 ml ($TCID_{50}$); 250 mg/ml (TC_0)	Ribavirin	Increases the percentage of CD3+ in peripheral blood T lymphocyte subsets, regulates the ratio of CD4/CD8, enhances the immune function of mice, and shows antiviral activity against H5N1 virus.	Sornpet et al., 2017; Wang et al., 2019
	Ethanol extraction of Andrographis paniculata	*In vitro*: influenza virus A/Chicken/Thailand/CUK2/04 (H5N1) in MDCK	8.2 g/ml	8.2 μg/ml (CD_{50})	–		
	Water extraction of Andrographis paniculata			380.3 μg/ml (CD_{50})			
Lonicera japonica Thunb. (Jinyinhua)	Chlorogenic acid, caffeic acid	In vitro: influenza A (H3N2) in MDCK	5 mg/ml	236.28 ± 15.37 μg/ml (IC_{50})	Ribavirin	Inhibits the influenza virus proliferation and neuraminidase activity, increases the content of IFN-γ in serum, and decrease the lung index.	Shen et al., 2012; Zhu et al., 2018; Zhao et al., 2020
		In vitro: Influenza A (H1N1) in MDCK		290.50 ± 34.82 μg/ml (IC_{50})			
Scutellaria baicalensis Georgi (Huangqin)	Baicalin	In vitro: A/FM1/1/47 (H1N1) in MDCK/A549	20, 30, 40, 60, 80 μg/ml (in MDCK); 5, 10, 20, 30, 40 μg/ml (in A549)	43.3/40.3 μg/ml (EC_{50})	Ribavirin	Inhibits neuraminidase activity, reduces virus replication, and decreases the protein expression of TLR3 and NF-κB and mRNA expression of TRIF, the protein and gene expression of proinflammatory cytokines TNF-α, IL-1 and IL-6 in lung tissue, and increases the protein and gene expression of anti-inflammatory cytokine IL-10 and antiviral factor IFN-γ in lung tissue after infection.	Ding et al., 2014; Wang et al., 2014; Zhang and Yu, 2010; Xu et al., 2019
		In vitro: A/Beijing/32/92 (H3N2) in MDCK or A549		104.9/100.1 μg/ml (EC_{50})			
		In vivo: A/FM1/1/47 (H1N1) in mice	50, 100, 200 mg/kg/day	52.3 μmol/L (IC_{50})			
		In vivo: A/Beijing/32/92 (H3N2) in mice		85.8 μmol/L (IC_{50})			
Coptis chinensis Franch. (Huanglian) and *Magnolia officinalis* Rehder & E.H.Wilson (Houpo)	Berberine	In vitro: influenza virus neuraminidase (NA)	32, 16, 8.0, 4.0, 2.0, 1.0, 0.5 mg/ml	21.10 mg/ml (IC_{50})	Oseltamivir	Inhibits neuraminidase activity, restrain influenza A (H1N1) virus, reduce the lung index of infected mice and ameliorates the lung pathological changes, suppresses the viral infection-induced up-regulation of TLR7 signaling pathway, such as TLR7, MyD88, and NF-κB (p65), at both the mRNA and protein levels, and inhibits the viral infection-induced	Wu et al., 2014; Chen et al., 2017; Enkhtaivan et al., 2018; Yan et al., 2018
	Berberine: magnolol (1:5)			19.09 mg/ml (IC_{50})			
	Berberine: magnolol (2:5)			15.39 mg/ml (IC_{50})			
	Berberine: magnolol (2:3)			3.80 mg/ml (IC_{50})			
	Berberine: magnolol (1:1)			3.53 mg/ml (IC_{50})			
	Berberine: magnolol (3:2)			13.66 mg/ml (IC_{50})			
	Berberine: magnolol (5:2)			8.90 mg/ml (IC_{50})			
	Berberine: magnolol (5:1)			19.04 mg/ml (IC_{50})			

(Continued)

TABLE 1 | Continued

Name of Herb	Components contained/ product	Model/Strains	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_{50}, CD_{50} *	Control	Actions and mechanisms	References
	Magnolol			19.37 mg/ml (IC_{50})		increase in Th1/Th2 and Th17/Treg ratios as	
	Berberine: magnolol (1:1)	*In vivo*: A/PR/8/34 (H1N1) in mice	8 g/kg	$10^{-2.5}$/100 μl (LD_{50})	Ribavirin	well as the production of inflammatory cytokines.	
Ephedra sinica Stapf (Mahuang)	(+) - Catechin	In vitro: A/California/07/ 2009 (H1N1) in MDCK	25 μg/ml	18.4 ± 0.7 μg/ml (EC_{50})	Amantadine	Suppresses the proliferation of influenza virus H1N1 and neuraminidase activity, and inhibits	Mantani et al., 2001; You et al., 2018;
	Water extract of Ephedra sinica	*In vitro*: respiratory syncytial virus (RSV) in Hela	5.00, 4.00, 3.20, 2.56 mg/ml	3.74 mg/ml (EC_{50})	Ribavirin	the adsorption and penetration of respiratory syncytial virus.	Zhu, 2008
Forsythia suspensa (Thunb.) Vahl (Lianqiao)	Ethanol extract: Forsyshiyanins A-B,	*In vitro*: A/PR/8/34 (H1N1) in MDCK	10 μM	18.4-26.2 μM (IC_{50})	Ginkgolide B	Forsyshiyanins A-B inhibit NP gene expression of influenza A virus after transfection.	Duan et al., 2012; Law et al., 2017;
	Forsythiaside (Phillyrin)	*In vitro*: respiratory syncytial virus (RSV) Long in Hep-2		10.5-14.4 μM (EC_{50})		Forsythoside A reduces the viral titers of different influenza virus subtypes in cell cultures and increases the survival rate of the mice in an *in vivo* influenza virus infection model, and reduces the influenza M1 protein, which in turn intervenes the budding process of the newly formed virions.	Zhao et al., 2020
Morus alba L. (Sangbaipi)	Cortex mori polysaccharide, total flavonoids of Cortex mori	*In vitro*: respiratory syncytial virus (RSV) Long in Hep-2	–	$10^{-2.25}$/100 μl ($TCID_{50}$)	Ribavirin	Inhibits respiratory syncytial virus, reduces the infiltration of inflammatory cells in alveolar wall to ameliorate the inflammatory status of lung tissue, promotes cell immune adjustment in mice infected by respiratory syncytial virus, decreases the protein expression of PI3K, Akt1/ 2 and NF-κBp65 in lung tissue of mice as well as the IL-4 and INF-γ in serum.	Dong et al., 2016a; Dong et al., 2016b; Liu, 2016
	Cortex mori polysaccharide	*In vivo*: respiratory syncytial virus (RSV) Long in mice	91 mg/kg/ day	$10^{-1.92}$/100 μl (LD_{50})			
	Total flavonoids of Cortex mori		114 mg/kg/ day				
Houttuynia cordata Thunb. (Yuxingcao)	Quercetin, isoquercetin	*In vitro*: Influenza A (H3N2) in MDCK	5 mg/ml	428.97 ± 38.54 μg/ml (IC_{50})	Ribavirin	*Houttuynia cordata* Thunb. extract, quercetin and cinanserin inhibit the activity of murine coronavirus and the protein expression of NF-κB, and restrains the replication of influenza A virus *in vitro*. Quercitrin inhibites both viral replication and TLR signaling in cells. Flavonoids from Houttuynia cordata attenuate H1N1-induced acute lung injury in mice *via* inhibition of influenza virus and Toll-like receptor signaling.	Chiow et al., 2016; Zhu et al., 2018; Ling et al., 2020
		In vitro: Influenza A (H1N1) in MDCK		522.28 ± 36.48 μg/ml (IC_{50})			
	Ethyl acetate (EA) fraction of *Houttuynia cordata* Thunb.	*In vitro*: Murine coronavirus	0.24-3.91 mg/ml	0.98 μg/ml (IC_{50})	Rutin		
	Quercetin		15.63-500.00 mg/ml	125.00 μg/ml (IC_{50})			
	Cinanserin (1 dpi)Cinanserin (2 dpi)		3.91-125.00 mg/ml	31.25 μg/ml (IC_{50})62.50 μg/ml (IC_{50})			
Cinnamomum cassia (L.) J.Presl (Guizhi)	Cinnamic aldehyde	*In vitro*: A/PR/8/34 (H1N1) in MDCK	0.132, 0.264 mg/kg	5.31×10^{-5} mg/ml (IC_{50})	Ribavirin	Inhibits the proliferation of influenza A virus (H1N1) in MDCK cells, restrains the infection by interfering with endocytosis, kills influenza virus, and increases the content of IFN-α and IFN-β in the serum of H1N1-infected mice.	Liu et al., 2012; Gou et al., 2013; Liu et al., 2013; Zhuang et al., 2009
	Volatile oil of Cassia twig		0.174, 0.348 mg/kg	5.80×10^{-5} mg/ml (IC_{50})			
	Cinnamomi Cortex extract	*In vitro*: wild-type SARS-CoV in Vero E6 cells	0.1, 0.2, 0.3 mg/ml	43.1± 2.8 μg/ml (IC_{50})	–		
	Ethanol extract of Cinnamomi Cortex			10.7± 0.4 μg/ml (IC_{50})			
				7.8± 0.3 μg/ml (IC_{50})			

(Continued)

TABLE 1 | Continued

Name of Herb	Components contained/ product	Model/Strains	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_{50}, CD_{50} *	Control	Actions and mechanisms	References
	Butanol fraction of Cinnamomi Cortex						
	Aqueous fraction of Cinnamomi Cortex			39.7± 2.1 μg/ml (IC_{50})			
Syzygium aromaticum (L.) Merr. & L.M.Perry (Dingxiang)	Methanol extraction of Clove	*In vitro*: A/PR/8/34 (H1N1) in MDCK	30, 10, 10/3, 10/9,	9.1 μg/ml (IC_{50})	Zanamivir, ribavirin	Inhibits neuraminidase activity, restrains the infection by interfering with endocytosis, increases the levels of IFN-γ and IL-10 in serum, decreases the levels of TNF-α and IL-6, alleviates the pathological changes of lung tissue and decreases the lung index of mice infected by A/swine/Tianjing/14/2009(H1N1).	Zhuang et al., 2009; Li et al., 2013; Liu et al., 2018
	Eugeniin		10/27, 10/81 μg/ml	7.85 μg/ml (IC_{50})			
	CFE (Caryophylli Flos extract)	*In vitro*: wild-type SARS-CoV in Vero E6 cells	0.1, 0.2, 0.3 mg/ml	50.1± 3.5 μg/ml (IC_{50})	–		
Cibotium barometz (L.) J.Sm. (Gouji)	Ethanol extraction of Cibotium barometz	In vitro: SARS-CoV in Vero E6 cells	200, 100, 50, 25 μg/ml	8.42 μg/ml (EC_{50})	Valinomycin	Inhibits viral replication and the enzymatic activity of SARS-CoV 3CL protease, and inhibit respiratory syncytial virus induced cytopathic effect *in vitro*.	Li et al., 2008; Wen et al., 2011
	Methanol extraction of Cibotium barometz			>10 μg/ml (EC_{50})			
Taxillus chinensis (DC.) Danser (Sangjisheng)	N-hexane extraction of Taxillus chinensis			5.39 μg/ml (EC_{50})			
Dioscorea oppositifolia L. (Shanyao)	Methanol extraction of Dioscorea batatas			8.06 μg/ml (EC_{50})			
Senna tora (L.) Roxb. (Juemingzi)	N-hexane extraction of Cassia tora			8.43 μg/ml (EC_{50})			
Gentiana scabra Bunge (Longdan)	N-hexane extraction of Gentiana scabra			8.70 μg/ml (EC_{50})			
	Active compound RG2-1 from Gentiana scabra	*In vitro*: respiratory syncytial virus (RSV) Long in Hela cells	50, 25, 12.5, 6.25 mg/ml	11.07 mg/ml (TC_{50}); 0.42 mg/ml (EC_{50})	Ribavirin		
Glycyrrhiza uralensis Fisch. ex DC. (Gancao)	Glycyrrhizic acid	In vitro: SARS-associated coronavirus in Vero cells	1,000, 4,000 mg/ml	After virus adsorption: 600 mg/L (EC_{50}); during and after virus adsorption: 300 mg/L (EC_{50}); during virus adsorption: 2,400 mg/L (EC_{50})	Mycophenolic acid, pyrazofurin and ribavirin	Inhibits virus replication, adsorption and membrane penetration, up-regulates the mRNA expression of IFN-γ and its immunomodulatory function against influenza virus infection, down-regulates the mRNA expression of TNF-α, and decreases the inflammatory reaction induced by TNF-α to reduce the host immune damage.	Cinatl et al., 2003; Xie Z. P. et al., 2007; Liu et al., 2006; Fu X. L. et al., 2020
	Active compound (GC3-1-4)	*In vitro*: Parainfluenza virus (type III) in Hela	25, 20, 16,12.8, 10.2 μg/ml	12.82 μg/ml (EC_{50}); 144.17 μg/ml (TC_{50})	Ribavirin		
		In vitro: respiratory syncytial virus (RSV) in Hela	100, 75, 56, 42, 32, 24 μg/ml	41.32 μg/ml (EC_{50}); 0.45 mg/ml (TC_{50})			
Rheum officinale Baill. (Dahuang)	Emodin (1,3,8-trihydroxy-6-methylanthraquinone), aqueous extract of Radixet Rhizoma Rhei	*In vitro*: SARS-CoV in Vero E6 cells	0.1, 0.5, 1, 5, 10, 50, 100 μg/ml	200 μM (IC_{50})	Promazine	Blocks the S protein and ACE2 interaction in a dose-dependent manner and also inhibits the infectivity of S protein-pseudotyped retrovirus to Vero E6 cells.	Ho et al., 2007

*****IC_{50}, *50% inhibiting concentration;* EC_{50}, *50% maximal effective concentration;* $TCID_{50}$, *50% tissue culture infectious dose;* TC_{50}, *median toxic concentration.* $TC_{O,}$ *Maximum nontoxic concentration;* LD_{50}, *median lethal dose;* CD_{50}, *50% cytotoxicity dose.*

for antiviral effect. Neuraminidase is a mushroom cloud tetramer glycoprotein located on the envelope of influenza virus and involved in virus release and spread. Jiawei Liu et al. used chromatographic separation technology to screen and isolate Eugeniin, an effective compound from *Syzygium aromaticum* (L.) Merr. & L.M.Perry, showing that it could inhibit neuraminidase activity of H1N1 *in vitro* (Liu et al., 2018). Studies by Kaotan Chen et al. showed that resveratrol, (E)-3,5,12-trihydroxystilbene-3-O-β-D-glucopy-ranoside-2'-(3″,4″,5″-trihydroxybenzoate) and Catechin-3-O-gallate, three extracts of *Reynoutria japonica* Houtt., could effectively inhibit neuraminidase activity (Chen et al., 2012). Baicalin is a flavonoid in *Scutellaria baicalensis* Georgi. Studies by Yue Ding et al. have confirmed that baicalin can significantly inhibit the neuraminidase activity of influenza A (H1N1) virus (p<0.05 or p<0.01) (Ding et al., 2014). Han-Bing Li et al. found that acidic sugars in Isatis tinctoria L. had higher inhibitory activity of neuraminidase than neutral sugars and total sugars. Moreover, acidic sugars were slightly stronger to inhibit activity of neuraminidase in H5N1 influenza virus than that in H1N1 virus (p<0.05) (Li et al., 2009). Xianying Yang et al. used UPLC-Q-TOF-MS (Ultra-high performance liquid chromatography coupled with a four-pole time-of-flight mass spectrometer) to detect the neuraminidase inhibitory activity of *Rhus chinensis* Mill., and found that ethyl acetate, ethanol extract, acyl-pentagalic glucose, ellagic acid and gallic acid from Wubeizi exhibit different levels of neuraminidase inhibitory activity (Yang et al., 2017). Studies by Shuang Lang et al. showed that the compound Iso-ginkgo biloba diflavone extracted from *Osmunda japonica* Thunb. has a significant inhibitory effect on the neuraminidase activity of influenza virus (Lang et al., 2019). Gao Chen et al. evaluated the ratio of combination of two Chinese medicines of *Coptis chinensis* Franch. and *Magnolia officinalis* Rehder & E.H. Wilson and found that the best inhibitory effect on neuraminidase was at 1:1 ratio (Chen et al., 2017). Traditional Chinese medical formulae and proprietary traditional Chinese medicine products such as Ephedra, Apricot Kernel, Gypsum and Licorice Decoction (Maxingshigan Tang), medicated serum of Folium Ginkgo and Ephedra Lung-clearing Capsule (Yinhuang Qingfei Jiaonang), as well as Heat-toxin-clearing Injection (Reduning Zhusheye) also exhibit inhibitory effect of neuraminidase (p<0.05 or p<0.01) (Hsieh et al., 2012; Sun et al., 2014; Li et al., 2015).

Indirect Inhibition of Viruses

Interferon is a glycoprotein produced by cells stimulated by viruses or other interferon inducers. After binding to interferon receptors, it can induce cells to produce antiviral proteins with enzyme activity, such as protein kinase and 2',5'-adenosine kinase (Der and Lau, 1995; Min and Krug, 2006), thereby inhibiting viral replication. Studies by Rong Liu et al. showed that the volatile oil and cinnamaldehyde contained in *Cinnamomum cassia* (L.) J.Presl could increase the content of IFN-α and IFN-β in serum of mice with viral pneumonia (p<0.05) (Liu et al., 2013). Ting He et al. documented that *Nepeta tenuifolia* Benth. can increase the levels of IFN-α, IFN-β and IL-2 in virus-infected mice (p<0.05 or p<0.01) (He et al., 2013). Hongri Xu et al. showed that *Scutellaria baicalensis* Georgi could increase the expression of the antiviral factor IFN-γ in lung tissue (p<0.05) (Xu et al., 2019). Cheng-Chuan Tsou pointed out that the antiviral effect of *Arctium lappa* L. was related to its ability to induce the organism to produce interferon (p<0.05 or p<0.01) (Tsou, 2007).

Regulatory Effects on Immune and Cellular Inflammatory Factors

Excessive immune response and release of inflammatory cytokines are important causes of viral pneumonia and lung injury. In patients infected with SARS, Influenza A (H1N1) virus and SARS-CoV-2 (Li et al., 2012; Casadevall and Pirofski, 2014; Huang et al., 2020), abnormally-elevated inflammatory cytokines so called cytokine storm can be detected and are closely related to disease severity. Therefore, inhibiting the overexpression of inflammatory factors and improving immune function have become important part in the treatment of viral pneumonia.

Regulation of the TLR-NF-κB Signaling Pathway

The TLR-NF-κB signaling pathway is an important pathway that mediates the expression of inflammatory factors. Toll-like receptor (TLR) is a transmembrane protein located on the cell membrane, which is composed of extracellular region, transmembrane region and intracellular region. At present, there are three kinds of TLR3, TLR7 and TLR8 which are closely related to the virus. These three receptors plus TLR9 have functional domain inside the cell, while the rest of the receptors are expressed outside the cell. Whether the different distribution of this functional domain is related to its antiviral effect needs further study. After virus invasion, both TLR3-mediated MyD88 independent signaling and TLR7-mediated MyD88-dependent signaling ultimately activate the nuclear transcription factor NF-κB, which induces and promotes the expression of preinflammatory factors (Sanjeewa et al., 2020). Yongfeng Wang et al. and Li Wang et al. established a mouse model of influenza viral pneumonia and found that gardenin from *Gardenia jasminoides* J.Ellis and baicalin from *Scutellaria baicalensis* Georgi could significantly reduce the expression of IL-6, TNF-α, TLR3 and TRIF mRNA in the lung tissue of mice (p<0.01) (Wang et al., 2014; Wang Y. F. et al., 2020). At the same time, studies have shown that gardenin and baicalin can also inhibit the TLR7/MyD88 pathway to reduce NF-κB activation (p<0.05 or p<0.01) (Zhang and Yu, 2010; Wan et al., 2014). Yuhuan Xie et al. found that essential oil of *Nepeta tenuifolia* Benth. can inhibit TRAF6 protein expression in the lung tissue of mice, and have a certain inhibitory effect on MyD88 to achieve anti-influenza virus pneumonia (p<0.05) (Xie Y. H. et al., 2007). Chinese medical formulas Sweet Wormwood and Scutellaria Gallbladder-Clearing Decoction (Haoqin Qingdan Tang), Pueraria Decoction (Gegen Tang), Wind-scatering and Lung-diffusing Formula Granule (Shufeng Xuanfei Fang Keli), Exterior-releasing and Interior-clearing Formula Granule (Jiebiao Qingli Fang Keli), and Lonicera, Forsythia, Bupleurum and Cinnamon Twig Formula II (Yinqiaochaigui Erhao Fang) all

could inhibited expression of TLR7, MyD88, NF-κB and decreased serum TNF-α, IL-1 and IL-6 levels ($p<0.05$ or $p<0.01$) (Lai et al., 2011; Liu et al., 2014; Li et al., 2018; Geng et al., 2019).

Regulation of the PI3K/Akt Signaling Pathway

The PI3K/Akt signaling pathway can also activate the nuclear transcription factor NF-κB, which induces the expression of inflammatory factors (Harikrishnan et al., 2018). Studies have shown that resveratrol [from *Morus alba* L. (Sangshen), *Reynoutria japonica* Houtt. (Huzhang), *Veratrum nigrum* L. (Lilu) or *Senna tora* (L.) Roxb. (Juemingzi)] can inhibit the expression of PI3K and NF-κB in the lung tissues of infected mice ($p<0.05$) (Li M. et al., 2020). Xiaoxue Liu showed that polysaccharides and flavonoids from *Morus alba* L. could significantly reduce the expressions of PI3K, AKT1/2 and NF-κBp65, as well as IL-4 and INF-γ in serum of respiratory syncytial virus (RSV)-infected mice ($p<0.05$) (Liu, 2016). Xiaoxue Liu et al. also pointed out that Lung-clearing and Collaterals-unblocking Ointment (Qingfei Tongluo Gao) applied on the back of mice could also inhibit the expression of PI3K and NF-κB proteins in lung tissue induced by respiratory syncytial virus, thereby reducing inflammation and protecting lung tissue ($p<0.05$) (Liu X. X. et al., 2016). Shuling Nan et al. found that Ascending and Descending Powder (Shengjiang San) can reduce the excessive expression of NF-κB protein in the lung tissue, significantly improve the content of lung sIgA, IL-10, IL-IRα and sTNFR, reduce the serum IL-1β, IL-6, TNF-α content, inhibit proinflammatory factor and induce suppression of inflammatory factor expression to reduce pulmonary inflammatory injury ($p<0.05$ or $p<0.01$) (Nan et al., 2016a; Nan et al., 2016b). In addition, researchers found that hypericin and hyperoside extracted from *Hypericum perforatum* L. could reduce the expression of IL-6 and TNF-α in lung tissue and serum of mice infected with influenza A virus, and increase the expression of IFN-γ and IL-10 protein ($p<0.05$ or $p<0.01$) (Wang et al., 2009). Wei Luo et al. found that the levels of TNF-α and IL-10 in serum and lung tissues of mice infected with influenza virus were reduced by electroacupuncture and moxibustion at bilateral Feishu (BL 13) on the back of mice ($p<0.01$) (Luo et al., 2014).

Regulation of Lymphocyte Subsets

As one of the three lines of defense, cellular immunity plays an important role in eliminating pathogens. Experimental studies have shown that *Andrographis paniculata* (Burm.f.) Nees and *Ilex asprella* (Hook. et Arn.) Champ. ex Benth. can increase the percentage of CD3+ lymphocytes in the T-lymphocyte subsets in peripheral blood of mice infected with influenza virus, and regulate the CD4/CD8 ratio to enhance the immune function of mice (Chen et al., 2016; Wang et al., 2019). *Hypericum perforatum* L. extract can improve the immunologic function of influenza virus-infected mice by enhancing T and B lymphocyte conversion, phagocytic function of macrophages and NK killing activity ($p<0.05$ or $p<0.01$) (Xu et al., 2016). Gegen decoction can regulate the ratio of CD3+CD4+/CD3+CD8+ and CD4+IFN-γ+/CD4+IL-4+ in peripheral blood of virus-infected mice ($p<0.01$) (Geng et al., 2019). Shengjiang Power can increase the percentage of CD8+ in peripheral blood, regulate the radio of CD4+/CD8+, and improve the immune function of the body ($p<0.05$ or $p<0.01$) (Nan et al., 2016b). Other study results have shown that compared with ribavirin, the Haoqin Qingdan Decoction can improve the ratio of T lymphocyte subgroup and Th1/Th2 cell balance more effectively in rats with damp-heat syndrome of influenza viral pneumonia (Zhang et al., 2013).

TCM Protecting Host Cells

Some traditional Chinese medicines have been studied, which do not directly inhibit virus replication or regulate immune and inflammatory factors, but protect host cells and increase their tolerance to viruses. Shanshan Guo et al. found that the extract ZG from *Gardenia jasminoides* J. Ellis can improve the host cell membrane fluidity after infection of parainfluenza virus type 1 (PIV-1) ($p<0.01$) and maintain its normal function therefore to play an antiviral role (Guo et al., 2007).

Clinical Research

Compared with single herbs, traditional Chinese medical formulas are more widely used in the clinical prevention and treatment of viral pneumonia. Studies have shown that proprietary traditional Chinese medicine product or Chinese medical formula decoction plays a certain role in anti-inflammatory, immune regulation, inhibition of viral replication, prevention of viral cytopathic disease and improvement of pathology (See **Table 2**).

TCM for the Treatment of SARS Coronavirus Pneumonia

Pneumonia caused by SARS coronavirus is a highly infectious pneumonia that can involve multiple organ lesions. The main clinical manifestations are fever, cough, headache, fatigue, aching pain of muscle and joint, oppression in chest, and dyspnea, etc. Tietao Deng, a master of Chinese medicine, considered that it belongs to the category of spring epidemic and damp-heat pestilence diseases, which pathogeneses are accumulation of damp-heat toxin, consumption of Qi and damage of Yin easily, and existance of blood stasis. According to Tietao Deng, SARS can be divided into early, middle, extreme and recovery stages (Deng, 2003). In the process of clinical treatment, therapeutic outcomes of combined treatment with TCM and western medicine are usually better than that of Western medicine alone in terms of release of clinical symptoms, improvements of pneumonia and blood oxygen saturation as well as the count of lymphocyte and T cell subsets. For example, Ruilin Zhang et al. treated 49 SARS patients with integrated traditional Chinese and western medicine. Beside the basic treatment, patients were respectively given Formula I for SARS during Hyperpyrexia Peroid (Feidian Gaoreqi Yihao Fang) with high fever to clear heat and resolve toxins, scatter wind and diffuse the lung, Formula II for SARS during Panting Peroid (Feidian Kechuanqi Erhao Fang) with cough and panting to clear heat

TABLE 2 | Effects and mechanisms of Chinese medical formulas on antiviral pneumonia.

Name of Formulas	Ingredients	Efficacy	Usage Mode	Model/Strains (Registration number)	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_0, LD_{50}*	Control	Actions and mechanisms	References
Ephedra Decoction (Mahuang Tang)	*Ephedra sinica* Stapf (Mahuang) 9g, Cinnamomum cassia (L.) J. Presl (Guizhi) 6g, Prunus armeniaca L. (Xingren) 6g and Glycyrrhiza uralensis Fisch. ex DC. (Gancao) 3g.	Induce sweating to release the exterior, diffuse the lung and relieve panting.	Decoction	*In vitro*: influenza virus A/PR/8/34 (H1N1) in MDCK	5.0, 2.5, 1.25, 0.63, 0.31 g/L	1.59 g/L (EC_{50}); 61.66 g/L (TC_{50}); 5.66 g/L (TC_0)	Oseltamivir	Blocks the invasion of influenza virus into host cells, inhibits the biosynthesis of influenza virus in cells, and down-regulates the expression levels of TLR4, TLR7, MyD88 and TRAF6 mRNA in cells.	Wei et al., 2018
Three Substances Scutellaria Decoction (Sanwu Huangqin Tang)	*Sophora flavescens* Aiton (Kushen) 6g, *Scutellaria baicalensis* Georgi (Huangqin) 6g and *Rehmannia glutinosa* (Gaertn.) DC. (Gandihuang) 12g.	Clear heat and resolve toxins, nourish the blood and enrich *yin*.	Extract by water extraction and alcohol sedimentation	*In vitro*: influenza virus A/PR/8/34 (H1N1) in MDCK	0.06, 0.12, 0.24, 0.49, 0.98, 1.95 mg/ml	10^{-7}/100 μl ($TCID_{50}$); 12.76 mg/ml (TC_{50}); 1.95 mg/ml (TC_0)	Oseltamivir	Inhibits influenza A/PR/8/34 (H1N1) virus at different stages of viral replication *in vitro* and *in vivo*.	Ma et al., 2018
				In vivo: influenza virus A/PR/8/34 (H1N1) in mice	5.85, 11.70, 23.40 g/kg/day	$10^{-4.5}$/50 μl (LD_{50})			
Pueraria Decoction (Gegen Tang)	Pueraria montana var. lobata (Willd.) Maesen & S.M.Almeida ex Sanjappa & Predeep (Gegen) 96g, *Ephedra sinica* Stapf (Mahuang) 72g, Cinnamomum cassia (L.) J. Presl (Guizhi) 48g, Paeonia lactiflora Pall. (Baishao) 48g, Zingiber officinale Roscoe (Shengjiang) 72g, Ziziphus jujuba Mill. (Dazao) 176g and Glycyrrhiza uralensis Fisch. ex DC. (Gancao) 48g.	Induce sweating to release the exterior, promote fluid production and unblock the channels.	Water extraction of Pueraria Decoction	In vitro: influenza virus (H1N1) in MDCK	6.25 mg/ml	$1\times10^{4.7}$/100 μl ($TCID_{50}$); 1.81 mg/ml (IC_{50}); 10.88 mg/ml (TC_{50}); 6.25 mg/ml (TC_0)	Oseltamivir	Antagonizes the activity of H1N1 influenza virus, inhibits virus adsorption, restrain the expression of pro-inflammatory factors IL-1α, IL-6 and TNF-α, and downregulates TLR7 expression.	Geng et al., 2019
			Decoction	*In vivo*: influenza virus (H1N1) in mice	1 g/ml	$1\times10^{3.8}$/20 μl (LD_{50})			
Heat-toxin-clearing Injection (Reduning Zhusheye) (Patent medicine)	Artemisia annua L. (Qinghao), Lonicera japonica Thunb. (Jinyinhua) and Gardenia jasminoides J.Ellis (Zhizi).	Clear heat, scatter wind and resolve toxins.	Preparation solution of injection	*In vitro*: A/PR/8/34 (H1N1) in MDCK	400 μg/ml	46.49 ± 2.25 μg/ml (IC_{50})	Zanamivir	Inhibits neuraminidase activity of H1N1, H3N2 and B influenza, relieves symptoms such as panting, cough and short of breath, and decreases body temperature.	Sun et al., 2014; Li, 2013
				In vitro: A/Sydney/5/97 (H3N2) in MDCK		49.77 ± 1.77 μg/ml (IC_{50})			
				In vitro: B/Jiangsu/10/2003(B) in MDCK		45.33 ± 5.32 μg/ml (IC_{50})			
			Injection	Controlled study *in vivo*: children with viral pneumonia	0.5-0.6 ml/(kg.day)	–	Ribavirin		
Wind-scatering and Lung-diffusing Formula Granule (Shufeng Xuanfei Fang Keli) (Hospital preparation)	Lonicera japonica Thunb. (Jinyinhua), Forsythia suspensa (Thunb.) Vahl (Lianqiao), Persicaria tinctoria (Aiton) Spach (Daqingye), Arctium lappa L. (Niubangzi), Isatis tinctoria L. (Banlangen), Periostracum Cicadae (Chantui), Fritillaria thunbergii Miq. (Zhebeimu), Scutellaria baicalensis Georgi (Huangqin), Nepeta tenuifolia Benth. (Jingjie), Glycine max (L.) Merr. (Dandouchi), Imperata cylindrica (L.) P.Beauv.	Clear heat and resolve toxins, and vent the exterior with acrid-cool (medicinals).	Preparation solution of granule	*In vitro*: influenza virus A1/Qianfang/166/85 (H1N1) in A549	10, 5, 2.5, 1.25, 0.63 μg/ml	$10^{-3.78}$/0.1 ml ($TCID_{50}$); 10.20 μg/ml (TC_0); 38.56 μg/ml (TC_{50}); 2.36 μg/ml (IC_{50})	Oseltamivir	Reduces the mortality of mice infected with virus and prolong the average survival time of mice, and downregulates the expression of TLR3, TLR7, MyD88 and IL-6, and increases the expression of IL-4 and IFN-γ.	Liu et al., 2014; Ge et al., 2015; Zhang et al., 2015
			Granular solution	*In vivo*: influenza virus A1/Qianfang/166/85 (H1N1) in mice	3.24, 1.62, 0.81 g/kg	–			

(Continued)

TABLE 2 | Continued

Name of Formulas	Ingredients	Efficacy	Usage Mode	Model/Strains (Registration number)	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_0, LD_{50}*	Control	Actions and mechanisms	References
	(Baimaogen) and Glycyrrhiza uralensis Fisch. ex DC. (Gancao).								
Exterior-releasing and Interior-clearing Formula Granule (Jiebiao Qingli Fang Keli) (Hospital preparation)	Ephedra sinica Stapf (Mahuang), Perilla frutescens (L.) Britton (Zisuye), Nepeta tenuifolia Benth. (Jingjie), Angelica biserrata (R.H.Shan & C.Q.Yuan) C.Q.Yuan & R.H.Shan (Duhuo), Hansenia forbesii (H.Boissieu) Pimenov & Kljuykov (Qianghuo), Gypsum Fibrosum (Shigao), Artemisia annua L. (Qinghao), Scutellaria baicalensis Georgi (Huangqin), Aster tataricus L.f. (Ziwan), Prunus armeniaca L. (Xingren), Platycodon grandiflorus (Jacq.) A. DC. (Jiegeng) and Glycyrrhiza uralensis Fisch. ex DC. (Gancao).	Release the exterior and clear the interior.	Granular solution	*In vitro*: influenza virus A1/Qianfang/166/85 (H1N1) in A549 *In vivo*: influenza virus A1/Qianfang/166/85 (H1N1) in mice	10, 5, 2.5, 1.25, 0.63 μg/ml 3.82, 1.91, 0.96 g/kg	$10^{-3.78}$/0.1 ml ($TCID_{50}$); 9.91 μg/ml (TC_0); 38.88 μg/ml (TC_{50}); 2.46 μg/ml (IC_{50}) –	Oseltamivir	Downregulates the protein expression of TLR7 and NF-κB, prolongs the average survival time of mice, decreases the mRNA and protein over-expressions of IL-1, TNF-α, IL-6, MCP-1, reduces inflammation, and restores stability and balance of immune function.	Liu et al., 2014; Ge et al., 2015; Zhang et al., 2015
Lonicera, Forsythia, Bupleurum and Cinnamon Twig Granule (Yinqiaochaigui Keli) (Hospital preparation)	Lonicera japonica Thunb. (Jinyinhua), Forsythia suspensa (Thunb.) Vahl (Lianqiao), Bupleurum chinense DC. (Chaihu), Cinnamomum cassia (L.) J.Presl (Guizhi), Platycodon grandiflorus (Jacq.) A. DC. (Jiegeng), Paeonia lactiflora Pall. (Baishao), Scutellaria baicalensis Georgi (Huangqin), Ephedra sinica Stapf (Mahuang), *Paris polyphylla* var. chinensis (Franch.) H.Hara (Chonglou) and Scrophularia ningpoensis Hemsl. (Xuanshen).	Harmonize ying and wei levels, release the exterior and clear heat toxin.	Granular solution diluted in DMEM	*In vitro*: influenza virus H1N1 (FM1) in MDCK *In vitro*: influenza virus H1N1 (PR8) in MDCK *In vitro*: influenza virus A III in MDCK	5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039 mg/ml	2.18 mg/ml (TC_{50}); 0.52 mg/ml (IC_{50}) 3.88 mg/ml (TC_{50}); 1.08 mg/ml (IC_{50}) 3.39 mg/ml (TC_{50}); >5 mg/ml (IC_{50})	Ribavirin	Decreases the content of TNF-α, IL-6 in BALF, increases the content of SOD in lung homogenate, decreases the content of MDA, and increases the ratio of T lymphocyte subsets, and inhibits TLR7-MyD88-NF-κB signaling pathway.	Xu, 2014; Li et al., 2018
Sweet Wormwood and Scutellaria Gallbladder-Clearing Decoction (Haoqin Qingdan Tang)	Artemisia annua L. (Qinghao) 6g, Scutellaria baicalensis Georgi (Huangqin) 6g, Citrus aurantium L. (Zhiqiao) 5g, Bambusa tuldoides Munro (Zhuru) 9g, Citrus aurantium L. (Chenpi) 5g, Pinellia ternata (Thunb.) Makino (Banxia) 5g, Poria cocos (Schw.) Wolf (Fuling) 9g, Talcum (Huashi) 6g, Glycyrrhiza uralensis Fisch. ex DC. (Gancao) 1g and Isatis tinctoria L. (Qingdai) 2g.	Clear gallbladder heat and drain dampness, dissolve phlegm and harmonize the stomach.	Preparation solution of decoction Decoction	*In vitro*: influenza virus A1/Jingke 96-25, A3/Jingke 92-32, B/Jingfang 93-184 in allantoic cavity of chicken embryo *In vivo*: influenza virus (H1N1) in mice Randomized controlled study: patients with influenza viral pneumonia	0.22, 0.12, 0.06 g/ml 36.92 mg/(kg.day) 0.18 g/ml, 300ml/day	2 g/ml (LD_0) –	Shuanghuanglian Oral Liquid Ribavirin	Inhibits influenza virus, and improves lung index and pathological changes and reduces the mRNA expression of NF-κB.	Mo et al., 2005; Lai et al., 2011; Sang et al., 2014
Ascending and Descending Powder (Shengjiang San)	Periostracum Cicadae (Chantui) 10g, Bombyx Batryticatus (Jiangcan) 10g, *Rheum officinale* Baill. (Shengdahuang) 6g and Curcuma longa L. (Jianghuang) 9g.	Clear heat and resolve toxins, dissolve phlegm and dissipate blood stasis.	Decoction	*In vivo*: influenza virus FM1 in mice	4.55 g/kg	$10^{-3.5}$/100 μl (LD_{50})	Ribavirin	Inhibits ICAM-1 and NF-κB overexpression in mouse lung, increases SIgA secretion and the expression of IL-10 and IL-1Rα, and decreases	Nan et al., 2016a; Nan et al., 2016b

(Continued)

TABLE 2 | Continued

Name of Formulas	Ingredients	Efficacy	Usage Mode	Model/Strains (Registration number)	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_0, LD_{50}*	Control	Actions and mechanisms	References
								content of IL-1β, IL-6, and TNF-α.	
Wind-scattering Toxins-resolving Capsule (Shufeng Jiedu Jiaonang) (Patent medicine)	Reynoutria japonica Houtt. (Huzhang), Forsythia suspensa (Thunb.) Vahl (Lianqiao), Isatis tinctoria L. (Banlangen), Bupleurum chinense DC. (Chaihu), Patrinia scabiosifolia Link (Baijiangcao), Verbena officinalis L. (Mabiancao), Phragmites australis subsp. australis (Lugen) and Glycyrrhiza uralensis Fisch. ex DC. (Gancao).	Scatter wind and clear heat, resolve toxins and relieve sore throat.	Solution after capsule dissolution	*In vitro*: influenza virus H1N1 (FM1, PR8, Jiangxixiushui, B10, B59), RSV, parainfluenza virus (Xiantai) in A549	5.55, 2.94, 1.52, 0.77 g/L	FM1: 0.56 g/L (IC_{50}); PR8: 0.56 g/L (IC_{50}); Jiangxixiushui: 0.56 g/L (IC_{50}); Brisbane-10: 0.56 g/L (IC_{50}); Brisbane-59: 0.56 g/L (IC_{50}); RSV: 0.32 g/L (IC_{50}); parainfluenza virus (Xiantai): 0.14 g/L (IC_{50})	Ribavirin	Inhibits the protein expression of PGE2, TNF-α, IL-1α, IL-1β, and IL-6, and restrains novel coronavirus by inhibiting MAPK/NF-κB signaling pathway.	Bao et al., 2019; Qu X. K. et al., 2020
Epidemic-pathogen-antagonizing Beverage (Kangli Yin) (Hospital preparation)	Artemisia annua L. (Qinghao) 10g, Scutellaria baicalensis Georgi (Huangqin) 15g, Pinellia ternata (Thunb.) Makino (Banxia) 10g, Periostracum Cicadae (Chantui) 6g, Bombyx Batryticatus (Jiangcan) 10g, Citrus aurantium L. (Chenpi) 5g, Citrus aurantium L. (Zhishi) 10g, Bambusa tuldoides Munro (Zhuru) 10g, Poria cocos (Schw.) Wolf (Fuling) 20g, Curcuma longa L. (Jianghuang) 10g, Pogostemon cablin (Blanco) Benth. (Huoxiang) 10g, *Rheum officinale* Baill. (Dahuang) 5g, Houttuynia cordata Thunb. (Yuxingcao) 15g, Eupatorium fortunei Turcz. (Peilan) 10g and Glycyrrhiza uralensis Fisch. ex DC. (Zhigancao) 5g.	Clear heat and drain dampness, harmonize the stomach and dissolve phlegm.	Decoction solution diluted in DMEM	*In vitro*: influenza virus H1N1 (FM1) in MDCK	22, 11, 5.5, 2.75, 1.375, 0.6875, 0.344, 0.172 mg/ml	9.38 mg/ml (TC_0); 21.33 mg/ml (TC_{50}); >22 mg/ml (IC_{50})	Ribavirin	No significant inhibitory effect on influenza virus H1N1 and RSV Long *in vitro*.	Deng, 2006; Sun, 2006
				In vitro: influenza virus RSV Long in MRC-5	7.62, 3.81, 1.905, 0.953, 0.476, 0.238, 0.119, 0.0595 mg/ml	5.50 mg/ml (TC_0); 7.62 mg/ml (TC_{50}); >7.62 mg/ml (IC_{50})			
			Decoction solution	*In vivo*: influenza virus H1N1 (FM1) in mice	5 g/kg, 2.5 g/kg	–		Reduces lung tissue lesions, increases the levels of CD3+, CD4+, IL-2 and IFN-γ, and inhibits the excessive production of CD8+, IL-6 and TNF-α.	
Two Roots Lung-clearing Beverage (Ergen Qingfei Yin)	Imperata cylindrica (L.) P. Beauv. (Baimaogen) 20g, Phragmites australis subsp. australis (Lugen) 20g, Scutellaria baicalensis Georgi (Huangqin) 10g, Gypsum Fibrosum (Shengshigao) 30g, Prunus armeniaca L. (Xingren) 10g, Ephedra sinica Stapf (Zhimahuang) 6g, Anemarrhena asphodeloides Bunge (Zhimu) 10g, Taraxacum mongolicum	Clear heat and dissolve phlegm, diffuse the lung and direct lung qi downward.	Decoction	Randomized controlled study with random number table: patients with influenza A (H1N1) viral pneumonia	0.923 g/ml, 200 ml/day	–	Oseltamivir	Relieves the symptoms of cough, expectoration, dry mouth, vexation, fever, panting and pulmonary rales, increases the level of IL-10 and decreases the level of IL-6, IL-8, TNF-α	Tian et al., 2019

(Continued)

TABLE 2 | Continued

Name of Formulas	Ingredients	Efficacy	Usage Mode	Model/Strains (Registration number)	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_0, LD_{50}*	Control	Actions and mechanisms	References
	Hand.-Mazz. (Pugongying) 10g, Isatis tinctoria L. (Banlangen) 15g, Morus alba L. (Sangbaipi) 10g, Houttuynia cordata Thunb. (Yuxingcao) 10g, Platycodon grandiflorus (Jacq.) A. DC. (Jiegeng) 10g and Aster tataricus L.f. (Ziwan) 10g.							and C-reactive protein in serum.	
Sweet Dew Toxin-Removing Elixir (Ganlu Xiaodu Dan)	Talcum (Huashi) 45g, *Scutellaria baicalensis* Georgi (Huangqin) 30g, Artemisia capillaris Thunb. (Yinchen) 30g, Acorus calamus var. angustatus Besser (Shichangpu) 18g, Fritillaria cirrhosa D.Don (Chuanbeimu) 15g, *Akebia trifoliata* (Thunb.) Koidz. (Mutong)15g, Pogostemon cablin (Blanco) Benth. (Huoxiang) 12g, Forsythia suspensa (Thunb.) Vahl (Lianqiao) 12g, *Wurfbainia vera* (Blackw.) Skornick. & A.D.Poulsen (Baidoukou) 12g, *Mentha canadensis* L. (Bohe) 12g and Iris domestica (L.) Goldblatt & Mabb. (Shegan) 12g.	Drain dampness and remove turbidity, clear heat and resolve toxins.	Decoction	*In vivo*: influenza virus A/PR/8/34 (H1N1) in mice	56 g/kg/day	–	Ribavirin	Decrease the level of serum IL-4 and expression of H1N1 mRNA and Aquaporin 1 (AQP1) protein in mice model with viral pneumonia.	Bi et al., 2019; Chen L. et al., 2020
				Retrospective study: patients with SARS-CoV-2 infection	0.71 g/ml	–	Arbidol, Moxifloxacin		
Folium Ginkgo and Ephedra Lung-clearing Capsule (Yinhuang Qingfei Jiaonang) (Patent medicine)	Lepidium apetalum Willd. (Beitinglizi), Ephedra sinica Stapf (Mahuang), Prunus armeniaca L. (Kuxingren), Fritillaria thunbergii Miq. (Zhebeimu), Eriobotrya japonica (Thunb.) Lindl. (Pipaye), Persicaria tinctoria (Aiton) Spach (Daqingye), Acorus calamus var. angustatus Besser (Shichangpu), Dioscorea nipponica Makino (Chuanshanlong), Aconitum brachypodum Diels (Yizhihao), Ginkgo biloba L. (Yinxingye), Schisandra chinensis (Turcz.) Baill. (Wuweizi), Citrus aurantium L. (Zhishi), Gypsum Fibrosum (Shengshigao) and Glycyrrhiza uralensis Fisch. ex DC. (Gancao).	Clear lung heat and dissolve phlegm, relieve cough and calm panting.	Solution after capsule dissolution	*In vivo*: A/PR/8/34 (H1N1) in rat	100, 200, 400 mg/kg	–	Bairui Capsule	Inhibits neuraminidase activity and influenza virus proliferation *in vivo*, improves pneumonia symptoms and histopathological changes, and increases the level of FGF2 and protein expression of FGFR1 in the lung.	Li et al., 2015; Peng et al., 2016; Qiu et al., 2018
			Medicated serum	*In vitro*: A/PR/8/34 (H1N1) in MDCK	50%, 25%, 12.5%, 6.25%, 3.125% (serum concentration)	25% of the containing serum solution (TC_0)	Ribavirin		
				In vitro: respiratory syncytial virus (RSV) in Hep-2	12.5%, 6.25%, 3.13%, 1.56%, 0.78% (serum concentration)	12.5% of the containing serum solution (TC_0); 10^{-8}/ 0.1 ml ($TCID_{50}$)			
Ephedra, Apricot Kernel, Gypsum and Licorice Decoction (Maxingshigan Tang)	Ephedra sinica Stapf (Mahuang) 6g, Prunus armeniaca L. (Xingren) 6g, Gypsum Fibrosum (Shengshigao) 24g and Glycyrrhiza uralensis Fisch. ex DC. (Gancao) 6g.	Release the exterior with acrid-cool (medicinals), clear lung heat and relieve panting.	Decoction	*In vivo*: respiratory syncytial virus (RSV) Long in rat	1.87, 2.8, 4.2, 6.3 g/kg	3.992 g/kg (ED_{50})	Ribavirin	Inhibits neuraminidase activity and influenza virus proliferation.	Hsieh et al., 2012; Cui et al., 2019
Lung-clearing and Collaterals-unblocking	*Rheum officinale* Baill. (Dahuang) and Scutellaria baicalensis Georgi (Huangqin) and *Allium sativum* L. (Dasuan).	Clear heat and resolve toxins.	Paste	*In vivo*: respiratory syncytial virus (RSV) Long in rat	0.6 g/paste	10^{-3}/0.1 ml ($TCID_{50}$)	–	Reduces the scope of lung lesions and alveolar exudates, and inhibits	Liu X. X. et al., 2016;

(Continued)

TABLE 2 | Continued

Name of Formulas	Ingredients	Efficacy	Usage Mode	Model/Strains (Registration number)	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_0, LD_{50}*	Control	Actions and mechanisms	References
Ointment (Qingfei Tongluo Gao) (Hospital preparation)								PI3K/Akt/NF-κB signaling pathway in intervention of RSV pneumonia.	Zhang et al., 2016
Lung-clearing Oral Liquid (Qingfei Koufuye)	Ephedra sinica Stapf (Mizhimahuang) 4g, Prunus armeniaca L. (Kuxingren) 10g, Angelica decursiva (Miq.) Franch. & Sav. (Qianhu) 10g, Gypsum Fibrosum (Shengshigao) 24g, Morus alba L. (Mizhisangbaipi), Lepidium apetalum Willd. (Tinglizi) 6g, Bistorta officinalis Delarbre (Quanshen) 12g, Bombyx Batryticatus (Baijiangcan) 6g, Reynoutria japonica Houtt. (Huzhang) 12g and Salvia miltiorrhiza Bunge (Danshen) 6g.	Diffuse the lung and dissolve phlegm, resolve toxins and invigorate blood.	Medicated serum	*In vitro*: respiratory syncytial virus (RSV) Long or R6 in Hep-2	20, 10, 5, 2.5, 1.25, 0.625 mg/ml	Medicated serum: 1:9 (TC_0); $10^{-3.5}$/ 50 μl ($TCID_{50}$)	Ribavirin	Relieves the symptoms of fever, cough and panting, regulates the Treg/Th17 balance, increases IL-10 cytokines and decreases IL-17 cytokines in RSV infected mice.	Wang et al., 2008; Dong et al., 2015; Wang et al., 2016
				In vivo: respiratory syncytial virus (RSV) Long in mice	4, 1.33 mg/ml				
			Oral liquid	Randomized controlled study: 507 cases of children virus pneumonia.	10, 20, 30 ml/day, (10 g/ml)	–			
Lonicera and Forsythia Epidemic-Clearing Capsule (Lianhua Qingwen Jiaonang) (Patent medicine)	Forsythia suspensa (Thunb.) Vahl (Lianqiao), Lonicera japonica Thunb. (Jinyinhua), Ephedra sinica Stapf (Zhimahuang), Prunus armeniaca L. (Kuxingren), Gypsum Fibrosum (Shengshigao), Isatis tinctoria L. (Banlangen), Dryopteris crassirhizoma Nakai (Mianmaguanzhong), Houttuynia cordata Thunb. (Yuxingcao), Pogostemon cablin (Blanco) Benth. (Guanghuoxiang), *Rheum officinale* Baill. (Dahuang), Rhodiola rosea L. (Hongjingtian), menthol and Glycyrrhiza uralensis Fisch. ex DC. (Gancao).	Clear the epidemic and resolve toxins, diffuse the lung and discharge heat.	Solution after capsule dissolution	*In vitro*: influenza virus H1N1 (FM1, PR8, Jiangxixiushui, B10, B59), RSV, parainfluenza virus (Xiantai) in A549	10, 5.55, 2.94, 1.52 g/L	FM1: 1.12 g/L (IC_{50}); PR8: 1.12 g/L (IC_{50}); Jiangxixiushui: 1.12 g/L (IC_{50}); Brisbane-10: 1.12 g/L (IC_{50}); Brisbane-59: 1.12 g/L (IC_{50}); RSV: 0.50 g/L (IC_{50}); Xiantai: 0.28 g/L (IC_{50})	Ribavirin	Inhibits the activity of influenza virus H1N1, parainfluenza virus, RSV, MERS- CoV or SARS-CoV *in vitro* as well as influenza A virus (H1N1) or MERS- CoV infection *in vivo*, suppresses virus-induced NF-κB activation, alleviates virus-induced gene expression of IL-6, IL-8, TNF-a, IP-10, and MCP-1, and also inhibits SARS-CoV-2 replication in Vero E6 cells and markedly reduced pro-inflammatory cytokines (TNF-α, IL-6, CCL-2/ MCP-1 and CXCL-10/IP-10) production at the mRNA levels.	Zhu et al., 2003; Ding et al., 2017; Guan et al., 2018; Bao et al., 2019; Gao et al., 2020; Li R. F. et al., 2020; Xiao et al., 2020
				In vitro: Middle East respiratory syndrome coronavirus (MERS-CoV) in Vero cells	600, 900, 1200, 1500, 1800, 2100, 2500 μg/ml	8472 μg/ml (IC_{50})	Arbidol		
				In vitro: novel coronavirus (SARS-CoV-2) in Vero E6 cells	150, 300, 600 μg/ml	411.2 μg/ml (IC_{50})	Remdesivir		
				Randomized controlled study: patients with COVID-19.(Registration number of clinical trials: ChiCTR2000029601).	0.3 g/kg	–	Oseltamivir and arbidol	Relieves clinical symptoms, reduces utilization rate of anti-infective drugs, and improves patient prognosis.	

(Continued)

TABLE 2 | Continued

Name of Formulas	Ingredients	Efficacy	Usage Mode	Model/Strains (Registration number)	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_0, LD_{50}*	Control	Actions and mechanisms	References
Formula I for SARS During Hyperpyrexia Peroid (Feidian Gaoreqi Yihao Fang)	Ephedra sinica Stapf (Mahuang) 5g, Prunus armeniaca L. (Xingren) 12g, Gypsum Fibrosum (Shengshigao) 45g, Anemarrhena asphodeloides Bunge (Zhimu) 10g, Lonicera japonica Thunb. (Jinyinhua) 15g, Forsythia suspensa (Thunb.) Vahl (Lianqiao) 12g, Gardenia jasminoides J.Ellis (Zhizi) 12g, Scutellaria baicalensis Georgi (Huangqin) 12g, Perilla frutescens (L.) Britton (Zisuye) 10g, Artemisia capillaris Thunb. (Yinchen) 15g, Pueraria montana var. lobata (Willd.) Maesen & S.M.Almeida ex Sanjappa & Predeep (Gegen) 15g and Pseudostellaria heterophylla (Miq.) Pax (Taizishen) 15g.	Clear heat and resolve toxins, scatter wind and diffuse the lung.	Decoction	Parallel controlled study: patients with severe acute respiratory syndrome (SARS)	0.593 g/ml, 300 ml/day	–	Ribavirin	Improves the total number of white blood cells and lymphocyte absolute value and the time of absorption of patchy shadow on chest X-ray.	Zhang R. L. et al., 2003
Formula II for SARS During Panting Peroid (Feidian Kechuanqi Erhao Fang)	Panax quinquefolius L. (Xiyangshen) 15g, Ophiopogon japonicus (Thunb.) Ker Gawl. (Maidong) 10g, Schisandra chinensis (Turcz.) Baill. (Wuweizi) 10g, Cornus officinalis Siebold & Zucc. (Shanzhuyu) 12g, Lepidium apetalum Willd. (Tinglizi) 15g, Aster tataricus L.f. (Ziwan) 15g, Eriobotrya japonica (Thunb.) Lindl. (Pipaye) 12g, Pheretima (Dilong) 12g, Salvia miltiorrhiza Bunge (Danshen) 12g, Paeonia anomala subsp. veitchii (Lynch) D.Y.Hong & K.Y.Pan (Chishao) 12g, Trollius chinensis Bunge (Jinlianhua) 8g, Scutellaria baicalensis Georgi (Huangqin) 10g and Trichosanthes kirilowii Maxim. (Gualoupi) 15g.	Clear heat and invigorate blood, boost qi and nourish yin, relieve cough and calm panting.	Decoction	Parallel controlled study: patients with severe acute respiratory syndrome (SARS)	0.527 g/ml, 300 ml/day	–	Ribavirin	Improves the total number of white blood cells and lymphocyte absolute value and the time of absorption of patchy shadow on chest X-ray.	Zhang R. L. et al., 2003
Formula III for SARS During Convalescence (Feidian Huifuqi Sanhao Fang)	Pseudostellaria heterophylla (Miq.) Pax (Taizishen) 15g, Ophiopogon japonicus (Thunb.) Ker Gawl. (Maidong) 15g, Atractylodes macrocephala Koidz. (Baizhu) 15g, Eriobotrya japonica (Thunb.) Lindl. (Zhipipaye) 15g, Wurfbainia villosa var. xanthioides (Wall. ex Baker) Skornick. & A.D.Poulsen (Sharen) 6g, Hordeum vulgare L. (Jiaomaiya) 15g, Crataegus pinnatifida Bunge (Jiaoshanzha) 15g, Astragalus mongholicus Bunge (Shenghuangqi) 15g, Pueraria montana var. lobata (Willd.) Maesen & S.M.Almeida ex Sanjappa & Predeep (Gegen) 15g, Salvia miltiorrhiza Bunge (Danshen) 15g, Citrus aurantium L. (Chenpi) 6g and Polygonatum cyrtonema Hua (Huangjing) 15g.	Boost qi and nourish yin, fortify the spleen and harmonize the stomach.	Decoction	Parallel controlled study: patients with severe acute respiratory syndrome (SARS)	0.59 g/ml, 300 ml/day	–	Ribavirin	Promotes the recovery of immune function and improves the lung inflammatory damage, improves clinical symptoms, reduces hormone dosage and shortens the course of treatment.	Zhang R. L. et al., 2003

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TABLE 2 | Continued

Name of Formulas	Ingredients	Efficacy	Usage Mode	Model/Strains (Registration number)	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_0, LD_{50}*	Control	Actions and mechanisms	References
SARS-Formula-I (Feidian Yihao Fang)	Gypsum Fibrosum (Shengshigao) 45g, Bupleurum chinense DC. (Chaihu) 15g, Anemarrhena asphodeloides Bunge (Zhimu) 10g, Fritillaria thunbergii Miq. (Zhebeimu) 10g, Scutellaria baicalensis Georgi (Huangqin) 15g, Artemisia annua L. (Qinghao) 15g, Paeonia suffruticosa Andrews (Mudanpi) 10g, Paeonia anomala subsp. veitchii (Lynch) D.Y.Hong & K.Y.Pan (Chishao) 12g, Forsythia suspensa (Thunb.) Vahl (Lianqiao) 15g, Cornus officinalis Siebold & Zucc. (Shanzhuyu) 30g, Atractylodes lancea (Thunb.) DC. (Cangzhu) 15g, Pogostemon cablin (Blanco) Benth. (Huoxiang) 15g, Coix lacryma-jobi var. ma-yuen (Rom.Caill.) Stapf (Yiyiren) 15g and Prunus armeniaca L. (Chaoxingren) 10g.	Clear heat and resolve toxins, dispel dampness and remove turbidity.	Decoction	Simple stratified randomized controlled study: patients with severe acute respiratory syndrome (SARS) in the early mild stage.	0.723 g/ml, 300 ml/day	–	Glucocorticoid, antibiotics, thymosin, and gamma globulin	Shortens the time of fever, slows down the symptoms of systemic poisoning caused by fever, promotes the absorption of pulmonary inflammation, and accelerates the reduction of glucocorticoid.	Zhang Y. L. et al., 2003; Zhang X. M. et al., 2003
				Prospective study: patients with severe acute respiratory syndrome (SARS) in the mild or severe stage.	0.58 g/ml, 400 ml/day		Glucocorticoid, ganciclovir, levofloxacin, Rocephin, Sulperazon, and thymosin		
SARS-Formula-II (Feidian Erhao Fang)	Scutellaria baicalensis Georgi (Huangqin) 15g, Artemisia annua L. (Qinghao) 15g, Trichosanthes kirilowii Maxim. (Gualou) 30g, Salvia miltiorrhiza Bunge (Danshen) 15g, Inula japonica Thunb. (Xuanfuhua) 10g, Curcuma aromatica Salisb. (Yujin) 10g, Acorus calamus var. angustatus Besser (Shichangpu) 10g, Dioscorea collettii var. hypoglauca (Palib.) S.J.Pei & C.T.Ting (Bixie) 12g, Faeces Bombycis (Cansha) 15g, Atractylodes lancea (Thunb.) DC. (Cangzhu) 15g, Atractylodes macrocephala Koidz. (Baizhu) 15g, Polyporus umbellatus (Pers.) Fries (Zhuling) 15g, Poria cocos (Schw.) Wolf (Fuling) 15g, Coix lacryma-jobi var. ma-yuen (Rom.Caill.) Stapf (Yiyiren) 15g, Prunus armeniaca L. (Chaoxingren) 10g, Plantago asiatica L. (Cheqianzi) 10g and Cornus officinalis Siebold & Zucc. (Shanzhuyu) 30g.	Clear and dispel damp-heat, diffuse the lung and direct counterflow downward.	Decoction	Simple stratified randomized controlled study: patients with severe acute respiratory syndrome (SARS) in the early mild stage.	0.757 g/ml, 300 ml/day	–	Glucocorticoid, antibiotics, thymosin, and gamma globulin	Shortens the average fever time, alleviates the systemic symptoms caused by fever, promotes the absorption of lung inflammation and accelerates the reduction of glucocorticoid.	Zhang Y. L. et al., 2003; Zhang X. M. et al., 2003
				Prospective study: patients with severe acute respiratory syndrome (SARS) in the mild or severe stage.	0.63 g/ml, 400 ml/day		Glucocorticoid, ganciclovir, levofloxacin, Rocephin, Sulperazon, and thymosin		
SARS-Formula-III (Feidian Sanhao Fang)	Panax quinquefolius L. (Xiyangshen) 30g, Astragalus mongholicus Bunge (Shenghuangqi) 30g, Cornus officinalis Siebold & Zucc. (Shanzhuyu) 30g, Ophiopogon japonicus (Thunb.) Ker Gawl. (Maidong) 15g, Anemarrhena asphodeloides Bunge (Zhimu) 10g, Fritillaria thunbergii Miq. (Zhebeimu) 10g, Patrinia scabiosifolia Link (Baijiangcao) 30g, Forsythia suspensa (Thunb.) Vahl (Lianqiao) 15g, Salvia miltiorrhiza Bunge (Danshen) 15g,	Boost qi and nourish yin, dissolve phlegm and invigorate blood, drain dampness and direct turbidity downward.	Decoction	Simple stratified randomized controlled study: patients with severe acute respiratory syndrome (SARS) in the early mild stage.	0.923 g/ml, 300 ml/day	–	Glucocorticoid, antibiotics, thymosin, and gamma globulin	Shortens the average fever time, alleviates the systemic symptoms caused by fever, promotes the absorption of lung inflammation and accelerates the reduction of glucocorticoid.	Zhang Y. L. et al., 2003; Zhang X. M. et al., 2003
				Prospective study: patients with severe acute respiratory	0.755 g/ml, 400 ml/day		Glucocorticoid, ganciclovir, levofloxacin,		

(Continued)

TABLE 2 | Continued

Name of Formulas	Ingredients	Efficacy	Usage Mode	Model/Strains (Registration number)	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_0, LD_{50}*	Control	Actions and mechanisms	References
	Dioscorea collettii var. hypoglauca (Palib.) S.J.Pei & C.T.Ting (Bixie) 12g, Faeces Bombycis (Cansha) 15g, Coix lacryma-jobi var. ma-yuen (Rom.Caill.) Stapf (Yiyiren) 15g, Polyporus umbellatus (Pers.) Fries (Zhuling) 15g, Poria cocos (Schw.) Wolf (Fuling) 15g, Trichosanthes kirilowii Maxim. (Gualou) 30g and Aster tataricus L.f. (Ziwan) 15g.			syndrome (SARS) in the mild or severe stage.			Rocephin, Sulperazon, and thymosin		
Compound Forsythia and Taraxaci Granule (Fufang Lianpu Keli) (Patent medicine)	Forsythia suspensa (Thunb.) Vahl (Lianqiao), Taraxacum mongolicum Hand.-Mazz. (Pugongying), Lonicera japonica Thunb. (Jinyinhua), Scutellaria baicalensis Georgi (Huangqin) and Isatis tinctoria L. (Banlangen).	Release the exterior with acrid-cool (medicinals), clear heat and resolve toxins.	Water solution	*In vitro*: novel coronavirus (SARS-CoV) BJ01 in Vero E6 cells	56 mg/ml	0.49 mg/ml (EC_{50})	Ribavirin	Inhibits SARS-CoV cultured in Vero-E6 cells.	Zhu et al., 2003
Six Spirits Capsule (Liushen Jiaonang) (Patent medicine)	*Calculus Bovis (Niuhuang), Moschus (Shexiang), Borneolum Syntheticum (Bingpian), Venenum Bufonis (Chansu), Margarita (Zhenzhu) and Realgar (Xionghuang).*	Clear heat and resolve toxins, reduce inflammation and relieve pain.	Triturated and prepared in dimethyl sulfoxide (DMSO)	*In vitro*: SARS-CoV-2 (MT123290.1) in Vero E6 cells	2.00, 1.00, 0.50, 0.25 μg/ml	0.6024 μg/ml (IC_{50}); 4.930 μg/ml (TC_{50}); 10^{-6}/100 μl ($TCID_{50}$)	Remdesivir	Inhibits SARS-CoV-2 virus infection *via* downregulating the expression of inflammatory cytokines induced virus and regulating the activity of NF-κB/MAPK signaling pathway *in vitro*.	Ma et al., 2020
Lung-clearing and Toxin-expelling Decoction (Qingfei Paidu Tang)	Ephedra sinica Stapf (Mahuang) 9g, Glycyrrhiza uralensis Fisch. ex DC. (Zhigancao) 6g, Prunus armeniaca L. (Xingren), Gypsum Fibrosum (Shengshigao) 10g (30g for fever), Cinnamomum cassia (L.) J.Presl (Guizhi) 9g, Alisma plantago-aquatica subsp. orientale (Sam.) Sam. (Zexie) 9g, Polyporus umbellatus (Pers.) Fries (Zhuling) 9g, Atractylodes macrocephala Koidz. (Baizhu) 9g, Poria cocos (Schw.) Wolf (Fuling) 15g, Bupleurum chinense DC. (Chaihu) 16g, Scutellaria baicalensis Georgi (Huangqin) 6g, Pinellia ternata (Thunb.) Makino (Jiangbanxia)9g, Aster tataricus L.f. (Ziwan) 9g, Zingiber officinale Roscoe (Shengjiang) 9g, Tussilago farfara L. (Kuandonghua) 9g, Iris domestica (L.) Goldblatt & Mabb. (Shegan) 9g, Asarum sieboldii Miq. (Xixin) 6g, Dioscorea oppositifolia L. (Shanyao) 12g, Citrus aurantium L. (Zhishi) 6g, Citrus aurantium L. (Chenpi) 6g and Pogostemon cablin (Blanco) Benth. (Huoxiang) 9g.	Dredge the sanjiao, clear lung heat and expel toxins, calm panting and relieve cough.	Decoction	Comparison before and after treatment: patients with COVID-19. Clinical retrospective controlled study: patients with COVID-19.(Registration number of clinical trials: ChiCTR2000029778 and registration number of TCM clinical trial registry: ChiMCTR2000003003).	0.985 or 1.085 g/ml, 200 ml/day 0.4925 or 0.5425 g/ml, 400 ml/day	>1,600 mg/kg (LD50)	– Interferon, lopinavir, or arbidol	Relieves cough, nasal congestion, runny nose, fatigue, anorexia, sore throat, diarrhea and other symptoms, and shows anti-inflammatory effects compared with those of only Western medicine in patients with mild and moderate COVID-19, and tends to mitigate the extent of multi-organ impairment.	Chen J. et al., 2020; Wang R. Q. et al., 2020; Xin et al., 2020; Zhou et al., 2020

(Continued)

TABLE 2 | Continued

Name of Formulas	Ingredients	Efficacy	Usage Mode	Model/Strains (Registration number)	Dosage/ Duration	IC_{50}, EC_{50}, $TCID_{50}$, TC_{50}, TC_0, LD_0, LD_{50}*	Control	Actions and mechanisms	References
Venting-releasing Epidemic-dispelling Granule (Toujie Quwen Keli)	Forsythia suspensa (Thunb.) Vahl (Lianqiao) 30g, *Pleione yunnanensis* (Rolfe) Rolfe (Shancigu) 20g, Lonicera japonica Thunb. (Jinyinhua) 15g, Scutellaria baicalensis Georgi (Huangqin) 10g, Persicaria tinctoria (Aiton) Spach (Daqingye) 10g, Bupleurum chinense DC. (Chaihu) 5g, Artemisia annua L. (Qinghao) 10g, Periostracum Cicadae (Chantui) 10g, Angelica decursiva (Miq.) Franch. & Sav. (Qianhu) 5g, Fritillaria cirrhosa D.Don (Chuanbeimu)10g, Fritillaria thunbergii Miq. (Zhebeimu) 10g, Prunus mume (Siebold) Siebold & Zucc. (Wumei) 30g, Scrophularia ningpoensis Hemsl. (Xuanshen) 10g, Astragalus mongholicus Bunge (Huangqi) 45g, Poria cocos (Schw.) Wolf (Fuling) 30g and Pseudostellaria heterophylla (Miq.) Pax (Taizishen) 15g.	Clear heat and resolve toxins, vent the exterior and scatter wind, boost qi and nourish yin.	Water solution	Randomized parallel controlled study: 65 cases of patients with COVID-19.	0.883 g/ml, 300 ml/day	–	Arbidol, moxifloxacin	Reduces the symptoms of patients with novel coronavirus pneumonia, and regulates the expression of peripheral blood inflammatory markers, decreases the level of CRP and increases the level of CD+ to antagonize novel coronavirus.	Fu X. X. et al., 2020
Lung-clearing, Pathogen-venting and Healthy-qi-reinforcing Formula (Qingfei Touxie Fuzheng Fang)	Ephedra sinica Stapf (Zhimahuang) 6g, Gypsum Fibrosum (Shengshigao) 20g, Prunus armeniaca L. (Xingren) 10g, Forsythia suspensa (Thunb.) Vahl (Lianqiao) 15g, Phragmites australis subsp. australis (Lugen) 30g, Lonicera japonica Thunb. (Jinyinhua) 30g, Coix lacryma-jobi var. ma-yuen (Rom.Caill.) Stapf (Yiyiren) 30g, Bombyx Batryticatus (Jiangcan) 10g, Periostracum Cicadae (Chantui) 10g, Reynoutria japonica Houtt. (Huzhang) 15g, Curcuma longa L. (Jianghuang) 10g, Paeonia lactiflora Pall. (Baishao) 10g, Pseudostellaria heterophylla (Miq.) Pax (Taizishen) 20g and Glycyrrhiza uralensis Fisch. ex DC. (Shenggancao) 15g.	Clear and diffuse lung heat, and reinforce healthy qi to consolidate the exterior.	Decoction	Randomized controlled study: Novel coronavirus pneumonia patients	0.77 g/ml; 300 400 ml/ day	–	Interferon-α	Relieves the clinical symptoms of fever, cough and expectoration, chest tightness and shortness of breath, promotes the absorption of lung lesions and improves oxygenation, and reduces the content of CRP, ESR and IL-6, and increases the expression of IFN-γ to antagonize novel coronavirus.	Ding et al., 2020

**IC_{50}, 50% inhibiting concentration; EC_{50}, 50% maximal effective concentration; $TCID_{50}$, 50% tissue culture infectious dose; TC_{50}, median toxic concentration. TC_0, Maximum nontoxic concentration; LD_{50}, median lethal dose; LD_0, lethal dose.*

and invigorate blood, boost qi and nourish yin, relieve cough and calm panting, Formula III for SARS during Convalescence (Feidian Huifuqi Sanhao Fang) with recovery to boost qi and nourish yin, fortify the spleen and harmonize the stomach. The results showed that the remission time of clinical symptoms and reduced hormone usage in the integrated TCM western medicine group were 2.52 days and 222.69 mg respectively, shorter than those in the control group, and the difference was statistically significant difference (p<0.05). Moreover, TCM also played an important role in promoting the recovery of immune function and reducing pulmonary inflammatory injury (Zhang R. L. et al., 2003). Yunling Zhang et al. employed integrated TCM and western medicine to treat 65 SARS patients, prescribing SARS-Formula-I (Feidian Yihao Fang) at the high fever stage to clear heat and resolve toxins, dispel dampness and remove turbidity; SARS-Formula-II (Feidian Erhao Fang) at panting and oppression stage to clear and dissolve damp-heat, diffuse the lung and direct counterflow downward; and SARS-Formula-III (Feidian Sanhao Fang) at the absorbing stage to boost qi and nourish yin, dissolve phlegm and invigorate blood, drain dampness and direct turbidity downward, respectively. The results showed that the treatment of integrated traditional Chinese and western medicine had advantages over the western medicine alone in terms of reducing fever, relieving clinical symptoms, absorbing pulmonary inflammatory lesions and reducing hormone usage (p<0.001 or p<0.05) (Zhang Y. L. et al., 2003). Jianping Liu et al. performed meta-analysis on the treatment of SARS with integrated Chinese and western medicine, and found that the combined Chinese and western medicine treatment could shorten the clinical symptoms and fever time, reduce secondary fungal infection, and relieve pulmonary inflammation (Liu et al., 2005).

TCM for the Treatment of Influenza Virus Pneumonia

Influenza virus pneumonia is a common pulmonary infection disease in clinic. Its symptoms often see fever, cough, bitter taste in the mouth, dry throat, throat pain, even visible high fever, heavy panting, profuse sweating, etc. Shouchuan Wang et al. used Lung-clearing Oral Liquid (Qingfei Koufuye) with effects of diffusing the lung and dissolving phlegm, resolving toxins and invigorating blood to treat infantile viral pneumonia with a pattern of phlegm-heat blocking the lung. The results showed the efficacy of Lung-clearing Oral Liquid was better than ribavirin injection in term of reducing fever, cough, asthma and inflammation (p<0.05 or p<0.01) (Wang et al., 2016). Based on the conventional treatment, Youzhong Tian et al. gave Two Roots Lung-clearing Beverage (Ergen Qingfei Yin) and Phlegm-heat-clearing Injection (Tanreqing Zhusheye) to treat patients with A (H1N1) viral pneumonia. The results showed that compared with the western medicine control group, combining Chinese and western medicine treatment could significantly reduce the content of serum inflammatory cytokines, such as TNF-alpha, IL-6, IL-8 and c-reactive protein, and the antipyretic and antitussive effect was better than that of the control group (p<0.01) (Tian et al., 2019). Fengmei Sang et al. used Sweet Wormwood and Scutellaria Gallbladder-Clearing Decoction (Haoqin Qingdan Tang) to treat patients with virus pneumonia with a pattern of damp-heat for a week as the observation group; the level of CD3+ and CD4+ was significantly higher than that of the control group patients (receiving conventional treatment); and the level of NF-κB was significantly lower than that of the control group patients (p<0.05). The total effective rate of the observation group was higher than that of the control group, with statistical significance (p<0.05) (Sang et al., 2014).

TCM for the Treatment of Coronavirus Induced Disease 2019 (COVID-19)

Coronavirus induced disease 2019 is a novel coronavirus pneumonia and characterized by fever, dry cough and fatigue as the main symptoms, accompanied by nasal congestion, runny nose, sore throat, muscle soreness and pain, etc. In severe cases, breathing difficulties and hypoxemia will occur, or patients develop into acute respiratory distress syndrome, septic shock, uncorrectable metabolic acidosis, coagulation dysfunction, and multi-organ failure and so on (General Office of National Health Commission of the People's Republic of China and Office of National Administration of TCM, 2020). TCM classifies COVID-19 as "epidemic disease". Raoqiong Wang et al. applied Lung-clearing and Toxin-expelling Decoction (Qingfei Paidu Tang) to treat 98 patients with COVID-19 and found that Lung-clearing and Toxin-expelling Decoction could significantly improve the liver and kidney functions of patients such as ALT and AST, recover the D-dimer, plasma C-reactive protein and erythrocyte precipitation, significantly reduce fever, cough (dry cough), asthma, pharyngeal pain, fatigue, anorexia and other symptoms, as well as relieve adverse reactions of antiviral drugs (p<0.01) (Wang R. Q. et al., 2020). Compared to 36 COVID-19 patients treated with oral abidor tablets and ambroxol tablets as the control group, Xiaoxia Fu et al. applied Venting-releasing Epidemic-dispelling Granule (Toujie Quwen Keli) to 37 COVID-19 patients as the treatment group. The results showed that compared with the western medicine treated control group, the combination of Chinese and western medicine treatment group can increase the absolute lymphocyte value and decrease C-reactive protein. CD4+ count and CD4+/CD8+ ratio were better than those in the control group (p<0.05) (Fu X. X. et al., 2020). Yunfei Qu et al. used the modified Ephedra, Apricot Kernel, Gypsum and Licorice Decoction (Maxingshigan Tang) [*Ephedra sinica* Stapf (Mahuang), *Prunus armeniaca* L. (Xingren), *Gypsum Fibrosum* (Shengshigao), *Platycodon grandiflorus* (Jacq.) A. DC. (Jiegeng), *Eriobotrya japonica* (Thunb.) Lindl. (Pipaye), *Atractylodes macrocephala* Koidz. (Baizhu), *Poria cocos* (Schw.) Wolf (Fuling), *Fritillaria cirrhosa* D.Don (Chuanbeimu), *Scutellaria baicalensis* Georgi (Huangqin), *Morus alba* L. (Sangbaipi) and *Glycyrrhiza uralensis* Fisch. ex DC. (Zhigancao)] with conventional western medicine to treat 40 patients with ordinary COVID-19, and found that after three days of treatment, IL-6 level significantly decreased compared with that before the treatment (p<0.05), and levels of AST, ALT and creatinine were normal. After 7 days of treatment, IL-6 level

decreased to normal, hypersensitive C-reactive protein level decreased significantly, CD4+T and CD8+T cell count increased significantly compared with that before treatment ($p<0.05$), levels of AST, ALT and creatinine were still normal. The results showed that this modified decoction had a significant effect on common COVID-19 without significant hepatorenal toxicity (Qu Y. F. et al., 2020). Ming Liu et al. evaluated the combination of traditional Chinese and western medicine for treatment of COVID-19. Based on the treatment with Lung-clearing, Pathogen-venting and Healthy-qi-reinforcing Formula (Qingfei Touxie Fuzheng Fang), Wind-scattering Toxins-resolving Capsule (Shufeng Jiedu Jiaonang), and Lonicera and Forsythia Epidemic-Clearing Granule (Lianhua Qingwen Keli) and so on respectively, the combination of traditional Chinese medicine and western medicine had better outcome than western medicine alone in several clinical aspects such as reducing severe conversion rate, shortening hospitalization time and improving the patients' clinical symptoms such as fever, cough, fatigue and oppression in chest (Liu et al., 2020). Furthermore, there are many other proprietary traditional Chinese medicine products also play an important therapeutic role by direct antiviral actions, antipyretic and analgesic, immune-regulation, anti-inflammation, and anti-acute lung injury, such as Agastache Qi-Correcting Capsule (Huoxiang Zhengqi Jiaonang), Lonicera Fever-clearing Granule (Jinhua Qinggan Keli), Wind-scattering Toxin-resolving Capsule (Shufeng Jiedu Jiaonang), Xiyanping Injection (Xiyanping Zhusheye), Blood-clearing Injection (Xuebijing Zhusheye), Ginseng and Aconite Injection (Shenfu Zhusheye), Ginseng and Ophiopogon Injection (Shenmai Zhusheye), Peaceful Palace Bovine Bezoar Pill (Angong Niuhuang Wan), etc. (Zhuang et al., 2020).

DISCUSSION

In the treatment of viral pneumonia through syndrome differentiation, TCM plays a variety of roles in inhibiting the proliferation, replication, adsorption and membrane penetration of the virus, promoting the expression of interferon *in vivo*, inhibiting inflammatory reaction, enhancing immunity, etc., which is one of the theoretical bases for the clinical application of TCM in the prevention and treatment of viral pneumonia. Viruses with RNA genetic material, such as influenza virus and coronavirus, are more likely to mismatch and cause mutations in the replication process than DNA viruses (Woolhouse et al., 2016). Their high variability makes it more difficult to develop vaccines and more susceptible to drug resistance to single chemical drugs. Traditional Chinese herbal medicine and compound medicinals are characterized by multi-component, multi-pathway and multi-pathway complex networks. Therefore, drug resistance is relatively rare in the clinical practice of TCM. Moreover, in the process of diagnosis and treatment of TCM, treatment based on differentiation of symptoms and signs, especially treatment based on classification of symptoms and signs, can best reflect the overall concept of TCM. TCM has precise therapeutic activity and less adverse reactions. Accumulating evidence has demonstrated the competent therapeutic effects of TCM against viral pneumonia with a prominent safety profile. TCM has obvious characteristics and great advantages on syndrome differentiation for the prevention and treatment of viral pneumonia before specific antiviral drugs and vaccines are developed and produced. However, TCM in treatment of viral pneumonia still have some problems. First, theoretical study of viral pneumonia in TCM, especially regarding the pathogenesis and changes of the virus are not comprehensive, systematic and in-depth; second, the complexity of traditional Chinese herbal medicine composition and its compound makes it difficult to understand mechanism and action and less specific; third, in the process of treatment of viral pneumonia, it is usually carried out by traditional and macroscopic methods, with strong subjectivity, which cannot be considered as microscopic and specific as modern medical diagnostic standards. There is still a lack of recognized and unified standards for the classification of TCM Syndrome of viral pneumonia; fourth, the basic research on prevention and treatment of SARS-CoV-2 and COVID-19 with TCM is less developed, which may be related to that case collection of infectious disease in different countries is different or may not be allowed, and/or lack of laboratories that meet the requirements for conducting research of contagious diseases. Furthermore, the TCM treatment was mainly based on decoction, which makes difficult to set up control group therefore, generate greater varieties.

The basic treatment of medical formula and proprietary traditional Chinese medicine product are mainly based on the analysis of the whole process of the etiology and pathogenesis of viral pneumonia, which include grasping the basic pathogenesis, establishing the basic treatment method, and combining the viewpoint of modern medicine, formulating the basic prescriptions, or adding or subtracting along with the syndromes, or further changing the dosage form, and developing the treatment method for patent medicine. Although this kind of treatment often lacks the concept of TCM syndrome and the flexibility of syndrome differentiation and treatment, it has been proved to be effective in practice due to its grasp of the basic pathogenesis of the disease and the application of various methods. Moreover, it has been reported successful many times and seems to become a distinct alternative choice alongside the classical approach. Furthermore, in addition to oral administration of TCM decoction or pills, intravenous administration of TCM and other methods have been reported as another treatment of viral pneumonia. In addition, there are also many reports about the external treatment of patients with viral pneumonia, such as the atomizing inhalation of traditional Chinese medicine extract, external application of Chinese medicine powder or paste, foot reflexology, infantile massage, etc., all which reveal some new ideas and ways for TCM treatment of viral pneumonia.

In recent years, researches on viral pneumonia by TCM mainly focus on influenza virus, mainly on mice or cell models infected by influenza A (H1N1) virus, while researches on SARS-CoV and MERS-CoV are few. Studies on such viruses as SARS-

CoV, MERS-COV, H1N1 and other viruses should be conducted in P3 laboratory (biosafety level 3 laboratory) or higher biosafety laboratory. Extensive and in-depth studies on the prevention and treatment of viral diseases with TCM are subject to certain conditions. Fortunately, in 2020, the Ministry of Science and Technology of China issued the "Guidance on Strengthening the Biosafety Management of Novel Coronavirus High-level Virus Microbiology Laboratory", requiring the laboratory to play a role as a platform to serve the needs of scientific and technological research. This will provide strong policy support for the in-depth study of the antiviral effect and mechanism of TCM. Although the research on the antiviral activity of TCM has been performed with molecular biology, the specific therapeutic effects of traditional Chinese herbal medicine or compound on virus and pneumonia remains to be further investigated because of its complex components. Therefore, in order to better treat viral pneumonia with TCM based on syndrome differentiation and the overall concept of theoretical system, we should adhere to the theory of TCM as the basis, actively combine with modern or western medicine, complement each other, and use modern science and technology to explore the role and mechanism of viral pneumonia and traditional Chinese medicine in a more comprehensive, systematic and in-depth way, deeply analyze the characteristics of viral pneumonia syndromes, unify evidence pattern classification standards, further standardize and unify the evaluation criteria of syndrome differentiation and efficacy in order to facilitate the communication of clinical and scientific research work, use new diagnostic techniques to prevent misdiagnosis and missed diagnosis, and establish positive drug control in a standardized way in the process of clinical research to improve the credibility of TCM treatment. In the future, the TCM treatment theory and clinical application of viral pneumonia should pay special attention to strengthen experimental research, especially the effective Chinese medicine compounds. The precise mechanism of Chinese medicine in the treatment of viral pneumonia should be scientifically clarified to achieve the synchronization of clinical research and experimental research. In this way, Chinese medicine can be better to treat patients with viral pneumonia in a scientific and standardized manner based on syndrome differentiation.

CONCLUSIONS

TCM has been widely used in basic and clinical researches of virus diseases especially viral pneumonia in human. Some Chinese medicine has shown certain therapeutic effect, but high-quality experimental design and randomized clinical controlled study are still needed. A wide variety of antiviral traditional Chinese herbal medicines also provides potential opportunity for further development in specific therapeutic agents to treat viral pneumonia around the world.

AUTHOR CONTRIBUTIONS

YL and SX wrote the manuscript. YL and LY helped in searching for related articles. Y'aY, LQ, TL, and SX proofread the manuscript. SX and YG guided the writing and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Potential Therapeutic Effect of Traditional Chinese Medicine on Coronavirus Disease 2019: A Review

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The Coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 has been rapidly spreading globally and has caused worldwide social and economic disruption. Currently, no specific antiviral drugs or clinically effective vaccines are available to prevent and treat COVID-19. Traditional Chinese medicine (TCM) can facilitate syndrome differentiation and treatment according to the clinical manifestations of patients and has demonstrated effectiveness in epidemic prevention and control. In China, TCM intervention has helped to control the epidemic; however, TCM has not been fully recognized worldwide. In this review, we summarize the epidemiology and etiological characteristics of severe acute respiratory syndrome coronavirus 2 and the prevention and treatment measures of COVID-19. Additionally, we describe the application of TCM in the treatment of COVID-19 and the identification of small molecules of TCM that demonstrate anti-coronavirus activity. We also analyze the current problems associated with the recognition of TCM. We hope that, through the contribution of TCM, combined with modern technological research and the support of our international counterparts, COVID-19 can be effectively controlled and treated.

Keywords: SARS-CoV-2, COVID-19, traditional Chinese medicine, therapeutic effect, technology

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Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel and pathogenic coronavirus, has developed into a public health emergency of international concern (World Health Organization, 2020). As of August 6, 2020, more than 19 million confirmed cases have been reported across more than 216 countries and territories, resulting in more than 700,000 deaths (according to data from Johns Hopkins University) and causing a great negative impact on people's health and economic development. COVID-19 is the worst global health crisis since the Spanish flu pandemic of 1918, and no specific antiviral agent or effective vaccine has been found (Gupta et al., 2020). As the global COVID-19 pandemic continues to escalate rapidly, an urgent need exists to identify safe and effective drugs or potential adjuvant therapy. Accordingly, we briefly review the epidemiology, pathogenesis and key targets, multi-organ damage and conventional preventive treatment of SARS-CoV-2, focusing on the application of traditional Chinese medicine (TCM) in the treatment of COVID-19 patients. Additionally, some opinions on the difficulties and solutions to the modernization of TCM in China are expressed.

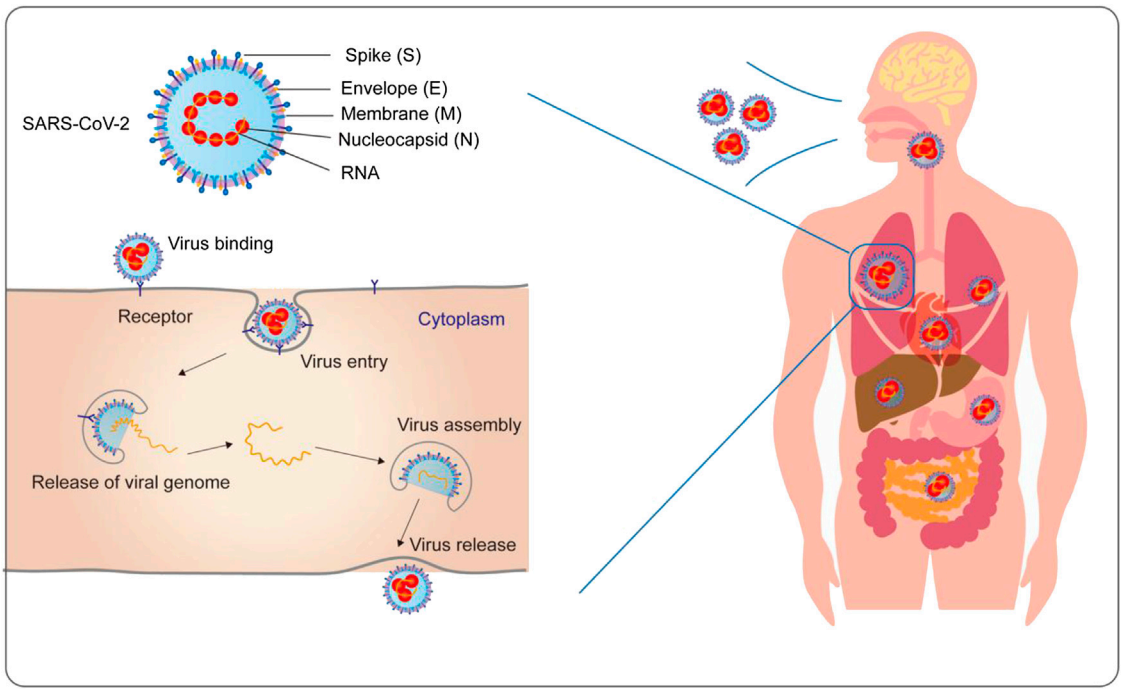

FIGURE 1 | Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2) is an enveloped RNA virus, comprising RNA and four major structural proteins [the nucleocapsid (N) protein, membrane (M) glycoprotein, envelope (E) protein, and spike (S) glycoprotein]. The pathogenic mechanism includes the four steps: attachment and entry, replication and transcription, assembly, and release. The virus mainly invades the host through the respiratory tract and directly or indirectly causes systemic multiple organ damage by identifying specific receptors on the host cell membrane.

EPIDEMIOLOGY OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2

Understanding the epidemiology of this virus is a key element to develop strategies for preventing COVID-19. Based on the findings of phylogenetic analysis, SARS-CoV-2 may have originated from bats or bat droppings associated with pollutants in the market or surrounding areas (Wu F. et al., 2020). SARS-CoV-2 has three types of hosts (natural, intermediate, and final) that can be transmitted between human hosts via respiratory droplets and contact routes (Li Q. et al., 2020). Moreover, the existing evidence of SARS-CoV-2 infecting intestinal epithelial cells reminds us to focus on the possibility of fecal-oral transmission (Lamers et al., 2020). Both asymptomatic and symptomatic patients are communicators; however, in the case of symptomatic patients, an increased viral load was observed (Kim et al., 2020). Studies have shown that adults are more susceptible to infection than children, especially the elderly with basic diseases such as hypertension and diabetes, among whom 80.9% have mild to moderate disease, and the mortality rate of confirmed cases is about 2.3% (Chang et al., 2020; Wu and McGoogan, 2020).

PATHOGENESIS AND KEY TARGETS OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2

SARS-CoV-2 is a positive-sense, single-stranded RNA virus with a genome of 29.9 kb and a diameter ranging from 80 to 160 nm, and is a novel beta-coronavirus belonging to the *Sarbecovirus* subgenus of the *Coronaviridae* family(Chan et al., 2020; Zhu N. et al., 2020). Its structure comprises a helical nucleocapsid formed by the binding of nucleic acid to the nucleocapsid (N) protein and a lipid envelope studded with structural proteins, including the membrane (M) glycoprotein, envelope (E) protein, and spike (S) glycoprotein (Boopathi et al., 2020). The pathogenic mechanism of coronavirus includes four steps: attachment and entry, replication and transcription, assembly and release (Fehr and Perlman, 2015). SARS-CoV-2 binds to the receptor angiotensin converting enzyme 2 (ACE2) with the help of S protein to enter cells and releases RNA that translates two polyproteins and structural proteins; thereafter, the viral genome begins to replicate (Yan R. et al., 2020). Genomic RNA and nucleocapsid proteins combine to form nucleocapsids, and then the vesicles containing the virus particles fuse with the plasma membrane to release the virus (**Figure 1**) (Knoops et al., 2008; Fehr and Perlman, 2015). SARS-CoV-2 S proteins

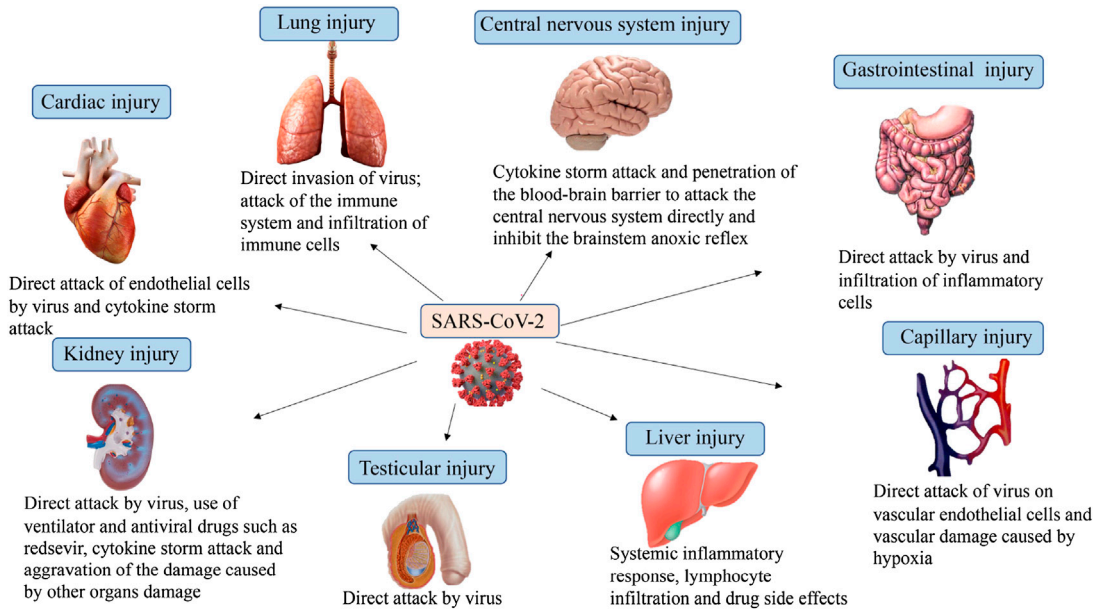

FIGURE 2 | Multi-organ dysfunction caused by SARS-CoV-2 infection includes heart injury, kidney injury, liver injury, lung injury, central nervous system injury, testicular injury, capillary injury, and gastrointestinal injury. Most organ damage is caused by direct virus attack and cytokine storm attack.

recognize ACE2 for entry and the serine protease TMPRSS2 for S protein priming (Hoffmann et al., 2020). Nevertheless, more studies have focused on nonstructural proteins such as the papain-like protease (PLpro), the 3C-like protease (3CLpro) and the RNA-dependent RNA polymerase (RdRp), which are critical for viral replication (Dong et al., 2020). These key proteins could be potential targets for diagnostic or therapeutic application.

MULTI-ORGAN DAMAGE OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2

After infecting the host, the SARS-CoV-2 stimulates humoral and cellular immunity, causing cytokine storms, which trigger a violent attack by the body's immune system (Sun et al., 2020). ACE2 has been identified as the functional host receptor for SARS-CoV-2 and is widely expressed in various human organs, including the oral and nasal mucosa, nasopharynx, lung, stomach, small intestine, skin, spleen, liver, kidney, and brain (**Figure 2**) (Li M.-Y. et al., 2020; Wang Q. et al., 2020). Among them, ACE2 immunostaining is the most abundant in alveolar epithelial cells; thus, the lung is a target organ that is most easily affected (Hamming et al., 2004). Autopsy reports from China and the United States both revealed diffuse alveolar injury and chronic inflammatory edema of the bronchial mucosa, confirming the occurrence of ARDS in COVID-19 patients whose main clinical manifestations are fever, cough and progressive dyspnea (Barton et al., 2020; Tian et al., 2020). Histological analysis of pulmonary vessels in patients with COVID-19 showed extensive thrombosis with microvascular lesions, with the amount of new vessel growth 2.7 times higher than that in patients with influenza (Ackermann et al., 2020). ACE2 is highly expressed not only in lung cells but also throughout the gastrointestinal tract (Bourgonje et al., 2020). Some patients experience gastrointestinal symptoms, including vomiting, abdominal pain and diarrhea (Cheung et al., 2020; Cholankeril et al., 2020), abdominal pain and diarrhea; imaging findings have suggested intestinal abnormalities (Bhayana et al., 2020). A case report revealed that SARS-CoV-2 enterocolitis continues to expel the virus for approximately two weeks after recovering from diarrhea (Hosoda et al., 2020), while studies from China have also found viral nucleocapsid protein in gastric, duodenal, and rectum glandular epithelial cells (Xiao et al., 2020). However, more evidence is needed to determine whether the virus has the possibility of fecal-oral transmission (Wu Y. et al., 2020). Several reports have indicated that the elevation of transaminase in patients suggests liver damage should be given attention (Feng et al., 2020; Wang Y. et al., 2020). SARS-CoV-2 infection is also associated with various diseases of the cardiovascular system, including myocarditis, cardiomyopathy and excessive vasoconstriction (Craver et al., 2020; Moderato et al., 2020). Some critically ill patients have abnormal hemagglutination with high D-dimer levels and elevated fibrinogen, which may cause vascular embolism, resulting in pulmonary embolism and stroke (Hess et al., 2020; Lax et al., 2020). If the virus appears in cutaneous blood vessels in patients with COVID-19, they may cause corresponding pathologic changes (Gianotti et al., 2020; Llamas-Velasco et al., 2020; Torres and Puig, 2020). The presence of the virus in the nerve and capillary endothelial cells in the frontal lobe tissue of an infected patient indicates that the virus can penetrate the blood-brain barrier to attack the

central nervous system directly (Paniz-Mondolfi et al., 2020; Reichard et al., 2020). Therefore, some patients have nervous system manifestations such as anosmia, dysgeusia, ataxia, and an altered mental status (Baig, 2020). Additionally, research data have shown that the mortality of patients with COVID-19 is related to the prevalence of kidney disease on admission (Cheng et al., 2020), and virus particles have been found in renal tubular epithelial cells under ultrastructure, providing evidence of direct infection of the kidney by SARS-CoV-2 (Farkash et al., 2020). In addition to the direct virulence of SARS-CoV-2, factors contributing to acute kidney injury include systemic hypoxia, abnormal coagulation, and possible drug or hyperventilation-relevant rhabdomyolysis (Su et al., 2020). Notably, Xixi Liu et al. found that ACE2-expressing cells exist in almost all testicular cell types, and sertoli cells have the highest expression level and positive cell ratio (Liu X. et al., 2020). Zhengpin Wang et al. also confirmed that the human testis is a potential target for SARS-CoV-2 from the level of single-cell transcription (Wang and Xu, 2020). Thus, SARS-CoV-2 causes damage to multiple organs of the host through various factors, especially hypertension, the elderly, obesity, male individuals with severe cardiovascular disease and those with blood group A (Menter et al., 2020). Therefore, the integration of multiple disciplines to carry out comprehensive diagnoses and treatment for patients with COVID-19 is warranted.

CONVENTIONAL PREVENTIVE TREATMENT OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2

Although SARS-CoV-2 is widespread and causes multiple organ damage, no specific antiviral drugs or vaccines are currently available. Prevention and supportive care are the main treatment strategies for COVID-19 (Jin et al., 2020). As one of the first affected countries, the epidemic in China has been well controlled, proving the importance of non-drug intervention (NPI), including the isolation of ill persons, contact tracing, quarantine of exposed persons, travel restrictions, school and workplace closures, cancellation of mass gatherings, and hand washing, among others (Lai et al., 2020). At present, there are mainly the following categories of drugs used to treat COVID-19, including antiviral drugs (e.g., remdesivir), antibodies (e.g., convalescent plasma), anti-inflammatory drugs (e.g., dexamethasone), and targeted immunomodulatory therapy (e.g., tocilizumab) (Gautret et al., 2020). Most antiviral drugs currently used to treat COVID-19 were initially developed for influenza, Ebola, or SARS/MERS. Convalescent plasma might be a potential therapy for critically ill patients infected with SARS-CoV-2 (Zhang and Liu, 2020). As a monoclonal antibody against inflammatory cytokines, tocilizumab has emerged as an alternative treatment for COVID-19 patients with a risk of cytokine storms (Luo P. et al., 2020). In addition, for the use of corticosteroids in the treatment of COVID-19, a study has been found that corticosteroids can reduce pulmonary immune inflammatory responses, but delay viral clearance, and systemic use is not recommended in patients with SARS-CoV-2 infection (Russell et al., 2020). However, another controlled, open-label trial found that dexamethasone use resulted in lower 28-days mortality in patients randomized to invasive mechanical ventilation or oxygen alone, compared with no benefit in patients with shorter duration of symptoms and no need for supplemental oxygen (Horby et al., 2020). The development of vaccines is the key to halting the spread of the virus. Thus, clinical trials have been carried out in many countries, among which the fastest progress concerns adenovirus vector vaccines, mRNA vaccines, DNA vaccines and inactivated vaccines, which have entered the clinical stage (Amanat and Krammer, 2020).

APPLICATION OF TRADITIONAL CHINESE MEDICINE TO PREVENT AND TREAT CORONAVIRUS DISEASE 2019

According to the History of Chinese Epidemics published by the Chinese Academy of Traditional Chinese Medicine, 321 epidemics have occurred in China in the past 2000 years since the Western Han Dynasty and have been resisted effectively by TCM (Medicine, 2003.06). The efficacy of TCM has been confirmed in the process of fighting severe acute respiratory syndrome (SARS) in 2003 (Chen and Nakamura, 2004; Zhang et al., 2004). TCM has achieved phased victories in China. According to statistics released by the China Administration of Traditional Chinese Medicine, 92.58% of the confirmed COVID-19 patients nationwide were treated with TCM, and the effective rate of TCM participation reached 95.10% up to March 3 (Medicine NAoTC, 2020h). TCM has therapeutic effects on all clinical stages of COVID-19 (Xiang et al., 2020). For mild and ordinary stages, TCM can relieve clinical symptoms (fever, shortness of breath, cough, diarrhea), shorten the course of the disease, improve the cure rate, and prevent the disease from becoming severe. For severe and critical cases, combined with supportive therapy such as oxygen therapy and fluid replacement in modern medicine, TCM can enhance pulmonary ventilation function and inhibit lung injury and an excessive immune response. In the rehabilitation stage, patients usually still have deficiencies in qi and yin. Thus, taking TCM compound decoctions with the effects of supplementing qi, nourishing yin and strengthening the spleen and tonifying the lung can help to restore physical strength, promote the absorption of pulmonary inflammation and reduce pulmonary interstitial fibrosis (Ang et al., 2020a; Liu, 2020). The successful experience of fighting COVID-19 has shown that TCM has a unique advantage in infectious diseases.

IDENTIFICATION OF ANTI-NOVEL CORONAVIRUS COMPOUNDS ISOLATED FROM TRADITIONAL CHINESE MEDICINE

The development of new agents against COVID-19 is not realistic to pass safety and toxicity tests in a short period and is both time-

TABLE 1 | Compounds with anti-coronavirus activity.

Compounds	Pharmacological action	Mode of action	References
Hesperetin	Antioxidation, anti-inflammation	3CLpro, Mpro, RBD- Spro	(Parhiz et al., 2015; Adem et al., 2020)
Baicalin	Anti-inflammatory, antioxidant, neuro-protective	ACE2	(Hwang et al., 2005; Chen and Du, 2020b)
Curcumin	Antioxidant, anti-inflammatory, anti-virus, anti-cancer	PLpro, RBD-Spro, protease domain-ACE2	(Moghadamtousi et al., 2014; Goswami et al., 2020)
Glycyrrhizic acid	Antiviral, antioxidant, immunomodulatory, cell membrane stabilization	ACE2	(Pang et al., 2016; Chen and Du, 2020b)
Luteolin	Antioxidation, anti-inflammation, anti-tumor	3CLpro, RdRp, PLpro, Spro	(Xiong et al., 2017; Yu et al., 2020)
Rutin	Anti-inflammation, anti-virus, anti-oxidation, neuroprotective effects	Mpro	(Chua, 2013; Huynh et al., 2020)

consuming and costly. Therefore, active compounds targeting viruses or host targets in existing Chinese herbal medicines were screened by many scientists. Currently, various natural compounds have been found to potentially possess anti-SARS-CoV-2 activity. In this review, we focus on six compounds: hesperetin, baicalin, curcumin, glycyrrhizic acid, luteolin, and rutin. The pharmacological action and targeting targets of these six compounds are shown in **Table 1**.

Hesperetin

Hesperetin, a flavonoid from the pericarp of citrus, has the biological characteristics of antioxidation and anti-inflammation (Parhiz et al., 2015). Hesperetin inhibited the cleavage activity of SARS-CoV 3C-like protease (3CLpro) in a dose-dependent manner, in which the IC_{50} was 8.3 μmol/l in the Vero cell lysis assay (Lin et al., 2005). Using computer virtual screening, hesperetin showed good binding affinity for 3CLpro (Wu et al., 2020a). Additionally, it binds well to COVID-19 Main protease (Mpro) (Adem et al., 2020) and may destroy the interaction between the receptor-binding domain (RBD) and ACE2 by binding to RBD in SARS-CoV-2 S protein (Wu et al., 2020a).

Baicalin

Baicalin is a flavonoid compound extracted and isolated from *Scutellaria baicalensis* Georgi, which has multiple biological activities, including anti-inflammatory, antioxidant, and neuro-protective effects (Hwang et al., 2005; Shi et al., 2017; Guo et al., 2019). Scientists have proven that baicalin has anti-SARS-CoV virus activity *in vitro* using the fetal rhesus kidney-4 (fRhK-4) cell line, in which the EC_{50} was 12.5 μg/ml at 48 h and the Vero-E6 cell line in which the EC_{50} was 100 μg/ml at 48 h; in the plaque reduction assay, using Vero cell line, the EC_{50} of baicalin was 11 μg/ml (Chen et al., 2004). The results of molecular docking showed that baicalin has a strong binding affinity to the ACE2 receptor (Chen and Du, 2020b).

Curcumin

Curcumin, a known phenolic compound extracted from the rhizome of turmeric, showed broad pharmacological effects, including antioxidant, anti-inflammatory, anti-virus and anti-cancer activities (Anand et al., 2008; Aggarwal and Sung, 2009; Moghadamtousi et al., 2014). Curcumin has activity against SARS-CoV replication with concentrations between 3.3 and 10 μmol/l, and inhibitory effects on 3CLpro were observed with IC_{50} values of 40 μmol/l (Wen et al., 2007). The release of IL-1, IL-6 and TNF-α in the treatment of patients with a cytokine storm was blocked by curcumin (Sordillo and Helson, 2015). The results of molecular docking using virtual screening showed that curcumin possess good affinity to PLpro, the RBD of spike glycoprotein (RBD-S), and the ACE2 receptor at the protease domain (PD-ACE2) (Goswami et al., 2020; Utomo and Meiyanto, 2020).

Glycyrrhizic Acid

Glycyrrhizic, also called glycyrrhizin, is one of the most important active ingredients in *Glycyrrhiza glabra* L., which has many

biological activities such as antiviral, antioxidant, immunomodulatory and cell membrane stabilization (Yong and Haji, 2010; Akman et al., 2015; Pang et al., 2016). In addition to inhibition of SARS-CoV replication, glycyrrhizin inhibits the adsorption and penetration of the virus and is less effective during the adsorption period (EC_{50}, 600 mg/l) and after the virus adsorption period (EC_{50}, 2,400 mg/l) and most effective both during and after the adsorption period (Cinatl et al., 2003). Chen et al. used systems biology tools to identify that a novel combination of vitamin C, curcumin and glycyrrhizic acid (VCG Plus) may regulate the immune response against SARS-CoV-2 infections by acting on NOD-like and Toll-like signaling pathways and inhibit excessive inflammatory responses to prevent the onset of a cytokine storm by inhibiting PI3K/AKT, NF-κB and MAPK signaling pathways (Chen L. et al., 2020). The activity of ACE2 in cells may be regulated by the binding of glycyrrhizin to ACE2, 3CLpro, Spike, PLpro, and RdRp (Huang F. et al., 2020; Chen and Du, 2020b).

Luteolin

As a natural flavonoid, luteolin not only has multiple effects such as antioxidation and anti-inflammation but also inhibits the proliferation of tumor cells (Xiong et al., 2017; Yu et al., 2019). Luteolin dose-dependently inhibited the cleavage activity of SARS coronavirus, and the EC_{50} value was 10.6 μmol/l (Yi et al., 2004). An *in vitro* study showed that luteolin could inhibit SARS-CoV 3CLPro, with an IC_{50} of 20.2 μmol/l (Ryu et al., 2010). Through molecular docking, luteolin showed strong interactions with the targets of SARS-CoV-2, including 3CLpro, RdRp, PLpro, and Spro (Yu et al., 2020).

Rutin

Rutin, a type of flavonoid, is an effective component of Lianhua Qingwen, which has many effects, such as anti-inflammation, anti-virus, anti-oxidation, and neuroprotective effects (Javed et al., 2012; Chua, 2013). Various RNA viruses, including influenza A virus (IAV) and enterovirus A71 (EV-A71), are inhibited by rutin (Savov et al., 2006; Lin et al., 2012). Molecular dynamics simulation suggests that rutin can bind stably to the Mpro' pocket of SARS-CoV-2 and block the binding of substrate in space (Huynh et al., 2020).

Through the enumeration of several compounds, it was found that flavonoids (e.g., hesperetin, baicalin and luteolin) have shown outstanding in fighting against the COVID-19. Flavonoids, a class of secondary metabolites produced by plants in the process of long-term natural selection, have a variety of pharmacological activities including antiviral, anti-inflammatory, cardiovascular and cerebrovascular disease prevention, antioxidation and anti-tumor, etc (Panche et al., 2016; Brodowska, 2017). Its unique and common chemical structure, i.e., C6-C3-C6 consisting of 2 aromatic rings (A and B) linked by a three-carbon chain (Kumar and Pandey, 2013). The hydroxylation pattern of the B ring of certain flavonoids enhanced the inhibitory effect of mast cells and macrophages on cytokine secretion (Ginwala et al., 2019). Flavonoids exerts express their anti-inflammatory activity by inhibiting the synthesis and activity of pro-inflammatory mediators (e.g., eicosanoids and cytokines), inhibiting the activation of transcription factors (e.g., NF-kappaB and activating protein-1) and modulation of proinflammatory gene expression (Kim et al., 2004; Serafini et al., 2010). High affinity binding between flavonoids and S protein, helicase, and protease sites on ACE2 resulting in conformational change, thereby inhibiting viral entry of SRAS-CoV-2. In addition, saikosaponins have great potential in the treatment of COVID-19 through immunomodulatory, anti-inflammatory and antiviral activities. On the one hand, saikossaponins exhibit anti-inflammatory effect by dose-dependently inhibiting the production of several inflammatory mediators which are responsible for the cytokine storm of severe COVID-19 patients, and immunomodulatory effect by inhibiting the proliferation of activated T lymphocytes (Bahbah et al., 2020). On the other hand, it can also directly bind to ACE2 to play the role of anti-SARS-CoV-2 (Yan Y.-M. et al., 2020). In short, most of the naturally active compounds reviewed by us show antiviral and anti-inflammatory effects and may possess anti-SARS-CoV-2 effects according to the results of computer simulation and *in vitro* experiments, but further *in vivo* and clinical trials are needed to verify.

APPLICATION OF ANTI- CORONAVIRUS DISEASE 2019 TRADITIONAL CHINESE MEDICINE HERBAL FORMULAS

In addition to compounds, many TCM herbal formulas are also widely used in the treatment of COVID-19. The formula therapy of TCM diagnosis and treatment has the advantages of multi-target and multi-link treatment. TCM herbal formulas such as Qingfei Paidu decoction, Huashi Baidu recipe, Lianhua Qingwen capsule and Xuebijing injection have demonstrated curative effects on COVID-19 (**Table 2**) (Medicine NAoTC, 2020h). Among them, the clinical stages are mainly based on the Guideline on Diagnosis and Treatment of Coronavirus disease 2019 in China (Medicine NAoTC, 2020f).

Jin Hua Qing Gan Granule

Jin Hua Qing Gan granule (JHG) have antiviral and immune regulation effects (Jimilihan, 2020). However, according to the current study, JHG plays an anti-COVID-19 effect mainly through modulating cytokine storm associated with COVID-19 mortality and acting directly on the virus itself (Ruan et al., 2020). The arachidonic acid (AA) metabolic pathway is mainly used to synthesize inflammatory cytokines; thus, inhibiting the AA metabolic pathway is beneficial to reduce the "cytokine storm." Ren Y et al. showed that JHG might be anti-COVID-19 by treating the cytokine storm based on the AA metabolic pathway (Ren et al., 2020). A recent retrospective analysis test showed JHG can effectively shorten the duration of nucleic acid detection and promote the absorption of pneumonia inflammatory exudate without obvious adverse reactions in patients with COVID-19 (Liu Z. et al., 2020). A systematic analysis of multiple prospective randomized controlled trials provide evidence to determine that JHG is an efficacy and safety treatment for COVID-19 (Chen H. et al., 2020). JHQ is

mainly used for COVID-19 patients with fever, cough, fatigue, headache and runny nose (Zhuang et al., 2020). At present, no adverse reactions reported. According to the recommended dosing methods of National Administration of Traditional Chinese Medicine, JHG is dissolved in boiling water and a bag or two 3 times a day for 5 or 7 days (Medicine NAoTC, 2020c).

Lian Hua Qing Wen Capsule

Lian Hua Qing Wen capsule (Lianhua Qingwen) is developed from the two classical formulas Fang Ma Xing Shi Gan Tang and Yinqiao Powder. In addition to improving the clinical symptoms of COVID-19 patients through anti-inflammation, Lianhua Qingwen can also act directly on the virus itself. *In vitro* studies have shown that Lianhua Qingwen can significantly inhibit the replication of SARS-CoV-2 in Vero E6 cells and significantly reduce the production of proinflammatory cytokines such as TNF-α, IL-6, CCL-2/MCP-1 and CXCL-10/IP-10. On the other hand, it affects the morphology of the virus at the mRNA level (Runfeng et al., 2020). The efficacy of Lianhua Qingwen in the treatment of H1N1 infection is similar to that of oseltamivir in terms of disease duration and virus shedding (Duan et al., 2011). A prospective, multicenter, open-label, randomized controlled trial including 284 confirmed COVID-19 (142 each in the treatment and control groups) displayed that add-on Lianhua Qingwen led to a shorter recovery time of fever, fatigue and coughing, a higher rate of improvement in chest computed tomographic manifestations and higher clinical cure; meanwhile, no serious adverse reactions were reported (Hu et al., 2020). Furthermore, Lianhua Qingwen was effective in improving clinical symptoms such as fever, shortness of breath, anorexia, fatigue and cough in COVID-19 patients, reported on two retrospective cohorts (Lu et al., 2020; Yao et al., 2020). In summary, Lianhua Qingwen was effective in improving the fever, cough, and fatigue of COVID-19, and no serious or adverse drug reactions were reported. The recommended dose is four capsules three times a day for 14 days (Hu et al., 2020; Medicine NAoTC, 2020c).

Xue Bi Jing Injection

The main components of Xue Bi Jing injection (XBJ) are *Carthamus tinctorius* L., *Paeonia lactiflora* Pall., *Conioselinum anthriscoides "Chuanxiong," Salvia miltiorrhiza* Bunge and *Angelica sinensis* (Oliv.) Diels, which have been widely used for sepsis with no significant adverse events (Yin and Li, 2014). XBJ can not only anti-inflammatory but also direct action on SARS-CoV-2 in treating COVID-19. In terms of anti-inflammatory, XBJ injection reduces inflammatory reaction by down-regulating the expression of TLR4 and NF-κB, preventing lung injury caused by DDVP poisoning (He et al., 2018). XBJ improves survival in septic shock, and its mechanism is also related to inhibition of the immune response by preventing cytokine storm attack and regulating the balance of Tregs/Th17 cells (Chen et al., 2018). Therefore, treatment of COVID-19 with XBJ may be related to cytokine storm treatment (Ren et al., 2020). In terms of acting on the virus, *in vitro* experiments showed that XBJ could block the proliferation of SARS-CoV-2 and protect cells from SARS-CoV-2-induced cell death (Wen et al., 2020). Treating severe community-acquired pneumonia with XBJ can improve the pneumonia severity index, reduce the mortality, reduce the time of mechanical ventilation and shorten the hospitalization time of ICU stay (Song et al., 2019). Randomized controlled clinical trials revealed that XBJ may improve lung injury in patients with severe or critical COVID-19 (Wen et al., 2020). Another clinical research showed that, XBJ can effectively improve the inflammatory markers (such as white blood cell, lymphocyte count and C-reactive protein) and prognosis of severe COVID-19 patients (Wen et al., 2020). XBJ is mainly applied to treat sepsis, infection-induced systemic inflammatory response syndrome, and multiple organ dysfunction syndrome (Medicine NAoTC, 2020c). Now, there was no reports of any adverse reactions to the treatment. Its usage and dosage is 100 ml XBJ plus 100 ml 0.9% sodium chloride injection for intravenous drip every 12 h for 7 days(Ma et al., 2020b; Medicine NAoTC, 2020c).

Qing Fei Pai Du Decoction

Qing Fei Pai Du decoction (QFPDD) is made by adding and subtracting Ma Xing Shigan Tang, Wuling San, Shegan Mahuang Tang and Xiao Chaihu Tang in Zhang Zhongjing's "typhoid fever." Among them, Ma Xing Shigan decoction interferes with SARS-CoV-2 infection by regulating various complement and coagulation cascades and the thrombin system *in vivo* (Yang et al., 2020). The mechanism of QFPDD against COVID-19 may be related to the regulation of anti-viral, anti-inflammatory activity and metabolic programming. Recently, clinical observation in four provinces of China showed that the total effective rate of Qingfei Paidu decoction in the treatment of COVID-19 patients is more than 90%, in which more than 60% of the patients' symptoms and imaging manifestations improved significantly (n = 214) (Medicine NAoTC, 2020g). Observation of the curative effect of 1,262 cases of COVID-19 in 66 designated units in China demonstrated that it blocks disease progression in critical patients (Medicine NAoTC, 2020e). QFPDD decoction, a only general prescription, can treat all stages (light, ordinary, severe and critical), which has the characteristics of definite curative effect, convenient use, no side effects, and low cost (Medicine NAoTC, 2020d). Recommended treatment options is one dose daily with half of the dose taken in the morning and half in the evening (40 min after meal) with warm water for 3 days, and a course of three doses. The first course of treatment should use the original prescription. If the symptoms improve but are not cured, take the second course, in which the prescription can be modified according to the actual situation. The treatment should be stopped with the symptoms disappear (National Health Commission, 2020; Medicine NAoTC, 2020d).

Other Formulas

The theory in Traditional Chinese Medicine that "lung being connected with large intestine" is associated with the gut-lung axis. TCM alleviates and cures lung diseases by regulating the balance of the intestinal microenvironment of COVID-19 patients (Luo et al., 2020b). Huo Xiang Zheng Qi powder has therapeutic effects on gastrointestinal diseases (Zhao et al., 2018). The plaque reduction test explained that Liu Shen capsule (LS)

TABLE 2 | Clinical application of traditional Chinese medicine in the treatment of Coronavirus disease 2019.

TCM herbal formulas	Constituent	Clinical stage	Therapeutic effect	References
Qing Fei pai Du decoction	*Ephedra sinica* Stapf, *Glycyrrhiza glabra* L., *Prunus amygdalus* Batsch, Gypsum Fibrosum, *Cinnamomum cassia* (L.) J.Presl, *Alisma plantago-aquatica subsp. orientale* (Sam.) Sam., *Polyporus umbellatus* (Pers) Fr., *Atractylodes macrocephala* Koidz., *Thespesia populnea* (L.) Sol. ex Corrêa, *Bupleurum falcatum* L., *Scutellaria baicalensis* Georgi, *Zingiber officinale* Roscoe, *Aster tataricus* L.f., *Tussilago farfara* L., *Iris domestica* (L.) Goldblatt and Mabb., *Asarum sieboldii* Miq., *Dioscorea alata* L., *Citrus* × *aurantium* L., *Pogostemon cablin* (Blanco) Benth	mild, ordinar, severe, critical	"Clear the lung and calm panting" according to TCM theory. Reportedly has anti-inflammatory and lung injury reduction effects	(Xu et al., 2020)
Hua Shi Bai Du recipe	*Ephedra sinica* Stapf, *Pogostemon cablin* (Blanco) Benth., Gypsum Fibrosum, *Prunus amygdalus* Batsch, *Pinellia ternata* (Thunb.) Makino, *Magnolia officinalis* Rehder and E.H.Wilson, *Atractylodes lancea* (Thunb.) DC., *Lanxangia tsao-ko* (Crevost and Lemarié) M.F.Newman and Skornick., *Thespesia populnea* (L.) Sol. ex Corrêa, *Astragalus mongholicus* Bunge, *Paeonia lactiflora* Pall., *Descurainia sophia* (L.) Webb ex Prantl, *Rheum officinale* Baill., *Glycyrrhiza glabra* L	mild, ordinary, severe	"Clear heat and detoxifying, removing dampness" according to TCM theory. Reportedly has cough symptom relief effect	(Chinadaily, 2020; Medicine NAoTC, 2020b)
Huo Xiang Zheng Qi powder	*Perilla frutescens* (L.) Britton, *Thespesia populnea* (L.) Sol. ex Corrêa, *Pinellia ternata* (Thunb.) Makino, *Atractylodes macrocephala* Koidz., *Citrus* × *aurantium* L., *Areca catechu* L., *Angelica dahurica* (Hoffm.) Benth. and Hook.f. ex Franch. and Sav., *Magnolia officinalis* Rehder and E.H.Wilson, *Platycodon grandiflorus* (Jacq.) A.DC., *Pogostemon cablin* (Blanco) Benth., *Glycyrrhiza glabra* L	mild, ordinary	"Harmonize the exterior and interior, and remove dampness" according to TCM theory. Reportedly has anti-inflammation, immune protection and gastrointestinal motility regulation effects	(Zhao et al., 2019)
Jin Hua Qing Gan granule	*Lonicera japonica* Thunb., Gypsum Fibrosum, *Ephedra sinica* Stapf, *Prunus amygdalus* Batsch, *Scutellaria baicalensis* Georgi, *Forsythia suspensa* (Thunb.) Vahl, *Fritillaria thunbergii* Miq., *Anemarrhena asphodeloides* Bunge, *Arctium lappa* L., *artemisia annua* L., *Mentha* × *piperita* L., *Glycyrrhiza glabra* L	mild, ordinary	"Clear heat and detoxifying, and diffuse the lung" according to TCM theory. Reportedly has antiviral and immune regulation effects	(Jimilihan, 2020)
Lian Hua Qing Wen capsule	*Forsythia suspensa* (Thunb.) Vahl, *Lonicera japonica* Thunb., *Ephedra sinica* Stapf, *Isatis tinctoria* L., Gypsum Fibrosum, *Mentha* × *piperita* L., *Pogostemon cablin* (Blanco) Benth., *Houttuynia cordata* Thunb., *Rheum officinale* Baill., *Prunus amygdalus* Batsch, *Glycyrrhiza glabra* L	mild, ordinary	"Clear heat and diffuse the lung, and detoxifying" according to TCM theory. Reportedly has antiviral, anti-inflammatory and immune regulation effects	(Ye et al., 2020)
Xuan Fei Bai Du granule	*Ephedra sinica* Stapf, *Prunus amygdalus* Batsch, *Coix lacryma-jobi* L., *Atractylodes macrocephala* Koidz., *Pogostemon cablin* (Blanco) Benth., *artemisia annua* L., Gypsum Fibrosum, *Reynoutria japonica* Houtt., *Verbena officinalis* L., *Phragmites australis subsp. australis*, *Citrus maxima* (Burm.) Merr., *Descurainia sophia* (L.) Webb ex Prantl, *Glycyrrhiza uralensis* Fisch. ex DC.	mild, ordinary	"Detoxify and remove blood stasis, diffuse the lung, removing dampness, clear heat" according to TCM theory	(Chinadaily, 2020)

(Continued on following page)

TABLE 2 | (*Continued*) Clinical application of traditional Chinese medicine in the treatment of Coronavirus disease 2019.

TCM herbal formulas	Constituent	Clinical stage	Therapeutic effect	References
Xue Bi Jing injection	*Carthamus tinctorius* L., *Paeonia lactiflora* Pall., *Conioselinum anthriscoides "Chuanxiong"*, *Salvia miltiorrhiza* Bunge, *Angelica sinensis* (Oliv.) Diels	Severe, critical	"Dissolve stasis and detoxifying." Immune regulation	(Chen et al., 2018)
Shen Fu injection	*Panax ginseng* C.A.Mey., *Aconitum carmichaeli* Debeaux	Severe, critical	"Restoring yang to rescue collapse and replenishing qi to prevent collapse." Reportedly has anti-inflammatory and immune regulation effects	(Liu et al., 2019)
Tan Re Qing injection	*Scutellaria baicalensis* Georgi, Fel Ursi, *Lonicera japonica* Thunb., Corne Caprae Hirci, *Forsythia suspensa* (Thunb.) Vahl	Severe, critical	"Clear heat and detoxifying, and resolving phlegm" according to TCM theory. Reportedly has antiviral, anti-inflammatory and immune regulation effects	(Jiang et al., 2009)

could significantly inhibit the replication of SARS-CoV-2 in Vero E6 cells (IC_{50} = 0.6024 μg/ml) and reduce the production of pro-inflammatory cytokines, a finding that may be related to the regulation of the expression of key proteins in the NF-κB/MAPK signaling pathway (Ma et al., 2020a). In a clinical study, Tan-re-qing injection was reported to have therapeutic effects on acute lung injury (ALI), reducing the levels of the serum inflammatory factors TNF-α, IL-6 and IL-8, delaying the progress of systemic inflammatory response syndrome (SIRS), slowing down the progress of SIRS and reducing the degree of respiratory distress (Yang et al., 2010). Postresuscitation lung injury was attenuated by Shen-fu injection *in vivo* by inhibiting lung cell apoptosis and improving energy metabolism and antioxidant capacity (Zhang et al., 2012). In addition, through *in vivo* and clinical trials confirmed that miRNA in honeysuckle decoction can be effectively absorbed through drinking, and effectively inhibits SARS-CoV-2 replication *in vivo*, and accelerates the negative conversion of COVID-19 patients (Zhou L.-K. et al., 2020).

COVID-19 belongs to the category of "epidemic" in TCM because of its strong infectivity and fast spread with the characteristics of "dampness, poison and epidemic", whose main site is the lung, involving the spleen and stomach (Luo et al., 2020a). Cold-dampness and dampness-heat in the lung symptoms are shown in mild cases of COVID-19, dampness toxin and cold-dampness obstructing the lung symptoms in ordinary, epidemic toxin obstructing the lung and blazing of both qi and nutrient symptoms in severe, and internal block and external collapse symptoms in critical (Ho et al., 2020). In TCM theory, the properties of herbal drugs include four fundamental characters: cool, cold, warm, and heat and five fundamental tastes: pungent, sweet, bitter, sour, and salty. Among the formulas of COVID-19 issued in China, the four flavors of the drugs for COVID-19 are mainly warm, cold, and flat, the five tastes were mainly bitter, hot, and sweet, and the meridians were mainly lung, stomach, and spleen (Zhou Z. et al., 2020). The pungent medicines are mostly used in the early stage, which the bitter drugs are valued in the middle and severe stage, and the sweet medicines are mostly found in the recovery stage (Gu et al., 2020). For example, JHG granule, Huo Xiang Zheng Qi powder, Lianhua Qingwen capsule and Xuan Fei Bai Du granule are widely used in mild and ordinary patients, with *Ephedra sinica* Stapf, *Pogostemon cablin* (Blanco) Benth and *Carthamus tinctorius* L. and other pungent herbal drugs. XBJ injection, Shen-fu injection and Tan re qing injection are valued in severe and critical patients. It is worth pointing out that Hua Shi Bai Du recipe and QFPDD have the characteristics of mild in nature and taste, having therapeutic effects on all periods. In fact, most of these herbal drugs have the effects of resolving dampness and detoxifying. Among them, *Glycyrrhiza glabra* L., *Scutellaria baicalensis* Georgi, *Ephedra sinica* Stapf, *Pogostemon cablin* (Blanco) Benth, *Carthamus tinctorius* L., *Prunus amygdalus* Batsch and *Magnolia officinalis* Rehder and E.H.Wilson are used more frequently (Gu et al., 2020; Wang C. et al., 2020; Zhou Z. et al., 2020). *Pogostemon cablin* (Blanco) Benth and *Thespesia populnea* (L.) Sol. ex Corrêa are frequently used to treat medical ailments, such as removing dampness (Yang et al., 2016; Lv et al., 2018). *Lonicera japonica* Thunb. and *Glycyrrhiza glabra* L. are popular with efficacy of clearing heat and detoxifying used in TCM (Nomura et al., 2002; Cao et al., 2012). However, presently studies on the treatment of COVID-19 TCM herbal formulas were mostly *in vivo* and *in vitro* studies, retrospective studies and case-control trials; few rigorous randomized controlled trials (RCTs) were carried out; thus, more RCTs should be carried out.

Integrated Traditional Chinese and Western Medicine for Coronavirus Disease 2019

Due to the different research methods and theoretical systems, traditional Chinese medicine and western medicine have their unique characteristics and advantages. TCM focuses on an overall approach to the analysis of illness and the patient's condition, and carries out diagnosis and treatment from integrative and holistic points of view. Different from TCM, western medicine focuses on the common law of diseases and treats it through analysis. Integrated traditional Chinese and western medicine (abbreviated as "integrated medicine") for COVID-19 has been successful in China. Respiratory support and circulatory support are important treatment methods in western medicine, while TCM has shown beneficial effects in improving clinical symptoms, immune regulation and organ protection (Huang Y.-F. et al., 2020). Clinical studies have shown that integrated medicine tends to decrease the mortality rate of SARS (Zhang et al., 2004) and is superior to western medicine in improving the

clinical symptoms of COVID-19 patients (Zhang et al., 2020). Nelfinavir in combination with spilanthol to treat COVID-19 was found to enhance its ability to control viral proliferation and improve the risk of disease progression and transmission (Ohashi et al., 2020). A systematic review and meta-analysis of the efficacy and safety of integrated medicine for COVID-19 including 11 studies from six databases revealed that the overall response rate ($p = 0.000$), cure rate ($p = 0.002$), severity illness rate ($p = 0.012$), hospital stay ($p = 0.002$) and clinical symptom improvement rate ($p < 0.05$) were better than those of western medicine alone (Liu M. et al., 2020). Another systematic review and meta-analysis showed that integrated medicine significantly improves the total effective rate, cough symptom disappearance rate, and sputum production symptom disappearance rate (Ang et al., 2020b). Therefore, integrated medicine can improve the clinical efficacy and has good prospects.

Application of Modern Science and Technology in the Treatment of Coronavirus Disease 2019 With Traditional Chinese Medicine

Due to the limitations of P3/P4 experimental conditions, the use of computer network technology has increased in drug research and development, such as network pharmacology, artificial intelligence, and CRISPR technology, which can accelerate drug research and development, reduce research costs and save research resources.

Study on the Mechanism of Network Pharmacology in Traditional Chinese Medicine

Network pharmacology, a new discipline based on the theory of systems biology and biological system network analysis, clarifies the mechanisms of multi-component, multi-target, and multi-pathways. Both TCM and network pharmacology emphasize a comprehensive understanding of diseases. Kaempferol, baicalein and oroxylin A in JHG might regulate various signaling pathways (such as PTGS2, BCL2 and CASP3) by binding to ACE2, thus playing a therapeutic role in the treatment of COVID-19 (Jimilihan, 2020). The mechanism of Lianhua Qingwen in the treatment of COVID-19 may be to improve human immunity by participating in T-cell and B-cell receptor signal transduction and natural killer cell-mediated cytotoxicity, as well as to exert anti-inflammatory effects through Fc epsilon RI, ErbB, ErbB, MAPK and other signal pathways (Ye et al., 2020). The protein interaction map screened 66 potential targets (e.g., Nsp1, Orf10, Spro, and Npro) and 69 potential drugs (e.g., chloroquine and azithromycin) of SARS-CoV-2 (Gordon et al., 2020).

Artificial Intelligence Helps Coronavirus Disease 2019 Traditional Chinese Medicine Research

Artificial intelligence (AI) has gradually become an important factor affecting the development of the pharmaceutical industry and has been widely used in numerous medical fields, such as intelligent medical robots, voice intelligence diagnosis and treatment, medical and health management systems, and drug research and development. AI is widely used in the prevention and control, diagnosis and treatment of COVID-19. Regarding prevention and control, through establishing a scientific model of an infectious disease transmission mechanism, a significant effect of community prevention and control policies will occur when 40–60% of the group abide by the policy. Additionally, a travel restriction policy can effectively reduce the risk of disease transmission caused by an insufficient proportion of people who abide by the policy (St-Onge et al., 2020). In terms of diagnosis, an AI model using CT images to assist in the diagnosis of COVID-19 was successfully established, with a total accuracy of 83% and that is rapid and efficient (Wang S. et al., 2020). Concerning treatment, AI is mainly used in the design and screening of small-molecule drugs. Baritinib, an effective immunosuppressant approved by the U.S. Food and Drug Administration (FDA), has been predicted to reduce the ability of novel coronavirus to infect lung cells (Richardson et al., 2020). Glycyrrhizin significantly inhibits the replication of SARS-CoV-2 in Vero E6 cells by imitating type I interferon (EC_{50} = 2.39 μmol/l) (Zhu J. et al., 2020). Professor Li Hua's research team proved that *Platycodon grandiflorum* saponins D and baicalin had high affinity with PLpro, and andrographolide and its derivatives with 3CLpro and RdRp by gene sequence comparison, homologous modeling and computer virtual target screening (Wu et al., 2020b).

Other Modern Science and Technologies

Many other modern science and technologies are also used in COVID-19 in addition to network pharmacology and AI. Molecular docking techniques have been used to predict the ACE2-binding abilities of baicalin, scutellarin, hesperetin, nicotine, and glycyrrhizin, which are possible therapeutic agents for COVID-19 (Chen and Du, 2020a). A prophylactic antiviral CRISPR in human cells (PAC-MAN) for viral inhibition has been developed that can effectively degrade RNA from SARS-CoV-2 sequences and live influenza A virus (IAV) in human lung epithelial cells (Abbott et al., 2020). The proteome and metabolite profiles of 99 serum samples (53 control vs. 46 COVID-19) were obtained by high-resolution mass spectrometry, and the results showed that molecular changes in the serum of patients with severe COVID-19, such as macrophage dysfunction, platelet degranulation, the complement system pathway and global metabolic inhibition (Shen et al., 2020). Moreover, Watanabe et al. (2020) used a site-specific mass spectrometric approach to map the glycan-processing states of SARS-CoV-2 S protein. Proteomics revealed changes in the protein levels and pathways in host cells infected with SARS-CoV-2 and demonstrated that SARS-CoV-2 replication in cells could be prevented by inhibiting compounds of these pathways (Bojkova et al., 2020). Single-cell RNA sequencing (scRNA-seq) used to analyze the peripheral immune response of patients with severe COVID-19, the results showed that peripheral immune cell phenotype was reconfigured and peripheral blood monocytes and lymphocytes did not express a large number of pro-inflammatory cytokines indicating that they were not involved in the cytokine storm (Wilk et al., 2020). Using RNAscope technique, it was found that the expression

levels of ACE2 and TMPRSS2 in nasal epithelial cells were significantly higher than the lower respiratory tract indicating the nasal susceptibility to SARS-CoV-2. Furthermore, the increase of $CD68^{+}$ and CD163 + macrophages in inflammatory infiltrating pulmonary parenchyma cells was observed based on RNAscope technique in a rhesus macaque model of SARS-CoV-2 infection (Chandrashekar et al., 2020).

Modern science and technology have not only played important roles in fighting the COVID-19 epidemic but have also provided an objective theoretical basis for the clinical application of TCM.

DIFFICULTIES AND SOLUTIONS FACED BY THE MODERNIZATION OF TRADITIONAL CHINESE MEDICINE

TCM is not only one of the greatest treasures of ancient Chinese science but also the key to the treasure house of Chinese civilization. Different from western medicine, a very important point is that TCM upholds the theory of "treat disease before it arises." The theory emphasizes disease prevention, as well as early diagnosis and treatment to prevent aggravation of the disease. TCM has been administered early, widely and in many participants, reflecting its unique value in the prevention and control of COVID-19 epidemic. TCM can inhibit the growth of the virus in the body, relieve patients' symptoms and prevent serious development of the disease with the functions of clearing heat, removing dampness and detoxification (Medicine NAoTC, 2020e). According to Chinese news reports, TCM has been used in more than 90% of COVID-19 patients (Liu, 2020), and the effective rate of integrated medicine is more than 92% in Beijing (Medicine NAoTC, 2020a). Because of the lack of convincing and strict experimental data, the therapeutic effect and safety of TCM have been questioned and is the main problem in the development and modernization of TCM.

Presently, most clinical studies of TCM are case reports and case-control studies, with a lack of rigorous and scientific randomized controlled trials (RCTs). Thus, the findings lack strong scientific evidence to be widely recognized. To address this problem, the TCM Clinical Evidence database (http://www.tcmevd.com/evidence/index) has been launched on April 22, 2020. Additionally, attention should be focused on standardized clinical studies of TCM, and communication between evidence-based medicine fields domestically and abroad should be strengthened. Moreover, the mysterious veil of TCM can be lifted, and the modern medical systems can be enriched and improved with the help of modern science and technology. TCM has many ingredients, unclear effective activity and complex mechanisms; thus, it is difficult to effectively guarantee its safety. Fortunately, these drawbacks can be clarified with modern science and technology, such as network pharmacology, molecular docking technology and data mining (Ye et al., 2020). The process and dynamic changes of TCM *in vivo* can be confirmed through pharmacokinetic and metabonomic studies. AI has contributed to the improvement of the service system of modern traditional Chinese medicine (Wang S. et al., 2020). Importantly, the lack of objective evaluation criteria for the diagnosis and treatment of TCM is a serious problem. TCM diagnosis refers to collecting information on clinical symptoms and signs using four diagnostic processes—looking, listening and smelling, asking and cutting—which are very subjective and closely related to personal experience. Thus, the information in the process of diagnosis should be standardized and digitized, and a scientific diagnostic standard and a systematic evaluation system should be established. TCM is gradually being adopted worldwide because of its unique advantages and efforts by the Chinese, and is expected to make positive contributions to the health of all people globally.

CONCLUSION AND PROSPECT

With the outbreak of COVID-19 epidemic, great efforts are being made to understand the pathogenesis and clinical characteristics of the disease to identify effective drugs and vaccines. However, no specific antiviral drugs and vaccines are available currently. Symptomatic and supportive therapies are the main treatment approaches, including general therapeutic measures, antiviral therapy and respiratory support. The history of TCM fighting against epidemics has spanned thousands of years. In China, TCM has been widely and deeply involved in the diagnosis and treatment of COVID-19 and has played a positive role in this war. Treatment using integrated traditional Chinese and western medicines has achieved success, and modern science and technology have made significant contributions. COVID-19 treatment results have shown that TCM is effective in relieving symptoms, improving the cure rate, reducing mortality, and promoting the rehabilitation of convalescent people, without world recognition. Presently, TCM diagnosis and treatment still lack objective evaluation criteria, its efficacy lacks strong scientific evidence, and its mechanisms of action are unknown. To realize the modernization of TCM, we should focus on evidence-based medicine, combined with science technology, and establish standards of diagnosis and treatment. We hope that, through the contribution of TCM, combined with modern technological research and the support of our international counterparts, COVID-19 can be effectively controlled and treated.

AUTHOR CONTRIBUTIONS

LL conceived and designed the review; QQ, FH, XHL, XLL, and LL wrote the manuscript; LL, YH, HL, and LC reviewed the paper and provided comments, and all of the authors reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.570893/full#supplementary-material

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REVIEW
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Phytochemicals: Potential Therapeutic Interventions Against Coronavirus-Associated Lung Injury

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Since the outbreak of coronavirus disease 2019 (COVID-19) in December 2019, millions of people have been infected and died worldwide. However, no drug has been approved for the treatment of this disease and its complications, which urges the need for finding novel therapeutic agents to combat. Among the complications due to COVID-19, lung injury has attained special attention. Besides, phytochemicals have shown prominent anti-inflammatory effects and thus possess significant effects in reducing lung injury caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Also, the prevailing evidence reveales the antiviral effects of those phytochemicals, including anti-SARS-CoV activity, which could pave the road in providing suitable lead compounds in the treatment of COVID-19. In the present study, candidate phytochemicals and related mechanisms of action have been shown in the treatment/protection of lung injuries induced by various methods. In terms of pharmacological mechanism, phytochemicals have shown potential inhibitory effects on inflammatory and oxidative pathways/mediators, involved in the pathogenesis of lung injury during COVID-19 infection. Also, a brief overview of phytochemicals with anti-SARS-CoV-2 compounds has been presented.

Keywords: coronaviruses, lung injury, phytochemicals, COVID-19, signaling pathway

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INTRODUCTION

The complex pathophysiological mechanisms behind viral diseases, along with the associated side effects of the present conventional drugs, urge the need for introducing alternative treatments. Among viral infections, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome (MERS-CoV), and the newest human CoVs (HCoVs) associated with the outbreak of coronavirus disease 2019-SARS-CoV-2 (COVID-19) have caused acute respiratory distress syndrome (Sharma et al., 2020). Based on the pathological findings, the inflammatory cytokines/signaling pathways lead to pulmonary edema and, ultimately, lung injury in COVID-19 patients (Merad and Martin, 2020). Considering their potential effects in targeting several dysregulated mediators, phytochemicals could be auspicious agents in the treatment/management of various diseases (Mani et al., 2020). The medicinal plants and phytochemicals target multiple proinflammatory and oxidative mediators such as tumor necrosis factor-α (TNF-α),

interleukin- (IL-) 1β, IL-6, IL-8, matrix metalloproteinases (MMPs), nuclear factor-kappa B (NF-κB), mitogen-activated protein kinase (MAPK), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and reactive oxygen species (ROS). Therefore, owing to the involvement of inflammation and oxidative stress in the pathogenesis of lung injury, phytochemicals have attracted particular attention to providing novel agents in combating coronaviruses and related complications (Bellik et al., 2012; Cornélio Favarin et al., 2013b). This article presents an overview of phytochemicals, including alkaloids, coumarins, polyphenols, especially flavonoids, quinones, and terpenes to show noticeable effects against lung injury. Therefore, they could be introduced as ameliorative agents against SARS-CoV-2-induced lung injury. Moreover, based on their simultaneous antiviral and preventive effects against lung injury, some phytochemicals such as matrine, cepharanthine, osthole, wogonin, myricetin, and triptolide have been also provided as promising candidates in the management of COVID-19. In general, this review article aims to introduce phytochemicals as potential therapeutic agents against coronavirus complications, focusing on lung injury.

CORONAVIRUSES AND PATHOGENESIS: FOCUSING ON LUNG INJURY

In striking contrast to the history of HCoVs, as relatively harmless respiratory pathogens, the outbreak of SARS and the emergence of MERS pose the CoVs as important pathogens in respiratory tract infections. SARS-CoV, MERS-CoV, and SARS-CoV-2 can cause clinical complications leading to severe diseases presented as acute respiratory distress syndrome (ARDS) (Yin and Wunderink, 2018). HCoVs contain single-stranded, polycistronic RNA genomes of positive polarity (~30 kb). These viral genomes are translated into multiple nonstructural proteins (ORF1a and ORF1b), structural proteins (S, E, M, and N), and lineage-specific accessory proteins showing differences in these viruses. For instance, in the case of SARS-CoV, accessory proteins include ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8a, ORF8b, and ORF9b (Fung et al., 2020).

The most common clinical symptoms in SARS-CoV-2 include fever, cough, dyspnea, fatigue, headache, myalgia, and diarrhea. Some patients afterward suffer from shortness of breath and recurrent or ongoing fever. In nearly 13% of patients, intense care treatment (e.g., mechanical ventilation) should be applied (Lai et al., 2020; Wang D. et al., 2020). The pathobiology of SARS-CoV-2 and related molecular mechanisms behind the coronavirus-associated lung injury are not yet completely understood; however, the role of some key molecular intermediates are not deniable (Marini and Gattinoni, 2020). Among those signaling mediators, TNF-α, IL-1, IL-6, IL-8, and IL-1β, NF-κB, MMPs, MAPK, and COX-2 seem to play critical roles in the pathogenesis of COVID-19 and associated lung injury (Fakhri et al., 2020b; Liu Y. et al., 2020; Merad and Martin, 2020). In terms of ROS, iNOS, as well as nuclear factor erythroid 2-like 2 (Nrf2), autophagy-related molecules (LC-3II, Atg5, and Beclin1), and Janus kinase-signal transducers and activator of transcription (JAK/STATs) pathway have shown an important role (Seif et al., 2017). From the other point of view, the extracellular signal-regulated kinase (ERK) and protein kinase B (Akt) signaling pathways are of the other dysregulated mediators following lung injury (Mo et al., 2014; Tsai et al., 2015; Jin et al., 2018). In COVID-19 patients, angiotensin-converting enzyme 2 (ACE2) receptor, located on alveolar epithelial cells, has attracted growing attention, as a high-affinity receptor and cotransporter for SARS-CoV-2 entrance to the lung (South et al., 2020; Ziai et al., 2020). Dysregulation of ACE2/Ag (1–7)/Mas receptor and ACE1/Ag II/Ag II type 1 receptor pathways could enhance ACE2, thereby increasing the chances of the viral entry (Rico-Mesa et al., 2020; South et al., 2020). Besides, the dysregulation of ACE2 by SAR-CoV-2 infection inhibits the degradation of Ag II into angiotensin (Ag) (1–7), exacerbates inflammation, and leads to vascular permeability, as well as cardiovascular/lung complications (Leung et al., 2020; South et al., 2020). Based on the pathological findings, an edematous lung with increased weight was also observed in this disease (Ding et al., 2003; Nicholls et al., 2003). Large multinucleated cells (macrophages and pneumocytes) and atypical enlarged pneumocytes comprise large nuclei, prominent nucleoli, and amphophilic granular cytoplasm, which have often been observed in the lungs of SARS patients. However, none of these signs can be considered as a unique feature of SARS-related pathology. The other pathological features usually observed in SARS include squamous metaplasia of bronchial and alveolar epithelial cells; cilia loss of bronchiolar epithelial cells; subpleural multiplication of fibrogranulative tissue in small airways and airspaces; vascular injury hemophagocytosis in residing mononuclear cells in pulmonary tissue; and apoptosis in epithelial cells, lymphocytes, monocytes/macrophages, and pneumocytes (Gu and Korteweg, 2007). Apart from a respiratory infection, gastrointestinal and central nervous system (CNS) infection was also reported in some patients suffering from SARS (Fung et al., 2020). Additionally, in most SARS autopsies, both extensive necrosis of the spleen and atrophy of the white pulp were reported. Reduction of $CD4^+$, $CD8^+$, and $CD20^+$ lymphocytes, dendritic cells, macrophages, and natural killer cells residing in the spleen, as well as atrophy and decrement of the lymph nodes lymphocytes, were often observed. The presence of SARS-CoV was also confirmed in circulating monocytes and T lymphocytes and to some degree in B lymphocytes and natural killer cells (Chong et al., 2004; Gu et al., 2005). The liver is another organ that is affected during the course of this disease. For example, the increment of serum alanine aminotransferase level in SARS patients was associated with some adverse outcomes. Besides, hemophagocytosis or bone marrow hypoplasia, destruction of epithelial cells in the thyroid glands, myofiber necrosis and atrophy of skeletal muscle tissue, and necrosis and vacuities of the adrenal medulla can occur in some SARS patients (Gu and Korteweg, 2007).

PHYTOCHEMICALS AGAINST CORONAVIRUSES

Since the outbreak of COVID-19 happened, several researchers have focused on the use of natural compounds for the treatment of related complications. Most of those studies are *in vitro* and *in vivo* screening of phytochemicals against coronaviruses (especially SARS-CoV-2), computer docking models studies on predicting the anti-CoVs effects of these compounds against the coronavirus family members such as SARS-CoV, MERS-CoV, and SARS-CoV-2 (Mani et al., 2020; Zhang D.-h. et al., 2020). According to those studies, natural polyphenol compounds such as quercetin (Chiow et al., 2016), kaempferol (Schwarz et al., 2014), myricetin (Yu et al., 2012), apigenin (Ryu et al., 2010a), and resveratrol (Wahedi et al., 2020) have prominent activities against coronaviruses. Cho and coworkers showed that the geranylated flavonoids (tomentin A-E) isolated from *Paulownia tomentosa* (Thunb.) Steud. (Paulowniaceae) inhibited the papain-like protease as a vital enzyme for SARS-CoV propagation (Cho et al., 2013). In addition, three flavonoid compounds including apigenin-7-O-rhamnoglucoside, herbacetin, and pectolinarin at the concentration of 20 μM blocked the crucial enzyme for SARS-CoV replication, 3C-like protease (Jo et al., 2020). Also, 3C-like protease was inhibited with ten polyphenols isolated from *Broussonetia papyrifera* (L.) L'Hér. ex Vent. (Moraceae), especially with papyriflavonol A at 3.7 μM (Park et al., 2017). On the other hand, the molecular docking study on traditional Chinese medicinal compounds against SARS-CoV-2 showed that the theaflavin, as a flavonoid compound isolated from black tea, *Camellia sinensis* (L.) Kuntze (Theaceae), via the inhibition of the SARS-CoV-2 RNA-dependent RNA polymerase, can exert anticoronavirus activities (Lung et al., 2020). Also, hesperidin, which is abundant in citrus, has shown a potential inhibitory effect on ACE2; thereby it could be a good candidate for clinical trials on SARS-CoV-2 (Haggag et al., 2020). Besides, alkaloids have also shown antiviral effects against coronaviruses. Lycorine, as an indolizidine alkaloid isolated from *Lycoris radiata* (L'Hér.) Herb. (Amaryllidaceae), showed anti-SARS-CoV activities at 15.7 nM (Li et al., 2005). Gyebi and coworkers introduced the 10-hydroxyusambarensine, an indole alkaloid isolated from *Strychnos usambarensis* Gilg ex Engl. (Loganiaceae), and 6-oxoisoiguesterin, a bisnorterpenes isolated from *Salacia madagascariensis* (Celastraceae) as the anti-SARS-CoV-2 agents, through their highest affinity in binding to 3C-like protease obtained from a docking study (Gyebi et al., 2020). Tetrandrine (0.33 μM), fangchinoline (1.01 μM), and cepharanthine (0.83 μM), bisbenzylisoquinoline alkaloids of *Stephania tetrandra* S. Moore (Menispermaceae), are other phytochemicals that showed the anti-HCoV activities (Kim et al., 2019). Sanguinarine, palmatine, berberine, chelidonine, jatrorrhizine, ipecac alkaloids, and emetine are other alkaloids that are suggested as anti-SARS-CoV-2 agents (Bleasel and Peterson, 2020; Wink, 2020). Besides, among other anti-CoVs phytochemicals saikosaponins (A, B2, C, D) as triterpene saponin glycosides of Bupleurum spp. (Apiaceae) have shown hopeful results. The saikosaponin B_2 at 6 μM, in addition to possessing an anti-CoV effect, also showed inhibitory effects on the virus propagation stages (Cheng et al., 2006). Glycyrrhizin is another triterpene saponin, obtained from *Glycyrrhiza glabra* L. (Fabaceae), with anti-CoV activity and blocking effects on several steps of viral replication such as permeation and adsorption (Bailly and Vergoten, 2020; Cinatl et al., 2003). Triterpenoids of *Euphorbia neriifolia* L. (Euphorbiaceae) also indicated anti-HCoV effects. Among these triterpenoids, friedelane derivatives showed the greatest effect (Chang et al., 2012). The phytochemicals in essential oils are among the other anti-HCoV natural compounds (Nadjib, 2020). Jensenone and 1,8-cineole, as monoterpenes in *Eucalyptus* spp. (Myrtaceae) essential oil, showed anti-CoV effects in docking studies (Sharma and Kaur, 2020a; Sharma and Kaur, 2020b). Savinin, a lignoid isolated from *Chamaecyparis obtusa* (Siebold & Zucc.) Endl. (Cupressaceae), and betulinic acid, a triterpenoid of *Betula spp.* (Betulaceae), showed anti-SARS-CoV activities via the inhibition of 3CL protease at 25 and 10 μM, respectively (Wen et al., 2007). Also, quinones such as emodin, aloe-emodin, and *Tripteryguim regelii* (Celastraceae) quinones including celastrol, pristimererin, tingenone, and iguesterin showed the anti-SARS-CoV activities. The emodin, aloe-emodin, and iguesterin inhibited the 3CL protease at 20, 366, and 2.6 μM, respectively (Lin et al., 2005; Ryu et al., 2010b; Schwarz et al., 2011). In addition, emodin, isolated from *Rheum officinale* Baill. and *Polygonum multiflorum* (Thunb.) Moldenke (Polygonaceae), inhibited interaction between ACE2 and S protein at 200 μM. With mention to the above, it can be said that phytochemicals are potential sources for the discovery of effective drugs against coronaviruses, especially anti-SARS-CoV-2. For this purpose, several clinical trials on phytochemicals such as polyphenols (NCT04400890), hesperidin and diosmin (NCT04452799), resveratrol (NCT04542993, NCT04536090, and NCT04377789), quercetin (NCT04468139 and NCT04377789), artemisinin and curcumin (NCT04382040), epigallocatechin gallate (NCT04446065), glycyrrhizin (NCT04487964), colchicine (NCT04527562, NCT04392141, NCT04375202, NCT04355143, and NCT04360980), berberine (NCT04479202), and tetrandrine (NCT04308317) have been designed and are going on. The structures of some anti-CoV phytochemicals are shown in **Figure 1**.

PHYTOCHEMICALS AS POTENTIAL AGENTS FOR CORONAVIRUS-ASSOCIATED LUNG INJURY

Medicinal plants and isolated phytochemicals can cover multiple therapeutic targets at the same time and lie in the fact that they are widely used in the treatment of various diseases, including viral diseases and related complications. Since infection with any of the viruses of the Coronaviridae family, including SARS-CoV-2, can cause severe damage to the pulmonary system (Ding et al., 2003), the plant-derived secondary metabolites can play a significant role in reducing these pulmonary complications. The phytochemicals with different molecular targets and signaling mechanisms, including reducing proinflammatory and oxidant

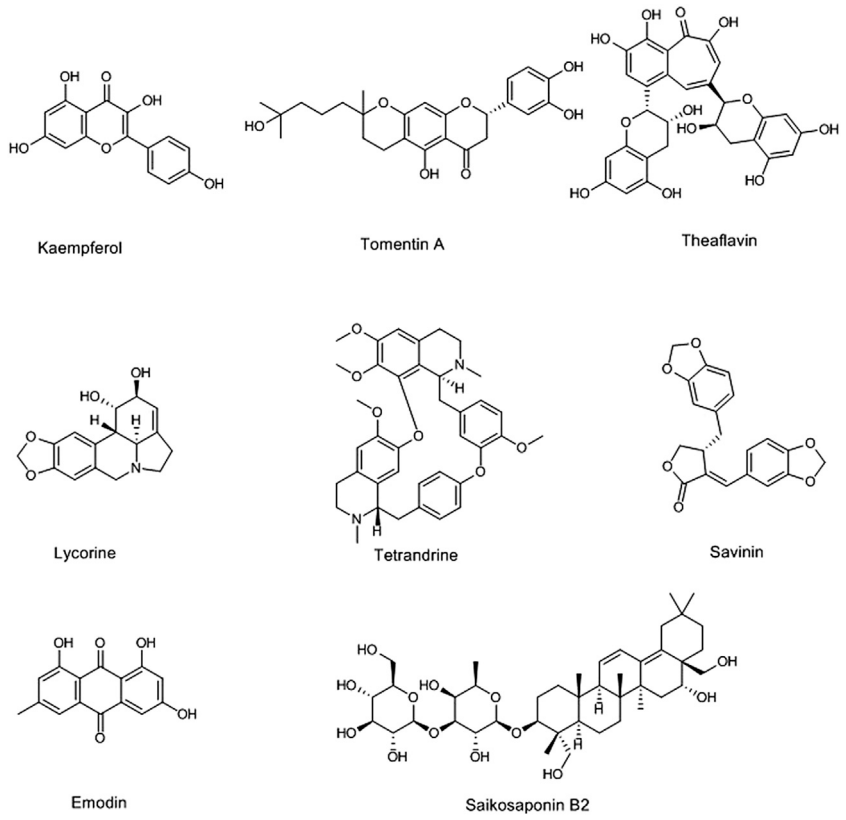

FIGURE 1 | Selected chemical structures of some phytochemicals with potential anti-CoV effects.

mediators such as TNF-α, IL-1, IL-6, IL-8, IL-1β, NF-κB, MMPs, iNOS, MAPK, COX-2, and ROS, minimize lung injury. Therefore, protective effects on lung injury, along with other effects, including antiviral (especially anti-CoVs) effects, have attracted the attention of many researchers on the use of phytochemicals as potential strategies for discovering new anti-CoV agents regarding controlling related complication (Bellik et al., 2012; Cornélio Favarin et al., 2013b).

Alkaloids

Alkaloids are one of the largest classes of natural products that are mainly found in several plant families such as Solanaceae, Ranunculaceae, Rubiaceae, Papaveraceae, Amaryllidaceae, and Fabaceae. The main feature of this group is the presence of the nitrogen atom in their structure (Yang and Stöckigt, 2010). Several studies showed that alkaloids have the potential of reducing lung injury induced by different methods. Sinomenine (**Figure 2**) is an isoquinoline alkaloid that is isolated from the stem and rhizome of *Sinomenium acutum* (Thunb.) Rehder & E.H.Wilson (Menispermaceae). It reduced the lung injury induced by lipopolysaccharides (LPS) and *Escherichia coli*, via regulation of inflammatory signaling pathway, including the downregulation of IL-6, IL-1β, TNF-α, NF-κB, iNOS, and COX-2 and upregulation of the protective anti-inflammatory adenosine A2A receptor. Sinomenine also inhibited oxidative stress markers, including the increase of the superoxide dismutase (SOD) and the decrease of the malondialdehyde (MDA) (Li et al., 2013; Liu S. et al., 2018). Besides, sinomenine [100 mg/kg, intraperitoneally (i.p.)] upregulated the expression of Nrf2 and autophagy-related molecules (LC-3II, Atg5, and Beclin1), as critical mediators in increasing cell resistance against oxidative stress and inflammation, 1 h after inducing lung injury by LPS (8 mg/kg) in mice. Moreover, lung wet/dry (W/D) ratio, pulmonary edema, and the protein leakage into bronchoalveolar lavage fluid (BALF), as the pathological markers of lung injury, were decreased by sinomenine (Wang X. et al., 2019). In addition, six isosteroid alkaloids (imperialine, verticinone, verticine, imperialine-3-β-D-glucoside, delavine, and peimisine) and total alkaloid extraction isolated from bulbs of *Fritillaria cirrhosa* D.Don (Liliaceae) showed the protective effects on lung injury, induced by LPS and cigarette smoke, increase the expression of Nrf2 and heme oxygenase (HO-1), and reduce ROS production, IL-6, and TNF-α expression *in vivo* and *in vitro* (Wang et al., 2016; Liu S. et al., 2020).

Toll-like receptor 4 (TLR4) is an inflammatory signaling pathway whose expression is increased in acute lung injury (Yang H.-Z. et al., 2012). Sophocarpine (25 and 50 mg/kg, i.p.), quinolizidine alkaloid isolated from the seeds of *Sophora alopecuroides* L. (Fabaceae), reduced LPS-induced lung injury in mice by the inhibition of TLR4 expression (Lu et al., 2019).

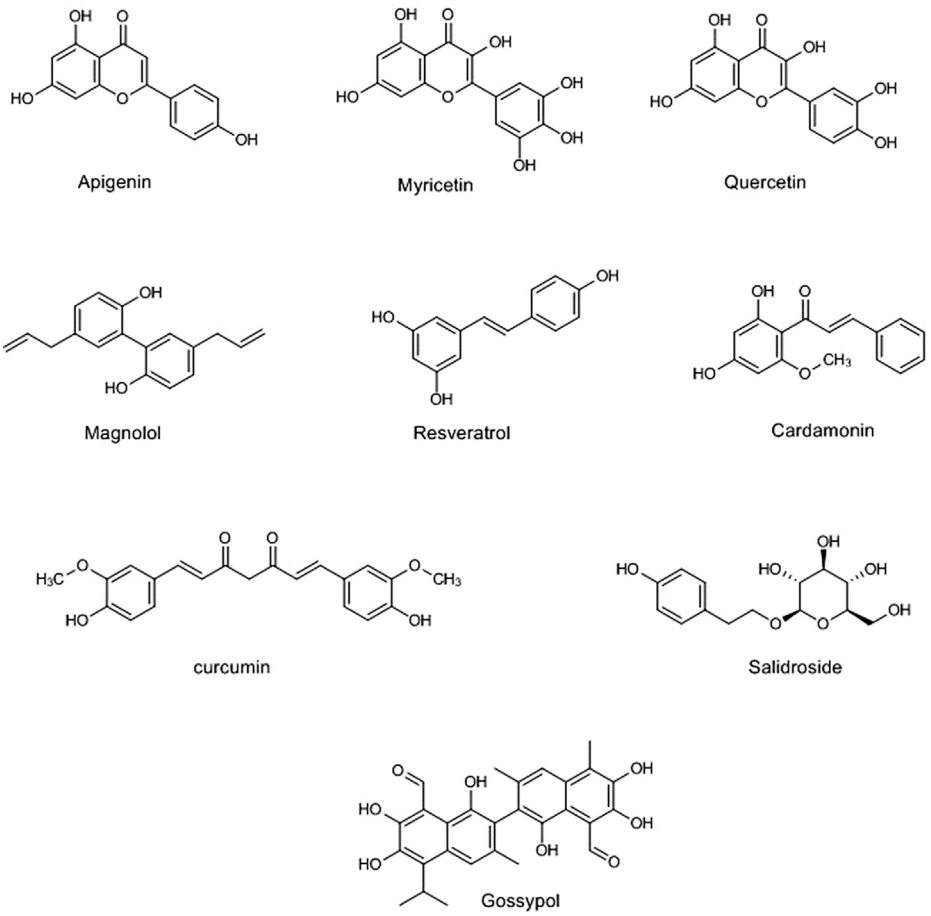

FIGURE 2 | Selected chemical structures of polyphenols with potential protective effects against lung injury.

Zhang et al. reported that tabersonine, as a monoterpenoid indole alkaloid isolated from the root of *Catharanthus roseus* (L.) G.Don (Apocynaceae), has shown a protective effect on lung injury induced by LPS *in vivo* (20 mg/kg, i.p.) and *in vitro* (mouse bone marrow-derived macrophages, 10 μM). This study showed that tabersonine decreased the expression of TNF receptor-associated factor 6 (TRAF6) and thereby blocked p38MAPK-activated protein kinase 2 (MAPK/MK2) and NF-κB activities. The amelioration of the aforementioned signaling pathways/mediators leads to the inhibition of proinflammatory mediators and the reduction of pathological indices of lung injury such as total protein concentrations in BALF ameliorated lung injury (Zhang et al., 2018).

Berberine, an isoquinoline alkaloid isolated from different species such as *Berberis vulgaris* L. (Berberidaceae) and *Coptis chinensis* Franch. (Ranunculaceae), has indicated protective effects on LPS-induced lung injury via activating Nrf2 and increasing the expression of HO-1 in C57BL/6 mice at 10 mg/kg (i.p., 24 and 2 h before injection of LPS, 2.5 mg/kg), as well as the *in vitro* manner on the human bronchial epithelial cell line at 5 and 10 μM concentrations. Berberine also reduced the pulmonary edema and the protein leakage into BALF of mice (Liang et al., 2019).

Matrine (tetracycloquinolizindine) (Li W. W. et al., 2019), antidesmone (tetrahydroquinoline) (Lu et al., 2017), cepharanthine (bisbenzylisoquinoline) (Huang et al., 2014), epigoitrin (pyrrolidine) (Luo et al., 2019), isotetrandrine (bisbenzyltetrahydroisoquinoline) (Liang et al., 2014), neferine (bisbenzylisoquinline) (Zhao et al., 2010), and oxysophoridine (quinolizidine) (Fu et al., 2015) are other alkaloids that have shown anti-lung injury effects evaluated by *in vivo* and *in vitro* experiments. Accordingly, they regulated the proinflammation mediators and oxidative markers (**Table 1**). show the chemical structures and schematic diagram of the possible mechanisms of action of some alkaloids and other phytochemicals with protective effects against lung injury, respectively. Generally, the alkaloids especially quinolines and quinazolines have shown therapeutic effects on lung injury via inhibiting the MAPKs pathway and their interconnected mediators, including TLR4, and inflammatory cytokines such as IL-1β, IL-6, and TNF-α. These agents have also been shown to enhance the Nrf2/HO-1 pathway, glutathione, and SOD as antioxidative stress markers. Therefore, this impressive role on lung injury, along with the other beneficial roles of the alkaloids especially their antiviral effects, introduces these compounds as the multitarget agents for the treatment of coronavirus infection and their complications.

TABLE 1 | Phytochemicals and their mechanisms of action against lung injury.

Phytochemical class	Compounds	Natural source	Mechanisms	Type of study	Lung injury model	Antiviral activity	References
Alkaloid	Antidesmone	*Antidesma membranaceum* Müll.Arg. (Euphorbiaceae)	↓TNF-α, ↓IL-6, ↓IL-1β, ↓NF-κB, ↓MAPK, ↓COX-2, ↓iNOS, ↓wet/dry ratio of lungs, ↓JNK, ↓ p38	*In vitro/In vivo*	LPS	NR	Lu et al. (2017)
Alkaloid	Berberine	*Berberis vulgaris* L. (Berberidaceae)	↑Nrf2, ↑HO-1, ↓MPO, ↓TGF-β1, ↓ROS, ↓wet/dry ratio of lungs	*In vitro/In vivo*	LPS, radiation	Yes	Yan et al. (2018), Liang et al. (2019)
Alkaloid	Cepharanthine	*Stephania cepharantha* Hayata (Menispermaceae)	↓TNF-a, ↓IL-6, ↓IL-1β, ↓NF-κB, ↓IκBα, ↓ERK, ↓MAPK,↓ MPO	*In vitro/In vivo*	LPS	Yes	Zhang et al. (2005), Huang et al. (2014)
Alkaloid	Epigoitrin	*Isatis tinctoria L.* (Brassicaceae)	↓Viral replications, ↓MFN2, ↑MAVS, ↑IFN-b, ↑IFITM3, ↓TNF-a, ↓IL-1β	*In vitro/In vivo*	Influenza virus	Yes	Luo et al. (2019)
Alkaloid	Isotetrandrine	*Fritillaria cirrhosa* D.Don (Liliaceae)	↓TNF-a, ↓IL-6, ↓IL-1β, ↓NF-κB, ↓NF-κB. ↓MAPK, ↓MPO, ↓wet/dry ratio of lungs	*In vitro/In vivo*	LPS	NR	Liang et al. (2014)
Alkaloid	Matrine	*Sophora flavescens* Aiton (Fabaceae)	↓TNF-a, ↓IL-6, ↓HMGB1, ↓MPO, ↓wet/dry ratio of lungs, ↓MDA, ↓ROS, ↓NF-κB	*In vitro/In vivo*	LPS	Yes	Yang Y. et al. (2012), Li W. W. et al. (2019)
Alkaloid	Neferine	*Nelumbo mucifera* Gaertn. (Nelumbonaceae)	↑SOD,↑MDA, ↓MPO, ↓TNF-a, ↓IL-6, ↓NF-κB, ↓TGF-b1	*In vitro/In vivo*	Bleomycin	NR	Zhao et al. (2010)
Alkaloid	Oxysophoridine	*Siphocampylus verticillatus* (Cham.) G.Don (Campanulaceae)	↓TNF-α, ↓IL-6, ↓IL1β, ↓wet/dry ratio of lungs, ↓NF-κB, ↓pulmonary cell apoptosis	*In vivo*	LPS	Yes	Fu et al. (2015), Zhang Y.-N. et al. (2020)
Alkaloid	Sinomenine	*Sinomenium acutum* (Thunb.) Rehder & E.H.Wilson (Menispermaceae)	↓IL-6, ↓IL-1β, ↓TNF-α, ↓NF-κB, ↓iNOS, ↓COX-2, ↑SOD, ↓MDA, ↑Nrf2, ↑ LC-3II, ↑Beclin1, ↓lung wet/dry ratio, ↓pulmonary edema, ↓BALF	*In vivo*	LPS, sepsis	NR	Li et al. (2013), Liu S. et al. (2018), Wang W. et al. (2020)
Alkaloid	Tabersonine	*Catharanthus roseus* (L.) G.Don (Apocynaceae)	↓TRAF6, ↓MAPK/MK2, ↓NF-κB, ↓TNF-α, ↓IL-6, ↓IL-1β, ↓MPO, ↓iNOS, ↓NO	*In vitro/In vivo*	LPS	NR	Zhang et al. (2018)
Anthocyanin	Cyanidin	*Vaccinium corymbosum* L. (Ericaceae)	↓TNF-α, ↓IL-6,↓IL-1β, ↓NF-κB, ↓COX-2, ↓PGE2	*In vivo*	Sepsis	Yes	Liu et al. (2015), Yan et al. (2015), Pour et al. (2019)
Anthocyanin	Malvidin	*Vaccinium corymbosum* L. (Ericaceae)	↓Bax/Bcl-2, ↓Caspase-3, ↓IL-1β, ↓TNF-α	*In vivo*	Radiation	Yes	Liu et al. (2015), Yan et al. (2015), Pour et al. (2019)
Carbohydrate	Polysaccharides	*Houttuynia cordata* Thunb. (Saururaceae)	↓TNF-α, ↓wet/dry ratio of lungs, ↓TLR4,.↓TNF-α, ↓IL-6, ↓IL-1β, ↓MPO	*In vivo*	LPS	NR	Cheng et al. (2012), Xu et al. (2015)
Chalcone	Cardamonin	*Alpinia katsumadai* K.Schum. (Zingiberaceae)	↓TNF-α, ↓IL-6, ↓IL-1β, ↓P38 MAPK, ↓MPO, ↓wet/dry ratio of lungs	*In vitro/In vivo*	Sepsis	NR	Wei et al. (2012)
Coumarin	Anomalin	*Saposhnikovia divericata* (Turcz. ex Ledeb.) Schischk (Apiaceae)	↓IL-1β, ↓IL-6, ↓TNF-α, ↑GST, ↑GSH, ↑catalase, ↓MDA, ↓NO, ↓wet/dry ratio of lungs	*In vitro/In vivo*	LPS	NR	Khan et al. (2019)
Coumarin	Daphnetin	*Daphne* spp. (Thymelaeaceae)	↓NF-κB, ↓TNF-α, ↓IL-6, ↓IL-1β, ↓JAK/STATs, ↓ROS, ↓MPO, ↓MAPK	*In vitro/In vivo*	LPS	NR	Yu et al. (2014), Seif et al. (2017), Shen et al. (2017)
Coumarin	Esculetin	*Artemisia capillaris* Thunb. (Asteraceae)	↓IL-23, ↓TNF-α, ↓IL-6, ↓IL-1β, ↓MAPK, ↓neutrophils, ↓NF-κB, ↓macrophages, ↓ERK, ↓Akt	*In vivo*	LPS	Yes	Galabov et al. (1996), Lee et al. (2020)
Coumarin	Fraxin	*Fraxinus chinensis* subsp. *Rhynchophylla* (Hance) A.E.Murray (syn. *Fraxinus rhynchophylla* Hance) (Oleaceae)	↓NLRP3, ↓wet/dry ratio of lungs, ↓NF-κB, ↓MPO, ↓MDA, ↓SOD, ↓IL-1β, ↓ IL-6, ↓TNF-α	*In vivo*	LPS	NR	Li W. et al. (2019)

(Continued on following page)

TABLE 1 | (*Continued*) Phytochemicals and their mechanisms of action against lung injury.

Phytochemical class	Compounds	Natural source	Mechanisms	Type of study	Lung injury model	Antiviral activity	References
Coumarin	Isofraxidin	*Sarcandra glabra* (Thunb.) Nakai (Chloranthaceae)	↓PGE2, ↓COX-2, ↓NF-κB, ↓IL-1β, ↓IL-6, ↓MIP-2, ↓wet/dry ratio of lungs, ↓MPO, ↓MAPK, ↓AKT	*In vitro/In vivo*	LPS, influenza virus	Yes	Jin et al. (2020), Majnooni et al. (2020)
Coumarin	Osthole	*Cnidium monnieri* (L.) Cusson (Apiaceae)	↓IL-1β, ↓IL-6, ↓TNF-α, ↓NF-κB, ↓ERK, ↓Akt, ↓wet/dry ratio of lungs	*In vitro/In vivo*	LPS, neutrophil oxidative stress, hemorrhagic shock, intestinal ischemia reperfusion	Yes	Shi et al. (2013), Shokoohinia et al. (2014)
Coumarin	Praeruptorin D and E	*Peucedanum praeruptorum* Dunn (Apiaceae)	↓NF-κB, ↓IL-6, ↓TNF-α, ↓neutrophils, ↓cells infiltration in BALF, ↓MPO	*In vivo*	LPS, hydrochloric acid	NR	Yu et al. (2013)
Coumarin	Psoralidin	*Cullen corylifolium* (L.) medik. (syn. *Psoralea corylifolia* L.) (Fabaceae)	↓COX-2, ↓5-LOX, ↓IL-1β, ↓IL-6, ↓TNF-α, ↓TGF-b	*In vitro*	Ionizing radiation	Yes	Yang H. J. et al., (2011), Kim et al., (2014)
Coumarin	Scoparone	*Artemisia capillaris* Thunb. (Asteraceae)	↓wet/dry ratio of lungs, ↓TLR4, ↓NF-κB, ↓IL-1β, ↓IL-6, ↓TNF-α, ↓MPO	*In vitro/In vivo*	LPS	NR	Niu et al. (2014)
Coumarin	Umbelliferone	*Petroselinum crispum* (Mill.) Fuss (Apiaceae)	↓MCP-1, ↓MPO, ↓MDA, ↑SOD, ↓TLR4, ↓MyD88, ↓NF-κB, ↓wet/dry ratio of lungs	*In vivo*	LPS	NR	Wang D. et al. (2019)
Flavonoid	Apigenin	*Citrus* × *aurantium* L. [syn. *Citrus sinensis* (L.) Osbeck] (Rutaceae)	↓TNF-α, ↓wet/dry ratio of lungs, ↓IL-6, ↓IL-1β, ↓NF-κB, ↓TLR4, ↓ MPO	*In vivo*	LPS	Yes	Shibata et al. (2014), Li et al. (2018)
Flavonoid	Breviscapine	*Erigeron breviscapus* (Vaniot) Hand.-Mazz (Asteraceae)	↓ICAM-1, ↓IL-18	*In vivo*	Left heart ischemic reperfusion	NR	Wang et al. (2013)
Flavonoid	Daidzein	*Glycine max* (L.) Merr (Fabaceae)	↓TLR4, ↓MyD88, ↓NF-κB, ↓MPO, ↓wet/dry ratio of lungs, ↓IL-6, ↓TNF-α	*In vitro/In vivo*	LPS	Yes	Feng et al. (2015), Seo et al. (2016)
Flavonoid	Eriodictyol	*Dracocephalum rupestre* Hance (Lamiaceae)	↑Nrf2, ↓MPO, ↓TNF-α, ↓IL-6, ↓IL-1β, ↓MIP-2, ↓wet/dry ratio of lungs	*In vivo*	LPS	NR	Zhu et al. (2015)
Flavonoid	Fisetin	*Fragaria x ananassa* (Duchesne ex Weston) Duchesne ex Rozier (Rosaceae)	↓Neutrophils, ↓macrophages, ↓TNF-α, ↓IL-6, ↓IL-1β, ↑Nrf2, ↑GPx, ↑SOD, ↑CAT	*In vivo*	Cigarette smoke	Yes	Lin et al. (2012), Hussain et al. (2019)
Flavonoid	Hesperetin	*Citrus* × *aurantium* L. [syn. *Citrus sinensis* (L.) Osbeck] (Rutaceae)	↓TNF-α, ↓IL-6, ↓MPO, ↓LDH, ↑SOD, ↓TLR4, ↓MyD88, ↓NF-κB	*In vivo*	LPS	NR	Wang X. et al. (2019)
Flavonoid	Hyperin	*Abelmoschus manihot* (L.) medik (Malvaceae)	↓inflammatory cell infiltration, ↓MPO activity, ↓TNF-α, ↓IL-6, ↓ IL-1β, ↓NF-κB, ↓wet/dry ratio of lungs	*In vivo*	LPS	Yes	Wu et al. (2007), Hu et al. (2019)
Flavonoid	Isorhamnetin	*Hippophae rhamnoides* L. (Elaeagnaceae)	↓Pulmonary edema, ↓TNF-α, ↓IL-6, ↓IL-1β, ↓ERK, ↓JNK, ↓NF-κB	*In vitro/In vivo*	LPS	Yes	Dayem et al. (2015), Chi et al. (2016)
Flavonoid	Kaempferol	*Malus domestica* (Suckow) Borkh. (Rosaceae)	↓Pulmonary edema, ↓TNF-α, ↓IL-6, ↓IL-1β, ↓alveolar wall thickness, ↓alveolar ↓hemorrhage, ↓leukocytes infiltration, ↑SOD,↓NF-κB,↓MAPKs, ↓MPO, ↓wet/dry ratio of lungs	*In vivo*	LPS	Yes	Guan et al. (2012), Schwarz et al. (2014)
Flavonoid	Luteolin	*Lonicera japonica* Thunb. (Caprifoliaceae)	↓Neutrophil chemotaxis, ↓MPO, ↓respiratory, ↓Akt, ↓ERK, ↑Nrf2, ↓NF-κB, ↑GPx, ↑SOD, ↑CAT	*In vivo*	Mercuric chloride, LPS	Yes	Lee et al. (2010), Liu B. et al. (2018), Yan et al. (2019)
Flavonoid	Myricetin	*Solanum lycopersicum* L. (Solanaceae)	↓TLR4, ↓MyD88, ↓NF-κB, ↓MPO,↓inflammatory cell migration, ↑SOD, ↑GPx, ↑CAT, ↓MPO, ↓wet/dry ratio of lungs, ↓IL-6, ↓TNF-α	*In vivo*	LPS	Yes	Ong and Khoo (1997), Mao and Huang (2017)
Flavonoid	Naringenin	*Citrus* × *aurantium L.* (syn. *Citrus paradisi* Macfad.) (Rutaceae)	↓PI3K, ↓Akt, ↓MAPK, ↓pulmonary edema, ↓ROS, ↓TNF-α, ↓MPO, ↓IL-6, ↓IL-1β	*In vitro/In vivo*	LPS, acid	Yes	Lee et al. (1999), Mao and Huang (2017), Zhao et al. (2017), Yu et al. (2020)

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TABLE 1 | (*Continued*) Phytochemicals and their mechanisms of action against lung injury.

Phytochemical class	Compounds	Natural source	Mechanisms	Type of study	Lung injury model	Antiviral activity	References
Flavonoid	Quercetin	*Myrsine melanophloeos* (L.) R.Br. ex Sweet (syn. *Rapanea melanophloeos* (L.) Mez) (Primulaceae)	↓NF-κB, ↓JNK/SAPK, ↓p38, ↓p44/p42, ↑caspase-3	*In vivo*	Radiation	Yes	Wang J. et al. (2015), Chiow et al. (2016)
Flavonoid	Rutin	*Fagopyrum esculentum* Moench (Polygonaceae)	↓NF-κB, ↓MAPK, ↑GPx, ↑SOD, ↑CAT, ↓MIP, ↓MMP-9, ↓Akt	*In vivo*	LPS	Yes	Lin et al. (2012), Chen et al. (2014), Yeh et al. (2014)
Flavonoid	Silymarin	*Silybum marianum* (L.) Gaertn (Asteraceae)	MPO	*In vitro/In vivo*	Paraquat	Yes	Özçelik et al. (2011), Zhao et al. (2015)
Flavonoid	Wogonin	*Scutellaria baicalensis* Georgi (Lamiaceae)	↓NO, ↓TNF-α, ↓IL-6, ↓IL-1β, ↓iNOS, ↓NF-κB,↓ MPO	*In vitro/In vivo*	LPS	Yes	Guo et al. (2007), Yao et al. (2014)
Iridoid	Geniposide	*Gardenia jasminoides* J.Ellis (Rubiaceae)	↓NF-κB, ↓MAPKs,↓ TNF-α, ↓IL-6, ↓alveolar hemorrhage, ↓MPO, ↓wet/dry ratio of lungs	*In vivo*	LPS	Yes	Xiaofeng et al. (2012), Zhang et al. (2017b)
Isothiocyanate	Sulforaphane	*Brassica* spp. (Brassicaceae)	↑Nrf2, ↓PGE2, ↓COX-2, ↓MMP-2, ↓NO, TNF-α, ↓IL-6	*In vivo*	LPS	Yes	Qi et al. (2016), Yu et al. (2016)
Phenolic acid	Caffeic acid	*Coffea arabica* L. (Rubiaceae)	↓MDA, ↑SOD, ↑CAT	*In vivo*	Radiation	Yes	Yildiz et al. (2008), Özçelik et al. (2011)
Phenolic acid	Chicoric acid	*Echinacea purpurea* (L.) Moench (Asteraceae)	↑Nrf2, ↑HO-1, ↓wet/dry ratio of lungs, ↓MPO, ↓MAPK,↓ NLRP3, ↑SOD, ↓NF-κB	*In vivo*	LPS	Yes	Lin et al. (1999), Ding et al. (2019)
Phenolic acid	Chlorogenic acid	*Coffea arabica* L. (Rubiaceae)	↓iNOS, ↓NO, ↓leukocytes, ↓MPO	*In vivo*	LPS	Yes	Zhang et al. (2010), Özçelik et al. (2011)
Phenolic acid	Ellagic acid	*Camellia sinensis* (L.) Kuntze (Theaceae)	↓NF-κB, ↓COX-2, ↑IL-10, ↓IL-6, ↓TNF-α, ↓IL-1β, ↓NF-κB	*In vitro/In vivo*	LPS, hydrochloric acid	Yes	Cornélio Favarin et al. (2013a), Park et al. (2014), Guan et al. (2017)
Phenolic acid	Rosmarinic acid	*Rosmarinus officinalis* Spenn. (Lamiaceae)	↓ERK/MAPK, ↓IL-6, ↓TNF-α, ↓IL-1β, ↑SOD	*In vivo*	LPS	Yes	Petersen and Simmonds (2003), Chu et al. (2012)
Phenolic compound	Apocynin	*Picrorhiza kurroa* Royle ex Benth. (Plantaginaceae)	↓TNF-α, ↑ SOD, ↓pulmonary vascular permeability, ↓MDA, ↓NADPH	*In vivo*	LPS	NR	Xu et al. (2014)
Phenolic glycoside	Salidroside	*Rhodiola rosea* L. (Crassulaceae)	↓IL-6, ↓TNF-α, ↓IL-1β, ↓wet/dry ratio of, ↓MPO, ↓NF-κB, ↓TGF-β1	*In vivo*	LPS, paraquat	Yes	Wang et al. (2009), Guan et al. (2012), Zhang et al. (2014)
Phenolic terpene	Cannabidiol	*Cannabis sativa* L. (Cannabaceae)	↓MPO, ↓TNF-α, ↓IL-6	*In vivo*	LPS	Yes	Ribeiro et al. (2012), Ribeiro et al. (2015), Lowe et al. (2017)
Polyphenol	Curcumin	*Curcuma longa* L. (Zingiberaceae)	↓NF-κB, ↓PGE2, ↓inflammatory responses, ↓TGF-β, ↓TNF-α, ↓IL-6, ↓MMP-9, ↓PGE2	*In vitro/In vivo*	Diabetes, bleomycin, LPS	Yes	Sun et al. (2008), Smith et al. (2010), Suresh et al. (2012), Zorofchian Moghadamtousi et al. (2014), Zhang et al. (2015)
Polyphenol	Gossypol	*Gossypium herbaceum* L. (Malvaceae)	↓IL-6, ↓TNF-α, ↓IL-1β, ↓wet/dry ratio of lungs, ↓p46-p54, ↓JNK, ↓p42, ↓p44 ERK, ↓p38, ↓NF-κB	*In vivo*	LPS	Yes	Liu Z. et al. (2013), Keshmiri-Neghab and Goliaei (2014)
Polyphenol	Magnolol	*Magnolia officinalis* Rehder & E.H.Wilson (Magnoliaceae)	↓IL-6, ↓TNF-α, ↓IL-1β, ↓NF-κB, ↓TLR4, ↓wet/dry ratio of lungs, ↓MPO	*In vivo*	LPS	Yes	Yunhe et al. (2012), Singla (2014)

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TABLE 1 | (*Continued*) Phytochemicals and their mechanisms of action against lung injury.

Phytochemical class	Compounds	Natural source	Mechanisms	Type of study	Lung injury model	Antiviral activity	References
Polyphenol	Resveratrol	*Vitis* spp. (Vitaceae)	↓MAPK, ↓PI3K, ↓AKT, ↓MyD88, ↓TLR4, ↓Nrf2, ↓HO-1, ↓wet/dry ratio of lungs, ↓NF-κB, ↓IL-1β, ↓IL-6, ↓TNF-α, ↓ROS, ↓iNOS, ↓ SOD, ↓MDA, ↓MIP-2, ↓IL18, ↓MPO	*In vivo*	LPS, hypoxia, sepsis, staphylococcal enterotoxin B, nickel, methamphetamine, bleomycin, chest trauma, cigarette smoke	Yes	Sovak (2001), Kolgazi et al. (2006), Şener et al. (2007), Cao et al. (2011), Rieder et al. (2012), Bao et al. (2013), Mo et al. (2014), Yu et al. (2014), Jiang et al. (2016), Lin et al. (2017), Torun et al. (2017), Liu S. et al. (2018), Wang et al. (2018), Yang et al. (2018), de Oliveira et al. (2019), Zhu et al. (2019), Özdemir et al. (2019), Cao et al. (2020), Wang X. et al. (2020)
Polyphenol	Tannic acid	*Camellia sinensis* (L.) Kuntze (Theaceae)	↓Wet/dry ratio of lungs, ↓MPO, ↓IL-6, ↓ TNF-α, ↓IL-1β, ↓NF-κB, ↓PGE2	*In vivo*	LPS, sepsis	Yes	Orlowski et al. (2014), Zhang et al. (2019)
Quinone	Emodin	*Rheum palmatum* L. (Polygonaceae)	Activating autophagy, ↓TNF-α, ↓IL-1β, ↓MPO, ↓NO	*In vivo*	LPS	Yes	Schwarz et al. (2011), Dong et al. (2019)
Quinone	Shikonin	*Lithospermum erythrorhyzon* Siebold & Zucc. (Boraginaceae)	↓NF-KB, ↓IL-6, ↓ TNF-α, ↓IL-1β, ↓NO, ↓COX 2, ↓ neutrophil infiltration, ↓wet/dry ratio of lungs	*In vivo*	LPS	Yes	Bai et al. (2013), Liang et al. (2013), Zhang et al. (2017a)
Quinone	Tanshinone IIA	*Salvia miltiorrhiza* Bunge (Lamiaceae)	↓NLRP3, ↓wet/dry ratio of lungs, ↓CO_2 partial pressure, ↑O_2 partial pressure	*In vivo*	Oleic acid	Yes	Chen T. et al., (2019), Sun et al. (2019)
Quinone	Thymoquinone	*Nigella sativa* L. (Ranunculaceae)	↓Alveolar infiltration, ↓alveolar edema, ↓iNOS	*In vivo*	Chronic toluene	Yes	Kanter (2011), Fröhlich et al. (2017)
Saponin	Dioscin	*Dioscorea* spp. (Dioscoreaceae)	↓TNF-α, ↓IL-6, ↓IL-1β, ↓MPO, ↓NF-κB	*In vivo*	LPS	Yes	Liu C. et al. (2013), Zeng et al. (2018)
Saponin	Ginsenoside Rg1	*Panax ginseng* C.A.Mey. (Araliaceae)	↓Wet/dry ratio of lungs, ↓proteins, ↓M2 macrophage, ↓pulmonary edema, ↓NF-κB, TNF-α, ↓IL-6, ↓IL-1β	*In vivo*	LPS	Yes	Song et al. (2014), Bao et al. (2015)
Saponin	Ginsenoside Rg3	*Panax ginseng* C.A.Mey. (Araliaceae)	↓NF-κB, ↓COX-2, TNF-α, ↓IL-6, ↓IL-1β, ↓wet/dry ratio of lungs	*In vivo*	LPS	Yes	Song et al. (2014), Cheng and Li (2016)
Saponin	Sodium aescinate	*Aesculus hippocastanum* L. (Sapindaceae)	↓Wet/dry ratio of lungs, ↑SOD, ↑MDA,↓MMP2	*In vivo*	Oleic acid	NR	Menegazzi et al. (2008)
Saponin	Soyasaponin	*Glycine* max (L.) Merr (Fabaceae)	↓COX-2, ↓iNOS, ↓TNF-α, ↓IL-6, ↓IL-1β, ↓NO	*In vivo*	LPS	Yes	Hayashi et al. (1997), Lin et al. (2016)
Terpenoid	Andrographolide	*Andrographis paniculata* (Burm.f.) nees (Acanthaceae)	↓IL-1β, ↑GPx, ↑Nrf2, ↑SOD	*In vivo*	Cigarette smoke	Yes	Guan et al. (2013), Gupta et al. (2017)
Terpenoid	Artemisitene	*Artemisia annua* L. (Asteraceae)	↑Nrf2, ↓TGF-β, ↓MCP-1, ↓IL-6	*In vivo*	Bleomycin	NR	Chen et al. (2016)
Terpenoid	Betulinic acid	*Betula* spp. (Betulaceae)	↑CAT, ↑SOD, ↓iNOS, ↓NO	*In vivo*	Sepsis	Yes	Aiken and Chen (2005), Lingaraju et al. (2015)
Terpenoid	Costunolide	*Lactuca sativa* L. (Asteraceae)	↓TNF-α, ↓IL-6, ↓IL-1β, ↓iNOS, ↓MAPKs	*In vivo*	Heat-killed *S. aureus* (HKSA)	Yes	Chen et al. (1995), Chen Y.-t. et al. (2019)
Terpenoid	Eugenol	*Syzygium aromaticum* (L.) Merr. & L.M.Perry (Myrtaceae)	↓Wet/dry ratio, ↑SOD1, ↑CAT, ↑Gpx1, ↑GST, ↓NF-KB, ↓MPO, ↓IL-6,↓ TNF-α	*In vivo*	LPS	Yes	Pramod et al. (2010), Huang et al. (2015)
Terpenoid	Farnesol	*Prunus persica* (L.) Batsch (Rosaceae)	↑Nrf2, ↑ HO-1, ↓MAPKs, ↓TNF-α, ↓IL-6, ↓IL-1β, ↑GSH, ↑H_2O_2, ↓LPO	*In vivo*	Cigarette smoke	Yes	Qamar and Sultana (2008), Ryabchenko et al. (2008)

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TABLE 1 | (*Continued*) Phytochemicals and their mechanisms of action against lung injury.

Phytochemical class	Compounds	Natural source	Mechanisms	Type of study	Lung injury model	Antiviral activity	References
Terpenoid	Geraniol	*Rosa* × *damascena* Herrm. (Rosaceae)	↓Wet/dry ratio of lungs, ↓MPO, ↓IL-6,↓TNF-α, ↓IL-1β, ↓ iNOS, ↓COX-2, ↓TLR4, ↓NF-κB, ↓Bax/Bcl-2 ratio	*In vitro/In vivo*	LPS	NR	Jiang et al. (2017)
Terpenoid	Glycyrrhizin	*Glycyrrhiza glabra* L. (Fabaceae)	↑ICAM-1, ↓TNF-α,↓IL-1β, ↓MPO, ↓LPO, ↓NF-κB	*In vivo*	Carrageenan	Yes	Menegazzi et al. (2008), Ashfaq et al. (2011)
Terpenoid	Isoforskolin	*Plectranthus hadiensis* (Forssk.) Schweinf. ex Sprenger (syn. *Coleus forskohlii* (Willd.) Briq.) (Lamiaceae)	↓IL-6, ↓TNF-α, ↓IL-1β, ↓wet/dry ratio of lungs, ↓MPO, ↑SOD	*In vivo*	LPS	NR	Yang W. et al. (2011)
Terpenoid	Linalool	*Citrus x aurantium* L. (Rutaceae)	↓IL-6, ↓TNF-α, ↓p38, ↓MAPK, ↓ERK, ↓JNK	*In vitro/In vivo*	LPS	NR	Huo et al. (2013)
Terpenoid	Oridonin	*Isodon rubescens* (Hemsl.) H.Hara (syn. *Rabdosia rubescens* (Hemsl.) H.Hara) (Lamiaceae)	↓NLRP3, ↓NF-KB, ↑Nrf2, ↑HO-1, ↑SOD, ↑GSH	*In vitro/In vivo*	LPS	Yes	Guo et al. (2013), Yang et al. (2019)
Terpenoid	*p*-Cymene	*Protium* spp. (Burseraceae)	↓IL-6, ↓TNF-α, ↓IL-1β, ↓MPO, ↓NF-KB, ↓MAPKs	*In vivo*	LPS	Yes	Xie et al. (2012), Sharifi-Rad et al. (2017)
Terpenoid	Rubriflordilactone A	*Schisandra sphenanthera* Rehder & E.H.Wilson (syn. *Schisandra rubriflora* Rehder & E.H.Wilson (Schisandraceae)	↓MMP9, ↓iNOS, ↓IL-6, ↓wet/dry ratio of lungs	*In vitro/In vivo*	LPS	Yes	Cassels and Asencio (2011), Lingaraju et al. (2015)
Terpenoid	Taraxasterol	*Taraxacum officinale* F.H.Wigg. (Asteraceae)	↓MPO, TNF-α, ↓IL-6, ↓IL-1β, ↓p65, ↓p38, ↓ ERK, ↓JNK, ↓NF-κB	*In vivo*	LPS	Yes	Chowdhury et al. (1990), San et al. (2014)
Terpenoid	Thymol	*Thymus vulgaris* L. (Lamiaceae)	↓NF-KB, ↓IL-6, ↓TNF-α, ↓IL-1β, ↑SOD, ↓MDA, ↓MPO	*In vivo*	LPS	Yes	Sharifi-Rad et al. (2017), Wan et al. (2018)
Terpenoid	Triptolide	*Tripterygium wilfordii* Hook.f. (Celastraceae)	↑Nrf2, ↑HO-1, ↓TLR4, ↓NF-KB, ↓IL-6, ↓TNF-α, ↓IL-1β, ↓MAPKs, ↓MPO	*In vivo*	LPS	Yes	Wan and Chen (2014), Wang et al. (2014)
Terpenoid	Zerumbone	*Zingiber zerumbet* (L.) Roscoe ex Sm (Zingiberaceae)	↑Nrf2, ↑HO-1, ↓LPO, ↓MPO, ↓MMP-9, ↑SOD, ↑GPx, ↑CAT	*In vivo*	LPS	Yes	Dai et al. (1997), Leung et al. (2017)

5-LOX, 5-Lipoxygenase; Akt, protein kinase B; BALF, bronchoalveolar lavage fluid; Bcl-2/Bax, B-cell lymphoma protein 2/associated X; CAT, catalase; CO_2, carbon dioxide; COX-2, cyclooxygenase-2; ERK, extracellular signal-regulated kinase; GPx, glutathione peroxidase; GSH, glutathione; GST, glutathione S-transferase; HMGB1, high-mobility group box 1 protein; HO-1, heme oxygenase-1; ICAM-1, intercellular adhesion molecule 1; IFITM3, interferon-induced transmembrane protein 3; IFN-b, interferon Beta 1; IL, interleukin; iNOS, inducible nitric oxide synthase; IκBα, inhibitor of nuclear factor-kappa B α; JAK/STATs, janus kinase-signal transducers and activator of transcription; JNK, jun N-terminal kinases; JNK/SAPK, JNK/stress-activated protein kinases; LDH, lactate dehydrogenase; LPS, lipopolysaccharides; MAPK, mitogen-activated protein kinase; MAPK/MK2, MAPK/activated protein kinase 2; MAVS, mitochondrial antiviral signaling; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MFN2, mitofusin-2; MIP-2, macrophage inflammatory protein 2; MMPs, matrix metalloproteinases; MPO, myeloperoxidase; MyD88, myeloid differentiation factor 88; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor-κB; NLRP3, nucleotide-binding oligomerization domain-like receptors pyrin domain-containing protein 3; NR, not reported; Nrf2, nuclear factor erythroid 2- related factor two; O_2, oxygen; PGE2, prostaglandin E2; PI3K, phosphoinositide 3-kinases; ROS, reactive oxygen species; SOD, superoxide dismutase; TGF-β1, transforming growth factor β1; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-α; TRAF6, TNF receptor-associated factor six.

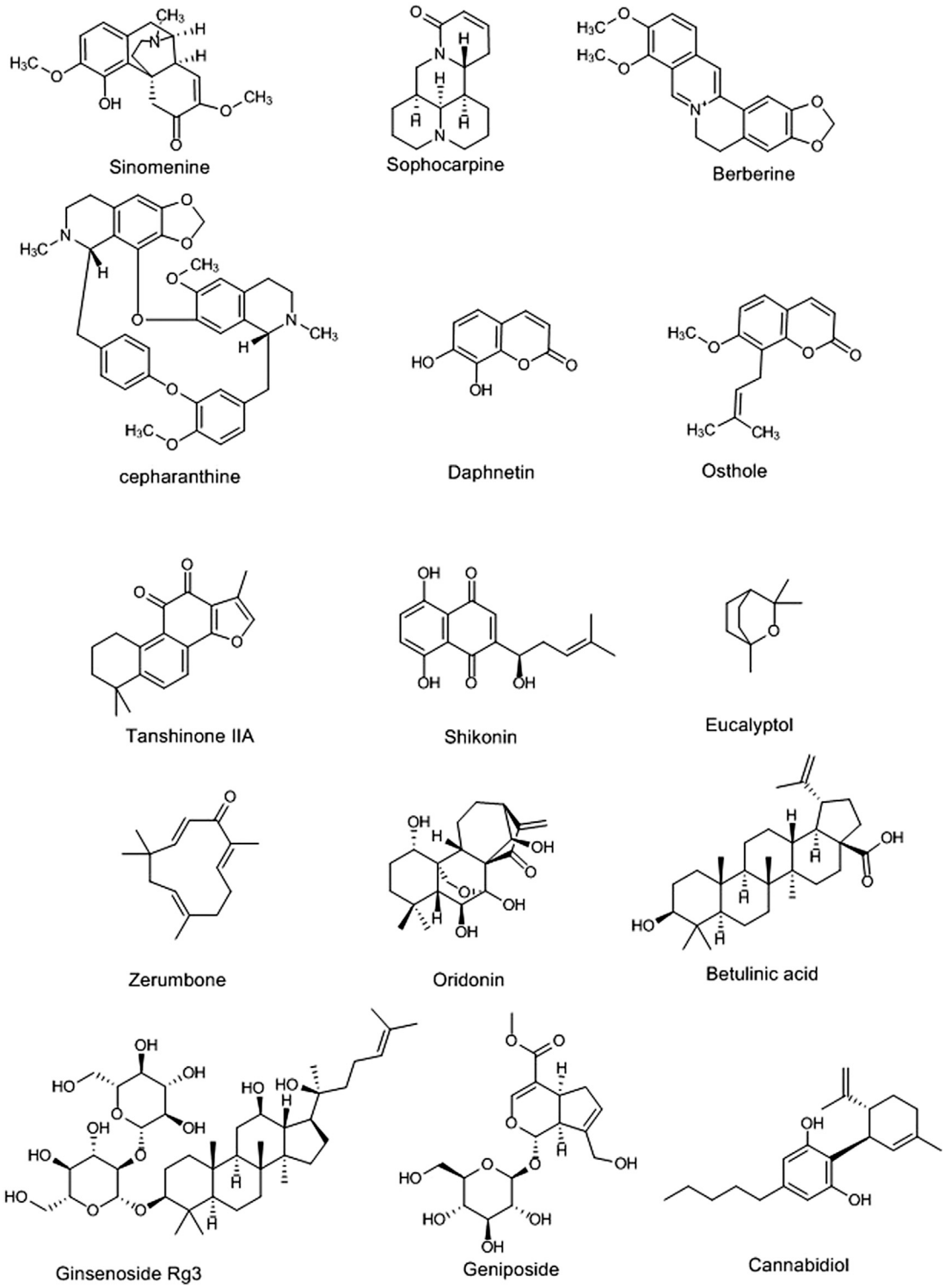

FIGURE 3 | Selected chemical structures of alkaloids, coumarins, terpenes, quinones, and other phytochemicals with potential protective effects against lung injury.

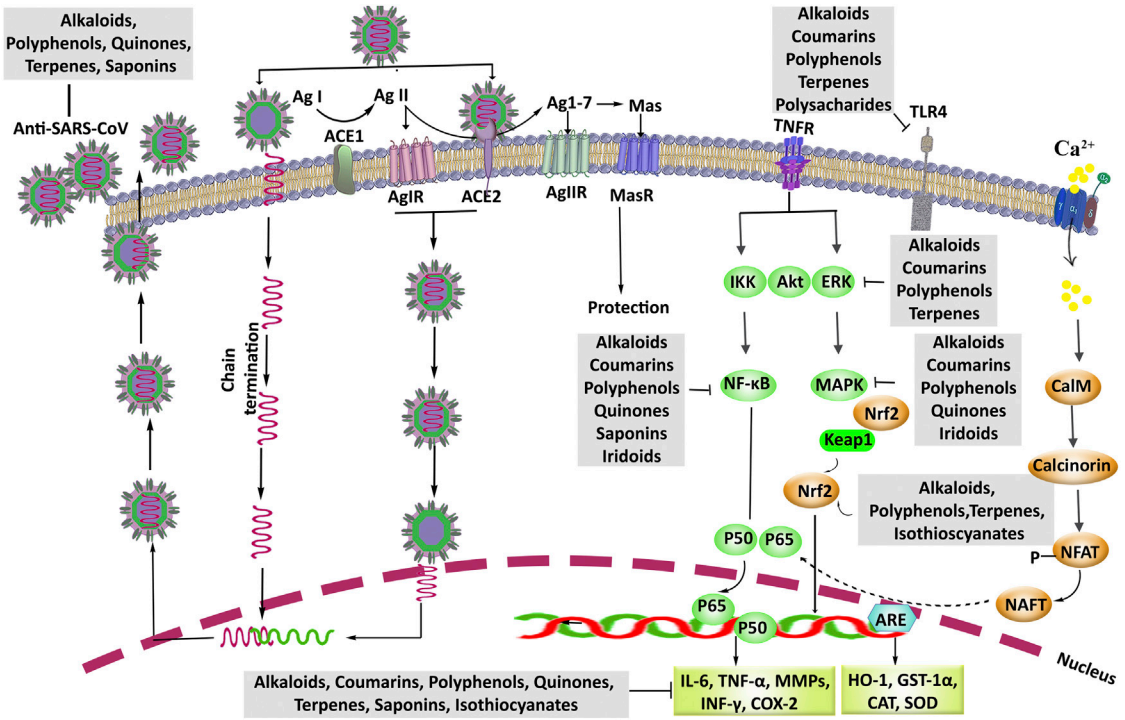

FIGURE 4 | The pharmacological mechanisms and therapeutic targets of phytochemicals against coronavirus-associated lung injury. ACE, angiotensin-converting enzyme; Ag, angiotensin; Akt, protein kinase B; ARE, antioxidant response element; Anti-SARS-CoV, anti-severe acute respiratory syndrome coronavirus; Ca^{2+}, calcium; CalM, calmodulin; NAFT, nuclear factor of activated T cells; CAT, catalase; COX-2, cyclooxygenase-2; ERK, extracellular signal-regulated kinase; GSH, glutathione; GST-1α, glutathione S-transferase; HO-1, heme oxygenase-1; IFNγ, interferon γ; IL, interleukin; IKK, inhibitor of nuclear factor-κB (IκB) kinase; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloproteinases; NF-κB, nuclear factor-κB; Nrf2, nuclear factor erythroid 2-related factor 2; SOD, superoxide dismutase; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-α; TNFR, TNF receptor.

Coumarins

Coumarins are the heterocyclic phytochemicals with 2*H*-1-benzopyran-2-one chemical structure. The Apiaceae is one of the greatest plant families that coumarins are isolated from its species (Ribeiro and Kaplan, 2002). Anti-inflammatory and antioxidant properties are two prominent effects of coumarins along with other pharmacological and biological activities such as cytotoxic and anticancer, antiviral, antiangiogenic, anticoagulant, edema-protective, and anxiolytic effect (Fylaktakidou et al., 2004; Venugopala et al., 2013; Srikrishna et al., 2018). The downregulation of inflammatory mediators, including NF-κB, TNF-α, iNOS, and MAPKs pathway, and inhibiting oxidative factors such as ROS and free radicals are critical mechanisms of coumarins. Therefore, coumarinic compounds are thought to exert anti-inflammatory effects on therapeutic applications against lung injury induced by LPS and other destructive inducers (Fylaktakidou et al., 2004; Bansal et al., 2013).

Daphnetin, as a hydroxy coumarin isolated from *Daphne* spp., showed protective effects on lung injury that are induced by LPS *in vivo* and *in vitro*. Daphnetin downregulated the NF-κB pathway via increasing the expression of NF-κB, and TNF-α-induced protein 3, in lung tissues of the C57BL/6 mice (5 mg/kg, i.p.) and the murine peritoneal macrophages (RAW264.7, 160 μM). In another study, pretreatment of mice with 5 mg/kg i.p. of daphnetin significantly reduced protein and cytokines (TNF-α, IL-6, and IL-1β) leakage into BALF that is stimulated with LPS (1 mg/kg). Consequently, daphnetin (80 and 160 μM) suppressed the expression of IL-6 and TNF-α in adenocarcinoma human lung epithelial cell lines (A549), which is induced by LPS (100 ng/ml) (Yu et al., 2014). Also, the regulation of JAK/STATs pathway has a critical role in the production of proinflammatory mediators such as TNF-α, iNOS, COX-2, IL6, and IL-1β (Seif et al., 2017). Daphnetin inhibited the LPS-induced cytokines expression through downregulating the JAK/STATs signaling in C57/BL6 mice (5 mg/kg, i.p.) and RAW264.7 cells (5, 10, and 20 μM). Daphnetin also reduced ROS production in these doses (Shen et al., 2017).

Another study showed that the praeruptorins D and E (80 mg/kg, gavage), as pyranocoumarins found in *Kitagawia praeruptora* (Dunn) Pimenov (syn. *Peucedanum praeruptorum*) roots, similar to daphnetin inhibited NF-κB and interconnected inflammatory cytokines (IL-6 and TNF-α) in male BALB/c mice with lung injury induced by intranasal administration of LPS (40 μg/ml) and hydrochloric acid (0.1 N). The total protein level, neutrophils, and cell infiltration in BALF were also reduced at 40 and 80 mg/kg of daphnetin (Yu et al., 2013).

IL-17 as one of the prominent inflammatory cytokines is produced by T lymphocyte helper cells whose production is

regulated by retinoic acid-related orphan receptor gamma t ($ROR\gamma t$). Esculetin (20 and 40 mg/kg, i.p.) as a hydroxycoumarin is widely found in *Fraxinus* spp., with the potential of reducing lung injury via inhibiting the $ROR\gamma t$ and then the suppression of IL-17 in mice. At the same doses, esculetin also inhibited MAPKs and neutrophils/macrophages entry in mice lung (Lee et al., 2020).

Besides, the protective effects of osthole, as prenylated coumarins purified first from the fruit of *Cnidium monnieri* (L.) Cusson (Apiaceae), were reported in several *in vivo* and *in vitro* studies (**Table 1**). Reducing the expression of cytokines (IL-1β, IL-6, and TNF-α) and blocking the NF-κB and ERK and Akt signaling pathway are of the critical protective mechanisms of osthole on lung injury (Mo et al., 2014; Tsai et al., 2015; Jin et al., 2018). Also, Shi and coworkers proposed that inhibition of ACE2 and Ag (1–7) depletion in lung tissues are other protective mechanisms of osthole (40 mg/kg, gavage) against the lung injury induced by LPS (Shi et al., 2013). Also, ACE2 has shown ameliorating effects on lung injury complications induced by acid, LPS, and viruses, including SARS coronavirus and influenza (Gu et al., 2016).

Isofraxidin is another hydroxycoumarin, isolated from *Fraxinus* spp., with prominent anti-inflammatory effects, especially pulmonary inflammations induced by influenza virus (Jin et al., 2020; Majnooni et al., 2020). Also, isofraxidin (5, 10, and 15 mg/kg, i.p.) showed improving effects on LPS-induced lung injury via reducing the production of inflammatory cytokines (IL-6 and TNF-α) and prostaglandin E_2 (PGE2). Consequently, it blocked the secretion of PGE2 in mice serum and BALF, also reduced COX-2 gene expression, and led to further improvement of lung damage (Niu et al., 2015).

Consistently, anomalin (Khan et al., 2019), fraxin (Li W. et al., 2019), psoralidin (Yang H. J. et al., 2011), scoparone (Niu et al., 2014), and umbelliferone (Wang D. et al., 2019) are other coumarinic compounds which have ameliorating effects on lung injury (**Table 1**).

According to the prominent anti-inflammatory effects of natural coumarins, along with their other pharmacological effects, these compounds can be introduced as one of the new sources of drug discovery for the protection and treatment of lung injury.

Flavonoids and Other Polyphenol Compounds

Structurally, polyphenols are divided into several categories, including flavonoids (flavonols, flavones, flavanones, flavanols, anthocyanins, and isoflavones), phenolic acids (hydroxybenzoic acid and hydroxycinnamic acids), stilbenes, catechins, tannins, and lignans (Pietta et al., 2003) provided in **Figure 2**. From the mechanistic point of view, the inhibition of MAPKs cascade, phosphatidylinositol 3-kinase (PI3K)/Akt, Src family kinase-Bruton's tyrosine kinase-Vav, myeloid differentiation factor 88-TLR4, Nrf2/HO-1, and NF-κB (Yu et al., 2014; Liu S. et al., 2018; Wang et al., 2018; de Oliveira et al., 2019; Tsai et al., 2019; Cao et al., 2020), enzymes involved in the arachidonic acid pathway, inflammatory cytokines, and NF-κB signaling pathway are among the main targets of polyphenols in combating inflammation (Santangelo et al., 2007; Guo et al., 2009). They have also shown to suppress the expression of macrophage inflammatory proteins 1α and 2, IL-1β, IL-6, and TNF-α as inflammatory cytokines (Yang et al., 2018; de Oliveira et al., 2019), decrease ROS production and iNOS expression (Cao et al., 2011; Zhu et al., 2019), decrease SOD activity and MDA levels (Mo et al., 2014), and increase the activity of sirtuin 1 as an antioxidant and anti-inflammatory factor (Wang X. et al., 2020), in *in vitro* and *in vivo* studies at different doses and routes of administration. Resveratrol is a stilbenoid widely found in *Vitis vinifera* L. (Vitaceae) fruits and has shown prominent protective effects on lung injury, induced by various methods such as LPS (Cao et al., 2011; Jiang et al., 2016), hypoxia (Özdemir et al., 2014), sepsis (Kolgazi et al., 2006), staphylococcal enterotoxin B (Rieder et al., 2012), nickel (Cao et al., 2020), methamphetamine (Wang X. et al., 2020), bleomycin (Şener et al., 2007), chest trauma (Torun et al., 2017), and cigarette smoke (Bao et al., 2013). Wang and coworkers showed that resveratrol ameliorated sepsis-induced lung injury after 30 mg/kg dose of i.p. administration. In their study, the level of Nrf-2, HO-1, *p*-Akt, IL-10, SOD, and caspase-3 activities as antioxidant and anti-inflammatory markers increased in lung tissue after treatment by resveratrol. Resveratrol was also able to decrease MIP-2, IL-18, and neutrophil leakage in BALF (Wang et al., 2018).

Flavonoids are another class of polyphenolic compounds whose effects on lung injuries have been extensively studied. Li et al. reported that apigenin C-glycoside, a trihydroxyflavone extracted from *Microcos paniculata* L. (Malvaceae), showed protective effects against LPS-induced lung injury in BALB/c mice at 20 and 40 mg/kg oral doses. In their study, the inhibition of inflammatory cytokines and NF-κB signaling pathway were found as the main mechanisms of apigenin (Li et al., 2018). Daidzein (2, 4, and 8 mg/kg, i.p.), an isoflavone widely found in *Glycine max* (L.) Merr. (soybeans, Fabaceae), and myricetin (10, 20, and 40 mg/kg, i.p.), a hexahydroxyflavone widely found in black tea, inhibited TLR4/MyD88/NF-κB cascade and thereby showed protective effects against LPS-induced lung injury in rats (Feng et al., 2015; Mao and Huang, 2017). Also, naringenin and its inhalation pharmaceutical dosage form improved the LPS-induced lung injury in rats. This trihydroxyflavanone compound showed protective effects at 100 mg/kg oral administration and 3 mg/rat inhalant administration doses via downregulating the PI3K/Akt and MAPKs pathways (Zhao et al., 2017; Yu et al., 2020). Besides, anthocyanins such as malvidin derivatives and cyanidin-3-*O*-glucoside, with similar structures to flavonoids, have also shown protective effects on lung injuries (Liu et al., 2015; Yan et al., 2015). Also, wogonin (Yao et al., 2014), rutin (Yeh et al., 2014), quercetin (Wang J. et al., 2015), luteolin (Liu B. et al., 2018), kaempferol (Chen et al., 2012), isorhamnetin (Chi et al., 2016), hyperin (Hu et al., 2019), hesperetin (Wang N. et al., 2019), fisetin (Hussain et al., 2019), breviscapine (Wang et al., 2013), eriodictyol (Zhu et al., 2015), and cardamonin (Wei et al., 2012) are other flavonoid compounds with protective effects on lung injury through different mechanisms (**Table 1**). Besides, such aforementioned flavonoids, as kaempferol (Schwarz et al., 2014), quercetin (Yang et al., 2020), and myricetin (Yu et al.,

2012), in addition to possessing a protective effect on lung injuries, have shown anticoronavirus effects, which increases the importance of their use in the treatment of COVID-19.

Phenolic acid derivatives such as curcumin, chlorogenic acid, caffeic acid, salidroside, rosmarinic acid, and apocynin are other compounds with protective effects on lung injury with various mechanisms (Yildiz et al., 2008; Zhang et al., 2010; Chu et al., 2012; Suresh et al., 2012; Xu et al., 2014; Zhang et al., 2014). Zhang and colleagues showed that curcumin, isolated from *Curcuma longa* L. (Zingiberaceae), at 200 mg/kg, i.p. dose, protected the LPS-induced lung injury in diabetic rats through suppressing NF-κB pathway (Zhang et al., 2015). Also, chlorogenic acid (50 mg/kg) and rosmarinic acid (5, 10, and 20 mg/kg), found in many herbs, decreased LPS-induced lung injury complications via inhibiting neutrophils and cells infiltration in BALF and downregulating ERK/MAPK pathway and increasing antioxidant activities (Zhang et al., 2010; Chu et al., 2012). Salidroside, isolated from *Rhodiola rosea* L. (Crassulaceae), is another phenolic acid compound that showed the protective effects on lung injury. Salidroside at 10 and 120 mg/kg, i.p., inhibited the expression of proinflammatory cytokines, including IL-6, TNF-α, IL-1β, and transforming growth factor-β1 through suppressing LPS-induced lung injury in mice, and paraquat-induced lung injury in rats, respectively (Guan et al., 2012; Zhang et al., 2014). Also, silibinin, as a flavonolignan mixture found in *Silybum marianum* L, showed a potential effect in blocking STAT pathway and reducing proinflammatory cytokines; thereby it could be a promising agent for the treatment of lung injuries in patients with COVID-19 (Bosch-Barrera et al., 2020). Silymarin, magnolol, thearubigin, gossypol, tannic acid, chicoric acid, and ellagic acid are other polyphenol compounds with protective effects on lung injury. The mechanisms, main natural source, and other related information are presented in **Table 1**. In summary, silymarin, thearubigin, and chicoric acid via upregulating the Nrf2/HO-1 (Zhao et al., 2015; Ding et al., 2019; Wang X. et al., 2019) and magnolol, tannic acid, gossypol, and ellagic acid through downregulating NF-κB pathways improved lung injury (Yunhe et al., 2012; Liu Z. et al., 2013; Guan et al., 2017; Zhang et al., 2019). In addition, silymarin is an undergoing clinical trial study for the treatment of SARS-CoV-2 lung injury (NCT04394208).

In general, due to the anti-inflammatory and antioxidant effects of polyphenol compounds, as well as their antiviral effects (**Table 1**), this category of secondary metabolisms of plants has the potential to treat COVID-19 and its complications, including lung injuries. However, the pharmacokinetic parameters of these compounds should be considered (Yu et al., 2020).

Quinones

Quinones are another class of phytochemicals with an aromatic ring attached to two carbonyl groups in their structure, including anthraquinones, benzoquinones, naphthoquinones, phenanthrenequinones, and polycyclic quinones derivatives (**Figure 3**). Several investigations showed that the quinones derivatives have demonstrated protective effects on lung injury by various mechanisms. Chen and coworkers reported that a phenanthrenequinone isolated from *Salvia miltiorrhiza* Bunge (Lamiaceae), Tanshinone IIA, suppressed the nucleotide-binding oligomerization domain-like receptors pyrin domain-containing protein 3 (NLRP3), as an inflammatory signaling pathway, at 10 mg/kg i.v. in rats, thereby reducing the oleic acid-induced lung injury (Chen T. et al., 2019). Also, emodin, an anthraquinone found in different laxative plants such as *Rheum rhabarbarum* L. (Polygonaceae), showed protective effects on LPS-lung injury via activating autophagy pathways at 20 mg/kg i.p. in rats (Dong et al., 2019). Shikonin (a naphthoquinone) and thymoquinone (a benzoquinone) are other quinones with protective effects on lung injury (Kanter, 2011; Liang et al., 2013) (**Table 1**).

Terpenoids and Saponins

Terpenoids are natural carbohydrate compounds, divided into seven categories based on the number of carbons in their structure (**Figure 3**), including hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), and polyterpenes (>C30). These compounds have shown several biological and pharmacological effects, including antioxidant, anticancer and cytotoxic, anti-inflammation, hypoglycemic, antiviral, and analgesic, antimicrobial, and anti-Alzheimer disease effect (Tholl, 2015; Jaeger and Cuny, 2016). In addition, these compounds have shown protective effects on lung injury with different mechanisms. Eucalyptol, thymol, linalool, eugenol, *p*-cymene, and geraniol, as monoterpenes isolated from the essential oils of various plants, have improved the lung injuries induced by cigarette smoke and LPS (**Table 1**). In terms of mechanism, these effects are exerted through inhibiting the expression of the anti-inflammatory cytokines and suppressing TLR4/NF-κB and MAPKs pathways, along with decreasing the infiltration of proteins, neutrophils, and cells to BALF. They have also indicated protective effects by reducing the Bax/Bcl-2 ratio and increasing the antioxidant activities (Xie et al., 2012; Huo et al., 2013; Huang et al., 2015; Jiang et al., 2017; Wan et al., 2018; de Lima Gondim et al., 2019). On the other hand, oridonin, a diterpenoid found in *Isodon rubescens* (Hemsl.) H.Hara [syn. *Rabdosia rubescens* (Hemsl.) H.Hara] (Lamiaceae), suppressed the NLRP3 signaling pathway, and NF-κB, as well as activating the Nrf2/HO-1 pathway, and thereby showed protective effects on lung injury at 2.5, 5, and 10 μM in *in vitro* study and 20 and 40 mg/kg, i.p. in rats (Yang et al., 2019). Triptolide (Wang et al., 2014), isoforskolin (Yang W. et al., 2011), carnosol (Tian et al., 2010), and andrographolide (Guan et al., 2013) are other diterpenes that are present in various herbal medicines with protective effects on lung injury via activating antioxidant pathways such as Nrf2/HO-1, as well as inhibiting TLR4/NF-κB, proinflammatory cytokines expression, and MAPKs pathways (**Table 1**). Also, sesquiterpenes, such as artemisitene isolated from A*rtemisia annua* L. (Asteraceae) at 10 mg/kg, i.p., in mice (Chen et al., 2016), zerumbone, presented in *Zingiber zerumbet* (L.) Roscoe ex Sm. (Zingiberaceae) at 10 μM/kg, i.p. (Leung et al., 2017), costunolide, isolated from *Lactuca sativa* L. (Asteraceae) at 30 mg/kg, i.p., in C57BL/6J mice (Chen Y.-t. et al., 2019), and farnesol, isolated from *Cymbopogon commutatus* (Steud.) Stapf (Poaceae) at 100 mg/kg orally (Qamar and Sultana, 2008),

showed protective effects against lung damage by activating Nrf2/HO-1 pathway and inhibiting MAPKs pathway and suppressing TNF-α, IL-6, and IL-1β expression. In addition, parthenolide as *Tanacetum parthenium* sesquiterpene lactones inhibited the cytokine storm in inflammatory conditions. Therefore, it can be a good candidate for clinical trial studies of lung injuries induced by SARS-Cov-2 (Bahrami et al., 2020). On the other hand, triterpenoids such as rubriflordilactones A, betulinic acid, and taraxasterol also have shown ameliorating effects on lung injuries induced by LPS and sepsis (San et al., 2014; Lingaraju et al., 2015; Wang Y.-Y. et al., 2015) (**Table 1**). Wang and coworkers showed that the rubriflordilactone, isolated from *Schisandra sphenanthera* Rehder & E.H.Wilson (syn. *Schisandra rubriflora* Rehder & E.H.Wilson) (Schisandraceae), improved the LPS-lung injury at 10 nM/kg in rats and 10 nM/ml on mouse lung epithelial cell lines (MLE-15) through increasing the expression of sirtuin 1 and suppressing inflammatory markers expression, including MMP9, iNOS, and IL-6 (Wang Y.-Y. et al., 2015). Saponins are other natural compounds, which are classified in the category of terpenoids. Ginsenoside Rg3 (20 and 30 mg/kg) and Rg1 (40 and 200 mg/kg) are two triterpenes saponins, isolated from *Panax ginseng* C.A.Mey. (Araliaceae), with improving effects on LPS-lung injury in mice. These compounds inhibited the infiltration of neutrophils and proteins and M2 macrophage in BALFs and reduced pulmonary edema. Their main mechanism of action is through the suppression of NF-κB (Bao et al., 2015; Cheng and Li, 2016). Increasing the expression of heat shock protein 70 leads to the inhibition of TLR4/MyD88 pathway. The later mechanism is the main protective mechanism of dioscin against lung injury as a steroidal saponin from *Dioscorea* spp. (Dioscoreaceae) (Zeng et al., 2018). Soyasaponin (Lin et al., 2016), glycyrrhizin (Menegazzi et al., 2008), and sodium aescinate (Menegazzi et al., 2008) are other saponin compounds with improving effects on lung injury (**Table 1**).

Miscellaneous Natural Compounds

Geniposide (20, 40, or 80 mg/Kg, i.p., mice), as an iridoid found in *Gardenia jasminoides* J.Ellis (Rubiaceae), improved the LPS-induced lung injury via suppressing the NF-κB and MAPKs (Xiaofeng et al., 2012). Sulforaphane, as an isothiocyanate isolated from *Brassica oleracea* L. (Brassicaceae), activated the Nrf2 pathway and inhibited the PGE2, COX-2, and MMP-2 at 50 mg/kg, i.p., in BALB/c mice, thereby ameliorating LPS-induced lung injury (Qi et al., 2016). Consequently, cannabidiol (**Figure 3**), as cannabinoid derivative of *Cannabis sativa* L. (Cannabaceae), and polysaccharides of *Houttuynia cordata* Thunb. (Saururaceae) showed similar effects through inhibiting the expression of proinflammatory cytokines (TNF-α and IL-6) and reducing the infiltration of cells and proteins in BALF (Ribeiro et al., 2015; Xu et al., 2015). Also, cannabidiol inhibited the cytokines storm-induced by viral infection at 5 mg/kg in C57BL/6 mice (Khodadadi et al., 2020). Besides, a clinical trial is underway on cannabidiol and its derivatives for the treatment of lung injury in patients with COVID-19 (NCT04467918).

In general, due to the protective effects of the aforementioned phytochemicals on lung injuries, these compounds can be used as a protector and treatment in lung injuries leftover from coronavirus activity, including COVID-19. Given the antiviral effects (especially anticoronavirus) of some of the compounds listed in **Table 1**, this role could lead researchers to find much more effective multitarget compounds in the treatment of patients with COVID-19 and its complications.

CONCLUSION

Since the World Health Organization (WHO) announced the pandemic of COVID-19 disease (March 11, 2020), no effective treatment or vaccine has been introduced to treat this disease. Besides, to eliminate the SARS-CoV-2, conventional medications have either failed or been used taking them in doses higher than their therapeutic index leading to side effects (Ianevski et al., 2020; Sharma et al., 2020). On the other hand, due to their multitarget character, phytochemicals have always been of the options for discovering drug molecules to treat complicated diseases, including viral diseases and their complications. On the other hand, lung injury is the main COVID-19 complication that happens with inflammatory cascades by SARS-CoV-2 (Fakhri et al., 2020b; Merad and Martin, 2020). In the present review, we described the candidate phytochemicals with protective effects on lung injuries induced by various methods, as well as their pharmacological mechanisms (**Figure 4**). In addition, we showed some phytochemicals possessing protective effects against lung injury, with a focus on cepharanthine, epigoitrin, isofraxidin, osthole, resveratrol, apigenin, kaempferol, myricetin, quercetin, chlorogenic acid, chicoric acid, emodin, thymoquinone, betulinic acid, eucalyptol, oridonin, zerumbone, glycyrrhizin, and sulforaphane and their antiviral activities (**Table 1**). On the other hand, despite the effectiveness of natural secondary metabolites in combating viral diseases, providing the novel drug delivery systems helps to drawback their pharmacokinetic limitations (Abbaszadeh et al., 2020; Fakhri et al., 2020a). Such reports could pave the way for discovering alternative drugs with anti-CoV effects and the potential in controlling the complication of COVID-19. Additional studies are needed to reveal the precise dysregulated pathways in COVID-19 and clarify the potential effects of phytochemicals on humans.

AUTHOR CONTRIBUTIONS

MM and MF contributed to conceptualization; MM, SF, and MF contributed to designing the structure of the paper; MM and SF contributed to software; MM, SF, YS, NK, KS, PM, MG, MF, and JE contributed to drafting the manuscript; and MM, SF, MF, and JE contributed to reviewing and editing the paper.

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REVIEW
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An Evaluation of Traditional Persian Medicine for the Management of SARS-CoV-2

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A new coronavirus causing severe acute respiratory syndrome (SARS-CoV-2) has emerged and with it, a global investigation of new antiviral treatments and supportive care for organ failure due to this life-threatening viral infection. Traditional Persian Medicine (TPM) is one of the most ancient medical doctrines mostly known with the manuscripts of Avicenna and Rhazes. In this paper, we first introduce a series of medicinal plants that would potentially be beneficial in treating SARS-CoV-2 infection according to TPM textbooks. Then, we review medicinal plants based on the pharmacological studies obtained from electronic databases and discuss their mechanism of action in SARS-CoV-2 infection. There are several medicinal plants in TPM with cardiotonic, kidney tonic, and pulmonary tonic activities, protecting the lung, heart, and kidney, the three main vulnerable organs in SARS-CoV-2 infection. Some medicinal plants can prevent "humor infection", a situation described in TPM which has similar features to SARS-CoV-2 infection. Pharmacological evaluations are in line with the therapeutic activities of several plants mentioned in TPM, mostly through antiviral, cytoprotective, anti-inflammatory, antioxidant, and anti-apoptotic mechanisms. Amongst the primarily-introduced medicinal plants from TPM, rhubarb, licorice, garlic, saffron, galangal, and clove are the most studied plants and represent candidates for clinical studies. The antiviral compounds isolated from these plants provide novel molecular structures to design new semisynthetic antiviral agents. Future clinical studies in healthy volunteers as well as patients suffering from pulmonary infections are necessary to confirm the safety and efficacy of these plants as complementary and integrative interventions in SARS-CoV-2 infection.

Keywords: herbal medicine, coronavirus, Traditional Persian medicine, antioxidant, phytochemical

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INTRODUCTION

Coronavirus 2019 (SARS-CoV-2) is a new member of the Coronaviridae family which has caused a global outbreak of a disease called COVID-19 (Wu et al., 2020). Despite the current pharmacotherapies, including different antiviral agents used for the management of hospitalized patients like remdesivir, lotinavir, ritonavir, and ribavirin, a growing number of deaths still occur all over the world (World Health Organization (2020) situation report 197) which has made scientists seek better therapeutic agents. Plants have always been an important source of medicinal

ingredients, including antiviral agents (Lin et al., 2014; Bahramsoltani et al., 2016). Although there is no approved drug for this disease, it is worth mentioning that oseltamivir (Tamiflu), an antiviral agent currently used in some SARS-CoV-2 infected patients, is based on shikimic acid from *Illicium verum* Hook.f. (star anise) fruit as the precursor (Patra et al., 2020). Additionally, the clinical effectiveness of several medicinal plants such as licorice and garlic has been demonstrated in the treatment of severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS) epidemics (Xiu-hui et al., 2003; Chen and Nakamura, 2004; Cui et al., 2020). A dramatic reduction of mortality from 52% to 1-4% was observed during the SARS epidemic in Beijing due to the addition of Traditional Chinese Medicine to conventional therapies (Chen and Nakamura, 2004). Thus, plant-derived natural agents provide a valuable list of compounds with possible antiviral properties against SARS-CoV-2 which can be the focus of future investigations. Furthermore, the use of complementary and alternative medicine, including different traditional medicines, can be cost-saving and decrease the prescription of conventional drugs, providing further reasons for scientists to undertake studies on natural products (World Health Organization (2013) WHO traditional medicine strategy: 2014-2023).

Traditional Persian Medicine (TPM) is one of the most ancient medical doctrines mostly known through manuscripts by Persian scientists such as *The Canon of Medicine* by Avicenna and *The Great Continens* by *Rhazes* (Hamedi et al., 2013). Additionally, TPM owes several other scientists with valuable manuscripts regarding anatomy and physiology, disease diagnosis, surgery instruments, and single and compound natural medicines (El-Seedi et al., 2019). TPM has several recommendations for the management of organ damage due to various infections. One of the main approaches in the primary and secondary treatment of diseases in TPM is to protect the four body humors, which are blood, phlegm, bile, and melancholy, from infection (Emtiazy et al., 2012; Kopaei et al., 2016). Many types of infections or the so-called "humor infections" with different clinical manifestations have been described in TPM manuscripts and textbooks, some of which have similar features to that of SARS-CoV-2. One of the pathological conditions explained in TPM for the humors is "humor excitation" which is equivalent to the activation of inflammatory pathways (Aghili Khorasani, 1771).

An examination of pathological conditions similar to COVID-19 along with the therapeutic approaches that are described in TPM manuscripts could pave the way for designing a series of natural products for the management of SARS-CoV-2 infection and related complications (Siahpoosh, 2020). Taking concepts from TPM to treat severe infective pulmonary disorders can help us to select medicinal plants that are potentially useful for SARS-CoV-2 (Kenari et al., 2018). The TPM approach includes lifestyle modifications, along with the administration of some medicinal plants to modify the quality and quantity of the four humors from a pathologic situation into the physiologic condition. The main organs affected in COVID-19 are the lung, heart, and kidney (Su et al., 2020; Zheng et al., 2020). Accordingly, another approach, which extracts TPM suggestions for the management of the disease is to focus on the natural agents that are specifically recommended as a tonic for these three organs. A "tonic", e.g. a cardiotonic medicine, in TPM is defined as a medicine by which an ideal condition is provided for the physiological functions of an organ so that it is less vulnerable to the pathological conditions (Aghili Khorasani, 1771). This study aims to introduce some of the medicinal plants that TPM claims are effective in the management of symptoms similar to COVID-19 and present current evidence on their efficacy.

METHODS

This study used several texts, including the *Makhzan-al-Adviah*, written by MH Aghili Khorasani in 1772 A.D. (Aghili Khorasani, 1771 AD), which is the most recent and complete encyclopedia of medicinal materials in TPM, as well as *The Great Continens* (Rhazes, 960) and *The Canon of Medicine* (Avicenna, 1025). We searched these books/manuscripts for references to medicinal plants that are described as having protective properties against humor infection and excitation, as well as tonifying and protecting effects on the lungs, heart, and kidney.

Our interpretation and translation of old Persian names into scientific names was based on a book by Ghahreman and Okhovvat (2009), which provides accounts of the most relevant scientific names according to morphological descriptions. The list of plants retrieved during this process was then searched in electronic databases including PubMed, Scopus, Cochrane Library, and Web of Science. Data were collected from inception until March 2020. Only published articles were included in this review and unpublished works were not considered. Language restriction was performed, and English language articles were included. The search terms were the scientific names and common names of each plant combined with "antiviral", "influenza", "lung", "pulmonary", "alveolar infiltration", "cardiac", "heart", "cardiomyopathy", "renal", "kidney", or "immunomodulatory". The inclusion criteria were any *in vitro*, *in vivo*, or clinical evidence on the antiviral activity of the selected plants against human pathogenic RNA viruses. Additionally, any study about the beneficial effects of these plants or their isolated phytochemicals on the heart, lungs, and kidneys was included. Studies on animal/plant viral pathogens were excluded. This review does not include *in silico* antiviral analyses, however, they are referred to in the discussion of the mechanisms and pharmacological effects reported in experiments and studies. The references of the included articles were also searched in order to find additional relevant studies. The plants discussed in the TPM sources and their protective mechanisms are listed in **Table 1** and the pharmacological evidence obtained from published papers are summarized in **Table 2**. **Table 3** shows a quality assessment of animal studies based on the Animal Research: Reporting of In vivo Experiments (ARRIVE) guidelines (McGrath and Lilley, 2015).

TABLE 1 | Medicinal plants with possible beneficial effects in treating COVID-19 based on Traditional Persian Medicine.

Scientific name/common name	Persian names	Part	Preventing the infection of humors	Preventing the excitation of humors	Cardiotonic properties	Pulmonary tonic properties	Kidney tonic properties	Reference
Acacia nilotica (L.) Delile/Gum Arabic	Samgh-e-Arabi	Gum				+		(Avicenna 1025; Aghili Khorasani, 1771)
Allium sativum L./Garlic	Soom, Sir	Bulb	+		+		+	(Aghili Khorasani, 1771)
Alpinia galanga (L.) Willd., *A. officinarum* Hance/Galangal	Khoulanjān	Rhizome				+	+	(Rhazes, 960; Avicenna 1025; Aghili Khorasani, 1771)
Aquilaria malaccensis Lam./Agar wood	Oud-e-Hendi	Wood			+			(Avicenna 1025; Aghili Khorasani, 1771)
Berberis vulgaris L./Barberry	Zereshk, ambarbāris (fruit), Arghis (root)	Fruit, root	+ (root)		+ (fruit)			(Aghili Khorasani, 1771)
Cicer arietinum L./Pea	Nokhod	Seed				+		(Avicenna 1025; Aghili Khorasani, 1771)
Cichorium intybus L./Chicory	Kāsni, Hendabā	Seed, root, leaf	+	+	+		+	(Avicenna 1025; Aghili Khorasani, 1771)
Commiphora myrrha (Nees) Engl./ Myrrh	Morr-e-Macci	oleo-gum resin	+					(Aghili Khorasani, 1771)
Coriandrum sativum L./Coriander	Geshniz, Kozboreh	Fruit			+			(Rhazes, 960; Aghili Khorasani, 1771)
Crocus sativus L./Saffron	Zaferān	Stigma			+	+	+	(Rhazes, 960; Avicenna 1025; Aghili Khorasani, 1771)
Cydonia oblonga Mill./Quince	Safarjal, Beh	Fruit			+			(Aghili Khorasani, 1771)
Cymbopogon schoenanthus (L.) Spreng./Lemon grass	Ezkher	Leaf				+	+	(Rhazes, 960; Avicenna 1025; Aghili Khorasani, 1771)
Echium amoenum Fisch. & C.A.Mey./ Red Feathers	Lesān-al-sour, gāv zaban	Flower				+		(Avicenna 1025; Aghili Khorasani, 1771)
Elettaria cardamomum (L.) Maton/ Cardamom	Hel	Seed			+			(Aghili Khorasani, 1771)
Ficus carica L./Fig	Anjeer, Tin	Fruit				+		(Avicenna 1025; Aghili Khorasani, 1771)
Fumaria parviflora Lam., *F. vaillantii* Loisel./Fumitory	Shātareh	Aerial parts		+				(Aghili Khorasani, 1771)
Gentiana lutea L./Yellow Gentian	Gentianā	Root			+			(Aghili Khorasani, 1771)
Glycyrrhiza glabra L./Licorice	Shirin bayan, sous	Root				+		(Rhazes, 960; Avicenna 1025; Aghili Khorasani, 1771)
Hordeum vulgare L./Barley	Shaeer, Jo	Seed				+		(Rhazes, 960; Avicenna 1025; Aghili Khorasani, 1771)
Inula helenium L./Elecampane inula	Rāsan	Root			+	+		(Rhazes, 960; Aghili Khorasani, 1771)
Lallemantia royleana (Benth.) Benth.	Bālangu	Seed			+			(Aghili Khorasani, 1771)
Laurus nobilis L./Bay laurel	Ghār, Barg-e-Bou	Leaf, fruit	+ (leaf)			+ (fruit)		(Avicenna 1025; Aghili Khorasani, 1771)
Malus domestica Borkh./Apple	Sib, Toffāh	Fruit			+			(Avicenna 1025; Aghili Khorasani, 1771)
Melissa officinalis L./Lemon balm	Bādranjbouyeh	Leaf			+			(Aghili Khorasani, 1771)
Nymphaea alba L./Water lily	Niloufar	Flower				+		(Avicenna 1025; Aghili Khorasani, 1771)
Phyllanthus emblica L./Amla	Ameleh	Fruit	+		+			(Rhazes, 960; Avicenna 1025; Aghili Khorasani, 1771)
Pistacia lentiscus L./Mastic	Mastaki	Oleo-gum-resin					+	(Aghili Khorasani, 1771)
Plantago major L., *P. lanceolata*/ Plantain	Bārhang	Seed					+	(Avicenna 1025; Aghili Khorasani, 1771)
Rheum palmatum L./Chinese rhubarb	Reevand	Root				+		(Aghili Khorasani, 1771)

(Continued)

TABLE 1 | Continued

Scientific name/common name	Persian names	Part	Preventing the infection of humors	Preventing the excitation of humors	Cardiotonic properties	Pulmonary tonic properties	Kidney tonic properties	Reference
Rosa × *damascena* Herrm./Damask rose	Vard, Gol-e-Mohammadi	Flower			+	+	+	(Rhazes, 960; Avicenna 1025; Aghili Khorasani, 1771)
Salix aegyptiaca L./Musk willow	Beedmeshk	Flower			+		+	(Aghili Khorasani, 1771)
Santalum album L./Sandal wood	Sandal	Wood			+			(Avicenna 1025; Aghili Khorasani, 1771)
Syzygium aromaticum (L.) Merr. & L.M.Perry/Clove	Gharanfol, Mlkhak	Flower bud			+		+	(Aghili Khorasani, 1771)
Tamarindus indica L./Tamarind	Tamr-e-Hendi	Fruit		+	+			(Aghili Khorasani, 1771)
Trigonella foenum-graecum L./ Fenugreek	Holbeh, Shanbalileh	Seed				+		(Rhazes, 960; Avicenna 1025; Aghili Khorasani, 1771)
Vitis vinifera L./Grape (fresh), raisin (dried)	Enab, angour (fresh), Maveez (dried)	Fruit juice				+	+	(Avicenna 1025; Aghili Khorasani, 1771)
Ziziphus jujuba Mill./Jujube	Onnab	Fruit		+				(Avicenna 1025; Aghili Khorasani, 1771)

RESULTS

Amla (*Phyllanthus emblica* L.)

Amla is the fruit of a tree from the family Phyllanthaceae, which is used as fresh fruit, jam, or electuary (Yadav et al., 2017). In TPM, amla fruit is considered as a cardiotonic and cardioprotective medicine that is useful for treating cardiovascular problems. Amla also prevents humors from infection and thus acts as a general tonic, i.e. strengthens the body against infection (Aghili Khorasani, 1771 AD).

The antiviral activity of amla has been demonstrated against the human immunodeficiency virus (HIV) (**Table 2**). Polyphenols such as kaempferol and quercetin glycosides, gallotannins, and putranjivain A, a potent non-competitive inhibitor of HIV reverse transcriptase (RT), are known as the main anti-HIV components of amla. The higher inhibitory activity of putranjivain A (IC_{50} = 3.9 μM) in comparison to the other isolated phytochemicals (IC_{50}>200 μM) from this plant seems to be the result of the hexahydroxydiphenoyl functional group (el-Mekkawy et al., 1995).

In addition to the direct antiviral activity of amla which can suggest natural molecular backbones for possible antiviral agents against SARS-COV-2, there are several reports on the protective effects of the plant on the main organs damaged in SARS-COV-2 infection (**Table 2**). Pyrogallol, a small gallotanin of amla, demonstrated *in vitro* protective effect on bronchial epithelial cells of cystic fibrosis infected with *P. aeruginosa*. The compound could significantly prevent bacterial pulmonary inflammation, evident from the reduced production of neutrophil chemokines, pro-inflammatory interleukins (IL), and intercellular adhesion molecule (ICAM)-1, an important contributor in leukocyte chemotaxis (Nicolis et al., 2008).

Additionally, amla could prevent immunotoxicity induced by chromium and arsenic through modulating the phagocytic properties of immune cells, as well as restoring their ability to produce interferon (IFN)-γ, a critical mediator of the immune system (Sai Ram et al., 2002; Sai Ram et al., 2003).

The cardioprotective effect of amla fruit has been demonstrated in several studies (**Table 2**). It has also been reported that it prevents the myocardial depletion of creatine kinase-MB (CK-MB), a marker of cardiac damage, and improves hemodynamic parameters, further showing its cardioprotective effects (Patel and Goyal, 2011; Ojha et al., 2012). Emblicanin-A and B, two small-sized hydrolysable gallotanins, have exhibited a protective effect in ischemia-reperfusion (I/R)-induced cardiac damage in rats. Emblicanins could represent cardioprotective properties mostly through the induction of endogenous enzymatic antioxidant defense mechanisms and the prevention of lipid peroxidation. The effect of 50 mg/kg of this compound was equal to 200 mg/kg of vitamin E (standard antioxidant); whereas 100 mg/kg of emblicanins showed a higher potency compared with vitamin E (Bhattacharya et al., 2002). These compounds have also represented nephroprotective activity in an animal model of cisplatin-induced nephrotoxicity through the same mechanism (Malik et al., 2016). In terms of the cardioprotective effects of amla extract, another target, the phosphoinositide 3-kinase/glycogen synthase kinase 3β (PI3K/

TABLE 2 | Pharmacological studies on the medicinal plants predicted to be useful in SARS-CoV-2 infection based on traditional Persian medicine.

General category of therapeutic activity	Scientific name/ preparation	Model/Design	Dosage and duration of treatment	Mechanisms	Reference
Antiviral activity					
	Allium sativum/diallyl disulfide, diallyl sulfide, alliin	*In vitro* antiviral activity against DENV-2 NGC virus in human liver & macrophage cells	10-1000 μM	↓TNF-α, IL-8, IL-10, LPO, iNOS	(Hall et al., 2017)
	***Alpinia galanga*/ acetoxychavicol acetate**	*In vitro* antiviral activity against influenza virus (H1N1)	–	↓Nuclear export of viral ribonucleoprotein complex (IC_{50} = 12.8 μM), ↓virus production (IC_{50} = 2μM, SI=2.8)	(Watanabe et al., 2011)
	Alpinia galanga/ acetoxychavicol acetate	*In vitro* inhibition of HIV-1 Rev	5-20 μM	↓Nuclear transport of Rev by direct binding of Cys-529 in chromosomal region maintenance-1 *via* direct binding to the nuclear export signal of Rev	(Tamura et al., 2009)
	***Alpinia officinarum*/ diarylheptanoids**	*In vitro* antiviral activity against RSV, poliovirus, measles virus	–	RSV: EC_{50} = 5-42 μg/ml, SI=0.9->6.1 Poliovirus: EC_{50} = 3.7-44 μg/ml, SI=1-5.5 measles virus: EC_{50} = 6-47 μg/ml, SI= 1.3-5.5	(Konno et al., 2011)
	***Alpinia officinarum*/ diarylheptanoids**	*In vitro* antiviral activity against influenza A & B different subtypes, influenza A/PR/8/34-induced pulmonary infection in mouse	*In vivo*: 30, 100 mg/kg/day, 6 days	↓Viral messenger RNA & antigens, No effect on virus adsorption or invasion, EC_{50} of plaque reduction= 16-96 μM, ↑survival, ↓Body weight loss & virus titer is BALF	(Sawamura et al., 2010)
	Crocus sativus/aqueous extract, picrocrocin, crocin	*In vitro* antiviral activity against HIV-1	–	↓Viral replication by crocin & picrocrocin: Crocin: anti-HIV-1: IC_{50}: 8 μM, SI: >187.5 Picrocrocin: anti-HIV-1: IC_{50}: 5 μM, SI: >600 No significant antiviral effect by the extract	(Soleymani et al., 2018)
	Glycyrrhiza glabra/aqueous & alkaline extracts	*In vitro* antiviral activity against HIV-1	–	Higher anti-HIV effect with alkaline extract (EC_{50} = 54-167 μg/ml, SI=3-9)	(Fukuchi et al., 2016)
	***Glycyrrhiza* spp./ glycyrrhizin**	*In vitro* antiviral activity against influenza A H5N1-infection in human airway epithelial A549 cells	25-200 μg/ml	↓Apoptosis, viral replication, IL-6, MCP-1, CCL5, CXCL10, ROS, ↓NF-κB, JNK, & p38 activation	(Michaelis et al., 2011)
	***Glycyrrhiza* spp./ glycyrrhizin**	*In vitro* antiviral activity against clinical isolates of SARS coronavirus (FFM-1 & FFM-2)	–	↓Viral replication (EC_{50} = 300, 600 mg/l, SI=33, 67), Higher activity when added during & after virus adsorption, ↓viral antigens expression	(Cinatl et al., 2003)
	Glycyrrhiza spp./MeOH extract & isolated compounds	*In vitro* antiviral activity against HCV	–	IC_{50} (μg/ml): *G. uralensis* MeOH extract: 20, chloroform fraction:8, glycycoumarin:8.8, liquiritigenin:16.4, licochalcone A:2.5, glycyrin:7.2, glabridin:6.2, glycyrol:4.6, isoliquiritigenin:3.7, glycyrrhizin:180, glycyrrhizic acid monoammonium: 320, Inhibition is mostly in post-entry step	(Adianti et al., 2014)
	***Glycyrrhiza uralensis*/ aqueous extract, 18β-glycyrrhetinic acid, & glycyrrhizin**	*In vitro* RSV-induced inflammation in human airway epithelial A549 & HEp-2 cells	–	18β-glycyrrhetinic acid: ↓Plaque formation (IC_{50}≈71, 75 μg/ml & TI≈71, 76), Extract: ↓Plaque formation (IC_{50}≈4 μg/ml & TI≈27), ↓viral attachment & internalization, ↑IFNβ	(Feng Yeh et al., 2013)
	Phyllanthus emblica/aqueous & MeOH extract	*In vitro* HIV-RT inhibition assay	–	IC_{50} = 9 μg/ml for aqueous & 10 μg/ml for MeOH extracts. putranjivain A from MeOH extract showed IC_{50} = 3.9 μM	(el-Mekkawy et al., 1995)
		In vitro antiviral activity against HIV-1	–		(Esposito et al., 2016)

(Continued)

TABLE 2 | Continued

General category of therapeutic activity	Scientific name/ preparation	Model/Design	Dosage and duration of treatment	Mechanisms	Reference
	Rheum palmatum & *Rheum officinale*/hydromethanolic extract & isolated compounds			IC_{50}: RNase H: *R. palmatum*=0.9 μg/ml, *R. officinale*=0.25 μg/ml, sennoside A=1.9 μM, sennoside B=2.1 μM RDDP: sennoside A=5.3 μM, sennoside B=2.3 μM, Integrase: sennoside A=3.8 μM, sennoside B=87 μM, ↓viral replication	
	Rheum palmatum/EtOH extract	*In vitro* antiviral activity against CVB_3, CVB_3-induced infection in mouse	*In vitro*: 2-10 μg/ml, *In vivo*: 0.18-0.5 mg/kg/day, i.p., 5 days	*In vitro*: ↓viral replication (IC_{50} = 4 μg/ml, SI=10), *In vivo*: ↑survival, ↓viral titer	(Xiong et al., 2012)
	Rheum spp./emodin	*In vitro* antiviral activity against EV71	29.6 μM	↓Viral replication, maturation, & virulence, Lower effect of viral protein expression, ↓cell cycle arrest at S phase	(Zhong et al., 2017)
	Rosa damascena/MeOH & aqueous extracts, purified flavonoids	*In vitro* antiviral activity against HIV	–	↓Infectivity (EC_{50} = 4- >250 μg/ml, SI=5- >100), ↓gp 120 binding to CD4, viral protease	(Mahmood et al., 1996)
	Syzygium aromaticum/ aqueous & MeOH extracts	*In vitro* inhibition of HCV protease	100 μg/ml	76% % 90% inhibition by the MeOH & aqueous extracts, IC_{50} = 33 μg/ml for aqueous extract	(Hussein et al., 2000)
	***Syzygium aromaticum*/ eugenol from essential oil**	*In vitro* CPE inhibitory assay against influenza A virus	5 μg/ml	↓Virus-induced autophagy & cell death, virus replication, ↓ROS, NO, LPO, IL-1, IL-6, IL-8, TNF-α, ↓activation of ERK1/2, p38MAPK, & IKK/NF-kB pathways but not JNK1, ↑GSH, GR, SOD	(Dai et al., 2013)
	Vitis vinifera/extract	*In vitro* antiviral activity against HCV	2.5-20 μg/ml	↓HCV replication, COX-2, NF-κB & MAPK/ERK/JNK signaling, Synergistic effect with conventional anti-HCV drugs	(Chen et al., 2016)
	***Vitis vinifera*/ proanthocyanidin extract**	*In vitro* RSV-induced inflammation in human airway epithelial A549 cells	5, 10 μg/ml	↓Viral replication, viral nucleoprotein & fusion protein, ↓MUC1, MUC2, MUC5B, MUC8 expression & mucin synthesis, Suppression of AP-1 & NF-κB *via* p38 MAPKs/JNK	(Lee et al., 2017)
	***Zizyphus jujuba*/betulinic acid**	*In vitro* antiviral activity against influenza A/PR/8 virus, Antiviral activity in mouse infected with influenza A/PR/8 virus	*In vitro*: 0.4-50 μg/ml *In vivo*: 10 mg/kg/day, i.p., 7 days	*In vitro*: ↓viral infection, *In vivo*: ↓pulmonary necrosis, inflammation, edema, leukocytes infiltration, ↓IFNγ, No effect on TNF-α, IL-1β, & virus replication	(Hong et al., 2015)
Cardioprotective activity	*Allium sativum*/allicin	DOX-induced cardiotoxicity in rat	20 mg/kg/day, p.o., 14 days	↑CAT, SOD, Gpx, ↓LPO, LDH, CK-MB, NO, TNF-α, IL-1β, COX-2, Casp-3	(Abdel-Daim et al., 2017)
	Allium sativum/alliin	I/R-induced cardiotoxicity in mouse	100 mg/day, i.p., two doses	↓Cardiomyocyte apoptosis & infarct size, ↑autophagic flux, LC3II/LC3I, beclin-1, Atg9b	(Zhao et al., 2019)
	Allium sativum/diallyl trisulfide	*In vitro* cytoprotection in H9c2 murine cardiocyte, I/R-induced cardiotoxicity in STZ-induced diabetic rat	*In vitro*: 10 μM, *In vivo*: 20 mg/kg/day, p.o., 3 days	*In vitro* & *in vivo*: ↓Apoptosis, ↑AMPK-mediated AKT/GSK-3β/HIF-1α activation	(Yu et al., 2017)
	Allium sativum/homogenate	Fructose-induced cardiotoxicity in diabetic rat	250 mg/kg/day, p.o., 8 weeks	↓NF-κB, ROS, LPO, NO, ↑CAT, GSH, Gpx, SOD, Modulation of PI3K/AKT/Nrf2-Keap1 pathway	(Padiya et al., 2014)
	Alpinia galanga/Cardamonin	DOX-induced cardiotoxicity in mouse			(Qi et al., 2020)

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TABLE 2 | Continued

General category of therapeutic activity	Scientific name/ preparation	Model/Design	Dosage and duration of treatment	Mechanisms	Reference
			20-80 mg/kg/day, p.o., 28 days	Improvement of cardiac function, ↑Nrf-2 signaling, HO-1, NQO-1, GCLM, SOD, GSH, CAT, ↓LPO, ROS, & apoptosis	
	Crocus sativus/aqueous extract	ISO-induced cardiotoxicity in rat	200-800 mg/kg/ day, p.o., 28 days	Improvement of hemodynamic function of heart, ↑Cardiac SOD, CAT, GSH, ↓LPO, LDH & CK-MB leakage	(Sachdeva et al., 2012)
	Crocus sativus/aqueous extract, safranal	ISO-induced cardiotoxicity in rat	20-160 mg/kg/ day of extract or 0.025-0.075 of safranal, i.p., 9 days	↓LDH, CK-MB, LPO, Improvement of myocardium morphological changes	(Mehdizadeh et al., 2013)
	Crocus sativus/crocin	*In vitro* LPS-induced cardiotoxicity in H9c2 murine cardiocyte	10-40 μM	↑Viability, thiol content ↓TNF-α, PGE2, IL-1β, and IL-6, NO, ↓TNF-α, COX-2, IL-1β, IL-6, & iNOS gene expression	(Rahim et al., 2019)
	Crocus sativus/crocin	DOX-induced cardiotoxicity in rat	20, 40 mg/kg/day, i.p., 20 days	Improvement of heart function, ECG, & histopathological damages	(Razmaraii et al., 2016)
	Crocus sativus/ hydromethanolic extract	*In vitro* I/R+DOX-induced toxicity in H9c2 murine cardiocyte	10 μg/ml	↑Viability, α-actinine, troponine C & MLC, AKT/P70S6K & ERK1/2 activity, ↓Casp-3, LDH, mitochondrial dysfunction	(Chahine et al., 2016)
	Glycyrrhiza glabra/aqueous extract	*In vitro* DOX-induced toxicity in H9c2 murine cardiocyte	20-200 μg/ml	↓ROS, DNA damage, mitochondrial dysfunction, ↑membrane integrity, actin stability, SIRT-1, PPARγ & PPARα	(Upadhyay et al., 2020)
	Glycyrrhiza glabra/ hydroalcoholic extract	I/R-induced cardiotoxicity in rat	400 mg/kg, p.o., 30 days	↑SOD, Gpx, CAT, GSH, Restoration of LDH, CK-MB, & hemodynamic cardiac function, ↓LPO	(Ojha et al., 2013)
	Glycyrrhiza spp./glycyrrhizic acid	ISO-induced cardiotoxicity in rat	10, 20 mg/kg, p.o., 2 days	Modulation of ECG & morphology, ↓CK-MB, LDH, Reversible inhibition of L-type Ca^{2+} channels in isolated rat cardiomyocytes (EC_{50} = 145.54 μg/mL)	(Li et al., 2019)
	Phyllanthus emblica/aqueous extract	I/R-induced cardiotoxicity in rat	100 mg/kg/day, p.o., 30 days	Upregulation of PI3K/Akt/GSK3β/β-catenin & Bcl-2, ↑eNOS phosphorylation, ↓myocardiocyte apoptosis,	(Thirunavukkarasu et al., 2015)
	Phyllanthus emblica/ emblicanin-A & -B enriched fraction	I/R-induced cardiotoxicity in rat	100 & 200 mg/kg/ day, p.o., 14 days	↑Cardiac SOD, CAT, Gpx, ↓LPO	(Bhattacharya et al., 2002)
	Phyllanthus emblica/ hydroalcoholic extract	ISO-induced cardiotoxicity in rat	100-500 mg/kg/ day, p.o., 30 days	Restoration of hemodynamic parameters & cardiac function, ↑Cardiac SOD, CAT, Gpx, GSH, ↓LPO, ↓LDH & CK-MB leakage from myocardium	(Ojha et al., 2012)
	Phyllanthus emblica/juice	STZ-induced diabetic myocardial dysfunction in rat	1 ml/kg/day, p.o., 8 weeks	Restoration of hemodynamic parameters, ↓LDH & CK-MB	(Patel and Goyal, 2011)
	Rheum officinale/emodin	Isolated perfused beating rabbit atria	10-100 μM	↑Atrial natriuretic peptide, ↓atrial pulse pressure & stroke volume, Involvement of L-type Ca^{2+} & K^+ channel, No change in muscarinic system	(Zhou et al., 2014)
	Rheum palmatum/ chrysophanol		*In vitro*: 1-20 μM, *In vivo*: 5-40 mg/	*In vitro*: ↓apoptosis, cleavage & activation of PARP1, Casp-3, cytochrome c release from mitochondria to cytoplasm,	(Lu et al., 2019)

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TABLE 2 | Continued

General category of therapeutic activity	Scientific name/ preparation	Model/Design	Dosage and duration of treatment	Mechanisms	Reference
		In vitro DOX-induced toxicity in H9c2 murine cardiocyte, DOX-induced cardiotoxicity in rat	kg/day, p.o., 7 days	↑Bcl-2/Bax, *In vivo*: modulation of ECG, ↓fibrosis, apoptosis, mitochondrial damage	
	Rheum palmatum/rhein	I/R-induced cardiotoxicity in H9c2 murine cardiocyte	1 μg/ml	↓Apoptosis, ROS, p-P38, ↑AKT & GSK3β phosphorylation	(Liu et al., 2018)
	Rosa damascena/ hydroethanolic extract	Isolated guinea pig heart un-treated or pretreated with propranolol, methacholine, & diltiazem	0.1-1 mg%	↑Heart rate & contractility, Higher activity of the extract vs. isoprenaline	(Boskabady et al., 2013)
	Trigonella foenum-graecum/ digoxigenin-3-O-rutin	ISO-induced cardiotoxicity in rat	2.5-10 mg/kg/ day, p.o., 10 days	↓CK-MB, Cr, LDH, AST, ALT, LPO, ↑GSH, Gpx, GST, SOD, CAT, Na^+-K^+-ATPase	(Panda and Kar, 2010)
	Trigonella foenum-graecum/ polysaccharide	*In vitro* cytoprotection in H9c2 murine cardiocyte, Thiamethoxam-induced cardiotoxicity in rat	0.01-1 mg/ml, 100 & 200 mg/kg/ day, p.o., 30 days	*In vitro*: ↓H9c2 necrosis & apoptosis, *In vivo*: ↓LDH, CPK, AST, troponin-T, LDL, TAG, LPO & protein oxidation, ↑GSH & NPSH	(Feki et al., 2019)
	Trigonella foenum-graecum/ powder	ISO+ hypercholesterolemic diet-induced cardiotoxicity in rat	10% of diet, p.o., 8 weeks	↓LDH, CK-MB, AST, ALT, Improvement of the lipid profile & histology of the heart muscle	(Mukthamba and Srinivasan, 2015)
	Trigonella foenum-graecum/ seed powder	STZ-induced cardiotoxicity in diabetic rats	9 g/kg/day, p.o., 30 days	↑Activity of cardiac SOD, CAT, GSH, GST, ↓LPO	(Tripathi and Chandra, 2009)
	Trigonella foenum-graecum/ seed powder	STZ-induced cardiotoxicity in diabetic rats	10% of the diet weight, 6 weeks	↓RAS activity, type IV collagen, fibronectin, Bax, 4-hydroxynonenal, iNOS, nitrate/nitrite, ↑PUFA/SFA ratio	(Pradeep and Srinivasan, 2018)
	Trigonella foenum-graecum/ trigonelline	ISO-induced cardiotoxicity in rat	20-80 mg/kg/day, p.o., 20 days	↓Infarction area, ↓CK-MB, LDH, LPO, ALT, ↓Hsp27 & αB crystallin, ↑GSH, Gpx, GST, SOD, CAT	(Panda et al., 2013)
	Vitis vinifera/proanthocyanidin extract	DOX-induced cardiotoxicity in rat	70 mg/kg, p.o., 10 days	Modulation of ECG, ↓CK-MB, LDH, LPO, ↑SOD, CAT	(Ammar el et al., 2013)
	Vitis vinifera/proanthocyanidin extract	*In vitro* I/R-induced cardiotoxicity in H9c2 murine cardiocyte	50-200 μg/ml	↑Viability, ↓LDH, GRP78, CHOP, phosphorylated PERK, eIF2α, endoplasmic reticulum stress-induced apoptosis	(Wang X. et al., 2017)
	Zizyphus jujuba/Jujuboside A	ISO-induced cardiotoxicity in H9c2 murine cardiocytes	5-20 μM	↑Viability, phosphorylation of PI3K, Akt, & mTOR, ↓LC3-II/I	(Han et al., 2016)
	Zizyphus jujuba/polyphenols	ISO-induced cardiotoxicity in rat	300 mg/kg/day, p.o., 5, 10 days	↓LPO, Ca^{2+} & Mg^{2+}-ATPase activity, ↑SOD, Gpx, Na^+K^+-ATPase, LDH & CK activity, Modulation of ECG	(Cheng et al., 2012)
Immunomodulatory activity	*Allium sativum*/aged extract	Randomized, double-blind, placebo-controlled trial in healthy individuals	2.6 g/day, p.o., 90 days	↑Proliferation of γδ-T cells & NK cells, ↓severity of cold & flu symptoms	(Nantz et al., 2012)
	Allium sativum/essential oil & organosulfur compounds	*In vitro* human neutrophils	–	↑Ca^{2+} flux (EC_{50} = 9-22 μM), ↑ROS production *via* PI3K activation, CREB, ERK1/2, & GSK-3α/β phosphorylation (200-500 μM of 1,3-dithiane)	(Schepetkin et al., 2019)
	Allium sativum/ polysaccharides	*In vitro* immunostimulation RAW 264.7 macrophages	5-200 μg/ml	↑Proliferation, phagocytosis, secretion of NO, IL-6, IL-10, TNF-α, IFNγ, Higher activity by the fresh sample	(Li et al., 2017)
			0.3-10 μM	↓IFNβ mRNA expression, NF-κB activation, NO production *via* TLR-3	(Ando et al., 2005)

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TABLE 2 | Continued

General category of therapeutic activity	Scientific name/ preparation	Model/Design	Dosage and duration of treatment	Mechanisms	Reference
	Alpinia galanga/ acetoxychavicol acetate	*In vitro* LPS-activated murine macrophage			
	Alpinia galanga/galangin	*In vitro* LPS-activated RAW 264.7 macrophages	12.5- 50 μM	↓iNOS, NO, IL-6, IL-1β, ERK & NF-κB-p65 phosphorylation	(Jung et al., 2014)
	Cichorium intybus/EtOH extract	EtOH-induced immunotoxicity in mouse	300 mg/kg/day, p.o., 28 days	↑Circulating leukocytes, splenic plaque forming cells, hemagglutination titers to SRBC, secondary IgG response to bovine serum albumin, phagocytes activity, NK cells, IFNγ, delayed-type hypersensitivity	(Kim et al., 2002)
	Cichorium intybus/powder	Innate immune response in growing piglets	4% as dietary supplement, p.o., 21 days	↓Apolipoprotein C-II complement component C6, CRP, CD14 antigen, C4b binding protein α & β chains, fibrinogen	(Lepczynski et al., 2015)
	Crocus sativus/hydroethanolic extract	Non-stimulated & phytohemagglutinin-stimulated lymphocytes	50-500 μg/ml	Stimulated cells: ↓IFNγ, IL-10, Non-stimulated cells: ↑IFNγ, IL-4 ↑Th1/Th2 balance	(Boskabady et al., 2011)
	Crocus sativus/powder	Randomized double-blind placebo-controlled in healthy men	100 mg, p.o., 6 weeks	Week 3: ↑IgG, monocyte percentage, ↓IgM, basophil percentage, Week 6: all results returned to baseline	(Kianbakht and Ghazavi, 2011)
	Glycyrrhiza spp./glycyrrhizin	*In vitro* LPS-induced inflammation in RAW 264.7 macrophage, LPS-induced endotoxemia in mouse	*In vitro*: 0.5-2 μM, *In vivo*: 200 mg/ kg, i.p., single dose	↑HO-1, nuclear translocation of Nrf2, ↓HMGB1, iNOS, Involvement of p38, but not ERK or JNK	(Kim et al., 2015)
	Phyllanthus emblica/EtOH extract	*In vitro* Chromium (VI)-induced immunosuppression in rat lymphocyte	10-1000 μg/ml	Restoration of IL-2 & IFNγ production ↑cell survival, GSH, Gpx, ↓LDH leakage, ROS, LPO, DNA fragmentation	(Sai Ram et al., 2002)
	Phyllanthus emblica/ hydroalcoholic extract	*In vitro* Chromium (VI)-induced immunosuppression in J-774 macrophage	250 μg/ml	Restoration of phagocytosis & IFNγ production, ↑cell survival, Gpx & GSH, ↓ROS	(Sai Ram et al., 2003)
	Syzygium aromaticum/biflorin	*In vitro* LPS-induced inflammation in RAW 264.7 macrophage, LPS-induced endotoxemia in mouse	5 & 10 mg/kg, i.p., single dose	↓iNOS, NO, COX-2, PG-E2, TNF-α, IL-6, p-STAT1, p-p38	(Lee et al., 2016)
	Syzygium aromaticum/ essential oil	Cyclophosphamide-induced immunosuppression in mouse under SRBC challenge	100-400 mg/kg/ day, p.o., 7 days	↑WBC, Stimulation of both humoral & cell-mediated immunity	(Carrasco et al., 2009)
	Syzygium aromaticum/ hydromethanolic extract & eugenol	*In vitro* LPS-induced inflammation in murine peritoneal macrophage	5-100 μg/well	↓IL-1β by the extract, ↓IL-6 & IL-10 by the extract & eugenol	(Bachiega et al., 2012)
	Trigonella foenum-graecum/ powder	Burn wound induced in cyclophosphamide-immunosuppressed rat	0.5 & 1 g/kg/day, p.o., 28 days	↓Neutropenia & lymphopenia, ↑weight & cellularity of thymus, spleen, bone marrow, ↑γ-globulin, delayed-type hypersensitivity response, & burn wound healing rate	(Ramadan et al., 2011)
	Vitis vinifera/proanthocyanidin extract	Aflatoxin-induced immunotoxicity in mouse	50, 100 mg/kg/ day, p.o., 3 weeks	↓Weight loss, ↓splenic LPO, IL–1β, IL-6, TNF-α, IFNγ, ↑CAT, GSH, Gpx, SOD	(Long et al., 2016)
	Zizyphus jujuba cv. *Huizao*/ acidic polysaccharides	Immunomodulatory effect in healthy mouse	50-200 mg/kg/ day, p.o., 7 days	↑Spleen & thymus indices, hemagglutination titers to SRBC, delayed-type hypersensitivity, phagocytes activity	(Zou et al., 2018)
Lung protective activity	*Allium sativum*/aqueous extract	Lambda-cyhalothrin-induced pulmonary damage in rat	100 mg/kg/day, i.p., 21 days	↓Cough, nasal discharge, alveolitis, lung inflammation & hyperplasia	(Mohi El-Din et al., 2014)

(Continued)

TABLE 2 | Continued

General category of therapeutic activity	Scientific name/ preparation	Model/Design	Dosage and duration of treatment	Mechanisms	Reference
	Allium sativum/S-allyl-L-cysteine	Bleomycin-induced pulmonary toxicity in mouse	5, 10 mg/kg, i.p., single dose	↓Pulmonary fibrosis *via* α-SMA, TNF-α, fibronectin, collagen I & III, leukocytes infiltration into BALF, iNOS, AKT & NF-κB p65 phosphorylation	(Nie et al., 2019)
	Allium sativum/S-allylmercaptocysteine	LPS-induced lung inflammation in mouse	10-60 mg/kg, p.o., single dose	↓Lung edema, MPO, TNF-α, IL-1β, IL-6, iNOS, COX-2, NF-κB, LPO, ↑GSH, SOD, HO-1, Modulation of Keap1/Nrf2 pathways	(Mo et al., 2020)
	Alpinia galanga/ acetoxychavicol acetate	OVA-induced airway inflammation in mouse	25, 50 mg/kg/day, i.p., 5 days	↓Leukocyte infiltration in BALF, airway hyperresponsiveness, goblet cells hyperplasia, ↓anti-OVA IgG, IL-4, IL-13, IL-12α, IFNγ	(Seo et al., 2013)
	Alpinia galanga/galangin	LPS-induced airway inflammation in mouse	1.5, 15 mg/kg/ day, i.p., single dose	↑HO-1 & oxygenation, ↓lung edema, NF-κB, MPO, IL-6, TNF-α	(Shu et al., 2014)
	Alpinia galanga/galangin	*In vitro* TNF-α-induced inflammation in normal human airway smooth muscle cells, OVA-induced airway inflammation in mouse	10 μM *in vitro* 5, 15 mg/kg/day, i.p., 3 days	*In vitro*: ↓MCP-1, nuclear translocation of p65, eotaxin, CXCL10, and VCAM-1, *In vivo*: ↓leukocyte infiltration in BALF, airway hyperresponsiveness, goblet cells hyperplasia, ↓anti-OVA IgG, IL-4, IL-5, IL-13, iNOS, VCAM-1, NF-κB-related inflammation	(Zha et al., 2013)
	Crocus sativus/crocin	*In vitro* cytoprotection in HUVEC cells, LPS-induced lung inflammation in mouse	*In vitro*: 20 μM, *In vivo*: 15-60 mg/ kg, i.p., 7 days	*In vitro* & *in vivo*: ↓NF-κB & MAPK activity, MMP-9, heparanase, ↑Pulmonary vascular permeability	(Zhang et al., 2020b)
	Crocus sativus/crocin	Bleomycin-induced pulmonary toxicity in rat	20 mg/kg/day, p.o., 5 weeks	↓Pulmonary inflammation, fibrosis, leukocytes infiltration into BALF ↓LDH, LPO, NO, TNF-α, TGF-β1, TLR-4, IL-10, ↑SOD, GSH, TAC, HO-1, Nrf2	(Zaghloul et al., 2019)
	Crocus sativus/powder	Randomized, triple-blind, placebo-controlled trial in patients with mild & moderate persistent asthma	100 mg/day, p.o., 8 weeks	Improvement of spirometry parameters, ↓CRP, anti-Hsp70 antibody	(Hosseini et al., 2018)
	Crocus sativus/safranal	OVA-induced airway inflammation in guinea pigs	4-16 μg/ml of drinking water	↓NO, nitrite, IL-4, tracheal response to methacholine & OVA, ↑IFNγ/IL-4	(Boskabady et al., 2014)
	Glycyrrhiza glabra/glycyrrhizic acid	OVA-induced airway inflammation in mouse	10-40 mg/kg/day, p.o., 30 days	↑Regulatory T cells, IFNγ, Foxp3 protein, ↓leukocytes infiltration into BALF, IL-4, IL-5, IL-13, OVA-specific IgE	(Ma et al., 2013)
	Glycyrrhiza glabra/glycyrrhizin	*In vitro* TGF-α-induced mucus production in NCI-H292 cells, LPS & IL-4-induced airway inflammation in mouse	*In vitro*: 10-1000 μM, *In vivo*: 15-135 mg/kg, s.c., 6 days	*In vitro*: ↓MUC5AC protein and mRNA expression *In vivo*: ↓Goblet cell hyperplasia & MUC5AC mRNA expression	(Nishimoto et al., 2010)
	Glycyrrhiza glabra/aqueous extract	Randomized, double-blind, placebo-controlled trial in 235 patients with postoperative sore throat & postextubation coughing	0.5 g/30 ml, as gargle,	↓Sore throat & incidence of coughing	(Ruetzler et al., 2013)
	Glycyrrhiza uralensis/ glycyrrhizic acid	*In vitro* LPS-induced inflammation in RAW 264.7 macrophage, LPS-induced lung inflammation in mouse	*In vitro*: 100 μg/ ml, *In vivo*: 200 mg/ kg, i.p., single dose	↑viability, LC3-II/I and Beclin-1, autophagy *via* PI3K/AKT/mTOR pathway, ↓TNF-α, IL-1β, HMGB1	(Qu et al., 2019)

(Continued)

TABLE 2 | Continued

General category of therapeutic activity	Scientific name/ preparation	Model/Design	Dosage and duration of treatment	Mechanisms	Reference
	Phyllanthus emblica/EtOH extract & pyrogallol	*In vitro* cytoprotective effects against *P. aeruginosa* damage in IB3-1 bronchial epithelial cells	500 μg/ml of the extract, 2, 20, 200 μM pyrogallol	↓IL-6, IL-8, GRO-α, GRO-γ, & ICAM-1, No effect on bacterial adhesion	(Nicolis et al., 2008)
	Rheum officinale/emodin	*In vitro* TGF-β1-induced toxicity in human embryo lung fibroblasts, Bleomycin-induced pulmonary toxicity in rat	*In vitro*: 15-60 μM, *In vivo*:10-40 mg/kg/day, p.o., 21 days	*In vitro*: ↓α-SMA, collagen IV, fibronectin, Smad2/3 & STAT3 activation *In vivo*:↓Pulmonary edema & fibrosis, TNF-α, IL-6, TGF-β1, α-SMA, HSP-47	(Guan et al., 2016)
	Rheum palmatum/aqueous extract	Randomized, controlled trial in patients with acute respiratory distress syndrome treated with the extract+ conventional drugs or only conventional drugs	10 g/30 ml, TDS, p.o., 7 days	↑Oxygenation, ↓Extravascular lung water index, pulmonary vascular permeability index	(He et al., 2017)
	Rheum palmatum/ chrysophanol	*In vitro* TNF-α-induced toxicity in human pulmonary epithelial BEAS-2B cells, OVA-induced airway inflammation in mouse	*In vitro*: 2, 20 μM, *In vivo*: 0.1-10 mg/kg/day, i.p., 4 days	*In vitro*: inhibition of NF-κB pathway, *In vivo*: ↓IL-4, IL-5, IL-13, TNF-α, iNOS, pulmonary α-SMA expression & airway remodeling, NF-κB p65 activation & nuclear translocation, ↓autophagy	(Song et al., 2019)
	Rheum palmatum/rhein	RSV-induced pulmonary damage in mouse	30-120 mg/kg/day, p.o., 5 days	Improvement of lung index, ↓IL-1β, IL-6, IL-18, IL-33, TNF-α, ↓NF-κB-dependent NLRP3 inflammasome activation	(Shen et al., 2019)
	Rosa damascena/EtOH extract & essential oil	*In vitro* KCl, methacholine, & methacholine + propranolol + chlorpheniramine-induced contraction in tracheal chains of guinea pig	0.25-1%	Relaxation in KCl & methacholine-induced tracheal contraction, Higher activity by the essential oil vs. theophylline	(Boskabady et al., 2006)
	Syzygium aromaticum/ aqueous extract	*In vitro* cytoprotective effect on human neutrophil, LPS-induced lung inflammation in mouse	200 mg/kg, two doses, i.p.	↓MPO in neutrophils, ↓neutrophil count, protein leakage in alveoli, MMP-2 & -9 activity	(Chniguir et al., 2019)
	Syzygium aromaticum/ eugenol	LPS-induced lung inflammation in mouse	160 mg/kg, i.p.	Improvement of lung function, ↓alveolar collapse, collagen fibers, & neutrophil influx, ↓NF-κB activation & TNF-α	(Magalhaes et al., 2010)
	Trigonella foenum-graecum/ hot water extract as syrup	Randomized controlled trial in patients with mild asthma	10 ml, BD, 4 weeks	Improvement of spirometry parameters & quality of life, ↓IL-4	(Emtiazy et al., 2018)
	Trigonella foenum-graecum/ hydroalcoholic extract	Bleomycin-induced pulmonary toxicity in rat	5-40 mg/kg/day, p.o., 28 days	Improvement of lung function & hematological parameters, ↓BALF differential cells, ↑peripheral blood oxygen content, SOD, GSH, CAT, Bcl-2, TAC, ↓NO, HO-1, LPO, Nrf-2, IL-1β, IL-6, IL-8, TNF-α, hydroxytriptamine, hydroxyproline, histamine, TGF-β, LDH, ALP, collagen-1, ET-1, NF-κB, VEGF, Smad-3, Bax, Casp-3	(Kandhare et al., 2015)
	Vitis vinifera/polyphenolic extract	Bleomycin-induced pulmonary toxicity in mouse	50, 100 mg/kg/day, p.o., 21 days	↓Leukocytes infiltration in BALF, hydroxyproline, TGF-β1, MMP-9, collagen 1-α1, fibronectin-1, ↑E-cadherin	(Liu et al., 2017)
	Vitis vinifera/proanthocyanidin extract	*In vitro* RSV-induced inflammation in human airway epithelial A549 cells	5, 10 μg/ml	↓IL–1β, IL–6, IL–8 mRNA & protein expression	(Kim et al., 2019)
	Vitis vinifera/proanthocyanidin extract	*In vitro* As-induced toxicity in human lung epithelial BEAS-2B cells, As-induced pulmonary toxicity in mouse	*In vitro*: 25, 50 mg/ml, *In vivo*: 400 mg/kg/day, p.o., 5 weeks	*In vitro* & *in vivo*: ↓Apoptosis, LPO, ROS, IL–1β, IL–6, CRP, TNF-α, NF-κB activation, ↑IL-10, ↓lung inflammation & edema *in vivo*	(Hu et al., 2019)

(Continued)

TABLE 2 | Continued

General category of therapeutic activity	Scientific name/ preparation	Model/Design	Dosage and duration of treatment	Mechanisms	Reference
	Vitis vinifera/proanthocyanidin extract	Pb-induced pulmonary toxicity in rat	200 mg/kg/day, p.o., 5 weeks	↑AMPK/Nrf2/p62 signaling activation, ↑GSH, SOD, γ-GCS, Bcl-2, NQO1, ↓Pb pulmonary concentration, apoptosis, LPO, Bax, p53, TNF-α, NF-κB nuclear translocation, HO-1	(Lu et al., 2018)
	Vitis vinifera/proanthocyanidin extract	Carrageenan-induced pulmonary inflammation in mouse	25-100 mg/kg, p.o., single dose	↓IL-17A & GITR expressing cells, ↓IL-17A, IL-1β, IL-2, IL-6, IL-12, IFNγ, ICAM-1, TNF-α, MCP-1, ↑TGF-β1, IL-4, IL5, IL-10	(Ahmad et al., 2014)
Nephroprotective activity	*Allium sativum*/diallyl trisulfide	As-induced nephrotoxicity in rat	80 mg/kg/day, p.o., 28 days	↓BUN, Cr, ↓renal As concentration, membranes bound ATPases, Bax, Cyt C, Nox2, p47phox & Nox4, TNF-α, IL-1β, IL-6, iNOS, NF-κB, Casp-3, ↑renal SOD, CAT, GST, Gpx, GR, G6PD, GSH, TSH, vitamin C & E, ↑Akt, PI3K & their phosphorylated form, Bcl-2	(Miltonprabu et al., 2017)
	Allium sativum/S-allylmercaptocysteine	*In vitro* cisplatin-induced cytotoxicity in human kidney HK-2 cells, Cisplatin-induced nephrotoxicity in rat	*In vitro*: 50-100 μM, *In vivo*: 10-30 mg/kg/day, i.p., 20 days	*In vitro*: ↓apoptosis, cleaved PARP, p53, ↑Bcl-2, *In vivo*: ↓tubular damage, NF-κB, LPO, TNF-α, IL-1β, TGF-β1, COX-2, ↑Nrf2, NQO1, CAT, SOD, GSH	(Zhu et al., 2017)
	Alpinia galanga/galangin	High-fructose diet-induced nephrotoxicity in rat	50-200 μg/kg/day, p.o., 60 days	↓LPO, Micro-albuminuria & tubular glomerular damage, ↑renal & plasma SOD, CAT, Gpx, GSH, vitamin C & E	(Sivakumar et al., 2010)
	Cichorium intybus/Aqueous extract	*In vitro* cytoprotection in HCK cells, Adenine + yeast-induced chronic kidney disease in rat	*In vitro*: 100-400 μg/ml, *In vivo*: 6.6, 13.2 g/kg/day, p.o., 5 weeks	*In vitro*: ↓transmembrane transport of uric acid, *In vivo*: ↓serum uric acid & Cr, microalbuminuria, GLUT-9 protein expression,	(Jin et al., 2018)
	Cichorium intybus/Aqueous extract	STZ-induced diabetic nephropathy in rat	125 mg/kg/day, i.p., 21 days	↓serum uric acid & Cr, microalbuminuria, & renal morphological damage	(Pourfarjam et al., 2017)
	Crocus sativus/aqueous extract	EtOH-induced nephrotoxicity in rat	40-160 mg/kg/day, p.o., 4 weeks	↓Renal LPO, TNF-α, IL-6, Casp-3, Casp-8, Casp-9, Bax/Bcl2, ↑GSH	(Rezaee-Khorasany et al., 2019)
	Crocus sativus/crocin	Tartrazine-induced nephrotoxicity in rat	50 mg/kg/day, p.o., 21 days	↓BUN, Cr, renal LPO, ↑GSH, TAC, SOD, CAT	(Erdemli et al., 2017)
	Crocus sativus/crocin	STZ-induced diabetic nephropathy in rat	20 mg/kg/day, p.o., 21 days	↓Tubular necrosis, inflammation, & desquamation, ↓BUN, Cr, LPO, xanthine oxidase activity ↑GSH	(Altinoz et al., 2015)
	Glycyrrhiza spp./glycyrrhizic acid	*In vitro* LPS-induced toxicity in rat mesangial cells, LPS-induced nephrotoxicity in rat	*In vitro*: 50, 100 μM, *In vivo*: 25, 50 mg/kg, i.p., single dose	*In vitro*: ↓apoptosis, Casp-3, iNOS, NO, COX-2, PGE2, ROS, NF-κB activation, ↑Bcl-2/Bax, HO-1, *In vivo*: ↓BUN, Cr, TNF-α, MCP-1, ICAM-1, VCAM-1	(Zhao et al., 2016)
	Phyllanthus emblica/ emblicanin-A & -B enriched extract	Cisplatin-induced nephrotoxicity is rat	150-600 mg/kg/day, p.o., 10 days	↑Renal CAT, GSH, SOD, ↓Inflammation & apoptosis, ↓LPO, MAPK phosphorylation	(Malik et al., 2016)
	Rheum officinale/different extracts	Adenine-induced chronic kidney disease in rat	200-800 mg/kg/day, p.o., 6 weeks	↓Renal α-SMA, collagen-I & collagen-III, ↓BUN, Cr, TGF-β1, TGF-β receptor I & II, Smad-2, Smad-3, Smad4, vimentin ↑Smad7, E-cadherin	(Zhang et al., 2018) (Tu et al., 2017)

(Continued)

TABLE 2 | Continued

General category of therapeutic activity	Scientific name/ preparation	Model/Design	Dosage and duration of treatment	Mechanisms	Reference
	Rheum palmatum/aqueous extract, rhein	*In vitro* Hank's balanced salt solution-induced autophagy in NRK-52E normal rat kidney cells, Adenine-induced chronic kidney disease in rat	*In vitro*: 1, 10 μM, *In vivo*: 1 g/kg/day, p.o., 3 weeks	*In vitro*: ↓autophagy *via* AMPK-dependent mTOR signaling pathway, Erk & p38 MAPKs by rhein *In vivo*: ↓Renal fibrosis, collagen-1, fibtonectin, LC3 conversion by the extract	
	Syzygium aromaticum/ aqueous extract	Infectious pyelonephritis in rat	500 mg/kg/day, p.o., 28 days	↓Leukocyte count, Normalization of histomorphological changes	(Nassan et al., 2015)
	Trigonella foenum-graecum/ seed powder	STZ-induced diabetic nephropathy in rat	10% of the diet weight, 6 weeks	↓Renal Glut-1 & -2, ACE, iNOS, & NO, ↓renal AST, ALT, alkaline & acid phosphatases, Na^+, K^+, ouabain-sensitive, Mg^{2+}ATPase, & Ca^{2+}ATPase, fructose 1,6-diphosphatase, G6 Pase, LDH activity, ↑renal hexokinase & G6PD activity, ↓urinary excretion of proteins, ↓renal polyol pathway enzyme activity, ↓podocyte damage & morphological changes	(Pradeep et al., 2019)
	Vitis vinifera/proanthocyanidin B2	*In vitro* glucosamine-induced nephrotoxicity in rat mesangial cells	2.5, 10 μg/ml	↓Apoptosis & mitochondrial dysfunction, ↑Gpx, SOD, PGC-1α, SIRT1, AMPK, NRF1,	(Bao et al., 2015)
	Vitis vinifera/proanthocyanidin extract	STZ-induced diabetic nephropathy in rat	250 mg/kg/day, p.o., 16 weeks	No significant change in BUN & Cr, ↓renal index, urinary albumin, endoplasmic reticulum stress-induced apoptosis *via* Casp-12	(Gao et al., 2018)
	Vitis vinifera/proanthocyanidin extract	As-induced nephrotoxicity in mouse	400 mg/kg/day, p.o., 5 weeks	↓NF-κB activation, IL–1β, IL–6, CRP, TNF-α, ↑IL-10	(Wang C. et al., 2017)
	Vitis vinifera/proanthocyanidin extract	Diatrizoate-induced nephrotoxicity in rat	100 mg/kg/day, p.o., 8 days	↓BUN, Cr, Casp-1 & -3, calpain-1, iNOS, eNOS, Better effect than N-acetylcysteine	(Ulusoy et al., 2014)

SOD, superoxide dismutase; CAT, catalase; Gpx, glutathione peroxidase; GSH, glutathione; GR, glutathione reductase; GST, glutathione-S-transferase; LPO, lipid peroxidation; p.o., oral; I/R, ischemia-reperfusion; HIV, human immunodeficiency virus; RT, reverse transcriptase; IC_{50}, inhibitory concentration 50%; SI, selective index=cytotoxic concentration 50%/virus inhibitory concentration 50%; CPE, cytopathic effect; GRO, growth-regulated oncogene; IL, interleukin; ICAM, intercellular adhesion molecule; CK-MB: ISO, isoproterenol; STZ, streptozotocin; Cr, creatinine; BUN, blood urea nitrogen; IFN, interferon; TAC, total antioxidant capacity; NO, nitric oxide; iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; LPS, lipopolysaccharide; MPO, myeloperoxidase; MMP, matrix metalloproteinase; PG, prostaglandin; COX, cyclooxygenase; STAT, signal transducer and activator of transcription; NF-kB, nuclear factor-κB; WBC, white blood cells; LDH, lactate dehydrogenase; CPK, creatinine phosphokinase; AST, aspartate transaminase; ALT, alanine transaminase; G6PD, glucose 6 phosphate dehydrogenase; LDL, low-density lipoprotein cholesterol; TAG, triacylglycerol; ACE, angiotensin converting enzyme; NPSH, Non protein thiol; RAS, renin angiotensin system; PUFA, poly unsaturated fatty acids; SFA, saturated fatty acid; Ig, immunoglobulin; Casp, caspase; SRBC, sheep red blood cells; DOX, doxorubicin; DNP, dinitrophenyl; HO, heme oxygenase; MCP-1, monocyte chemoattractant protein; VCAM, vascular cell adhesion molecule; RSV, respiratory syncytial virus; BALF, bronchoalveolar lavage fluids; TLR, Toll-like receptor; NQO1, NAD(P)H:quinone oxidoreductase 1; PI3K, phosphatidylinositol-3 kinase; CREB, cAMP response element binding; GSK-3a/b, glycogen synthase kinase 3a/b; ERK, extracellular signal-regulated kinase; As, arsenic; TSH, total sulfhydryl groups; CRP, C-reactive protein; Pb, lead; gGCS, g-glutamylcysteine synthetase; GRP78, glucose–regulated protein 78; PERK, protein kinase RNA–like ER kinase; eIF2a, eukaryotic translation initiation factor–2; JNK, c-Jun N-terminal kinase; AP-1, activator protein-1; CXCL10, interferon-g-inducible protein 10; OVA, ovalbumin; EV, enterovirus; mTOR, mammalian target of rapamycin; RDDP, Reverse Transcriptase-associated DNA Polymerase; RNase H, Ribonuclease H; a-SMA, asmooth muscle actin; STAT, signal transducer and activator of transcription; HSP, heat shock protein; NOX4, NADPH oxidase 4; PPAR, Poly(ADP) ribose polymerase; HMGB, High Mobility Group Box.
Bold studies are antiviral assessments against viral lung pathogens.

TABLE 3 | Quality assessment of animal studies on the pharmacological activity of traditional Persian medicine-suggested plant possibly beneficial in COVID-19 according to Animal Research: Reporting of *In vivo* Experiments (ARRIVE) guideline.

Reference	Validity	Ethical statement	Animals	Experimental procedures	Housing & husbandry	Numbers analyzed	Interpretation & scientific implications	Generalizability/ translation
(Sawamura et al., 2010)	−	+	+	+	+	+	−	−
(Xiong et al., 2012)	−	+	+	+	−	+	−	−
(Hong et al., 2015)	+	+	+	+	−	−	−	−
(Abdel-Daim et al., 2017)	+	+	+	+	+	+	−	+
(Zhao et al., 2019)	+	+	+	+	−	+	−	+
(Yu et al., 2017)		+	+	+	+	+	−	+
(Padiya et al., 2014)	−	+	+	+	+	+	−	−
(Qi et al., 2020)	+	+	+	+	+	+	−	+
(Sachdeva et al., 2012)	+	+	+	+	+	+	−	+
(Mehdizadeh et al., 2013)	+	−	+	+	+	+	−	−
(Razmaraii et al., 2016)	+	+	+	+	+	+	−	+
(Ojha et al., 2013)	+	+	+	+	+	+	−	+
(Li et al., 2019)	+	+	−	+	+	+	−	+
(Thirunavukkarasu et al., 2015)	+	+	+	+	−	−	−	−
(Bhattacharya et al., 2002)	−	−	+	+	+	+	−	+
(Ojha et al., 2012)	+	+	+	+	+	+	−	+
(Patel and Goyal, 2011)	+	+	+	+	+	+	−	+
(Lu et al., 2019)	+	+	+	+	−	+	−	+
(Panda and Kar, 2010)	+	+	+	+	+	+	−	−
(Feki et al., 2019)	+	+	+	+	+	+	−	−
(Mukthamba and Srinivasan, 2015)	+	+	+	+	+	+	−	−
(Tripathi and Chandra, 2009)	+	+	+	+	+	+	−	+
(Pradeep and Srinivasan, 2018)	+	+	+	+	+	+	−	−
(Panda et al., 2013)	+	+	+	+	+	+	−	−
(Ammar el et al., 2013)	+	+	+	+	+	+	−	+
(Cheng et al., 2012)	+	+	+	+	+	+	−	−
(Kim et al., 2002)	+	−	+	+	+	+	−	−
(Lepczynski et al., 2015)	+	+	+	+	−	−	−	−
(Ramadan et al., 2011)	+	+	+	+	−	−	−	+
(Long et al., 2016)	+	+	+	+	+	+	−	−
(Zou et al., 2018)	−	+	+	+	+	−	−	+
(Mohi El-Din et al., 2014)	+	+	+	+	+	−	−	−
(Nie et al., 2019)	+	+	+	+	−	+	−	+
(Mo et al., 2020)	+	+	+	+	+	+	−	+
(Seo et al., 2013)	−	+	−	+	−	+	−	+
(Shu et al., 2014)	−	+	+	+	+	+	−	−
(Zha et al., 2013)	−	+	+	+	+	+	−	+
(Zhang et al., 2020b)	−	+	+	+	+	+	−	+
(Zaghloul et al., 2019)	+	+	+	+	−	+	−	+
(Boskabady et al., 2014)	+	+	+	+	−	+	−	+
(Ma et al., 2013)	+	+	+	+	−	+	−	+
(Nishimoto et al., 2010)	−	+	+	+	−	+	−	+
(Qu et al., 2019)	+	+	+	+	−	+	−	+
(Guan et al., 2016)	+	+	+	+	+	+	−	+
(Song et al., 2019)	−	+	+	+	+	−	−	+
(Shen et al., 2019)	+	+	+	+	+	+	−	+
(Chniguir et al., 2019)	−	+	+	+	−	+	−	−
(Magalhaes et al., 2010)	+	+	+	+	−	+	−	+
(Kandhare et al., 2015)	+	+	+	+	+	+	−	−
(Liu et al., 2017)	+	+	+	+	+	+	−	+
(Hu et al., 2019)	+	−	−	+	−	+	−	+
(Lu et al., 2018)	+	+	+	+	+	+	−	+
(Ahmad et al., 2014)	+	+	+	+	+	+	−	−
(Miltonprabu et al., 2017)	+	+	+	+	+	+	−	+
(Zhu et al., 2017)	+	+	+	+	+	+	−	+
(Sivakumar et al., 2010)	+	+	+	+	+	+	−	−
(Jin et al., 2018)	+	+	+	+	+	+	−	−
(Pourfarjam et al., 2017)	+	+	+	+	+	−	−	−
(Rezaee-Khorasany et al., 2019)	+	+	+	+	+	+	−	−
(Erdemli et al., 2017)	+	+	+	+	+	+	−	+

(Continued)

TABLE 3 | Continued

Reference	Validity	Ethical statement	Animals	Experimental procedures	Housing & husbandry	Numbers analyzed	Interpretation & scientific implications	Generalizability/ translation
(Altinoz et al., 2015)	+	+	+	+	+	−	−	−
(Zhao et al., 2016)	+	+	+	+	+	+	−	−
(Malik et al., 2016)	+	+	+	+	+	+	−	+
(Zhang et al., 2018)	+	+	+	+	−	+	−	−
(Tu et al., 2017)	−	+	+	+	+	−	−	+
(Nassan et al., 2015)	−	−	+	+	+	−	−	+
(Pradeep et al., 2019)	+	+	+	+	+	+	−	+
(Gao et al., 2018)	+	+	+	+	−	+	−	+
(Wang C. et al., 2017)	+	+	+	+	+	+	−	+
(Ulusoy et al., 2014)	+	+	+	+	+	−	−	+

GSK3β) pathway, was identified in the I/R model. The phosphorylation of Akt and subsequently GSK3β leads to the release of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and β-catenin nuclear translocation which further activates anti-apoptotic signaling pathways like B cell lymphoma-2 (Bcl-2) and endothelial Nitric oxide synthase (eNOS), finally resulting in cardioprotection (Thirunavukkarasu et al., 2015).

The effective animal dosage of amla is 100-500 mg/kg/day for the aforementioned therapeutic activities. This dose is equal to a relatively high human dose; however, the plant has an acceptable safety profile and is routinely taken in several countries of the world. Thus, these studies suggest that amla could be a functional food, useful for primary and secondary prevention of COVID-19 (Pingali Usharani and Muralidhar, 2013; Upadya et al., 2019; Kapoor et al., 2020). Interestingly, amla fruit is the second richest source of vitamin C (nearly 600-700 mg in each fruit), which WHO has recommended people take to protect the immune system against SARS-CoV-2 infection (Goraya and Bajwa, 2015).

Chicory (*Cichorium intybus* L.)

Chicory is a cosmopolitan herbaceous plant from the Compositae (Asteraceae) family, used both as a medicinal plant and a food additive because it tastes similar to coffee. All parts of the plant, including roots, aerial parts, and seeds, are used due to its medicinal properties. Chicory is well-known as a hepatoprotective plant in different complementary and alternative medicines (Street et al., 2013). In TPM, it is also considered as a tonic for kidney and heat-related heart diseases, as well as a modulator of overall health through prevention of humor infection.

Chicory extract was effective in the prevention of diabetic nephropathy following three weeks of administration to rats. At the dose of 125 mg/kg, the effect of chicory was approximately equal to 100 mg/kg of metformin in several parameters (Pourfarjam et al., 2017). The nephroprotective effect of the extract was confirmed in the human kidney cell line through inhibition of GLUT-9 expression, an important transporter of uric acid in kidneys. Chicory extract could also decrease renal damage in an experimental model of chronic renal failure; however, the administered doses of the extract (6.6 and 13.2 g/kg) were dramatically higher than benzbromarone (20 mg/kg) as the standard drug (Jin et al., 2018).

Chicory is also demonstrated to have immunomodulatory activities. In the animal model of ethanol-induced immunotoxicity, chicory increased both circulating leukocytes and the weight of lymphatic organs, showing an improvement in immune system function (Kim et al., 2002). Additionally, chicory as a dietary supplement could affect the plasma protein profiles, resulting in the lower level of pro-inflammatory markers such as the C-reactive protein (CRP) (Lepczynski et al., 2015). Chicory root is a rich source of inulin-type fructans, a group of carbohydrates considered as prebiotics (Lepczynski et al., 2015). Today, the remarkable role of the normal flora of different body organs in various diseases, including immunological problems, has attracted the attention of scientists (Pretorius et al., 2018). Thus, aside from the direct immunomodulatory effects of chicory, the presence of such prebiotics in this plant may also have a modulatory effect on normal flora. The indirect protective effect of chicory against pathologic conditions can be hypothesized in future studies.

Clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry)

The flower buds of clove, from the family Myrtaceae, have long been used in both medicine and for culinary purposes. The phenylpropanoids of the essential oil, mainly eugenol, are considered to be the main active compounds of the plant and are responsible for several pharmacological activities (Chaieb et al., 2007). Clove is known as a tonic for the cardiovascular system in TPM and is thought to improve blood supply to both the heart and the brain. Moreover, it is mentioned to be specifically useful in chronic coughs, shortness of breath, and palpitations (Aghili Khorasani, 1771).

The methanolic and aqueous extracts of clove have shown inhibitory effects on HCV protease enzyme with an IC_{50} of 33 μg/ml (Hussein et al., 2000). Eugenol has exhibited antiviral effects against the influenza A virus through direct reduction of viral replication, as well as inhibition of autophagy, a supporting mechanism for viral replication and cell death which results in acute lung damage. It should be mentioned that the potency of eugenol at the concentration of 5 μg/ml was equal or higher than 25 μg/ml of ribavirin as the gold standard antiviral agent, recommending eugenol as a potent antiviral compound

(Dai et al., 2013). Furthermore, eugenol could regulate cellular inflammatory cascades such as nuclear factor-κB (NF-κB) and extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathways, nitric oxide (NO), the release of pro-inflammatory ILs, and endogenous antioxidant defense mechanisms (**Table 2**).

Clove has demonstrated modulatory effects on the function of murine white blood cells and macrophages damaged through inflammation/oxidative stress damage (**Table 2**). In the animal model of immunosuppression, one-week administration of clove essential oil (400 mg/kg) could improve both humoral and cell-mediated immunity with equal efficacy to 50 mg/kg of levamisole (Carrasco et al., 2009). Clove extract and its major ingredient, eugenol, have shown an anti-inflammatory effect on the lipopolysaccharide (LPS)-induced damage in macrophages. No statistically significant difference was observed between eugenol (100 μg/well) and dexamethasone (10^{-4} mol/l) (Bachiega et al., 2012). The same effect is also reported with a single dose of the flavonoid biflorin (Lee et al., 2016), suggesting this compound is a fast-acting agent that may be useful in acute inflammations such as cytokine storm in COVID-19.

LPS-induced lung inflammation was also relieved with clove aqueous extract and eugenol through reduction of tumor necrosis factor (TNF)-α and inhibition of NF-κB signaling, as well as improvement in alveolar damage (Magalhaes et al., 2010; Chniguir et al., 2019).

Moreover, the aqueous extract has demonstrated a protective effect on an animal model of infectious pyelonephritis (Nassan et al., 2015), a condition also reported in COVID-19 patients (Su et al., 2020).

Aside from the above-mentioned features of clove essential oil, it has shown strong antibacterial effects, even against the infections of immunosuppressed hospitalized patients (Chaieb et al., 2007). Thus, the essential oil can be a valuable option to prevent secondary bacterial infections in COVID-19 patients.

Damask Rose (*Rosa* × *damascena* Herrm.)

Damask rose, from the family Rosaceae, is one of the most valued medicinal plants in TPM due to its modulatory effects on the function of almost all body organs and consequently, overall health (Nayebi et al., 2017). It helps the body to excrete abnormal watery phlegm humor which is highly susceptible to infection and thus, it is a tonic of the lungs. Furthermore, TPM texts discuss that it alleviates infectious fevers (Aghili Khorasani, 1771).

An *in vitro* study on the antiviral properties of damask rose has demonstrated significant activity against HIV infection. Flavonoids purified from the methanolic extract including quercetin, kaempferol, and two of its analogues, showed the highest activity *via* the inhibition of viral protease and gp 120 binding to CD4 glycoprotein. The compounds were not as potent as azidothymidine (zidovudine) as a standard anti-HIV agent in regard to SI and IC_{50}; however, they showed an additive effect in combination with this drug (Mahmood et al., 1996).

Damask rose has shown cardiotonic properties on perfused guinea pig heart and reversed bradycardia by increasing heart contractility (Boskabady et al., 2013). Both ethanolic extract and the essential oil represented an antispasmodic effect on guinea pig tracheal chains (Boskabady et al., 2006).

Fenugreek (*Trigonella foenum-graecum* L.)

Fenugreek is a member of the family Leguminosae and the seeds are frequently used as a lung tonic in TPM due to the moderate heat, causing a mucolytic activity on pulmonary mucosa. This effect helps to remove the thick phlegm humor and causes a soothing effect on lung injuries, accelerating the healing procedure (Aghili Khorasani, 1771).

Fenugreek seed extract has demonstrated significant anti-inflammatory properties in an animal model of pulmonary inflammation, evident from the reduction of leukocytes infiltration to the bronchoalveolar lavage fluid (BALF) and lung fibrosis. Pro-inflammatory cytokines and endogenous enzymatic and non-enzymatic antioxidants were also restored to near normal levels. Stimulation of nuclear factor E2-related factor 2 (Nrf2) by fenugreek, an antioxidant cascade, prevents the hemeoxigenase-1 (HO-1) overproduction, subsequently ameliorating pulmonary fibrosis. The effectiveness of the extract with 5-40 mg/kg dose range in most of the investigated parameters were equal or higher than 10 mg/kg of methylprednisolone as the gold standard drug (Kandhare et al., 2015). Likewise, the antiasthmatic effect of fenugreek seed syrup, designed based on the lung protecting properties of the plant in TPM, was assessed in a clinical trial. Four weeks of treatment with the syrup caused a significant improvement in spirometry parameters in comparison to the baseline values (Emtiazy et al., 2018).

There are also several studies on the cardioprotective effects of fenugreek (**Table 2**). Dietary fenugreek powder (Mukthamba and Srinivasan, 2015), trigonelline (a pyridine alkaloid), and digoxigenin-3-O-rutin (a cardiac glycoside) have reversed isoproterenol-induced cardiotoxicity in rats (Panda and Kar, 2010; Panda et al., 2013). Both compounds exerted modulatory effects on the cardiac biomarkers of oxidative stress and inflammation, including CK-MB, lactate dehydrogenase (LDH), and lipid peroxidation. Trigonelline showed the highest pharmacological activity at a dose of 40 mg/kg. On the other hand, digoxigenin-3-O-rutin was effective at 2.5-10 mg/kg which was comparable with 5 mg/kg of digoxin as the gold standard drug (Panda and Kar, 2010). Furthermore, trigonelline could reduce the level of heat shock protein (HSP)-27 and αB-crystallin, two novel biomarkers of oxidative damage, in the myocardium damage, further confirming the cardioprotective activity of fenugreek (Panda et al., 2013). Polysaccharides as another important category of fenugreek components have demonstrated significant cardioprotective activity both *in vitro* and *in vivo*, possibly due to their antioxidant activity and prevention of DNA damage (Feki et al., 2019). Aside from antioxidant activity (Tripathi and Chandra, 2009), inhibition of pathologic NO production by inducible nitric oxide synthase (iNOS) and markers of cardiac fibrosis, such as fibronectin and collagen, seem to be the main cardioprotective mechanisms of fenugreek demonstrated in streptozotocin (STZ)-induced cardiac damage (Pradeep and Srinivasan, 2018).

In a cyclophosphamide-induced animal model of immunosuppression, fenugreek could prevent lymphopenia and neutropenia, and improve the cellularity of bone marrow, spleen, and thymus, proposing an immunostimulatory effect for this plant (Ramadan et al., 2011).

Galangal (*Alpinia galanga* (L.) Willd., *A. officinarum* Hance)

Galangal species belong to the family Zingiberaceae, a valuable plant family comprising of several important medicinal plants such as ginger and turmeric (Abubakar et al., 2018). In TPM, the plant is known to have nephroprotective properties and lung tonic activity and is used for the treatment of cough. Aside from the essential oil, the most important secondary metabolites in this plant family are diarylheptanoids with significant anti-inflammatory properties (Abubakar et al., 2018).

Seven diarylheptanoids from lesser galangal (*A. officinarum*) have demonstrated significant *in vitro* antiviral effects on RSV, poliovirus, and measles virus. The lowest IC_{50} values were 5, 3.7, and 6.3 μg/ml against RSV, poliovirus, and measles virus, respectively; however, they were all higher than those of the gold standards, ribavirin and acyclovir (Konno et al., 2011). In another study, the antiviral effects of two galangal diarylheptanoids were assessed against several types of influenza virus, one of which showed remarkable activity. The active compound was not only effective *in vitro* against all virus types, including oseltamivir-resistant type, but also showed *in vivo* protective effects on the murine model of influenza. The compound showed a dose-dependent inhibition of viral RNA and antigen expression; while it was ineffective on the viral adsorption or invasion. The *in vitro* inhibition of viral growth by 60 μg/ml of the compound was higher than 20 μg/ml of ribavirin (Sawamura et al., 2010). 1′-Acetoxychavicol acetate, a phenylpropanoid from the greater galangal (*A. galanga*) also exhibited anti-influenza activity with an IC_{50} of 2 μM (460 ng/ml). This effect was mediated through the inhibition of viral ribonucleoprotein complex nuclear export, an important part of the viral life cycle that controls the transcription and replication (Watanabe et al., 2011). Furthermore, the compound has demonstrated an inhibitory effect on the nuclear export of HIV-Rev protein. Analysis of the structure-activity relationship revealed that the presence of 10-acetoxyl-20-ene moiety, two acetyl functional groups, along with a 10-S configuration is crucial for its antiviral activity (Tamura et al., 2009). It is worth mentioning that a molecular docking analysis showed the effectiveness of galangal compounds against SARS-CoV-2; however, experimental studies are needed to confirm this hypothesis (Zhang et al., 2020a).

1′-Acetoxychavicol acetate has been proved to have a protective effect on the lungs, as well. In an ovalbumin-induced mouse model of asthma, the compound reduced the infiltration of eosinophils into the BALF. The secretion of pro-inflammatory cytokines by both types 1 and 2 T cells was also significantly decreased and the effect of the higher dose (50 mg/kg) was equal to 1 mg/kg of dexamethasone (Seo et al., 2013). Galangin, a flavonoid of the greater galangal, has represented anti-inflammatory effects in the same animal model *via* inhibition of the NF- κB pathway. Furthermore, galangin decreased the monocyte chemoattractant protein (MCP)-1 and vascular cell adhesion molecule (VCAM)-1 of lung tissue, both of which participate in leukocytes chemotaxis (Zha et al., 2013). It should be mentioned that in most evaluated parameters, the effect of 15 mg/kg of galangin was equal or higher than dexamethasone (3 mg/kg), showing a high anti-inflammatory potency (Zha et al., 2013). Moreover, galangin has demonstrated anti-inflammatory effects in LPS-induced acute lung damage (Shu et al., 2014) and macrophage stimulation (Jung et al., 2014) through the suppression of NF-κB downstream signaling.

In addition to galangin, 1′-acetoxychavicol acetate has also shown immunoregulatory properties in stimulated murine macrophages *via* the prevention of NF-κB activation and IFN-β mRNA expression, subsequently inhibiting NO production by iNOS. Despite the important role of NO in physiological status, its overproduction by iNOS is involved in several pathologic inflammatory conditions (Ando et al., 2005).

Likewise, cardamonin, another flavonoid from *A. galanga*, decreased the cardiotoxicity of doxorubicin (DOX) *via* inhibition of both inflammation and oxidative stress through the Nrf2 pathway. Nrf2 has a close cross-talk with NF-κB and thus, the antioxidant and anti-inflammatory effect of galangal *via* these pathways is further confirmed (Qi et al., 2020).

Galangin exhibited nephroprotective effects in the high-fructose diet-induced renal damage by inhibition of oxidative damage (Sivakumar et al., 2010).

Taken together, galangal species seem to have direct antiviral properties, as well as protective effects on the main organs damaged in SARS-CoV-2 infection and may be suitable complementary therapies in this infection.

Garlic (*Allium sativum* L.)

Even though garlic, from the family Amaryllidaceae, is one of the most ancient medicinal plants there is always has something new to say about it medicinally. In TPM, it is useful for the primary and secondary prevention of different infections and is recommended that it be used during epidemic infectious diseases. Moreover, it has been proposed as a blood thinner and used for the management of several types of cardiovascular events (Aghili Khorasani, 1771). Garlic owes several of its significant pharmacological activities to the organosulfur compounds which are also responsible for the strong flavor and fragrance of the plant (Li et al., 2013).

A recently published molecular docking analysis demonstrated the high inhibitory effects of garlic volatile organosulfur compounds on the invasion of SARS-CoV-2. This effect was mediated through the inhibition of ACE2, a participant in SARS-CoV-2 infection. Allyl disulfide and allyl trisulfide, the major components of garlic essential oil, showed the highest antiviral activity (Thuy et al., 2020). Garlic has demonstrated therapeutic activity against the Dengue virus, a member of the Flaviviridae family causing a lethal hemorrhagic fever. Diallyl disulfide, diallyl sulfide, and alliin could decrease the inflammatory markers in infected cells through the inhibition

of oxidative damage (Hall et al., 2017). There are several other reports on the antiviral effects of garlic against influenza viruses A and B, rhinovirus, rotavirus, HIV, and viral pneumonia. Most of these investigations date back to more than twenty years ago when current, more precise techniques were not available (Bayan et al., 2014). Thus, the results of these antiviral assessments need to be reconfirmed with newly developed laboratory methods.

Garlic aqueous extract could effectively ameliorate pulmonary interstitial alveolitis and macroabscesses in lung damage (Mohi El-Din et al., 2014). In LPS-induced acute pulmonary inflammation, oral administration of S-allylmercaptocysteine could dose-dependently inhibit lung damage. The effect of 60 mg/kg of S-allylmercaptocysteine was equal to or higher than 500 mg/kg of N-acetylcysteine (positive control), showing a high potency. This compound could suppress pro-inflammatory cytokines, reducing macrophage and neutrophils infiltration into BALF, inhibiting NF-κB activation, and improving endogenous enzymatic and non-enzymatic antioxidants. Furthermore, Nrf2 and its downstream signals, HO-1 and NAD(P)H: quinone oxidoreductase 1 (NQO1), a cytoprotective mediator in oxidative damage, were increased by S-allylmercaptocysteine (Mo et al., 2020). The effect of garlic essential oil and organosulfur components on human neutrophils was also demonstrated *in vitro*, where they could improve neutrophils function as an immunomodulatory response (Schepetkin et al., 2019). Moreover, S-allyl-L-cysteine has shown inhibitory effects on bleomycin-induced pulmonary inflammation and fibrosis at 10 mg/kg; though, the potency cannot be accurately judged, since no positive control drug was used (Nie et al., 2019). In addition to animal studies, aged garlic extract (garlic soaked in alcohol) has represented beneficial properties in reducing the number and duration of symptoms in subjects with cold/flu. These results also showed an improved proliferation of natural killer (NK) cells and γδ-T lymphocytes in response to pathogen-associated molecular patterns (Nantz et al., 2012).

In another study, black garlic, another popular product prepared through garlic fermentation, was compared with fresh raw garlic regarding its immunostimulatory effects on macrophages. While fresh garlic could significantly improve the phagocytic activity of macrophages and production of cytokines, black garlic showed only negligible effects on these parameters. This significant variation is attributed to the different polysaccharides in the two extracts, specifically fructans which are degraded in black garlic (Li et al., 2017).

Garlic is also widely used for different cardiologic problems and myocardial protection (Bradley et al., 2016). In this regard, raw garlic homogenate showed protective effects on fructose-induced oxidative stress in cardiac tissue *via* the elevation of cardiac H_2S and preventing myocardial injury. Additionally, PI3K/AKT signaling, a critical pathway in cell survival linked with Nrf2, was activated by garlic (Padiya et al., 2014). Alliin has demonstrated modulatory effects on autophagy, a mechanism involved in cytoprotection and apoptosis of different cells, including myocardium (Zhao et al., 2019). Similarly, allicin has exhibited anti-inflammatory and antioxidant properties in DOX-induced cardiotoxicity (Abdel-Daim et al., 2017). Allicin is produced from alliin by alliinase after chopping garlic and is further metabolized into diallyl trisulfide. The latter compound has exerted cardioprotective effects through AMP-activated protein kinase (AMPK), GSK-3β, and hypoxia-inducible factors (HIF)-1α (Yu et al., 2017). Considering the doses of alliin, allicin, diallyl trisulfide (20, 100 mg/kg) versus garlic homogenate (250 mg/kg), it can be hypothesized that the purified compounds are more potent than garlic. However, a study on all of these components in the same setting is required to clarify this hypothesis.

Diallyl trisulfide has also exhibited nephroprotective activity in arsenic-induced kidney damage. In addition to the improvement of BUN and creatinine as routine markers of renal function, the compound could modify several markers of inflammation, apoptosis, and oxidative stress (**Table 2**) (Miltonprabu et al., 2017). A similar activity was observed with *in vitro* and *in vivo* administration of S-allylmercaptocysteine in cisplatin-induced kidney injury. Furthermore, p-53, an initiator of apoptosis, and its downstream proteins B-cell associated X protein (Bax) and Bcl-2 were regulated by this compound. Poly ADP ribose polymerase (PARP), a DNA repairing enzyme during apoptosis which is also under regulation of p-53, was decreased as well, revealing the reversal of cisplatin-induced DNA damage (Zhu et al., 2017).

In general, garlic and its organosulfur compounds act as a multitargeted therapy in several tissues susceptible to SARS-CoV-2 injury and may be beneficial as a primary/secondary prevention in these patients.

Grape and Raisin (*Vitis vinifera* L.)

Grape, from the family Vitaceae, is one of the most respected fruits in TPM since it produces a series of physiologically balanced humors and thus, is recommended for the general population. In addition to its high nutritional value, modern pharmacological investigations have focused on the remarkable antioxidant properties of this fruit. This attention is due to polyphenolic compounds including proanthocyanidins in the flesh and resveratrol in the fruit seed/peel (Nassiri-Asl and Hosseinzadeh, 2016). Intriguingly, it is emphasized in TPM to take grape with its seed for the urologic problems, showing the knowledge of Persian physicians about the specific effects of the seeds.

In a virtual screening considering influenza A virus vRNA promoter, as well as an *in vitro* evaluation, procyanidin, a major component of grape extract, revealed significant antiviral activity (Dai et al., 2012). Grape seed extract has shown inhibitory effects on HCV replication *via* suppression of virus-induced cyclooxygenase (COX)-2 overexpression. It has also shown a synergistic effect by co-administration with conventional anti-HCV medicines including telaprevir, daclatasvir, sofosbuvir (Chen et al., 2016). Suppression of the MAPK/JNK pathway is also involved in the antiviral effect of grape seed extract against both HCV (Chen et al., 2016) and RSV (Lee et al., 2017). Moreover, the extract has prevented ILs, MAPK/JNK, and NF-κB pro-inflammatory cascades and regulated mucin production

via reducing the expression of several mucin MUC genes in the airway epithelium. Interestingly, this effect is in line with the lung protecting properties of grape as described in TPM, by cleaning the pathological viscous pulmonary mucosa (Lee et al., 2017; Kim et al., 2019).

In lead and arsenic-induced lung inflammation, the extract reduced pulmonary levels of pro-inflammatory cytokines, oxidative damage, and pro-apoptotic markers (Lu et al., 2018; Hu et al., 2019). In carrageenan-induced acute lung inflammation, a single oral dose of grape seed extract could significantly balance the level of several pro-inflammatory and anti-inflammatory cytokines. In addition, it modulated tumor necrosis factor receptor, a co-activator of effector T lymphocytes, upregulated during lung inflammation (Ahmad et al., 2014). In pulmonary fibrosis induced by bleomycin, the extract prevented leukocytes infiltration and markers of fibrosis including matrix metalloproteinase and collagen deposition and increased the anti-fibrotic marker E-cadherin. The potency of the higher extract dose, 100 mg/kg, was equal to 0.5 mg/kg of dexamethasone in most parameters (Liu et al., 2017).

The immunomodulatory effect of grape was assessed in the subchronic immunotoxicity by aflatoxin B1, a fungal toxin causing oxidative stress and subsequent cellular damages. Grape seed extract at the dose of 100 mg/kg showed a significant improvement of endogenous antioxidants and decreased pro-inflammatory cytokines to a similar level to those of the healthy animals (Long et al., 2016).

The cardioprotective effect of the extract was also demonstrated in DOX-induced cardiotoxicity, evident from the modulation of electrocardiogram (ECG) and an improvement of endogenous antioxidants activity (Ammar el et al., 2013). Another specific cardioprotective mechanism that has been reported for grape proanthocyanidins is the modification of protein kinase RNA–like ER kinase (PERK) and eukaryotic translation initiation factor−2 (eIF2α). In endoplasmic reticulum oxidative stress conditions, overactivation of PERK/eIF2α stimulates C/EBP–homologous protein (CHOP) and subsequently, apoptosis. Grape seed extract could reverse the I/R-induced upregulation of these pathways and prevent the apoptosis of cardiomyocytes (Wang X. et al., 2017).

The inhibition of endoplasmic reticulum-dependent apoptosis was also involved in the nephroprotective effects of grape in diabetic nephropathy (Gao et al., 2018). In another study in diabetic nephropathy, procyanidin B2 could exhibit antioxidant activity *via* the prevention of mitochondrial dysfunction, a phenomenon accompanied by increased production of ROS as the byproduct of cell metabolism (Bao et al., 2015). Grape seed extract has represented protective effects against kidney damage *via* the reduction of caspase enzymes, an important category of participants in apoptosis, as well as inhibition of endothelial and inducible forms of NOS. The potency of 100 mg/kg of the grape extract was equal or higher than the same dose of N-acetylcysteine as the positive control (Ulusoy et al., 2014). The extract has also shown anti-inflammatory properties against arsenic-induced nephrotoxicity through the regulation of pro-inflammatory and anti-inflammatory cytokines, as well as deactivation of NF-κB (Wang C. et al., 2017).

Considering the above-mentioned beneficial effects of grape, in addition to its nutritional value and safety, we suggest that this fruit to be part of the diet of patients with COVID-19. Since the fresh fruit, having several minerals and natural vitamins, is easily available in almost all parts of the world, the juice can be taken orally or even administered *via* nasogastric tube.

Jujube (*Ziziphus jujuba* Mill.)

Jujube fruit from the family Rhamnaceae is one of the valuable, yet not well-recognized medicinal foods mostly grown in Iran and China (Shahrajabian et al., 2019). It is a popular plant as a traditional remedy for different types of coughs. In TPM, it is mostly considered as a modulator of the quality of humor, mainly by preventing the negative effect of excess heat. It is also described as having soothing effects on pulmonary inflammations and shortness of breath (Aghili Khorasani, 1771). In addition to its high nutritive value, jujube is a rich source of bioactive secondary metabolites such as polyphenols, polysaccharides, and terpenoids (Gao et al., 2013).

Betulinic acid, a triterpene constituent of jujube fruit, has shown antiviral activity against influenza A virus both *in vitro* and *in vivo*. At the concentration of 50 μM, betulinic acid showed a 98% inhibition of virus cytopathic effects; while it was not toxic for the host cells. Despite the remission of symptoms in the infected animals, no significant change was observed in viral replication and pro-inflammatory cytokines (except for IFNγ). Thus, further mechanistic investigations are needed to establish the antiviral properties of this compound (Hong et al., 2015).

Acidic polysaccharides are another category of active phytochemicals of jujube fruit with immunostimulatory properties. These effects were evident from the increased indices of main lymphatic organs, i.e. spleen and thymus in animals, showing an improved proliferation of immune cells. Additionally, these polysaccharides contain a series of metal ions which possibly participate in biological activities (Zou et al., 2018).

Two categories of polyphenols of jujube peel, free phenols, and bond phenols, were assessed regarding their cardioprotective activity in ISO-induced heart injury in rats. Both types of phenols demonstrated prophylactic antioxidant properties and modulatory effects on different cardiac ion channels with no significant difference between the two polyphenol categories (Cheng et al., 2012). Jujuboside A, another major triterpenoid of this plant, has represented *in vitro* cardioprotection *via* regulating of the PI3K/AKT/mTOR autophagic pathway (Han et al., 2016).

Despite the valuable properties of the jujube plant, the number of high-quality studies on the different medicinal aspects of this plant are limited. Further investigations regarding the active components of the plant and their mechanisms of action are necessary.

Licorice (*Glycyrrhiza glabra* L., *G. uralensis* Fisch.)

Licorice from the family Leguminosae is a valuable medicinal plant in TPM, as well as several other doctrines of traditional

medicines. The sweet taste of the roots is mostly due to the triterpenoid glycyrrhizin and the plant has several valuable secondary metabolites such as saponins and flavonoids (Pastorino et al., 2018). In TPM, licorice is highly respected as an antitussive medicine for different types of coughs and lung diseases and is also recommended for chronic fevers due to infections (Aghili Khorasani, 1771).

Licorice is a well-studied plant in terms of its antiviral activity (Wang et al., 2015). In addition to its remarkable antiviral properties against some RNA-viruses like HCV and HIV (Adianti et al., 2014; Fukuchi et al., 2016), licorice has demonstrated remarkable antiviral effects on respiratory viruses. Glycyrrhizin has shown a high inhibitory effect on the *in vitro* replication of two clinical isolates of SARS (Cinatl et al., 2003). Accordingly, fifteen semisynthetic derivatives of this compound were virtually screened against the virus. Glycoside, amide, and carboxyl moieties can significantly increase the activity in comparison to the original backbone; however, the cytotoxicity was also increased in these new structures (Hoever et al., 2005). In influenza A H5N1-infected lung cells, glycyrrhizin inhibited both viral replication and host cell inflammatory and apoptotic response to the infection, a condition responsible for severe flu symptoms (Michaelis et al., 2011). In silico analysis has demonstrated the ability of twelve licorice components to inhibit influenza neuraminidase (NA), a viral surface enzyme involved in the release of replicated viruses from infected host cells (Grienke et al., 2014). Aqueous licorice extract, glycyrrhizin, and one of its metabolites, 18β-glycyrrhetinic acid were investigated against human RSV. While the extract and 18β-glycyrrhetinic acid revealed significant antiviral activity, glycyrrhizin was inactive (Feng Yeh et al., 2013). This observation forms the hypothesis that glycyrrhizin acts as a pro-drug which turns into active metabolites such as 18β-glycyrrhetinic acid but future investigations are required to examine this idea.

Licorice has been traditionally used for cough in the form of a medicinal candy or lozenge. In a clinical study, patients who underwent thoracic surgery and post-operative double-lumen endotracheal intubation gargled a liquid licorice preparation as a prophylaxis for post-extubation coughing. The incidence of cough and sore throat was significantly lower with licorice compared with placebo (simple sugar syrup) (Ruetzler et al., 2013). In ovalbumin-induced lung inflammation, glycyrrhizic acid caused a dose-dependent regulation of cytokine production by types 1 and 2 of helper T lymphocytes, and the effect of the highest dose (40 mg/kg) was equal to 2 mg/kg of dexamethasone in several parameters (Ma et al., 2013). In LPS-induced inflammation in murine macrophages, glycyrrhizic acid increased autophagy markers such as LC3-II/I and Beclin-1 *via* PI3K/AKT/mTOR pathway. Likewise, the compound demonstrated *in vivo* protective effects on LPS-induced pulmonary inflammation through the reduction of pro-inflammatory cytokines. It also decreased the high mobility group box (HMGB)-1, a product of damaged cells further activating pro-inflammatory pathways (Qu et al., 2019). The same mechanism was also reported for glycyrrhizin (Kim et al., 2015). Moreover, glycyrrhizin represented a stimulatory effect on the nuclear translocation of Nrf2 and HO-1 expression in macrophages *via* the p38 MAPK pathway, an important intracellular signaling modulating apoptosis and autophagy in response to pathologic conditions (Kim et al., 2015). Glycyrrhizin could reduce abnormally-increased mucus production both *in vitro* and *in vivo via* suppression of MUC5AC mRNA expression, confirming the mucolytic properties of licorice mentioned in TPM. The effect of 45 mg/kg of glycyrrhizin on goblet cell hyperplasia was nearly equal to 1 mg/kg of dexamethasone, while 135 mg/kg of this compound was higher than dexamethasone (Nishimoto et al., 2010).

G. glabra extracts have shown cardioprotective properties both *in vitro* and *in vivo via* the improvement of endogenous antioxidant defense mechanisms (Ojha et al., 2013; Upadhyay et al., 2020). In DOX-induced toxicity in cardiomyocytes, licorice aqueous extract has improved sirtuin (SIRT)-1, a cardioprotective transcription factor, and its downstream proteins, peroxisomes proliferator-activated receptors (PPAR)-α/γ (Upadhyay et al., 2020). Glycyrrhizic acid has demonstrated an inhibitory effect on the long-lasting (L)-type calcium channels of cardiomyocytes. Although this compound could prevent calcium overload in ISO-induced cardiotoxicity, its potency was lower than verapamil even at the highest (20 mg/kg) dose (Li et al., 2019). Both hydroethanolic licorice extract and glycyrrhizic acid decreased the release of CK-MB and LDH, two indicators of myocardial damage (Ojha et al., 2013; Li et al., 2019).

Glycyrrhizic acid has also exhibited a nephroprotective effect on the LPS-induced renal inflammation *via* modulation of several apoptosis markers such as Bax, Bcl-2, and caspase-3, as well as the pro-inflammatory cytokines and iNOS activity. The reduction of COX-2 activity and its product, prostaglandin E2 (PGE2), was also involved in the nephroprotective activity of this compound (Zhao et al., 2016).

From the above-mentioned studies, it can be inferred that licorice is a multipotential medicinal plant with both direct antiviral properties and protective effects on vulnerable organs in SARS-CoV-2 infection and thus, can be further investigated as an adjuvant therapy in this disease.

Rhubarb (*Rheum palmatum* L., *R. officinale* Baill.)

Several species of the genus *Rheum* from the family Polygonaceae are commonly consumed as rhubarb. More than the purgative effects that make it a suitable detoxifying agent, rhubarb has pulmonary tonifying activity from the view of TPM. It helps the body to excrete abnormal (pathologic) phlegm and consequently, reduces the organ's susceptibility to infections (Aghili Khorasani, 1771; Zheng et al., 2013).

R. palmatum extract has demonstrated antiviral activity against CVB3, the main reason for viral myocarditis. Interestingly, the viral titers in the internal organs of infected animals treated with 0.3 g/kg of the extract were lower than those that received 0.01 g/kg ribavirin as the gold standard. Serum viral RNA was not detected on the last day of the experiment in the

extract-treated group; while it remained positive in the ribavirin group (Xiong et al., 2012). Rheum emodin, an anthraquinone of rhubarb, has also shown antiviral activity against EV-71, mostly through the suppression of viral maturation and virulence and to a lower extent, viral genome levels and protein expression (Zhong et al., 2017). Sennosides A and B, two other anthraquinones of rhubarb, as well as the extracts of two rhubarb species, *R. palmatum* and *R. officinale*, were assessed against HIV infection. The compounds were assessed regarding their inhibitory effects on three main HIV-1 enzymes, i.e. reverse transcriptase (RT)-associated DNA polymerase (RDDP), integrase, and ribonuclease H (RNase H). *R. officinale* extract and sennoside A showed a higher potency toward the inhibition of viral RNase H. Additionally, sennoside A had a lower IC_{50} for RDDP and integrase, showing a higher activity against viral replication which was also confirmed in the cell-based assay (Esposito et al., 2016).

Rhein, another anthraquinone structure of rhubarb, has shown protective effects on RSV-induced lung infection *via* modulation of the host inflammatory response, evident from the reduced levels of pro-inflammatory cytokines and NLRP3. NLRP3 inflammasome is a downstream protein complex of the NF-κB pathway, activating caspase-1 and consequently, apoptosis. It should be mentioned that rhein at a dose of 120 mg/kg showed similar efficacy to 46 mg/kg of ribavirin (Shen et al., 2019). In both *in vitro* and *in vivo* models of pulmonary fibrosis, emodin caused a decrease in α-smooth muscle actin and collagen production. Furthermore, the compound reduced TGF-β1-dependent phosphorylated Smad2/3, a pro-fibrotic mediator inhibiting fibroblast differentiation to myofibroblasts (Guan et al., 2016). Signal transducer and activator of transcription (STAT)-3, another TGF-β1-dependent stimulator of fibroblast activation, as well as HSP-47, a collagen-specific heat shock protein and an indicator of pulmonary fibrosis, were also decreased by emodin, further showing its antifibrotic activity (Guan et al., 2016). Chrysophanol, another anthraquinone from rhubarb, has proved anti-inflammatory properties mostly *via* the inhibition of NF-κB activity and the prevention of lung fibrosis. Moreover, this compound could reduce ovalbumin-induced autophagy and inflammation (Song et al., 2019). In a randomized, controlled clinical trial in patients with acute respiratory distress syndrome (ARDS), a life-threatening condition also observed in COVID-19, a liquid rhubarb preparation was administered *via* nasogastric tube for one week. In comparison to the control group, the adjuvant rhubarb administration could significantly improve oxygenation. Additionally, it could decrease the extravascular lung water index and pulmonary vascular permeability index (He et al., 2017), confirming the results of the preclinical studies in a clinical setting. It should be noted that no identification process was considered for rhubarb and thus, it is not clear which species of the plant were used in this trial (He et al., 2017).

Rhubarb anthraquinones have also demonstrated cardioprotective properties. Emodin could increase the secretion of atrial natriuretic peptide (ANP), a molecule secreted by cardiomyocytes with a multitargeted role in cardioprotection (Zhou et al., 2014). Rhein could restore the downregulation of the PI3K/GSK3β cardioprotective pathway in I/R-induced cardiotoxicity (Liu et al., 2018). Chrysophanol has shown an inhibitory effect on PARylation, the process of PARP attachment to its target proteins, overactivation of which participates in DOX-induced cardiotoxicity. In addition, apoptotic markers, as well as mitochondrial damage were reduced by chrysophanol both *in vitro* and *in vivo* (Lu et al., 2019).

Rhubarb extract could inhibit adenine-induced renal damage *via* the suppression of the TGF-β/Smad pathway (Zhang et al., 2018), a mechanism also reported for its protective effects on the lung (Guan et al., 2016). Furthermore, several other fibrosis biomarkers including E-cadherin, collagen, α-smooth muscle actin and vimentin were decreased in rats treated with rhubarb (Zhang et al., 2018). The aqueous *R. palmatum* extract and rhein have demonstrated nephroprotective activity in cellular and animal models of chronic kidney disease. This effect was mediated *via* suppression of autophagy-related pathways and renal fibrosis (Tu et al., 2017).

The result of preclinical studies on rhubarb show the pharmacological activity of this plant to be highly attributed to its anthraquinones through several therapeutic targets in organs damaged in SARS-CoV-2 infection. Especially, a clinical study of the protective effects of this plant in patients with ARDS represents the significant efficacy and acceptable safety of its use in treating a condition remarkably similar to COVID-19. Thus, rhubarb could be one of the possible choices that are clinically assessed in treating this disease.

Saffron (*Crocus sativus* L.)

Saffron, known as the red gold, is the stigma of an herbaceous plant from the family Iridaceae, native to Iran, with several pharmacological activities. The most investigated components of the plant are carotenoid structures including crocins and their metabolites, crocetin responsible for the saffron color. The terpene glycoside picrocrocin, as well as the monoterpene aldehyde safranal, a volatile compound causing the specific saffron aroma are other important components of saffron (Boskabady and Farkhondeh, 2016). In TPM, Saffron is considered to be a tonic of the lung and kidneys. It is highly valued not only as a cardiotonic medicine, but also as a means of enhancing the delivery of other medicinal ingredients to the heart. Thus, saffron is one of the most frequently used ingredients in TPM multicomponent preparations for heart diseases (Aghili Khorasani, 1771; Sadati et al., 2016).

The aqueous extract of saffron, as well as crocin and picrocrocin, were evaluated in terms of *in vitro* antiviral activity against HIV-1. Both carotenoids showed antiviral activity with a relatively low IC_{50} (5 and 8 μM) and high SI (>187 and 600)(**Table 2**), showing these compounds to be potent antiviral agents. On the other hand, the aqueous extract showed no considerable antiviral activity, revealing that the antiviral activity of the plant is mostly due to its lipophilic compounds such as carotenoids (Soleymani et al., 2018).

Safranal has demonstrated anti-inflammatory effects on OVA-induced airway inflammation *via* modulation of type 1

and 2 helper T lymphocytes balance, evident from the reduced serum IL-4 and elevated IFNγ (Boskabady et al., 2014). This modulatory effect on cytokine production was also observed *in vitro* in T lymphocytes (Boskabady et al., 2011). Interestingly, even the effect of the lowest dose (4 μg/ml of drinking water) was higher than 50 μg/ml of dexamethasone, showing a significantly higher potency for this compound in alleviating pulmonary inflammation (Boskabady et al., 2014). In bleomycin-induced pulmonary damage, crocin has reduced tissue inflammation *via* the reduction of pro-inflammatory cytokines and markers of fibrosis, improvement of endogenous antioxidant mechanisms, and the induction of the Nrf2 cytoprotective pathway. Likewise, the compound was an inhibitor of Toll-like receptor (TLR)-4, a receptor participating in leukocytes infiltration, neutrophils activation, TNF-α-dependent inflammation, and TGF-β-dependent fibrosis. The antifibrotic effect of crocin at 20 mg/kg was higher than 0.2 mg/kg of halofuginone (Zaghloul et al., 2019). Additionally, crocin has been shown to relieve the effects of LPS-induced acute lung injury by suppressing NF-κB and MAPK pro-inflammatory cascades. Matrix metalloproteinase 9 (MMP-9), heparanse, and two glycocalyx shedding enzymes overactivated in inflammatory lung diseases were also decreased by crocin (Zhang et al., 2020b). In a randomized, triple-blind, placebo-controlled clinical trial in patients with mild to moderate asthma, a saffron powder supplement was administered for two months. Spirometry parameters, including forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), FEV1/FVC ratio, and forced expiratory flow 25-75% were significantly improved in comparison to placebo. Furthermore, serum levels of CRP and the anti-HSP70 antibody were significantly decreased. A direct correlation between the severity of asthma symptoms and anti-HSP70 antibody was observed and thus, its reduction is an indicator of decreased pulmonary inflammation (Hosseini et al., 2018).

Saffron aqueous extract exhibited cardioprotective effects on an animal model of ISO-induced cardiac damage *via* reduction of CK-MB and LDH leakage from myocardial cells and improvement of endogenous antioxidants in cardiac tissue (Sachdeva et al., 2012). A similar result was obtained with safranal from a significantly lower effective dose in comparison to the aqueous extract, suggesting these effects are partially due to this saffron component (Mehdizadeh et al., 2013). In DOX plus I/R-induced cardiotoxicity, saffron could restore the level of contractile proteins including α-actinine, myosin light chain, and troponine C. It could prevent mitochondrial dysfunction and recover the phosphorylation level of the AKT/P70S6K and ERK1/2 cardioprotective pathways (Chahine et al., 2016). These effects may be partially mediated by crocin since this compound has shown to have protective activity with respect to ECG in an animal model of DOX-induced cardiotoxicity (Razmaraii et al., 2016). Similarly, crocin was effective in the reduction of LPS-induced cardiotoxicity *via* a decrease in the protein level and gene expression of pro-inflammatory cytokines. COX-2 enzyme and its product, PGE2, which are increased in LPS-induced damages were returned to the normal level by crocin administration (Rahim et al., 2019).

Saffron extract has shown detoxifying effects on alcohol-induced renal damage mostly through the inhibition of pro-apoptotic mediators including caspase enzymes and Bax/Bcl-2 signaling, as well as pro-inflammatory cytokines production (Rezaee-Khorasany et al., 2019). Crocin has demonstrated nephroprotective properties in an animal model of diabetes-induced nephrotoxicity (**Table 2**). Aside from general antioxidant properties (Erdemli et al., 2017), this compound could decrease hyperurocemia *via* the inhibition of xanthine oxidase (Altinoz et al., 2015). Moreover, a clinical trial using 100 mg/day of saffron in healthy subjects showed this plant to have a short-term immunopotentiation, considering the altered levels of immunoglobulins and the leukocytes count (Kianbakht and Ghazavi, 2011).

DISCUSSION AND CONCLUSIONS

This paper has reviewed the pharmacological mechanisms of medicinal plants with possible beneficial effects on SARS-CoV-2 infection and related organ damage based on the suggestions of TPM. Most of the medicinal plants included in this review show multitargeted activity and protective mechanisms in the tissues damaged in SARS-CoV-2 infection.

The most important effects of the medicinal plants and their isolated phytochemicals include anti-inflammatory activity and antioxidant properties (**Figure 1**). These two mechanisms comprise several cellular and subcellular pathways such as NF-κB, Nrf2, pro-inflammatory and anti-inflammatory cytokines balance, and endogenous enzymatic/non-enzymatic antioxidant defense mechanisms. It should be considered that such mechanisms are general cytoprotective cascades observed in nearly all body organs and that the beneficial effects of phytochemicals in one tissue can be extrapolated to other tissues. For instance, if a compound has shown a stimulatory effect on Nrf2 signaling in myocardial cells, the same activity could be expected in lung tissue or kidney tubules. This is in line with the holistic view of TPM, which encompasses the idea that treatments should improve the overall health of the human body as a whole, instead of focusing on the damaged organ. It is believed in TPM that reinforcement of the body's inner power is one of the best ways to combat a disease. Most of the discussed medicinal plants stimulate cytoprotective mechanisms to face pathogenic factors, which can be considered as equivalent to these ideas of improving the body's inner power.

One of the key pro-inflammatory cytokines affecting clinical manifestations of COVID-19 is IL-6. In patients with COVID-19, organ failures have been observed and the subsequent deaths of many patients are often due to cytokine storm, an exaggerated inflammatory response (Mehta et al., 2020). IL-6 has a major role in this event and the IL-6 inhibitor tociluzumab (Actemra®) has been evaluated in several clinical studies in patients. Another systematic review has also discussed the value of this agent in treating COVID-19, highlighting that current evidence suggests that the drug could be beneficial in this infection (Alzghari and Acuña, 2020). Another important cytokine in this infection is

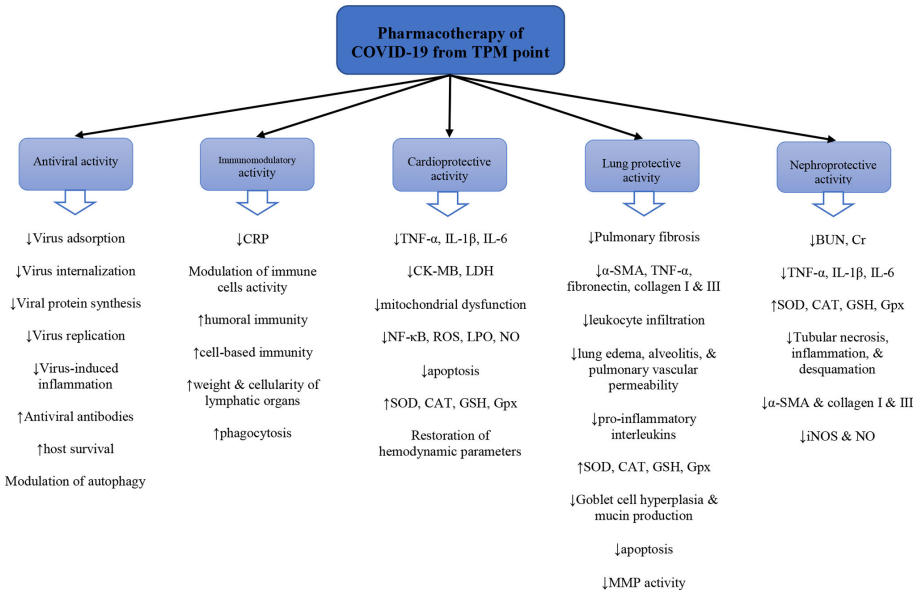

FIGURE 1 | Mechanisms of medicinal plants introduced based on traditional Persian medicine for the management of SARS-CoV-2 infection.

TNF-α and the inhibitors of this cytokine are also currently being assessed as therapeutic options for SARS-CoV-2 patients (Feldmann et al., 2020). A considerable number of the plants discussed in this present review have been shown to reduce IL-6 and TNF-α levels and/or activities (**Table 2**). Major phytochemicals of these plants should be further investigated in the design and development of plant-derived cytokine inhibitors in future studies.

Edible plants, particularly grape, jujube fruit, amla, and damask rose, are well-known and used medicinally every day by people all over the world, which indicates that they are safe. Such plants can be prepared as a juice or in a standardized liquid dosage form for COVID-19 patients. Where the guidelines on effective drugs for treating this infection vary day-by-day, increasing amounts of new data are being released from ongoing clinical trials regarding the pros and cons of the medicines recommended. In such a situation, physicians may be willing to stay on the safe side and avoid using multi-ingredient complementary medicinal preparations with several possible side effects/drug interactions. As a more acceptable and safer approach, natural tonics that are based on these popular food plants could be recommended as part of the healthy daily diet for people prone to or who are vulnerable to the disease. This includes healthcare providers caring for COVID-19 patients in hospitals, the family members of the patients in home-quarantine, and people with underlying diseases such as cardiovascular problems or diabetes who are more vulnerable to this infection. Studies on healthy populations are encouraged due to the lower risk of adverse effects and the fact that they would indicate the safety and efficacy of natural products, with use in patients as the second step in developing these adjunct therapies.

On the other hand, some phytochemicals have revealed direct antiviral activity *via* blockade of different stages of the virus life cycle including fusion, replication, protein synthesis, and viral particle release from the host cells. Although the life cycle and target proteins of SARS-CoV-2 may differ from the assessed viruses to some extent, there would be similarities that are worth assessing in terms of the antiviral activity of these compounds against the virus. As has been reported in virtual screenings (Zhang et al., 2020a), some of these compounds have shown significant interactions with SARS-CoV-2 structures, further confirming their antiviral activity. In this regard, molecular docking analyses would help in selecting structures with the highest binding affinity and the ability to inhibit viral enzymes. The virtually selected antiviral phytochemicals can then be evaluated in cell-based and animal studies. Likewise, they can be considered as molecular backbones in the design of new semisynthetic structures and in creating new compounds with higher safety and antiviral efficacy.

Some of the included compounds such as quercetin, pyrogallol, and fructan polysaccharides are considered ubiquitous, i.e. they can be found in several foods and spices of the human diet and do not belong to a specific medicinal plant. Despite the abundance of these compounds, their pharmacological activity cannot be denied and further interpretation of the results of studies is of great importance. For example, in an *in vitro* study by Mahmood et al. (1996) quercetin showed the highest antiviral activity against HIV amongst several evaluated phytochemicals. This does not mean that any quercetin-containing plant can be effective against viral infection. Instead, the effective dose of this compound should be measured in preclinical studies and a dose translation calculation is needed to suggest accurate human doses of quercetin and to observe related pharmacological activity.

An important limitation of the studies included in this review is that many of them lacked a positive control group. Some of the reports used dexamethasone as a standard anti-inflammatory agent in antiviral evaluations, however, most of the studies did not consider a standard treatment. This methodological problem makes any judgment regarding the potency of the assessed materials difficult. Some antiviral phytochemicals have significantly lower SI compared with conventional antiviral agents which excludes them from further assessment. Lack of a positive control makes such comparisons difficult since SI reported in other studies do not provide an accurate comparison due to these methodological differences. The same problem exists in the evaluation of the other pharmacological properties of the plants. Thus, future studies should consider the use of a gold standard drug for a better presentation of the potency of test agents.

One of the concerns in the clinical use of medicinal plants is the possibility of herb-drug interaction. Phytochemicals can have inducing (Soleymani et al., 2017) or inhibitory effects (Bahramsoltani et al., 2017) on drug metabolizing enzymes such as hepatic cytochrome P450 and intestinal P-glycoprotein and thus, can affect the pharmacokinetics of conventional drugs. Consequently, the serum level of conventional antiviral agents administered to COVID-19 patients may be affected by concomitant administration of herbal supplements. Such pharmacokinetic interactions may increase serum level and adverse effects, or decrease serum concentration and clinical response, both of which can have life-threatening outcomes in patients.

Another discussion can be raised concerning the plants that contain ACE inhibitor compounds. ACE inhibitors do not have a direct effect on SARS-CoV-2 but they can increase the expression of the ACE2 receptors. This receptor acts as a co-receptor in the invasion of the virus and thus, a hypothesis is formed that ACE inhibitors may increase susceptibility to SARS-CoV-2 (Danser et al., 2020).

Most of the studies included in this review were animal and cell-based evaluations, providing only foundational evidence for the efficacy of the aforementioned plants in human studies. Only six clinical studies were considered in this paper and higher levels of evidence are essential to examine the medicinal suggestions of TPM in clinical settings. Although there are several ongoing clinical trials on the effect of TPM formulas on COVID-19, no published data is available. Despite the challenges in decision-making for the clinical assessment of natural products in SARS-CoV-2, high-quality trials have evaluated the effect of such products with valuable outcomes (Yang et al., 2020), with over fifty clinical trials designed to assess the effect of self-made and commercial TCM formulas. The National Health Commission of China has also added some TCM recommendations to the latest guidelines for the management of COVID-19 patients, even in severe cases (Yang et al., 2020). Thus, the trial design and inclusion/exclusion criteria for patients, as well as drug preparation can be defined in a way that is comprehensively approved. Natural compounds may have some advantages in comparison to conventional drugs. For instance, there are concerns about the currently available TNF-α inhibitors because they can suppress the immune system, leading to secondary bacterial/viral infections (Feldmann et al., 2020). On the other hand, a remarkable number of natural antiviral compounds have immunomodulatory and antibacterial properties. Further studies in healthy individuals that assess the prophylactic activity of these supplements as well as trials in patients with less severe symptoms would pave the way for further clinical evaluations.

In conclusion, medicinal plants have great potential value and can be recommended for treatment of COVID-19 based on the therapeutic approaches of TPM, several of which have also been confirmed by pharmacological studies in modern medicine. The currently available data, regarding these medicinal plants, provide foundational evidence. Future preclinical mechanistic studies as well as clinical trials are necessary to confirm the safety and efficacy of these plants for the management of SARS-CoV-2 infection.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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Deciphering the Pharmacological Mechanisms of Ma Xing Shi Gan Decoction against COVID-19 through Integrating Network Pharmacology and Experimental Exploration

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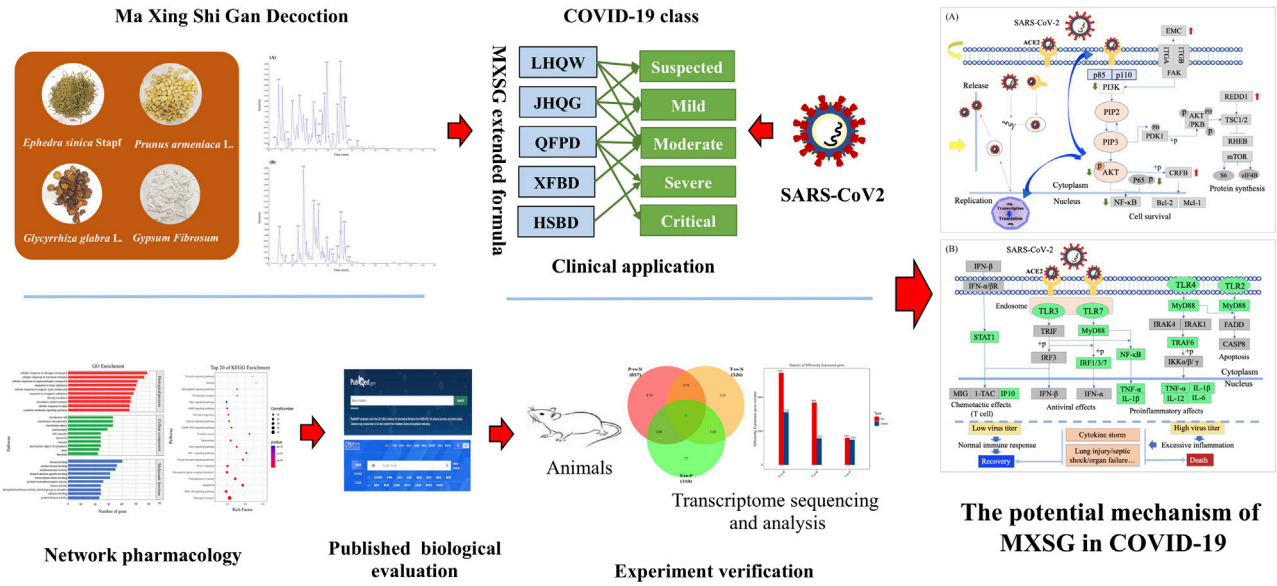
The outbreak of new infectious pneumonia caused by SARS-CoV-2 has posed a significant threat to public health, but specific medicines and vaccines are still being developed. Traditional Chinese medicine (TCM) has thousands of years of experience in facing the epidemic disease, such as influenza and viral pneumonia. In this study, we revealed the efficacy and pharmacological mechanism of Ma Xing Shi Gan (MXSG) Decoction against COVID-19. First, we used liquid chromatography–electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) to analyze the chemical components in MXSG and identified a total of 97 components from MXSG. Then, the intervention pathway of MXSG based on these components was analyzed with network pharmacology, and it was found that the pathways related to the virus infection process were enriched in some of MXSG component targets. Simultaneously, through literature research, it was preliminarily determined that MXSG, which is an essential prescription for treating COVID-19, shared the feature of antiviral, improving clinical symptoms, regulating immune inflammation, and inhibiting lung injury. The regulatory mechanisms associated with its treatment of COVID-19 were proposed. That MXSG might directly inhibit the adsorption and replication of SARS-CoV-2 at the viral entry step. Besides, MXSG might play a critical role in inflammation and immune regulatory, that is, to prevent cytokine storm and relieve lung injury through toll-like receptors signaling pathway. Next, in this study, the regulatory effect of MXSG on inflammatory lung injury was validated through transcriptome results. In summary, MXSG is a relatively active and safe treatment for influenza and viral

Abbreviations: A549, human endothelial lung cells; ACE2, angiotensin-converting enzyme 2; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; BALB, Bagg's albino mice; COVID-19, Corona Virus disease 2019; DEGs, differentially expressed genes; DPEP, D-pseudo-ephedrine; GL, glycyrrhizin; GO enrichment, gene ontology enrichment; H1N1, influenza A virus hemagglutinin; Hfl-1, human lung fibroblast cells; HSBD, Hua Shi Bai Du decoction; JHQG, Jin Hua Qing Gan granules; KM, Kunming mice; LC-ESI MS/MS, liquid chromatography-electrospray ionization tandem mass spectrometry; LEP, L-ephedrine; LHQW, Lian Hua Qing Wen capsules; Lico A, licochalcone A; LMEP, L-methylephedrin; LPS, lipopolysaccharide; MDCK, Madin-Darby canine kidney; MERS-CoV, Middle East Respiratory Syndrome Coronavirus; MXSG, Ma Xing Shi Gan decoction; PAMPs, pathogen-associated molecular patterns; PI3K/AKT, phosphatidylinositide 3-kinases/protein kinase B; PPAR, peroxisome proliferators-activated receptor; PRRs, pattern recognition receptors; QFPD, Qing Fei Pai Du decoction; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SD, Sprague-Dawley rats; T cell, T-lymphocyte; TCM, Traditional Chinese Medicine; TLRs, Toll-like receptors; WT, wild type mice; XFBD, Xuan Fei Bai Du decoction.

pneumonia, and its therapeutic effect may be attributed to its antiviral and anti-inflammatory effects.

Keywords: Ma Xing Shi Gan decoction, COVID-19, Traditional Chinese Medicine, immunomodulatory, antiviral



Graphical Abstract | Study design:the workflow for the whole study

INTRODUCTION

The coronavirus disease 2019 (COVID-19) epidemic is a global public health crisis, with considerable mortality and morbidity exerting pressure on health care and the economy. Unfortunately, there are currently no drugs or vaccines available to treat specific antivirals (Zumla et al., 2016; Xie et al., 2020). The majority of patients infected with SARS-CoV-2 show symptoms of pneumonia, fever, dry cough, fatigue, and other symptoms such as myalgia, and diarrhea (Henry and Vikse, 2020; Stower, 2020; Xiong et al., 2020). Like SARS-CoV, the spine (S) protein of SARS-CoV-2 enters human alveolar epithelial cells by binding the angiotensin-converting enzyme 2 (ACE2) receptor (Zhou et al., 2020). At low viral titers, the human immune response may be characterized by the antiviral response to type I interferon and the $CD4^+$ and $CD8^+$ T-cell response, leading to viral clearance. Severe infection and excessive immune-inflammatory response caused by high viral titer have been proved to be the leading cause of progression to acute lung injury (ALI), acute respiratory distress syndrome, respiratory and circulatory failure, and even death (Chen et al., 2020; Narasaraju et al., 2020; Wang et al., 2020). Patients with COVID-19 exhibit pathogenesis; clinical manifestations are similar to the symptoms of SARS-CoV and H1N1 infections. Therefore, although the pathogenesis of COVID-19 is poorly understood, the similar mechanisms of SARS-CoV and H1N1 can give us a great deal of information on the pathogenesis of SARS-CoV-2 infection to promote our recognition (Li et al., 2020c).

Based on clinical observation of COVID-19 and experience in treating SARS and H1N1, the guideline on diagnosis and treatment of COVID-19 has proposed that combines modern medicine with TCM in China (Liu et al., 2012; Li and Peng, 2013; Dei-Cas et al., 2020). According to the Press Conference of the Joint Prevention and Control Mechanism of State Council, 74,187 people have used Chinese medicine in confirmed cases of COVID-19 in China. It accounted for 91.5% of the total cases. Clinical observation shows that the total effective rate of TCM has reached more than 90% (National Health Commission of the People's Republic of China, 2020). Ma Xing Shi Gan (MXSG) decoction, was the basic formula of three drugs and three formulas including Lian Hua Qing Wen capsules (LHQW), Jin Hua Qing Gan granules (JHQG), Qing Fei Pai Du decoction (QFPD), Hua Shi Bai Du decoction (HSBD), and Xuan Fei Bai Du decoction (XFBD) promulgated by China's National Health Commission for the treatment of COVID-19. It has been applied to COVID-19 patients in both suspected cases and confirmed individuals with mild cases, moderate cases, severe cases, and critical cases. A previous study has demonstrated that MXSG exhibits similar antiviral activity to oseltamivir and broad-spectrum inhibitory activity in mice infected with influenza A

virus (Li et al., 2017; Zou et al., 2018). This activity has also been confirmed in the LHQW and JHQG treatment of H1N1 patients (Duan et al., 2011; Wang et al., 2011). Also, MXSG can downregulate chemokines, inhibit inflammation response, and ameliorate the ALI in model rats (Ma et al., 2014; Fei et al., 2019). However, the overall understanding of the therapeutic effect and potential mechanisms of MXSG in the COVID-19 remains elusive.

Here, we dissected the chemical components of MXSG by liquid chromatography–mass spectrometry (LC-ESI-MS/MS) and analyzed the intervention pathways of MXSG based on components detected through network pharmacology. At the same time, the therapeutic effect of MXSG on COVID-19 was explained through published articles, and the relevant regulatory mechanism was proposed. Then, in this study, the regulatory effect of MXSG on inflammatory lung injury was validated through transcriptome results. In summary, our study suggests that MXSG inhibits viral invasion, proliferation, and mitigation of virus-induced lung injury, which may be a key mechanism of its therapeutic effect on COVID-19. These results provide experience for the treatment of infectious diseases and lung injury.

RESULTS

Characterization of Chemical Constituents in Ma Xing Shi Gan Decoction

Representative total ion chromatograms obtained by LC-ESI-MS/MS are shown in **Figure 1**. After peak integration, 126 peaks were detected as individual compounds. Based on the MetWare database and the published database of metabolite information, qualitative

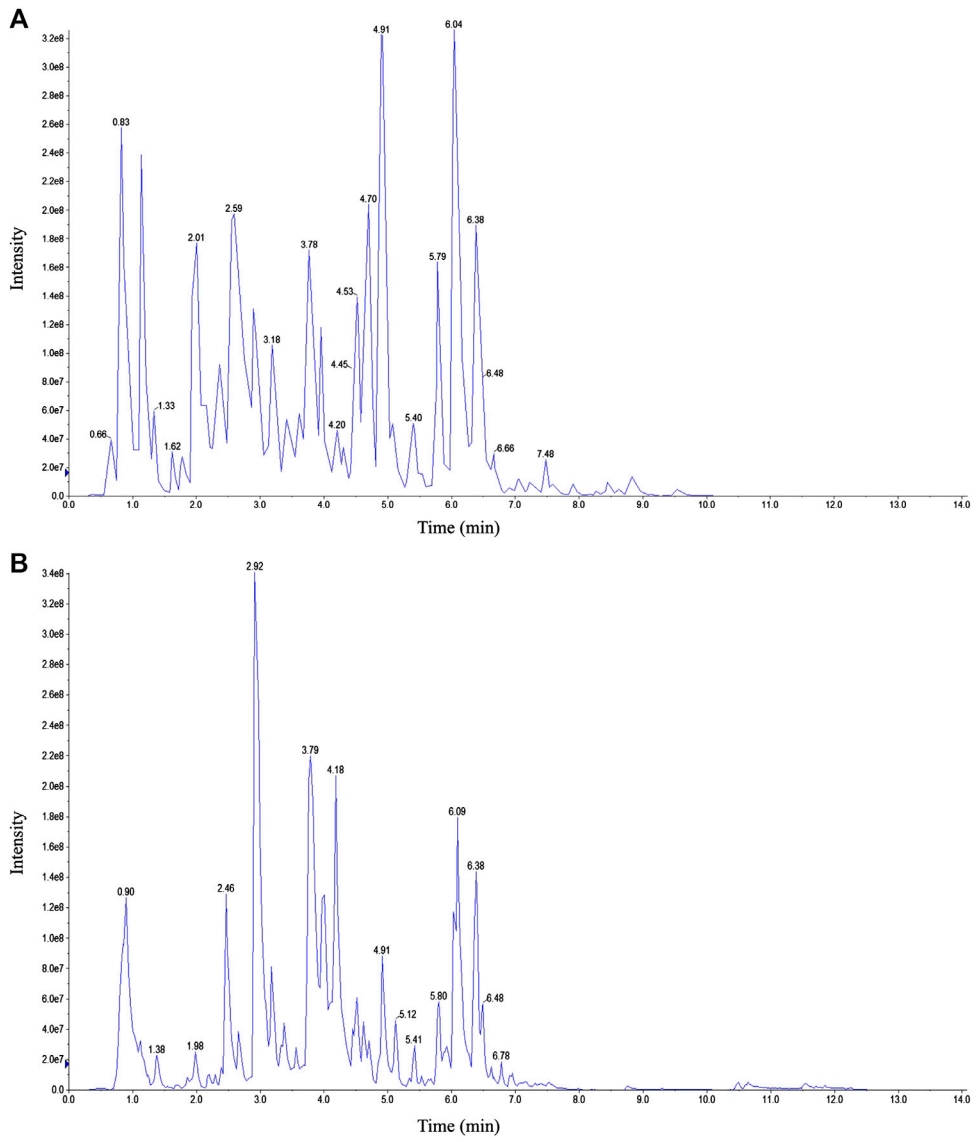


FIGURE 1 | Representative total ion chromatogram MXSG. **(A)** Positive mode. **(B)** Negative mode.

analysis was conducted on the primary and secondary spectrum data of mass spectrometry. As a result, 97 components were identified from MXSG. The compounds identified from MXSG were listed in **Supplementary Table S1**, including 40 flavonoids, 18 phenolic acids, 16 alkaloids, 10 terpenes, five lignans and coumarins, one quinone, and other types of compounds.

Characterization of Potential Therapeutic Targets of Ma Xing Shi Gan Decoction

The potential therapeutic target network of MXSG was presented in **Figure 2**. We first collected the targets of compounds identified by LC-ESI-MS/MS. Among the 97 compounds detected, 54 corresponding targets were obtained through database retrieval, and a total of 204 targets were obtained after merging and deleting. Then, GO and KEGG enrichment analysis was performed on the target information (**Figure 2**). In the GO enrichment analysis results, MXSG mainly played an intervention role by interfering with cellular processes and metabolic processes. The intervention mainly affects the cell membrane structure, and the main target molecules participate in the protein binding process and catalytic function. KEGG analysis showed that the role of MXSG was mainly to interfere with tumor-related pathways and viral infection–related pathways. The target information is in **Supplementary Table S2**.

Efficacy of Ma Xing Shi Gan Decoction Against COVID-19 From Published Research Evaluation

MXSG is one of the most frequently used and valid prescriptions for COVID-19 prevention and control programs. It is the fundamental component of three drugs and three formulas, including LHQW, JHQG, QFPD, HSBD, and XFBD promulgated by China's National Health Commission. We made a summary of MXSG and its extended formula formulation, effects, and clinical features (**Supplementary Table S3**).

And then, we made a summary of therapeutic effects of MXSG or its extended formula for SARS-CoV-2 or H1N1 infection. The results showed that MXSG was effective in treating influenza or viral pneumonia in both animal and clinical studies. The animal research involved three animal models of type A influenza virus infection, including BALB/c mice, KM mice, and WT mice. In the clinical studies, a total of 4,596 cases patients with SARS-CoV-2 or H1N1 infection were involved, including clinical observation studies, prospective cohort studies, retrospective studies, double-blinded randomized control trials, and randomized double-blind positive controlled clinical trial studies (**Table 1**).

MXSG has been showing the effects of antiviral (reduction of the duration of viral shedding), ameliorated the clinical symptoms (fever, fatigue, coughing, etc.), inhibited progression (reducing the deterioration of the disease), regulated immune inflammation (alleviating inflammatory, improving the lymphocyte count, inhibiting the release of inflammatory cytokine, etc.), and depressed lung injury (pathological scores, alveolar-capillary barrier damage, pulmonary edema, and inflammatory factors were reduced.) in basic and clinical studies. In particular, the effect of MXSG for improving viral lung injury was close to oseltamivir. Moreover, in these studies, no noticeable drug-related adverse reactions were found between MXSG or its extended formula (**Table 1**).

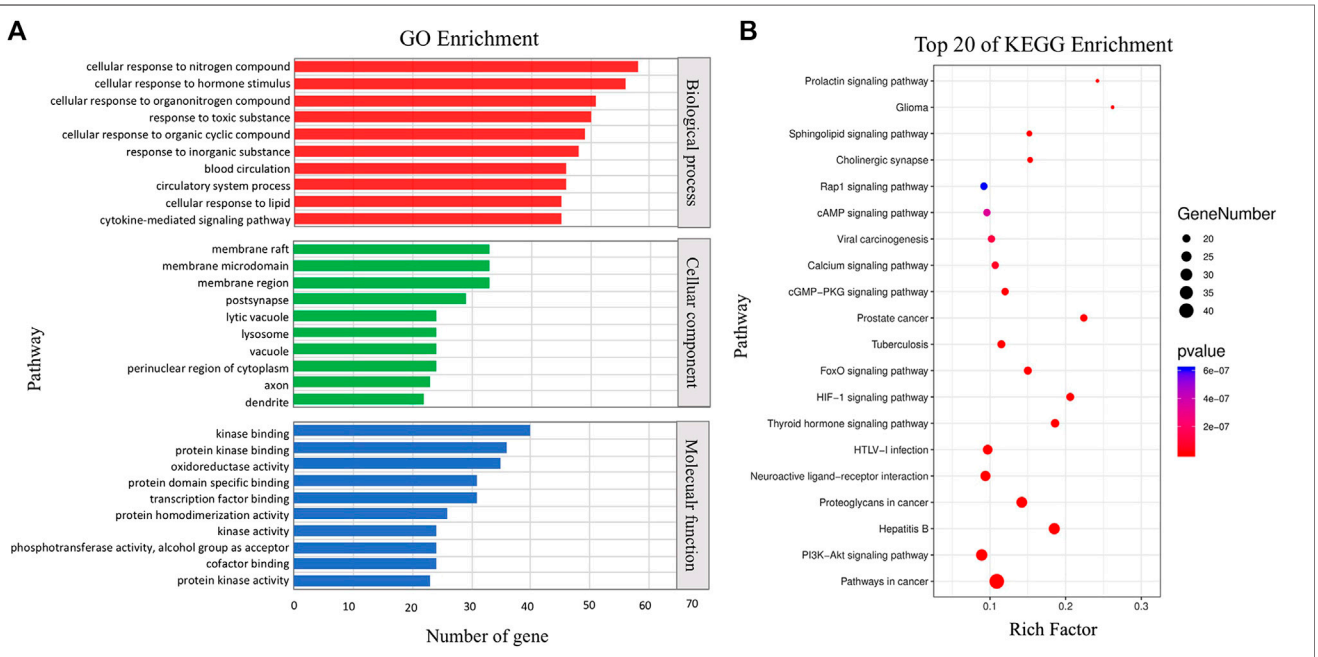


FIGURE 2 | Functional analysis of MXSG. **(A)** GO enrichment of related genes. **(B)** Top 20 pathways enriched by the KEGG method.

TABLE 1 | Efficacy evaluation of MXSG or its extended formula for influenza virus infection.

Drug	Therapeutic effects	Methodology	References
MXSG	Alleviated lung inflammatory, reduced lung weight index	Animal studies: MXSG treatment in type A influenza virus infection in BALB/c mice	Zhang et al. (2013)
	Alleviated colon tissue pathological injury induced by influenza virus lung infection	Animal studies: MXSG treatment in type A influenza virus infection in KM mice	Zou et al. (2018)
	Antiviral, improved lung inflammation and cytokines balance, protected the immune organ	Animal studies: MXSG treatment in type A influenza virus infection in WT mice	Li et al. (2017)
	Definite curative effect, no obvious adverse reaction	Clinical observation: 40 COVID-19 were treated with usual treatment combined with MXSG	Qu et al. (2020)
LHQW	Increased the symptom recovery rate and median time (fever, fatigue, and coughing), and improved the rate of chest CT manifestations and clinical cure	Prospective cohort study: 284 patients with COVID-19 were randomly divided into two groups (142 in each treatment group and control group), which received usual treatment alone or in combination with LHQW	Hu et al. (2020)
	Compared with oseltamivir, similar therapeutic effects were achieved, with shorter duration of disease and viral shedding, and reduced the severity of illness and the duration of symptoms	Randomized, double blind, positive controlled clinical trial: 244 patients with influenza A (H1N1) virus, were randomized to two treatment groups (112 cases in each group). Each group assigned to receive either LHQW or oseltamivir	Duan et al. (2011)
	Significantly improve the symptoms, no obvious adverse reaction	Retrospective study: 101 COVID-19 suspected case, 63 cases were received usual treatment and combination with LHQW, 38 cases were received usual treatment	Lv et al. (2020)
JHQG	Oseltamivir and JHQG, alone and in combination, reduced the duration of the fever	Prospective cohort study: 410 cases with confirmed H1N1, were randomly assigned receive oseltamivir/JHQG treatment alone or in combination (control 103 cases, oseltamivir 102 cases, JHQG 103 cases, and oseltamivir plus JHQG 2,013 cases)	Wang et al. (2011)
	The clinical symptoms of fever, cough, fatigue, and expectoration were reduce compared with control group; psychological anxiety of patients was relieved	Clinical observation: 123 COVID-19 patients were randomly divided (1:2) into routine treatment alone or combined with JHQG	Duan et al. (2010)
	Routinely low dose JHQG was effective and safe in treating patients with influenza	Double blinded randomized control trial: 136 influenza patients were randomized by stratification into three groups, high-dose JHQG group (44 cases), low-dose JHQG group (45 cases), and placebo control group (47 cases)	Li et al. (2013a)
	Definite curative effect, improve the clinical symptoms, reduce the deterioration of the disease, also has the effect on the immunological index	Clinical observation: 102 mild cases and moderate cases with Covid-19, were randomized to receive usual treatment alone or in combination with JHQG. Retrospective study: 80 COVID-19 patients were received routine treatment in combination with JHQG	National Health Commission of the People's Republic of China (2020), Liu et al. (2020)
QFPD	Reduced the length of hospital stay, improved clinical symptoms, stopped the deterioration of the disease, reduced the death rate, and weakened the harm of the epidemic	Retrospective study: 60 COVID-19 patients were received usual treatment alone (30 cases) or in combination with QFPD (30 cases). Clinical observation: 1,263 cases with Covid-19, 57 severe cases with Covid-19, patients were received usual treatment in combination with QFPD	National Health Commission of the People's Republic of China (2020), Li et al. (2020b)
XFBD	In mild and normal patients, improved clinical symptoms, controlled the progression of the disease, alleviated inflammatory, and improved the lymphocyte count	Clinical observation: 1,120 cases with Covid-19 (XFBD group 70 cases, control 50 cases). 240 mild cases and moderate cases with Covid-19. 3,500 mild and moderate cases with Covid-19. Patients were randomized to receive usual treatment alone or in combination with XFBD	National Health Commission of the People's Republic of China (2020)
HSBD	The effectiveness and safety were determined, improved pulmonary inflammation and clinical symptoms, and shortened duration of viral shedding and hospital stay. No drug-related adverse reactions were found	Clinical observation: 175 severe cases with Covid-19. 2,124 moderate cases with Covid-19. 3,894 mild cases and moderate cases with Covid-19 (HSBD group 452 cases). Patients were randomly divided into single routine treatment or combined HSBD	National Health Commission of the People's Republic of China (2020)

TABLE 2 | Experimental evidence of MXSG or its herbal/active ingredients for anti-influenza virus.

MXSG/ingredient	Target	Mechanism	Methodology	References
MXSG	AKT phosphorylation↓, PI3K↓	Inhibited both viral adsorption and penetration; induced disruption of the viral particle	MXSG against influenza virus A/WSN/33 in MDCK cells	Hsieh et al. (2012)
	Neuraminidase↓	Prevented the proliferation of influenza virus	MXSG against type A influenza virus infection in BALB/c mice	Zhang et al. (2013)
MH	Acidification of endosomes and lysosomes↓	Inhibited virus growth	MH against influenza A/PR/8 virus in MDCK cells	Mantani et al. (1999)
LMEP, LEP and DPEP	NA	Inhibited the proliferation	MXSG treatment in influenza A in MDCK cells and male ICR mice	Wei et al. (2019)
(+)-Catechin	Acidification of endosomes and lysosomes↓	Inhibited virus growth	(+)-Catechin treatment in influenza A/PR/8 virus in MDCK cells	Mantani et al. (2001)
Glycyrrhizin	NA	Lower membrane fluidity and inhibited virus entry	Glycyrrhizin treatment in influenza A/Aichi/2/68 virus in MDCK cells	Harada (2005)
	NA	Reduced cell membrane endocytotic activity and reduced virus uptake	Glycyrrhizin treatment in influenza A virus (IAV) in MDCK/A549/Hfl-1	Wolkerstorfer et al. (2009)
	NA	Inhibited virus proliferation, adsorption and penetration	Glycyrrhizin treatment in SARS-associated coronavirus in Vero cell culture (FFM-1 and FFM-2)	Cinatl et al. (2003)

MH, Ephedra sinica *Stapf; GC,* Glycyrrhiza glabra *L.; LMEP, L-methylephedrin; LEP, L-ephedrine; DPEP, D-pseudo-ephedrine; MDCK, Madin-Darby canine kidney; A549, human endothelial lung cells; Hfl-1, human lung fibroblast cells.*

Molecular Targets and Mechanism From Published Biological Evaluation

Ma Xing Shi Gan Decoction Inhibits the Adsorption and Replication of Virus

MXSG might directly inhibit the adsorption and replication of SARS-CoV-2 at the viral entry step. LMEP, LEP, DPEP, and (+)-catechin are active ingredients of *Ephedra sinica* Stapf. Glycyrrhizin is an active ingredient of *Glycyrrhiza glabra* L. We summarized the action of MXSG or its active components on influenza and coronavirus to validate our hypothesis (**Table 2**). MXSG or its active ingredients could inhibit both viral adsorption and penetration by inducing disruption of the viral particle or affecting the interacts with the cell membrane. Moreover, they also have a potent inhibitory effect on virus replication.

Ma Xing Shi Gan Decoction Inhibits the Inflammatory Response Through Toll-Like Receptor Signaling Pathway

TLRs are at the interface of innate immune activation in an infected environment by responding to a variety of microorganisms and endogenous ligands (Mollen et al., 2006). MXSG could target TLRs and the inflammatory response triggered by TLRs. Resulting in multiple phenotype changes, such as inhibiting the release of inflammatory cytokines, reduces lung inflammation. Experimental evidence that MXSG or its active ingredients for inhibiting inflammatory lung injury were summarized (**Table 3**).

Validation From *In Vivo* Transcriptome of Lipopolysaccharide-Induced Lung Injury

Differential Expression Analysis

Differential expression analysis revealed a total of 310 differential genes after treatment with MXSG. There were 160 upregulated genes and 150 downregulated genes (p value < 0.05, |log2FC| > 1; **Figures 3A,B**). According to the MA and volcano plot of differentially expressed genes (DEGs) between the MXSG group and the pneumonia group, the upregulated and downregulated DEGs showed significant differences (**Figures 3C,D**). The cluster analysis of the DEG level also showed that the biological function significantly changes after the intervention of MXSG (**Figure 3E**).

Differentially Expressed Genes' KEGG Pathway Enrichment Analysis

DEGs' KEGG pathway enrichment analysis indicated that 20 pathways of the transcription genes in lung tissues were enriched after the intervention of MXSG, including retinol metabolism, steroid hormone biosynthesis, complement and coagulation cascades, chemical carcinogenesis, herpes simplex infection, arachidonic acid metabolism, linoleic acid metabolism, NOD-like receptor signaling pathway, metabolism of xenobiotics by cytochrome P450, influenza A, drug metabolism–cytochrome P450, antigen processing and presentation, primary bile acid biosynthesis, ascorbate and alternate metabolism, PPAR signaling pathway, graft versus host disease, phenylalanine metabolism, pentose and glucuronate interconversions, drug metabolism—other enzymes, and allograft rejection (**Figure 4**). According to the KEGG secondary classification,

TABLE 3 | MXSG or its active ingredients for inhibiting the inflammatory lung injury.

MXSG/ingredient	Target	Mechanism	Methodology	References
MXSG	TNF-α, IL-1β and IL-6↓, TLR4, MyD88, and TRAF6↓	Inhibited TLR4-MyD88-TRAF6 signaling pathway and release of inflammatory cytokines, alleviated the inflammation reaction	MXSG against type A influenza virus infection in WT mice	Li et al. (2017)
	MCP-1↓	Inhibited inflammation reaction	MXSG treatment in type A influenza virus infection in KM mice	Zou et al. (2018)
	TNF-α, IL-1β and IL-6↓, ICAM-1, TLR4, cav-1, Src and NF-κB↓, claudin-5, JAM-1 and occludin↑, p-cav-1↓, and MPO↓	Inhibited the release of inflammatory cytokines and alleviated the inflammation reaction	MXSG posttreatment in LPS-induced male Sprague–Dawley rats ALI	Ma et al. (2014)
	TNF-α, IL-1β and IL-6↓, MPO↓, HMGB1, TLR4, MyD88, and p-p65↓	Inhibited HMGB1/TLR4/NF-κB signaling and release of inflammatory cytokines, and alleviated the inflammation reaction	MXSG treatment in PM2.5 induced male Sprague-Dawley rats ALI	Fei et al. (2019)
Glycyrrhizin	F12, F13b, F9, and AT3	These proteins were involved in the conversion of zymogen to serine protease, affecting the regulation of innate immunity	MXSG treatment in LPS-induced rats ALI	Yang et al. (2020)
	NA	Stimulation of IFN-gamma production by T cells	GL treatment in mice infected with influenza virus A2	Utsunomiya et al. (1997)
	TNF-α, IL-1β, and IL-6↓, TLR4, COX-2, MPO, iNOS, and NF-κB↓	Inhibition of the TLR-4/NF-κB signaling pathway	GL treatment in LPS-induced BALB/c mice ALI	Lee et al. (2019)
	Tlr2↑, MIP-2, KC, IL-4, IL-6, GM-CSF, NF-κB, and IFN-γ↓	Inhibition of the TLR signaling pathway	GL treatment in LPS-induced BALB/c nude mice ALI	Kong et al. (2019)
	TLR2, MyD88, and NF-κB↓	Downregulate TLR2 signaling inhibit I/R-induced inflammatory response	GL could ameliorate I/R induced male BALB/C mice lung injury	Fei et al. (2017)
Licochalcone A	TNF-α, IL-1β, and IL-6↓	Anti-inflammation reaction and alleviated inflammatory lung injury	Lico A treatment in LPS-induced male BALB/c mice ALI	Chu et al. (2012)
LEP, DPEP	IL-1β, TNF-α, TLR3, TLR4, TLR7, MyD88, NF-κB p65, and RIG-1, IFN-γ, and IL-10↓	Adjusting the TLRs and RIG-1 pathways alleviating lung injury	LEP, DPEP treatment in influenza A in male ICR mice	Wei et al. (2019)

GL, glycyrrhizin; LMEP, L-methylephedrin; LEP, L-ephedrine; DPEP, D-pseudo-ephedrine; Lico A, licochalcone A; LPS, lipopolysaccharide; ALI, acute lung injury; TLRs, toll-like receptors; I/R, ischemia–reperfusion.

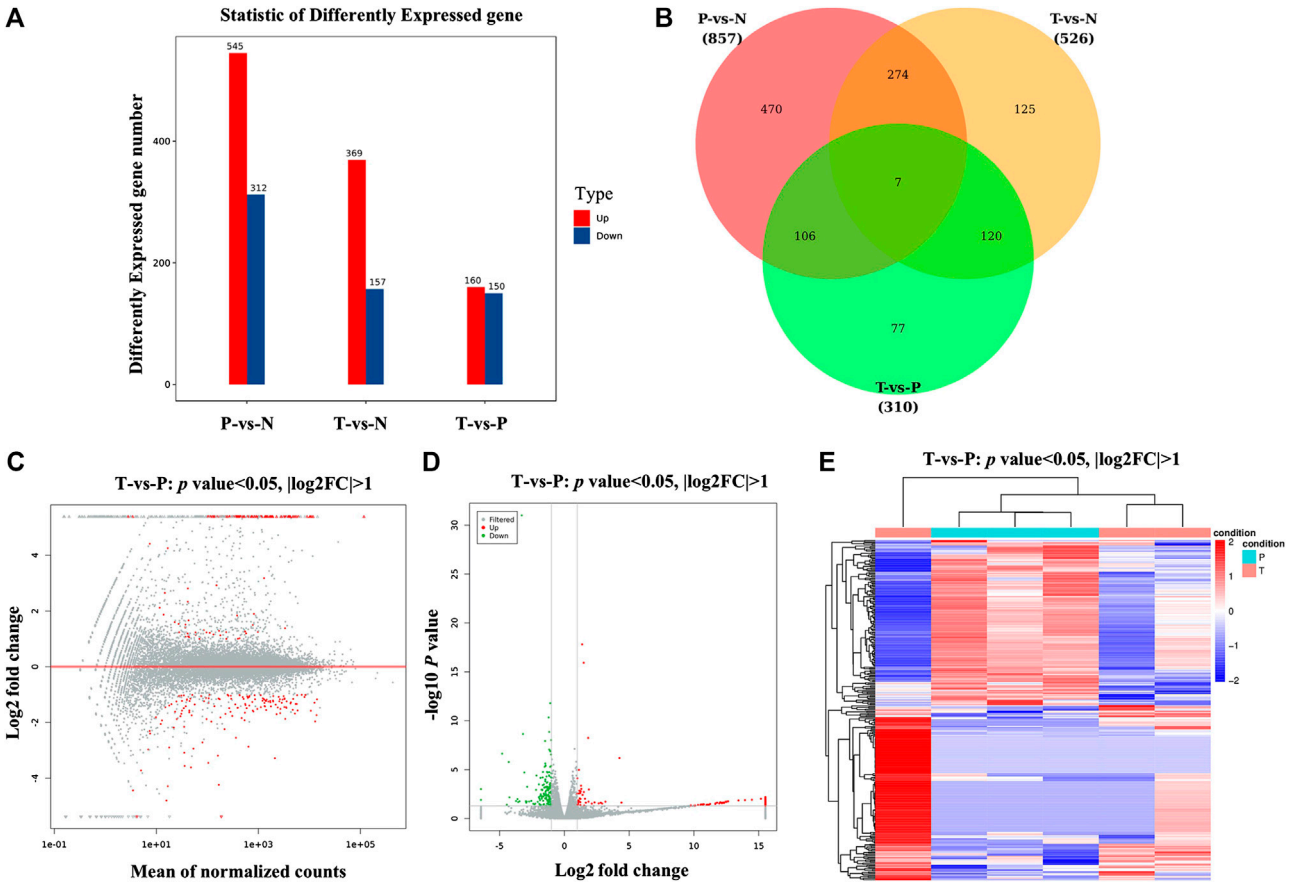


FIGURE 3 | Screening and hierarchical clustering analysis of DEGs. **(A)** Histogram of DEGs in groups. The horizontal axis is the comparison groups. The vertical axis is the number of DEGs in the comparison group, in which Up is the number of significantly upregulated genes and Down is the number of significantly downregulated genes. **(B)** Venn plots of DEGs in groups. **(C)** MA plots of DEGs, the horizontal axis indicated standardized expression mean in all samples, and vertical axis indicated log2 fold change. Red dots indicated significant DEGs. **(D)** Volcano plot of DEGs between MXSG group and pneumonia group. Red and green dots indicated significantly upregulated and downregulated genes. Gray dots indicated nonsignificant DEGs. The horizontal axis indicated where log2 fold change, and vertical axis indicated where –log10 *p* value. **(E)** Clustering analysis of DEGs between MXSG group and pneumonia group. MXSG group and pneumonia group were shown as red and cyan along the horizontal bar, and genes were shown along the vertical bar. Upregulated genes were shown as red and downregulated genes were shown as green. N, normal group; P, pneumonia model group; T, MXSG group.

the genes with more differences were correlated with the endocrine system, immune system, lipid metabolism, metabolism of cofactors and vitamins, and infectious diseases (**Figure 4**).

The Enrichment of Differentially expressed genes in Phosphatidylinositide 3-Kinases/Protein Kinase and Toll-Like Receptor Signaling Pathways

According to the KEGG enrichment pathway, the upregulated genes in the PI3K/Akt signaling pathway include REDD1, ECM, and CREB, downregulated genes. In the toll-like receptor signaling pathway, downregulated genes include IRF7, STAT1, and IP-10, and treatment with MXSG (**Supplementary Figure S1**).

DISCUSSION

TCM theory of MXSG in the treatment of COVID-19. Chinese medical specialist confirms that COVID-19 belongs to the category of epidemic disease in TCM. Dampness toxin pestilence and vacuity of right qi are the main cause. The toxin combining with the dampness pathogen is the main TCM pathogenesis of COVID-19. It also includes cold pathogens and hot pathogens. The pathological evolution of SARS-CoV-2 in TCM can be summarized as pathogenic factors invading defense exterior in early stage, and then influences the lungs and spleen function, finally involves heart, liver and kidney. (Gu et al., 2020; Luo et al., 2020). It will cause some typical lung symptoms, including fever and cough. Besides, a few people also develop symptoms of the spleen, such as diarrhea and fatigue (Fan et al., 2020; Tong et al., 2020) (**Figure 5**). MXSG has a history of 1,800 years, from Treatise on Cold Damage. It is the core prescription of TCM to treat cough and asthma, and has the functions of clearing heat and preventing asthma, dispersing lung, and relieving cough (**Supplementary Table S3**). In this decoction, *Ephedra sinica* Stapf is a warm and dissipating acridity drug, adept in dispersing lung qi, opening the

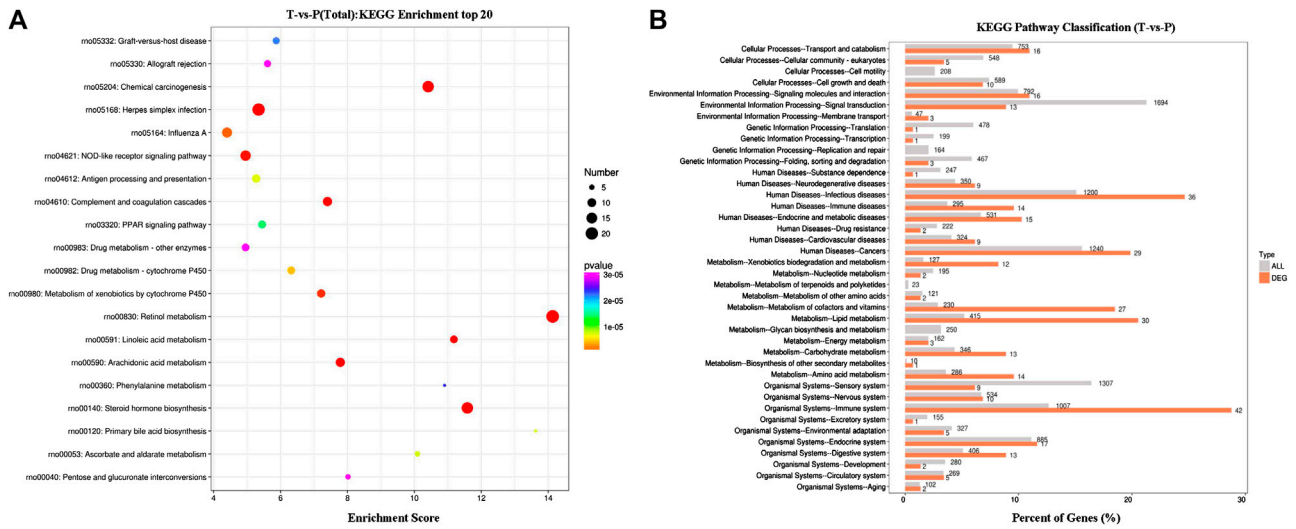


FIGURE 4 | KEGG enrichment results. **(A)** Bubble map of the top20 KEGG pathways. **(B)** Comparison of histogram of KEGG level 2 distribution between DEGs and all gene. The horizontal axis is the ratio (%) of the genes annotated to each level 2 metabolic pathway and the total number of all genes annotated to the KEGG pathway. The vertical axis represents the name of level 2 pathway, and the number to the right of the column represents the number of DEG notes under the level 2 pathway.

interstice structures, effusing wind cold. *Gypsum fibrosum* (calcium sulfate) has a medicinal property to treat cold, good at clearing lung fire, releasing flesh, and abating heat. *Prunus armeniaca* L., as a descending qi with the bitter-warm drug, has the effect of cough-suppressing phlegm transforming. *Glycyrrhiza glabra* L. (licorice) is a harmonizing drug, relaxing the middle and supplementing vacuity, and clearing heat and detoxification. Studies show that MXSG is applied as a basic prescription in the treatment of SARS, H1N1, or MERS, and has achieved satisfactory efficacy (Wang et al., 2011; Li et al., 2013b; Zhang et al., 2013; Zou et al., 2018). In the clinical prevention and treatment of COVID-19 (Li et al., 2020a; Tang et al., 2020), MXSG has the highest frequency in China's the national and provincial prevention and treatment programs and applied to COVID-19 patients' both suspected cases, and confirmed individuals with mild cases, moderate cases, severe cases, and critical cases, and has become the core prescription of pulmonary infection.

The efficacy of MXSG in the treatment of COVID-19. Clinical manifestations of COVID-19 include fever, cough,

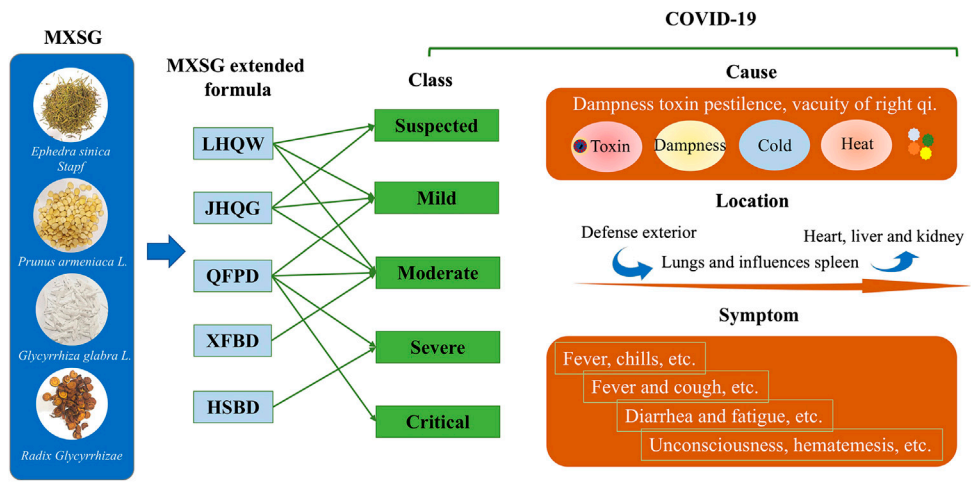


FIGURE 5 | TCM theory of MXSG in the treatment of COVID-19. Information is from the latest guideline for the diagnosis and treatment of COVID-19 issued by the National Health Commission of the People's Republic and the published literatures (Fan et al., 2020; Luo et al., 2020; Tong et al., 2020). The green arrows indicated this drug is recommended for the corresponding phase of COVID-19 treatment in the latest guideline for the diagnosis and treatment of COVID-19.

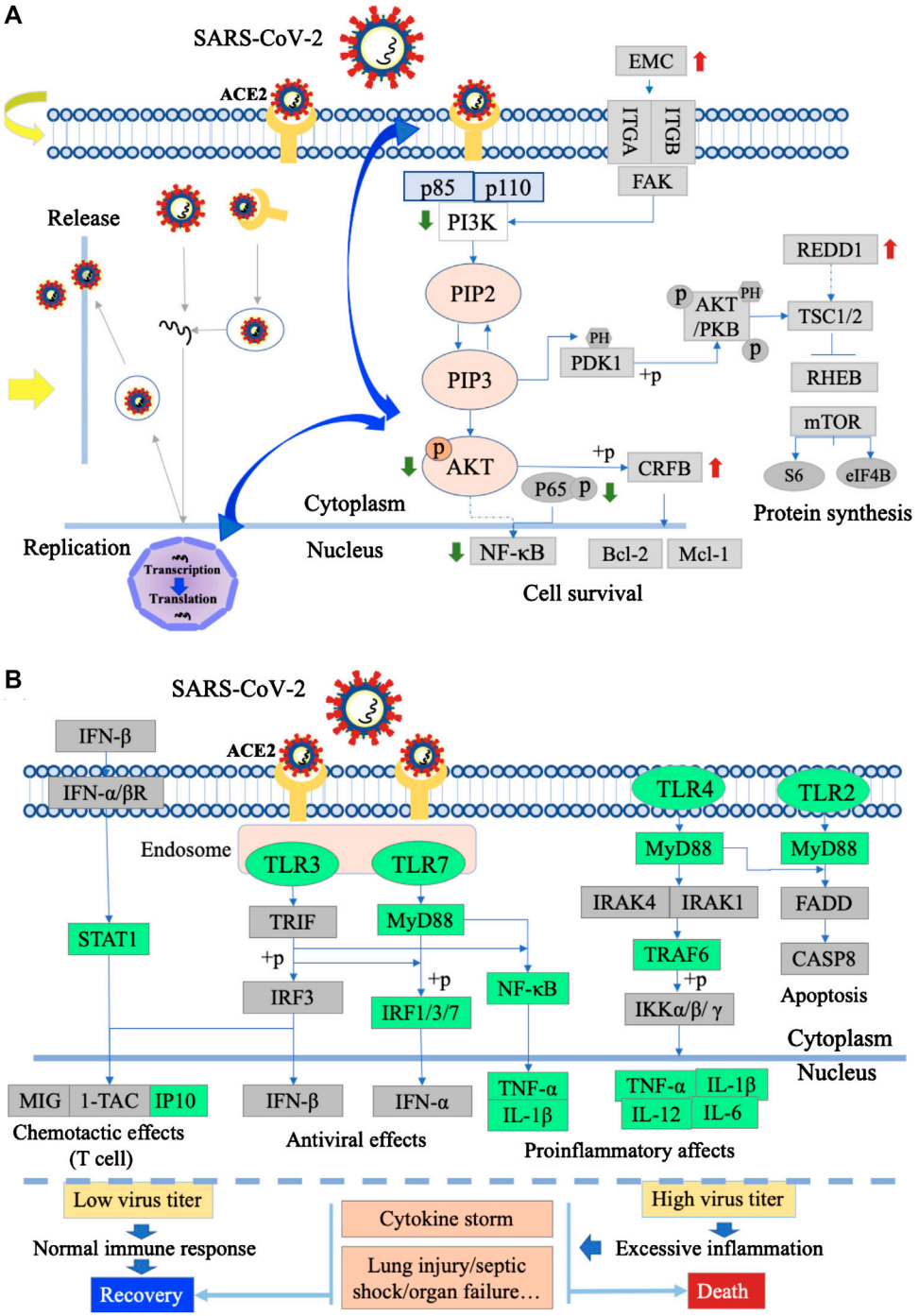


FIGURE 6 | Action mechanism diagram of MXSG. **(A)** SARS-CoV-2 entry, replication, and PI3K/AKT signaling pathway infected with SARS-CoV-2. The green arrows indicated downregulated genes, and the red arrows indicated upregulated genes with MXSG or its ingredients intervention. The yellow arrows indicated MXSG regulates the intrusion and replication of viruses. The blue arrows indicated MXSG might play antiviral effect in regulating the interaction between PI3K/AKT and virus invasion and replication. **(B)** Toll-like receptors signaling pathway infected with the severe/mild SARS-CoV-2. The green box indicated downregulated genes with MXSG or its ingredient intervention.

fatigue, myalgia, diarrhea, normal or decreased white blood cell counts, and radiological evidence of pneumonia (Henry and Vikse, 2020; Li and De Clercq, 2020; Stower, 2020; Xiong et al., 2020). Severe patients usually develop dyspnea and/or hypoxemia 1 week after onset. In severe cases, acute respiratory distress syndrome, sepsis, refractory metabolic

acidosis, coagulation disorders, and multi-organ failure may develop rapidly. These symptoms are similar to those of SARS-COV, H1N1, and MERs-CoV infections. MXSG and the extended formula have been showing the effects of antiviral, ameliorated the clinical symptoms, inhibited progression, regulated immune inflammation, and depressed lung injury in basic and clinical studies (**Table 1**). In particular, the effect of MXSG for improving viral lung injury was close to that of oseltamivir in animal studies (Zhang et al., 2013; Li et al., 2017; Zou et al., 2018). And similarly, JHQG alone and in combination, oseltamivir reduced time to fever resolution in patients with influenza A (H1N1) virus (Wang et al., 2011). Compared with oseltamivir, LHQW also achieved a similar therapeutic effectiveness reduction of the duration of symptoms and viral shedding, and reduced the severity of illness in patients with influenza A (H1N1) virus (Duan et al., 2011). Moreover, in these studies, no noticeable drug-related adverse reactions were found between MXSG and its extension. These show that MXSG is a relatively safe and effective treatment for influenza and viral pneumonia.

The mechanism of MXSG in the treatment of COVID-19. Besides chemical methods and literature surveys, network pharmacology is also an effective way to decipher the effective components and comprehensive information of Chinese medicine (Jiang et al., 2020). Although the web-based pharmacology strategy has the limitations of database itself defects and the uncertainty of active ingredient function prediction (Huang et al., 2020), the strategy will facilitate the mechanistic investigations of these clinically effective TCMs on COVID-19 to some extent (Jiang et al., 2020).

In this study, we analyzed the chemical composition of MXSG using LC-ESI-MS/MS and carried out GO and KEGG enrichment analysis on the targets of its composition. The results showed that MXSG mainly interfered with cellular and metabolic processes. The intervention mainly affects the cell membrane structure, and the main target molecules are involved in the protein binding process and catalytic function. KEGG analysis showed that the central role of MXSG was to interfere with the viral infection–related pathway and the PI3K/AKT signaling pathway (**Figure 2**). In the result of summarization, MXSG and its ingredients can inhibit influenza/coronavirus virus replication and invasion (**Table 2**). Hsieh et al. (2012) reported that MXSG could inhibit the synthesis of both viral RNA and protein, disrupt viral surface structure, and block the virus entry phase. More interestingly, in this study, it was demonstrated that virus intrusion is regulated by the PI3K/AKT signaling pathway, which was inhibited by MXSG. In addition, MH, GC, LMEP, LEP, and DPEP have been reported to prevent virus entry or proliferation (Mantani et al., 1999; Mantani et al., 2001; Cinatl et al., 2003). Thus, MXSG might directly inhibit the adsorption and replication of SARS-CoV-2 at the viral entry step.

MXSG can effectively alleviate inflammatory lung injury. The early immune inflammatory response is essential for virus clearance. Pattern recognition receptors recognize the pathogen-associated molecular patterns. The initiation of the inflammatory response depends on the recognition of pattern recognition receptors. TLRs have been reported, which enables the recognition of influenza viruses by pattern recognition receptors. It can have both protective and detrimental effects during infection. Innate responses are armaments that the host can use to prevent or slow viral replication early in infection (Krammer et al., 2018) and, however, are the cause of severe conditions such as lung damage caused by excessive inflammation (Short et al., 2014; Biondo et al., 2019) (**Figure 6B**). Various studies revealed that MXSG and its ingredients could inhibit inflammation reaction by intervening TLR signaling (**Table 3**). This effect effectively reduced the level of inflammatory cytokines and improved lung injury (Chu et al., 2012; Ma et al., 2014; Fei et al., 2017; Li et al., 2017; Wei et al., 2019). In this study, the regulatory effect of MXSG on inflammatory lung injury was validated through transcriptome results. After MXSG intervention, several genes on the toll-like receptor signaling pathway were found to be significantly altered. Thus, MXSG may inhibit SARS-CoV-2 inflammatory lung injury by regulating the TLR signaling pathway. However, a more precise regulatory mechanism still needs to be demonstrated in future studies.

This study incorporates chemical methods, literature surveys, and network pharmacology ways to decipher the effective components and comprehensive information of MXSG. Through this study, the active components of MXSG were analyzed, the curative effect of MXSG on COVID-19 was proved, and the possible mechanism of MXSG in the treatment of COVID-19 was proposed. Then, transcriptome experiments were used to preliminarily verify the possible mechanism. Undeniably, there are some limitations in this study, including that the proposed mechanism of MXSG has not been effectively verified, and the selection of animal models cannot be fully recognized. Therefore, our research group will carry out further research in the future.

CONCLUSION

In this study, we analyzed the main active components of MXSG and predicted its intervention mechanism. Through literature analysis, it is preliminarily determined that MXSG is an essential prescription for the treatment of COVID-19. It has the curative effect of antivirus, improving clinical symptoms, regulating immune inflammation, and inhibiting lung injury. Further, we found that MXSG might directly inhibit the adsorption and replication of SARS-CoV-2 at the viral entry step. In addition, it may play an anti-inflammatory and immune regulatory role to prevent cytokine storm, relieving lung injury through TLR signaling pathway. However, the specific mechanism of MXSG in the treatment of COVID-19 still needs further research.

MATERIALS AND METHODS

Component Detection, Target Prediction, and Functional Analysis of Ma Xing Shi Gan Decoction

The Pharmacy Department provided all crude drugs of Ma Xing Shi Gan Decoction, Dongfang Hospital Affiliated to Beijing University of Chinese Medicine (Beijing, China). They were purchased from the Beijing Tcmages Pharmaceutical Co., Ltd., (Shunyi district, Beijing, Datong Road). *Ephedra sinica* Stapf [Ephedraceae] (Ma Huang) were collected from the province of Henan, China; *Prunus armeniaca* L. [Rosaceae] (Ku Xing Ren) from the province of Hebei, China; *Glycyrrhiza glabra* L. [Fabaceae] (Gan Cao; licorice) from the province of Ningxia, China; and *Gypsum fibrosum* (Shi Gao; calcium sulfate) from the province of Shanxi, China. The quality of crude drugs was strictly performed according to Good Manufacturing Practice for Drugs to guarantee quality control (Chinese FDA). Furthermore, these species were authenticated by Prof. Xiaohong Gu (Beijing University of Chinese Medicine) before use. Voucher specimens (no. BUCM-LI-2019001 for *Ephedrae herba*; no. BUCM-LI-2019002 for *Armeniacae amarum semen*; no. BUCM-LI-2019003 for *Glycyrrhizae radix preparata*; no. BUCM-LI-2019004 for *Gypsum fibrosum*) were deposited in the School of Traditional Chinese medicine, Beijing University of Chinese Medicine (Beijing, China). The Pharmacy Department provided the test samples of Ma Xing Shi Gan Decoction according to the prescription proportion of "Treatise on Cold Damage" (*Ephedra sinica* Stapf:*Prunus armeniaca* L.:*Glycyrrhiza glabra* L.:*Gypsum fibrosum* = 2:1.5:1:4), Dongfang Hospital Affiliated to Beijing University of Chinese Medicine (Beijing, China). A method of decoction was used to extract the herb, and then the extracts were concentrated and dried to form granules (Dongfang Hospital Affiliated to Beijing University of Chinese Medicine). The characteristics of each component in MXSG, based on traditional prescription theory, were shown in **Supplementary Table S4**.

The reagents methanol, ethanol, and acetonitrile of HPLC grade were provided by Merck Chemicals (Darmstadt, Germany). Standard (DMSO) of HPLC grade was provided from BioBioPha (http://www.biobiopha.com/) and Sigma-Aldrich (St. Louis, MO, United States). A Milli-Q system (Millipore Corp, Millipore, MA, United States) was used to provide ultrapure water.

The freeze-dried MXSG was crushed using a mixer mill (MM 400, Retsch) with a zirconia bead for 1.5 min at 30 Hz. 100 mg powder was weighted and extracted overnight at 4°C with 1.0 ml 70% aqueous methanol. Following centrifugation at 10,000g for 10 min, the extracts were absorbed (CNWBOND Carbon-GCB SPE Cartridge, 250 mg, 3 ml; ANPEL, Shanghai, China, www.anpel.com.cn/cnw) and filtrated (SCAA-104, 0.22 μm pore size; ANPEL, Shanghai, China, http://www.anpel.com.cn/) before LC-ESI-MS/MS analysis.

The sample extracts were analyzed using an LC-ESI-MS/MS system (HPLC, Shim-pack UFLC SHIMADZU CBM30A system, www.shimadzu.com.cn/; MS, Applied Biosystems 6500 Q TRAP, www.appliedbiosystems.com.cn/). The analytical conditions were as follows, HPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm × 100 mm); solvent system, water (0.04% acetic acid): acetonitrile (0.04% acetic acid); gradient program, 100:0V/V at 0 min, 5:95V/V at 11.0 min, 5:95V/V at 12.0 min, 95:5V/V at 12.1 min, 95:5V/V at 15.0 min; flow rate, 0.40 ml/min; temperature, 40°C; injection volume: 2 μL. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (Q TRAP)-MS.

LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (Q TRAP), API 6500 Q TRAP LC/MS/MS system, equipped with an ESI Turbo Ion-Spray interface, operating in a positive ion mode and controlled by Analyst 1.6.3 software (AB Sciex). The ESI source operation parameters were as follows: an ion source, turbo spray; source temperature 500°C; ion spray voltage (IS) 5,500 V; ion source gas I (GSI), gas II (GSII), curtain gas (CUR) was set at 55, 60, and 25.0 psi, respectively; the collision gas (CAD) was high. Instrument tuning and mass calibration were performed with 10 and 100 μmol/L polypropylene glycol solutions in QQQ and LIT modes, respectively. QQQ scans were acquired as MRM experiments with collision gas (nitrogen) set to 5 psi. DP and CE for individual MRM transitions were done with further DP and CE optimization. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.

Based on the self-established MetWare database and the common database of metabolite information, qualitative analysis was conducted on the primary and secondary spectrum data of mass spectrometry. In the qualitative analysis of some substances, isotopic signals, repeated signals containing K^+ ions, Na^+ ions, and NH_4^+ ions, as well as repeated signals of fragments of other substances with larger molecular weight, are removed. Metabolite structure analytical reference MassBank (http://www.massbank.jp/), KNAPSAcK (http://kanaya.naist.jp/KNApSAcK/), HMDB (http://www.hmdb.ca/), MoTo DB (http://www.ab.wur.nl/moto/), and METLIN (http://metlin.scripps.edu/index.php), and other existing mass spectrometry public database.

Retrieval of the compounds detected targets from the Symmap database (http://www.symmap.org) and TCMSP database (http://lsp.nwsuaf.edu.cn/tcmsp.php). Then, target genes were uploaded to the Metascape platform (http://metascape.org/gp/index.html) for GO analysis and then uploaded to the DAVID platform (https://david.ncifcrf.gov/) for KEGG analysis. The results are shown by bar and bubble diagrams.

Therapeutic Effects of Ma Xing Shi Gan Decoction

Information of MXSG or its extended formula is from corresponding drug labels and the latest guideline for the diagnosis and treatment of COVID-19 issued by the National Health Commission of the People's Republic. Therapeutic effects of MXSG or its extended formula, and potential action mechanisms of MXSG and its ingredients for COVID-19 were summarized from published evaluation in PubMed (https://pubmed.ncbi.nlm.nih.gov/). Since some of the research was published only in Chinese, we also added a few published evaluations from CNKI (https://www.cnki.net). Since the symptoms of COVID-19 and H1N1 are highly similar, the therapeutic effects of MXSG or its extended formula were summarized to include the treatment of H1N1.

In Vivo Transcriptome Experiment of Ma Xing Shi Gan Decoction

Eight- or nine-week-old male SD rats (110 g ± 10 g) were purchased from SPF (Beijing) Biotechnology Company. Experimental animals were maintained under specific pathogen-free conditions according to agency guidelines. The rats were kept with a 12-h light/dark cycle and with access to water and food *ad libitum*. The experimental procedures were approved by the Ethical Committee on Animal Research at the Beijing University of Chinese Medicine (BUCM-4-2019082701-3040) and conducted following the Guide for the Care and Use of Laboratory Animals established by the US National Institutes of Health. After 3 days of adaptive breeding, 30 rats were randomly divided into three groups (n = 10): normal group, pneumonia model group, and MXSG group. Next, the pneumonia model group and MXSG group were received, given 0.5 mg/ml LPS nebulization intervention, 30 min per day for three consecutive days. After 3 days, the MXSG group were intragastrically administered of MXSG once a day for 3 consecutive days (according to clinical guidelines, all doses were converted according to the equivalent dose of 0.018 for human and rat). The model group and the control group received an equal volume of saline accordingly. Rats were sacrificed after drug treatment on the condition of free drinking but without food for 12 h, then anesthetized with 10% chloral hydrate. Lung was collected for quick freezing. Furthermore, the samples were stored at −80°C refrigerator.

RNeasy Mini-Kit (QIAGEN, Valencia, CA) was used to simultaneously extract total RNA from the lungs of three groups of rats (normal, model, and MXSG). Later, DNA was digested using DNase and enriched with Oligo (dT) magnetic beads. Then, the destruction reagent was added to decompose the mRNA into short fragments, using the destroyed mRNA as a template and randomly using six bases. Primers synthesized single-stranded cDNA, and then the double-stranded reaction system was prepared to synthesize double-stranded cDNA and purify double-stranded cDNA. The purified double-stranded cDNA was terminal repaired, a tail was added, attached to the sequencing adapter, and finally, PCR amplification was performed. After the library was detected by Agilent 2100 Bioanalyzer, Illumina HiSeq™ two sequencer was used to sequence the library, and 125 bp or 150 bp double-ended data were generated. After quality inspection, Illumina sequencer was used for sequencing, and bioinformatics analysis was conducted according to the sequencing results.

DESeq software was used to standardize the counts of each sample gene (the basement value is used to estimate the expression quantity) and calculate the multiple difference. NB (negative binomial distribution test) was used to test the different significance of read numbers. Finally, significantly, DEGs were screened according to the difference in multiple and different significance test results. p value < 0.05 and fold change > 2 or fold change < 0.5 was set as the selection condition. Pathway analysis of differential expression was performed using the KEGG database (combined with KEGG annotation results), and the hypergeometric distribution test was used to calculate the significance of enrichment of DEGs in each pathway entry. The formula for calculating p value and enrichment fraction by hypergeometric distribution test is as follows:

$$P = 1 - \sum_{i=1}^{m-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}.$$

$$\text{Enrichment score} = \frac{m/n}{M/N},$$

where N is the number of KEGG annotated genes in all genes, n is the number of genes with KEGG annotation in DEGs in N, M is the number of genes annotated with specific KEGG pathways in all genes, and m is the number of genes differentially expressed by specific KEGG pathways.

DATA AVAILABILITY STATEMENT

The RNA seq data generated in this study have been submitted to the NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?accGSE158832) under accession number GSE158832.

ETHICS STATEMENT

The animal study was reviewed and approved by the Experimental Animal Health Ethics Committee of Beijing University of Chinese Medicine.

AUTHOR CONTRIBUTIONS

XG, JC, CB, QL, and TL conceived and designed the experiments. CB, RY, WX, and SL performed the experiments. CB, QL, and XP analyzed and interpreted the data. QL organized the original draft. XG, CB, and TL edited and reviewed the article. All authors have read, revised, and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.581691/full#supplementary-material

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Inhibition of SARS-CoV-2 by Highly Potent Broad-Spectrum Anti-Coronaviral Tylophorine-Based Derivatives

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Tylophorine-based compounds and natural cardiotonic steroids (cardenolides and bufadienolides) are two classes of transmissible gastroenteritis coronavirus inhibitors, targeting viral RNA and host cell factors, respectively. We tested both types of compounds against two types of coronaviruses, to compare and contrast their antiviral properties, and with view to their further therapeutic development. Examples of both types of compounds potently inhibited the replication of both feline infectious peritonitis virus and human coronavirus OC43 with EC_{50} values of up to 8 and 16 nM, respectively. Strikingly, the tylophorine-based compounds tested inhibited viral yields of HCoV-OC43 to a much greater extent (7–8 log magnitudes of p.f.u./ml) than the cardiotonic steroids (about 2–3 log magnitudes of p.f.u./ml), as determined by end point assays. Based on these results, three tylophorine-based compounds were further examined for their anti-viral activities on two other human coronaviruses, HCoV-229E and SARS-CoV-2. These three tylophorine-based compounds inhibited HCoV-229E with EC_{50} values of up to 6.5 nM, inhibited viral yields of HCoV-229E by 6–7 log magnitudes of p.f.u./ml, and were also found to inhibit SARS-CoV-2 with EC_{50} values of up to 2.5–14 nM. In conclusion, tylophorine-based compounds are potent, broad-spectrum inhibitors of coronaviruses including SARS-CoV-2, and could be used for the treatment of COVID-19.

Keywords: HCoV-OC43, HCoV-229E, FIPV, tylophorine, SARS-CoV-2, ouabain, COVID-19, coronavirus

INTRODUCTION

COVID-19, the disease caused by infection with SARS-CoV-2, has now spread to more than 187 countries, areas or territories; infected 26,011,421 people; and caused 863,966 deaths with a global mortality rate of 3.32% (as of September 03, 2020, https://www.cdc.gov.tw/) since the first reports of patient cases emerged from WuHan, China in December 2019 (Ali and Alharbi, 2020; Velavan and

Meyer, 2020). COVID-19 has been the subject of frenzied research activity, and a handful of potential treatments have now been identified, including novel drugs such as remdesivir (Amirian and Levy, 2020), and repurposed older ones such as ciclesonide (Iwabuchi et al., 2020), chloroquine (Gao et al., 2020; Touret and de Lamballerie, 2020), and the combination of hydroxychloroquine and azithromycin (Gautret et al., 2020a; Gautret et al., 2020b). However, there remains an urgent unmet need for additional therapies, especially given case reports of COVID-19 patients who recovered from the disease only to test positive again—perhaps due to their discontinuation of antiviral drugs (Wu et al., 2020); or their viral load being below the threshold of detection and/or restricted to particular tissues, and therefore difficult to detect with regular diagnostic methods (Hoang et al., 2020; Kang et al., 2020; Long et al., 2020). Accordingly, drugs that directly target the SARS-CoV-2 virus and diminish the patient's viral load alone or in combination with other drugs should be further pursued, in addition to those designed to mitigate the symptoms of COVID-19.

In previous work, we established the potent anti-coronaviral activity of tylophorine-based compounds against transmissible gastroenteritis virus (TGEV), severe acute respiration syndrome coronavirus (SARS-CoV), or murine hepatitis virus (MHV) (Yang et al., 2010; Lee et al., 2012; Yang et al., 2017a); and cardiotonic steroids (cardenolides and bufadienolides), against TGEV but not MHV (Yang et al., 2017a). The underlying mechanism of action for these two classes of antiviral compounds are different: tylophorine based compounds target the viral ribonucleoprotein complex (Yang et al., 2017b), whereas cardiac steroids interfere with host factors via either augmenting PI3K_PDK1 signaling or downregulating JAK1 (Yang et al., 2017a; Yang et al., 2018; Yang et al., 2020a).

Here, we tested these tylophorine-based compounds and cardiotonic steroids against a wider variety of coronaviruses (feline inflammatory peritonitis virus (FIPV) and the human coronaviruses HCoV-OC43, HCoV-229E, and SARS-CoV-2) and assessed their effect on viral yields/load by end point and TCID50 assays (Reed and Muench, 1938), which determine the infectious titer over a period of 4 (for HCoV-229E) or 6 (for HCoV-OC43) days. All of the compounds tested were found to be highly potent inhibitors of HCoV-OC43 (low EC_{50} values at low nM concentration ranges), as measured by IFA against viral nucleocapsid protein, but their safety indices and the extent to which they diminished viral yields were more variable. Finally, we demonstrated that the tylophorine-based compounds are highly potent inhibitors of SARS-CoV-2, and therefore may be of merit as treatments for COVID-19.

MATERIALS AND METHODS

Chemicals and Antibodies for IFA Analyses

DMSO (≧99.5%), digoxin (D6003, ≧95%, HPLC), digitoxin (D5878, ≧92%, HPLC), digitoxigenin (D9404, 99%, TLC), ouabain (O3125, ≧95%, HPLC), oleandrin (O9640, ≧98%, HPLC), crystal violet (C0775, dye content ≥90%), and methylcellulose (#M0387) were purchased from Sigma-Aldrich (St. Louis, MO, United States); bufalin (15725, ≧ 98%, HPLC) from Cayman Chemical (Ann Arbor, MI, United States); digoxin-BSA (80-ID10, HPLC) from Fitzgerald Industries (Acton, MA, United States); rostafuroxin (T2621, ≧99%, HPLC) from Target Molecule Corp. (Boston, MA, United States); istaroxime hydrochloride (HY-15718A, ≧99%, HPLC) from MedChem Express (Monmouth Junction, NJ, United States); and Remdesivir (GS-5734) (S8932, 99.3%, HPLC) and GS-441524 (S6814, 99.3%, HPLC) were from Selleckchem (Houston, TX, United States). The antibody against nucleocapsid proteins of HCoV-OC43 (Mab9013) was purchased from Merck Millipore (Burlington, MA, United States), and fluorescein isothiocyanate (FITC)-conjugated anti-mouse immunoglobulin (#55499) from MP Biomedicals (Irvine, CA, United States). Anti-SARS-CoV-2 N protein antibodies were provided by Dr. An-Suei Yang of the Genomics Research Center, Academia Sinica. Goat anti-human IgG-Alexa Fluor 488 (A11013) and DAPI (D1306) were purchased from Invitrogen. 10% formaldehyde solution was purchased from Marcon™ Chemicals (#H121-08).

Tylophorine Based Compounds

dbq33b (99.0%, HPLC), dbq33b4p7 (99.4%, HPLC), PI09 (95.5%, HPLC), PQ09 (95.5%, HPLC), dbq29a (98.0%, HPLC) and 031p13-3 (>95%, TLC) were synthesized as previously reported (Yang et al., 2010; Lee et al., 2012) with some modifications which will be published elsewhere. NMR data of all these compounds has been previously reported (Yang et al., 2010; Lee et al., 2012) except those of 031p13-3 and dbq33b4p7, which are disclosed below.

031p13-3

Yellow crystal; ^{1}H-NMR (300 MHz, $CDCl_3$): 2.01–2.04 (1H, m), 2.20–2.26 (2H, m), 3.16 (1H,d, J = 15.0 Hz), 3.30–3.39 (1H, m), 3.45–3.54 (2H, m), 3.71–3.81 (2H, m), 3.93 (3H, s), 3.97 (3H, s), 3.98 (3H, s), 4.06 (3H, s), 4.09 (3H, s), 4.85 (1H, d, J = 15.3 Hz), 5.35 (1H, d, J = 15.0 Hz), 6.87 (1H, s), 7.08 (1H, s), 9.16 (1H, s). ESI-MS m/z 440 $(M + H)^+$.

dbq33b4p7

White crystal; ^{1}H-NMR (400 MHz, $CDCl_3$): 1.01 (3H, t, J = 7.2 Hz), 1.41 (3H, d, J = 6.4 Hz), 2.52 (1H,qd, J = 6.4, 4.4 Hz), 2.78 (2H,qd, J = 7.2, 4.4 Hz), 3.12 (1H,d, J = 15.2 Hz), 3.28 (1H,d, J = 15.2 Hz), 4.66 (1H, s), 6.26 (1H, s), 7.21 (1H,dd, J = 9.2, 2.4 Hz), 7.06 (1H, s), 7.73 (1H,d, J = 2.4 Hz), 8.29 (1H,d, J = 2.4 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$): 9.3, 15.8, 46.1, 51.3, 55.5, 55.7, 55.8, 57.3, 68.4, 102.8, 103.3, 104.4, 114.8, 123.9, 124.1, 124.6, 126.4, 128.7, 130.5, 148.6, 148.9, 157.6. LRMS (EI^+) m/z (rel intensity) 381 (M^+, 14%) and 310 (100%). HRMS calcd for $C_{23}H_{27}NO_4$ (M^+) 381.1940; found, 381.1930.

Cells, Viruses, Immunofluorescent Assay, Cytopathic Effect, Plaque Assay and Cytotoxicity Assay

Felis catus whole fetus-4 (Fcwf-4) cells (ATCC®CRL-2787) were maintained in Dulbecco's modified Eagle's medium (DMEM, Hyclone Laboratories, Logan, UT, United States) containing 10% fetal bovine serum (FBS) with 1% penicillin/streptomycin at 37°C

with 5% CO_2. The serotype II FIPV Taiwan isolate NTU156 strain, a kind gift from National Taiwan University, was propagated and titrated in Fcwf-4 cells (Lin et al., 2009). The EC_{50} for anti-FIPV activity and CC_{50} for cell cytotoxicity were determined as previously described (Yang et al., 2020b).

Human colon adenocarcinoma cell line HCT-8 (ATCC® CCL-244™) was obtained from American Type Culture Collection (ATCC) and passaged within 6 months of receipt. It was established as stock in the cell bank at an early passage, to ensure cell line-specific characteristics. HCoV-OC43 (ATCC® VR1558™) was grown and propagated in HCT-8 cells cultured with DMEM and 2% FBS. For compound treatment studies, cells were seeded in 96-well plates and then cultured in DMEM medium containing 2% FBS. Cells were pretreated with compounds each in a series of five concentrations at five-fold dilution for 1 h prior to HCoV-OC43 infection at an MOI of 0.05. The supernatants at 72 h.p.i. from OC43 infected HCT-8 cells treated with the test compounds were subjected to the end-point assay and TCID50 determination at 6 d.p.i. to measure the viral-yield inhibition of each treatment. This procedure was performed to quantify how much infectious virus was present in a preparation. The resultant adherent cells (72 h.p.i.) were then fixed with 80% acetone and subjected to IFA analyses with an antibody against OC43 N protein and the EC_{50} values determined as described (Yang et al., 2020b). The viabilities of HCT-8 cells culture in media containing 10% FBS treated with compounds each in a series of eight concentrations at two-fold dilution for 72 h were determined using the CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay kit (MTS) (Promega, Madison, WI, United States); CC_{50} values were determined as previously described (Yang et al., 2020b). In addition, for visualization of cytopathic effects of HCoV-OC43 on HCT-8 cells infected at an MOI of 0.05, the cells were fixed by 80% acetone at 6 d.p.i. and stained with crystal violet.

Human lung fibroblasts cells, MRC-5 (ATCC® CCL-171™) were cultured in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum and penicillin/streptomycin at 37°C with 5% CO_2. HCoV-229E (ATCC® VR-740™) were grown and propagated in MRC-5 cells cultured with MEM and 2% FBS. For the compound treatment studies, cells were then cultured in MEM medium containing 2% FBS. MRC-5 cells were seeded the day before compound treatment and HCoV-229E infection. The tested compounds were added to the wells 1 h prior to the addition of HCoV-229E at an MOI of 0.05. At 4 d.p.i., the resultant supernatants were subjected to the end point assay and TCID50 determination at 5 d.p.i.; the remaining cells (at 4 d.p.i.) were stained by crystal violet for visualization of cytopathic effects prior to being dissolved in 100% MeOH for quantification of the absorbance at 560 nm. The viabilities of MRC-5 cells (cultured in medium containing 10% FBS) and CC_{50} values were assayed and determined as for the MRC-5 cells (*vide supra*).

End Point Dilution Assay for Determining Virus Titers

The end point dilution experiment was performed as previously described (Yang et al., 2017a). Viral titers of the supernatants obtained from the culture of HCoV-OC43 infected HCT-8 cells or HCoV-229E infected MRC-5 (MOI of 0.05) treated with the indicated compounds or vehicle DMSO were determined using an end point dilution assay. The Reed Muench method was used to determine TCID50 (Tissue Culture Infective Dose). The virus titer was then calculated as 1 TCID = 0.69 PFU (Plaque-Forming Units) (Reed and Muench, 1938).

mRNA Isolation and RT-qPCR

These experiments were performed as described (Yang et al., 2007b). Mock or HCoV-229E infected (MOI of 1) lysates were processed and prepared at 30 h.p.i. without or with compound treatment. The total RNA was extracted with TRIzol reagent (Invitrogen). The relative viral RNA expression levels were determined by semi-RT-qPCR, analyzed with the ImageJ Analyzer program (http://imagej.nih.gov/ij/index.html), and normalized with the housekeeping gene GAPDH. The primers used to amplify the PCR products were 5′-GGCGAGGTGGAATTTGTTTA-3′ and 5′-ACCTTTAAGCCA CCATGTGC-3′ for ORF1; 5′-TCTGCCAAGAGTCTTGCTCG-3′ and 5′-AGCATAGCAGCTGTTGACGG-3′ for ORF N. The amplification of target cDNA was conducted under the following conditions: 25 cycles of 95°C for 30 s, 55°C for 20 s, and 72°C for 30 s for ORF N; 29 cycles of 95°C for 30 s, 55°C for 20 s, and 72°C for 30 s for ORF1, and 35 cycles of 95°C for 30 s, 55°C for 20 s, and 72°C for 30 s for GAPDH. The final PCR products were subjected to electrophoresis on 2% agarose gel containing ethidium bromide along with DNA markers.

CPE and IFA for SARS-CoV-2

CPE was performed as described (Wu et al., 2004). For IFA, Vero E6 cells (BCRC number: 60476; derived from ATCC CRL-1586) were treated with each compound at the indicated concentration for 1 h at 37°C. The cells were adsorbed with SARS-CoV-2 (TCDC#4) (sequence available on the GISAID website) at an MOI = 0.01 for 1 h at 37°C. After virus adsorption, the cells were washed with PBS and fresh medium containing each compound at the indicated concentration was added. After 2 days, cells were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. The cells were stained with anti-SARS-CoV-2 N protein antibody, provided by Dr. An-Suei Yang of the Genomics Research Center, Academia Sinica, and goat anti-human IgG-Alexa Fluor 488 (A11013, Invitrogen) (in green). The nuclei were counter stained with DAPI (in blue) (D1306, Invitrogen). The N protein expression was measured using a high-content image analysis system (Molecular Devices). The cell viability was determined by Cell Counting Kit-8 (CCK-8) (Sigma-Aldrich, cat #96992). EC_{50} and CC_{50} values were calculated by Prism software.

Plaque Assay for SARS-CoV-2

A plaque assay was performed in triplicate in 24-well tissue culture plates. The Vero E6 cells were seeded in DMEM with 10% FBS and antibiotics 1 day before infection. SARS-CoV-2 was added to the cell monolayer for 1 h at 37°C. Subsequently, viruses were removed and the cell monolayer was washed once with PBS before covering with media containing 1% methylcellulose (Sigma, cat #M0387) and the test compounds at the indicated concentrations. After 5–7 days, cells were fixed with 10% formaldehyde solution (Marcon™ Chemicals, cat #H121-08)

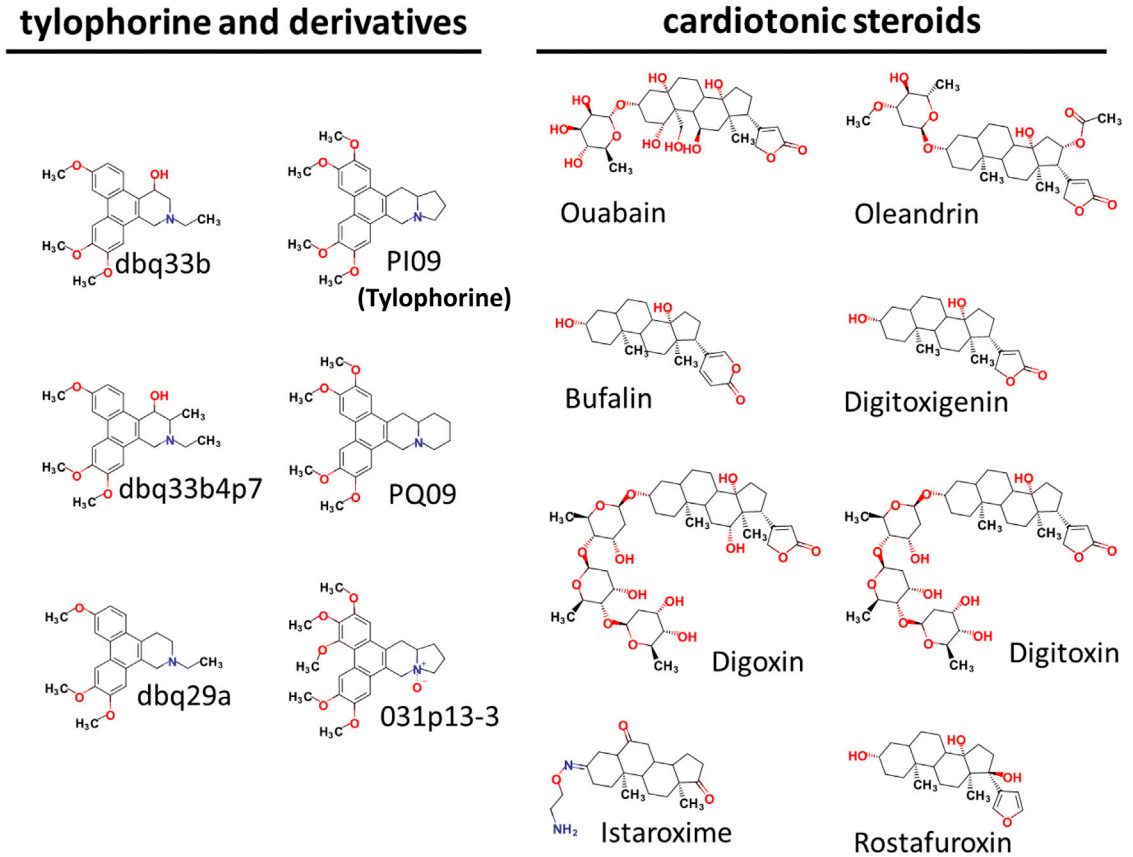

FIGURE 1 | Chemical Structures of tylophorine-based compounds and cardiotonic steroids.

TABLE 1 | The inhibitory activities of tylophorine-based compounds and cardenolides against FIPV and HCoV-OC43.

Compound name	Feline infectious peritonitis virus[a]			Human coronavirus OC43[a]		
	EC_{50} (nM)[b] CPE/visualization	CC_{50} (nM)[c] Visualization	Selectivity index	EC_{50} (nM)[b] (IFA)	CC_{50} (nM)[c] (MTS)	Selectivity index
dbq33b	8 ± 2	3,125 ± 1,083	390.6	16 ± 5	>10,000	>610
dbq33b4p7	20 ± 7	6250 ± 2165	312.5	56 ± 6	>10,000	>179
PI09	62 ± 25	6375 ± 2250	102.8	68 ± 3	>10,000	>147
PQ09	17 ± 6	3094 ± 886	182.0	52 ± 6	>10,000	>193
dbq29a	352 ± 77	4313 ± 1125	12.3	213 ± 62	>10,000	>46.9
031p13-3	1,556 ± 638	>50,000	>32.1	1892 ± 228	>10,000	>5.3
Ouabain	1,556 ± 638	6250 ± 2165	4.0	71 ± 7	504 ± 11	7.1
Bufalin	19 ± 9	542 ± 213	28.5	56 ± 4	900 ± 180	16.1
Digoxin	212 ± 96	1188 ± 603	5.6	269 ± 45	1396 ± 350	5.2
Digoxin-BSA	115 ± 36	669 ± 242	5.8	61 ± 1	461 ± 129	10.0
Digitoxin	97 ± 6	825 ± 195	8.5	88 ± 16	909 ± 123	10.3
Digitoxigenin	458 ± 18	3563 ± 375	7.8	323 ± 20	>2500	>7.7
Istaroxime	2,156 ± 563	7125 ± 750	3.3	5416 ± 268	32,050 ± 1484	5.9
Oleandrin	729 ± 244	2847 ± 1069	3.9	56 ± 5	211 ± 62	3.8
Remdesivir[d]	ND[e]	ND	ND	947 ± 320	>50,000	>52.8
GS441524[d]	ND	ND	ND	6,929 ± 430	>50,000	>7.2

[a]*Data are means ± SD from three rounds of experiments, each in triplicate (FIPV); and means ± SD from three independent experiments, each in duplicate (HCoV-OC43).*
[b]*EC50: The values of 50% maximal effective concentration.*
[c]*CC50: The values of 50% maximal cytotoxic concentration.*
[d]*Remdesivir used as a control reference compound.*
[e]*ND, not determined.*

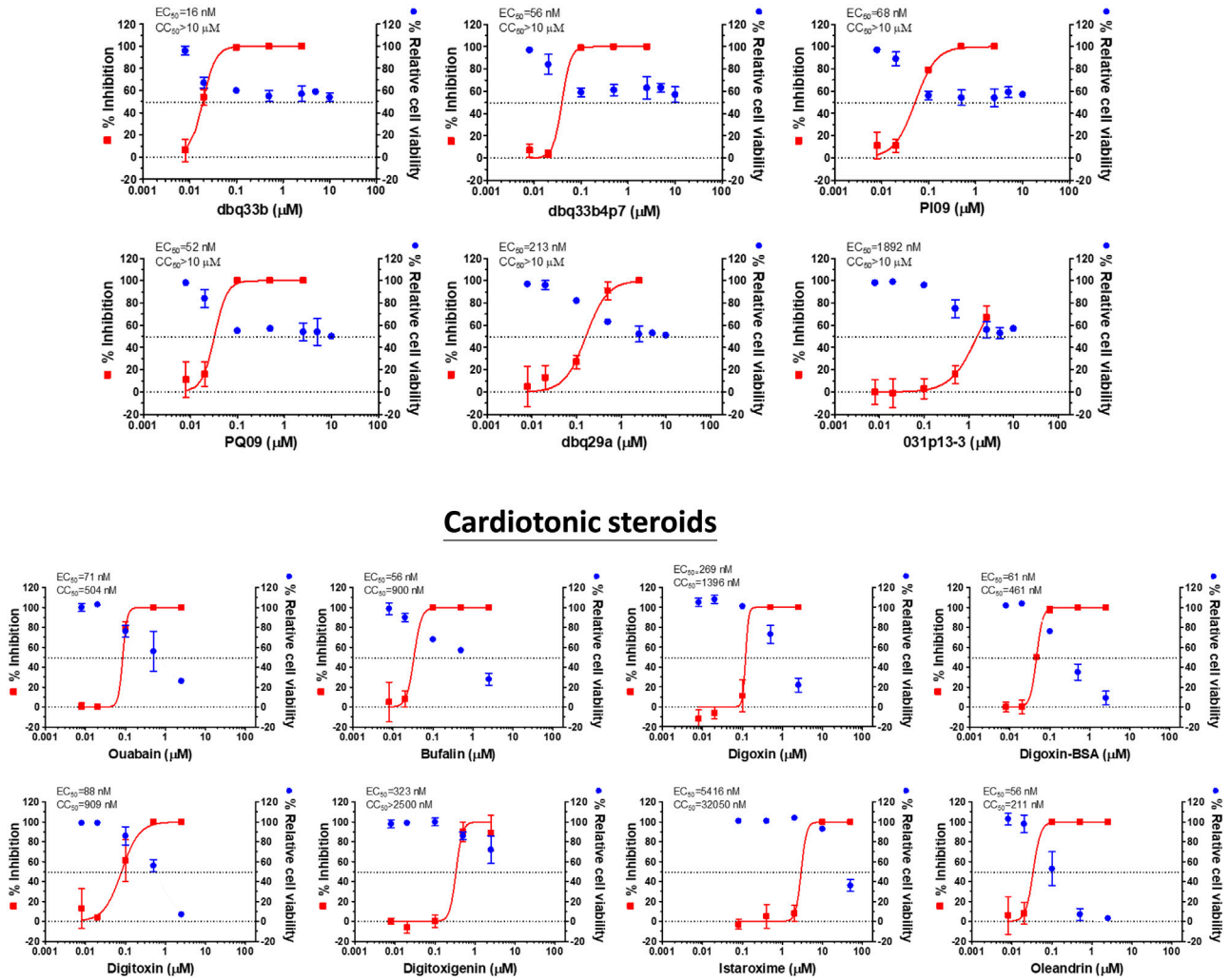

FIGURE 2 | Dose response curves of tylophorine-based compounds and cardiotonic steroids on the viral inhibition in HCoV-OC43 infected HCT-8 and cell viability.

overnight. After removal of overlay media, the cells were stained with crystal violet and the plaques were counted. The percentage of inhibition was calculated as [1 – (VD/VC)] × 100%, where VD and VC refer to the virus titer in the presence and absence of the test compound, respectively.

RESULTS

The Tylophorine-Based Compounds and Cardenolides Tested Were Potent Inhibitors of FIPV and HCoV-OC43 Replication

Tylophorine-based compounds have a plethora of biological activities including anti-inflammatory, anti-cancer, and anti-viral activities (Yang et al., Yang et al., 2006; Yang et al., 2007a; Wu et al., 2009; Yang et al., 2010; Lee et al., 2011; Lee et al., 2012; Yang et al., 2013; Qiu et al., 2015; Yang et al., 2017b; Lee et al., 2020). Cardenolides and bufadienolides are steroids incorporating a five- or six-membered lactone ring, and are best known for their inhibition of the Na^+/K^+ ATPase (Agrawal et al., 2012; Yang et al., 2017a), although they have a multitude of other biological activities as well, and may be useful for treating cardiac arrhythmias and human cancers, and reducing viral production (Prassas and Diamandis, 2008; Su et al., 2008; Diederich et al., 2017; Yang et al., 2017a). We identified six tylophorine-based compounds and eight cardiotonic steroids (seven cardenolides and one bufadienolide) (**Figure 1**. Chemical structures), and assessed their inhibition of FIPV (*alphacoronavirus*) by visual

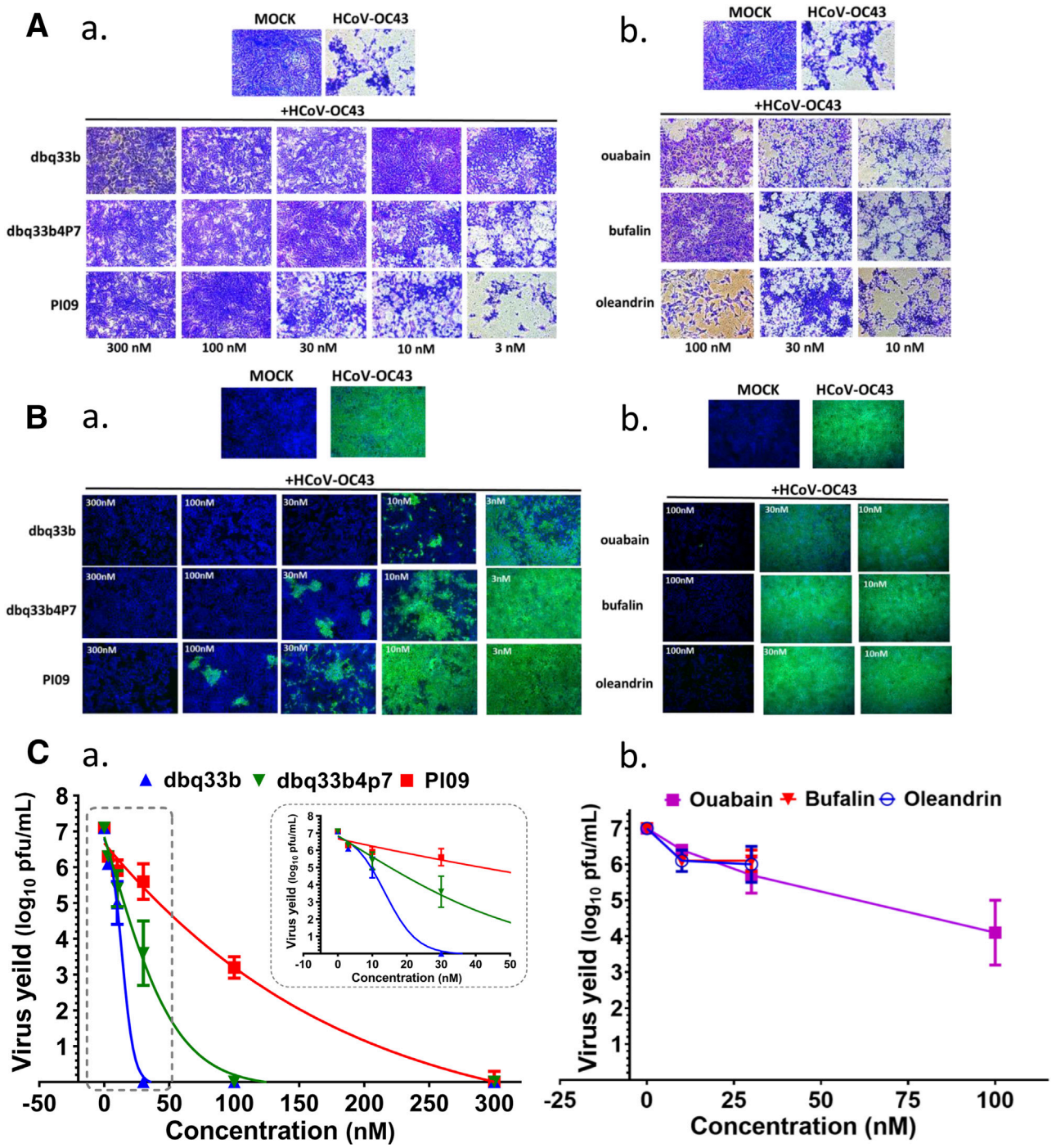

FIGURE 3 | Anti-HCoV-OC43 activities of tylophorine-based compounds and cardenolides. **(A)** HCoV-OC43-induced cytopathy in HCT-8 cells protected by tylophorine-based compounds **(A-a)** or cardiotonic steroids **(A-b)**. Cells were stained by crystal violet for visualization of cytopathic effects. **(B)** The immunofluorescent assays of tylophorine-based compounds **(B-a)** and cardenolide compounds **(B-b)** against HCoV-OC43 N (green) and Hoechst staining (blue) for the host live cells. **(C)** Dose dependent effects of tylophorine-based compounds **(C-a)** and cardenolide compounds **(C-b)** on reducing HCoV-OC43 yields/titers. Indirect immunofluorescent assay (IFA) with antibodies against N protein (green) of HCoV-OC43 in HCoV-OC43 (0.05 MOI) infected HCT-8 cells at 72 h.p.i. treated with vehicle (0.5% DMSO) or chemicals as indicated. Nuclei (blue) were stained with Hoechst dye. HCT-8 cells were seeded the day before compound treatment or HCoV-OC43 infection. The tested compounds were added to the wells 1 h prior to the addition of HCoV-OC43 at an MOI of 0.05. The resultant cultures were then incubated for an additional 72 h at 37°C. The supernatant of cells in each specific treatment was collected and subjected to viral titer determination via an end-point dilution assay conducted with HCT-8 cells at 6 d.p.i. Images shown are representative of three independent experiments.

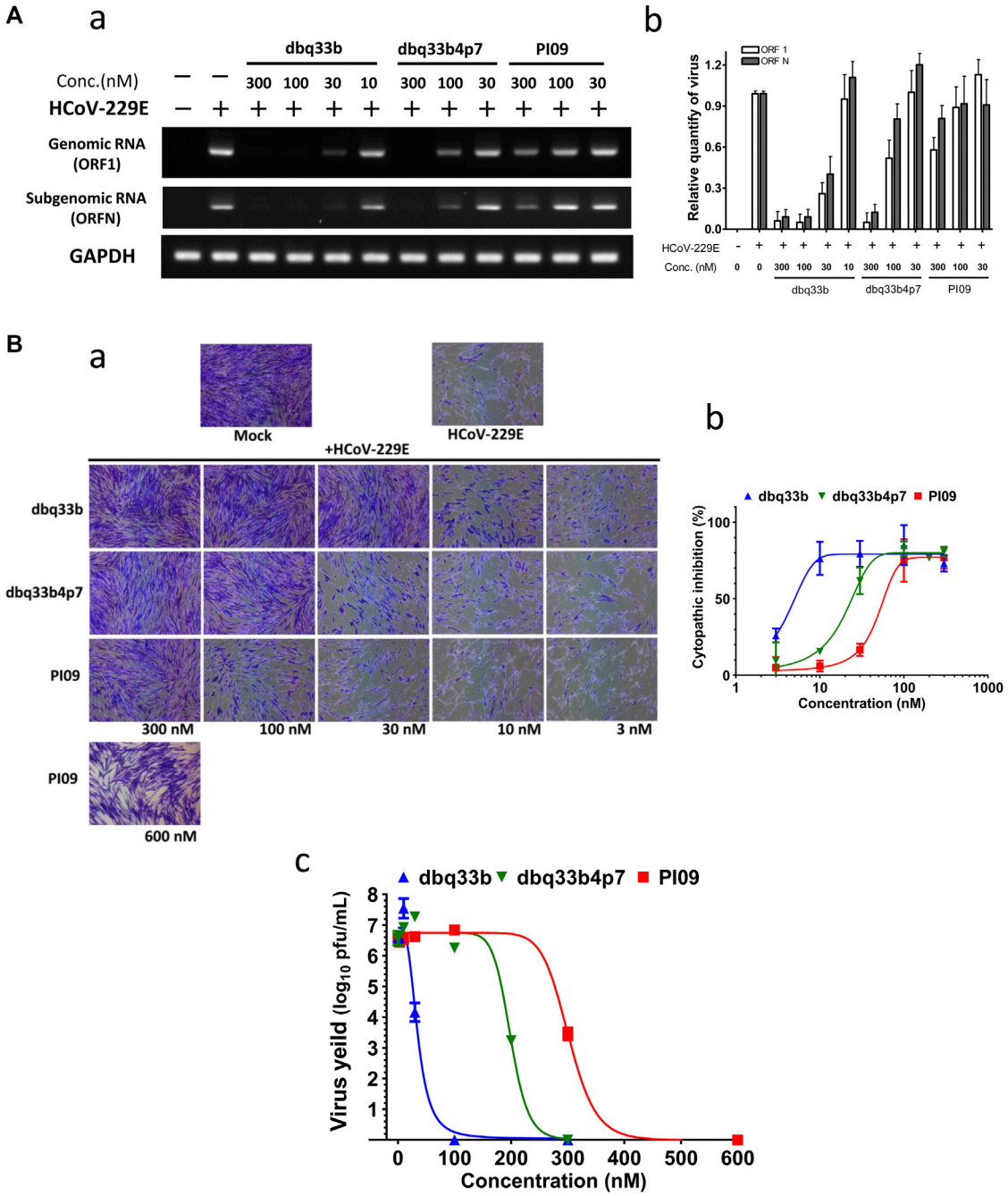

FIGURE 4 | Anti-HCoV-229E activities of tylophorine-based compounds. **(A)** Tylophorine-based compounds significantly diminished the viral RNA levels in HCoV-229E infected MRC-5 cells in a dose dependent manner. Mock or HCoV-229E infected (MOI of 1) lysates were processed and prepared at 30 h.p.i. without or with compound treatment as indicated. The total RNA was extracted with TRIzol reagent (Invitrogen). The relative viral RNA expression levels were determined by semi-RT-qPCR, analyzed with the ImageJ Analyzer program (http://imagej.nih.gov/ij/index.html), and normalized with the housekeeping gene GAPDH. The final PCR products were subjected to electrophoresis on 2% agarose gel containing ethidium bromide along with DNA markers and the representative from three independent experiments was shown in the left panel **(A-a)**. Averages ± SD of relative expression levels from three independent experiments were shown in the right panel **(A-b)**. **(B)** Tylophorine-based compounds mitigated cytopathy and eliminated viral yields of HCoV-229E in infected MRC-5 cells. Cytopathic effects in MRC-5 cells induced by HCoV-229E were mitigated by tylophorine-based compounds **(B-a)** and the averages ± SD from three independent experiments were also shown **(B-b)**. The reduction of HCoV-229E yields/titers depended on the dose of the tylophorine-based compound that was present **(B-c)**. MRC-5 cells were seeded the day before compound treatment and HCoV-229E infection. The tested compounds were added to the wells 1 h prior to the addition of HCoV-229E at an MOI of 0.05. At 4 d.p.i. the resultant supernatants were subjected to the end point assay and TCID50 determination at 5 d.p.i.; the remaining cells were stained by crystal violet for visualization of cytopathic effect prior to being dissolved by 100% MeOH for quantitation of the absorbance at 560 nm. Shown are averages ± SD from three independent experiments.

TABLE 2 | The inhibitory activities of tylophorine-based compounds against HCoV-229E and SARS-CoV-2.

Compound name	Human coronavirus 229E[a]			SARS-CoV-2[a]					
	EC_{50} (nM)[b]	CC_{50} (nM)[c]	Selectivity index	EC_{50} (nM)[b]	CC_{50} (nM)[c]	Selectivity index	EC_{50} (nM)[b]	CC_{50} (nM)[c]	Selectivity index
	CPE[d]/ crystal violet	MTS		CPE[d]/ visualization	Visualization		IFA[e]	CCK-8	
dbq33b	6.5 ± 1.0	1,712 ± 161	264	2.5	1,250	500	13.9	5,104	367
dbq33b4p7	25.4 ± 3.1	7,362 ± 288	290	20	2,500	125	31.9	2,973	93
PI09	71.4 ± 13.2	6,635 ± 578	93	78	5,000	77	76.8	3,546	46

[a]*Data are means mean ± SD from three independent experiments, each in duplicate (HCoV-229E); and means from duplicate for SARS-CoV-2.*
[b]*EC50: The values of 50% maximal effective concentration which were determined by CPE by crystal staining measurement or by visualization and by IFA as indicated.*
[c]*CC50: The values of 50% maximal cytotoxic concentration which were determined by MTS, visualization or cell counting CCK-8 as indicated.*
[d]*CPE: cytopathic effect by visualization (for SARS-CoV-2) or crystal violet stained and correspondent absorption measured at 560 nm (HCoV-229E).*
[e]*IFA: immunoflurorescent assay with antibody against virus N-protein.*

observation of cytopathic effects, and of HCoV-OC43 (*betacoronavirus*) by an immunofluorescent assay (IFA) against HCoV-OC43 nucleocapsid (N) protein. EC_{50} values for the tylophorine-based compounds ranged from 8 nM to 1.6 μM against FIPV and 16 nM to 1.9 μM against HCoV-OC43, with selectivity indices ranging from 610 to 5.3 (**Table 1**). EC_{50} values for the cardiotonic steroids ranged from 19 nM to 2.2 μM for FIPV and 56 nM to 5.4 μM for HCoV-OC43, with selectivity indices ranging from 28.5 to 3.3 (**Table 1**; **Figure 2**). In addition, the ouabain antagonist rostafuroxin did not exhibit any inhibitory activity against either FIPV or HCoV-OC43 at concentrations of up to 50 μM. Three tylophorine-based compounds (dbq33b, dbq33b4p7, and PI09) were highly potent inhibitors of HCoV-OC43, with EC_{50} values of 16 ± 4.7 nM, 56 ± 6.2 nM, and 68 ± 2.7 nM, respectively. In addition, two cardenolides (ouabain and oleandrin) and a bufadienolide (bufalin) were also potent inhibitors of HCoV-OC43, with EC_{50} values of 71 ± 7 nM, 56 ± 5 nM, and 56 ± 4 nM, respectively. All six of these compounds were then tested for their effect on HCoV-OC43 viral yields/titers.

Tylophorine-Based Compounds and Cardenolide Compounds All Reduced HCoV-OC43 Virus Titers in a Dose Dependent Manner but the Magnitude of Their Inhibition Was Highly Variable

The inhibition of HCoV-OC43 by three representative tylophorine-based compounds (dbq33b, dbq33b4p7, and PI09) and representative cardiotonic steroids (ouabain, oleandrin, and bufalin) was assessed by visual observation of their cytopathic effects at 6 d.p.i. (**Figure 3A**) and assessed by IFA against HCoV-OC43 N protein at 3 d.p.i. (**Figure 3B**), with a subsequent end point assay and TCID50 determination, to establish if these inhibitory activities on viral titers were dose dependent.

End point assays were conducted to measure the titers remaining in supernatants of HCoV-OC43 infected HCT-8 cells treated with various concentrations of tylophorine-based compounds and cardiotonic steroids. After HCoV-OC43 infection at an MOI of 0.05 for 72 h, viral yields reached ~10^7 p.f.u./ml. The tylophorine-based compounds dbq33b, dbq33b4p7, and PI09 all significantly blocked viral replication and diminished viral yields; complete reduction of viral yield by ~7–8 log of magnitudes was accomplished at concentrations of 30, 100, and 300 nM, respectively (**Figure 3C-a**). Ouabain, oleandrin, and bufalin also blocked viral replication and diminished viral yields, but only by a maximum of ~2–3 orders of magnitudes [at concentrations of 10, 30, and 100 nM (**Figure 3C-b**), respectively].

Tylophorine-Based Compounds Significantly Reduced HCoV-229E Virus Titers and Suppressed Viral Genome Replication in a Dose Dependent Manner

The activities of the tylophorine-based compounds (dbq33b, dbq33b4p7, and PI09) against another human coronavirus, HCoV-229E (an alpha-coronavirus), in fetus lung fibroblast MRC-5 cells were also ascertained.

First, HCoV-229E viral genome replication was examined by RT-PCR with specific primers against its open reading frame 1 (ORF1) and ORF nucleocapsid (ORFN). MRC-5 cells were inoculated with HCoV-229E at an MOI of 1, and the infected cells with or without drug treatment were analyzed at 30 h.p.i. by RT-PCR with specific primers. Results shown that dbq33b, dbq33b4p7, and PI09 inhibited HCoV-229E viral genome replication and subgenomic viral RNA syntheses at concentrations of 10–300 nM in a dose dependent manner (**Figure 4A**).

Second, the cytopathic effects of HCoV-229E on infected MRC-5 cells (MOI of 0.05) at 4 d.p.i. were also visualized (**Figure 4B-a**) and the resultant supernatants of each compound treatment were subjected to the TCID50 end point assays. Cells were stained with crystal violet, to determine the EC_{50}. In addition, CC_{50} values were also determined, and selectivity indices calculated (**Figure 4B-b**; **Table 2**).

Third, it was found that HCoV-229E viral yields reached 10^6–10^7 p.f.u./ml at 5 d.p.i, and treatments with dbq33b, dbq33b4p7, or PI09 all significantly blocked viral replication and eliminated viral yields, resulting in a complete reduction of viral yield by 6–7 log of magnitudes at concentrations of 100 nM, 300 and 600 nM (**Figure 4B-c**), respectively in HCoV-229E infected MRC-5 cells.

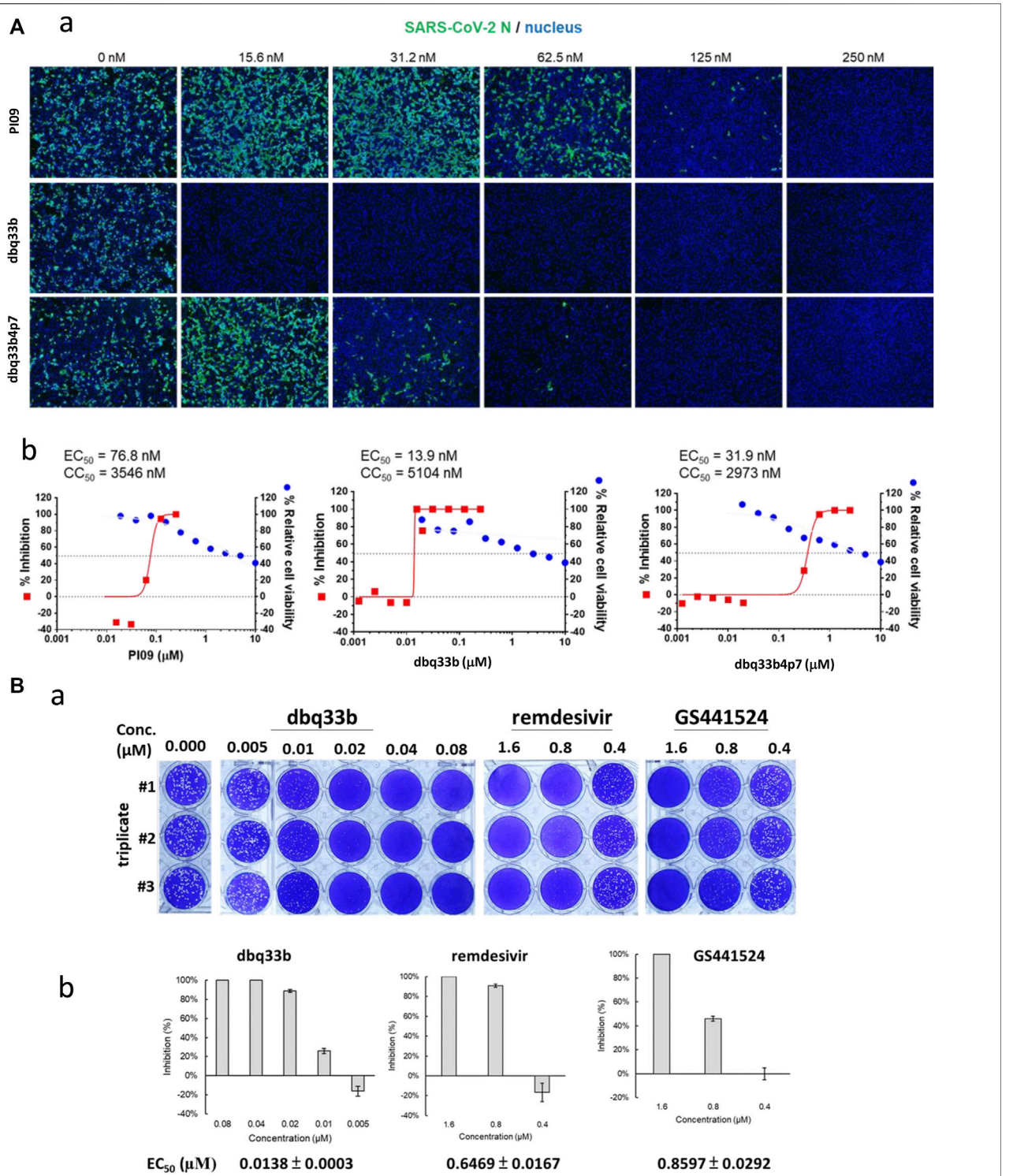

FIGURE 5 | (Continued)

FIGURE 5 | Anti-SARS-CoV-2 activities of tylophorine-based compounds. **(A)** The immunofluorescent assays against SARS-CoV-2 N (green) and DAPI staining (blue) for the Vero E6 host live cells. Vero E6 cells were treated with each compound at the indicated concentrations for 1 h at 37°C. The cells were adsorbed with SARS-CoV-2 (TCDC#4) at MOI = 0.01 for 1 h at 37°C. After virus adsorption, the cells were washed with PBS and fresh medium with each compound added at the indicated concentrations and then incubated for 2 days. The cells were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. The cells were stained with anti-SARS-CoV-2 N protein antibody and anti-human IgG-488 (green) and the nuclei were counter stained with DAPI (blue) as shown **(A-a)**. The N protein expression was measured by using a high-content image analysis system (Molecular Devices); the cell viability was determined by Cell Counting Kit-8 (CCK-8); and EC_{50} and CC_{50} were calculated by Prism software **(A-b)**. Shown results are representative of two independent experiments. **(B)** Tylophorine-based compound dbq33b profoundly diminished the plaque formation caused by SARS-CoV-2 in Vero E6 cultured plaque assays. Plaque assay was performed in triplicate in 24-well tissue culture plates. The Vero E6 cells were seeded in DMEM with 10% FBS and antibiotics 1 day before infection. SARS-CoV-2 was added to the cell monolayer for 1 h at 37°C. Subsequently, viruses were removed and the cell monolayer was washed once with PBS before covering with media containing 1% methylcellulose (Sigma, cat #M0387) and test compounds at indicated concentrations for 5–7 days. The cells were fixed with 10% formaldehyde solution (Marcon™ Chemicals, cat #H121-08) overnight. After removal of overlay media, the cells were stained with crystal violet and the plaques were counted **(B-a)**. Remdesivir and GS441524 were used as control reference compounds. The percentage of inhibition was calculated as [1 − (VD/VC)] × 100%, where VD and VC refer to the virus titer in the presence and absence of the test compound, respectively **(B-b)**. Shown results are representative of two independent experiments.

Tylophorine-Based Compounds Show Potent anti-SARS-CoV-2 Activities in Vero E6 Cells

The tylophorine-based compounds dbq33b, dbq33b4p7, and PI09 were examined for their inhibitory activity against SARS-CoV-2 in Vero E6 cells. The cytopathic effects of SARS-CoV-2 on Vero E6 cells in the presence of the test compounds at a two-fold dilution and a series of 12 concentrations were visualized, and values of EC_{50} and CC_{50} calculated (**Table 2**). All three compounds were found to be highly potent inhibitors of SARS-CoV-2, with EC_{50} values of 2.5, 20, and 78 nM, respectively (**Table 2**). In addition, an IFA assay at 2 d.p.i. was also performed using an antibody against SARS-CoV-2 N protein; the corresponding EC_{50} values were determined to be 14, 32, and 77 nM respectively – comparable to those obtained by cytopathy assay. As expected, dbq33b, dbq33b4p7, and PI09 were highly potent with selectivity indices of 376, 93, and 46, respectively (**Table 2**; **Figure 5A**).

The inhibitory potency of dbq33b, the most potent compound, was additionally determined by plaque assay in SARS-CoV-2 infected Vero E6 cells. An EC_{50} of 14 nM was obtained (**Figure 5B**), consistent with the cytopathic effect assay and IFA results (**Table 2**).

DISCUSSION

Coronaviruses are grouped into four genera: *Alphacoronaviruses*, *Betacoronaviruses*, *Gammacoronaviruses* and *Deltacoronaviruses*, of which those belonging to the former two are infectious to humans (Chen et al., 2020). The tylophorine-based compounds tested herein exerted potent, broad-spectrum antiviral activity against multiple types of coronavirus (Yang et al., 2010; Lee et al., 2012; Yang et al., 2017b), **Tables 1** and **2**; **Figures 1**–**5**), including SARS-CoV, SARS-CoV-2, HCoV-OC43, and MHV (*betacoronavirus*) and TGEV, FIPV, and HCoV-229E (*alphacoronavirus*). Accordingly, they should amongst the first compounds to be tested as therapies against novel coronaviruses in the future.

Basically, the structure-activities relationships of tylophorine-based derivatives and cardiotonic steroids have been proposed or discussed as published previously (Yang, et al., 2010; Lee, et al., 2012; Yang, et al., 2017a). The results obtained herein are in consistent with previous structure-activities relationships or conclusion.

The cardiac steroids tested were also highly potent inhibitors of these coronaviruses, but their safety indices were only up to ~16 (for HCoV-OC43, assayed by IFA) and or ~28 (for FIPV, assayed by visualization of CPE) (**Table 1**). Furthermore, the inhibition of HCoV-OC43 was limited to two to three magnitudes of viral load, as the p.f.u./ml decreased from ~10^7 to ~10^5 or 10^4 (**Figure 3C-b**) by end point assay and TCID50 determination. On the contrary, the tylophorine based compounds were all highly potent inhibitors of all the coronaviruses tested, demonstrating complete elimination of viral load/yield by ~10^7 p.f.u./ml (**Figure 3C-a**) and good safety indices of up to >610 (for HCoV-OC43, assayed by IFA) and ~390 (for FIPV, by visualization of CPE) (**Table 1**). Therefore, the tylophorine-based compounds tested are concluded to be inherently superior to the cardiac steroids for the purpose of combating COVID-19. These differences may reflect their differential underlying mechanisms and pharmacological targets – the tylophorine-based compounds targeting viral RNA (Yang et al., 2017b), and the cardiotonic steroids affecting host cell factors (Yang et al., 2017a; Yang et al., 2018).

The tylophorine-based compounds also had the property of cell growth inhibition, with the selectivity index depending on the design of the experiment and the methods used to measure cytotoxicity (Yang et al., 2017a). Thus, selectivity indices of tylophorine compounds, CC_{50}/EC_{50}, may be easily unintentionally underestimated. Moreover, coronavirus-infected cells treated with tylophorine compounds usually expand their size (area in the culture plates) and are protected from the formation of syncytia or multinucleated giant cells resulting from virus-induced fusion of cell membranes (Yang et al., 2010; Lee et al., 2012).

Tylophorine-based compounds block viral replication as demonstrated in three ways: 1) by directly targeting and interacting with genomic/subgenomic RNAs and the nucleocapsid protein; 2) by colocalizing with coronaviral RNA and RNA-dependent RNA polymerase in the viral replication-transcription complexes (RTCs) surrounding nuclei of infected

cells; and 3) by effectively blocking syntheses of coronaviral antigens and genomic/subgenomic RNAs (Yang et al., 2017b). Coronavirus N protein forms complexes with genomic and subgenomic RNAs and plays a crucial role in virus replication, transcription and translation (McBride et al., 2014). Furthermore, N protein exclusively binds to nonstructural protein 3 (NSP3), a component of RTCs, for recruitment to RTCs is crucial and critical for viral RNA syntheses (Cong et al., 2020). Therefore, by directly targeting viral replication-transcription machinery, tylophorine-based compounds are highly effective inhibitors of coronaviral replication. Accordingly, they merit further development into an approved therapeutic agent.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

C-WY, Y-ZL, and H-YH performed most of the biochemistry, and molecular biology experiments. J-TJ, T-TP, J-JL, C-CL, T-LC, Y-HP, H-CK, C-PC, G-HN, and S-HW performed part of the biochemistry, and molecular biology experiments. S-JL, C-WY, Y-ZL, H-YH, J-TJ, Y-LL, and S-YC designed experiments and analyzed the obtained results; interpreted the data and wrote the manuscript. R-BY, W-ZH, J-HL, H-KS and C-TC were involved in composition of the manuscript. S-JL supervised the experimental design, the interpretation of the data, and the composition of the manuscript.

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Potential Simultaneous Inhibitors of Angiotensin-Converting Enzyme 2 and Transmembrane Protease, Serine 2

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Outbreak of coronavirus disease 2019 occurred in Wuhan and has rapidly spread to almost all parts of world. GB-1, the herbal formula from Tian Shang Sheng Mu of Chiayi Puzi Peitian Temple, is used for the prophylaxis of SARS-CoV-2 in Taiwan. In this study, we investigated that the effect of GB-1 and the index compounds of GB-1 on the ACE2 and TMPRSS2 expression through *in vitro* and *in vivo* study. In our result, GB-1 can inhibit ACE2 and TMPRSS2 protein expression in HepG2 cells, 293T cells, and Caco-2 cells without cytotoxicity. For the mouse model, GB-1 treatment could decrease ACE2 and TMPRSS2 expression levels of the lung and kidney tissue without adverse effects, including nephrotoxicity and hepatotoxicity. In the compositions of GB-1, 0.5–1 mg/ml of *Glycyrrhiza uralensis* Fisch. ex DC. extract could not inhibit *ACE2* mRNA and protein expression in HepG2 cells. In addition, theaflavin-3-gallate could inhibit protein expression of ACE2 and TMPRSS2 without significant cytotoxicity. Our results suggest that GB-1 and theaflavin-3-gallate could act as potential candidates for prophylaxis or treatment of SARS-CoV-2 infection through inhibiting protein expression of ACE2 and TMPRSS2 for the further study.

Keywords: SARS-CoV-2, ACE2, TMPRSS, theaflavin 3-gallate, theaflavin, (+)-catechin 4

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INTRODUCTION

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–induced coronavirus disease 2019 (COVID-19) was recorded in December 2019. About ten million patients across the globe were infected within several months. Angiotensin-converting enzyme 2 (ACE2) is a membrane-associated enzyme for catalyzing the cleavage of angiotensin I to angiotensin (1–9) (Donoghue et al., 2000). Its peptidase domain can directly bind to the receptor-binding domain of the spike protein on the surface of the SARS-CoV-2 viral envelope, thus promoting viral entry into host cells (Hoffmann et al., 2020b; Walls et al., 2020; Yan et al., 2020). After binding to the ACE2

Abbreviations: ACE2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane protease, serine 2; STAT3, signal transducer and activator of transcription 3; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SARS-CoV, severe acute respiratory syndrome coronavirus; COVID-19, coronavirus disease 2019; IHC, immunohistochemistry; GU, *Glycyrrhiza uralensis* Fisch. ex DC.; T3G, theaflavin-3-gallate.

receptor, transmembrane protease, serine 2 (TMPRSS2), and furin of host cells can cleave and activate the SARS-CoV-2 spike protein (Hoffmann et al., 2020a; Hoffmann et al., 2020b).

ACE2 expression and distribution in different parts of the human body might indicate infection routes of SARS-CoV-2. Studies have identified high ACE2 expression in the lung, kidney, intestines, heart, and brain (Baig et al., 2020; Zou et al., 2020). These organs should be regarded as high-risk sites for potential SARS-CoV-2 infection. A recent study described high ACE2 expression in the oral cavity, indicating that the oral route is particularly relevant to SARS-CoV-2 infection (Xu et al., 2020). Therefore, ACE2 and TMPRSS2 are potential antiviral intervention targets for the prevention or SARS-CoV-2 infection or related treatment (Hoffmann et al., 2020b; Danser et al., 2020; Gurwitz, 2020; Morse et al., 2020).

Some herbal formulas are used for the prophylaxis and treatment of SARS-CoV-2 (Li et al., 2020; Ren et al., 2020; Runfeng et al., 2020; Zhang et al., 2020). However, the actual inhibitory mechanisms preventing SARS-CoV-2 entry into the host cells and the efficiency of these formulas remain unclear. In this study, we investigated the effect of GB-1, a formula from Tian Shang Sheng Mu of Chiayi Puzi Peitian Temple, and their index compounds, theaflavin-3-gallate, theaflavin, and (+)-catechin, on the protein and mRNA expression of ACE2 and TMPRSS2.

MATERIALS AND METHODS

Cell Culture and Treatment

The design of the GB-1 formula was obtained from Tian Shang Sheng Mu of Chiayi Puzi Peitian Temple: dry roots of *Glycyrrhiza uralensis* Fisch. ex DC. (25 g; Chang Gung Memorial Hospital, Taiwan) and *Camellia sinensis* var. assamica (black tea, 5 g; Chang Gung Memorial Hospital, Taiwan). This GB-1 or 10 g of the dry root of *G. uralensis* Fisch. ex DC. alone was soaked in 2,000 ml of boiling hot water for 25 min in thermal flasks. The samples were then filtered using a filter paper to remove particulate matter. The obtained water extracts were then evaporated under reduced pressure to obtain viscous masses of 4 g for GB-1 and 3 g for *G. uralensis* Fisch. ex DC. alone. These samples were stored at −80 °C until further experimentation. For all experiments, the final concentrations of the test compounds were prepared by diluting the viscous masses with water. We purchased theaflavin-3-gallate and theaflavin from ChromaDex (Irvine, CA, United States) and (+)-catechin from Sigma-Aldrich.

HepG2 cells, Caco-2 cells, and 293T cells were obtained from the Bioresource Collection and Research Center, Taiwan (passage number of both cell lines = 10–15). HepG2 cells and 293T cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (FBS) at 37 °C and 5% CO_2. Caco-2 cells were cultured in Eagle's minimal essential medium supplemented with 10% fetal bovine serum (FBS) at 37 °C and 5% CO_2. Before treatment, the cells were cultured to 60–70% confluence, after which the medium was replaced with the same fresh medium in water or methanol at the indicated concentrations. The cells treated with water or vehicle alone were used as untreated controls, whereas the parental cells without any treatment were used as blank controls.

XTT Assay

The indicated cells were plated at a density of 1×10^3 cells/well in 96-well plates with medium containing 10% FBS. After the cells attached to culture dishes, the medium was replaced with fresh medium containing 10% FBS. The cells were then treated with the indicated drugs for the indicated hours. Thereafter, the cells were subjected to an XTT assay (Roche, catalog number: 11465015001) performed according to the manufacturer's instructions. The absorbance of the formed XTT–formazan complex was quantitatively measured at 492 nm using an enzyme-linked immunoassay reader (Bio-Rad Laboratories, Inc.).

Western Blot Analysis

The treated cellular protein extracts were prepared as described previously (Lin et al., 2017; Lee et al., 2018). In brief, equal amounts of protein were separated on an 8–10% SDS-PAGE gel and transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat dried milk for 30 min and then incubated with primary antibodies for 6–12 h at room temperature. The following primary antibodies were used: anti-ACE2 (1:1,000; Cell Signaling), anti-STAT3 (1:1,000; Cell Signaling), anti-TMPRSS2 (1:1,000; Abcam), anti-β-actin (1:10,000; Santa Cruz), and anti-GAPDH (1:10,000; Santa Cruz) antibodies. All the primary and secondary antibodies were diluted with 1% nonfat dried milk in Tris-buffered saline with 0.1% Tween 20 detergent. The membranes were washed using 0.1% Tris-buffered saline with Tween-20 and incubated in horseradish peroxidase–conjugated secondary anti-mouse or anti-rabbit antibodies (Santa Cruz, ratio: 1:5,000) for 1 h at room temperature. The membranes were washed for 1 h at room temperature. Chemiluminescent protein signals were detected by applying the SuperSignal West Pico PLUS chemiluminescent substrate (Pierce, catalog number: 34087).

Quantitative Real-Time Polymerase Chain Reaction

Quantitative real-time polymerase chain reaction (PCR) was performed as described previously (Lin et al., 2017; Lee et al., 2018). Total RNA of the indicated cells was extracted using the illustra™ RNAspin Mini RNA Isolation Kit (GE Healthcare, catalog number: 25-0500) and then reverse transcribed using the Superscript first-strand synthesis kit (Invitrogen, catalog number: 11904018)—all according to the manufacturer's instructions. Quantitative real-time PCR analysis was performed using the comparative cycle threshold method on an ABI PRISM 7700 Sequence Detection System by using the SYBR Green PCR Master Mix kit, according to the manufacturer's instructions. After initial incubation at 5°C for 2 min and then at 95°C for 10 min, 40 amplification cycles were performed at 95°C for 20 s, followed by 65°C for 20 s, and then 72°C for 30 s. *GAPDH* was used as the housekeeping gene for data normalization. Primers used were *ACE2* forward, 5'- TCC ATT

GGT CTT CTG TCA CCCG-3' and *ACE2* reverse, 5'-AGA CCA TCC ACC TCC ACT TCTC-3', and *GAPDH* forward, 5'-TGC ACC ACC AAC TGC TTAGC-3' and *GAPDH* reverse, 5'-GGC ATG GAC TGT GGT CATGA-3'.

Protein Quantification

For protein quantification, Western blot band images were analyzed using AlphaEase®FC according to the manufacturer's instructions. After a band was selected for each group, the background was subtracted and the band densities were calibrated automatically. The density of the untreated group was used as the standard to calculate protein ratios for the other groups.

Mice

All current procedures involving mice were approved by the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital (Approval number 2017081601). Surgery was performed under sodium pentobarbital anesthesia.

Ten 5- to 7-week-old male C57BL/6 mice (weight = 18–20 g), obtained from BioLASCO Taiwan, were randomized into two groups of five: one group received the vehicle (water), whereas the other received oral GB-1 at 200 mg/kg/day. Mouse weights were measured every 1–2 days for 1 week. At 1 week after treatment, the mice were sacrificed. Subsequently, mouse blood samples were tested for serum creatinine, aspartate aminotransferase, and alanine aminotransferase levels.

Immunohistochemistry Assessment

Immunohistochemical (IHC) analysis was performed as described previously (Liu et al., 2019). Lung and kidney tissue specimens obtained from our mice were fixed with 4% formalin, embedded in paraffin, sectioned, and stained with primary antibodies against ACE2 (1:100; Bioss Antibodies) or STAT3 (1:100; Cell Signaling) or TMPRSS2 (1:1,000; Abcam). For IHC assessment, we used peroxidase-linked goat anti-rabbit secondary antibodies and the Rabbit Probe HRP Labeling Kit (catalog number: TAHC03D; BioTnA). The photomicrographs were observed under the Nikon TE3000 microscope. The ImageJ system (1.50 days, United States) was used to quantify the integrated optical density per stained area (IOD/area) for the staining of ACE2, STAT3, and TMPRSS2.

High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) analysis was performed on the LC-10Avp system (Shimadzu) equipped with a Supelco Discovery® C18 column (5-μm particle size, 150-mm length × 4.6-mm internal diameter; Supelco); 0.1% phosphoric acid was used as the mobile phase; the flow rate was 0.6 ml/min; the detection wavelength was 280 nm; and the column temperature was 25°C.

Statistical Analyses

All values are presented as means ± standard errors of the means of the replicate samples (n = 3–6, depending on the experiment). All experiments were repeated at least three times. Differences between the two groups were assessed using the unpaired two-tailed Student's t test, and among those, more than two groups were examined using analysis of variance (ANOVA). For testing the significance of pairwise group comparisons, Tukey's test was used as a post hoc test in ANOVA. For all comparisons, p values of <0.05 were considered to indicate statistical significance. SPSS (version 13.0; SPSS, Chicago, IL, United States) was used for all statistical analyses.

RESULTS

Effect of GB-1 on HepG2, 293T, and Caco-2 Cell Growth and ACE2 and TMPRSS2 Expression

In previous studies, HepG2 cells and Caco-2 cells showed high ACE2 protein expression and were used as a model of SARS-CoV and SARS-CoV-2 entry models (Inoue et al., 2007; Liao et al., 2013; Bojkova et al., 2020). In addition, 293T cells have also been used as a model of SARS-CoV-2 entry (Letko et al., 2020). To investigate the effect of GB-1 on ACE2 and TMPRSS2 expression, we used HepG2 cells, 293T cells, and Caco-2 cells as cellular models. First, using an XTT assay, we investigated a potential cytotoxic effect of GB-1 on HepG2 cells, 293T cells, and Caco-2 cells. After treatment at a concentration range of 10–250 μg/ml, GB-1 had not inhibited the proliferation of HepG2 cells (**Figure 1A**) or 293T cells (**Figure 2A**) or Caco-2 cells (**Figure 2D**), suggesting that this concentration range of GB-1 has no considerable cytotoxic effect on HepG2 cell, 293T cell, and Caco-2 cell growth.

Because the SARS-CoV-2 spike protein can bind directly to ACE2 and is primed by TMPRSS2 (Hoffmann et al., 2020b; Walls et al., 2020; Yan et al., 2020), we examined the effect of GB-1 on ACE2 and TMPRSS2 expression in host cells. Twenty-four hours of treatment with GB-1 significantly reduced ACE2 and TMPRSS2 protein expression in HepG2 cells in a dose-dependent manner (**Figures 1B,D**). GB-1 also inhibited the protein expression of ACE2 and TMPRSS2 in 293T cells at a concentration of 250 μg/ml (**Figure 2B**). In addition, GB-1 inhibited the protein expression of ACE2 and TMPRSS2 in Caco-2 cells at a concentration of 250 μg/ml (**Figures 2E,F**). GB-1 also inhibited *ACE2* mRNA expression in HepG2 cells and 293T cells after 24 h (**Figures 1C**, **2C**). In a recent study, silencing STAT3 (signal transducer and activator of transcription 3) can affect ACE2 expression (Shamir et al., 2020). In addition, inhibition of STAT3 with galiellalactone significantly reduced the expression of TMPRSS2 in benign tissue cultures (Handle et al., 2018). For investigating the mechanism, we examined the effect of GB-1 on STAT3 expression in host cells. The treatment with GB-1 had not affected STAT3 protein expression in HepG2 cells (**Figure 1D**). Overall, these results suggest that GB-1 can affect ACE2 and TMPRSS2 protein expression in HepG2

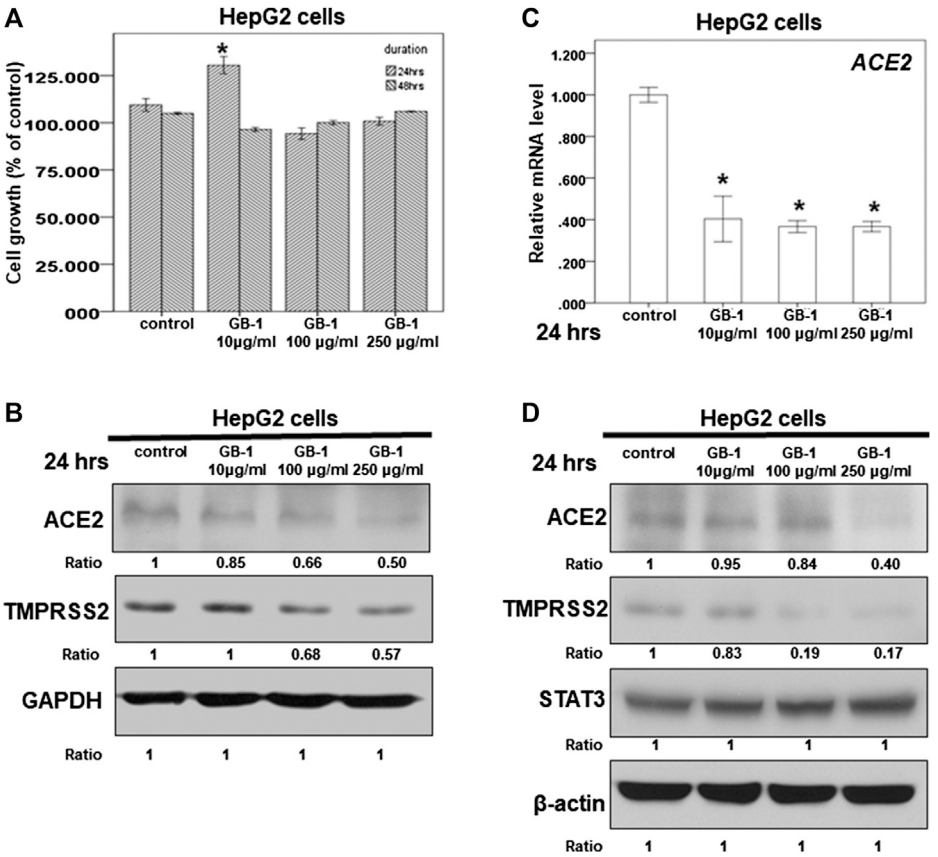

FIGURE 1 | Effect of GB-1 on ACE2 and TMPRSS2 expression in HepG2 cells. **(A)** HepG2 cells were measured by XTT assay after indicated hours of culturing in the presence of GB-1. **(B,D)** Total cell extracts of HepG2 cells were harvested from untreated cells and cells treated with GB-1 for 24 h. The protein was immunoblotted with polyclonal antibodies specific for ACE2 or TMPRSS2 or STAT3. GAPDH or β-actin was used as an internal loading control. **(C)** Total mRNA was extracted from the HepG2 cells after treating with GB-1 for 24 h. The coding regions of human ACE2 were used as probes for real-time polymerase chain reaction analysis. All the results are representative of at least three independent experiments. (Error bars = mean ± S.E.M. * denotes samples significantly different from the control group with $p < 0.05$).

cells, 293T cells, and Caco-2 cells, without a significant cytotoxic effect.

In Vivo Effect of GB-1 on the Mouse Model

To investigate the effects of GB-1 *in vivo*, we used mice as our model. From the XTT assay, we revealed that 10–250 μg/ml of GB-1 had no significant cytotoxic effect on HepG2 cell, 293T cell, and Caco-2 cell growth (**Figures 1A**, **2A**,**D**). We treated the mice with 200 mg/kg of GB-1 through oral administration every day. After 1 week of treatment, no significant alteration in either the activity or the body weight of the mice had been observed, and no mice had died (**Figure 3A**). Moreover, creatinine, alanine aminotransferase, and aspartate aminotransferase levels of serum in the GB-1 group were not elevated compared with those in the control group (**Figures 3B–D**), indicating that GB-1 did not cause significantly acute nephrotoxicity and hepatotoxicity in the mice.

As the lungs and kidneys have higher ACE2 expression and are the major target organs for SARS-CoV and SARS-CoV-2 infection (Hamming et al., 2004; Baig et al., 2020; Lukassen et al., 2020; Zou et al., 2020), we investigated the effect of GB-1 on ACE2 protein expression in the lung and kidney tissue. After 1 week of GB-1 treatment (200 mg/kg, oral administration), IHC data of the lung and kidney tissue showed that the ACE2 expression level in the GB-1 group was markedly reduced compared with that in the control group (**Figure 4**). In addition, the TMPRSS2 expression level in the lung and kidney tissue decreased substantially in the GB-1 group compared with the control group (**Figure 4**). However, the STAT3 expression level in the lung and kidney tissue showed no significant alteration between the GB-1 group and the control group (**Figure 4**). These results suggest that GB-1 may protect against SARS-CoV and SARS-CoV-2 infection through inhibiting ACE2 and TMPRSS2 expression in both lung and kidney tissues, without inducing significant nephrotoxicity or hepatotoxicity.

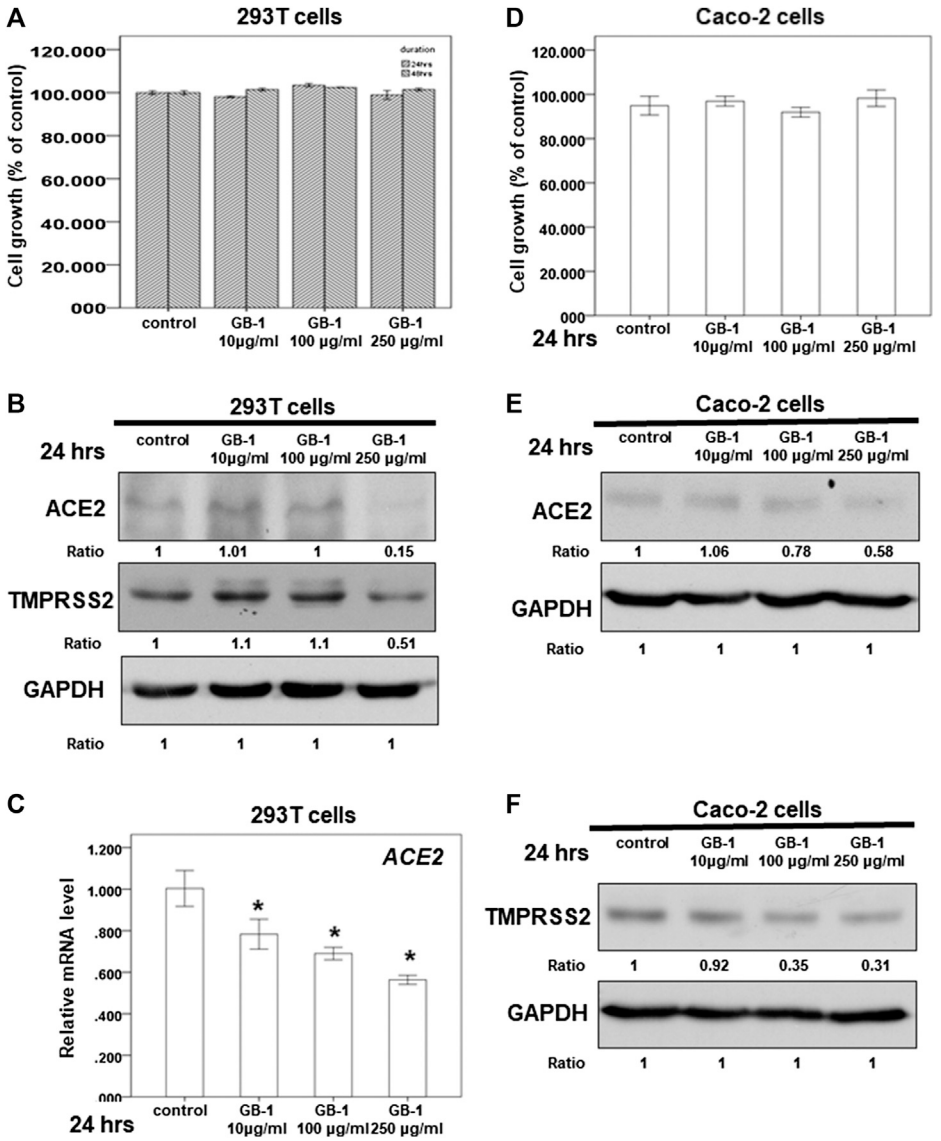

FIGURE 2 | Effect of GB-1 on ACE2 and TMPRSS2 expression in 293T cells and Caco-2 cells. **(A**,**D)** 293T cells **(A)** or Caco-2 cells **(D)** were measured by XTT assay after indicated hours of culturing in the presence of GB-1. **(B**,**E,F)** Total cell extracts of 293T cells **(B)** or Caco-2 cells **(E,F)** were harvested from untreated cells and cells treated with GB-1 for 24 h. The protein was immunoblotted with polyclonal antibodies specific for ACE2 or TMPRSS2. GAPDH was used as an internal loading control. **(C)** Total mRNA was extracted from the 293T cells after treating with GB-1 for 24 h. The coding regions of human ACE2 were used as probes for real-time polymerase chain reaction analysis. All the results are representative of at least three independent experiments. (Error bars = mean ± S.E.M. * denotes samples significantly different from the control group with $p < 0.05$).

Effect of *Glycyrrhiza uralensis* Fisch. ex DC. on ACE2 Expression

The dry root of *Glycyrrhiza uralensis* Fisch. ex DC. (GU) and *Camellia sinensis* var. assamica extract are the major components of GB-1 and are commonly consumed in Taiwan and around the world for food and medicine. Our results indicate that 0.5–1 mg/ml of GU had no significant cytotoxic effect on HepG2 cell growth (**Figure 5A**). However, the same concentration range of GU could not inhibit *ACE2* mRNA and protein expression in HepG2 cells (**Figures 5B**,**C**). These results suggest that GU may not be responsible for the inhibitory effect of GB-1 on *ACE2* mRNA and protein expression in HepG2 cells.

Effect of (+)-Catechin on ACE2 and TMPRSS2 Expression

Because we suspected that *Camellia sinensis* var. assamica extract might play a role in inhibiting the expression of ACE2 and TMPRSS2, we investigated the effect of (+)-catechin (**Figure 6A**), an index compound of *Camellia*

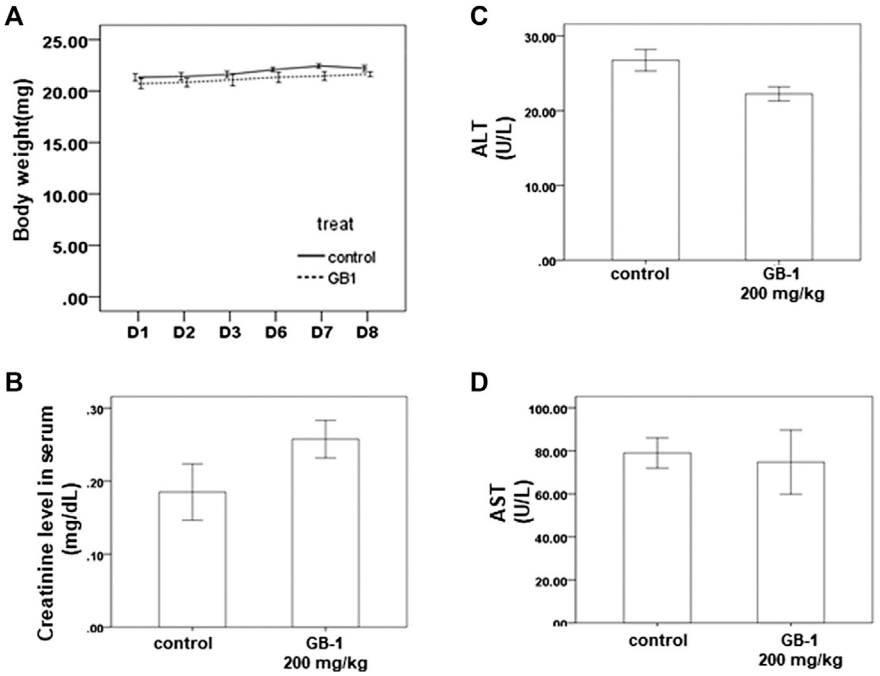

FIGURE 3 | *In vivo* effect of GB-1 on the mouse model. **(A)** Average mice weights with vehicle/200 mg/kg/day GB-1 every day by oral administration over a time course of 1 week. **(B–D)** Creatinine **(B)**, ALT **(C)**, and AST **(D)** levels in serum of mice after the treatment of vehicle/GB-1. (n = 5 per group, error bars = mean ± S.E.M. * denotes samples significantly different from the control group with $p < 0.05$).

sinensis var. assamica extract, on HepG2 cells. We first assessed the potential cytotoxicity of (+)-catechin in HepG2 cells at a concentration range of 10–50 μg/ml. Our results showed that (+)-catechin could not inhibit the growth of HepG2 cells (**Figure 6B**). However, 50 μg/ml of (+)-catechin inhibited ACE2 but not TMPRSS2 protein expression in HepG2 cells (**Figure 6C**). These results suggest that higher concentrations (≥50 μg/ml) of (+)-catechin can inhibit ACE2 protein expression in HepG2 cells.

Effect of Theaflavin on ACE2 and TMPRSS2 Expression

Next, we investigated the effect of theaflavin (**Figure 6D**), another index compound of *Camellia sinensis* var. assamica extract, on HepG2 cells. We tested the potential cytotoxicity of theaflavin in HepG2 cells at a concentration range of 10–50 μg/ml. At 50 μg/ml, theaflavin had a mild cytotoxic effect on the growth of HepG2 cells (**Figure 6E**). Moreover, only 50 μg/ml of theaflavin inhibited ACE2 and TMPRSS2 protein expression in HepG2 cells (**Figure 6F**). These results suggest that higher concentrations (≥50 μg/ml) of theaflavin can inhibit the protein expression of ACE2 and TMPRSS2 in HepG2 cells.

Effect of Theaflavin-3-Gallate on ACE2 and TMPRSS2 Expression

We also investigated the effect of theaflavin-3-gallate (**Figure 7A**), another index compound of *Camellia sinensis* var. assamica extract, on HepG2 cells. In our HPLC result, the concentration of theaflavin-3-gallate in GB-1 is 0.3087017% (w/w) (**Figures 7B–D**). We tested the cytotoxicity of theaflavin-3-gallate in HepG2 cells and 293T cells and found that the theaflavin-3-gallate was not cytotoxic to the growth of HepG2 cells and 293T cells at a concentration of 10–50 μg/ml (**Figures 7E,G**). However, 50 μg/ml of theaflavin-3-gallate inhibited the protein expression of ACE2 and TMPRSS2 in HepG2 cells and 293T cells (**Figures 7F,H**). These results suggest that 50 μg/ml of theaflavin-3-gallate contributes to the inhibition of the protein expression of ACE2 and TMPRSS2.

DISCUSSION

The present study demonstrates that TMPRSS2 expression in host cells can activate SARS-CoV-2 and promote its spread (Hoffmann et al., 2020b). Camostat mesylate, an inhibitor of TMPRSS2 approved for clinical use, can block the SARS-CoV-2 infection of lung cells (Hoffmann et al., 2020b). However, studies involving mouse models of acute respiratory distress syndrome have reported that ACE2 knockout resulted in more severe symptoms (Imai et al., 2005). Higher levels of ACE2 in lung cells are associated with less severe acute respiratory distress syndrome (Wosten-van Asperen et al., 2013). The disruption of the physiological balance between ACE/ACE2 and the angiotensin II/angiotensin system by SARS-CoV infection plays a pathogenic role in SARS-CoV–induced lung injury (Kuba et al., 2006; Yamamoto et al., 2006; Imai et al.,

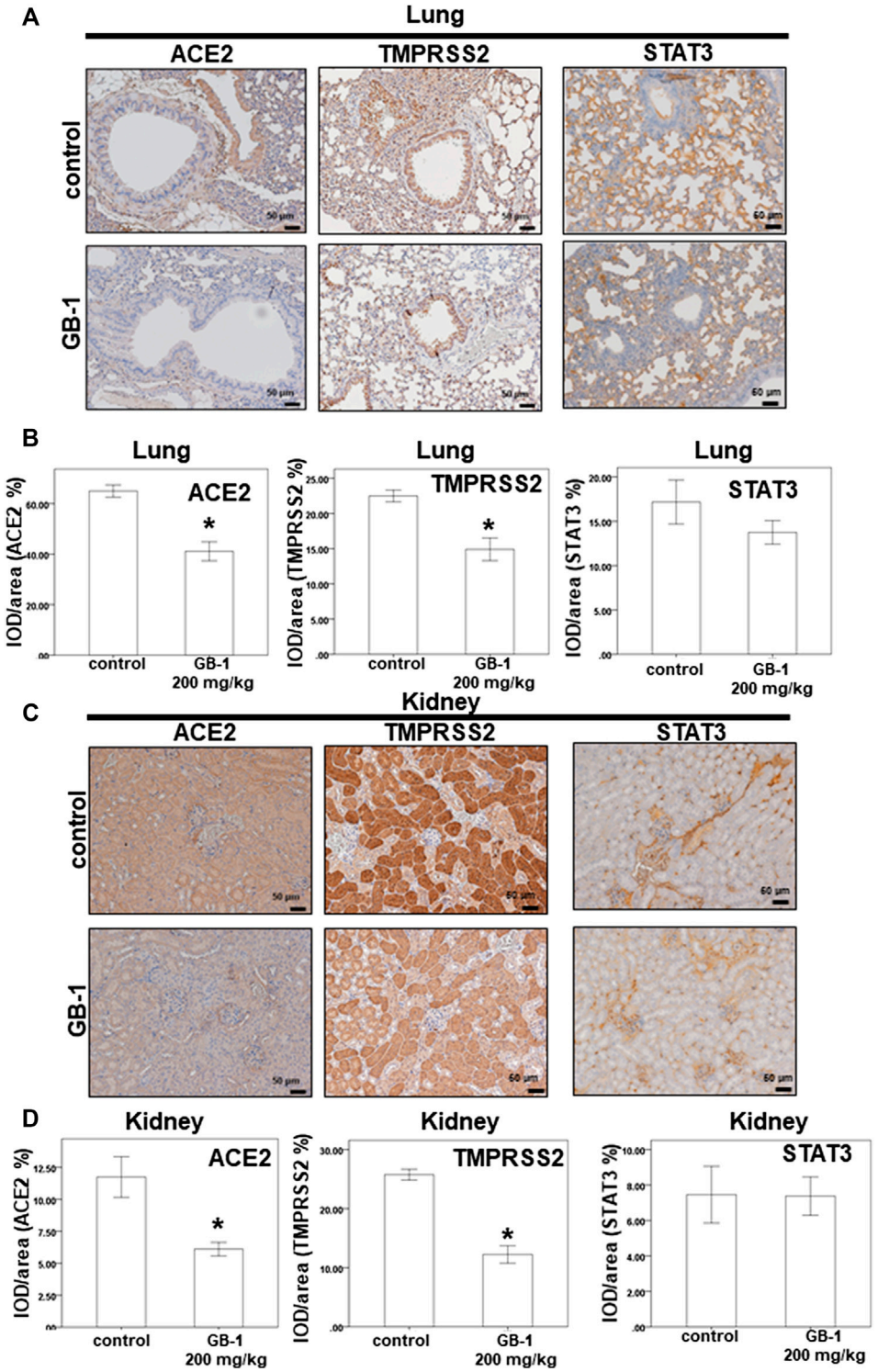

FIGURE 4 | Effect of GB-1 on ACE2 and TMPRSS2 and STAT3 expression on the mouse model. **(A**,**C)** Representative IHC staining photomicrographs of the lung tissue **(A)** and kidney tissue **(C)** in mice. **(B**, **D)** Quantitative results of IHC staining, which were presented as the IOD/area and were proportional to the levels of ACE2 and TMPRSS2 and STAT3. (n = 5 per group, error bars = mean ± S.E.M. * denotes samples significantly different from the control group with $p < 0.05$).

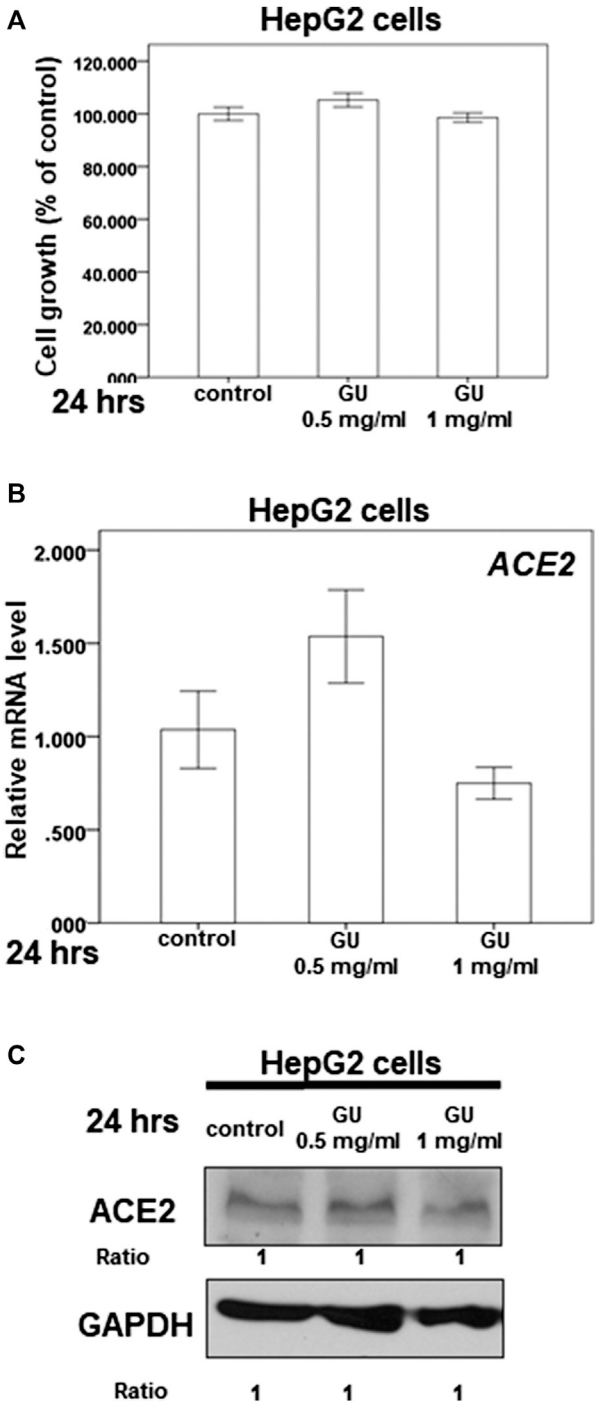

FIGURE 5 | Effect of *Glycyrrhiza uralensis* Fisch. ex DC. extract (GU) on ACE2 expression. **(A)** HepG2 cells were measured by XTT assay after indicated hours of culturing in the presence of GU. **(B)** Total mRNA was extracted from the HepG2 cells after treating with GU for 24 h. The coding regions of human *ACE2* were used as probes for real-time polymerase chain reaction analysis. **(C)** Total cell extracts of HepG2 cells were harvested from untreated cells and cells treated with GU for 24 h. The protein was immunoblotted with polyclonal antibodies specific for ACE2 or TMPRSS2. GAPDH was used as an internal loading control. All the results are representative of at least three independent experiments. (Error bars = mean ± S.E.M. * denotes samples significantly different from the control group with $p < 0.05$).

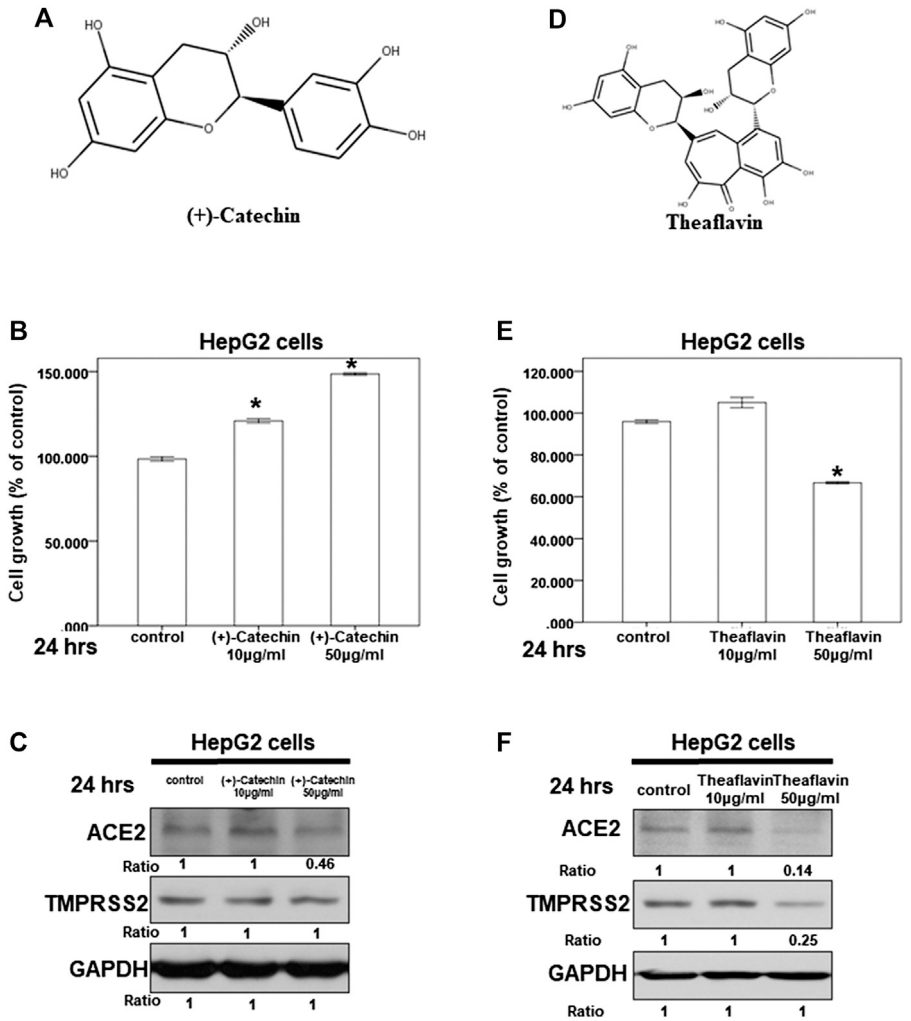

FIGURE 6 | Effect of (+)-catechin and theaflavin on ACE2 and TMPRSS2 expression. **(A,D)** The structure of (+)-catechin **(A)** and theaflavin **(D)**. **(B,E)** HepG2 cells were measured by XTT assay after indicated hours of culturing in the presence of (+)-catechin **(B)** or theaflavin **(E)** **(C,F)** Total cell extracts of HepG2 cells were harvested from untreated cells and cells treated with (+)-catechin **(C)** or theaflavin **(F)** for 24 h. The protein was immunoblotted with polyclonal antibodies specific for ACE2 or TMPRSS2. GAPDH was used as an internal loading control. All the results are representative of at least three independent experiments. (Error bars = mean ± S.E.M. * denotes samples significantly different from the control group with $p < 0.05$).

2008; Wu, 2020). Therefore, developing simultaneous inhibitors of both ACE2 and TMPRSS2, rather than only ACE2 inhibitors, may be a more suitable strategy for blocking SARS-CoV-2 infection. Our finding that GB-1 and theaflavin-3-gallate can inhibit the protein expression of both ACE2 and TMPRSS2 indicates that they could be candidates for the prophylaxis or treatment of SARS-CoV-2 infection in the further. In previous studies, STAT3 can affect both the expression of ACE2 and TMPRSS2 (Handle et al., 2018; Shamir et al., 2020). However, our studies showed GB-1 had not affected STAT3 protein expression in HepG2 cells and lung and kidney tissues of mice (**Figures 1D**, **4**). These results suggested GB-1 might affect the expression of ACE2 and TMPRSS2 through other signal pathways.

In our previous study, we discovered that theaflavin has a potential chemical structure of anti-SARS-CoV-2 RNA-dependent RNA polymerase (Lung et al., 2020). In addition, 50 μg/ml of theaflavin was responsible for inhibiting the protein expression of ACE2 and TMPRSS2 in HepG2 cells. Chen et al. (2005) reported that theaflavin-3-gallate inhibited the 3C-like protease activity of SARS-CoV. In another study, (−)-catechin gallate and (−)-gallocatechin gallate demonstrated remarkable inhibition of SARS-CoV nucleocapsid protein (Roh, 2012). Nguyen et al. (2012) also reported that epigallocatechin gallate and gallocatechin gallate, which belong to the catechin family, had good inhibition of the 3C-like protease of SARS-CoV. In our study, 50 μg/ml of theaflavin-3-gallate, theaflavin, and (+)-catechin inhibited the protein expression of ACE2, and 50 μg/ml of theaflavin-3-gallate and theaflavin inhibited

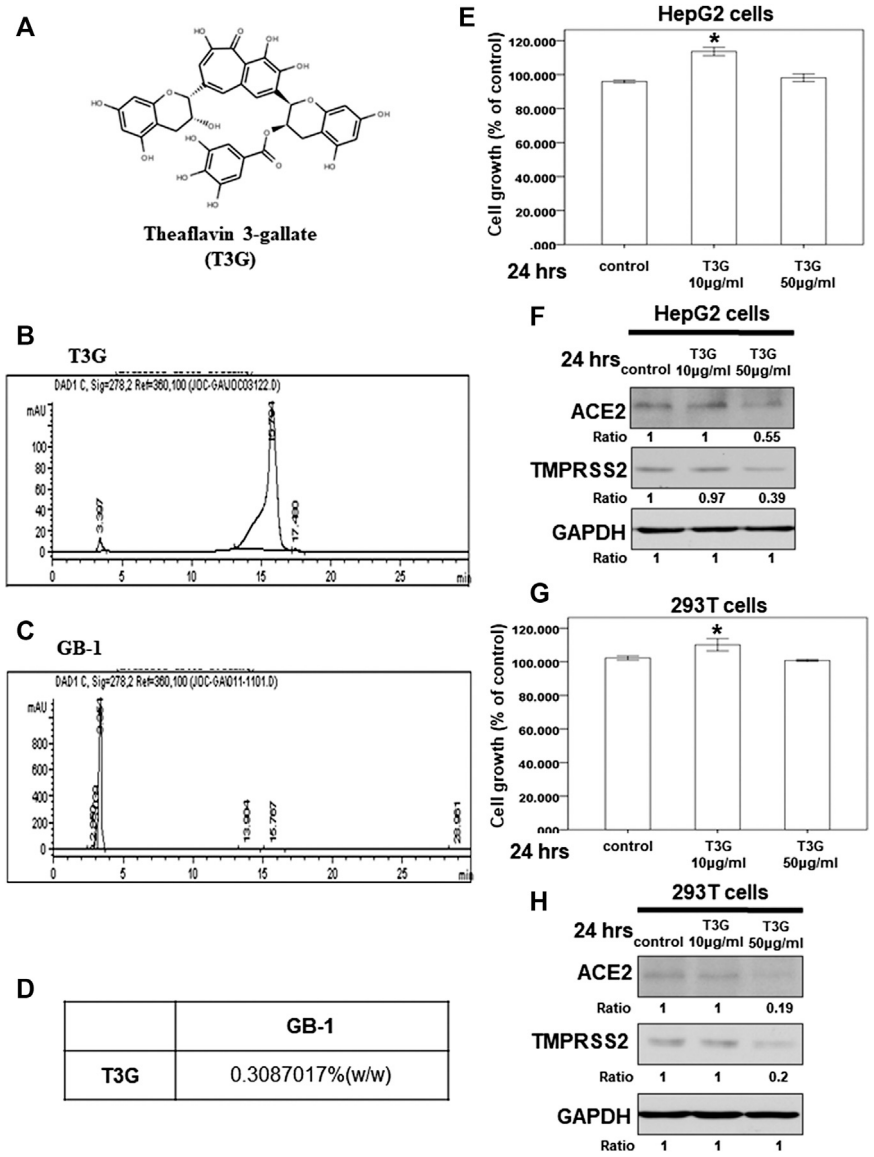

FIGURE 7 | Effect of theaflavin-3-gallate on ACE2 and TMPRSS2 expression. **(A)** The structure of theaflavin-3-gallate. **(B,C)** HPLC chromatograms of theaflavin-3-gallate **(B)** and GB-.1 **(C) (D)** The concentration of theaflavin-3-gallate in GB-1. **(E,G)** HepG2 cells **(E)** and 293T cells **(G)** were measured by XTT assay after indicated hours of culturing in the presence of theaflavin-3-gallate. **(F,H)** Total cell extracts of HepG2 cells HepG2 cells **(F)** and 293T cells **(H)** were harvested from untreated cells and cells treated with theaflavin-3-gallate for 24 h. The protein was immunoblotted with polyclonal antibodies specific for ACE2 or TMPRSS2. GAPDH was used as an internal loading control. All the results are representative of at least three independent experiments. (Error bars = mean ± S.E.M. * denotes samples significantly different from the control group with $p < 0.05$).

TMPRSS2 protein expression. Glycyrrhizin, the bioactive compound in GU, inhibited SARS-CoV replication (Cinatl et al., 2003; Hoever et al., 2005). Our study discovered GU extract was not responsible for inhibiting *ACE2* mRNA and protein expression in HepG2 cells in the concentration range of 0.5–1 mg/ml. However, many compounds in GB-1, including GU and *Camellia sinensis* var. assamica, remain unstudied. Some of these compounds might play important roles in the effect of ACE2 and TMPRSS2. These compounds may work together to inhibit the protein expression of ACE2 and TMPRSS2.

In conculsion, GB-1 is a potential candidate for prophylaxis of SARS-CoV-2 infection through inhibiting protein expression of ACE2 and TMPRSS2. Some index compounds of *Camellia sinensis* var. assamica, including theaflavin-3-gallate, theaflavin, and (+)-catechin, may be essential to inhibiting the protein expression of ACE2 and TMPRSS2. However, the exact clinical effect remains unclear; further studies are necessary to confirm the protective effects of GB-1 and theaflavin-3-gallate against SARS-CoV-2 entry.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital (Approval number 2017081601).

AUTHOR CONTRIBUTIONS

C-YW conceived the idea and designed experiments and wrote manuscript. Y-SL prepared GB-1 and performed the experiments; L-HS, Y-CC, and H-TL analyzed the data. Y-HY revised English writing of the manuscript. All authors reviewed and approved the final version.

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frontiers in Pharmacology

REVIEW
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A Review on Plant Bioactive Compounds and Their Modes of Action Against Coronavirus Infection

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The rapid outbreak of coronavirus disease 2019 (COVID-19) has demonstrated the need for development of new vaccine candidates and therapeutic drugs to fight against the underlying virus, severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). Currently, no antiviral treatment is available to treat COVID-19 as treatment is mostly directed to only relieving the symptoms. Retrospectively, herbal medicinal plants have been used for thousands of years as a medicinal alternative including for the treatment of various viral illnesses. However, a comprehensive description using various medicinal plants in treating coronavirus infection has not to date been described adequately, especially their modes of action. Most other reports and reviews have also only focused on selected ethnobotanical herbs such as Traditional Chinese Medicine, yet more plants can be considered to enrich the source of the anti-viral compounds. In this review, we have screened and identified potential herbal medicinal plants as anti-coronavirus medication across major literature databases without being limited to any regions or ethnobotanic criteria. As such we have successfully gathered experimentally validated *in vivo*, *in vitro,* or *in silico* findings of more than 30 plants in which these plant extracts or their related compounds, such as those of *Artemisia annua* L., *Houttuynia cordata* Thunb., and *Sambucus formosana* Nakai, are described through their respective modes of action against specific mechanisms or pathways during the viral infection. This includes inhibition of viral attachment and penetration, inhibition of viral RNA and protein synthesis, inhibition of viral key proteins such as 3-chymotrypsin-like cysteine protease ($3CL^{pro}$) and papain-like protease 2 (PL^{pro}), as well as other mechanisms including inhibition of the viral release and enhanced host immunity. We hope this compilation will help researchers and clinicians to identify the source of appropriate anti-viral drugs from plants in combating COVID-19 and, ultimately, save millions of affected human lives.

Keywords: COVID-19, drug, herb, SARS, Traditional Chinese medicine (TCM), medicinal plant, natural products, viral infection

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INTRODUCTION

Coronaviruses are known to infect various hosts such as mice (mouse hepatitis virus, MHV), pigs (porcine epidemic diarrhea virus, PEDV), birds (avian coronavirus, IBV) and even human (human coronavirus, HCoV including severe acute respiratory syndrome coronavirus (SARS-CoV), SARS-CoV-2, Middle East Respiratory Syndrome-CoV (MERS-CoV), HCoV-OC43, HCoV-NL63 and HCoV-229E) with different disease severity (Vellingiri et al., 2020; Zhu et al., 2020). On January 30,

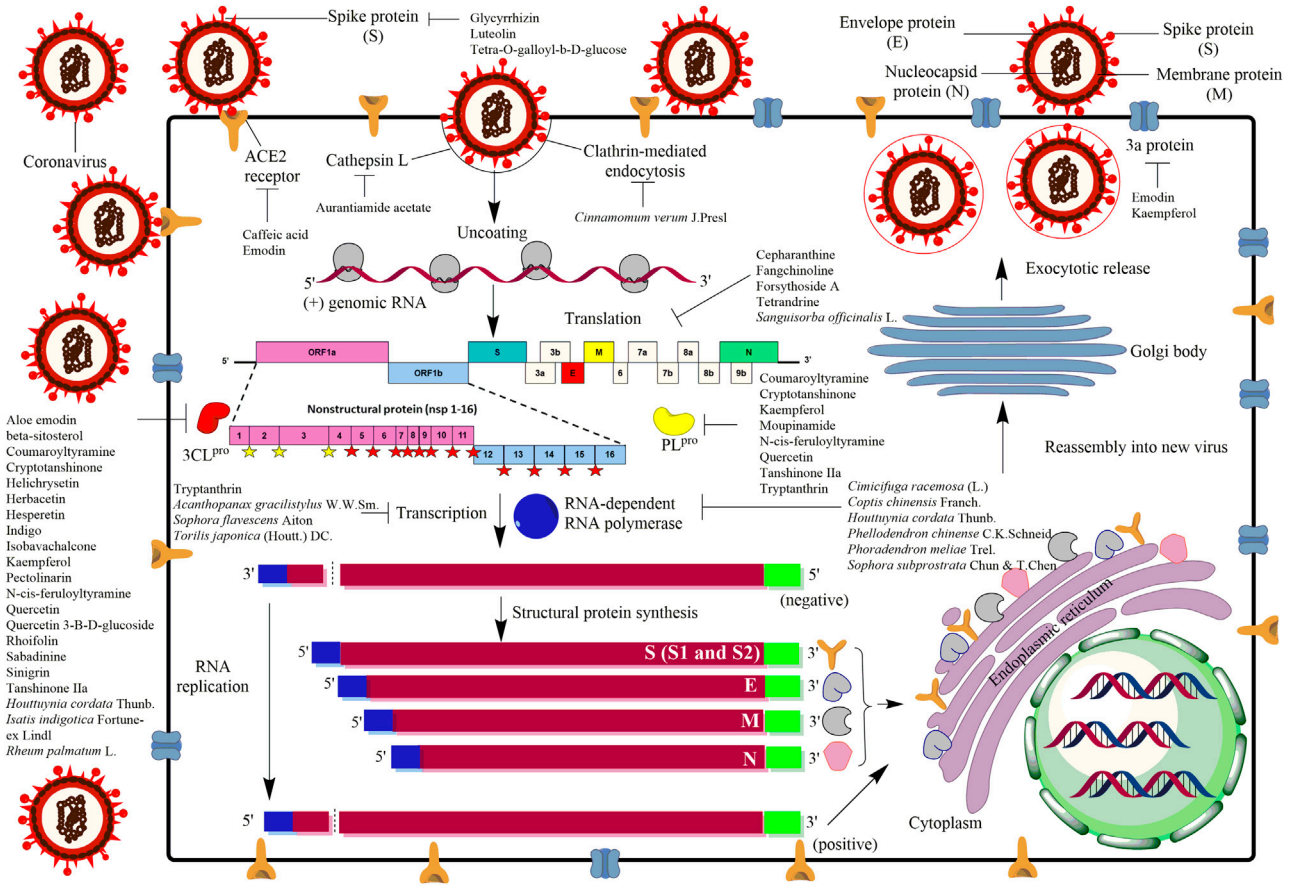

FIGURE 1 | Mechanism of actions of antiviral agents/compounds from plants against coronavirus infection. As the virus initially infects an eukaryotic cell, several stages in the infection process occur which can be the potential sites of attack by the antiviral compounds. The life cycle of the virus begins when spike protein (S) of coronavirus binds to the angiotensin-converting enzyme 2 receptor (ACE2) located on the membrane of the host cells. The receptor binding facilitates viral envelope fusion with the host cell membrane before releasing the viral RNA into the host cell. The viral RNA encodes four structural proteins and two large non-structural polyproteins called ORF1a and ORF1b. These two non-structural polyproteins are translated and cleaved into 16 non-structural proteins (nsp) by two proteinases, 3-chymotrypsin-like cysteine protease ($3CL^{pro}$) and papain-like protease 2 (PL^{pro}). The yellow filled stars are the sites predicted to be cleaved by PLpro whereas the red filled stars are the sites predicted to be cleaved by $3CL^{pro}$. Besides that, the coronavirus genome also encodes structural proteins, including spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. After replication, transcription and translation, viral proteins and genome RNA are subsequently assembled into virions in the endoplasmic reticulum (ER) and Golgi body before they will be transported via vesicles to be released out of the cell as new virus progenies.

2020, the World Health Organization (WHO) declared the outbreak of COVID-19, caused by the SARS-CoV-2, to be a global pandemic, which requires an international public health emergency (Patel and Jernigan, 2020). Ever since its outbreak in December 2019 (Yang et al., 2020), the SARS-CoV-2 virus has spread and caused more than 15.3 million cases and 631,000 deaths worldwide as of July 23, 2020 (https://virusncov.com/). The outbreak originated from the Hunan seafood market in Wuhan, a main city of China which frequently sold live exotic animals such as bats, frogs, snakes, dogs, civets, and marmots (Wang et al., 2020a). Although the zoonotic source of COVID-19 is yet to be verified, however, sequence-based analysis of isolates from infected patients has indicated that bats may serve as the primary reservoir, of which over 80% of the viral genome sequences is identical to the previous human SARS-coronavirus (Wu et al., 2020). Most COVID-19 patients initially suffered from fever, cough, and fatigue while developing other symptoms including muscle pain, headache, shortness of breath, and diarrhea, of which, in extreme cases, severe inflammatory responses may lead to fatality (Chen et al., 2020).

SARS-CoV-2 belongs to the order *Nidovirales*, family *Coronaviridae* and genus *Coronavirus*, which is comprised of single stranded positive RNA sense viruses with 29.7 kb in length (Ksiazek et al., 2003; Marra et al., 2003; Ruan et al., 2003). Such RNA encodes two large non-structural polyproteins and four major structural proteins (**Figure 1**). The non-structural polyproteins, known as ORF1a and ORF1b with protein sizes of 486 and 790 kDa, respectively (Ksiazek et al., 2003; Marra et al., 2003), will undergo co-translational processing by two viral-encoded proteases called 3-chymotrypsin-like cysteine protease ($3CL^{pro}$) and a papain-like protease (PL^{pro}) (Gao et al., 2003; Snijder et al., 2003; Thiel et al., 2003). The processing of ORF1a is carried out by PL^{pro} at three sites (yellow filled star) which release nsp1, nsp2, and nsp3, whereas the rest of the sites are processed by $3CL^{pro}$ (red filled star) releasing nsp4 until nsp16 (Lindner

et al., 2005). Besides that, the viral RNA encodes for the structural proteins, including nucleocapsid (N), envelope (E), membrane (M), and spike (S) proteins (**Figure 1**) (Du et al., 2016; Zhou et al., 2018; Wang et al., 2020b). The N protein is a soluble protein that contains the nucleocapsid packages of the RNA genome. Whereas, E is a small protein, around 76–109 amino acids containing a single predicted hydrophobic domain that is essential for interaction with M protein (Ruch and Machamer, 2012) which is the most prevalent membrane protein in the virion envelope (Masters, 2006; Hogue and Machamer, 2014). Meanwhile, the S protein serves as an important mechanism for viral attachment and fusion to the host cells (**Figure 1**). In general, upon S protein attachment to the host receptor called angiotensin-converting enzyme 2 (ACE2), the coronavirus will enter into the host cell through endocytosis (**Figure 1**). The virus will then release the genomic RNA before the genetic codes are being translated into non-structural polyproteins including $3CL^{pro}$, PL^{pro} and RNA-dependent RNA polymerase (RdRp), followed by other structural proteins (N, M, E and S) (Kumar et al., 2020). Eventually, the viral progenies will bud out with all the complete components including replicated genomic RNA to infect other neighboring regions (**Figure 1**).

Due to its infectivity, scientists have been racing to comprehend the origin and transmission of the virus including elucidating the mechanism for its disease pathogenesis (Liu et al., 2020). A number of comprehensive research have unraveled viable viral genes and proteins as promising future targets for the development of therapeutic agents and vaccines for COVID-19 or other related human coronaviruses (Guo et al., 2020; Liu et al., 2020). However, there are still no clinically approved vaccine or specific antiviral drugs to fight the infection as of July 2020, although more than nine pharmaceutical companies are currently working on this disease (https://www.forbes.com/sites/moneyshow/2020/06/16/9-pharmaceutical-companies-racing-for-a-covid-19-vaccine/#3acd13c576ad). Current treatments used by physicians mainly depend on managing the symptoms such as breathing support using mechanical ventilation, and corticosteroids to reduce lung swelling, as well as the current available antiviral and antibiotics medications (Cascella et al., 2020).

Alternatively, herbal medicine has been used for thousands of years to treat various viral-related illnesses and may become a valuable source of anti-coronovirus treatment (Ling, 2020). Evidently, in 2003, patients who suffered from severe acute respiratory syndrome (SARS) and received Traditional Chinese Medicine (TCM) treatment were reported to have short-term hospitalization (inpatient care), reduced steroid-related side effects, and improvement from the viral symptoms (World Health Organization, 2004). Thus, most previous publications including review papers have mainly focused on the use of TCM and other ethnobotanical herbs (Dudani and Saraogi, 2020; Khanna et al., 2020; Ling, 2020; Vellingiri et al., 2020) or the use of drugs and other dietary supplements that are currently in clinical trials (Di Matteo et al., 2020; Kumar et al., 2020; Stahlmann and Lode, 2020). This may not cover most, if not all, potential plant species or extracts available globally. Besides that, the mechanism of these plant extracts and compounds, especially their modes of action against the viral infection, are still not comprehensively described (Dudani and Saraogi, 2020; Ling, 2020). Therefore, in this review, we have gathered previous studies on medicinal herbs with some evidence against coronavirus infection, either through *in vivo*, *in vitro,* or *in silico* studies, and classified them based on their different modes of actions. This review highlights the potential use of herbal plants and related compounds in treating the coronavirus infection, in the hope that it could lead to a potential source of anti-viral drugs for curbing the COVID-19 outbreak.

All manuscripts were obtained from various databases such as Scopus, PubMed, and Web of Science by using key words (herb* OR herbal) AND (virus OR viral) AND corona* in the search field. We have identified and included research studies starting from year 2004 to year 2020. Relevant papers were selected after critical evaluation on their significance and were further divided based on their experimental designs, whether *in vitro*, *in vivo,* or *in silico* (**Tables 1–3**). However, we acknowledge that the recorded assay values, for examples IC50 and SI values from the *in vitro* analysis (**Table 1**), are not comparable between cell types/lines nor coronavirus types (Kalliokoski et al., 2013) due to their inherent molecular and physiological differences. Thus, the plant herbal extracts/compounds are only discussed within specific application (cell and virus types) of their original reported studies, to avoid over interpretation. Furthermore, the potential use of various herbs are described in this review according to their modes of action including inhibition of viral attachment and penetration, inhibition of RNA and protein synthesis, inhibition of viral proteases, inhibition of viral release and enhancement of host immunity as well as other mechanisms.

Inhibition of Viral Attachment and Penetration

The inhibition of viral penetration and attachment is an effective way to curb coronavirus infection. Infectious virion binds to cell membrane receptors, permeates the cellular membranes and removes the virion's protein coat once entered into the cell cytoplasm, releasing viral nucleic acid (**Figure 1**) (Smith et al., 1980). Coronavirus S protein plays a critical role in viral attachment, fusion, and entry, making it a potential target in the development of vaccines, antibodies, and inhibitors (Liu et al., 2004; Du et al., 2009, 2017; Wang et al., 2016). The S protein modulates the viral penetration through the first binding on the host cells using receptor-binding domain in the S1 subunit and then fusion into host cells via the S2 subunit through the host ACE2 receptor (Liu et al., 2004). However, different subtypes of coronavirus have been described to recognize different receptors. For examples, SARS-CoV specifically recognizes the ACE2 receptors, while MERS-CoV identified dipeptidyl peptidase 4 (DPP4) receptors (Li et al., 2003; Raj et al., 2013). The current SARS-CoV-2 binds ACE2 receptor (Zhou et al., 2020), thus inhibition at these S proteins or ACE2 may inhibit the viral attachment from entering host cells (**Figure 1**).

Among 121 herbal compounds that were screened by Wu et al. (2004), tetra-O-galloyl-β-D-glucose (TGG) and luteolin, isolated from *Rhus chinensis* Mill. and *Veronica linariifolia* Pall. ex Link,

TABLE 1 | *In vitro* antiviral activity of various herbal extracts against coronavirus infection.

No.	Herbs	Extract/Compound	Coronavirus type	CC50 (conc.) or cell survivability (%)	IC50	SI	References
1.	*Acanthopanax gracilistylus* W.W.Sm.	Methanol extract	MHV-A59	170.0 ± 6.4 μg/ml	0.9 ± 0.1 μg/ml	188.9	Kim et al. (2010)
2.	*Artemisia annua* L.	Ethanol extract	SARS-CoV BJ-001	1,053.0 ± 92.8 μg/ml	34.5 ± 2.6 μg/ml	31	Li et al. (2005)
			SARS-CoV BJ-006	1,053.0 ± 92.8 μg/ml	39.2 ± 4.1 μg/ml	27	
3.	*Cimicifuga racemose* (L.) Nutt.	Methanol extract	MHV-A59	239.0 ± 44.4 μg/ml	19.4 ± 7.0 μg/ml	12.3	Kim et al. (2008)
4.	*Cinnamomum verum* J.Presl (cortex)	Butanol extract	wtSARS-CoV	180.0 ± 6.0 μg/ml	7.8 ± 0.3 μg/ml	23.1	Zhuang et al. (2009)
			HIV/SARS-CoV	444.0 ± 13.7 μg/ml	85.3 ± 7.5 μg/ml	5.2	
		Procyanidin A2	wtSARS-CoV	1,116.7 ± 60.3 μM	29.9 ± 3.3 μM	37.35	
			HIV/SARS-CoV	796.6 ± 63.7 μM	120.7 ± 13.1 μM	4.08	
		Procyanidin B1	wtSARS-CoV	648.2 ± 43.4 μM	41.3 ± 3.4 μM	15.69	
			HIV/SARS-CoV	656.2 ± 36.7 μM	161.1 ± 20.3 μM	4.08	
		Cinnamtannin B1	wtSARS-CoV	184.7 ± 15.5 μM	32.9 ± 3.9 μM	5.61	
			HIV/SARS-CoV	242.3 ± 14.8 μM	32.9 ± 2.8 μM	7.36	
5.	*Coptis chinensis* Franch.	Methanol extract	MHV-A59	71.3 ± 7.2 μg/ml	2.0 ± 0.5 μg/ml	34.9	Kim et al. (2008)
6.	*Epimedium koreanum* Nakai	Water extract	PEDV, SM98, TGE	ND	>90% at 1.5 mg/ml	ND	Cho et al. (2012)
7.	*Euphorbia neriifolia* L.	3β-friedelanol	HCoV	NC	132.4% at 5 μg/ml	NC	Chang et al. (2012)
		3β-acetoxy friedelane	HCoV	NC	80.9% at 5 μg/ml	NC	
		Friedelin	HCoV	NC	109.0% at 5 μg/ml	NC	
		Epitaraxerol	HCoV	NC	111.0% at 5 μg/ml	NC	
8.	*Forsythia suspensa* (Thunb.) Vahl	Forsythoside A	IBV	<1.28 mM	0.64 mM	ND	Li et al. (2011)
9.	*Glycyrrhiza glabra* L.	Glycyrrhizin	SARS-CoV	>20,000 mg/ml	300 mg/ml	>67	Cinatl et al. (2003), Hoever et al. (2005), Hoever et al. (2005)
		Glycyrrhizin	SARS-CoV	>24,000 μM	365 ± 12 μM	>65	
		Glycyrrhetinic acid	SARS-CoV	>20 μM	20 ± 5 μM	NC	
		Derivative GL 1	SARS-CoV	>3,000 μM	40 ± 13 μM	>75	
		Derivative GL 2	SARS-CoV	1,462 ± 50 μM	35 ± 7 μM	41	
		Derivative GL 3	SARS-CoV	215 ± 18 μM	139 ± 20 μM	2	
		Derivative GL 9	SARS-CoV	44 ± 6 μM	8 ± 2 μM	6	
		Derivative GL 10	SARS-CoV	250 ± 19 μM	50 ± 10 μM	5	
		Derivative GL 11	SARS-CoV	15 ± 3 μM	5 ± 3 μM	3	
		Derivative GL 12	SARS-CoV	66 ± 8 μM	16 ± 1 μM	4	
10.	*Houttuynia cordata* Thunb.	Water extract	IBV	250 mg/ml	62.5 mg/ml	4	Yin et al. (2011)
		Water extract	SARS-CoV	NC	1,000 μg/ml	NC	Lau et al. (2008)
		Water extract	SARS-CoV	NC	50, 100, 200, 400 and 800 μg/ml	NC	Lau et al. (2008)
		Ethyl acetate	MHV	>3.91 μg/ml	0.98 μg/ml	>4.00	Chiow et al. (2016)
		Quercetin	MHV	116.52 μg/ml	125 μg/ml	0.93	Chiow et al. (2016)
11.	*Isatis indigotica* Fortune ex Lindl.	Water extract	SARS-CoV	>5,000 μg/ml	191.6 ± 8.2 μg/ml	>26	Lin et al. (2005)
		Indigo	SARS-CoV	7,375 μM	752 μM	9.8	
		Sinigrin	SARS-CoV	>10,000 μM	217 μM	>46	
		Beta-sitosterol	SARS-CoV	1,475 μM	1,210 μM	1.21	
		Aloe emodin	SARS-CoV	11,592 μM	366 μM	31.67	
		Hesperetin	SARS-CoV	2,718 μM	8.3 μM	327.47	
12.	*Lindera aggregata* (Sims) Kosterm.	Ethanol extract	SARS-CoV BJ-001	1,374.0 ± 39.0 μg/ml	88.2 ± 7.7 μg/ml	16	Li et al. (2005)
			SARS-CoV BJ-006	1,374.0 ± 39.0 μg/ml	80.6 ± 5.2 μg/ml	17	
13.	*Lycoris radiata* (L'Hér.) Herb.	Ethanol extract	SARS-CoV BJ-001	886.6 ± 35.0 μg/ml	2.4 ± 0.2 μg/ml	370	Li et al. (2005)
			SARS-CoV BJ-006	886.6 ± 35.0 μg/ml	2.1 ± 0.2 μg/ml	422	
		Lycorine	SARS-CoV	14,980 nM	15.7 nM	954.14	
14.	*Phoradendron meliae* Trel.	Methanol extract	MHV-A59	334.3 ± 7.0 μg/ml	13.0 ± 1.4 μg/ml	25.6	Kim et al. (2008)
15.	*Pelargonium sidoides* DC.	Aqueous extract (EPs® 7630)	HCoV-229E	>100 μg/ml (87%)	44.5 μg/ml	>2.3	Michaelis et al. (2011)
16.	*Phellodendron chinense* C.K.Schneid.	Methanol extract	MHV-A59	139.5 ± 81.3 μg/ml	10.4 ± 2.2 μg/ml	13.4	Kim et al. (2008)

(Continued on following page)

TABLE 1 | (*Continued*) *In vitro* antiviral activity of various herbal extracts against coronavirus infection.

No.	Herbs	Extract/Compound	Coronavirus type	CC50 (conc.) or cell survivability (%)	IC50	SI	References
17.	*Polygonum multiflorum* Thunb. (vine)	Water extract	SARS-CoV	NC	1–10 μg/ml	NC	Ho et al. (2007)
	Polygonum multiflorum Thunb. (root)	Emodin	SARS-CoV	NC	200 μM	NC	Ho et al. (2007)
		Water extract	SARS-CoV	NC	1–10 μg/ml	NC	Ho et al. (2007)
18.	*Pyrrosia lingua* (Thunb.) Farw.	Chloroform	SARS-CoV BJ-001	2,378.0 ± 87.3 μg/ml	43.2 ± 14.1 μg/ml	55	Li et al. (2005)
			SARS-CoV BJ-006	2,378.0 ± 87.3 μg/ml	40.5 ± 3.7 μg/ml	59	
19.	*Rheum officinale* Baill. (root)	Water extract/Emodin	SARS-CoV	NC	1–10 μg/ml	NC	Ho et al. (2007)
20.	*Rheum palmatum* L.	Ethyl acetate extract	SARS-CoV	NC	13.76 ± 0.03 μg/ml	NC	Luo et al. (2009)
21.	*Rhus chinensis* Mill.	Tetra-O-galloyl-β-D-glucose (TGG)	SARS-CoV	1,080 μM	4.5 μM	240	Wu et al. (2004)
22.	*Sanguisorba officinalis* L.	Methanol extract	MHV-A59	388.4 ± 4.5 μg/ml	3.7 ± 1.4 μg/ml	105.0	Kim et al. (2010)
23.	*Sambucus formosana* Nakai	Ethanol extract	HCoV-NL63	180.62 ± 63.04 μg/ml	1.17 ± 0.75 μg/ml	154.37	Weng et al. (2019)
		Caffeic acid	HCoV-NL63	>500 μM	3.54 ± 0.77 μM	NC	
		Chlorogenic acid	HCoV-NL63	>500 μM	43.45 ± 6.01 μM	NC	
		Gallic acid	HCoV-NL63	>500 μM	71.48 ± 18.40 μM	NC	
24.	*Sophora flavescens* Aiton	Methanol extract	MHV-A59	556.8 ± 2.9 μg/ml	0.8 ± 0.2 μg/ml	696.0	Kim et al. (2010)
25.	*Sophora subprostrata* Chun & T.Chen	Methanol extract	MHV-A59	307.3 ± 6.6 μg/ml	27.5 ± 1.1 μg/ml	11.1	Kim et al. (2008)
26.	*Stephania tetrandra* S.Moore	Tetrandrine	HCoV-OC43	13.41 ± 0.36 μM	0.33 ± 0.03 μM	40.19	Kim et al. (2019)
		Fangchinoline	HCoV-OC43	11.54 ± 0.46 μM	1.01 ± 0.07 μM	11.46	
		Cepharanthine	HCoV-OC43	11.26 ± 0.69 μM	0.83 ± 0.07 μM	11.63	
27.	*Strobilanthes cusia* (Nees) Kuntze	Methanol extract	HCoV-NL63	>100 μg/ml	0.64 ± 0.43 μg/ml	156.25	Tsai et al. (2020)
		Tryptanthrin	HCoV-NL63	>400 μM	1.52 ± 0.13 μM	263.16	
		Indigodole B	HCoV-NL63	>400 μM	2.60 ± 0.11 μM	153.85	
28.	*Toona sinensis* (Juss.) M.Roem.	Water extract	SARS-CoV	>500 μg/ml	30–43 μg/ml	17	Chen et al. (2008)
29.	*Torilis japonica* (Houtt.) DC.	Methanol extract	MHV-A59	156.5 ± 2.6 μg/ml	0.8 ± 0.0 μg/ml	195.6	Kim et al. (2010)
30.	*Veronica linariifolia* Pall. ex Link	Luteolin	SARS-CoV	10,600 μM	10.6 μM	14.62	Wu et al. (2004)
31.	Various (Chinese medicinal herbs)	Emodin	SARS-CoV	NC	50% at 20 μM	NC	Schwarz et al. (2012)
		Kaempferol	SARS-CoV	NC	20% at 20 μM	NC	
		Kaempferol glycosides	SARS-CoV	NC	>50% at 20 μM	NC	
32.	Various (used flavonoid library)	Herbacetin	MERS & SARS-CoV	NC	33.17–40.59 μM	NC	Jo et al. (2019), Jo et al. (2020)
		Rhoifolin	SARS-CoV	NC	27.45 μM	NC	Jo et al. (2020)
		Pectolinarin	SARS-CoV	NC	37.78 μM	NC	Jo et al. (2020)
		Isobavachalcone	MERS-CoV	NC	35.85 μM	NC	Jo et al. (2019)
		Quercetin 3-β-d glucoside	MERS-CoV	NC	37.03 μM	NC	Jo et al. (2019)
		Helichrysetin	MERS-CoV	NC	67.04 μM	NC	Jo et al. (2019)

CC50 is the cytotoxicity concentration of the extracts or compounds that can inhibit 50% population of host cells. IC50 is the inhibitory concentration of the extracts or compound that can cause 50% of virus inhibition or reduction after it has been treated with extract/compound. Selectivity index (SI) is calculated based on the ratio of CC50/IC50. The higher the value of SI, the lesser cytotoxicity for the host and hence being potentially safe to be applied as a future antiviral agent. NC is non-calculable, and ND is data not determined/shown from respective articles. Abbreviation: MHV, mouse hepatitis virus; wtSARS-CoV; wild-type SARS coronavirus; HIV, human immunodeficiency virus; PEDV, porcine epidemic diarrhea virus; TGE, transmissible gastroenteritis coronavirus; HCoV, human coronavirus; IBV, avian coronavirus; $3CL^{pro}$, 3-chymotrypsin-like cysteine protease; PL^{pro}, papain-like protease 2; RdRp, RNA-dependent RNA polymerase; SARS, severe acute respiratory syndrome; MERS, middle east respiratory syndrome

respectively, were identified to have the highest affinity to the S2 subunit of the virus, thus postulating that these compounds may interfere with the viral cell fusion process (**Figures 1**, **2**) (Wu et al., 2004). TGG and luteolin exhibit significant anti-SARS-CoV activity with IC_{50} of 4.5 and 10.6 μM, respectively, as well as very high selectivity index of 240.0 and 14.62, respectively (**Table 1**). Selectivity index (SI) is determined based on the proportion of cytotoxicity concentration of the extracts or compounds that can inhibit 50% population of host cells (CC_{50}) to inhibitory concentration of the extracts or compounds that can cause 50% of virus inhibition (IC_{50}). The higher the value of SI, the lesser cytotoxicity for the host and hence it being potentially safe to be applied as a future antiviral agent (**Table 1** and **Figure 2**) (Wu et al., 2004). Thus, this suggested that both compounds can be effective against the coronavirus and, more importantly, safe for human application.

TABLE 2 | *In silico* antiviral activity of various herbal extracts against coronavirus protein target.

No.	Herbs	Compounds	Inhibition target	Docking binding energy (kcal/mol)		References
				PLpro	3CLpro	
1.	Various (Traditional Chinese Medicine Systems Pharmacology Database)	Coumaroyltyramine	PL^{pro} and $3CL^{pro}$	−3.22	−4.18	Zhang et al. (2020)
		Cryptotanshinone	PL^{pro} and $3CL^{pro}$	−5.25	−6.23	
		Desmethoxyreserpine	Replication, $3CL^{pro}$, $3CL^{pro}$	Not significant	−3.52	
		Dihomo-γ-linolenic acid	S protein	Not significant	−3.88	
		Kaempferol	Replication, $3CL^{pro}$	−2.15	−6.01	
		Lignan	PL^{pro}	Not significant	−4.27	
		Moupinamide	PL^{pro} and $3CL^{pro}$	−3.05	Not significant	
		N-cis-feruloyltyramine	PL^{pro} and $3CL^{pro}$	−3.11	−4.31	
		Quercetin	Replication, $3CL^{pro}$	−4.62	−6.25	
		Sugiol	PL^{pro} and $3CL^{pro}$	Not significant	−6.04	
		Tanshinone IIa	PL^{pro} and $3CL^{pro}$	−5.02	−5.17	
2.	Various (Traditional Chinese Medicine Database)	Aurantiamide acetate	SARS-CoV cathepsin-L (−50.767 kcal/mol)	Not carried out	Not carried out	Wang et al. (2007)
3.	Various (flavonoid library)	Herbacetin	SARS & MERS-CoV $3CL^{pro}$	Not carried out	−9.263 & −10.246	Jo et al. (2020)
		Rhoifolin	SARS-CoV $3CL^{pro}$	Not carried out	−9.565	
		Pectolinarin	SARS-CoV $3CL^{pro}$	Not carried out	−8.054	
		Isobavachalcone	MERS-CoV $3CL^{pro}$	Not carried out	−9.364	Jo et al. (2019)
		Quercetin 3-β-d glucoside	MERS-CoV $3CL^{pro}$	Not carried out	−9.751	
		Helichrysetin	MERS-CoV 3CLpro	Not carried out	−9.953	
4.	*Veratrum sabadilla* Retz.	Sabadinine	$3CL^{pro}$	Not carried out	−11.6	Toney et al. (2004)

TABLE 3 | *In vivo* antiviral activity of various herbal extracts against coronavirus infection.

No.	Herbs	Extract/ Compound	Coronavirus type	Animal model	Concentration	Observation, survivability and/or biopsy	References
1.	*Allium sativum* L.	Water extract	IBV	Chicken embryo	0.1 ml of extract	Less dwarfism observed in chicken embryos treated with garlic	Mohajer Shojai et al. (2016)
2.	*Epimedium koreanum* Nakai	Water extract	PEDV	Pig	0.6% of extract	No disease symptoms such as diarrhoea Biopsy results showed clean intestine	Cho et al. (2012)
3.	*Houttuynia cordata* Thunb.	Water extract	IBV	Chicken embryo	62.5 mg/ml	Fully protected the chicken embryos and more than 50% protection rate in chickens	Yin et al. (2011)

In addition, glycyrrhizin isolated from *Glycyrrhiza glabra* L. (licorice) also has been identified to inhibit viral attachment and penetration (**Figure 1**) (Cinatl et al., 2003). In addition, licorice root has been known historically as a powerful antiviral herb (Fiore et al., 2008), used thousands of years ago as a folk medicine for the treatment of throat infection, asthma, bronchitis, peptic ulcers, inflammation, and allergies (Das et al., 1989; Krausse et al., 2004; Nassiri Asl and Hosseinzadeh, 2008). Glycyrrhizin or its other name glycyrrhizic acid (**Figure 2**) is the principal bioactive component of triterpenoid glycoside in this herb, having demonstrated promising inhibition of SARS-CoV (Chen et al., 2004). Evidently, the compound was found more effective (SI = over 67; **Table 1**) against the replication of clinical isolates of SARS-CoV than several synthetic antivirals, such as ribavirin, pyrazofurin, 6-azauridine, and mycophenolic acid (Cinatl et al., 2003). Glycyrrhizin also has been shown to suppress inflammation through downregulation of proinflammatory mediators (Ramos-Tovar and Muriel, 2019; Khanna et al., 2020), but its effectiveness in combating COVID-19 severe inflammation including cytokine storm requires further investigation.

Another study conducted on 312 controlled traditional herbs successfully identified two species from the family *Polygonaceae* to inhibit the interaction of coronavirus S protein and ACE2 (Ho

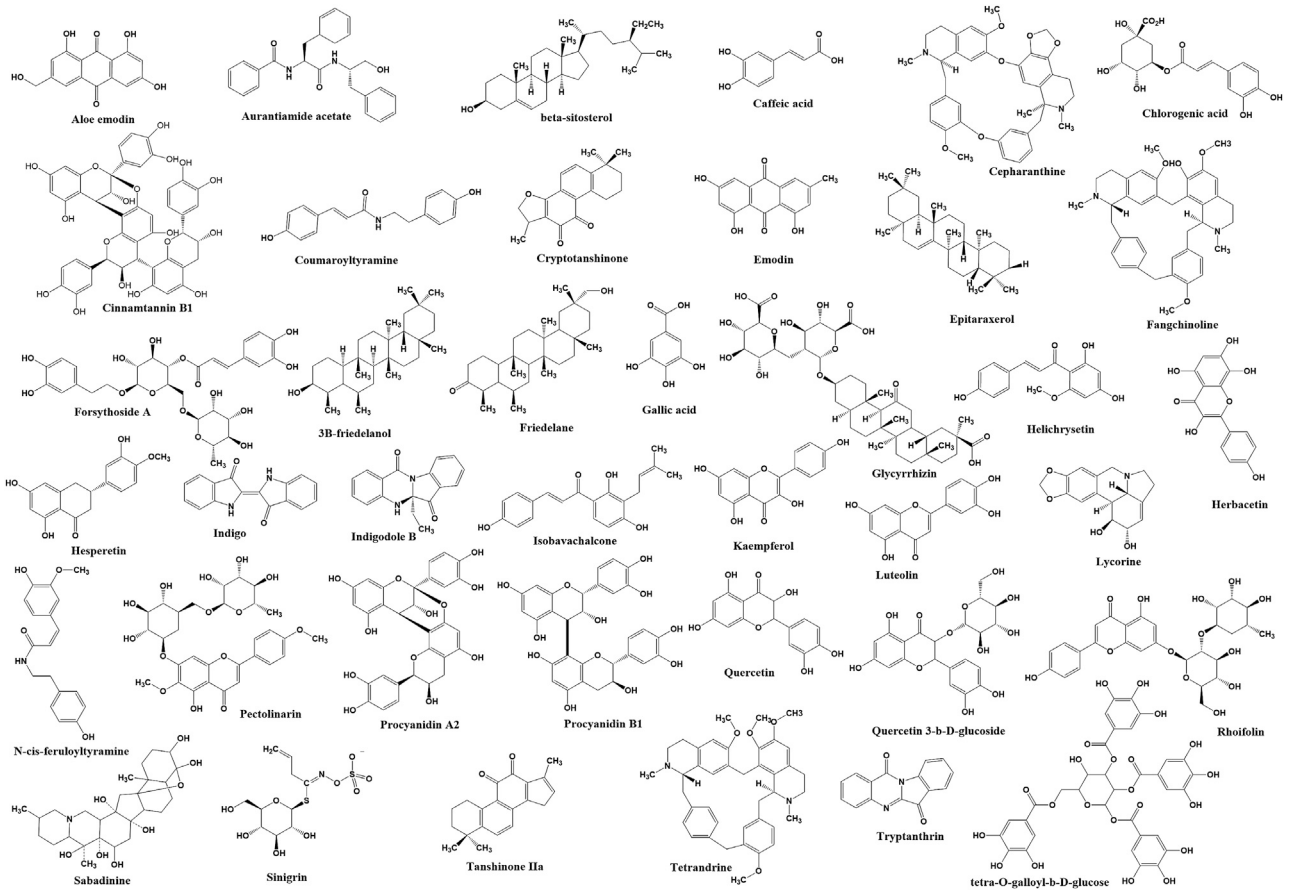

FIGURE 2 | The chemical structure of bioactive compounds identified from plants that have inhibition effects on coronavirus infection.

et al., 2007). Water extracts from the root tubers of *Rheum officinale* Baill., as well as the vines and root tubers of *Polygonum multiflorum* Thunb., showed IC_{50} range of 1–10 μg/ml for the viral inhibition (**Table 1**) (Ho et al., 2007). Emodin, an anthraquinone glycoside compound found from both species (**Figure 2**), significantly obstructed the S protein and ACE2 interaction (**Figure 1**) in a dose-dependent way (Ho et al., 2007). This compound also inhibited the S protein-pseudotyped retrovirus infectivity in Vero E6 cells (Ho et al., 2007), thus showing promising potential for blocking the viral entry (**Figure 1**) (Ho et al., 2007).

Besides that, ethanol extract of *Sambucus formosana* Nakai, a species of elderberry, also has been identified to have very potent anti-viral effect as it has very low IC50 at 1.17 μg/ml and high SI of 154.37, against the coronavirus (**Table 1**) (Weng et al., 2019). Caffeic acid isolated from the plant extract (**Figure 2**) also demonstrated potential anti-coronavirus activity through viral entry inhibition. This compound was able to impair the binding interaction of human coronavirus NL63 (HCoV-NL63) with ACE2 receptor (**Figure 1**) (Chiou et al., 2017) and heparan sulphate proteoglycans (co-receptor) (Milewska et al., 2014) on the host cell surface. In addition to caffeic acid, two other phenolic acid constituents from this plant extract, chlorogenic acid and gallic acid, were found to exhibit antiviral effect by reducing the development of HCoV-NL63 particles *in vitro* (**Table 1** and **Figure 2**) (Weng et al., 2019). The IC50 of these two compounds showed promising viral inhibition (chlorogenic acid IC50 = 43.45 μM and gallic acid IC50 = 71.48 μM) but the caffeic acid was more potent (IC50 = 3.54 μM) (**Table 1**) (Weng et al., 2019).

The coronavirus S-protein also utilizes endosomal cathepsin L-protease enzymatic activity for viral entry (**Figure 1**) (Simmons et al., 2005; Huang et al., 2006). Cathepsin L stimulates the S protein-mediated membrane fusion by promoting receptor dependent and acid-dependent conformational changes in the S2 domain in which a low pH permits optimal proteolytic activity in endosomes (Huang et al., 2006; Bosch et al., 2008). This indicates that the S protein may be triggered proteolytically during the entry of the virus into infected cells throughout the endocytic route (Simmons et al., 2005; Huang et al., 2006). Screening of various natural compounds based on the Traditional Chinese Medicine (TCM) database has identified aurantiamide acetate derived from *Artemisia annua* L. plant (**Figure 2**) (Wang et al., 2007) to inhibit the SARS cathepsin L with the lowest docking binding energy (−50.767 kcal/mol) (**Table 2**), thus possibly blocking viral entry (**Figure 1**) (Wang

et al., 2007). This plant is originally known to exhibit many therapeutic efficacies especially against flu-like symptoms, such as antitracheitis (inflammation at trachea), cough relief, phlegm removal, asthma relief and others (Zhou et al., 2011). These findings suggest the compound could be implemented as medication to prevent SARS-CoV infection, although this should be evaluated further in animal studies in future (Du et al., 2009).

SARS-CoV also utilizes endocytosis route mediated by clathrin to invade host cells (**Figure 1**) (Inoue et al., 2007). Clathrin-based endocytosis has been well characterized to use growth factor receptors such as the transferrin receptor (TfR) (Mellman, 1996), epidermal growth factor receptor (Stang et al., 2004), and the keratinocyte growth factor receptor (Belleudi et al., 2003). Butanol fraction of *Cinnamomum verum* J. Presl (cortex) was shown to inhibit wild-type SARS-CoV (wtSARS-CoV) infection and HIV/SARS-CoV S pseudovirus infections by inhibiting clathrin-mediated endocytosis pathway through TfR receptor (**Table 1** and **Figure 1**) (Zhuang et al., 2009). Besides that, procyanidin A2, procyanidin B1, and cinnamtannin B1 successfully fractionated from this plant (**Figure 2**) showed moderate anti-wtSARS-CoV activity (IC50s of 29.9, 41.3 and 32.9 μM, respectively, and SIs of 37.35, 15.69, and 5.61, respectively) (**Table 1**) (Zhuang et al., 2009). However, the procyanidins have not been shown to inhibit the internalization of the virus (Zhuang et al., 2009) and hence more studies needs to be performed to elucidate their exact mechanism of inhibition.

Inhibition of RNA and Protein Synthesis

Several plants such as *Sanguisorba officinalis* L., *Stephania tetrandra* S. Moore, and *Strobilanthes cusia* (Nees) Kuntze also have anti-viral activity towards RNA and protein synthesis of the coronavirus. For instance, N protein expression of mouse hepatitis virus, MHV-A59 was reduced in *Sanguisorba officinalis* L. treatment, suggesting this plant extract might negatively affect the viral nucleocapsid formation (**Figure 1**) (Kim et al., 2010). Besides that, natural bis-benzylisoquinoline alkaloids such as tetrandrine, fangchinoline, and cepharanthine isolated from *Stephania tetrandra* S.Moore (**Figure 2**) were able to suppress the expression of viral S and N proteins, thus inhibiting the replication of human coronavirus OC43 (HCoV-OC43) infection in MRC-5 human lung cells (**Figure 1**) (Kim et al., 2019). In addition to that, *Strobilanthes cusia* (Nees) Kuntze leaf methanol extract strongly suppressed HCoV-NL63-infected cells with IC50 of 0.64 μg/ml (**Table 1**), and the HCoV-NL63 infection was potently inhibited in a concentration-dependent manner (Tsai et al., 2020). Tryptanthrin and indigodole B (5aR-ethyltryptanthrin) are among the various bioactive compounds present in the extract of *S. cusia* (Nees) Kuntze (**Figure 2**) that exhibited significant antiviral activity in minimizing the cytopathic effect and development of viral progeny with IC_{50} values of 1.52 and 2.60 μM, respectively (**Table 1**) (Tsai et al., 2020). Different modes of time-of-addition/removal assay demonstrated that tryptanthrin prevented the replication of HCoV-NL63 in the early and the late replication stages, mainly by blocking genome synthesis of viral RNA (**Figure 1**) (Tsai et al., 2020).

Furthermore, intracellular viral RNA concentrations were also reduced in the extracts of *Sophora flavescens* Aiton, *Acanthopanax gracilistylus* W.W.Sm., and *Torilis japonica* (Houtt.) DC. with a comparable reduction in viral protein and MHV-A59 production (Kim et al., 2010). Moreover, the extracts reduced the replication of other subtypes of coronavirus such as the John Howard Mueller strain of MHV and porcine epidermic diarrhea virus (PEDV) *in vitro* (Kim et al., 2010). Besides that, an active ingredient of forsythoside A isolated from the fruits of *Forsythia suspensa* (Thunb.) Vahl has been identified to reduce the viral yield and decreased the expression of infectious bronchitis virus (IBV) N gene (**Figure 1**) in contrast to untreated IBV infected cells (Li et al., 2011). Forsythoside A is completely able to inhibit IBV in primary chicken embryo kidney cells and abrogated the virus progeny *in vitro* at concentration of 0.64 mM (**Table 1** and **Figure 2**) (Li et al., 2011). This compound also effectively inhibited pathogenic bacteria including *Staphylococcus aureus* (Nishibe et al., 1982). *Forsythia suspensa* (Thunb.) Vahl is typically used to prevent inflammation, pyrexia and emesis in traditional Chinese medicine (Li et al., 2011).

RNA-dependent RNA polymerase (RdRp) is a replicase enzyme that is crucial for the transcription and replication of coronavirus (Thiel et al., 2003). Since RdRp has been identified to play a vitally important role for the virus life cycle, several polymerase inhibitors such as Remdesivir have been implemented for the treatment of varying viral infections such as human immunodeficiency virus type 1 (HIV-1), human hepatitis B virus (HBV), hepatitis C virus (HCV), Zika virus, and herpes virus (Korba et al., 2006; Mercorelli et al., 2018; Gordon et al., 2020). Thus, the inhibition of this enzyme can potentially be used for the discovery of anti-SARS-CoV agent (Lau et al., 2008). For example, *H. cordata* Thunb. has been identified to act on RdRp activity (**Figure 1**) (Lau et al., 2008). A study on the polymerase activity with different concentration levels of *H. cordata* Thunb. water extract (50, 100, 200, 400 and 800 μg/ml) showed a marked reduction in RdRp activity (**Table 1**) (Lau et al., 2008). Moreover, the oral acute toxicity test showed that *H. cordata* Thunb. was not harmful toward mice after implementing oral administration at 16 g/kg (Lau et al., 2008) and 2000 mg/kg (Chiow et al., 2016), thus it would be potentially safe for human consumption. Several plant extracts from *Cimicifuga racemosa* (L.) Nutt., *Phellodendron chinense* C.K.Schneid., *Sophora subprostrata* Chun & T.Chen, *Phoradendron meliae* Trel., and *Coptis chinensis* Franch. were also known to inhibit RdRp activity (**Figure 1**) (Kim et al., 2008). Methanol extracts from these plants decreased the production of intracellular viral RNA and protein expression in murine coronavirus (MHV) with IC_{50} values between 2.0 and 27.5 μg/ml (**Table 1**) (Kim et al., 2008). These extracts also significantly decreased PEDV production *in vitro,* a coronavirus that infects the cell lining of pig small intestine (Kim et al., 2008).

Inhibition of Viral Proteases, $3CL^{pro}$ and PL^{pro}

Viral proteases such as $3CL^{pro}$ and PL^{pro} also play a prominent role in the replication of coronavirus and presently have become the potential drug target for the development of anti-coronavirus agents (Anand et al., 2003; Han et al., 2005; Gan et al., 2006; Zhang et al.,

2020). These two proteases (PL^{pro} and $3CL^{pro}$) are responsible for the synthesis and maturation of the various viral polyproteins as shown in **Figure 1**, and hence they are vital for the biogenesis of the virus replication complex (Lindner et al., 2005). We identified several plants and compounds that have significant activities to inhibit these enzymes. However, compared to PL^{pro}, many more studies have been conducted on $3CL^{pro}$, possibly as it was able to generate 12 important non-structural proteins (nsp 4 to nsp 16), including the viral RdRp (nsp 12) and helicase (nsp 13) (Rut et al., 2020).

In silico docking analysis has identified 13 herbal compounds such as coumaroyltyramine, cryptotanshinone, kaempferol, N-cis-feruloyltyramine, quercetin, and tanshinone IIa that are able to inhibit $3CL^{pro}$ and PL^{pro} (**Figures 1**, **2** and **Table 2**) (Zhang et al., 2020). Furthermore, tryptanthrin isolated from the extract of *S. cusia* (Nees) Kuntze (**Figure 2**, **Table 1**) also has been identified to inhibit PL^{pro} activity of the HCoV-NL63 *in vitro* (**Figure 1**) (Tsai et al., 2020). Another *in silico* study has identified several other plant compounds such as helichrysetin, herbacetin, isobavachalcone, pectolinarin, quercetin 3-β-D-glucoside, and rhoifolin (**Figure 2**) to effectively interrupt the enzymatic activity of $3CL^{pro}$ of coronavirus (**Figure 1** and **Table 2**) (Jo et al., 2019, 2020). Moreover, sabadinine, a naturally occurring bioactive compound originally isolated from the Lily plant *Veratrum sabadilla* Retz. (**Figure 2**) (Sayre, 1907), was also shown able to dock into the active site of $3CL^{pro}$ (**Table 2**) (Toney et al., 2004).

Besides that, *H. cordata* Thunb., *Isatis indigotica* Fortune ex Lindl. and *R. palmatum* L. also exhibited significant inhibitory effects on $3CL^{pro}$ of SARS-CoV in *in vitro* experiments (**Figure 1** and **Table 1**) (Lin et al., 2005; Lau et al., 2008; Luo et al., 2009). The activity of $3CL^{pro}$ was reduced to 50% at the maximum concentration of 1,000 μg/ml of *H. cordata* Thunb. water extract, thereby indicating that polar molecules are responsible for the enzyme inhibition (**Table 1**) (Lau et al., 2008). Furthermore, compounds isolated from *I. indigotica* Fortune ex Lindl. (a member of broccoli family) such as sinigrin, beta-sitosterol, and indigo (**Figure 2**) significantly inhibited cleavage activities of the $3CL^{pro}$ (**Figure 1**) (Lin et al., 2005). Sinigrin with an IC_{50} of 217 μM was found more effective than indigo compound (IC_{50}: 752 μM) or beta-sitosterol (IC_{50}: 1,210 μM) in inhibiting the cleavage processing of the $3CL^{pro}$ in a cell-based assay (**Table 1**) (Lin et al., 2005). In addition, aloe emodin and hesperetin, which are phenolic compounds from *I. indigotica* Fortune ex Lindl. (**Figure 2**), also dose-dependently inhibited the cleavage activity of $3CL^{pro}$, in which the IC_{50} for aloe emodin was 366 and 8.3 μM for hesperetin (**Table 1**) (Lin et al., 2005). Moreover, *R. palmatum* L. ethyl acetate extract also had anti-SARS-$3CL^{pro}$ activity with the IC_{50} being 13.76 μg/ml (**Table 2**) and the level of inhibition being up to 96% (Luo et al., 2009). This high rate of $3CL^{pro}$ inhibitory activity from different plants suggests that these extracts or isolated compounds may represent a potential therapeutic agent against coronavirus.

Inhibition of Viral Release and Enhancement of Host Immunity

Plant compounds also have been identified to specifically target the proteins involved in the release mechanism of the virus such as 3a ion-channel proteins (**Figure 1**) (Krüger and Fischer, 2009; Fischer et al., 2010; Wang et al., 2011; Kelly et al., 2003; Montal, 2003; Lu et al., 2006). Thereby, herbal medicine that suppressed the channel protein could thus be anticipated to prevent the viral spread to other cells (Schwarz et al., 2012). Employment of the anthraquinone emodin that has been used as alternative therapy in treatment of SARS has demonstrated that it can block the 3a ion channel, thus inhibiting the release of virus at concentration of 20 μM (**Figures 1**, **2** and **Table 1**) (Schwarz et al., 2012). Besides that, the flavonols, kaempferol and kaempferol glycosides (**Figure 2**) also showed potent inhibition towards the 3a channels (**Table 1**) (Schwarz et al., 2012), which suggests their potential role in inhibiting coronavirus release.

Another strategy to fight against the viral infection is by boosting the host immunity (Lau et al., 2008; Cho et al., 2012). The host with better immunity may act as physiological resistance to protect itself from any infection such as increase in the formation of white blood cells which are able to destroy the viruses rapidly. Evidently, the *in vivo* study of *Epimedium koreanum* Nakai on PEDV showed this herbal treatment protects against disease symptoms such as treating diarrhea and ensuring clean intestine in pigs (**Table 3**) (Cho et al., 2012). It has been suggested that the antiviral effect of *E. koreanum* Nakai is modulated by immune responses including macrophage and lymphocyte stimulation (Cho et al., 2012). Quercetin and icariin are the main compounds of *E. koreanum* Nakai (Cho et al., 2012), and interestingly, a previous study has identified that the quercetin also may be able to inhibit the replication of PEDV through specific viral induced reactive oxygen species pathway (Song et al., 2011). Besides that, an *in vitro* study of this herb also showed that this plant exhibited antiviral effect against other coronavirus subtypes such as SM98 and transmissible gastroenteritis coronavirus (TGE) viruses (**Table 1**) (Cho et al., 2012). This extract is not toxic within the host cells, hence indicating that it can be safely delivered for possible infection treatment (Cho et al., 2012). Besides that, *H. cordata* Thunb. study on mice has identified that the water extract from this plant was able to increase immune system of the mice through significantly stimulating the proliferation of mouse spleen lymphocytes (Lau et al., 2008). It was also revealed that *H. cordata* Thunb. increased the CD4 + and CD8 + T cell proportion and also caused a significant increment of mouse splenic lymphocyte secretion of interleukin-2 (IL-2) and interleukin-10 (IL-10) (Lau et al., 2008). These findings demonstrated that *H. cordata* Thunb. extract could exhibit immunostimulatory effect which may aid to suppress the coronavirus infection. Various other studies have also shown that diets including micronutrients such as vitamin C and D have the potential to prevent or treat COVID-19 by fortifying immune system, some of which have entered clinical trial phase (Di Matteo et al., 2020).

Other Mechanisms

There are also other studies that have provided excellent evidence regarding natural herbs and traditional medicine as future anti-coronavirus compounds, even though its exact mechanism is still unknown. For instance, plants such as *Euphorbia neriifolia* L. and

Pelargonium sidoides DC. were identified as having appreciable antiviral activity against human coronavirus infection. Several compounds such as 3β-friedelanol, 3β-acetoxy friedelane, friedelin, and epitaraxerol (**Figure 2**) that successfully isolated from the ethanolic extract of *E. neriifolia* L. leaves exhibited potent anti-human coronavirus (HCoV) activity on human fibroblasts (MRC-5) infected cells, as opposed to the positive control, actinomycin D (**Table 2**) (Chang et al., 2012). Structure-activity relationship further demonstrated the importance of friedelane skeleton for developing a new anti-HCoV-229E treatment (Chang et al., 2012). Moreover, *Pelargonium sidoides* DC. extract EPs® 7630 that is approved for treating acute bronchitis has broad spectrum antiviral agent activity toward various respiratory viruses including human coronavirus (**Table 1**) (Michaelis et al., 2011). Infected cells treated with the extract showed high survivability which was up to 87% (**Table 1**) (Michaelis et al., 2011). Besides that, the *P. sidoides* DC. roots have been used for generations in Southern Africa for the medication of different diseases including airways infections (Conrad et al., 2007; Brendler and van Wyk, 2008; Kolodziej, 2008) and in 2005, the Federal Institute of Drugs and Medical Devices approved its standardized extract for the use of acute bronchitis in Germany (Conrad et al., 2007). However, more work may be needed to establish the extract efficacy in treating the coronavirus infection.

Lycoris radiata (L'Hér.) Herb. extract also was identified as having potent anti-coronovirus activity based on a screening through an *in vitro* study (Li et al., 2005). Ethanol extract of *L. radiata* (L'Hér.) Herb. that has been treated on SARS-CoV BJ-006 strain showed the highest SI (370-422) compared with the other plants such as *Lindera aggregata* (Sims) Kosterm. (SI: 16-17), *Pyrrosia lingua* (Thunb.) Farw. (SI: 55-59), and *Artemisia annua* L. (SI: 27-31) (**Table 1**) (Li et al., 2005). Further structure and activity analysis resulted in a single bioactive compound called lycorine being identified as an active component of anti-SARS-CoV in *L. radiata* (L'Hér.) Herb. with an IC_{50} value of 15.7 nM (**Table 1** and **Figure 2**). This compound also has a CC_{50} value of 14,980 nM in cytotoxicity assay and a selective index (SI) greater than 900 (**Table 1**) (Li et al., 2005). This finding has shown that lycorine may serve as a great candidate for the new anti-coronavirus treatment (Li et al., 2005).

Besides that, other plants such as *Toona sinensis* (Juss.) M. Roem. (Chinese mahogany) have been identified to possess antiviral activity toward coronavirus infection too. *T. sinensis* (Juss.) M. Roem (also known as *Cedrela sinensis*, belongs to the family Meliacceae) is popular in Taiwan, China, and Malaysia, has also been tested for SARS-CoV inhibition *in vitro* analysis and the water extract was shown to inhibit SARS coronavirus replication with an SI of 17 (**Table 2**) (Chen et al., 2008). However, the main compound responsible for inhibiting the SARS-CoV is still unknown, although many bioactive compounds having been isolated from its leaves including beta-sitosterol, beta-sitosteryl-glucoside, (+)-catechin, (−)-epicatechin, gallic acid, kaempferol, kaempferol-d-glucoside, methyl gallate, phytol, quercetin, quercitrin, rutin, stigmasterol, stigmasterol glucoside and toosendanin (Chia, 2007).

Additionally, *in vivo* study of chicken embryo that received treatment from garlic extract (*Allium sativum* L.) showed inhibitory effects against IBV, the coronavirus that contributes to tremendous economic loss in the poultry industry around the world (Mohajer Shojai et al., 2016). These treated embryos showed less dwarfism compared to the untreated group (**Table 3**) (Mohajer Shojai et al., 2016). The metabolite profiling of this extract also showed highly abundant compounds such as diallyl disulphide, disulfide di-2-propenyl and methyl 2 propenyl disulphide that might be responsible for its bioactivity (Mohajer Shojai et al., 2016). Furthermore, *H. cordata* Thunb. water extracts have been shown to have an inhibitory effect on the IBV (**Table 3**) (Yin et al., 2011). *In vitro* and *in vivo* study of *H. cordata* Thunb. conducted on specific pathogen free (SPF) chicken embryos and chickens demonstrated inhibitory activity of more than 90% against IBV infection in Vero cells and chicken embryo kidney cells as well as reduction of more than 90% of apoptosis-inducing cell death resulting from IBV infection (Yin et al., 2011). Besides that, *H. cordata* Thunb. also protected chickens against the virus during pre-treatment (62.5 mg/ml extract given before IBV infection) or post-treatment (62.5 mg/ml given after IBV infection), curing more than 50% of the infected chickens (**Tables 2** and **3**) (Yin et al., 2011). Additionally, ethyl acetate fraction of *H. cordata* Thunb. demonstrated anti-MHV activity at an IC_{50} of 0.98 μg/ml (**Table 1**) without any appreciable cytotoxic effects on CCL9.1 cells (Chiow et al., 2016). The flavonoids *of H. cordata* Thunb. which is quercetin (**Figure 1**) also significantly inhibit MHV activity with IC_{50} of 125 μg/ml (**Table 1**) (Chiow et al., 2016). Interestingly, quercetin is currently being tested in a clinical trial due to its strong antioxidant and prophylactic effect against COVID-19 (Di Matteo et al., 2020). Although these evidence suggest the use of these plant extracts for anti-coronaviral treatments, more in-depth studies are needed to elucidate their exact mechanism in targeting the viral infection.

CONCLUSION

A considerable amount of research strongly indicates that both excellently characterized and less familiar medicinal plants around the world, either crude extracts or bioactive compounds from these plants, are very convincing as therapy for the new emerging coronavirus infection. Several compounds such as TGG, emodin, glycyrrhizin, aurantiamide acetate, and caffeic acid have been identified to have inhibition on viral attachment and penetration. Besides that, several plants and compounds such as *Sanguisorba officinalis* L., *Stephania tetrandra* S. Moore (tetrandrine, fangchinoline, and cepharanthine), and *Strobilanthes cusia* (Nees) Kuntze (tryptanthrin), and *F. suspensa* (Thunb.) Vahl (forsythoside A) were able to inhibit the viral RNA and protein synthesis. Other compounds such as kaempferol, N-cis-feruloyltyramine, and quercetin have targeted and inhibited the viral proteases such as $3CL^{pro}$ and PL^{pro}, important enzymes for the co-translation of non-structural proteins. We also identified a few compounds that may act on the viral release mechanism such as through the 3a ion

channel (emodin and kaempferol) as well as enhancing the host immune systems (*E. koreanum* Nakai and *H. cordata* Thunb. extracts). Last but not least, several plants such as *L. radiata* (L'Hér.) Herb., *A. sativum* L., *E. neriifolia* L., *P. sidoides* DC., and *T. sinensis* (Juss.) M. Roem. have also been discovered to have anti-coronavirus activity, yet their exact mechanisms in targeting the coronavirus infection are still unknown, thus becoming exciting candidates for the development of new anti-coronavirus agents. Perhaps the most promising extracts for this current pandemic are from *L. radiata* (ethanol extract and lycorine) and *S. cusia* (methanol extract, tryptanthrin and indigodole B) and *I. indigotica* (hesperetin) with excellent *in vitro* IC50 and SI values against human coronaviruses, as well as quercetin and kaempferol from various herbs as evidenced from in silico experimentation. However, more preclinical and clinical studies are needed to justify their use and efficacy against the current SARS-CoV-2 virus. Altogether, this compilation will aid researchers or clinicians to better evaluate some targeted plant extracts and bioactive compounds for an effective potential treatment against this devastating pandemic.

AUTHOR CONTRIBUTIONS

JR analysed, interpreted and reviewed the research articles as well as drafting the article. WA designed the research framework and critically revised the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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REVIEW
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Targeting Neurological Manifestations of Coronaviruses by Candidate Phytochemicals: A Mechanistic Approach

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The novel coronavirus 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has made a wide range of manifestations. In this regard, growing evidence is focusing on COVID-19 neurological associations; however, there is a lack of established pathophysiological mechanisms and related treatments. Accordingly, a comprehensive review was conducted, using electronic databases, including PubMed, Scopus, Web of Science, and Cochrane, along with the author's expertize in COVID-19 associated neuronal signaling pathways. Besides, potential phytochemicals have been provided against neurological signs of COVID-19. Considering a high homology among SARS-CoV, Middle East Respiratory Syndrome and SARS-CoV-2, revealing their precise pathophysiological mechanisms seems to pave the road for the treatment of COVID-19 neural manifestations. There is a complex pathophysiological mechanism behind central manifestations of COVID-19, including pain, hypo/anosmia, delirium, impaired consciousness, pyramidal signs, and ischemic stroke. Among those dysregulated neuronal mechanisms, neuroinflammation, angiotensin-converting enzyme 2 (ACE2)/spike proteins, RNA-dependent RNA polymerase and protease are of special attention. So, employing multi-target therapeutic agents with considerable safety and efficacy seems to show a bright future in fighting COVID-19 neurological manifestations. Nowadays, natural secondary metabolites are highlighted as potential multi-target phytochemicals in combating several complications of COVID-19. In this review, central pathophysiological mechanisms and therapeutic targets of SARS-CoV-2 has been provided. Besides, in terms of pharmacological mechanisms, phytochemicals have been introduced as potential multi-target agents in combating COVID-19 central nervous system complications.

Keywords: coronaviruses, COVID-19, SARS-CoV-2, neurology, nervous system, phytochemicals, pharmacology, signaling pathways

INTRODUCTION

Phylogenetic studies on the genomic structure, introduced various types of coronaviruses (CoVs), including NL63, 229E, OC43, HKU1, middle east respiratory syndrome (MERS)-CoV, severe acute respiratory syndrome coronavirus (SARS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Gurung et al., 2020a; Vellingiri et al., 2020), divided into four groups of alpha (229E and NL63), beta (OC43 and HKU1), gamma and delta coronaviruses. Among coronaviruses, alpha and beta groups cause respiratory manifestations in human (Gurung et al., 2020b; Gurung et al., 2020c; Rasool et al., 2020). Recently, a new strain of coronaviruses, namely SARS-CoV-2 has been found, belonging to a distinct class of beta coronaviruses (Divani et al., 2020). SARS-CoV-2 made a deadly disease, termed coronavirus disease 2019 (COVID-19) with devastating manifestation all over the world (Fitriani et al., 2020; Nemoto et al., 2020). The large and positive sense RNA genome with a size of 27–32 kb, as well as an envelope with spike (S1 and S2)/conjugated proteins (Holmes and Lai, 1996; Davidson et al., 2020) are associated with COVID-19 symptoms over a period of 2–14 days. Studies have revealed that when viruses enter to the lung tissue cells and proliferate, cause alveolar and interstitial inflammatory secretion and edema that leads to alveolar gas exchange impairment and hypoxia in the central nervous system (CNS), thereby increases anaerobic metabolism in the mitochondria of brain cells (Wu et al., 2020c). Besides, SARS-CoV enters the nasal passage and triggers neural inflammatory responses through dysregulation of the immune system. The entry factors for SARS-CoV-2 are highly expressed in nasal epithelial cells (Sungnak et al., 2020). As a consequence, CoVs enters the brain via the olfactory tract in the early stages of nasal vaccination or infection (Mori, 2015; Wu et al., 2020c; Desforges et al., 2020). Accordingly, this virus is not limited to the respiratory system but invades peripheral nerves and enters the CNS then causes/aggravates neurodegenerative disorders (Matsuda et al., 2004; Vellingiri et al., 2020). Research has shown the presence of SARS-CoV in cortex, hippocampus, spinal cord, brain stem, cerebellum, striatum, colliculus superior, and hypothalamus (Jacomy and Talbot, 2003). Consequently, COVID-19 patients have shown neurological symptoms, including headache, dizziness, hypogeusia, nausea, vomiting, and anosmia (Ahmad and Rathore, 2020; Vellingiri et al., 2020).

From the pathophysiological point of view, the spike protein in the morphology of COVID-19 bind to angiotensin-converting enzyme (ACE)-receptors on alveolar epithelial cell type 2 (AT2), primed by transmembrane protease serine 2 (TMPRSS2) to allow coronavirus entry (Marchetti et al., 2018; Li et al., 2019b; Wang et al., 2020b; Vallamkondu et al., 2020). Experimental evidence indicated that COVID-19 enters the lung via the respiratory tract and invades AT2 cells to generate a surfactant regarding declining related tension within alveoli to alleviate collapsing pressure. Also, ACE2 is presented in kidney, heart, enterocytes, pancreas, endothelial cells and widely distributed in brain to facilitate the SARS-CoV-2 entry into the cells (Li et al., 2003; Diao et al., 2020). The neural distribution of ACE2 was controversial at first. While a quantitative real-time RT-PCR study showed low levels of ACE2 mRNA in the human brain (Harmer et al., 2002), immunohistochemistry results indicated that ACE2 protein expression was restricted to arterial and endothelial smooth muscle cells (Hamming et al., 2004). Additionally, the predominant expression of ACE2 in glial cells was shown in brain primary cell cultures (Gallagher et al., 2006). Complementary evidence showed the widespread presence of ACE2 mRNA and protein throughout the brain (Doobay et al., 2007) or brainstem (Lin et al., 2008). Finding SARS-CoV in brains of infected patients also confirmed related distribution of ACE2 (Ding et al., 2004; Gu et al., 2005; Xu et al., 2005; Xia and Lazartigues, 2008).

As a critical sign of COVID-19, neuroinflammation occurs through elevated levels of neuronal interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, tumor necrosis factor-α (TNF-α), interferon-gamma (IFN-γ), granulocyte colony-stimulating factor (GMCSF), IFN-γ-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP1), macrophage inflammatory protein 1α (MIP1α), and T cell expression (Xu et al., 2005; Yarmohammadi et al., 2020). The coronaviruses release inflammatory mediators to stimulate macrophages. These macrophages activate IL-1, IL-6, TNF-α, C-X-C motif chemokine ligand 10 (CXCL10) and chemokine ligand 2 (CCL2). Prevailing evidence is showing that CoVs reach the neurons, astrocytes, and/or microglia in CNS. Consequently, microglia and astrocytes play major roles in neuroinflammation and released inflammatory mediators (Murta et al., 2020). These cytokines and chemokines causes vasodilation and also increased capillary penetrance that causes declined surfactant stage in AP-2 cells which in turn lead to alveolar collapse and perturbation in gaseous exchange (Zaki et al., 2012; Wu et al., 2020a; Guan et al., 2020; Yang et al., 2020). In the other level of disease there is an increased level of inflammatory mediators via $CD4^+$ and also increased generation of neutrophils and macrophages using IL-17, IL-21, and IL-22 that causes difficulties in breathing, hypoxemia, and cough (de Wit et al., 2016; Gao et al., 2020; Wan et al., 2020). In addition to the elevation of neuronal inflammatory mediators in CNS and related neuronal associations, ACE2/spike proteins and downstream mediators, RNA-dependent RNA polymerase (RdRP)/proteases, seem to be golden targets in stopping related neuronal signs.

Unfortunately, up to now there is no antiviral drug or vaccine for the treatment of coronaviruses infection, although candidate phytochemicals can be promising factors with antiviral potentials for the treatment of infection (Liu and Du, 2012; Gurung et al., 2020b). Some previous research has indicated neurological manifestations in coronaviruses (Ahmed et al., 2020; Yavarpour-Bali and Ghasemi-Kasman, 2020). Besides, limited studies suggested natural products and candidate phytochemicals as helpful agents for the prevention and treatment of coronaviruses (Hasan et al., 2020; Majnooni et al., 2020; Zhou and Huang, 2020). In this study, an extensive review was performed on neurological manifestations of coronaviruses, as well as the effects of candidate phytochemicals on the aforementioned signaling pathways. Additionally, this is the first review on highlighting phytochemicals with antiviral and

neuroprotective effects, which targets the neural pathogenic pathways of CoVs (termed candidate phytochemicals) regarding the prevention and treatment of COVID-19 neuronal signs.

STUDY DESIGN

We used electronic databases (e.g., Scopus, PubMed, Medline, and Web of Science) and related articles in other sources, to conduct a comprehensive review on the neurological manifests of coronaviruses, as well as the phytochemicals effects. The keywords ("Severe Acute Respiratory Syndrome" OR "SARS" OR "Middle East Respiratory Syndrome" OR "MERS" OR "Coronavirus disease 2019" OR "COVID-19" OR "SARS-CoV" OR "SARS-CoV-2") AND ("neurological sign" OR "neurological manifestation" OR "neuron" OR "nerve" OR "central nervous system" OR "CNS" OR "brain" OR "neurology" OR "neuropathy" OR "stroke" OR "multiple sclerosis" OR "encephalitis" OR "encephalopathy") [title/abstract/keywords] were used. All the phytochemicals possessing both the antiviral and neuroprotective activities with the keywords ("phytochemical" OR "secondary metabolite" OR "plant" OR "polyphenol" OR "phenolic compound" OR "flavonoid" OR "alkaloid" OR "terpen" OR "terpenoid" OR "quinone") were also searched in the whole text. Overall, the phytochemicals with reported antiviral and neuroprotective effects possessing the potential of modulating coronaviruses pathophysiological mechanisms were included. Data were collected without language and date restrictions until October 2020. The screening of retrieved articles was also done. on the reference lists/citation. Regarding completing review on the electronic databases, hand searching also was done relying on the authors' expertize on the SARS-CoVs pathophysiological mechanisms in CNS and candidate phytochemicals.

NEURONAL MANIFESTATIONS OF CORONAVIRUSES

Experimental evidence showed two types of neurological manifestations referring to the CNS and peripheral nervous system (PNS). Of the PNS, there are various neurological associations such as hypogeusia, hyposmia, impaired eye movement, trigeminal neuropathy, Miller-Fisher syndrome, polyneuritis cranialis, rhabdomyolysis, Guillain-Barré Syndrome, and olfactory dysfunction (Ahmad and Rathore, 2020; de Freitas Ferreira et al., 2020; Mochan and Modi, 2020; Nordvig et al., 2020; Pascual-Goñi et al., 2020; Yavarpour-Bali and Ghasemi-Kasman, 2020). COVID-19 also causes CNS impairment such as cerebrovascular disorders, acute ischemic stroke (1–3%), intracranial haemorrhage (0.5%), encephalitis (brain inflammation), demyelination, meningitis, polyneuritis cranialis, vasculitis, and skeletal muscular damage (Li et al., 2016; Dorche et al., 2020; Filatov et al., 2020; Mao et al., 2020; Moriguchi et al., 2020; Zhou et al., 2020). It has been shown that 229E and OC43 coronavirus strains invade to neuroblastoma, neuroglioma, astrocytoma, microglial, and oligodendrocytic cell cultures (Cheng et al., 2020b) toward revealing neuronal complications. Werner and co-workers have indicated additional symptoms of several cases, such as acute necrotizing encephalopathy, neck stiffness, bilateral ankle clonus, positive Brudzinski, left Babinski, and right Chaddock signs (Werner et al., 2020). Other neuronal symptoms of COVID-19 are ataxia, refractory status epilepticus (Xu et al., 2005), neuron denaturation/necrosis, broad gliocytes hyperplasia with gliosome formation (Yassin et al., 2020), myalgia, dyspnea (Prakash et al., 2020), taste and smell dysfunctions, acute cerebrovascular and oculomotor nerve palsy (Nepal et al., 2020). Mao *et al.* indicated that elevated creatine phosphokinase (CPK), C-reactive protein (CRP), D-dimer, necrotizing myopathy, thick filament myopathy, critical illness myopathy (nonspecific), and acute quadriplegic myopathy are other neural manifestation of COVID-19 (Mao et al., 2020; Suri et al., 2020; Warner, 2020). Reports have also shown other neurological manifestations such as Bickerstaff's encephalitis, critical illness myopathy, severe lymphopenia, thrombocytopenia and uremia, facial diplegia, and toxin associated myopathy and neuropathy (Wu et al., 2017; Gulati et al., 2020; Zheng et al., 2020). Of the clinical behavioral signs, headache, syncope, agitation, delirium, dysgeusia, fatigue, dizziness, acute confusion, sleep disorders, changed the level of consciousness, and altered mental status, have been observed in COVID-19 patients (Stewart et al., 1992; Dessau et al., 2001; Lau et al., 2004; Wang et al., 2020a; Wang et al., 2020c; Wu et al., 2020c; Dorche et al., 2020; Helms et al., 2020; Mochan and Modi, 2020; Nalleballe et al., 2020). The aforementioned neurological signs are being manifested in 84% of patients with COVID-19 (Wang et al., 2020c; Helms et al., 2020).

Severe respiratory syndrome as one of the critical impairment of COVID-19 may result in systemic hypoxia, hypercarbia, and anaerobic metabolism resulting in neuronal swelling and brain edema/damage (Suri et al., 2020). SARS-CoV-2 also invades to the spinal cord and causes acute inflammation of gray and white matter in the spinal cord (myelitis), which was recognized by the acute flaccid myelitis of lower limbs, urinary and bowel incontinence (Zhao et al., 2020). Evidence has shown a close relationship between COVID-19 and Parkinson disease, increased motor symptoms (e.g., tremor), freezing of gait or dyskinesias, and declined the efficacy of dopaminergic medication (Macht et al., 2007; Zach et al., 2017; Ehgoetz Martens et al., 2018). Interestingly, it seems to be a near linkage between dopamine synthesis pathway and COVID-19 pathophysiology. In this line, dopa decarboxylase, as a regulatory enzyme in dopamine pathway is meaningfully co-expressed with ACE2 receptor. On the other hand, SARS-COV virus downregulates ACE2 in consistent with dopamine synthesis alteration (Kuba et al., 2005). Besides, as dopamine is expressed in the alveolar epithelial cells, it also contributes in lung immunity, as well as what ACE2 does (Bone et al., 2017). Accordingly, considering the critical role of dopamine deficiency in Parkinson's disease, the SARS-CoV-2 virus may cause such sporadic signs COVID-19 patients (Rietdijk et al., 2017).

Additionally, evidence indicated that CoVs may play an essential function in the pathogenesis of multiple sclerosis

(Saleki et al., 2020). The CoVs isolated from multiple sclerosis patients were neutralized using the patients' serum. This revealed the destructive role of CoVs in the pathogenesis of multiple sclerosis (Burks et al., 1980). Growing studies are evaluating the use of immunomodulatory/disease-modifying agents in multiple sclerosis patients with COVID-19. Results declared an increased risk of COVID-19 complications in those treated patients (Baysal-Kirac and Uysal, 2020; Ramanathan et al., 2020). Decision on continuing/stopping the immunotherapy in these patients is closely dependent on disease severity and activity (Giovannoni et al., 2020).

Orsucci and co-others have revealed that there are olfactory and gustatory function impairments as common neural disorders in patients of COVID-19 (Orsucci et al., 2020). It has shown in CNS-CoV disease, there is a lower level of lymphocytes, eosinophils and a higher level of neutrophils as well as monocyte (Saleki et al., 2020). Also, Toscano *et al.* observed Guillain–Barré syndrome, lower limb weakness and paresthesia, facial diplegia followed by ataxia and paresthesia, flaccid tetraparesis or tetraplegia in COVID-19 (Toscano et al., 2020). Researchers in several cases observed tonic-clonic seizure, anxiety, psychotic symptoms, meningeal irritation signs, extensor plantar response, encephalitis, dysphagia, dysarthria, bulbar impairment and massive hemorrhagic conversion (Wang et al., 2020a).

THE PATHOPHYSIOLOGICAL MECHANISTIC PATHWAYS OF CORONAVIRUSES IN CENTRAL NERVOUS SYSTEM

Experimental evidence has indicated that coronaviruses invade to neurons and glial cells to induce an unfolded protein response (UPR) regarding necroptosis in neuronal cells (Meessen-Pinard et al., 2017). As previously mentioned, coronaviruses caused neuronal damages and death along with related neuroinflammatory responses (Morfopoulou et al., 2016). There are multiple mechanisms by which SARS-CoV-2 enters the CNS and causes associated complications. Those mechanisms are blood-mediated contamination (hematogenous), neuronal-mediated infection (neurogenic), immunodeficient related damage, direct respiratory infection, and hypoxic injury (Ahmed et al., 2020) which are described as following. During the hematogenous manner, CoVs crossed the blood-brain barrier (BBB) and entered the brain. This occurs via two mechanisms, by direct penetration of the virus particle crossing the BBB or by hijacking peripheral blood cells (Bohmwald et al., 2018). In the latter way of invasion, Human coronavirus OC43 (HCoV-OC43) accesses the CNS via the neurogenic way to be appeared in the cell bodies and dendrites of olfactory neurons, then spread in hippocampus, cortex and spinal cord (Niu et al., 2020a). During the viremia phase of illness, BBB disruption causes a direct virus entrance to the brain. Spreading/disseminating of SARS-CoV-2 from the cribriform bone in nearby proximity to the olfactory bulb, and brain causes in seven days (Baig et al., 2020). Besides, peripheral invasion of nerve terminals by CoVs through the connected synapse leads to the virus entry to the CNS (Ahmad and Rathore, 2020). Additionally, systemic hypoxia resulted from severe pneumonia causes vasodilatation, anaerobic metabolism, hypoxia and accumulation of toxic compounds lead to brain damage (Tu et al., 2020).

One of the most widely accepted neuropathological mechanisms of SARS-CoV-2 is hyper-inflammatory state (Yavarpour-Bali and Ghasemi-Kasman, 2020). Accordingly, the immune-mediated damage is resulted from cytokine storms, as well as the activation of T lymphocytes, endothelial cells, and macrophages which leads into vascular leakage, coagulation, and end-organ damage (Mehta et al., 2020; Tveito, 2020). It was shown that coronavirus triggers innate immunity associated with the release of microglial-induced INF-α/β (Savarin et al., 2018). In this regard, several cytokines and chemokines are released by microglia and astrocytes such as IL-1α, IL-1β, IL-6, IFN-γ, TNF-α, and CXCL10 (Joseph et al., 1993). Li *et al.* indicated the increased levels of many inflammatory mediators in the cerebrospinal fluid, including IL-6, IL-8, MCP-1, and granulocyte-macrophage colony-stimulating factor (GM-CSF) in COVID-19 patients (Li et al., 2016). During early stage of CoV neuroinfection, CXCL10 and CXCL9 are present in the peripheral blood of patient affected by IFN-γ (Jiang et al., 2005). Experiment has shown that CoVs play a destructive role in acute disseminated encephalomyelitis (ADEM) correlated with increased inflammatory mediators such as IL-6, IFN-γ, TNF-α, CXCL9, and CXCL10 (Kothur et al., 2016). It has been demonstrated that there is a direct correlation between the levels of IL-1β, IL-6, IL-8, TNF-α, IL-10 and COVID-19 central inflammatory complications such as neuromyelitis optica (also known as Devic's disease), transverse myelitis, acute disseminated encephalomyelitis, amyotrophic lateral sclerosis, herpes simplex encephalitis, Parkinson's disease, traumatic brain injury, epilepsy, and stroke (Vezzani et al., 2002; Rodney et al., 2018; Vezzani et al., 2019; West et al., 2019). In this line, it has shown that IL-2 and IL-2 receptors (IL-2R) have important signals for T cell activation via Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway (Fu et al., 2018; Shi et al., 2020). The transcription factor nuclear factor-κB (NF-κB) is another essential regulator in immune system, which is activated in lung inflammatory immunopathology-induced by SARS-CoV (DeDiego et al., 2014; Catanzaro et al., 2020).

As previously mentioned, studies have suggested several mechanisms for entering the SARS-CoV-2 to the nervous system, although the exact mechanism is not clear (Yavarpour-Bali and Ghasemi-Kasman, 2020). Scientists have suggested that coronaviruses enter the olfactory bulb/epithelium, then penetrates to CNS. So, make the anosmia or hyposmia as the neural manifestation of COVID-19. Recently additional studies have suggested different mechanisms for anosmia in COVID-19, such as olfactory cleft syndrome, mucosal obstruction, direct damage of olfactory sensory neurons, impairment of the olfactory perception center and cytokine storm in the brain (Yazdanpanah et al., 2020). Released inflammatory factors altered the penetrance of the

BBB and increased inflammatory cascade (Singhi, 2011). Studies have also shown that deficiency in neuronal endoplasmic reticulum (ER) leads to the activation of UPR-induced by SARS-CoV (Chan et al., 2006; Ron and Walter, 2007). Until now, some related signaling pathways have shown functional roles in the UPR processing, such as ATF6, phospho-extracellular signal-regulated kinase (p-ERK)/eIF2-alpha and IRE1/XBP1 (Ron and Walter, 2007). Favreau *et al.* indicated that HCoV-OC43 induced UPR and causes neuronal death by caspase-3 activation and nuclear fragmentation (Favreau et al., 2009). From another mechanistic point, studies suggested that SARS-CoV-2 induces severe inflammation that leads to thrombosis. SARS-CoV-2 also binds to toll-like receptors (TLR) and causes the synthesis and liberation of IL-1. As a matter of fact, TLRs activate biochemical cascade by inflammasome activation as well as type I interferon (IFN) which is released as an important player against viral infection (Marchetti et al., 2018; Conti et al., 2020; Vaninov, 2020).

ROLE OF RENIN-ANGIOTENSIN SYSTEM IN THE NEURONAL MANIFESTATIONS OF CORONAVIRUSES

It has been shown that SARS-CoV-2 mainly enters the CNS via the ACE2 or TMPRSS2 receptors. These receptors are expressed in the glial cells of brain/spinal cord and thereby facilitates the invasion of coronavirus to the spinal cord, which is essential for the host cell entry of SARS-CoV-2 and also plasma membrane fusion (El Tabaa and El Tabaa, 2020; Nemoto et al., 2020). Also, it has been indicated that when coronavirus enters the cells, ACE2 will break and shed by ADAM Metallopeptidase Domain 17 (ADAM17) into the membrane space (Li and De Clercq, 2020). Studies suggested that phosphorylation of ACE2 at Ser680 inhibits ubiquitination of ACE2 and also increase related membrane expression (Amraei and Rahimi, 2020). It has been indicated that renin-angiotensin system (RAS), including angiotensin II (Ang II), ACE, ACE2, angiotensin type-1 receptor (AT1R), angiotensin type-2 receptor (AT2R), and Mas receptor (MAS), plays critical physiological functions. Research suggested that Ang II prevents COVID-19 infection through binding to ATR1 and activating ACE2 internalization, then declining ERK1/2 and p38 mitogen-activated protein kinase (MAPK) pathway (Koka et al., 2008; Fernandes et al., 2011; Divani et al., 2020). Recent reports indicated that Ang II act via two G protein-coupled receptors (GPCR) such as AT1R, angiotensin type-2 receptor (AT2R) which expressed in human lung tissue. Besides, the activations of Ang II can be mediated by AT1R through enhancing several signaling pathways such as MAPK/ERK, IP3/diacylglycerol, tyrosine kinases, and NF-κB (Balakumar and Jagadeesh, 2014; El Tabaa and El Tabaa, 2020). In a parallel way, AT1 stimulates monocytes, macrophages and vascular smooth muscle cells to generate TNF-α and IL-6 (Balakumar and Jagadeesh, 2014). Additionally, Ang II promotes vasoconstriction, released pro-inflammatory cytokine, vascular endothelial dysfunction, and platelet aggregation (Nakashima et al., 2006; Shatanawi et al., 2011). There is also a relationship between Ang II and endothelin-1 (ET-1). Indeed ET-1 has an important function in Ang II-induced endothelial dysfunction and platelet activation through inducing IL-6 release (Touyz and Schiffrin, 1993; Browatzki et al., 2000). In order to reduce SARS-CoV-2 entry and related side effects, ACE2 activity should be declined. It has been found that ACE2 is a critical enzyme in the RAS, which has a critical function role in the human body. In this pathway, renin generated in the kidneys cleaves angiotensinogen from the liver, producing Ang I and then is cleaved by ACE into Ang-II (the substrate of ACE2). Ang I binds to the AT1R and AT2R as well as the RAS system has an important function in SARS-CoV-2 infection (Battagello et al., 2020).

In addition to the critical role of blood, hypoxia, ACE2, neuroinflammation in the neuronal pathogenesis of COVID-19, modulating RdRP/3-chymotrypsin-like protease (3CLpro) and papain-like protease (PLpro), as critical enzymes involved in the replication of SARS-CoV-2, is of great importance. There are also several receptors, namely CD209L (L-SIGN), CD209 (DC-SIGN), neuropilin receptors (NRPs), and CD147/Basigin, which facilitate SARS-CoV-2 entry (Amraei and Rahimi, 2020). As described, there is a close interconnection between the aforementioned dysregulated signaling pathways. In this line, providing multi-target agents capable of a simultaneous modulation of the aforementioned targets could pave the road against COVID-19 neurological manifestations.

IMPORTANCE OF NATURAL PRODUCTS IN COMBATING COVID-19 GENERAL MANIFESTATIONS

The widespread pandemic of COVID-19 disease, by infecting millions of people, and thousands of killing around the world, has triggered researchers to make a diligent effort regarding finding potential drugs or vaccines against SARS-CoV-2. However, these efforts have not yet reached credible drugs due to the inherent complexity of the SARS-CoV-2 pathogenicity/complications (Sharma et al., 2020). Due to their simultaneous effects on multi-therapeutic targets and low side effects, phytochemicals including alkaloids, flavonoids, polyphenols, quinones, and terpenoids are of the most promising options for finding effective treatment against SARS-CoV-2 (Efferth and Koch, 2011; Mani et al., 2020).

Recent studies showed that three main targets, including main proteases, as well as S protein interaction with ACE2, have attracted the most attention of researchers to discover effective drugs against SARS-CoV-2 from phytochemicals. Additionally, phytochemicals potentially target neuroinflammation to combat related neuronal signs in COVID-19.

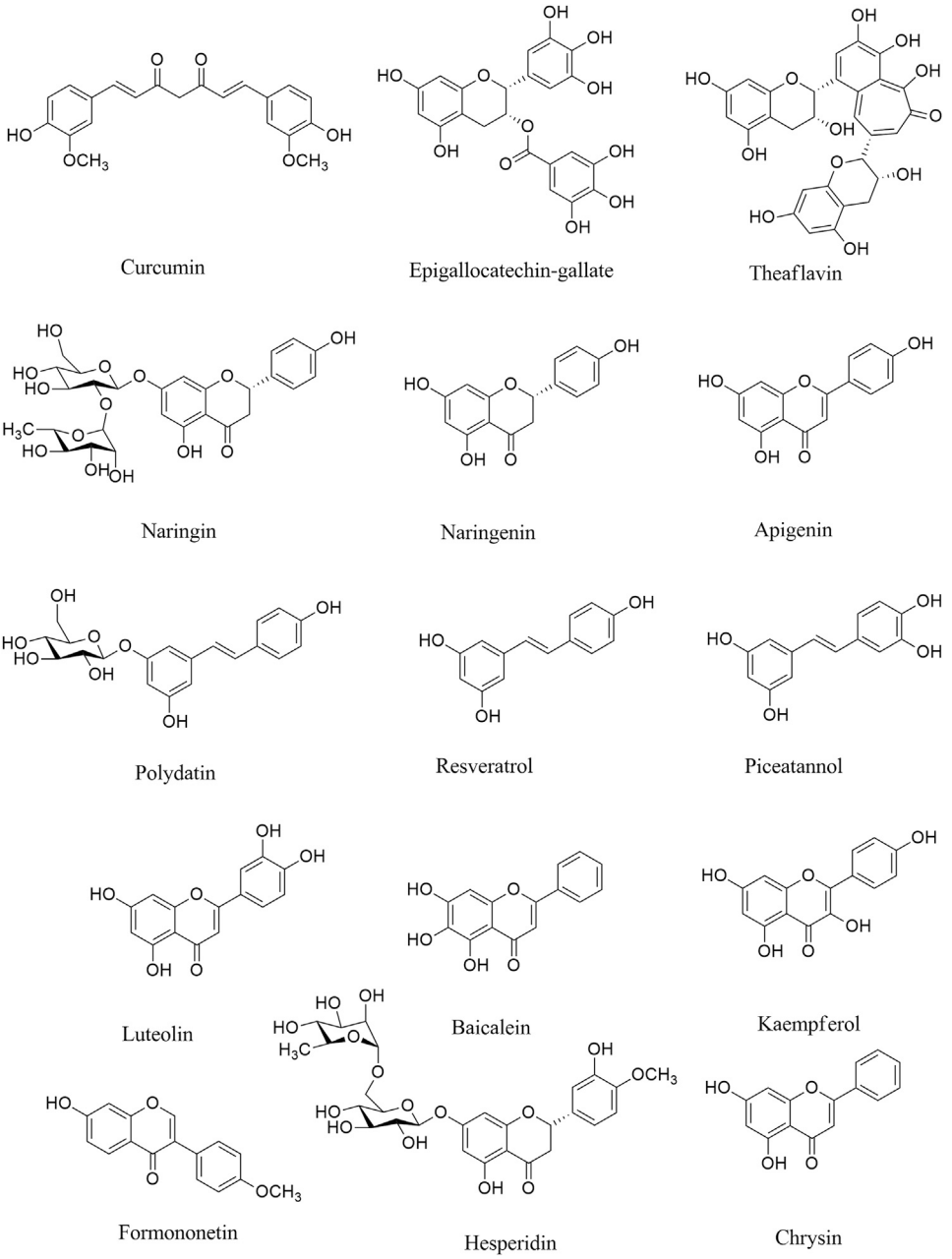

FIGURE 1 | Chemical structures of selected polyphenols/flavonoids with the potential of being used against COVID-19 neurological manifestations.

POTENTIAL OF PHYTOCHEMICALS AGAINST COVID-19 NEUROLOGICAL ASSOCIATIONS

Recently, no drug or vaccine has been developed for the treatment/prevention of SARS-CoV-2. Phytochemicals have shown to play critical antiviral biological activities and health benefits in CNS (Kähkönen et al., 1999). As previously mentioned, there are several major targets for phytochemicals against coronavirus such as ACE2, spike protein, TMPRSS2, 3CLpro, RdRp and PLpro, which among them ACE2 plays an important role regarding the initial stage of SARS-CoV-2 invasion into the cells/neurons (Huang et al., 2020b). Also, 3CLpro and PLpro play vital roles in SARS-CoV-2 maturation and replication (Xue et al., 2008; Ryu et al., 2010a).

The potential of phytochemicals in suppressing neuroinflammation induced by SARS-CoV-2 has also made them promising agents in combating neuronal signs of COVID-19.

Phytochemicals Inhibit Neuroinflammation and Neural Manifestations in COVID-19

As previously mentioned, hyper-inflammation is one of the critical neuropathological mechanisms of SARS-CoV-2 in line with the release of IL-2, IL-6, IL-7, IL-10 and TNF-α (Yang et al., 2020). Studies also suggested elevated levels of IL-8, MCP-1, IFN-γ, CXCL9, CXCL10 and GM-CSF in COVID-19 patients (Li et al., 2016; Marchetti et al., 2018; Conti et al., 2020; Vaninov, 2020) regarding triggering the neuronal manifestations. Systemic inflammation following the leukocyte activation prior to its BBB migrating is another major mechanism toward viral neurological complications (Campbell et al., 2014). The released inflammatory agents change the BBB permeability, triggers the neuroinflammatory flows and drive neuronal hyper-excitability through the activation of glutamate receptors, leading to acute seizure (Libbey et al., 2011; Yavarpour-Bali and Ghasemi-Kasman, 2020). Considering the crucial role of inflammation in the neuropathogenesis of COVID-19, phytochemicals with neuronal anti-inflammatory effects could pave the road in combating related neuronal manifestations. Recent reports also have declared the critical role of phytochemicals in health care through their antiviral (Fitriani et al., 2020) and the inhibition of neuroinflammatory-interconnected pathways (Abbaszadeh et al., 2020). Phytochemicals with potential antioxidant and anti-inflammatory effects (e.g., carotenoids and polyphenols) interact with major transcription factors such as Nrf2 and NF-κB (Iddir et al., 2020).

Naringin (**Figure 1**) is a phenolic phytochemical belonging to the flavonoid class, possessing anti-neuroinflammatory (Chen et al., 2016; Chtourou et al., 2016; Ngwa et al., 2020), and antiviral (Ng et al., 1996) effects with the potential of being used in the prevention/treatment of COVID-19 (Dabaghian et al., 2020). Naringin also inhibits the expression level of cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), IL-1β and IL-6 via suppressing high mobility group box 1 (HMGB1) in COVID-19 (Park et al., 2003; Huang et al., 2020a). It also declined the expression level of p38MAPK to inhibit HMGB1 generation of inflammatory mediators and associated lung injury (Gil et al., 2016; Kim et al., 2019b). According to the critical destructive role of inflammatory mediators in the neurological signs of COVID-19, naringin seems to be a hopeful anti-inflammatory/antiviral candidate in combating related neuronal manifestations. As an aglycone form of naringin, naringenin has similarly shown anti-neuroinflammatory (Nouri et al., 2019; Alberca et al., 2020), and antiviral effects with the potential of being used against COVID-19 (Tutunchi et al., 2020). We have previously shown the neuroprotective potential of naringenin through modulation of inflammatory mediators (NF-κB, TNF-α, IL-1β, etc) and microglia activation in the CNS (Nouri et al., 2019), thereby it could mitigate the neuronal signs of COVID-19 mediated by the inflammatory mediators. As another phenolic compound, resveratrol has shown promising beneficial effects against COVID-19, through the activation of ERK1/2 and SIR1 signaling pathways related to survival, DNA protection (Levy et al., 2020; Ma and Li, 2020), and anti-neuroinflammatory responses (Bastianetto et al., 2015). It also inhibits neuroapoptosis by reducing FGF-2 and suppressing NF-κB (Xu et al., 2018). Considering the critical role of the aforementioned inflammatory mediators in COVID-19 (Yarmohammadi et al., 2020), resveratrol could potentially decline neuroinflammatory signs of COVID-19 patients (Chen et al., 2005). As a major natural derivative of resveratrol, polydatin potentially decline the neural levels of NF-κB, TNF-α, IL-1β, IL-6, IL-8, prostaglandin E2 (PGE2), NO, COX-2, iNOS, matrix metalloproteinase (MMP)-3 and MMP-9, thereby could be a novel agents in combating neuronal inflammatory manifestation in COVID-19 (Lo Muzio et al., 2020). A recent study by Bonucci *et al.*, has also introduced polydatin as a protective phytochemical against COVID-19 (Bonucci et al., 2020). So, focusing on their ameliorating effects against neuroinflammation, as well as related antiviral properties, resveratrol and plydatin derivative could be of candidate phytochemicals in combating neuronal signs of COVID-19.

Consistently, evidence has shown that epigallocatechin gallate (EGCG), as a natural polyphenolic compound, plays important functions such as antitumorigenic, anti-inflammatory, antibacterial, antioxidative, and antiproliferative effects (Chacko et al., 2010; Ge et al., 2018; Mhatre et al., 2020). The anti-neuroinflammatory effects through inhibiting microglia activation, and suppressing inflammatory mediators (Abbaszadeh et al., 2020), as well as antiviral effects of EGCG (Steinmann et al., 2013) make it a potential polyphenol for the treatment of neurological symptom in COVID-19. Green tea with the prominent phytochemicals of such polyphenols, including EGCG, epicatechin gallate, epicatechin and catechin plays both the antiviral (Chojnacka et al., 2020), anti-SARS-CoV-2 (Ghosh et al., 2020) and anti-neuroinflammatory activities (Calis et al., 2020), thereby could play promising role in combating COVID-19 neural complications. EGCG has employed several other mechanisms to suppress SARS-CoV-2 in different steps of virus life cycle (Jang et al., 2020).

As another polyphenol, formononetin declined neuroinflammation by decreasing the levels of TNF-α, IL-6, IL-1β, PGE2, iNOS, and COX-2. Evidence indicated that formononetin inhibited neuroinflammation through suppressing NF-κB signaling pathway, thereby could be a novel drug for the neurological manifestation of COVID-19 (El-Bakoush and Olajide, 2018; An et al., 2020). Formononetin was shown to modulate MAPK, ERK, p38, JNK pathway and downstream mediators to play antiviral effects and inhibit infection-induced inflammation (Wang et al., 2015; Lalani and Poh, 2020). Recent reports also have considered the formononetin as one of major plant-derived secondary metabolites with acceptable effectiveness against COVID-19 (Mirzaie et al., 2020). Consistently, theaflavins are other phenolic compounds with antiviral, anti-inflammatory, antioxidative, and antibacterial effects (Higdon and Frei, 2003; Lambert and Yang, 2003). Theaflavins also suppressed the levels of inflammatory mediators such as COX-2, TNF-α, intercellular adhesion molecule 1 (ICAM-1), and NF-κB mRNA (Mhatre et al., 2020). The aforementioned effects of theaflavins, as well as its antiviral potentials (Zu et al., 2012), could introduce it as a useful

treatment against the neurological sign of COVID-19, via modulation of neuronal IL-1β, IL-6, TNF-α, IL-10, glial fibrillary acidic protein and Bax. As well as related interaction with ACE2/spike proteins, and main proteases. Based on molecular dynamic analysis Kumar *et al.* indicated that some other phenolic compounds play important roles in the inhibition of SARS-CoV-2 such as rosmarinic acid, ferulic acid, ursonic acid, piperine, gingerol, curcumin, and silymarin (Kumar et al., 2020). Previously the neuroprotective effects of such plant-derived secondary metabolites have been reported through inhibiting the inflammatory-interconnected mediators (Abbaszadeh et al., 2020; Fakhri et al., 2020b). Among the aforementioned phytochemicals, ferulic acid, silymarin and curcumin possess particular anti-neuroinflammatory effects, in addition to related antiviral effects (Dutta et al., 2009; Borah et al., 2013; Ghosh et al., 2017). The anti-neuroinflammatory effects of curcumin is applied through suppressing microglia cells (Ghasemi et al., 2019). Other flavonoids like luteoloside and baicalein also possess potential modulatory effects against neuroinflammation, toward antiviral effects (Nagai et al., 1995; Cao et al., 2016; Li et al., 2019a; Welcome, 2020).

Among other classes of phytochemical compounds, phytosterols also have shown potential anti-inflammatory effects (Dash et al., 2020). Of those compounds, stigmasterol and β-sitosterol reduced the expression of COX-2, TNF-α, iNOS, IL-6, IL-1β, PGE2 and NF-κB (Philip et al., 2018). Consequently, Krupanidhi *et al.* indicated the antiviral effects of stigmasterol and β-sitosterol against the SARS-CoV-2 by computational studies. So, considering the antiviral and anti-inflammatory potential of stigmasterol and β-sitosterol, they could be potential agents in combating COVID-19 neurological signs (Krupanidhi et al., 2020).

Additionally, several lines of evidence indicated that asiaticoside (a saponin), borneol (a terpene), catalpol (an iridoid) as other phytochemicals declined the neuronal levels of TNF-α, IL-6, TLR4, NF-κB, IL-β and IL-8, thus may be hopeful agents against neurological symptoms in COVID-19 (Welcome, 2020). In fact, since inflammation triggers several cascades of CNS pathogenesis in COVID-19, suppressing related mediators could potentially ameliorate related symptoms. Among other phytochemicals, some alkaloids also show promising anti-inflammatory and antiviral effects (Chen et al., 2015; Powers and Setzer, 2016), with the potential of being used against COVID-19 (Bleasel and Peterson, 2020). This effect of alkaloids was also confirmed by a recent *in silico* study by Garg and Roy. In their study, two alkaloids of sophaline D and thalimonine indicated potential antiviral activities by suppressing main viral proteases (Garg and Roy, 2020) and inflammatory pathways (Varadinova et al., 1996; Pour et al., 2019).

Several other phytochemicals play important roles in the inhibition of SARS-CoV-2 such as sarsasapogenin (a steroidal sapogenin), novobiocin (a coumarin), and alpha terpinyl acetate (a terpenoid) (Kumar et al., 2020). Previously the neuroprotective effects of such plant-derived secondary metabolites have been reported through inhibiting the inflammatory-interconnected mediators (Abbaszadeh et al., 2020; Fakhri et al., 2020b).

Cannabinoids also possess critical anti-inflammatory roles in viral diseases (Walter and Stella, 2004; Rizzo et al., 2020). These compounds are major constituents of the cannabis plant. The physiological roles of cannabinoids and cannabis are primarily mediated by the cannabinoid receptors (CB1R and CB2R), endocannabinoids, and related metabolic enzymes which are widely distributed throughout the body, especially CNS. The mediators of cannabinoid receptors are being considered as potential targets for numerous disorders, including those correlated with inflammation and autoimmune dysregulation (Rizzo et al., 2020). Prevailing evidence are indicating the pivotal anti-inflammatory and immunoregulatory effects of cannabis-derived cannabinoids, through suppressing cytokines, inhibition of immune cell migration/proliferation (Almogi-Hazan and Or, 2020). Besides, selective cannabinoid agonists present a novel way regarding the treatment of virus-associated neuroinflammation. Considering their growing global acceptance for medicinal uses (Onaivi et al., 2020), cannabinoids seem to be of potential agents against inflammatory cytokine and related mortality in COVID-19 (Onaivi and Sharma, 2020).

Overall, phytochemicals with the potential of modulating the immune system and attributed neuronal cytokine storm could pave the road in combating COVID-19 neuronal complications.

Phytochemicals Inhibit ACE2, and Spike Protein Thereby Neural Manifestations in COVID-19

As previously mentioned, SARS-CoV-2 enters the CNS via the ACE2 or TMPRSS2 receptors (El Tabaa and El Tabaa, 2020; Nemoto et al., 2020). In order to decline SARS-CoV-2 entry to neural cells, ACE2 activity should be declined (Battagello et al., 2020). Spike (S) glycoprotein as the main SARS-CoV-2 structural protein with a critical role in binding to the host cell and protecting the virus against some of the host species antibodies, is another target of phytochemicals (Schoeman and Fielding, 2019).

ACE2 is an enzyme found in the outer membrane of the human cell that acts as a binding site for the S protein. Several studies have shown that there is a strong interaction between ACE2 and S protein. So, blocking ACE2 is also another phytochemical strategy to fight SARS-CoV-2 (Li et al., 2005).

Flavonoids reduce the ACE2 expression through activating Nrf2, thereby combat SARS-CoV-2 (Mendonca and Soliman, 2020; Muchtaridi et al., 2020). Based on the molecular docking mutagenesis study and experimental verification results, hesperidin, chrysin and emodin can be used for COVID-19 treatment (Basu et al., 2020). An *in silico* study indicated that kaempferol, quercetin, and fisetin bind to the hACE2-S-protein complex, near the interface of hACE2 and S protein binding (Pandey et al., 2020). In a recent study by Rebas et al., 2020 the neuroprotective effects of the aforementioned compounds have been shown. So, kaempferol, quercetin and fisetin are of promising flavonoids against COVID-19 neurological signs. Two *in silico* studies showed that quercetin, quercetin 3-glucuronide-7-glucoside, quercetin 3-vicianoside, absinthin, glabridin, and gallic acid gave better binding energy (BE) with

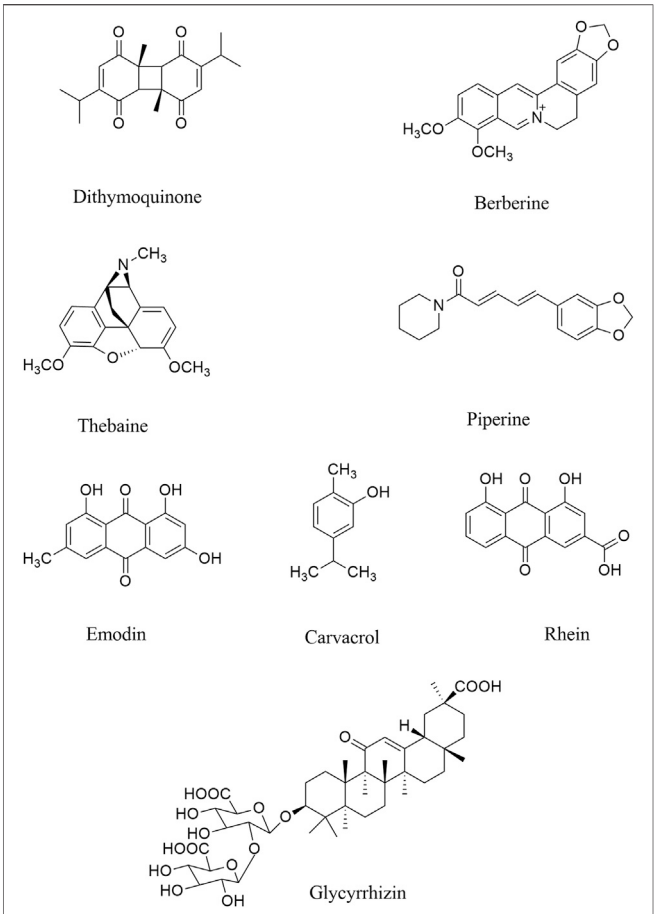

FIGURE 2 | Chemical structures of selected alkaloids/terpenes/quinones with the potential of being used against COVID-19 neurological manifestations.

ACE2 (Joshi et al., 2020) toward inhibiting COVID-19 (Joshi et al., 2020).

Through the same molecular docking analysis piceatannol also has shown neuroprotective responses (Zhang et al., 2018; Talebi et al., 2020) with the potential of binding to ACE2, thereby playing a critical role in the prevention and treatment of COVID-19 (Wahedi et al., 2020; Ahmad et al., 2020). The phytochemicals, baicalin, scutellarin, and hesperetin, also bind to ACE2, regarding reducing neurological symptoms in COVID-19 (Cheng et al., 2020a). Several *in silico* studies showed that the binding energy of hesperidin with the SARS-CoV-2 spike protein, and main proteases are lower than that of ritonavir, lopinavir, and indinavir. It could introduce hesperidin as an effective antiviral agent. Hesperidin also has shown to counteract the cell damaging induced by virus infection, inflammation and free radicals (Bellavite and Donzelli, 2020). Many of other phenolic compounds, including naringenin, hesperetin, hesperidin, and baicalin, showed potential inhibitory effects on ACE2 activity, thereby showed potential effects on COVID-19 and related neural manifestations (Muchtaridi et al., 2020). In another study, stilbene-based compounds especially resveratrol, are promising candidate phytochemicals acting via disrupting spike protein and human ACE2 receptor complex (Wahedi et al., 2020).

EGCG and theaflavin gallate seem to be of promising phytochemicals in targeting spike-protein central channel of SARS-CoV-2 (Maiti and Banerjee, 2020). In a recent study by Kulkarni et al., 2020 some terpenoids such as carvacrol, geraniol, anethole, L-4-terpineol,cinnamyl acetate, thymol and pulegone, and other phenolic as cinnamaldehyde were effective antiviral agents with potential inhibitory effects on viral spike protein. In this line, nimbin (a triterpenoid) and curcumin (polyphenol) showed high binding affinity regarding interacting with ACE2 and the S protein (Maurya et al., 2020). Consistently, Chen and Due estimated the BE of ACE2 interaction with the flavonoid glycoside scutellarin and the triterpenoid glycyrrhizin as a -14.9 and -9 kcal/mol, respectively, that were more strong than other studied phytochemicals including baicalin, hesperetin, and nicotianamine (Chen and Du, 2020). A study by Vardhan *et al.*, showed that one hundred fifty-four analogous of limonoids and triterpenoids showed potential inhibitory effects on ACE2, 3CLpro, PLpro, spike protein, and RdRp. Another *in silico* study also showed that limonin, obacunone, ursolic acid, glycyrrhizic acid, 7-deacetyl-7-benzoylgedunin, maslinic acid, and corosolic acid effectively target SARS-CoV-2 proteins (Vardhan and Sahoo, 2020).

Evaluated by molecular docking analysis, dithymoquinone (a quinone, **Figure 2**) showed neuroprotective responses (Zhang et al., 2018; Talebi et al., 2020) through binding to ACE2, to show key roles in the prevention and treatment of COVID-19 (Wahedi et al., 2020; Ahmad et al., 2020). As a potential phytochemical of *Nigella sativa L.* (Ranunculaceae), dithymoquinone, with binding affinity of -8.6 kcal/mol, showed a higher potential of binding at SARS-CoV-2 ACE2 (Ahmad et al., 2020). According to the molecular modeling results on SARS-CoV-2, a new indazole alkaloid from the seeds of *N. sativa*, nigellidine meaningfully bind to active sites of SARS-CoV-2 (Maiti et al., 2020).

Parvez and co-workers, in an *in silico* study, showed that two chalcones azobechalcone (binding energy [BE], −14.4 kcal/mol) and isolophirachalcone (BE, −12.8 kcal/mol) as well as two alkaloids fangchinoline (BE, −12.5 kcal/mol) and tetrandrine (BE, −12.6 kcal/mol) have shown high binding affinity to S protein of SARS-CoV-2 (Parvez et al., 2020). Also, three alkaloids, including cepharanthine, fangchinoline, and tetrandrine inhibited the S protein of Human-CoV-OC43 expression at 5 μM (Kim et al., 2019a), as previously showed anti-inflammatory roles in viral diseases. In another study, Ho and co-workers showed that anthraquinone emodin (IC_{50}, 200 μM) blocked the interaction between ACE2 and S protein (Okamoto et al., 2001; Ho et al., 2007).

In a survey by Niu *et al.* glabridin, genistein, chrysoeriol, and tectorigenin have been introduced as phytochemicals affecting miRNAs of ACE2 (Niu et al., 2020b). *In vitro* investigation showed that rhoifolin, δ-viniferin, myritilin, homoflavone A, lactucopicrin15-oxalate, nympholide A, afzelin, biorobin, phyllaemblicin B, cyanidin, baicalin, scutellarin, glycyrrhizin, tangeretin, pro-cyanidin, nobiletin, brazilein, galangin, acetoxychavicol acetate (ACA) and delphinidin are among

TABLE 1 | Selected/candidate phytochemicals with inhibitory effects on ACE2, spike proteins, protease, and RdRP in combating COVID-19 neurological signs.

Phytochemical class	Compound	Study type	References
		ACE2 interaction	
Alkaloid	Nicotianamine	*In silico*	(Chen and Du, 2020)
Flavonoid	Baicalin	*In silico*	(Cheng et al., 2020a; Chen and Du, 2020)
Flavonoid	Chrysin	*In silico*	(Basu et al., 2020)
Flavonoid	Fisetin	*In silico*	(Pandey et al., 2020)
Flavonoid	Hesperetin	*In silico*	(Chen and Du, 2020)
Flavonoid	Kaempferol	*In silico*	(Pandey et al., 2020)
Flavonoid	Naringenin	*In silico*	(Muchtaridi et al., 2020)
Flavonoid	Quercetin	*In silico*	(Joshi et al., 2020; Williamson and Kerimi, 2020)
Flavonoid	Scutellarin	*In silico*	(Chen and Du, 2020)
Polyphenol	Curcumin	*In silico*	(Maurya et al., 2020)
Polyphenol	Piceatannol	*In silico*	(Wahedi et al., 2020)
Polyphenol	Resveratrol	*In silico*	(Wahedi et al., 2020)
Quinone	Dithymoquinone	*In silico*	(Ahmad et al., 2020)
Terpenoid	Glycyrrhizin	*In silico*	(Chen and Du, 2020)
Terpenoid	Nimbin	*In silico*	(Maurya et al., 2020)
		Spike protein interaction	
Alkaloid	Berberine	*In silico*	(Maurya et al., 2020)
Alkaloid	Cepharanthine	*In vitro*	(Kim et al., 2019a)
Alkaloid	Piperine	*In silico*	(Rout et al., 2020)
Alkaloid	Thebaine	*In silico*	(Maurya et al., 2020)
Alkaloid	Fangchinoline	*In silico, In vitro*	(Kim et al., 2019a; Parvez et al., 2020)
Alkaloid	Tetrandrine	*In silico, In vitro*	(Kim et al., 2019a; Parvez et al., 2020)
Flavonoid	Epigallocatechin gallate	*In silico*	(Maiti and Banerjee, 2020)
Flavonoid	Fisetin	*In silico*	(Pandey et al., 2020)
Flavonoid	Isolophirachalcone A	*In silico*	(Parvez et al., 2020)
Flavonoid	Quercetin	*In silico*	(Pandey et al., 2020)
Flavonoid	Theaflavin	*In silico*	(Maiti and Banerjee, 2020)
Phenolic	Cinnamaldehyde	*In silico*	(Kulkarni et al., 2020)
Polyphenol	Curcumin	*In silico*	(Maurya et al., 2020)
Polyphenol	Resveratrol	*In silico*	(Wahedi et al., 2020)
Quinone	Emodin	*In vitro*	(Okamoto et al., 2001; Ho et al., 2007; Ho et al., 2007)
Terpenoid	Carvacrol	*In silico*	(Kulkarni et al., 2020)
Terpenoid	Glycyrrhizin	*In silico*	(Chen and Du, 2020)
Terpenoid	Nimbin	*In silico*	(Maurya et al., 2020)
Terpenoid	Saikosaponin	*In silico*	(Sinha et al., 2020)
		RdRP blockers	
Alkaloid	6-Acetonyldihydrochelerythrine	*In silico*	(Pandeya et al., 2020)
Alkaloid	Allocryptopine	*In silico*	(Pandeya et al., 2020)
Alkaloid	Cepharanthine	*In silico*	(Ruan et al., 2020)
Alkaloid	Fangchinoline	*In silico*	(Parvez et al., 2020)
Alkaloid	Protopine	*In silico*	(Pandeya et al., 2020)
Alkaloid	Tetrandrine	*In silico*	(Parvez et al., 2020)
Flavonoid	Apigenin	*In silico*	(Rameshkumar et al., 2020)
Flavonoid	Cyanidin	*In silico*	(Rameshkumar et al., 2020)
Flavonoid	Delphinidin	*In silico*	(Rameshkumar et al., 2020)
Flavonoid	Hesperidin	*In silico*	(Singh et al., 2020)
Flavonoid	Isolophirachalcone A	*In silico*	(Parvez et al., 2020)
Flavonoid	Myricetin	*In silico*	(Singh et al., 2020)
Flavonoid	Theaflavin	*In silico*	(Lung et al., 2020; Singh et al., 2020)
Polyphenol	Epigallocatechin gallate	*In silico*	(Singh et al., 2020)
Polyphenol	Gallic acid	*In silico*	(Abd El-Aziz et al., 2020)
Polyphenol	Resveratrol	*In silico*	(Abd El-Aziz et al., 2020)
		Main protease inhibitors	
Alkaloid	Berberine	*In silico*	(Narkhede et al., 2020)
Alkaloid	Fangchinoline	*In silico*	(Parvez et al., 2020)
Alkaloid	Solanine	*In silico*	(Hasan et al., 2020)
Alkaloid	Triptanthrine	*In silico*	(Narkhede et al., 2020)
Flavonoid	Amentoflavone	*In vitro*	(Ryu et al., 2010a)

(Continued on following page)

TABLE 1 | (*Continued*) Selected/candidate phytochemicals with inhibitory effects on ACE2, spike proteins, protease, and RdRP in combating COVID-19 neurological signs.

Phytochemical class	Compound	Study type	References
		ACE2 interaction	
Flavonoid	Apigenin	*In vitro*	(Ryu et al., 2010a)
Flavonoid	Fortunellin	*In silico*	(Panagiotopoulos et al., 2020)
Flavonoid	Hesperidin	*In silico*	(Adem et al., 2020a)
Flavonoid	Isolophirachalcone	*In silico*	(Parvez et al., 2020)
Flavonoid	Luteolin	*In vitro*	(Ryu et al., 2010a)
Flavonoid	Narcissin	*In silico*	(Owis et al., 2020)
Flavonoid	Naringenin	*In silico*	(Kim et al., 2019b)
Flavonoid	Oolonghomobisflavan-A	*In silico*	(Bhardwaj et al., 2020)
Flavonoid	Papyriflavonol	*In vitro*	(Park et al., 2017)
Flavonoid	Quercetin	*In vitro*	(Ryu et al., 2010a; Nguyen et al., 2012)
Flavonoid	Rutin	*In silico*	(Adem et al., 2020a)
Iridoid	Geniposide	*In silico*	(Rahman et al., 2020)
Lignan	Savinin	*In vitro*	(Wen et al., 2007)
Polyphenol	Dieckol	*In vitro*	(Park et al., 2013)
Polyphenol	Gallocatechin-3-gallate	*In silico*	(Ghosh et al., 2020)
Quinone	Rhein	*In silico*	(Narkhede et al., 2020)
Quinone	Tanshinone I	*In vitro*	(Park et al., 2012)
Terpenoid	1,8-cineole	*In silico*	(Sharma and Kaur, 2020)
Terpenoid	Andrographolide	*In silico*	(Enmozhi et al., 2020)
Terpenoid	Betulinic acid	*In vitro*	(Wen et al., 2007)

other phytochemicals which inhibit ACE to suppress COVID-19 (Maroli et al., 2020; Muchtaridi et al., 2020).

Recent reports confirmed that there are several other phytochemicals, which inhibited ACE2 activity, including neohesperidin, nobiletin, scutellarin, nicotinamin, and glycyrinodin (Muchtaridi et al., 2020). As another natural product with antiviral properties, glycyrrhizic acid binds to ACE2, thereby could be used for treatment of COVID-19 neurological signs (Pilcher, 2003). Luteolin also inhibited furin proteins which breakdown the S protein in SARS-CoV. Similarly, herbacetin inhibited the interaction between S protein and ACE2. Accordingly, these phytochemicals can be useful for treating/managing neurological manifestation of COVID-19 by targeting the ACE2/spike proteins to suppress the penetration/attachment of SARS-CoV-2 to the CNS cells, what triggers the neurological signs (Wu et al., 2020b). Overall, evidence has shown berberine, thebaine, piperine (as alkaloids), withaferin A (steroidal lactone), nimbin, embelin, cafestol, murrayanine, murrayaquinone-A and andrographolide are phytochemicals with the potential antiviral effects for example through binding to spike protein in SARS-CoV-2, as well as ACE2 receptor (Grover et al., 2011; Boukhatem and Setzer, 2020; Gupta et al., 2020; Parida et al., 2020). Consistent docking results showed the same acceptable inhibitory effects against SARS-CoV-2.

The main phytochemicals with reported inhibitory effects on ACE2 and spike proteins are presented in **Table 1**.

Phytochemicals Inhibit RdRp, 3CLpro and PLpro, Thereby Neural Manifestations in COVID-19

Ongoing studies are consisting on the key role of RdRp, 3CLpro and PLpro, in the neuropathogenesis of SARS-CoV-2. Proteases especially 3CLpro and PLpro, play critical roles in SARS-CoV-2 maturation and replication, and are of the main targets of anti-SARS-CoV-2 phytochemicals (Xue et al., 2008; Ryu et al., 2010a; Shamsi et al., 2016). Polyphenols, especially flavonoids, are among the phytochemicals with anti-SARS effects through inhibiting proteases (Senthilvel et al., 2013; Shamsi et al., 2016; Annunziata et al., 2020). Adem and co-workers, in a molecular docking study on 80 flavonoids showed that 24 of them had suitable interaction with the main protease of SARS-CoV-2, of which hesperidin and rutin had the highest interaction (Adem et al., 2020a). In another *in silico* report, four hundred fifty-eight flavonoids were screened, which among them apigenin 7-(6″-malonylglucoside), cyanidin-3-(*p*-coumaroyl)-rutinoside-5-glucoside, delphinidin 3-*O*-beta-D-glucoside 5-*O*-(6-coumaroyl-beta-D-glucoside), albireodelphin, and (-)-Maackiain-3-*O*-glucosyl-6″-*O*-malonate possessed the most potential in inhibiting SARS-CoV-2. The aforementioned flavonoids showed the highest binding energy values against RdRP, and S proteins of SARS-CoV-2 (Rameshkumar et al., 2020). Another study on twenty-three flavonoids and twenty-five chalcones compounds, showed that the compounds were capable of blocking main proteases. In their study, cyanidin inhibited RNA polymerase and, quercetin blocked the viral spike. As previously mentioned, RdRp catalyzes SARS-CoV-2 RNA replication and thereby is considered an important target for antiviral drug design. Molecular docking investigation revealed that EGCG, theaflavin, theaflavin-3′-*O*-gallate, theaflavin-3′-gallate, theaflavin 3,3′-digallate, hesperidin, quercetagetin, and myricetin bind to the active site of RdRp (Singh et al., 2020). Overall, flavonoids and indole chalcones could combat SARS-CoV-2 (Vijayakumar et al., 2020). Additional evidence confirmed that quercetin and kaempferol possess beneficial anti-inflammatory, antioxidant, antiviral, antiallergic effects which potentially inhibits SARS-CoV 3CLpro, PLpro, and S protein (Di Pierro et al., 2020).

Accordingly, docking evidence indicated quercetin and kaempferol as promising compounds against SARS-CoV-2. So, these phytochemicals could decline neurological manifestations in COVID-19 patients (Ryu et al., 2010b). In a recent *in silico* report by Gorla et al. (2020) silymarin, and biochanin A were proposed as bioflavonoids possessing the most acceptable interaction with ACE2/spike protein of SARS-CoV-2. Also, an *in silico* study indicated that naringenin inhibited 3CLpro chains, thereby may be a promising phytochemical for alleviating neurological symptoms in COVID-19 patients (Kim et al., 2019b). Papyriflavonol A as a prenylated flavone inhibited the PLpro and 3CLpro of SARS-CoV at 3.7 and 103.6 μM, respectively (Park et al., 2017). Also, Ryu and co-workers showed that a biflavonoid, amentoflavone, blocked the 3CLpro at 8.3 μM while apigenin, luteolin, and quercetin inhibited the enzyme at 280.8, 20.2, and 23.8 μM, respectively (Ryu et al., 2010a; Yao et al., 2018; Istifli et al., 2020). Oolonghomobisflavan-A (Bhardwaj et al., 2020), narcissin (Owis et al., 2020), isolophirachalcone (Parvez et al., 2020), fortunellin (Panagiotopoulos et al., 2020), dieckol (Park et al., 2013), gallocatechin-3-gallate (Ghosh et al., 2020) are other polyphenols with inhibitory effects on SARS-CoV-2 proteases.

Theaflavins, a group of polyphenols formed after the fermentation of green tea, have a very strong affinity to bind to RdRp (Lung et al., 2020; Singh et al., 2020). Lung and co-workers reported that theaflavin had a high affinity for RdRp of SARS-CoV2, SARS-CoV, and MERS-CoV (Lung et al., 2020). Also, Singh et al. (2020) showed that theaflavin-3,3′-digallate, theaflavin-3′-gallate, theaflavin-3′-*O*-gallate, and theaflavin had the highest affinity for RdRp with -9.9, -9.6, -9.6, and -9.3 kcal/mol bonding energy, respectively. EGCG and hesperidin (Singh et al., 2020), isolophirachalcone A (Parvez et al., 2020), gallic acid and resveratrol (Abd El-Aziz et al., 2020) are other polyphenols with anti-SARS-CoV-2 activities through the high binding affinity to RdRp.

Of other classes of phytochemicals, solanine is a steroidal alkaloid that interacts with two clusters of amino acids of the C3-like protease. The first cluster consists His163, His164, Met165, and Pro168 and the latter contains Asp187, Gln189, and Ala191 (Hasan et al., 2020). There are several other alkaloid that interact with C3-like protease such as solasurine, omatidenol, cycloartanol, diosgenin, lupeol and purpurin (Hasan et al., 2020). Besides, the alkaloids including cepharanthine (Ruan et al., 2020), fangchinoline and tetrandrine (Parvez et al., 2020), protopine, 6-Acetonyldihydrochelerythrine, and allocryptopine (Pandeya et al., 2020) showed strong binding to SARS-CoV-2 RdRp in docking studies.

Nsp15 is responsible for protein interference with the innate immune response, which is essential in the function of coronavirus. Studies indicated that sarsasapogenin, ursonic acid, apigenin, curcumin, ajmalicine, novobiocin, silymarin, alpha amyrin, pomolic acid, carnosol, asiatic acid, reserpine, betulinic acid, platanic acid, taspine, alphitolic acid, taxifolin, wogonin, chlorogenic acid, afromosin, gliotoxin, psoralen, carinatine rhinacanthin, caffeic acid, coriandrin, scopoletin, cordycepin, ricinoleic acid, alpha asarone, allicin and aranotin as other phytochemicals, can bind to Nsp15 protein, thereby could be useful factors for inhibitors of COVID-19 (Kumar et al., 2020; Umesh et al., 2020). In a research by Adem *et al.*, showed the beneficial effects of caffeic acid derivatives were shown as inhibitors of SARS-CoV-2, via inhibition of COVID-19 Nsp15, main proteases, and spike protein (Adem et al., 2020b).

In addition to alkaloids and flavonoids, terpenoids and quinones are other phytochemicals with inhibitory effects on main proteases of SARS-CoV-2. In an *in silico* study, some natural products against SARS-CoV-2 anthraquinones such as rhein and crysophanic acid as well as the alkaloids such as indican, indigo, berberine, tryptanthrine and terpenes (e.g., bicylogermecrene and glycyrrhizin) showed a strong interaction with SARS-CoV-2 main protease. In their study based on the lowest binding energy, rhein (BE, −8.9 kcal/mol) and tryptanthrine (BE, −8.2 kcal/mol) were introduced as suitable candidates against SARS-CoV-2 (Narkhede et al., 2020). Andrographolide (Enmozhi et al., 2020), 1,8-cineole (Sharma and Kaur, 2020), betulinic acid and savinin (Wen et al., 2007), geniposide (Rahman et al., 2020), and tanshinone I (Park et al., 2012) are other phytochemicals with anti-SARS-CoV-2 activities via the blocking the SARS-CoV-2 proteases. In a similar study, silibinin, dihydrorobinetin, peonidin, robinetin, 5-deoxygalangin, scutellarein, purpurin, isorhamnetin, tricetin, gossypetin, norathyriol, coumestrol, isosakuranetin, pectolinarigenin, tangeritin, nobiletin, pratensein, hispidulin, baicalein, morin, urolithin A, acacetin, pelargonidin, irilone, pinocembrin, malvidin, dalbergin, butein, biochanin A, fustin, 5-hydroxyflavone, pinostrobin, pinobanksin, datiscetin, galangin, cyanidin, daidzein, glycitein, wogonin, phloretin, urolithin B, angolensin, pinosylvin, formononetin, liquiritigenin, prunetin, alpinetin, biochanin A, rhapontigenin, equol, piceatannol, isorhapontigenin, danshensu, eugenin, sinapic acid, pterostilbene, pyrogallol, resacetophenone, syringic acid, *p*-coumaric acid, paeonol, protocatechuic acid, tyrosol, catechol, 4-ethylphenol and cinnamic acid as natural product binding to SARS-CoV-2 RdRp (Kurokawa et al., 2001; Bosch-Barrera et al., 2020; Singh et al., 2020). Consistently, based on an study of Umesh et al. (2020) carnosol, rosmanol, and arjunglucoside-I, as natural phytochemicals have shown potential inhibitory effects on SARS-CoV main protease using molecular docking approach. In a recent study by Chojnacka *et al.*, some biologically active phytochemicals like quercetin, betulinic acid, luteolin, indigo, aloeemodine, and quinomethyl triterpenoids, or gallates were of potential key antiviral agents in blocking viral proteases (Chojnacka et al., 2020). Additional studies have shown several phytochemicals such as 18-hydroxy-3-epi-alphayohimbine, vincapusine, alloyohimbine, and gummadiol, toward the inhibition of SARS-CoV 3CLpro, SARS-CoV-2 3CLpro, and MERS-CoV 3CLpro toward the treatment of COVID-19 neuronal associations (Bhardwaj et al., 2020). Phytochemicals with the potential of inhibiting RdRP and proteases are also presented in **Table 1**.

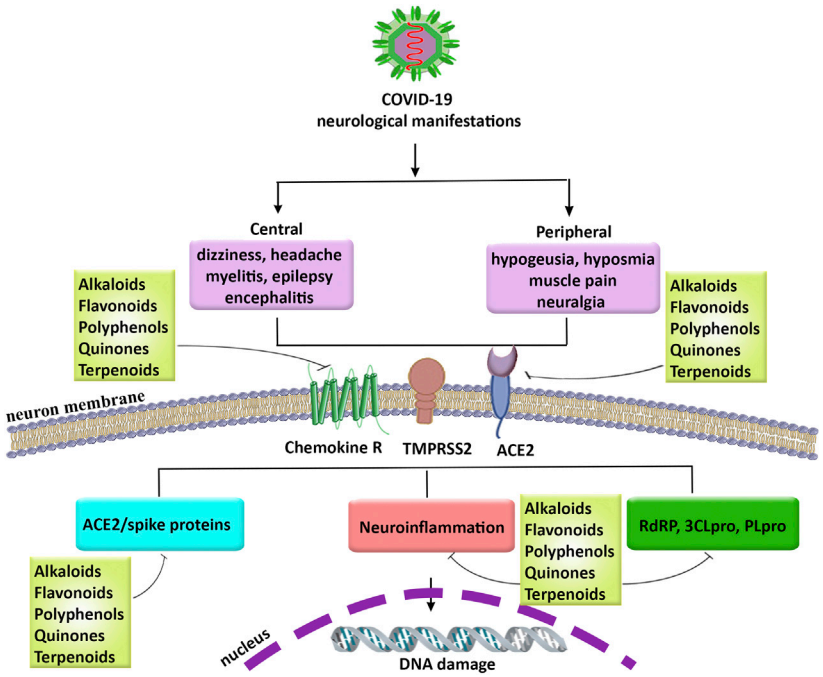

FIGURE 3 | The neurological manifestations in COVID-19, related pathophysiological mechanisms, and promising role of phytochemicals. COVID-19: coronavirus 2019, PLpro: papain-like protease, RdRP: RNA-dependent RNA polymerase, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, TMPRSS2: transmembrane protease, serine 2, 3CLpro: 3-chymotrypsin-like cysteine protease.

PHARMACOKINETIC INTERACTION AND BBB PERMEABILITY OF PHYTOCHEMICALS: AN APPROACH TO NOVEL DELIVERY SYSTEMS

However, the neuroprotective effects of such phytochemicals have been provided in several studies, estimations of the permeability through the BBB of the phytochemicals were assessed by the SwissADME program (Daina et al., 2017). Information on the estimations of permeability through the BBB, as well as predict absorption, distribution, metabolism, and excretion (ADME) parameters, pharmacokinetic properties, druglike nature and medicinal chemistry friendliness are shown in **Supplementary Table S1**. Among the fifty-five phytochemicals, the screening of BBB permeability gave fifteen compounds with a positive effect. Among these are the monoterpenoids 1,8-cineole and carvacrol; the alkaloids 6-acetonyldihydrochelerythrine, allocryptopine, berberine, piperine, protopine, thebaine, and triptanthin; the flavonoid chrysin; the quinones dithymoquinone and tanshinone I; the phenolic compounds resveratrol and cinnamaldehyde; and the lignan savinin. To overcome the aforementioned pharmacokinetic drawbacks of some phytochemicals, novel delivery systems are being applied regarding increasing their penetration to BBB. Accordingly, nano-formulations, polymeric micelles, nano-/micro-emulsions, nano-gels, solid lipid nano-particles, polymer composites, and liposome/phospholipid have been studied so far (Abbaszadeh et al., 2020; Fakhri et al., 2020a).

As previously mentioned, inflammatory conditions play critical roles during the pathogenesis of COVID-19 disease. It is worth noting that inflammation could increase the BBB penetration of phytochemicals to facilitate their central permeation. This pathophysiological condition simplifies the CNS penetration of those phytochemicals with limitations in their penetration.

DISCUSSION

COVID-19 pandemic is an important threat to human life. Up to now, no effective drug or vaccination has been provided to combat various complications in COVID-19. So, finding therapeutic agents to combat related manifestations in COVID-19, is of great importance. Among different complications of COVID-19 the neurological manifestations have attracted particular attention. Growing evidence is highlighting the involvement of multiple dysregulated mechanisms behind the pathophysiology of COVID-19 neurological manifestations, including hypoxia, neuroinflammation, ACE2/spike proteins, and related enzymes in virus proliferation (e.g., RdRP, 3CLpro, and PLpro). So, providing multi-target agents could pave the road in combating associated neuronal manifestations in COVID-19. For many years, the plant kingdom has shown promising antiviral, and anti-neuroinflammatory results. Accordingly, the hope regarding identifying new applications for the candidate phytochemicals has a successful history in

complementary/alternative medicine. We previously showed the antiviral approaches and therapeutic targets of plant-derived secondary metabolites in various steps of viruses life cycle, including penetration, uncoating, replication, and release (Pour et al., 2019). In the present study, potential phytochemicals with antiviral effects and modulatory potentials against neuroinflammation, ACE2/spike protein, and related main proteases in the virus life cycle have been highlighted regarding inhibiting the penetration/attachment and replication phases of coronaviruses (**Figure 3**). Among the aforementioned phytochemicals, *in silico*/*in vitro* results introduced polyphenols (mainly flavonoids), alkaloids, and terpenes/terpenoids as potential candidates in counteracting the neurological signs of COVID-19. Although the BBB limits the CNS penetration of some phytochemicals, the disease-related inflammatory conditions as well as novel delivery systems could potentially overcome the BBB dynamic and drawback the limitation. As the results, flavonoids like naringin and it aglycone (naringenin), theaflavins, silymarin, curcumin, EGCG, polyphenol resveratrol and its derivative (polydatin), as well as some phytosterols and cannabinoids showed the most simultaneous anti-neuroinflammatory and antiviral potentials in combating SARS-CoV-2 neural complications. To suppress the viral penetration/attachment the flavonoids hesperidin, chrysin, kaempferol, quercetin, fisetin, baicalin, naringenin, EGCG, and theaflavin as well as some terpenes chalcones, glycyrrhizin, nimbin and alkaloids like berberin, thebaine, piperine as well as terpenoids have shown a more potential future in targeting ACE2/spike proteins. Consequently, regarding targeting the main proteases of coronaviruses flavonoids apigenin, cyaniding, delphinidin, EGCG, theaflavin, naringenin, hesperidin, quercetin and kaempferol, as well as some chalcones, steroidal alkaloid, terpenoids, and quinones are of potential candidates in inhibiting the main proteases of coronaviruses. Overall, the aforementioned phytochemicals have shown growing evidence to be of potential agents in combating neurological signs of COVID-19 through attenuation of neuroinflammation, ACE2/spike proteins, and main proteases.

Such studies could pave the road regarding finding novel therapeutic agents in combating neurological manifestations in COVID-19. Further reports are required to reveal the precise dysregulated pathways responsible for COVID-19 neurological signs, as well as potential therapeutic phytochemicals.

AUTHOR CONTRIBUTIONS

Conceptualization, SF, MF, and JE; drafting the manuscript, SF, SP, MM; Software: SF, review and editing the paper: SF, MF, and JE; All authors have read, revised and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.621099/full#supplementary-material.

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REVIEW
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Calming the Storm: Natural Immunosuppressants as Adjuvants to Target the Cytokine Storm in COVID-19

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The COVID-19 pandemic has caused a global health crisis, with no specific antiviral to treat the infection and the absence of a suitable vaccine to prevent it. While some individuals contracting the SARS-CoV-2 infection exhibit a well coordinated immune response and recover, others display a dysfunctional immune response leading to serious complications including ARDS, sepsis, MOF; associated with morbidity and mortality. Studies revealed that in patients with a dysfunctional immune response, there is a massive cytokine and chemokine release, referred to as the 'cytokine storm'. As a result, such patients exhibit higher levels of pro-inflammatory/modulatory cytokines and chemokines like TNFα, INFγ, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-9, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, MCSF, HGF and chemokines CXCL8, MCP1, IP10, MIP1α and MIP1β. Targeting this cytokine storm is a novel, promising treatment strategy to alleviate this excess influx of cytokines observed at the site of infection and their subsequent disastrous consequences. Natural immunosuppressant compounds, derived from plant sources like curcumin, luteolin, piperine, resveratrol are known to inhibit the production and release of pro-inflammatory cytokines and chemokines. This inhibitory effect is mediated by altering signal pathways like NF-κB, JAK/STAT, MAPK/ERK that are involved in the production and release of cytokines and chemokines. The use of these natural immunosuppressants as adjuvants to ameliorate the cytokine storm; in combination with antiviral agents and other treatment drugs currently in use presents a novel, synergistic approach for the treatment and effective cure of COVID-19. This review briefly describes the immunopathogenesis of the cytokine storm observed in SARS-CoV-2 infection and details some natural immunosuppressants that can be used as adjuvants in treating COVID-19 disease.

Keywords: COVID-19, cytokine storm, immunomodulatory agents, plant-derived immunosuppressants, adjuvant

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) caused by a novel ß-coronavirus, SARS-CoV-2 was first reported in Wuhan, China in December 2019 (Wang et al., 2020a). Spreading rapidly across the globe, the outbreak was declared as a Public Health Emergency of International Concern on 30 January 2020 by the World Health Organization (World Health Organization, 2020a). The reality

and challenge of the present situation is that there is no specific drug to treat and cure the disease; neither is there a vaccine for prevention from it (at the time of submission of this review) (World Health Organization, 2020b).

One characteristic feature of the COVID-19 disease is the complex immune dysregulation observed in patients. This immune dysfunction causes deleterious clinical manifestations that lead up to organ injury, consequent organ failure and ultimately mortality (Giamarellos-Bourboulis et al., 2020; Li et al., 2020a). The hallmark of the immune dysregulation observed is an exaggerated immune response; manifested as hyperinflammation and hypercytokinemia (cytokine storm syndrome/ cytokine storm) in the COVID-19 patients. The factors that contribute to the state of hyperinflammation are persistent lymphopenia, neutrophilia, over-activation of complement components C3, C3a, C5, C5a and mannose binding lectin-associated serine protease (MASP2); in addition to the cytokine storm (Girija et al., 2020). The cytokine storm observed is the most dangerous and potentially life-threatening event in the COVID-19 disease. This is because it plays a crucial role in disease aggravation by promoting acute respiratory distress syndrome (ARDS) and multiple organ failure (MOF) (Coperchini et al., 2020; Ye et al., 2020). Several studies suggest that in addition to the use of antiviral drugs for the treatment of COVID-19, downregulation of the cytokine storm would prove to be an efficient treatment strategy to successfully combat the disease (Rahmati and Moosavi, 2020; Soy et al., 2020; Zhao, 2020). Plants, plant extracts and their derivatives have been known to possess immunomodulatory properties. Additionally, some plant-derived bioactive compounds have been evaluated for their ability to suppress the cytokine storm associated with inflammation and disease. This paper discusses the cytokine storm syndrome observed in COVID-19 patients in brief and emphasizes on some natural immunosuppressant agents derived from plants sources that can play an important role in targeting and mitigation of the cytokine storm observed in COVID-19.

COVID-19 AND THE CYTOKINE STORM

Introduction to COVID-19

COVID-19 is a viral disease caused by a beta coronavirus, SARS-CoV-2; an enveloped, non-segmented RNA virus (Astuti and Ysrafil, 2020; Wang et al., 2020b). The disease outbreak, declared as an ongoing pandemic by the WHO is widespread; affecting 220 countries, areas/ territories across the world with a total of 55,928,327 confirmed cases and with 1,344,003 confirmed deaths reported (World Health Organization, 2020c). Transmission of SARS-CoV-2 occurs primarily through respiratory droplets and contact routes (World Health Organization, 2020d). The spike protein plays an important role for SARS-CoV-2 to gain access to the intracellular compartment of the host. The receptor-binding domain (RBD) of the S1 subunit of the spike protein binds to the hACE2 receptor with high affinity, facilitating viral entry and infection in the lower respiratory tract cells (Hemmat et al., 2020; Shang et al., 2020). Once SARS-CoV-2 enters the host cell, its RNA is translated and viral replication for the production of mature newly synthesized virons occurs, which are released out of the infected cells (Shereen et al., 2020). The mean incubation period for SARS-CoV-2 is 3–7 days (Li et al., 2020b). Symptoms develop post the incubation period (Wujtewicz et al., 2020). Some infected individuals are asymptomatic, others are symptomatic with mild disease and, a third group is symptomatic with severe disease (Tabata et al., 2020). The symptoms of the disease are fever, cough, fatigue, headache, myalgia, sore throat, shortness of breath, sputum production and diarrhea. Other symptoms include chest pain, chills, nasal congestion, rhinorrhea and nausea (Fu et al., 2020a). Clinical presentations include elevated levels of lactate dehydrogenase, creatine kinase, alanine transaminase and aspartate aminotransferase (Wang et al., 2020a). Lymphocytopenia, high exhaustion levels and reduced functional diversity of T-cells ($CD4^+$ and $CD8^+$ T-cells) in peripheral blood is also another clinical feature of COVID-19 patients (Zheng et al., 2020a; Zheng et al., 2020b; Zhao et al., 2020). Higher levels of serum IgM, IgG and IgA can be detected in patients; severely ill SARS-CoV-2 patients have significantly higher antibody levels than mildly ill patients (Ma et al., 2020). The platelet and neutrophil levels are also elevated in patients (Zhou et al., 2020). Elevated D-dimer levels, prolonged prothrombin time (PT) and altered levels of fibrinogen observed point to the state of pulmonary intravascular coagulopathy that develops in patients (Zhang et al., 2020a; Lancet Haematol, 2020; Connors and Lavy, 2020; Long et al., 2020; McGonagle et al., 2020). Severe COVID-19 patients exhibit widespread complement activation which is characterized by C3 activation, C3a generation, C3 fragment deposition and increased serum C5a levels (Mastaglio et al., 2020; Noris et al., 2020; Risitano et al., 2020). Inflammatory markers like erythrocyte sedimentation rate (ESR), IL-6, C-reactive protein (CRP) and procalcitonin (PCT) are also elevated; especially in the patients with severe disease (Zeng et al., 2020). Elevated levels of all these inflammatory markers have been associated with disease severity; indicating the hyper-immune inflammatory state existing in the body resulting in higher morbidity and mortality in this patient group (Chen et al., 2020a; Chen et al., 2020b; Lippi and Plabani, 2020; Sun et al., 2020). Patients also exhibited higher levels of pro-inflammatory cytokines TNFα, IFNγ, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-9, IL-12, IL-13, IL-17, G-CSF, GM-CSF, MCSF and chemokines CXCL8, MCP1, IP10, MIP1α, MIP1β. As in the case of inflammatory markers, the levels of serum cytokines and chemokines are higher in patients with severe disease. A diverse cytokine profile is observed in the two groups of patients; with critically ill patients showing higher levels of IL-1, IL-6, IL-2, IL-7, IL-10, IL-17, G-CSF, IP10, MCP1, MIP1α and TNFα. Among the cytokines, IL-6, IL-10 and CXCL10 are predictive of high risk patients of disease deterioration. As a consequence of the surging cytokine levels, the epithelial lining of the lungs gets injured that leads to deterioration of the alveolar cellular barriers. Changes to the microvasculature are also observed owing to damage in the endothelial cells. These changes results in the development of ARDS (Schett et al., 2020a; Han et al., 2020; Laing et al., 2020; Lingeswaran et al., 2020; Lingeswaran et al., 2020). The immunopathology gradually

progresses to respiratory failure, establishment of secondary infections, sepsis and septic shock, multiple organ failure (kidney, liver); an overall poor prognosis that can end up in mortality (Gabriella et al., 2020; Guo et al., 2020; Hou et al., 2020).

Cytokine Storm/ Hypercytokinemia/ Cytokine Storm Syndrome

Cytokine storm and hypercytokinemia are the two terminologies used to describe a severe, life-threatening condition that can occur as a result of an infection, autoimmune condition, or other disease. The National Cancer Institute defines cytokine storm as a severe immune reaction in which the body releases too many cytokines into the blood too quickly (National Cancer Institute, 2020). This condition is also referred to as cytokine storm syndrome sometimes (Shimabukuro-Vornhagen et al., 2018). These cytokines are a diverse group of small, non-structural, low molecular weight protein signaling molecules that are secreted and released by cells that have a complex regulatory influence on immunity and inflammation (Zhang and An, 2007; Ray et al., 2016). They play a significant role in regulating the immune response in health and disease (Duque and Descoteaux, 2014). Escalated cytokine production is associated with disease conditions (Orzechowski et al., 2014). Although the clinical presentations of cytokines vary for different diseases, they are characterized by broadly similar cytokine profiles. Cytokines generally associated with cytokine storm are interferons, interleukins, chemokines, colony stimulating factors (CSFs) and tumor necrosis factors (TNFs) (Tisoncik et al., 2012).

Immunopathogenesis of Cytokine Storm in COVID-19

The immune system plays a crucial role in the control and resolution of SARS-CoV-2 infection. An out-of-control immune response to the pathogen can lead to immunopathogenesis that can prove to be fatal; causing excessive inflammation and even death (Guo et al., 2020; Qin et al., 2020). In most of the individuals infected with SARS-CoV-2, the immune system is primed and cells are recruited which clears the infection in the lung. The immune response then gradually recedes and the patients recover successfully. However, there are some patients wherein a dysfunctional immune response is observed, that triggers a massive cytokine and chemokine release; 'cytokine storm', mediating widespread inflammation in the lung (Catanzaro et al., 2020; Tay et al., 2020). One of the major causes of the ARDS and MOF observed in severe SARS-CoV-2 infection is the cytokine storm (Lingeswaran et al., 2020; Ye et al., 2020). Studies revealed that the progression of SARS-CoV-2 infection is associated with cytokine storm; higher level of cytokine storm is associated with more severe disease development (Tang et al., 2020a; Han et al., 2020). Plasma levels of cytokines IL-1β, IL-1RA, IL-7, IL-9, IL-10, FGF, G-CSF, GM-CSF, PDGF, VEGF, IFNγ, TNF and chemokines CXCL8, IP10, MCP1, MIP1α, MIP1β are significantly increased in patients with COVID-19 compared to healthy individuals. Furthermore, pro-inflammatory cytokines IL-2, IL-7, IL-10, G-CSF, TNF and chemokines IP10, MCP1, MIP1α are increased in severe patients compared with mildly-infected patients (Tang et al., 2020b).

Cytokines and chemokines are essential immune system mediators that have a significant role to play in maintenance of anti-viral immunity (Melchjorsen et al., 2003; Sládková and Kostolansky 2006). SARS-CoV-2 activates both the innate and adaptive immune response (Catanzaro et al., 2020). Initially after the entry of the virus into host cells, pathogen recognition receptors (PRRs) like TLR7, TLR8, RLRs and NLRs expressed by epithelial cells and antigen presenting cells like alveolar macrophages facilitate the recognition of virus by recognizing PAMP compromised nucleic acids, carbohydrate moieties, glycoproteins, lipoproteins, intermediate products such as dsRNA and small molecules found in the structural components of virus. TLR3, TLR7 and TLR8 are the first to identify the virus and this is responsible for an enhanced interferon production. Viral recognition triggers the stimulation of the innate immune response that leads to the activation of several signal transduction cascades and consequent downstream transcription factors resulting in the expression of genes encoding pro-inflammatory cytokines and chemokines; the SARS-CoV-2 signature characterized by induction of IL-1Rα, IL-1β, IL-6 and TNF. The chemokines produced serves to attract more innate immune response cells; NK cells, dendritic cells, polymorphonuclear leukocytes and monocytes; which in turn produce chemokines; MIG, IP10 and MCP-1 that are capable of recruiting lymphocytes. These recruited T-lymphocytes serve to recognize the antigen that is presented by the dendritic cells. The primed CD4 T-cells then execute the highly specific adaptive immune response by giving the signal to the B-cells for the production of antibodies and to the cytotoxic CD8 T-cells that are capable of targeting and eliminating the SARS-CoV-2. The antibodies that are produced; mainly IgG, IgM and IgA are highly specific and are directed toward the SARS-CoV-2 surface glycoproteins; primarily the spike glycoprotein and the nucleocapsid protein. This constitutes the humoral immune response to SARS-CoV-2 and these antibodies are vital to neutralize the viral infection of the human cells and tissues expressing ACE2. The cellular immune response to SARS-CoV-2 is mediated by the T-helper cells; the CD4 T-cells that help B-cells produce neutralizing antibodies, the cytotoxic CD8 T-cells; aided by helper T-cells that directly kill SARS-CoV-2-infected cells, the other T-cells; including the Th17-cells that drive the subsequent inflammatory response observed and the regulatory T-cells that are responsible for containing the immune response to preventing an exaggerated response (García, 2020; Herb et al., 2020; Hotez et al., 2020; Poland et al., 2020; Ragab et al., 2020; Shah et al., 2020; Song et al., 2020; Soy et al., 2020; Tay et al., 2020; Vabret et al., 2020).

Studies have revealed that the transition from the innate to adaptive immune response is critical for the clinical progress of the SARS-CoV-2 infection. Though the immune regulatory events in play during this transition are poorly understood at this point, it is clear that it either leads to a protective immune response in some patients or a dysfunctional immune response in others. Another contributing factor is the dysregulated interferon

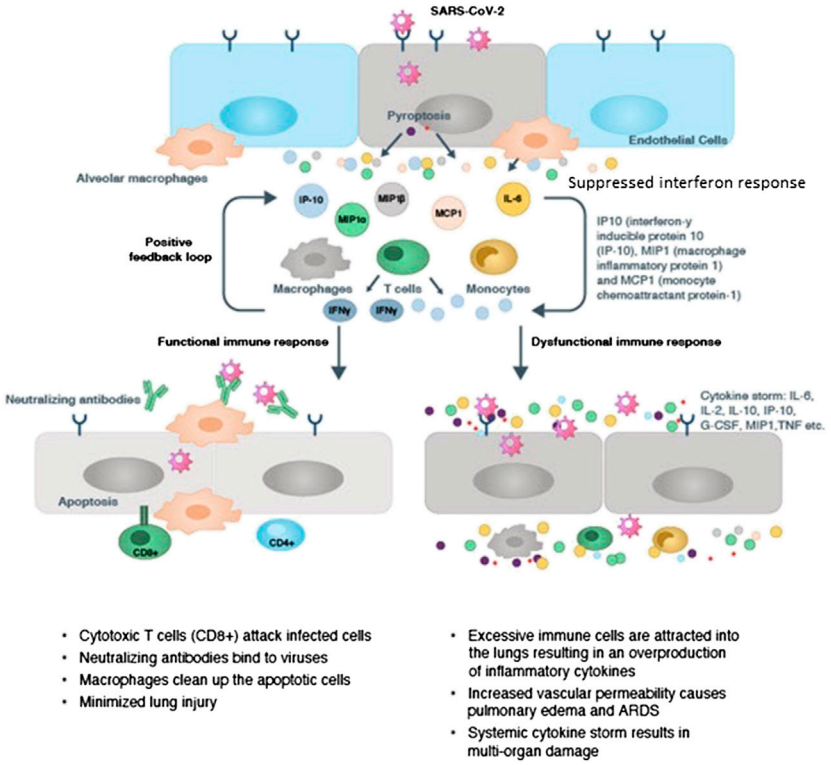

Figure 1 | Immunopathology of Cytokine Storm in COVID-19 (CAS, 2020).

responses. SARS-CoV-2 replication during the incubation period in the host cells is stealthy; without detectably triggering interferon production resulting in high viral load. As a result, there is an initial, suppressed interferon response in early stage of infection. In severe COVID-19 patients, however, robust IFN-1 responses have been reported. Such a dysfunctional immune response results in failure to effectively clear the pathogen and persistent viral replication ensuing an exacerbated inflammatory response and elevated cytokine production. Together, impaired viral clearance, low levels of type I interferons in the initial stage of infection, increased neutrophil extracellular traps and triggered NK cell activation constitute the plausible predisposing factors that drive the cytokine storm or cytokine storm syndrome in COVID-19 contributing to the aggressive inflammatory response. Other contributing factors to the cytokine storm syndrome include pyroptosis; an inflammatory form of programmed cell death and ACE2 receptor mediated-increase in cytokines IL-6 and MCP1 triggered by an increased angiotensin II following the disruption of the rennin-angiotensin system; caused when S1 subunit of spike protein binds to hACE2 in the alveolar macrophages (Acharya et al., 2020; Fu et al., 2020b; Golonka et al., 2020; Iwasaki et al., 2020; Lee and Shin, 2020; Lee et al., 2020; Mahmudpour et al., 2020).

Initially, there is a lag in the production of cytokines and chemokines by the innate immune cells in the COVID-19 disease. This is followed by a sudden acute increase in the pro-inflammatory cytokine and chemokine production by the activated macrophages, monocytes and other recruited lymphocytes. The surge in levels of pro-inflammatory cytokines and chemokines augments infiltration of neutrophils, macrophages, monocytes and T-cells to the site of infection, bringing about an intense and violent inflammatory response in the bronchi and alveoli. This leads to disruption of air-blood barrier, endothelial and epithelial cell damage, breakdown of the alveolar-epithelial barrier, diffuse alveolar damage leading to ARDS and pulmonary fibrosis (PF). In addition to the local damage at the site of infection, the cytokine storm also has ripple effects across the body; contributing to viral sepsis, MOF and finally death in critically-ill patients (Schett et al., 2020b; Li et al., 2020c; Lingeswaran et al., 2020; Matthay et al., 2020; Nile et al., 2020; Prompetchara et al., 2020; Ragab et al., 2020; Risitano et al., 2020; Spagnolo et al., 2020; Tay et al., 2020; Vabret et al., 2020). A diagrammatic representation of the cytokine storm observed in COVID-19 is found in **Figure 1** (CAS, 2020).

IMMUNOMODULATORY AGENTS-TARGETING THE CYTOKINE STORM IN COVID-19

Despite intense scientific study and research, there are no specific drugs or vaccine available for the treatment or prevention of COVID-19. More than 600 clinical trials have been undertaken, with no promising results to date (at the time of submission of this review). The current standard care for COVID-19 being

practiced is just symptomatic, palliative and supportive treatment (Catanzaro et al., 2020; Risitano et al., 2020). A diverse spectrum of pharmaceutical agents is being employed in an attempt to treat and efficiently manage COVID-19 infection. Antiviral agents, other chemical agents, monoclonal antibodies and other drugs are part of the current treatment regime. The antiviral drugs in use are lopinavir, ritonavir, remdesivir, oseltamivir, favipiravir, arbidol, ribavirin, ganciclovir and amantadine. Other chemical agents used for treatment are hydroxychloroquine, chloroquine, azithromycin, ivermectin, colchicine, thalidomide and glucocorticoids like methylprednisolone and dexamethasone. Monoclonal antibody tocilizumab, convalescent plasma interferons and intravenous immunoglobulin therapy are some of the other treatment methods in vogue. Additionally, antibiotics like cephalo-sporins, quinolones, carbapenems, linezolid or antifungal agents may be given (Chakraborty et al., 2020a; National Institutes of Health, 2020a; Saha et al., 2020a; Sethi et al., 2020; Tobaiqy et al., 2020).

While the role played by specific antiviral drugs and other pharmaceutical agents that can effectively target SARS-CoV-2 and reduce the infection is significant and cannot be downplayed, the use of immunomodulatory therapy is potentially effective, considering that SARS-CoV-2 drives immune dysfunction and induces hyperinflammation and a cytokine storm in patients (Iannaccone et al., 2020; Shi et al., 2020; Tufan et al., 2020; Zhong et al., 2020). A treatment strategy goes that beyond antiviral therapy alone, using immunomodulatory agents as adjuvants is a holistic therapeutic approach that takes into account the immune response of the host, hence checking the acute immune/ inflammatory response and preventing ARDS and PF (Alijotas-Reig et al., 2020; Liang et al., 2020). The cytokine storm is one prominent feature of the severe immune aberrations observed in COVID-19 patients (Yazdanpanah et al., 2020). Targeting the cytokine storm in order to ameliorate the state of hyperinflammation is proposed to be a novel therapeutic approach in the treatment of COVID-19 patients (Felsenstein et al., 2020; Prompetchara et al., 2020; Roshanravan et al., 2020a; Vabret et al., 2020). Several synthetic drugs, monoclonal antibodies and stem cell therapies have been proposed and are being explored to treat the cytokine storm in COVID-19 and thus reduce the state of hyperinflammation (Alijotas-Reig et al., 2020). Some of the therapeutic approaches being used currently and are also under clinical trials are discussed as follows.

Anti-malarial drugs hydroxychloroquine and chloroquine that are know to decrease Th17-related cytokines; IL-6, IL-17, IL-22, TNFα and IL-1β have been evaluated for efficacy in COVID-19 treatment in several clinical trials (Felsenstein et al., 2020; da Silva et al., 2020; Karres et al., 2020; Meo et al., 2020). Selective cytokine blockade with monoclonal antibodies tocilizumab and sarilumab that are antagonists of IL-6 receptors are being studied in clinical trials in certain countries (Atal and Fatima, 2020; Chakraborty et al., 2020b; Saha et al., 2020b). Calcineurin inhibitors reduce both the calcium-production of IL-2 and the expression of IL-2 receptor thus reducing T-cell activation. Two such inhibitors; cyclosporine and tacrolimus are being considered as potential drug candidates to reduce the cytokine storm syndrome observed in COVID-19 (Mejia et al., 2014; Cure et al., 2020; Rudnicka et al., 2020; National Institutes of Health, 2020b). The IL-1 receptor antagonist anakinra and the anti-IL-1β monoclonal antibody canakinumab are two drug candidates that efficiently treat cytokine storm syndromes observed in other hyper-inflammatory conditions like Still's disease that are being evaluated for efficacy against the SARS-CoV-2-induced cytokine storm (Huet et al., 2020; Filocamo et al., 2020; National Institutes of Health, 2020c). Infliximab and adalimumab that are anti-TNF antibodies are being considered for modulating the hyperinflammation observed in COVID-19 by specifically targeting TNF (Roshanravan et al., 2020b). Individual studies and clinical trials to evaluate efficacy of low-molecular weight unfractionated heparin in COVID-19 patients with coagulopathy are underway (Tang et al., 2020b; National Institutes of Health, 2020d). Another immunomodulatory treatment molecule being evaluated is the use of intravenous immunoglobulin (IVIG). A clinical trial studying the benefit of administering intravenous immunoglobulin when compared to standard care alone is currently underway (National Institutes of Health, 2020e). Individual studies evaluating the efficacy of high dose of IVIGs as a therapeutic option in deteriorating patients have been undertaken, yielding mixed results (Xie et al., 2020; Cao et al., 2020). Hyperimmune immunoglobulin treatment for COVID-19 is also being explored (Alwis et al., 2020). A clinical trial that compares the efficacy of convalescent plasma to anti-COVID-19 human immunoglobulin in hospitalized patients is currently in progress (National Institutes of Health, 2020f). The JAK/STAT pathway is the main signaling pathway involved in the control of cytokine production (Bagca and Avci, 2020; Seif et al., 2020). Ruxolitinib selectively inhibits Janus kinase (JAK) 1 and 2 and has a modest to marked selectivity against tyrosine kinase (TYK) 2 and JAK 3. Studies and clinical trials are being conducted to evaluate the efficacy of ruxolitinib to target the excess production of cytokines in COVID-19 patients with hyperinflammation and ARDS (La Rosée et al., 2020; National Institutes of Health, 2020g; National Institutes of Health, 2020h). Baricitinib is another inhibitor that binds to JAK 1/2 and inhibits its activation and consequent cytokine release (National Institutes of Health, 2020i). The use of baricitinib for targeting the COVID-19 cytokine storm is being explored in pilot studies and phase 2 and 3 clinical trials (Cantini et al., 2020; National Institutes of Health, 2020j; National Institutes of Health, 2020k). Corticosteroids which are known for their ability to modulate the inflammatory response and have been used in other viral outbreaks are another option for COVID-19 treatment (Russell et al., 2020). Clinical trials testing the prophylactic action, safety and efficacy of dexamethasone and methylprednisolone in reducing the cytokine storm in COVID-19 patients are ongoing (Horby et al., 2020; National Institutes of Health, 2020l; National Institutes of Health, 2020m; Nature, 2020). Statins are a class of drugs that have immunomodulatory and anti-inflammatory properties and are being considered for treating the cytokine storm in COVID-19 (Castiglione et al., 2020; Lee et al., 2020; National Institutes of Health, 2020n). The entry of SARS-CoV-2 into host cells is mediated by hACE2, which is also involved in the virus-induced acute lung injury (Liu et al., 2020). Clinical trials are ongoing, to check the safety and

efficacy of recombinant hACE2 (rhACE2) to mediate direct (via restoration of rennin-angiotensin system) and indirect (the chimeric receptor effect) therapeutic effects in SARS-CoV-2 induced inflammation and ARDS (Barone et al., 2020; National Institutes of Health, 2020o; National Institutes of Health, 2020p). The use of multipotent mesenchymal stem cell (MSCs) to inhibit the exaggerated immune response caused by the cytokine storm in COVID-19 patients is also being studied (Rajarshi et al., 2020).

Though the benefits of these synthetic therapeutic immunomodulatory agents cannot be disputed, their use is frequently associated with adverse side effects. Cardiomyopathy, neurological and gastrointestinal side effects are reported with chloroquine and hydroxychloroquine treatment and as COVID-19 patients are more vulnerable because of co-morbidities, there are concerns about safety (Colafrancesco et al., 2020; U.S. Food and Drug Administration, 2020; Gevers et al., 2020). Treatment of COVID-19 patients with IL-6 antagonists with tocilizumab and sarilumab also raises safety concerns as these are known to cause endocrinological, hematological, gastrointestinal and cardiovascular adverse effects (Atal and Fatima, 2020). IL-2 blockers cyclosporine and tacrolimus are known to cause side effects and can be extremely harmful (Cure et al., 2020; Xia et al., 2020). Anakinra and canakinumab, IL-1 blockers have a reasonably good safety profile, but can cause elevation of liver enzyme levels, myopathy and mild leukopenia (Colafrancesco et al., 2020). Anti-TNF treatment adverse effects are reported to be infrequent (Favalli et al., 2020). Severe drug reactions were reported for JAK/STAT inhibitor, ruxolitinib administered to COVID-19 patients; thus prompting the early stoppage of this drug in the study undertaken (Gaspari et al., 2020). There is also a risk of developing serious infections with the use of another oral JAK/STAT inhibitor, baricitinib (Lilly, 2020). Previously conducted clinical trials on statin treatment in ARDS and sepsis patients was met with negative outcomes, thus causing reluctance in considering them as adjuncts for COVID-19 therapy (Lee et al., 2020). In a study of rhACE2 treatment for ARDS patients, hypernatremia and dysphagia were the noted side effects (Barone et al., 2020). In case of improper administration of MSCs, there is a probability of lethal adverse thrombotic complications and reactions necessitating a thorough understanding of this treatment option and application routes (Moll et al., 2020).

NATURAL IMMUNOSUPPRESSANT AGENTS- ADJUVANTS TO TARGET THE CYTOKINE STORM IN COVID-19

The discovery of immunomodulatory agents from plants sources with enhanced bioavailability and essentially devoid of toxic side effects is a ray of hope and opens up a novel approach to mitigate the cytokine storm syndrome observed in COVID-19 (Grigore, 2017; Cena and Chieppa, 2020). Several plants and plant-derived bioactive compounds have been studied for their immunomodulatory activity and many have been identified for their immunosuppressive activity in particular (Sagrawat and Khan, 2007; Sahoo and Banik, 2018). These natural immunosuppressive agents provide an alternative therapeutic strategy for the efficient management SARS-CoV-2-associated illness; the cytokine storm and the resulting hyperinflammation. Targeting the cytokine storm with plant-derived immunosuppressants is a very promising strategic treatment method. This is because the individual mediators of the inflammatory cascade; cytokines including IL-1β, IL-6, TNFα and chemokines IP10, MCP1 are neutralized or inhibited, rather than a broad immune suppression that can negatively effect the viral clearance. This specific targeting of cytokines is achieved by the inhibitory action on specific signaling cascades. With their good safety profiles in addition to their capacity to keep a check on the pro-inflammatory cytokine levels all the while without compromising on the ability of the immune system to respond to the SARS-CoV-2; plant-derived immunosuppressant are the need of the hour. Some immunosuppressant agents derived from plants proven to reduce inflammation by downregulating the levels of pro-inflammatory cytokines that can be used as adjuvants in COVID-19 treatment are discussed as follows.

Andrographolide

Andrographolide is a labdane diterpenoid; 4-hydroxy-3-[2-[6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl]ethylidene]oxolan-2-one. It is isolated from the medicinal plant Andrographis paniculata (Brum.f.) Nees and has a wide range of therapeutic properties; anti-inflammatory, anti-allergic, anti-platelet aggregation, antineoplastic, anti-HIV and hepatoprotective activity (Jayakumar et al., 2013; National Center for Biotechnology Information, 2020a). Andrographolie is also a potent immunomodulator; known to significantly stimulate the immune response, regulate the production of NK cells and cytokines and stimulate the production of cytotoxic T-lymphocytes (Varma et al., 2011). Andrographolide efficiently brought about a dose-dependent reduction in the levels of inflammatory cytokines TNFα, IL-12, IL-1β, IL-6, IL-18 in LPS/ IL-4-activated murine macrophages (Wang et al., 2010). Andrographolide treatment suppresses inflammatory mediators IL-1β, TNFα, prostaglandin E2 (PGE2), NADPH oxidase 2 (NOX2) and inducible nitric oxide synthase (iNOS) in ischemic brain tissues after pMCAO stimulation (Lu et al., 2019). In LPS-stimulated RAW264.7 cells, andrographolide treatment resulted in a similar dose-dependent reduction in levels of pro-inflammatory cytokines TNFα, IL-1β and IL-6 and their corresponding mRNA expression levels. This reduction in pro-inflammatory cytokine secretion is because andrographolide suppresses LPS-induced NF-κB and MAPK pathways; andrographolide reduced the levels of p65 and IkBα phosphorylation in the NF-κB pathway and the levels of *p*-JNK, *p*-ERK1/2 and p-p38 in MAPK pathway (Li et al., 2017). Andrographolide derivatives were found to inhibit TNFα/ NF-κB and TLR4/ NF-κ signaling pathways by inhibiting the nuclear translocation of the NF-κB p65 subunit and attenuating the phosphorylation of p65 and IκBα, thus decreasing the levels of serum pro-inflammatory cytokines and chemokines (Nie et al.,

2017). In a study, oral administration of andrographolide significantly attenuated mouse cortical chemokine levels from the C-C (CCL2, CCL5) and C-X-C (CXCL1, CXCL2, CXCL10) subfamilies. Andrographolide also abrogated LPS-induced chemokines (CCL2, CCL5, CXCL1, CXCL5, CX3CL1) and TNFα in astrocytes (Wong et al., 2016). In a study carried out using Jurkat cells stimulated with phorbol myristate acetate and ionomycin (PMA/ionomycin), andrographolide was found to reduce the production of IL-2 and reduce the activity of NF-κB. It also brought about a decrease in the ERK1 and ERK5 phosphorylation induced by anti-CD3 or PMA/ionomycin (Carretta et al., 2009). Andrographolide significantly reduced the production of INFγ and partially inhibited IL-2 production in murine T-cells stimulated with concanavalin A. This reduced INFγ production was associated with a significant decrease in the ERK1/2 phosphorylation on treating the cells with andrographolide. In the same study, andrographolide was also found to reduce hydrocortisone/PMA-induced apoptosis in thymocytes (Burgos et al., 2005). Docking studies carried out revealed that andrographolide is also a potent inhibitor of the main protease of SARS-CoV-2 (Mpro). Moreover, andrographolide is safe and does not interfere with the metabolism of other therapeutic drugs (Enmozhi et al., 2020). This dual beneficial role of andrographolide; as a potent immunosuppressive agent to alleviate the abnormal cytokine and chemokine production and as a potential inhibitor of SARS-CoV-2 by targeting the main protease make andrographolide a promising natural agent to be considered for COVID-19 targeted therapy (Banerjee et al., 2020).

Allicin

Allicin is a thiosulphate; 3-prop-2-enylsulfinylsulfanylprop-1-ene which is a constituent of garlic oil responsible for the typical smell and taste associated with freshly cut garlic (Allium sativum L.). Allicin is also found in garden onion (Allium cepa L.) and in other species in the family Alliaceae (Borlinghaus et al., 2014; National Center for Biotechnology Information, 2020b). Allicin is known to possess antioxidant, antimicrobial, antiviral, anti-inflammatory, anti-tumor and anti-diabetic properties (Batiha et al., 2020). In a study undertaken, allicin brought about a marked inhibition in the spontaneous and TNF-induced secretion of IL-1β, CXCL8 and IP10 in a dose-dependent manner in two different cell lines of intestinal epithelial cells. The mRNA levels of the IL-1β cytokine and CXCL8 chemokine are also reduced. This reduction in cytokine production is because of the effect of allicin on the NF-κB pathway; it suppresses the degradation of IκB (Lang et al., 2004). Allicin treatment to BALB/c mice post Plasmodium yoelii infection resulted in improved defense and survival due to an increase in the production of IFNγ and expansion of $CD4^+$ T-cells. Allicin supplementation along with tamoxifen treatment to Ehrlich ascites carcinoma (EAC) cells resulted in marked decrease in TNFα levels, showing its beneficial role as an adjuvant (Arreola et al., 2015). In clinical trials evaluating the safety of allicin treatment, no adverse effects were reported (Sharifi-Rad et al., 2019). In HT-29 and Caco-2 cells stimulated with TNFα, allicin brought about a marked inhibition in the secretion of IL-1β, IP10, CXCL8 and MIG in a dose-dependent manner. RNA protection assay revealed that allicin reduced the expression of IL-1β and CXCL8 mRNA levels (Lang et al., 2004). Allicin was also responsible for suppressing the degradation of IkappaB. In LPS-treated blood samples, allicin drastically reduced the release of IL-10 (Keiss et al., 2003). Allicin successfully attenuated the LPS-induced inflammatory responses in a study with cultured human umbilical vein endothelial cells (HUVECs). Significant decrease in the levels of TNFα, CXCL8 and NFκB activity were observed on treatment with allicin. Additionally, treatment with allicin also reduced LPS-induced apoptosis in the cultured HUVECs (Zhang et al., 2017). With its ability to alter NF-κB signaling for reduction in cytokine secretion potentially targeting the cytokine storm, improve defense by increasing the IFNγ production for increased antiviral defense and improve expansion of $CD4^+$ T-cells thus targeting the lymphocytopenia, allicin is a promising natural immunomodulatory adjuvant that can be used in conjunction with antiviral therapy in COVID-19 patients. However, its safe use in COVID-19 patients necessitates clinical trials to evaluate safety profile and pharmacokinetics (with antiviral agents in use) in these patients.

Colchicine

Colchicine is a secondary metabolite (alkaloid) extracted from Colchicum autumnale L. and Gloriosa superb L. (Varsha et al., 2017). Chemically, colchicine is N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-6,7-dihydro-5H-benzo[a]heptalen-7-yl]acetamide (National Center for Biotechnology Information, 2020c). It is known to possess anti-inflammatory and immunomodulatory properties and is used in the treatment of gout, FMF and Beçhet's syndrome. Colchicine is known to inhibit the production of IL-1 (Soy et al., 2020). Microtubules form an important part of the cytoskeleton, which is involved in cellular processes like secretion of cytokines and chemokines and cell migration. Colchicine blocks the polymerization of microtubule by binding to the *ß*-tubulin subunit with high affinity, thus preventing its assembly and affecting the process of cytokine and chemokine secretion and the migration of inflammatory cells like monocytes and neutrophils. Colchicine also disrupts the activation of inflammasome, suppressing activation of caspase-1 and subsequent release of IL-1β and IL-18 (Montealegre-Gómez et al., 2020). In an experimental study on patients with acute coronary syndromes, colchicine was found to bring a significant reduction in the levels of inflammatory cytokines IL-1β, IL-6 and IL-18 (Martínez et al., 2015). Colchicine was also found to bring about the inhibition of the assembly of the NLRP3 inflammasome thus resulting in the inhibition of the expression of pro-inflammatory cytokines including IL-1β. Given the increased NLRP3 inflammasome activation that contributes to hypercytokinemia observed in SARS-CoV-2 patients, colchicine is definitely an agent of interest to suppressing the NLRP3 inflammasome and thus ameliorate the state of hypercytokinemia observed (Papadopoulos et al., 2020; Ribeiro et al., 2020; Shah, 2020; Vitiello et al., 2020). Individual studies have been undertaken to evaluate the efficacy of cholchicine treatment for COVID-19. Colchicine was observed to bring about a reduction in the cytokine levels, activation of neutrophils and

macrophages and inflammasome in SARS-CoV-2 patients. Adverse effects in patients were minimal, mild diarrhea was only effect reported in few patients (Della-Torre et al., 2020; Montealegre-Gómez et al., 2020). Clinical trials are underway to consider the clinical utility, safety and efficacy of colchicine-adjuvant therapy for COVID-19 (Soy et al., 2020).

Curcumin

Curcumin (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) is a polyphenol pigment derived from the perennial plant Curcuma longa L., commonly known as the turmeric spice. It is a natural antioxidant, possesses anti-inflammatory, neuroprotective and hepatoprotective activity, and also inhibits tumor cell proliferation. Curcumin is also an immunomodulator (Hewlings and Douglas, 2017; National Center for Biotechnology Information, 2020d). The ability of curcumin to inhibit the production and release of different pro-inflammatory cytokines and chemokines IL-1β, IL-2, IL-6, IL-12, TNFα, CXCL8, IP10, MCP1, MIP1α and IFNγ has been proved in several studies. This immunosuppressive action of curcumin is mediated by its effect on different signaling pathways. Curcumin regulates the NF-κB pathways at multiple stages; inhibiting activation of IKKβ, blocking cytokine-mediated NF-κB activation by inhibiting IκBα degradation, blocking NF-κB signaling by activating AMPK, disturbing the NF-κB pathway by acting on p65 and as a consequence downregulating transcription of cytokine genes. On the other hand, curcumin positively regulates anti-inflammatory cytokines, particularly IL-10. IL-10 reduces levels of TNFα, and ICAM 1. Curcumin was found to increase the expression, production and activity of IL-10 (Jain et al., 2009; Sordillo and Helson., 2015; Shimizu et al., 2019; Liu et al., 2020). A study on the effect of curcumin on the pro-inflammatory cytokine IL-18 in LPS-stimulated murine macrophage-like cells RAW264.7 revealed that curcumin significantly inhibited the production of IL-18 (Yadav et al., 2015). In another study on monocyte culture exposed to pre-eclamptic plasma, curcumin caused a significant reduction in the levels of pro-inflammatory cytokines IL-1α, IL-6 and TNFα and also brought about a decrease in the level of nuclear NF-κB p50 (Rahardjo et al., 2014). In Iran, a randomized, double-blind, placebo-controlled study was carried out on COVID-19 patients form the Imam Reza Hospital of Tabriz University of Medical Sciences was carried out. This study aimed to evaluate the efficacy of nano-curcumin (dose- 160 mg of in four 40 mg capsules daily for 14 days) in modulating the levels of inflammatory cytokines IL-1β, IL-6, TNFα and IL-18 in 40 patients. Decreased expression and secretion of IL-1β and IL-6 was observed in the COVID-19 patients. However, there was no positive effect on the IL-18 mRNA expression and TNFα concentration with the treatment of nano-curcumin (Babaei et al., 2020; Valizadeh et al., 2020; Zahedipour et al., 2020). Being a natural spice extract, curcumin is safe and has low toxicity. All these properties motivate further clinical investigations for the use of curcumin as an immunomodulatory adjuvant to mitigate the cytokine storm, hyperinflammation and ARDS observed as a result of SARS-CoV-2 infection (Sordillo and Helson., 2015; Liu and Ying, 2020; Liu et al., 2020).

Eugenol

An aromatic oil, eugenol is 2-methoxy-4-prop-2-enylphenol. Although eugenol is also called clove oil as it is the main component of clove buds (Syzygium aromaticum (L.) Merr. and L.M. Perry), it is found many other plants like cinnamon bark and leaves, tulsi leaves, turmeric, pepper, ginger and organic herbs like oregano, thyme, basil, bay, marjoram, mace and nutmeg (Khalil et al., 2017; National Center for Biotechnology Information, 2020e). Eugenol is known to possess multiple beneficial pharmacological properties. It is a potent antioxidant, analgesic, antimicrobial, anticonvulsant and anticancer agent. Additionally it possesses anti-inflammatory and anti-viral properties and is an immunomodulatory agent (Pramod et al., 2010; Dibazar et al., 2015). In a study, eugenol inhibited the production of IL-6 and IL-10 *in vitro* (Bachiega et al., 2012). Administration of eugenol prevented increase in IL-4 and IL-5, reduced NF-κB signaling pathways and ultimately protected lung tissue from OVA-induced eosinophilia. In another study, eugenol caused the downregulation of pro-inflammatory cytokines IL-6 and TNFα; reduced the expression of NF-κB signaling, thus reducing leukocyte recruitment and inflammation in the lung tissue. Eugenol also reduced the TNFα and IL-1β as well as the NF-κB, ERK1/2, and p38 MAPK signaling pathways in LPS-induced macrophages (Barboza et al., 2018). When used in low doses, eugenol has few adverse effects; local irritation and rare allergic reactions. In case of accidental overdose, tissue injury and damage to liver and kidney can result (National Institute of Diabetes and Digestive and Kidney Diseases, 2020). Treatment with polymeric nanocarriers of eugenol was found to bring about a significant reduction in the TPA-induced IL-6 levels in Swiss mice (de Araújo Lopes et al., 2018). In a LPS-induced inflammatory model in porcine intestinal epithelial cells, eugenol enervated the inflammatory response by reducing both, the CXCL8 and TNFα mRNA levels significantly (Hui et al., 2020). Another study carried out on LPS-challenged macrophages revealed that eugenol suppressed the production of IL-6 and IL-10. However, there was no effect observed on the production of IL-1β (Bachiega et al., 2012). Mouse peritoneal macrophages were subject to activation with a bacterial LPS in order to evaluate the effect of eugenol on pro-inflammatory mediator genes NF-κB1 and TNFα. Since LPS did not induce the expression of NF-κB1, the effect of eugenol could not be ascertained. In case of TNFα, hypoexpression of was observed on treatment with eugenol in LPS-activated cells (Porto Mde et al., 2014). The effect of eugenol treatment on the two important inflammatory markers IL-6 and TNFα was examined in adult male Wistar rats. The levels of both IL-6 and TNFα were elevated post thioacetamide-induced hepatic injury. Pretreatment with eugenol brought about a significant decrease in the levels of these inflammatory markers (Yogalakshmi et al., 2010). With the good safety profile of eugenol and its ability to downregulate key pro-inflammatory cytokines like IL-6 and TNFα consequently reducing leukocyte recruitment in lung tissue, it can be a suitable natural

immunosuppressant that can be used as an adjuvant along with antiviral agents to suppress the hypercytokinemia and hyperinflammation observed in COVID-19.

Gallic Acid

Gallic acid is a phenolic secondary metabolite found in abundance in plants; in the bark, wood, leaf, fruit, root and seeds. Chemically, it is 3,4,5-trihydroxybenzonic acid (Fitzpatrick and Woldermariam, 2017). It is a natural antioxidant that also has anticancer, antifungal, antimicrobial and anti-inflammatory properties. Studies revealed that gallic acid suppresses the levels of pro-inflammatory cytokines IL-1, IL-6, IL-12, IL-17, IL-23, TGFβ, TNFα and chemokines CCL2 and CCL7 in TNBS-induced ulcerative colitis and RA FLS. This anti-inflammatory activity of gallic acid is exerted by inhibiting of NF-κB pathway (Huang et al., 2019; Zhu et al., 2019). Another study revealed that gallic acid selectively suppressed Th2 cytokines IL-4 and IL-5 but did not suppress the Th1 cytokine IFNγ in anti CD3-stimulated spleen cells (Kato et al., 2001). Treatment with gallic acid was found to suppress the activity of NF-κB and inhibited pro-inflammatory cytokine release in high glucose-induced human monocytes (THP-1 cells) (Lee et al., 2015). The inhibitory effect of gallic acid on the production of pro-inflammatory cytokines TNFα and IL-6 was evaluated in human mast cells (HMC-1). HMC-1 cells were stimulated with PMA plus A23187 which caused the secretion of both TNFα and IL-6. Gallic acid treatment significantly blocked the secretion of both these cytokines in the stimulated HMC-1 cells (Kim et al., 2006). Gallic acid treatment was also found to successfully inhibit the PMA plus A23187-induced degradation of IκBα and the nuclear translocation of p65 NF-κB. The inflammatory regulatory effect of gallic acid was assessed in LPS-induced endometriosis cells. Gallic acid was responsible for a significant decrease in the expression of NF-κB and in the secretion profile of pro-inflammatory cytokine IL-6 (Bustami et al., 2018). The effect of polygallic acid, enzymatically produced from gallic acid on the secretion of pro-inflammatory cytokines IL-6, TNFα and IL-1β was evaluated in human monocytes exposed to PMA. A substantial decrease in the production of all three cytokines under study was observed on treatment with polygallic acid (Zamudio-Cuevas et al., 2020). This suggests that gallic acid can be used as an immunosuppressive adjuvant, to selectively suppress pro-inflammatory cytokines thus targeting the cytokine release syndrome observed in SARS-CoV-2 patients without negatively affecting the ability of the host to produce interferons.

Gingerol

A constituent of fresh ginger (Zingiber officinale Roscoe), gingerol is a beta-hydroxy ketone that is 5-hydroxydecan-3-one substituted by a 4-hydroxy-3-methoxyphenyl moiety at position 1 (5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one (National Center for Biotechnology Information, 2020f). Gingerol is also found in the rhizomes of several members of the Zingiber species. Gingerol possesses antioxidant, anti-inflammatory, anti-metastatic, anti-angiogenic, anti-diabetic, analgesic and antipyretic activity. Additionally, gingerol is also known to possess immunomodulatory properties and anti-allergic activity (Sharifi-Rad et al., 2017). 6-gingerol was found to exert an inhibitory effect on the production of pro-inflammatory cytokines IL-1β, IL-12, TNFα in LPS-stimulated macrophages, without interfering with their antigen presentation ability (Tripathi et al., 2007). In another study, treatment with an effective dose of 50 μM of 6-gingerol was found to reduce the V. cholerae-infection triggered levels of cytokines IL-1α, IL-1β, IL-6 and chemokine CXCL8. The respective mRNA levels were also reduced. This reduction in the levels of pro-inflammatory cytokines was attributed to the down-regulation of NF-κB pathway because of increase in phosphorylated IKBα and downregulation of p65 (Saha et al., 2016). Another study reported that 6-gingerol reduced IL-1β-induced inflammation by reducing the mRNA titers of IL-6 and CXCL8, reducing the COX2 over-expression and the NF-κB activity (Li et al., 2013). In a DSS-induced mouse colitis model, 6-gingerol treatment was found to successfully suppress the DSS-elevated production of pro-inflammatory cytokines IL-1β, IL-12 and TNFα. An *in vitro* study on DSS-treated Caco-2 cells subject to 6-gingerol therapy revealed AMPK activation following treatment; suggesting that the anti-inflammatory effect of 6-gingerol could be exerted through AMPK activation (Chang et al., 2015). The production of pro-inflammatory cytokines TNFα, IL-1β and IL-12 in LPS stimulated murine peritoneal macrophage was inhibited on treating with 6-gingerol (Tripathi et al., 2007). In another study carried out on C6 astroglioma cells treated with LPS, treatment with 6-gingerol was found to attenuate the production of pro-inflammatory cytokines in a dose dependent manner. Additionally, in the *in vivo* studies, 6-gingerol also inhibited the LPS-induced increase of TNFα levels in the rat brain (Zhang et al., 2018). The effect of 6-gingerol on cytokine production was evaluated in a DSS-induced colitis mouse model. 6-gingerol inhibited the DSS-stimulated rise in the serum levels of cytokines IL-6 and IL-17 and their respective mRNA levels. Moreover, it also inhibited the DSS-induced decrease in the serum levels and mRNA levels of the anti-inflammatory cytokine. IL-10 (Sheng et al., 2020). Gingerol is a potential natural immunomodulatory agent that may prove to be useful in regulation of the cytokine storm observed in COVID-19 when used in conjunction with standard antiviral therapy.

Luteolin

A naturally occurring flavonoid, luteolin is 2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one. It is found in many vegetables (celery, parsley, broccoli, carrots, peppers, and cabbages); fruits (apple skins); flowers (chrysanthemum) and in medicinal herbs. It is a potent antioxidant, inhibits tumor cell proliferation and suppresses metastasis. It is also an anti-inflammatory agent and an immune system modulator (Lin et al., 2008; National Center for Biotechnology Information, 2020g). Luteolin exhibits a concentration-dependent inhibition of the production of TNFα and IL-1β in LPS/IFN-induced primary microglia and BV-2 microglial cells. This inhibitory effect results from an inhibition on NF-κB, STAT1 and IRF1; all essentially required for the transcription of pro-inflammatory genes (Kao et al., 2011). In another study, a significant inhibition of TNFα, IL-6, CXCL8, GM-CS and suppression of NF-κB

activation was observed with luteolin treatment on PMA plus A23187-induced HMC-1 cells (Kang et al., 2010). In human monocytes under hyperglycemic condition, luteolin brought about a significant reduction in the release of IL-6 and TNFα by suppressing NF-κB activity (Kim et al., 2014). In a study with human whole blood incubated with LPS, luteolin effectively inhibited the production of IL-1β, IL-6, TNFα and IFNγ (Ribeiro et al., 2010). Primary murine microglia and BV-2 microglial cells stimulated with LPS exhibited significant increase in levels of inflammatory cytokine, IL-6. Pretreatment with luteolin was found to attenuate this increase in IL-6; both at mRNA and protein level. While luteolin was found to bring about a marked reduction in the binding activity of AP1 transcription factor and inhibit JNK phosphorylation, it had no significant effect on the LPS-induced increase in NF-κB DNA binding activity nor the LPS-induced IκBα degradation. Furthermore, the *in vivo* studies on mice treated with luteolin revealed that it successfully reduced the plasma levels of IL-6 and the mRNA levels of IL-6 in the hippocampus, but not in the cortex or cerebellum. This inhibitory activity of luteolin on the LPS-induced production of IL-6 can be attributed to its ability to inhibit the both JNK signaling pathway and the activation of AP1 in the microglia (Jang et al., 2008). Luteolin was also found to significantly reduce the serum levels of pro-inflammatory cytokines IL-1β, IL-6 and TNFα in MSU induced-inflammation in rats (Lodhi et al., 2019). Luteolin effectively inhibited the production of TNFα and IL-6 in LPS/INFγ stimulated RAW264.7 cells. NF-κB expression was also suppressed in a dose-dependent manner, following luteolin treatment (Lee et al., 2016). Luteolin pretreatment was found to bring a marked decrease in the mRNA expression and the release of IL-6, IL-8 and VEGF in a dose dependent manner in human HaCaT and primary keratinocytes on TNF stimulation. The TNF-induced phosphorylation, nuclear translocation and DNA binding of NF-κB was significantly brought down by treatment with luteolin. Furthermore, the TNF-induced mRNA expression of the NFKB1 and RELA genes which encode the two NF-κB subunits (NF-κB p50 and NF-κB p65, respectively) was also reduced by luteolin treatment, suggesting that luteolin reduces pro-inflammatory cytokine production by decreasing NF-κB induction (Weng et al., 2014). Luteolin also has broad anti-viral activity, inhibits mast cells and potentially inhibits SARS-CoV-2 main protease (3CLpro) as revealed in recent in docking studies (Theoharides, 2020). Taken together with its immunomodulatory properties, luteolin is an important natural agent for consideration as a potential therapeutic that can be further evaluated as an adjuvant to ameliorate the cytokine storm observed in COVID-19 and to potentially inhibit SARS-CoV-2 infection. Further clinical trails in this regard to ascertain the safety, efficacy and dosage for COVID-19 treatment needs to be conducted.

Melatonin

Melatonin is a bioactive compound reported to be found in numerous plants species. Chemically, it is a tryptophan-derived substituted indolamine; N-acetyl-5-methoxytryptamine. It is a universal amphiphilic antioxidant and an anti-inflammatory molecule (Salehi et al., 2019). Melatonin is found in the roots of Huang-qin (*Scutellaria baicalensis Georgi.*), curcuma (*Curcuma aeruginosa Roxb.*); leaves and flowers of St. John's wort (*Hypericum perforatum L.*); leaves of *Tanacetum parthenium* (L.) Sch. Bip., black pepper (*Piper nigrum* L.); seeds of corn (*Zea mays* L.), rice (*Oryza sativa* L.), black mustard (*Brassica nigra* (L.) K.Koch), white mustard (*Brassica hirta Moench*), wolf berry (*Lycium barbarum* L.), fennel (*Foeniculum vulgare Mill.*), sunflower (*Helianthus annuus L.*), fenugreek (*Trigonella foenum-graecum* L.), barley (*Hordeum vulgare L.*), almonds (*Prunus amygdalus Batsch*), coriander (*Coriandrum sativum L.*), celery (*Apium graveolens* L.), anise (*Pimpinella anisum* L.), poppy (*Papaver somniferum* L.), flax (*Linum usitatissimum* L.) and in the bean of coffee (Coffea canephora Pierre. Ex A.Froehner, *Coffea arabica* L.). The presence of melatonin has been reported in many plant species belonging to the families Rosaceae, Vitaceae, Poaceae, Apiaceae and Brassicaceae (Nawaz et al., 2016). Melatonin has been reported to possess indirect anti-viral activity due to its anti-inflammatory activity. It down-regulates the production and release of pro-inflammatory cytokines, inflammation and cell recruitment. This down-regulation and consequent anti-inflammatory activity of melatonin is because it suppresses the activation of NF-κB, a key transcription factor involved in the production of pro-inflammatory cytokines. In addition, studies revealed that melatonin is able to cause significant decrease in serum levels of IL-6, TNFα, IL-1β (Tarocco et al., 2019; Zhang et al., 2020b). Melatonin efficiently suppressed the production of IL-8 in acrolein-induced human pulmonary fibroblasts (Kim et al., 2012). Treatment with melatonin was found to significantly suppress pro-inflammatory cytokine production and inhibit NLRP3 inflammasome activation in a study carried out in cadmium–induced liver injury in male C57BL/6 mice (Cao et al., 2017). In another study carried out on BV2 murine microglial cells, the LPS-induced production of chemokines MCP1, CCL5 and CCL9 mRNA expression was significantly inhibited by melatonin treatment. Melatonin also inhibited the AkT phosphorylation and NF-κB activation induced by LPS exposure. Moreover, LPS-induced STAT1/3 phosphorylation and interferon-gamma activated sequence (GAS)-driven transcriptional activity was also inhibited as a result of melatonin treatment. This suggests that the anti-inflammatory activity of melatonin is mediated by inhibiting the NF-κB and STAT/GAS activation in LPS-stimulated BV2 murine microglial cell line (Min et al., 2012). A recent mechanistic analysis suggested that supplementation with melatonin can efficiently downregulated the cytokine storm in COVID-19 by reversing aerobic glycolysis in immune cells (Reiter et al., 2020). Short-term usage of melatonin is safe; melatonin has a high safety profile. Infrequently occurring side-effects reported are only occasional dizziness, headache, nausea and sleepiness. These findings support the use of melatonin as an immunosuppressive adjuvant to reduce the cytokine storm syndrome observed in COVID-19 (Zhang et al., 2020b).

Morphine and Codeine

An opiate alkaloid obtained from the plant *Papaver somniferum L.*, morphine (4R,4aR,7S,7aR,12bS)-3-methyl-2,4,4a,7,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline-7,9-diol) binds to and activates the δ, μ, κ opiate receptors involved in controlling different brain functions. Morphine is an analgesic, brings about sedation, respiratory depression and gastrointestinal system smooth muscle contraction (National Center for Biotechnology Information, 2020h). The immunosuppressive activity of morphine has been known in clinical medicine for over hundred years (Dinda et al., 2005). Several studies have established the immunosuppressive activity of morphine with respective to cytokine production. *In vivo* studies have revealed that morphine inhibits the production of TNFα, IFNγ, MCP 1, IL-12 and IL-6 (Clark et al., 2007; Fukada et al., 2012; Cruz et al., 2017). Morphine has the ability to reduce the production of TNFα, IL-1 and IL-6 (Chang et al., 2011). Another study evaluated the effect of morphine on the intraperitoneal release of TNFα and MCP1 in Swiss–Webster, C57BL/6J, mast cell deficient Kit Wsh/Wsh (W-sh) and mast cell reconstituted (W-sh-rec) mice. While morphine inhibited the LPS-induced TNFα release, it had no significant effect on the MCP1 release in the intraperitoneal cavity (Madera-Salcedo et al., 2011). Morphine treatment resulted in significant reduction in the levels of TNFα, IL-1, IL-2 and MIP2 in the bronchoalveolar lavage fluids and in lung tissue in CB6F1 male mice. Morphine treatment also inhibited transcription factor NF-κB in lung resident cells (Wang et al., 2005). Codeine (4R,4aR,7S,7aR,12bS)-9-methoxy-3-methyl-2,4,4a,7,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinolin-7-ol) is a naturally occurring phenanthrene alkaloid and an opioid agonist also obtained from P. somniferum L., also known for its analgesic, anti-diarrheal and antitussive properties (National Center for Biotechnology Information, 2020i). Treatment with codeine was found to significantly suppress the production of IL-2 in ConA-stimulated splenocytes extracted from male Swiss mice (Sacerdote et al., 1997). Morphine and codeine bind directly to ACE2 with high affinity, as revealed by docking studies. This can hypothetically reduced receptor-mediated cytokine release. This makes morphine and codeine candidate immunosuppressant adjuvants for regulating the cytokine storm observed in COVID-19 (Roshanravan et al., 2020b). However, further studies need to be conducted to study the use of morphine or codeine for COVID-19, given that the timing of morphine administration greatly effects its influence on cytokine production; late administration increases cytokine production and that codeine was found to induce the production of cytokines and chemokines by mast cells *in vitro* (Sheen et al., 2007; Fukada et al., 2016).

Nicotine

Nicotine is a plant alkaloid found in the tobacco plant (*Nicotiana tabacum L.*). Chemically, it is 3-(1-methylpyrrolidin-2-yl) pyridine. It is an immunomodulator, a peripheral nervous system drug and a psychotropic drug (National Center for Biotechnology Information, 2020j). Nicotine is can also be found in some members of the families Asclepiadaceae, Crassulaceae, Solanaceae (Dawson et al., 1960). It is used clinically in the treatment of ulcerative colitis to counteract inflammation. As it is a cholinergic agonist, nicotine is an inhibitor of pro-inflammatory cytokines acting through the cholinergic anti-inflammatory pathway via a7-nicotinic acetylcholine receptor (a7-nAChRs). Nicotine inhibits TNF, IL- 1 and IL-6 production. The underlying mechanism of action involves the activation of a7-nAChRs on inflammatory cells like macrophages and neutrophils that induces the suppression of the NF-κB activation and consequently suppression of the secretion of pro-inflammatory cytokines and chemokines from inflammatory cells. Nicotine brought about an inhibition in the secretion of IFNγ, TNFα, IL-1β and IL-2 but had no effect on the production of IL-6 in as study carried out on PBMC triggered by HT-29 colon carcinoma cells. However, nicotine treatment in the in PBMC triggered by RKO colon carcinoma cells showed no suppressive effect on the cytokine production (Djaldetti and Bessler, 2017). Nicotine inhibits the LPS-induced production of TNFα and NF-κB in human macrophages and splenocytes. This inhibitory effect is attributed to the ability of nicotine to activate JAK2 and STAT3 and is mediated by tristetrapolin (TTP) expression in macrophages (Lakhan and Kirchgessner, 2011). Studies have showed that nicotine has the ability to inhibit the production of IL-1, IL-6, IL-12, INFγ, MIP1, TNFα. Nicotine inhibits the transcriptional activity of NF-κB by suppressing the phosphorylation of I-κB (Cloëz-Tayarani and Changeux, 2007; Piao et al., 2009). Nicotine is also an accessible, approved treatment. Hence, it is hypothesized that nicotine is a suitable adjuvant that can ameliorate the cytokine storm in COVID-19 and can likely reduce the rising mortality in the short term (Farsalinos et al., 2020; Gonzalez-Rubio et al., 2020).

Piperine

Piperine is an alkaloid obtained from the plant Piper nigrum L. and other plants from the family Piperaceae. Chemically, it is N-acylpiperidine that is piperidine substituted by a (1E,3E)-1-(1,3-benzodioxol-5-yl)-5-oxopenta-1,3-dien-5-yl group at the nitrogen atom (2E,4E)-5-(1,3-benzodioxol-5-yl)-1-piperidin-1-ylpenta-2,4-dien-1-one (National Center for Biotechnology Information, 2020k). Piperine is known to possess numerous therapeutic effects; antioxidant, anti-inflammatory, antimicrobial, anti-metastatic and hepatoprotective activities, to name a few. It is also an immunomodulator and is known for its bioavailability enhancement property of therapeutic drugs (Gorgani et al., 2017). Piperine was reported to inhibit the production of Th2 cytokines IL-4 and IL-5 in an ovalbumin-induced asthma model (Kim and Lee, 2009). Another study reported that piperine inhibits LPS-induced expression and production of pro-inflammatory cytokines IL-1β, IL-6, IL-10 and TNFα by inhibiting NF-κB activation (Dzoyem et al., 2017). Pipeine also protects macrophages from pyroptosis and suppresses the release of IL-1β by suppressing ATP-induced AMPK activation in murine macrophages (Liang et al., 2016). In another study carried out using human PBMC, piperine was found to inhibit the production of IL-2 and IFNγ in a dose-dependent manner by suppressing the IL-2 and IFNγ mRNA

expression, as revealed by ELISA assay and RT-PCR data (Chuchawankul et al., 2012). Piperine was found to selectively suppress the expression of S. aureus-induced pro-inflammatory cytokines IL-1β, IL-6 and TNFα and increase the expression of the anti-inflammatory cytokine IL-10 by inhibiting the NF-κB and MAPK pathways in an experimental study in mice (Zhai et al., 2016). The IL-6 expression by IL-1β stimulated fibroblast-like synoviocytes derived form patients with rheumatoid arthritis was successfully inhibited by piperine treatment. Additionally, piperine could inhibit the migration of activator protein 1 (AP-1), but not nuclear factor (NF)κB into the nucleus (Bang et al., 2009). Piperine was found to bring about a reversal in the LPS-induced increase of pro-inflammatory cytokines IL-1β, IL-6 and TNFα production in a study carried out on human colonic epithelial cells. This attenuation of cytokine release was attributed to the ability of piperine to activate IκBα and consequently suppress NF-κB expression (Buagaew et al., 2020). Significant reduction in the levels of IL-1β, IL-6, TNFα and GM-CSF in B16F-10 melanoma cells was observed on treatment with piperine. Additionally, piperine treatment also reduced the nuclear translocation of p65, p50, c-Rel subunits of NF-kappaB and other transcription factors such as ATF-2, c-Fos and CREB. Piperine inhibits the activation of NF-κB as it suppresses the degradation of IκBα and the translocation of p65 from the cytosol to the nucleus (Pradeep and Kuttan, 2004; Chung, 2019; Yang et al., 2019). Additionally, recent docking studies revealed that piperine exhibits significant binding affinity toward the spike glycoprotein of SARS-CoV-2 and the ACE2 receptor. Hence, piperine may prove to be a useful therapeutic agent not only for restricting attachment of virus to the host cells but also for targeting the cytokine storm by suppressing the activity of NF-κB and MAPK pathways and subsequent pro-inflammatory cytokines release (Maurya et al., 2020).

Quercetin

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one) is a polyphenolic flavonoid found in abundance in many plants including broccoli, red onions, eggplant, potatoes and green leafy vegetables including celery, lettuce; fruits including apples, citrus fruits, red grapes, tomatoes; berries including cranberries and raspberries. The extract of quercetin has been used for prevention and treatment of rheumatic disease, cardiovascular disease, hypercholesterolemia, infections and cancers (Li et al., 2016; National Center for Biotechnology Information, 2020l). Quercetin was found to effect the production of cytokines; it was found to decrease the production of pro-inflammatory cytokines TNFα, IL-6, G-CSF, GM-CSF and VEGF and chemokines IP10 and MCP1 in mouse macrophages induced with polyinosinic-polycytidylic acid, and bring about an increase in the level of anti-inflammatory cytokine IL 27 in influenza A-treated MDCK cells (Kim and Park, 2016; Mehrbod et al., 2018). It was also found to stimulate T-helper cells to produce IFNγ and downregulate Th2-derived IL-4 in cultured blood peripheral mononuclear cells (Biancatelli et al., 2020). Carrageenin-induced production of IL-1β was significantly reduced with quercetin treatment (Valério et al., 2009). Quercetin was responsible for a marked reduction in the production of TNFα, in a dose-dependent manner in a study in normal PBMC. This inhibitory effect of quercetin on the pro-inflammatory cytokine TNFα is mediated via the modulation of NF-κB1 and IκB (Nair et al., 2006). Quercetin treatment was found to bring about a reduction in the LPS-induced increasing levels of inflammatory factors IL-1β, IL-6 and TNFα in RAW 264.7 cells. The ability of quercetin to suppress the production of pro-inflammatory cytokines is mediated by its suppression of the activation of ERK and p38 MAP kinase and NF-κB/IκB signal transduction pathways (Tang et al., 2019). In a study on LPS-stimulated RAW 264.7 cells, quercetin decreased the activation of phosphorylated ERK kinase and p38 MAP kinase. No significant effect on JNK MAP kinase was observed. Additionally, quercetin also inhibited the activation of NF-κB/IκB complex and the degradation of IκB (Cho et al., 2003). Quercetin brought about a dose-dependent reduction in the levels of IL-6, IL-8, MCP1 and ICAM1 in IL-1β-stimulated human retinal pigment epithelial (ARPE-19) cells. Quercetin was found to inhibit the signaling pathways related to the inflammatory process; including inhibition of the phosphorylation of mitogen-activated protein kinases (MAPKs), inhibiting the nuclear factor κ-B kinase (IKK)α/β, c-Jun, cAMP response element-binding protein (CREB), activating transcription factor 2 (ATF2) and nuclear factor (NF)-κB p65, and blocking the translocation of NF-κB p65 into the nucleus (Cheng et al., 2019). Another study on patients with coronary artery disease showed that treatment with quercetin brought about a reduction in the levels of IL-1β, TNFα and the levels of IL-10 tended to decrease. This was attributed to a decrease in the transcriptional activity of NF-κB (Chekalina et al., 2018). Oral supplementation with quercetin treatment is relatively safe, causing no significant adverse effects; headache and temporary peripheral paresthesia were the only effects observed in two members out of thirty in a study. The use of quercetin as an adjuvant to concurrently fortify the immune response by promoting IFNs production and modulating the levels of pro- and anti-inflammatory cytokines may complement currently used interventions in treating COVID-19 (Biancatelli et al., 2020).

Resveratrol

A polyphenolic phytoalexin, resveratrol (5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol) is found in more than 70 species of plants, such as grapes, cranberry, blueberry, mulberry, peanuts, jackfruit, soy and wine. Resveratrol is known to posses a number of beneficial health effects; it is has antioxidant, anticancer, anti-inflammatory, antiviral, anti-aging and life-prolonging effects (Bansal et al., 2018; National Center for Biotechnology Information, 2020m). Resveratrol is an efficient immunomodulator; it interferes with immune cell regulation, pro-inflammatory cytokine synthesis and gene expression, thus regulating immunity (Malaguarnera, 2019). In a study carried out, resveratrol caused an irreversible inhibition of IFNγ and IL-2 by splenic lymphocytes and the production of TNFα and IL-12 by peritoneal macrophages. The activation of NF-κB was blocked without affecting the basal NF-κB activity (Gao et al., 2001). In another study inflammatory cytokine and chemokine release (GM-CSF and CXCL8) by stimulated alveolar

TABLE 1 | Plant-derived Immunosuppressants and their Effect on Pro-inflammatory Cytokines.

S. No	Natural Immunomodulatory Agent	Plant Source	Immuno-Action	Effect on Cytokines	Probable Mechanism of Action	Study	References
1	Citral	Citrus fruits, lemongrass	Immunosuppressant	Inhibits IL-1β and IL-6 release, IL-10 production	Inhibition of NF-κB	Macrophages challenged with LPS	Bachiega et al. (2011)
				Reduced IL-1β, IL-4, INFγ, TNFα production; inhibits NLRP3 inflammasome activation	Inhibits ATP-induced caspase-1 activity; inhibits NF-κB, p65 activation	LPS-induced mouse ASLN model	Ka et al. (2015)
2	Ginsenoside	Panax ginseng C.A.Mey., P. notoginseng (Burkill) F.H.Chen., P. quinquefolius L., *P. japonicus* var. major (burkill) C.Y.Wu. and feng (and other members of panax genus)	Immunosuppressant	Reduced TNFα, IL-6, IL-1β, IL-12p40, CXCL2 mRNA expression; augmented IL-10 expression	Inhibits NF-κB and MAPK pathways	LPS-activated BMDMs	Paik et al. (2019)
				Downregulates IL-6, TNFα and IL-10 expression	Inhibits NF-κB activation	II/R induced lung injury *in vivo*	Jiang et al. (2014)
3	Kaempferol	Delphinium, witch-hazel, grapefruit, and other plant sources	Immunosuppressant	Reduced overproduction of TNF α, IL-1β, IL-6, ICAM1, VCAM1	Negative regulation in TLR4, NF-κB and STAT signaling	LPS-induced rat intestinal microvascular endothelial cells (RIMVECs)	Bian et al. (2019)
				Inhibited release of IL-6, IL-8 and TNFα	Inhibited activation of PKC θ	C57BL/6 anti-BDF1 MLC	Kempuraj et al. (2005)
4	Withaferin A	Withania somnifera (L.) Dunal and other members of solanaceae family	Immunosuppressant	Inhibits IL-1α, IL-1β, IL-2, IL-3, IL-5, IL-10, IL-12p70, IL-13, IL-18, IP10, GM-CSF, CCL2/MCP-1, CCL17/TA RC, SDF1α/CXCL12 and CCL20/MIP3α	Targets NF-κB and the inflammasome complex (blocks the nuclear translocation of NF-kB; inhibits caspase-1 activation)	ATP-stimulated monocyte-derived THP-1 cells	Dubey et al. (2018)
				Dose-dependent reduction in IL-6, IL-8, IL-1α, MIP1α, MIP1β, G-CSF and IP10	NF-κb inhibition	Mouse and human islet cells; *in vitro*	SoRelle et al. (2016)

macrophages from COPD patients was blocked by resveratrol (Culpitt et al., 2003). Resveratrol blocks the increased secretion of IL-6 and TNFα in EV71-infected RD cells (Zhang et al., 2015). In yet another study, treatment with resveratrol induced a dose-dependent inhibition of IL-1α, IL-6 and TNFα *in vitro* and downregulated the production of IL-17 in HTLV-1 infected cells (Fuggetta et al., 2016). Resveratrol treatment was found to be associated with downregulation of NF-κB in the inflammatory cells of the lungs (Rieder et al., 2012). LPS-induced phosphorylation and degradation of IκBα is blocked in macrophages subject to resveratrol treatment decreasing the NF-κB DNA binding activity. The inhibitory effect of resveratrol on the NF-κB pathway correlates with its ability to bring about a reduction in IKK activity (Yamamoto and Gaynor, 2001). In a study evaluating the efficacy of resveratrol dry suspension in pigs, it was found that resveratrol upregulated the release of IFNγ and downregulated the release of TNFα; thus regulating the humoral immune responses (Fu et al., 2018). A dose-dependent reduction in the levels of pro-inflammatory cytokines IL-6, IL-8 and MCP1 was observed with resveratrol treatment in adipocytes under inflammatory conditions (Zagotta et al., 2015). The inhibitory effect of resveratrol on TNFα, IL-6 and MIP2 levels was established in a study on rabbit model of acute pharyngitis. Resveratrol reduced the protein expression of IL-1β and IL-18. Furthermore, treatment with resveratrol resulted in suppression of TLR4 and myeloid differentiation primary response protein 88 protein expression, reduced p-NF-κB and increased *p*-IκB protein expression (Zhou et al., 2018). A decreased production of cytokines IL-1β, IL-6, IL-8, IL-12 and TNFα was observed on treatment with resveratrol in a dose- and time-dependent (Rizzo et al., 2012). Resveratrol is considered safe when taken at supplemental doses. Resveratrol can be an adjunctive agent to consider for SARS-CoV-2 infection, to mitigate the cytokine storm and consequently reduce inflammation (Marinella et al., 2020).

A few more plant-derived immunosuppressants that can be potentially used as adjuvants in COVID-19 therapy are described in **Table 1**.

CONCLUSION

The COVID-19 pandemic is affecting millions of people across the globe. In some patients infected with SARS-CoV-2, the immunological reaction involves mobilization and sustained production of several pro-inflammatory cytokines and chemokines, giving rise to a cytokine storm. This cytokine storm appears to be one of the common causes of mortality in this disease. This necessitates a suitable therapeutic strategy that can efficiently attenuate this excessive host immune response. Different synthetic immunomodulatory agents such as corticosteroids, interleukin receptor antagonists, JAK/STAT inhibitors and monoclonal antibodies like tocilizumab, sarilumab, infliximab and adalimumab that target specific cytokines are being evaluated for treatment of COVID-19 patients. Unfortunately, most of these immunomodulators are known to cause adverse effects, thus limiting their use. Plant-derived immunosuppressants present an invaluable alternative to manage the cytokine storm syndrome observed in COVID-19. They have a good safety profile and specifically target the production and release of certain cytokines by suppressing definite reactions in the signaling cascades. Additionally, some of these agents also exhibit antiviral properties against coronaviruses and some others can potentially inhibit the main protease of SARS-CoV-2. Clinical trails to investigate the dosage, administration timing, bioefficacy, bioavailability, mechanism of action and safety with respect to COVID-19 is the need of the hour to capitalize on this promising therapeutic approach. A combination treatment including plant-derived immunosuppressants along with antiviral agents and other drugs will be effective in tackling SARS-CoV-2 infection, rather than the use of a single drug alone. Such a synergistic therapeutic approach with the inclusion of natural immunosuppressants to ameliorate the cytokine storm holds great treatment potential.

AUTHOR CONTRIBUTIONS

AEP conceived the idea, surveyed the literature and wrote the manuscript; BVS, BGR, and VLK were involved in manuscript revision, read and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GLOSSARY

ACE Angiotensin-converting enzyme
AMPK 5'AMP-activated protein kinase
ARDS Acute respiratory distress syndrome
ASLN Accelerated and severe lupus nephritis
BMDM Bone-marrow derived macrophage
COPD Chronic obstructive pulmonary disease
COVID-19 Coronavirus disease 2019
COX Cyclooxygenase
CRP C-reactive protein
DSS Dextran sulfate sodium
EAC Ehrlich ascites carcinoma
ELISA Enzyme-linked immunosorbent assay
ESR Erythrocyte sedimentation rate
FMF Familial Mediterranean fever
FLS Fibroblast-like synoviocytes
G-CSF Granulocyte colony-stimulating factor
GM-CSF Granulocyte-macrophage colony-stimulating factor
HGF Hepatocyte growth factor
HIV Human immunodeficiency virus
HMC Human mast cell line
ICAM Intercellular adhesion molecule
IFN Interferon
II/R Intestinal ischemia and reperfusion
IkBα I-kappa B-alpha
IKKβ Inhibitor of nuclear factor kappa-B kinase subunit beta
IL Interleukin
iNOS Inducible nitric oxide synthase
IP Interferon gamma-induced protein
IRF Interferon regulatory factor
IVIG Intravenous immunoglobulin
JAK Janus kinase
LPS Lipopolysaccharide
MAPK Mitogen activated protein kinase
MASP Mannose-binding lectin serine protease
MCP Monocyte chemoattractant protein
MCSF Macrophage colony-stimulating factor
MIG Monokine induced by gamma; CXCL9
MIP Monocyte chemoattractant protein
MOF Multiple organ failure
MSC Mesenchymal stem cell
MSU Monosodium urate
NADPH Nicotinamide adenine dinucleotide phosphate
NF-κB Nuclear factor kappa-light-chain-enhancer of activated B cells
NK Natural killer
NLR NOD-like receptor
NOX2 NADPH oxidase 2
NOD Nuclear-binding and oligomerization domain
OVA Ovalbumin
PAMP Pathogen-associated molecular pattern
PBMC Peripheral blood mononuclear cells
PCT Procalcitonin time
PDGF Platelet-derived growth factor
pERK Phosphorylated extracellular signal-regulated kinase
PF Pulmonary fibrosis
PGE2 Prostaglandin E2
pJNK Phosphorylated c-Jun N-terminal kinase
PKC protein kinase C
PMA Phorbol 12-myristate 13-acetate
pMACO Permanent middle cerebral artery occlusion
PT Prothrombin time
RA Rheumatoid arthritis
RBD Receptor binding domain
RD Rhabdosarcoma
rhACE recombinant hACE
RIG Retinoic acid-inducible gene I
RLR Rig-I-like receptor
RNA Ribonucleic acid
RT-PCR Reverse transcription polymerase chain reaction
SARS-CoV-2 Severe acute respiratory syndrome coronavirus 2
STAT Signal transducer and activator of transcription
TLR Toll-like receptor
TNBS 2,4,6-trinitrobenzene sulfonic acid
TNF Tumor necrosis factor
VEGF Vascular endothelial growth factor

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REVIEW
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Research Advance on Qingfei Paidu Decoction in Prescription Principle, Mechanism Analysis and Clinical Application

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Since the sudden epidemic of coronavirus disease 2019 (COVID-19), the State Administration of Traditional Chinese Medicine immediately organized experts to formulate and screen the effective prescriptions of traditional Chinese medicine according to the characteristics of the novel coronavirus infection. Qingfei Paidu decoction (QFPDD) has been proven to be effective in multi-provincial clinical trials, and has been selected as a general prescription for the treatment of COVID-19 in different stages that was later promoted to be used nationwide. This review highlights the latest advances of QFPDD, focusing on the TCM theory, mechanism analysis, clinical application of QFPDD and its future perspectives. Moreover, an in-depth discussion of some valuable issues and possible development for future research on QFPDD is also discussed, aiming to provide a novel guide to combat the global epidemic COVID-19.

Keywords: qingfei paidu decoction, novel coronavirus pneumonia, prescription principle, mechanism analysis, clinical application

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1 INTRODUCTION

As of November 1, 2020, novel coronavirus pneumonia (COVID-19), has spread over 211 countries around the world including all the continents, except Antarctica with around 46.43 million cumulative confirmed cases and 1.2 million deaths due to its strong infectiousness. The prevalence of COVID-19 has surpassed that of SARS in 2003, and is recognized as a severe health menace worldwide.

Since December 1, 2019, COVID-19 was emerged in Wuhan, Hubei province, China. Subsequently, the epidemic broke out throughout the country with the floating population during the Spring Festival. The mode of transmission for COVID-19 was soon recognized to be the inhalation of droplets from sneezing and coughing or the physical contact with the mucous secretions from infected individuals. People were generally susceptible and contracting the COVID-19 infection at exponentially high rate. Due to the sudden rise in the number of COVID-19 cases, China immediately launched the nationwide strict epidemic prevention and control guidelines. According to the Law of the People's Republic of China on the Prevention and Treatment of Infectious Diseases, the COVID-19 epidemic is listed in class-B infectious disease while it is managed in accordance with Class A infectious diseases (Xue et al., 2020). Until now, the number of cases infected by COVID-19 continues to grow around the globe, and it is predicted to be continued for longer period of time (Guan et al., 2020). Still until now, proper and effective targeted therapy, drugs

or vaccines, for COVID-19 epidemic control has not been identified. The accessibility of traditional drugs based on natural origin with effective therapeutic potential and the valuable historical treatment experience provide a more prominent therapeutic approach against COVID-19. Traditional Chinese medicine (TCM) has accumulated rich experience in the long-term practice of epidemic prevention and treatment, and it is characterized by broad-spectrum immunity, universal adaptability, foresight and so on. The unique advantages of TCM have attracted more and more attention to the epidemic prevention and treatment of COVID-19 (Yang et al., 2020a). Therefore, on January 27, 2019, the National Administration of Traditional Chinese Medicine launched the "Clinical screening for effective prescriptions of TCM for the prevention and treatment of pneumonia caused by novel coronavirus (2019-nCoV) infection" under the criteria "urgent, practical and effective"; nationwide. Qingfei Paidu Decoction (QFPDD), a multi-component herbal formula, was used clinically to treat 214 confirmed cases of COVID-19 with for three consecutive days as a course of treatment in four different pilot provinces in China from January 27, 2020 to February 5, 2020. The total effective rate was more than 90%, among them more than 60% of the cases showed significant improvement of symptoms and imaging manifestations and 30% of the patients showed stability of symptoms without aggravation or worsening (Yao et al., 2020). On February 18, 2020, the National Health Commission and National Administration of TCM jointly issued document No. 145 Diagnosis and Treatment Program of Novel Coronavirus Pneumonia (Trial Sixth edition). The document proposed to officially include TCM, QFPDD, in the clinical treatment of confirmed COVID-19 cases. QFPDD has been recommended as a general treatment prescription of TCM treatment for COVID-19 and has been promoted to the whole country for its remarkable clinical effect in the clinical prescription screening (Qin et al., 2020). Throughout the country, around 28 provinces, autonomous regions, and cities have been using this prescription, which is suitable for all periods and symptoms of COVID-19. Currently its use has been extended to treat suspected cases which has also been found effective and the feedback received is good.

QFPDD is formulated by the combination of syndrome differentiation and innovation based on the four classical prescriptions in *Treatise on Febrile Diseases* according to the pathogenic characteristics and development laws of COVID-19 (Jin, 2020). QFPDD has manifested its potential advantages and beneficial effects for the treatment of COVID-19. In this review, after summarizing the extant literature including CNKI, PubMed, Springer, Taylor & Francis, Google Scholar, and Baidu Scholar databases and other scientific resources e.g., Chinese Pharmacopoeia, 2020 edition, postgraduate research (PhD and MSc thesis, etc.), we have systematically summarized the TCM theory, modern mechanism analysis, clinical practice and application of QFPDD, hoping that it could offer some enlightenment for the further development and propel the research forward for efficiency, safety and controllable quality of QFPDD, so as to provide strong support for the global fight against the COVID-19 (**Supplementary Figure**).

1.1 TCM Theory and Prescription Principle of QFPDD

According to TCM theory, the experts have reached a consensus that COVID-19 belongs to a category of phytophthora blight (Li et al., 2020a), however, different experts have different understandings of COVID-19, including damp-toxin epidemic, cold-damp epidemic, and damp-heat epidemic. Wang and Miao et al. (2020) proposed COVID-19 as a damp-toxin epidemic caused by the damp toxin that belongs to yin, with the injury of *Yang* as the mainline (Miao et al., 2020; Wang et al., 2020c). Some believed that COVID-19 is a cold-damp epidemic caused by noxious dampness, and the basic pathogenesis is characterized by dampness, poison, blood stasis, and closure (Tong et al., 2020; Wang et al., 2020c; Xue et al., 2020). Luo and Zeng (2020) considered that COVID-19 is a damp-heat type caused by damp-heat epidemic toxin, and the main pathogenesis is the dampness, heat block of the Qi movement, endogenesis of phlegm and its transformation into fire and toxin, cremation of toxin and the combination of heat and blood stasis (Luo et al., 2020; Zeng and Sun, 2020).

Based on comprehensive analysis of COVID-19 clinical manifestations and syndrome types issued by the National Health Commission of PRC and various provinces in response to local conditions (**Table 1**), it is considered that COVID-19 is a damp-heat lung plague caused by damp-heat and epidemic toxin, and the pathogenesis and evolution process can be dry, and fire, and wind. At the very beginning, the pestilence attacks from the *Taiyang* meridian into the *Yangming* meridian quickly, or straight into the three *Yang* meridians, which is called *concurrent disease of three yang meridians*. But sometimes there is cold-dampness surrounding the exterior along with intense interior pathogenic fire. Or at the beginning, the exogenous pathogenic factors invade into three *Yin* meridians quickly, which may conduce to the syndrome of internal blockade and external collapse or syncope and collapse syndrome. The intermingled dampness and heat block the Qi movement, turbid phlegm hence appears inside and transforms into fire and toxin, intermingled heat and stasis is its main pathogenesis. Therefore, the treatment should be focused on dispelling dampness, heat and damp toxin, clearing dampness in triple warmer as well as strengthening vital Qi to eliminate pathogenic factors (Luo and Chen, 2020; Zhou, 2020). The most typical syndrome of COVID-19 is concurrent disease of three *Yang* meridians, which is often common in mild, moderate and part of severe cases. Hence, QFPDD is prescribed especially for this kind of syndrome.

QFPDD is composed of 21 TCMs, including *Herba Ephedrae* (*Ephedra sinica* Stapf; 9 g), *Radix Glycyrrhizae* (*Glycyrrhiza uralensis* Fisch.; 6 g; baked), *Semen Armeniacae Amarum* (*Prunus armeniaca* L.; 9 g), *Raw Gypsum* (15–30 g; first decocted), *Ramulus Cinnamomi* (*Cinnamomum cassia* (L.) J.Presl; 9 g), *Rhizoma Alismatis* (*Alisma plantago-aquatica* Linn.; 9 g), *Polyporus Umbellatus* (*Polyporus umbellaru* (Pers.) Fr.; 9 g), *Rhizoma Atractylodis Macrocephalae* (*Atractylodes macrocephala* Koidz.; 9 g), *Poria* (*Poria cocos* (Schw.) Wolf.;

TABLE 1 | TCM Syndrome Types of COVID-19 in national and various provinces' prevention and control programs.

Countries and regions	Mild	Moderate/Ordinary	Severe	Critical	Convalescence
National health commission	Cold-dampness retention lung syndrome; damp-heat retention lung syndrome	Pathogenic dampness retention lung syndrome; cold-dampness stagnating the lung	Syndrome of epidemic toxin obstructing lung; syndrome of flaring heat in qifen and yingfen	Syndrome of internal blockade and external collapse	Lung and spleen qi deficiency, deficiency of both qi and yin
Hubei province	Syndrome of heat-toxin invading lung	Pathogenic dampness retention lung syndrome	Syndrome of accumulated dampness-toxicity	Syndrome of blazing heat-toxin	NA
Heilongjiang province	Damp warm retention lung syndrome	Phlegm-heat retention lung syndrome	Syndrome of pathogenic toxin obstructing lung	Syndrome of pathogenic toxin clouding orifices	Pathogenic factors residue, deficiency of both qi and yin
Beijing province	Syndrome of epidemic toxin invading lungs	NA	Epidemic toxin retention lung syndrome	Syndrome of epidemic toxin obstructing lung	Deficiency of both qi and yin
Shanghai province	Noxious dampness retention lung syndrome	NA	Syndrome of heat-toxin obstructing lung	Syndrome of internal blockade and external collapse	Lung and spleen qi deficiency, deficiency of both qi and yin
Guangdong province	Pathogenic dampness stagnating the lung, cardinal disadvantageous; syndrome of pathogenic heat congesting lung, impairment of the ascending and descending function of the lung	NA	Pathogenic heat obstructing lung syndrome, obstruction of fu-qi; warm-heat obstructing lung syndrome	Syndrome of internal blockade and external collapse	Pathogenic factors residue, deficiency of both qi and yin, deficiency of both lung and spleen
Jiangxi province	Noxious dampness retention lung syndrome, cardinal disadvantageous	Heat-toxin with dampness syndrome, impairment of the ascending and descending function of the lung	Syndrome of heat-toxin obstructing lung, obstruction of fu-qi	Syndrome of internal blockade and external collapse	NA
Shanxi province	Syndrome of exterior tightened by cold-dampness, impairment of fluid due to heat retention; syndrome of heat-toxin invading lung; external-cold and internal-heat	NA	Heat-toxin retention lung syndrome	Syndrome of internal blockade and external collapse	Syndrome of lingering heat, deficiency of both qi and yin
Tianjin province	Syndrome of heat-toxin invading lung	Pathogenic dampness retention lung syndrome	Syndrome of accumulated dampness-toxicity	Syndrome of blazing heat-toxin	NA
Yunnan province	Dampness-heat retention lung syndrome	Pathogenic heat retention lung syndrome	Syndrome of pathogenic toxin obstructing lung	Syndrome of internal blockade and external collapse	NA
Sichuan province	Wind heat with dampness syndrome; wind chill with dampness syndrome	Pathogenic dampness retention lung syndrome; dampness-heat retention lung syndrome	Pathogenic heat retention lung syndrome; epidemic toxin obstructing lung syndrome	Syndrome of internal blockade and external collapse	Pathogenic factors residue, deficiency of both qi and yin
Gansu province	Syndrome of warm pathogen attacking lung	Warm-heat retention lung syndrome	Syndrome of warm toxin obstructing lung	Syndrome of internal blockade and external collapse	NA

15 g), *Radix Bupleuri* (*Bupleurum chinensis* DC.; 16 g), *Radix Scutellariae* (*Scutellaria baicalensis* Georgi; 6 g), *Rhizome Pinelliae Preparata* (*Pinellia ternata* (Thunb.) Breit.; 9 g; processed with ginger), *Rhizoma Zingiberis Recens* (*Zingiber officinale* Roscoe; 9 g), *Radix Asteris* (*Aster tataricus* Linn. f.; 9 g), *Flos Farfarae* (*Tussilago farfara* Linn.; 9 g), *Rhizoma Belamcandae* (*Iris domestica* (L.) Goldblatt & Mabb.; 9 g), *Herba Asari* (*Asarum sieboldii* Miq.; 6 g), *Rhizoma Dioscoreae* (*Dioscorea oppositifolia* L.; 12 g), *Fructus Aurantii Immaturus* (*Citrus sinensis* Osbeck; 6 g), *Pericarpium Citri Reticulatae* (*Citrus aurantium* L.; 6 g), and *Herba Pogostemonis* (*Pogostemon cablin (Blanco)* Benth.; 9 g). This prescription is mainly composed of Maxing Shigan decoction, Shegan Mahuang decoction, Xiaochaihu decoction and Wuling powder. In addition, it also incorporates Daqinglong decoction, Juzhijiang decoction, Fuling Xingren Gancao decoction, etc. QFPDD is a syncretic innovation of classical prescriptions from *Treatise on Febrile Diseases*, which act on different stages and viscera of water, dampness, phlegm, and fluid (Fan et al., 2020). This formula is suitable for the pathogenesis of COVID-19, affecting cold, dryness, damp toxin and dampness, and can effectively improve the symptoms. TCM theory and composition mechanism of QFPDD are summarized in **Figure 1**. The meridian tropisms of drugs in QFPDD are shown in **Figure 2**, where the top meridian tropism in QFPDD is lung meridian, indicating that drugs in QFPDD are mainly specific for lung diseases. The prescriptions of QFPDD are synergistic and complementary and the prescription principle of QFPDD is shown in **Figure 3**. Maxing Shigan decoction is to relieve exterior *Taiyang* syndrome, relieve superficies and ventilate lung Qi, clear heat and relieve panting; Shegan Mahuang decoction (*Fructus Jujubae* and *Fructus Schisandra chinensis* were taken out) is for lowering the adverse Qi and resolving fluid ventilate lung Qi, dispelling phlegm and relieving cough; Xiaochaihu decoction is for harmonizing half-superficies and half-interior *Shaoyang* syndrome, and large dose of raw gypsum is used to clear interior heat of the *Yangming* meridian, and Wuling powder is to warm the triple energizer and transform Qi and remove dampness by promoting diuresis; Juzhijiang decoction can activate Qi and dispel phlegm; *Herba Pogostemonis* can exorcise toxins and eliminate dampness; and *Rhizoma Dioscoreae* can strengthen the spleen and supplement the lung (Shen et al., 2020; Wang and Jin, 2020). The combination of *Ramulus Cinnamomi* and *Radix Glycyrrhizae* can nourish *Yang* and support healthy energy. QFPDD is not made up of drugs but multiple concordant prescriptions contributing to get twice the result with half the effort, so that the damp-heat and epidemic toxin can be quickly discharged (Wang et al., 2020a).

1.2 Mechanism Analysis of QFPDD

As described earlier, QFPDD contains a total of 21 TCMs, therefore, it is difficult to clearly explain the complex mechanisms of QFPDD in the treatment of COVID-19. Modern research on TCM holds that Chinese herbal compound formula plays an omnidirectional and overall regulatory role in the body due to the characteristics of multi-components, multi-targets and multi-path of the formula.

Recently, based on the reported components of QFPDD, several research groups have adopted the method of network pharmacology, molecular docking, and computer-aided drug design to provide data and clues for the multi-directional exploration of the material basis and pharmacodynamic mechanism of QFPDD in the treatment of COVID-19. Xu et al. (2020b) used the network pharmacology to screen significant effective compounds and key targets. Using TCMSP database, 148 related targets of 302 bioactive components in QFPDD were screened. Another database, GeneCards, using "COVID-19", "2019-nCoV" and "Novel Coronavirus Pneumonia" as keywords, was used to screen 362 COVID-19 related targets where a total of 23 intersection targets were obtained by Venn analysis. By using the CentiScaPe plug-in of Cytoscape software, the network topology diagram of the 10 significant effective compounds, i.e., quercetin, luteolin, naringenin, kaempferol, beta-sitosterol, stigmasterol, baicalein, isorhamnetin, nobiletin, and wogonin (**Table 2**); and five pivotal targets, i.e., PTGS2, NOS2, PPARG, MAPK14, and PTGS1 were analyzed (**Table 3**). The results of molecular docking of the above most significant compound, quercetin, and target, PTGS2, with the highest degree value showed that the binding and interaction ability between these molecules was strong. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the key targets were done using the Cluster Profiler package of R software, which showed that significant compounds such as quercetin, luteolin, naringin, kaempferol, and baicalein have expectorant, antitussive, antiviral and anti-inflammatory effects in various degrees. The key targets were mainly concentrated in 144 related signaling pathways including IL-17, tuberculosis, human cytomegalovirus infection, TNF, MAPK, Hepatitis B, etc. (**Table 4**). It contained 28 biological effects including cytokine receptor binding, MAP kinase activity and phosphatase binding to regulate and control metabolism, immune regulation, lung function, inflammation, and other physiological processes (Xu et al., 2020b). Xu et al. (2020a) showed that 217 related targets of 186 active components and 200 COVID-19 related targets were screened, and 51 common drug-disease targets were obtained by Venn analysis. Then, five significantly effective compounds i.e., quercetin, luteolin, kaempferol, naringin, and isorhamnetin were obtained by using the CentiScaPe plug-in of Cytoscape software to further construct the network topology diagram. The GO and KEGG pathway enrichment analysis indicated that the key targets were mainly concentrated in 30 related signal pathways such as IL-17, NF-κB, TNF, MAPK, Th17, etc. It involved several biological functions such as inflammation, immune regulation, neuroprotection, reduction of lung injury, and other physiological processes (Xu et al., 2020a).

Zhao et al. revealed that 464 compounds of QFPDD corresponded to 790 different putative targets, of which 232 targets were co-expressed with angiotensin-converting enzyme 2 (ACE2), the receptor of 2019-nCoV. Main signaling pathways regulated by key targets of QFPDD are shown in **Table 3**, where the main targets are concentrated on two types of disease pathways i.e., virus infection and lung injury. In addition, 48 important targets interacted densely

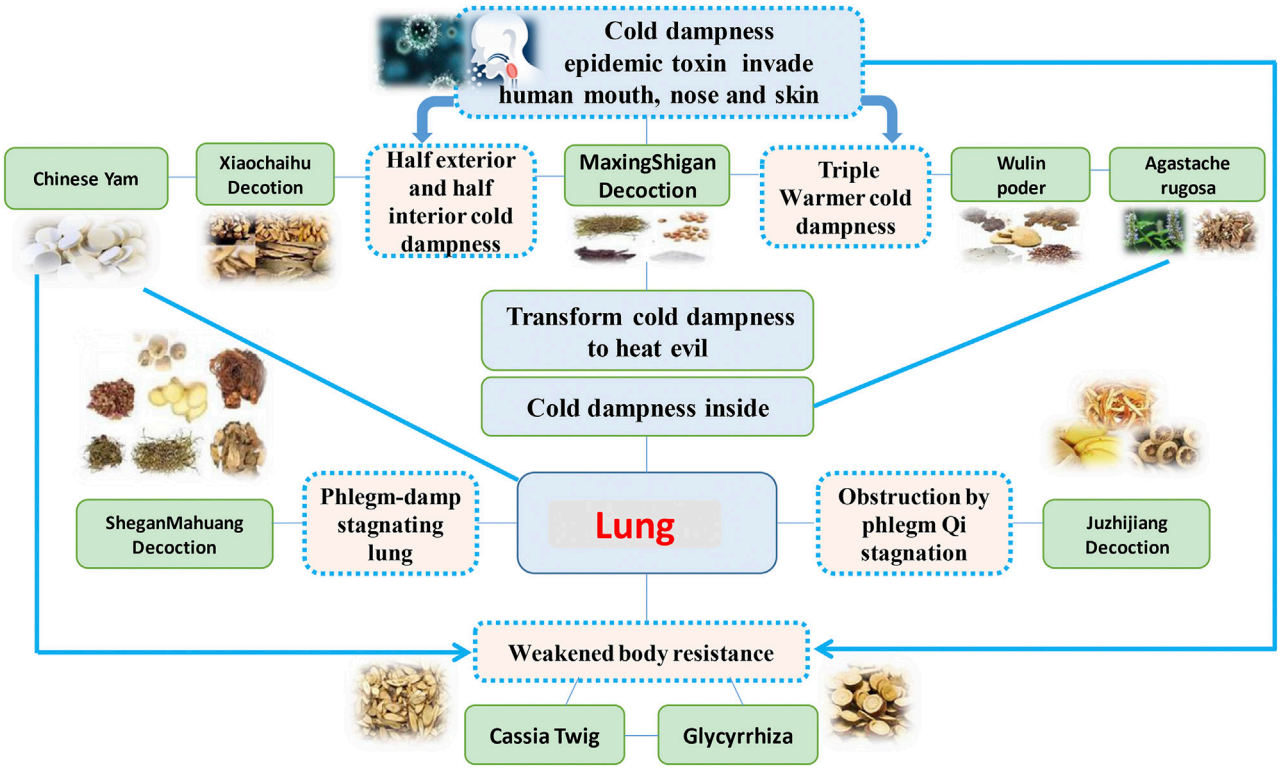

FIGURE 1 | TCM theory and composition mechanism of durgs in QFPDD.

with six proteins of HIV, indicating its potential antiviral effect. Key targets regulated a series of signaling pathways in biological processes such as endocrine system, immune system, translation, nervous system, and signal transduction (Zhao et al., 2020).

Wu et al. (2020b) showed that the QFPDD compound-pneumonia target network contained 292 compounds and 214 corresponding potential targets and the top five pivotal targets were AKT serine/threonine kinase 1 (AKT1), interleukin-6 (IL-6), mitogen-activated protein kinase 8 (MAPK8), mitogen-

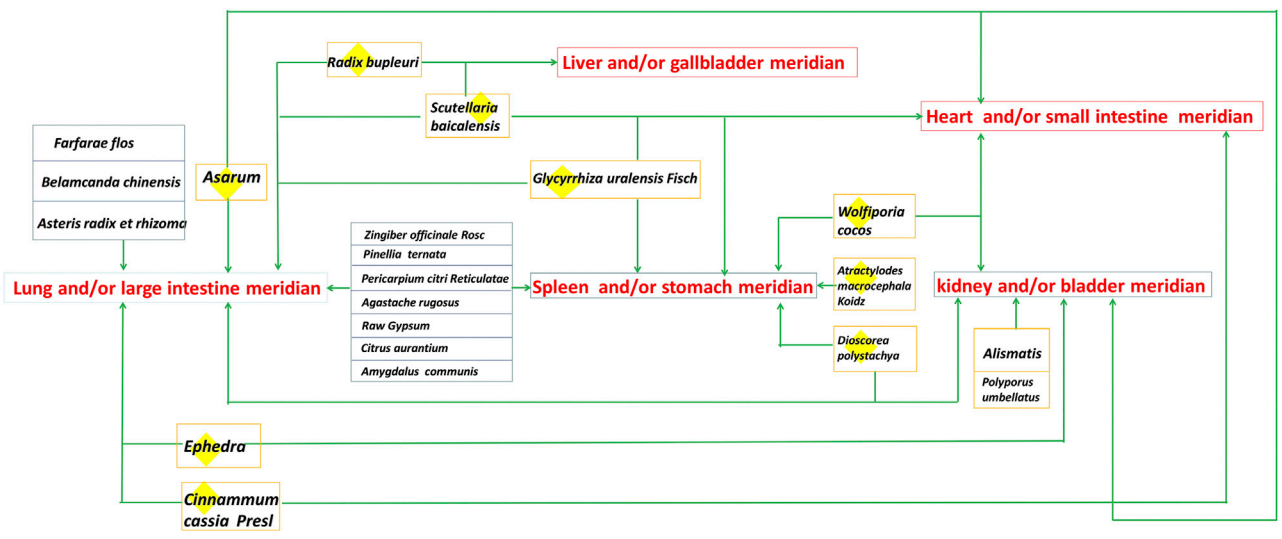

FIGURE 2 | Drug-meridian network of QFPDD.

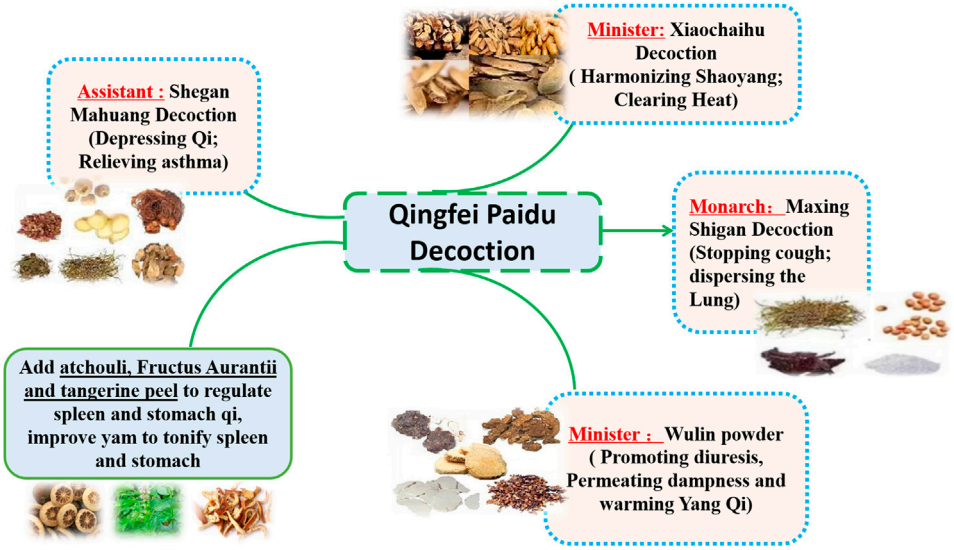

FIGURE 3 | Compatibility of QFPDD in prescription.

activated protein kinase 1 (MAPK1), and jun proto-oncogene (JUN). The GO and KEGG enrichment analysis and screening yielded 122 related signaling pathways, including non-small cell lung cancer, small cell lung cancer, hypoxia inducible factor-1, toll-like receptor signaling pathway, T cell receptor signaling pathway and other pathways related to pneumonia. Moreover, the same enrichment analysis also included TNF signaling pathway, P13k-Akt signaling pathway, MAPK signaling pathway, B cell receptor signaling pathway, apoptosis, and other pathways related to the reduction of lung injury (**Table 4**). The molecular docking results indicated that some core compounds such as ergosterol, shionone, tussilagone, etc. of the TCMs present in QFPDD had a certain degree of binding activity for 2019-nCoV 3C-like protease (3CLpro) and ACE2. It was worthwhile pointing out that ergosterol is the only one that can form a hydrogen bond with 3CLpro of 2019-nCoV (Wu et al., 2020b). In another study by Yan et al. (2020) QFPDD compound-2019-nCoV and COVID-19 target-biological function network was screened, it contained 163 active ingredients, 10 protein targets, and 42 biological functions such as renin-angiotensin regulation of blood volume and systemic arterial blood pressure to treat COVID-19. The results of preliminary molecular docking showed that the core ingredients had a good affinity with SARS-CoV-2 3CL hydrolase to form complexes with stable conformations and high binding energy, indicating that QFPDD might treat COVID-19 through RAS signaling pathway (Yan et al., 2020). Cytokine storm is considered one of the central causes of clinical sudden deterioration of COVID-19. It has been reported that QFPDD had an inhibitory effect on cytokine storm in the treatment of COVID-19 by acting on multiple targets and pathways with multiple components (Zhou et al., 2020). Duan et al. (2020) revealed that QFPDD had a potential common action mechanism in the treatment of SARS, MERS, and COVID-19. 337 corresponding targets of 246 components in QFPDD and 148 common disease-related targets for SARS, MERS, and COVID-19 were screened, and 44 common drug-disease targets were obtained by Venn analysis. The GO and KEGG pathway enrichment analysis of the key targets indicated that the key targets were mainly concentrated in 77 related signal pathways such as pertussis, tuberculosis, MAPK, FoxO, TNF, NOD-like receptor signaling pathways, and other pathways related to viral pneumonia. biological angiogenesis, immune

TABLE 2 | The key active compounds of QFPDD.

Compounds	References	Compounds	References
Quercetin	Xu et al. (2020a), Zhou et al. (2020), Duan et al. (2020), Xu et al. (2020b), Wu et al., 2020b	Beta-sitosterol	Xu et al. (2020b), Wu et al., 2020b
Luteolin	Xu et al. (2020a), Zhou et al. (2020), Duan et al. (2020), Xu et al. (2020b), Wu et al., 2020b	Wogonin	Zhou et al. (2020), Duan et al. (2020), Xu et al. (2020b)
Kaempferol	Xu et al. (2020a), Zhou et al. (2020), Duan et al. (2020), Wu et al., 2020b, Xu et al. (2020b)	Baicalein	Xu et al. (2020b)
Naringenin	Xu et al. (2020a), Zhou et al. (2020), Duan et al. (2020), Xu et al. (2020a), Xu et al. (2020b)	Nobiletin	Xu et al. (2020b)
Isorhamnetin	Xu et al. (2020a), Xu et al. (2020b)	Stigmasterol	Xu et al. (2020b)

TABLE 3 | The main key targets of QFPDD in the treatment of COVID-19.

Key targets	References	Key targets	References
Cell tumor antigen p53 (TP53)	Peng et al. (2020), Duan et al. (2020), Zhou et al. (2020)	Caspase 3 (CASP3)	Peng et al. (2020), Xu et al. (2020a), Yan et al. (2020), Duan et al. (2020), Zhou et al. (2020)
Protein kinase B1(Akt1)	Peng et al. (2020), Wu et al., 2020b	Janus kinase 2 (JAK2)	Peng et al. (2020)
Nuclear factor nuclear transcription factor-κB p105 subunit (NFKB1)	Peng et al. (2020)	Nuclear factor transcription factor-κB p100 subunit (NFKB2)	Peng et al. (2020)
Nuclear factor p65 subunit (RELA)	Peng et al. (2020)	Calmodulin 1 (CALM1)	Peng et al. (2020), Xu et al. (2020a)
Adenylate cyclase type 1 (ADCY1)	Peng et al. (2020)	Eukaryotic translation initiation factor 2, subunit 3 (EIF2S3)	Peng et al. (2020)
Adenylate cyclase type 2 (ADCY2)	Peng et al. (2020)	B-cell CLL/lymphoma 2 (BCL2)	Peng et al. (2020), Xu et al. (2020a), Zhou et al. (2020)
Heat shock protein α A1 (HSP90AA1)	Peng et al. (2020)	Protein kinase C-delta (PRKCD)	Peng et al. (2020)
Adenylate cyclase type 5 (ADCY5)	Peng et al. (2020)	Jun proto-oncogene (JUN)	Peng et al. (2020), Wu et al., 2020b
Recombinant human glucocorticoid receptor (NR3C1)	Peng et al. (2020)	Prostaglandin-endoperoxide synthase 2 (PTGS2)	Xu et al. (2020a), Xu et al. (2020b), Duan et al. (2020), Zhou et al. (2020)
Mitogen-activated protein kinase 8 (MAPK8)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020), Wu et al., 2020b	Prostaglandin-endoperoxide synthase 1 (PTGS1)	Xu et al. (2020a), Xu et al. (2020b), Zhou et al. (2020)
Mitogen-activated protein kinase 3 (MAPK3)	Peng et al. (2020), Xu et al. (2020a), Zhou et al. (2020)	Dipeptidyl peptidase-4 (DPP4)	Xu et al. (2020a), Yan et al. (2020)
Human NK-κB inhibited protein α (NFKBIA)	Peng et al. (2020)	V-rel reticuloendotheliosis viral oncogene homolog A (RELA)	Xu et al. (2020a), Zhou et al. (2020)
Bcl2-associated X protein (BAX)	Xu et al. (2020a), Zhou et al. (2020)	V-fos FBJ murine osteosarcoma viral oncogene homolog (FOS)	Xu et al. (2020a), Zhou et al. (2020)
Apolipoprotein D (APOD)	Xu et al. (2020a)	Lymphocyte specific tyrosine kinase (LCK)	Peng et al. (2020)
Peroxisome proliferative activated receptor, gamma (PPARG)	Xu et al. (2020a), Xu et al. (2020b), Zhou et al. (2020)	Signal transducerand activator of transcription 1(STAT1)	Xu et al. (2020a), Zhou et al. (2020)
Nitric oxide synthase (NOS2)	Xu et al. (2020a), Xu et al. (2020b), Zhou et al. (2020)	Retinoblastoma 1 (RB1)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)
Mitogen-activated protein kinase 14 (MAPK14)	Xu et al. (2020a), Xu et al. (2020b), Duan et al. (2020), Zhou et al. (2020)	Interleukin-6 (IL-6)	Xu et al. (2020a), Zhou et al. (2020), Wu et al., 2020b
Heme oxygenase (decycling) 1 (HMOX1))	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)	Apoptosis-related cysteine peptidase (CASP8)	Xu et al. (2020a), Zhou et al. (2020)
Intercellular adhesion molecule 1 (ICAM1)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)Z	Superoxide dismutase 1 (SOD1)	Xu et al. (2020a), Zhou et al. (2020)
Epidermal growth factor receptor (EGFR)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)	Protein kinase C alpha type (PRKCA)	Xu et al. (2020a), Zhou et al. (2020)
Bcl-2-like protein 1 (BCL2L1)	Xu et al. (2020a), Zhou et al. (2020)	Heat shock 70 kDa protein 5 (HSPA5)	Xu et al. (2020a), Zhou et al. (2020)
Mitogen-activated protein kinase 1 (MAPK1)	Xu et al. (2020a), Duan et al. (2020), Wu et al., 2020b	Interleukin-1β (IL-1β)	Xu et al. (2020a), Duan et al. (2020)
Chemokine (C-C motif) ligand 2 (CCL2)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)	Protein kinase C beta type (PRKCB)	Xu et al. (2020a), Zhou et al. (2020)
Serine protease inhibitor protein E1 (SERPINE1)	Xu et al. (2020a), Zhou et al. (2020)	Nitric oxide synthase 3 (NOS3)	Xu et al. (2020a), Zhou et al. (2020)
Interleukin-2 (IL-2)	Xu et al. (2020a), Zhou et al. (2020)	Heat shock 27 kDa protein 1 (HSPB1)	Xu et al. (2020a), Zhou et al. (2020)
Interleukin-1α (IL-lα)	Xu et al. (2020a), Zhou et al. (2020)	Poly ADP-ribose polymerase 1 (PARP1)	Xu et al. (2020a), Zhou et al. (2020)
Chemokine CXC motif ligand 2 (CXCL2)	Xu et al. (2020a), Zhou et al. (2020)	Chemokine CXC motif ligand 11 (CXCL11)	Xu et al. (2020a), Zhou et al. (2020)
C-reactive protein (CRP)	Xu et al. (2020a), Zhou et al. (2020)	Chemokine CXC motif ligand 10 (CXCL10)	Xu et al. (2020a), Zhou et al. (2020)
CD40 ligand (CD40LG)	Xu et al. (2020a), Zhou et al. (2020)	BCL2-antagonist of cell death (BAD)	Xu et al. (2020a), Zhou et al. (2020)
Interferon regulatory factor 1 (IRF1)	Xu et al. (2020a), Zhou et al. (2020)	Catalase (CAT)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)
Phospholipase A2 (PLA2G4A)	Xu et al. (2020a), Zhou et al. (2020)	cAMP responsive element binding protein 1 (CREB1)	Xu et al. (2020a), Zhou et al. (2020)
Cyclin D3 (CCND3)	Xu et al. (2020a)	Myeloid cell leukemia sequence 1 (MCLl)	Xu et al. (2020a)
Interleukin-4 (IL-4)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)	Cyclin-dependent kinase 4 (CDK4)	Xu et al. (2020a), Zhou et al. (2020)
Angiotensin I-converting enzyme (ACE)	Yan et al. (2020)	Glucose-6-phosphate dehydrogenase (G6PD)	Xu et al. (2020a), Zhou et al. (2020)
Angiotensin I-converting enzyme 2 (ACE2)	Yan et al. (2020)	Furin (FURIN)	Yan et al. (2020)
Angiotensin II type 1 receptor (AT1R/ AGTR1)	Yan et al. (2020)	Caspase 6 (CASP6)	Yan et al. (2020)
Myeloid cell Leukemia sequence 1 (MCL1)	Yan et al. (2020), Zhou et al. (2020)	Polymerase (DNA directed), delta 1, catalytic subunit 125 kDa (POLD1)	Yan et al. (2020)
Tumor necrosis factor (TNF)	Yan et al. (2020), Zhou et al. (2020)	Interleukin- 10 (IL-10)	Duan et al. (2020), Zhou et al. (2020)
Interferon, gamma (IFNG)	Duan et al. (2020), Zhou et al. (2020)	Interleukin-8 (IL-8)	Duan et al. (2020), Zhou et al. (2020)
Transforming growth factor, beta 1 (TGFB1)	Zhou et al. (2020)		

TABLE 4 | Main enriched signaling pathways of QFPDD in the treatment of COVID-19.

Pathway name	References	Pathway name	References
Adherens junction	Zhao et al. (2020)	AGE-RAGE signaling pathway in diabetic complications	Xu et al. (2020b), Xu et al. (2020a)
Focal adhesion	Zhao et al. (2020)	C-type lectin receptor signaling pathway	Xu et al. (2020b), Xu et al. (2020a)
Osteoclast differentiation	Zhao et al. (2020), Xu et al. (2020b), Wu et al., 2020b	HIF-1 signaling pathway	Xu et al. (2020b), Xu et al. (2020a), Wu et al., 2020b
Estrogen signaling pathway	Zhao et al. (2020)	Toxoplasmosis	Xu et al. (2020b), Wu et al., 2020b, Xu et al. (2020a), Duan et al. (2020)
Thyroid hormone signaling pathway	Zhao et al. (2020), Wu et al., 2020b	*Yersinia* infection	Xu et al. (2020b)
Relaxin signaling pathway	Zhao et al. (2020)	Hepatitis B	Xu et al. (2020b), Xu et al. (2020a), Wu et al., 2020b
Prolactin signaling pathway	Zhao et al. (2020), Wu et al., 2020b	NOD-like receptor signaling pathway	Xu et al. (2020b), Wu et al., 2020b, Duan et al. (2020), Zhou et al. (2020)
Oxytocin signaling pathway	Zhao et al. (2020)	Kaposi sarcoma-associated herpesvirus infection	Xu et al. (2020b), Xu et al. (2020a)
Glucagon signaling pathway	Zhao et al. (2020)	Pertussis	Xu et al. (2020b), Wu et al., 2020b, Xu et al. (2020a), Duan et al. (2020)
Th17 cell differentiation	Zhao et al. (2020), Xu et al. (2020b)	Leishmaniasis	Xu et al. (2020b), Wu et al., 2020b, Xu et al. (2020a), Duan et al. (2020)
B cell receptor signaling pathway	Zhao et al. (2020), Wu et al., 2020b	Endocrine resistance	Xu et al. (2020b)
T cell receptor signaling pathway	Zhao et al. (2020), Wu et al., 2020b	FoxO signaling pathway	Xu et al. (2020b), Wu et al., 2020b, Duan et al. (2020)
Neurotrophin signaling pathway	Zhao et al. (2020)	Prion diseases	Xu et al. (2020b)
Dopaminergic synapse	Zhao et al. (2020)	Pancreatic cancer	Wu et al., 2020b, Xu et al. (2020b)
ErbB signaling pathway	Zhao et al. (2020), Wu et al., 2020b	Hepatitis C	Wu et al., 2020b, Duan et al. (2020)
MAPK signaling pathway	Zhao et al. (2020), Duan et al. (2020), Zhou et al. (2020)	Ras signaling pathway	Wu et al., 2020b
PI3K-Akt signaling pathway	Zhao et al. (2020), Xu et al. (2020b), Wu et al., 2020b	Bladder cancer	Wu et al., 2020b
TNF signaling pathway	Zhao et al. (2020), Wu et al., 2020b, Xu et al. (2020b), Duan et al. (2020), Zhou et al. (2020)	Prostate cancer	Wu et al., 2020b
Wnt signaling pathway	Zhao et al. (2020)	Melanama	Wu et al., 2020b
VEGF signaling pathway	Zhao et al. (2020), Xu et al. (2020b), Wu et al., 2020b	Thyroid hormone signaling pathway	Wu et al., 2020b
Ribosome	Zhao et al. (2020)	Chronic myeloid leukemia	Wu et al., 2020b
IL-17 signaling pathway	Xu et al. (2020b), Xu et al. (2020a)	Glioma	Wu et al., 2020b
Chagas disease (American trypanosomiasis)	Xu et al. (2020b), Wu et al., 2020b, Xu et al. (2020a), Duan et al. (2020)	Endometrial cancer	Wu et al., 2020b
Tuberculosis	Xu et al. (2020b), Wu et al., 2020b, Xu et al. (2020a), Duan et al. (2020)	Influenza A	Wu et al., 2020b, Xu et al. (2020b), Duan et al. (2020)
Human cytomegalovius infection	Xu et al. (2020b), Xu et al. (2020a)	Toll-like receptor signaling pathway	Wu et al., 2020b, Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020), Yang et al. (2020a)
Epithelial cell signaling in helicobacter pylori infection	Wu et al., 2020b	*Salmonella* infection	Wu et al., 2020b, Duan et al. (2020)
Melanoma	Wu et al., 2020b	Colorectal cancer	Wu et al., 2020b
RIG-I-like receptor signaling pathway	Wu et al., 2020b	Small cell lung cancer	Wu et al., 2020b
Herpes simplex infection	Wu et al., 2020b, Duan et al. (2020)	Non-alcoholic fatty liver disease	Wu et al., 2020b
Shigellosis	Wu et al., 2020b	HTLV-I infection	Wu et al., 2020b
Cytosolic DNA-sensing pathway	Wu et al., 2020b	Apoptosis	Xu et al. (2020a)
Acute myeloid leukemia	Wu et al., 2020b	Human immunodeficiency virus 1 infection	Xu et al. (2020a)
Measlea	Xu et al. (2020a)	Proteoglycans in cancer	Wu et al., 2020b
Non-small cell lung cancer	Wu et al., 2020b	Glutamatergic synapse	Jin et al. (2020)
Amphetamine addiction	Jin et al. (2020)	Long-term potentiation	Jin et al. (2020)
Long-term depressio	Jin et al. (2020)	Retrograde endocannabinoid signaling	Jin et al. (2020)
Cocaine addiction	Jin et al. (2020)	Nitrogen metabolism	Jin et al. (2020)
Nicotine addiction	Jin et al. (2020)	Neuroactive ligand-receptor interaction	Jin et al. (2020), Chen et al. (2020a)
Interleukin-4 and interleukin-13 signaling	Peng et al. (2020)	Interleukin-1 processing	Peng et al. (2020)
Adrenoceptors	Peng et al. (2020)	IκBα variant leads to EDA-ID	Peng et al. (2020)
CLEC7A/inflammasome pathway	Peng et al. (2020)	DEx/H-box helicases activate type I IFN and inflammatory cytokines production	Peng et al. (2020)
G alpha (s) signaling events	Peng et al. (2020)	G alpha(z) signaling events	Peng et al. (2020)

(Continued on following page)

TABLE 4 | (*Continued*) Main enriched signaling pathways of QFPDD in the treatment of COVID-19.

Pathway name	References	Pathway name	References
Tp53 regulates transcription of DNA repair	Peng et al. (2020)	RIP-mediated NF-κB activation via ZBP1	Peng et al. (2020)
Interleukin-21 signaling	Peng et al. (2020)	PI5P, PP2A and IER3 regulate PI3K/Akt signaling	Peng et al. (2020)
Interleukin-2 signaling	Peng et al. (2020)	Signaling by SCF-KIT	Peng et al. (2020)
Erythropoietin activatesPphosphoinositide-3-kinase (PI3K)	Peng et al. (2020)	Activation of the AP-1 family of transcription factors	Peng et al. (2020)
Interleukin-10 signaling	Peng et al. (2020)	Interleukin receptor SHC signaling	Peng et al. (2020)
Adenylate cyclase inhibitory pathway	Peng et al. (2020)	Calmodulin induced events	Peng et al. (2020)
Inflammatory bowel disease (IBD)	Duan et al. (2020)	Cytokine-cytokine receptor interaction	Duan et al. (2020), Zhou et al. (2020)
Rheumatoid arthritis	Duan et al. (2020)	Amebiasis	Duan et al. (2020)
African trypanosomiasis	Duan et al. (2020)	Malaria	Duan et al. (2020)
Dteroid biosynthesis	Chen et al. (2020a)	PPAR signaling pathway	Chen et al. (2020a)
Adipocytokine signaling pathway	Chen et al. (2020a)	Steroid hormone biosynthesis	Chen et al. (2020a)

response, nitric oxide synthesis and cell apoptosis might be the potential common mechanisms of QFPDD in the treatment of SARS, MERS, and COVID-19 (Duan et al., 2020).

In addition, Peng et al. (2020) constructed the interaction network of *Formula-Herb-Disease-Targets-Pathways* based on the three main clinical symptoms of COVID-19: pneumonia, fever, and cough. The research results indicated that key-targets such as cell tumor antigen p53 (tp53), protein kinase B1 (Akt1), nuclear factor nuclear transcription factor-κB (NK-κB) p105 subunit (NFKB1), nuclear factor p65 subunit (RELA), human NK-κB inhibited protein α (NFKBIA), etc. were mainly related to the regulation of apoptosis and immune response, inflammatory response, improving lung function, etc. The GO and KEGG pathway enrichment analysis indicated that the 103 key targets were mainly concentrated in the signal pathways such as interleukin signaling, adrenoceptors, seven members of the family of c-type lectin domains A (CLEC7A)/inflammasome pathway, phosphoinositide-3-kinase (PI3K)/protein kinase B (Akt) inflammatory signaling pathway, tp53 regulates transcription of DNA repair, etc. which might be the main pathways related to QFPDD's effect on the treatment of COVID-19 accompany with lung injury, fever, cough, and other symptoms (Peng et al., 2020).

Based on computer-aided drug design, Jin et al. (2020) systematically explored and analyzed the material basis and molecular mechanism of QFPDD in the three aspects of detoxification, anti-inflammatory storm, and diuresis-removing dampness. Molecular docking virtual screening was performed based on the 2,740 compounds in QFPDD and the targets including ACE2, interleukin-6 receptor (IL-6R), and aquaporins (APQ). The mechanism of action was predicted by reverse target prediction, GO and KEGG pathway enrichment analysis for *Atractylodes macrocephala, Polyporus umbellatus, Poria cocos, and Alisma plantago-aquatica*. Research showed that Xiaochaihu decoction ranked the first in the number of potentially active compounds to block the virus and suppress inflammatory storm among the five classic prescriptions of QFPDD. The top three most prominent drugs to block the key binding sites of the virus were *Radix Glycyrrhizae, Herba Ephedrae*, and *Citrus aurantium*, while the top three to suppress inflammatory storm were *Radix Glycyrrhizae, Radix Asteris, and Radix Bupleuri*. Quercetin and its derivatives, the potential dual-target active compounds, had a high binding ability to ACE2 and IL-6R targets. *Atractylodes macrocephala, Cinnamomum cassia, Poria cocos, Polyporus umbellatus, and Alisma plantago-aquatica* lacked compounds that blocked viruses and suppressed inflammatory storms, but dehydroburicoic acid, scopoletin, alismoxide and alpha-D-galactose contained in the above drugs had the potential binding ability with AQP4. Each component of the sub-medical prescription is reasonably compatible and plays a role in prevention and treatment through multi-point cooperation and complementary advantages. The interaction between these targets can form a molecular network, and it is found that many active components of QFPDD play a role in virus invasion, virus replication, and multiple organ damage (Jin et al., 2020). Furthermore, Chen et al. (2020a) divided QFPDD into five functional units (four sub-medical prescriptions and the rest) in the light of the compatibility theory of TCM. Results showed that all the five functional units had a positive effect on COVID-19 independently, and it involved physiological processes such as inflammation, bacterial and viral responses, immune system, signaling transduction, etc (Chen et al., 2020a). Yang et al. (2020c) also reported the chemical composition and pharmacological mechanism of QFPDD which indicated the thrombin and Toll-like receptor (TLR) signaling pathway were suggested to be main pathways for Maxing Shigan decoction mediated anti-inflammatory effects (Yang et al., 2020c).

1.3 *In Vivo* Distribution and Metabolomics of QFPDD

Liu et al. (2020b) investigated the main chemical constituents in QFPDD and the tissues distribution of the main absorbed constituents in mice following oral administration of QFPDD. As shown in **Table 5**, a total of 39 compounds were identified from QFPDD using UHPLC-Q-Orbitrap HRMS. After administered QFPDD in mice (2.6 g/100 g, ig), 12, 9, 10, 8, 9,

TABLE 5 | Components of QFPDD distribution in the organs.

Name	CAS No	Distribution					
		Serum	Liver	Heart	Spleen	Lung	Kidney
Synephrine	94-07-5	-	-	-	-	-	-
Dihydroxyacetone	96-26-4	-	-	-	-	-	-
Gallic acid monohydrate	5,995-86-8	-	-	-	-	-	-
Neochlorogenic acid	906-33-2	-	-	-	-	-	-
(1R,2S)-2-(Methylamino)-1-phenylpropan-1-ol	299-42-3	+	+	+	+	+	+
Pseudoephedrine	90-82-4	+	+	+	+	+	+
Caffeic acid	331-39-5	-	-	-	-	-	-
Chlorogenic acid	327-97-9	-	-	-	-	-	-
Cryptochlorogenic acid	905-99-7	-	-	-	-	-	-
(R)-amygdalin	29,883-15-6	+	+	+	+	+	+
Benzeneacetonitrile, a-(b-D-glucopyranosyloxy)-, (aR)-	99-18-3	+	+	+	+	+	+
(-)-3,5-Dicaffeoyl quinic acid	89,919-62-0	-	-	-	-	-	-
Ferulic acid	1,135-24-6	-	-	-	-	-	-
Liquiritin	551-15-5	+	+	+	+	+	+
Isochlorogenic acid B	14,534-61-3	-	-	-	-	-	-
3,5-Dicaffeoylquinic acid	2,450-53-5	+	-	-	-	+	+
Hyperoside	482-36-0	+	+	+	-	+	+
Rutin	153-18-4	-	-	-	-	-	-
Resveratrol	501-36-0	-	-	-	-	-	-
Naringen	4,493-40-7	-	-	-	-	-	-
Hesperiden	520-26-3	+	+	+	+	+	+
Isochlorogenic acid C	57,378-72-0	-	-	-	-	-	-
Cinnamaldehyde	14,371-10-9	-				-	-
Baicalin	21,967-41-9	+	+	+	+	+	+
Quercetin	117-39-5	-	-	-	-	-	-
Luteolin	491-70-3	-	-	-	-	-	-
Kaempferol	520-18-3	-	-	-	-	-	-
Irisflorentin	41,743-73-1	+	+	+	+	+	+
Gingerol	23,513-14-6	-	-	-	-	-	-
2,5,7-trimethoxyphenanthren-3-ol	51,415-00-0	-	-	-	-	-	-
Asarinin	133-04-0	-	-	-	-	-	-
Glycyrrhizic acid	1,405-86-3	+	-	-	-	-	-
(8)-Gingerol	23,513-08-8	-	-	-	-	-	-
Atractylenolide I	73,069-13-3	-	-	-	-	-	-
Saikosaponin A	20,736-09-8	-	-	-	-	-	-
Tussilagone	104012-37-5	-	-	-	-	-	-
(10)-Gingerol	23,513-15-7	-	-	-	-	-	-
Alisol B,23-acetate	19,865-76-0	+	-	-	-	-	-
Pachymic acid	29,070-92-6	-	-	-	-	-	-

and 10 constituents were identified in serum, heart, lung, spleen, liver, and kidney, respectively. The results showed that these nine constituents (ephedrine, pseudoephedrine, amygdalin, prunasin, liquiritin, hyperoside, hesperidin, baicalin, and risflorentin) could be quickly absorbed into the circulation system and then widely distributed in various tissues. At 0.5 h, except baicalin, the exposure of the other eight target components reached a peak in serum and tissues. The exposure of baicalin was peaked at 2 or 4 h. At 0.5 h, the exposure of target components to lung tissue was ranked as follows: ephedrine (2,759.11 ± 784.39 ng/g), prunasin (1819.7 ± 427.28 ng/g), pseudoephedrine (880.6 ± 287.97 ng/g), amygdalin (304.43 ± 234.7 ng/g), hesperidin (78.33 ± 38.38 ng/g), risflorentin (8.62 ± 4.66 ng/g), baicalin (8.53 ± 1.91 ng/g), hyperoside (7.72 ± 1.63 ng/g), liquiritin (7.68 ± 5.19 ng/g). At 2 h, ephedrine (776.61 ± 148.4 ng/g), prunasin (173.77 ± 58.21 ng/g), pseudoephedrine (84.68 ± 59.04 ng/g), baicalin (49.33 ± 17.06 ng/g), amygdalin (1.26 ± 0.26 ng/g) (Liu et al., 2020b). Furthermore, Wu et al. (2020a) indicated that treatment with QFPDD (1.5, 6 g/kg/day, p.o.) for continued 5 days, could significantly regulate the host metabolism and gut microbiota composition in rats such as enriched romboutsia, turicibacter, and *clostridium*_sensu_stricto_1, and decreased norank_f_Lachnospiraceae. The results from GC-MS and LC-MS/MS identified a total of 23 and 43 differential metabolites respectively that were altered by QFPDD. The metabolic pathways of these differential metabolites included glycerophospholipid metabolism, linoleic acid metabolism, TCA cycle, and pyruvate metabolism (Wu et al., 2020a).

1.4 Clinical Application and Practice of QFPDD

QFPDD is taken as water decoction, once a day, administrated in the morning and at night separately, 40 min after meals and total of three doses as a course of treatment (Jiang and Chen, 2020). If possible, half a bowl of rice water can be taken after taking the decoction every time, and those suffering from body fluid deficiency can take one bowl of rice water (Tian et al., 2020).

Diagnosis and Treatment Program of COVID-19 (Seventh edition) issued by National Health Commission of the PCR has clearly stated that TCM treatment requires syndrome differentiation and treatment based on the local climate characteristics and different physical constitution. QFPDD, as a general prescription, could not take into account individual differences and may bring some related adverse reactions. Common adverse reactions of QFPDD include nausea and vomiting, dizziness, dermatitis, etc. Wang et al. (2020b) collected information about the entire diagnosis and treatment of 98 confirmed cases of COVID-9 treated with QFPDD in Sichuan province, and found that during the course of QFPDD treatment, four patients had nausea and vomiting, two patients had dizziness, one patient had a rash, and the incidence of adverse reactions was 7.14% (Wang et al., 2020b). In addition, Hu et al. revealed the observation on clinical effect of Qingfei Paidu granules in the treatment of 76 confirmed cases of COVID-9 in Hubei province, and found that during the course of Qingfei Paidu granules treatment, two patients had mild diarrhea, one patient had nausea and vomiting, one patient suffered from pruritus, and the incidence of adverse reactions was 5.26%, but above adverse reaction symptoms were mild and disappeared without special treatment (Hu et al., 2020). As shown in **Table 6**, some clinical observation of Qingfei Paidu prescription with different dosage forms in the treatment of COVID-19 indicated that QFPDD could effectively improve the symptoms and the effective rate is above 80%. The specific clinical indicators of TCM syndromes and main laboratory indices and safety observation which reflect the efficacy of QFPDD are shown in **Table 7** and **Table 8**, respectively. For each course of treatment, clinicians should objectively evaluate the efficacy and actual adverse reactions of QFPDD to adjust the prescription appropriately.

If the patient does not have a fever, the dosage of raw gypsum should be reduced, otherwise, the dosage of raw gypsum should be increased. If the symptoms are improved but not cured, the second course should be added. If the patient has other basic diseases, the second course of the prescription shall be modified according to the actual situation. (You et al., 2020). If the symptoms disappear, the patients can stop taking the medicine in the second course of treatment. For patients with obvious deficiency of spleen *Yang*, 15 g of raw gypsum can be used in the prescription; for patients with the deficiency of stomach *Yin*, the method of nourishing *Yin* and eliminating dampness can be followed for empirical treatment and for those with excessive sweating, high blood pressure, palpitation, and insomnia, the dosage of the prescription can be appropriately reduced, or the dosage of yam can be increased. In the case of hepatic insufficiency, clinicians should analyze the causes of hepatic insufficiency, stop taking drugs if necessary, or add liver protection therapy (Dong et al., 2020; Lai et al., 2020). As for the dosage of *Herba Asari*, QFPDD is used up to 6 g, although it does not follow "the dosage of *Herba Asari* is not more than 5 g", it is still in the range of commonly used clinical dosage and its fluctuation, which is more suitable for the patients with cold-dampness-yang injury and severe deficient cold. For those with severe heat and dampness, the dosage of *Herba Asari* should be

TABLE 6 | Observation on clinical effect of Qingfei Paidu prescription with different dosage forms in the treatment of COVID-19.

No	The number of cases	Pharmaceutical dosage form	Course of treatment	Cure rate (%)	Total effective rate (%)	Province	References
1	76 cases	Granules	5 days as a course of treatment, three courses of treatment	65.79%	88.16%	Hubei province	Hu et al. (2020)
2	98 cases	Decoction	3 days as a course of treatment, three courses of treatment	41.13%	92.09%	Sichuan province	Wang et al., 2020b
3	30 cases	Decoction	3 days as a course of treatment, three courses of treatment	NA	83.335	Hubei province	Li et al., 2020b
4	151 cases	Mixture	3 days as a course of treatment, three courses of treatment	43.70%	90.07%	Sichuan province	Lai et al. (2020)
5	108 cases	Decoction	3 days as a course of treatment, three courses of treatment	NA	91.67%	Hubei province	Meng et al. (2020)
6	214 cases	Decoction	3 days as a course of treatment, three courses of treatment	NA	90%	Shanxi, Hebei, Shaanxi, Heilongjiang province	General Office of National Health Commission, State Administration of Traditional Chinese Medicine (2020)

TABLE 7 | Clinical symptom rating scale of TCM syndromes of COVID-19.

Primary symptoms	Normal (0 point)	Slight (2 points)	Medium (4 points)	Severe (6 points)
Fever	≤37.2°C	37.2°C–38.2°C	38.3°C–39.0°C	>39.0°C
Cough	None	Occasionally, with a single cough	Often, but does not affect work and rest	Cough frequently with more than one cough, cause vomiting, affects work and rest
Asthma	The respiration is stable and the frequency is within the normal range of the corresponding age	Exceeding the upper limit of the normal value of the corresponding age (≤10 times/min), there is no flaring of nares and three concave sign	Exceeding the upper limit of the normal value of the corresponding age (11–20times/min), and/or intermittent wheezing, flapping of nasal wings, three concave sign	Exceeding the upper limit of the normal value of the corresponding age (≥21times/min), and/or continuous wheezing, flaring of nares, three concave sign
Expectoration	None	There is an occasional sound of phlegm in the throat and a small amount of sputum	The phlegm sound in the throat is hissing and the phlegm is yellow	There is a roar of phlegm sound in the throat and a large amount of yellow-phlegm
Nasal obstruction	None	Occasionally. It doesn't affect breathing through the nose	Patients often have the nasal obstruction during the day	Obvious nasal obstruction patients have to breathe through the mouths
Nasal discharge	None	Occasionally	Patients have runny nose in the morning and at night	Continuously
Dry mouth	None	Occasionally	Sometimes	Continuously
Pharyngalgia	None	Slightly	Dry pain, pain when swallowing	Burning pain, sharp pain when swallowing
Hypodynamia	Normal	Slightly	Obvious	General weakness
Anorexia	Normal	Poor appetite	Loss of appetite	The appetite is extremely poor, or the patients refuse to eat
Diarrhea	None	Less than 3 times a day Loose stool	Three to six times a day Loose stool	More than 7 times a day The stool is watery
Secondary symptoms	**Normal (0 point)**	**Slight (1 point)**	**Moderate(2 points)**	**Severe (4 points)**
Complexion	Normal	Flushing of face and lusterless complexion	Flushing of face and dim complexion	Pallor and dim complexion
Palpitation	None	Mildly	Sometimes	Continuously
Abdominal distension	None	Occasional abdominal distension or postprandial abdominal distension	Abdominal distension is severe, up to 6 hours a day	Abdominal distension all day long
Aversion to cold	None	Slightly	Moderately	Shivering
Cyanosis	None	Slight cyanosis [$P(O_2)$50 mmHg–80 mmHg,SaO_2 80%–90%]	Moderate cyanosis [$P(O_2)$30 mmHg–50 mmHg,SaO_2 60%–80%]	Severe cyanosis [$P(O_2)$<30 mmHg,SaO_2 <60%]
Hyperhidrosis	None	Usually the skin is slightly moist or occasionally hot and sweating	Usually the skin is moist, sweating if you move a little; hectic fever on the chest and back, sweating repeatedly	Sweat usually and sweat like washing with moving
Short breath	None	Slightly	Shortness of breath increases after exercise	Obviously affecting work and daily life
Insomnia	Normal	Difficulty falling asleep	Difficulty falling asleep, sleep lightly	Hard to sleep
Urination	Normal	Slightly yellow	Dark yellow	Dark urine
Tongue manifestation	**Normal (0 point)**		**Abnormal (2 points)**	
Tongue property	Light red tongue		Red or dark-red tongue, or with ecchymosis, or prickly tongue	
Coated tongue	The tongue coating is thin and white		Tongue coating is yellow, thick, greasy, etc.	
Pulse	**Normal (0 point)**		**Abnormal (2 points)**	
Pulse	Normal pulse		Irregular-rapid pulse, irregularly intermittent pulse, regularly intermittent pulse, etc.	

TABLE 8 | The main laboratory indices and safety observation in the treatment of COVID-19.

Detection of laboratory indices	References	Detection of laboratory indices	References	Safety observation	References
White blood cell count (WBC)	Hu et al. (2020), Wang et al., 2020b, Meng et al. (2020)	Lactate dehydrogenase (LDH)	Wang et al., 2020b	Throat swab nuclei cacid detection	Hu et al. (2020), Wang et al., 2020b
Lymphocyte percentage (LYMPH%)	Hu et al. (2020), Wang et al., (2020b), Meng et al. (2020)	Creatine kinase isozyme (CK-MB)	Wang et al., 2020b	Chest computed tomography	Hu et al. (2020), Wang et al., 2020b
Neutrophil percentage (NEUT%)	Hu et al. (2020), Wang et al., (2020b), Meng et al. (2020)	Creatine kinase (CK)	Wang et al., 2020b	Blood biochemistry	Hu et al. (2020), Wang et al., 2020b
Aspartate aminotransferase (AST)	Wang et al., 2020b, Meng et al. (2020)	C-reactive protein (CRP)	Hu et al. (2020), Wang et al., 2020b, Meng et al. (2020)	Electrocardiogram	Hu et al. (2020), Wang et al., 2020b
Erythrocyte sedimentation rate (ESR)	Hu et al. (2020), Wang et al., 2020b	Procalcitonin (PCT)	Hu et al. (2020), Wang et al., 2020b	Observation of adverse reactions	Hu et al. (2020), Wang et al., 2020b
Albumin (ALB)	Hu et al. (2020), Meng et al. (2020)	D-dimer (D-dimer)	Hu et al. (2020), Wang et al., 2020b		
Urea (UREA)	Hu et al. (2020), Wang et al., 2020b, Meng et al. (2020)	Alanine aminotransferase (ALT)	Hu et al. (2020), Wang et al., 2020b, Meng et al. (2020)		
Creatinine (CREA)	Hu et al. (2020), Wang et al., 2020b, Meng et al. (2020)				

reduced as appropriate (Liu et al., 2020a). Some scholars also have recommended the modified QFPDD combined with western medicine such as alpha-interferon, oseltamivir, chloroquine phosphate, arbidol, ribavirin in the treatment of COVID-19, and found that it was more effective than the treatment of western medicine alone, which could significantly shorten the patient's hospitalization time, the time of clinical symptom improvement and the time of lung CT improvement (Fang et al., 2020; Li et al., 2020b; Yang et al., 2020b).

2 CONCLUSION AND PERSPECTIVES

COVID-19 is a new type of infectious disease. Western medicine mainly focuses on symptomatic relief. TCM has been applied for treating epidemics for thousands of years, and many clinicians have conducted in-depth research on COVID-19 etiology, pathogenesis, and syndrome differentiation. Since TCM played a huge role in the treatment of SARS in China in 2003, the National Health Commission and the National Administration of TCM jointly issued the "New Coronavirus Infection Pneumonia Diagnosis and Treatment Program (Fourth, Fifth, Sixth, Seventh and Trial Eighth edition)", which advocated the integration of Chinese and Western medicine, strived to shorten the course of the disease, improve clinical efficacy and reduce the incidence and mortality of critically ill patients (Lu and Lu, 2020; Xie, 2020).

In the process of the treatment of COVID-19, under the guidance of TCM theory, based on clinical practice and patient-oriented principle combined with data mining and basic research of modern biology and pharmacology, China established treatment methods for different stages and syndromes in different regions by systematically sorting out several classic and effective prescriptions and quickly put them into the clinical application (Zhang et al., 2020). Given the current epidemic situation of COVID-19, early intervention of TCM has played an important role in this epidemic control. Chinese and western advantages complement each other, which has a definite curative effect in reducing fever and other symptoms, controlling disease progression and reducing complications. QFPDD was selected and recommended by the National Administration of TCM as a general prescription for treating different stages of COVID-19. QFPDD is combined with multiple prescriptions and has the properties and flavors of pungent-warm and pungent-cool, aiming at the pathogenesis of COVID-19, including cold, dampness, heat, toxin, and deficiency (Chen et al., 2020b). QFPDD has the functions of dispelling cold and dampness, eliminating heat and turbidity, promoting and nourishing lung and spleen, detoxifying and removing pathogenic factors, etc. Modern pharmacologic studies have also confirmed the anti-inflammatory, antiviral and immunological functions of QFPDD which is attributed to the multi-component, multi-target, and multi-pathway characteristics of TCM. QFPDD is also a widely accepted prescription for treating COVID-19 based on its successful and effective clinical observations. The successful use of QFPDD in this novel viral pneumonia epidemic has confirmed the advantages of TCM in treating emergencies. However, at present, the mechanism of QFPDD is still unclear. It is necessary to further

comprehensively evaluate the efficacy and safety of QFPDD and clearly explain the complex mechanisms of QFPDD in the treatment of COVID-19 through systematic reviews and meta-analysis (Gao et al., 2020). Currently, there is a lack of extended research with sufficient breadth and depth and the current research has just focused on QFPDD TCM theory, clinical experience, network pharmacology, etc., with only a small number of clinical research samples. In the follow-up research, it is not only essential to carry out more comprehensive chemical composition characterization, pharmacokinetic and pharmacodynamic studies *in vitro* and *in vivo*, but also extended clinical data should be evaluated to elucidate the material basis and systematically explain the effectiveness of QFPDD against COVID-19, and further provide a theoretical basis for the clinical scientific and rational application of QFPDD in the prevention and clinical treatment of COVID-19.

AUTHOR CONTRIBUTIONS

RW, YM, RQW, LP, GL, and SJY conceived and designed the review; QS, QGP, LD and MM reviewed the literature; RW and YM wrote the manuscript.

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SUPPLEMENTARY MATERIAL

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frontiers in Pharmacology

SYSTEMATIC REVIEW
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Molecular Mechanism of Action of Repurposed Drugs and Traditional Chinese Medicine Used for the Treatment of Patients Infected With COVID-19: A Systematic Scoping Review

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Background: The emergence of COVID-19 as a pandemic has resulted in the need for urgent development of vaccines and drugs and the conduction of clinical trials to fight the outbreak. Because of the time constraints associated with the development of vaccines and effective drugs, drug repurposing and other alternative treatment methods have been used to treat patients that have been infected by the SARS-CoV-2 virus and have acquired COVID-19.

Objective: The objective of this systematic scoping review is to provide an overview of the molecular mechanism of action of repurposed drugs or alternative treatment medicines used to attenuate COVID-19 disease.

Method: The research articles or gray literature, including theses, government reports, and official news online, were identified from four databases and one search engine. The full content of a total of 160 articles that fulfilled our inclusion criteria was analyzed and information about six drugs (ritonavir, lopinavir, oseltamivir, remdesivir, favipiravir, and chloroquine) and four Traditional Chinese Medicines (*Shuang Huang Lian Kou Fu Ye*, TCM combination of *Bu Huan Jin Zheng Qi San* and *Da Yuan Yin, Xue Bi Jing Injection*, and *Qing Fei Pai Du Tang*) was extracted.

Results: All of the repurposed drugs and complementary medicine that have been used for the treatment of COVID-19 depend on the ability of the drug to inhibit the proliferation of the SARS-CoV-2 virus by binding to enzyme active sites, viral chain termination, or triggering of the molecular pathway, whereas Traditional Chinese Medicine plays a pivotal role in triggering the inflammation pathway, such as the neuraminidase blocker, to fight the SARS-CoV-2 virus.

Keywords: COVID-19, alternative medicine, repurposed drugs, SARS-CoV-2, molecular mechanism

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INTRODUCTION

In December 2019, a novel type of viral pneumonia was discovered in Wuhan, Hubei Province, China. The disease has been officially named "COVID-19 (CoronaVirus Disease 2019)" and the virus has been named SARS-CoV-2 by The International Committee of Taxonomy of Viruses (Gorbalenya et al., 2020). The new coronavirus has rapidly spread among humans all over the world and has led to more than 10 million cases within 6 months. On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a pandemic, which is defined as "worldwide spread of a new disease." As of July 8, 2020, the number of confirmed cases of COVID-19 globally was 11,669,259, and the total number of deaths has reached 539,906 (4.6%) people (World Health Organization, 2020).

The causation of COVID-19, SARS-CoV-2, is from the family of CoVs, single-stranded RNA viruses that look like a crown under a microscope (coronam is the Latin word for crown) and contain spike glycoproteins on the envelope. It is categorized as a betaCoV, which has an elliptic or round and often pleomorphic form, with a diameter of 60–140 nm. Acute respiratory illness appears to be the most common manifestation of COVID-19 infection. The stages of symptomatic infection range from mild to severe; most infections are not severe (Zhou et al., 2020; Huang et al., 2020; Wang D. et al., 2020):

Mild Clinical Symptoms

Mild symptoms are present at the time of inoculation and the incubation period, such as malaise, a dry cough, and fever. During this time, the SARS-CoV-2 virus multiplies and primarily establishes residence in the respiratory system. The virus binds to its target through the angiotensin-converting enzyme 2 (ACE2) receptor in human cells (Wan et al., 2020).

Moderate Clinical Symptoms

Localized inflammation in the lungs and viral multiplication occur in the second stage of the disease. Patients develop a viral pneumonia, with symptoms including fever, cough, and hypoxia. A chest roentgenogram or computerized tomography usually shows bilateral infiltrates or ground glass opacities. Increased lymphopenia and neutrophil-lymphocyte ratio (NLR) are evident in blood tests (Siddiqi and Mehra, 2020).

Severe Clinical Symptoms

Approximately one out of every six patients transition into the third stage of the illness, which is the most severe and is manifested as an extrapulmonary systemic hyperinflammation syndrome. Systemic inflammation is present during this stage, as well as a decrease in suppressor, helper, and regulatory T cell counts (Qin et al., 2020). Shock, respiratory failure, vasoplegia, and cardiopulmonary collapse are discernible as well as systemic organ involvement and myocarditis.

The latest trend shows that human-to-human spread is the main mode of transmission, which occurs through respiratory droplets resulting from sneezing and coughing. Erosol transmission could also occur in closed areas. Infection might also happen if someone touches a contaminated surface and then touches their own eyes, nose, or mouth (Cascella et al., 2020; van Doremalen et al., 2020). The most frequent source of spread of COVID-19 is people with symptoms; however, the possibility of transmission before symptoms develop, or even from individuals who remain asymptomatic, cannot be excluded. Moreover, the period during which an individual with COVID-19 is infectious is uncertain. The duration of viral shedding is also variable (Cascella et al., 2020; Rothe et al., 2020; Yu et al., 2020). Data and modeling indicate that the use of social distancing is the best way to control this pandemic. Several countries have taken measures such as mobility restrictions, drastic social distancing, school closures, and travel bans, which could significantly disrupt economic and social stability.

At this moment, the therapeutic approaches to handle COVID-19 are only supportive. There is neither a vaccine to prevent infections nor clinically approved antiviral drugs to treat COVID-19. Therefore, the identification of drug treatment options is critical for responding to the pandemic. Clinical trials for vaccines are currently underway in many countries. However, the efficacy of the vaccines, how long immunity will last, or if infection can occur even if a person possesses a high level of antibodies will not be clear for at least 1 year after injection (Callaway, 2020). Furthermore, the safety of the developed vaccines is unknown because laboratory tests are being conducted in parallel with clinical trial phase 1 owing to the emergence of COVID-19 as a pandemic. The unknown efficacy and safety of the vaccines used might cause disease enhancement, by which vaccinated subjects might develop an even more severe form of disease than the subjects that have not been vaccinated, which has been shown in studies of SARS vaccines, in which vaccinated ferrets developed damaging inflammation in their livers after they were infected with the virus (Weingartl et al., 2004).

According to a previous study, disruption of the liver has been reported in patients diagnosed with SARS (Chau et al., 2004) and MERS (Alsaad et al., 2018). This might occur in patients diagnosed with COVID-19 owing to the genome sequence similarity (Zhou et al., 2020) and it has been proven that approximately 2–11% patients had liver comorbidities and 14–53% possess abnormal alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level which occurred more in severe cases of COVID-19 (Zhang et al., 2020). This symptom might be caused by the binding of SARS-CoV-2 to angiotensin-converting enzyme 2 (ACE2) receptor (Wan et al., 2020) to dysregulate liver function and drug hepatotoxicity according to large difference observed from various cohort studies (Zhang et al., 2020). Until now, no drugs have been successfully developed for the control of COVID-19 (Xu et al., 2020); however, numerous efforts are underway worldwide (Lu, 2020).

Drug repurposing has become a model for the rapid development of drugs against infectious diseases, especially in the current global emergency, as it allows the saving of time as well as being a more cost-effective form of drug development. Drug repurposing involves the uncovering of existing therapeutic agents for the treatment of new illnesses or the identification of new therapeutic targets for existing drugs (Pantziarka and André, 2019), including approved, discontinued, shelved, or

experimental drugs (Talevi and Bellera, 2020). This strategy could shorten the timeline in drug development because the existing drugs have undergone a scrutinous and extensive process to prove their efficacy and safety to use on humans before marketing surveillance. This strategy has been particularly utilized in oncology and has shown some successes. A notable candidate is aspirin, which is best known for its therapeutic effect in cardiovascular disease but has been shown to have antitumor properties through the suppression of tumors through the inhibition of COX-1 by preventing binding of platelets on tumor cells (Ishida et al., 2016). Prior successes indicate that drug repurposing has a high potential to be the solution for current pandemic while waiting for long-lasting efficacy of vaccination. Therefore, drug repurposing as well as existing alternative medicine could be effective methods for the treatment of patients with COVID-19.

Several previous studies have reviewed drug repurposing for the treatment of COVID-19. However, most reviews have only focused on the mechanism of action of a single drug. In this review, we included 10 medications, comprising six drugs and four complementary medicines. These medications were selected based on their initial successful treatments of COVID-19 patients at the beginning of the COVID-19 outbreak, before it was declared as a pandemic. A few months after the pandemic was declared, the six drugs in this study were officially announced by WHO to be included in a multicountry trial known as SOLIDARITY; the four complementary medicines were included in the national guideline for management of COVID-19 by China, the first country infected by COVID-19. In this comprehensive systematic review of these 10 medications, we discuss the mode of action from a molecular mechanism perspective to attenuate COVID-19 in the human system. By understanding different molecular mechanisms of 10 important drugs instead of a single drug molecular mechanism, researchers could gain a deeper insight of the pivotal genes or mechanisms that should be targeted for future study to ameliorate this pandemic condition, which can also lead to the development of new and effective drugs for the treatment of COVID-19.

MATERIALS AND METHODS

A systematic scoping review was conducted from January 1, 2020, until March 18, 2020, by including papers that were published and not published before March 18, 2020, on reported drug repurposing and Traditional Chinese Medicine (TCM) as treatment options for COVID-19 used in patients from different countries to reduce publication bias, increase the comprehensiveness and timeliness of the review, and foster a balanced picture of available evidence (Paez, 2017). The review was performed according to criteria using Preferred Reporting Items for Systematic Reviews (PRISMA) statement (Moher et al., 2009). The list of publications was obtained from the listed databases and search engines: PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), Google Scholar (https://scholar.google.com/), Science Direct (https://www.sciencedirect.com/), and Semantic Scholar (https://www.semanticscholar.org/). Gray literature search such as guidelines, conference papers, theses and dissertations, government reports, research and committee reports, and abstracts were obtained from WHO, Chinese Clinical Trial Registry (http://www.chictr.org.cn/abouten.aspx), U.S National Library of Medicine, China Center for Disease Control and Prevention, China Dissertation Database, China Important Conference Papers Database, and online official news websites. Most of the gray literatures were from China because China is the first country to encounter this disease and provides more data to tackle this disease. Different combinations of the following keywords: NCov-2019, COVID-19, 2019-nCoV, SARS-CoV-2, 2019 novel coronavirus, Wuhan virus, drug repurposing, medication, treatment, traditional Chinese medicine, and alternative and complementary medicine, were used in the literature search through a three-level search strategy based on standardized descriptors defined by the Medical Subject Headings algorithm. A secondary search was based on screening of the reference list of all the relevant studies identified in the direct search. The entire potentially relevant studies were evaluated after title screening to exclude irrelevant information. Only studies that reported the repurposing of drugs and TCMs for COVID-19 and contained information about the structure of the chemical constituents, *in vivo* or *in vitro* studies, case reports, treatment of patients diagnosed with COVID-19, and molecular mechanisms, were extracted and assessed.

RESULTS

The primary search identified 8,074 published and unpublished papers, of which 2,132 were from PubMed, 2,775 from ScienceDirect, 720 from Google Scholar, 1,190 from Semantic Scholar, and 1,257 from Google search engine. A total of 3,798 duplicates were excluded, leaving 4,276 articles for screening by title analysis. 841 eligible published and unpublished papers were identified after excluding 3,435 papers that had a different theme from this systematic scoping review. In conclusion, the content of 841 papers was fully analyzed, of which 681 were excluded according to our exclusion criteria, which was medication that was not administrated as treatment for COVID-19 before March 18, 2020. To establish any differences within a drug class, the drug classes were divided into different subclasses and individual drugs. Repurposed drugs used for treatment of COVID-19 consisted of six drugs (ritonavir, lopinavir, oseltamivir, remdesivir, favipiravir, and chloroquine) and four TCMs (*Shuang Huang Lian Kou Fu Ye*, TCM combination of *Bu Huan Jin Zheng Qi San* and *Da Yuan Yin*, *Xue Bi Jing Injection*, and *Qing Fei Pai Du Tang*). The 133 articles in this review consist of five guidelines, 16 clinical trial registries, 11 from official news, 42 *in vitro* and *in vivo* studies, 40 reports presenting *in vitro* and *in vivo* outcomes, and 19 clinical findings and treatment efficacy. A flowchart of the progressive study selection and numbers at each stage is shown in **Figure 1**.

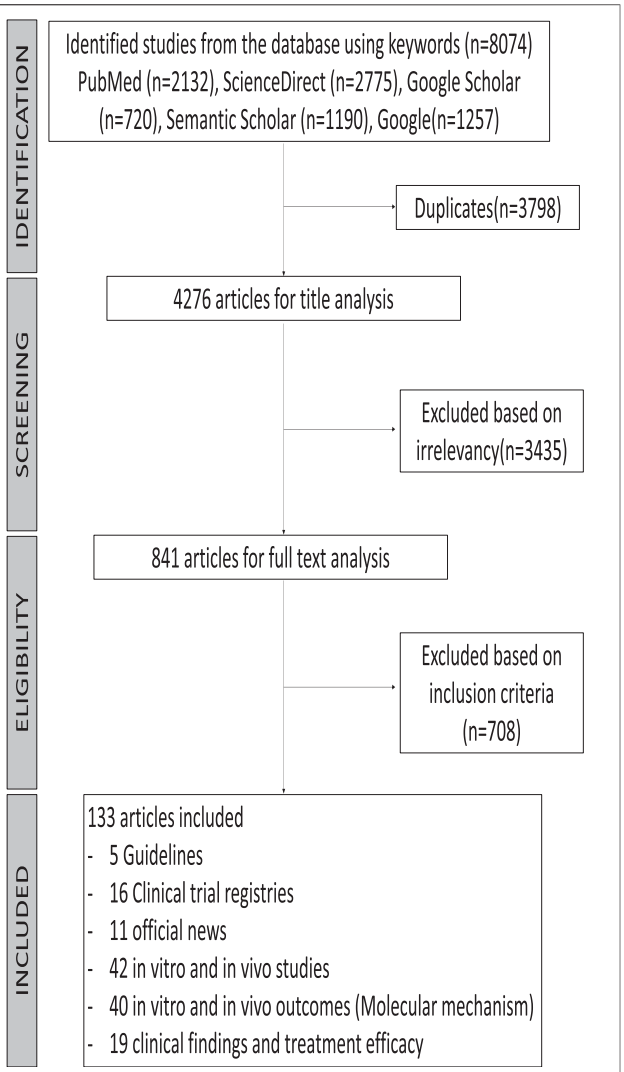

FIGURE 1 | Flow diagram of the systematic search according to the guidelines for Preferred Reporting Items for Systematic Reviews (PRISMA).

DISCUSSION

In this systematic scoping review, we provide an overview of previous studies in which existing drugs or Traditional Chinese Medicines (TCMs) have been repurposed for the treatment of COVID-19 and discuss the initial mechanism of action of these drugs from a molecular perspective for the treatment of coronaviruses or other viruses. A summary of the mode of action of all the drugs in this study is shown in **Table 1** and **Figure 2**. WHO reported the launch of a multiarm and multicountry clinical trial of four drugs, remdesivir, lopinavir, and ritonavir (Kaletra), interferon beta, and chloroquine, on March 18, 2020 (Branswell, 2020). Even though our systematic scoping review literature search method covered repurposed drugs used until March 18, 2020, we have covered the majority of repurposed drugs that were used in the SOLIDARITY clinical trial launched by WHO. We did not focus on other drugs that have been recently used for clinical trials because they were not used before the analysis performed for this scoping review. Furthermore, the recent clinical trials have not been multicountry trials; therefore situations such as differences in drug response of the population and adverse drug reactions might exist (Bachtiar and Lee, 2013) and the result cannot represent the efficacy of the drugs for the whole population.

Repurposed Drugs as Drug Candidates in the Solidarity Trial

HIV Protease Inhibitors and Anti-Influenza Drugs Used for COVID-19 Treatment

Initially, lopinavir and ritonavir were developed as a standalone antiviral agent for the treatment of HIV infections; however they are combined to obtain a more efficient drug response and sold under the brand name Kaletra (Wishart et al., 2018). Both of the drugs were used for the treatment of HIV-1 and HIV-2 infections through reversible inhibition of the HIV proteases by blocking access to the proteases' active site, thus preventing the processing of the HIV Gag and Gag-Pol polyproteins (Kempf, 2007). This results in the formation of immature HIV particles that are not infectious. However, the extremely high mutation rate of HIV-1 *in vivo* (Cuevas et al., 2015) has given rise to a strain of HIV-1 that is resistant to ritonavir. The rise of ritonavir-resistant HIV strains has led to the development of more effective drugs for combating HIV infections, one of which is lopinavir. Compared with ritonavir, lopinavir was more effective *in vitro* at a lower amount (17 nM) (Lv et al., 2015); however it undergoes oxidative metabolism by the cytochrome P450 3A4 enzyme in human liver microsomes (van Waterschoot et al., 2010), thus reducing the bioavailability, which is why it was combined with ritonavir for better efficacy.

These drugs were the first repurposed drugs that were used to treat patients diagnosed with COVID-19 (Wipatayotin, 2020). The administration of ritonavir and lopinavir for the treatment of COVID-19 might be because the similarity of the genome sequence to SARS-CoV is approximately 79.6% and it originated from the same genus as SARS-CoV-2, SARS-CoV, and MERS-CoV (*Betacoronavirus*) (Zhou et al., 2020). Therefore, therapies and drugs that have been developed for the treatment of SARS-CoV and MERS-CoV could also be used for the development of COVID-19 drugs, assuming that the mechanism of SARS-CoV-2 is similar to its family members (Yao et al., 2020). Lopinavir/ritonavir have been administered to patients with moderate stage COVID-19 symptoms, which is the second stage of established pulmonary disease and viral multiplication (Siddiqi and Mehra, 2020). Successful treatment of COVID-19 patients with lopinavir and ritonavir has also been reported in India (a 69-year-old male and a 70-year-old female) (Harrison, 2020) and Spain (62-year-old male) (Boyd, 2020).

A molecular dynamics simulation suggested that lopinavir and ritonavir can inhibit the SARS-CoV $3CL^{pro}$ enzyme by binding to the enzyme's active site, with neither of them having a higher binding affinity than the other (Nukoolkarn et al., 2008). A recent

Table 1 | Treatment of patients during the start of the COVID-19 pandemic.

Type of Drugs	Therapeutic agent	Compounds or components	Mode of action	Reference
Repurposed drugs	Lopinavir Ritonavir	HIV protease inhibitor	Inhibit the SARS-CoV $3CL^{pro}$ enzyme	Nukoolkarn et al. (2008)
	Oseltamivir	Neuraminidase inhibitor	Inhibits the viral neuraminidase in influenza, however the mechanism in SARS-CoV-2 is still in progress by AbbVie Inc	Harrison, (2020), Mulangu et al. (2019)
	Remdesivir Favipiravir	Nucleotide analogues	Inhibits viral RNA synthesis through chain termination	Sangawa et al. (2013), Abdelnabi et al. (2017)
	Chloroquine	9-Aminoquinoline	Inhibitory effects on SARS-CoV-2 at the cellular level	Wang M. et al. (2020)
Alternative/ complementary medicine	Shuang Huang Lian Kou Fu Ye	*Lonicera japonica Thunb.* (chlorogenic acid), *Scutellaria baicalensis Georgi*, (baicalin) and *Forsythia suspensa (Thunb.) Vahl* (forsythoside A)	Inhibit angiotensin-converting enzyme (ACE) by baicalin	Yang et al. (2020)
	Bu Huan Jin Zheng Qi San and Da Yuan Yin	*Atractylodes lancea (Thunb.) DC.*, *Citrus × aurantium L.*, *Magnolia officinalis* Rehder and E. H. Wilson, *Agastache rugosa* (Fisch. and C. A. Mey.) *Kuntze*, *Lanxangia tsao-ko* (Crevost and Lemarié) M. F. Newman and Skornick., *Ephedra sinica S.*, *Hansenia weberbaueriana* (Fedde ex H. Wolff) Pimenov and Kljuykov, *Zingiber officinale Roscoe*, and *Areca catechu L.*	Altering TLR7 signaling pathway through regulation of TLR7, MyD88, TNFR6, and IFN-β mRNA in influenza A, yet mechanism of SARS-CoV-2 still needs further investigation	Cheng et al. (2016)
	Xue Bi Jing Injection	*Conioselinum anthriscoides "Chuanxiong," Salvia miltiorrhiza Bunge*, *Paeonia lactiflora Pall*, *Carthamus tinctorius L.*, and *Angelica sinensis (Oliv.) Diels*	Prevent development of a systemic inflammatory response syndrome	Qi et al. (2011)
	Qing Fei Pai Du Tang	*Ephedra sinica S.*, *Glycyrrhiza uralensis Fisch. ex DC.*, *Prunus armeniaca L.*, Gypsum, *Cinnamomum cassia (L.) J. Presl*, *Alisma plantago-aquatica L.*, *Polyporus umbellatus (Pers.) Fries*, *Atractylodes macrocephala Koidz.*, *Poria cocos (Schw.) Wolf*, *Bupleurum chinense DC.*, *Scutellaria baicalensis Georgi*, *Pinellia ternata (Thunb.) Makino*, *Zingiber officinale Roscoe, Aster tataricus L. f.*, *Tussilago farfara L.*, *Iris domestica (L.)* Goldblatt and Mabb., *Asarum sieboldii M.*, *Dioscorea oppositifolia L.*, *Citrus trifoliata L.*, *Citrus × aurantium L.*, and *Agastache rugosa* (Fisch. and C. A. Mey.) *Kuntze*.	Reportedly have anti-SARS-CoV activity Inhibit angiotensin-converting enzyme (ACE) by baicalin	Yang et al. (2020), Chen et al. (2017)

COVID-19 study revealed that SARS-CoV-2 utilizes the same cell entry method used by SAR-CoV, namely, relying on the ACE2 receptor and priming of the spike protein by TMPRSS2 (Zhang and Yap, 2004). The authors also suggested that antibodies produced against SARS-CoV could potentially be used to combat SARS-CoV-2, albeit at a lower efficiency. Antibodies recovered from recovered COVID-19 patients could be used to combat COVID-19, although only as a preventive measure or in the early stages of the infection (Hoffmann et al., 2020). However, this method was successfully used to treat seriously ill patients in China, with patients showing improvement within 24 h (Bloomberg, 2020).

Even though lopinavir and ritonavir showed their ability to inhibit SAR-CoV during cell entry, a binding analysis showed that half of the lopinavir remained outside of the catalytic site and one of the side benzene side chains of ritonavir might be too long to perfectly fit the substrate binding pocket. This would lead to ritonavir and lopinavir having poor efficacy, and this is reflected in weak *in vitro* activity against SARS-CoV (Zhang and Yap, 2004). In addition, a study conducted on lopinavir's and ritonavir's effectiveness against MERS-CoV also revealed that their effectiveness is lower than interferon beta and remdesivir although lopinavir and ritonavir have antiviral activity against MERS-CoV (Sheahan et al., 2020). Another study suggests that both lopinavir and ritonavir have no effect on SARS-CoV replication, with nelfinavir being the only inhibitor that has an effect on SARS-CoV replication (Yamamoto et al., 2004). Despite not manifesting any effect on SARS-CoV replication owing to its low bioavailability, lopinavir possesses antiviral activity against SARS-CoV, with one study suggesting lopinavir has a synergistic effect when used with ribavirin (Chu et al., 2004).

Influenza Drug Administered in Combination With an HIV Protease Inhibitor Provides Better Outcome

Oseltamivir is another synthetic prodrug used for the treatment of COVID-19. This drug is initially capable of inhibiting the

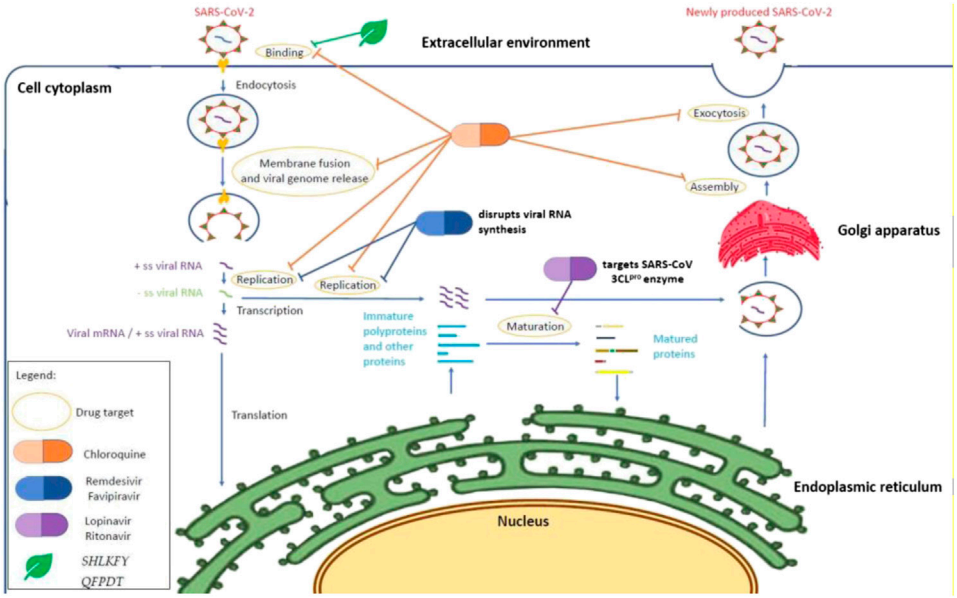

FIGURE 2 | Mechanism of action of repurposing drugs against SARS-CoV-2 within a host cell. Chloroquine inhibits SARS-CoV-2 at the cellular level (Wang M. et al., 2020) when the pH environment is disrupted. HIV protease inhibitors such as lopinavir and ritonavir may demonstrate an antiviral effect through binding to the SARS-CoV $3CL^{pro}$ enzyme (Nukoolkarn et al., 2008), whereas nucleotides analogues (remdesivir and favipiravir) disrupt the viral RNA synthesis through chain termination (Sangawa et al., 2013; Abdelnabi et al., 2017). On the other hand, *Shuang Huang Lian Kou Fu Ye* and *Qing Fei Pai Du Tang* are suspected to inhibit the binding on angiotensin-converting enzyme (ACE2) owing to the presence of baicalin from *Scutellaria baicalensis* (Yang et al., 2020). The action of oseltamivir and the other two alternative and complementary medicines (Combination of *Bu Huan Jin Zheng Qi San* and *Da Yuan Yin* and *Xue Bi Jing Injection*) remained unknown and are suspected to inhibit the viral neuraminidase according to their previous antiviral effect in influenza virus particles (Mulangu et al., 2019; Harrison, 2020) and altering of the TLR7 signaling pathway (Cheng et al., 2016).

neuraminidase enzymes on the surface of influenza virus particles. Oseltamivir is administered orally in its prodrug form, oseltamivir phosphate, for the treatment and prophylaxis of influenza A and influenza B infections (Jones et al., 2014). Oseltamivir phosphate is readily absorbed in the gastrointestinal tract, after which it is converted by hepatic esterases into its active form, oseltamivir carboxylase, a competitive inhibitor to neuraminidase found in influenza A and influenza B (Bachtiar and Lee, 2013). It reversibly binds to the active site of the neuraminidase, preventing the neuraminidase from cleaving the sialic acid residues (McNicholl and McNicholl, 2001) found on the surface of the host cell. This prevents the entry of the virus into uninfected cells and the release of newly formed virions from the infected cells and reduces both viral shedding and infectivity of the virus (Bachtiar and Lee, 2013).

On February 18, 2020, a 74-year-old Chinese woman with COVID-19 in Thailand was treated at Rajvitjhi Hospital for COVID-19-related pneumonia with a cocktail of HIV and flu drugs (Yao et al., 2020). The patient was first given ritonavir and lopinavir for 5 days. After failing to show signs of recovery, oseltamivir was administered to relieve the cough and fever symptoms and reduce the severity of these symptoms in the second stage of COVID-19 infection (Siddiqi and Mehra, 2020). This led to a marked improvement in her pneumonia condition in 8–12 h, with the patient testing negative for COVID-19 after 48 h (Mulangu et al., 2019). The drug cocktail was administered for the next 10 days, and subsequent tests for COVID-19 over the next 20 days gave negative results. However, the synergistic effect of the combination of these drugs is unclear because oseltamivir does not inhibit SARS-CoV (Tan et al., 2004) and MERS-CoV like lopinavir and ritonavir (Al-Tawfiq et al., 2014). The repurposing of ritonavir, lopinavir, and oseltamivir for the treatment of COVID-19 is currently being studied by companies such as AbbVie Inc. (Harrison, 2020).

Chain Termination of Viral RNA Synthesis by Nucleotide Analogues to Combat COVID-19

Remdesivir (RDV; development code GS-5734) is a 1′-cyano-substituted adenosine nucleotide analogue prodrug (**Figure 3**) that was developed by Gilead Sciences in 2017 as a treatment for Ebola virus infection (Tchesnokov et al., 2019). Several studies have revealed that it has broad-spectrum antiviral activity against RNA viruses such Ebola virus (EBOV), SARS-CoV, MERS-CoV, Marburg, Nipah virus (NiV), respiratory syncytial virus (RSV), and Hendra virus (Dörnemann et al., 2017; Lo et al., 2017; Sheahan et al., 2017).

The antiviral mechanism interferes with the action of viral RNA polymerase, causing delayed chain termination and leading to decreased viral RNA production (Lo et al., 2017; Gordon et al., 2020). Based on an *in vitro* test utilizing primary human lung epithelial cell cultures, remdesivir was potently antiviral against coronaviruses that consisted of Bat-CoVs, zoonotic Bat-CoVs, SARS-CoV, MERS-CoV, and circulating contemporary human-

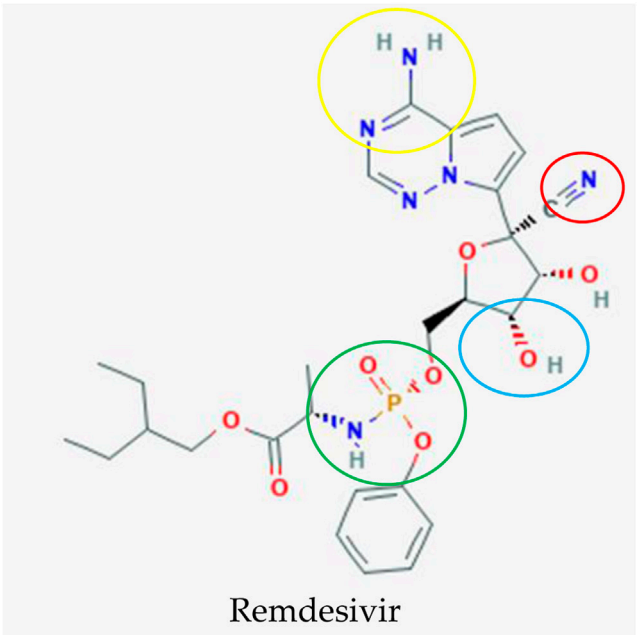

FIGURE 3 | Potential drug candidate to treat COVID-19 manufactured by Gilead Sciences to treat COVID-19, remdesivir (Kim et al., 2019). The addition of 3″ hydroxyl group (blue circle) in remdesivir inhibits the replication of the MERS RNA strand after a few cycles of nucleotide addition, which shields this antiviral drug from removal by coronavirus proofreading enzymes that abolish nucleotide analogs whereas the nitrogens (yellow circle) function as a proton donor and acceptor for a hydrogen bond to a uracil base as an identical binding site of double stranded RNA in adenosine. The phosphate group (green circle) is created as a protecting group or McGuigan ProTide to transport this antiviral compound into cells through phosphorylation, which is identical to a normal nucleotide triphosphate and recognized by polymerases in the cell. The important and unique part for this nucleotide analog is the addition of the 1′ cyano group (red circle) to eliminate the side effect of blockage of the mitochondrial RNA polymerase exhibited in mice.

CoVs (Dörnemann et al., 2017; Agostini et al., 2018; Brown et al., 2019). Remdesivir displayed superior antiviral activity *in vivo* in a transgenic mouse with a humanized MERS-CoV receptor (dipeptidyl peptidase 4, hDPP4) and *in vitro* using Calu-3 cells with MERS-nanoluciferase compared with other antiviral drugs such as lopinavir, ritonavir, and interferon beta (Dörnemann et al., 2017). The study stated that both prophylactic and therapeutic remdesivir improved pulmonary function and reduced lung viral loads and severe lung pathology, and an efficacy test of prophylactic and therapeutic remdesivir treatment in a nonhuman primate model of MERS-CoV infection, the rhesus macaque, was performed (de Wit et al., 2020). The induction of clinical disease by MERS-CoV was completely prevented with inhibition of MERS-CoV formation and lung lesion formation after prophylactic treatment initiated 24 h prior to inoculation. The data also strongly suggested a clinical benefit for therapeutic treatment with reduced virus replication and severity of lung lesions.

The improvement of severe lung pathology from *in vitro* and *in vivo* study by Sheahan and colleagues (Yamamoto et al., 2004) might help to improve the illness of patients in severe category of COVID-19, which is manifested as an extrapulmonary systemic hyperinflammation syndrome (Qin et al., 2020), and make remdesivir a drug candidate with a high potential for combating COVID-19. A recent study reported chloroquine and remdesivir effectively inhibit SARS-CoV-2 infection in Vero E6 cells (Wang M. et al., 2020). This drug was even used to treat the first US patient from Washington, who was diagnosed with COVID-19 owing to a pneumonia condition (Holshue et al., 2020). Because the use of remdesivir dramatically improved the patient's condition, a phase III clinical trial in China, Hong Kong, United States, Singapore, Republic of Korea, and France has been approved to evaluate the efficacy and safety of the drug in patients with COVID-19 (National Institute of Health, 2020). Patients received an initial dose of 200 mg remdesivir followed by a daily dose of 100 mg remdesivir through intravenous infusion in addition to standard of care therapy, whereas patients in the control group received standard of care therapy and the same dose of a remdesivir placebo according to the clinical trial data from U.S National Library of Medicine in 2020.

Favipiravir (FPV; T-705) is another nucleotide analogue developed by Toyama Chemicals for treatment of influenza virus infections (Furuta et al., 2002; Furuta et al., 2013) that has been used to treat COVID-19. It has been demonstrated to have antiviral activity against a wide range of RNA viruses such as norovirus (Rocha-Pereira et al., 2012), Zika virus (Zmurko et al., 2016), foot-and-mouth disease virus (FMDV) (Santos et al., 2006), rabies (Yamada et al., 2016), and Ebola virus (Sissoko et al., 2016; Bixler et al., 2018; Kerber et al., 2019). Similar to remdesivir, favipiravir inhibits viral RNA synthesis through chain termination (Sangawa et al., 2013; Abdelnabi et al., 2017). Favipiravir is metabolized into ribofuranosyl 5′-triphosphate (RTP) and incorporated in the growing RNA strand. The extension of an RNA strand was partially prevented by the incorporation of a single favipiravir-RTP molecule, and the incorporation of two favipiravir-RTP molecules completely blocked further extension (Abdelnabi et al., 2017). This mechanism efficiently inhibits the viral RNA-dependent RNA polymerase function (Smee et al., 2009; Jin et al., 2013; Arias et al., 2014).

To study the effect of favipiravir on COVID-19, favipiravir was approved for the treatment of COVID-19 disease on February 15, 2020. in China. A pilot study of a nonrandomized control trial at The Third People's Hospital of Shenzhen reported significantly better treatment effects in terms of disease progression and viral clearance compared with lopinavir/ritonavir treatment (Cai et al., 2020). A randomized clinical trial to compare the efficacy and safety of favipiravir and arbidol for the treatment of COVID-19 patients was conducted at three hospitals in China; Zhongnam Hospital of Wuhan University, Leishenshan Hospital, and The Third People's Hospital of Hubei Province (Chen C. et al., 2020). The trial recruited a total of 240 patients and followed up from Feb 20, 2020, to Mar 12, 2020. Patients in the experimental group received various doses of 1,600–2,400 mg of favipiravir and were compared with patients treated with other multiple antiviral drugs: Kaletra, oseltamivir, and hydroxychloroquine. Favipiravir-treated patients were found to have a higher clinical recovery rate and more effectively reduced incidence of fever and cough (Chen C. et al., 2020) which manifested as mild and moderate symptoms of COVID-19 where during this initial stage, SARS-CoV-2 multiplies

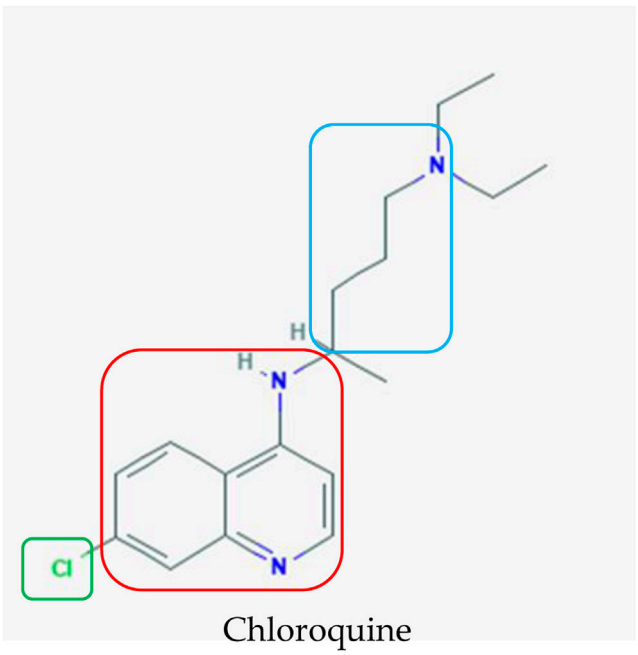

FIGURE 4 | Chemical structure of chloroquine, which was initially used as an antimalarial agent (Kim et al., 2019). The terminal nitrogens and spacer (blue box) were designed to impart parasite resistance whereas the 4-amino quinolone nucleus (red box) and small electron withdrawing group (green box) are pivotal for binding to hematin and halting the formation of hemozoin, respectively, when chloroquine is administered to treat malaria.

and binds to angiotensin-converting enzyme 2 (ACE2) receptor on human cells (Siddiqi and Mehra, 2020; Wan et al., 2020). Other clinical trials of favipiravir monotherapy or combination drug therapy are currently ongoing in China and Thailand to further evaluate the efficacy and safety of favipiravir for the treatment of COVID-19 disease.

Chloroquine Antimalarial Drug With a New Effect on COVID-19

In contrast to remdesivir and favipiravir, chloroquine (CQ) is an antimalarial drug considered as one of the drug candidates that exhibit good inhibitory effects on SARS-CoV-2 at the cellular level (Wang D. et al., 2020). Chloroquine is a 9-aminoquinoline that was synthesized in 1934 as an effective substitute for natural quinine used against malaria (**Figure 4**) (Powell, 1982; Winzeler, 2008; Parhizgar and Tahghighi, 2017). Studies have also reported its versatile antiviral activity against RNA viruses as diverse as the rabies virus, poliovirus, HIV, hepatitis viruses, influenza viruses, and Ebola virus (Kronenberger et al., 1991; Boelaert et al., 2001; Vigerust and McCullers, 2007; Mizui et al., 2010; Dowall et al., 2015; Devaux et al., 2020).

The potential activity of chloroquine against coronaviruses has been demonstrated in different *in vitro* studies. Chloroquine successfully inhibited viral replication of HCoV-229E, SARS-CoV, MERS-CoV, and EBOV in various cell lines (Blau and Holmes, 2001; Savarino et al., 2003; Vincent et al., 2005; Johansen et al., 2013; Madrid et al., 2013; Colson et al., 2020). Conversely, animal studies have revealed mixed results. Treatment with chloroquine showed no significant protection against SARS-CoV and EBOV with reports of high toxicity in mouse and hamster models (Barnard et al., 2006; Falzarano et al., 2015). However, other studies have reported positive results against HCoV-OC43 and EBOV when treated with chloroquine (Keyaerts et al., 2009; Madrid et al., 2013). The contradicting results in animal studies could be owing to the range of doses tested, whereby higher doses could be necessary to produce consistently positive results. However, this could result in a poor outcome owing to an increase in drug-related toxicity. Furthermore, chloroquine may be more effective as a prophylactic treatment owing to its activity during the early stages of a viral cycle, during which it establishes residence in the host through replication of SARS-CoV-2 during the incubation period in patients in the initial stage of the disease, which is a mild condition (Wan et al., 2020).

Recently, Wang M. et al. (2020) reported that the antiviral drugs remdesivir and chloroquine were effective in preventing replication of a clinical isolate of SARS-CoV-2. A clinical trial of over 100 patients also demonstrated that chloroquine phosphate was superior to the control treatment for the inhibition of the exacerbation of pneumonia, promoting a virus negative conversion and shortening the course of the disease, which are symptoms during the severe stage of illness in COVID-19 (Qin et al., 2020). However, the data should be carefully considered before drawing definitive conclusions, because no other results have been published to support this trial. There are a number of clinical trials for the treatment of COVID-19 using CQ registered in the Chinese Clinical Trial Registry (ChiCTR2000029939, ChiCTR2000029935, ChiCTR2000029899, ChiCTR2000029898, ChiCTR2000029868, ChiCTR2000029837, ChiCTR2000029826, ChiCTR2000029803, ChiCTR2000029762, ChiCTR2000029761, ChiCTR2000029760, ChiCTR2000029741, ChiCTR2000029740, ChiCTR2000029609, ChiCTR2000029559, ChiCTR2000029542) (Chinese Clinical Trial Register (ChiCTR), 2020; Kearney, 2020). The requests to conduct these clinical trials have been approved and the findings from chloroquine might explore and investigate the mechanism of action of chloroquine to inhibit SARS-CoV-2.

Even though the trial is still ongoing, there has been a report that long-term usage of chloroquine might contribute to cardiac disorder (Chatre et al., 2018). This drug accumulates in the body and can induce cardiac toxicity if the treatment is longer than 5 years and the cumulative dose is higher than 460 g (Chatre et al., 2018). Despite the fact that the toxicity is rare owing to the variability and nonspecificity, the monitoring of the patients treated with chloroquine to alleviate COVID-19 is essential.

Alternative and Complementary Medicine Used for the Treatment of COVID-19

Traditional Chinese Medicine Can Suppress SARS-CoV-2 *In Vitro*

Complementary medicine has also been used to fight this pandemic disease as an alternative medication. One of the complementary or alternative medicines that have been used is

Shuang Huang Lian Kou Fu Ye. It was officially used on January 23, 2020, by Beijing Administration of Traditional Chinese Medicine (Morse et al., 2020). It is a Traditional Chinese Medicine (TCM) comprised of three medicinal plants: 375 g of *Lonicera japonica Thunb.*, 375 g of *Scutellaria baicalensis Georgi*, and 750 g of *Forsythia suspensa (Thunb.) Vahl*, according to Pharmacopoeia of People's Republic of China (Zhang et al., 2013; Xu, 2020).

This complementary medicine was sold out after the announcement made by Wuhan Institute of Virology through articles from Shanghai Institutes for Biological Sciences, CAS, who claimed that this medicine can suppress the SARS-CoV-2 in a cell culture according to a study in collaboration with Shanghai Institute of Materia Medica (Xu, 2020). However, this TCM was not included in the guideline launched by National Health Commission of the People's Republic of China for prevention of COVID-19 which have been updated to version 7 and known as Guidelines of Diagnosis and Treatment for COVID-19 version 4 report in China for prevention of COVID-19, as reported by China News (Morse et al., 2020; Yang et al., 2020). This is because the findings were limited to an initial laboratory phase and insufficient data were available to confirm that this TCM can suppress SARS-CoV-2. A clinical trial is still essential to verify its efficacy. As a result, Shanghai Public Health Clinical Center and Wuhan Tongji Hospital have initiated a clinical trial on *Shuang Huang Lian Kou Fu Ye* (Yue, 2020). Although there is still insufficient scientific evidence that this TCM can be used to control COVID-19, it is still widely used and the Beijing Administration of Traditional Chinese Medicine claims that it is one of the TCM that can be used to prevent COVID-19. Furthermore, the medicinal plants in this TCM are known for their antiviral function and have been utilized to alleviate influenza and restrain SARS coronavirus. Because both SARS-CoV and SARS-CoV-2 are from the coronaviruses family, the strategies used for the treatment of SARS could be relevant for COVID-19 (Chen Y. et al., 2020).

The antiviral effect is due to the main components from the three plants which are chlorogenic acid, baicalin, and forsythoside A (Shang et al., 2011; Ding et al., 2014; Ding et al., 2017; Zhao et al., 2019). They are found to play a role as neuraminidase blocker (Ding et al., 2017) to inhibit H1N1 and H3N2 from releasing newly formed virus particles from infected cells and by activating the JAK/STAT-1 signaling pathway (Muluye et al., 2014) by inducing IFN-γ production in human $CD4^+$ and $CD8^+$ T cells and NK cells and by attenuating miR-146a, a pivotal key in the replication of H1N1 and H3N2, by targeting the TNF-receptor-associated factor 6 (TRAF6) (Ding et al., 2014; Li and Wang, 2019; Li et al., 2019; Zhao et al., 2019). These chemical constituents, chlorogenic acid, baicalin, and forsythiaside A, share a common characteristic of being antiviral against influenza; however, their antiviral activity against coronaviruses requires further investigation for further clarification.

Traditional Chinese Medicine Recommended by China CDC According to Severity of Clinical Symptoms

Based on the Guidelines of Diagnosis and Treatment for COVID-19 Version 5 by the National Health Commission (NHC) of the People's Republic of China on February 8, 2020, three TCMs have been used depending on the severity of the condition of COVID-19 and symptom differentiation (Yang et al., 2020).

Treatment of Mild Clinical Symptoms

For patients that showed mild clinical symptoms such as a dry cough, fatigue, chest tightness, nausea, mild cold fever, or no fever, the TCM consisted of 15 g of *Atractylodes lancea (Thunb.) DC.*, 6 g of *Citrus × aurantium L.*, 10 g of *Magnolia officinalis* Rehder and E. H. Wilson, 9 g of *Agastache rugosa* (Fisch. and C. A. Mey.) *Kuntze*, 6 g of *Lanxangia tsao-ko* (Crevost and Lemarié) M.F.Newman and Skornick., 9 g of *Ephedra sinica S.*, 10 g of *Hansenia weberbaueriana* (Fedde ex H. Wolff) Pimenov and Kljuykov, 9 g of *Zingiber officinale Roscoe*, and 10 g of *Areca catechu L.* (Guo et al., 1989). This decoction is used a combination of the prescribed TCM *Bu Huan Jin Zheng Qi San* and *Da Yuan Yin* adapted from a medical encyclopedia "Gu Jin Yi Tong Da Quan" chapter 76 and "Wen Yi Lun" (Epidemic Diseases) by Wu Youke, respectively, from Dynasty Ming (Wang et al., 2018). Atractylon is a chemical compound present in the chief ingredient, *Atractylodes lancea (Thunb.) DC.*, which attenuated the influenza A virus within 5 days through the TLR7 signaling pathway by upregulating the Toll-like receptor 7 (TLR7), MyD88, tumor necrosis factor receptor-associated factor 6, and IFN-β mRNA expression in the lung tissue of mice infected with influenza A virus (Cheng et al., 2016). The inhibition of coronaviruses by the synergistic effect of this TCM could be similar in action to the inhibition of influenza through triggering of the TLR7 signaling pathway. However, further studies are needed to prove the mechanism of action of this decoction on COVID-19.

Treatment of Mild to Severe Clinical Symptoms

Recently, *Qing Fei Pai Du Tang* has been applied for the treatment of patients with any clinical symptoms of COVID-19 ranging from mild to severe cases (incubation period of SARS-CoV-2, viral multiplication, and extrapulmonary systemic hyperinflammation syndrome) (Qin et al., 2020; Siddiqi and Mehra, 2020; Wan et al., 2020) and even used as a preventative medicine for this disease. 1,102 of 1,261 confirmed cases in 10 Chinese provinces were reported to be cured and discharged after the treatment with this TCM (Zhang, 2020). Similarly, the China government has reported that, from 108 patients diagnosed with mild COVID-19 cases, the number of cases that evolved from mild to severe was approximately 10% when given Western medicine alone compared with approximately 4.1% when integrated Chinese and Western medicine treatment was used (Yang et al., 2020). *Qing Fei Pai Du Tang* is comprised of 20 medicinal plants and one mineral: 9 g of *Ephedra sinica S.*, 6 g of *Glycyrrhiza uralensis Fisch. ex DC.*, 9 g of *Prunus armeniaca L.*, 15–30 g of Gypsum, 9 g of *Cinnamomum cassia (L.) J. Presl*, 9 g of *Alisma plantago-aquatica L.*, 9 g of *Polyporus umbellatus* (Pers.) Fries, 9 g of *Atractylodes macrocephala Koidz.*, 15 g of *Poria cocos* (*Schw.*) *Wolf* , 16 g of *Bupleurum chinense DC.*, 6 g of *Scutellaria baicalensis Georgi*, 9 g of *Pinellia ternata (Thunb.) Makino*, 9 g of *Zingiber officinale Roscoe,* 9 g of *Aster tataricus L. f.*, 9 g of *Tussilago farfara L.*, 9 g of *Iris domestica (L.)* Goldblatt and Mabb., 6 g of *Asarum sieboldii*

M., 12 g of *Dioscorea oppositifolia L.*, 6 g of *Citrus trifoliata L.*, 6 g of *Citrus* × *aurantium L.*, and 9 g of *Agastache rugosa* (Fisch. and C. A. Mey.) *Kuntze*. This TCM is a combination of four combinations of well-known prescribed classic TCMs from Treatise on Cold Damage Diseases, which are *Ma Xing Shi Gan Tang, She Gan Ma Huang tang, Xiao Cai Hu Tang*, and *Wu Ling San* (Zhang et al., 2000; Zhang and Zhang, 2020; Zhang et al., 2020).

Ma Xing Shi Gan Tang (MXSGT) is known for antipyretic effects and is commonly used to treat pneumonia, influenza, and other respiratory diseases (Gong, 2018). A systematic review found that the combination of MXSGT with Western medicine significantly increased the effective rate of treatment to treat pneumonia ($p < 0.00001$) (Li et al., 2009) and showed significant improvement ($p < 0.05$) on day 7 of consumption of the decoction besides being effective and safe for the treatment of community-acquired pneumonia (Gong, 2018). This effectiveness is mediated by β2-adrenoceptors on bronchial smooth muscle to inhibit neutrophil from entering the respiratory airway, block acetyl-cholinergic and histaminergic receptor-induced bronchial contraction, and finally reduce neutrophilic inflammation (Kao et al., 2001; Eng et al., 2019). Furthermore, it plays roles in decreasing IL-4, IL-8, and TNF-α, yet increase IFN-γ in a COPD rat model (Zhang et al., 2006). This decoction was also used to regulate the pathogenesis of influenza virus A in infected RAW264.7 cells by the attenuation of LC3, the autophagy marker protein (Li et al., 2019).

A classic decoction used in China and Japan, *She Gan Ma Huang tang* (SGMHT), inhibits mast cells from releasing substances during inflammation, regulates the viscera's function, and promotes the apoptosis of eosinophils (Zhu, 2014). mRNA expression levels of Th2 cytokines were decreased and associated with Th1 cytokines upregulation and direct attenuation of the pulmonary edema and suppression of the NF-kB pathway through two herbs (*Aster tataricus L. f.* and *Iris domestica (L.)* Goldblatt and Mabb.) from a modified version of SGMHT (Eng et al., 2019). In contrast to the other TCMs mentioned above, the other two TCM formulas (*Xiao Cai Hu Tang* and *Wu Ling San*) function differently and did not exhibit function against acute airway obstruction. *Xiao Cai Hu Tang* (XCHT) is known as a TCM for liver treatment, particularly chronic hepatitis B. This TCM modulates STAT3 expression and indirectly suppresses the hepatitis B virus according to western blot analyses and real time PCR results (Chen et al., 2017). *Wu Ling San* (WLS) has been used to treat impairments of the regulation of body fluid homeostasis in Japan, China, and Korea (Ahn et al., 2012) through affecting the signal transduction pathway such as NF-kB, MAPKs, and HO-1 to demonstrate anti-inflammatory effects like MXSGT to treat pneumonia or respiratory diseases in lipopolysaccharide stimulated macrophages (Oh et al., 2014).

The derived formulation of *Qing Fei Pai Du Tang* from the four combinations of these classic TCM showed its ability to reduce the symptoms of COVID-19 patients by restoring the normal body temperature in 94.6% of 112 patients and stopping coughing in 80.6% of 214 patients (Gu, 2020). As a result, this TCM has been listed as one of the treatment options by the National Health Commission (NHC) of the People's Republic of China in Guidelines of Diagnosis and Treatment for COVID-19 Version 7 (National Health Commission of the People's Republic of China, 2020; Yang et al., 2020). The effectiveness of *Qing Fei Pai Du Tang* can reach 97.78% with 1,102 patients being cured from 1,261 patients from 10 districts in China until March 13, 2020 (Yang et al., 2020; Zhang et al., 2020). Until now, none of the cases became severe from mild conditions after the consumption of *Qing Fei Pai Du Tang*. However, any pharmaceutical drugs can induce side effect or cause adverse events and TCM herbs are not an exception (Chow et al., 2019). There is evidence that one of the herbs (*Glycyrrhiza uralensis Fisch.*) used in this concoction possesses hepatoxicity in a clinical trial from an extensive literature review study through standardized causality assessment algorithms, Roussel Uclaf Causality Assessment Method (Chow et al., 2019). As a circumstance, the toxicology of the TCM concoction should be determined to ensure the safety of patients, even though so far there has been no report of side effects caused by the alternative or complementary medicine mentioned in this study.

Treatment of Severe Clinical Symptoms

Xue Bi Jing Injection, an established TCM in China, has been recommended for severe symptoms of COVID-19 patients (National Health Commission of the People's Republic of China, 2020; Yang et al., 2020). Unlike other drugs that undergo a conventional clinical trial phase, it proceeded from bedside to bench and finally back to bedside before approval was obtained from China Food and Drug Administration in 2004 (Zhang et al., 2018). *Xue Bi Jing Injection* consists of numerous compounds, including Senkyunolide I, safflor yellow A, paeoniflorin, ferulic acid, galloylpaeoniflorin, anhydrosafflor yellow B, oxypaeoniflorin, caffeic acid, albiflorin, uridine, gallic acid, guanosine, danshensu, protocatechuic aldehyde, and hydroxysafflor yellow A, which are extracted from five medicinal plants, *Conioselinum anthriscoides "Chuanxiong," Salvia miltiorrhiza Bunge, Paeonia lactiflora Pall*, *Carthamus tinctorius L.*, and *Angelica sinensis (Oliv.) Diels* (Gong et al., 2015).

It was initially developed for activating blood circulation to remove blood stasis, cooling the blood, and clearing toxic heat (Zhang et al., 2018). However, this TCM has been used to fight SARS-CoV-2 because of its effectiveness in treating severe pneumonia, the severe stage of COVID-19, by significantly reducing mortality by approximately 15.9% and elevating the improvement of the pneumonia severity index by approximately 60.8% (Zhang et al., 2018). The *Xue Bi Jing Injection* relieved or reduced severe pneumonia by triggering the inflammation pathway through downregulation of TNF-α, IL-6, and IL-8 on the 3rd, 7th, and 14th day after treatment, although it did not significantly influence the release of leptin (Qi et al., 2011). This suggests that an antiendotoxin effect was deployed by halting the release of TNF-α, IL-6, and IL-8, endogenic inflammatory mediators. As a result, blocking the development of a systemic inflammatory response syndrome occurred through the disruption of the inflammation vicious cycle (Qi et al., 2011). This mechanism of action of the *Xue Bi Jing Injection* reduces the severity of pneumonia in COVID-19 patients and lowers the side effects on the organ functions.

These results indicate that TCMs can be used as complementary medicine for the treatment of patients during

this pandemic disease; however, the mechanism of action of these TCMs still requires further investigation and validation.

CONCLUSION

The treatment of COVID-19 with the six repurposed drugs discussed in this study is dependent on the ability of the drug to inhibit the proliferation by binding to the enzyme active sites, viral chain termination, and triggering of molecular pathways. In contrast to the six drugs, the four TCMs discussed in this review were initially used to treat influenza and SARS by acting as neuraminidase blockers and trigger the inflammation pathway. In this review, we provide a framework for better understanding of the mechanism of action of repurposed drugs and TCMs and their involvement in the molecular pathway for inhibiting viral replication such as SARS or MERS.

Instead of focusing on one drug mechanism of action, we analyzed repurposed drugs that are currently involved in worldwide clinical trials to elucidate their molecular mechanism. The inclusion of gray literature provides more data to better understand the properties and effects of repurposed drugs. However, there are limitations to our findings because the quality of the evidence and risk of bias were not evaluated due to the nature of a scoping review. Furthermore, there is still a lack of concrete evidence for the mechanisms of action of the drugs and their curative effect on COVID-19 because the clinical trial is still ongoing. Further experimental validation is needed to provide more concrete evidence.

Understanding the different molecular mechanisms of these drugs instead of just one drug molecular mechanism can provide insights on the pivotal genes or mechanisms that should be targeted in future studies and lead to the development of effective drugs for the treatment of COVID-19 in the future.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: No restrictions. Requests to access these datasets should be directed to FFL, lemfuifui@moh.gov.my.

ETHICS STATEMENT

Ethical clearance is exempted, as approved by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (NMRR-20-347-53302), because no personal information or samples from human subjects are used in this systematic scoping review.

AUTHOR CONTRIBUTIONS

FFL was responsible for conceptualization, supervision, formal analysis, methodology, data curation, and writing. FTC was responsible for methodology and writing of original draft. DL was responsible for writing of original draft. FO was responsible for data curation and writing of original draft. LFP was responsible for writing of original draft, writing of review, and editing. SNC was responsible for conceptualization, validation, writing of original draft, and funding acquisition.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.585331/full#supplementary-material.

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REVIEW
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The Potential Therapeutic Effect of RNA Interference and Natural Products on COVID-19: A Review of the Coronaviruses Infection

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The SARS-CoV-2 virus was reported for the first time in Wuhan, Hubei Province, China, and causes respiratory infection. This pandemic pneumonia killed about 1,437,835 people out of 61,308,161cases up to November 27, 2020. The disease's main clinical complications include fever, recurrent coughing, shortness of breath, acute respiratory syndrome, and failure of vital organs that could lead to death. It has been shown that natural compounds with antioxidant, anticancer, and antiviral activities and RNA interference agents could play an essential role in preventing or treating coronavirus infection by inhibiting the expression of crucial virus genes. This study aims to introduce a summary of coronavirus's genetic and morphological structure and determine the role of miRNAs, siRNAs, chemical drugs, and natural compounds in stimulating the immune system or inhibiting the virus's structural and non-structural genes that are essential for replication and infection of SARS-CoV-2.

Keywords: coronavirus, miRNA, siRNA, natural products, phytochemicals, SARS-CoV-2

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INTRODUCTION

Over the past 50 years, a wide range of human and animal diseases have been caused by coronaviruses (CoVs). Since the emergence of Severe Acute Respiratory Syndrome (SARS-CoV) Coronavirus in 2003, a considerable number of new Human coronaviruses (H-CoVs) have been identified (Ding et al., 2003). The appearance of Middle East Respiratory Syndrome (MERS-CoV) coronavirus in 2012 and the continuous occurrence of human cases further intensified attention to the importance of studying these viruses from different aspects (Centers for Disease Control and Prevention, 2012). Tolerance of new mutations and recombination has led to the evolution of this group of viruses, giving them the ability to transmit between species. In most cases, CoV infections are self-limiting, and after completing a reasonable period, the body recovers from the illness (Enjuanes et al., 2000). However, some strains of this family cause severe infections and have been the cause of widely spread epidemics during the last two decades (Mahase, 2020).

The SARS-CoV-2 virus was reported for the first time in Wuhan, Hubei Province, China, and causes respiratory infection. According to WHO statistics, on November 27, 2020, Coronavirus

disease 2019 (COVID-19) killed about 1,437,835 people out of 61,308,161cases, and the most significant number of deaths and infected people were in the Americas (Chen et al., 2020b). In recent years, many treatment strategies such as prescribing antibiotics, the application of antiviral drugs (including Human Immunodeficiency Virus 1 (HIV-1) protease inhibitors, oseltamivir, and ribavirin), different corticosteroids, interferons, and natural human immunoglobulin have been used for treating patients suffering from H-COVs. Recently, most treatment strategies for CoVs such as MERS-CoV, SARS-CoV, and Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2) have been based on inhibiting viral agents involved in the replication, infection, or induction of host immune system agents to combat viral infection (Li and De Clercq, 2020; Prajapat et al., 2020). Numerous studies are being conducted today to prove the role of various biomolecules, including RNA interference (RNAi), fusion inhibitors, neutralizing antibodies, plant, or microbial metabolites, as antiviral compounds (Prajapat et al., 2020).

This review article tries to present some information about the biology, replication, infusion, and pathogenesis of coronavirus. Also, it has decided to explain the existing treatment strategies based on chemical drugs, natural compounds, small interfering RNA (siRNAs), and microRNA (miRNAs) that can be used to fight against coronavirus infection, especially SARS-CoV-2. For this purpose, keywords including natural product, flavonoid, polyphenols, phytochemicals, microRNA, siRNA, Coronavirus, COVID-19 up to August 2020 were searched and evaluated using Scopus, PubMed, WOS, and Google Scholar databases.

CORONAVIRUS

Coronavirus Classification and Taxonomy

Genome organization, genome homology, reproduction strategies in a host, and the virion's structural traits are criteria for CoVs classification by the International Committee for Taxonomy of Viruses (ICTV) (Casals et al., 1980). In terms of phylogenetics, CoVs belong to Coronaviridae, as the class of Nidovirals. Coronaviridae family includes two sub-families: Orthocoronavirinae and Torovirinae. The sub-family of Orthocoronavirinae consists of four genera: Alpha, Beta, Gamma, and Delta coronaviruses that are responsible for infection on a vast range of hosts, from mammalians to birds (King et al., 2012). Human infection with CoVs was first reported in 1965 (Hamre and Procknow, 1966). H-CoV 229E and CoV-NL63 are human pathogens of the genus Alpha-CoV, causing common cold (Mcintosh et al., 1970; Monto, 1974). Severe Acute Respiratory Syndrome Coronavirus 1 (SARS-CoV-1), MERS-CoV, and SARS-CoV-2 are phylogenetically classified in the Beta-CoVs genus and have caused a high percentage of mortality in the human population during the last two decades (Zhou et al., 2020). The most common CoVs genes used for phylogenetic studies are nsp12 (RNA dependent RNA polymerase), nsp5 (chymotrypsin-like protease), nsp13 (helicase), nucleocapsid (N), and spike protein (S) (ICTV, 2009).

Genomic Structure

CoVs are positive single-strand RNA with 32–46% G + C content. They have the biggest genome size among all known RNA viruses (about 26.4–31.7 kb) (Lai and Cavanagh, 1997; Enjuanes et al., 2000). Depending on the strain, the genome in CoVs contains a different number of open reading frames (ORF). Nevertheless, ORF1a, ORFb1, envelope (E), protein S, protein N, and membrane protein (M) are present in all Human-CoV strains (Cavanagh, 1995; Enjuanes et al., 2000). The SARS-CoV-2 genome size varies from 29.8 to 29.9 kb and encodes 9,860 amino acids. The ORF1ab in SARS-CoV-2 is over 21 KB in length and covers two-thirds of the entire genome (Cascella et al., 2020). According to recent reports, the ORF portion of the SARS-CoV-2 genome has a lower CG dinucleotide percentage than other coronaviruses, so ORF RNA translation is highly efficient (Wang Y. et al., 2020).

Coronavirus Replication Process

The replication cycle of CoVs occurs in several stages (**Figure 1**). In the initial stage, the virus binding proteins are attached to appropriate receptors on the host cell's surface. Then membrane fusion occurs, which finally leads to virus genome insertion in the host cell. In the second step, the RNA polymerase-dependent RNA gene is activated to translate the virus genome into structural proteins (Ziebuhr, 2005). During these steps, in addition to viral factors, some host cell factors can also enhance or inhibit this process.

Attachment; Entry and Cellular Factors Involvement

The first stage of virus entry starts with the attachment of S proteins to host ligands. Most HCoVs use endocytosis pathways for their entrance, while others can use direct membrane fusion (Gallagher and Buchmeier, 2001). The ectodomain S protein consists of a receptor-binding subunit (S1) and a membrane subunit (S2). After binding to the host cell surface receptors, the S1 subunit leads to a structural change in S2. The S2 proteolytic domain then fuses the virus membrane into the host membrane, leading to the viral genome's entry into the host cell. A partial mutation in the S protein that changes the amino acid sequence can significantly affect the virus's pathogenesis and tissue or cell susceptibility to infection (Li, 2016).

HCoV-NL63, SARS-CoV, and SARS-CoV-2, use Angiotensin-Converting Enzyme 2 (ACE2) to contaminate cells (Moore et al., 2004; Zhang et al., 2020a). Both types of alveolar cells (I and II types) and the endothelial cells express ACE2. It has been shown that MERS-CoV uses Dipeptidyl peptidase 4 (DPP4), a physiological ligand for adenosine deaminase, which is extensively expressed by the endothelial cells in various tissues (Raj et al., 2013). However, many host factors can limit the entry of the virus. According to cell culture mechanisms, a family of Interferon-Inducible Transmembrane proteins (IFITM) could limit S protein-dependent access in HCoV-229E and HCoV-NL63, which would also lead to intense reduction of the infection in SARS-CoV and MERS-CoV (Huang et al., 2011; Wrensch et al., 2014). It seems that IFITM inhibits the membrane fusion by

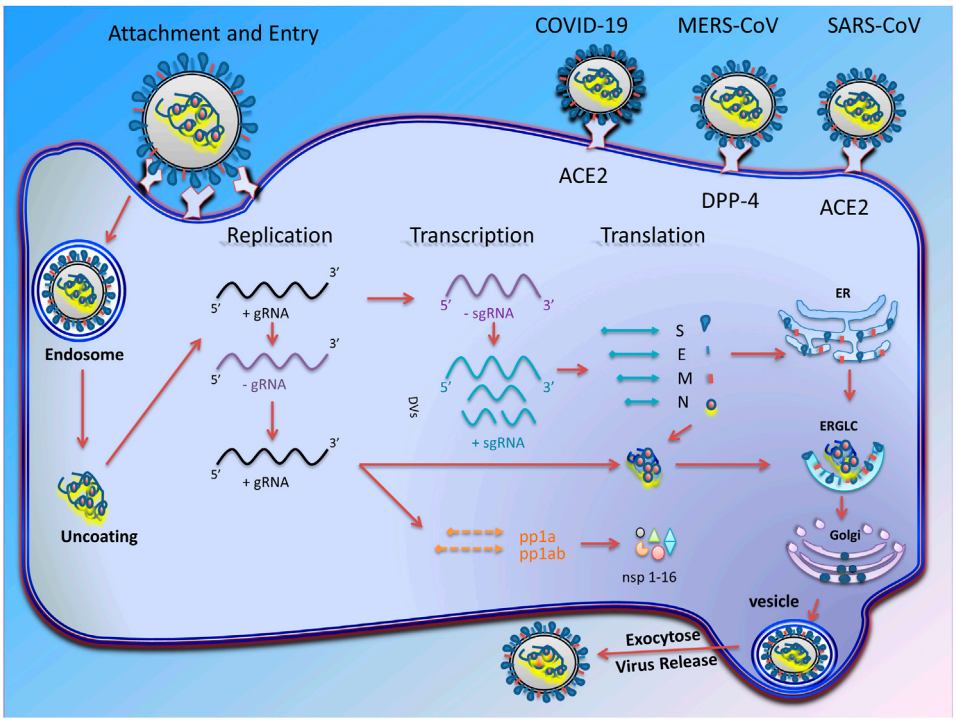

FIGURE 1 | Transcription and replication of coronavirus. Schematic picture showing the coronavirus infection starts with attachment of virus S proteins to receptors on the host cells. After successful fusion, ribonucleocapsid enters the cell cytoplasm and loses its coat to mRNA is released. RNA-dependent RNA synthesis in CoVs includes two different genome replication processes to achieve multiple copies of genomic RNAs (gRNAs) and transcription of sub-genomic RNAs (sg mRNAs) coding structural and accessory proteins. The gRNA eventually producing 16 non-structural proteins (nsp-16) that are involved in virus replication. After assembly and budding, the full virus particles are transferred to the cytoplasmic membrane and finally released through the exocytosis process.

preventing the virus fusion to the cell membrane, endosomal membranes, or through membrane fluidity. Moreover, it was shown that human coronavirus HCoV-OC43 utilizes IFITM2 and IFITM3 as receptors to facilitate cell entry (Zhao et al., 2014).

Transcription and Replication

In CoVs, RNA-dependent RNA polymerase amplifies the virus genome in two ways and eventually produces several copies of genomic RNAs (gRNAs) and subgenomic RNAs (sg mRNAs) (Ziebuhr, 2005). Generally, CoVs replication is initiated with the translation of ORF1a to produce polyprotein1a (pp1a) with 4,382 amino acids and polyprotein1ab (pp1ab) with 7,073 amino acids. Then, the ORF1b is translated through the ribosomal frameshifting mechanism (Lai, 1990; Ziebuhr, 2005). Eventually, each of these polyproteins is cleaved to produce 16 non-structural proteins (nsp-16), which are involved in the virus replication cycle (Mcintosh and Peiris, 2009). Some of the non-structural proteins (**Table 1**) that have critical roles in the virus's life cycle include helicase (nsp13), RNA-dependent RNA polymerase (RdRP) (nsp12 polymerase), 3-chymotripsin-like protease ($3CL^{pro}$) (nsp5 protease), and papain-like protease (PL^{pro}) (nsp3 protease) (Lai, 1990; Sawicki et al., 2005).

Virus Assembly and Egress

The structural proteins (**Table 2**) and some membrane accessory proteins are translated inside ER-bound ribosomes, while N protein is translated via free ribosomes in the cytoplasm (Nal et al., 2005). One of the distinct characteristics of CoVs is virion aggregation inside the ER and budding toward the Golgi apparatus (Cawood et al., 2007). Moreover, E, S, and N proteins interpolation to virions is modulated by M protein heterotypic interaction in the budding locus (Opstelten et al., 1995). After assembly and budding, the full virus particles are transferred to the cytoplasmic membrane and finally are released through the exocytosis process (**Figure 1**).

Coronaviruses Pathogenesis

Clinically, the pathogenesis of coronaviruses is divided into three stages. In the viremia phase, the virus enters the peripheral blood after the lungs are infected. Thus, the virus can reach its target tissues, such as the heart, kidneys, and gastrointestinal tract, through the bloodstream. The second phase is the pneumonia phase. Then the patient enters the recovery phase if the immune system can overcome the virus's attack at this stage. However, in patients with immunodeficiency, high blood pressure, diabetes, and the elderly, the immune system cannot effectively manage the infection, and a critical stage of the disease occurs (Peiris et al., 2003; Guan et al., 2020).

TREATMENT

Vaccination is the best way to control the COVID-19 pandemic, and many efforts are being made to produce it. Nevertheless, its

TABLE 1 | Biological activities of coronavirus non-structural proteins.

Proteins	Biological functions in virus particle	Effect on the host cellular response	Refs.
Nsp1	A virulence factor. The genus-specific marker. Highly divergent among CoVs	Interact with the 40S subunit of ribosome to prevent host mRNA translation.	Woo et al. (2010)
Nsp2	Not reported.	Interaction with two host proteins, PHB1 and PHB2 and disruption of intracellular host cell surviving signaling pathway.	Cornillez-Ty et al. (2009)
Nsp3	Cysteine-type endopeptidase activity at the N-terminus of the replicase polyprotein.	Plays a role in host membrane rearrangement. Downregulate mRNA levels of pro-inflammatory cytokines including CCL5, and CXCL10.	Lei et al. (2018)
	Nucleic acid binding by interaction with N protein, RNA-directed 5′-3′ RNA-directed 5′-3′ RNA polymerase activity.	Responsible for suppressing IFN induction. Antagonize IFN production	
Nsp4	Marker for the coronavirus-induced DMVs. Interacts with nsp3 and nsp6 to formation of the replication complexes.	Not reported.	Angelini et al. (2013)
Nsp5	Cleaves the C-terminus of replicase polyprotein at 11 sites	Bind an ADRP, may cleave host ATP6V1G1 and modifying host vacuoles intracellular pH	Lin et al. (2005)
Nsp6	Necessary for viral replication.	Plays a role in the initial induction of autophagosomes from host reticulum endoplasmic.	Angelini et al. (2014)
	Mediates the DMV formation by rearrangement of host membrane.	Limits the expansion of these phagosomes that are no longer able to deliver viral components to lysosomes.	
Nsp7	A primase in the form of heterohexadecamer dsRNA-encircling as ring structure.	Not reported.	Te Velthuis et al. (2012)
Nsp8	A processivity factor for the RdRP.		
Nsp9	May participate in viral replication by acting as a ssRNA-binding protein.	Not reported.	Miknis et al. (2009)
Nsp10	Interact with nsp1, nsp7, nsp14, and nsp16. Implicated in the regulation of polyprotein processing. Plays a pivotal role in viral transcription by stimulating both nsp14 3′-5′ exoribonuclease, and nsp16 2′-*O*-methyltransferase activities	Not reported.	Bouvet et al. (2012)
Nsp12	Responsible for replication and transcription of the viral RNA genome.	Not reported.	Ahn et al. (2012)
Nsp13	Magnesium dependent helicase activity. Displaying RNA and DNA duplex-unwinding activities with 5′ to 3′ polarity.	Not reported.	Tanner et al. (2003)
Nsp14	An exoribonuclease acting on both ssRNA and dsRNA in a 3′ to 5′ direction.	Interacts with DDX1 via N-terminus.	Denison et al. (2011)
	Acts as a proofreading exoribonuclease for RNA replication.	Modulation of the innate immune response.	
Nsp15	Mn^{2+}-dependent, uridylate-specific enzyme, which leaves 2′-3′-cyclic phosphates 5′ to the cleaved bond.	Essential to evade dsRNA sensors.	Ricagno et al., 2006)
Nsp16	Mediates mRNA cap 2′-O-ribose methylation to the 5′-cap structure of viral mRNAs.	Essential to evade MDA5 recognition. Negatively regulating innate immunity. IFN antagonism.	Bouvet et al. (2010)

production is time-consuming because it must first be proven to be immunogenic and effective. Therefore, prevention is currently the best treatment. There are two essential strategies for treatment this disease. The first step is a general treatment that reduces the symptoms and clinical complications of the patients, and the second is drug treatment.

General Treatment

The available treatment options for these patients include measuring and monitoring of vital signs such as heart function, kidney, liver, respiratory rate, and oxygen therapy if necessary. Moreover, patients must be getting enough water, sufficient calories, balance for electrolytes, and use of fever medicines such as acetaminophen and ibuprofen (Wang J. et al., 2020; Zimmermann and Curtis, 2020). Although the use of corticosteroids such as methylprednisolone in the SARS-CoV epidemic has been shown to improve some clinical symptoms, it is not common in COVID-19. Nevertheless, it could be used only temporarily in patients with severe illness such as dyspnea and Acute Respiratory Distress Syndrome (ARDS) (Huang et al., 2020; Russell et al., 2020; Zhang et al., 2020b). Herbal medicines have the property to be used as a dietary supplement for relieving and reduce respiratory symptoms and other symptoms of COVID-19. They can improve the general condition of patients with mild disease severity. *Althaea officinalis* L. (Malvaceae), *Commiphora myrrha* (T.Nees) Engl. (Burseraceae), *Glycyrrhiza glabra* L. (Fabaceae), *Hedera helix* L. (Araliaceae), and *Sambucus nigra* L. (Viburnaceae) are some of the herbal medicines can be used as adjunctive therapy for mild COVID-19 (Silveira et al., 2020).

Coronavirus Drug Treatment

SARS-CoV-2 is a new virus from the Coronaviruses family, with 79% genomic similarity to SARS-CoV and 51.8% to MERS-CoV.

TABLE 2 | The biological functions of structural proteins of SARS and SARS-CoV 2.

Protein	Post-translational modification	Biological functions in virus particle	Effect on the host cellular response	Refs.
S	Disulfide bridge, Palmitoylation, N-glycosylation	Virulence factor. Responsible for recognition of the cellular receptor. Fusion of virus membrane with host endosome membrane.	Physically interaction with eIF3F. Modulation the expression of the pro-inflammatory cytokines IL 6 and 8 at a later stage of infection.	Bosch et al. (2003) and Nal et al. (2005)
M	O-glycosylation, N-glycosylation	Plays a central role in virus morphogenesis. Maintain the structure and assembly via its interactions with other viral proteins such as 3a and 7a, forms a complex with HE and S proteins. Promotes membrane curvature, binds to the nucleocapsid and participates in RNA packaging into the virus	Pro-apoptotic mediated activation of caspases 8 and 9. Suppress IFN I production mediated by RIG-I and inhibiting the translocation of IRF3 into the nucleus, IFN antagonism	Nal et al. (2005) and Siu et al. (2009)
E	Palmitoylation, glycosylation	Plays a central role in virus morphogenesis and assembly. Responsible for the curvature of the viral envelope. A virulence factor trafficking within the infected cells and budding of the virion.	Induction of the cell stress response and mitochondrial-mediated apoptosis. Disruption of the lung epithelium, Potential B cell antagonism.	An et al. (1999) and Liu et al. (2007)
N	*O*-glycosylation, ADP-ribosylation, Sumoylation, Phosphorylation	An RNA chaperone, associates with the viral genome in a helical nucleocapsid. Plays a fundamental role during virion assembly through its interactions with the viral genome and membrane protein M, E and nsp3. Plays an important role in enhancing the efficiency of sgRNA transcription and viral replication.	Modulate transforming growth factor-beta signaling by binding host smad3. Interfere with the function of IRF3. Inhibition of IFN I response. Induction of apoptosis.	Zhao et al. (2006) and Wu et al. (2009)

Therefore, since the risk of SARS-CoV-2 vertical infection is the same as these two types of viruses, their treatment can be similar. Consequently, drugs that were previously effective for the treatment of SARS-CoV and MERS-CoV may also have therapeutic potential for the treatment of COVID-19 (Ren et al., 2020). Although a definitive cure for this new virus has not yet been discovered, previous studies suggest that drugs such as western medicines and natural products may have potential efficiency against COVID-19 and could be used to reduce the severity of the disease.

Interferon

Interferon type I is a member of the innate immune system produced and secreted in response to viral infections, including alpha and beta-interferon. Interferons exert their antiviral activity in two ways 1) cytotoxic T lymphocytes and macrophages stimulate the immune system to kill the virus by increasing or stimulating natural killer cells, 2) or inhibit virus replication in the host cell (Samuel, 2001; Sadler and Williams, 2008; Fensterl and Sen, 2009). Previous studies have shown that interferon-α, particularly its recombinant form (INF- α2b), can alleviate symptoms and shorten the disease course of respiratory tract viral infections in the early phase (caused by influenza, SARS-CoV, and MERS-CoV) through inhibiting virus replication and decreasing the virus load. Therefore, these molecules may be useful in the treatment of COVID-19 (Zheng et al., 2004; Danesh et al., 2011; Falzarano et al., 2013; Khalid et al., 2015). However, according to the latest update (3.2020) of the COVID-19 Treatment Guidelines Panel, there is no comment about its therapeutic effect on COVID-19 (COVID-19 Treatment Guidelines Panel).

Lopinavir/Ritonavir

Lopinavir/ritonavir is a protease inhibitor that has previously been used against HIV due to its antiviral activity. Nevertheless, later in the SARS-CoV (an epidemic that occurred in 2003), it was discovered that lopinavir/ritonavir by $3CL^{pro}$-inhibitor action could be useful for coronavirus treatment (Chu et al., 2004; Su et al., 2019). Due to the structural similarity of $3CL^{pro}$ in SARS-CoV2 and SARS-CoV, previous studies have suggested that this drug may be useful for treating SARS-CoV2 (Uzunova et al., 2020). Nevertheless, later it was found that these drugs do not have the desired effect in treating this infectious disease, and the coronavirus disease 2019 treatment guidelines (last updated: November 3, 2020) announced that its use is not recommended, except in a clinical trial (COVID-19 Treatment Guidelines Panel, 2019).

Ribavirin

Ribavirin is a nucleoside analog that has antiviral activity. Co-administration of lopinavir/ritonavir has a significant therapeutic effect against SARS-CoV and may reduce ARDS risk (Chu et al., 2004). However, according to the latest update (November 3, 2020) of the COVID-19 Treatment Guidelines Panel, there is no comment about its therapeutic effect on COVID-19 (COVID-19 Treatment Guidelines Panel).

Chloroquine

Chloroquine is an antiparasitic drug used against malaria, but recently it has been shown to have antiviral activity by blocking virus infection and could suppress SARS-CoV-2 infection *in vitro* (Wang M. et al., 2020; Colson et al., 2020). Although many physicians and specialists initially considered this drug to treat

COVID-19, its use is no longer recommended today (COVID-19 Treatment Guidelines Panel).

Favipiravir

One of the promising drugs in the treatment of COVID-19 is favipiravir. This drug has an inhibitory effect on RNA polymerase, and its early clinical trial showed that not only is the antiviral activity of this drug more significant than lopinavir/ritonavir, but it has fewer side effects (Cai et al., 2020). However, according to the latest update (November 3, 2020) of the COVID-19 Treatment Guidelines Panel, there is no comment about its therapeutic effect on COVID-19 (COVID-19 Treatment Guidelines Panel).

Remdesivir

Remdesivir is one of the drugs that can be used against RNA viruses such as SARS-CoV/MERS-CoV. *In vivo* and *in vitro* studies have shown that this adenosine analog by inhibiting RdRP has a therapeutic effect against COVID-19 and could be a choice for treating this new pandemic infectious disease (Holshue et al., 2020; Wang M. et al., 2020). This drug is currently recommended to treat COVID-19 according to the latest update (November 3, 2020) of the COVID-19 Treatment Guidelines Panel (COVID-19 Treatment Guidelines Panel).

Arbidol

Arbidol is an antiviral compound for prophylaxis and treatment of influenza. An *in vitro* study has proved that arbidol has a direct antiviral activity on SARS-CoV replication (Wang X. et al., 2020). A clinical trial of this drug on SARS-CoV-2 has also shown that it reduces high flow nasal catheter (HFNC) oxygen therapy, enhances the process of viral clearance and improves focal absorption on radiologic images (Xu et al., 2020a). Arbidol could inhibit host cell adhesion and SARS-CoV-2 spike glycoprotein trimerization. Furthermore, the simultaneous use of arbidol by Lopinavir/ritonavir has a more significant therapeutic effect than the Lopinavir/ritonavir group (Deng et al., 2020). However, according to the latest update (November 3, 2020) of the COVID-19 Treatment Guidelines Panel, there is no comment about its therapeutic effect on COVID-19 (COVID-19 Treatment Guidelines Panel).

Antithrombotic Therapy

Anticoagulants, like heparins, play a vital role in preventing arterial thromboembolism in patients with heart arrhythmias. One of the leading causes of death in patients with COVID-19 is myocardial and stroke infarction for unknown reasons. Therefore, if hospitalized patients with COVID-19 due to hemophilia or similar diseases do not have anticoagulant contraindications, administration of a prophylactic dose plays a vital role in reducing the complications COVID-19 in patients with symptoms of blood coagulation (such as low levels of antithrombin, increased fibrinogen and di-dimer) (Godino et al., 2020).

Natural Products as Antiviral Agents

Natural products are biochemical mixtures produced by living organisms in nature The effectiveness of natural compounds in ancient medicine was introduced thousands of years ago (Ji et al., 2009). These compounds, with various pharmacological traits, have antioxidant, anticancer, anti-inflammatory, and antiviral activities. Today, many of the chemical drugs used in medicine are derived from natural compounds (Frabasile et al., 2017). The therapeutic strategy for natural products and chemical drugs is to target the protein molecules needed for each stage of the virus's life cycle (Dias et al., 2012). This section discusses the natural compounds' role in the inhibition of coronavirus infection (**Table 3**; **Figure 2**; **Figure 3**; and **Figure 4**). It is important to emphasize that within the compounds listed in **Table 3**, only epigallocatechin-3-gallate (NCT04446065) and resveratrol and zinc picolinate combination therapy (NCT04542993) has been investigated in clinical phase-2 trials against COVID-19 infection.

Targeting S Protein, ACE2, and TMPRSS2 to Inhibit Cell Attachment and Entry

When a coronavirus enters the host cell, it must first, through its S protein, bind to the specific receptors at the surface of host cells. The particular receptor for SARS-CoV and SARS-CoV-2 is the ACE2 protein. To reduce the viral entry strength, the expression of the virus S protein can be inhibited or the expression of ACE2 in host cells can be reduced by natural products. Studies have shown that the amino acid sequence of SARS-CoV Spike protein is approximately 76.47%, similar to SARS-CoV-2 (Xu et al., 2020b). Since virus S protein is highly glycosylated, the use of plant-derived lectins, which tend to bind to glycosylated proteins, can prevent the virus from binding to receptors on the cell surface. Previous studies have shown that herbal compounds could be used as inhibitors for the human immune deficiency virus and SARS-CoV feline (Pyrc et al., 2007). Emodin belongs to the anthraquinones compounds (a group of natural compounds), which have anti-inflammatory, anticancer, and antioxidant activities (Izhaki, 2002). In a dose-dependent manner, emodin could significantly inhibit the ACE2 and S protein interaction in the biotinylated Enzyme-Linked Immunosorbent Assay (ELISA) method and Vero E6 cell line. Therefore, we can consider this compound as an antiviral therapeutic agent in the treatment of SARS-CoV (Ho et al., 2007). Some drugs and substances could reduce the expression of the ACE2 receptor. As seen, interleukin-4 and interferon-γ decreased this receptor in Vero E6 cells (De Lang et al., 2006). Molecular docking and structure-based drug design study reveal that berberine, thebaine, mangiferin, piperine, nimbin, and curcumin have an inhibitory effect against ACE2 receptor and spike glycoprotein of SARS-CoV-2. Among these, curcumin and nimbin have shown the highest interaction with ACE2 receptors and spike glycoprotein than other natural compounds and chemical drugs such as hydroxychloroquine, nafamostat, and captopril (Maurya et al., 2020).

The citrus peel is rich in flavonoid and alkaloid compounds (such as naringin, naringenin, hesperetin, and hesperidin) that play an essential role in maintaining gastrointestinal health and improving the immune system. Naringin is an anti-inflammatory substance that decreases the inflammatory effect in LPS-induced RAW 264.7 macrophages and LPS-treated rats. Therefore, it can inhibit the increase of pro-inflammatory cytokines such as IL-6,

TABLE 3 | Different types of phytochemicals by their sources and function in the coronavirus infection.

Compound name	Phytochemical class	Source	Function	Clinical trial stage	References
Baicalein	Flavonoid	*Scutellaria baicalensis* Georgi, *Scutellaria lateriflora* L. (Lamiaceae)	Inhibit $3CL^{pro}$ of SARS-CoV-1	—	Su et al. (2020)
			Decreased the levels of IL-1β and TNF-α in serum during the SARS-CoV-2 infection	—	Song et al. (2020)
Broussochalcone B	Polyphenol	*Broussonetia papyrifera* (L.) L'Hér. ex Vent. (Moraceae)	Inhibit PL^{pro} of SARS-CoV-1 and MERS-CoV	—	Park et al. (2017)
Curcumin	Polyphenol	*Curcuma longa* L. (Zingiberaceae)	Inhibit the ACE2 receptor and spike glycoprotein of SARS-CoV-2	—	Maurya et al. (2020).
Emodin	Anthraquinone	*Rheum palmatum* L. (Polygonaceae)	Inhibit the interaction of SARS-CoV-1 S protein and ACE2	—	Ho et al. (2007)
			Inhibit 3a protein channel of SARS-CoV-1	—	Schwarz et al. (2011)
Epigallocatechin-3-gallate	Polyphenol	*Camellia sinensis* (L.) Kuntze (Theaceae)	Inhibit $3CL^{pro}$ of SARS-CoV-1 and SARS-CoV-2	Phase 2 (SARS-CoV-2)	Jang et al., (2020) and Nguyen et al. (2012)
Gallocatechin gallate	Polyphenol	*Camellia sinensis* (L.) Kuntze (Theaceae)	Inhibit $3CL^{pro}$ of SARS-CoV-2	—	Jang et al. (2020)
			Inhibit PL^{pro} and CL^{pro} of SARS-CoV-1	—	Nguyen et al. (2012) and Park et al. (2016)
Herbacetin	Flavonoid	*Linum usitatissimum* L. (Linaceae)	Block the proteolytic activity of SARS-CoV-2 $3CL^{pro}$	—	Jo et al. (2020a)
Hirsutanonol	Phenol	*Alnus glutinosa* (L.) Gaertn. (Betulaceae)	Inhibit PL^{pro} and $3CL^{pro}$ of SARS-CoV-1	—	Park et al., 2012a
Hirsutenone	Phenol	*Alnus japonica* (Thunb.) Steud. (Betulaceae)	Inhibits PL^{pro} activity of SARS-CoV-1	—	Park et al. (2012a) and Park et al. (2012b)
Iodobananin	Oligo-oxa-adamantane	Natural product derivative	Inhibit NTPase/Helicase of SARS-CoV-1	—	Tanner et al. (2005)
Isobavachalcone	Flavonoid	*Cullen corylifolium* (L.) Medik. (syn. *Psoralea corylifolia*) (Fabaceae)	Inhibit PL^{pro} of SAR-CoV-1	—	Kim et al. (2014)
Kaempferol 3,7-diglucoside	Glycosyloxyflavone	*Asplenium ruta-muraria* L., *Asplenium scolopendrium* L. (syn. *Asplenium altajense*) (Aspleniaceae)	Block virus ion channels in SARS-CoV-1	—	Schwarz et al. (2014)
			Target SARS-CoV2-S spike protein	—	Pan et al. (2020)
Kazinol F	Polyphenol	*Broussonetia papyrifera* (L.) L'Hér. ex Vent. (Moraceae)	Inhibit PL^{pro} of SARS-CoV-1 and MERS-CoV	—	Park et al. (2017)
Myricetin	Polyphenol	*Myristica fragrans* Houtt. (Myristicaceae)	Inhibit helicase and nsP13 of SARS-CoV-1	—	Yu et al. (2012)
Naringin	Flavonoid	*Citrus x aurantium* L., (Rutaceae)	Inhibit $3CL^{pro}$ of SARS-CoV-1	—	Nguyen et al. (2012)
Naringenin	Flavonoid	*Citrus* fruits (Rutaceae)	Two-Pore Channels (TPCs) inhibitor MERS-CoV	—	Pafumi et al. (2017)
			Block the enzymatic activity $3CL^{pro}$ in SARS-CoV-1	—	Jo et al. (2020b)
Pectolinarin	Flavonoid	*Cirsium chanroenicum* (Nakai) Nakai (Asteraceae)	Block the enzymatic activity of SARS-CoV-1 $3CL^{pro}$	—	Jo et al. (2020b)
Papyriflavonol A	Flavonoid	*Broussonetia papyrifera* (L.) L'Hér. ex Vent. (Moraceae)	Inhibit PL^{pro} of SARS-CoV-1 and MERS-CoV	—	Park et al. (2017)
Quercetin	Glycosyloxyflavone	*Allium cepa* L. (Amaryllidaceae)	Target SARS-CoV2-S spike protein	—	Pan et al., 2020
Quercetin 3-β-D glucoside	Flavonoid	*Passiflora subpeltata* Ortega (Passifloraceae)	Inhibit $3CL^{pro}$ of MERS- CoV	—	Jo et al. (2019)
Resveratrol	Polyphenol	*Vitis vinifera* L. (Vitaceae)	Inhibit nucleocapsid protein translation in and reduced the MERS-CoV-mediated apoptosis	Phase 2 (SARS-CoV-2)[a]	Lin et al. (2017)
Rhoifolin	Flavonoid	*Toxicodendron succedaneum* (L.) Kuntze (syn. *Rhus succedanea*) (Anacardiaceae)	Inhibit $3CL^{pro}$ SARS-CoV-1	—	Nguyen et al. (2012)
Rubranoside A	Diarylheptanoids	*Alnus hirsuta* (Spach) Rupr. (syn. *Alnus sibirica*) (Betulaceae)	Inhibit $3CL^{pro}$ and PL^{pro} SARS-CoV-1	—	Park et al. (2016)
Rubranoside B	Diarylheptanoids	*Alnus japonica* (Thunb.) Steud. (Betulaceae)	Inhibit $3CL^{pro}$ and PL^{pro} SARS-CoV-1	—	Park et al. (2016)
Scutellarein	Flavonoid	*Scutellaria lateriflora* L. (Lamiaceae)	Inhibit helicase, nsP13 SARS-CoV-1	—	Yu et al. (2012)
Vanillinbananin	Oligo-oxa-adamantane	Natural product derivative	Inhibit NTPase/Helicase of SARS-CoV-1	—	Tanner et al. (2005)

[a]*Resveratrol and Zinc Picolinate combination therapy.*

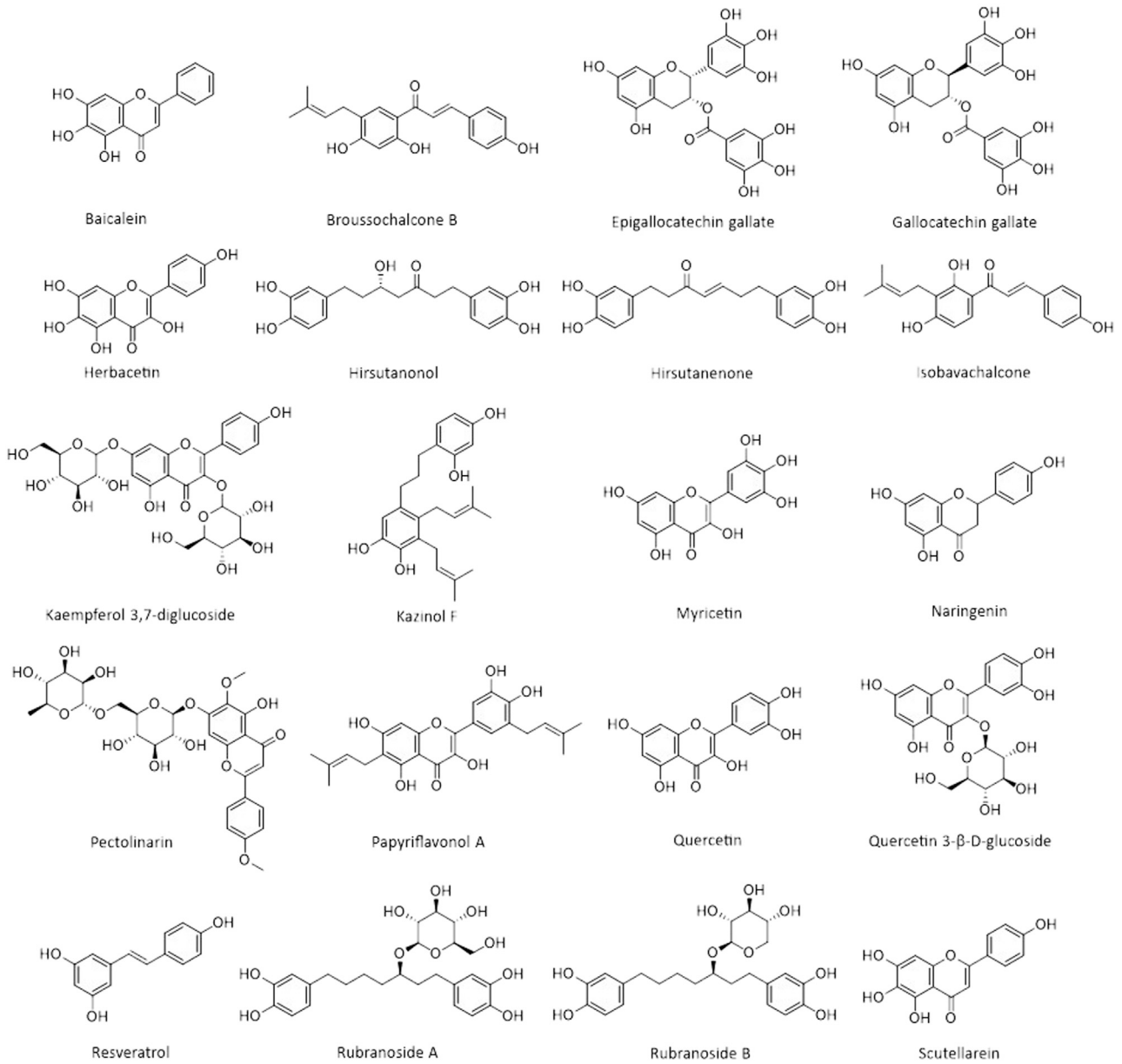

FIGURE 2 | Chemical structures of some natural products introduced in this study.

IL-1β, iNOS, and COX-2 induced by LPS treatment. Molecular docking investigation showed that naringin, naringenin, and hesperetin could bind to ACE2, such as chloroquine. Furthermore, these compounds can bind to ACE2 at lower binding energy levels than chloroquine (docking energy of 5.7 kcal/mol). However, more *in vitro* and *in vivo* experimentation is needed to determine whether these compounds are more effective than chloroquine (da Silva Antonio et al., 2020). However, it should be noted that inhibition of ACE2 due to its important physiological activities (such as homeostasis of blood pressure, protection against pulmonary cell destruction, electrolyte retention, and water) may have detrimental effects on patients' life. In addition to ACE2, Transmembrane Protein Serine 2 (TMPRSS2) is another essential protein for SARS-CoV-1 and SARS-CoV-2 infection. TMPRSS2 induces viral fusion with the host cell membrane through irreversible structural changes in viral S protein (Hoffmann et al., 2020). As a result, the inhibition of TMPRSS2 in the animal model's airways decreases the severity of lung damage after infection by SARS-CoV and MERS-CoV (Chikhale et al., 2020). Therefore, one of the strategies that could block the entry and restrict the pathogenesis of SARS-CoV-2 is the inhibition of TMPRSS2. The results of two independent studies using computational biology and molecular docking showed that some natural compounds such as neohesperidin, myricitrin, quercitrin, naringin, icariin, citicoline,

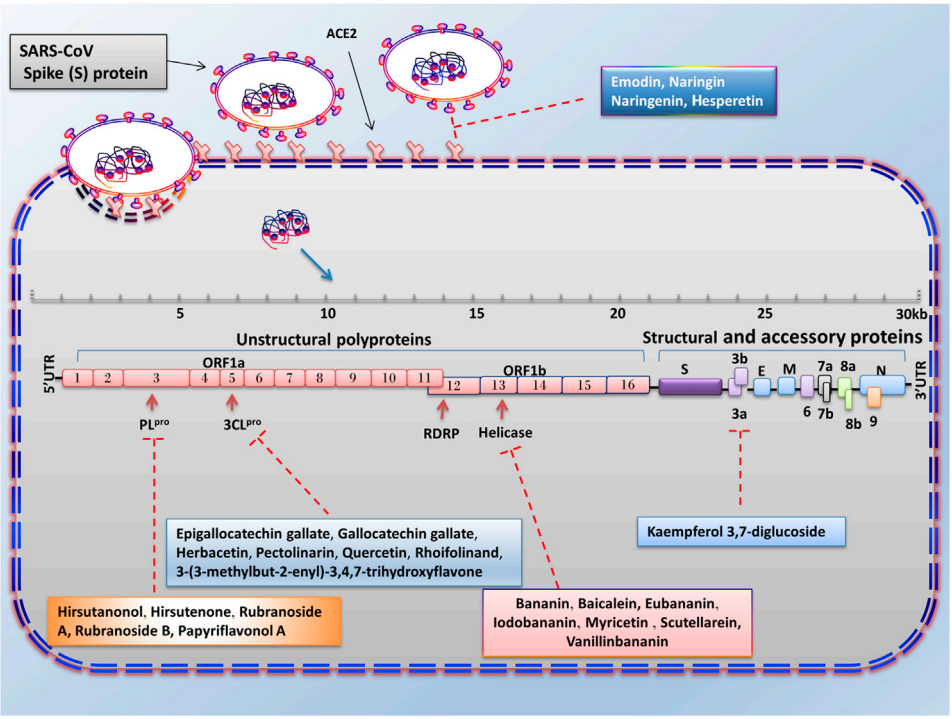

FIGURE 3 | Advantages of employing natural compounds against infection and replication of SARS-CoV. Some of the natural compounds by reducing the expression of $3CL^{pro}$, PL^{pro}, helicase, and 3a genes can play a therapeutic role in inhibiting the SARS-CoV infection.

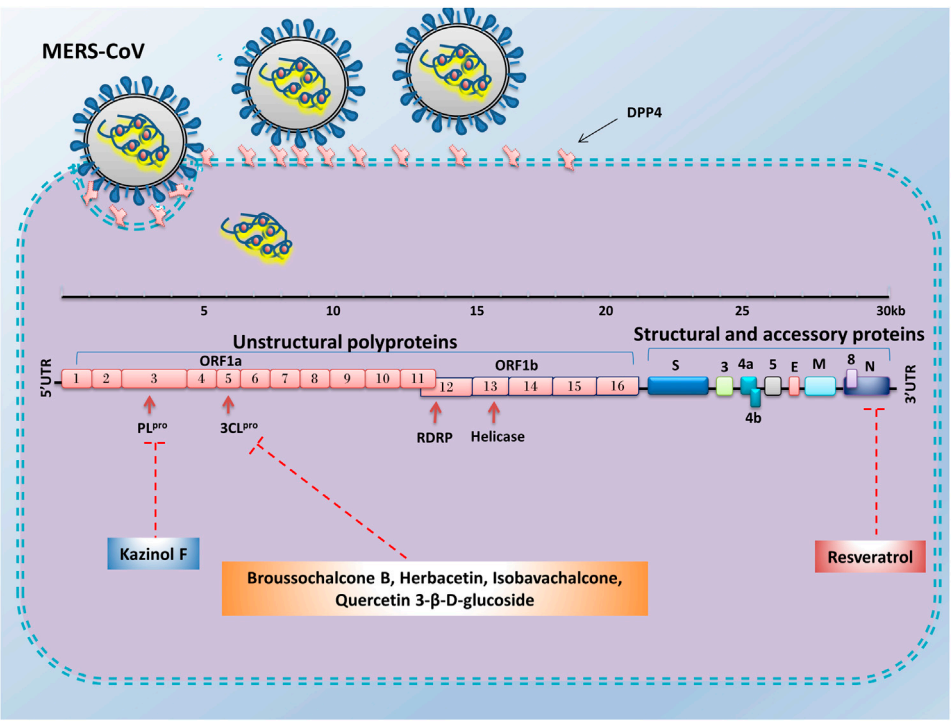

FIGURE 4 | Advantages of employing natural compounds against infection and replication of MERS-CoV. Some of the natural compounds by reducing the expression of nucleocapsid, $3CL^{pro}$, and PL^{pro} genes could play a therapeutic role in inhibiting the MERS-CoV infection.

bianthraquinone, isogemichalcone B, and (-)-epigallocatechin-(3-*O*-methyl) gallate could be used as a TMPRSS inhibitor (Chikhale et al., 2020; Rahman et al., 2020).

Targeting Helicase and Inhibiting Virus Replication

SARS-CoV-2 Helicase protein is encoded by the nsp 13, a gene located downstream of the RdRP. The inhibition of this protein could reduce virus replication (Ivanov et al., 2004). Helicases could separate the double-stranded Nucleic Acid (NA) by using free energy that is obtained from the hydrolysis of Nucleoside Triphosphate (NTP). Therefore, this protein is a potential target for antiviral drug development. Previous studies revealed that various natural products could suppress helicase (unwinding or ATPase) activity. In 2005, Tanner et al. investigated the effect of adamantane-derived bananins on SARS-CoV helicase.

Their results showed that iodobananin, vanillinbananin, bananin, and eubananin had an inhibitory effect on the helicase protein's ATPase activity with IC_{50} rates in the range 0.5–2.8 μM. Furthermore, using Fluorescence Resonance Energy Transfer (FRET) it was found that these four compounds could also inhibit unwinding helicase activity. The authors also observed that these compounds were ineffective against *E. coli* helicase but only affected SARS helicase, so they did not have a general helicase inhibitor activity. Finally, the results of cytopathic effects in fetal rhesus kidney-4 cells (FRhK-4) and RT-PCR demonstrated that these compounds could reduce virus replication without being toxic to the cell (Tanner et al., 2005). In 2012, the results of a study by Yu et al. showed that of the sixty-four natural compounds (flavonoids), only myricetin and scutellarein could reduce SARS-CoV helicase activity. The events showed that these two compounds could reduce Helicase ATPase activity by up to 90% at a dose of 10 μM without any toxicity effects on the healthy breast cell (MCF10A). Moreover, further analysis has shown that they do not change the helicase unwinding activity (Yu et al., 2012). Baicalein is another natural product that has an inhibitory effect on SARS-CoV (nsp13) helical activity. Studies have shown that this compound has no inhibitory effect on the dsDNA-unwinding activity of nsP13; however, it can decrease helicase ATPase activity up to 60% with an IC_{50} value 0.47 μM (Keum et al., 2013).

Targeting $3CL^{PRO}$, PL^{PRO} Protease and Inhibiting Virus Protein Processing

Another important antiviral strategy is the use of specific inhibitors against viral proteases such as $3CL^{pro}$ and PL^{pro}, which play an essential role in the processing and maturation of proteins and virus replication. The SARS-CoV-2 genome (positive single-stranded RNA), after translation, is capable of producing two polypeptides, pp1a and pp1ab. Finally, these two polypeptides, cleaved by $3CL^{pro}$ or PL^{pro} activity, produce sixteen non-structural proteins (Thiel et al., 2003; Muramatsu et al., 2016). Flavonoids are a group of phenolic compounds found in plants with anti-inflammatory, antiviral, antioxidant, and anticancer activities. There have been many reports of reduced coronavirus infection with these compounds (Tapas et al., 2008). *In vitro* studies in *Pichia pastoris* GS115 showed that epigallocatechin-3-gallate, quercetin, and gallocatechin gallate could reduce the expression of SARS-CoV $3CL^{pro}$. Molecular docking experiments and kinetic enzyme studies also revealed that these three compounds with IC_{50} rates in the range 47–73 μM could reduce protease activity up to 80%. In the meantime, gallocatechin gallate's effect was more significant than the other compounds (Nguyen et al., 2012). Rhoifolinand, herbacetin, and pectolinarin are other flavonoid compounds that have an adverse influence on $3CL^{pro}$. Studies with FRET protease assays and absorption spectroscopic studies have shown that these compounds can bind to $3CL^{pro}$ and significantly inhibit its protease activity (drug concentration less than 40 μM in IC_{50}) (Jo et al., 2020b). In another study, Jo et al. examined the inhibitory effects of several flavonoids compounds against MERS-CoV $3CL^{pro}$. Their results reveal the isobavachalcone, quercetin 3-β-D-glucoside, and herbacetin had noticeable inhibitory actions with IC_{50} values of 35.85, 37.03, 40.59 μM, respectively (Jo et al., 2019). The $3CL^{pro}$ of SARS-CoV-2 is highly conserved among all CoVs and has approximately 96% similarity with SARS-CoV-1. Due to its essential role in viral replication, it is a potential therapeutic target for COVID-19 (Xu et al., 2020c). Since natural products derived from microbial sources have a unique chemical diversity compared to plant products, more than 50% of FDA-approved natural compound-based medicines are derived from microbial compounds. The virtual screening and molecular binding by Sayed et al. showed that citriquinochroman, holyrine B, proximicin C, and several other microbial compounds could inhibit $3CL^{pro}$ of SARS-CoV-2 (Sayed et al., 2020). In addition to microbial compounds, molecular docking and MD simulation studies have shown that some marine natural products including hydroxypentafuhalol, pentaphlorethol B, and luteolin-7-rutinoside (ΔG about −14.6 to −10.7 kcal/mol) can also inhibit $3CL^{pro}$ of SARS-CoV-2 (Gentile et al., 2020).

The Papain-like protease (PL^{pro}), is another protease that controls the proliferation of SARS-CoV-2 and is known as a potential target for treating this virus. Previous studies have shown that *Alnus japonica* (Thunb.) Steud. (Betulaceae) has anticancer, anti-inflammatory, and anti-influenza properties. The study of Kim et al. showed that the natural phenolic compounds (diarylheptanoids) prepared from this plant could change the proteolytic activity of PL^{pro}. The fluorometric assay results showed that among the nine extracted substances, hirsutenone, hirsutanonol, rubranoside B, and rubranoside A had a dose-dependent inhibitory effect against PL^{pro}. Among these compounds, hirsutenone had a remarkable inhibitory effect on SARS-CoV PL^{pro} (IC_{50} = 4.1 μM) and $3CL^{pro}$ (IC_{50} = 36.2 μM) enzyme activity. Further study showed that this substance, containing an α, β-unsaturated carbonyl group with a catechol moiety in the backbone, and the presence of this structure played an essential role in its inhibitory effects (Park et al., 2012a). In addition, polyphenols derived from the root of *Broussonetia papyrifera* (L.) L'Hér. ex Vent. (Moraceae) have been shown to have good inhibitory potential against SARS-CoV and MERS-CoV proteases. Park et al. investigated the inhibitory effect of ten

natural compounds derived from this plant on SARS-CoV and MERS-CoV proteases. The effect of these compounds on SARS-CoV proteases showed that the papyriflavonol A (Broussonol E) was the most effective inhibitor of PL^{pro} (IC_{50} value 3.7 μM) and 3-(3-methylbut-2-enyl)-3,4,7-trihydroxyflavone was the most useful inhibitor of $3CL^{pro}$ (IC_{50} value of 30.2 μM). Additionally, broussochalcone B (Bavachalcone), with a concentration of 27.9 μM (IC_{50}) and kazinol F with a concentration of 39.5 μM (IC_{50}), were able to reduce the activity of MERS-CoV $3CL^{pro}$ and MERS-CoV PL^{pro}, respectively (Park et al., 2017).

Targeting Nucleocapsid (N) Protein to Inhibit Virus Infection and Replication

Resveratrol is a natural compound with anti-inflammatory, antioxidant, and anticancer properties (Yeung et al., 2019; Filardo et al., 2020). The previous study demonstrated that this compound has antiviral activity and can inhibit viral infections caused by Herpes Simplex Virus (HSV), Respiratory Syncytial Virus (RSV), and Epstein-Barr Virus (EBV) (Faith et al., 2006; Zang et al., 2011; De Leo et al., 2012). Also, resveratrol by repressing the expression of MERS-CoV N protein in the Vero E6 cell line, could reduce RNA expression, viral yield, and replication of MERS-CoV. Additionally, it significantly decreases the virus's infection and enhances infected cells' survival by repressing Caspase 3 cleavage (Lin et al., 2017). Other natural compounds that can reduce coronavirus infection risk include the alkaloids fangchinoline, cepharanthine, and tetrandrine. The results of a study by Kim et al. showed that these compounds could inhibit the expression of pro-inflammatory cytokines (IFN-α1, IL-6, IFN-β1, IL-8, and IL-1) caused by human coronavirus OC43 infection in the MRC-5 cell line. These three natural compounds can also decrease the OC43 replication by inhibiting N protein expression and reducing the cytotoxic effect of this virus in the MRC-5 cell line, and increasing the proliferation and survival of MRC-5 human lung cells (Kim et al., 2019).

Targeting 3A Protein and Inhibiting Virus Release

The production and release of the virus require some ion channels in the host cell membrane. Therefore, inhibition of these ion channels played a significant role in inhibiting viral infections, so one antiviral strategy is to use compounds that can restrain these channels (Liang and Li, 2010). One of these ion channels is the cation-selective channel (3a protein) generated by the ORF3a of the SARS-CoV genome. Kaempferol glycoside is a natural flavonol found in a variety of plants. Previous studies have shown that this compound has antiviral properties (IC_{50} value of 2.3 μM) and can inhibit the expression of 3a protein SARS CoV (an ion channel) in *Xenopus* oocyte as a model system (Schwarz et al., 2014).

RNA INTERFERENCE

RNA interference (RNAi) are RNA molecules found in many eukaryotes that inhibit gene expression by targeted 3UTR of mRNA molecules. Today, siRNA and miRNA are the most common type of RNAi used for gene silencing. RNAi origin can be endogenous (originating in the cell) and exogenous (coming from a virus or laboratory tools). Exogenous RNAi can be transmitted to cells using electroporation, viral vectors, liposomes, and calcium phosphate (Sohrab et al., 2018). When inserting into the cell, synthetic siRNAs are cleaved by Dicer in the cytoplasm. After placement in the RNA-Induced Silencing Complex (RISC) and Ago2, RNAi molecules (**Figure 5**) become single-stranded RNA and can destroy the target mRNA or inhibit its translation (Ding et al., 2018).

The Therapeutic Effect of siRNA on Coronaviruses

Synthetic siRNAs have about 21–23 bp length and perform their role by inhibiting gene expression at the post-transcription level. Unlike miRNAs, each siRNA is designed against a specific gene, so it can only impede that gene expression (Taxman et al., 2006). The siRNAs are first inserted into the cell as long double-stranded RNAs and then are cleaved by RNase III (Dicer) in the cytoplasm to become small dsRNAs of approximately twenty-one base pairs. This dsRNA later enters the RISC and converts to single-stranded RNA (ssRNA). If RISC and siRNAs complex could find the specific target site on mRNA, it could cleave the mRNA, and cellular exonucleases could invade to destroy the target mRNA (Wu and Chan, 2006). Studies have shown that this type of RNAi has the potential to act as antiviral agent to reduce replication and infection of many viruses such as HIV, Flock House Virus (FHV), Hepatitis C Virus (HCV), and Hepatitis B Virus (HBV) (Hamasaki et al., 2003; Liu et al., 2017; Shahid et al., 2017; Taning et al., 2018). Previous studies have shown that siRNA could target different parts of the virus genome to reduce the replication and infection of SARS-CoV (**Figure 6A**). For example, targeting the nsp1 gene (from nucleotides 250–786) by siRNA is one of the best ways to control SARS-CoV. Because targeting this area of the genome inhibits virus propagation, pathogenesis, and replication in Vero E6 cells (Ni et al., 2005). Another strategy that can help to suppress infection and replication of SARS-CoV is inhibiting the virus S protein or its specific receptor ACE2.

The SARS-CoV Spike protein is an essential viral surface glycoprotein for identifying target cells and interacting with ACE2 host cell receptors (Gallagher and Buchmeier, 2001). A study by Zhang et al. reveals that inhibiting expression of S protein using specific siRNAs could reduce the viral titers, infection, and replication of SARS-CoV in Vero E6 and 293T cells (Zhang et al., 2004). RNAi technology is also a useful instrument to suppress ACE2 expression at the host cells' surface to counteract SARS-CoV infection. In addition to the lungs, ACE2 is expressed on the surface of the bronchial, renal, duodenum, colon, gastrointestinal, and cardiovascular cells (Donoghue et al., 2000). Inhibition of the ACE2 protein in Vero E6 cell lines using siRNA could reduce replication, copies number, and infection of severe acute respiratory syndrome-associated coronavirus (Lu et al., 2008). Li et al. investigated the effect of siSC5 (nsp12 region) and siSC2 (spike protein) against SARS-CoV on *in vitro* and *in vivo* models. Their results showed

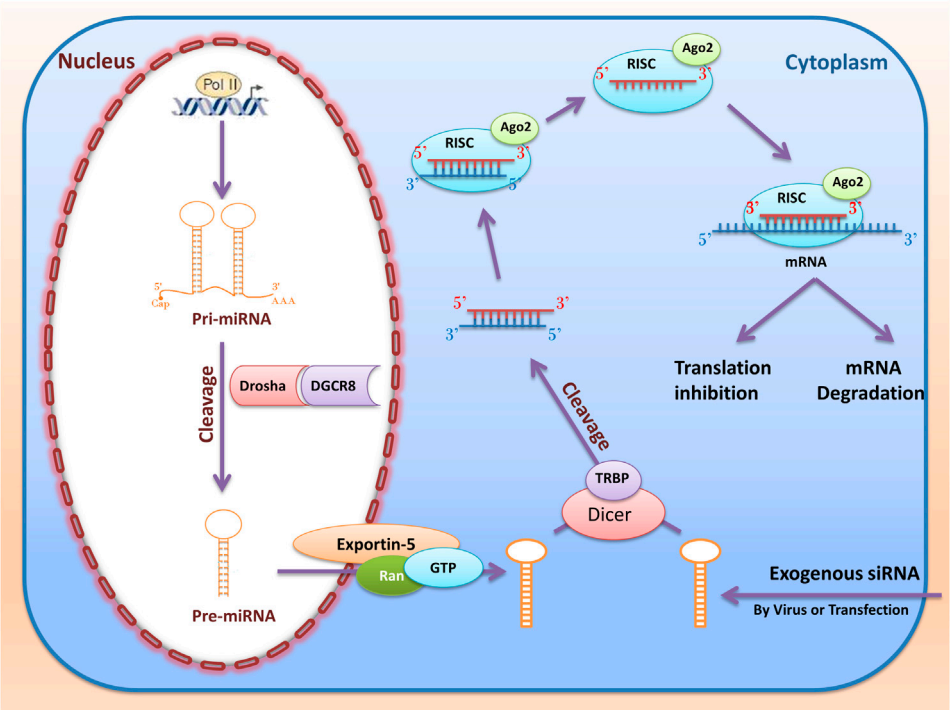

FIGURE 5 | RNA interference (miRNAs and siRNAs) biogenesis and function. The miRNAs first transcribed from the nucleus genome as pri-miRNA. Then pri-miRNA cleavage with Drosha and DGCR and converted to pre-miRNA. Then, RanGTP and exportin 5 cause the pre miRNA to be transported from the nucleus to the cytoplasm and cleavage by Dicer and TRBP. Ultimately, after entering the RISC complex, mature miRNA can be attached to the target mRNA and perform their function by destroying mRNA or inhibiting the translation. When inserting into the cell, synthetic siRNAs are cleaved by Dicer in the cytoplasm. This dsRNA enters RISC, and if cold finds the target mRNA, the mRNA is cleaved by the RISC and Ago2.

that these two siRNAs by inhibited virus replication in FRHK-4 cells could reduce the virus infection's effects and symptoms without having any toxicity effect on *Rhesus macaque* (dosages of 10–40 mg/kg) (Li et al., 2005a).

The spike protein structure determines the type of host cells which could be infected by a virus. However, variations of S protein are seen among the various strains of coronavirus. Therefore, one of the essential strategies for controlling viral infections targets the conserved genome areas between various coronaviruses (Kuo et al., 2000). Hence, Wang et al. tried to use two specific siRNAs against the conserved sequence of the SARS-CoV. The study results showed that by targeting two regions (14,450–14,468 and 15,877–15,895), encoding RNA polymerase could inhibit expression of RNA polymerase, N protein, $3CL^{pro}$ and also reduce replication and cytopathic effect of the virus (Wang et al., 2004). Among the two COVID-19 proteases, the nsp3 sequence encoding papain-like protease is less conserved, while the nsp5 sequence encoding $3CL^{pro}$ is highly conserved and can be selected as a potential target of siRNA for COVID-19 treatment (Liu et al., 2020).

The RdRP gene encodes a key enzyme for the replication of the virus. In SARS-CoV-2, this gene is located in ORF1b by 645 bp long and has been reported to be highly conserved, so it has the potential to target several siRNAs. Also, investigations of the RdRP sequence have shown that this gene has no genetic similarity to human genes and other coronaviruses (Wu et al., 2020). Lu et al.'s investigation shows using specific siRNA can reduce SARS-CoV RdRP expression by more than 90% in HeLa and 239T cell lines, leading to inhibition of plaque formation in Vero E6 cells. Therefore, its suppression could be considered a suitable therapeutic target in COVID-19 patients (Lu et al., 2004). The siRNAs could target other essential coronavirus genes. For example, by designing three different siRNAs, Shi et al. were able to reduce the expression of N, E, and M genes of SARS-CoV in Vero E6 cells (Yi et al., 2005). Furthermore, the replication of SARS-CoV could be inhibited by targeting the leader sequence using specific siRNA in Vero E6 cells (Li et al., 2005b).

Although previous studies have shown that RNAi has antiviral potential, it appears that viruses can use these molecules to their advantage. MERS-CoV could infect both human and bat cell lines. Nevertheless, its pathogenic power and replication are varied between the bat (*Eptesicus fuscus*) cells and human (A549, MRC5, and Huh7) cell lines. In human cells, MERS-CoV shut-down interferon antiviral responses in the innate immunity system, unlike in bat cells by inhibition of IRF3 (a critical activator for INF β expression) (Banerjee et al., 2019).

Complemented palindromic small RNAs (cpsRNAs) are a group of small RNAs produced by mammalian and invertebrate viruses. There are sequences in the SARS-CoV (ORF3b) genome that have the origin of a cpsRNA called SARS-CoV-cpsR-19. The apoptosis assay events indicate that SARS-CoV-cpsR-19 could induce apoptosis in HeLa cells by

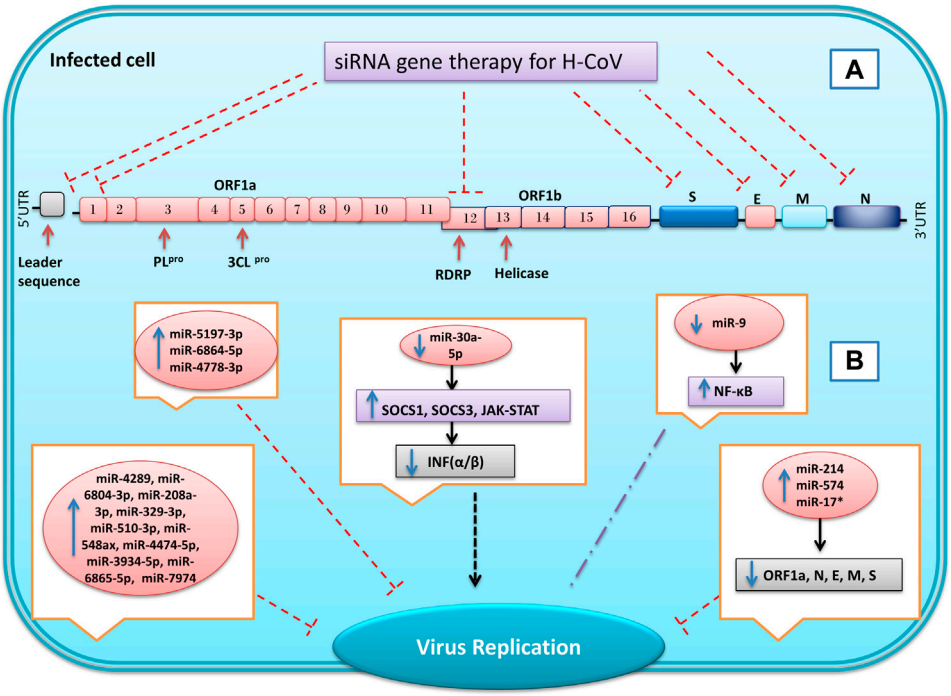

FIGURE 6 | Inhibition of genes expression of SARS coronavirus using miRNAs and synthetic small interfering RNAs (siRNA). By designing specific siRNAs, it is possible to inhibit the expression of the virus's structural and non-structural genes for reduce the replication and infusion of the virus **(A)**. In coronavirus infection, the microRNAs expression of host cells changes in response to infection. Some of these changes are the cell's response to the infection, and others are caused by the virus, which can eventually lead to a reduction or increase in virus replication and infection **(B)** Inhibition ⊤, Induction ⇣, not define ⋮, Up-regulation ↑, Down-regulation ↓.

increasing the caspase 3 and BAX/BCL2 ratio and may play an essential role in SARS-CoV pathogenesis (Liu et al., 2018). It has been shown that some viruses (such as the Ebola virus and influenza A) could preserve themselves from RNAi-based immune systems and facilitate their replication by using Viral Suppressors of RNA silencing (VSR). Studies of Cui et al. have shown a short hairpin RNA (a novel VSR) in the SARS-CoV nucleocapsid protein sequence that defeats RNAi-triggered suppression (Cui et al., 2015). Moreover, N protein overexpression in Neuro-2a cells efficiently inhibits Dicer-mediated dsRNA cleavage and could increase replication and titration of MHV-A59 (a close relative to SARS-CoV in Coronaviridae family) viruses. The SARS-CoV-2 (N) nucleocapsid protein can also act as an escape agent from the immune system and contribute to its pathogenicity. N proteins have high homology (94%) of the amino acid sequences among coronaviruses. A recent study has shown that the N protein of SARS-CoV-2 has VSR activity that can antagonize RNAi in both effectors (recognition and cleavage of viral dsRNA by Dicer) and initiation (siRNA biogenesis) steps (Mu et al., 2020).

MicroRNAs

The miRNAs are a group of small non-coding RNAs with twenty-two nucleotides of length. These molecules are first transcribed from the nucleus genome (exons, introns, and intergenic regions) as pri-miRNA with a length of two hundred nucleotides to several thousand nucleotides (Booton and Lindsay, 2014). It is noteworthy that each pri-miRNA can be a precursor to several mature miRNAs. Then, first processing of pri-miRNA starts with RNase III (Drosha) and its cofactor (DGCR) to form pre-miRNA, a hairpin with a length of about sixty nucleotides. In the next step, RanGTP and exportin 5 cause the pre-miRNA to be transported from the nucleus to the cytoplasm. Dicer and TRBP performed the second processing in the cytoplasm to create a double-stranded RNA molecule with a length of about twenty-two nucleotides (Bartel, 2004). Ultimately, after entering the RISC complex, one of the strings (passenger strand) is destroyed, and mature miRNA (guide strand) can be attached to the target mRNA. MiRNAs perform their function by destroying mRNA or inhibiting the translation (Vazquez, 2006). Previous studies have shown that a miRNA alone can regulate the expression of several different genes by its function. Meanwhile, various miRNAs can simultaneously control the expression of one mRNA. However, about 60% of human genes can be regulated by these molecules (Kalhori et al., 2020). For the first time, Lecellier et al. (2005) reported that cellular miR-32 could reduce virus replication of the primate foamy virus (PFV-1) by targeting the viral RNA genome (Lecellier et al., 2005).

MicroRNA Regulates the Innate Immune System, Virus Replication, and Pathogenesis of Coronavirus

The innate immune system is the body's first defense against viruses and bacteria. This system's principal cells include macrophages, dendritic cells, natural killer cells, monocytes, and granulocytes

(Leon-Icaza et al., 2019). Viruses, to increase replication, suppress the host's innate immune system via reducing INF α/β production. For example, Japanese Encephalitis Virus (JEV), Dengue Virus (DENV), and Enterovirus 71 (EV71) are able to inhibit the overexpression of INF α/β in response to viral infection by enhancing the expression of miR-146a in infected cells (Wu et al., 2013; Ho et al., 2014; Sharma et al., 2015). Viruses can also decrease the innate immune system by inhibiting the expression or function of some miRNAs. For instance, in oligodendroglioma cells, the Borna Disease Virus (BDV) can inhibit the expression of miR-155 by its specific phosphoprotein, thus inhibiting INF α/β overexpression in response to viral infection and reducing the innate immune system (Zhai et al., 2013). Therefore, one of the main antiviral components of the intrinsic immune system is type 1 interferons (INF α/β).

Coronaviruses can prevent the induction of the immune system in response to viral infection with different strategies. *In vivo* and *in vitro* studies of Ma et al. on the Transmissible Gastroenteritis Virus (TGEV), a member of the *alpha-coronavirus* family, showed that this virus could downregulate miR-30a-5p expression. Furthermore, they found that virus replication was facilitated by reducing IFN-I signaling cascades via removing the inhibitory effect of miR-30a-5p on INF negative regulators (such as SOCS1, SOCS3, and JAK-STAT) (Ma et al., 2018). Therefore, the overexpression of miRNAs in infected cells could increase the innate immune system and may be considered as a therapeutic approach for treatment.

Although it has previously been reported that miRNAs play a significant role in regulating the eukaryotic gene, subsequent studies showed that these nano molecules can also alter the virus's replication to increase or decrease its infection (**Figure 6B**). For example, miR-122 plays an essential role in the pathogenesis and replication of the Hepatitis C virus. The miR-122, to increase the virus's stability and replication bind to the 5′ non-translated regions (NTRs) of the virus and repress RNA degradation via exonucleases. Therefore, the knockout of this miRNA in Huh-7 cells could reduce HCV replication (Jopling et al., 2005). In contrast, miR-32 could negatively alter the replication of the PFV-1 by targeting viral genes in the human HEK-293T cell line (Lecellier et al., 2005).

Some viruses have sequences (hairpin) in their genomes similar to miRNAs and can regulate the gene expression of the host cell or virus (Grundhoff and Sullivan, 2011; Kincaid and Sullivan, 2012). A computational approach study by Hassan et al. showed there are several hairpins in the genome of the MERS that could act as a precursor for thirteen miRNAs, which were significantly similar to human miRNAs. Their study showed ten miRNAs (miR-4289, miR-6804-3p, miR-208a-3p, miR-329-3p, miR-510-3p, miR-548ax, miR-4474-5p, miR-3934-5p, miR-6865-5p, and miR-7974) of these, miRNAs do not have any known specific biological function in humans or animals at all. Nevertheless, miR-18a, miR-628, and miR-342-3p had a biological role in humans related to Basal Cell Carcinoma (BCC) of the skin, malignant glioblastoma, and late-stage prion disease, respectively (Hasan et al., 2014). Numerous studies and reports suggest that some miRNAs have antiviral activity and can be used against influenza, HIV, HBV, and poliovirus (PV) (Sanghvi and Steel, 2012; Zhang et al., 2013; Shim et al., 2016; Hamada-Tsutsumi et al., 2019). On the other hand, viruses could alter the gene and miRNA expression profile in the host cell. For example, miR-146a and miR-130b upregulated by the human T Cell Leukemia Virus (HTLV-1) in PBMC cells (Bouzar and Willems, 2008).

Nucleocapsid (N) protein is a structural protein that has the same function in all coronaviruses. The human coronavirus CoV-OC43 could inhibit miR-9 function by its N protein and increase NF-κB expression in 293T cells. However, it is unclear whether upregulation NF-κB is a suitable response for virus replication or a secondary inhibitor for virus replication (Lai et al., 2014). Infection of bronchoalveolar stem cells (BASICs) by SARS-CoV reveals that this virus could upregulate the expression of miR-574-5p, miR-214, and miRNAs-17* 2–4 fold. Moreover, overexpression of these miRNAs could repress SARS-CoV replication by targeting the four viral structure proteins (E, S, M, N), and orf1a (Mallick et al., 2009). Unfortunately, to date, not many *in vivo* and *in vitro* studies have been performed on RNAi's role in inhibiting the COVID-19, and most studies have been performed base on bioinformatics and *in silico* studies. Qingfei Paidu decoction (QFPD) contains twenty-one traditional Chinese medicines that have been used to treat COVID-19 since February 7, 2020. Chen et al.'s molecular docking study revealed that QFPD can bind to structural and non-structural proteins of COVID-19. They also found that miR-183 and miR-130A/B/301 predict targets of QFPD, and QFPD by these microRNAs may exert anti-SARS-CoV-2 activity (Chen et al., 2020a). In a bioinformatics approach study performed by Khan et al., it was found that several miRNAs can have antiviral properties in infections caused by SARS-CoV-1 and SARS-CoV-2. For example, evidence has shown miR-323a-5p, miR-622, miR-198, and miR-654-5p for SARS-CoV-1 and miR-323a-5p, miR-20b-5p, miR-17-5p for SARS-CoV-2 have antiviral roles by targeting the ORF1ab and the S region (Khan et al., 2020).

Therefore, it is likely miRNA with low side effects can be used as a therapeutic agent for the COVID-19 treatment. Differences in miRNA expression profiles in individuals are probably one reason why COVID-19 causes death in some people and causes only brief symptoms in others. As a result, microRNAs have recently emerged as a critical factor in increasing or inhibiting the potential of viral infection. We hope that clinical and preclinical research can use them in gene therapy as antiviral agents soon.

CONCLUSION

Today, the whole world is suffering from a pandemic disease called COVID-19, which has caused deaths in many developed and developing countries. Despite all the advances in human medicine, we have not yet been able to find a suitable treatment for this viral disease. The use of molecular or pharmacological methods to control infection or virus replication requires identifying essential genes involved in infection and replication of the virus. Two strategies are suggested to treat this disease. The first step is to reduce the virus's infection by preventing the virus from attaching to its specific receptor. The next step is to reduce the

virus's replication by inhibiting the virus's structural and non-structural genes. Medicinal plants and natural products are a good option for preventing and treating viral infections, especially COVID-19, due to their lower cost, lower side effects, and natural origin compared with chemical drugs. These compounds could increase efficiency and strengthen the host immune system against many infections and diseases due to their inherent properties. In the treatment of COVID-19, these compounds can reduce virus infection or replication by repressing the virus's coupling to the host cell's receptors or by inhibiting the expression of structural and non-structural genes. Moreover, RNAi (siRNA and miRNA) could inhibit viral infections, especially COVID-19, by inhibiting essential virus genes or inducing a host immune system. Therefore, the simultaneous use of natural compounds and RNAi can play a critical role in the treatment of SARS-CoV-2 and restraining this pandemic pneumonia.

AUTHOR CONTRIBUTIONS

The study was conceptualized by MS and EA. The methodology was given by EA and MF. Writing and original draft preparation was done by MK and FS. Writing, review, and editing were done by MF, IA, and JE. Funding acquisition was provided by MK and JE. All authors contributed to the article and approved the submitted version.

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Prospective: Evolution of Chinese Medicine to Treat COVID-19 Patients in China

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During the outbreak of the novel coronavirus disease (COVID-19), the Chinese government took a series of public health measures to tackle the outbreak and recommended six traditional Chinese medicine (TCM) evolved formulas, collectively referred to as "3-drugs-3-formulas", for the treatment. In this prospective article, we will discuss how these six formulas evolved from TCM and what their underlying mechanisms of actions may be by evaluating the historical usage of the component formulas, the potential targeted pathways for the individual herbs used by STAR (signal transduction activity response) database from our laboratory, and the pathogenesis of COVID-19. Five of the six recommended formulas are administered orally, while the sixth is taken as an injection. Five classic categories of herbs in the six formulas including "Qing-Re", "Qu-Shi", "Huo-Xue", "Bu-Yi" and "Xing-Qi" herbs are used based on different stages of disease. All five oral formulas build upon the core formula Maxingshigan Decoction (MD) which has anti-inflammatory and perhaps antiviral actions. While MD can have some desired effects, it may not be sufficient to treat COVID-19 on its own; consequently, complementary classic formulas and/or herbs have been added to potentiate each recommended formula's anti-inflammatory, and perhaps anti-renin-angiotensin system (RAS)-mediated bradykinin storm (RBS) and antiviral effects to address the unique medical needs for different stages of COVID-19. The key actions of these formulas are likely to control systemic inflammation and/or RBS. The usage of Chinese medicine in the six formulas is consistent with the pathogenesis of COVID-19. Thus, an integrative systems biology approach—combining botanical treatments of conventional antiviral, anti-inflammatory or anti-RBS drugs to treat COVID-19 and its complications – should be explored.

Keywords: COVID-19, Chinese medicine, 3-drugs-3-formulas, pathogenesis, cytokine storm, RAS-mediated bradykinin storm

INTRODUCTION

In late December 2019, pneumonia clusters from unknown causes were reported in Wuhan, China. A novel β-coronavirus strain, belonging to the same family as the SARS-associated coronavirus (SARS-CoV) and Middle East Respiratory Syndrome coronavirus (MERS-CoV), was identified as the cause of these pneumonia outbreaks (Petrosillo et al., 2020). This novel β-coronavirus was named 2019 novel coronavirus (2019-nCoV). Its entire viral genome sequence was uploaded to virological.org and GenBank by a consortium led by Yong-Zhen Zhang (Zhang and Holmes, 2020) on January 11, 2020. Subsequently, the International Committee on Taxonomy of Viruses (ICTV) renamed the strain "Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)" on February 11, 2020 and the World Health Organization (WHO) announced "COVID-19" as the official name of this new disease.

At the start of the COVID-19 outbreak, there were no effective treatments available. The Chinese government referenced and applied lessons learned from the SARS outbreak in 2003 and took several bold actions to control COVID-19. First, to slow down and prevent infections, on January 23, 2020, the Chinese government unprecedently locked down a city. Wuhan, with a population of 10 million people, required mask-wearing and enforced a stay-at-home order. This policy was then extended to all high-risk areas across China. Second, local governments constructed new hospitals and added hospital beds to treat severe COVID-19 patients, and remodeled large public facilities into quarantine centers for asymptomatic carriers and mild COVID-19 patients. Third, Chinese scientists isolated and characterized SARS-CoV-2 and then developed and mass-produced detection kits for SARS-CoV-2 to speed up screening. Fourth, researchers from different institutions developed *in vitro* and cell culture methods to test all available current medicines and TCM herbs (based on their historical usages) to identify potential treatment candidates for the range of unmet clinical needs of COVID-19 patients at different stages of disease progression. Researchers also developed new TCM based treatment protocols. Clinicians began examining the potential of herbal formulas to prevent infection and to treat COVID-19. Fifth, scientists began using the viral sequence to explore vaccine approaches. After three months of concerted effort, China was able to significantly reduce COVID-19 deaths. On March 19th, Wuhan reported no new COVID-19 cases.

Currently, the Chinese government recommends six formulas for the treatment of COVID-19, collectively referred to as "3-drugs-3-formulas". Of these formulas, one is an injectable form and five are administered orally. In this discussion, we will share our perspective on how the six formulas evolved from TCM and we will discuss some scientific basis to support the ways in which these formulas could treat different stages of COVID-19.

PATHOGENESIS AND CONVENTIONAL TREATMENT OF COVID-19

COVID-19 patients may exhibit a wide range of symptoms as the disease progresses. Common mild and moderate symptoms include fever, dry cough, fatigue, loss of smell, and diarrhea (Farah Yusuf Mohamud et al., 2020). In severe and critical stages, patients may develop pulmonary symptoms such as acute respiratory distress syndrome (Jin et al., 2020). Patients with pre-existing conditions, such as hypertension, obesity, and diabetes, would exhibit a higher risk of disease progression and lower survival rate (Jordan et al., 2020). Men infected with COVID-19 have lower immune response ability and a higher mortality rate compared to women (Scully et al., 2020). This could also be attributed to sex-determining gene expression, chromosomes, and/or hormones (Li et al., 2020). When COVID-19 progresses to a severe stage, significantly higher plasma levels of inflammatory related cytokines, including IL-1, IL-6, IL-7, G-CSF, IP-10, MCP1, MIP1α and TNFα were found, resulting in cytokine storms (**Figure 1A**). Studies have suggested that cytokine storms correlate directly with lung injury, multi-organ failure, and an unfavorable prognosis of severe COVID-19 cases (Huang et al., 2020; Ragab et al., 2020; Ruan et al., 2020). Therefore, tackling cytokine storms in the later-stages of COVID-19 might be key to shortening the course of the disease, decreasing mortality rate, and improving the prognosis of COVID-19 patients. Recent research suggests that renin-angiotensin system (RAS)-mediated bradykinin storm (RBS) plays a key role in the pathogenesis of COVID-19 (Garvin et al., 2020). This is now being further investigated.

Physical life support systems such as ventilators, extracorporeal membrane oxygenation (ECMO), artificial liver support systems (ALSS), and blood purification systems, are commonly used to support the life of COVID-19 patients. Drugs used for treatment of COVID-19 in different parts of the world, particularly Western medicine-practicing countries, may vary greatly due to the nature of, and the principles behind the medicines used.

Treatment of COVID-19 in countries practicing Western medicine are based on a pathogenesis perspective. Disease progression can be separated into three stages: early infection, pulmonary phase, and severe hyperinflammation and RBS phase (**Figure 1A**). The antiviral drug, Remdesivir, demonstrates its benefit when used as a treatment during the pulmonary phase, while the anti-inflammatory drug, dexamethasone, combined with oxygen, has been used to treat the pulmonary to hyperinflammation and RBS stages (IIB amd III) of COVID-19 (**Figure 1A**) (Siddiqi and Mehra, 2020). There is some dispute over the efficacy of Remdesivir as some clinical trials have failed to show any clinical benefits (Wang et al., 2020c; Spinner et al., 2020). However, Beigel et al. suggested that Remdesivir was superior to placebo in shortening recovery time in adults hospitalized with COVID-19. They also showed evidence of Remdesivir reducing respiratory tract infection (Beigel et al., 2020). Convalescent plasma and monoclonal antibodies may be helpful for severely ill patients as a passive antibody treatment; however, more rigorous clinical trials are needed to prove this (Roback and Guarner, 2020). Recently, a new hypothesis, RBS, has been developed to explain the wide range of symptoms caused by COVID-19 infection. The RBS theory could account for the increased vascular permeability that causes fluid leakage into lung tissue (Garvin et al., 2020). Thus, it has been suggested that existing FDA-approved pharmaceuticals for treating RBS including Vitamin D may be useful for reversing or treating COVID-19. It should be pointed out that angiotensin-converting enzyme 2 (ACE2) is not only a SARS-CoV-2 receptor but it is also

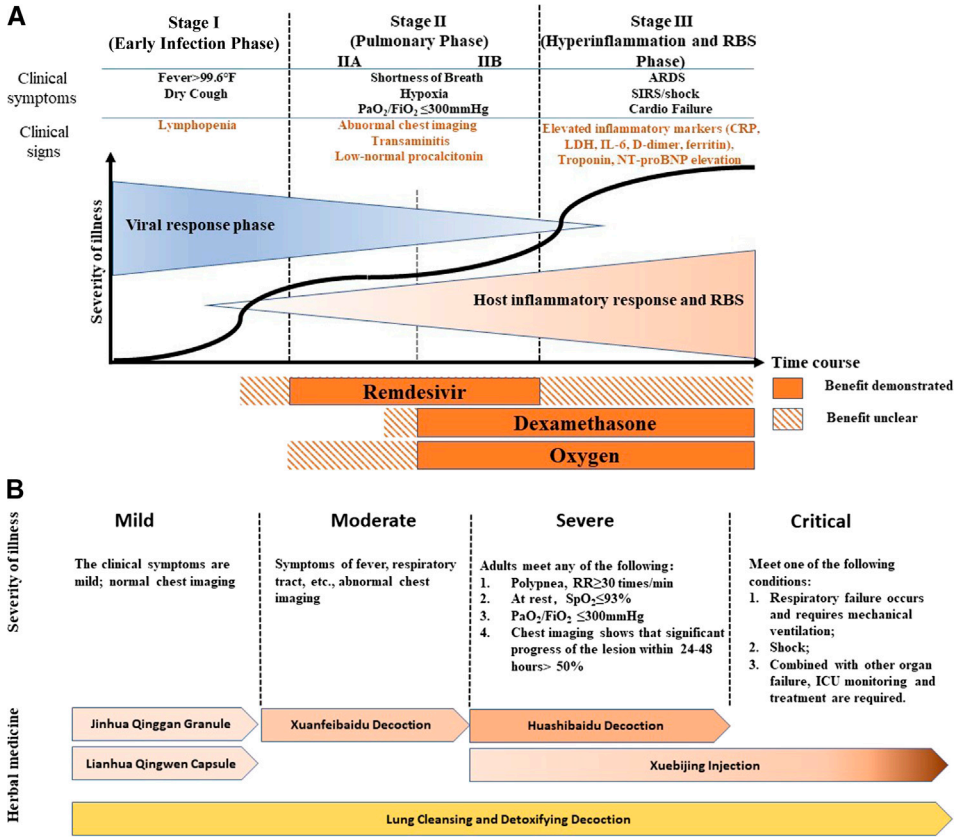

FIGURE 1 | COVID-19 Therapies at Different Stages **(A)** Modified from publication by Siddiqi, H.K., and Mehra, M.R. (2020). COVID-19 illness in native and immunosuppressed states: A clinical-therapeutic staging proposal. J Heart Lung Transplant 39**,** 405–407 **(B)** Modified from China, N.H.C.O.T.P.S.R.O. (2020a). "Diagnosis and Treatment of COVID-19 (8th trial edition)", (ed.) N.H.C.O.T.P.S.R.O. China (Beijing: National Health Commission of the People's Republic of China), and Wang et al., (2020). Comprehensive Analysis of TCM Diagnosis and Treatment Schemes for COVID-19 in All Regions of China. Modernization of Traditional Chinese Medicine and Materia Medica-World Science and Technology 22**,** 257–263. The diagnostic criteria of children in severe stage is deleted in this figure.

part of the RAS-bradykinin axis. Some treatments targeting ACE2 may not only impact viral life cycle but may also play into RBS.

"3-DRUGS-3-FORMULAS" FOR TREATING DIFFERENT STAGES OF COVID-19

In China, COVID-19 belongs to the "plague" category in TCM and is classified into four stages (mild, moderate, severe, and critical) based on the severity of illness and the symptoms that present (**Figure 1B**) (China, 2020a). According to China's guidelines (China, 2020a; Wang et al., 2020), the following six treatments are used for different stages of COVID-19 (**Figure 1B**): Jinhua Qinggan Granule (JQG) and Lianhua Qingwen Capsule (LQC) are recommended for mild cases, Xuanfeibaidu Decoction (XD) for moderate cases, Huashibaidu Decoction (HD) for severe cases, Xuebijing Injection (XI) for severe and critical cases, and Lung Cleansing and Detoxifying Decoction (LCDD) for all stages (**Figure 1B**) (China, 2020a; Wang et al., 2020). In addition, patients may be prescribed supplementary herbal medicines based on their individual condition. This strategy of treatment, based on the specific stage of disease progression, demonstrates the concept of "precision medicine" or "individualized treatment" and is a strategy that is commonly practiced by TCM practitioners.

The "3 drugs" in 3-drugs-3-formulas are JQG, LQC, and XI. These drugs were previously approved in China for treating respiratory diseases. The "3 formulas" are LCDD, HD, and XD. These formulas were created to treat COVID-19 by combining several classical formulas and adding complementary herbs. All of the recommended formulas are comprised of traditional formulas that have been used to treat pulmonary and respiratory diseases in China for thousands of years. A summary of disease progression and usage of recommended treatment are shown in **Figure1B**.

ALL FIVE ORAL FORMULAS (DRUGS OR DECOCTIONS) HAVE MORE THAN ONE HERB BELONGING TO THE CATEGORY OF "QING-RE HERBS"- KNOWN FOR THEIR ANTI-INFLAMMATORY PROPERTIES

Zhang, et al. executed *in silico* screen to identify Chinese medical herbs that contain compounds that might directly inhibit SARS-

TABLE 1 | Biological activities of 3-drugs-3-formulas.

Formulas	Inflammation						Innate Immunity		Anti-Oxidation	Fibrosis	Anti-Viral	Anti-Bacteria
	TNFα	IL6	IFNγ	COX2	iNOS	GRE	TLR2	TLR4	NRF2	TGFβ	Direct antiviral	Type III protein secretion
Jinhua Qinggan Granule (JQG)	↓↓↓↓	↓↓↓↓↓	↓↓↓↓↓	↓↓↓↓↓↓	↓↓↓↓	↓↓	↓↑↑↑	↓↑↑↑↑	↑↑↑	↓↓	↑↑↑↑ (Zhang et al., (2020))	↓↓
Lianhua Qingwen Capsule (LQC)	↓↓	↓↓↓	↓↓↓↓	↓↓↓↓↓	↓↓↓↓↓	↓↓	↑↑↑↑	↑↑↑↑↑↑	↑↑↑	↓	↑↑↑↑ (Zhang et al., (2020))	↓↓↓↓
Xuanfeibaidu Decoction (XD)	DK[3]	↓↓	↓↓↓	↓↓↓↓	↓↓↓	↓	↑↑↑↑↑	↑↑↑↑↑	↑	↓↓	↑↑ (Zhang et al., (2020))	↓
Huashibaidu decoction (HD)	↓	↓↓↓↓	↓↓↓↓	↓↓↓	↓↓↓↓↓	↓↓↓↑	↑↑↑	↑↑↑(↓↑)	↑	↓	↑↑↑ (Zhang et al., (2020))	↓
Lung Cleanshing and Detoxifying Decoction (LCDD)	↓	↓↓↓	↓↓↓	↓↓	↓↓↓	↓↓↓↑	↑↑↑↑↑↑↑↑↓	↑↑↑↑↑↑↑↓	↑	↓↓	↑↑↑↑↑ (Zhang et al., (2020))	↓
Xuebijing Injection (XI)	↓	↓↓	↓↓	↓↓	↓↓↓↓	↓↑	↑↑↑	↑↑(↑↓)↓	DK[1]	↓↓	DK[2]	↓

Not all herbs of formulas were examined. The direction of arrows (↑or ↓) in the table represents known ↑ stimulation or ↓ inhibition activity; each arrow represents an herb that has this activity (for example ↓↓↓, represents three (3) herbs having inhibition activity on a given target, whereas ↓↓↓↓↓ represents five (5) herbs having inhibition activity). (↑↓) represents an herb in the formula have biphasic activity, DK1: indicates negative results when 5 herbs of XI were examined in NRF2 luciferase assay , DK2: indicates negative results when 5 herbs of XI were examined against COVID-19 (Zhang et al., 2020), DK3: indicates negative results when 11 out of 13 herbs in XD were examined in TNFα-NFκB luciferase assay and 2 out of 13 herbs have not yet been examined in our lab. Direct antiviral was done by others (Zhang et al., 2020). COX-2 and iNOS are enzymatic reactions. Gram (-) bacteria Type III protein secretion results were published (Tsou et al., 2016). Methods to determine effects of herbs on luciferase activity of reporter cells induced by their corresponding ligands: TNFα- NFκB, IL6-STAT3, IFNγ-STAT1, TGFβ-SMAD2/3, LPS-TLR4- NFκB, PGN-TLR2- NFκB, or COX2/iNOS activities were shown in previous reports (Lam et al., 2018; Lam et al., 2010).

CoV-2. Interestingly, All five oral formulas contain herbs that had anti-SARS-CoV-2 activity as has been suggested by Zhang, et al. (Zhang et al., 2020).

We have developed the STAR (Signal Transduction, Activity and Response) Drug Discovery Platform—a rich database of 300 commonly used medicinal herbs, tested across more than 30 signaling pathways using luciferase reporter assays, enzymes, and other bioassays—now licensed to Yiviva, Inc. Using STAR, we examined the activities of the herb(s) used in the "3-drugs-3-formulas" against TNFα, IL-6, IFN-γ, TGFβ, TLR2, TLR4 pathways, COX-2 and iNOS enzyme activities as well as against Type III protein secretion of Gram (-) bacteria (Lam et al., 2010; Tsou et al., 2016; Lam et al., 2018). Each of these formulas have herbs that inhibit one or more and cover all of these pathways (**Table 1**). The biological activities of the herbs could be the potential mechanisms of action for these formula treatments to control COVID-19 disease progression. In our own studies we have found that these formulas inhibit multiple pathways of inflammation, induce NRF2 anti-oxidation, and have anti-fibrosis and anti-bacteria activities. These activities could help to explain the mechanisms of action for each formula in treating COVID-19.

To better understand each of these formulas from the perspective of Chinese medicine, we dissect the components of each. We categorize the components by their TCM classification. A summary of the components of the 3-drugs-3-formulas is shown in **Table 2**.

In TCM, herbs categorized as "Qing-Re" (translated as "remove heat") are used when "heat" symptoms, which cover current inflammation symptoms, have been diagnosed. With the exception of Xuebijing Injection (XI), the five oral formulas in the "3-drugs-3-formulas" all contain "Qing-Re" herbs. Eight of 12 herbs in JQG are "Qing-Re" herbs, nine of 13 herbs in LQC, five of 13 herbs in XD, three of 14 herbs in HD, and two of 21 herbs in LCDD. We previously demonstrated that "Qing-Re" herbs often have multiple mechanisms of anti-inflammatory activity, but 80% of all herbs examined also have one to two actions against six inflammatory mechanisms studied (Guan et al., 2018). Although XI does not contain any "Qing-Re" herbs and all five of its herbs belong to the category of "Huo-Xue" herbs, some of the "Huo-Xue" herbs within XI also promote one or more anti-inflammatory activities (Yu et al., 2013). Thus, the five herbs administered together could potentiate anti-inflammatory activity that may be more powerful than any single herb in the formula. For example, LCDD contains two herbs belonging to "Qing-Re" herbs and 19 herbs belonging to other categories. Together, LCDD exhibits anti-inflammatory activities on all six key inflammatory mechanisms (**Table 1**). Thus, the common mechanism for all six "3-drug-3-formulas" targets the inflammatory process. The anti-inflammatory activity of these formulas should be considered holistically and sequestered to individual herbs.

As the COVID-19 disease progresses and new symptoms emerge, additional categories of medicinal herbs have been added to the base formulas to improve their efficacy. JQG and LQC are commonly used for mild stages and the majority of herbs used in their formulas are "Qing-Re" herbs. In moderate stages of COVID-19, herbs categorized as "Qu-Shi" translated as "remove dampness have also been included to treat symptoms such as excess mucus secretion and edema, which can cause shortness of breath and hypoxia in patients. Interestingly, many "dampness" symptoms diagnosed by practitioners of Chinese medicine are similar to those of RBS in the advanced pulmonary stage of

TABLE 2 | Compositions of 3-drugs-3-formulas.

Treatment	Stage	Key Formulation	Additional Formulations	Additional Herbs	Number of Herbs
Jinhua Qinggan Granule (JQG)	Mild	Maxingshigan Decoction • *Ephedra sinica* Stapf.[a]1, Stir-fried *Prunus armeniaca*.[b]2, Gypsum Fibrosum, *Glycyrrhiza glabra* L.3	Yinqiao Poder • *Arctium lappa* L.[b]2, *Lonicera japonica Thunb.*4, *Forshythia suspensa* (Thunb.) Vahl.2, *Mentha canadensis*L.12, *Glycyrrhiza glabra* L.3	*Fritillaria thunbergii* Miq.[b]5, *Scutellaria baicalensis* Georgi, *Anemarrhena asphodeloides* Bunge.8, *Artemisia annua* L.6	12 (1^a+3^b+8)
Lianhua Qingwen Capsule (LQC)	Mild	Maxingshigan Decoction • *Ephedra sinica* Stapf.[a]1, Stir-fried *Prunus armeniaca*.[b]2, Gypsum Fibrosum, *Glycyrrhiza glabra* L.3	Yinqiao Powder • *Lonicera japonica* Tgunb. 4, *Forsythia suspensa*(Thunb.) Vahl. 2, l-Menthol, *Glycyrrhiza glabra* .3	*Isatis tinctoria* L.7, *Dryopteris crassirhizoma* Nakai.8, *Rheum palmatum* L.8, *Houttuynia cordata* Thunb.6, *Pogostemon cablin* (Blanco) Benth. 12, *Rhodiola crenulata*(Hook.f. and Thomson) H.Ohba 3	13 (1^a+1^b+9+1+1)
Xuanfeibaidu Decoction (HD)	Moderate	Maxingshigan Decoction • *Ephedra sinica* Stapf.[a]1, Stir-fried *Prunus armeniaca*.[b]2, Gypsum Fibrosum, *Glycyrrhiza glabra* L.3	Maxingyigan Decoction • *Ephedra sinica* Stapf.[a]1, Armeniacae Semen Amarum[b]2, *Glycyrrhiza glabra*L.3, *Coix lacryma-jobi var. ma-yuen*(Rom.Caill) Stapf.2, Qianjinweijing Decoction • *Phragmites austalis* subsp. *australis*.8 Tinglidazao Xiefei Decoction • *Descurainia sophia* (L.) Webb ex Prantl.[b]2	*Artemisia annua* L.6, *Reynoutria japonica* Houtt.3, Verbena officinalis L.12, *Atractylodes lancea*(Thunb.) DC.8, *Pogostemon cablin* (Blanco) Benth.12, *Citrus* × *aurantium* L.13	13 (1^a+2^b+5+4+1)
Huashibaudu Decoction (HD)	Severe	Maxingshigan Decoction • *Ephedra sinica* Stapf.[a]1, Stir-fried *Prunus armeniaca*.[b]2, Gypsum Fibrosum, *Glycyrrhiza glabra* L.3	Huopoxiefei Decoction • Gingered *Pinellia ternata* (Thunb.) Makini.[b]9, *Pogostemon cablin* (Blanco) Benth.12. Poria cocos (Schw.) Wolf. *Magnolia officinalis* Rehder and E.H. Wilson15,	*Descurainia sophia* (L.) Webb ex Prantl.[b]2, *Rheum palmatum* L.8, *Atractylodes lancea* (Thunb.) DC.8, *Lanxangia tsao-ko*(Crevost and Lemarié) M.F.Newman and Skornick.2, *Paeonia Lactiflora* Pall.7, *Astragalus mongholicus* Bunge,7	14 (1^a+3^b+3+4+1+1+1)
Lung Cleansing and Detoxifying Decoction (LCDD)	All stages	Maxingshigan Decoction • *Ephedra sinica* Stapf.[a]1, Stir-fried *Prunus armeniaca*.[b]2, Gypsum Fibrosum, *Glycyrrhiza glabra* L.3	Sheganmahuang Decoction • *Ephedra sinica lStapf.*[a]*1, Asarum sieboldii* Miq.[a]3, *Iris domestica*(L.) Goldblatt and Mabb.[b]8, *Aster tataricus* L.f.[b]3, *Tussilago farfara* L.[b]4 Xiaochaihu Decoction • *Bupleurum chinese* DC.[a], *Zingiber officinale* Rosco.[a], Gingered *Pinellia ternata*(Thunb.) Makino.[b]9, *Scutellaria baicalensis* Georgi7, Honeyed *Glycyrrhiza glabra* L.3 Wuling Powder • *Cinnamomum cassia*(L.)J.Presl.[a]11, Poria cocos (Schw.) Wolf, *Polyporus umbellatus* (Pers.) Fries, *Alisma plantago-aquatica*L.9, *Attractylodes macrocephala* Koidz.8, Juzhihiang Decoction • *Zingiber officinale* Rosco.[a]8, *Citrus* × *aurantium*L.13, *Citrus trifoliata*L.2	*Pogostemon cablin* (Blanco) Benth.12, *Dioscorea polystachya* Turcz.9	21 (5^a+2^b+2+5+2+2)
Xuebijing (XI)	Severe or critical			*Carthamus tinctorius*L.14, Paeonia lactiflora Pall.7, *Conioselinum anthriscoides* 'Chuanxiong'.8, *Salvia miltiorrhiza* Bunge.7, *Angilica sinensis*(Oliv.) Diels. 7	5

The stages of coronavirus disease-19 are defined by the diagnosis and treatment (8th trial edition) in China (China, 2020a). The unique symbols or text colors correspond to different categories of herbs used in the formulations; [a]: "Jie-Biao" herbs; [b]: "Huatan-Zhike-Pingchuan" herbs; green: "Qing-Re" herbs; orange: "Qu-Shi " herbs; red: "Huo-Xue" herbs; blue: "Bu-Yi" herbs; purple: "Xing-Qi" herbs; some herbs in the table are repeated. Numbers represent medicinal parts of Chinese medicines in the table; 1: herbaceous stem; 2: fruit; 3: root and rhizome; 4: bud; 5: bulb; 6: aerial parts; 7: root; 8: rhizome; 9: tuber; 10: sclerotium; 11: twig; 12: whole herb; 13: peel; 14: flower; 15: bark.

COVID-19 (Garvin et al., 2020). Studies have suggested that COVID-19 patients requiring time in an intensive care unit (ICU) tend to present severe hypercoagulability along with a severe inflammatory state. Moreover, fibrin formation and polymerization may predispose patients to thrombosis and may correlate with a worse patient outcome (Panigada et al., 2020; Spiezia et al., 2020). Giuseppe et al. also suggested that controlling coagulation disorder may be the key to lowering mortality rates (Magro, 2020). In severe and critical stages, "Huo-Xue" herbs, translated as "activate blood" and "Bu-Yi" herbs, translated as "tonics" are added to the formulas. In TCM, Huo-Xue herbs are used to improve blood circulation, local hypoxia, blood rheology and coagulation; increase local blood flow; promote fibrinolysis, anticoagulation, and antithrombotic activity; eliminate microcirculation obstacles; and inhibit platelet activity (Yu et al., 2013). "Huo-Xue" herbs could be useful in limiting the degree of hypercoagulability and may improve patient outcome for those in severe or critical COVID-19 stages. "Huo-Xue" herbs are used in LQC and HD and injectable XI. "Qing-Re" and "Qu-Shi" herbs are key categories of herbs used in the other three oral formulas to treat patients in moderate to critical stages. "Bu-Yi" herbs are claimed to be useful in improving the physical state of individuals such as fatigue— a major symptom of patients in the severe and critical stages (Yang et al., 2019). Additionally, another category of herbs, "Xing-Qi" herbs, are claimed to "promote the circulation of *qi*" and treat indigestion and loss of appetite. "Bu-Yi" and "Xing-Qi" herbs were added together into the formula of HD and LCDD for treating severe stage patients. The potential synergetic action of these two categories of herb could be interesting to explore.

ALL FIVE ORAL FORMULAS SHARE MAXINGSHIGAN DECOCTION (MD) AS A COMMON CORE FORMULA

With the exception of the injected treatment formula, all five oral treatments (drugs or decoctions) consist of MD. MD is a classic formula described 1800 years ago in *Treatise on Febrile Caused by Cold and Miscellaneous Diseases* (Shang Han Za Bing Lun), a book that introduced the pathogenesis and treatment of infectious diseases in ancient times. The formula consists of four traditional Chinese medicines, *Ephedra sinica* Stapf., herbaceous stem, *Prunus armeniaca* L., fruit, stir-fried, gypsum fibrosum (Shi Gao) and *Glycyrrhiza glabra* L., root and rhizome, raw or honeyed. Historically, MD was used to treat febrile diseases with symptoms of perspiration, panting with no fever, or mild fever. In addition to being used to treat diseases, MD is used today by clinicians to control radio-chemotherapy induced lung injury, acute lung injury, asthma, influenza infection, viral pneumonia, and severe community-acquired pneumonia (Lin, 2015; Li L. et al., 2018; Song et al., 2018; Li, 2020a; Zhou X. et al., 2020). *Ephedra sinica* Stapf., herbaceous stem has been claimed to be effective for allaying asthma and for inducing diaphoresis and diuresis, but it could have severe adverse effects (Commission, 2010). *Glycyrrhiza glabra* L., root and rhizome, raw or honeyed (GG) is included in the formula as it is claimed to decrease adverse effects of *Ephedra sinica* Stapf., herbaceous stem (Wei et al., 2016a; Wei et al., 2016b). GG has been used for centuries in TCM for the treatment of cough and influenza virus (Lin et al., 2016). Flavanone liquiritigenin and its precursor and isomer chalcone isoliquiritigenin are the main bioactive constituents of GG, and have also been suggested to have anti-inflammatory activities (Ramalingam et al., 2018). In addition, when used in silico screening, GG was claimed to have the potential to directly inhibit SARS-CoV-2 (Zhang et al., 2020). *Prunus armeniaca* L., fruit, stir-fried is used as an anti-asthmatic, a mucolytic, an expectorant, and a laxative agent. Amygdalin, a cyanogenic diglucoside found in *Prunus armeniaca* L., fruit, stir-fried, could be metabolized in the human body to produce hydrocyanic acid which could inhibit the respiratory center in the brain to render smoother breathing, thereby gradually reducing cough and asthma (Shi and Liu, 2018). Gypsum fibrosum is mainly composed of calcium sulfate dihydrate, with the chemical formula $CaSO_4{\cdot}2H_2O$. It is used to treat febrile diseases, dysphoria and thirst in TCM practice. A study indicated that gypsum fibrosum exerted anti-inflammatory effects, but the mechanisms are still unclear (Yin, 2019). MD has been suggested to be able to regulate viral infection and immune inflammatory response by promoting TH17 cell differentiation and T-cell homeostatic proliferation to inhibit virus proliferation, and to negatively regulate immune inflammatory factors such as interleukin, TNFα and integrin, thus decreasing the degree of cytokine storm in patients with COVID-19 (Zhang et al., 2020). Zhang et al. (Zhang et al., 2013) also found that MD could significantly reduce the inflammatory response in lungs of mice infected with influenza virus. The mechanism may be related to the inhibition of neuraminidase activity and prevention of viral proliferation. MD not only has anti-inflammatory and antiviral effects, but it is also known for its antitussive and antipyretic properties (Lin et al., 2016) which could alleviate cough or fever symptoms in COVID-19 patients. Recently Li, et al. suggested that MD might directly inhibit the absorption and replication of SARS-CoV-2, prevent cytokine storm and relieve lung injury. However, the difference of spectrum of actions between MD and the other four formulas based on MD wasn't mentioned (Li et al., 2020c). This formula of MD alone may not be sufficient for treating COVID-19, but it appears to be the core formula in the five oral formulas of the "3-drugs-3-formulas". It should be noted that *Ephedra sinica* Stapf., herbaceous stem is banned in several regions of the world due to its severe adverse effects. Low-dose *Ephedra sinica* Stapf., herbaceous stem extracts can reduce body weight and can improve athletes' physical performance. However, high doses or long-term use of Ephedra extracts can cause various adverse effects (Gardner et al., 2003; Worley and Lindbloom, 2003; Miao et al., 2020). Consequently, the formulas containing *Ephedra sinica* Stapf., herbaceous stem will need to overcome regulatory hurdles in order to be used in countries where its usage is heavily regulated. At relevant dosage, MD may not have the adverse effects listed above that are attributed to *Ephedra*

sinica Stapf., herbaceous stem, due to the proportionally low concentration of *Ephedra sinica* Stapf., herbaceous stem used relative to the other herb components. Nevertheless, the adverse effects of *Ephedra sinica* Stapf., herbaceous stem should be further studied in light of its potential benefits.

FOR TREATMENT OF THE MILD STAGE OF COVID-19 - JINHUA QINGGAN GRANULE (JQG) AND LIANHUA QINGWEN CAPSULE (LQC)

Both JQG and LQC are approved drugs in China. Their formulas share MD, as well as another common TCM formula: Yinqiao Powder (YP), which was first described in the *Treatise on Differentiation and Treatment of Epidemic Febrile Disease* (Wen Bing Tiao Bian) published in 1798. Historically, YP was used for treating early stages of epidemic febrile diseases with symptoms of aversion to "heat", however, not to treat "cold" and "thirst" symptoms. In modern times, YP is mainly used to treat upper respiratory diseases, laryngopharyngeal inflammation, suppurative tonsillitis, and viral infection. An *in vivo* study suggested that YP could inhibit the release of pro-inflammatory cytokines of IL-1β and TNFα in the early stage of sepsis (He et al., 2016). Lei et al. suggested that YP may boost the mucosal immune system to prevent and treat upper respiratory diseases by improving lysozymal activity and by increasing SIgA levels in saliva (Lei et al., 2013). Additionally, its components, *Lonicera japonica* Thunb., bud and *Forsythia suspensa* (Thunb.) Vahl., fruit, were suggested to have direct inhibitory potential on SARS-CoV-2 and on anti-inflammatory activities (Guo et al., 2015; Zhang et al., 2020). YP is widely used for preventing and treating upper respiratory diseases. Ultimately, YP could enhance the anti-inflammatory and antiviral activities of MD.

The key differences in herbal components of JQG and LQC are as follows. The drug formula JQC has additional "Qing-Re" herbs *Scutellaria baicalensis* Georgi., root, *Anemarrhena asphodeloides* Bunge., rhizome, *Artemisia annua* L., aerial parts; "Jie-Biao" herbs *Arctium lappa* L., fruit; "Huatan-Zhike-Pingchuan" herbs *Fritillaria thunbergii* Miq., bulb; while the drug formula LQC, adds "Qing-Re" herbs *Isatis tinctoria* L., root, *Dryopteris crassirhizoma* Nakai., rhizome, *Rheum palmatum* L., rhizome, *Houttuynia cordata* Thunb., aerial parts; "Qu-Shi" herbs *Pogostemon cablin* (Blanco) Benth., whole herb, "Huo-Xue" herbs *Rhodiola crenulata* (Hook.f. and Thomson) H. Ohba., root and rhizome.

JQC consists mainly of three categories of herbs: "Jie-Biao" herbs, "Huatan-Zhike-Pingchuan" herbs and "Qing-Re" herbs. LQC includes additional "Qu-Shi" herbs and "Huo-Xue" herbs to enhance anti-inflammatory activities and to improve symptoms related to "dampness" such as excessive mucus secretion, edema or "yellow-greasy coating of tongue" in COVID-19 patients. Moreover, LQC is better at resolving fever. Compared to LQC, JQG has a stronger antitussive effect due to its use of *Fritillaria thunbergii* Miq., bulb and *Arctium lappa* L., fruit, which are herbs commonly used for pharyngeal diseases to improve expectoration and to relieve cough and sore throat. In summary, JQG and LQC are two oral drugs that are used for treating patients who fall under the mild state of COVID-19. Duan, et al. (Duan et al., 2020) suggested that JQC could significantly reduce fever time, alleviate clinical symptoms of cough, fatigue and expectoration, and relieve psychological anxiety in patients with mild COVID-19. Hu, et al. (Hu et al., 2020) conducted a multicenter, prospective, randomized controlled trial (RCT) upon the efficacy and safety of Lianhua Qingwen capsules involving 284 patients randomized to receive usual treatment alone or in combination with LQC. Results suggested that LQC could be considered to ameliorate clinical symptoms of Covid-19 including shortening recovery of fever, fatigue and coughing. These two drugs share two common classic formulas, MD and YP, as well as different complementary herbs. So, each formula has its own advantage. JQG more effectively treats COVID-19 patients with a cough, likely due to the presence of *Fritillaria thunbergii* Miq., bulb and *Arctium lappa* L., fruit, while LQC is used to treat patients with fevers, likely due to the presence of additional "Qing-Re" herbs.

FOR TREATMENT OF THE MODERATE STAGE OF COVID-19-XUANFEIBAIDU DECOCTION (XD)

XD is composed of MD, plus three additional traditional formulas (Maxingyigan Decoction, Qianjinweijing Decoction, Tinglidazao Xiefei Decoction), and six additional herbs. It is suitable for the treatment of moderate stages of COVID-19, including symptoms of "dampness-toxin stagnating in the lung syndrome". Clinical manifestations include fever, cough with little sputum or yellow sputum, airway obstruction and short of breath, abdominal distension and inhibited defecation, "dark-red plump tongue with yellow greasy or yellow dry coating", and "slippery rapid or wiry slippery pulse" (China, 2020a). Five of 13 herbs in XD are "Qing-Re" herb and four of 13 are "Qu-Shi" herbs. "Qu-Shi" herbs are often used for treating mucus secretion, sputum, and edema. According to China's COVID-19 guidelines (China, 2020a), abnormal chest imaging starts to present in moderate stage cases, indicating disease progression spreading of the lesion from the upper respiratory tract to the lungs. A series of studies (Liu and Liang, 2014; Li et al., 2019; Ma et al., 2020) suggest that XD component herbs, *Coix lacryma-jobi var. ma-yuen (Rom.Caill.)* Stapf., fruit, *Phragmites australis* subsp. *australis*, rhizome, *Atractylodes lancea* (Thunb.) DC., rhizome and *Pogostemon cablin* (Blanco) Benth., whole herb, all promote anti-inflammatory activity. Moreover, *Coix lacryma-jobi var. ma-yuen (Rom.Caill.)* Stapf., fruit and *Pogostemon cablin* (Blanco) Benth., whole herb have been suggested to have analgesic, immune enhancement, and anti-microbial properties. A forty-two patients randomized clinical trial suggested that XD combined with conventional medicine may significantly improve patient's clinical symptoms, increase the number of white blood cells and lymphocytes to improve immunity, and also significantly reduce C-reactive protein and erythrocyte sedimentation rate to exert anti-inflammatory effect (Xiong et al., 2020). Network pharmacology and molecular docking studies have revealed that XD may inhibit viral

invasion and viral replication by binding to ACE2 receptors and to 3CLPro of SARS-CoV-2 through its flavonoids and phytosterols. XD may have direct antiviral activities. Additionally, *Descurainia sophia* (L.) Webb ex Prantl, fruit, a component of XD has also been suggested to have direct anti-coronavirus effects (Zhang et al., 2020). Moreover, XD may play a role in the treatment of COVID-19 by regulating key targets such as MAPK3, MAPK1, CCL2, EGFR, and NOS2 after viral infection of cells, exerting an anti-cytokine storm against IL-6 and IL-1β, having an anti-oxidation effect, and regulating the body's immunity. In summary, XD includes more herbs, in addition to MD, that promote anti-inflammatory and immune enhancing activities, ultimately enhancing the anti-inflammatory properties of the XD formula to treat moderate cases of COVID-19. In addition, ACE2 is also involved in the bradykinin storm pathway. The XD formula has been claimed to relieve dampness symptoms which mimic RBS symptoms (Guo, 2020). Thus, the XD formula may also interact with ACE2 or COX-2 as well as NF-κB pathways which could relieve RBS. This should be further explored.

FOR TREATMENT OF SEVERE STAGE OF COVID-19—HUASHIBAIDU DECOCTION

HD is composed of MD, Huopoxialing Decoction, *Descurainia sophia* (L.) Webb ex Prantl., fruit-also in XD, *Paeonia lactiflora* Pall., root, *Rheum palmatum* L., rhizome, *Astragalus mongholicus* Bunge., root, *Atractylodes lancea* (Thunb.) DC., rhizoma, *Lanxangia tsao-ko* (Crevost and Lemarié) M.F.Newman and Skornick., fruit. HD is recommended for treating COVID-19 patients with shortness of breath. Clinical manifestations include "fever with flushed face", "cough with little yellow sticky sputum, or blood-stained sputum", airway obstruction and shortness of breath, lassitude, "dryness, bitterness, and stickiness in the mouth", nausea and loss of appetite, inhibited defecation, "scanty dark urine", "red tongue with white greasy coating", and "slippery rapid pulse" (China, 2020a). Intriguingly, HD (as well as XD) includes additional "Qing-Re" herb: *Rheum palmatum* L., rhizome, raw, "Huo-Xue" herb: *Paeonia lactiflora* Pall., root. and "Bu-Yi" herb: *Astragalus mongholicus* Bunge., root. *Descurainia sophia* (L.) Webb ex Prantl., fruit, *Astragalus mongholicus* Bunge., root. and *Glycyrrhiza glabra* L., root and rhizome, raw or honeyed were also suggested to have direct anti-coronavirus effects (Zhang et al., 2020). Its composite Huopoxialing Decoction is a formula used in TCM for its strong ability to "resolve dampness". HD's formula appears to be designed to resolve symptoms in severe COVID-19 patients such as a cough with an increase in sputum secretion, airway obstruction or shortness of breath, and fatigue. In TCM, the manifestations of "dampness" appear to be related to excess interstitial fluid accumulation, such as mucus secretion and edema. A pathological investigation of two severe COVID-19 patients, indicated excessive mucus secretion with serous and fibrinous exudation. The exudation could aggravate the dysfunction of ventilation and may be one of the pathogenic mechanisms responsible for hypoxemia (Wang et al., 2020b). At this stage, symptoms appear to mimic those associated with RBS-pulmonary edema, shortness of breath, and gastrointestinal (GI) disorders, all of which fall under the classification of "dampness" syndrome. One RCT (ChiCTR2000030988) with sample size of 204 evaluated the effectiveness of HD comparing with Western medicine. Three additional studies on HD had been carried out in Jinyintan Hospital (75 severe cases), Dongxihu Fangcang Hospital (124 moderate cases), and Jiangjunlu Street Health Center (894 mild and moderate cases), respectively (Luo et al., 2020b). The results suggested significant improvement in symptoms and CT images of lungs, shortening hospital stay and reducing rate of viral clearance by polymerase chain reaction, and no adverse events or liver and kidney damage were found (China, 2020b). Network pharmacology studies and molecular docking analyses have demonstrated that signaling pathways involving HD include TNFα, PI3K–Akt, NOD-like receptor, MAPK, and HIF-1. Baicalein and quercetin are the top two compounds of HD, with high affinity for ACE2 (Tao et al., 2020). Another study has suggested that quercetin not only impairs the binding of viral S-protein to ACE2 receptor, but also has virus neutralizing effect of quercetin on SARS-CoV-2 (Pan et al., 2020). As mentioned, ACE2 is a key component of the bradykinin storm in addition to serving as a receptor of SAR-CoV-2. Baicalein and quercetin are major compounds of *Scutellaria baicalensis* Georgi., root, a key herb in JQG and LCDD formulas. In summary, MD is the core formula of HD, Huopoxialing Decoction is included to "resolve dampness", and additional herbs eliminate or relieve symptoms of cough with sputum secretion, airway obstruction, short of breath, and fatigue in severe COVID-19 cases. The impact of the HD formula in relieving bradykinin storm should be further studied.

FOR TREATMENT OF ALL STAGES—LUNG CLEANSING AND DETOXIFYING DECOCTION (LCDD)

LCDD was claimed to have excellent efficacy in the clinic by National Administration of Traditional Chinese Medicine of China and has been recommended as a universal formula to treat all stages of COVID-19 in China (China, 2020a). It has been shown to relieve cough, shortness of breath, and panting in COVID-19 patients. An *in vivo* study suggested that Xiaochaihu Decotion, decreased TNFα, IL-1β, and IL-6 level in plasma of LPS-induced inflammation mice. This indicates that Xiaochaihu Decoction has anti-inflammatory properties. *Bupleurum chinense* DC., root and *Scutellaria baicalensis* Georgi., root, two components of Xiaochaihu Decoction, contain flavonoids that are known to be effective antiviral agents (Zakaryan et al., 2017). Moreover, polysaccharides extracted from Bupleurum have been suggested to have an immunomodulatory effect via the NF-κB signaling pathway (Song et al., 2017). Another component formula, Wuling Powder, is a classic formula known for "resolving dampness" by promoting urination. Modern pharmacological research also suggests that Wuling Powder can eliminate edema, improve gastrointestinal function, remove free radicals, reduce lipid

peroxide levels, increase superoxide dismutase activity, reduce the production of inflammatory cytokines, and regulate the body immune function, etc. (Yao et al., 2020). Therefore, Wuling Powder may improve lung tissue edema, protect gastrointestinal function, and have anti-oxidation and anti-inflammatory effects, reducing COVID-19 damage to target tissues, and regulating immunity to improve the body's antiviral ability to facilitate disease recovery. In another component formula, Juzhijiang Decoction, *Citrus* × *aurantium* L., peel has antithrombotic and anti-inflammatory actions; *Citrus trifoliata* L. fruit may improve myocardial metabolism and also has anti-inflammatory activity (Yao et al., 2020). Two additional herbs, *Pogostemon cablin* (Blanco) Benth., whole herb and *Dioscorea polystachya* Turcz., tuber. *Dioscorea polystachya* Turcz., tuber are included to relieve gastrointestinal symptoms in COVID-19 patients, such as abdominal distention, loss of appetite, and diarrhea. *Glycyrrhiza glabra* L., root and rhizome, raw or honeyed and *Dioscorea polystachya* Turcz., tuber belong to "Bu-Yi" herbs in TCM and are used to treat patients with fatigue – one of the main symptoms in COVID-19 patients (Li et al., 2020b), known to worsen throughout COVID-19 disease progression and to hinder patient recovery. In summary, in addition to reducing lung damage, LCDD may reduce other tissue damage, such as in the kidney, liver, heart and brain. Components of LCDD, *Aster tataricus* L. f., root and rhizome, *Tussilago farfara* L., bud, *Bupleurum chinense* DC., root, *Glycyrrhiza glabra* L., root and rhizome, raw or honeyed, may have direct anti-SARS-CoV-2 effects (Zhang et al., 2020). Six registered trials evaluated the effect of LCDD: One (ChiCTR2000030810) was a registered RCT comparing LCDD versus Western medicine with sample size of 100; one (ChiCTR2000029778) was a controlled clinical trial comparing LCDD plus Western medicine versus Western medicine only with sample size of 600; Three (ChiCTR2000030864, ChiCTR2000030883, and ChiCTR2000032767) Were single-arm studies; one (ChiCTR2000030806) was a retrospective study evaluating the effectiveness of LCDD plus ulinastatin, a human urinary trypsin inhibitor. A controlled clinical trial suggested that the combination of LCDD with Western medicine demonstrated effects of anti-inflammatory and mitigating the extent of multi-organ impairment compared with those of Western medicine alone in patients with mild and moderate COVID-19 (Xin et al., 2020).

LCDD builds upon MD with four additional component formulas to enhance the anti-inflammatory activity of MD and to relieve symptoms occurring in the cardiovascular system, gastrointestinal system, or kidneys, which are out of the typical therapeutic scope of MD and are essential in fulfilling the clinical needs of COVID-19 patients. *Asarum sieboldii* Miq., root and rhizome, gingered is also a restricted herb in the United States, Germany, and other regions. *Asarum sieboldii* Miq., root and rhizome, gingered contains aristolochic acid which may be carcinogenic, mutagenic, and/or nephrotoxic (Han et al., 2019). Thus, it is necessary to study the importance of *Asarum sieboldii* Miq., root and rhizome, gingered action as a component of of LCDD to allow most efficient use. We compared LCDD, with and without *Asarum sieboldii* Miq., root and rhizome, gingered, using the LPS-induced inflammation model of mice. Our preliminary results clearly indicated that the full formula of LCDD better protects lung and systemic inflammation than LCDD without *Asarum sieboldii* Miq., root and rhizome, gingered(data not shown). This suggests that *Asarum sieboldii* Miq., root and rhizome, gingered. is an essential component of LCDD action and confirms the challenge that will exist in bringing LCDD to the market in many countries. Further studies will be required to demonstrate the effects of LCDD used at the clinically relevant dosage and to further look at the carcinogenic, mutagenic, and nephrotoxic characteristics of *Asarum sieboldii* Miq., root and rhizome, gingered.

FOR TREATMENT OF SEVERE AND CRITICAL COVID-19 STAGES—XUEBIJING INJECTION (XI)

Xuebijing Injection (XI) is the only injectable formula among the 3-drugs-3-formulas. A published meta-analysis of the efficacy and safety of XI combined with conventional treatment of sepsis (Li C. et al., 2018), including 16 high-quality randomized controlled trials (Jadad score ≥3 points), a total of 1,144 sepsis patients showed that compared with conventional treatment, combined XI could reduce 28-days mortality, APACHE II score, body temperature, white blood cell count and other indicators of sepsis patients, and no obvious adverse reactions. XI is composed of five herbs (**Table 2**), all of which belong to the "Huo-Xue" category of herbs. In TCM, "Hue-Xue" herbs are used to treat traumatic injury, menstrual disorders, amenorrhea, dysmenorrhea, rheumatism caused pain, cardiovascular disease, etc. "Huo-Xue" herbs have been claimed to have anti-inflammatory, antibacterial, analgesia, antitumor, cardiovascular regulatory, anti-asthmatic, anti-myocardial ischemia and antithrombotic properties (Yu et al., 2013). Many patients with severe cases of COVID-19 present coagulation abnormalities that mimic other systemic coagulopathies associated with severe infections, such as disseminated intravascular coagulation (DIC) or thrombotic microangiopathy (Levi and Scully, 2018). Mounting studies have revealed that abnormal coagulation in severe COVID-19 cases is associated with a poor prognosis and increased risk of death (Tang et al., 2020a; Tang et al., 2020b). "Huo-Xue" herbs have shown substantial antithrombotic effects (Yu et al., 2013); however, the mechanisms of action may differ depending on the herb used from the category. "Huo-Xue" herbs may decrease risk of death in severe and critical COVID-19 patients with abnormal coagulation by anti-myocardial ischemia, antithrombotic actions, etc. It should be noted that some of the herbs used in XI such as *Rhodiola crenulata* (Hook.f. and Thomson) H. Ohba., root and rhizome are components of LQC. *Paeonia lactiflora* Pall., root is also in HD. The capabilities of the XI formula could also include its potential anti-viral, anti-inflammatory, and anti-RBS properties.

CONCLUSION AND PERSPECTIVES

While China was once the epicenter of COVID-19, it has essentially contained the outbreak of COVID-19. It is highly likely that TCM played a pivotal role in treating COVID-19 patients and thus in containing the outbreak. The 3-drugs (JQC, LQC and XI) are established formula drugs in China that have been used for the treatment of respiratory diseases; the 3-formulas (LCDD, HD and XD) are new formulas that evolved by the combining different formulas to treat different stages of COVID-19. The five oral formulas JQG, LQC, LCDD, HD and XD all build upon the core formula of Maxingshigan Decoction (MD). While MD has anti-inflammatory activities, it is likely not sufficient to serve as a standalone treatment. Thus, additional formulas or herbs with unique activities have been added to increase anti-inflammatory activity and to relieve other symptoms caused by infection. "Qing-Re" herbs play a key anti-inflammatory role, while the four other herbal categories, "Qu-Shi", "Huo-Xue", "Bu-Yi", and "Xing-Qi" herbs are included to treat symptoms such as excessive mucus secretion, edema, coagulation, fatigue, loss of appetite and indigestion, etc. It should be noted that *Ephedra sinica* Stapf., herbaceous stem and *Asarum sieboldii* Miq., root and rhizome, gingered, two "Jie-Biao" herbs in the six formulas, can cause a decrease in symptoms of high fever and can also cause weight-loss (Worley and Lindbloom, 2003; Perwaiz, 2014). Whether most of "Jie-Biao" herbs share similar metabolic properties should be explored. Among the six formulas, only HD and LCDD, the two formulas used to treat severe stages of COVID-19, utilize "Bu-Yi" herbs to resolve fatigue. The usage of different categories of herbs depend on the severity of disease progression. Some herbs may have a direct or indirect antiviral effect. Herb components with a direct antiviral effect may target ACE2, the SARS-CoV-2 receptor and key component of the bradykinin axis, or the serine protease TMPRSS2. An indirect antiviral effect may induce type I interferon (IFN-α and β). It should be noted that in early stages, IFN-α and IFN-β could be critical for innate and adaptive immune responses against SARS-CoV-2. Some of these formulas could also induce IFN-α and β expression (data not shown). However, IFN-α and IFN-β may also facilitate the occurrence of hyper-inflammation and may play a central pathogenic role in severe and critical patients with COVID-19 (Ruscitti et al., 2020). Thus, it is critical that the appropriate drug is chosen for each stage of COVID-19.

Although we focused on discussing "3-drugs-3-formulas", there are many more formulas and drugs used in the prevention and treatment of COVID-19 in China and beyond (Luo et al., 2020a; Zhao et al., 2020). To expand the use of these treatments beyond China and to the rest of the world, three key steps must be taken. First, rigorous and objective quality control (QC) is needed to ensure consistent, high-quality preparations. Due to the complexity of these formulas, chemical analysis may not be powerful enough to predict biological activity among batches. Our lab has developed an advanced mechanism-based quality control platform (Mech QC) designed to assess the quality and batch-to-batch consistency of complex mixtures focusing biological activity (Lam et al., 2018). Mech QC could be applied to assess and control the consistency of these formulas. The five oral formulas contain, on average, 17 herbs. Efforts should be made to simplify the formulas and to replace the restricted herbs while ensuring the same clinical results. Second, stringent clinical trials are needed. Ideally, multiregional, placebo-controlled, double-blind, randomized clinical studies should be conducted to explore the effects of gender, ethnicity, region, lifestyle, and diet on COVID-19. Moreover, the design of clinical trials is critical. Additional add-on clinical studies would be more suitable for investigating the effects of TCM treatment candidates. Potential adverse effects for each treatment must be investigated. As mentioned previously, Ephedra sinica Stapf., herbaceous stem and Asarum sieboldii Miq., root and rhizome, gingered may have adverse effects at certain dosages. These two herbs have been considered safe when used in certain formulas, where it is possible that either the dosages were low enough to minimize adverse effects, or other herbs in these formulas reduced potential adverse effects. Further studies are needed. In addition, for optimal treatment, one may need to consider the role of circadian rhythms due to circadian clocks' role in regulating physiology pharmacokinetics and efficacy of many therapeutics (Ruben et al., 2019). Drug interactions should also be carefully monitored. Many of the clinical trials integrating "Western" and Chinese medicine to treat COVID-19 have not been randomized-double blind trials. These trials also lack details about the dosages and categories of Western medicines used, and they do not explain the randomization methodology. In a recent publication, the status of clinical trials using herbal medicines was reported (Zhou Z. et al., 2020). Third, the mechanisms of action and active compounds for each formula need to be further explored. This knowledge would explain how these formulas work, may be helpful in simplifying the formulas, and could improve quality control.

Traditional medicines, including TCM, have evolved with human usage over time and across cultures. The experienced-based nature and systems biology approach of traditional medicines can and should be developed into modern evidence-based medicines to treat current unmet medical needs. The systems biology effect of botanical medicines should be considered a component of the treatment regimen in light of their anti-inflammatory and perhaps anti-RBS and positive cardiovascular impact on different organs. The usage of these formulas could be most effective in combination with reductionist, antiviral, anti-inflammatory, or anti-RBS drugs. Many groups have used network pharmacology to understand the multiple actions of herbs. It should be kept in mind that this approach is only helpful in forming hypotheses; evidence-based studies are still needed to test those hypotheses.

While COVID-19 treatment is critical, prevention will be the best solution in controlling the disease. In China, TCM is utilized in national policies as preventative medicine. More rigorous studies and concrete evidence are needed to validate the efficacy of these preventative formulas and drugs. Given the many environmental, social, and genetic factors that

influence our health, now more than ever, our diverse needs necessitate new and different preventative measures. After multiple SARS-CoV-2 strains isolated from different regions were sequenced, research teams from various countries applied the information to begin developing a vaccine. To date, ten coronavirus vaccines have been approved (Craven, 2020). In theory, a vaccine for an RNA virus such as SARS-CoV-2 would be ideal. However, in practice, a single vaccine may not be sufficient given industry experiences developing vaccines for past influenza viruses. The influenza vaccine must be updated yearly to account for viral mutation or draft. One study shows that SARS-CoV-2 could exist in patients who have virus-specific immunoglobulin G (IgG) for an unexpectedly short time (36–50 days). This raises the question of whether patients with antibodies are still at risk for reinfection (Wang et al., 2020a). There are many unknowns regarding the efficacy and long-term adverse effects of a future COVID-19 vaccine and its ability to benefit enough patients for a sustained period of time. Modern botanical drugs could be used to aid in prevention and treatment of COVID-19 and could serve as adjuvant therapy for vaccines. This should be pursued.

COVID-19 patients recovering from viral infection may develop long-term health issues such as fatigue, cardiovascular, neurological, renal, and pulmonary issues (Gan et al., 2020; Gupta et al., 2020). It is important that these long-term risks be taken into consideration during treatment. By nature, Chinese medicine acts on many targets and therefore may be such a solution to address the long-term health risks. Currently, Western and Eastern practices take two fundamentally different approaches to medicine. Western (W) medicine takes a potent, targeted approach with a narrow spectrum of action while Eastern (E) medicine (including TCM) utilizes multiple chemicals, acting on several targets through a broad spectrum, systems biology approach. The treatment and prevention of COVID-19 would benefit greatly from a more effective, integrated, WE Medicine approach in order to address the complexities and pathogenesis of SARS-CoV-2.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

Y-CC and WL put forward the ideas and designed the experiments; JW gathered the materials and wrote the paper. †BS and LH contributed equally; BS contributed as a consultant in traditional Chinese medicine and participated in the preliminary animal studies together with FG, LW and JW; LH contributed to the discuss; PC and SS helped write the manuscript.

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frontiers in Pharmacology

SYSTEMATIC REVIEW
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Chinese Herbal Medicine Used With or Without Conventional Western Therapy for COVID-19: An Evidence Review of Clinical Studies

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Objective: To present the evidence of the therapeutic effects and safety of Chinese herbal medicine (CHM) used with or without conventional western therapy for COVID-19.

Methods: Clinical studies on the therapeutic effects and safety of CHM for COVID-19 were included. We summarized the general characteristics of included studies, evaluated methodological quality of randomized controlled trials (RCTs) using the Cochrane risk of bias tool, analyzed the use of CHM, used Revman 5.4 software to present the risk ratio (RR) or mean difference (MD) and their 95% confidence interval (CI) to estimate the therapeutic effects and safety of CHM.

Results: A total of 58 clinical studies were identified including RCTs (17.24%, 10), non-randomized controlled trials (1.72%, 1), retrospective studies with a control group (18.97%, 11), case-series (20.69%, 12) and case-reports (41.38%, 24). No RCTs of high methodological quality were identified. The most frequently tested oral Chinese patent medicine, Chinese herbal medicine injection or prescribed herbal decoction were: Lianhua Qingwen granule/capsule, Xuebijing injection and Maxing Shigan Tang. In terms of aggravation rate, pooled analyses showed that there were statistical differences between the intervention group and the comparator group (RR 0.42, 95% CI 0.21 to 0.82, six RCTs; RR 0.38, 95% CI 0.23 to 0.64, five retrospective studies with a control group), that is, CHM plus conventional western therapy appeared better than conventional western therapy alone in reducing aggravation rate. In addition, compared with conventional western therapy, CHM plus conventional western therapy had potential advantages in increasing the recovery rate and shortening the duration of fever, cough and fatigue, improving the negative conversion rate of nucleic acid test, and increasing the improvement rate of chest CT manifestations and shortening the time from receiving the treatment to the beginning of chest CT manifestations improvement. For adverse events, pooled data showed that there were no statistical differences between the CHM and the control groups.

Conclusion: Current low certainty evidence suggests that there maybe a tendency that CHM plus conventional western therapy is superior to conventional western therapy alone. The use of CHM did not increase the risk of adverse events.

Keywords: traditional Chinese medicine, Chinese herbal medicine, novel coronavirus pneumonia, coronavirus disease 2019, COVID-19, SARS-CoV-2, review, clinical study

INTRODUCTION

Novel coronavirus pneumonia (NCP), officially named as Coronavirus Disease 2019 (COVID-19) by the World Health Organization (WHO) (World Health Organization, 2020a), is an acute respiratory infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) which has affected the general population. The main symptoms of COVID-19 are fever, cough and fatigue, and may be accompanied by nasal congestion, runny nose, sore throat, diarrhea, or loss of taste and smell anosmia (National Health Commission of the People's Republic of China, 2020a). In traditional Chinese medicine, COVID-19 is classified within the pestilential (Yibing, 疫病) category. The National Health Commission of the People's Republic of China has incorporated COVID-19 into the category B infectious diseases as stipulated in the Law of the People's Republic of China on the Prevention and Control of Infectious Diseases, and carried out prevention and control management following category A infectious diseases. On 11 March 2020, the director-general of World Health Organization (WHO), Dr Tedros Adhanom Ghebreyesus, declared that COVID-19 was now characterized as a pandemic (World Health Organization, 2020b), that is, COVID-19 had spread worldwide, and posed a great challenge and threat to the existing public health resources.

At present, there is no specific and effective therapy for the treatment and prevention of this disease (Chandan et al., 2020; Torequl et al., 2020). Traditional Chinese medicine (TCM) has accumulated thousands of years of experience on the use of Chinese herbal medicine (CHM) to prevent and treat infectious diseases (Jiang 2011). Its success was initially substantiated by modern human clinical research on severe acute respiratory syndrome (SARS) and H1N1 influenza epidemics, suggesting that using historical CHM experience may be a worthwhile approach (Luo et al., 2020). As this current epidemic escalated into a pandemic, the National Health Commission of the People's Republic of China has released multiple editions of guidelines for the diagnosis and treatment of COVID-19 (hereinafter referred to as GDT of COVID-19). In the third edition (National Health Commission of the People's Republic of China, 2020b), CHM was recommended for the treatment of COVID-19, and all relevant medical institutions were required to actively encourage of the use of CHM in the treatment of COVID-19. The early application of CHM during the COVID-19 pandemic and appeared to have a potentially beneficial effects. CHM has increasingly shown its potential in the treatment and prevention for infectious diseases, and has received widespread attention.

To further probe the role of CHM used with or without conventional western therapy on the treatment of COVID-19, an evidence-based approach was employed to systematically collate, analyze and evaluate clinical studies on the therapeutic effects and safety of using CHM for COVID-19.

MATERIALS AND METHODS

Inclusion and Exclusion Criteria of Studies

The following criteria were used to identify relevant studies.

Inclusion criteria were as follows: 1) Clinical studies which aimed to evaluate the therapeutic effects and/or safety of CHM used with or without conventional western therapy in patients with COVID-19; 2) There were no limits on the study design and could be randomized controlled trials (RCT), non-randomized controlled trials (non-RCT), cohort studies, case series, case reports or other study designs; 3) Participants were patients diagnosed with COVID-19. Disease severity could be mild, common, severe or critical, as prescribed in the guideline for the diagnosis and treatment of COVID-19 formulated by the National Health Commission of the People's Republic of China. There was no limitation on participants' age, gender and their ethnicity, or the setting of the studies; 4) The interventions in the experimental group were CHM and included prescribed herbal decoctions, oral Chinese patent medicines (capsules, tablets or granules) or Chinese herbal medicine injection, or CHM combined with comparators. For controlled clinical studies, comparators could be conventional western therapy or placebo.

Exclusion criteria were: 1) The full text of the studies could not be obtained; 2) Any duplicated articles; 3) Registered clinical studies but had not started or completed; 4) Clinical studies that had been registered and completed but had not published research data, and the data which could not be obtained by contacting the authors; 5) If the registered protocol and the publication(s) were from the same study, the protocol was excluded.

Retrieval Platforms and Search Strategies of Studies

Studies were retrieved through nine electronic databases including: China National Knowledge Infrastructure (CNKI, as of April 30, 2020), Wanfang Database (from January 1 to April 30, 2020), the China Science Technology Journal Database (VIP, from January 1 to April 30, 2020), SinoMed (from January 1 to April 30, 2020), PubMed (from January 1 to April 30, 2020), Embase (from January 1 to April 30, 2020), BioRxiv (as of April 30, 2020), MedRxiv (as of April 30, 2020), arXiv (as of April 30, 2020) and clinical trial registration platforms (CTRPs) including

ClinicalTrials.gov (www.clinicaltrials.gov, as of April 30, 2020) and Chinese Clinical Trial Registry (ChiCTR, www.chictr.org/cn, as of April 30, 2020).

For the databases/CTRPs with COVID-19 thematic platforms, including CNKI and ClinicalTrials.gov, the search was performed directly in the COVID-19 thematic platform. For Wanfang, VIP, SinoMed, PubMed and Embase, search terms were used. The search terms included Xinxing Guanzhuang Bingdu Bing (新型冠状病毒病), Xinguan Feiyan (新冠肺炎), 2019 Guanzhuang Bingdu Bing (2019冠状病毒病), coronavirus disease-19, COVID-19, 2019 novel coronavirus, 2019-nCOV, NCP, Zhongyi (中医), Zhongyao (中药), Caoyao (草药), Tangji (汤剂), Zhongchengyao (中成药), Zhusheji (注射剂), Zhongxiyi Jiehe (中西医结合), Chinese medicine, traditional Chinese medicine, herbal medicine, decoction, patent medicine, injection, integrated Chinese and western medicine. For ChiCTR, title search was carried out using Xinxing Guangzhuang Bingdu (新型冠状病毒) and COVID-19 as search terms. For BioRxiv, MedRxiv and arXiv, title or abstract search was carried out using COVID-19 as search terms. Appendix 1 shows the search strategies for the nine electronic databases and CTRP.

Before submission, we updated the search and included the latest published studies that met the inclusion criteria.

Study Selection and Data Extraction

Published studies were screened according to the inclusion/exclusion criteria by titles, abstracts and (or) full texts of the published articles. Registered studies were screened according to the inclusion/exclusion criteria by reading the titles and details of registered protocols. SBL, YYZ, CS, CHL, YQL, BYL and ZYT were responsible for the selection of articles.

Excel 2010 was used to provide the data sheets for extraction. Extracted items include first author's name or registered protocol's ID, study titles, the country in which the study was carried out, study design, characteristics of participants (such as sample size, age, gender, severity of COVID-19, etc.), details of interventions and outcomes, etc. For each included study, two authors independently extracted and checked the data. The inconsistencies were resolved by the two authors through consultation. If any disagreements, a third author (JPL) was consulted. SBL, YYZ, YQL, CS, BYL, ND, YJ, XWZ, CHL, YPZ and MX participated in data extraction in pair.

Outcomes

Primary outcomes included cure rate, mortality rate and aggravation rate (the change in the disease severity category, or patients were admitted to the ICU, et al.).

Secondary outcomes included the recovery rate or the duration (time to recovery) of main symptoms (including fever, cough and fatigue), negative conversion rate of nucleic acid test for SARS-CoV-2, improvement or recovery of chest CT manifestations, length of hospitalization and adverse events.

For outcomes reported at multiple timepoints, we used the longest reported follow-up timepoint.

Design of This Review and Data Synthesis

This is an evidence review of clinical studies on the therapeutic effects and safety of CHM used with or without conventional western therapy for COVID-19. Initially, we summarized the general characteristics of the included studies and then the methodological quality of included RCTs was assessed by SBL and YQL using the Cochrane risk of bias tool (Higgins et al., 2011). Subsequently, counts and percentages were applied to analyze the use of CHM. Lastly, we evaluated the therapeutic effects and safety of CHM used with or without conventional western therapy for COVID-19. For studies without control group, such as case series and case reports, we only presented these findings qualitatively as they were not sufficient to probe the therapeutic effects of CHM for COVID-19 due to the absence of control and a high risk of bias in case selection. For studies with control group, we used Cochrane Collaboration Review Manager 5.4 (Revman 5.4) software to conduct meta-analysis of the data. We presented binary data as a risk ratio (RR) with its 95% confidence interval (CI), and continuous data as a mean difference (MD) with its 95% CI. Considering potential sources of clinical heterogeneity, the random-effect model (REM) was used for meta-analysis. We planned to conduct the following subgroup analysis for the primary outcomes if data were available: 1) subgroup analysis based on the severity of COVID-19, to detect whether the therapeutic effects of CHM is related to the severity; 2) subgroup analysis based on the use of CHM with or without conventional western therapy, to detect whether CHM alone or whether CHM plus conventional western therapy is more beneficial for the treatment of COVID-19.

RESULTS

Search Results

Figure 1 shows the flow diagram for the searching and screening of published articles. A total of 4763 published articles were retrieved from the above-mentioned nine open electronic databases, of which 102 articles were selected by reading full-texts and 54 were removed for various reasons. Finally, 48 published articles (representing 48 completed studies) met the inclusion criteria. Before submission, we updated the search and included 10 further completed studies that met the inclusion criteria. **Figure 2** shows the flow diagram for searching and screening of registered clinical studies. A total of 1669 registered protocols were retrieved from the above-mentioned two CTRPs and 50 registered protocols (50 registered clinical studies) meeting the inclusion criteria. However, all the 50 registered studies were excluded due to their status as 'not yet started' or "in progress."

Therefore, 58 published articles (representing 58 completed studies) were included in our review.

The Characteristics of Included 58 Clinical Studies

All the 58 clinical studies were conducted in China. Of these, 52 were published in Chinese and six were in English. Among the

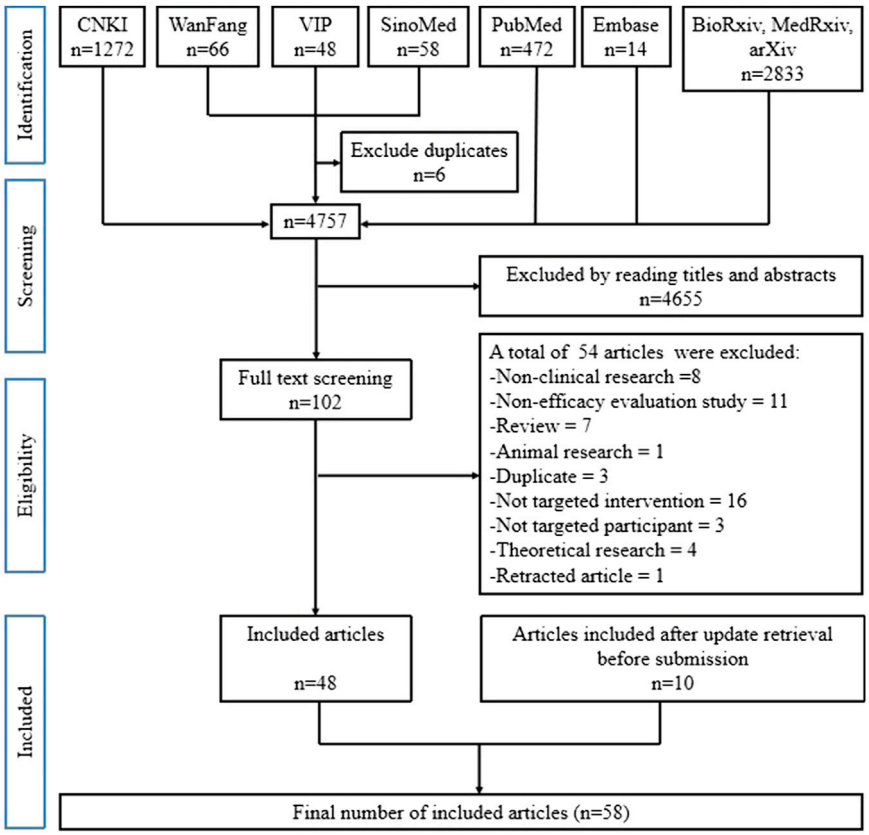

FIGURE 1 | Flow diagram for searching and screening of published articles.

included studies, 10 (17.24%) were RCTs, one (1.72%) was non-RCT, 11 (18.97%) were retrospective studies with a control group, 12 (20.69%) were case-series, 24 (41.38%) were case-reports.

Of 2773 COVID-19 patients involved in the included studies, 1921 (69.28%) received CHM. The level of severity of COVID-19 involved non-serious (including mild and common) and serious (including severe and critical). Of the included 58 studies, 29 (50.00%) studies included only non-serious patients, 12 (20.69%) studies included only serious patients, 11 (18.97%) included both non-serious and serious patients, and the remaining 6 (10.34%) studies did not report the level of severity of COVID-19.

Of the included 58 studies, 8 (13.79%) involved only the use of CHM, and 51 (87.93%) involved CHM used in combination with conventional western therapy (such as abidor, ganciclovir, lopinavir, oxygen inhalation, nutritional support, etc.). The course of treatment varied from 4 to 15 days.

Table 1 shows the characteristics of the 58 included studies.

Methodological Quality of RCTs

In terms of the random sequence generation methods of the included 10 RCTs, six RCTs (Fu et al., 2020a; Wang et al., 2020c; Duan et al., 2020; Qiu et al., 2020; Sun et al., 2020; Yu et al., 2020) used random number tables, two trials (Ding et al., 2020; Ye, 2020) used a simple random allocation method and the remaining two RCTs (Zhang et al., 2020a; Fu et al., 2020b) only mentioned random without describing the detailed randomization method. Two RCTs (Wang et al., 2020c; Ye, 2020) performed allocation concealment. Therefore, the risk of selection (allocation) bias was unclear for the majority of the included RCTs due to lack of information on allocation concealment. Due to no trials used blinding to participants and personnel, the performance bias of all the included trials was judged as high-risk. Two RCTs (Wang et al., 2020c; Ye, 2020) performed outcome assessor blinding and the remaining eight RCTs (Fu et al., 2020a; Zhang et al., 2020a; Fu et al., 2020b; Ding et al., 2020; Duan et al., 2020; Qiu et al., 2020; Sun et al., 2020; Yu et al., 2020) did not report relevant information, thus the detection bias for the majority of the included RCTs was judged as unclear-risk. In terms of attrition bias, eight RCTs (Fu et al., 2020a; Zhang et al., 2020a; Wang et al., 2020c; Ding et al., 2020; Duan et al., 2020; Qiu et al., 2020; Sun et al., 2020; Ye, 2020) were assessed as low-risk of bias due to complete outcome data or incomplete outcome data being adequately addressed, two RCTs (Fu et al., 2020b; Yu et al., 2020) were assessed as high-risk due to incomplete outcome data that were not adequately addressed. Two RCTs (Wang et al., 2020c; Ye, 2020) registered the study protocol and reported the registration information. By comparison, we found that there was no selective reporting of outcomes in these two RCTs, so their reporting bias was evaluated as low-risk. Since the protocols or registration information of the other eight included RCTs (Yu et al., 2020; Duan et al., 2020; Sun et al.,

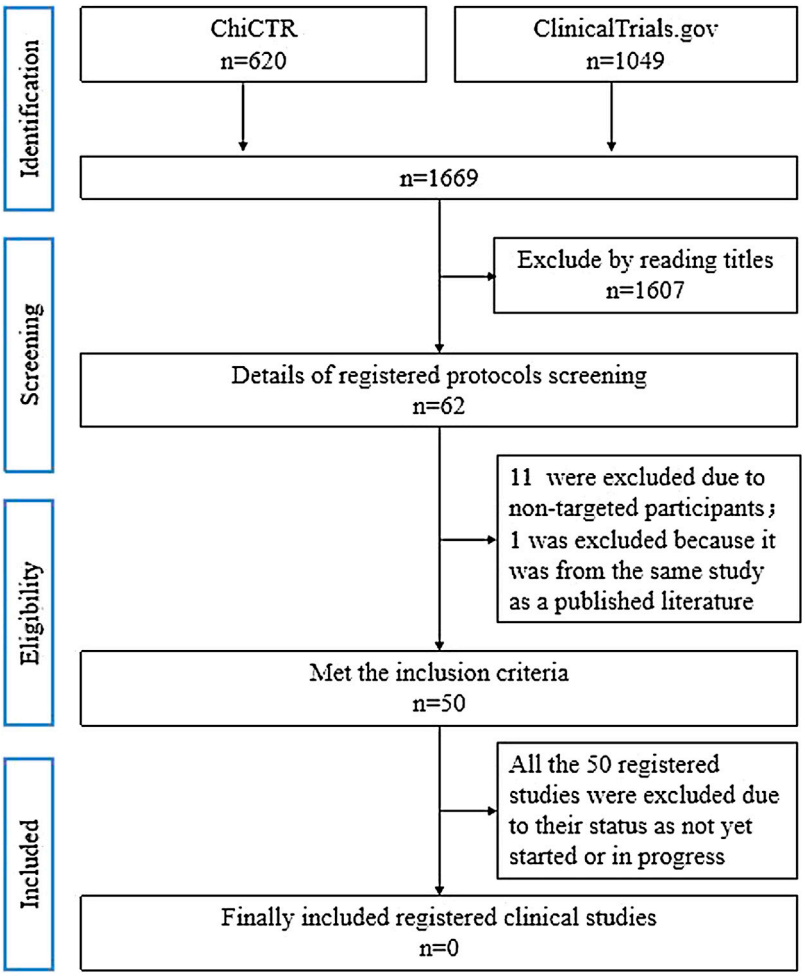

FIGURE 2 | Flow diagram for searching and screening of registered clinical studies.

2020; Fu et al., 2020a; Fu, et al., 2020b; Ding et al., 2020; Qiu et al., 2020; Zhang et al., 2020a) were not available, the selective reporting of outcomes in these RCTs could not be judged and the reporting bias of these was assessed as unclear-risk. All 10 RCTs reported the comparability of baseline data, so they were assessed as having a low-risk of other bias.

Figure 3 demonstrates the risk of bias of included 10 RCTs.

Analysis of the use of CHM

For the type of CHM, 24 (41.38%) studies tested oral Chinese patent medicine, 40 (68.97%) studies tested prescribed herbal decoction, and 7 (12.07%) studies tested Chinese herbal medicine injection.

The top ten CHMs used were Maxing Shigan Tang [麻杏石甘汤, 15.52% (9/58)], Lianhua Qingwen granule/capsule [连花清瘟颗粒/胶囊, 15.52% (9/58)], Xuebijing injection [血必净注射剂, 8.62% (5/58)], Dayuanyin [达原饮, 8.62% (5/58)], Shufeng Jiedu capsule[疏风解毒胶囊, 8.62% (5/58)], Qingfei Paidu Tang [清肺排毒汤, 6.90% (4/58)], Xiaochaihu Tang [小柴胡汤, 6.90% (4/58)], Ganlu Xiaodu Dan [甘露消毒丹, 5.17% (3/58)], Liujunzi Tang [六君子汤, 5.17% (3/58)] and Toujie Quwen granule [透解祛瘟颗粒, 5.17% (3/58)]. Of which, the most frequently used oral Chinese patent medicine, Chinese herbal medicine injection and prescribed herbal decoction were Lianhua Qingwen granule/capsule [连花清瘟颗粒/胶囊], Xuebijing injection [血必净注射剂], and Maxing Shigan Tang [麻杏石甘汤], respectively.

Table 2 lists the CHM used at least twice.

Therapeutic effects and Safety of CHM in the Treatment or Adjuvant Treatment of COVID-19

Analysis for Studies with Control Group

Primary Outcomes

Cure Rate. Six studies including one RCT (Fu et al., 2020b) and five retrospective studies with a control group (Qu et al., 2020a; Li et al., 2020c; Yang et al., 2020c; Xia et al., 2020; Shi et al., 2020a) reported this outcome. Of which, one study (Shi et al., 2020) was not enrolled into the meta-analysis due to no assessment criteria of cure rate in it's publication. All the other five studies adopted the judgment criteria of the GDT of COVID-19 for cure: 1) the body temperature returned to normal for longer than three days; 2) the respiratory symptoms

TABLE 1 | The characteristics of included studies of Chinese herbal medicine for COVID-19.

Study ID	Sample size (M/F)	Age (year)	The severity (*) of COVID-19	Type of Chinese herbal medicine	Conventional western therapy (Yes/No)	Course of CHM treatment	Outcomes	Author's conclusion towards the role of Chinese herbal medicine in the treatment or adjuvant treatment of COVID-19 (positive/ negative)
Study type 1: randomized controlled trials (10, 17.24%)								
Yu et al. (2020)	T:82/65, C:89/59	T:48.27±9.56, C: 47.25±8.67	Non-serious	Chinese patent medicine	Yes	7 days	②③⑪⑬	Positive
Duan et al. (2020)	T:39/43, C:23/18	T:51.99±13.88, C: 50.29±13.17	Non-serious	Chinese patent medicine	Yes	5 days	②④⑤⑥⑬	Positive
Sun et al. (2020)	T:17/15, C:11/14	T:45.4±14.10, C: 42.0±11.70	Non-serious	Chinese patent medicine	Yes	14 days	②④⑤⑥⑧⑪	Positive
Fu et al. (2020a)	T:17/15, C:19/14	T:43.26±7.15, C: 43.68±6.45	Non-serious	Chinese patent medicine	Yes	10 days	②⑪⑬	Positive
Fu et al. (2020b)	T:19/18, C:19/17	T:45.26±7.25, C: 44.68±7.45	Non-serious	Chinese patent medicine	Yes	15 days	①②⑬	Positive
Ding et al. (2020)	T:39/12, C:39/10	T:54.7±21.3, C: 50.8±23.5	T: 46 (non-serious) / 5 (serious), C: 11 (non-serious) / 4 (serious)	Prescribed herbal decoction	Yes	10 days	④⑤⑪⑬	Positive
Ye (2020)	T:2/26, C:4/10	T:53.5–69, C:47–67	Serious	Prescribed herbal decoction	Yes	7 days	②③	Positive
Qiu et al. (2020)	T:13/12, C:14/11	T: 53.35±18.35, C: 51.32±14.62	Non-serious	Prescribed herbal decoction	Yes	10 days	②⑦⑧⑪	Positive
Zhang et al. (2020a)	T:9/13, C:10/13	T:53.7 ±3.5, C: 55.6±4.2	Non-serious	Prescribed herbal decoction	Yes	7 days	⑦⑧⑨⑪⑬	Positive
Wang et al. (2020c)	T:14/10, C:12/11	T:46.8±14.4, C: 51.4±17.6	Non-serious	Prescribed herbal decoction	Yes	14 days	②③⑦⑬	Positive
Study type 2: Non-randomized controlled trial (1, 1.72%)								
Xiao et al. (2020)	T:64/36, C:66/34	T:60.90±8.70, C: 62.20±7.50	Non-serious	Chinese patent medicine	Yes	14 days	⑦⑧⑨⑪⑬	Positive
Study type 3: Retrospective studies with a control group (11, 18.97%)								
Cheng et al. (2020)	T:26/25, C:27/24	T:55.5±12.3, C: 55.8±11.6	Non-serious	Chinese patent medicine	Yes	7 days	②④⑤⑥⑦⑧⑨⑪	Positive
Liu et al. (2020c)	T:21/23, C:16/20	T:50.73, C:51.75	T: 37 (non-serious) / 7 (serious), C: 28 (non-serious) / 8 (serious)	Chinese patent medicine	Yes	7 days	⑪⑬	Positive
Zhang et al. (2020b)	T:10/12, C:12/10	T:25–73, C:19–67	Non-serious	Chinese herbal medicine injection	Yes	7 days	⑩⑪⑬	Positive
Li et al. (2020c)	T:15/15, C:13/17	T:53.600±0.259, C: 50.433±0.338	T: 3 (serious)/27(not reported) , C: 2 (serious)/28(not reported)	Prescribed herbal decoction	Yes	Not reported	①②⑦⑧⑪⑬	Positive
Yang et al. (2020c)	T:28/23, C: 24/28	T:61.57±1.84, C: 66.35±1.82	Serious	Prescribed herbal decoction + Chinese herbal medicine injection	Yes	Not reported	①③⑪⑫⑬	Positive
Qu et al. (2020a)	T:25/15, C:16/14	T:40.65±8.23, C: 39.82±6.40	Non-serious	Chinese patent medicine	Yes	10 days	①⑦⑧⑨⑩⑬	Positive

(Continued on following page)

TABLE 1 | (*Continued*) The characteristics of included studies of Chinese herbal medicine for COVID-19.

Study ID	Sample size (M/F)	Age (year)	The severity (*) of COVID-19	Type of Chinese herbal medicine	Conventional western therapy (Yes/No)	Course of CHM treatment	Outcomes	Author's conclusion towards the role of Chinese herbal medicine in the treatment or adjuvant treatment of COVID-19 (positive/ negative)
Xia et al. (2020)	T:17/17, C:6/12	T:54.18±13.08, C: 53.67±12.70	T: 27 (non-serious) / 7 (serious) , C: 13 (non-serious) / 4 (serious)	Chinese patent medicine + Chinese herbal medicine injection + prescribed herbal decoction	Yes	5-10 days	①②③⑦⑪⑫⑬	Positive
Yao et al. (2020)	T:16/5, C:12/9	T:57.1±14.0, C: 62.4±12.3	Non-serious	Chinese patent medicine	Yes	Not reported	④⑤⑥⑦	Positive
Shi et al. (2020a)	T:26/23, C:10/8	T:47.94±14.46, C: 46.72±17.40	T: 41 (non-serious) / 8 (serious) , C: 15 (non-serious) / 3 (serious)	Chinese patent medicine + prescribed herbal decoction	Yes	Not reported	①②⑪⑫	Positive
Yang et al. (2020a)	T:16/10, C:9/14	T:50.35±13.37, C: 47.17±16.57	Non-serious	Chinese patent medicine	Yes	7 days	②⑩⑪⑬	Positive
Chen et al. (2020)	T:14/20, C:15/19	T:65.06±10.63, C: 64.35±10.34	Non-serious	Chinese patent medicine	Yes	7 days	②④⑤⑥⑦⑧⑨⑪⑫⑬	Positive
Study type 4: Case-series (12, 20.69%)								
Zhang et al. (2020c)	9/15	49.96±12.79 (27-69)	Non-serious	Prescribed herbal decoction	Yes	6-14 days	NA	Positive
Wang et al. (2020d)	52/46	42.70±16.86	87 (non-serious) / 11 (serious)	Prescribed herbal decoction	No	9 days	NA	Positive
Xie et al. (2020a)	8	35–79	Serious	Prescribed herbal decoction	Yes	Not reported	NA	Positive
Li et al. (2020d)	3/3	42–79	Serious	Chinese patent medicine + Chinese herbal medicine injection + prescribed herbal decoction	Yes	Not reported	NA	Positive
Ba et al. (2020)	243/208	43–66	399 (non-serious) / 46 (serious)	Prescribed herbal decoction	Yes	Not reported	NA	Positive
Liu et al. (2020a)	36	NR	Not reported	Prescribed herbal decoction	Yes	14 days	NA	Positive
Huang et al. (2020)	38/33	41.3±16.7	Non-serious	Chinese patent medicine + Chinese herbal medicine injection + prescribed herbal decoction	Yes	Not reported	NA	Positive
Xie et al. (2020b)	27	2–68	Non-serious	Prescribed herbal decoction	Yes	Not reported	NA	Positive
Cheng and Li (2020)	29/25	60.1±16.98 (25–95)	Non-serious	Chinese patent medicine	Yes	8. 0 ± 4. 10 days	NA	Positive
Zhou et al. (2020)	17/23	19–68	Non-serious	Prescribed herbal decoction	Yes	14 days	NA	Positive
Qu et al. (2020b)	23/17	61.2±16.5 (24–79)	Non-serious	Prescribed herbal decoction	Yes	7 days	NA	Positive

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TABLE 1 | (*Continued*) The characteristics of included studies of Chinese herbal medicine for COVID-19.

Study ID	Sample size (M/F)	Age (year)	The severity (*) of COVID-19	Type of Chinese herbal medicine	Conventional western therapy (Yes/No)	Course of CHM treatment	Outcomes	Author's conclusion towards the role of Chinese herbal medicine in the treatment or adjuvant treatment of COVID-19 (positive/ negative)
Shi et al. (2020c)	15/25	43.9±16.3 (20–94)	32 (non-serious) / 8 (serious)	Prescribed herbal decoction	Yes	Not reported	NA	Positive
Study type 5: Case-reports (24, 41.38%)								
Fu et al. (2020)	1/1	32, 46	Non-serious	Chinese patent medicine	Yes	10/14 days	NA	Positive
Tian et al. (2020)	2/3	24, 28, 36, 40, 49	2 (non-serious) / 3 (serious)	prescribed herbal decoction + Chinese patent medicine	Yes	9 days	NA	Positive
Dong et al. (2020)	1 M	56	Not reported	Prescribed herbal decoction	No	11 day	NA	Positive
Shi et al. (2020b)	2 M	45, 48	Non-serious	Prescribed herbal decoction + Chinese herbal medicine injection	Yes	7/18 days	NA	Positive
Li et al. (2020b)	1/1	35, 36	1 (non-serious) / 1 (serious)	Prescribed herbal decoction	No	4/6 days	NA	Positive
Zhao et al. (2020)	1 F	41	Not reported	Prescribed herbal decoction	Yes	9 days	NA	Positive
He et al. (2020)	2 M	25, 29	Serious	Prescribed herbal decoction	Yes	8/6 days	NA	Positive
Yang and Niu (2020)	1 F	74	Serious	Prescribed herbal decoction	Yes	15 days	NA	Positive
Wang et al. (2020e)	2 M	33, 54	1 (non-serious) / 1 (serious)	Prescribed herbal decoction	Yes	Not reported	NA	Positive
Li et al. (2020e)	1 F	71	Serious	Prescribed herbal decoction	Yes	Not reported	NA	Positive
Feng et al. (2020)	1 F	51	Serious	Prescribed herbal decoction	Yes	15 days	NA	Positive
Xu et al. (2020)	1 M	35	Non-serious	Prescribed herbal decoction	Yes	12 days	NA	Positive
Liu et al. (2020b)	1 F	38	Non-serious	Prescribed herbal decoction	Yes	7 days	NA	Positive
Li et al. (2020f)	2 F	17, 45	Non-serious	Chinese patent medicine + prescribed herbal decoction	No	9 days	NA	Positive
Lin et al. (2020)	1 F	35	Not reported	Prescribed herbal decoction	Yes	12 days	NA	Positive
Hu et al. (2020)	1 F	61	Serious	Chinese patent medicine + prescribed herbal decoction	Yes	11 days	NA	Positive
Wang et al. (2020f)	3/1	19 , 32 , 63 , 63	2 (non-serious) / 2 (serious)	Chinese patent medicine	Yes	Not reported	NA	Positive

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TABLE 1 | (*Continued*) The characteristics of included studies of Chinese herbal medicine for COVID-19.

Study ID	Sample size (M/F)	Age (year)	The severity (*) of COVID-19	Type of Chinese herbal medicine	Conventional western therapy (Yes/No)	Course of CHM treatment	Outcomes	Author's conclusion towards the role of Chinese herbal medicine in the treatment or adjuvant treatment of COVID-19 (positive/ negative)
Deng et al. (2020)	1 F	39	Serious	Prescribed herbal decoction	Yes	Not reported	NA	Positive
Ni et al. (2020)	1/2	27, 51, 53	Serious	Chinese patent medicine	1 Yes / 2 No	Not reported	NA	Positive
Gao et al. (2020)	1F	42	Non-serious	Chinese patent medicine	No	7 days	NA	Positive
Li et al. (2020a)	1/1	68, 47	Non-serious	Prescribed herbal decoction	No	Not reported	NA	Positive
Lai et al. (2020)	1/2	56, 61, 60	1 (non-serious) / 2 (not reported)	Prescribed herbal decoction	No	6/7 days	NA	Positive
Wang et al. (2020a)	1/1	45, 32	Serious	Chinese herbal medicine injection + prescribed herbal decoction	Yes	12/14days	NA	Positive
Wang et al. (2020b)	1/1	63, 49	Non-serious	Prescribed herbal decoction	Yes	10/14 days	NA	Positive

Note: *M, male; F, female; T, treatment group involving Chinese herbal medicine; C, controlled group not involving Chinese herbal medicine; Yes, the intervention involved in this study was Chinese herbal medicine combined with conventional western therapy; No, the intervention involved in this trial was Chinese herbal medicines alone, not combined with conventional western therapy; NA, not applicable; Positive, Chinese herbal medicine has benefits on the treatment or adjuvant treatment of COVID-19; negative, Chinese herbal medicine has no benefits on the treatment or adjuvant treatment of COVID-19, or can even make the disease worse.*

The severity (*) was classified according to the guidelines for the diagnosis and treatment of COVID-19 released by the National Health Commission of the People's Republic of China. We divide them into two categories of non-serious (including mild and common) and serious (including severe and critical).

Although the article (Wang et al., 2020c) did not specify the severity of COVID-19, since all participants in this trial were screened from suspected COVID-19 patients, we considered the severity of COVID-19 of these participants as non-serious.

Outcomes: ① cure rate; ② aggravation rate; ③ mortality rate; ④ the recovery rate of fever; ⑤ the recovery rate of cough; ⑥ the recovery rate of fatigue;⑦ the duration of fever; ⑧ the duration of cough; ⑨ the duration of fatigue; ⑩ negative conversion rate of nucleic acid test; ⑪ improvement or recovery of chest CT manifestations; ⑫ Length of hospitalization; ⑬ adverse events.

Although one trial (Yu et al., 2020) reported the outcome of aggravation rate, we did not enrolled the data on this outcome in the statistical analysis due to the inconsistency between the data presented in the table and in the text of the trial's publication.

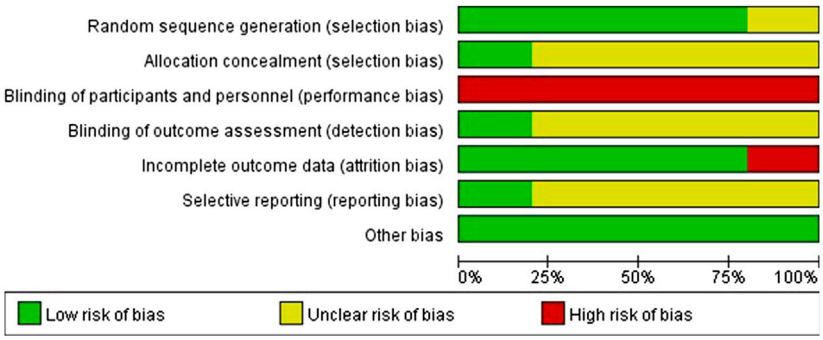

FIGURE 3 | Risk of bias graph of included 10 RCTs.

improved significantly; 3) the pulmonary imaging showed that the inflammation has obviously disappeared; 4) the respiratory pathogenic nucleic acid, (the sampling time interval of two tests was at least 1 day or 24 h), and the results were both negative.

All five studies compared CHM plus conventional western therapy with conventional western therapy. After analyzing separately according to the study design, the results (see **Figure 4**) regardless of RCTs or retrospective studies with a control group showed that there was no statistical difference between the experimental and control groups (RR 1.42, 95% CI 0.76 to 2.62, 1 RCT (Fu et al., 2020b); RR 1.20, 95% CI 0.98 to 1.48, 4 retrospective studies with a control group (Li et al., 2020; Qu et al., 2020a; Xia et al., 2020; Yang et al., 2020c)).

Aggravation Rate. A total of 14 studies that compared CHM plus conventional western therapy with conventional western therapy reported on this outcome. Of these, two retrospective studies with a control group (Shi et al., 2020a; Yang et al., 2020a) reported that there were no patients who experienced aggravation in either the experimental or control group. Although one study (Yu et al., 2020) reported this outcome in their trial, we did not enrolled the data on this outcome in the statistical analysis due to the inconsistency between the data presented in the table and in the text. After analyzing separately according to the study design of the remaining 11 studies, the results of RCTs or retrospective studies with a control group both showed that CHM plus conventional western therapy was better than conventional western therapy alone in reducing aggravation rate (RR 0.43, 95% CI 0.23 to 0.80, 7 RCTs (Fu et al., 2020a; Wang et al., 2020c; Duan et al., 2020; Qiu et al., 2020; Sun et al., 2020; Ye, 2020; Fu et al., 2020b); RR 0.37, 95% CI 0.22 to 0.64, 4 retrospective studies with a control group (Chen et al., 2020; Cheng et al., 2020; Li et al., 2020c; Xia et al., 2020)). **Figure 5** illustrates the details of these results.

Mortality Rate. Five studies that compared CHM plus conventional western therapy with conventional western therapy reported this outcome. After analyzing separately according to the study design, the results (see **Figure 6**) regardless of RCTs or retrospective studies with a control group showed that there was no statistical difference between the experimental and control groups (RR 0.45, 95% CI 0.09 to 2.13, 3 RCTs (Wang et al., 2020c; Ye, 2020; Yu et al., 2020); RR 0.66, 95% CI 0.35 to 1.27, 2 retrospective studies with a control group (Yang et al., 2020c; Xia et al., 2020)).

Secondary Outcomes

The results on secondary outcomes are shown in **Table 3**.

The recovery Rate and the Duration of Main Symptoms (Fever, Cough and Fatigue).

a.The recovery rate of main symptoms A total of six studies including 3 RCTs (Ding et al., 2020; Duan et al., 2020; Sun et al., 2020) and 3 retrospective studies with a control group (Chen et al., 2020; Cheng et al., 2020; Yao et al., 2020) reported the recovery rate of main symptoms. All studies compared CHM plus conventional western therapy with conventional western therapy. Of these, the number of studies that reported the recovery rate of fever, cough and fatigue was six (Chen et al., 2020; Cheng et al., 2020; Ding et al., 2020; Duan et al., 2020; Sun et al., 2020; Yao et al., 2020), six (Chen et al., 2020; Cheng et al., 2020; Ding et al., 2020; Duan et al., 2020; Sun et al., 2020; Yao et al., 2020) and five (Chen et al., 2020; Cheng et al., 2020; Duan et al., 2020; Sun et al., 2020; Yao et al., 2020), respectively.

Regarding studies which explored the recovery rate for fever, after analyzing separately according to the study design, although the pooled data of retrospective studies with a control group showed that CHM plus conventional western therapy was better than conventional western therapy alone (RR 1.34, 95% CI 1.13 to 1.58, 3 retrospective studies with a control group), the pooled result of RCTs showed that there was no statistical difference between the experimental and control groups (RR 1.18, 95% CI 0.91 to 1.54, 3 RCTs, I^2 = 64%).

Regarding studies which investigated the recovery rate of cough, the results of RCTs or retrospective studies with a control group both showed that CHM in combination with conventional western therapy was superior to conventional western therapy alone (RR 1.36, 95% CI 1.15 to 1.62, 3 RCTs; RR 1.82, 95% CI 1.22 to 2.71, 3 retrospective studies with a control group).

For studies reporting the recovery rate of fatigue following COVID-19, the results regardless of RCTs or retrospective studies with a control group showed that CHM plus conventional western therapy had a higher recovery rate than conventional western

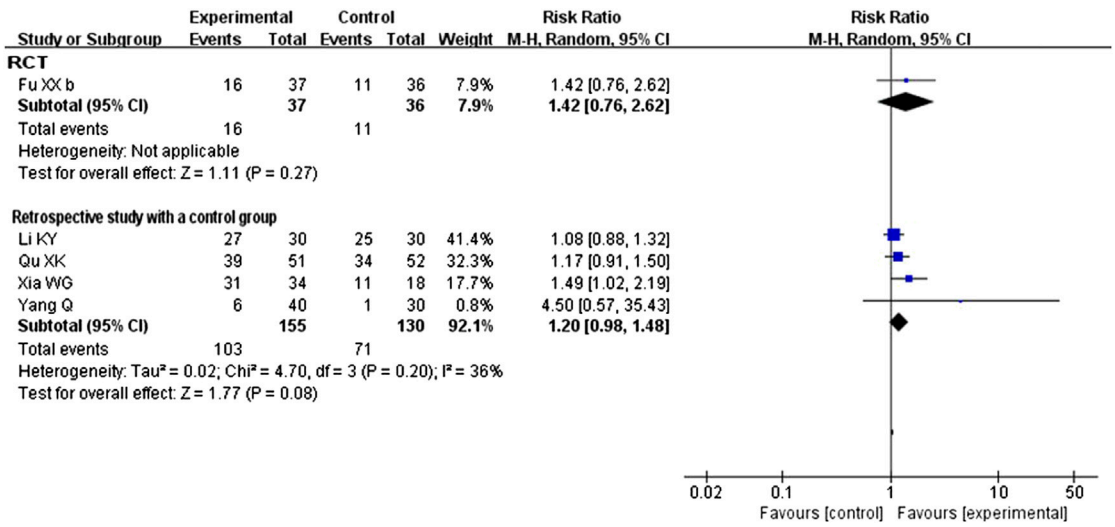

FIGURE 4 | Forest plot of cure rate: CHM plus conventional western therapy vs. conventional western therapy.

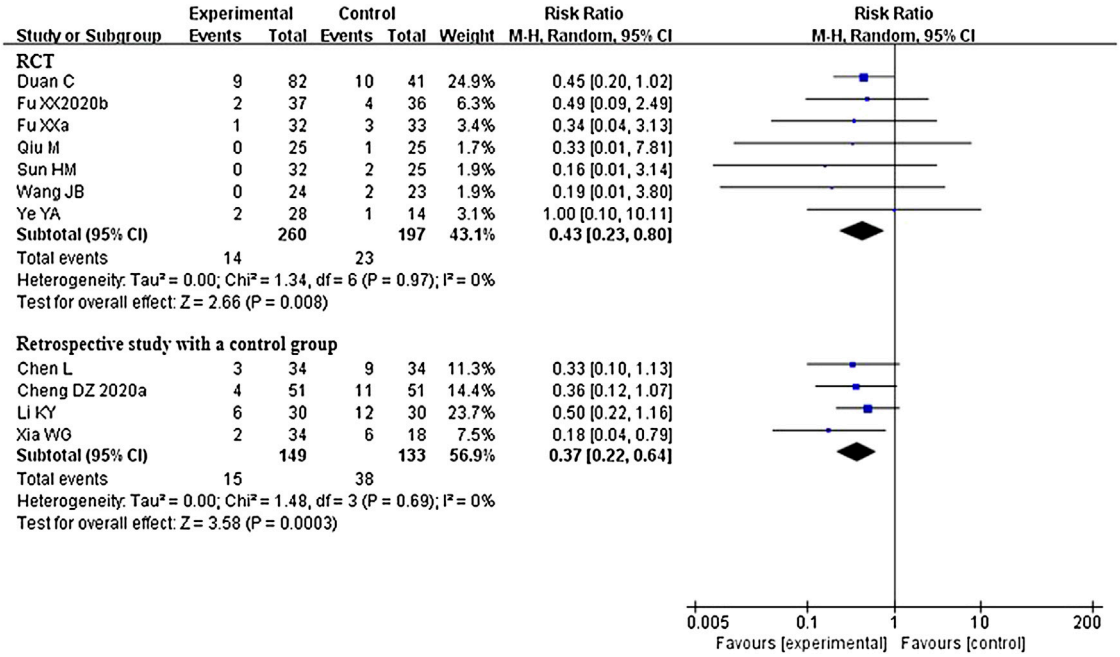

FIGURE 5 | Forest plot of aggravation rate: CHM plus conventional western therapy vs. conventional western therapy.

therapy alone (RR 1.33, 95% CI 1.03 to 1.71, 2 RCTs; RR 1.48, 95% CI 1.14 to 1.93, 3 retrospective studies with a control group).

b. *The duration (time to recovery) of main symptoms* A total of 11 studies including 4 RCTs (Zhang et al., 2020a; Wang et al., 2020c; Qiu et al., 2020; Sun et al., 2020), 1 non-RCT (Xiao et al., 2020) and 6 retrospective studies with a control group (Qu et al., 2020a; Chen et al., 2020; Cheng et al., 2020; Li et al., 2020c; Xia et al., 2020; Yao et al., 2020) reported the duration of main symptoms and all of them compared CHM plus conventional western therapy with conventional western therapy. Of these, the number of studies that reported the duration of fever, cough and fatigue was ten (Qu et al., 2020a; Zhang et al., 2020a; Chen et al., 2020; Cheng et al., 2020; Li et al., 2020c; Wang et al., 2020c; Qiu et al., 2020; Xia et al., 2020; Xiao et al., 2020; Yao et al., 2020), eight (Qu et al., 2020a; Zhang et al., 2020a; Chen et al., 2020; Cheng et al., 2020; Li et al., 2020c; Qiu et al., 2020; Xiao et al., 2020; Sun et al., 2020) and five (Qu et al., 2020a; Zhang et al., 2020a; Chen et al., 2020; Cheng et al., 2020; Xiao et al., 2020), respectively.

TABLE 2 | Chinese herbal medicine used twice or more frequently.

The name of Chinese herbal medicine (CHM)	Frequency (N)	Percentage (%)
Type 1 of CHM: Prescribed herbal decoction		
Maxing Shigan Tang [麻杏石甘汤]	9	15.52
Dayuanyin [达原饮]	5	8.62
Qingfei Paidu Tang [清肺排毒汤]	4	6.90
Xiaochaihu Tang [小柴胡汤]	4	6.90
Ganlu Xiaodu Dan [甘露消毒丹]	3	5.17
Liujunzi Tang [六君子汤]	3	5.17
Sanren Tang [三仁汤]	2	3.45
Feiyan No.1 Fang [肺炎1号方]	2	3.45
Xiaoqinglong Tang [小青龙汤]	2	3.45
Wulingsan [五苓散]	2	3.45
Type 2 of CHM: Oral Chinese patent medicine		
Lianhua Qingwen granule/capsule [连花清瘟颗粒/胶囊]	9	15.52
Shufeng Jiedu gapsule[疏风解毒胶囊]	5	8.62
Toujie Quwen granule [透解祛瘟颗粒]	3	5.17
Jinhua Qinggan granule [金花清感颗粒]	2	3.45
Shuanghuanglian oral liquid [双黄连口服液]	2	3.45
Type 3 of CHM: Chinese herbal medicine injection		
Xuebijing injection [血必净注射剂]	5	8.62
Xiyanping injection [喜炎平注射液]	2	3.45
Tanreqing injection [痰热清注射液]	2	3.45
Shenfu injection [参附注射液]	2	3.45
Shengmai injection [生脉注射液]	2	3.45

Note: Frequency refers to the number of included studies using the CHM. Such as, the frequency of Maxing Shigan Tang is 9, which means that nine included studies used Maxing Shigan Tang.

*Percentage = (N/58) * 100%*

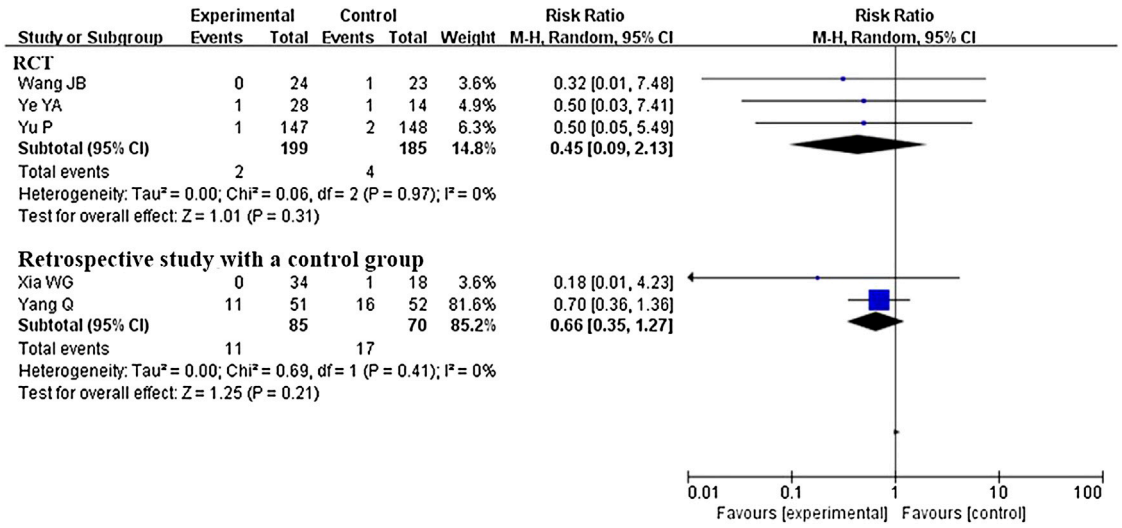

FIGURE 6 | Forest plot of mortality rate: CHM plus conventional western therapy vs. conventional western therapy.

For the duration of fever, one study (Wang et al., 2020c) reported that, the CHM group exhibited a significant improvement in time to fever resolution (p = 0.035) compared with the control group. After analyzing separately in light of the other nine studies' design, the results regardless of RCTs, non-RCT or retrospective studies with a control group showed that CHM plus conventional western therapy was better than conventional western therapy alone in shortening the duration of fever (MD-2.08 days, 95% CI-2.90 to-1.26, 2 RCTs, I^2 = 60%; MD-0.83 days, 95% CI-1.22 to-0.44, 1 non-RCT; MD-1.54 days, 95% CI-1.82 to-1.26, 6 retrospective studies with a control group).

In shortening the duration of cough, one trial (Sun et al., 2020) reported that CHM group was superior to conventional western therapy alone in shortening the duration of cough (P < 0.5). After analyzing separately based on the studys' design, the results

TABLE 3 | The pooled results of secondary outcomes of CHM used with or without conventional western therapy for COVID-19.

Comparisons and outcomes	Design of the included study	Number of study	Number of participant	The pooled results
Chinese herbal medicine + conventional western therapy vs. conventional western therapy				
●The recovery rate of fever	RCT	3	207	RR 1.18, 95% CI 0.91 to 1.54, I^2 = 64%
	Retrospective study with a control group	3	163	RR 1.34, 95% CI 1.13 to 1.58
●The recovery rate of cough	RCT	3	231	RR 1.36, 95% CI 1.15 to 1.62
	Retrospective study with a control group	3	156	RR 1.82, 95% CI 1.22 to 2.71
●The recovery rate of fatigue	RCT	2	108	RR 1.33, 95% CI 1.03 to 1.71
	Retrospective study with a control group	3	126	RR 1.48, 95% CI 1.14 to 1.93
●The duration of fever	RCT	2	95	MD -2.08 days, 95% CI -2.90 to -1.26, I^2 = 60%
	Non-RCT	1	200	MD -0.83 days, 95% CI -1.22 to -0.44
	Retrospective study with a control group	6	322	MD -1.54 days, 95% CI -1.82 to -1.26
●The duration of cough	RCT	2	95	MD -2.34 days, 95% CI -3.32 to -1.37, I^2 = 56%
	Non-RCT	1	200	MD 0.28 days, 95% CI -0.40 to 0.96
	Retrospective study with a control group	4	214	MD -1.68 days, 95% CI -1.92 to -1.43
●The duration of fatigue	RCT	1	45	MD -2.35 days, 95% CI -2.91 to -1.79
	Non-RCT	1	200	MD -0.33 days, 95% CI -0.78 to 0.12
	Retrospective study with a control group	3	136	MD -1.75 days, 95% CI -2.01 to -1.49
●Negative conversion rate of nucleic acid test	Retrospective study with a control group	3	163	RR 1.32, 95% CI 1.05 to 1.66
●The improvement rate of chest CT manifestations	RCT	6	607	RR 1.28, 95% CI 1.10 to 1.49
	Non-RCT	1	200	RR 1.21, 95% CI 1.05 to 1.40
	Retrospective study with a control group	7	484	RR 1.22, 95% CI 1.03 to 1.45, I^2 = 60%
●The recovery rate of chest CT manifestations	RCT	2	355	RR 1.42, 95% CI 1.00 to 2.02
	Retrospective study with a control group	3	251	RR 1.50, 95% CI 0.97 to 2.31
●The time from receiving treatment to the beginning of chest CT manifestations improvement	Retrospective study with a control group	2	140	MD -2.23 days, 95% CI -2.46 to -2.00
●Length of hospitalization	Retrospective study with a control group	4	290	MD -0.42 days, 95% CI -3.49 to 2.64, I^2 = 95%
●Adverse events	RCT	3	270	RR 2.06, 95% CI 0.34 to 12.38
	Non-RCT	1	200	RR 1.00, 95% CI 0.21 to 4.84
	Retrospective study with a control group	4	276	RR 0.87, 95% CI 0.26 to 2.93
Chinese herbal medicine vs. conventional western therapy		None		

Note: RR, risk ratio; MD, mean difference; CI, confidence interval; RCT, randomized controlled trial; Non-RCT, non-randomized controlled trial.

regardless of RCTs or retrospective studies with a control group showed that CHM plus conventional western therapy was superior to conventional western therapy alone (MD-2.34 days, 95% CI-3.32 to-1.37, 2 RCTs, I^2 = 56%; MD-1.68 days, 95% CI-1.92 to-1.43, 4 retrospective studies with a control group). However, the results from one non-RCT showed that there was no statistical difference between the experimental and control groups (MD 0.28 days, 95% CI -0.40 to 0.96, 1 non-RCT).

Regarding those studies reporting the duration of fatigue as secondary outcome, both RCTs and retrospective studies with a control group showed better effects for the CHM plus conventional western therapy when compared with conventional western therapy alone (MD -2.35 days, 95% CI-2.91 to-1.79, 1 RCT; MD-1.75 days, 95% CI-2.01 to-1.49, 3 retrospective studies with a control group). However, the result from one non-RCT showed that there was no statistical difference between the two groups (MD-0.33 days, 95% CI-0.78 to 0.12, 1 non-RCT).

Negative Conversion Rate of Nucleic Acid Test for SARS-Cov-19. A total of three retrospective studies with a control group (Qu et al., 2020a; Yang et al., 2020a; Zhang et al., 2020b) reported this outcome and all compared CHM plus conventional western therapy with conventional western therapy. Pooled data from 3 studies showed that CHM in combination with conventional western therapy was superior to conventional western therapy alone (RR 1.32, 95% CI 1.05 to 1.66) in improving the negative conversion rate of nucleic acid test for SARS-Cov-19.

Improvement or Recovery of Chest CT Manifestations. A total of 16 studies (Yu et al., 2020; Sun et al., 2020; Fu et al., 2020a; Ding et al., 2020; Xiao et al., 2020; Cheng et al., 2020; Liu et al., 2020c; Zhang et al., 2020b; Li et al., 2020c; Yang et al., 2020c; Xia, et al., 2020; Shi et al., 2020a; Yang et al., 2020a; Qiu et al., 2020; Zhang et al., 2020a; Chen et al., 2020) reported this outcome and all compared CHM plus conventional western therapy with conventional western therapy.

Of these, 14 studies (Fu et al., 2020a; Shi et al., 2020a; Yang et al., 2020a; Zhang et al., 2020a; Zhang et al., 2020b; Chen et al., 2020; Cheng et al., 2020; Yang et al., 2020c; Ding et al., 2020; Qiu et al., 2020; Sun et al., 2020; Xia et al., 2020; Xiao et al., 2020; Yu et al., 2020) the improvement rate of chest CT manifestations (improvement rate = the number of patients with improvement of chest CT manifestations/the total number of patients in experimental or control group × 100%). After analyzing separately according to the study design, the results regardless of RCTs, non-RCT or retrospective studies with a control group showed that CHM plus conventional western therapy was better than conventional western therapy alone in increasing the improvement rate of chest CT manifestations (RR1.28, 95% CI 1.10 to 1.49, 6 RCTs; RR 1.21, 95% CI 1.05 to 1.40, 1 non-RCT; RR 1.22, 95% CI 1.03 to 1.45, 7 retrospective studies with a control group). Five studies (Fu et al., 2020a; Yu et al., 2020; Chen et al., 2020; Yang et al., 2020c, Liu et al., 2020c) reported the recovery rate of chest CT manifestations (recovery rate = the number of patients with recovery of chest CT manifestations / the total number of patients in experimental or control group × 100%). After analyzing separately according to the studys' design, the results demonstrated that there was no statistical difference between the two groups in increasing the recovery rate of chest CT manifestations (RR 1.42, 95% CI 1.00 to 2.02, 2 RCTs; RR 1.50, 95% CI 0.97 to 2.31, 3 retrospective studies with a control group).

The other two retrospective studies with a control group (Li et al., 2020c; Liu et al., 2020c) reported the time from receiving the treatment to the beginning of chest CT manifestations improvement and the pooled analysis from the two studies showed that CHM plus conventional western therapy was superior to conventional western therapy alone in shortening the time (MD-2.23 days, 95% CI-2.46 to -2.00, two retrospective studies with a control group).

Length of Hospitalization. A total of four retrospective studies with a control group (Shi et al., 2020a; Yang et al., 2020c; Qiu et al., 2020; Xia et al., 2020) reported length of time in hospital as an outcome. All four studies compared CHM plus conventional western therapy with conventional western therapy. The pooled analysis from the four studies showed that there was no statistical difference between the experimental and control groups (MD -0.42 days, 95% CI -3.49 to 2.64, I^2 = 95%) in shortening the length of hospitalization.

Adverse Events. A total of 16 studies reported this outcome and all compared CHM plus conventional western therapy with conventional western therapy. Of these, eight studies (Fu et al., 2020a; Yang et al., 2020a; Zhang et al., 2020a; Fu et al., 2020b; Chen et al., 2020; Liu et al., 2020c; Xia et al., 2020; Yu et al., 2020) reported that no adverse events occurred in either the experimental or control group. Pooled data from the other eight studies (Qu et al., 2020a; Li et al., 2020c; Wang et al., 2020c; Yang et al., 2020c; Zhang et al., 2020c; Ding et al., 2020; Duan et al., 2020; Xiao et al., 2020) showed that there was no statistical difference between the experimental and control groups (RR 2.06, 95% CI 0.34 to 12.38, three RCTs (Duan et al., 2020; Ding et al., 2020; Wang, et al., 2020c); RR 1.00, 95% CI 0.21 to 4.84, one non-RCT (Xiao et al., 2020); RR 0.87, 95% CI 0.26 to 2.93, four retrospective studies with a control group (Zhang et al., 2020a; Li et al., 2020c; Yang, et al., 2020c; Qu et al., 2020a)). The adverse events reported in these eight studies were mild abdominal pain, diarrhea, nausea, vomiting and drug allergy, et al.

Subgroup Analysis

As all controlled studies compared CHM plus conventional western therapy with conventional western therapy, we failed to perform the subgroup analysis based on the use of CHM with or without conventional western therapy. Therefore, we only conducted the subgroup analysis based on the level of severity of COVID-19 (non-serious, serious or a mix of non-serious and serious) for primary outcomes.

With regard to cure rate, although a pooled data of five studies that reported this outcome showed that CHM plus conventional western therapy was superior to conventional western therapy in improving it (RR 1.21, 95% CI 1.01 to 1.45), the results (see Supplement-**Figure 1**) of the subgroup analysis based on the level of severity of COVID-19 showed that there was no statistical difference between the experimental and control groups (RR 1.69, 95% CI 0.72 to 3.92, two studies (Qu et al., 2020a; Fu et al., 2020b) involving 143 non-

serious patients; RR 1.17, 95% CI 0.91 to 1.50, one study (Yang et al., 2020c) involving 103 serious patients; RR 1.23, 95% CI 0.87 to 1.72, two studies (Li et al., 2020c; Xia et al., 2020) involving 112 patients, a mix of non-serious and serious, I^2 = 62%).

Regarding aggravation rate, a total of 11 studies (Fu et al., 2020b; Duan et al., 2020; Sun et al., 2020; Fu et al., 2020a; Ye, 2020; Cheng et al., 2020; Li, et al., 2020c; Xia et al., 2020; Qiu et al., 2020; Chen et al., 2020; Wang et al., 2020c) that reported this outcome were used to conduct meta-analysis, and the results (see **Supplement Figure-2**) from the 11 studies showed that CHM plus conventional western therapy was better than conventional western therapy alone in reducing aggravation rate (RR 0.40, 95% CI 0.26 to 0.59). Of which, seven studies (Duan et al., 2020; Sun et al., 2020; Fu et al., 2020a; Cheng et al., 2020, Qiu et al.,2020 ; Chen et al., 2020; Wang et al., 2020c) included only patients with non-serious COVID-19, and pooled data from the seven studies showed that CHM plus conventional western therapy was better than conventional western therapy (RR 0.37, 95% CI 0.22 to 0.63, seven studies). One study (Ye, 2020) included only patients with serious COVID-19, the results showed that there was no statistical difference between the experimental and control groups (RR 1.00, 95% CI 0.10 to 10.11, one study). The remaining three studies (Fu et al., 2020b; Li et al., 2020c; Xia et al., 2020) included both non-serious patients and serious patients with COVID-19, and the results from the three studies showed a lower aggravation rate in the experimental group compared with the control group (RR 0.40, 95% CI 0.21 to 0.79, three studies).

For mortality rate, a total of five studies (Wang et al., 2020c; Yang et al., 2020c; Xia et al., 2020; Ye, 2020; Yu et al., 2020) were included, and pooled data from five studies showed that there was no statistical difference between the experimental and control groups (RR 0.62, 95% CI 0.34 to 1.14) in reducing mortality rate. The results (see Supplement-**Figure 3**) of the subgroup analysis based on the level of severity of COVID-19 showed that there was also no statistical difference between the two groups (RR 0.43, 95% CI 0.06 to 2.86, two study (Wang et al., 2020c; Yu et al., 2020) involving 342 non-serious patients; RR 0.69, 95% CI 0.36 to 1.31, two studies (Yang et al., 2020c; Ye, 2020) involving 145 serious patients; RR 0.18, 95% CI 0.01 to 4.23, one study (Xia et al., 2020) involving 52 patients, a mix of non-serious and serious).

Analysis of Case Series and Case Reports

A total of 12 case series and 24 case reports were included in our review. Of which, one case series and 7 case reports involving 111 patients only used CHM, and 11 case series and 19 case reports involving 828 patients used CHM plus conventional western therapy. The authors of the 36 articles concluded that CHM with or without conventional western therapy was beneficial for the treatment of COVID-19.

With regard to 111 patients who received CHM treatment for a period of time from 4 to 11 days, one case series and one case report involving 100 patients reported that 42 patients were cured (42/100), 7 case reports involving 13 patients reported that 13 patients were negative for nucleic acid test (13/13), one case series and 6 case reports involving 54 patients reported that 30 patients with the recovery of fever (30/54), one case series and one case report involving 71 patients reported that 17 patients with the recovery of cough (17/71), one case series involving 75 patients reported that 20 patients with the recovery of fatigue (20/75), one case series and 5 case reports involving 96 patients reported that 87 patients (87/96) showed improvement or recovery of chest CT manifestations.

For 828 patients who received CHM plus conventional western therapy for a period of time from 6 to 15 days, 4 case series and 6 case reports involving 641 patients reported that 561 patients were cured (561/641), 6 case series and 16 case reports involving 182 patients reported that 179 patients were negative for nucleic acid test (179/182), 5 case series and 13 case reports involving 271 patients reported that 258 patients with the recovery of fever (258/271), 5 case series and 3 case reports involving 437 patients reported that 284 patients with the recovery of cough (284/437), 5 case series and 2 case reports involving 327 patients reported that 212 patients with the recovery of fatigue (212/327), and 3 case series and 11 case reports involving 525 patients reported that 483 patients (483/525) showed improvement or recovery of chest CT manifestations. In addition, there were 3 case series which reported adverse events. Of these, 2 case series reported that no adverse events occurred, and the remaining reported that seven patients with the treatment of CHM plus conventional western therapy experienced adverse events including vomiting (4), dizziness 2) and rash (1).

DISCUSSION

Although RCT is the gold standard to evaluate the therapeutic effects of interventions, it cannot answer all the important questions about a given intervention (Black, 1996). Considering the characteristics of sudden acute infectious diseases and the practical problems of ethics and informed consent, the implementation of RCT faces more challenges under conventional medical conditions (Yang et al., 2020b). Many questions in medical research are investigated in observational studies having a role in research into the benefits and harms of medical interventions (Black, 1996; Glasziou et al., 2004), having an important reference for the preliminary evaluation of the therapeutic effects of CHM and clinical decision-making. In this case, other types of studies (e.g., non-RCT, retrospective studies, case-series) were included in our review.

Summary of the Main Findings

A total of 58 clinical studies whose purpose were to evaluate the therapeutic effects of CHM used with or without conventional western therapy for COVID-19 were included. The included studies involved RCTs, non-RCT, retrospective studies with a control group, case-series and case-reports. In total the studies involved 2773 COVID-19 patients, 1921 (69.28%) of them received CHM. The severity of COVID-19 varied from non-serious (mild and common) and serious (severe and critical). Most of the studies used a combination of CHM and conventional western therapy. Analysis of the frequency of different CHM indicated that the most frequently used oral Chinese patent medicine, Chinese herbal medicine injection and prescribed herbal decoction were Lianhua Qingwen granule/capsule, Xuebijing injection, and Maxing Shigan Tang, respectively.

This review suggested that CHM in combination with conventional western therapy appeared better than conventional western therapy alone in reducing aggravation rate, increasing the

recovery rate or shortening the duration of main symptoms (fever, cough and fatigue), improving the negative conversion rate of nucleic acid test, increasing the improvement rate of chest CT manifestations and shortening the time from receiving the treatment to the beginning of chest CT manifestations improvement. For the primary outcomes, subgroup analyses were conducted based on the level of severity of COVID-19 and suggested that CHM in combination with conventional western therapy had more significant effect than conventional western therapy in reducing aggravation rate for non-serious patients.

In terms of reducing mortality rate and shortening the length of hospitalization, there was no statistical difference between the CHM combined conventional western therapy group and the conventional western therapy group. Although some studies have reported adverse events (e.g., mild abdominal pain, diarrhea, nausea and vomiting) in the CHM plus conventional western therapy group, but there was also no statistical difference between the experimental and control groups. This suggests that the use of CHM did not increase the risk of adverse events.

Although in this review there were no pooled results for CHM used alone from controlled studies for COVID-19, one case-series and seven case-reports that were included reported that CHM alone may play a positive therapeutic role in the treatment of COVID-19.

Strengths and Limitations

This review systematically collected the evidence from clinical studies whose purpose was to evaluate the therapeutic effects and safety of CHM with or without conventional western therapy for COVID-19. Relevant clinical studies were analyzed from the aspects of general characteristics, quality assessment, analysis of the use of CHM, therapeutic effects and safety of CHM for COVID-19 patients, providing important evidence for future related research.

However, this review did not summarize the specific administration methods of CHM in all the included studies, especially considering the complexity of prescribed herbal decoction use, which may require further specific research in the future. Therefore, this review cannot be directly used to guide clinical practice. In addition, all included studies were conducted in China, whether this evidence is equally applicable to other countries outside China needs further international study.

Implications for Further Research

The benefits for the use of CHM for COVID-19 needs to be verified by more rigorous designed and implemented clinical trials, especially randomized controlled trials. The following points should be noted when conducting relevant RCTs: 1) Clear reporting of random allocation and random concealment; 2) Application of blinding to participants, personnel (doctors), outcome evaluators and statistical analysts; 3) Design and register the study protocol; 4) Definition of important outcomes, such as time to cure, aggravation and mortality; 5) Selection of CHM: considering the difficulty in the use of herbal decoction (e.g., dosage of herbal medicien, especially about its use outside China), we suggest that trials of oral Chinese patent medicine or Chinese herbal medicine injection should be given priority to verify the therapeutic effects and safety of these two, so as to find safe, effective and convenient medications to cure more COVID-19 patients as soon as possible. Unfortunately, in our this research, we did not to perform subgroup analysis on oral Chinese patent medicine, Chinese herbal medicine injection and prescribed Chinese herbal medicine decoction.

CONCLUSION

Current low certainty evidence suggests that there maybe a tendency that CHM plus conventional western therapy is superior to conventional western therapy alone. The use of CHM did not increase the risk of adverse events.

AUTHOR CONTRIBUTIONS

J-PL and S-BL conceived and designed the review. S-BL, Y-YZ, CS, Y-QL, B-YL, C-HL, and Z-YT were responsible for the searching, screening and selecting studies. S-BL, Y-YZ, Y-QL, CS, B-YL, ND, YJ, X-WZ, C-HL, Y-PZ, and MX participated in data extraction. S-BL and Y-QL assessed the risk of bias of the included trials. S-BL performed the statistical analysis. Y-YZ, Y-QL, B-YL, and ND helped to perform the statistical analysis. S-BL drafted the manuscript. J-PL, YZ, NR, CS, and Y-YZ were all involved in critically revising the manuscript. All authors have read and approved the final manuscript. All authors approved the final version of the article, including the authorship list.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.583450/full#supplementary-material.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

APPENDIX 1

SEARCH STRATEGIES FOR THE NINE ELECTRONIC DATABASES AND CLINICAL TRIAL REGISTRATION PLATFORMS (CTRP).

Databases/CTRP	Search strategy	Time limit
CNKI	Since CNKI has set up a thematic platform for COVID - 19, the "treatment" section of the platform was selected for manual retrieval	As of April 30, 2020
VIP	#1: M = Xinxing Guangzhuang Bingdu Bing(新型冠状病毒病) OR Xinguan Feiyan (新冠肺炎) OR 2019 Guanzhuang Bingdu (2019冠状病毒病) OR COVID-19 OR 2019-nCOV OR NCP #2: M = Zhongyi (中医) OR Zhongyao (中药) OR Caoyao (草药) OR Tangji (汤剂) OR Zhongchengyao (中成药) OR Zhusheji (注射剂) OR Zhongxiyi Jiehe (中西医结合) #3: #1 AND #2	From January 1 to April 30, 2020
Wanfang	#1: Major Topic: "Xinxing Guangzhuang Bingdu Bing (新型冠状病毒病)" + "Xinguan Feiyan (新冠肺炎)" + " 2019 Guanzhuang Bingdu Bing (2019冠状病毒病)" + "COVID-19" + "2019-nCOV" + "NCP" #2: Major Topic: "Zhongyi (中医)" + "Zhongyao (中药)" + "Caoyao (草药)" + "Tangji (汤剂)" + "Zhongchengyao (中成药)" + "Zhusheji (注射剂)" + "Zhongxiyi Jiehe (中西医结合)" #3: #1 AND #2	From January 1 to April 30, 2020
SinoMed	#1: ("Xinxing Guangzhuang Bingdu Bing (新型冠状病毒病)"[标题:智能] OR "Xinguan Feiyan (新冠肺炎)"[标题:智能] OR "2019 Guanzhuang Bingdu Bing (2019冠状病毒病)"[标题:智能] OR "COVID-19"[标题:智能] OR "2019-nCOV"[标题:智能] OR "NCP"[标题:智能]) #2: ("Zhongyi (中医)"[标题:智能] OR "Zhongyao (中药)"[标题:智能] OR "Caoyao (草药)"[标题:智能] OR "Tangji (汤剂)"[标题:智能] OR "Zhongchengyao (中成药)"[标题:智能] OR "Zhusheji (注射剂)"[标题:智能] OR "Zhongxiyi Jiehe (中西医结合)"[标题:智能]) #3: #1 AND #2	From January 1 to April 30, 2020
PubMed	(((Corona virus disease-19 OR COVID-19 OR 2019 novel coronavirus OR 2019-nCOV OR NCP[MeSH Major Topic]) AND (Chinese medicine OR traditional Chinese medicine OR herbal medicine OR decoction OR patent medicine OR injection OR integrated Chinese and western medicine[MeSH Major Topic]) AND ("2020/01/01"[Date-Publication] : "2020/04/30"[Date-Publication])	From January 1 to April 30, 2020
Embase	#1: ab,ti: corona virus disease-19 OR COVID-19 OR 2019 novel coronavirus OR 2019-nCOV OR NCP #2: ab,ti: Chinese medicine OR traditional Chinese medicine OR herbal medicine OR decoction OR patent medicine OR injection OR integrated Chinese and western medicine #3: #1 AND #2	From January 1 to April 30, 2020
ChiCTR	Title search was carried out using Xinxing Guangzhuang Bingdu (新型冠状病毒) and COVID-19 as search terms	As of April 30, 2020
ClinicalTrials.gov	Searched in covid-19 special registration section	As of April 30, 2020
BioRxiv, MedRxiv, arxiv	Title or abstract search was carried out using COVID-19 as search terms	As of April 30, 2020

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REVIEW
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Indian Medicinal Plants and Formulations and Their Potential Against COVID-19–Preclinical and Clinical Research

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The cases of COVID-19 are still increasing day-by-day worldwide, even after a year of its first occurrence in Wuhan city of China. The spreading of SARS-CoV-2 infection is very fast and different from other SARS-CoV infections possibly due to structural differences in S proteins. The patients with severe diseases may die due to acute respiratory distress syndrome (ARDS) caused by systemic inflammatory reactions due to the excessive release of pro-inflammatory cytokines and chemokines by the immune effector cells. In India too, it is spreading very rapidly, although the case fatality rate is below 1.50% (https://www.statista.com), which is markedly less than in other countries, despite the dense population and minimal health infrastructure in rural areas. This may be due to the routine use of many immunomodulator medicinal plants and traditional AYUSH formulations by the Indian people. This communication reviews the AYUSH recommended formulations and their ingredients, routinely used medicinal plants and formulations by Indian population as well as other promising Indian medicinal plants, which can be tested against COVID-19. Special emphasis is placed on Indian medicinal plants reported for antiviral, immunomodulatory and anti-allergic/anti-inflammatory activities and they are categorized for prioritization in research on the basis of earlier reports. The traditional AYUSH medicines currently under clinical trials against COVID-19 are also discussed as well as furtherance of pre-clinical and clinical testing of the potential traditional medicines against COVID-19 and SARS-CoV-2. The results of the clinical studies on AYUSH drugs will guide the policymakers from the AYUSH systems of medicines to maneuver their policies for public health, provide information to the global scientific community and could form a platform for collaborative studies at national and global levels. It is thereby suggested that promising AYUSH formulations and Indian medicinal plants must be investigated on a priority basis to solve the current crisis.

Keywords: COVID-19, AYUSH medicine, indian medicinal plants, indian traditional medicine, immunomodulators, antiviral agents

1 INTRODUCTION

A novel coronavirus-induced pneumonia, which was later called coronavirus disease 2019 (COVID-19), has rapidly increased to an epidemic scale and affected whole human population globally (WHO, 2020a). In India, the first case of COVID-19 was an imported case from Wuhan, China on January 30, 2020 traced in Kerala (Sahasranaman and Kumar, 2020) and the death rate of COVID-19 in India is 1.45%, as of 12th December, 2020 (Worldometers, 2020). Severe acute respiratory syndrome-related coronavirus (SARS-CoV-2) has become a pandemic hazard to global public health worldwide.

Coronaviruses (CoVs) are large viruses comprising of four genera, namely alpha, beta, gamma, and delta. The beta-coronavirus class includes severe acute respiratory syndrome (SARS) virus (SARS-CoV), Middle East respiratory syndrome (MERS) virus (MERS-CoV), and the COVID-19 causative agent SARS-CoV-2. (Li G. et al., 2020). The novel SARS-CoV-2 is a beta CoV that shows 88% similarity to two bat-derived SARS-like CoVs (bat-SL-CoVZC45 and bat-SL-CoVZXC21), about 50% identical to the sequence of MERSCoV, and 70% similarity in genetic sequence to SARS-CoV (Cheng and Shan, 2020). Although there is an extremely high resemblance between SARS-CoV and the novel SARS-CoV-2, the SARS-CoV-2 is spreading rapidly as compared to the SARS-CoV, which may be explained by structural differences in the S proteins (Rabaan et al., 2020).

The SARS-CoV-2 S protein has been found as a significant determinant of virus entry into host cells using angiotensin converting enzyme 2 (ACE2) receptor similar to SARS-CoV. Whereas the binding affinity of virion S glycoprotein and ACE2 is reported to be 10–20 folds higher in SARS-CoV-2 as compared to that of SARS-CoV (Song et al., 2018).

Severe cases of COVID-19 are reported to have increased plasma concentrations of pro-inflammatory cytokines, including interleukins (IL-6 and IL-10), tumor necrosis factor (TNF)-α granulocyte-colony stimulating factor (G-CSF), monocyte chemoattractant protein 1 (MCP1), and macrophage inflammatory protein (MIP)1α (Yuki et al., 2020). Akin to the common viral infections, the antibody profile against the SARS-CoV virus manifests a typical pattern of IgM and IgG antibody production. The IgG antibody is believed to play a protective role, as the SARS-specific IgG antibodies last for a longer time while IgM antibodies disappear at the end of 12 weeks. The latest reports show a significant reduction in the number of $CD4^+$ and $CD8^+$ T cells in the peripheral blood of SARS-CoV-2-infected patients, besides activation of other pro-inflammatory cytokines such as nuclear factor-κB (NF-κB), interferon regulatory factor 3 (IRF3) and type I Interferons (IFN-α/β) (Li G. et al., 2020). A recent report shows that many patients died from acute respiratory distress syndrome (ARDS) caused by the cytokine storm, which is a deadly uncontrolled systemic inflammatory response resulting from the release of large amounts of pro-inflammatory cytokines and chemokines by immune effector cells in SARS-CoV infection (Guo et al., 2020).

Although the pathogenesis of COVID-19 is still not clear, patients with COVID-19 show non-specific symptoms ranging from no symptoms (asymptomatic) to severe pneumonia and death. However, the most common symptoms include fever, non-productive cough, dyspnea, myalgia, fatigue, diarrhea, lung damage, normal or decreased leukocyte counts, and radiographic evidence of pneumonia, which are similar to the symptoms of SARS-CoV and MERS-CoV infections (WHO, 2020b; Rothan and Byrareddy, 2020). Complications include ARDS, acute heart injury, and secondary infections (Guo et al., 2020).

The present conventional strategy of the disease control includes isolation of cases and tracing their contacts, providing optimal care to these infected cases, reducing chances of secondary infections by early diagnosis, and rapid development of effective diagnostic, preventive and therapeutic strategies, including vaccines (WHO, 2020b). The treatment approach for COVID-19 is supportive care, which is supplemented by the combination of broad-spectrum antibiotics, antivirals, corticosteroids and convalescent plasma (Yang et al., 2020).

Scientists are working hard to develop effective treatments. As of October 18, 2020, more than 3611 clinical trials (with more than 100 complementary medicines) on COVID-19 are either ongoing or enrolling patients, and new ones are being added every day, as the case count skyrockets globally. The drugs being tested range from repurposed flu treatments to failed ebola drugs, to malaria treatments that were first developed decades ago (Lythgoe and Middleton, 2020). There is scale-up development of vaccines across the world by many pharmaceutical companies as well as research organizations. These treatments undergoing trials may require months or years to develop and hit the market, meaning that an immediate treatment or control mechanism should be found, if possible (**Table 1**).

Considering the current situation, various treatment modalities have been well-thought-out, including traditional medicine, which has been widely used during the past epidemic outbreaks, including SARS and H1N1 influenza (Luo et al., 2020). Until now three countries including India, China, and South Korea, have issued guidelines on traditional regimens for the prevention and management of COVID-19 (Ang et al., 2020).

The Indian Traditional System of Medicine is one of the oldest systems of medical practice in the world and has played an essential role in providing health care service to human civilization, right from its inception. India has the exclusive distinction of its own recognized traditional medicine; Ayurveda, Yoga, Unani, Siddha, and Homoeopathy (AYUSH) (Adhikari and Paul, 2018). These systems are based on definite medical philosophies and represent a way of achieving a healthy lifestyle with conventional and established ideas on the prevention of diseases and the promotion of health. The basic treatment approach of all these systems is holistic and the pharmacological modalities are based on natural products of plants, animals, or mineral origin. Given this, there is a resurgence of interest in AYUSH systems, which have helped the nation in the pandemic crisis due to plague, cholera, Spanish flu, etc. in the past. Hence, by repurposing the traditional uses of Indian medicinal plants and formulations, new treatment options

TABLE 1 | Details of clinical trials completed on AYUSH drugs for COVID-19 (Source: www.ctri.nic.in).

Ctri No./Treatment details	Study title	Type of trial (design of study) Recruitment status	Remarks
CTRI/2020/04/024883 ZINGIVIR-H	Clinical research on safety and efficacy of ZingiVir-H as an add on therapy in COVID-19 patients	Interventional (Other) Completed	Zingivir H consumption with standard of care in COVID 19 confirmed patients showed a remarkable recovery compared to that of placebo
CTRI/2020/05/025161/Herbal formulation-aayudh advance	To study the effectiveness of herbal formulation - aayudh advance as a supplementary treatment for the corona virus 2019 (COVID-19) infected patients	Interventional (randomized, parallel group, active controlled Trial) Completed	"Aayudh advance", when given concomitantly with standard of care, was found to be 100% safe, devoid of any drug-drug interaction, effective as virucidal to reduce viral load, and increased the recovery rate when compared to standard of care alone when tested in mild symptomatic COVID-19 patients
CTRI/2020/05/025215/Kabasura kudineer	Effectiveness of siddha medicine, kabasura kudineer and vitamin c-zinc supplementation in the management of mild COVID-19 patients	Interventional (randomized, parallel group Trial) Completed	The role of vitamin C with zinc supplementation in the management of COVID 19 is still not clear. Therefore, study will compare the effect of kabasura kudineer and vitamin C with zinc supplementation in terms of negative conversion of SARS CoV-2 infection
CTRI/2020/05/025275/Ayurveda rasayana along with conventional guidelines for health care workers	Role of chyawanprash in the prevention of COVID-19 in health care workers	Interventional (randomized, parallel group Trial) Completed	No adverse effect was found in the study
CTRI/2020/05/025276/Ayurveda protocol	Effect of ayurvedic intervention in COVID-19 positive cases	Interventional (single arm Trial) Completed	Ayurveda treatment protocol includes sanshamani, nagaradi kwath, amalaki churna and golden milk improved the strength of the patient
CTRI/2020/05/025397/Purified aqueous extract of cocculus hirsutus (AQCH)	A study to evaluate the effect and safety of a phytopharmaceutical drug in treatment of coronavirus infection	Interventional (randomized, parallel group Trial) Completed	Clinical improvement was observed in covid patients in terms of disease severity
CTRI/2020/05/025425/Chayapanprash (an ayurvedic herbal preparation)	Ayurvedic intervention (chyawanprash) in the prevention of COVID-19 pandemic among health care personnel	Interventional (single arm Trial) Completed	This remedy was found to be a possible safe prophylactic remedy for COVID-19
CTRI/2020/06/025527/Amrta karuna syrup	Clinical trial on immunity and antiviral for quarantine patients of COVID-19	Interventional (randomized, parallel group, active controlled Trial) Closed to recruitment	The formulation was found to be immunomodulatory
CTRI/2020/06/025556/Virulina® along with standard treatment protocol	A clinical trial to know the effect of Virulina® along with standard treatment in COVID-19 positive patients	Interventional (randomized, parallel group, placebo controlled Trial) Completed	The formulation was found to boost the immunity of the patients and help ease the symptoms
CTRI/2020/06/025590/Astha 15 capsule	A clinical trial to evaluate safety and efficacy of polyherbal capsule Astha-15 used as an add on therapy with standard care of therapy as an immunity booster in the suspected and COVID-19 diagnosed patients	Interventional (randomized, parallel group, placebo controlled Trial) Completed	A better recovery rate was observed
CTRI/2020/06/025592/Immunity kit	Use of herbal medicine like tulsi, amruth (giloy), turmeric, ashwagandha as add on treatment in COVID-19 patients	Interventional (single arm Trial) Completed	Upon using the ayurvedic formulation as add on treatment, the recovery was better in terms of signs and symptoms of COVID-19 patients
CTRI/2020/06/026221/Arogya Kashayam-20	Intervention of ayurvedic medicine (arogya kashayam) in COVID-19 positive cases (asymptomatic and mild symptomatic)	Interventional (randomized, parallel group, active controlled Trial) Completed	The unani regimen was found to be effective against the mild symptoms of covid 19
CTRI/2020/06/026227/Khameera marwareed Tiryaq-e-Arba Unani joshanda/ decoction behidana (*Cydonia oblonga*) 3 gm, unnab (*Zizyphus jujube*) 5 in number, sapistan (*Cordia myxa*) 9 in numbers	A study on unani regimen for prevention of high/moderate risk population of COVID-19	Interventional (non-randomized, multiple arm Trial) Completed	Improvement was found in immune status of covid patients
CTRI/2020/06/025801/Tab. Bresol and tab. Septilin	Role of herbal immunomodulators in mild COVID-19 confirmed cases	Interventional (randomized, parallel group, active controlled Trial) Completed	Use of herbal immunomodulators as add on treatment, improved the recovery rate of COVID-19 patients
CTRI/2020/07/026337/Add-on personalized ayurveda intervention to ICMR guideline on Covid-19	The COVID-19 study with ayurveda add-on to ICMR guideline	Interventional (randomized, parallel group trial) completed	Efficacy of treatment was measured in terms of average stay of patients in the hospital to become covid negative

(Continued on following page)

TABLE 1 | (*Continued*) Details of clinical trials completed on AYUSH drugs for COVID-19 (Source: www.ctri.nic.in).

Ctri No./Treatment details	Study title	Type of trial (design of study) Recruitment status	Remarks
CTRI/2020/07/026371/1. Kabasura kudineer 2.Shakti drops 3.Turmeric plus tablets	Kabasura kudineer, shakti drops and turmeric plus in the management of COVID-19	Interventional (Others) Completed	Better recovery rate was observed in terms of signs and symptoms of stage 1 and 2 of COVID-19 cases on addition of ayurvedic medicines, thereby improving the quality life of stage 1 and 2 of COVID-19 patients
CTRI/2020/07/026433/1. Dashamula kwatha and pathyadi kwatha with trikatu churna 2. Sansamani vati 3. AYUSH 64 4. Yastimadhu Ghanavati	Effect of ayurveda medicine in COVID-19 mild symptoms	Interventional (randomized, parallel group, active controlled Trial) Completed	No adverse reaction was observed and improvement in signs and symptoms
CTRI/2020/07/026570/Cap. IP	Safety and efficacy of ayurvedic capsule in mild to moderate COVID-19 infection	Interventional (randomized, parallel group Trial) Completed	Improvement was observed in respiratory symptoms of covid patients

can be identified to combat the current deadly pandemic. In view of the COVID-19 outbreak, the entire human race across the globe is perturbed. While there is no medicine for COVID-19 as of now, it is imperative to take preventive measures such as practicing self-hygiene, social distancing and boosting immunity. Many safe traditional formulations of AYUSH, which are well known immunity modulators, have been used for centuries in respiratory disorders and in allergic conditions. The Ministry of AYUSH (Govt of India) has listed out such formulations and recommended their use as a prophylactic measure in red zones, containment zones, as well as for corona warriors. Many of them are now under clinical trial in COVID-19 patients (**Table 1**).

Similarly, there are many medicinal plants indigenous to India and used in the Indian Systems of Medicine which have been reported as potent antiviral with immunomodulatory and anti-allergic/anti asthmatic activities. Many of these medicinal plants are also an integral part of several traditional formulations that have been in use for a long time.

This review discusses the possible alternative strategies for the management of the SARS-CoV-2 infection by reducing its morbidity in patients as an adjuvant to modern therapy and also by providing prophylactic management. Further, potential testing targets of botanicals from Indian medicinal plants need to be explored against SARS-CoV-2 infection and categorized on a priority basis in view of their reported antiviral, immunomodulatory and other related activities.

2 POTENTIAL TRADITIONAL INDIAN/ AYUSH FORMULATIONS FOR THE MANAGEMENT OF COVID-19

There is plenty of data supporting the effectiveness of herbs in treating the viral infection. For instance, in controlling the contagious disease spread in the Guangdong Province of China during the 2003 SARS outbreak (Zhang et al., 2020). There are convincing pieces of evidence to establish that traditional Chinese medicine (TCM) has favorable effect in the treatment or prevention of SARS (Yang et al., 2020). A combination of modern and traditional therapy might reduce the severity of the disease, intensity of symptoms, death rate, and side effects. Similar are the observations for *Shuanghuanglian* (A Chinese medicine) a liquid composed of a blend of honeysuckle, Chinese skullcap, and forsythia, which is claimed to have antiviral, antibacterial, and immunomodulatory effects (https://www.bioworld.com/). Since AYUSH encompasses five different systems of medicine, rich in a variety of traditional formulations, it is likely to have a better chance than other systems to come up with a satisfactory solution to the COVID-19 crisis.

Ayurveda means 'Science of life'. It provides a complete system to have a long and healthy life. It is derived from the concepts of "*Dinacharya*" - daily regimes and "*Ritucharya*" - seasonal regimes to maintain a healthy life. Uplifting and maintaining the immunity is duly emphasized across the Ayurveda's classical scriptures.

The Unani system of medicine, known as Greco-Arab Medicine, is built on the four conditions of living (hot, sodden, frosty, and dry) and four humors of Hippocratic hypothesis namely, blood, yellow bile, dark bile, and mucus. Epidemics, referred to as waba in the Unani system of medicine, are thought to occur if any contagion or ajsam-i -khabitha, finds a place in air and water. Furthering the view, Ibn-e-Sina (980–1035 CE) stated that epidemics spread from one person to another, and one city to another 'like a message' (Sina, 1878).

AYUSH systems of medicine propagate general preventive measures aimed at preventing the spread of infection such as social distancing, hygiene and anti-septic measures (sanitization of surroundings), improvement of immunity, and promotion of general health (dietary modifications and herbal drugs). The present article elucidates some traditional Indian AYUSH formulations with proven antiviral, anti-asthmatic, and immunomodulatory activities, however their role in combating COVID-19 needs to be established. Clinical trials of AYUSH medicines like Ashwagandha, Yashtimadhu, Guduchi, Pippali, and AYUSH-64 on patients, health workers, and those working in high-risk areas have been initiated in India by the Ministry of AYUSH, Ministry of Health and Family Welfares, and the Council of Scientific and Industrial Research (CSIR) with the technical support of Indian Council of Medical Research (ICMR) (**Table 1**).

TABLE 2 | AYUSH recommended prophylactic approach through Ayurvedic formulations. Ref: AYUSH Ministry of Health Corona Advisory-D.O. No. S. 16030/18/2019-NAM; dated: 06th March, 2020. Ref: AYUSH Ministry of Health Corona Advisory -F.No. Z 25.23/09/2018–2020-DCC (AYUSH); dated: 24th April, 2020.

Name of the formulation	Composition	Proof of activity related to COVID-19	References
Anuthaila	*Leptadenia* reticulate (Retz.) Wight and Arn. (root/stem bark)	A,C	Pravansha et al. (2012), Mohanty et al. (2015)
	Cedrus deodara (Roxb. ex D.Don) G.Don (stem)	B	Raghavendhar et al. (2019)
	Vetiveria zizanioides (L.) Nash (root)	B	Lavanya et al. (2016)
	Ocimum sanctum L. (leaves)	A,B,C	Goel et al. (2010), Ghoke et al. (2018), Soni et al. (2015)
	Berberis aristata DC. (bark)	A,B,C	Yan et al. (2018), Wang et al. (2017), Kumar et al. (2016)
	Glycyrrhiza glabra L. (root rhizome)	A,B,C	Mitra Mazumder et al. (2012), Ashraf et al. (2017), Patel et al. (2009)
	Cyperus rotundus L. (rhizome)	A,B,C	Soumaya et al. (2013), Xu et al. (2015), Jin et al. (2011)
	Asparagus racemosus Willd. (root)	A	Gautam et al. (2009)
	Aegle marmelos (L.) Correa (stem bark)	A,C	Patel and Asdaq (2010), Kumari et al. (2014)
	Solanum indicum L. (leaves)	C	Kaunda and Zhang (2019)
	Solanum xanthocarpum Schrad. and Wendl (fruit)	B	Kumar and Pandey (2014)
	Uraria picta (jacq.) DC. (whole plant)	C	Nagarkar et al. (2013)
	Embelia ribes Burm.f. (fruit)	B,C	Mahendran et al. (2011)
	Cinnamomum verum J.Presl. (bark)	A,B,C	Niphade et al. (2009), Brochot et al. (2017), Kandhare et al. (2013)
	Elettaria cardamomum (L.) Maton (fruit)	B	Rahman et al. (2017)
	Vitex negundo L. (leaves)	A,B,C	Lad et al. (2016), Kannan et al. (2012), Chattopadhyay et al. (2012)
	Sesamum indicum L. (seed oil)	A,C	Khorrami et al. (2018), Nagpurkar and Patil (2017)
Agasthaya hareetaki	*Aegle marmelos* (L.) Correa (root/stem bark)	A,C	Patel and Asdaq (2010), Kumari et al. (2014)
	Oroxylum indicum (L.) Kurz (root/stem bark)	B	Zaveri et al. (2008)
	Gmelina arborea Roxb. (root/stem bark)	B	Panda et al. (2017)
	Stereospermum suaveolens (Roxb.) DC. (root/stem bark)	C	Balasubramanian et al. (2010)
	Premna mucronta Roxb. (root/stem bark)	A,C	Dianita and Jantan (2017)
	Desmodium gangeticum (L.) DC. (whole plant)	A	Gulati et al. (2002)
	Uraria picta (jacq.) DC. (whole plant)	C	Nagarkar et al. (2013)
	Solanum indicum L. (whole plant)	C	Kaunda and Zhang (2019)
	Solanum surattense Burm.f. (whole plant)	C	Kaunda and Zhang (2019)
	Tribulus terrestis L. (whole plant)	B,C	Malik et al. (2018), Kang et al. (2017)
	Mucuna pruriens (L.) DC. (seed)	B,C	Lampariello et al. (2012)
	Convolvulus pluricaulis Choisy (whole plant)	A,B,C	Agarwal et al. (2014)
	Hedychium spicatum Sm. (rhizome)	A,C	Uttara and Mishra (2009), Ghildiyal et al. (2012)
	Sida cordifolia L. (root)	A,C	Tekade et al. (2008), Singh S. et al. (2011)
	Piper chaba Hunter (fruit)	C	Sireeratawong et al. (2012)
	Achyranthes aspera L. (root)	A,B,C	Narayan and Kumar (2014), Mukherjee et al. (2013), Khuda et al. (2013)
	Piper longum L. (root)	A,B,C	Tripathi et al. (1999), Jiang et al. (2013), Kaushik et al. (2012)
	Plumbago zeylanica L. (root)	B	Gebre-Mariam et al. (2006)
	Clerodendron serratum Spr. (root)	A	Juvekar et al. (2006)
	Inula racemosa Hook.f. (root)	A,C	Mishra et al. (2016), Vadnere et al. (2009)
	Hordeum vulgare L. (seed)	C	Gul et al. (2014)
	Terminalia chebula Retz. (pulp)	A,B,C	Shivaprasad et al. (2006), Kesharwani et al. (2017), Haq et al. (2013)
Samshamani vati	*Tinospora cardifolia* (Willd.) Miers (stem)	A,B,C	Alsuhaibani and Khan (2017), Pruthvish and Gopinatha (2018), Tiwari et al. (2014)
AYUSH-64	*Alstonia scholaris* (L.) R.Br. (bark)	A,B,C	Iwo et al. (2000), Antony et al. (2014), Zhao et al. (2017)
	Picrorhiza kurroa Royle ex Benth. (rhizome)	A,B,C	Sharma et al. (1994), Win et al. (2019), Sehgal et al. (2013)
	Swertia chirayita (Roxb.) H.Karst. (whole plant)	B,C	Woo et al. (2019), Khan et al. (2012)
	Caesalpinia crista L. (seed pulp)	C	Ramesh et al. (2014)
AYUSH kwath	*Ocimum sanctum* L. (leaves)	A,B,C	Goel et al. (2010), Ghoke et al. (2018), Soni et al. (2015)
	Cinnamomum verum J.Presl. (stem bark)	A,B,C	Niphade et al. (2009), Brochot et al. (2017), Kandhare et al. (2013)
	Zingiber officinale Roscoe (rhizome)	A,B,C	Zhou et al. (2006), Chang et al. (2013), Khan et al. (2015)
	Piper nigrum L. (fruit)	A,B,C	Majdalawieh and Carr (2010), Mair et al. (2016), Tasleem et al. (2014)

Note: A = Immunomodulators; B = Antiviral; C = Anti-allergic/Anti-asthmatic/Anti-inflammatory/Respiratory disorders.

3 AYUSH RECOMMENDATIONS FOR MANAGEMENT OF COVID-19

Based on the different systems of Indian Medicine, separate recommendations have been issued from time to time from the Ministry of AYUSH (Government of India) for the management of COVID-19. These different approaches are being followed by the Hospitals as per their specialization, mainly as adjuvants to modern medicine, which could be potentially relevant for COVID 19 treatment. Details of recommended formulations are described below and depicted in **Table 2** (Ayurveda), **Table 3** (Unani) and **Table 4** (Siddha).

TABLE 3 | AYUSH recommended prophylactic approach through Unani formulation. Ref: AYUSH Ministry of Health Corona Advisory–D.O. No. S. 16030/18/2019- NAM; dated: 06th March, 2020.

Name of the formulation	Composition	Proof of activity related to COVID-19	References
Arq-e-Ajeeb	Camphor	B,C	Chen et al. (2013), Ziment and Tashkin (2000)
	Menthol	B,C	Taylor et al. (2020), Ziment and Tashkin (2000)
	Thymol	C	Al-Khalaf (2013)
Asgandh safoof	*Withania somnifera* (L.) Dunal (root)	A,B,C	Rasool and Varalakshmi (2006), Pant et al. (2012), Sahni and Srivastava (1993)
Habb-e-Bukhar	*Cinchona officinale* L. (bark)	B	Devaux et al. (2020)
	Tinospora cordifolia (Willd.) Miers (stem)	A,B,C	Alsuhaibani and Khan (2017), Pruthvish and Gopinatha (2018), Tiwari et al. (2014)
	Bambusa bambos (L.) Voss (stem)	A	Sriraman et al. (2015)
	Acacia arabica (Lam.) Willd. (gum)	C	Roqaiya et al. (2015)
Habb-e-Hindi zeeqi	*Aconitum chasmanthum* Stapf ex Holmes (root)	C	Alamgeer et al. (2018)
	Calotropis procera (Aiton) W.T.Aiton (root)	A,C	Bagherwal (2011), Arya and Kumar (2005)
	Zingiber officinale Roscoe (rhizome)	A,B,C	Zhou et al. (2006), Chang et al. (2013), Khan et al. (2015)
Habb-e-Mubarak	*Myrica esculenta* Buch.-Ham. ex D.Don (stem bark)	A,C	Kabra et al. (2019)
	Caesalpinia bonduc (L.) Roxb. (cotyledon)	A,C	Shukla et al. (2010), Arunadevi et al. (2015)
Khamira-e-Banafsa	*Viola odorata* L. (flower)	B,C	Gerlach et al. (2019), Koochek et al. (2003)
Khameera marwareed	*Mytilus margaritiferus* (pearl)	A	Khan et al. (2009), Beaulieu et al. (2013)
	Bambusa bambos (L.) Voss (stem)	C	Muniappan and Sundararaj (2003)
	Vateria indica L. (gum)	B,C	Meena and Ramaswamy (2015)
	Santalum album L. (stem)	B,C	Paulpandi et al. (2012), Gupta and Chaphalkar (2016)
	Rosa × *damascena* Mill. (flower)	B,C	Mahmood et al. (1996), Boskabady et al. (2011)
Laooq-e-Katan	*Linum usitatissimum* L. (seed)	A,C	Liang et al. (2019), Rafieian-kopaei et al. (2017)
Laooq-e-Sapistan	*Cordia myxa* L. (fruit)	A,B,C	Ali et al. (2015), Rashed (2014), Ranjbar et al. (2013)
	Ziziphus jujuba Mill. (fruit)	A,B,C	Yu et al. (2016), Hong et al. (2015), Mesaik et al. (2018)
	Viola odorata L. (flower)	B,C	Gerlach et al. (2019), Koochek et al. (2003)
	Althea officinalis L. (seed)	C	Bonaterra et al. (2020)
	Cassia fistula L. (seed)	A,B,C	Laxmi (2015), Indrasetiawan et al. (2019), Antonisamy et al. (2019)
	Cassia angustifolia M. Vahl (leaves)	A	Jassim and Naji (2003)
	Fraxinus ornus L. (flower)	C	Al-Snafi (2018)
	Prunus amygdalus Batsch (seed oil)	B,C	Musarra-Pizzo et al. (2019), Masihuddin et al. (2019)
Roghan-e-Baboona	*Matricaria chamomilla* L. (flower)	A,C	Amirghofran et al. (2000), Singh O. et al. (2011)
Sarbat-e sadr	Bombyx mori (cocoons)	A	Soumya et al. (2019)
	Ziziphus jujuba Mill. (fruit)	A,B,C	Yu et al. (2016), Hong et al. (2015), Mesaik et al. (2018)
	Tachyspermum ammi (L.) Sprague (seed)	A,B	Shruthi et al. (2017), Roy et al. (2015)
	Glycyrrhiza glabra L. (root)	A,B,C	Mitra Mazumder et al. (2012), Ashraf et al. (2017), Patel et al. (2009)
	Foeniculum vulgare Mill. (fruit)	C	Rather et al. (2016)
	Adhatoda vasica Nees (leaves)	A,B,C	Vinothapooshan and Sundar (2011), Singh et al. (2010), Gibbs (2009)
	Onosma bracteatum Wall. (leaves)	C	Patel et al. (2011)
	Malva sylvestris L. (seed)	C	Martins et al. (2017)
	Hyssopus offloinalis L. (whole plant)	B	Behbahani (2009)
	Ficus carica L. (fruit)	A,B,C	Patil et al. (2010), Camero et al. (2014), Abe (2020)
	Cordia myxa L. (fruit)	C	Oza and Kulkarni (2017)
	Papaver somniferum L. (flower)	B,C	Chattopadhyay and Naik (2007)
	Onosma bracteatum Wall. (flower)	C	Patel et al. (2011)
Sharbat-e-Toot siyah	*Morus nigra* L. (fruit)	A,C	Lim and Choi (2019)
Triyaq-e-Araba	*Laurus nobilis* L. (berries)	A	Aurori et al. (2016)
	Bergenia ciliata (haw.) Sternb. (stem)	A	Rajbhandari et al. (2009)
	Aristolochia indiaca L. (root)	C	Mathew et al. (2011)
	Commiphora myrrha (Nees) Engl. (gum)	C	Su et al. (2015)

Note: AProvide the references Mallik and nayak (2014), Sengottuvelu et al. (2012), and Weili et al. (2011)= Immunomodulators; B = Antiviral; C = Anti-allergic/Antiasthmatic/Anti-inflammatory/Respiratory disorders.

3.1 Ayurvedic Approaches

3.1.1 AYUSH Kwath

Ministry of AYUSH promotes the use of AYUSH kwath, which is a ready-made formulation for health promotion of the masses. The formulation is made of four herbs *Ocimum sanctum* L. leaves, *Cinnamomum verum* J. Presl. stem barks, *Zingiber officinale* Roscoe rhizomes and *Piper nigrum* L. fruits. The formulation is sold in the market with different names like 'AYUSH Kwath', 'AYUSH Kudineer' or 'AYUSH Joshanda'. It is available in powder and tablet forms in the market. These herbs are

TABLE 4 | AYUSH recommended prophylactic approach through formulations of Siddha system of medicine. Ref: AYUSH Ministry of Health Corona Advisory–D.O. No. S. 16030/18/2019-NAM; dated: 06th March, 2020.

Name of the formulation	Composition	Proof of activities related to COVID-19	References
Ahatodai manapagu (siddha)	*Adhatoda vasica* Nees (leaves)	A,B,C	Vinothapooshan and Sundar (2011), Singh et al. (2010), Gibbs (2009)
	Saccharum officinarum L.	C	Cheavegatti-Gianotto et al. (2011)
Kabasura kudineer (siddha)	*Zingiber officinale Roscoe* (rhizome)	A,B,C	Zhou et al. (2006), Chang et al. (2013), Khan et al. (2015)
	Piper longum L. (fruit)	A,B,C	Tripathi et al. (1999), Jiang et al. (2013), Kaushik et al. (2012)
	Syzygium aromaticum (L.) Merr. and L.M. Perry (fruit)	A,C	Dibazar et al. (2015), Chniguir et al. (2019)
	Tragia involucrate L. (leaves)	B,C	Kumar et al. (2019), Alagar Yadav et al. (2015)
	Anacyclus pyrethrum (L.) Lag. (root)	A,B	Sharma et al. (2010), Kumar et al. (2019)
	Adhatoda vasica Nees (leaves)	A,B,C	Vinothapooshan and Sundar (2011), Singh et al. (2010), Gibbs (2009)
	Tinospora cordifolia (Willd.) Miers (stem)	A,B,C	Alsuhaibani and Khan (2017), Pruthvish and Gopinatha (2018), Tiwari et al. (2014)
	Andrographis paniculata (Burm.f.) Nees (whole plant)	A,B,C	Wang et al. (2010), Wintachai et al. (2015), Bao et al. (2009)
	Sida acuta Burm.f. (root)	C	Arciniegas et al. (2017)
	Cyperus rotundus L. (rhizome)	A,B,C	Soumaya et al. (2013), Xu et al. (2015), Jin et al. (2011)
	Terminalia chebula Retz. (pulp)	A,B,C	Shivaprasad et al. (2006), Kesharwani et al. (2017), Haq et al. (2013)
Nilavembu kudineer (siddha)	*Andrographis paniculata* (Burm.f.) Nees (whole plant)	A,B,C	Wang et al. (2010), Wintachai et al. (2015), Bao et al. (2009)
	Plectranthus vettiveroides (Jacob) N.P.Singh and B.D.Sharma (root)	A,B	Kavinilavan et al. (2017)
	Vetiveria zizanioides (L.) Nash (root)	B	Lavanya et al. (2016)
	Zingiber officinale Roscoe (rhizome)	A,B,C	Zhou et al. (2006), Chang et al. (2013), Khan et al. (2015)
	Piper Nigrum L. (fruit)	A,B,C	Majdalawieh and Carr (2010), Mair et al. (2016), Tasleem et al. (2014)
	Cyperus rotundus L. (rhizome)	A,B,C	Soumaya et al. (2013), Xu et al. (2015), Jin et al. (2011)
	Santalum album L. (stem)	B,C	Paulpandi et al. (2012), Gupta and Chaphalkar (2016)
	Trichosanthes cucumerina L. (whole plant)	B,C	Kumar et al. (2019), Arawwawala et al. (2010)
	Mollugo cerviana (L.) Ser. (whole plant)	A,B,C	Ferreira et al. (2003), Jain et al. (2019), Sadique et al. (1987)

Note: A = Immunomodulators; B = Antiviral; C = Anti-allergic/Antiasthmatic/Anti-inflammatory/Respiratory disorders.

reported to boost immunity (Carrasco et al., 2009; Niphade et al., 2009; Alsuhaibani and Khan, 2017; Bhalla et al., 2017) and are active remedies to various viral diseases (Mair et al., 2016; Ghoke et al., 2018; Pruthvish and Gopinatha, 2018).

3.1.2 Samshamani Vati

Samshamani vati (Guduchi ghana vati) is an ayurvedic formulation used in all types of fevers. It is also used as an antipyretic and anti-inflammatory remedy (Patgiri et al., 2014). Samshamani vati is made of aqueous extract of *Tinospora cordifolia* (Willd.) Miers (family Menispermaceae), and reported to be an immunomodulator (More and Pai, 2011) due to the synergistic effect of the various compounds present. It is also effective in various viral diseases (Sachan et al., 2019).

3.1.3 AYUSH-64

AYUSH-64 tablet is composed of *Alstonia scholaris* (L.) R. Br. bark, *Picrorhiza kurroa* Royle ex Benth. rhizomes, *Swertia chirayita* (Roxb.) H. Karst. whole plant, and *Caesalpinia crista* L. seed pulp. Because of its antimalarial activity, AYUSH-64 is considered to be effective among the high-risk coronavirus population. Researchers have reported that each of its constituents is effectively antiviral, anti-asthmatic, and immunoboosting (Sharma et al., 1994; Siddiqui et al., 2012; Sehgal et al., 2013; Panda et al., 2017; Win et al., 2019; Woo et al., 2019).

3.1.4 Agasthaya Hareetaki

Agastya Haritaki Rasayana is a popular 'Avaleha kalpana', used in the management of various respiratory infection and comprises more than 15 herbal ingredients. Most of its ingredients showed antiviral, anti-asthmatic, anti-inflammatory, and immunomodulatory activities (Mouhajir et al., 2001; Tripathi and Upadhyay, 2001; Balasubramanian et al., 2007; Vadnere et al., 2009; Patel and Asdaq, 2010; Pathak et al., 2010; Jain et al., 2011; Kumar et al., 2011; Lampariello et al., 2012; Jiang et al., 2013). The above literature suggests the symptomatic management of COVID-19 by Agastya Haritaki.

3.1.5 Anuthaila

Anuthaila consists of about twenty ingredients and out of them *Leptadenia reticulate* (Retz.) Wight and Arn. has been reported in allergic response, treatment of asthma, bronchitis, and throat trouble (Mohanty et al., 2017). Similarly, *Ocimum sanctum* L. is recommended for a wide range of conditions including, cough, asthma, fever, and malaria (Cohen, 2014) and *Sesamum indicum* L. oil for dry cough, asthma, migraine, and

respiratory infections (Nagpurkar and Patil, 2017). There are reports on *S. indicum* seeds with *Tachyspermum ammi* (L.) Sprague seeds for dry cough, asthma, lung diseases, and common cold (Patil et al., 2008). On the basis of above literature, Anuthaila justifies its use in corona virus pandemic condition (**Table 2**).

3.2 Unani Approaches

3.2.1 Triyaq-e-Araba

Triyaq-e-Araba is an important Unani formulation used as a detoxifying agent. It contains *Laurus nobilis* L. berries, *Bergenia ciliate* (Haw.) Sternb. stem, *Aristolochia indica* L. roots and *Commiphora myrrha* (Nees) Engl. It has been reported by several authors as a potent antiviral agent (Aurori et al., 2016), including against SARS-CoV (Loizzo et al., 2008). Further, *B. ciliata* is found to be effective against the influenza virus-A and herpes simplex virus-1 (HSV-1) (Rajbhandari et al., 2003), whereas its active principal, bergenin, has been found to be effective against hepatitis C virus (HCV) and HIV virus (Ahmad et al., 2018). On the basis of this literature, Triyaq-e-Araba could be one of the effective antiviral medicine and certifies its use against COVID-19.

3.2.2 Roghan-e-Baboona

Roghan-e-Baboona is an Unani remedy utilized as an anti-asthmatic and for the treatment of inflammatory complaints. Flowers of *Matricaria chamomilla* L. are the main ingredient of Roghan-e-Baboona. It is composed of the flowers of *M. chamomilla*, which is found effective for acute viral nasopharyngitis (Srivastava et al., 2010), as well as for sore throat (Kyokong et al., 2002).

3.2.3 Arq-e-Ajeeb

Arq-e-Ajeeb is a liquid preparation that contains thymol, menthol, and camphor. Thymol is a promising candidate for topical application as an antiviral agent for herpetic infections (Lai et al., 2012; Sharifi-Rad et al., 2017). Menthol has been reported as an anti-inflammatory agent (Zaia et al., 2016). The Unani physicians have a very successful history of treating Nazla wabai (Swine flu) using Arq -e-Ajeeb. These studies support the use of Arq-e-Ajeeb for COVID-19.

3.2.4 Khamira-e-Banafsha

Khamira-e-Banafsha is a semi-solid Unani formulation prepared by adding decoction of flowers of *Viola odorata* L. to a base of sugar or sugar with honey and used for cold-cough as expectorant and for the treatment of ailments of respiratory system and chest diseases, bronchitis, whooping cough, fever, expectorant, antipyretic etc. Further, *V. odorata* has been reported to suppress the viral load and increase antiretroviral drug efficacy (Gerlach et al., 2019), decrease the thickness of the alveolar wall, hemorrhage area, and alter the epithelial lining of bronchioles of the lungs (Koochek et al., 2003). The above literature supports its use for the management of COVID-19.

3.2.5 Laooq-e-Sapistan

Laooq-e-Sapistan is a semisolid sugar-based polyherbal Unani formulation extensively used by the masses in India for the treatment of cold and cough, whooping cough, and phlegm. It reduces inflammation of the pharynx, tonsils, and irritation or infection. The jelly like sticky mass of ripe fruit of *Cordia myxa* L. is the main ingredient, which has been reported as antiviral and antitussive (Jamkhande et al., 2013). Another important constituent is Ziziphus fruit, which contains betulinic acid. Literature showed the down-regulation of IFN-γ level by betulinic acid in mouse lung, thus enhancing immunity and suggested as potential therapeutic agent for viral infections (Hong et al., 2015). Aqueous extract also reported increasing thymus and spleen indices as well as enhance the T-lymphocyte proliferation, hemolytic activity, and natural killer (NK) cell activity (Yu et al., 2016). *Viola odorata* L., one of its ingredients, suppresses the viral load (Gerlach et al., 2019). Hence, the literature supports the use of AYUSH formulation Laooq-e-Sapistan in COVID-19.

3.2.6 Sharbat-e-Sadar

Sharbat-e- Sadar is an Unani polyherbal syrup formulation and is widely used for common cold, cough and respiratory diseases. *Trachyspermum ammi* (L.) Sprague, an important ingredient, reported to neutralize antibodies for Japanese encephalitis virus (Roy et al., 2015), and a glycoprotein was found to proliferate B-cells (Shruthi et al., 2017). *Adhatoda vasica* Nees inhibits HIV-Protease (Singh et al., 2010), *Bombyx mori* was reported to increase immune responses against viral infection (Lü et al., 2018). Other ingredients such as *Glycyrrhiza glabra* L., *Ficus carica* L., *Onosma bracteatum* Wall., and *Ziziphus jujuba* Mill. also possess the antiviral and immunomodulatory activities, as summarized in **Table 5**.

3.2.7 Khameera Marwareed

Khameera marwareed is a compound, sugar-based, semisolid Unani formulation used as an immunomodulator. It has been reported to stimulate the immune system through T helper 1 (Th1) type cytokine response and maintains the body in a healthier position to fight against viral infections (Khan et al., 2009). Its ingredients showed powerful antiviral activities by inhibiting replication (Benencia and Courrèges, 1999).

3.2.8 Asgandh Safoof

Asgand (*Withania somnifera* (L.) Dunal) is a very popular Indian medicinal plant. The root powder is used in the Unani system of medicine as an immunomodulator. It is reported that the root's extract significantly increases the $CD4^+$ and $CD8^+$ counts (Bani et al., 2006) and blood profile, especially WBC and platelet counts (Agarwal et al., 1999). Aqueous suspension showed potent inhibitory activity toward mitogen-induced proliferative response of T-lymphocytes and prevent SARS-CoV-2 entry by disturbing connections between viral S-protein receptor binding domain and host ACE2 receptor (Balkrishna et al., 2020). The above literature supports the preventive use of Asgandh safoof against COVID-19.

3.2.9 Habb-e-Bukhar

Habb-e-Bukhar is a polyherbal tablet formulation of Unani system of medicine, prescribed in elephantiasis and malarial fever. The main ingredient of Habb-e-Bukhar is cinchona bark. Its active constituent quinine is being used by some

TABLE 5 | List of Indian Medicinal Plants/AYUSH drugs with proven immunomodulatory, antiviral and anti-allergic/anti-inflammatory/anti-asthmatic activity having potential for exploring against COVID 19 categorized for prioritization on the basis of their earlier reports.

Category. Sl no	Botanical name/Common name/Family/Part	Immunomodulatory activity	Anti-viral activity	Anti-allergic/anti asthmatic/anti-inflammatory/respiratory disorders
C1.1	*Acacia catechu* (L.f.) Willd./Khadira/Fabaceae/Leaves, bark, heartwood	Aqueous and alcoholic extract increased phagocytic response showed by peritoneal macrophages. The extracts inhibited TNF-α and the production of NO, IL-10. **Dose: 100 and 200 mg/kg** Sunil et al. (2019)	Aqueous, hydroalcoholic and n-butanol extract showed anti HIV-1 activity by inhibiting viral protein and Tat **IC_{50}: 1.8 μg/ml** Nutan et al. (2013)	Aqueous extract of leaves showed inhibitory effects on histamine synthesis in rat peritoneal as well as mast cells. **Dose: 100 mg/kg** Prasad et al. (2009), Negi and Dave (2010)
C1.2	*Adhatoda vasica* Nees/Adusa/Acanthaceae/ Leaves	Methanolic extract of leaves inhibit DTH reactiveness, increased the percentage neutrophil adhesion, promoting increased phagocytic activity vis-à-vis increased concentration of lytic enzymes for more effective killing.**Dose: 400 mg/kg** Vinothapooshan and Sundar (2011)	Ethanolic leaf extract inhibit the activity of HIV-Protease. HIV-protease plays a significant part in the replication cycle. Singh et al. (2010)	Alcoholic extract inhibited IgE-dependent basophil mediator release. **Dose: 20 mg/kg** Gibbs (2009), Hossain and Hoq (2016)
C1.3	*Aegle marmelos* (L.) Correa/Bael/Rutaceae/Root, stem bark, fruits	Alcoholic extract stimulates immune system by acting through cellular and humoral immunity.**Dose: 100 and 500 mg/kg** Patel and Asdaq (2010)	Purified seselin showed inhibitory potential over multiple SARS-COV-2 targets and holds a high potential to work effectively as a novel drug for COVID-19. Nivetha et al. (2020)	Aqueous extract inhibit production of nitric oxide (NO) by rat peritoneal cells, anti-histamine effect, and membrane stabilization activity. **Dose: 200 mg/kg** Kumari et al. (2014)
C1.4	*Anacyclus pyrethrum* (L.) Lag./Akkal kadha/ Asteraaceae/Root	Petroleum ether extract showed cellular and humoral immunity.**Dose: 50–100 mg/kg** Sharma et al. (2010)	Pyrethrin act as ligands to bind with viral proteins to prevent the binding of host receptors preventing the fusion lead viral replication in COVID 19 Kumar et al. (2019)	-
C1.5	*Andrographis paniculata* (Burm.f.) Nees/ Kalmegh/Acanthaceae/Leaves	Isolated compound of andrographolide modulate immune responses by regulating macrophage phenotypic polarization and MAPK and PI3K signaling pathways regulate macrophage polarization. **Dose: 10 μg/ml (*In vitro*) and 1 mg/kg (*In vivo*)** Wang et al. (2010)	Alcoholic extract inhibit the viral titer in A549 cells transfected with SRV. They showed the activity through p38 MAPK/Nrf2 pathway.**Dose: 50 μg/ml** Churiyah et al. (2015), Wintachai et al. (2015)	Andrographolide attenuate allergic asthma by inhibition of the NF-kappaB signaling pathway. **Dose: 0.1, 0.5, and 1 mg/kg** Bao et al. (2009)
C1.6	*Carica papaya* L./Papaya/Caricaceae/Leaves, fruits	Alcoholic extract of fruit pulp and seed enhanced phagocytic activity of peritoneal macrophages is correlated with T helper 1 cytokine response. Interferon-gamma increases the phagocytosis process.**Dose: 0.11 g/ml extract every day using a gastric cannula** Amin et al. (2019)	Aqueous extract of the *C. papaya* leaves increases the expression of the envelope and NS1 proteins in DENV-infected THP-1 cells.**IC_{50}: 100 μg/ml** Sharma N. et al. (2019)	Alcoholic extract of leaves in mouse model of ovalbumin- (OVA) induced allergic asthma down regulates IL-4, IL-5, eotaxin, TNF-α, NF-κB, and iNOS levels thus exhibits anti-inflammatory effect. **Dose: 100 mg/kg** Inam et al. (2017)
C1.7	*Cassia occidentalis* L./Kasunda/Fabaceae/Aerial part, seeds	Isolated rhein suppresses the functional responses of the T- and B-lymphocytes and also suppresses lymphoproliferation in splenocytes.**Dose: 10 μM** Panigrahi et al. (2016)	Alcoholic extract showed that the plant possessed an anti-HIV property through inhibition of viral reverse transcriptase activity.**IC_{50}: >100 mg/ml** Estari et al. (2012)	Isolated anthraquinone showed anti-asthmatic potential by decreasing mRNA expression of Th1/Th2 cytokine in lung tissue. **Dose: 250, 500 and 2000 mg/kg** Xu et al. (2018)
C1.8	*Cocculus hirustus* (L.) Diels/Patalagarudi/ Menispermaceae/Whole plant	Methanolic extract showed significantly enhanced specific and non-specific activity on various immune paradigm in cyclophosphamide induced immunosuppresed animals.**Dose: 200 mg/kg** Mallik and nayak (2014)	Found effective against all strains of dengue virus and SARS CoV 2 in *in vitro* studies, hence under phase 2 clinical trial as phytopharmaceutical drug against COVID 19 at 12 centers. (https://www.clinicaltrialsarena.com/news/sun-pharma-covid-19-trial/)	The methanolic leaf extract showed significant analgesic activity in mice as well as significant anti- inflammatory activity using *in vitro* and *in vivo* rat models. **Dose: 100 mg/kg** Sengottuvelu et al. (2012)
C1.9	*Cordia myxa* L./Sapistan/Boraginaceae/Fruits	Aqueous extract of *C. myxa* fruits significantly increased the delayed type hypersensitivity (DTH), mitotic index (MI) of bone marrow and spleen cells Ali et al. (2015)	Dichloromethane, ethyl acetate, and methanol stem extracts showed anti-viral potential against HIV-1 using the syncytia formation assay. **IC_{50}: 21.8 μg/ml** Rashed (2014)	Hydroalcoholic extract inhibit the oxidant stress factors that lead to progression of colitis.**Dose: 100 mg/kg** Ranjbar et al. (2013)

(Continued on following page)

TABLE 5 | (*Continued*) List of Indian Medicinal Plants/AYUSH drugs with proven immunomodulatory, antiviral and anti-allergic/anti-inflammatory/anti-asthmatic activity having potential for exploring against COVID 19 categorized for prioritization on the basis of their earlier reports.

Category. Sl no	Botanical name/Common name/Family/Part	Immunomodulatory activity	Anti-viral activity	Anti-allergic/anti asthmatic/anti-inflammatory/respiratory disorders
C1.10	*Curcuma longa* L./Haldi/Zingiberaceae/Rhizome	Lyophilized turmeric was found to decrease spleen weight, decrease the proportion of $CD4^-$, $CD8^+$ T cells, and decrease phagocytic activity. **Dose: 1 and 2% (w/w)** Kim et al. (2014). Polysaccharide fraction of aqueous extract of *C. longa* inhibiting the secretion of IL-12 and PGE2. **Dose: 0.8–500 μg/ml** Chandrasekaran et al. (2013)	Aqueous extract of *C. longa* suppressed the HBV replication and the transcription of HBV genes in HepG2 cells which produce HBV particles. **Dose: 200 mg/L and 500 mg/L** Kim et al. (2009). Isolated curcuminoids from aqueous extract of curcuma longa exhibited significant inhibitory activity against the neuraminidases from novel influenza H1N1 (WT) and oseltamivir-resistant novel H1N1 (H274Y mutant) expressed in 293 T cells. **IC_{50}: 6.18 ± 0.64 to 40.17 ± 0.79 μg/ml** Dao et al. (2012). Virtual screening of curcumin and its analogue found its activity SARS CoV 2 surface proteins and is under clinical trial. (https://chemrxiv.org/articles/Virtual screening of curcumin and its analogs against the spike surface glycoprotein of SARS-cov-2 and SARS-cov/12142383)	Alcoholic extract of *C. longa* ameliorates food allergy by maintaining Th1/Th2 immune balance in ovalbumin challenged mice. **Dose: 100 mg/kg** Shin et al. (2015)
C1.11	*Cynodon dactylon* (L.) Pers./Doorva/Poaceae/Whole plants	Fresh juice of the grass increased humoral antibody response upon antigen challenge, significant increase in antibody titer in the haemagglutination antibody assay and plaque forming cell assay. **Dose: 250 and 500 mg/kg** Mangathayaru et al. (2009)	Alcoholic dried extract showed virustatic and virucidal activity against porcine reproductive and respiratory syndrome virus (PRRSV) and also significantly inhibits replication of PRRSV. **Dose: 0.78 mg/ml** Pringproa et al. (2014)	Chloroform extract of whole plant produces a bronchodilation via antimuscarinic calcium channel blocking activators and phosphodiesterase inhibition activity. **Dose: 5, 10, 50 and 100 mg/kg** Patel et al. (2013)
C1.12	*Jatropha curcas* L./Euphorbiaceae/Leaves, roots	Phytoconstituents of hydroalcoholic extract ameliorated both cellular and humoral antibody response. **Dose: 0.25, 0.5, 1 mg/kg** Abd-Alla et al. (2009)	Successive extract of *J. curcas* was evaluated by inhibition of HIV replication as determined by HIV p24 antigen ELISA showed 100% inhibition by methanolic and 97.19% inhibition by aqueous extract. **IC_{50}: 0.0255–0.4137 mg/ml (aqueous) and 0.00073–0.1278 mg/ml (Methanolic)** Dahake et al. (2013)	Isolated jatrophacine showed anti-inflammatory potential by inhibiting production of nitric oxide in LPS-induced RAW 264.7 macrophages. **IC_{50}: 0.53 μM** Yang et al. (2019)
C1.13	*Mollugo cerviana* (L.) Ser./Grishmasundara/Molluginaceae/Whole plant	Alcoholic extracts increase NO release by peritoneal cells. **Dose: 25 μg/ml** Ferreira et al. (2003)	Alcoholic extract exhibits antiviral properties for both chikungunya virus and dengue virus. **Dose: 1.8 mg/ml** Jain et al. (2019)	Hydroalcoholic extract inhibit the levels of lipid peroxides, acid phosphatase, and gamma-glutamyl transpeptidase activity. **Dose: 1 mg/g** Sadique et al. (1987)
C1.14	*Nigella sativa* L./Kalonji/Ranunculaceae/Seeds	Aqueous extract of *N. sativa* enhance the proliferative capacity of splenocytes and T lymphocytes, suppression of IFNγ secretion from splenocytes. **Dose: 10, 50, and 100 g/ml** Majdalawieh et al. (2010)	Nigellidine and α- hederin found to have the best potential to act as COVID-19 treatment in docking studies. (https://chemrxiv.org/articles/Identification of compounds from nigella sativa as new potential inhibitors of 2019 novel corona virus Covid-19 molecular docking study/12055716/1)*N. sativa* seeds oil possesses a striking antiviral effect against MCMV infection. **Dose: 100 mg/100 ml/mouse** Umar et al. (2016)	Aqueous extract of seed showed sensory receptors mediating reflex bronchoconstriction and tachykinin receptor antagonists. **Dose: 3.3% w/w extract** Boskabady et al. (2003)

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TABLE 5 | (*Continued*) List of Indian Medicinal Plants/AYUSH drugs with proven immunomodulatory, antiviral and anti-allergic/anti-inflammatory/anti-asthmatic activity having potential for exploring against COVID 19 categorized for prioritization on the basis of their earlier reports.

Category. Sl no	Botanical name/Common name/Family/Part	Immunomodulatory activity	Anti-viral activity	Anti-allergic/anti asthmatic/anti-inflammatory/respiratory disorders
C1.15	*Ocimum sanctum* L./Tulsi/Lamiaceae/Leaves	Aqueous extract of leaves showed regulation of IL-2 production and exhibited leukocytosis and augmentation of T& B cells. **Dose: 250 mg/kg** Goel et al. (2010)	Hydroalcoholic extract showed promising antiviral properties against H9N2 virus by inhibition of a stage in viral intracellular multiplication and non-specific interference with virus-cell interactions. **Dose: 135, 67, 33 mg/0.1 ml** Ghoke et al. (2018)Tulsinol and dihydroeugenol have been found effective against SARS CoV 2 in molecular docking studies. (https://papers.ssrn.com/sol3/papers.cfm abstract id = 3554371)	Alcoholic extracts showed anti-asthmatic potential through inflammatory mechanism by inhibiting LTC4, LTA4 and COX-2 in HL-60 cell lines and reduction in inflammation in asthma mice model. **IC_{50}: 1–10 μg/mlDose: 100 mg/kg** Soni et al. (2015)
C1.16	*Phyllanthus emblica* L./Amla/Phyllanthaceae/Fruits	Alcoholic extract of fruits stimulate B and T lymphocyte and restored the interleukin production considerably. **Dose: 10 mg to 1 mg/ml** Sai Ram et al. (2002)	Fractionated alcoholic extract inhibit HIV reverse transcriptase activity. **IC_{50}: >100 mg/ml** Estari et al. (2012)	Alcoholic extract exhibits anti-inflammatory and anti-oxidant activity by protecting RAW264.7 cells from oxidative damage by increasing glutathione content and total superoxide dismutase activity, suppressing MDA content and decreasing release of pro-inflammatory mediators. **IC_{50}: 0.677 ± 0.029 mg/ml** Li W. et al. (2020)
C1.17	*Solanum nigrum* L./Makoi/Solanaceae/Seeds, barriers	Isolated polysaccharides of significant increment in the percentage of $CD4^+$ T lymphocyte and a decrease in the percentage of $CD8^+$ T lymphocyte of tumor-bearing mice peripheral blood. **Dose: 90, 180, 360 mg/kg** Li et al. (2009)	Chloroform extract decreased the expression or function of HCV NS3 protease in a dose dependent manner and GAPDH remained constant. **Dose: 100 μg/μL** Javed et al. (2011)	Petroleum ether extract of berries inhibits asthma by inhibiting increase in leukocyte and eosinophil count, protection against mast cell degranulation and resisting contraction due to presence of β-sitosterol. **Dose: 50, 100 and 200 mg/kg** Nirmal et al. (2012)
C1.18	*Valeriana wallichii* DC./Valerianaceae/Roots	Alcoholic root extract inhibited HCV by binding with HCV NS5B protein.**Dose: 250 μg/ml** Ganta et al. (2017)	Alcoholic extract and its fraction inhibit HCV by binding with HCV NS5B protein. **Dose: 200 μg/ml** Ganta et al. (2017)	Crude extract showed protection against airway disorders through relax ion of the low K^+ (25 mM)-induced contractions with a mild effect on the contractions induced by high K^+ (80 mM). **Dose: 0.03–3.0 mg/ml** Khan and Gilani (2012)
C1.19	*Vitex negundo* L./Renuka/Verbanaceae/Leaves	Hydroalcoholic extract of leaves of *V. negundo* activate the phagocytic cells such as macrophages and neutrophils. **Dose: 200 mg/kg** Lad et al. (2016)	Alcoholic extract of leaves inhibits HIV-1 reverse transcriptase activity in *in vitro* assay thus exhibits anti-HIV activity. **Dose: 200 μg/ml** Kannan et al. (2012)	*V. negundo* leaf oil inhibit COX-2 without much interfering COX-1 pathways. **Dose: 500 μL/kg** Chattopadhyay et al. (2012)
C1.20	*Withania somnifera* (L.) Dunal/Asgand/Solanaceae/Roots	Aqueous suspension of root showed potent inhibitory activity toward mitogen induced proliferative response of T-lymphocyte and delayed-type hypersensitivity reaction. **Dose: 1000 mg/kg** Rasool and Varalakshmi (2006)	Hydro-alcoholic root extract of *W. somnifera* showed antiviral properties against IBD virus by cytopathic effect reduction assay. **Dose: 25 μg/ml** Pant et al. (2012). Withanone and withaferin a have been found effective against SARS CoV 2 in bioinformatics studies and asgandh extract is under clinical trial. (https://www.researchsquare.com/article/rs-17806/v1), (http://www.bioinformation.net/016/97320630016411.pdf)	Aqueous extract of withania root inhibit histamine and 5-HT in early phase and prostaglandins in delayed phase of inflammatory reaction. **Dose: 1000 mg/kg** Sahni and Srivastava (1993)

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C1.21	*Zingiber officinale* Roscoe/Sunthi/Zingiberaceae/ Rhizome	Volatile oil of ginger influences both cell-mediated immune response and nonspecific proliferation of T lymphocyte. **Dose: 0.125, 0.25, and 0.5 g/kg** Zhou et al. (2006)	Aqueous extract effective against HRSV-induced plaque formation on airway epithelium by blocking viral attachment and internalization. **IC_{50}: >150 μg/ml** Chang et al. (2013)	Aqueous and alcoholic extract showed anti-asthmatic effect by reducung inflammation through suppression of Th2-mediated immune response. **Dose: 500 mg/kg (alcoholic extract)720 mg/kg (aqueous extract)** Khan et al. (2015)
C2.1	*Abutilon indicum* (L.) Sweet/Tuthi/Malvaceae/ Aerial parts	Alcoholic extract showed stimulatory effect on T lymphocytes. Increasing doses of showed higher HA titer value, restoration of WBC count. It also increased lymphocyte and E-rosette formation. **Dose: 200 and 400 mg/kg** Gaikwad and Krishna Mohan (2012)	Alcoholic extract of leaves showed anti-MCV and anti-HSV activities. **Dose: 0.4 μg/ml** Vimalanathan et al. (2009)	Methanolic extract of aerial part showed mast cell stabilizing and anti-inflammatory activity. **Dose: 250 and 500 mg/kg** Mehta and Paranjape (2008)
C2.2	*Achyranthes aspera* L./Apamarga/ Amaranthaceae/Root	Polyphenolic compounds of hydroalcoholic extract showed cytokine based immunomodulatory role. **Dose: 100 mg/kg** Narayan and Kumar (2014)	Alcoholic extract showed potential activity against herpes simplex virus type-1 and type-2 by inhibiting the early stage of multiplication in vero cells. Mukherjee et al. (2013)	Ethyl acetate fraction from methanolic extract showed *in vitro* anti-inflammatory activity. **IC_{50}: 50 = 76 ± 0.14** Khuda et al. (2013)
C2.3	*Aloe vera* (L.) Burm.f./Ghrit kumari/ Asphodelaceae/Roots, leaves	Aloe vera gel administration did not increase ovalbumin (OVA)- specific cytotoxic T lymphocyte (CTL) generation in normal mice. **Dose: 100 mg/kg** Im et al. (2010)	Isolated anthraquinone showed anti-viral activity by inhibiting virus replication. **IC_{50}: 13.70 ± 3.80 to 62.31 ± 3.05** Borges-Argáez et al. (2019)	Polysaccharide isolated from gel showed anti-allergy potential by inhibition of type 2 helper T cell (Th_2) immune response, increase in IL-10 production and stimulating type 1 regulatory T (Tr1) cells activation. **Dose: 50 and 100 mg/kg** Lee D. et al. (2018)
C2.4	*Alstonia scholaris* (L.) R.Br./Saptaparni/ Apocynaceae/Bark	Aqueous extract enhanced phagocytic activity. **Dose: 50 mg/kg** Iwo et al. (2000)	Aqueous and alcoholic plant extract showed anti-viral potential against coxsackie B2, polio virus and herpes simplex virus. **Dose: 2.8 mg/kg** Antony et al. (2014)	Alcoholic extract inhibited inflammatory response by through reduction in ovalbumin-provoked airways allergic inflammatory stress. **Dose: 10, 25, and 50 mg/kg** Zhao et al. (2017)
C2.5	*Azadirachta indica* A.Juss./Neem/Meliaceae/ Leaves	Dried powdered leaves significantly enhanced the antibody titers against new castle disease virus (NCDV) antigen. **Dose: 2 g/kg** Sadekar et al. (1998)	Isolated polysaccharides from aqueous extract of the leaf virucidal aginst Poliovirus-1 (inhibiting initial stage of viral replication). **IC_{50}: 80 μg/ml and 77.5 μg/ml** Faccin-Galhardi et al. (2012)	Aqueous leaves extract showed anti-inflammatory and analgesic activity by in chemical and thermal induced pain models in albino rats. **Dose: 500 mg/kg** Buchineni et al. (2014)
C2.6	*Berberis aristata* DC./Daruharidra/ Berberidaceae/Bark	Isolated berberine inhibited the suppressed viral infection-induced up-regulation of TLR7 signaling pathway. **Dose: 20 mg/kg** Yan et al. (2018)	Isolated compound of berberine inhibited EV71 replication by down regulating autophagy and MEK/ERK signaling pathway. **IC_{50}: 7.43 to 10.25 μM** Wang et al. (2017)	Hydroalcoholic extract showed anti-inflammatory potential, which may be attributed to its inhibitory activity on macrophage-derived cytokine and mediators. **Dose: 50, 100, and 200 mg/kg** Kumar et al. (2016)
C2.7	*Bergenia ciliata* (Haw.) Sternb./Pashanbheda/ Saxifragaceae/Stem	Alcoholic extract stimulated the expression of CD69 on lymphocytes. **Dose: 3.13 and 6.25 mg/ml** Tumova et al. (2018)	Alcoholic extract showed potent anti-viral activity against both influenza virus a and HSV-1. **IC_{50}: >6.25 μg/ml** Rajbhandari et al. (2009)	Alcoholic extract exhibited significant anti-inflammatory activity in carrageenan-induced rat paw oedema manner. **Dose: 300 mg/kg** Sinha et al. (2001)
C2.8	*Camellia sinensis* (L.) Kuntze/Chary/Thecae/ Leaves	Aqueous extract of *C. sinensis* changes hematological profile, immuno potentiating cells, cellular response in splenectomised mice. **Dose: 250 and 500 mg/kg** Gomes et al. (2014)	Hydroalcoholic extract of *C. sinensis* inhibited ADV replication in post-adsorption stage. **IC_{50}: 6.62 μg/ml** Karimi et al. (2016)	Aqueous extract showed anti-asthmatic potential by increasing expression of Th1 cell-specific anti-asthmatic biomarkers (tumor necrosis factor-ß and interferon-γ) and decreasing the expression of anti-asthmatic cytokines in the lungs. **Dose: 25 μg/ml** Heo et al. (2008)

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C2.9	*Cannabis sativa* L./Vijaya/Cannabaceae/Leaves	Cannabinoids indicating mainly immunosuppressive effects on macrophages, NK cells, T lymphocytes and their ability to produce cytokines. **Dose: 5 mg/day** Killestein et al. (2003)	*C. sativa* inhibited viral DNA synthesis. It inhibit the replication cycle of various types of DNA or RNA viruses Jassim and Naji (2003) Hot water extract *C. sativa* reduced the plaque forming ability. **Dose: 300–500 μg/ml** Kurokawa et al. (1993)	Oil extract of *C. sativa* protective effect against COPD through affecting the expression of specific airway epithelial cell genes that modulate Th1 processes using *in vitro* assay. **Dose: 2.4, 1.2, and 0.6 μg/ml** Mamber et al. (2020)
C2.10	*Cassia fistula* L./Amaltas/Fabaceae/Bark	Hydro alcoholic extract of *C. fistula* increased antibody titer against salmonella typhimurium 'O' antigen and significant enhancement in skin thickness in DNCB sensitized albino rats. **Dose: 125 mg/kg, 250 mg/kg and 500 mg/kg** Laxmi (2015)	Hydroalcoholic extract of *C. fistula* suppressed extracellular HBV DNA production. **Dose: 100 μg/ml** Indrasetiawan et al. (2019)	Isolated rhein showed anti-inflammatory activity by modulating levels carrageenan-induced hind paw edema, croton oil-induced ear oedema, cotton pellet-induced granuloma and acetic acid-induced vascular permeability models. **Dose: 10 mg/kg** Antonisamy et al. (2019)
C2.11	*Cinnamomum verum* J.Presl./Daarchini/ Lauracea/Stem, bark	Bark suspension increased the phagocytic index in carbon clearance test, neutrophil adhesion and serum immunoglobulin levels and antibody titer values. **Dose: 10 and 100 mg/kg** Niphade et al. (2009)	Aqueous extract provide treatment against influenza virus infections in vero cells transfected with H7N3 influenza. Brochot et al. (2017)	Isolated procyanidine showed reduction in the elevated levels of total protein, albumin, goblet cell hyperplasia and inflammatory cell infiltration in lung tissue. **Dose: 10, 30, and 100 mg/kg** Kandhare et al. (2013)
C2.12	*Cissampelos pareira* L./Akamai/ Menispermaceae/Aerial parts, roots	Isolated alkaloid fraction of alcoholic extract modulate both T and B cell mediated immune response. **Dose: 100 mg/kg** Bafna and Mishra (2010)	Alcoholic extract of aerial part of *C. pareira* inhibit the viral replication and ability to down-regulate the production of TNF-α, a cytokine implicated in severe dengue disease. **IC_{50}: ≥125 μg/ml** Sood et al. (2015)	Alkaloids fraction suppressed the production of nitric oxide, a critical mediator in inflammation. **Dose: 100 mg/kg** Bafna and Mishra (2010)
C2.13	*Cyperus rotundus* L./Musta/Cyperaceae/ Rhizome	Aqueous, alcoholic, ethyl acetate and total oligomer flavonoids (TOF) extracts of *C. rotundus* influence humoral-mediated immunity by stimulating B and T cell proliferation. **Dose: 1–1000 μg/ml** Soumaya et al. (2013)	Aqueous, alcoholic and ethyl acetate extract of *C. rotundus* inhibited the HBV DNA replication in HepG2.2.15 cell line. **IC_{50}: 29.0, 21.5, 263.4** Xu et al. (2015)	Isolated sesquiterpenes from alcoholic extract showed anti-allergic potential against immediate-type as well as delayed-type hypersensitivity. **Dose: 300 μg/ml (*in vitro*) and 50–300 mg/kg (*in vivo*)** Jin et al. (2011)
C2.14	*Daphne gnidium* L./Lota/Thymelaeaceae/Aerial part	Dichloromethane extract of the aerial exhibited strong antiretroviral activity by interference with HIV co-receptors, CCR5 and CXCR4. Vidal et al. (2012)	Dichloromethane extract of the aerial parts exhibited strong antiretroviral activity and absence of cytotoxicity and pure compounds were active against multidrug-resistant viruses irrespective of their cellular tropism. **Dose: 10 μg/ml** Vidal et al. (2012)	Ethyl acetate extract showed anti-inflammatory effects by inhibiting macrophage proinflammatory function by reducing LPS-induced production of IL-1β, TNF-α, COX-2-derived PGE2 and iNOS-II-synthesized NO. **Dose: 1–100 μg/ml** Harizi et al. (2011)
C2.15	*Ficus carica* L./Anjeer/Moraceae/Leaves, latex	Administration of extract ameliorated both cellular and humoral antibody response Patil et al. (2010)	Resuspension of latex in DMEM containing 1% ethanol able to interfere with the replication of CpHV-1. **IC_{50}: 100 μg/ml** Camero et al. (2014)	Tea infusion of leaves showed anti-allergy potential through promotion of dissociation of IgE from FcεRI receptors. **Dose: 10 ml/kg** Abe (2020)
C2.16	*Glycyrrhiza glabra* L./Mulethi/Fabaceae/Roots, rhizome and leaves	Aqueous root extract showed leukocyte count and phagocytic index increased as well as cellular immune response study, an enhancement in foot pad thickness was observed. **Dose: 1.5 g/kg** Mitra Mazumder et al. (2012)	Aqueous and alcoholic extracts of *G. glabra* verified hemagglutination (HA) test data through which amount of virus is quantified from the allantoic fluid of chicken embryos. **Dose: 300 μg/ml** Ashraf et al. (2017)	Saponin fraction showed anti-asthmatic potential in triple antigen sensitized rats by inhibition of mast cell degranulation. **Dose: 100 mg/kg** Patel et al. (2009)
C2.17	*Illicium verum* Hook.f./Takkola/Magnoliaceae/ Fruit	Isolated lectins from *I. verum* showed immunomodulatory action by stimulating phagocytic function. **Dose: 30 and 50 mg/kg** Bouadi et al. (2015)	Aqueous, alcoholic and hydroalcoholic extracts exhibited inhibitory effects against NDV and avian reovirus. **Dose: 0.24–3.9 mg/ml** Alhajj et al. (2020)	70% alcoholic extract exert antiasthmatic effects through upregulation of $Foxp3^+$ regulatory T cells and inhibition of Th2 cytokines. **Dose: 50, 100, and 200 mg/kg** Sung et al. (2017)

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C2.18	*Mentha* × *piperita* L./Peppermint/Lamiaceae/ Leaves	Hydrodistillate fractions of *M. piperita* affect the functional responses of human PMNs and PBMCs. **Dose: 2 mM and 12 μL** Cosentino et al. (2009)	Alcoholic extract showed antiviral activity against RSV with a high selectivity index, and significantly decreased the production of NO, TNF-α, IL-6, and PGE2 in lipopolysaccharide-stimulated RAW 264.7 cells. **IC_{50}: 10.41 μg/ml** Li et al. (2017)	Essential oil showed antispasmodic activity by regulating prostaglandins and nitric oxide synthase on rat trachea. **Dose: 1–300 μg/ml** de Sousa et al. (2010)
C2.19	*Mentha spicata* L./Spearmint/Lamiaceae/Leaves	Essential oil from *M. spicata* proliferate T-cells, IL-2 and potently inhibit the production of pro-inflammatory cytokine TNF-α production Orhan et al. (2016)	Aqueous extract exhibits anti-viral potential against porcine parvovirus (PPV) *in vitro* by efficiently killing them and control their multiplication in cells. **IC_{50}: 0.0340 mg/ml** Weili et al. (2011)	Ethyl acetate soluble fraction of leaves by inhibit antigen stimulated rat basophile. Prasad et al. (2009)
C2.20	*Momordica charantia* L./Bitter guard/ Cucurbitaceae/Leaves, fruits and seed	Alcohol and diethyl ether extract has been found that the exposure of neutrophils and macrophages stimulates both their capacity to ingest foreign particles and their intracellular killing activities. **Dose: 250, 500, 1000 mg/kg** Mahamat et al. (2020)	Crude protein fraction of *M. charantia* strongly inhibited H1N1, H3N2 and H5N1 subtypes. **IC_{50}: 40–200 ?g/ml** Pongthanapisith et al. (2013)	Alcoholic extract showed the highest reduction of LPS-induced NO, iNOS and prostaglandin E2 production and down regulates pro-interleukin-1β and NF- κB activation expression in RAW 264.7 macrophages Lii et al. (2009)
C2.21	*Morus alba* L./Sahatoot/Moraceae/Leaves, fruits	Isolated water soluble polysaccharides stimulates murine RAW264.7 macrophage cells to release chemokines and proinflammatory cytokines. Lee et al. (2013). Alcoholic extract of leaves significant increase in the phagocytic index and adhesion of neutrophils. **Dose: 100 mg/kg and 1 g/kg** Bharani et al. (2010)	*M. alba* fruits juice and its fractions inhibit internalization and replication of MNV-1, whereas it may influence adherence or internalization of FCV-F9 virions. **EC_{50}: 0.005 (MNV-1) and 0.25–0.30 (FCV-F9)** Lee et al. (2014)	Juice of *M. alba* fruits inhibit production of NO and proinflammatory cytokines (TNF-α, IL-6), as well as the expression of NOS2 and PTGS2 in LPS-stimulated RAW264.7 macrophages. **Dose: 0.1, 0.5, and 1 μg/ml** Jung et al. (2019)
C2.22	*Nyctanthes arbor-tristis* L./Parijata/Oleaceae/ Leaves, flowers and seeds	Immunostimulant activity of NAFE seems to be mediated through splenocytes proliferation and increased production of cytokines, especially IL-2 and IL-6 of aqueous extract of Nyctanthes arbor-tristis. **Dose: 400 and 800 mg/kg** Bharshiv et al. (2016)	n-Butanol fraction of alcoholic extract of protected encephalomyocarditis virus (EMCV) infected mice against semliki forest virus (SFV). **Dose: 125 mg/kg** Gupta et al. (2005)	Alcoholic extract showed anti-asthmatic and anti-tussive activity against histamine and acetylcholine cocktail induced asthma and citric acid induce cough in Guinea pig. **Dose: 100, 200, and 300 mg/kg** Mathur et al. (2016). Extracted polysaccharide from leaves aqueous extract reduce the number of cough efforts without influencing the specific airway resistance, it triggers cough reflex provocation. **Dose: 25 and 50 mg/kg** Ghosh et al. (2015)
C2.23	*Ocimum basilicum* L./Basil/Lamiaceae/Leaves	Hydroalcoholic extract of leaves increased the IFN-γ/IL-4 ratio and decreasing BALF levels of IgE, PLA_2 and TP. **Dose: 50,300, 600 mg/kg** Eftekhar et al. (2019b)	Alcoholic extract inhibit ZIKV replication in vero E6 cells. The extract seems to inhibit the virus at the step of attachment and entry into the host cell. **IC_{50}: 1:134** Singh et al. (2019)	Hydroalcoholic extract showed therapeutic effect on asthma by reducing eosinophil's, monocytes, neutrophils percentage and increase in percentage of lymphocytes and antioxidant biomarkers levels. **Dose: 0.75, 1.50, and 3.00 mg/ml** Eftekhar et al. (2019a)
C2.24	*Oleo europea* L./Zaitoon/Oleaceae/Leaves	Isolated oleuropein from hydroalcoholic extract showed lymphocyte activation and proliferation properties. Oleuropein exhibited a high degree of lymphocyte aggregation. **Dose: 540 μg/ml** Randon and Attard (2007)	Aqueous leaves extract showed anti-viral potential against newcastle disease virus by restricting replication. **Concentration: 1000 μg/ml** Salih et al. (2017)	Essential oil from leaves inhibit NFB activation in monocytes and monocyte derived macrophages. Lucas et al. (2011)

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C2.25	*Panax ginseng* C.A.Mey./Ginseng/Araliaceae/ Roots	Ginsenosides increased the number of spleen plaque-forming cells, the titers of sera hemagglutinins as well as the number of antigen-reactive T-cells and splenocyte natural killer activity. **Dose: 10 mg/kg** Buriana et al. (1990)	Fermented extract improved the survival of human lung epithelial cells, inhibits RSV replication, suppressed the expression of RSV-induced inflammatory cytokine genes and the formation of ROS in epithelial cell cultures. **Dose: 25 mg/kg** Wang et al. (2018)	*P. ginseng* extract showed anti-asthmatic activity by restoring EMBP(eosinophil major basic protein), Muc5ac, CD40, and CD40L expression and mRNA and protein levels of IL-1, IL-4, IL-5, and TNF-α. **Dose: 20 mg/kg** Kim and Yang (2011)
C2.26	*Peganum harmala* L./Harmal/Nitrariaceae/Aerial parts and seeds	Alcoholic extract (80%) of seed showed effects on zymosan-A activated neutrophils (PMNs). **Dose: 25, 50, and 100 μg/ml** Koko et al. (2008)	Alcoholic extract inhibits viral RNA replication and viral polymerase activity. **IC_{50}: 9.87 μg/ml** Moradi et al. (2017)	Alkaloid fraction of alcoholic extract showed potent antitussive, expectorant and bronchodilating activities in cough models of mice and Guinea pigs. **Dose: Total extract (1650 mg/kg) and alkaloid fraction (90 mg/kg)** Liu et al. (2015)
C2.27	*Phyllanthus amarus* Schumach. and Thonn./Bhui amla/Phyllanthaceae/Whole plant	Alcoholic extract of aerial parts exhibited potent inhibitory action on both phagocytic and CD18 expression of phagocytes. **Dose: 6.25–100 μg/ml** Jantan et al. (2014)	Aqueous extract inhibited cellular proliferation and suppressed HBsAg production in human hepatoma cells. **Dose: 1 mg/ml** Yeh et al. (1993)	Alcoholic extract attenuates asthma by modulating oxido-nitrosative stress SOD, GSH, MDA, and NO), immune-inflammatory makers (HO-1, TNF-α, IL-1β, and TGF-β1), and Th2 cytokines. **Dose: 100 and 200 mg/kg** Wu et al. (2019)
C2.28	*Picrorhiza kurroa* Royle ex Benth./Kutki/ Plantaginaceae/Rhizome, leaves	Hydroalcoholic extract stimulate cell-mediated and humoral immunities, along with complement activity and phagocytic function. **Dose: 25, 50, 100 mg/kg** Sharma et al. (1994)	Isolated iridoids from chloroform fractionated alcoholic extract of inhibit expression of vpr in TREx-HeLa-vpr cells and these iridoid are naturally occurring vpr inhibitors. **Dose: 5 and 10 μg/ml** Win et al. (2019)	Alcoholic extract showed anti-asthmatic potential by exhibiting relaxation effect against histamine and acetylcholine induced contraction model in Guinea pigs.**Dose: 25 mg/kg (*in vivo*)1, 10 and 100 mg/ml (*in vitro*)** Sehgal et al. (2013)
C2.29	*Piper longum* L./Pipli/Piperaceae/Fruits	Aqueous extract possessed a demonstrable immunostimulatory activity, both specific and nonspecific, as evident from the standard test parameters such as haemagglutination titer, macrophage migration index and phagocytic index. **Dose: 225 mg/kg** Tripathi et al. (1999)	Butanol fraction of alcoholic extract possessed remarkable inhibitory HBV activity, against the secretion of hepatitis B virus surface antigen (HBsAg) and hepatitis B virus e antigen (HBeAg). **IC_{50}: 0.15 mM for HBsAg and 0.14 mM for HBeAg** Jiang et al. (2013)	Aqueous and pet ether extract showed anti-asthmatic potential by protecting against histamine induced bronchospasm, haloperidol induced catalepsy and passive paw anaphylaxis and by decreasing number of leukocytes in milk-induce leukocytes model. **Dose: 50, 100, and 200 mg/kg** Kaushik et al. (2012)
C2.30	*Piper nigrum* L./Marica/Piperaceae/Fruits	Aqueous extract of *P. nigrum* capable of promoting the proliferative signaling pathways in splenocytes and enhance murine splenocyte proliferation. **Dose: 50 and 100 μg/ml** Majdalawieh and Carr (2010)	Isolated piperamides from *P. nigrum* inhibit coxsackie virus type B3 (CVB3). It inhibit the proliferation of VSMCs. **IC_{50}: 21.6μM to10.6 μg/ml** Mair et al. (2016)	Isolated piperine acted partially through stimulation of pituitary adrenal axis. **Dose: 5, 10, 20, and 40 mg/kg** Tasleem et al. (2014)
C2.31	*Pongamia pinnata* (L.) Pierre/Karanj/Fabaceae/ Seeds	Isolated oil impact on immune cell signaling events needed for continued recruitment of neutrophils/other cells. **Dose: 0.3 or 0.5 g/kg** Muniandy et al. (2018)	Aqueous extract interfered with HBsAg and thus probably may prevent HBV entry. **Dose: 5 mg for 0.18 pg/ml concentrations of the virus** Mathayan et al. (2019)	Isolated isoflavone and showed inhibitory effects against NO production in LPS-stimulated BV-2 microglial cell thus anti-inflammatory effects. **IC_{50}: 9.0 μM** Wen et al. (2018)
C2.32	*Punica granatum* L./Anar/Punicaceae/Fruit, peel	Aqueous extract showed, a significant decrease in nitric oxide levels and TNF-α levels. A significant diminution of iNOS, TNF-α and NF-κB expression was also observed. **Dose: 0.65 g/kg** Labsi et al. (2016)	Alcoholic extract inhibited influenza a PR8 virus replication in the MDCK cell line, it could suppress the amplification of the infectious influenza viruses. **IC_{50}: 6.45 μg/ml** Moradi et al. (2019)	Isolated galloyl-hexahydroxydiphenoyl-glucose showed protective effect against acute lung injury and anti-inflammatory activity by inhibiting LPS-induced JNK and NF-κB activation and reduction in expression of the TNF-α, IL-6, and IL-1β genes in lungs. **Dose: 5, 50, and 100 mg/kg** Pinheiro et al. (2019)

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TABLE 5 | (*Continued*) List of Indian Medicinal Plants/AYUSH drugs with proven immunomodulatory, antiviral and anti-allergic/anti-inflammatory/anti-asthmatic activity having potential for exploring against COVID 19 categorized for prioritization on the basis of their earlier reports.

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C2.33	*Plantago major* L./Lahuriya/Plantaginaceae/ Whole plants, seeds	Aqueous extract increased lymphocyte proliferation and secretion of interferon-γ at low concentrations (<50 μg/ml), but at high concentrations, it can inhibit this property (<50 μg/ml). **Dose: 50 μg/ml** Chiang et al. (2003)	Isolated compound of caffeic acid from aqueous extract possesses interesting anti-HSV-1, anti-HSV-2 and anti-ADV-3 activities. Caffeic acid was found to inhibit HSV-1 replication. **EC_{50}: 15.3 μg/ml** Chiang et al. (2002)	Hydroalcoholic extract showed amelioration of asthma by increasing mean mast cells, alveolar epithelium thickness and glycoprotein accumulation. **Dose: 100 mg/kg** Farokhi and Khaneshi (2013)
C2.34	*Psoralea corylifolia* L./Babchi/Fabaceae/Seeds	Hydroalcoholic extract stimulate natural killer cell activity. A positive response was also observed in the ADCC activity of spleen cells. **Dose: 100 and 200 mg/kg** Latha et al. (2000)	Aqueous extract found more effective in suppressing the virosis and reduced the mortality against virosis cellular and biochemical changes. Kiran Kumar et al. (2012)	Extract showed novel agent for asthma by inhibiting eosinophils accumulation into airways and modulating Th1/Th2 cytokine balance. **Dose: 200 and 400 mg/kg** Lee and Kim (2008), Wen et al. (2018)
C2.35	*Rhodiola rosea* L./Rhodora/Crassulaceae/Whole plant	Isolated compound of could promote the activation of T lymphocytes, differentiate them into $CD4^+$ cell or $CD8^+$ cell, and implement their functions. **Dose: 12.5, 25, 50 μg** Guan et al. (2011)	Alcoholic extract inhibit the entry and infection of ebola and marburg viruses. **IC_{50}: 0.25 μg/ml (ebola virus)4.0 μg/ml (marburg virus)** Cui et al. (2018)	Isolated salidroside showed protective effect in acute lung injury by decrease in the *W/D* ratio, myeloperoxidase activity of lung, reducing protein concentration, macrophages in the bronchoalveolar lavage fluid and regulating inflammatory cytokines and NF-κB. **Dose: 120 mg/kg** Guan et al. (2012)
C2.36	*Santalum album* L./Sandalwood/Santalaceae/ Stem	Aqueous extract inhibited cell proliferation, nitric oxide production and CD14 monocyte. **Dose: 30 mg/ml** Gupta and Chaphalkar (2016)	β-Santalol from hexane extract exhibits anti-influenza A/HK (H3N2) virus by inhibition of viral mRNA synthesis. **Dose: 100 μg/ml** Paulpandi et al. (2012)	Alcoholic extract showed *in vitro* anti-inflammatory activity as compared to Diclofenac. **Dose: 500 mg/ml** Saneja et al. (2009)
C2.37	*Saussurea lappa* (Decne.) C.B.Clarke/Kutha/ Compositae/Roots	Isolated compound of costunolide and dehydrocostus lactone showed suppressive effect on the expression of the hepatitis B surface antigen (HBsAg) in Hep3B cells. **IC_{50}: 1.0–2.0 μM** Chen et al. (1995)	Hexane fraction of alcoholic extract suppress the HBsAg production by Hep3B cells. **IC_{50}: 1.0–2.0 μM** Chen et al. (1995)	SML0417, epiligulyl oxide and elecampane camphor isolated from roots ameliorates allergic asthma in murine model by inhibiting antigen-induced degranulation, reduction in inflammatory signs and mucin production and expression and secretion of Th2 cytokines. Lee B. K. et al. (2018)
C2.38	*Sphaeranthus indicus* L./Mundi/Asteriae/Leaves, flowers	Petroleum ether extract from the flower heads of *S. indicus* increasing phagocytic activity, hemagglutination antibody titer and delayed type hypersensitivity. **Dose: 200 mg/kg** Bafna and Mishra (2007)	Alcoholic extract exhibits anti-virus potential against herpes simplex virus (HSV) and mouse corona. **Dose: 0.4 μg/ml** Vimalanathan et al. (2009)	Alcoholic leaves extract inhibit prostaglandin synthesis. **Dose: 100,200, and 400 mg/kg** Meher et al. (2011)
C2.39	*Syzygium aromaticum* (L.) Merr. and L.M. Perry/ Lavang/Myrtaceae/Fruits	Aqueous and alcoholic suppressive effects on mouse macrophages and inhibit IL-1β, IL-6, and IL-10. **Dose: 1000 μg/ml** Dibazar et al. (2015). Essential oil increased the WBC count and enhanced DTH response in mice. Carrasco et al. (2009)	Hydroalcoholic extract exhibits anti- viral activity against herpes simplex virus-1 evaluated on vero cell line using MTT assay. **IC_{50}: 8.4 μg/ml** Moradi et al. (2018)	Aqueous extract decreases neutrophil count and proteins leakage into bronchoalveolar lavage fluid. **Dose: 200 mg/kg** Chniguir et al. (2019)
C2.40	*Terminalia chebula* Retz./Halala/Combretaceae/ Fruits	Aqueous extract increase in humoral antibody titer and delayed-type hypersensitivity in mice. **Dose: 100–500 mg/kg** Shivaprasad et al. (2006)	Hydroalcoholic extract of prevents the attachment as well as penetration of the HSV-2 to vero cells and efficacy to inhibit virus attachment and penetration to the host cells. **IC_{50}: 0.01 ± 0.0002 μg/ml** Kesharwani et al. (2017)	Carbohydrate polymer from aqueous extract of dried ripe fruit showed antitussive efficacy in citric acid-induced cough efforts. **Dose: 50 mg/kg** Nosalova et al. (2013). Ethyl acetate fraction showed antitussive efficacy on sulfur dioxide gas induced cough partially through modulation of opioid receptors. **Dose: 500 mg/kg** Haq et al. (2013)

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C2.41	*Tinospora cordifolia* (Willd.) Miers/Giloe/ Menispermaceae/Stem	Aqueous and alcoholic extract reduced bacterial load as compared to untreated macrophages. **Dose: 100, 200, and 500 μg/ml** Alsuhaibani and Khan (2017)	Methanol and ethyl acetate mixture extract inhibits the growth of HSV. **Dose: 50–100 μg/ml** Pruthvish and Gopinatha (2018)	Hydroalcoholic extract ameliorates asthma through decreasing oxidative stress and inflammation through modulating glutathione homeostasis and regulation of NFκB and pro-inflammatory genes. **Dose: 100 mg/kg** Tiwari et al. (2014)
C2.42	*Tribulus terrestis* L./Gokhru/Zygophyllaceae/ Whole plant	Saponin fraction increased phagocytic activity in dose dependent manner. **Dose: 50, 100, 200 μg/ml** Tilwari et al. (2011)	Alcoholic extract showed antiviral potential against newcastle disease virus evaluated by titering *in vivo* vero cell line culture. **Dose: 80 μg/ml** Malik et al. (2018)	Hydroalcoholic fruit extract activate mast cell. **EC_{50}: 1% extract with 0.1% HC** Kang et al. (2017)
C2.43	*Ziziphus jujuba* Mill./Unnab/Rhamnaceae/Fruits	Aqueous extract increase thymus and spleen indices as well as enhance the T-lymphocyte proliferation, hemolytic activity and NK cell activity. **Dose: 1.3, 2.6, and 5.2 g/kg** Yu et al. (2016)	Isolated betulinic acid showed antiviral activity on influenza virus by attenuating pulmonary pathology and down-regulation of IFN-γ level. **Concentration: 50 μM** Hong et al. (2015)	Alcoholic extract showed inhibition of expression and activity of COX-2. **Dose: 200, 400, and 600 mg/kg** Mesaik et al. (2018)
C2.44	*Zataria multiflora* Boiss./Satar/Lamiaceae/Whole plant, leaves	Obtained essential oils from hydrodistillation increase in the secretion of TNF-α, IFN-γ, IL-2 and decrease in IL-4. **Dose: 10mg/one BALB/c and 7mg/one C57BL/6** Jamali et al. (2020)	*Z. multiflora* destruction of virus infectivity or inhibition of early phases of viral proliferation cycle. Arabzadeh et al. (2013)	Hydro-alcoholic extract ameliorates allergic asthma by decreasing pro-inflammatory cytokines, increasing expression of anti-inflammatory cytokines gene and number of treg (FOXP3) in splenocytes. **Dose: 200, 400, and 800 μg/ml** Kianmehr et al. (2017)
C3.1	*Artemisia absinthium* L./Vilayati afsantin/ Asteraceae/Roots	Alcoholic extract modulates the percentage expression and fluorescent intensity of CD86, CD40 and MHC II molecules on DCs. **Dose: 100 μg/ml** Azeguli et al. (2018)	Decoction effectively suppressed HBV DNA, HBeAg, and HBsAg.**Dose: 15 ml (containing 1 g of dried extract**) Ansari et al. (2018)	-
C3.2	*Datura metel* L./Safed dhatura/Solanaceae/ Leaves, fruits and seeds	-	Aqueous and alcoholic extract performed in vero cell line using MTT assay showed good antiviral activity. **IC_{50}: 2.5 mg/ml** Roy et al. (2016)	Aqueous extract in ovalbumin challenged mice ameliorates asthma through promotion of naïve T cell development and reducing activated T cells. **Dose: 0.56 mg/kg** Rifa'i et al. (2014)
C3.3	*Elettaria cardamomum* (L.) Maton/Chotielaichi/ Zingiberaceae/Fruits	Essential oil overlapped with that of various canonical signaling pathways which support its immunomodulator activity. Han and Parker (2017)	-	The extract obtained from supercritical fluid extraction with carbon dioxide inhibit NF-kappa signaling pathway. **Dose: 0.03%** Souissi et al. (2020)
C3.4	*Embelia* ribes Burm.f./Baberang/Myrsinacea/ Fruits	-	Ethyl acetate extract exhibits antiviral activity MDCK cells infected with influenza virus A/Puerto rico/8/34 (H1N1). **IC_{50}: 0.2 μg/ml** Hossan et al. (2018)	Isolated embelin attenuates anti-inflammatory activity against carrageenan induced paw edema in rats. **Dose: 20 mg/kg** Mahendran et al. (2011)
C3.5	*Hedychium spicatum* Sm./Kapurkachri/ Zingiberaceae/Rhizome	Alcoholic extract increased phagocytosis, WBC and neutrophils count. **Dose: 200–500 mg/kg** Uttara and Mishra (2009)	-	Aqueous extract attenuates anti-histaminic action against histamine-induced bronchospasm in Guinea pig. **Dose: 200 mg/kg** Ghildiyal et al. (2012)
C3.6	*Hyssopus officinalis* L./Zoofa/Lamiaceae/ Flowers, leaves	Alcoholic extract of leaves inhibits plaque formation of both of the two strains of HSV-1 in vero E6 cells. **Dose: 125 mg/kg** Behbahani (2009)	Aqueous extract of flowers affect the levels of some cytokines (such as IL-4, IL-6, IL-17, and IFN-γ) in asthmatic mice. By detection of the expressions of MMP-9 and TIMP-1 and the morphological changes. **Dose: 0.04 g/10 g** Ma et al. (2014)	

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C3.7	*Inula racemosa* Hook.f./Puskara/Asteraceae/ Root	Polysaccharide fraction of water extract showed immunomodulatory action by stimulating phagocytic function. **Dose: 100–200 mg/kg** Mishra et al. (2016)	-	Pet. Ether extract shows anti-asthmatic potential by mast cell degranulation. **Dose: 50 and 100 mg/kg** Vadnere et al. (2009)
C3.8	*Lepidium sativum* L./Chansur/Cruciferae/Whole plants	Protein extract of lepidium sativum alter the proliferation induced by Con-A. Daoudi et al. (2013)	-	Isolated fractions from ethanol extract of whole plant inhibit bronchospasm induced by histamine and acetylcholine. Rehman et al. (2012), Prasad et al. (2009)
C3.9	*Leptadenia reticulata* (Retz.) Wight and Arn./ Meethi dodi)/Apocynaceae/Root, stem bark	Alcoholic extract increased haematological profile, GSH, SOD, CAT activity and decreased LPO levels in cyclophosphamide-induced rats. **Dose: 100–200 mg/kg** Pravansha et al. (2012)	-	Ethyl acetate fraction inhibit pro-inflammatory cytokines (IL-2, IL-6, TNF-α) and release of prostaglandin to prevent inflammation. **Dose: 600 mg/kg** Mohanty et al. (2015)
C3.10	*Magnolia officinalis* var. officinalis/Himchampa/ Magnoliaceae/Bark	-	Isolated compound magnolol and honokiol from petroleum ether extract of bark provoked IRF7 transcripts (magnolol) and reinforcing the host antiviral response via NF-κB pathways (Honokiol). **Dose: 35 mg/L** Chen et al. (2017)	Aqueous extract exhibits anti-allergic actions through inhibition of local immunoglobulin E, histamine release and TNF-α production in 48/80 induced systemic anaphylaxis in rats. **Dose: 0.001–1 g/kg** Shin et al. (2001). Polyphenolic rich extract of *Magnolia officinalis* suppressed the production of inflammatory mediators, NO, pro-inflammatory cytokines, TNF-α and IL-6, and inhibition of TLR3 and NF-κB activation. **Dose: 10 and 200 mg/kg** Fang et al. (2015)
C3.11	*Mucuna pruriens* (L.) DC./Kaunchbeej/Fabaceae/ Seeds	*M. pruriens* modulate the immune components like TNF-a, IL-6, IFN-l, IL-1b, iNOS and IL-2. Rai et al. (2017) Alcoholic extract of root influenced both humoral and cell mediated immunity. **Dose: 100, 200 and 400 mg/kg** Murthy and Mishra (2016)	-	Alcoholic extract of seeds of *M. pruriens* act on opoid receptor that located on airway passage and produce inhibitory effect. **Dose: 500 mg/kg** Nuzhat et al. (2013)
C3.12	*Piper betle* L./Paan/Piperaceae/Leaves	Alcoholic extract of *P. betle* leaves showed lymphocyte proliferation, interferon- γ receptors and the pro-duction of nitric oxide. It suppressed phytohaemagglutinin stimulated peripheral blood lymphocyte proliferation. **Dose: 500 mg/kg** Kanjwani et al. (2008)	-	Alcoholic extract of leaves decreased histamine and GM-CSF produced by an IgE-mediated hypersensitive reaction, and inhibited eotaxin and IL-8 secretion in a TNF-α and IL-4-induced allergic reaction. **Dose: 10 mg/ml** Wirotesangthong et al. (2008)
C3.13	*Sesamum indicum* L./Tila/Pedaliaceae/Seed	Essential oil suppress cellular immunity with the domination of Th2 responses and also modulate macrophages, dendritic cells proinflammatory functions. Dose:100 μg/ml Khorrami et al. (2018)	-	Aqueous extract reduce LPS induced inflammatory gene expression. **EC_{50}: 100 ng/ml** Deme et al. (2018)
C3.14	*Sida cordifolia* L./Beejband/Malvaceae/Seeds	*S. cordifolia* increased production of T-cell precursor and passive influences on the production of cytokines. **Dose: 2 gm/kg** Tekade et al. (2008)	-	Alcoholic extract of seed inhibit paw edema and granuloma formation. **Dose: 200 and 400 mg/kg** Singh S. et al. (2011)
C3.15	*Swertia chirayita* (Roxb.) H.Karst./Chirayata/ Gentianacea/Whole plant	-	Chloroform extract inhibit expression of viral protein R in hela cells harboring the TREx plasmid encoding full-length vpr (TREx-HeLa-vpr cells. **Dose: 10 μM** Woo et al. (2019)	Chloroform fraction exhibits bronchodilator effect by Ca^{2+} channel blockade. **Dose: 0.1–3.0 mg/ml** Khan et al. (2012)

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C3.16	*Tachyspermum ammi* (L.) sprague/Ajwain/ Umbelliferone/Seed	Isolated glycoprotein from aqueous extract of seed proliferate B-cell enriched murine splenocytes and activated macrophages in releasing NO and promoted phagocytosis. **Dose: 1 µg/ml** Shruthi et al. (2017)	Seed oil neutralize antibody for Japanese encephalitis virus. **Dose: 0.5 mg/ml** Roy et al. (2015)	-
C3.17	*Tylophora indica* (Burm.f.) Merr./Antamool/ Asclepiadaceae/Roots, leaves	Alkaloidal fraction inhibit proliferation of splenocytes and both macrophages and T cells were found to be vulnerable to tylophora alkaloids. Ganguly et al. (2001)	-	Alcoholic extract of aerial part showed anti-inflammatory effect against carrageenan induced paw oedema and cotton pellet induced granuloma. **Dose: 100, 200 and 400 mg/kg** Raj et al. (2006)
C3.18	*Viola odorata* L./Banafsha/Violaceae/Flowers	-	*V. odorata* effective against multiple protease inhibitors Gerlach et al. (2019)	Aqueous flower extract effectively reduced the hemorrhage area, alveolar wall thickness and septum rupture, and alteration of the epithelial lining of bronchioles of lungs. **Dose: 50 mg/kg** Koochek et al. (2003)

C1: Category 1, includes 21 "Most promising drugs" which have already shown activity against Coronaviruses/HIV/Dengue viruses with their immunomodulatory and anti-allergic/anti-inflammatory properties.
C2: Category 2, composed of 44 "Equally promising drugs" which reportedly have shown anti-viral, immunomodulatory and anti-allergic/anti-inflammatory activities.
C3: Category 2, represents 18 "Possibly promising drugs" which have been reported to show anti-viral, immunomodulatory and anti-allergic/anti-inflammatory activities.

countries as either experimental treatment or suggested as a drug with a promising profile against COVID-19 (Devaux et al., 2020). Another constituent, *Tinospora cordifolia* (Willd.) Miers is reported as potent antiviral agent against HSV (Pruthvish and Gopinatha, 2018) as well as suggested for immune-enhancing activity (Rastogi et al., 2020). Thus, literature supports Habb-e-Bukhar in the treatment of COVID-19.

3.2.10 Sharbat-e-Toot Siyah

Sharbat-e-Toot Siyah is composed of the juice of *Morus nigra* L. in a sugar base and is used to treat tonsillitis and sore throat. It has been reported as anti-inflammatory and analgesic and inhibits the pro-inflammatory cytokines (Chen et al., 2016). Very recently, it has been reported to enhance immunomodulatory activity (Lim and Choi, 2019).

3.2.11 Laook-e-Katan

Laook-e-Katan is a sugar-based semisolid Unani formulation composed of *Linum usitatissimum* L. seed, which contains alpha linolenic acid and has been reported to have antiviral, anti-inflammatory, and immunomodulatory activities (Leu et al., 2004; Erdinest et al., 2012; Miccadei et al., 2016). In Unani, it is recommended for respiratory disorders (**Table 3**).

3.3 Siddha Approaches

3.3.1 Nilavembu Kudineer

Nilavembu Kudineer is a polyherbal Siddha formulation prescribed for the prevention and management of viral infections and fevers. It acts as an immunomodulator and plays a defending role against dengue fever and chikungunya. Recent studies showed that formulation has antiviral and antimicrobial actions, which makes it suitable for viral fevers, malaria, and typhoid fever (Mahadevan and Palraj, 2016). Previously, studies proved that most of its constituents are effective as antiviral, anti-asthmatic, and immunobooster agents (Carrasco et al., 2009; Wang et al., 2010; Jin et al., 2011; Chang et al., 2013; Wintachai et al., 2015; Mair et al., 2016).

3.3.2 Ahatodai Manapagu

Ahatodai Manapagu is composed of *Adathoda vasica* Nees leaves, which contains alkaloids like vasicine, the active ingredient in various cough syrups. *A. vasica* has been used in the Indian medicinal system for thousands of years, to treat various types of respiratory disorders (Sampath Kumar et al., 2010). Vinothapooshan et al. suggested that its extract positively modulates the immunity of the host (Vinothapooshan and Sundar, 2011).

3.3.3 Kabasura Kudineer

Kabasura Kudineer is a traditional formulation used in the Siddha system of medicine for managing common respiratory complaints such as flu and cold. Siddha practitioners also recommended this formulation for severe phlegm, dry cough, and fever. It is made up of more than ten herbal ingredients, and each ingredient has a unique pharmacological activity in respiratory disorders. Hence, the ministry of AYUSH recommends its use for symptomatic management in COVID-

19 (Sampath Kumar et al., 2010; Jin et al., 2011; Vinothapooshan and Sundar, 2011; Chang et al., 2013).

In addition to Ayurvedic, Unani, and Siddha formulations recommended by AYUSH there are some homeopathic formulations such as Arsenium album, Brayonia alba, and Rhus toxicodendrum have been recommended which have not been included due to controversies over the use of homeopathic medicine**.** These formulations are prepared by dilutions in such a way so that no single detectable molecule is present in the final formulation, which results in controversy (Ernst, 2010). The criticism is due to nonevidential rationale to determine the biological effects of solutions containing unmeasurable starting material (Kaur, 2013).

Further, advancements in pathogenesis and understanding of diseases provide a wider platform to report the pharmacological limitations and opportunities of these highly diluted homeopathic medicines. Day by day, it is becoming more challenging for a pharmacologist to validate the therapeutic claims of homeopathic medicines through experiments. Low acceptance of homeopathic formulations is due to the absence of standardized protocols to justify their pharmacological potential. A major concern is to develop evidence-based validated methods and advancements in the homeopathic system to justify its measurable dilutions, which will help in understanding the mechanism of action and acceptability of homeopathic medicine (**Table 4**).

3.4 Routinely Used Common Indian Medicinal Plants for Exploring Against COVID-19

Ashwagandha, giloe, ginger, cinnamon, tulsi, black pepper, black cumin, amla, turmeric, garlic, and flax seeds have been traditionally used as herbal remedies for multiple diseases since ancient times. These herbs have been utilized in food preparations and traditional medicines in several countries. However, in India, their culinary use is very common and they are a part of kitchen in every house. Similarly, there are some traditional Indian formulations such as Chyawanprash, Triphala, and Rooh Afza etc. that are very commonly used in Indian territory as a part of daily used nutritional supplements. These plants and formulations are very common and at least one of them is being used daily by every Indian, irrespective of religion/community/financial status. The above-mentioned herbs and formulations have been proved potent scientifically for their immunomodulatory, antioxidant, and anti-infective properties, which might be one of the reasons behind the lower death rate of Indians per million of population due to COVID-19 even with minimum health infrastructure.

3.4.1 *Allium sativum* L. (Garlic)

Various research has been conducted *in vivo* to highlight the effect of *A. sativum* in immunomodulation using garlic oil extract. The results showed reduction in serum TNF-α, ICAM-1 and immunoglobulin (G and M) levels confirming the enhancement in immune system activity (Kamel and El-Shinnawy, 2015). Pre-treatment with aqueous garlic extract showed notable antiviral effects mainly by reduction in infectivity and titer of virus against the velogenic strain of Newcastle disease virus in embryonated chicken eggs (Arify et al., 2018). *A. sativum* also showed antiviral effect against avian influenza virus H_9N_2 on Vero cells (Rasool et al., 2017). Its defensive effect on allergen-induced airway inflammation in rodent model showed significant reduction in inflammatory cell count, eosinophil infiltration and serum IgE modulation of Th1, Th2, and Th3 cytokines, upregulation of Th-1, Th-3 and simultaneous down-regulation of Th-2 expression. (Hsieh et al., 2019). Old extract of *A. sativum* showed modulation of airway inflammation established in BALB/c mice by reduction in percentage of eosinophil, lavage and serum IgG1 levels, and perivascular inflammation. The study suggested the attenuation of allergic airway inflammation by aged garlic extract (Zare et al., 2008). It has been found that fresh raw garlic extract showed anti-inflammatory effects by decreasing production of prostaglandin E2 (PGE_2), IL-6, IL-1β, nitric oxide (NO), and leukotrienes (LT D_4 and E_4) in lipopolysaccharide activated RAW264.7 cells (Jeong et al., 2016).

3.4.2 *Cinnamomum verum* J.Presl. (Cinnamon) or *Cinnamomum zeylanicum* Blume

C. verum essential oil and powder exhibited anti-oxidant, immunostimulant, and antiviral activity in Newcastle disease virus in chickens mainly by modulating total protein, globulin, total antioxidant capacity, and lysozyme activity, and significantly increased phagocytic activity (Islam et al., 2017). Another study reported that *C. zeylanicum* essential oil when blended with other essential oils showed effective antiviral potential against H1N1 and HSV1 viruses. Reduction in virus infectivity has been observed with 99% at 60-min contact time and more than 99.99% after 60 min for both H1N1 and HSV1 viruses (Brochot et al., 2017). Its bark extract exhibited immunomodulatory activity and significantly increased serum immunoglobulins, phagocytic index, neutrophil adhesion, and antibody titer (Niphade et al., 2009). Procyanidine polyphenols (Type A) extracted from *C. zeylanicum* bark showed anti-inflammatory potential in edema induced by carrageenan (Vetal et al., 2013). Alcoholic extract of bark suppressed intracellular release of TNF-α (murine neutrophils) and leukocytes (pleural fluid) as well as inhibition of TNF-α gene expression in lipopolysaccharide-stimulated human peripheral blood mononuclear cells (Joshi et al., 2010).

3.4.3 *Curcuma longa* L. (Turmeric)

Aqueous extract of *C. longa* decreased relative spleen weight and modulation in hematological changes indicating the potential of *C. longa* as an immunomodulator in cyclophosphamide-immunosuppressed *in vivo* model. The study observed promising effects of turmeric as an immunomodulator by representing spleen cells in younger mice (Mustafa and Blumenthal, 2017). *C. longa* extract also showed antiviral potential against dengue virus in *in vitro* and *in vivo* studies on Huh7it-1 cells and a remarkable reduction in viral load has been observed by in *in vivo* model (Ichsyani et al., 2017). Water and ethanolic crude extracts have been found to be antiviral in

H5N1 also showed upregulated TNF-α as well as IFN-β mRNA expression, highlighting its promising role in the inhibition of the replication of viruses (Sornpet et al., 2017). Turmeric extract has been found to be anti-allergic in mice immunized with ovalbumin and alum. Attenuation of food allergy by maintaining balance of Th1/Th2 has been reported. Extract has been found to cause reduction in Th2 and increase in Th1 cell-related cytokines. Further, increased levels of IgE, IgG1 and mMCP-1 levels were also decreased proving effects of turmeric in allergic disorders mainly, asthma and food allergies (Shin et al., 2015). Various other studies also reported anti-inflammatory effects of *C. longa* either alone or in combination (Lee et al., 2020).

3.4.4 *Linum usitatissimum* L. (Flax Seed)

Heteropolysaccharide, extracted from flax seed hull possessed immunomodulatory activity and anti-hepatitis B virus potential. It significantly stimulated mRNA expression of TNF-α, NO and IL exhibiting immune responses in murine macrophages. Antiviral activity has been reported through inhibition of expression of surface antigen as well as envelop antigen and also interfered with DNA replication. The study suggested its promising potential as an immunostimulant and vaccine adjuvant (Liang et al., 2019). It showed anti-inflammatory and immunomodulatory potential in obesity-associated insulin resistance. Its oil in co-culture with 3T3-L1 adipocytes-RAW 264.7 macrophages of C57BL/6 mice reported shifting the cytokines toward anti-inflammatory with a decrement in TNF-α. Immunomodulation has been observed through an increase in levels of Th2-related cytokine (IL-4), serum anti-ova IgG1, and IgE, and a decrease in Th-1 related cytokines (TNF-α and IFN-γ) and anti-ova IgG levels (Palla et al., 2015). Another study reported the immunomodulatory activity of phenolic components of flax seed mainly through reduction in cell-mediated immune responses (Kasote et al., 2012).

3.4.5 *Nigella sativa* L. (Black Cumin)

Nigella sativa L.'s bioactive compounds have been observed as potential inhibitors of COVID-19 in molecular docking studies. Nigellidine gave energy complex at active site (6LU7) with energy scores closest to chloroquine and better than hydroxychloroquine and favipiravir whereas α-hederin gave energy complex at the active site (2GTB) with energy scores better than chloroquine, hydroxychloroquine, and favipiravir (Salim and Noureddine, 2020). The alcoholic seed extract has shown immunosuppressive activity on a phytohemagglutinin and immunostimulating effect on non-phytohemagglutinin (PHA) stimulated proliferation (Alshatwi, 2014). The thymoquinone-rich oil showed suppression of cytokine signaling molecules, and PGE_2 in T-lymphocytes as well as enhanced PGE2 release in adrenocarcinomic human alveolar basal epithelial A549 cells (Koshak et al., 2018).

3.4.6 *Ocimum sanctum* L. (Tulsi)

Hydro-alcoholic extract of *Ocimum sanctum* inhibited intracellular multiplication of virus. It also inhibits non-specific interference with virus-cell interactions in H9N2 viruses. (Ghoke et al., 2018). The immunomodulatory potential of alcoholic leaves extracts at IC_{50} value of 73.3 μg/ml showed reduction in hepatic parasite and, skewing of the humoral response toward Th1 type (Bhalla et al., 2017). *O. sanctum* inhibits leukotriene-C4-synthase, leukotriene-A4-hydrolase and cyclooxygenase-2 activities in cultured HL-60 cells and causes a significant reduction in OVA-induced lung inflammation (Soni et al., 2015).

3.4.7 *Phyllanthus emblica* L. (Amla)

Amla has been reported to significantly relieve chromium-induced immunosuppressive effect on lymphocyte proliferation and led to restoration in production of IL-2 and INFγ (Sai Ram et al., 2002). Phenolics from emblica has been found to increase splenocytes proliferation. Geraniin and isocorilagin showed significant immunostimulatory effects (Liu et al., 2012). Ethanolic extract of amla strongly reduced levels of pro-inflammatory cytokines and increased levels of anti-inflammatory cytokine (Bandyopadhyay et al., 2011). An isolated compound (1, 2, 4, 6-tetra-*O*-galloyl-β-d-glucose) of *P. emblica* showed antiviral potential against HSV by HSV-1 inactivation, which leads to inhibition of early infection indulging attachment and penetration of virus, suppression of intracellular growth and inhibited gene expression of HSV-1 E and L along with DNA replication (Xiang et al., 2011).

3.4.8 *Piper nigrum* L. (Black Pepper)

Piperamides isolated from *P. nigrum* fruits showed significant inhibition of coxsackie virus type B3 in a cytopathic effect inhibition assay (Mair et al., 2016). Aqueous extract of *P. nigrum* acted as a potent modulator of the macrophages and significantly enhanced splenocyte proliferation in a dose-dependent manner (Majdalawieh and Carr, 2010). The isolated alkaloid from *P. nigrum* exhibited anti-inflammatory effect in RAW 264.7 cells stimulated by LPS and significant inhibition in iNOS-mediated NO and IL-1β, IL-6, and TNF-α. It also demonstrated anti-inflammatory activity in edema induced by carrageenan (Pei et al., 2020). Reports have confirmed the improvement of ovalbumin-induced nasal epithelial barrier dysfunction in allergic rhinitis mouse model. Further, protection of epithelium integrity, enhancement in E-cadherin tight junction protein as well as inhibition of the degraded levels of zonula occludens-1 and occluding in the nasal passage have been reported. Additionally, enhancing the activation of Nrf2/HO-1 signaling showed anti-allergic and anti-asthma activities (Bui et al., 2020).

3.4.9 *Tinospora cordifolia* (Willd.) Miers (Giloe)

In vitro screening of *T. cordifolia* silver nanoparticles against chikungunya virus cell showed significant antiviral potential (Sharma V. et al., 2019). Alcoholic leaves extract of *T. cordifolia* significantly decreases intracellular reactive oxygen species (ROS) in chikungunya patients with high levels of intracellular ROS in persisting polyarthralgia by *ex vivo* treatment (Banerjee et al., 2018). An *in vitro* study revealed the antiviral potential of crude stem extract of *T. cordifolia* against HSV in Vero cell lines by inhibiting the growth of

HSV (Pruthvish and Gopinatha, 2018). Aqueous extract of *T. cordifolia* stem significantly increase INFγ and IL levels (IL-1, IL-2, IL-4) in isolated chicken peripheral blood mononuclear cells (PBMCs) against infectious bursal disease virus. Further, immunomodulatory potential via the toll like receptor (TLR)-mediated pathway was also concluded (Sachan et al., 2019). The hydro-alcoholic extract of *T. cordifolia* stem in drinking water caused enhancement of cellular immunity as well as humoral immunity in broiler chicks (Nety et al., 2017). Chloroform extract significantly prevented pro-inflammatory biomarkers (IL-6, IL-1β and PGE2) and decreased paw oedema ($p \leq 0.05$) with no toxicity reported when conducted in RAW264.7 macrophages (Philip et al., 2018).

3.4.10 *Withania somnifera* (L.) Dunal (Ashwagandha)

Multiple studies have proved that Ashwagandha has antiviral and immunomodulatory potential. Very recently, an *in silico* study concluded that Withaferin-A exhibits antiviral potential against SARS-CoV-2 through inhibiting RNA polymerase with higher binding energy than hydroxychloroquine and other drugs used against SARS-CoV-2. Another study on withanone showed blockage of SARS-CoV–2 entry and also its subsequent infection by interrupting electrostatic interactions between the RBD and ACE2 (Balkrishna et al., 2020). Grover and colleagues through molecular docking reported the potential of withaferin A against HSV through inhibition of DNA polymerase enzyme (Grover et al., 2011). *W. somnifera* molecular mechanism has been elucidated by using network ethnopharmacological technique and reported that withanolide-phytosterol combination is a good immunomodulator (Chandran and Patwardhan, 2017). *W. somnifera* formulation (supplemented with minerals) has been reported to improve both cellular and humoral immunity as well as hematological profile in addition to the significant inhibition in mouse splenocytes (Trivedi et al., 2017). Aqueous root extract of *W. somnifera* attenuates production of pro-inflammatory cytokines and transcription factor in collagen-induced arthritis (Khan et al., 2018). A study in 2018 showed that *W. somnifera* significantly inhibited mRNA expression of inflammatory cytokines and promotes the mRNA expression of the anti-inflammatory cytokine in HaCaT cells (Sikandan et al., 2018).

3.4.11 *Zingiber officinale* Roscoe (Ginger)

Fresh ginger aqueous extract showed antiviral activity against human respiratory syncytial virus in human respiratory tract cell lines (HEp-2 and A549) and decreased the plaque counts in a dose-dependent manner. It also stimulated the secretion of IFN-β that contributes to counteracting against viral infection (Chang et al., 2013). It also showed antiviral potential against avian influenza virus H9N2 on Vero cells in a dose-dependent manner (Rasool et al., 2017). Oral administration of Soft gel capsules containing a *Z. officinale* in combination showed immunomodulatory and anti-inflammatory properties parallel to those exerted by positive control, and gene expression data highlighted overall same transcriptional remodeling (Dall'Acqua et al., 2019). A study on essential oil of ginger reported immunomodulatory effects by improving the humoral immunity in cyclophosphamide-immunosuppressed mice in a dose-dependent manner (Carrasco et al., 2009). Oral administration of alcoholic ginger extract to allergic rhinitis patients showed significant reduction in total nasal symptom scores (TNSS), with overall improvement in rhino conjunctivitis quality of life questionnaire (Yamprasert et al., 2020). The aqueous and alcoholic extracts of rhizome decreased goblet cell hyperplasia, infiltration of inflammatory cells in airways with reduced total and differential counts of eosinophils and neutrophils in mouse model (Khan et al., 2015) (**Table 5**).

3.5 Routinely Used Indian Natural Health Supplements to Explore for Use Against COVID 19

3.5.1 Chyawanprash

Chyawanprash is an Ayurvedic polyherbal health supplement, which is made up of concentrated extracts of nutrient-rich herbs and minerals. Chyawanprash comes under Awaleha (electuaries/herbal jams) due to its consistency, and composed of Amla fruit as a base, which is considered as the most active Rasayana to improve strength, stamina, and vitality.

Although several types of research have been published on Chyawanprash to report its health benefits against various ailments, the study reports antioxidant (Anil and Suresh, 2011) free radical scavenging (Bhattacharya et al., 2002) antibacterial, antiviral, anti-inflammatory, antiallergic, and antithrombotic effects (Gupta et al., 2017). In a randomized controlled trial, it was found effective for pulmonary tuberculosis as an adjunct to antitubercular drugs. (Debnath et al., 2012; Sharma R. et al., 2019). An experimental study showed that Chyawanprash pre-treatment reduced plasma histamine levels and IgE release when rats and mice were challenged with allergen- and ovalbumin-induced allergy, suggesting its anti-allergic potential. NK cell activity was significantly increased by Chyawanprash treatment. On treating dendritic cells with Chyawanprash, there was a significant increase in immunity marker levels as well as phagocytic activity that proves its immunomodulatory activity (Sastry et al., 2011).

3.5.2 Triphala

Triphala is a well-known polyherbal Ayurvedic medicine consisting of equal proportions of fruits of *Phyllanthus emblica* L., *Terminalia bellerica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. in the form of powder for digestive and refreshing action. Triphala is associated with many of the therapeutic potentials such as antioxidants, antiinflammatory, antineoplastic, antimicrobial, antidiabetic, etc. (Peterson et al., 2017). Alcoholic extract of Triphala showed specific antimicrobial activity (Tambekar and Dahikar, 2011), broad-spectrum antimicrobial activity against antibiotic-resistant bacteria isolated from humans (Peterson et al., 2017).

Triphala extract was found more active than the NSAID drug, indomethacin, in improving arthritic and inflammatory effects and reduced expression of inflammatory mediators

through inhibition of NF-κB activation (Kalaiselvan and Rasool, 2015). In LPS-stimulated macrophages, Triphala inhibited the production of inflammatory mediators, intracellular free radicals, and inflammatory enzymes (Reddy et al., 2009; Kalaiselvan and Rasool, 2016). It has been shown to reduce multiple cell signaling pathways of inflammation and oxidative stress and prevented the noise-stress induced changes in rats thereby strengthening the cell-mediated immune response (Prasad and Srivastava, 2020). A clinical study of Triphala showed immunostimulatory properties on T cells and NK cells, however did not change the cytokine levels in healthy volunteers (Phetkate et al., 2012). The individual constituents of Triphala have also showed immunomodulatory activity (Aher and Wahi, 2011). The stated data on Triphala reveals that it is a powerful polyherbal formulation with countless therapeutic uses for maintaining homeostasis as well as the cure and management of various disease.

3.5.3 Sharbat Rooh Afza

Rooh Afza is a well-known refreshing formulation with global acceptance. It is a concentrated squash prepared as sugar syrup with distillates of numerous medicinal plants including seeds of khurfa (*Portulaca oleracea* L.), kasni (*Cichorium intybus* L.), angoor (*Vitis vinifera* L.), nilofar (*Nymphaea alba* L.), Neel Kamal (*Nymphaea nouchali* Burm. f.), kamal (*Nelumbo nucifera* Gaertn.), gaozaban (*Borago officinalis* L.), badiyan (*Coriandrum sativum* L.), fruits/juices of santara (*Citrus* × *sinensis* (L.) Osbeck), ananas (*Ananas comosus* (L.) Merr.), seb (*Malus domestica* (Suckow) Borkh.), berries (*Rubus fruticosus* L.), vegetables like palak (*Spinacia oleracea* L.), gazar (*Daucus carota* L.), and pudina (*Mentha arvensis* L.). Rooh Afza boosts the energy system of the body by naturally refreshing. Although there is no evidence on Rooh Afza revealing its therapeutic value, its constituents have been reported as potently antiviral, immunomodulatory, and antiallergic against respiratory disorders.

The flower extract of *P. oleracea* possessed significant antioxidant and protective effects against DNA damage induced by necrotic effects (Dogan and Anuk, 2019). *V. vinifera* fruits exhibit anti-asthmatic activity by inhibiting cellular response and subsequent production of inflammatory cytokines (Arora et al., 2016). A study on *N. alba* flower has been reported against inflammatory activity in Swiss Albino mice using acute inflammatory models in a dose-dependent manners (RS et al., 2013). The immunoregulatory and anti-HIV-1 enzyme activities of *N. nucifera* suggest that it could be potentially important against virus development (Jiang et al., 2011).

Thus, it can be perceived that Rooh Afza not only provides natural refreshness to the body but also has antioxidant, immunomodulatory, and anti-inflammatory/antiviral activities. However, to validate the scientific data on the therapeutic value of Rooh Afza, experimental research should be undertaken to prove its role in health benefits therapeutically.

The above studies encourage further investigations of traditional medicinal plants for their preventive use against coronavirus infection. The herbs could be taken individually or synergistically at appropriate concentrations as candidates for developing potential therapeutic tools against COVID-19.

3.6 Potential Indian Medicinal Plants for Exploring Against COVID-19

There are many other Indian medicinal plants, which are either part of AYUSH recommendations as such or as ingredients of formulations or are known for improving immunity with antiviral and anti-allergic/anti-inflammatory potential and can offer potential leads against COVID-19. **Table 5** provides a list of 83 medicinal plants categorized on a priority basis as per their reported properties. Category 1 (C1) includes 21 "*Most promising drugs*" which have already shown activity against Coronaviruses/ HIV/Dengue viruses with their immunomodulatory and anti-allergic/anti-inflammatory properties. Category 2 (C2) is composed of 44 *"Equally promising drugs"* which reportedly have shown anti-viral, immunomodulatory, and anti-allergic/ anti-inflammatory activities. Category 3 (C3) represents 18 *"Possibly promising drugs"* which have been reported to show anti-viral/immunomodulatory and/or anti-allergic/anti-inflammatory activities.

Listed medicinal plants and AYUSH recommended formulations could help as the potential alternate therapeutics for management and cure of COVID-19. However, this needs scientific explorations and validation of their preclinical and clinical studies. Since there is such a rich diversity, many other medicinal plants and their bioactive fractions need the attention of the scientific community to be explored against COVID-19.

4 CONCLUSION

The SARS-CoV-2 has become a threat to human population due to non-availability of approved vaccines or drugs for its treatment. Many herbs that have been reported to work as an immunity booster against other viral infections, and to possess anti-allergic/anti-inflammatory activities, need to be tested against COVID-19. Indian Traditional Medicines have a wide potential for being used in these tough times either for prophylaxis or as adjuvant, owing to their longstanding use in community, ancient references and scientific evidence about their safety and clinical efficacy. The AYUSH ministry, Govt of India has issued several advisories from time to time, considering the strength and evidence of these systems of medicines and making considerable efforts to encourage researchers to explore herbal products for COVID-19. Interventions and herbal formulations from different AYUSH systems have the support of evidence for their immunity-enhancing, anti-inflammatory and antiviral effects. These herbal remedies may, therefore, provide some respite until the availability of trial-tested drug or vaccine to combat the COVID-19 menace. Further, it was noted that a major portion of public and private funding were dedicated to AYUSH trials. More than 50% of these trials were sponsored by the government and various stakeholders associated with the Ministry of AYUSH. It is expected that the results of these clinical studies will be disseminated soon at the public platform so that the policymakers from the AYUSH systems

of medicines may reframe their policies for public health and provide information to the global scientific community, which could form a platform for collaborative studies at the national and global levels. The medicinal plant species discussed in this review and categorized for their preclinical and clinical investigation may be taken up by research organizations on priority basis, as this may result in the development of lead molecule against SARS-CoV-2 and COVID-19. Keeping in view the potential of AYUSH medicines and medicinal plants of India, the herbal drug, manufacturers, and the national and global research organizations should develop necessary strategies for furtherance of preclinical and clinical research on these promising therapeutic leads.

AUTHOR CONTRIBUTIONS

SA: conceptualization, methodology, writing - reviewing and editing; SZ and BP: data curation, writing - original draft preparation; PB, GG, and AP: visualization, investigation; RP and MA: software, validation.

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1,2,3,4,6-Pentagalloyl Glucose, a RBD-ACE2 Binding Inhibitor to Prevent SARS-CoV-2 Infection

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The outbreak of SARS-CoV-2 virus caused more than 80,155,187 confirmed COVID-19 cases worldwide, which has posed a serious threat to global public health and the economy. The development of vaccines and discovery of novel drugs for COVID-19 are urgently needed. Although the FDA-approved SARS-CoV-2 vaccines has been launched in many countries recently, the strength of safety, stringent storage condition and the possibly short-term immunized efficacy remain as the major challenges in the popularity and recognition of using vaccines against SARS-CoV-2. With the spike-receptor binding domain (RBD) of SARS-CoV-2 being responsible for binding to human angiotensin-converting enzyme 2 receptor (hACE2), ACE2 is identified as the receptor for the entry and viral infection of SARS-CoV-2. In this study, molecular docking and biolayer interferometry (BLI) binding assay were adopted to determine the direct molecular interactions between natural small-molecule, 1,2,3,4,6-Pentagalloyl glucose (PGG) and the spike-RBD of the SARS-CoV-2. Our results showed that PGG preferentially binds to a pocket that contains residues Glu 340 to Lys 356 of spike-RBD with a relatively low binding energy of -8 kcal/mol. BLI assay further confirmed that PGG exhibits a relatively strong binding affinity to SARS-CoV-2-RBD protein in comparison to hACE2. In addition, both ELISA and immunocytochemistry assay proved that PGG blocks SARS-CoV-2-RBD binding to hACE2 dose dependently in cellular level. Notably, PGG was confirmed to abolish the infectious property of RBD-pseudotyped lentivirus in hACE2 overexpressing HEK293 cells, which mimicked the entry of wild type SARS-CoV-2 virus in human host cells. Finally, maximal tolerated dose (MTD) studies revealed that up to 200 mg/kg/day of PGG was confirmed orally safe in mice. Our findings suggest that PGG may be a safe and potential antiviral agent against the COVID-19 by blockade the fusion of SARS-CoV-2 spike-RBD to hACE2 receptors. Therefore, PGG may be considered as a safe and natural antiviral agent for its possible preventive application in daily anti-virus hygienic products such as a disinfectant spray or face mask.

Keywords: 1, 2, 3, 4, 6-pentagalloyl glucose, RBD-ACE2 inhibitor, SARS-CoV-2, COVID-19, viral infection

INTRODUCTION

The worldwide pandemic Coronavirus disease 2019 (abbreviated as COVID-19) is a viral sickness caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has posed a serious threat to global public health and the economy (Paules et al., 2020; Zhao et al., 2020). Over the past 20 years, two highly pathogenic human coronavirus (HCoVs) including severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) emerging from animal reservoirs, have led to global epidemics with high morbidity and mortality (Paules et al., 2020). While MERS-CoV does not transmit easily between humans unless they are closely contacted with each other (Zaki et al., 2012), SARS-CoV-2 is more infectious than the above two highly pathogenic coronaviruses and can be transmitted *via* either asymptomatic or presymptomatic infection. As of December 30, 2020, the coronavirus has rapidly spread to 222 countries with 80,155,187 confirmed cases and 1,771,128 deaths all over the world according to the report of World Health Organization (WHO). Typical symptoms of all these infections include fever, dyspnea, muscle ache, dry cough, sore throat and diarrhea. As the disease progressed, bilateral pneumonia, multiplemottling and ground-glass opacity in transverse chest x-ray and CT image are observed (Cui et al., 2020). Although veklury (remdesivir) is the first approved drug for COVID-19 treatment in United States (Lamb, 2020), patients with COVID-19 treated with veklury experienced severe side effects including multiple organ dysfunction syndrome, septic shock, acute kidney injury and high blood pressure. On the other hand, since the approval and the start of the vaccination program in different countries, allergic reactions and side effects including cough, loss of appetite, vomiting and diarrhea were reported with suspected correlation with the vaccine. Up to now, effective clinical treatments or prevention strategies for the highly pathogenic SARS-CoV-2 still cannot meet the demand of the huge increasing number of infected patients. Furthermore, many countries including United Kingdom, Australia and Japan have notified the mutations of coronavirus with higher contagious and pathogenic properties (Li et al., 2020), suggesting that the developed vaccine may be not effective in preventing the pandemic of COVID-19.

SARS-CoV-2 is a positive chain enveloped β-coronavirus. Similar to SARS and MERS viruses, its genome can encode non-structural proteins, structural proteins and helper proteins (Naqvi et al., 2020). Non-structural proteins include 3-chymotrypsin like protease (3CLpro), papain like protease (PLpro), helicase and RNA-dependent RNA polymerase (RdRp), whereas viral spike (S) protein is the structural protein. Among them, 4 non-structural proteins are the key enzymes for virus proliferation and replication, and S proteins are essential for virus entry through membrane receptor interaction with host cells (Shang et al., 2020). During infection, the S protein is cleaved into the N-terminal S1 subunit and C-terminal S2 subunit by host proteases such as TMPRSS2 (Simmons et al., 2013). S1 and S2 comprise the extracellular domain and a single transmembrane helix and mediate receptor binding and membrane fusion, respectively (Hoffmann et al., 2020). S1, which consists of the N-terminal domain (NTD) and the receptor binding domain (RBD), is critical in determining tissue tropism and host ranges (Wrapp et al., 2020). The RBD is responsible for binding to angiotensin-converting enzyme 2 (ACE2), previously identified as the cellular receptor for SARS-CoV (Chi et al., 2020a), while the function of NTD is not well understood. While these 5 proteins as mentioned above are considered to be important targets for discovery of anti-viral drugs, the SARS-CoV-2 Spike protein–targeting small molecules with potent neutralizing activity are a focus in the development of therapeutic interventions for COVID-19. Many studies reported the functions and structures of SARS-CoV-2–neutralizing antibodies that target the RBD and inhibit the association between the spike protein and ACE2 (Chi et al., 2020a; b;Wang et al., 2020).

1,2,3,4,6-O-Pentagalloylglucose (PGG) is a natural polyphenolic compound isolated from many traditional medicinal herbs, such as *Paeonia lactiflora* pall, *Sanguisorba officinalis* L and *Mangifera indica* (Lee et al., 2006; Liu et al., 2011; Kim et al., 2020). It has been reported to inhibit a variety of viruses although whether it can inhibit coronaviruses are not known. PGG plays a major role in inhibition the 3′-processing of HIV-1 integrase in HIV disease (Ahn et al., 2002). It can also reduce the viral HBsAg expression, a key protein in HBV DNA released for Hepatitis B virus (HBV) (Lee et al., 2006). For hepatitis C virus (HCV), PGG efficiently blocks the entry of HCV to host cells during the viral attachment (Behrendt et al., 2017). In addition, PGG shows anti-influenza-virus activity, through reducing plasma membrane accumulation of nucleoprotein at the late stage of the replication cycle and inhibit progeny virus release from the infected cells (Liu et al., 2011). Although PGG did not disrupt the integrity of the virus directly, it participates in blocking viral entry, replication and offspring release. Natural small-molecules have been playing a significant role in the prevention and treatment of emerging respiratory infectious diseases such as H1N1 influenza (Notka et al., 2004). Silico screening has identified the potential active components from Chinese herbal medicines which may inhibit 2019 novel coronavirus (Zhang et al., 2020). In the present study, molecular docking was adopted to simulate the interaction between the three-dimensional structure and interaction of the Spike-RBD and PGG. Besides, the binding of PGG to RBD using Bio-layer interferometry (BLI) and Enzyme linked Immunosorbent Assay (ELISA) were validated. Finally, by using the RBD-pseudotyped lentivirus, our results have confirmed that PGG effectively inhibited the binding and infection of virus in ACE2 overexpressing human host cells. Therefore, with the continuous evolutionary change of the virus, while the development of novel antiviral drugs and specific vaccines are still remaining the major research focus of the world, specialized personal hygiene and protective products should also be developed or modified not only for the better prevention of the disease, but more convenient and safety daily usage. For example, although most of the alcohol-based sanitizers or disinfectants available in markets can kill virus effectively, the safety risks should also be considered with its flammable nature

during its usage or storage especially in the public transportations. In addition, excessive usage of bleach-based disinfectants also possess hazardous side effect to the human and the environment. Therefore, our results have discovered a non-alcoholic, safe and natural antiviral natural agent for its potential use as the active ingredient in the disinfectant spray or facial mask for neutralizing the viral spike-RBD to prevent SARS-CoV-2 infection.

MATERIALS AND METHODS

Target and Ligand Preparation

The RBD/ACE2-B0AT1 complex of SARS-CoV-2 was downloaded from the protein data bank (ID 6M17). The target was prepared using UCSF Chimera. To isolate the receptor binding domain (RBD) amino acid chains representing the sodium-dependent neutral amino acid transporter B (0)AT1, angiotensin-converting enzyme 2 (ACE2) and the second receptor binding domain were removed. Additionally, all non-standard residues (water, N-acetyl glucosamine and zinc) were also deleted. The PDB file was then loaded and processed using Flare (Cresset, version 3.0) software. Hydrogens were added and optimal ionization states were assigned for each residue. To maximize hydrogen bond interactions and minimize steric strain, the spatial positions of polar hydrogens were optimized. The orientation of His, Asn and Gln residue side chains were optimized. Unresolved side chains were detected and reconstructed. Finally, the processed target was saved in PDB format. PGG was downloaded from Pubchem (CID 65238) in SDF format. Energy minimization and conversion to mol2 file format was performed using OpenBabel software.

Molecular Docking

In order to predict the preferred binding pocket of corilagin on the RBD, blind docking was performed using SwissDock, a free web-based docking program. SwissDock generates all possible binding modes for the ligand under study (Grosdidier et al., 2011). The most favourable binding modes are generated and clustered at a specified pocket. All clusters were visualized with UCSF Chimera. A cluster is a predicted binding pocket on the target protein and each cluster was inspected for interacting amino acids. To determine the best pose at the predicted binding pocket, site specific docking was performed using Flare (Cresset, version 3.0) software. PGG in SDF file format was loaded and processed using default settings. The grid was selected to include the predicted binding pocket identified from the SwissDock results. The best binding pose of compound was selected for further analysis.

Molecular Dynamics Simulation

It is essential to understand the stability of the interaction of PGG with the SARS-CoV2 RBD to further evaluate the binding affinity. The top binding pose of PGG with SARS-CoV2 RBD from the molecular docking results was subjected to MD simulation using GROMACS package version 2020.3. The topology of SARS-CoV2 RBD protein was generated using the CAHRMM36 force field. The selected binding pose of PGG was converted to Mol2 file format using Avogadro software. The topology of PGG was generated using the CGenFF server. The protein-ligand complex was generated and then solvated in a dodcahedral water box using an explicit SPC water model. The system was neutralized by adding appropriated counter ions. To minimize the energy, the system was allowed to converge at the tolerance of 1,000 $kJ{\cdot}mol^{-1}{\cdot}\,nm^{-1}$ with 500 steps of steepest descent. The system was then equilibrated in two phases. The first phase was implemented for NVT equilibration at 300 K. The second phase was performed for NPT equilibration at 1 bar pressure. The MD simulation was executed to 10 ns time. Root mean-squared deviation (RMSD) and interaction energy were stored in the trajectory for every 1 ps and were analyzed using Grace Software.

Biolayer Interferometry

ACE2-His tag protein (Sino Biological, China) was immobilized onto the Ni-NTA probes (Fortebio, United States). SARS-CoV-2 RBD peptide (Sino Biological, China) were diluted to the different concentrations from 0.625 to 10 μg/ml. After a 60-s washing and baseline step with PBS containing 2% DMSO (Sigma, United States), respectively, biosensor tips were immersed into the wells containing SARS-CoV-2 RBD peptide with serial dilutions and allowed to associate for 300 s, followed by a dissociation step of 300 seconds. The KD value was calculated using a 1:1 binding model in Data Analysis Software 9.0 (Fortebio, United States). PGG were diluted to the different concentrations from 100 to 3.13 μM with PBS and purified SARS-CoV-2 RBD peptide (Sino Biological, China) were conjugated with biotin using EZ-Link™ Sulfo-NHS-Biotin (Genemore, China) following the manufacturer's protocol. Then, the biotinylated SARS-CoV-2 RBD peptide were immobilized onto Super Streptavidin (SSA) biosensors (Fortebio, United States). The following experiments were as described above.

Enzyme-Linked Immunosorbent Assay

Nickel-coated 96 well white microplates (Bioscience, United States) were coated with 1 μg/ml ACE2-His-tag protein (Bioscience, United States) following the manufacturer's protocol. Serial dilutions of PGG were added to wells designated "Test Inhibitor" and 2% DMSO in inhibitor buffer were added to wells as "Blank" and incubated at room temperature for 1 h with slow shaking. 1 ng/uL SARS-CoV-2 RBD protein were added to wells labeled "Positive Control" and incubated at room temperature for 1 h with slow shaking. After washing with 1× Immuno Buffer 1, the plates were incubated using Blocking Buffer 2 at room temperature for 10 min. After washing with 1× Immuno Buffer 1, secondary horseradish peroxidase (HRP)-labeled antibody 1 was added at the dilution of 1:1,000 with blocking buffer 2 and incubated at room temperature for 1 h with gentle shaking. After washing, ELISA ECL substrate solution was added to the microplate. The microplate was immediately read by a luminometer (Tecan, Männedorf, Switzerland) capable of reading chemiluminescence. The data was analyzed using

GraphPad Prism 7.0. Quantification bar chart represents the ELISA results from 5 independent experiments.

Cytotoxicity Assay

Cell viability and the half maximal inhibitory concentration (IC_{50}) were examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. PGG was dissolved in DMSO at a final concentration of 100 mmol/L and stored at −20°C before use. Briefly, Beas-2B, LO2 and HEK 293 cells were seeded in 96-well plates and then exposed to the tested compound at different concentrations or DMSO as a control for 72 h, respectively. Subsequently, 10-μL MTT was added to each well for 4 h followed by the addition of 100-μL solubilization buffer (10% SDS in 0.01 mol/L HCl) and overnight incubation. Absorbance at A570 nm was measured to cells viability on a plate reader (Tecan, Männedorf, Switzerland). The percentage of cell viability was calculated by the formula: Cell viability (%) = $(A_{treated} - A_{background})/(A_{control} - A_{background})$.

Immunocytochemistry Assay

For immunofluorescence analysis, HEK293 cells. cells were cultured in 10 cm culture dish and transfected with ACE2/EGFP construct (Vectorbuilder Inc.) for 24 h, 1×10^5 transfected cells were seeded on the cover glasses in the 24-wells plate. The next day, PGG and SARS-CoV-2-RBD-mFc protein (Sino Biological, China) were pre-incubated for 30 min, then added into the cells and incubated for another 40 min. Cells were washed 3 times with PBST and fixed with 4% PFA for 10 min. Subsequently, cells were washed for 3 times, and then blocked in PBS containing 3% BSA for 30 min. After that, the fixed cells were incubated with goat anti-mouse IgG Fc TRITC antibody (Invitrogen Inc.) for 2 h. Cells were washed for 5 min with PBST for 3 times. The cover glasses were mounted with FluorSave reagent (Calbiochem, United States). The cells were imaged by Leica SP8 confocal microscope. For semi-quantitative determination, fluorescence images were analyzed by the Image J, and the numbers of TRIC-positive cells for each well were counted to represent infection performance. The reduction (%) in the number of TRIC-positive cells in RBD-treated wells compared with that in un-treated control wells were calculated to show the neutralizing potency.

Pseudovirus-Based Viral Infection Assay

HEK 293 cells were seeded in 100 mm culture dish and cultured overnight. The cells were transfected with human ACE2/mCherry construct (Vectorbuilder Inc.) for 24 h. After that, 1×10^5 transfected cells were seeded on the cover glasses of 24-well plate. The next day, PGG, RBD-pseudotyped lentivirus (Vectorbuilder Inc.) and polybrene (Vectorbuilder Inc.) were premixed in blank medium, and then added into the cells. After 12h, the medium was replaced with fresh FBS-medium and continually incubated for 48 h. The cells were then washed 3 times with PBST and then fixed with 4% PFA. The cover glasses were mounted with FluorSave reagent (Calbiochem, United States). The cells were imaged by Leica SP8 confocal microscope. For semi-quantitative determination, cells were cultured as described before. Briefly, a standard calibration curve of viral infection using 46.6, 37.3, 23.3, 4.66, 0 $\times10^6$ TU of RBD-pseudotyped lentivirus were established. 3 points of cells fluorescence intensity for every titer of viral infection were calculated by Image J. Calibration curve was established for the evaluation of virus inhibition. Quantification bar chart represents the data from 5 independent experiments.

Viral infection formula: y = 286200x - 98,383, R^2 = 0.9729.

Maximum-Tolerate Dosage Study in C57BL/6 Mice

Male C57BL/6 mice at the age of 6–8 weeks were obtained from SPF (Beijing) Biotechnology Co., Ltd. All experiments were carried out in accordance with the "Institutional Animal Care and User Committee Guidelines" of the Macau University of Science and Technology. To determine the maximum-tolerate dosage of PGG, mice were randomly divided into 3 groups, which are control group (n = 2), low dose and high dose treatment groups (n = 4). PGG was dissolved in sterilized water contained 1% DMSO. Mice from the treated group were orally administered with 100 mg/kg or 200 mg/kg PGG, whereas the control group mice were received same volume of sterilized water contained 1% DMSO for 7 consecutive days. The health condition of mice was monitored based on their body weight and vital organs weight changes. Vital organs including liver, spleen and kidney were collected after scarification.

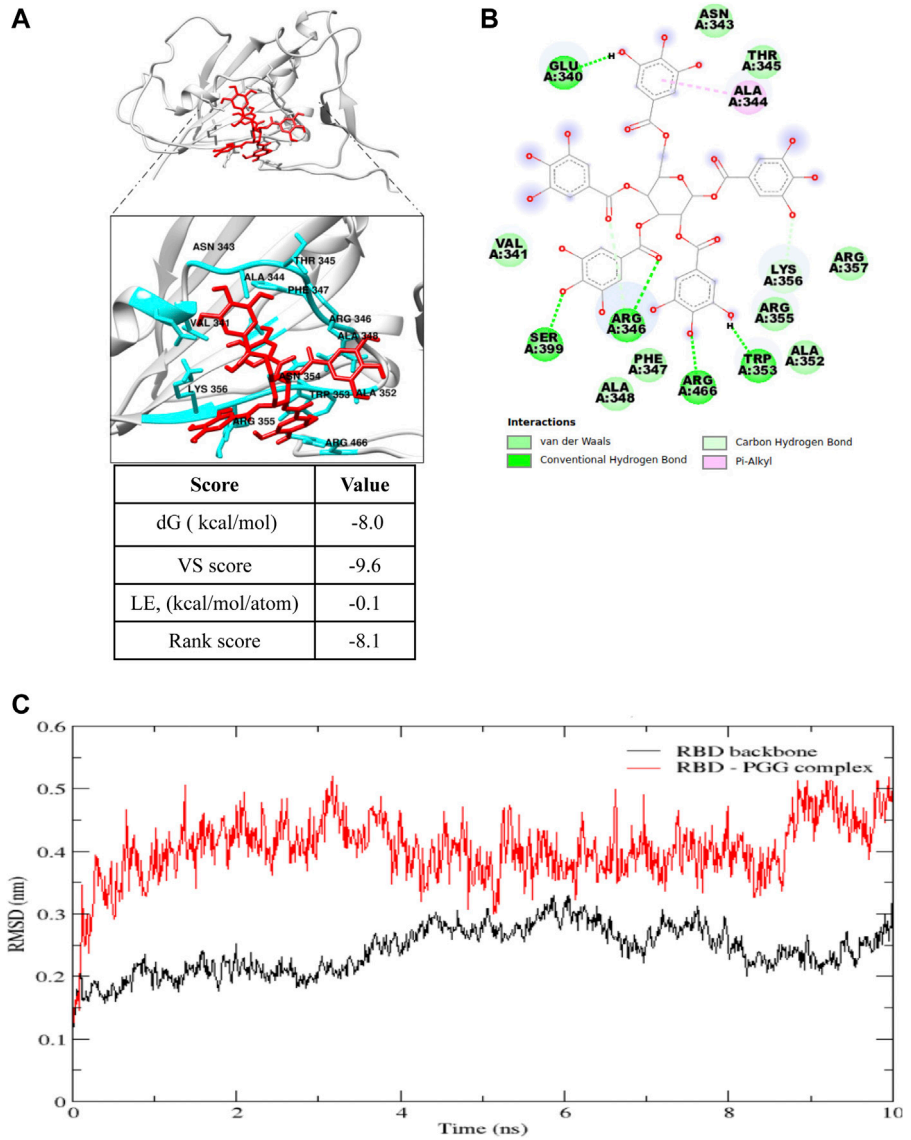
Data Statistical Analysis

All experiments were repeated for at least 3 times. Results are presented as mean ± S.D. All statistical analyses were performed using Prism 6 software (GraphPad Software Inc., San Diego, CA, United States). Statistical significance among multiple group comparisons were determined by one-way analysis of variance (ANOVA). P value < 0.05 was considered statistically significant.

RESULTS

PGG Binds and Interacts With Spike-RBD Domain of SARS-CoV-2 and ACE2 Receptor

PGG has been reported to show an anti-viral effect toward HIV, HBV, HCV and influenza-virus by blocking the viral entry and its replication (Lee et al., 2006), however, its effect toward SARS-CoV-2 remains unidentified. Therefore, the effect of PGG in interacting the viral infection of SARS-CoV-2 and thereby reducing the risk of COVID-19 was studied. To begin, the free web-based docking software SwissDock was used to predict the preferential binding site of PGG. The binding pocket with the highest number of clusters was specified as the preferential binding site (**Figure 1A**). As shown in **Figure 1B**, the preferential binding pocket for PGG involved residues Glu 340 to Lys 356. In order to specify the best binding pose more accurately, site specific docking was performed using Flare (Cresset) on slow but accurate mode. The grid was adjusted to include the predicted binding pocket. The dG, VS and LG scores were recorded for the best binding pose as shown in **Figure 1A**. The best binding pose was considered for analysis by molecular dynamics simulation.



Score	Value
dG (kcal/mol)	-8.0
VS score	-9.6
LE, (kcal/mol/atom)	-0.1
Rank score	-8.1

FIGURE 1 | Computational docking prediction of 1,2,3,4,6-Pentagalloyl glucose (PGG). **(A)** Molecular docking result showing the best binding pose of interaction, and **(B)** the residues involved and types of interaction of PGG with the receptor binding domain of the SARS-CoV-2 spike protein. **(C)** Root mean square deviation (RMSD) plot of the SARS-CoV-2 spike protein receptor binding domain (RBD) backbone alone and in complex with PGG during the 10 ns molecular dynamics simulation.

In molecular dynamics simulations, the system reaches an energy minimum when a plateau is reached in the RMSD of atomic distances as a function of time. The RMSD fluctuation along with the MD simulation time, and the considerable structural change was noticed within 2 ns time as shown in **Figure 1C**. The average interaction energies were calculated throughout the simulation from the elelctrostatic and Lennard-Jones interaction energies. Electrostatic interaction energy was found to be -214.6 ± 19 kJ/mol and the Lennard-Jones interaction energy was found to be -154.1 ± 7.6 kJ/mol. Collectively, computational docking and molecular dynamics simulations suggest that PGG may bind and interact appropriately with Spike-RBD of SARS-CoV-2.

To further validate the binding affinity of PGG on Spike-RBD protein, we tested the association of SARS-CoV-2 RBD and hACE2 accordingly. As shown in **Figure 2A**, SARS-CoV-2 RBD was dose-dependently associated with hACE2. The equilibrium dissociation constants (KD) of the interaction between SARS-CoV-2 RBD and hACE2 was 0.172 μg/ml, R2 = 0.9979 (Steady state analysis, lower panel), which confirmed that SARS-CoV-2 RBD targets and tightly binds to hACE2. After that, the Spike-RBD peptide was immobilized onto the biosensor coated with super streptavidin (SSA) *via* biotinylation and measured the binding affinity of PGG with the labeled probe using bio-layer interferometry (BLI) machine. The BLI analysis relied on the immobilization of the biotinylated Spike-RBD to the

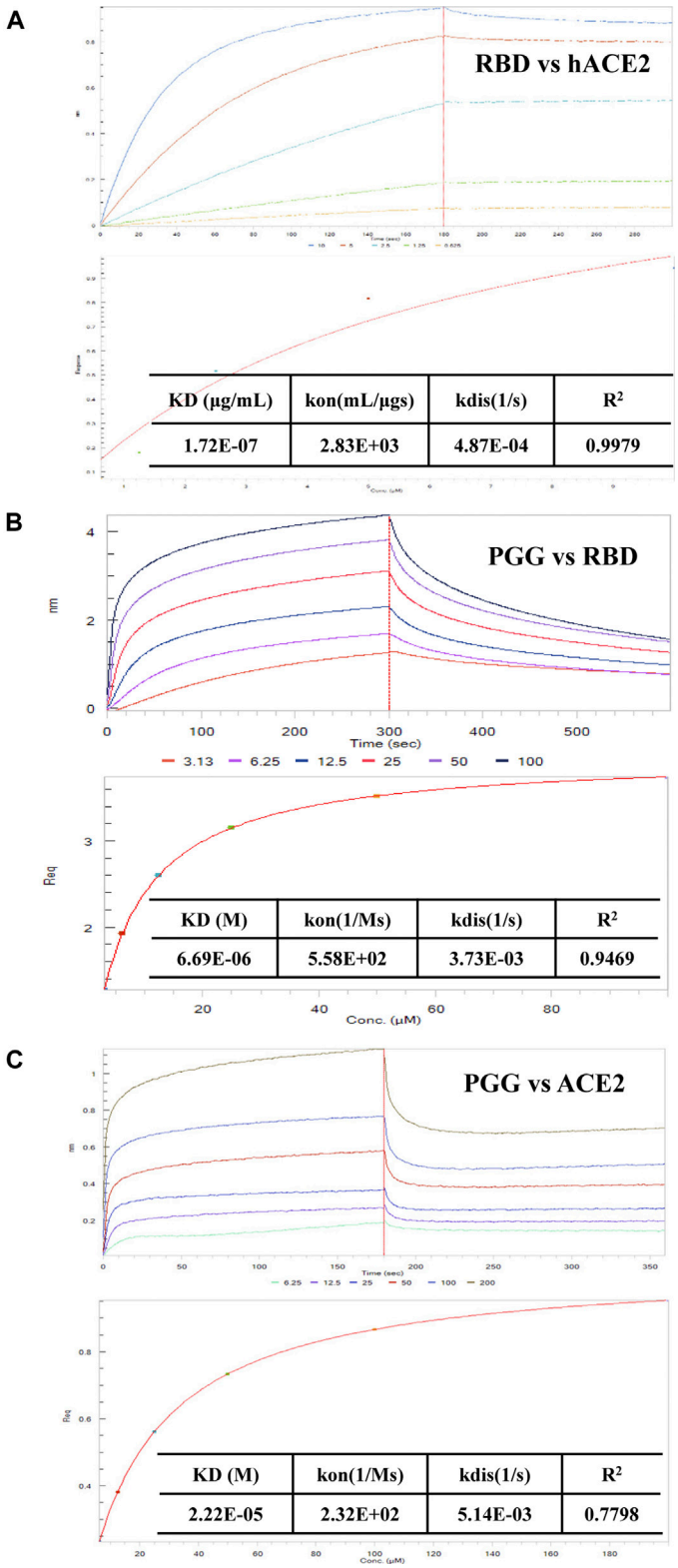


FIGURE 2 | Effect of PGG on the interaction of Spike-RBD peptide and hACE2 receptor. **(A)** BLI was used to monitor the binding association of SARS-CoV-2 RBD and hACE2. **(B)** The binding kinetics and steady-state analysis of the interaction between immobilized RBD and PGG at indicated concentrations. **(C)** The binding kinetics and steady-state analysis of the interaction between immobilized ACE2 and PGG at indicated concentrations. Representative results were shown from 3 independent experiments.

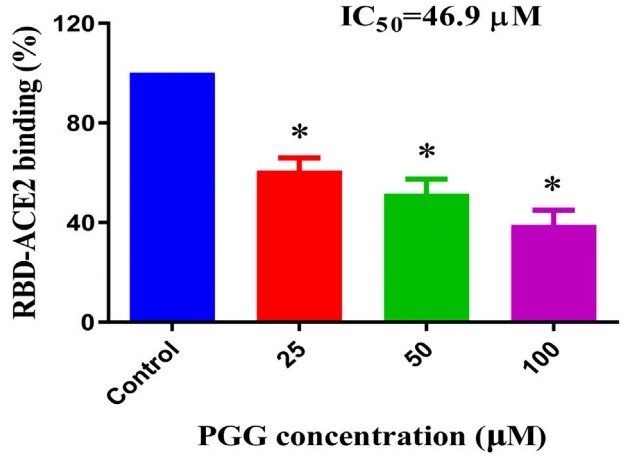


FIGURE 3 | PGG reduces the Spike-RBD and ACE2 interaction. ELISA assay was adopted to determine the binding inhibitory effect of PGG to the interaction of Spike-RBD protein and ACE2 receptor. Data were expressed as mean ± S.D., n = 5; *p < 0.05, one-way ANOVA analysis.

biosensor surface with subsequent exposure to various concentrations of PGG, whereby the association and dissociation phases were real-time recorded accordingly. As shown in **Figure 2B**, PGG was found to dose-dependently bind to Spike-RBD, whereas the binding curves of RBD suggested that a simple 1:1 binding mode occurred with R^2 = 0.9469. Consistent with the computational docking data, the equilibrium dissociation constants (KD) of the interaction between RBD and PGG was 6.69 μM (Steady state analysis, lower panel). On the other hand, ACE2 has been shown to be a functional receptor for SARS-CoV-2 to enter host target cells (Bourgonje et al., 2020). Small-molecules adhesion on ACE2 may also affect the attachment and viral infection of SARS-CoV-2. Therefore, His-tagged ACE2 immobilized onto the biosensor coated with nickel-nitrilotriacetic acid (Ni-NTA) was used to measure the binding affinity with PGG. Concomitantly, PGG was also found to dose-dependently bind to ACE2, whereas the binding curves of ACE2 suggested that a simple 1:1 binding mode occurred with R2 = 0.7798 (**Figure 2C**). The equilibrium dissociation constants (KD) of the interaction between ACE2 and PGG was 22.2 μM (Steady state analysis, lower panel), suggesting that PGG may bind and interact with both viral Spike-RBD and host cells ACE2 receptor for anti-viral infection.

PGG Directly Inhibits the Interaction of Spike-RBD Peptide and ACE2 Receptor

To examine whether the binding of PGG to Spike-RBD and ACE2 can affect the interaction of Spike-RBD-ACE2, the ELISA assay with immobilization of ACE2-His-tag protein on the nickel-coated 96 well white microplates was adopted. The inhibition (%) in the optical density of PGG-treated wells compared with that in un-treated control wells were calculated to obtain the half maximal inhibitory concentration (IC_{50}). As shown in **Figure 3**, PGG dose-dependently blocked the binding of Spike-RBD peptide to ACE2 at an IC_{50} of 46.9 μM, suggesting the possible inhibitory effect of PGG on the fusion between the viral Spike-RBD and host cells ACE2 receptor.

PGG Suppresses the Binding and Infection of Recombinant RBD Pseudovirus in Human ACE2 Overexpressing Cells

To visualize the Spike-RBD binding inhibitory effect and antiviral infectious potency of PGG *in vitro*, immunocytochemistry on RBD binding and RBD-pseudotyped lentivirus infection assay were conducted. As shown in **Figure 4**, mouse Fc (mFc)-fused SARS-CoV-2 Spike-RBD protein and hACE2-EGFP transfected cells were co-localized in untreated control, indicating the perfect binding of Spike-RBD on cell surface ACE2 receptor. Addition of PGG dose-dependently suppressed the binding and co-localization of Spike RBD on hACE2 (**Figure 4A**), indicating that the binding of PGG onto Spike-RBD protein prevented the fusion of Spike-RBD-hACE2 (**Figure 4B**). The inhibitory potency of PGG on viral infection was assessed by using the SARS-CoV-2 S-pseudotyped lentivirus. Consistent with the RBD binding ICC assay, HEK cells with hACE2 (mCherry) overexpression were completely infected by the S-pseudotyped lentivirus as indicated by co-localization of mCherry and EGFP fluorescence signal (**Figure 5A**). Moreover, viral infection assay further revealed that PGG dose-dependently inhibited SARS-CoV-2 RBD S-pseudotyped lentivirus infection (**Figure 5B**) as demonstrated by weak EGFP fluorescence signal. Taken together, PGG may be considered as a relatively safe SARS-CoV-2 entry inhibitor candidate to protect human cells from viral infection.

PGG Shows Relative Low Cytotoxic Effect in Human Normal Cells and Exhibits Non-Observable Toxic Effect in C57BL/6 Mice

To evulate the cytotoxicity of PGG, 3 human normal cell lines including lung epithelial cells (Beas-2B), normal liver hepatocytes (LO2) and human embryonic kidney 293 cells (HEK 293) were treated with PGG from 0 to 100 μM by MTT assay. Of note, PGG showed no significant cytotoxic effect on these human normal cell lines for a concentration of up to 100 μM (**Figure 6A**). To further investigate the *in vivo* toxic effect of PGG, maximum-tolerate dosage (MTD) of PGG was evaluated in C57BL/6 mice. As shown in **Figure 6B**, mice orally administrated with 100 mg/kg/day or 200 mg/kg/day of PGG indicated no toxic or harmful effect to animals as revealed by a survival rate of 100 %, no decline in body weight and organs weight after a 7-day treatment course.

DISCUSSION

Due the outbreak of COVID-19 at December 2019, well accepted preventive vaccine or antiviral therapeutic strategy are urgently needed to combat this deadly virus. With the long

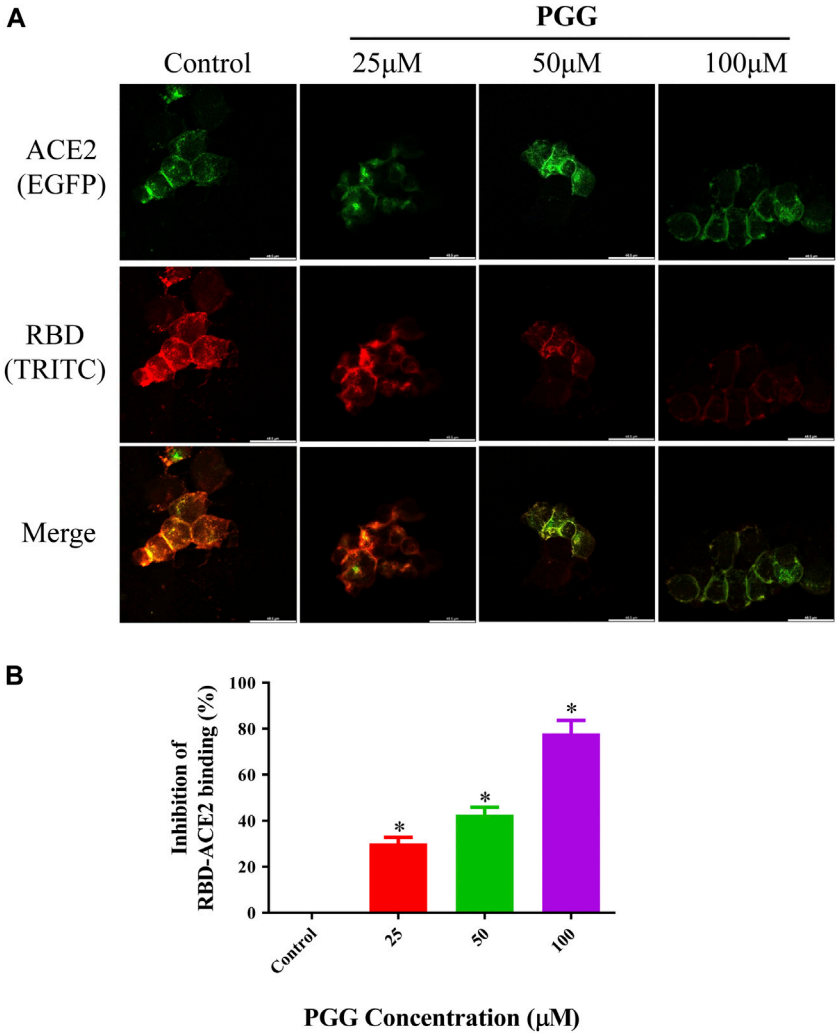


FIGURE 4 | PGG suppresses the binding of Spike-RBD on ACE2 receptor in HEK293 cells. **(A)** HEK293 cells were transiently transfected with hACE2-EGFP (green). After 24 h, the cells were incubated with supernatant containing mFc-tagged SARS-CoV-2-RBD with or without PGG (25–100 μM) for 40 min. The cells were subsequently fixed and detected with mouse IgG Fc TRITC antibody (red). All images were captured by confocal microscopy using a Leica SP8 (×40 oil immersion objective lens). **(B)** Images of Spike-RBD-ACE2 binding intensity were quantified by ImageJ. Data were expressed as mean ± S.D., n = 3; *$p < 0.05$, one-way ANOVA analysis.

history of Chinese medicine in treating various infectious diseases, many herbal formulations have been shown to process protective effect in the intervention of COVID-19, and has provided a novel insight and the source of new drug discovery for the prevention and treatment of COVID-19 and its complications. For instance, Jinhua Qinggan granules was recommended for the treatment of COVID-19 patients in the medical observation period according to the "Diagnosis and Treatment Scheme for New Coronavirus Infected Pneumonia" (Cao et al., 2020). Lian Hua Qing Wen Capsule, Shuang Huang Lian Oral Liquid, and Qingfei Paidu Decoction were reported to show beneficial effects on COVID-19 (Yang et al., 2020b). In the current study, the active compound 1,2,3,4,6-penta-O-galloyl-b-D-glucose (PGG) has been found in Chinese medicinal herb like geranium (Piao et al., 2008). PGG has aroused special scientific interest due to its therapeutic potential in anti-tumor, antiviral, antimicrobial, anti-inflammatory, antidiabetic and anti-oxidant effects (Torres-León, 2017). Notably, PGG has been reported to delay the nuclear transport process of Herpes simplex virus type 1 (HSV-1) and suppressed its nucleocapsid egress by inhibiting the expression and cellular localization of pEGFP-UL31 and pEGFP-UL34 (Jin et al., 2016). Beside, PGG can also inhibit Influenza A virus (IAV) infection by interacting with the viral hemagglutinin (Liu et al., 2011). Furthermore, PGG was shown to effectively inhibit the cell entry of human respiratory syncytial virus (hRSV), which viral particles carrying F proteins are resistant to BMS-433771 or palivizumab (Haid et al., 2015). However, whether PGG exhibits inhibitory effect on coronaviruses has not been illustrated yet.

Structure based drug design has become a valuable and indispensable tool in drug discovery (Anderson, 2003). It makes use of three-dimensional structural information gathered from biological targets for studying the interaction with small-molecules. For instance, computational docking and molecular dynamics are the most frequently used methods

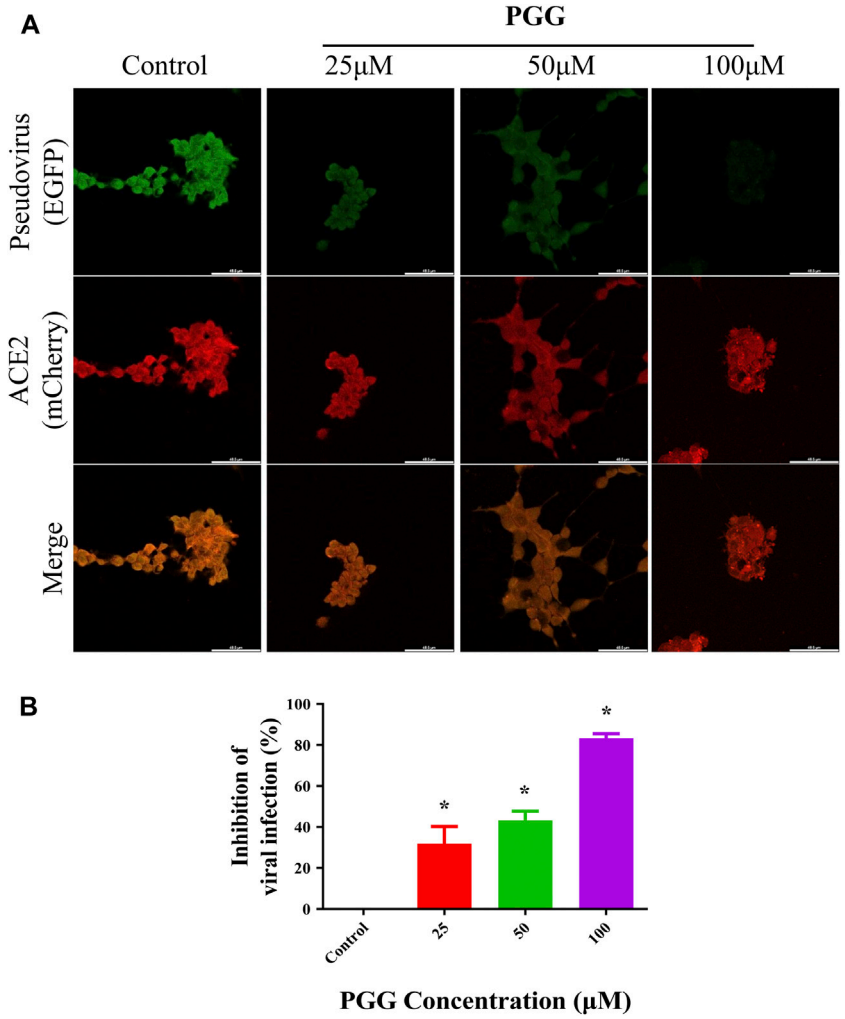


FIGURE 5 | PGG inhibits the infection of S-pseudotyped lentivirus in human ACE2 overexpressing cells. **(A)** HEK293 cells were transiently transfected with hACE2-mCherry (red). After 24 h, the hACE2 overexpressing cells were infected by RBD S-pseudotyped lentivirus (green) for 12 h. The infected cells were then replaced with fresh medium and continually incubated for 48 h. All images were captured by confocal microscopy using a Leica SP8 (×40 oil immersion objective lens). **(B)** Images of RBD S-pseudotyped lentivirus infection intensity were quantified by ImageJ. Data were expressed as mean ± S.D., n = 5; *$p < 0.05$, one-way ANOVA analysis.

for new drug prediction and discovery. These methods help in understanding the principles by which small-molecules recognize and interact with target macromolecules. In the present study, we adopted these 2 methods to predict the molecular interactions between PGG and the RBD of the SARS-CoV-2 protein. (Ferreira et al., 2015). We found that PGG preferentially bound to a pocket that involved residues Glu 340 to Lys 356. The results of the site-specific docking showed that the best binding pose of PGG interacts with a relatively low binding energy of -8 kcal/mol. It was found from the 10 ns molecular dynamics simulation that the selected binding pose was stable for the last 8 ns The binding interaction depended mainly on electrostatic interaction with minimal contribution of van der Waal's interactions. This could be explained by the 7 hydrogen bonds shown in **Figure 1**. A recent study on the cryo–electron microscopy structure of SARS-CoV-2 spike (S) glycoprotein revealed that the RBD tightly bound with linoleic acid in 3 composite binding pockets (Toelzer et al., 2020). Interestingly, our predicted binding pocket was found to be one of these binding pockets. A similar pocket is found in the previous severely pathogenic strains severe acute respiratory syndrome, namely, coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). Binding of linoloic acid to this pocket was shown to stabilize the S-protein in the closed conformation and inhibit its transformation into the open conformation necessary for its binding to its receptor ACE2. It is therefore expected that PGG would irreversibly lock the S-protein in the closed conformation and interfere with its interaction with the receptor. In light of the interactions between PGG with the SARS-CoV-2-RBD *via* molecular docking, we speculated that the PGG should bind to the RBD protein with strong affinity. To test this hypothesis, we performed a real-time biolayer interferometry (BLI) assay. We confirmed that PGG has a relatively strong binding affinity to SARS-CoV-2-RBD protein.

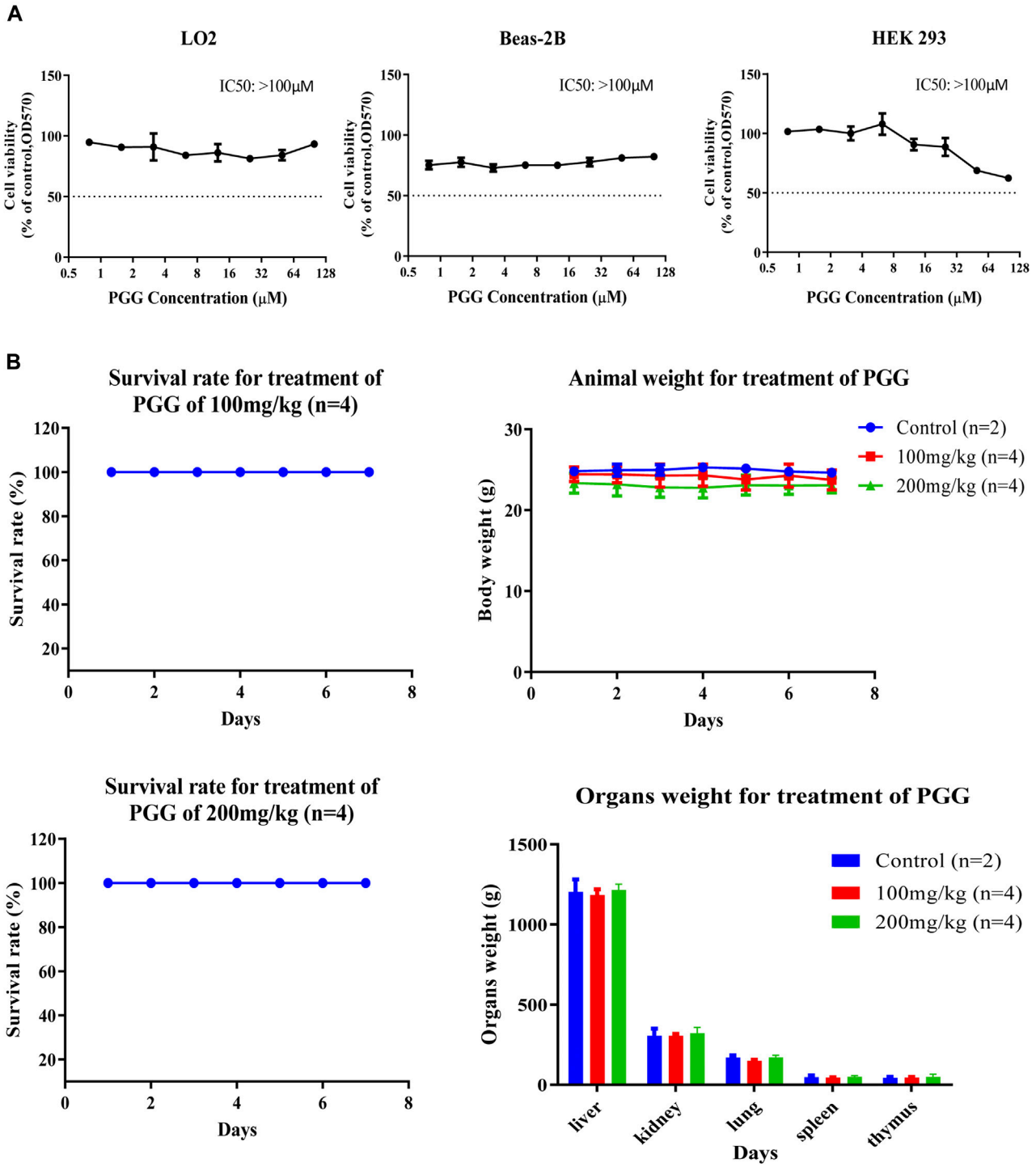


FIGURE 6 | PGG exhibits no cytotoxicity and toxicity on normal cell lines and C57BL/6 mice. **(A)** PGG exhibited relative low cell cytotoxicity towards three human normal cells (LO2, BEAS-2B and HEK293). Cell cytotoxicity was measured by MTT assay. Cells were seeded and treated with PGG from 0 to 100 μM for 72 h incubation. Then, 10 μL of MTT was added to each well. After 4 h incubation, 10% SDS in 0.01 mol/L HCl was added. After 24 h later, optical density was measured by plate reader at 570 nm. **(B)** Study on the sub-chronic lethal dose of PGG. C57BL/6 mice were orally administrated with 100 or 200 mg/kg/day of PGG for consecutive 7 days, the survival, body weight and organs weight of mice were monitored and recorded. Representative results were shown as mean ± S.D. from 3 independent experiments. IC_{50} values were calculated.

Analysis of ELISA assay validated PGG could dose dependently block SARS-CoV-2-RBD binding to hACE2 receptor. By ICC immuno-visualization, PGG was validated to block the binding of SARS-CoV-2 RBD protein to hACE2 receptor in cellular level. Of note, pre-incubation of PGG with RBD-pseudotyped lentivirus also abolished the infectious property of virus in hACE2

overexpressing HEK293 cells, which mimiced the entry of wild type SARS-CoV-2 virus in human host cells.

Owing to the strong infectivity, SARS-CoV-2 cannot be studied in most research laboratories without P3 standardized facilities, which may prohibit the development of new drugs. To solve this problem, the virus simulation experiment *in vitro* has been developed. The SARS-CoV-2 Spike-RBD protein is one of the most important protein for SARS-CoV-2 to enter cells, which mediates the attachment and fusion of virus to cells. A recent study characterized the cross-neutralizing activity of SARS-CoV-2, SARS-CoV and MERS-RBD protein in hACE2/293 cells and the results showed that SARS-RBD exhibits competitive inhibition with SARS-CoV-2-RBD, but not with MERS-RBD, indicating that SARS-CoV-2 and SARS-CoV have similar infection mechanism (Tai et al., 2020). In addition, SARS-CoV-2 Spike-RBD was used to develop a vaccine which can induce protective immunity (Yang et al., 2020a). Although SARS-CoV-2 Spike-RBD protein can be used to preliminarily determine whether drugs can inhibit viral ligands attach to hACE2 receptor, there are still huge variables in the biological level. Therefore, a pseudovirus containing SARS-CoV-2 Spike protein was developed. Similar to SARS-CoV-2 Spike-RBD protein, SARS-CoV-2 Spike pseudovirus initially played an important role in the identification of SARS-CoV-2 binding sites (Ou et al., 2020). In the development of vaccines, SARS-CoV-2 Spike pseudovirus has also become an important index to evaluate the viral inhibition *in vitro* (Hu et al., 2020). In our study, we utilized SARS-CoV-2 Spike-RBD protein and SARS-CoV-2 Spike pseudovirus for viral infection test. The results demonstrated that PGG had effect of competitive inhibition on SARS-CoV-2 Spike-RBD and inhibited 80% of RBD protein binding to hACE2/HEK-293 cells at 100 μM. Further experiments showed that PGG could inhibit SARS-CoV-2-Spike pseudovirus invasion by 85% at 100 μM, which was consistent with SARS-CoV-2-RBD protein binding test. In terms of safety issue, PGG was reported to inhibit the biofilm formation of *Staphylococcus aureus* and show no toxicity to human epithelial cells and fibroblasts (Lin et al., 2011). In addition, PGG can be used as a non-cytotoxic elastin stabilizer in the treatment of abdominal aortic aneurysm model rats (Isenburg et al., 2007). On the other hand, Feldman et al. reported that infusion of 50–60 mg PGG per rat (~200 g body weight) resulted in a precise and lethal drop in blood pressure within 30 min, whereas 30 mg per rat did not affect blood pressure or blood glucose levels (Feldman et al., 2001). Moreover, PGG is astringent which can inhibits human salivary α-amylase, potential negative effect on starch digestion and food taste (Hofmann et al., 2006). In our study, we found that PGG had no toxic effect on the 3 normal cell lines including BEAS-2B, LO2 and HEK293 cells. Meanwhile, we observed that there was no significant weight loss in animal body or their vital organs in C57BL/6 mice, suggesting that PGG could be considered safe for external use. Taken together, these results indicated that PGG might be further developed as an effective anti-viral agent for external use, e.g., anti-viral spray to fight against the COVID-19 by blockade of the binding of spike-RBD of SARS-CoV-2 to cellular ACE2 receptors.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Ethical Committee of Department of Health and Supervision, Macao Special Administrative Region of China.

AUTHOR CONTRIBUTIONS

RC and LY contribute equally to the works. Corresponding authors: VW and BL. RC, LY, and SH carried out the experiment. RC, LY and SH wrote the manuscript with support from VW and BL. VW, and BL conceived the original idea. SC, CL, and KZ edited the article. XG and CX discussed the results and contributed to the final manuscript.

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frontiers in Pharmacology

REVIEW
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Plant Products as Inhibitors of Coronavirus 3CL Protease

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Background: The ongoing COVID-19 pandemic has created an alarming situation due to extensive loss of human lives and economy, posing enormous threat to global health security. Till date, no antiviral drug or vaccine against SARS-CoV-2 has reached the market, although a number of clinical trials are under way. The viral 3-chymotrypsin-like cysteine protease ($3CL^{pro}$), playing pivotal roles in coronavirus replication and polyprotein processing, is essential for its life cycle. In fact, $3CL^{pro}$ is already a proven drug discovery target for SARS- and MERS-CoVs. This underlines the importance of 3CL protease in the design of potent drugs against COVID-19.

Methods: We have collected one hundred twenty-seven relevant literatures to prepare the review article. PubMed, Google Scholar and other scientific search engines were used to collect the literature based on keywords, like "SARS-CoVs-3CL protease," "medicinal plant and anti-SARS-CoVs-3CL protease" published during 2003–2020. However, earlier publications related to this topic are also cited for necessary illustration and discussion. Repetitive articles and non-English studies were excluded.

Results: From the literature search, we have enlisted medicinal plants reported to inhibit coronavirus 3CL protease. Some of the plants like *Isatis tinctoria* L. (syn. *Isatis indigotica* Fort.), *Torreya nucifera* (L.) Siebold and Zucc., *Psoralea corylifolia* L., and *Rheum palmatum* L. have exhibited strong anti-$3CL^{pro}$ activity. We have also discussed about the phytochemicals with encouraging antiviral activity, such as, bavachinin, psoralidin, betulinic acid, curcumin and hinokinin, isolated from traditional medicinal plants.

Conclusion: Currently, searching for a plant-derived novel drug with better therapeutic index is highly desirable due to lack of specific treatment for SARS-CoV-2. It is expected that in-depth evaluation of medicinally important plants would reveal new molecules with significant potential to inhibit coronavirus 3CL protease for development into approved antiviral drug against COVID-19 in future.

Keywords: coronavirus disease 2019, 3-chymotrypsin-like cysteine protease, main protease, SARS-CoV-2, plant products, plant-derived 3CLpro inhibitors

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INTRODUCTION

Recently, a novel coronavirus was discovered in patients suffering from respiratory ailment accompanied by fever, dry cough and tiredness. Other symptoms, like sore throat, nasal congestion, headache, conjunctivitis, diarrhea, loss of taste or smell, were usually mild, and appeared gradually. This was coronavirus disease 2019 (COVID-19), unknown to the world before its outbreak in December 2019 in Wuhan, China (Burki, 2020). The highly infectious virus started to spread rapidly among the population in many countries all over the world, and created a pandemic situation within a couple of months (Bedford et al., 2020). Reports have indicated that even asymptomatic people can transmit the virus, mainly through respiratory droplets that can cause human-to-human transmission. The extremely contagious novel coronavirus 2019 (nCoV-19) was responsible for about 1.76 million deaths and 79.67 million confirmed cases globally till date (WHO, 2020).

The major phenotype of COVID-19 is severe acute respiratory distress syndrome (ARDS), similar to that caused by SARS-CoV and MERS-CoV (Hirano and Murakami, 2020). At present, there is no specific treatment for COVID-19, while vaccines will take some more time to come to the market. Hence, clinical management of COVID-19 is currently limited to preventive and supportive treatments, and mostly designed to alleviate further complications and organ damage (Rodríguez-Morales et al., 2020). Clinical studies have shown promising results in patients using the protease inhibitor drug lopinavir in combination with ritonavir, commonly used to treat HIV (Lu, 2020). Also, hydroxychloroquine, an antimalarial drug, and remdesivir, a nucleoside analogue of SARS-CoVs, were used to treat COVID-19 patients (Lee and Hsueh, 2020; Wu et al., 2020).

SARS-CoV-2 encodes two proteases, a papain like protease (PL^{Pro}), and a 3- chymotrypsin-like cysteine protease ($3CL^{Pro}$) also known as viral main protease (M^{pro}), for proteolytic processing during viral maturation. The PL^{Pro} of coronavirus cleaves at no less than two sites on the pp1a polyprotein, whereas $3CL^{Pro}$ has at least eleven inter-domain sites on the pp1a and pp1ab polyproteins (Krichel et al., 2020). The functional importance of this proteolytic enzyme in the viral life cycle makes $3CL^{pro}$ a promising target for drug development against SARS-CoV-2 and other coronaviruses. Zhang et al. have successfully crystallized the 3CL protease from SARS-CoV-2 (Zhang et al., 2020a). This protease contains several highly conserved substrate-binding sites within the active site of the enzyme. Therefore, this is an opportunity for designing a wide variety of inhibitors against coronavirus $3CL^{pro}$. It is also exciting that the structures of $3CL^{pro}$ in SARS-CoV-2 and SARS-CoV differ by only twelve amino acids with comparable ligand binding efficiency (Macchiagodena et al., 2020). This demonstrated the possibility that inhibitors of SARS-CoV-$3CL^{pro}$ will be active against SARS-CoV-2-$3CL^{pro}$, too. In fact, protease inhibitors have drastically reduced the mortality against HCV/HIV, and maximized the therapeutic benefit (Kurt Yilmaz et al., 2016). Altogether, it is speculated that 3CL protease represents a potential target for the inhibition of CoV replication and this will definitely escalate the ongoing search for a new drug against SARS-CoV-2.

Natural products and their derivatives are used for treatment of various ailments since pre-historic times. A large number of herbal products and their constituents have shown promising inhibitory activity related to viral infections in humans (Ganta et al., 2017; Panda et al., 2017; Shen et al., 2019; Bahramsoltani and Rahimi, 2020). In 2002, during SARS-CoV outbreak in China, several clinical research projects were initiated on administration of Traditional Chinese Medicine (TCM) in combination with Western medicine for treatment of SARS-CoV infection (WHO, 2004). On verification of the clinical data, WHO declared that judicious use of TCM against SARS-CoV would help to reduce the mortality rate as compared to Western medicine alone. Furthermore, the combination therapy could lower the overall cost, and highlighted the importance of introducing plant products for the treatment of SARS-CoV (WHO, 2004).

In this background, it would be relevant to search for plant products with potential efficacy to inhibit coronavirus 3CL protease (**Figure 1**). In fact, SARS-CoV-$3CL^{Pro}$ was reportedly inhibited by phytochemicals abundant in green tea extracts (Chen et al., 2005). The therapeutic importance of green tea is evident in customary Indian and Chinese medicinal systems for prevention of various diseases (Haqqi et al., 1999; Kavanagh et al., 2001; Sueoka et al., 2001), and also for its, neuroprotective (Weinreb et al., 2004), anti-bacterial (Roccaro et al., 2004), and antiviral (Weber et al., 2003) properties. Flavanols and flavonols, including (-)-epigallocatechin-3-gallate (EGCG), are mainly responsible for health promoting effects of green tea (Higdon and Frei, 2003; Moyers and Kumar, 2004). Another traditional plant, *Angelica keiskei* (Miq.) Koidz (Umbelliferae), commonly used as a mild cathartic and diuretic tonic (Kimura and Baba, 2003), showed inhibitory activity against SARS-CoV-3CLpro with IC50 values of 11.4 μM (Park et al., 2016). This plant extract was also reported for its anti-bacterial, hepato-protective and other activities (Kil et al., 2017). *Cullen corylifolium* (L.) Medik. (syn. *Psoralea corylifolia* L.) is used in Chinese medicine and traditional Ayurveda against different types of skin diseases, such as leprosy, leukoderma and psoriasis (Sah et al., 2006). This plant is also known for its anti-inflammatory and antimicrobial properties (Khushboo et al., 2010). Later on, six aromatic compounds isolated from seeds of *Cullen corylifolium* (L)., Medik. were noted for their inhibitory activity against PL^{pro} (Kim et al., 2014); the isolated phytochemicals inhibited the enzyme in a dose-dependent manner with IC_{50} ranging from 4.2 to 38.4 μM. Similarly, many natural products have shown antiviral activity at nanomolar concentration against SARS-CoV (e.g., lycorine, homoharringtonine, silvestrol, ouabain, tylophorine and 7-methoxycryptopleurine), and might contribute to future drug discovery pipeline (Islam et al., 2020). In fact, clinical trials of a few herbal compounds against SARS-CoV-2-$3CL^{Pro}$ aroused hope for plant-derived anti-SARS-CoV-2 drugs. Very recently, 3CL protease inhibitor NLC-001, a plant product administered orally as dietary supplement, got US FDA approval. Presently, clinical studies of NLC-001 to treat COVID-19 is going on in Israel while the

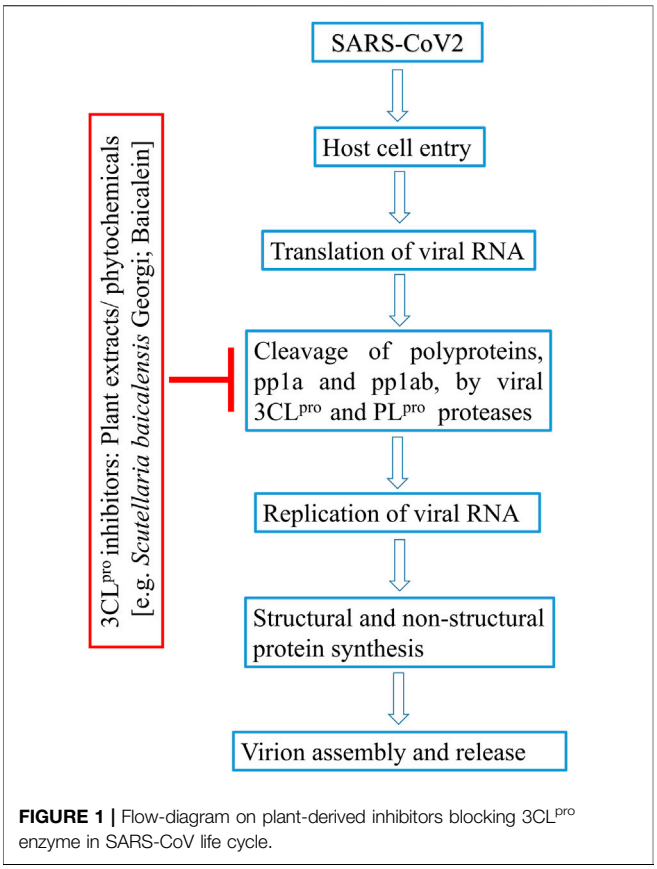

FIGURE 1 | Flow-diagram on plant-derived inhibitors blocking $3CL^{pro}$ enzyme in SARS-CoV life cycle.

pharmaceutical company Todos Medical Ltd. is evaluating its commercialization options worldwide (Golodetz, 2020). In another study, a natural product, baicalein, derived from TCM was identified as inhibitor of SARS-CoV-2-$3CL^{pro}$ (**Figure 1**). Clinical trial showed that baicalein was well tolerated in treatment of acute, or chronic hepatitis in China (clinical trials registration number CTR20132944) (Li et al., 2014b).

In this review, we provided a compilation of well-documented plant-derived compounds and their derivatives acting against coronavirus 3CL protease, and their current stage of investigation. A detailed insight into the active compounds with regard to their mode of inhibition have been presented along with predictive studies *in silico*. Finally, a discussion is focused on several plants with ethno-pharmacological reputation. Further, we have proposed a few selected phytochemicals for evaluation against this protease enzyme with a view to providing clues toward coronavirus drug discovery in future.

METHODOLOGY

Scientific search engines PubMed, Google Scholar, ScienceDirect, SpringerLink, Scopus, EMBASE, Medline were critically investigated to obtain research articles with the help of search strings, viz. "SARS-CoVs-3CL protease," "anti-SARS-CoV-2 drugs," "medicinal plant and anti-SARS-CoVs-3CL protease," "phytochemicals against SARS-CoVs-3CL protease," "medicinal plants and HIV/HCV proteases," etc. Research article inclusion criteria: 1) crude plant extracts, fractions or semi-purified fraction inhibiting coronaviruses, 2) isolated phytochemicals or its derivatives effective against coronaviruses, and 3) studies with medicinal plants acting against HIV/HCV proteases. Exclusion criteria: 1) literature duplication, 2) non-relevant articles, 3) studies involving herbal mixtures with undefined contents, 4) plant extracts with toxicity issues, 5) natural products not derived from plants. Total one hundred twenty-seven publications from 1994 to 2020 were cited in the present article (**Figure 2**). Mendeley desktop software was used for preparing the bibliography.

CORONAVIRUS LIFE CYCLE

SARS-CoV-2 is an enveloped, non-segmented, positive-sense RNA virus with genome size of ~30 kb, belonging to the *Nidovirales* order and *Coronaviridae* family (Khailany et al., 2020). The viral genome contains at least six open reading frames (ORFs). The first ORF (ORF1a/b) is about two-thirds of the whole genome length and encodes 16 nonstructural proteins (nsp1-16). The virus contains four major structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N), all located within the 3′ end of the viral genome. Spike protein is responsible for binding with cellular receptors as well as fusion of viral and cellular membrane. M protein plays a central role in the assembly of viruses, making cellular membranes as workshops where viruses and host factors come together to create new virus particles. E protein is necessary for viral pathogenesis and its smaller size (9–12 kDa) makes it easier for the virus to assemble before release (Bianchi et al., 2020). N protein is the only protein in nucleocapsid that binds to the viral genome in a conformation of the "beads-on-a-string" type (**Figure 3A**) (Gordon et al., 2020). SARS-CoV-2 was found with ~80% nucleotide identity to SARS coronavirus (Kim et al., 2020).

The attachment of SARS-CoV-2 to the host cell is initiated by interaction between S protein and its host cell receptor known as angiotensin-converting enzyme (ACE2) (**Figure 3B**) (Choudhary et al., 2020). This interaction is followed by a series of events leading to the delivery of viral genome into the cytoplasm. The 5′ end of the RNA genome, ORFs 1a and 1b, are translated into pp1a and pp1ab; upon entry into the cell pp1ab is translated through a frameshift mechanism. The non-structural proteins (nsps) 1–11 and 1–16 are encoded by polyproteins pp1a and pp1ab, respectively. These polyproteins are then cleaved into individual non-structural proteins by two viral proteases, namely, PLP and $3CL^{pro}/M^{pro}$. The PLP encoded in nsp3, cleaves between nsp1/2, nsp2/3 and nsp3/4, while $3CL^{pro}/M^{pro}$ encoded by nsp5 is responsible for the remaining 11 cleavage events (Krichel et al., 2020). Most of the non-structural proteins in the replicase–transcriptase complex (RTC) contribute to establish an environment favorable for RNA synthesis (Wang et al., 2020).

The synthesis of viral RNA produces genomic as well as sub-genomic RNAs. After viral RNA translation, the structural proteins S, E, and M are moved through secretory pathway into the endoplasmic reticulum–golgi intermediate compartment. The viral genomes, encapsulated in the compartment membrane,

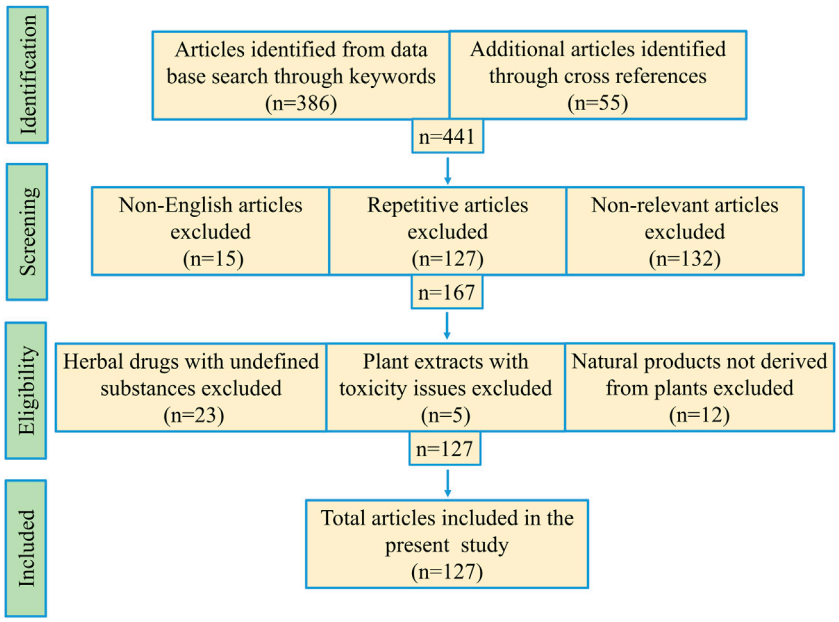

FIGURE 2 | Schematic representation of methodology used for this study.

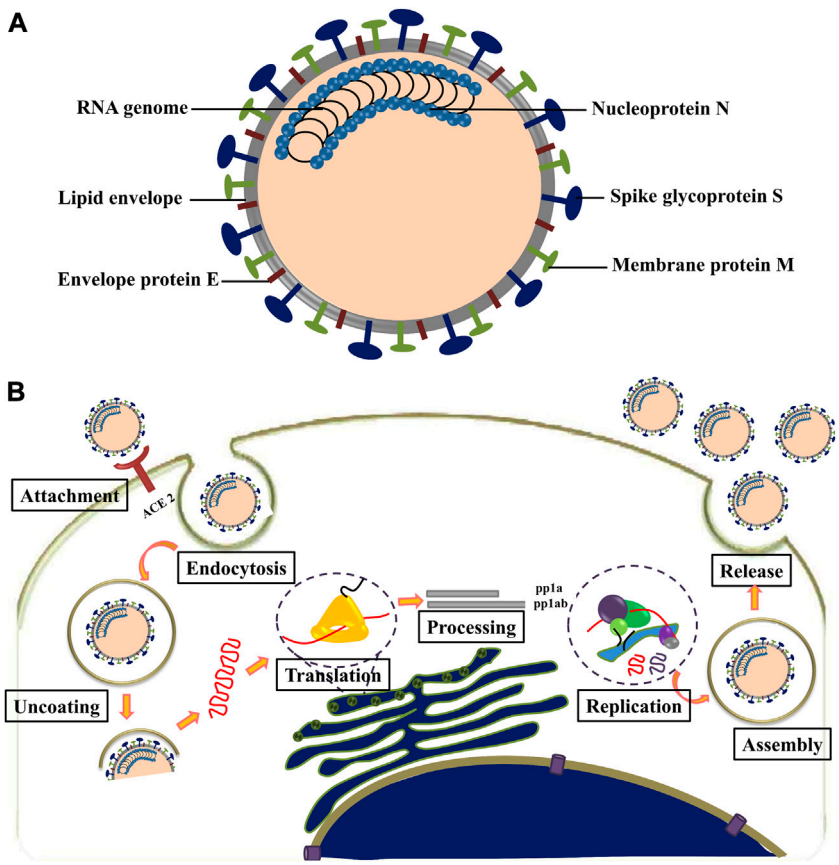

FIGURE 3 | (A) Structure of coronavirus. **(B)** Life cycle of coronavirus.

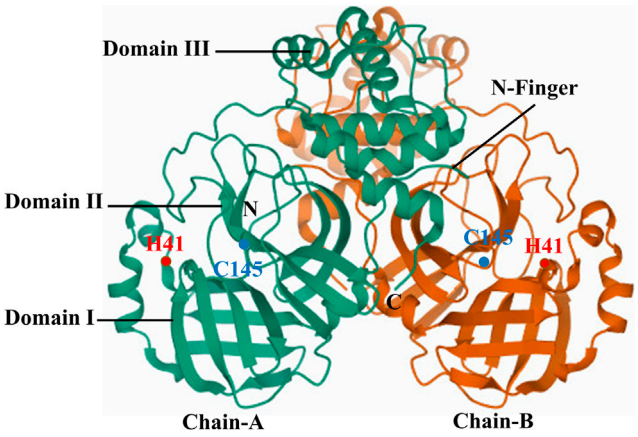

FIGURE 4 | Crystal structure of SARS-CoV-2-3CL^{pro}.

interact with the structural proteins to form mature virions and get transported to the cell surface to be released by exocytosis (Shereen et al., 2020).

STRUCTURE OF SARS-COV-3CL^{PRO}

SARS-CoVs are single-stranded RNA viruses with the largest known viral RNA genomes to date. The genome is a polyadenylated RNA of ~30 kb, and 41 percent of the residues are G or C rich (Rota et al., 2003). Functional polypeptides are released by extensive proteolytic processing from each polyprotein, primarily by 33.8-kDa 3C-like protease (Yang et al., 2003). The crystal structure of 3CL^{pro} (2.54 Å) reveals that the molecule is composed of three domains. Domains I, II and III contain amino acid residues 8 to 99, 100 to 183, and 184 to 303, respectively. Domains I and II are six-stranded antiparallel β barrels, and together mimic the architecture of chymotrypsin and 3C proteinases of picornavirus. Domain III is the substratum binding site found in a cleft between the two. A domain III loop connects domain II with C-terminal end of domain III (Anand et al., 2003). Domain III (residues 198–303), a globular cluster of five helices, regulates the dimerization of 3CL^{pro} through interaction of the salt-bridge between Glu290 of chain-A and Arg4 of chain-B (Shi and Song, 2006). The 3CL^{pro} active site contains a catalytic dyad in which a cysteine residue (Cys145) acts as a nucleophile, and histidine residue (His41) acts as a base (**Figure 4**) (Anand et al., 2003). SARS-CoV-2-3CL^{pro} has 96% structural similarity with SARS-CoV-3CL protease forming a dimeric structure for catalytic activity (Zhang et al., 2020a).

PLANTS WITH INHIBITORY ACTIVITY AGAINST 3CL PROTEASE

Natural products have always played a crucial role in drug development against various diseases. Therefore, traditional herbs from diverse geographical locations and various habitats could be considered as potential sources of new drugs for treatment of viral infections, including those caused by SARS-CoVs and its emergent mutants. For centuries, the medicinal plant *Isatis tinctoria* L. (Brassicaceae) have been esteemed in Europe, Central Asia, and in TCM for therapeutic and cosmetic application, and indigo-blue dyeing character (Speranza et al., 2020). All parts of this plant are utilized in complementary and alternative medicinal preparations against eruptive epidemic diseases, pharyngitis, laryngitis, hepatitis, various kinds of fevers, influenza and viral skin diseases. Currently, *I. tinctoria* root is recognized in European phytotherapy for medicinal properties, mostly due to its antiviral activities (Hamburger, 2002). During the outbreaks of SARS-CoV in China, Hong Kong, and Taiwan, *Isatis tinctoria L.* and several phenolic herbs were commonly used against the viral disease. *I. tinctoria* L. root contains phytochemicals such as indigo, indirubin, indican, β-sitosterol, sinigrin and γ-sitosterol. Seven other compounds from various sources, namely aloe-emodin, hesperetin, quercetin, naringenin, daidzein, emodin and chrysophanol were also tested for their SARS-CoV-3CL^{pro} inhibitory effect (vide **Table 1**; **Figure 5**). Among these, aloe-emodin, sinigrin and hesperetin reportedly inhibited cleavage activity of the 3CL^{pro} in a cell-based assay in dose-dependent manner (Lin et al., 2005).

Torreya nucifera (L.) Siebold and Zucc. is historically used as a medicinal herb in Asia. Ethanol extract of *T. nucifera* leaves showed promising inhibitory activity against SARS-CoV-3CL^{pro} (62% viral inhibition at 100 μg/ml). Eight diterpenoids and four biflavonoids were isolated and evaluated for SARS-CoV-3CL^{pro} inhibition using fluorescence resonance energy transfer analysis following bioactivity-guided fractionation. Out of these compounds, the most potent inhibitory effect on 3CL^{pro} was shown by a biflavone, namely, amentoflavone (IC_{50} = 8.3 μM). Three more biflavones, viz. apigenin, luteolin and quercetin inhibited 3CL^{pro} activity with IC_{50} values of 280.8, 20.2, and 23.8 μM, respectively (Bae et al., 2010).

Ethanolic extract of *Cullen corylifolium* (L.) Medik (syn. *Psoralea corylifolia* L.) seeds showed high activity against the SARS-CoV-PL^{pro} with an IC_{50} value of 15 μg/ml. Subsequently, bioactivity-guided fractionation of the ethanol extract resulted in six aromatic compounds known as bavachinin, neobavaisoflavone, isobavachalcone, 4′-O-methylbavachalcone, psoralidin and corylifol A. All the isolated flavonoids inhibited PL^{pro} to exhibit their inhibitory potency in a dose-dependent manner (Kim et al., 2014).

Another study with twenty phytocompounds, including abietane and labdane-type diterpenes, lupane-type triterpenes, lignoids and curcumin, were tested for their anti-SARS-CoVs activity. The tested phytocompounds exhibited substantial levels of anti-SARS-CoV activity at 10 μM. Betulinic acid, betulonic acid, hinokinin, curcumin, niclosamide and savinin were among the twenty phytocompounds showing substantial inhibition of 3CL protease. It was claimed as the first report to demonstrate that natural lupane-type triterpenes and lignan could block 3CL protease activity by competitive inhibition (Wen et al., 2007).

Various types of tea extracts including black tea, green tea, oolong tea, and pu'er tea were investigated for 3CL^{Pro} inhibitory activity. Results suggested that extracts from pu'er and black tea were more potent than those from green or oolong tea. Finally,

TABLE 1 | Plant products with inhibitory activity against coronavirus 3CL protease.

Plant name [family]	Active principle	IC_{50} value	References
Aloe vera (L.) Burm.f. [Asphodelaceae]	Aloe emodin	8.3 μM	Lin et al. (2005)
Citrus japonica Thunb. [Rutaceae]	Hesperetin	217 μM	
Isatis tinctoria L. [Brassicaceae]	Sinigrin	365 μM	
	Indigo	752 μM	
Torreya nucifera (L.) Siebold and Zucc. [Taxaceae]	Biflavone amentoflavone	88.3 μM	Bae et al. (2010)
	Apigenin	280.8 μM	
	Luteolin	20.2 μM	
	Quercetin	23.8 μM	
Cullen corylifolium (L.) Medik. [Leguminosae]	Bavachinin	38.4 ± 2.4 μM	Kim et al. (2014)
	Corylifo	32.3 ± 3.2 μM	
	Isobavachalcone	18.3 ± 1.1 μM	
	4′-O-methylbavachalcone	10.1 ± 1.2 μM	
	Neobavaisoflavone	18.3 ± 1.1 μM	
	Psoralidin	4.2 ± 1.0 μM	
Feretia apodanthera subsp. *apodanthera* [Rubiaceae]	Betulinic acid	10 μM	Wen et al. (2007)
Betula pendula Roth [Betulaceae]	Betulonic acid	>100 μM	
Curcuma longa L. [Zingiberaceae]	Curcumin	40 μM	
Phyllanthus niruri L. [Phyllanthaceae]	Hinokinin	>100 μM	
Pterocarpus santalinus L.f. [Fabaceae]	Savinin	25 μM	
Camellia sinensis (L.) Kuntze [Theaceae]	Theaflavin-3′-gallate (black tea)	7 μM	Chen et al. (2005)
	Tannic acid (black tea)	3 μM	
	Theaflavin-3,3′-digallate (black tea)	9.5 μM	
Scutellaria baicalensis Georgi [Lamiaceae]	Baicalein	0.39 μM	Liu et al. (2020)
	Scutellarein	5.80 ± 0.22 μM	
	Dihydromyricetin	1.20 ± 0.09 μM	
	Quercetagetin	1.27 ± 0.15 μM	
	Myricetin	2.74 ± 0.31 μM	
Rheum palmatum L. [Polygonaceae]	RH10 fraction	38.09 ± 1.70 (μg/mL)	Luo et al. (2009)
	RH11 fraction	22.30 ± 1.26 (μg/mL)	
	RH12 fraction	59.33 ± 6.52 (μg/mL)	
	RH121 fraction	13.76 ± 0.03 (μg/mL)	
	RH122 fraction	34.01 ± 5.68 (μg/mL)	
	RH124 fraction	52.43 ± 4.52 (μg/mL)	
	RH125 fraction	20.53 ± 3.20 (μg/mL)	

water extracts from different types of tea were prepared and tested for their inhibitory activities against $3CL^{Pro}$ (Chen et al., 2005). The authors found that $3CL^{Pro}$ inhibitors were tannic acid, theaflavin-3′-gallate and theaflavin-3, 3′-digallate, as revealed by the fluorogenic substrate assay.

The inhibitory effect of sixty-four purified natural compounds on the function of SARS-CoV helicase, nsP13, and HCV helicase was determined by double-strand DNA unwinding assay, or fluorescence resonance energy transfer, or colorimetry-based ATP hydrolysis assay. The study showed that a few selected natural flavonoids, including myricetin and scutellarein, may serve as potent inhibitors of SARS-CoV (Yu et al., 2012). Recently, traditional Chinese medicinal plant *Scutellaria baicalensis* Georgi, widely used as broad spectrum antiviral agent, demonstrated anti-SARS-CoV-2-$3CL^{pro}$ activity *in vitro* with EC_{50} value 0.74 μg/ml (Liu et al., 2020). The active molecule baicalein strongly inhibited the enzymatic activity with IC_{50} value 0.39 μM. Again, the authors identified four baicalein analogues, namely, scutellarein, dihydromyricetin, quercetagetin, and myricetin could inhibit SARS-CoV-2-$3CL^{pro}$ activity at micromolar concentration.

The alcoholic extract of Chinese medicinal plant *Rheum palmatum* L. roots and rhizomes were studied against the protease enzyme; the semi-purified fractions such as RH11, RH121, and RH125 significantly inhibited 3CL protease activity of SARS coronavirus (Luo et al., 2009).

IN SILICO STUDIES ON PHYTOCHEMICALS AGAINST 3CL-PROTEASE

Phytochemical screening *in silico* is particularly appealing because it can virtually screen thousands of compounds within a stipulated time, and scrutinize the prospect of drug-like molecules. Several *in silico* studies revealed potential anti-SARS-CoV-$3CL^{pro}$ natural compounds using molecular docking studies. Induced-fit docking analysis showed that three flavonoids, namely herbacetin, rhoifolin and pectolinarin could block the enzymatic activity of SARS-CoV-$3CL^{Pro}$ and suggested as templates for designing functionally improved inhibitors (Jo et al., 2020). Another recent study by Tahir ul Qamar et al. (2020) identified nine potential SARS-CoV-$3CL^{pro}$ phytochemicals, namely; 5,7,3′,4′-tetrahydroxy-2′-(3,3-dimethylallyl) isoflavone, myricitrin, calceolarioside B, methyl rosmarinate, 3,5,7,3′,4′,5′-hexahydroxy flavanone-3-O-beta-D-glucopyranoside, (2S)-eriodictyol 7-O-(6′-O-galloyl)-beta-D-glucopyranoside, myricetin 3-O-beta-D-glucopyranoside, licoleafol, and amaranthin after screening of a medicinal

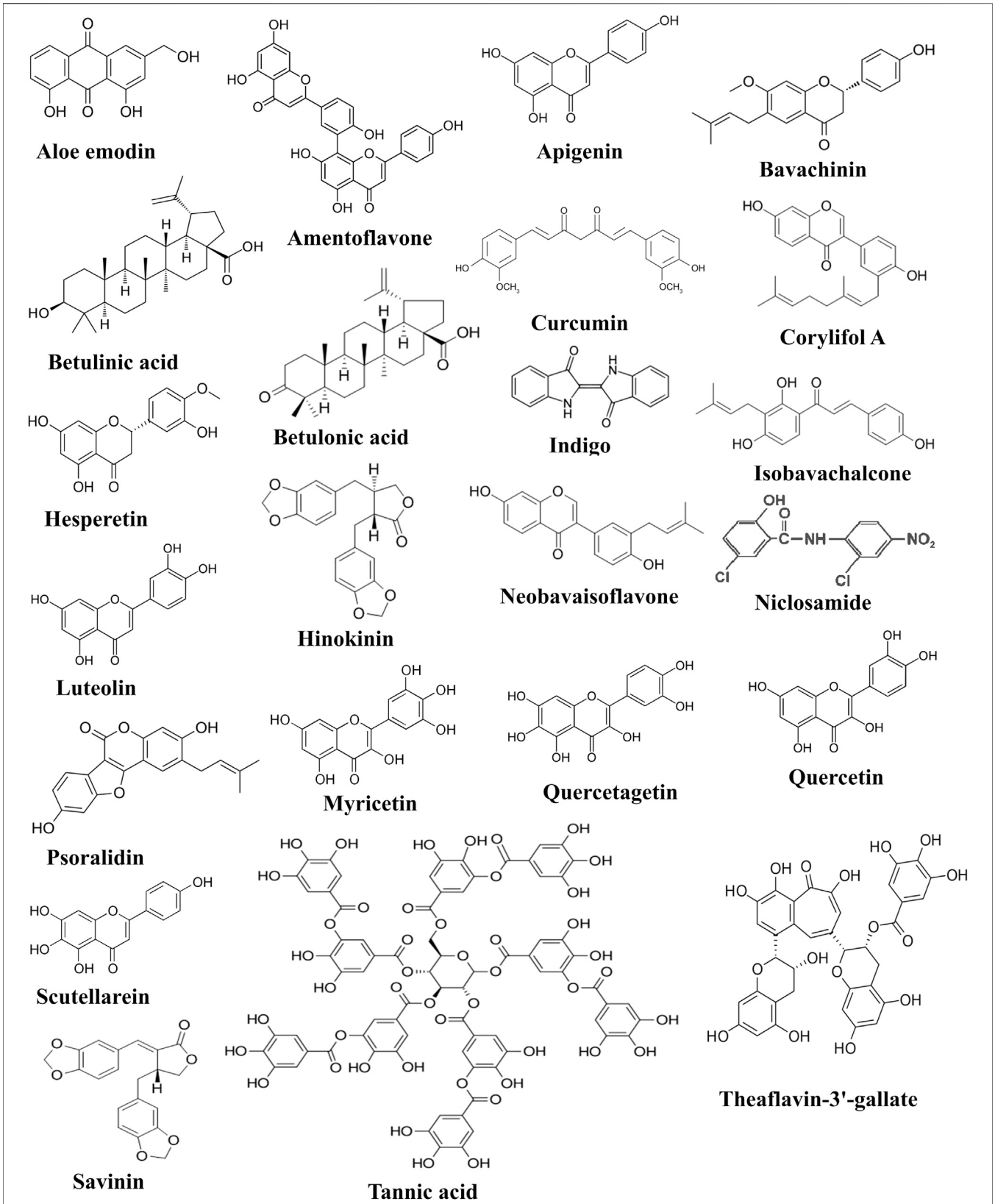

FIGURE 5 | Chemical structures of compounds active against SARS-CoV-2-3CL^{pro}.

plant database containing 32,297 potential antiviral phytochemicals (Tahir ul Qamar et al., 2020). Another *in silico* study demonstrated andrographolide as a potential inhibitor of coronavirus main protease, with good pharmacodynamic property and target accuracy (Enmozhi et al., 2020). Molecular docking study revealed that several flavonoids from *Salvadora persica* L. inhibited SARS-COV-2-3CL protease (Owis et al., 2020). Previously, the proteolytic activity of SARS-CoV-3CL^{pro} was found to be inhibited by apigenin, luteolin, quercetin, amentoflavone, daidzein, puerarin, epigallocatechin, epigallocatechin gallate, gallocatechin gallate and kaempferol (Bae et al., 2010; Thanh et al., 2012; Efferth and Schwarz, 2014). Similarly, several natural alkaloids and terpenoids could inhibit 3CL^{pro} of both SARS-CoV-2 and SARS-CoV with highly conserved inhibitory pattern. In another study, some African plants were screened using *in silico* approach to derive alkaloids and terpenoids as potential inhibitors of coronavirus 3CL^{pro} (Gyebi et al., 2020). Twenty alkaloids and terpenoids with high binding affinities to SARS-CoV-2-3CL^{pro} were additionally docked in SARS-CoV and MERS-CoV-3CL^{pro}. A strongly specified hit-list of seven compounds (10-hydroxyusambarensine, cryptoquindoline, 6-oxoisoiguesterine, 2-hydroxyhopan-3-one, cryptospirolepine, isoiguesterine, and 20-epibryonolic acid) were identified in the ligand-protein interaction analysis (Gyebi et al., 2020). In order to explore the antiviral activity of well-known phytochemicals, like kaempferol, quercetin, luteolin-7-glucoside, demethoxycurcumin, naringenin, apigenin-7-glucoside, oleuropein, curcumin, catechin, and epicatechin-gallate against COVID-19 M^{pro} protein, a molecular docking study was recruited, which strongly correlated the inhibitory activity of the selected phytochemicals (Khaerunnisa et al., 2020). A docking study was performed to find one hundred eighteen constituents, with high binding affinity toward SARS-CoV-2-3CL^{pro}, identified from Respiratory Detox Shot, a TCM prescription for COVID-19 control and prevention. Subsequently, *in vitro* assessment performed on the drug like candidates using the 3CL^{pro} inhibition assay could validate twenty-two active constituents (Zhang et al., 2020c).

FUTURE PROSPECT

Promising Plants and Related Natural Products Waiting to be Evaluated against SARS-CoV-2-3CL^{pro}

Considering the extensive global research focusing on antiviral plant products, the prospect of discovering anti-SARS-CoV-2 drugs remains hopeful. In fact, several bioactive constituents derived from traditional medicinal plants have now gained much attention as prophylactic immunity-boosters and/or adjuvant therapy for management of SARS-CoV-2. In the earlier sections, we have presented a number of phytochemicals and plant products inhibiting coronavirus 3CL protease. Now we shall enlist some of the prospective medicinal plants waiting for evaluation against coronavirus 3CL^{pro}, and present them in **Tables 2–6**. In future, it would be more meaningful if advanced technology is used for cross-screening of these plants, as shown in **Figure 6**, for their potential activity against SARS-CoV-2.

It has been reported that HIV protease inhibitors could be used against coronavirus by targeting SARS-CoV-2-3CL^{pro}. To deal with the COVID-19 pandemic, HIV protease inhibitors (lopinavir/ritonavir) are currently being used with some success. Both the drugs are active against SARS-CoV-2-3CL^{pro} (Chen et al., 2020). Hence, we searched for the plants which have already demonstrated inhibitory activity against HIV protease, for example, *Justicia adhatoda* L (Acanthaceae) leaf extract and *Andrographis paniculata* (Burm. f.) Nees listed in **Table 2**. Therefore, these plants should be specifically investigated for the probability of anti-SARS-CoV-2-3CL^{pro} activity as well. Again, mangiferin, isolated from *Mangifera indica* L., demonstrated efficacy against mutant strains of HIV-1 protease. Hence, mangiferin should be tasted against resistance strains of SARS-CoV-2-3CL^{pro}. Flavones/menthalactone isolated from *Mentha villosa* Huds. inhibited HIV-protease at post-translational level, therefore, may also inhibit polyprotein processing activity of SARS-CoV-2-3CL^{pro}. Similarly, natural products like camelliatanin H and maslinic acid could be tested for application against coronavirus.

Structural similarity of HCV NS3/4A protease and SARS-CoV-2-3CL^{pro} suggested a promising approach for finding useful plant products for COVID-19 therapy (Bafna et al., 2020). Both the proteases have a striking 3D-structural similarity, particularly in the key active site residues. Hence, plants which are already known to possess inhibitory activity against HCV NS3/4A protease need to be tested against coronavirus also (**Table 3**). Thus, plants like *Vachellia nilotica* (L.) P.J.H.Hurter and Mabb. (syn. *Acacia nilotica* (L.). Willd. ex Delile), *Boswellia carterii* Birdw., *Embelia ribes* Burm. f., *Phyllanthus amarus* Schumach. and Thonn. should be repurposed as coronavirus therapeutics.

Following the outbreak of SARS-CoV in 2002, research efforts to find new antiviral agents were rationally focused on traditional medicinal plants reputed in different parts of the world for treatment of pulmonary disorder, asthma and fevers related to cough and cold problems and bronchial infections. The plants enlisted in **Table 4** have been commonly used as anti-allergic/anti-histamine/bronchodilator/muscle relaxant in folk cultures, and well-known for immunomodulatory, anti-inflammatory and antioxidant properties. All of these features are useful for the treatment of respiratory disorders (Savithramma et al., 2007). Therefore, these medicinal plants should be evaluated for anti-SARS-CoV-2 activity, since respiratory disorder is an expected outcome of COVID-19 infection. For example, *Broussonetia papyrifera* L., and *Artemisia scoparia* Waldst. and Kit. are traditionally used for common cough, lung inflammation, bronchitis and asthma. Medicinal plants such as *Salvia officinalis* Linn. and *Carum carvi* L. pharmacologically act as bronchodilators, while *Ficus religiosa* L, *Mimosa pudica* Linn. and *Hyoscyamus niger* Linn. are used for anti-asthmatic purposes. Some such plants, enlisted in **Table 5**, are waiting to be explored for unknown antiviral constituents, as well as for their prospective anti-SARS-CoV-2-3CL^{pro} activity.

TABLE 2 | HIV protease inhibiting plants waiting to be evaluated against SARS-CoV-2-3CL^{pro}.

Name and family	Active part	Extract type/Molecule	EC_{50} (μg/ml)	CC_{50} (μg/ml)	References
Alisma plantago-aquatica subsp. *orientale* (Sam.) Sam. [Alismataceae]	Rh	Hot water	29.2	394.2	Xu et al. (1996)
Andrographis paniculata (Burm. f.) Nees [Acanthaceae]	WP	Water	7.2	210	Otake et al. (1995)
Camellia japonica L. [Theaceae]	Pc	Camelliatanin H	90	≥166.6	Park et al. (2002)
Crotalaria pallida Aiton [Fabaceae]	S, F	Methanol, Ethanol	23	127.0	Govindappa et al. (2011)
Erythroxylum citrifolium A.St.-Hil. [Erythroxylaceae]	Tr	Water	50	62	Govindappa et al. (2011)
Geum japonicum Thunb. [Rosaceae]	WP	Maslinic acid	ND	ND	Matsuse et al. (1998)
Helichrysum populifolium DC. [Asteraceae]	L, Ss	Ethanol	ND	ND	Xu et al. (1996)
Justicia adhatoda L. [Acanthaceae]	L	Water	6.7 ± 5.2	≤1,000	Singh et al. (2010)
Mangifera indica L. [Anacardiaceae]	L	Mangiferin	27.3 ± 2.3	≥500	Wan et al. (1996)
Mentha villosa Huds. [Lamiaceae]	L	Flavones/Menthalactone	1.8	32.6	Hinay and Sarol (2014)
Plectranthus amboinicus (Lour.) Spreng. [Lamiaceae]	L	Ethanol	123.7	726.2 ± 3.1	Thayil et al. (2016)
Rhus chinensis Mill. [Anacardiaceae]	Bb, G	Hot extract	ND	ND	Xu et al. (1996)
Waltheria indica L. [Malvaceae]	Br	Water	48	154.2	Matsuse et al. (1998)
Xylopia frutescens Aubl. [Annonaceae]	B	Methanol	46	56.6	Matsuse et al. (1998)

AP, erial part; B, bark; Bb, bulb; Br, branch; F, fruit; G, gall; L, leaf; ND, not determined; Pc, pericarp; Rh, rhizome; S, stem; Ss, seeds; Tr, trunk; WP, whole plant.

TABLE 3 | HCV protease inhibiting plants waiting to be evaluated against SARS-CoV-2-3CL^{pro}.

Plant name (family)	Active part	Extract type/Molecule	% of inhibition	IC_{50} (μg/ml)	References
Boswellia carterii Birdw. [Burseraceae]	R	Methanol	100.0 ± 0.0	23.0	Hussein et al. (2000)
		Water	71.6 ± 2.5	ND	
Embelia ribes Burm [Myrsinaceae]	F	Methanol	93.9 ± 0.4	38.0	Bachmetov et al. (2012)
		Embelin	96.6 ± 0.9	21	
		5-O-Methylembelin	77.9 ± 1.7	46	
Phyllanthus amarus Schumach. and Thonn. [Phyllanthaceae]	R	Methanol	ND	ND	Ravikumar et al. (2011)
Punica granatum L. [Punicaceae]	FP	Methanol	89.9 ± 0.9	ND	Hussein et al. (2000)
		Water	84.9 ± 1.8	ND	
Trichilia emetica Vahl [Meliaceae]	B	Methanol	57.3 ± 3.7	ND	Hussein et al. (2000)
Vachellia nilotica (L.) P.J.H. Hurter and Mabb. [Fabaceae]	B	Methanol	91.0 ± 0.0	ND	Hussein et al. (2000)
Vitis vinifera L. [Vitaceae]	R	Vitisin B	3.0	>10.0	Lee et al. (2016)

B, Bark; F, Fruit; FP, Fruit Pericarp; ND, not determined; R, root.

TABLE 4 | Medicinal plants, used in respiratory disorders, with potential activity against SARS-CoV-2-3CL^{pro}.

Name and family	Active part	Traditional use	Pharmacological effect	References
Broussonetia papyrifera (L.) L'Hér. ex Vent. [Moraceae]	F	Cough	Protection of bronchitis and inflammation in lungs	Ko et al. (2013)
Carum carvi L. [Asteraceae]	S, L	Bronchitis, cough	Anti-cholinergic, bronchodilator	Boskabady and Shahabi (1997)
Ficus religiosa L. [Papilionaceae]	F, L	Asthma	Mast cell stabilizing effect in bronchospasm animal model	Kapoor et al. (2011)
Hyoscyamus niger Linn. [Zygophyllaceae]	WP	Asthma, Whooping cough	Bronchodilatory effect through blockade of Ca^{2+} channels and muscarinic receptors	Gilani et al. (2008)
Mimosa pudica Linn. [Lamiaceae]	R, L	Asthma	Bronchodilatory effect in bronchospasm animal model	Mail et al. (2011)
Salvia officinalis Linn. [Liliaceae]	L	Cough, cold	Bronchodilatory effect through activation of K^+ channels and phosphodiesterase inhibition	Gilani et al. (2007)
Spinacia oleracea L. [Amarylliadaceae]	L	Cough	Anti-asthmatic effect in animal model	Yamada et al. (2010)
Vitex negundo Linn. [Verbenaceae]	L	Flu	Anti-inflammatory, anti-asthmatic, anti-allergic and bronchodilatory activity	Patel et al. (2011)

F, fruit; L, leaf; R, root; S, stem; WP, whole plant.

Further, evidences from *in silico* molecular docking studies have suggested potential anti-SARS-CoV-3CL^{pro} activity of plants, like *Allium sativum* L., *Anethum graveolens* L., *Citrus aurantium* L., *Curcuma longa* L, *Zingiber officinale* Roscoe, as given in **Table 5**. Molecular docking study showed that the binding energy of SARS-CoV-3CL^{pro} with luteolin-7-glucoside,

TABLE 5 | Phytochemicals selected for anti-SARS-CoV-2-3CL^{pro} activity *in silico*.

Plant name (family)	Active compound	Binding energy	References
Allium fistulosum L. [Amaryllidaceae]	Luteolin-7-glucoside	−8.17	Khaerunnisa et al. (2020)
Allium sativum L. [Amaryllidaceae]	Allicin	−4.03	Khaerunnisa et al. (2020)
Andrographis paniculata (Burm. f.) Nees [Acanthaceae]	Andrographolide	−3.09	Enmozhi et al. (2020)
Anethum graveolens L. [Umbellifers]	Kaempferol	−8.58	Khaerunnisa et al. (2020)
Averrhoa bilimbi L. (Juss.) M.Roem. [Oxalidaceae]	Apigenin-7-glucoside	−7.83	Khaerunnisa et al. (2020)
Citrus aurantium L. [Rutaceae]	Naringenin	−7.89	Khaerunnisa et al. (2020)
Curcuma longa L. [Zingiberaceae]	Demethoxycurcumin	−7.99	Khaerunnisa et al. (2020)

TABLE 6 | SARS-CoV inhibiting plants waiting for *in vitro* anti-SARS-CoV-2-3CL^{pro} activity.

Plant name (family)	Active part	Extract type/Molecule	EC_{50}	CC_{50} (μg/ml)	References
Capsella bursa-pastoris (L.) Medik [Brassicaceae]	WP	Butanol	43.1 ± 2.8 μg/ml	283.4 ± 16.3 μg/ml	Zhuang et al. (2009)
Houttuynia cordata Thunb. [Saururaceae]	L	Ethyl acetate	0.98 μg/ml	NC	Chiow et al. (2016)
Lycoris radiata (L'Hér.) Herb. [Amaryllidaceae]	L	Lycorine	15.7 ± 1.2 nM	14,980 ± 912 nM	Li et al. (2005)
Toona sinensis (Juss.) M.Roem. [Meliaceae]	L	Water	30 μg/ml	>500 μg/ml	Chen et al. (2008)
Veronica linariifolia Pall. ex Link [Plantaginaceae]	WP	Luteolin	9.02 μM	155 μM	Wu et al. (2004)
Vitis vinifera L. [Vitaceae]	Sk	Resveratrol	125–250 μM	>250 μM	Lin et al. (2017)

NC, no cytotoxicity at all tested concentrations; L, leaf; R, root; Sk, skin; WP, whole plant.

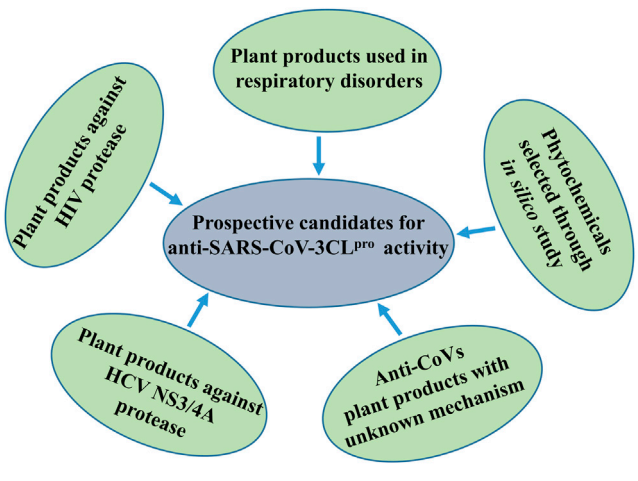

FIGURE 6 | Schematic demonstration of prospective candidates for anti-SARS-CoV-3CL^{pro} activity.

allicin, andrographolide, kaempferol, apigenin-7-glucoside, naringenin, and demethoxycurcumin, were −8.17, −4.03, −3.09, −8.58, −7.83, −7.89, and −7.99 kcal/mol, respectively. The docking analysis indicated the comparative inhibition potential of these compounds in the following order: kaempferol > luteolin-7-glucoside > demethoxycurcumin > naringenin > apigenin-7-glucoside > allicin > andrographolide. Hence, these phytochemical constituents of medicinal plants could be explored as potential inhibitors of SARS-CoV-3CL^{pro}.

Again, the specific mechanisms of antiviral action of certain plants, as shown in **Table 6**, are either unknown, or by way of some other inhibitory pathway, subject to experimental validation through anti-SARS-CoV-3CL^{pro} activity. Butanol extract of *Capsella bursa-pastoris* (L.) Medik showed inhibitory activity against SARS-CoV with IC_{50} value of 283.4 ± 16.3 μg/ml, and EC_{50} = 43.1 ± 2.8 μg/ml. Chen et al. (2008) reported for the first time that leaf extract of Toona sinensis Roem. can inhibit SARS-CoV. Another report described superior antiviral efficacy of ethyl acetate extract isolated from *Houttuynia cordata* Thunb. Therefore, these plants would serve as important clues awaiting further exploration on their putative anti-SARS-CoV-3CL^{pro} activity.

Lycorine isolated from *Lycoris radiata* (L'Hér.) Herb. is an effective antiviral compound against coronavirus. Again, resveratrol (terpenoid) and luteolin (flavonoid) extracted from *Veronica linariifolia* Pall. ex Link strikingly inhibited coronavirus entry by interfering with the binding between ACE2 receptor and spike protein of SARS-CoV. These compounds have antiviral potential against coronavirus and need experimental validation in order to confirm their anti-SARS-CoV-3CL^{pro} activity.

Selection of Phytochemicals Suitable for Future Development Against SARS-CoV-3CL^{pro}

Following the outbreak of SARS-CoV in 2002, research efforts were focused on the antiviral prospect of selected constituents from medicinal plants which are traditionally used for community health care in different parts of the world. Some of them are presented below with relevance to their development against COVID-19, in future.

Betulin Analogues

Betulin and betulinic acid (pentacyclic lupane-type triterpenes) are ubiquitous secondary metabolites found in a variety of medicinal plants, mainly of Betulaceae family, possessing a wide range of pharmacological activities. The bark extracts of several species of

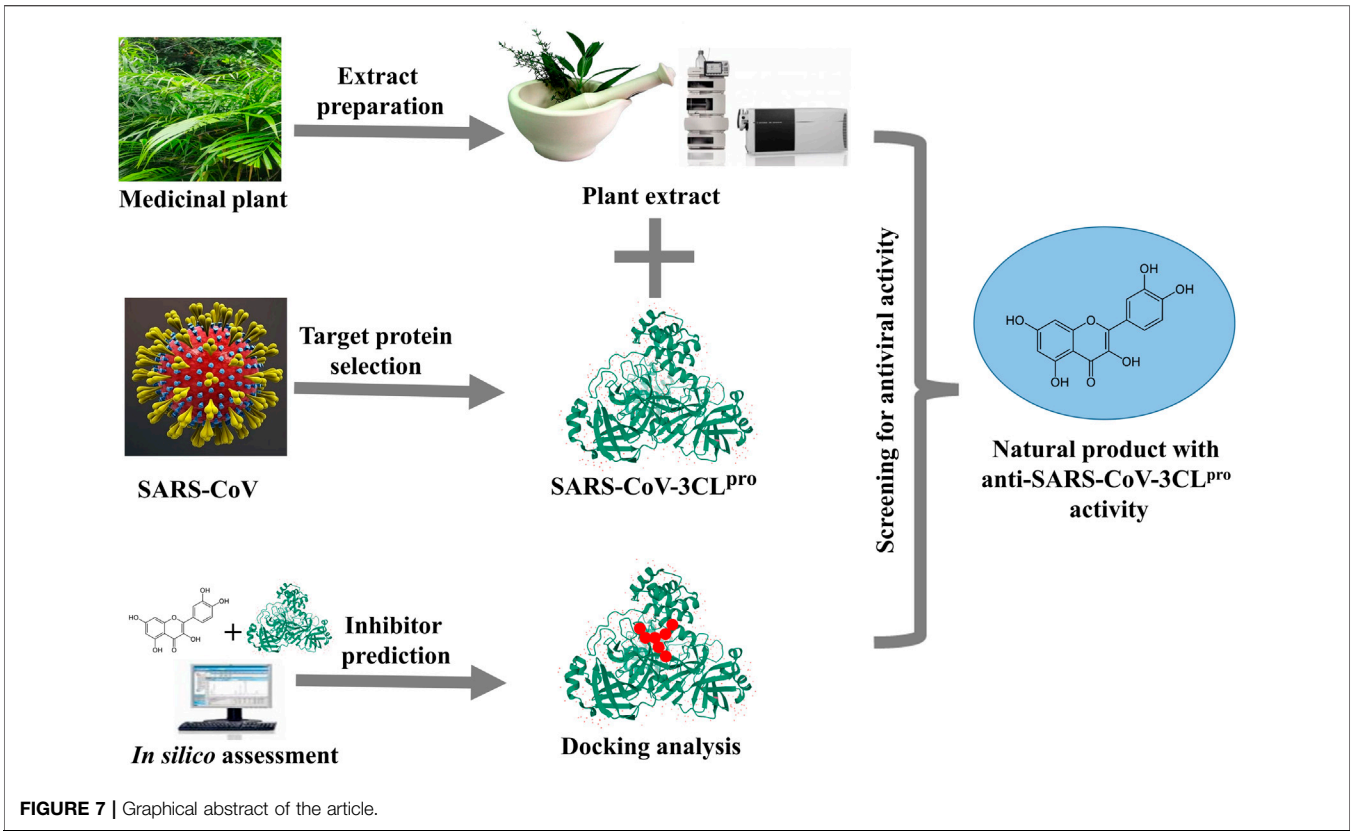

FIGURE 7 | Graphical abstract of the article.

birch trees (*Betula spp.*) have been recommended in traditional medicinal systems for the treatment of rheumatism and arthritis, hepatitis, skin rash, intestinal worms and scurvy (Rastogi et al., 2015). Plenty of investigations have been undertaken to clarify the ethnopharmacological aspects, which showed that betulinic acid and its analogues could be useful for the treatment of cancer, inflammatory diseases, metabolic disorders, cardiovascular conditions, and neurological ailments (Ebeling et al., 2014; Amiri et al., 2020). Moreover, betulin and betulinic acid were shown to be non-toxic, with favourable therapeutic index at doses up to 500 mg/kg body weight in mice (Jäger et al., 2008). The promising antiviral activity of betulin and betulinic acid was reported by several workers (Fujioka et al., 1994; Pavlova et al., 2003). Further studies indicated that betulinic acid acts in the early phase of HIV infection by preventing cleavage of Gag protein, thereby hindering maturation of the virus (Bildziukevich et al., 2019). Again, Navid et al., 2014 demonstrated effective inhibition of herpes virus by analogues of betulin against acyclovir-resistant clinical isolates of HSV-1; also, hepatitis B virus has been found to be susceptible to betulin analogues (Yao et al., 2009; Navid et al., 2014). In a study by Wen et al. (2007), phyto-compounds were evaluated for antiviral activity against SARS-CoV (Wen et al., 2007). Out of 221 compounds, betulinic acid was found to be the most effective inhibitor of the coronavirus. Then, on the basis of molecular modeling analysis, it was observed that the competitive inhibitory activity of betulinic acid on $3CL^{pro}$ activity was consistent with the formation of multiple hydrogen bond interactions between the compound and specific amino acid residues located at the active site of the pocket of the protease enzyme (Pillaiyar et al., 2016). Taken together, this indicated practical applicability of betulinic acid and its derivatives to provide a precise direction for development of novel anti-SARS-CoVs-$3CL^{pro}$ drugs from natural sources.

Griffithsin

Griffithsin, an algal protein, was isolated from an aqueous extract of *Griffithsia* sp (Ceramiaceae genus) of marine red alga (Rhodophyta). Traditionally, red algae have a long history of use as functional foods for their richness in protein, fatty acids, minerals and vitamins (Li et al., 2014a). The potent anti-HIV activity of griffithsin was found with EC_{50} at a pico-molar range, and much lesser toxicity to the host cells (Mori et al., 2005). Later on, griffithsin was considered for topical application as a pre-exposure prophylactic against HIV (O'Keefe et al., 2009). In fact, it has been found that the lectin could inactivate other enveloped viruses also, especially those with highly glycosylated proteins on their surface (Kouokam et al., 2016). The protein showed inhibitory activity against a broad spectrum of animal and human coronaviruses, as it could bind specifically to the SARS-CoV spike glycoprotein and inhibited entry of the virus (O'Keefe et al., 2010). The outstanding efficacy of griffithsin in SARS-CoV-infected mice suggested that it merits further investigation for prophylaxis or treatment of respiratory infection caused by emerging viruses of the Coronaviridae family. Thus, by virtue of its broad antiviral spectrum, griffithsin holds great promise for development into universal

antiviral therapeutics. Therefore, this molecule needs further exploration on its putative anti- SARS-CoV-3CL^{pro} activity.

Glycyrrhizin

Glycyrrhizin (or glycyrrhizic acid) is a major constituent of liquorice root obtained from *Glycyrrhiza glabra* L., and *Glycyrrhiza uralensis* Fisch. ex DC. from Fabaceae family of plants. Liquorice extract is a well-known phytomedicine in use since prehistoric times to alleviate common ailments like bronchitis, gastritis and jaundice. Currently, it is added as edible emulsifier and gel-forming agent in modern foodstuffs, and investigated for its anti-inflammatory, anti-allergenic, antimicrobial and antiviral properties in order to validate its traditional medicinal applications (Li et al., 2014a). Oriental medicinal systems recommended it as antitussive treatment of viral respiratory tract infections, such as dry cough or hoarse voice, and also for chronic fevers (Shibata, 2000). Actually, randomized controlled trials conducted with glycyrrhizin and its derivatives reduced hepatocellular damage in patients with HIV-1, and chronic hepatitis B and C virus infection. Additionally, animal studies on glycyrrhizin demonstrated a reduction of mortality in HSV, and influenza A virus (Fiore et al., 2008). *In vitro* studies revealed antiviral activity against HIV-1, SARS-CoV, respiratory syncytial virus, arboviruses, vaccinia virus and vesicular stomatitis virus (Baltina et al., 2015). Further studies on clinical isolates of coronavirus showed efficacy of glycyrrhizin to inhibit viral replication, as well as the adsorption and penetration of virus into host cell at non-cytotoxic concentration (Cinatl et al., 2003). Further, chemically modified glycyrrhizin derivatives exhibited enhanced inhibition of SARS-CoV replication *in vitro* (Hoever et al., 2005). Recent studies based on integrated computational approach and pharmacological aspects lend further support to glycyrrhizin playing an auxiliary role in COVID-19 treatment (Luo et al., 2020; Muhseen et al., 2020). In a timely review, Bailly and Vergoten (2020) have critically analyzed the prospective development of glycyrrhizin analogues not only as antiviral drugs, but also as adjuvant therapy for their protective effects on the vulnerable organs in patients suffering from SARS-CoV-2 infection (Bailly and Vergoten., 2020). Taking all this into consideration it is a high time to explore this time-tested safe phytochemical against SARS-CoV-2-3CL^{pro} to an approved drug in future.

Lycorine

Lycorine is a bioactive constituent of Amaryllidaceae family of plants, like *Hymenocallis littoralis* (Jacq.) Salisb., *Lycoris radiata* (L'Hér.) Herb., and *Narcissus pseudonarcissus* L. cv. Dutch Master, which are traditionally used in many countries for wound healing, and treatment of cancer and infectious diseases (Nair and Van Staden, 2014). In China, the bulbs of *Lycoris radiata* have been traditionally used in the treatment of laryngeal complications, wounds and carbuncles (He et al., 2013). Lycorine potently inhibited flaviviruses in cell culture mainly through suppression of viral RNA replication (Zou et al., 2009). In fact, this alkaloid has been found to exhibit a wide range of antiviral activities against ZIKA, HIV-1, HCV, and SARS-CoV (Liu et al., 2011). A high throughput screening study on two hundred herbal extracts demonstrated *Lycoris radiata* (L'Hér.) Herb. to be the most potent antiviral plant against SARS-CoV with EC_{50} = 2.4 ± 0.2 μg/ml. Further, meticulous fractionation and analysis of *L. radiata* extract led to the identification of lycorine as the active principle, with EC_{50} = 15.7 ± 1.2 nM (Li et al., 2005). In a recent study, the mechanism behind the anti-SARS-CoV-2 activity of lycorine has been attributed to modulating the host factors (Zhang et al., 2020b). Nevertheless, it would be interesting to explore this molecule as a candidate for anti-3CL^{pro} activity in future.

Tanshinone

Tanshinones are a class of phenanthrene-quinone diterpenoids that are the major lipophilic constituents of the root of *Salvia miltiorrhiza* Bunge, a well-known traditional Chinese medicinal herb (Danshen), primarily used for treating cardiovascular and cerebrovascular diseases. More than 40 types of tanshinone molecules have been characterized exhibiting a variety of biological activities in traditional clinical applications (Li et al., 2013), and pharmacological properties such as anti-cancer, antibacterial and antiviral effects (Jiang et al., 2019). Investigation on *S. miltiorrhiza* showed that its lipophilic fraction possesses marked inhibitory activity against both the proteases (3CL^{Pro} and PL^{Pro}) of SARS-CoV, and also found the inhibitory effects of dihydrotanshinone against viral entry in MERS-CoV (Park et al., 2012). In fact, dihydrotanshinone may play a dual role by blocking the entry of coronavirus as well as its post-attachment replication inside the host cell (Kim et al., 2018). Obviously, further validation and clinical trials are needed to establish its antiviral efficacy against SARS-CoV-3CL^{Pro}.

CONCLUSION

Presently, the world has witnessed the extraordinary outbreak of COVID-19 caused by SARS-CoV-2, raising widespread health concerns. Earlier, in 2003, soon after the emergence of SARS-CoV, the X-ray crystallographic structure of SARS-CoV-3CL^{pro} dimer with a covalently bound inhibitor was elucidated. Since then, numerous inhibitors of 3CL^{pro} enzyme, some of these originating from plants, have been identified, although none of them has reached the clinic till this day. In this perspective, we have discussed several traditional medicinal plant-products screened against 3CL^{pro} activity through *in vitro* and *in silico* studies (**Figure 7**). In addition, we have proposed promising plant-products which have not yet been explored for inhibition of 3CL^{pro}. Needless to say that all the prospective molecules would require *in vivo* antiviral assessment and pharmacokinetic evaluation before going for clinical trial. The structural similarity of HIV- and HCV- protease with SARS-CoV-3CL^{pro} is a promising approach to find useful plant products for COVID-19 therapy, as elaborated in this article. Also, the plants known for treatment of respiratory disorders have been suggested in this regard. Thus, it is a challenge to repurpose and develop these natural products into potent, low molecular weight inhibitors of SARS-CoV-3CL^{pro}, with minimum toxicity, to combat the

onslaught of emerging coronavirus diseases. We hope that this review will be useful for phytochemists and virologists targeting $3CL^{pro}$ to identify novel therapeutics against SARS-CoVs.

AUTHOR CONTRIBUTIONS

AM and BH designed the study and collected the information. AM and AJ wrote the article. AM revised the text under supervision of BH. All authors contributed to the article and approved the submitted version.

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REVIEW
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Fever and Antipyretic Supported by Traditional Chinese Medicine: A Multi-Pathway Regulation

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The coronavirus disease, 2019 (COVID-19), has spread rapidly around the world and become a major public health problem facing the world. Traditional Chinese medicine (TCM) has been fully committed to treat COVID-19 in China. It improved the clinical symptoms of patients and reduced the mortality rate. In light of the fever was identified as one of leading clinical features of COVID-19, this paper will first analyze the material basis of fever, including pyrogenic cytokines and a variety of the mediators of fever. Then the humoral and neural pathways of fever signal transmission will be described. The scattered evidences about fever recorded in recent years are connected in series. On this basis, the understanding of fever is further deepened from the aspects of pathology and physiology. Finally, combining with the chemical composition and pharmacological action of available TCM, we analyzed the mechanisms of TCMs to play the antipyretic effect through multiple ways. So as to further provide the basis for the research of antipyretic compound preparations of TCMs and explore the potential medicines for the prevention and treatment of COVID-19.

Keywords: COVID-19, fever, antipyretic, traditional chinese medicine, mechanism, bioactive components

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INTRODUCTION

COVID-19 has become a major threat to worldwide public health, having rapidly spread to more than 180 countries and infecting over 1.6 billion people. Fever, cough, diarrhea, and fatigue are the most common initial symptoms of COVID-19 (Luo et al., 2020a). However, fever was identified as leading clinical feature. A study of the clinical progression of COVID-19 patients in Shanghai, China, included 249 confirmed cases of COVID-19 from Jan 20 to Feb 6, 2020. The research found that as high as 94.3% of the patients, including those who were afebrile on admission had fever. The estimated median duration of fever in all the patients with fever was 10 days after onset of symptoms. Patients who were transferred to ICU had significantly longer duration of fever as compared to those who were stable, up to 31 days (Chen et al., 2020). Clinical data from another study showed that only 43.8% of patients presented with a fever, but 88.7% developed a fever after hospitalization, indicating the afebrile patients may be at the early stage of the disease (Guan et al., 2020). Therefore, preventive treatment should be carried out for a large number of suspected cases and their close contacts in order to reduce the possibility of infection and block the spread of COVID-19. In addition, timely treatment of confirmed patients can prevent further deterioration of the disease, reduce the chance of patients with mild symptoms becoming serious. In light of fever was the most common initial

TABLE 1 | Descriptive table of the Chinese herbal medicines mentioned in this paper.

Scientific name	English name	Common name	Local chinesename	Parts used
Bupleurum chinense DC. and *Bupleurum scorzonerifolium* Willd	Bupleuri radix	Chinese thorowax root	Chai-hu	Root
Scutellaria baicalensis Georgi	Scutellariae radix	Baical skullcap root	Huang-qin	Root
Conioselinum anthriscoides 'Chuanxiong' (syn. *Ligusticum chuanxiong* Hort)	Chuanxiong rhizome	Rhizoma ligustici wallichii	Chuan-xiong	Root
Cinnamomum cassia (L.) J.Presl	Cinnamomi ramulus	Cassia twig	Gui-zhi	Branch
Forsythia suspensa (Thunb.) Vahl	Forsythiae Fructus	Forsythia	Liao-qiao	Fruit
Lonicera japonica Thunb	Lonicera japonica Flos	Honeysuckle flower	Jin-yin-hua	Flower
Ephedra sinica Stapf, *Ephedra intermedia* Schrenk et C. A. Mey and *Ephedra equisetina* Bunge.	Ephedrae Herba	Ephedra erial parts	Ma-huang	Erial parts
	Gypsum Ustum	Gypsum	Shi-gao	
Pueraria montana var. lobata (Willd.) Maesen & S.M.Almeida ex Sanjappa & Predeep (syn. *Pueraria lobata* (Willd.) Ohw)	Puerariae Lobatae radix	Kudzu root	Ge-gen	Root
Bubalus bubalis Linnaeus	Bubali Cornu	Buffalo horn	Shui-niu-jiao	Horn
Houttuynia cordata Thunb	Houttuyniae Herba	Cordate houttuynia	Yu-xing-cao	Herb
Gardenia jasminoides J.Ellis	Gardeniae Fructus	Gardenia	Zhi-zi	Fruit
Andrographis paniculata (Burm.f.) Nees	Andrographis Herba	Herba andrographitis	Chuan-xin-lian	Herb

symptom in patients with COVID-19, it is of great significance to understand the mechanisms of fever and take antipyretic measures for diagnosis, treatment and prognosis of COVID-19 patients.

Currently, the treatment of fever includes physical cooling and antipyretic medications such as NSAIDs and Paracetamol. Research has shown that patients with moderate fever should avoid active cooling because it increases the metabolic rate, activates the autonomic nervous system, and provokes thermal discomfort (Lenhardt et al., 1999). In addition, on March 16th, 2020, the French Minister of Health has announced that NSAIDs may worsen clinical conditions of patients with COVID-19 based on the evaluation of four patients affected by the disease (Capuano et al., 2020). Some authors have suggested that NSAIDs, particularly Ibuprofen, may induce increased sensitivity to more severe clinical features in COVID-19 infection (Laura et al., 2020). They argued that coronaviruses bind to angiotensin-converting enzyme-2 (ACE-2), and ibuprofen administration can increase the bioavailability of ACE-2, thus potentiating and enhancing the infectious processes of coronaviruses (Fang et al., 2020). Since nowadays no scientific evidence establishes a correlation between NSAIDS and the worsening of COVID-19, patients should be advised against NSAIDs when COVID-19 like symptoms begins (Fang et al., 2020). Although it has been suggested that patients could take paracetamol to treat the symptoms of COVID-19, overdose or long-term use of Paracetamol can also produce the largely irreversible hepatotoxicity and incipient gastric toxicity (Whitehouse and Butters, 2014). Therefore, it is essential to explore a safe, effective, and low toxicity treatment method. TCM has attracted global attention due to its low toxicity and high efficacy. According to clinical observations, the common symptoms of fever subsided by more than 90% of the 3,698 patients with COVID-19 within 1.74 days on average when the indicated TCM formulation was commenced (Li et al., 2020). TCM has been used to treat fever for more than 2000 years. Many years of clinical observations and several published studies suggest single Chinese medicine and compound preparations of TCM have specific antipyretic effects; these include Bupleuri Radix (Idrisusman et al., 2010), Scutellariae Radix (Tsai et al., 2006), Lonicera Japonica Flo (Xie et al., 2009), Shuang-Huang-Lian injection (Huang et al., 2019), Qingkailing injection (Zhang et al., 2017) and so on.

In this paper, through the systematic analysis of the mechanisms of fever, combined with the chemical composition and pharmacological action of TCM (**Table 1**), we analyzed the material basis, mechanisms, and characteristics of the antipyretic action of single TCM and its compound preparations to deepen the theory of TCM from the micro-level, and to develop safer and effective antipyretic preparations of TCM, as well as improve the treatment level and promote the rehabilitation of patients with COVID-19.

THE DEFINITION OF FEVER

After three revisions, the Commission for Thermal Physiology of the International Union of Physiological Sciences defined fever as the elevation of the set-point of body temperature due to a change in the thermo controller characteristics. It is usually part of the defense response of organism (host) to the invasion of pathogenic or foreign living (microorganism) or inanimate substances. At this level, the core temperature will be maintained for a period of time (Sciences, 2003). In a normal healthy individual, the thermal regulatory network of the body maintains a temperature of 36.2–37.5°C (Prajitha et al., 2019). Many medical and clinical studies regard rectal temperature of ≥38°C or axillary temperature ≥37.5°C as indicative of fever (Shu et al., 2016). It is worth noting that not all temperature increases can be defined as fever. Clinically, there are 2 cases of typical temperature increase: fever and hyperthermia. Contrary to fever, in hyperthermia, the set-point is unchanged; it occurs in response to specific environmental, pharmacologic, or endocrine stimuli. The elevated body temperature that occurs in hyperthermia

syndrome can exceed 41.0°C (Niven et al., 2013). Hyperthermia does not respond to typical antipyretics since there are no pyrogenic molecules (Dewitt et al., 2017); this distinguishes fever from hyperthermia.

THE MATERIAL BASIS OF FEVER

Pyrogenic Cytokines

In 1948, Beeson obtained a substance from the granulocytes of the sterile peritoneal exudate of rabbits, which would raise the body temperature of normal rabbits after injection (Beeson, 1948). Subsequently, similar substances were discovered in other febrile animal models, resulting in the release of an elaborated endogenous pyrogen in consequence of the stimulation by exogenous pyrogens (Dinarello, 1999). Howeve, some endogenous substances, such as autoantibody complexes, inflammatory bile acids, may act as pyrogen without exogenous pyrogens induction (Sajadi, 2015). With the acceptance of the term cytokine, the term endogenous pyrogen is no longer appropriate. In order to differentiate cytokines that are intrinsically pyrogenic from those that are not, it is more appropriate to define them as pyrogenic cytokines (Dinarello, 2004).

The pyrogenic cytokine is a part of the autoimmune system. Invasion of a host by exogenous pyrogens triggers a series of immune responses through pathogen-associated molecular patterns (PAMPs), including LPS, lipoarabinomannans, lipoteichoic acid, and viral RNA (Evans et al., 2015; Kumar et al., 2011). PAMPs act through pattern recognition receptors, such as toll-like receptors (TLRs), on immune cells to induce the release of pyrogenic cytokines (Prajitha et al., 2018). The currently recognized major pyrogenic cytokines are interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor α (TNF-α) (Conti et al., 2004). Systemic injection of LPS in animal models can increase the release of these cytokines into the general circulation (Harré et al., 2002). LPS stimulate TLRs, specifically TLR4, inducing the release of pyrogenic cytokine that induce fever.

IL-1

IL-1 is a prototypical inflammatory cytokine for neuroimmune communication. Discovery of the actions of IL-1 in the macrophages, fibroblasts B cells, endothelium, and large granular lymphocytes, showed that IL-1 represents two different molecular forms (IL-1α and IL-1β) and an endogenous IL-1 receptor antagonist (IL-1RA) (Equils et al., 2020). The study demonstrated that IL-1 is essential in the induction of fever as a central injection or intraperitoneal injection of IL-1RA caused significant inhibition of LPS-induced fever (Miller et al., 1997; Smith and Kluger, 1992). In addition, it has been shown that many species can cause fever response to peripheral injection of IL-1α and IL-1β (Dinarello, 1996; Kluger, 1991). The present explanation for this is that IL-1 induces intermediates, prostaglandin E2 (PGE2), and cyclooxygenase-2 (COX-2), which are considered necessary downstream events that mediate peripheral IL-1-induced fever (Ching et al., 2007; Li et al., 2001). IL-1 induced COX-2 and PGE2 depends on the expression of the mitogen-activated protein kinase kinase kinase 7 (MAP3K7) also known as TAK1. It is most likely to induce COX-2 by activating MAPKP38 and c-Jun, which are necessary for fever induction (Ridder et al., 2011). IL-1 activates the inhibitor of nuclear factor B (IκB) kinase 2 (IKK2), which binds to polyubiquitin chains on several upstream molecules, includingTAK1 and TAB3. Activated IKK phosphorylates nuclear factor-κB (NF-κB) inhibitor α (IκBα) and then activates NF-κB (Weber et al., 2010). The rapid activation of the NF-κB pathway induced by IL-1 has been proven to be the cause of COX-2 production in cerebrovascular endothelial cells (Nadjar et al., 2005). Therefore, IL-1 can also induce the production of COX-2 and PGE2 by activating the NF-κB pathway.

IL-6

IL-6 is produced under a variety of stimuli including tissue damage, viruses, or other proinflammatory cytokines. It is secreted by innate immune cells, as well as endothelial cells, fibroblasts, astrocytes, and epithelial cells (Rincon, 2012). The IL-6 during inflammation and infection is induced via stimulation of cells by IL-1 or TNF-α or through stimulation of TLRSs after binding of PAMPs (Kang et al., 2019). Receptors for IL-6 exist in two forms: a soluble receptor (sIL-6R) and a membrane-bound receptor (IL-6R) (Uciechowski and Dempke, 2020). Studies have shown that IL-6 knockout mice, as well as in animals treated with IL-6 antiserum, produced no febrile response to the peripheral immune response, suggesting that the existence of IL-6 is very important for fever (Nilsberth et al., 2009). IL-6 produced by non-hematopoietic cells is the key component of LPS induced fever; IL-6 produced by hematopoietic cells plays a secondary role in the production of systemic IL-6. However, the phenotype of these cells is unknown and may involve multiple cell types, requiring further study (Hamzic et al., 2013). In recent years, a new study has shown that tissue macrophages are not involved in the early IL-6 response to LPS. CEACAM1, a molecule ubiquitously expressed in the epithelium, neutrophils, activated lymphocytes, negatively regulates the early response of IL-6 to LPS in murine monocytes through the RP105 signaling pathway (Zhang Z. et al., 2019). Therefore, CEACAM1 may be a potential drug target for antipyretic. The present study also sheds light on the issue of the central administration of IL-6 via PGE2 to induce fever (Harden et al., 2008). Recent studies have further confirmed this view that IL-6 binds to IL-6 receptors on brain endothelial cells, and ligand binding induces the expression of the prostaglandin synthase COX-2 through signals involving activator of transcription 3 (STAT3) pathway (Eskilsson et al., 2014).

TNF-α

TNF is a cytokine produced naturally by macrophages in response to bacterial infection or other immune sources. According to its source and structure, it can be divided into two types: TNF-α and TNF-β. The former is principally produced by macrophages, T cells, and natural killer cells (Zelová and Hošek, 2013). TNF-α is the first member of cytokine cascade

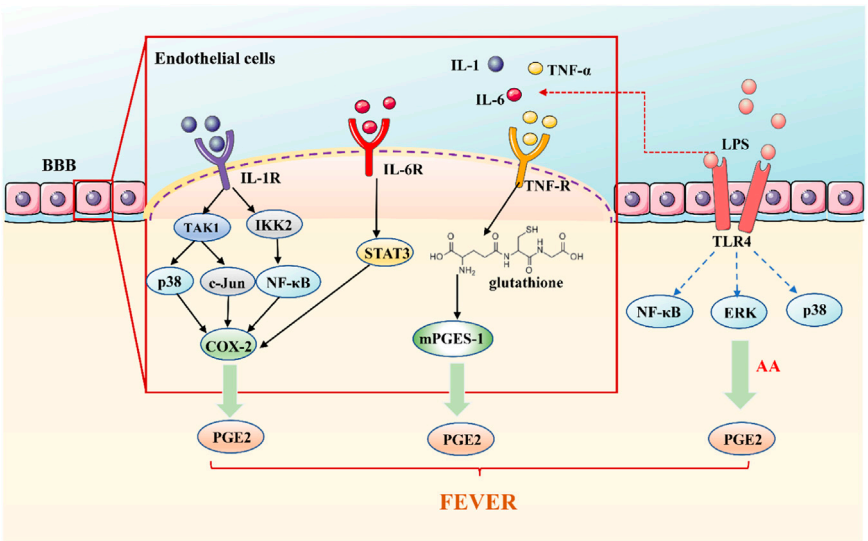

FIGURE 1 | The Humoral Transmission Pathway of Fever Signals. Circulating PAMPs, represented by LPS bind to TLR-4 on the fenestrated capillaries in the BBB. Triggering TLR-4 induces the transcription of COX-2 to converted into PGE2, causing fever; pyrogenic cytokines, TNF-α, IL-1, and IL-6, play a role outside the brain by activating cytokine receptors located on the CVO, resulting in the release of PGE2 to cause fever.

induced by injection of LPS (Roth and Blatteis, 2014). It has been reported that LPS mediated transcription of TNF-α can be divided into two main signaling pathways. The first proceeds through the NF-κB-inducing kinase route, which regulates the phosphorylation of the inhibitory-κB proteins. The second pathway is mediated by the extracellular signal-regulated kinase and MAPKp38 pathways (Haddad and Land, 2002). Peripheral injection of TNF-α in human and experimental animals can rapidly cause fever (Michie et al., 1988; Roth et al., 1998). Intravenous injection of human recombinant TNF (rhTNF) caused fever in rabbits, which also revealed that the pyrogen potential of rhTNF was associated with an increase in PGE2; the mechanism is related to glutathione. The regulation of TNF-α biosynthesis induced by LPS is redox-sensitive and requires the participation of the glutathione mediated signaling pathway (Wrotek et al., 2015). In the presence of glutathione, it can activate the activity of PGE synthase-1 (mPGES-1), to produce PGE2 (Saha et al., 2005; Thorén and Jakobsson, 2000). A number of researchers also reason that TNF-α induces IL-1 *in vivo*; therefore, TNF-α and IL-1 may play a synergistic role in fever production; however, no specific mechanism has been reported.

Others

In addition to the typical pyrogenic cytokines described above, some intrinsic cytokines play a role in fever production (Billiau and Matthys, 2009). Studies have shown that intravenous ET-1 can increase the body temperature of rats. In addition, injection of ET-1 into AH/POA also causes fever, indicating that ET-1 is important for fever response (Zampronio et al., 2015). Leptin is an adipocyte-derived hormone that induced the proinflammatory cytokine IL-1β in the brain, resulting in a prostaglandin-dependent fever (Wisse et al., 2004). Substance P (SP) belongs to tachykinin family. When SP is antagonized by peptide SP analogues, fever response induced by LPS for guinea pig and rat is blocked, which indicates the role of SP in fever (Pakai et al., 2018).

The Mediator of Fever

A standard linkage was shared by nearly all pyrogenic cytokines above: the production of PGE2. Previous studies have shown that COX-2 and PGE2 induction is required for fever (Pecchi et al., 2009). In addition to PGE2, other mediators can also induce the generation of fever. Free radicals, glutamate, and metabolic pathway disorders also play a role in fever.

PGE2 in Fever

The febrile response is characterized by an early rapid phase and a delayed late phase (Blatteis et al., 2004). In some experiments, the pyrogenic cytokines were released later, for example, TNF-α was detected 30 min after LPS IV injection, while the level of PGE2 increased rapidly after LPS IV injection, and then increased further 40 min later (Blatteis, 2006; Callery et al., 1991). PGE2 is a lipid-soluble substance that can pass through the blood-brain barrier (BBB), while pyrogenic cytokines are relatively large, lipophobic peptides, and cannot freely pass through the BBB. Therefore, pyrogenic cytokines may not be able to provide rapid fever signals and be involved in maintaining fever (Blatteis et al., 2005). We can speculate that PGE2 is the first to be initiated in the beginning stage of fever, and is the crucial mediator of fever.

Although it seems definite that PGE2 is an essential mediator of fever, it is not certain whether the PGE2 is of the peripheral or central origin. Which stages of febrile pathogenesis are mediated by the peripheral PGE2, and which stages are mediated by the central, these questions require further investigation. The study has shown that the immediate appearance of PGE2 in inferior vena cava plasma after the IV of LPS and puts forward a

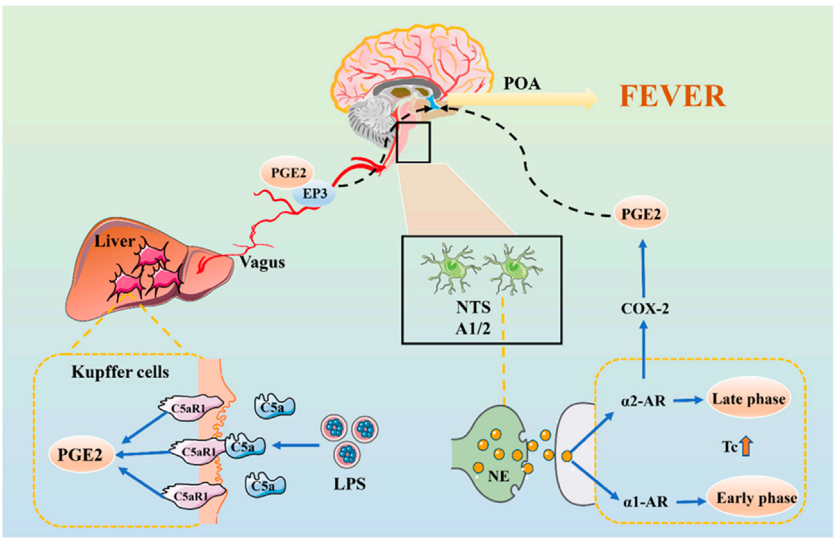

FIGURE 2 | The Neural Transmission Pathway of Fever Signals. The initial stage of fever may be mediated by peripheral PGE2, which is released by KC stimulated by LPS-activated C5a and binding to EP3 receptors. PGE2 is transmitted to the NTS via vagal afferents and is further transmitted via the ventral noradrenergic bundle to the POA, wherein NE is released.

hypothesis that LPS-activated complement triggers the release of PGE2 by KC (Perlik et al., 2005). The complement component 5a (C5a) is an essential mediator of the febrile response to LPS (Li et al., 2002). C5aR1 is expressed by KC and rapidly activates COX-1-catalyzed PGE2 production (Schieferdecker et al., 1999; Schieferdecker et al., 2001). PGE receptors have four subtypes, EP1, EP2, EP3, and EP4. Studies have shown that EP3 receptors are of great importance in the febrile response. The organum vasculosum of the lamina terminalis (OVLT) and preoptic-anterior hypothalamic area may be the sites where PGE2 acts on EP3 receptors to generate fever (Oka, 2004). In summary, the initial stage of fever may be mediated by peripheral PGE2, and the rapid transmission of fever signals may depend on neural pathways (see below).

PGE2 is also produced by endothelial cells in the brain and released from the arachidonic acid pathway. This pathway is mediated by the enzymes phospholipase A2 (PLA2), COX-2, and mPGES-1 (Blomqvist and Engblom, 2013; Wilhelms et al., 2014). Brain endothelial cells express IL-1 receptor type 1 (Konsman et al., 2004) and TNF-α receptor p55 (Nadeau and Rivest, 1999). IL-6R is normally absent in the brain endothelial cells, but also induced by inflammation. However, even if there is no membrane-bound IL-6R, soluble IL-6R in blood may participate in IL-6 signal transduction through gp130, a constitutively expressed IL-6 receptor signal sensor in endothelial cells (Vallières and Rivest, 1997). COX-2 and mPGES-1 are the target genes of NF-κB and STAT3, and the activation of NF-κB or STAT3 in brain endothelial cells is related to COX-2 and mPGES-1 in these cells (Rummel et al., 2006). Previous studies have stated that pyrogenic cytokines can induce the production of COX-2 and mPGES-1 through various signaling pathways, so the PGE2 produced by brain endothelial cells is primarily mediated by the effect of these pyrogenic cytokines. However, the generation of PGE2 by COX-2/mPGES-1 did not coincide with the fever response (Steiner et al., 2006b). Therefore, although peripheral synthesis of PGE2 may occur in initiating the fever, central synthesis of PGE2 may participate in its maintenance.

Oxygen Free Radicals and Glutamate in Fever

The oxygen free radicals include superoxide anion, hydrogen peroxide, and hydroxyl radical. Dose dependent increase of hydroxyl radical level and core temperature in OVLT induced by LPS or glutamate (Huang et al., 2006). It is suggested that the increase of body temperature caused by LPS or glutamate is related to the increase of hydroxyl radicals in OVLT. Pretreatment with hydroxyl radical scavengers significantly reduced the increase of hydroxyl radicals and fever induced by LPS. Glutamate excessively activation of N-methyl-D-aspartate (NMDA) receptor can produce reactive oxygen species (ROS) in the brain (Yang et al., 1996). After pretreatment with NMDA receptor antagonists, the fever and the increase of hydroxyl radicals in OVLT decreased significantly after LPS injection (Hou et al., 2011). In conclusion, LPS or glutamate may cause excessive accumulation of hydroxyl radicals in peripheral blood and CSF, which can be inhibited by hydroxyl radical scavengers or NMDA receptor antagonists. There is evidence that ROS can activate NF-κB as a second messenger, leading to over induction of COX-2 (Oh et al., 2004). After LPS injection, the ROS in the hypothalamus can stimulate the activation of NF-κB and the expression of COX-2, resulting in the excessive production of NO and PGE2, thus causing fever (Huang et al., 2006). The role of oxygen free radicals and glutamate in a fever not only provides a new theoretical basis for people to understand the mechanism of

fever but also suggests that anti-free radical injury could be a new way to study the mechanism of fever in the future.

Metabolic Disorders in Fever

A febrile response is a systemic pathological process. In recent years, metabonomics has been widely used in the study of febrile response to reveal the pathological mechanism of this system response. Three methods were used to establish fever model in rats, and the characteristics of plasma metabolism in febrile rats were studied: the TCM-induced rats with fever, yeast-induced rats with fever, and 2,4-dinitrophenol-induced rats with fever, to further investigate the common potential biomarkers in rats with fever (Liu et al., 2017b). The changes in plasma metabolites showed that amino acid, fatty acid amides, phospholipid, sphingolipid, fatty acid oxidation, and glycerolipid metabolisms; also, bile acid biosynthesis were related to fever. It has been confirmed that tryptophan metabolism is important in the metabolic disorder in the fever response (Gao et al., 2013b). Tryptophan, an essential amino acid, is the precursor of 5-hydroxy tryptamine (5-HT) (Guo et al., 2014). It was reported that the 5-HT in the hypothalamus was positively correlated with fever induced by yeast (Peindaries and Jacob, 1971). The increased level of tryptophan in the febrile rats leads to the enhancement of febrile response may be due to the increased ability of tryptophan to synthesize 5-HT. γ-aminobutyric acid (GABA) and phosphatidylinositol were also increased in the urine of febrile rats (Gao et al., 2013b). The increase of GABA could lead to an increase of temperature through the pathway of $Na^+/Ca2^+$-cAMP in the hypothalamus (Myers et al., 1976; Romei et al., 2012). The increased cAMP can inhibit the phosphoinositide signaling system and further lead to the significant increase of phosphatidylinositol. Therefore, the disorder of tryptophan metabolism in the process of fever can increase the synthesis of 5-HT and cAMP, and further induce the generation of fever.

Metabonomics is widely used in finding new biomarkers of diseases and revealing the potential mechanism of clinical drugs. At present, it is mainly based on the biomarkers of three kinds of biological liquid samples, serum, plasma, and urine, to reflect the changes in body metabolism. However, because the BBB can control the transfer of molecules between the brain and blood, it is suggested that the metabonomic methods of serum, plasma, and urine should be combined with the metabonomics methods of the brain to explore the antipyretic mechanism of TCM. The research results of metabonomics have created a foundation for the treatment of fever by TCM from different perspectives. They indicate whether we can take a nutritional supplement for the imbalance of essential amino acids, fatty acids, phospholipids, and other substances as a new way of fever treatment in the future.

TRANSMISSION PATHWAY OF FEVER SIGNALS

The fever activator stimulates the immune cells to produce the pyrogenic cytokines, which are needed to cause fever through PGE2. At the same time, other central media, independent of PGE2, will also act on the thermoregulatory center to cause fever. At present, with the study of these fever signals and their role when entering the center, it was concluded that the fever response was ultimately regulated in two ways: the humoral and neural pathways (Zeisberger, 1999).

The Humoral Transmission Pathway of Fever Signals

A classical theory of fever signal transmission is the theory of the humoral transmission pathway. Fever signals are carried by PAMPs or by pyrogenic cytokines in this pathway (**Figure 1**) (Ogoina, 2011). It is known that circulating PAMPs represented by LPS bind to TLR-4, which is located in the fenestrated capillaries of the circum ventricular organ in the BBB. Triggering TLR-4 induces the transcription of COX-2 via NF-κB, MAPKp38, and extracellular signal-regulated kinases (ERK1/2).Also cPLA2 phosphorylation and arachidonic acid mobilization, via MAPK or Mitogen- and stress-activated kinases (MSK1), which is, in turn, converted into PGE2 by COX-2 and released into the cell membrane (Salvi et al., 2016); which through the BBB, then activate the thermal neurons in the front of the hypothalamus, causing fever (Steiner et al., 2006a; Turrin and Rivest, 2004).

According to the classical concept, exogenous pyrogens stimulate peripheral mononuclear phagocytes to produce pyrogenic cytokines, principally TNF-α, IL-1, and IL-6, which are transported from the blood to the brain (Blatteis, 2006; Blatteis et al., 1998). Pyrogenic cytokines are unable to enter the center to stimulate the brain structures through the BBB (Rothwell et al., 1996). At present, there are several opinions on how pyrogenic cytokines are involved in the brain. Pyrogenic cytokines may directly penetrate the sensory circumventricular organs (CVOs) without BBB. Pyrogenic cytokines bind to and activate cytokines receptors on the fenestrated capillaries of the CVO which act outside the brain, leading to the release of PGE2 (Conti et al., 2004; Ogoina, 2011). Vascular endothelial cells and perivascular cells within the entire brain, have been shown to secrete IL-6, PGE2, and other mediators into the brain parenchyma after being stimulated by inflammation in the lumen (blood) side (Blatteis et al., 2000; Roth and Blatteis, 2014; Schiltz and Sawchenko, 2003). In the humoral pathway of fever signal transmission, pyrogenic cytokines can enter the brain directly through the tissue lacking BBB to cause fever or to release PGE2 outside the brain indirectly or stimulate the BBB cells to deliver fever medium to the brain's endocrine system and cause fever.

Due to initial detection of pyrogenic cytokines in the blood does not coincide with the fever caused by IV LPS, the hypothesis of the humoral pathway is not enough to explain the whole process of fever signal transmission. Therefore, an accelerated pathway of fever signal transmission, the neural pathway, has been proposed. The neural pathway, as a supplementary pathway of the humoral pathway, functions in the conduction of fever

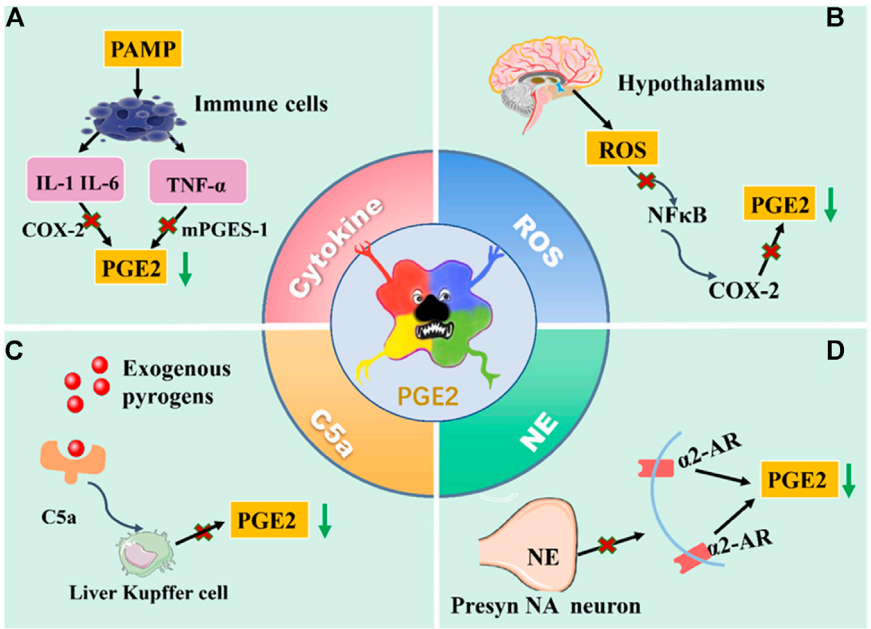

FIGURE 3 | The production way of PGE2. **(A)** PAMPs induces pyrogenic cytokines through pattern recognition receptors such as TLR on immune cells. IL-1and IL-6 can activate COX-2 to produce PGE2; TNF-α activates the mPGES-1 and produces PGE2. **(B)** In the fever state, the overproduction of ROS in the hypothalamus stimulates the activation of NF-κB and expression of COX-2, leading to the generation of PGE2. **(C)** LPS-activated complement can rapidly trigger KC to activate COX-1 to catalyze PGE2 production. **(D)** NE induces the production of COX-2/mPGES-1-dependent PGE2 in the POA via an α2-AR-mediated mechanism. Above, the antipyretic effect was achieved by blocking the production pathway of PGE2 and reducing the content of PGE2.

signals in coordination with the humoral pathway. The conduction process of the two pathways is complex, so the mechanism of their synergistic effect requires further study.

The Neural Transmission Pathway of Fever Signals

The activation of the neural pathway is believed to be another mechanism for fever (**Figure 2**). The idea of a neural transmission pathway concerned with fever emerged decades ago. In 1987, Morimoto speculated that peripheral nerves were involved in the development of fever (Morimoto et al., 1987). The messages, utilizing nerve fibers, have the advantages of rapid transmission speed and not being affected by the impedance of the BBB. The rapidity of neural communication between the immune system and the brain seems to be crucial at the beginning of fever (Romanovsky et al., 2000).

Previous studies have shown that bilateral truncal subdiaphragmatic vagotomy or intraperitoneal injection of low doses of capsaicin can desensitize the abdominal sensory nerve and inhibit the fever caused by IV LPS in rats (Romanovsky et al., 1997; Székely et al., 2000). The anterior and posterior branches of the vagus nerve are divided into five main branches under the diaphragm. We have shown that the selective hepatic branch vagotomy plays an important role in the early febrile phase (Simons et al., 1998). The circulating LPS activates KC to produce a factor that stimulates cognate receptors on hepatic vagal afferents, and quickly transmits its fever information to POA (Blatteis, 2006). In the early stage of fever, it may be mediated by peripheral PGE2, which is released by KC stimulated by LPS-activated C5a and binding to EP3 receptors on sensory hepatic vagal afferents.

There may be another mechanism for nerve conduction of fever signals. LPS induce c-fos expression in the nucleus of the solitary tract (NTS) (Wan et al., 1994). PGE2 is produced in the peripheral, introduced into the NTS through vagal afferents and is further transmitted to POA through the ventral noradrenergic bundle, in which norepinephrine (NE) is released (Roth and Blatteis, 2014). The role of the central noradrenergic system in thermoregulation has also been confirmed. NE induces two differentially mediated temperature rises: the first develops promptly and is mediated by the α1-adrenergic receptor (α1-AR), but not by PGE2 (Roth and Blatteis, 2014). The second is mediated by α2-AR and NE induces the production of COX-2/mPGES-1-dependent PGE2 in the POA via an α2-AR-mediated mechanism which is significantly later than the first (Blatteis, 2006). PGE2 also enhances the production of cAMP of hypothalamic cells, which can change the temperature set-point.

Generally speaking, fever is the result of complex and periodic interactions between the fever medium and the body. The mechanism of its occurrence is complex. From the material basis of fever and the transmission of fever signals, PGE2 is the vital medium of fever, whether infectious or noninfectious fever. Its means of production are 1) invasion of exogenous pyrogen to the host triggers a series of immune responses through PAMPs. PAMPs induces pyrogenic cytokines (IL-1, IL-6, and TNF- α) through pattern recognition receptors such as TLR on immune cells. IL-1 can activate MAPKp38 and c-Jun by TAK1 to induce COX-2 and produce PGE2; it can also induce

COX-2 and PGE2 by activating the NF-κB pathway. After binding IL-6 with IL-6R on brain endothelial cells, COX-2 expression can be induced by intracellular signaling of the STAT3 pathway to produce PGE2. TNF-α activates the mPGES-1 and produces PGE2 under the glutathione mediated signal pathway. 2) In the fever state, the overproduction of ROS in the hypothalamus stimulates the activation of NF-κB and expression of COX-2, leading to the generation of PGE2. 3) LPS-activated complement can rapidly trigger KC to activate COX-1 to catalyze PGE2 production. 4) NE induces the production of COX-2/mPGES-1-dependent PGE2 in the POA via an α2-AR-mediated mechanism (as shown in **Figure 3**).

The humoral pathway transmits the first two kinds of fever signals; the neural pathways mediate the rest. The above understanding of the fever mechanism also broadens a new way of thinking for antipyretic. The antipyretic effect can be achieved by inhibiting the production of PGE2 in various ways and blocking its role in the fever pathway. Recent research data on the fever medium indicates that some endogenous mediums may cause fever. These studies show that in addition to PGE2, the mediators produced by the center also participate in the fever response. At present, the corticotropin-releasing factor, endothelin, and macrophage inflammatory protein one are the most studied central agents of fever. However, whether the antipyretic effect is independent of PGE2 is still controversial. Therefore, the antipyretic mechanism needs further study.

SINGLE CHINESE HERBAL MEDICINES IN THE TREATMENT OF FEVER

Bupleuri Radix

Bupleuri Radix (BR), called Chaihu in Chinese, is the dried roots of *Bupleurum chinense* DC and *Bupleurum scorzonerifolium* Willd. The compounds of BR include essential oils, triterpenoid saponins, polyacetylenes, flavonoids, lignans, fatty acids, and sterols (Yang et al., 2017). Jin et al. (2014) researched the effect of BR water extract, BR saponin extract, and BR essential oil extract on the fever model of rats via subcutaneous injection of a yeast suspension. It was observed that the three extracts had a good antipyretic effect. Chen et al. (2010) extracted the essential oil from BR to prepare the gel and sprayed it into the nasal cavity of febrile rabbits that had undergone IV injected *Escherichia coli* endotoxin. The results showed that the essential oil of BR could play an antipyretic effect on the rabbit's fever by decreasing the concentration of cAMP in the cerebrospinal fluid. One study demonstrated that intracerebroventricular injection of saikosaponin A (SSA) reduces the core temperature of rats with fever caused by IL-1β after 30 min. At the same time, the content of cAMP in serum and protein kinase A (PKA) in the cytoplasm was significantly lower than those in the model group. The results showed that SSA could be used as an antipyretic by reducing cAMP secretion and PKA activity in the hypothalamus (Sun et al., 2016). The study demonstrated that SSA can significantly inhibit the expression of TNF-α, IL-1β, and IL-6; it also inhibits the activation of the NF-κB signaling pathway by suppressing the phosphorylation of inhibitory IκBα (Zhu et al., 2013). The above studies showed that BR could play an antipyretic role by inhibiting the secretion of cAMP, the expression of pyrogenic cytokines, the activity of PKA, and the activation of the NF-κB signaling pathway.

Scutellariae Radix

Scutellariae Radix (SR), known as Huangqin in Chinese, is the dried root of the Labiatae plant *Scutellariae baicalensis* Georgi (Bruno et al., 2002). Flavonoids, phenylethanoid glycosides, iridoid glycosides, alkaloids, phytosterols, and polysaccharides are the main compounds of SR (Li et al., 2011). SR has an antipyretic effect in the clinical setting. In recent years, the antipyretic mechanism of SR and its components have been studied intensely. The results show that baicalin and baicalein are main active components of SR for reducing fever (Yang and Meng, 2009). The body temperature of rats with fever-induced by yeast decreased significantly after the administration of baicalin by gavage. The contents of IL-6, IL-1β, and TNF-α in serum, hypothalamus, and CSF of rats decreased correspondingly, indicating that baicalin has an antipyretic effect by reducing the content of pyrogenic cytokines (Li and Ge, 2010). Baicalin was used to treat rats with fever induced by intraperitoneal injection of LPS. The results showed that baicalin significantly reduced the body temperatures of the fever rats. The mechanism may be that baicalin can inhibit the up-regulation of TLR4 mRNA and protein expression regulated by LPS, downregulate NF-κB activation and decrease the expression of TNF-α, IL-1β protein (Ye et al., 2015). Baicalin can inhibit the fever response induced by LPS and the excessive production of glutamate and hydroxyl radicals in the hypothalamus caused by central administration of TNF-α. These results suggest that the antipyretic effect of baicalin may be achieved by inhibiting the MNDA receptor-dependent hydroxyl radical pathways in the hypothalamus and circulating TNF-α accumulation during LPS-induced fever (Tsai et al., 2006). Nakahata et al. (1998) found that $Ca2^{+}$ ionophore A23187 caused phosphorylation of MAPK, resulting in the activation of cytosolic phospholipase A2 (cPLA2), baicalein reduces the A23187 induced PGE2 release by inhibition of AA liberation through the inhibition of the MAPK-cPLA2 pathway. Baicalein inhibit the expression of COX-2 induced by LPS in Raw 264.7 cells, through blockading C/EBPb DNA binding to the COX-2 promoter, thereby inhibiting the expression of COX-2 and the production of PGE2 (Woo et al., 2006). In conclusion, SR can play an antipyretic role by inhibiting the release of pyrogenic cytokines and PGE2, the activation of NF-κB, and the excessive production of hydroxyl radicals.

Chuanxiong Rhizome

Chuanxiong Rhizome (CX), also known as Chuanxiong in Chinese, the dried rhizome of Conioselinum anthriscoides 'Chuanxiong' (syn. *Ligusticum chuanxiong* Hort) The main effective components of CX are phthalides, terpenes, polysaccharides, alkaloids, and essential oil (Chen et al., 2018). A large number of studies have shown that the CX essential oil has an antipyretic effect. The body temperature of the rats with fever caused by subcutaneous injection of 20% yeast suspension was significantly decreased after CX essential oil administration;

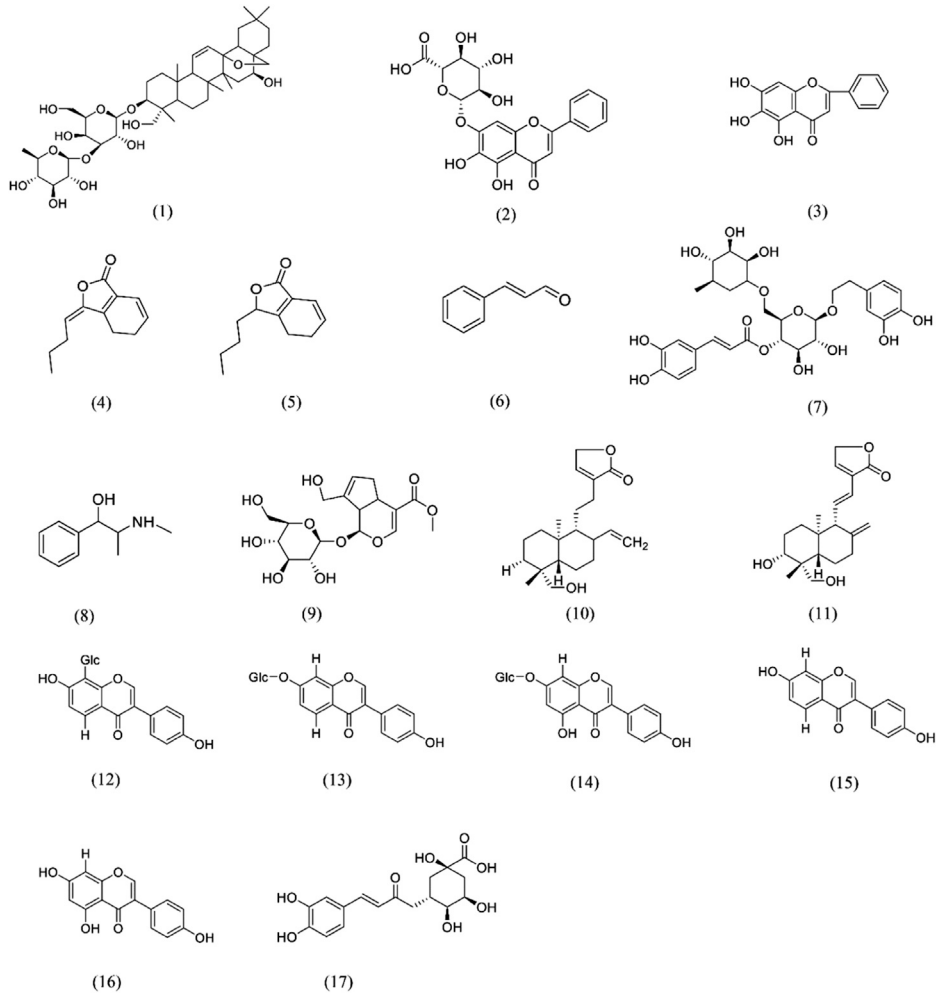

FIGURE 4 | Chemical structures of e phytochemicals that possess antipyretic activity (1)Saikosaponin A; (2) Baicalin; (3) Baicalein; (4) Z-ligustilide; (5) Senkyunolide A; (6) Cinnamaldehyde; (7) Forsythoside A; (8) Ephedrine; (9) Geniposide; (10) Andrographolide; (11) 14-deoxy-11,12-didehydroandrographolide; (12) Puerarin; (13) Daidzin; (14) Genistin; (15) Daidzein; (16) Genistein; (17) Chlorogenic acid.

TABLE 2 | The single Chinese herbal medicine of antipyretic.

Chinese herbal medicine	Scientific name	Bioactive components	Mechanisms
Bupleuri radix	*Bupleurum chinense* DC and *Bupleurum scorzonerifolium* Willd	Essential oilsaikosaponin a (1)	Decrease the concentration of cAMP; Reduce cAMP secretion and PKA activity in the hypothalamus; Inhibit the expression of TNF-α, IL-1β, IL-6 and the activation of the NF-κB signaling pathway
Scutellariae radix	*Scutellaria baicalensis* Georgi	Baicalin (2) Baicalein (3)	Inhibit the upregulation of TLR4 mRNA; Downregulated NF-κB activation with simultaneous decreases in TNF-α and IL-1β protein expression; Suppress glutamate and hydroxyl radicals in the hypothalamus; Inhibit the expression of COX-2 and PGE2
Chuanxiong rhizome	Conioselinum anthriscoides 'Chuanxiong' (syn. *Ligusticum chuanxiong* Hort)	Z-ligustilide (4) senkyunolide a (5)	Inhibit the expression of COX-2 and PGE2; Reduce the content of cAMP in the hypothalamus; Change of monoamine neurotransmitter content in the center; Suppressive effects on TNF-α-mediated NF-κB activation
Cinnamomi ramulus	*Cinnamomum cassia* (L.) J.Presl	Cinnamaldehyde (6)	Up-regulated the expression of TRPV1 in DRG neurons; Reduce the activity of COX-2 in brain endothelial cells and the content of PGE2 in the hypothalamus of febrile rats
Forsythiae Fructus	*Forsythia suspensa* (Thunb.) Vahl	Essential oilForsythoside a (7)	Reduce the content of cAMP in the hypothalamus; Suppress TRPV1 expression and activation, inhibiting MAPKs activation of the hypothalamus and DRG Inhibit TNF-α and NF-κB through blockade of the LPS/TLR4 signaling pathways
Lonicera japonica Flos	*Lonicera japonica* Thunb	—	Inhibit the synthesis of PGE2 by inhibiting the activity of COX-2; Inhibit EP3 expression in POAH

TABLE 3 | The other single Chinese herbal medicine of antipyretic.

Chinese herbal medicine	Scientific name	Bioactive components	Model	Drug delivery cycle	Mechanisms	The species investigated	References
Ephedrae Herba	*Ephedra sinica* Stapf, *Ephedra intermedia* Schrenk et C. A. Mey and *Ephedra equisetina* Bunge.	Ephedrine (8)	Subcutaneous injection of 20% yeast water suspension (10 ml/kg) to induce fever	Ephedrae Herba extracts were administered 8.1 g/kg	Reduce the level of 5-hydroxytryptamine and NE in the hypothalamus	Fifty Wistar rats (weighing 200 ± 20 g)	Wang et al. (2018a)
Gypsum Fibrosum	—	—	Subcutaneous injection of 15% yeast suspension (10 ml/kg) to induce fever	Intragastric administration of Gypsum suspension for 7 days (10 g/kg)	Reduce the synthesis of PGE2	Aged SD rats (weighing200–250 g)	TangZhishu and Bing (2012)
Gardeniae Fructus	*Gardenia jasminoides* J.Ellis	Geniposide (9)	15% saline suspension of yeast was injected in the back of rats (10 ml/kg)	Administered with Gardeniae Fructus at 4.5 g/kg (10 ml/kg)	Reduce the expression of IL-6 and TNF-α; Reduce the production of PGE2	Male SD rats (weighing 170 ± 10 g)	Zhang X. et al. (2019)
Bubali Cornu	*Bubalus bubalis* Linnaeus	—	Fever caused by subcutaneous injection of 20% yeast (10 ml/kg)	400 mg/kg Bubali Cornu powder extract was administrated orally with a dosage of 10 ml/kg	Change the metabolism of uric acid and cysteine; enhance the activity of antioxidant enzymes; reduce the level of TNF - α; reduce the ROS production and PGE2 synthesis	Aged SD rats (weighing 200 ± 20 g)	Liu et al. (2016)
Houttuyniae Herba	*Houttuynia cordata* Thunb	—	Fever caused by subcutaneous injection of 15% yeast suspension (10 ml/kg)	Three hours after the establishment of the model, 20, 10, and 5 ml/kg were administrated intravenously in the high, middle and low dose groups	Inhibit the production of 1L-1, TNF-α, and the expression of PGE2	Aged male SD rats	Zhang et al. (2010)
Andrographis Herba	*Andrographis paniculata* (Burm. f.) Nees	Andrographolide (10)14-deoxy11,12-didehydroandrographolide (11)	Rats were injected subcutaneously with yeast (0.135 g/kg)	Two hours after yeast injection,4 mg/kg of andrographis Herba extracts were injectedIntraperitoneally	Inhibit the expression of NF-kB, reducing the expression COX-2 and the level of PGE2	SD rats	Suebsasana et al. (2009)
Puerariae Lobatae radix	*Pueraria montana var. lobata* (Willd.) Maesen & S.M.Almeida ex Sanjappa & Predeep (syn. *Pueraria lobata* (Willd.) Ohw)	Puerarin (12) daidzin (13) Daidzein (14)Genistin (15) Genistein (16)	Fever caused by subcutaneous injection of LPS (50 mg/kg)	The experimental groups received 50 and 100 mg/kg of pueraria extract	Inhibit cyclooxygenase; inhibit PGE2 release From mouse peritoneal macrophages in vitro	Male mice (weighing 22–26 g)	Yasuda et al. (2005)

TABLE 4 | Chinese Patent Medicines and Complex Prescriptions of antipyretic.

Chinese patent medicine and complex prescriptions	Components	Scientific name	Model	Drug delivery cycle	Mechanisms	The species investigated	References
Jinxin oral liquid (JXOL)	Ephedra sinica, Descurain Semen, Mori Cortex, Armeniacae Semen Amarum, Gypsum Ustum, Peucedani Radix, Scutellariae Radix, Polygoni Cuspidati Rhizoma et Radix	*Ephedra sinica* Stapf, *Descurainia sophia* (L.) Webb ex Prantl, *Morus alba* L., *Prunus armeniaca* L., Gypsum Ustum, *Scutellaria baicalensis Georgi*, *Reynoutria japonica* Houtt	Fever caused by subcutaneous injection of 20% yeast (15 ml/kg)	Subcutaneous injection of 7.02 g/kg JXOL	Reduce the production of IL-1 β, PGE2 and the level of quinolinic acid and pantothenic acid; regulate the metabolism level of 3-phosphoglycerate, pyruvate and other metabolites	Male SD rats (weighing 80 ± 20 g)	Qian et al. (2019)
Yin Qiao San (YQS)	Lonicera Japonica Flos, Lophatheri Herba, Forsythiae Fructus, Platycodonis Radix, Sojae Semen Praeparatum, Arctii Fructus, Menthae Haplocalycis Herba, Schizonepetae Herba, Phyllostachys Rhizoma, Glycyrrhizae Radix et Rhizoma	*Lonicera japonica* Thunb, *Lophatherum gracile* Brongn, *Forsythia suspensa* (Thunb.) Vahl, *Platycodon grandiflorus* (Jacq.) A.DC., *Glycine max* (L.) Merr., *Arctium lappa* L., *Mentha canadensis* L., *Nepeta tenuifolia* Benth., *Phragmites australis* subsp. Australis, *Glycyrrhiza uralensis* Fisch. ex DC.	Subcutaneous injection of 20% yeast (20 ml/kg) to induce fever	Different doses of YQS solution were respectively gavage administration in fever rats	Reduce the cAMP level of the hypothalamus	Male SD rats (weighing 200 ± 20 g)	Huang and Chang (2016)
Hao Jia Xu Re Qing Granules (HJ)	Artemisiae Annuae Herba, Glycyrrhizae Radix et Rhizoma Trionycis Carapax, Rehmanniae Radix, Dendrobii caulis, Anemarrhenae rhizoma, Moutan cortex, Puerariae Lobatae Radix,	*Artemisia annua* L., *Glycyrrhiza uralensis* Fisch. ex DC., *Trionyx sinensis* Wiegmann, *Rehmannia glutinosa* (Gaertn.) DC., *Dendrobium nobile* Lindl., *Anemarrhena asphodeloides* Bunge, *Paeonia suffruticosa* Andrews, *Pueraria montana var. lobata* (Willd.) Maesen & S.M.Almeida ex Sanjappa & Predeep	Rats were subcutaneously injected with 10 mg/kg of 10% yeast suspension	1.44 g/kg, 0.72 g/kg, 0.36 g/kg of HJ were given by gavage after being injected with yeast	Inhibit the tryptophan metabolism;Reduce the level of 5-HT	SD rats (weighing 200 ± 20)	Yu et al. (2018)
Reduning injection (RDN)	Artemisiae Annuae Herba, Lonicera Japonica Flos, Gardeniae Fructus	*Artemisia annua* L., *Lonicera japonica* Thunb, *Gardenia jasminoides* J.Ellis	Rats were subcutaneously injected with 5 ml/kg of 20% yeast suspension	Rats were I.V.with 6 ml/kg RDN	Reduce the level of IL-1β, IL-6, PGE 2, TNF-α and cAMP in febrile rats; Change the regulation of amino acid metabolism, lipid metabolism and energy metabolism	Male SD rats (weighing180-220 g)	Gao et al. (2020)
Gegen Qinlian decoction (GQLD)	Puerariae lobatae Radix, Scutellariae Radix, Coptidis Rhizoma, Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle	*Pueraria montana var. lobata* (Willd.) Maesen & S.M.Almeida ex Sanjappa & Predeep, *Scutellaria baicalensis* Georgi, *Coptis teeta* Wall., *Glycyrrhiza glabra* L.,	Rats were subcutaneously injected with 10 ml/kg of 20% yeast suspension	Rats were orally administered with GQLD (1.728 g/kg)	Regulate the metabolisms of phospholipid, sphingolipid, fatty acid oxidation, fatty acid amides, amino acid and glycerolipid in vivo	Male Wistar rats weighing (240 ± 20 g)	Liu et al. (2019b)
Bai-Hu decoction (BHD)	Gypsum Ustum, Anemarrhena Rhizoma, Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle, Rice	Gypsum Ustum, *Anemarrhena asphodeloides* Bunge, *Glycyrrhiza glabra* L., Rice	Intravenous injection of LPS (200 ng/kg)	Orally administered with BHD 7 ml/kg	Reduce the content of IL-1β and TNF-α in serum, and TNF-α in hypothalamus	New Zealand rabbits (weighing 2.0–3.0 kg)	Jia et al. (2013)

TABLE 5 | Clinical trials of Chinese Patent Medicines of antipyretic.

Chinese patent medicines	Disease	Subject	Study design	Intervention	Length	Outcome	References
Shuang-Huang-Lian injection	Acute tonsillitis	90 subjects (56 men and 64 Women)	Randomized controlled trials	1 ml/kg·d SHLI diluted with 5% glucose injection 500 ml or physiological saline 500 ml, intravenous drip, once a day	5–7 days	Fever resolution	Yan et al. (2007)
Shuang-Huang-Lian lyophilized powder for injection	Acute upper respiratory tract infection	98 subjects (43 men and 55 Women)	Randomized controlled trials	3 g Shuang-Huang-Lian lyophilized powder diluted with physiological saline 500 ml, intravenous drip, once a day	4–7 days	Fever resolution	Ouyang et al. (2008)
Shuang-Huang-Lian oral liquid	Acute tonsillitis	72 children (36 male and 36 Female)	Randomized controlled trials	One for children aged 1–3, three times a day; two for children aged 4–7, three times a day	7 days	Fever resolution Reduce IL-6 and TNF-α in serum	Ye (2019)
Shuang-Huang-Lian injection (SHLI)	Acute tonsillitis	120subjects (61 men and 59 Women)	Randomized controlled trials	20 ml SHLI was diluted in 20 ml physiological saline for ultrasonic atomization inhalation treatment, twice a day	14 days	Fever resolution;Reduce IL-6 and TNF-α in serum	Fu et al. (2019)
Shuang-Huang-Lian lyophilized powder for injection	Acute tonsillitis	46 subjects (20 men and 26 Women)	Randomized controlled trials	3 g Shuang-Huang-Lian lyophilized powder diluted with physiological saline 500 ml, intravenous drip, once a day	5–7 days	Fever resolution	Jiang and Wang (2019)
Shuang-Huang-Lian oral liquid	Bacterial respiratory infection	46 subjects of both sexes	Randomized controlled trials	20 ml Shuang-Huang-Lian oral liquid, three times a day	3–5 days	Fever resolution	Yang (2018)
Qingkailing injection (QKLI)	Acute upper respiratory tract infection	46 subjects (24 men and 21 Women)	Randomized controlled trials	20 ml QKLI diluted with 5% physiological saline 250 ml, intravenous drip, once a day	7 days	Fever resolution	Fan and Liu (2011)
Qingkailing injection	Acute upper respiratory tract infection	80subjects (43 men and 37 Women)	Randomized controlled trials	20 ml QKLI diluted with 5% physiological saline 250 ml, intravenous drip, once a day	7 days	Fever resolution	Zhang (2019)
Qingkailing injection	Febrile convulsion in children	50 subjects of both sexes	Randomized controlled trials	15 ml for 3-4-year-old children and 30 ml for 5-6-year-old children, it was added into 10% glucose injection for intravenous drip once a day	4 days	Fever resolution; Reduce IL-β, cAMP and TNF-α	Yan and Fan (2019)
Qingkailing injection	Acute upper respiratory tract infection with high fever	54 subjects of both sexes	Randomized controlled trials	16–40 ml QKLI diluted with 5% glucose injection 250 ml or physiological saline 250 ml, intravenous drip once a day	3–7 days	Fever resolution	Xian et al. (2010)
ReduningInjection (RDNI)	Fever, rash, and ulcers in children	120 subjects of both sexes	Randomized, double-blind, parallel controlled, and multicenter clinical trial	Patients 1–5 years old,RDNI was given at 0.5 ml/kg per day with a maximal dosage of 10 ml; patients 6–10 years old, 10 ml RDNI was given; patients 11–13 years old, 15 ml RDNI was given once a day	3–7 days	Reduction in onset time of antifebrileEffect, an acceleration of body temperatureRecovery, and a stability of body temperature after fever reduction	Zhang et al. (2013a)
ReduningInjection (RDNI)	Acute upper respiratory tract infection with fever	123 subjects of both sexes	Randomized controlled trials	0.6 ml/kg RDNI diluted with physiologicalSaline100ml, intravenous drip once a day	3 days	Fever resolution	Li (2013)

(Continued on following page)

TABLE 5 | (*Continued*) Clinical trials of Chinese Patent Medicines of antipyretic.

Chinese patent medicines	Disease	Subject	Study design	Intervention	Length	Outcome	References
Yin Qiao San (YQS)	Acute upper respiratory tract infection with fever	327 subjects of both sexes	Randomized, double blind placebo-controlled trial	Two 7 g sachets, twice a day	10 days	Fever resolution	Wong et al. (2012)
Yin Qiao San	Viral influenza	124 subjects of both sexes	Randomized, single blind clinical trial	The herbs decoct until about 300 ml. A daily dose, with warm water, twice a day	5 days	Fever resolution	Huang et al. (2013)

the expression of COX-2 in the hypothalamus and the production of PGE2 in the central thermoregulation of rats were inhibited (Yang et al., 2009). It has also been shown that the antipyretic action of CX may reduce the content of cAMP in the hypothalamus of the rats with fever-induced by yeast, to make the temperature set-point decrease and reach an antipyretic effect (Yang et al., 2008). It was found that in rabbits with fever caused by ET, CX not only played an antipyretic role but also change the proportion of hypothalamus 5-HT and NE (Li et al., 2003). It is suggested that the change of monoamine neurotransmitter content in the center is one of the antipyretic mechanisms of CX essential oil. Two phthalide lactones, Z-ligustilide and senkyunolide A, were identified from CX essential oil and characterized as inhibitors of TNF-α production in monocytes by LPS. They can also exhibit significant suppressive effects on TNF-α-mediated NF-κB activation (Ran et al., 2011). In conclusion, the antipyretic effect of CX is related to the inhibition of the expression of COX-2, PGE2, the reduction of cAMP in the hypothalamus, the regulation of the neurotransmitter, and suppressive effects on TNF-α-mediated NF-κB activation.

Cinnamomi Ramulus

Cinnamomi Ramulus (CR), also called Guizhi in Chinese, is the dried twigs of *Cinnamomum cassia* (L.) Presl. It consists of phenylpropanoids, terpenoids, aliphatics, and its glycosides, sterols, flavonoids, and organic acids. Phenylpropanoids such as cinnamaldehyde have been considered the characteristic constituents of CR (Liu et al., 2019a). Cinnamaldehyde has been proved to have an antipyretic effect. An *in vitro* study showed that the expression of transient receptor potential vanilloid 1 (TRPV1) mRNA in the primary dorsal respiratory group (DRG) neurons was significantly upregulated at both 37°C and 39°C after incubation with different concentrations of cinnamaldehyde. The findings might explain the part of the mechanisms of the antipyretic action of cinnamaldehyde, which is achieved through a non-TRPA1 channel pathway (Sui et al., 2010). Rat cerebral microvascular endothelial cells (RCMEC) were cultured in M199 medium containing IL-1β in the presence or absence of cinnamaldehyde. The results showed that cinnamaldehyde inhibited IL-1β-induced PGE2 production through the inhibition of COX-2 activity in cultured RCMEC (Guo et al., 2006). It provides some pharmacological evidence for clinical use of CR in fever. By using a yeast-induced fever model and IL-1β stimulated rat brain microvascular endothelial cells (bEnd.3) as an experimental system to determine the content of PGE2 in the hypothalamus and the supernatant of bEnd.3 (Ma et al., 2008). The results showed that cinnamaldehyde could effectively inhibit the fever response induced by yeast in rats, significantly reduce the content of PGE2 in the hypothalamus of rats with fever, and also inhibit the release of PGE2 stimulated by IL-1. The above studies show that CR can not only relieve fever through the non-TRPA1 channel but also reduce the activity of COX-2 in brain endothelial cells and the content of PGE2 in the hypothalamus of febrile rats.

Forsythiae Fructus

Forsythiae Fructus (FF), also called Lianqiao in Chinese, is the fruit of *Forsythia suspensa* (Thunb.) Vahl. Currently, compounds have been found in FF, including lignans, phenylethanoid glycosides, flavonoids, terpenoids, cyclohexyl-ethanol derivatives, alkaloids, steroidals, and other compounds (Wang et al., 2018b). According to the clinical practice in TCM, FF has a significant antipyretic effect. Essential oil and forsythoside a (FTA) have been demonstrated to have an antipyretic effect (Dang et al., 2017). Subcutaneous injection of yeast caused fever in rats. The body temperature and the content of cAMP and PGE2 in the hypothalamus of the rats were observed after 2 h. The results showed that both the essential oil and the extract of FF significantly reduced the body temperature of rats with fever. The extract of FF can downregulate cAMP and PGE2 in the hypothalamus. The FF essential oil has an antipyretic effect by downregulating cAMP in the hypothalamus (Dang et al., 2017). Previous studies have suggested that FTA can reduce the temperature of the yeast-induced fever mice. FTA significantly downregulated the expression of TRPV1 in hypothalamus and DRG of the yeast-induced fever mice, inhibited MAPKs activation of the hypothalamus and DRG, and then decreased secretion ofPGE2 (Liu et al., 2017a). Recent studies have shown that forsythoside can significantly reduce TNF-α secretion in LPS-stimulated RAW 264.7 cells, suggesting that it can reduce TNF-α secretion to relieve fever (Guan et al., 2013). FTA can also suppress LPS-mediated induction of the TLR4 pathway. LPS combined with TLR4 activated NF-κB through the primary response gene-88 (MyD88)-independent pathways. Therefore, FTA may inhibit TNF-α and NF-κB by blocking LPS/TLR4 signaling pathway (Zeng et al., 2017). Generally, FF may play an

antipyretic role by reducing cAMP, PGE2, and TNF-α in the hypothalamus, suppressing TRPV1 expression, and the LPS/TLR4 signaling pathway.

Lonicera Japonica Flos

Lonicera Japonica Flos (LJ), also known as Jin Yin Hua, is the dry flower buds of *Lonicera japonica* Thunb. It is abundant with iridoids, essential oil, flavones, organic acids, and triterpenoid saponins (Shang et al., 2011). The aqueous extract from LJ has been used in TCM for treating fever for thousands of years (Xu et al., 2007). In rabbits with fever caused by IL-1β, based on observing the antipyretic effect of LJ, the expression of prostaglandin receptor EP3 in the preoptic-anterior hypothalamus (POAH) of New Zealand rabbits was detected. The results showed that LJ has an antipyretic effect, and its mechanism may be related to the inhibition of EP3 expression in POAH (Xie et al., 2009). The current study investigated the effect of LJ water extracts on inhibition of both COX-1 and COX-2 activity. The result showed that the inhibitory effect of LJ water extract on COX-2 activity after boiling was four times. The COX-2 transcriptional inhibition of boiled LJ extracts may be due to the action of bifidoflavonoids or similar compounds through NF-κB (Xu et al., 2007). The antipyretic effect of LJ may inhibit the synthesis of PGE2 by inhibiting the activity of COX-2, as well as inhibiting EP3 expression in POAH. Chlorogenic acid (CGA) is the main active component of LJ, however, CGA did not inhibit LPS induced fever even at the highest test dose (200 mg/kg) (Dos Santos et al., 2006). Therefore, CGA may lack antipyretic activity; the material basis of the antipyretic effect of LJ needs further study.

Other Single Chinese Herbal Medicines of Antipyretics

The above are six commonly used TCM in clinical practice. Their active components play an antipyretic role through different mechanisms. The chemical structure formula is shown in **Figure 4**. In addition to the above six commonly used TCM (**Table 2**), Ephedrae Herba, Gypsum Fibrosum, Gardeniae Fructus, Bubali Cornu, Houttuyniae Herba, Andrographis Herba, and Puerariae Lobatae Radix also have significant antipyretic effects (**Table 3**).

CHINESE PATENT MEDICINES AND COMPLEX PRESCRIPTIONS IN THE TREATMENT OF FEVER

Qingkailing Injection

Qingkailing injection (QKLI) is a composite formula of TCM with a significant antipyretic action (Gao et al., 2013a). It comprises eight TCMs or extracts thereof, including Isatidis Radix, Lonicera Japonica Flos, Gardeniae Fructus, Bubali Cornu, Margaritifera Concha, Baicalinum, Acidum Cholicum, and Acidum Hyodesoxy-cholicum (Yan et al., 2005). Plasma pharmacokinetics study of QKLI demonstrated that baicalin 2) and geniposide 9) might be the potential active antipyretic components of QKLI (Zhang et al., 2016). Baicalin and geniposide could be detected in the hypothalamic dialysate after IV administration of QKLI. However, metabolomics biomarkers of QKLI were highly correlated with baicalin while gardenoside has no statistically significant correlation with these biomarkers (Zhang et al., 2017). Therefore, baicalin may be the more important active component for QKLI to play the role of antipyretic. The network pharmacology study of the antipyretic effect of baicalin shows that baicalin can regulate fever-related molecules NO by targeting on caspase 3 (CASP3) and regulate cAMP, PGE2 to produce antipyretic effect (Zhang et al., 2017). The mechanism of antipyretic action of SR includes baicalin; it plays an antipyretic role by inhibiting the release of IL-6, IL-1β, TNF-α, and PGE2, the activation of NF-κB, and the excessive production of hydroxyl radicals. From QKLI, baicalin was the only antipyretic component screened; it alone cannot represent the antipyretic activity of QKLI. Other antipyretic components in QKLI that play their antipyretic role through humoral pathway. In the study of urine metabolomics, it was found that QKLI can reduce the content of tryptophan by 5-HT and repair the disorder of amino acid metabolism on yeast induced fever rats (Gao et al., 2013a). A plasma study shows that QKLI can correct the interference of amino acid metabolism and lipid metabolism to relieve fever (Qin et al., 2016). However, except baicalin, other antipyretic components of QKLI are seldom studied, and its antipyretic components need to be further studied.

Shuang-Huang-Lian Preparation

SHL is a famous TCM recipe, which was included in Chinese pharmacopoeia in 2015. It contains LJ, SR, and FF; it is clinically used to treat fever and infectious diseases such as acute upper respiratory tract infection (Gao et al., 2014a). SHL products are administered in a variety of different routes (e.g., oral, injectable, and pulmonary routes) (Gao et al., 2014c). Three effective ingredients, chlorogenic acid (17), baicalin (2), and forsythin (7), are officially recorded as quality control standards. Baicalin has an antipyretic effect by inhibiting the release of IL-6, IL-1β, TNF-α, and PGE2, the activation of NF-κB, and the excessive production of hydroxyl radicals (See 6.2). Forsythin may have an antipyretic effect by reducing cAMP, PGE2, and TNF-α in the hypothalamus, suppressing TRPV1 expression, and the LPS/TLR4 signaling pathway (See 6.6). The study first evaluated the antipyretic effect of SHL injection (SHLI) using UPLC-Q-TOF/MS-based metabolomics study to reveal the antipyretic mechanism of SHLI on the yeast-induced pyrexia rat model. The result shows that SHLI might contribute to the repair of lipid metabolism, amino acid metabolism, and energy metabolism to work antipyretic effects (Gao et al., 2014b). It reported that baicalin in SHL was metabolized to baicalein in a study, baicalin was used as a representative compound to study the pharmacokinetics of SHL. It was found that the compound prescription might prolong the effect of baicalin *in vivo* (Di et al., 2006). It suggests that the antipyretic effect of SHL may be better than that of SR. Another study was conducted to compare the antipyretic effect of LJ and SHL on rectal temperature changes induced by yeast. The results indicated

that SHL showed a better antipyretic effect than LJ (Gao et al., 2014). In conclusion, the antipyretic effect of SHL is better than that of single herb.

Others

In addition to the QKLI and SHL series preparations, Jinxin oral liquid, Yin Qiao San, Hao Jia Xu Re Qing Granules, and Reduning injection are used in clinical antipyretic treatment. Gegen Qinlian decoction and Bai-Hu decoction are two classical antipyretic prescriptions that have also been proved to have significant antipyretic effects (**Table 4**).

CLINICAL TRIALS

In the study of the antipyretic mechanism of TCM, various models have been developed to simulate the natural fever of experimental animals. However, fever is an important clinical manifestation of many diseases, so most of the clinical trials of antipyretics in TCM are focused on acute upper respiratory tract infection, acute tonsillitis, acute otitis media, and other diseases (Li, 2002; Zhang et al., 2013b). At present, there are a few clinical trials on the antipyretic effect of single Chinese herbal medicines (**Table 5**). The clinical trials of antipyretic Chinese patent medicine mainly include SHL series preparations and QKLI. It's worth noting that the clinical trials of antipyretic TCMs are concentrated on infectious fever, but high-quality clinical trials are lacking. Clinical trials study to evaluate the effect of antipyretic TCMs will use more strict protocols, concealment of allocation, and double-blinding, in order to ensure the compliance of international acceptable standards.

CONCLUSION AND PERSPECTIVES

Since the global outbreak of the infectious disease COVID-19 in 2019, China has taken strong measures to quickly engage in the fight against the COVID-19. TCM has played an important role in the prevention and treatment of COVID-19 because of its unique insights and experiences. For patients with mild symptoms, TCM early intervention can effectively prevent the disease from turning into severe. In severe cases, TCM improves the symptoms to win the rescue time for the patients. TCM has own characteristics such as holistic concept, syndrome differentiation and treatment, strengthening the body resistance to eliminate pathogenic factors. Judging from the current treatment plan, TCM treats patients with COVID-19 based on the idea of syndrome differentiation and treatment. In order to improve the fever in some patients, prescriptions need to be compatible with antipyretic TCMs.Therefore, this paper summarized the mechanisms of fever from two aspects of pathology and physiology. On this basis, combined with the chemical composition and pharmacological action of TCM, it analyzed the mechanisms of the antipyretic effects of TCM through various ways, so as to provide reference for the efficient utilization of existing drugs.

It must be pointed out that in the face of the spread of new epidemics, due to the particularity of the disease and the urgency of formulating effective diagnosis and treatment plans, suitable treatment models have been developing based on basic theories and clinical experiences. Therefore, it is important to choose drugs with a clear therapeutic effect and clinical basis. The single Chinese medicine and compound preparations of TCM which have specific antipyretic effects listed in this paper are widely used in clinical practice, and can effectively improve the symptoms of fever in acute respiratory infections and control infections. TCM has the unique properties of multi-components and multi-targets. The majority of these mentioned drugs may not only exert the effects of antipyretic, but also have the properties of anti-inflammation, and immunity enhancement; and some of them are antiviral, and may be promising medicines for the treatment or adjuvant treatment of COVID-19 patients.

AUTHOR CONTRIBUTIONS

D-KZ and R-CX put forward the idea; L-LM wrote the manuscript and made the figures; H-ML, C-HL, Y-NH, FW, and H-ZH collected the literature; LH and MY contributed to the revisions.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GLOSSARY

TCM traditional chinese medicine
NSAIDs nonsteroidal anti-inflammatory drugs
Poly-IC polyinosinic polycytidylic acid
ACE-2 angiotensin-converting enzyme-2
LPS lipopolysaccharides
PAMPs pathogen-associated molecular patterns
TLRs toll-like receptors
IL-1 interleukin-1
IL-6 interleukin-6
TNF-α tumor necrosis factor α
IL-1RA IL-1 receptor antagonist
PGE2 prostaglandin E2
Cox-2 cyclooxygenase 2
IKK2 IκB kinase 2
rh TNF human recombinant TNF
mPGES-1 PGE synthase-1
I.V intravenous
I.C.V intra cerebro-ventricular
BBB blood-brain barrier
Kc kupffer cells
C5a complement component 5a
PLA2 phospholipase A2;
IL-6R IL-6 receptor
NMDA N-methyl-D-aspartate
ROS reactive oxygen species
iNOS inducible nitric oxide synthetase
5-HT 5-hydroxy tryptamine
GABA γ-aminobutyric acid
OVLT organum vasculosum of lamina terminalis
POA preoptic-anterior hypothalamic area
AA arachidonic acid
CVOs circumventricular organs
NTS nucleus of the solitary tract
NE norepinephrine
PKA protein kinase A
IκBα NF-κB inhibitor α
RCMEC rat cerebral microvascular endothelial cells
bEnd.3 brain microvascular endothelial cells
FTA forsythoside a
DRG dorsal respiratory group
POAH preoptic anterior hypothalamus
CGA Chlorogenic acid
AURI acute upper respiratory tract infection

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PERSPECTIVE
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Medicinal Plants in COVID-19: Potential and Limitations

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Currently, the search to identify treatments and vaccines for novel coronavirus disease (COVID-19) are ongoing. Desperation within the community, especially among the middle- and low-income groups acutely affected by the economic impact of forced lockdowns, has driven increased interest in exploring alternative choices of medicinal plant-based therapeutics. This is evident with the rise in unsubstantiated efficacy claims of these interventions circulating on social media. Based on enquiries received, our team of researchers was given the chance to produce evidence summaries evaluating the potential of complementary interventions in COVID-19 management. Here, we present and discuss the findings of four selected medicinal plants (*Nigella sativa*, *Vernonia amygdalina*, *Azadirachta indica*, *Eurycoma longifolia*), with reported antiviral, anti-inflammatory, and immunomodulatory effects that might be interesting for further investigation. Our findings showed that only *A. indica* reported positive antiviral evidence specific to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) based on preliminary *in silico* data while all four medicinal plants demonstrated differential anti-inflammatory or immunomodulatory effects. The definitive roles of these medicinal plants in cytokine storms and post-infection complications remains to be further investigated. Quality control and standardisation of medicinal plant-based products also needs to be emphasized. However, given the unprecedented challenges faced, ethnopharmacological research should be given a fair amount of consideration for contribution in this pandemic.

Keywords: complementary therapy, ethnopharmacology, COVID-19, coronavirus, medicinal plants, herbs

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INTRODUCTION

The emergence of a new coronavirus, known as the SARS-CoV-2 has initiated a pandemic of COVID-19 (World Health Organisation, 2020b). More than 31 million infections with at least 960,000 COVID-19 associated deaths were reported by September 23, 2020 (World Health Organisation, 2020c). Since its first reported case in Wuhan, China in December 2019 (World Health Organisation, 2020c), new discovered evidence by both clinicians and researchers globally have helped shed some light on the disease pathogenesis and the nature of the virus itself. The availability of new information subsequently fed policy changes on transmission prevention strategies as well as development of preventative vaccines and therapeutic drug candidates. Enforced physical distancing, hand hygiene, and arguably proper usage of personal protective equipment including wearing a surgical mask remains the most effective way of controlling the spread of the disease, with most countries which adopted such measures reporting some success in curbing the disease spread (Chu et al., 2020; Sardar et al., 2020). However, several challenges remain

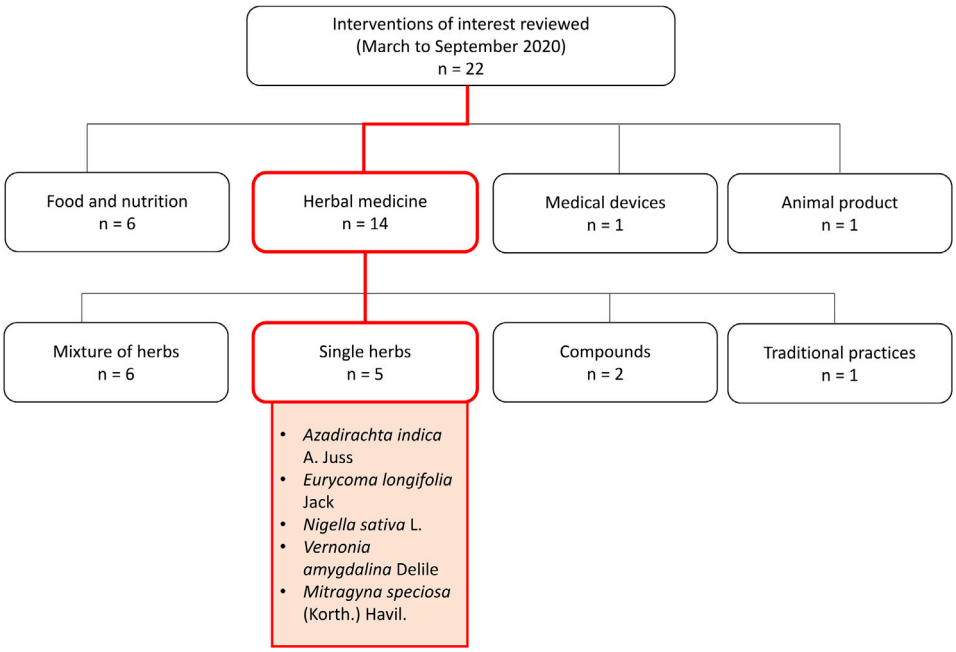

FIGURE 1 | Selection of single medicinal plants as interventions of interest.

in maintaining these drastic measures of enforced physical distancing for long periods of times. Resurgences of infection waves were reported in few countries after the relaxation of rules. In addition, the economic impact of prolonged lock down on social issues such as loss of income and increased poverty, especially for the low and middle-income countries, is evident (Bonaccorsi et al., 2020; United Nations Development Programme, 2020).

As the world looks towards science in search of an effective drug or vaccine, a few countries, such as China and India, with long histories of traditional medicine use (Li et al., 2020; Rastogi et al., 2020), have also started exploring the role of traditional and complementary, alongside conventional treatment. The Malaysian community, coming from a tropical multi-racial country rich in flora and fauna, also appears to be interested in venturing towards the use of herbal and complementary medicine, some of which are based on local traditional knowledge. During the Movement Control Order implemented by the Malaysian Government in March 2020 in attempts to curb the disease spread, the herbal medicine research arm of biomedical research institute in Malaysia has received numerous queries on the potential use of complementary remedies including single medicinal plants, traditional remedies, finished herbal products, supplements, food products, and medical devices against COVID-19. These queries were mainly submitted directly by the general public and persons with readily available herbal products, or identified through highly circulated messages on several social media platforms. From March to September 2020, 22 interventions of interest were reviewed through searches conducted on electronic databases such as PubMed, Web of Science, Google Scholar; as well as hand searching of grey literature, including books on herbal and traditional medicine available in institutional library resources. The predetermined search terms used are 'COVID-19', 'antiviral', 'anti-inflammatory', 'immune system', 'immunomodulatory', 'safety', 'toxicity', in combination with the name of the main intervention of interest or its synonyms. From these evidence summaries, five were single medicinal plants including *Azadirachta indica* A. Juss, *Eurycoma longifolia* Jack, *Nigella sativa* L., *Gymnanthemum amygdalinum* (Delile) Sch. Bip. (or *Vernonia amygdalina* Delile), and *Mitragyna speciosa* (Korth.) Havil (**Figure 1**).

Of the five individual medicinal plants, this review presents and discussed the available evidence of four selected plants (A. *indica*, *E. longifolia*, *N. sativa*, and *V. amygdalina*), considering their efficacy evidence as antiviral, anti-inflammatory, and immunomodulatory agents for use in COVID-19 management; as well as completeness of quality and safety data to be incorporated into human trials. *M. speciosa* was not further discussed here due to established reports on toxicity and dependance (Meireles et al., 2019). *M. speciosa* is also currently listed as a prohibited ingredient in natural products in Malaysia (National Pharmaceutical Regulatory Agency, Ministry of Health Malaysia, 2020). Although public interest in the use of the selected four medicinal plants (*A. indica*, *E. longifolia*, *N. sativa*, and *V. amygdalina*) for COVID-19 seemed strong, there are concerns on their efficacy and safety. As research in COVID-19 treatment intensifies, exploring the potential roles of medicinal plants, lobbying and extrapolating from known scientific evidence on safety and efficacy, can be beneficial.

TABLE 1 | Pharmacological properties and safety evidence of selected herbs and supplements (Koley and Lal, 1994; Talwar et al., 1995; Talwar et al., 1997; Badary et al., 1998; Hore et al., 1999; Salem and Hossain, 2000; Petrovsky, 2006; Bamosa et al., 2010; Datau et al., 2010; Iyyadurai et al., 2010; Momoh et al., 2010; Salem et al., 2011; Schumacher et al., 2011; Venugopalan et al., 2011; Aljindil, 2012; Choudhary et al., 2012; Momoh et al., 2012; Abdel-Moneim et al., 2013; Li et al., 2013; Mishra and Dave, 2013; Saalu et al., 2013; Adedapo et al., 2014; Nabukenya et al., 2014; Tran et al., 2014; Ulasli et al., 2014; Yee et al., 2014; Gholamnezhad et al., 2015; Majdalawieh and Fayyad, 2015; Ashfaq et al., 2016; George et al., 2016; Im et al., 2016; Omoregie and Pal, 2016; Rehman et al., 2016; Zakaria et al., 2016; Onasanwo et al., 2017; Salem et al., 2017; Tavakkoli et al., 2017; Asante et al., 2019; Onah et al., 2019; Ruan et al., 2019; Borkotoky and Banerjee, 2020; Dwivedi et al., 2020).

		***Azadirachta indica* A. Juss.**	***Eurycoma longifolia* Jack**	***Nigella sativa* L.**	***Gymnanthemum amygdalinum* (Delile) Sch. Bip.**
Pharmaco-logical properties	Antiviral	+[a]		++	
	Anti-inflammatory	+	+	++	+
	Immuno-modulatory	++	++	++	++
Safety	Preclinical	Leaf extract: arrhythmia, hypoglycemia, and blood pressure reduction Seed oils and extracts: abortifacient	Standardised aqueous extract (root): no-observed-adverse-effect-level (NOAEL) dose >1,000 mg/kg orally; minimal mammalian carcinogenicity; no genotoxicity	Thymoquinone: hypoglycaemia and hepatic impairment	Aqueous extract (Leaf): kidney congestion; Ethanolic extract (Leaf): testicular toxicity
	Clinical	Seed oils and extracts: acidosis, renal injury, anti-human chorionic gonadotropin effects	Safe dose (standardised aqueous extract) used in clinical trial: 200 mg/day	Seed: Safe up to 3 months of consumption	

+: positive preclinical evidence published; ++: positive clinical evidence published.
[a]*COVID-19 specific evidence.*

MEDICINAL PLANTS IN COVID 19: EFFICACY, SAFETY, AND RESEARCH GAPS

In the research of phytomedicine, it is common to observe multiple pharmacological properties from a single plant. It is now well understood that a single plant may contain a wide range of phytochemicals, making ethnopharmacology research both full of possibilities yet challenging (Süntar, 2019). Overall, these selected interventions of interest discussed here can be broadly categorised into those with 1) antiviral, 2) anti-inflammatory, 3) immunomodulatory effects, and more often 4) a combination of these effects, based on available evidence for efficacy (**Table 1**). Details on quality, efficacy, and safety of individual studies is presented in **Supplementary Table S1**. On top of exhibiting direct antiviral effects, medicinal plants with reported anti-inflammatory activities may have pleiotropic roles in COVID-19 management as the elevation of inflammatory markers such as interleukin (IL)-6, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) has been associated with severe disease with worse outcomes among COVID-19 patients, most likely related to cytokine storm (Zeng et al., 2020).

According to our evidence summaries, *N. sativa* (black cumin) seed was among one of the medicinal plants with most published positive evidence. Ethanolic extracts of *N. sativa* seeds demonstrated antiviral properties by decreasing viral load, alpha fetoprotein, and improved liver function parameters among hepatitis C infected patients (Abdel-Moneim et al., 2013). In animal studies, *N. sativa* seed oil presented both antiviral and immunomodulatory effects against cytomegalovirus, reducing viral loads to an undetectable level. It can also enhance the immune response by increasing CD3 and CD4 counts, as well as up-regulating interferon-gamma (IFN-γ) release from Natural Killer T-cells and macrophages (Salem and Hossain, 2000). In cell studies, ethanolic extracts of *N. sativa* seeds also demonstrated inhibitory activity against coronavirus species MHVA59 (mouse hepatitis virus-A59) replication by downregulating gene expressions of various leukocyte transient receptor proteins (TRP) such as the TRPA1, TRPC4, TRPM6, TRPM7, TRPM8 and TRPV4 genes (Ulasli et al., 2014). Traditionally, *N. sativa* is used for a diverse range of indications including in respiratory diseases such as asthma (Al-Jauziyah, 2003). The benefits of *N. sativa* supplementation (details of formulation unclear) in improving asthmatic symptoms have also been reported in a clinical trial, and is thought to be partially due to the anti-hypersensitivity and potentially anti-inflammatory properties (Salem et al., 2017). Positive preclinical and clinical evidence of *N. sativa's* immunomodulatory and anti-inflammatory effects have been collectively concluded in three separate review papers (Gholamnezhad et al., 2015; Majdalawieh and Fayyadet, 2015; Tavakkoli et al., 2017). More interestingly about *N. sativa* and its bioactive compound thymoquinone, is their immunomodulatory effects reported in respiratory diseases, including those of infectious origin. An *in vitro* study has reported that thymoquinone enhances survival of antigen-activated $CD8^+$ cells, highlighting the potential for adoptive T-cell therapies (Salem et al., 2011). The potential of *N. sativa* to modulate B cell-mediated immune response while balancing Th1/Th2 ratio to potentiate T cells-mediated immune response merits further investigation. This activity can be explored as an adjunct to potential vaccine candidates to mediate meaningful and sustained immune response post vaccination (Petrovsky, 2006), which is one of the main challenges with current

potential COVID-19 vaccines in development (Sahin et al., 2020). As for safety, long-term consumption (up to three months) of *N. sativa* seeds at 3 g/day in humans reported no significant adverse effects on both liver and kidney functions (Bamosa et al., 2010; Datau et al., 2010). However, precautions should be paid towards thymoquinone as animal toxicity studies at high doses of 2–3 g/kg have resulted in hypoglycaemia and hepatic enzyme derangements (Badary et al., 1998).

Another plant that has shown immune enhancing effects as adjunct to vaccines is *G. amygdalinum*, more commonly known as *V. amygdalina* or bitter leaf. This plant was reported to be traditionally used to relieve fever, diarrhoea, cough, and headache (Plant Resources of Tropical Africa, PROTA, 2004). Aqueous extracts of G. *amygdalinum* showed positive effects in enhancing immune response by increasing the levels of white blood cells and $CD4^+$ (Momoh et al., 2010; Momoh et al., 2012; Im et al., 2016). With the capability to increase the $CD4^+$ cell counts, this extract was reported to be adjuvant to antiretroviral therapy in HIV positive patients (Momoh et al., 2012). In addition, the aqueous extract also demonstrated potential immune augmenting effects as adjuvant to Hepatitis B vaccine by increasing levels of surface antigen of the Hepatitis B virus (rHBsAg)-specific antibodies immunoglobulin M, immunoglobulin G sub class 1, and immunoglobulin A (Onah et al., 2019). As a plant with various phytochemicals with the potential to exhibit multimodal mechanism of actions, ethanol, methanol, and acetone extracts also reported anti-inflammatory activity in laboratory animals via modulation of levels of inflammatory cytokines and mediators including the pro-inflammatory (prostaglandin-endoperoxide synthase 2, nuclear factor kappa B (NFκB), tumor necrosis factor-alpha (TNF-α), IL-1β, IL-6, IL-8, nitric oxide, CRP) and anti-inflammatory markers (Adedapo et al., 2014; Omoregie and Pal, 2016; Onasanwo et al., 2017; Asante et al., 2019). Despite the reported potent activity of this plant in regulating the immune and inflammation responses, its toxicity profile remains be ascertained. Although no mortality was reported in an acute toxicity study in animals (Zakaria et al., 2016), subacute administration of the aqueous extract (200 and 600 mg/kg body weight) in rats caused kidney congestion (Nabukenya et al., 2014) while testicular toxicities was reported with an ethanol extract (300 and 600 mg/kg) (Saalu et al., 2013). Currently, there is insufficient direct evidence on the efficacy of *V. amygdalina* in COVID-19, despite various reported antiviral, anti-inflammatory, and immunomodulatory effects.

The leaves of neem (*A. indica*), a popular Indian plant, is traditionally boiled and consumed for treatment of fever (Burkill, 1935), with reported anti-inflammatory effects in animal studies (Schumacher et al., 2011). *In vitro* and *in silico* docking studies demonstrated that neem leaves extracts and its phytochemicals such as flavonoids and polysaccharides have direct antiviral effects against various viruses including dengue (Dwivedi et al., 2020) and Hepatitis C Virus (Ashfaq et al., 2016). Specific to SARS-CoV-2, molecular docking studies have demonstrated that the neem derived compounds nimbolin A, nimocin, and cycloartanols have the potential to bind to envelope (E) and membrane (M) glycoproteins of the SARS-CoV-2 and act as inhibitors (Borkotoky and Banerjee, 2020). As for immunomodulatory effects, both neem seeds and leaves reported positive effects in enhancing immune response in animals (Venugopalan et al., 2011; Aljindil, 2012). In mice vaccinated with *Brucella* Rev-1 vaccine, neem seed extract given subcutaneously enhanced the production of IFN-γ post vaccination (Aljindil, 2012). However, the main issue with exploring neem's potential for COVID-19 is its safety profile. Although neem leaves have been used traditionally for a long time, well documented safety records are still insufficient. Several animal toxicity studies have reported variable adverse effects including arrhythmia, hypoglycaemia, and blood pressure reduction at high doses of neem leaf extracts (Koley and Lal, 1994; Hore et al., 1999). Human cases of acidosis and renal injury have also been reported on neem seed oil consumption (Iyyadurai et al., 2010; Mishra and Dave, 2013). In pregnant women, neem seed extracts should be avoided as animal studies have shown its abortifacient effects (Talwar et al., 1997) while human trials have reported its anti-human chorionic gonadotropin effects (Talwar et al., 1995). That being said, the traditional use of neem for medicinal purposes is largely focused on leaves consumption, boiled in water and drank (Mustapha et al., 2017). In view of safety concerns, studies establishing safe doses of neem leaves specific to the formulation intended for use is required prior to further investigations on efficacy.

The main challenges of phytopharmaceutical development for therapeutic claims is quality control, identification, and standardisation of the bioactive compounds of a plant-based product. Due to the inherent nature of natural products containing multiple bioactive and chemical markers, the quality control process to meet stringent regulatory standards of safety considerations is time consuming and lengthy (Tan et al., 2020). Tongkat Ali, or *E. longifolia*, a popular Malaysian plant traditionally used for improving men's health (Rehman et al., 2016) is among one of the few natural products with established standardisation and safety data available. Acute, subacute, and subchronic toxicity studies of the powdered root of *E. longifolia* in rats reported a calculated acceptable daily intake of up to 1.2 g/adult/day in humans (Li et al., 2013). Safety assessment of the standardised aqueous extract of *E. longifolia* (acute, subacute, and 90 days subchronic general toxicity studies) conducted according to the relevant Organisation for Economic Co-operation and Development (OECD) guidelines reported no toxic effects in rats (Choudhary et al., 2012). Specific toxicity studies of the same extract also reported low mammalian mutagenicity with no genotoxic effects (Yee et al., 2014). Although no direct antiviral effects were reported with standardised aqueous extract of *E. longifolia*, clinical data have shown its positive effects in enhancing immune response in the aging population by improving the $CD4^+$ counts, with a safe dose of 200 mg/day (George et al., 2016). Preclinical evidence of the anti-inflammatory properties of *E. longifolia* are also available. Among the potential bioactive anti-inflammatory compounds isolated from *E. longifolia* include eurycomalactone, 14,15β-dihydroklaieanone, and 13,21-dehydroeurycomanone with potent NF-κB inhibitory effects (Tran et al., 2014). Several phenolic compounds isolated from the roots of *E. longifolia* were also reported to significantly reduce expression of IL-6 in

lipopolysaccharide stimulated RAW264.7 macrophage (Ruan et al., 2019). Given its well established safety profile, future investigations on the potential anti-inflammatory effects of *E. longifolia* may be explored in the context of COVID-19. However, as many of the published studies were industrial sponsored (Choudhary et al., 2012; Yee et al., 2014; George et al., 2016), the potential for bias remains to be ascertained.

PERSPECTIVE: DEVELOPING HERBAL MEDICINE FOR COVID-19

One year into the pandemic, it has become apparent that developing an effective antiviral against the SARS-CoV-2 is challenging due to the virus infectivity and disease course. Viral life cycle modelling studies suggested that early administration of a highly potent antiviral is needed to effectively curb the infection and preserve host cells. This number also coincides with the average number of days for peak viral load to occur and symptoms onset, making it a challenge for timely administration of antivirals in community spreading (Goncalves et al., 2020). Though there have been many claims on antimicrobial properties of the selected medicinal plants discussed here, only one medicinal plant, neem, demonstrated preliminary *in silico* evidence of antiviral effects specific towards the SAR-CoV-2 (Borkotoky and Banerjee, 2020). Currently, the antiviral remdesivir is approved by the U.S Food and Drug Administration (FDA) for use in hospitalised patients with COVID-19 based on positive data from clinical trials (U.S. Food and Drug Administration, 2020). Although remdesivir improved clinical symptoms, there is insufficient evidence to support its benefits on mortality (Dyer, 2020). Remdesivir is thought to act via early termination of viral RNA synthesis hence inhibiting replication (Eastman et al., 2020). Based on viral kinetics modelling, a combination of various antivirals targeting multiple stages of the viral life cycle of infecting the host is suggested as a plausible strategy to effectively curb infection (Dodds et al., 2020). Hence, future investigations on the effects of compounds identified from neem such as nimbolin A, nimocin, and cycloartanols through a different targeted pathway (inhibition of E and M glycoproteins) (Borkotoky and Banerjee, 2020) from remdesivir may provide additional benefits.

Instead of antiviral properties, most of the medicinal plants discussed here demonstrated anti-inflammatory effects supported by *in vivo* preclinical evidence. At present, anti-inflammatory and immunomodulatory agents such as corticosteroids and IL-6 receptor antagonist are being utilised in the management of COVID-19 related cytokine storm associated with severe acute respiratory distress syndrome, in hopes to improve survival (Prescott and Rice, 2020; Saha et al., 2020). Medicinal plants such as V. *amygdalina* and *E. longifolia* demonstrated suppression effects on specific pro-inflammatory cytokines correlated with worsened COVID-19 outcome such as the IL-6 (Adedapo et al., 2014; Omoregie and Pal, 2016; Onasanwo et al., 2017; Asante et al., 2019; Ruan at al., 2019; Zeng et al., 2020). However, considering the treatment of cytokine storms are administered to patients who are severely ill, a majority of them on mechanical ventilation (Prescott and Rice, 2020), the administration of medicinal plant or an herbal formulation via the oral route will be challenging in intubated patients. Compatibility and potential of herbal formulations adsorption on nasogastric tubes also needs to be evaluated. Furthermore, as some medicinal plants such as the *E. longifolia* also reported immune-stimulating activity in older adults (George et al., 2016), the risk of worsening an existing cytokine storm needs to be evaluated. Consideration on optimal timing of administration during different disease stages to modulate the immune system is crucial to maximise the benefits versus risks of such agents (Nidadavolu and Walston, 2020; Nugraha et al., 2020). From a different perspective, it will be interesting to explore the potential role of medicinal plants with anti-inflammatory properties in post SARS-CoV-2 infection complications related to chronic inflammation such as lung fibrosis and neuropsychiatric symptoms (Fraser, 2020; Paterson et al., 2020), given the existing adverse effects associated with long term steroids use (Liu et al., 2013). As post COVID-19 complications remains a new field of study at present, investigation on long-term safety profile and pharmacokinetics of potential medicinal plants can be beneficial.

The time and processes required to develop an herbal medicine of high enough quality and consistency for therapeutic use with sufficient safety data is extremely protracted. This is due to the nature of medicinal plants containing multiple phytochemicals, which are also easily affected by agronomic factors (Süntar, 2019). In addition, identifying, isolating, and producing reference standards required for the standardisation of medicinal plants is challenging, compared to synthetic chemical entities, which are more straight forward. Standardisation of herbal products based on bioactive markers remains important to ensure batch-to-batch consistency and efficacy (Sachan et al., 2016). Due the variation in the formulations available for individual medicinal plants, adequate toxicity studies specific to the formulation of interest are required to ensure its safety (World Health Organization Regional Office for the Western Pacific, 1993). As a result of these challenges, it is highly unlikely to develop new products from scratch in time for emergency use during crises like the current COVID-19 pandemic. In times of emergency, accelerated approvals for therapeutic candidates of proven safety with minimal risk, as well as having the potential for benefits are often considered (Van Norman, 2020). These considerations drive the bulk of research to favour repurposing existing drugs, including remdesivir (Singh et al., 2020). The same concept may be applied to natural products, keeping in mind that each individual formulation and product though containing the same plant, is unique on its own. Although ideally the development of a most potentially efficacious agent is desired, in the case of considering herbal medicine for emergency use, the availability of a well-developed standardised herbal product with sufficient safety data is equally valuable. Compared to published reviews on herbal medicine in COVID-19 (Huang et al., 2020; Nugraha et al., 2020), some of the medicinal plants mentioned here including *E. longifolia* and *V. amygdalina* were not identified

in previous reviews. Among the four medicinal plants reviewed here, it appears that only one (*E. longifolia*) had extensive safety data on a marketed aqueous extract to be considered for a clinical trial. However, in these individual papers, the quality data on chemical fingerprinting and quantitative assessment were not reported (Choudary et al., 2012; Yee et al., 2014; George et al., 2016). Apart from quantitative assessment of phytochemical markers and intrinsic toxicity, additional quality assessments on the risk of extrinsic toxicities from external contaminants and adulteration are also important (Posadski et al., 2013).

In these unprecedented times where the pandemic has affected people worldwide in ways unimaginable, advancing science in herbal medicine for therapeutic claims should be included as an important contribution towards research in COVID-19. As part of the efforts in strengthening science and contribution of traditional herbal medicine in the current pandemic, the World Health Organisation, with the Africa Centre for Disease Control and Prevention, and the African Union Commission for Social Affairs have recently endorsed a protocol for conducting clinical trials on herbal medicine in COVID-19 (World Health Organisation, 2020a). For the medicinal plants discussed here, in addition to requiring more direct evidence of their role in COVID-19 management, other concerns that remains to be addressed include identification of bioactive ingredients, safe dose specific to formulations, and potential drug-herb interaction prior to entering a clinical trial. Innovative ways to utilise the antimicrobial properties of medicinal plants beyond systemic absorption, such as development of medicinal plant-coated antimicrobial mask (Wang et al., 2017), can be further examined.

CONCLUSION

In conclusion, the four medicinal plants (*A. indica*, *E. longifolia*, *N. sativa*, and *V. amygdalina*) discussed here collectively exhibited pleiotropic effects which can potentially provide a multimodal approach via antiviral, anti-inflammatory, and immunomodulatory effects in COVID-19 management. At present, it is evidently challenging to pool data from published studies due to variation in extracts selection and a lack of well-reported standardisation data of the investigated formulations. Still, it is quite clear that there is insufficient evidence of direct antiviral effects specific to the SARS-CoV-2. Further investigations on differential anti-inflammatory and immunomodulatory effects as well as quality and safety of herbal medicines are required to ascertain their role in COVID-19 management.

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AUTHOR CONTRIBUTIONS

XL is the first and corresponding author. XL, BT, and TT conceptualised the manuscript. XL, TT, and the HMRC COVID-19 Rapid Review Team contributed towards search strategy, literature search, and data interpretation of individual evidence summaries. XL, BT, and TT further extracted and interpreted the overall data based on individual evidence summaries, drafted, edited, reviewed, and approved of the final article to be published.

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SUPPLEMENTARY MATERIAL

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REVIEW
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Therapeutic Potentials of Antiviral Plants Used in Traditional African Medicine With COVID-19 in Focus: A Nigerian Perspective

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The coronavirus disease 2019 (COVID-19) pandemic is caused by an infectious novel strain of coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which was earlier referred to as 2019-nCoV. The respiratory disease is the most consequential global public health crisis of the 21st century whose level of negative impact increasingly experienced globally has not been recorded since World War II. Up till now, there has been no specific globally authorized antiviral drug, vaccines, supplement or herbal remedy available for the treatment of this lethal disease except preventive measures, supportive care and non-specific treatment options adopted in different countries via divergent approaches to halt the pandemic. However, many of these interventions have been documented to show some level of success particularly the Traditional Chinese Medicine while there is paucity of well reported studies on the impact of the widely embraced Traditional African Medicines (TAM) adopted so far for the prevention, management and treatment of COVID-19. We carried out a detailed review of publicly available data, information and claims on the potentials of indigenous plants used in Sub-Saharan Africa as antiviral remedies with potentials for the prevention and management of COVID-19. In this review, we have provided a holistic report on evidence-based antiviral and promising anti-SARS-CoV-2 properties of African medicinal plants based on *in silico* evidence, *in vitro assays* and *in vivo* experiments alongside the available data on their mechanistic pharmacology. In addition, we have unveiled knowledge gaps, provided an update on the effort of African Scientific community toward demystifying the dreadful SARS-CoV-2 micro-enemy of man and have documented popular anti-COVID-19 herbal claims emanating from the continent for the management of COVID-19 while the risk potentials of herb-drug interaction of antiviral phytomedicines when used in combination with orthodox drugs have also been highlighted. This review exercise may lend enough

credence to the potential value of African medicinal plants as possible leads in anti-COVID-19 drug discovery through research and development.

Keywords: COVID-19, phytomedicines, Traditional African Medicine, herbal immuno-stimulants, herb-drug interaction

INTRODUCTION

The current pandemic threatening the global community, a highly communicable viral infection otherwise known as Coronavirus disease 2019 (COVID-19), is caused by the Severe Acute Respiratory Syndrome Coronavirus two or SARS-CoV-2 (**Figures 1**, **2**) (Chan et al., 2020a). The sudden emergence of the disease was first noticed in Wuhan city, China, East Asia (Chan et al., 2020b; Guo et al., 2020). Social distancing, hand washing, alcoholic disinfectants or hand sanitizers, isolation/quarantine, travel restrictions, wearing of face mask, community containments and partial or total lockdown (World Health Organization, 2020) have continued to remain effective non-pharmaceutical preventive measures.

Despite all the divergent efforts to halt the spread and mortalities associated with COVID-19, the devastating micro-enemy has continued to spread causing more deaths and a lot of socio-economic implications. While most of the affected countries in Europe and America are relying solely on orthodox drugs, South-East Asia and in particular, China where the COVID-19 pandemic appear to have originated, has adequate documentation of successful outcomes following the integration of Traditional Chinese Medicine (TCM) with orthodox medicines in COVID-19 management (Chang et al., 2020; Gao et al., 2020). Interestingly, overwhelming literature evidence suggests that China and neighboring Asian territories practice a robust age-long traditional medicine system that has been favorably integrated with the western medicine; the TCM-western system of healthcare was therefore adopted to combat the earlier outbreak of SARS-CoV in Guangdong, China in 2002 leading to the reported defeat of the epidemic (Leung, 2007). Top among the well documented herbal recipes and formulations used as adjuvants alongside western medicines during the time included San Ren Tang, Yin Qiao San, Ma Xing Shi Gan Tang, Gan Lu Xiao Du Dan, and Qing Ying Tang, a polyherbal formulation containing many indigenous plants. In addition, Hong Kong has documented the traditional application of Sang Ju Yin and Yu Ping Feng San, *Isatis tinctoria* L. (Brassicaceae) and *Scutellaria baicalensis* Georgi (Lamiaceae), for prophylactic use among health workers against SARS-CoV infection (Hensel et al., 2020; Luo et al., 2020). Following the reported success with the use of herbal adjuvants during the previous outbreaks of viral infections in China, the outbreak of SARS-CoV2 received an immediate authorization of integral Traditional Chinese–Western medicines to treat COVID-19 (Gao et al., 2020). This means Traditional Chinese Medicine - TCMs (mainly plant-based) were co-administered with western drugs as adjuvants.

However, in Africa, the use of phytomedicines which is also referred to as herbal medicine or phytotherapy is well embraced in different Pan African territories where 80–90% of its rural populations rely on traditional medicines (mainly plant-based) for primary healthcare (Elujoba et al., 2005; Mahomoodally, 2013). The extensive use of the predominantly plant-derived traditional medicine in Africa otherwise referred to as Traditional African Medicine, has been described to be associated with African socio-economic and socio-cultural endowments (Elujoba et al., 2005). For this reason, the WHO has continued to sensitize African Member states toward the integration of TAM into their health system (Mahomoodally, 2013) as the body recognizes the relevance of traditional, complementary and alternative medicine to Africa which has a long history of TAM and knowledgeable indigenous practitioners. For instance, there has been an unprecedented use of phytomedicines in Africa following the outbreak and global spread of COVID-19 pandemic, a situation which has been compounded by lack of authorized medicines that are effective, affordable and accessible to the populations coupled with a relatively weak African health sector (Lone and Ahmad, 2020; WHO, 2020). Coincidentally, available evidence from Africa Center for disease Control and Prevention (Africa CDC) suggests that the African continent is the last to be hit by the viral pandemic and least affected continent whose mortality rate (2.1%) until July 21, 2020 was less than half of the reported global mortality (5%) rate. Hence, despite the vulnerability of the African continent, it accounts for only 5% of the globally reported cases of COVID-19. While several factors may be attributable to this seeming positive trend, the near absolute dependence on the obvious potentials of the African medicinal plants for COVID-19 management may not be ruled out. As a malaria endemic region, the Sub-Saharan Africa often co-administer herbal remedies alone or combined with orthodox drugs as adjuvants and many of these plant-based medicines have since been informally repurposed by various users for COVID-19 prevention and symptomatic management as simple home remedies. Unlike the Traditional Chinese Medicine, there is a paucity of well reported studies on the impact of the widely embraced TAM adopted so far for the prevention, management and treatment of COVID-19. This review is therefore aimed at the documentation of African medicinal plants and their therapeutic potentials in the prevention and management of COVID-19. The potential risks associated with herb-drug interaction of antiviral phytomedicines when used in combination with orthodox drugs have been highlighted. In addition, we document the pharmacokinetic considerations in developing potential anti-COVID19 herbal products.

METHODS

In this review, a literature search was carried out and popular scientific databases including PubMed, PubChem, Google

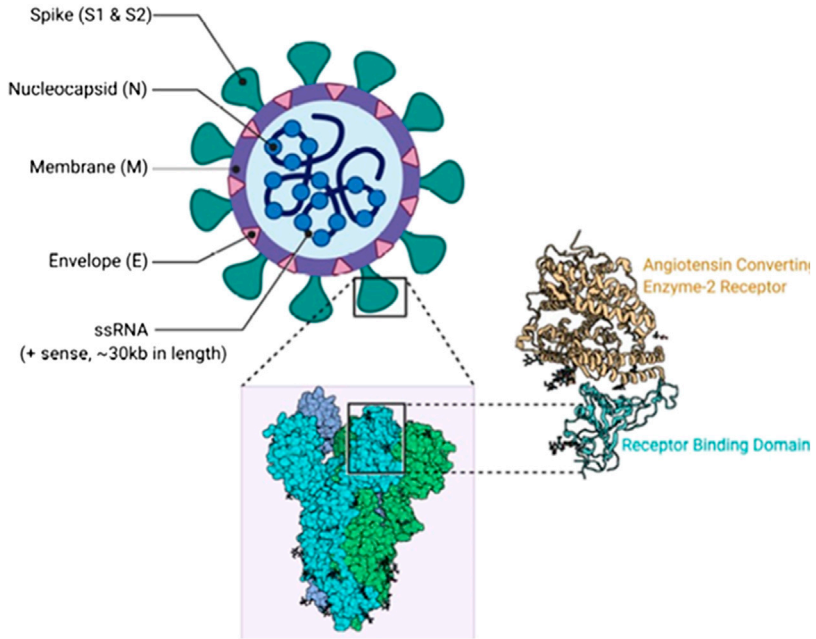


FIGURE 1 | SARS- CoV two Structure (Cascella et al., 2020). Contributed by Rohan Bir Singh, MD; Made with Biorender.com.

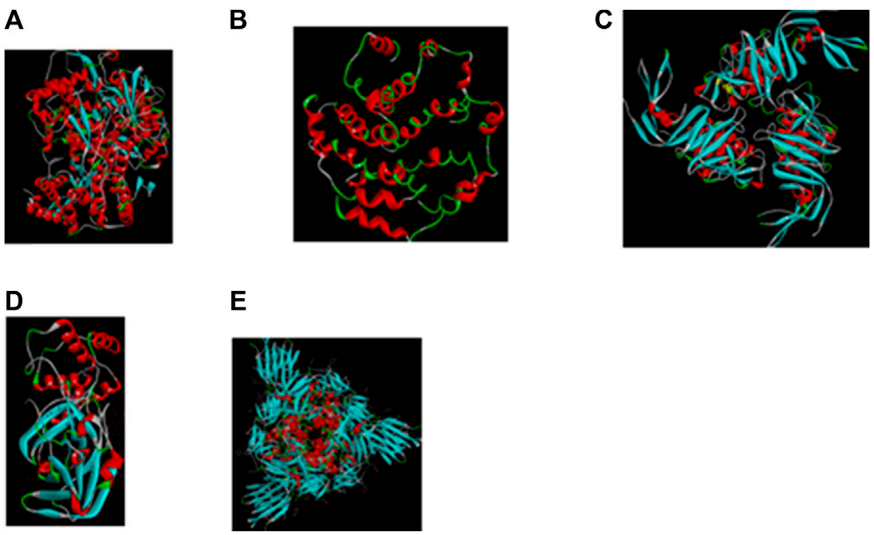


FIGURE 2 | Important molecular targets in SARS-CoV2 structure for interaction with antiviral compounds in phytomedicines. Many African herbal solutions are polyherbal with potentials for more than one therapeutic targets on the viral particle **(A)** PDB 6M71: Structure of the RNA-dependent RNA polymerase from COVID-19 virus (Gao et al., 2020); **(B)** PDB 5X29: NMR structure of the SARS Coronavirus E protein pentameric ion channel (Surya et al., 2018); **(C)** PDB 6W9C: The crystal structure of papain-like protease of SARS CoV-2 (Walls et al., 2020); **(D)** PDB 6MQ: SARS-CoV-2 3CL protease (3CL pro) apo structure (Su et al., 2010); **(E)** PDB 6VXX: Structure of the SARS-CoV-2 spike glycoprotein (closed state) (Osipiuk et al., 2020).

Scholar, HINARI; these were searched to retrieve scientific peer-reviewed publications on African traditional medicinal plants with antiviral potentials. Considering the framework of unveiling the role played by antiviral plants commonly used in Traditional African Medicine (TAM) in tackling deadly infectious diseases such as COVID 19, the traditional uses, bioactive metabolites, in silico, *in vitro*, *in vivo*, and clinical studies as well as the sustainable use of these plants in African ethnomedicine and associated challenges were considered and included. Articles published in English before July 2020 using the keywords; "Africa", "antiviral plants", "SARS COV", "COVID-19", "antiviral phytomedicines", "Traditional

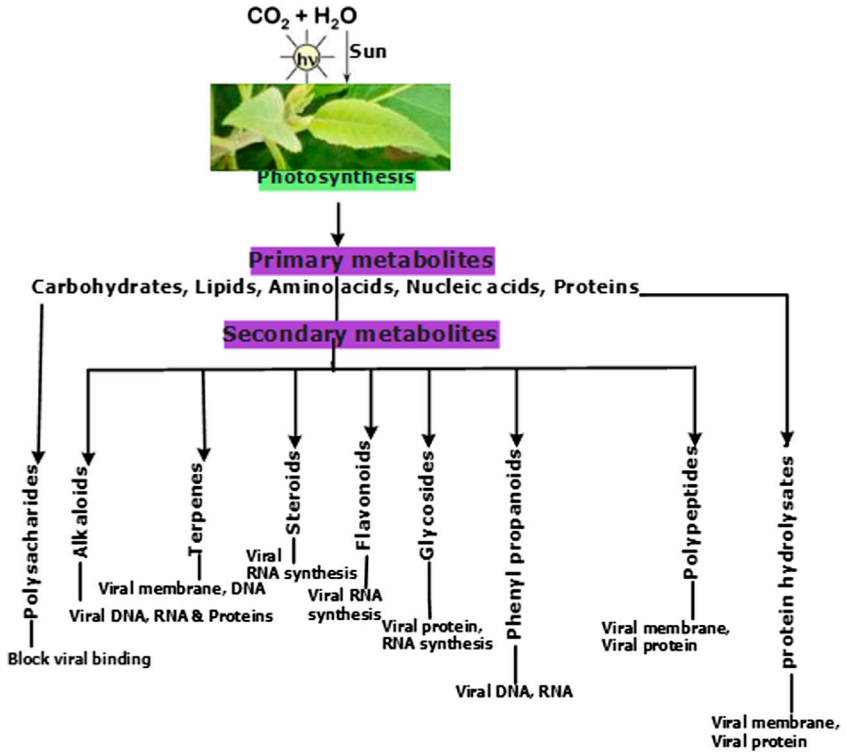


FIGURE 3 | The role of primary and secondary plant metabolites as antiviral agents.

African Medicine", "herbal immuno-stimulants", "herb-drug interaction" were subsequently retrieved. Generally accepted and popular anecdotal claims on plant-based COVID-19 treatment options have also been included wherever appropriate. Excluded from this review were studies carried out on plants not found in Africa, repetitive studies and publications that have failed to meet the inclusion criteria. Following the minimal impact of the much earlier outbreaks of the severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) epidemics on the African continent, SARS and MERS have not attracted a significant TAM-related research attention; and therefore are not a focus of this review.

In order to rightly place the claims made in proper context with regards to the availability of research data, we have defined and categorized the claims reported in this review based on the relevance of different plants and plant products in COVID-19 management; consequently, to reveal what level of evidence exists for a reported plant, the following classifications have been described;

Level I evidence - Evidence from at least one clinical study.
Level II evidence - Inferences supported by *in vivo* experiments.
Level III evidence - Detailed mechanistic and other *in vitro* evaluations support the conclusion.
Level IV evidence - Evidence from preliminary *in vitro* screening or in silico data (IV*).
Level V evidence - Claims are extrapolated from activities demonstrated against other similar viruses or in contextually related settings.

These defined levels of evidence are indicated in square brackets within the review, e.g [Level I] for claims derived from at least one human study.

Medicinal Plants of African Origin with Antiviral Activities

Africa, with one of the richest cultural heritage in the traditional application of plants in healthcare, is endowed with a vast plant biodiversity (Cunningham, 1997; Dzoyem et al., 2013). An estimated 68,000 plant species have been reported to grow within the continent, over half (35,000) of which are endemic to Africa (UNEP-WCMC, 2016).

The peculiar diversity and uniqueness of climatic, soil, rainfall and environmental factors have encouraged the growth of an extensive plant diversity, endemism and great variation in indigenous plants across the entire region (James et al., 2007). The proximity, accessibility and abundance of African medicinal plant resources may have informed their amazing acceptability and popularity by African populations for meeting primary healthcare needs (Neuwinger, 2000) especially during emergency scenarios as in COVID-19.

Diverse plants, with their isolated products and derivatives with antiviral properties including alkaloids, flavonoids, phenolic

FIGURE 4 | Family of Plants endemic to Africa expressing and accumulating antiviral primary and secondary metabolites.

compounds, terpenes, polysaccharides and polypeptides (**Figure 3**), have been reported (Badia-Boungou et al., 2019; Maroyi 2014). As nature's *biological* laboratories containing hundreds and thousands of bioactive metabolites, African medicinal plants abundantly accumulate phytochemical markers and defense compounds of chemotaxonomic significance in different plant families (**Figure 4**). This variation in bioactive chemical markers in different plants has facilitated and justified the use of some plants in some families more often than others following their superior efficacy for conditions they are meant to treat in Traditional African Medicine (TAM) including viral outbreaks. Plant families which accumulate antiviral classes of compounds have been summarized in **Figure 4**. The antiviral properties and immuno-modulatory activities of these compounds can be utilized in the prevention, treatment and management of COVID-19, which till date awaits effective, safe, affordable and accessible treatment options. The efficacy of some plants and derived phytochemicals of African origin have been established following their potential to interfere with the replication and transcription machinery of some causative agents of viral infections (Mehrbod et al., 2018a; Mehrbod et al., 2019). Documented antiviral potency of these medicinal plant extracts justifies their selection for further studies as potential agents for prophylactic administration or potential therapeutic intervention against COVID-19. However, an in-depth and rigorous analysis of their efficacy and safety using internationally acceptable protocols is germane during clinical trials prior to healthcare utilization.

Cos and colleagues (Cos et al., 2002a) reported some African plants that are active against poliomyelitis, coxsackie, semliki forest, measles, and vesicular stomatitis virus (VSV). The antiviral activity of the extracts investigated was determined as the reduction factor (RF) of the viral titer which is interpreted as the ratio of the virus titer in the absence and in the presence of the extract. The leaves of *Macaranga kilimandscharica* Pax (Euphorbiaceae) exhibited considerable *in vitro* effect against measles. The 80% ethanol extracts were found to block the viral replication of Coxsackie and Measles. The leaf extracts of *Guizotia scabra (Vis.)* Chiov. (Asteraceae) were active against Coxsackie and Polio, while *Pavetta ternifolia* Hiern (Rubiaceae) (leaves) was shown to display high activity against only Coxsackie. The leaves of *Eriosema montanum* Baker f. (Fabaceae) have been reported to have considerable activity against all the tested viruses. The stems of *Entada abyssinica (EnE)* Steud. ex A. Rich (Fabaceae) were highly potent against Polio while displaying intermediate efficacy against other viruses tested with the exception of Measles. The leaves of *EnE* have profound antiviral effects against Semliki forest virus (Cos et al., 2002a). The antiviral activity of aqueous extract of *Syzygium brazzavillense* Aubrév. and Pellegr. (Myrtaceae) against coxsackievirus (CV) and poliovirus type 1 was revealed by Badia- Boungou et al. The extract was found to inhibit replication of CVB4 in HEp-2 cell cultures and also limit the cytopathic effect (CPE) induced by type 1 polioviruses and by CVB2, CVB3, and CVB4 (Badia-Boungou et al., 2019) and may possibly interfere with SARS-CoV-2 replication. Active fractions and metabolites (such as flavonoids and terpenenoids) reported in these African plants have now been documented to be promising COVID-19 (da Silva Antonio et al., 2020; James et al., 2007; Nworu et al., 2017) remedies making these plants sustainable biomass for drug discovery against COVID-19 [Level V]. However, little is known about the toxicity of the plants while *in vivo* data as well as elaborate and mechanistic *in vitro* investigations will be required to support the current preliminary *in vitro* findings.

In another experiment, Cos et al. (Cos et al., 2002b) investigated the antiviral activity of certain Rwandan plants against human immunodeficiency virus type-1. They showed that ethanolic extract of *Aspilia pluriseta* Schweinf. ex Engl. (Asteraceae) exhibited pronounced antiviral activity by enabling an absolute cell-resilient against HIV-induced cytopathic effect compared to the controls. The selective index value of the extract was found to be greater than 12 (Cos et al., 2002b). Also, thiarubrine-A **(93)** isolated from the leaves of *A. pluriseta* demonstrated phototoxic activity against enveloped

viruses such as cytomegalovirus and Sindbis virus (Hudson et al., 1986). The ethanolic extract of *Rumex nepalensis* Spreng. (Polygonaceae) with a selective index of 11 was able to achieve 89% cell protection against HIV-induced cytopathic effect. The residue obtained when ethanolic extract of *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) was suspended in 60% methanol and was subsequently extracted with petroleum ether and ethyl acetate concurrently: it displayed significant inhibitory effect as anti-HIV-1 agent having a selective index greater than 461 (Cos et al., 2002b). In addition, the aqueous fraction showed pronounced anti-HIV-1 activities at concentrations of 200, 40, 8, 1.6 and 0.32 μg/ml. Furthermore, isolation of sesquiterpene lactones such as diversifolin **(91),** diversifolin methyl ether, and tirotundin **(92)** from *T. diversifolia* has been reported with relevant pharmacological properties (Asres et al., 2001; Cos et al., 2002b). The mode of action of these compounds is associated with decline in the production of inflammatory mediators including cytokines and chemokines. The reported mechanism involves the interference with the DNA binding activity of the transcription factor NF-κB (Rüngeler et al., 1998). The fact that some of these products exhibit biological activities involving host inflammatory response may indicate their potential treatment potentials in COVID-19 with its reported inflammatory undertone [**Level III, V**]. Another bioactive compound with anti-HIV-1 activity isolated from the mature stems of *T. diversifolia* is an artemisinic acid derivative (Bordoloi et al., 1996). Artemisinic acid is a sesquiterpenoid precursor of artemisinin and the semi-synthetic product 12-*N*-butyl deoxoartemisinin has been reported to inhibit HIV activity (Jung et al., 2012). It should however be pointed out that in-depth *in vivo* and clinical investigations will need to be conducted to objectively establish the clinical relevance of these plant products. Interestingly however, the major component of the Madagascar's COVID organics (CVO), a herbal formulation containing *Artemisia annua* L. (Asteraceae), is the antimalarial compound artemisinin. Although the efficacy and safety of CVO is yet to be clinically validated, the overwhelming willingness of other African countries to participate in the clinical trials highlights the priority accorded plant-derived medicines in Africa. The WHO and African Health Ministers have agreed to allow herbal and indigenous health products to go through requisite clinical trials to establish their efficacy and safety prior to adoption as treatment options for COVID-19 (WHO, 2020). Increasing evidence suggests that these plant-derived antimalarial sesquiterpene lactones, an active component *T. diversifolia* and *A. annua* may hold a promise in COVID-19 treatment provided further research attention is given to support efficacy and safety (Rahman et al., 2020; da Silva Antonio et al., 2020).

Helichrysum foetidum Moench (Asteraceae) is one of the selected Rwandan medicinal plants (Sindambiwe et al., 1999) whose ethanol extract (200 mg/ml), after a 10-fold dilution produced antiviral activity by limiting the extracellular viability of herpes simplex virus type 1 (HSV 1) and Semliki forest virus A7 (SF A7) while *Chamaecrista mimosoides* (L.) Greene (Fabaceae) and *Ipomoea involucrata* P.Beauv. (Convolvulaceae) under the same experimental conditions and concentration displayed high antiviral potential against HSV 1. *Ipomoea involucrata* was potent as a virucidal agent against vesicular stomatitis virus T2 (VSV T2), SF A7 and measles virus strain Edmonston A (MV-EA). Findings from this study showed that *C. mimosoides*, *Rotheca myricoides* (Hochst.) Steane and Mabb. (Lamiaceae) and *Helichrysum cymosum* (L.). D. Don ex G. Don (Asteraceae) demonstrated virucidal activity against HSV 1, measles virus strain Edmonston A (MV-EA), and Semliki forest virus A7. In addition, the study highlights the virucidal activity of *Maesa lanceolata* Forssk. (Primulaceae) against the screened enveloped viruses which was exceptional compared to the other tested plants, making this plant an interesting candidate for further research consideration against SARS-CoV-2 [**Level V**]. Also investigated is a mixture isolated from methanol extract of *M. lanceolata* (leaves) termed maesasaponin mixture A. This mixture was found to reduce the titer and infectivity of herpes simplex virus type 2 (HSV 2) at concentrations of 100 μg/ml and 250 μg/ml, respectively. More so, it incapacitated the virus at 500 μg/ml concentration. Maesasaponin mixture A also repressed the activity of vesicular stomatitis virus T2 (VSV T2) (Sindambiwe et al., 1999). Maesasaponin mixture A may be a promising potential source of active antiviral metabolites which may produce activity against SARS-CoV-2 following a more elaborate preclinical, clinical investigations and phytochemical standardization of extracts which are lacking in the study under review [**Level V**].

The extracts of *Pittosporum viridiflorum* Sims (Pittosporaceae) and *Rapanea melanophloeos* (L.) Mez (Primulaceae) were reported by Mehrbod et al. (Mehrbod et al., 2018a) to have inhibitory effect against influenza A virus (IAV). The activity of the extracts resulted in averages of 7.4 and five logs hemagglutination (HA) decrements for *R. melanophloeos* and *P. viridiflorum*, respectively. This shows the potency of the plants against IAV (Mehrbod et al., 2018a). In another study, Mehrbod and colleagues again evaluated the activity of a glycoside flavone **(73)** (quercetin-3-O-α-L-rhamnopyranoside) isolated from *R. melanophloeos* against IAV. Quercetin-3-O-α-L-rhamnopyranoside **(73)** was reported to decrease the virus titer at 150 μg/ml by directly inhibiting the virus replication, and modulation of cytokine production (Mehrbod et al., 2018b). Research evidence supports the antiviral activity and more specifically, anti-COVID-19 potentials of a combination of quercetin and vitamin C, some common components of the mainly polyherbal extracts used in TAM (Colunga Biancatelli et al., 2020) [**Level III**]. Interestingly, emerging evidence suggests that the anti-SARS-2 activity of glycosylated forms of flavonoids may be significantly higher than their respective aglycons while plant extracts and fractions may be significantly more effective than isolated pure compounds (Zakaryan et al., 2017; da Silva Antonio et al., 2020). However, the indigenous formulations containing these plant species require preclinical and clinical standardization for evidence-informed application and for a possible clinical use.

In a study demonstrating the antiviral activity of Ethiopian medicinal plants against both HIV-1 and HIV- 2, the methanol fraction obtained from the root bark of *Bersama abyssinica* Fresen. (Francoaceae) and the leaves of *Combretum paniculatum* Vent (Combretaceae) at median effective

concentrations (EC_{50}) of 3.1 and 5.2 μg/ml were the most potent in inhibiting the replication of HIV-1 having a selective index of 3.8 and 6.4, respectively [**Level V**]. The extracts obtained from the leaves of *Dodonaea viscosa subsp. angustifolia* (L.f.) J. G. West (Sapindaceae) and the stem bark of *Ximenia americana* L. (Olacaceae) were found to be slightly active against HIV-1 with EC_{50} values ranging from 8.3 to 27.7 μg/ml and selectivity indices that ranged from 3.9 to 4.9. The acetone fraction of *C. paniculatum* displayed an inhibitory potential against HIV-2 with a relatively high selectivity index of 32 at an EC_{50} value of 3.0 μg/ml while demonstrating moderate activity against HIV-1 with EC_{50} value of 15 and selectivity index of 6.4. Also, the replication in HIV-2 was altered by hydroalcohol fraction of *X. americana* at EC_{50} value of 27.1 μg/ml (Asres et al., 2001). In addition to lack of robustness and quality issues associated with these investigations, it remains to be determined if *in vitro* studies would suggest potential benefits of clinical relevance.

Another study involving human subjects, carried out in collaboration with herbal practitioners in different districts of Uganda, revealed that HIV-positive patients showed a treatment outcome with significant decrease in CD4 positive T-cell lymphocytes in the blood when treated with *Aloe spp*., *Erythrina abyssinica* Lam. (Fabaceae), *Nauclea latifolia* Sm (Rubiaceae), *Psorospermum febrifugum* Spach (Hypericaceae), *Mangifera indica* L. (Anacardiaceae), and *Warburgia salutaris* (G. Bertol.) Chiov. (Canellaceae) (Lamorde et al., 2010). The use of *Calendula officinalis* L([Asteraceae) have also been shown to result in progressive decline in viral loads and in CD4 T-cell counts in HIV-positive volunteers (Mills et al., 2005). However, these human studies lack adequate comparative data so that it remains unclear whether the patient recovered because of the use of particular herbal preparation or the general clinical care received. While findings from this study may be of interest, there is need for further investigation to establish an elaborate toxicological data, *in vivo* evidence and clinical proof of safety and efficacy. These documented antiviral African medicinal plants hold promise in the ambitious search for potent medicines to defeat the lethal COVID-19 pandemic [**Level V**].

African Plant-Derived Antiviral Metabolites, Immunomodulation and Molecular Targets

Phytomedicnes have shown potentials as immunoadjuvants for their ability to increase the effectiveness of vaccines while plant-derived chemical compounds including ellagic acid **(80)**, curcumin **(72)**, flavonoids and quercetin possess anti-infective properties that work either by directly attacking the pathogen or indirectly by stimulating innate and acquired defense mechanisms of the host (Sodagari et al., 2018; Afolayan et al., 2020). Chemically diverse antiviral compounds including primary plant metabolites such as polysaccharides, proteins, lectins, protein hyrolysates and aminoglycans (Monzingo et al., 1993; Bouckaert et al., 1996; Sankaranarayanan et al., 1996; Harata and Muraki, 2000; Meagher et al., 2005) as well as secondary metabolites including alkaloids, phenylpropanoids, tannins, flavonoids, lignans, coumarins, glycosides, steroids, terpenes, polypeptides, antimicrobial peptides, defensins, cyclotides (**1–7**) and many other plant-derived cystine-knot peptides (Kapoor et al., 2017; Rex et al., 2018; Younas et al., 2018; Berit et al., 2020; Ghildiyal et al., 2020; He et al., 2020) have been detected and isolated from African medicinal plants **(1–172)**. The role of these antiviral compounds and their main molecular target have been presented in **Figure 3** while plant families native to Africa which abundantly express and accumulate these phytocompounds that have found uses as anti-infective agents in TAM are presented in **Figure 4**.

Phytomedicines with a long history of use in traditional medicines and bioactive compounds obtained from them have been shown to exert antiviral, anti-inflammatory and immunomodulatory effects and these bioactivities have been proposed to be linked (Fialho et al., 2016), following their ability to modulate the immune response (Han et al., 1998; Zhang et al., 2002; Shivaprasad et al., 2006; Cruz et al., 2007; Sodagari et al., 2018) and in parallel reduce viral or parasite load (Omoregie and Pal, 2016; Michelini et al., 2018; Jasso-Miranda et al., 2019; Salinas et al., 2019; Afolayan et al., 2020). These desirable dual antiviral effects have been demonstrated in indigenous plants used in TAM for the treatment of various viral diseases (Goren et al., 2003; Esimone et al., 2005; Akram et al., 2014; Buba et al., 2016; Donma and Donma, 2020; Jacques et al., 2020; Kumar and Venkatesh, 2016; Kumar et al., 2015; Nawrot et al., 2014; Nworu et al., 2017; Osadebe and Omeje, 2007; Parvez et al., 2019; Raza et al., 2015). For instance, *Combretum micranthum* G. Don is one of the main constituents of an indigenous Nigerian antiviral phytomedicine called "Seven Keys to Power" used in the traditional management of smallpox, chicken pox, measles and HIV/AIDS (Esimone et al., 2005). In addition, *R. capparoides* has been used by herbalists in the eastern part of Nigeria for the treatment of chickenpox, smallpox and hepatitis, while *C. cajan* is used in ethnoveterinary medicine for the treatment of several viral diseases of cattles in Northern Nigeria (Esimone et al., 2005). However, rigorous, robust and well validated scientific investigations are needed to turn these potential antiviral remedies to clinical use. At this time, these data are not available, thus limiting their application.

A typical medicinal plant is a biological factory of a plethora of complex bioactive metabolites and most of the phytomedicines used in TAM are polyherbal with potential multiple targets in host and/or pathogen structure. Expectedly, complex phytotherapeutics which target both the pathogen as well as the host structure required for infection of viruses without a significant cytotoxicity to the host, could represent an alternative way to develop new and effective antiviral phytotherapies (Bekerman and Einav, 2015). Illustrated in **Figures 1**, **2** are some important molecular targets identified in SARS-CoV-2 and featuring druggable structural components capable of fostering interaction with nature-inspired antiviral metabolites biosynthesized from both primary and secondary metabolic pathways presented in **Figure 3** (da Silva Antonio et al., 2020; Ghildiyal et al., 2020). Bioactive protein hydrolysates and cysteine-rich polypeptides target viral membrane and proteins, alkaloids and glycosides target viral proteins and RNA, terpenes

TABLE 1 | Selected antiviral Angiosperm plants of African origin and the major class of phytochemicals present based on widespread use and documented evidence.

S/N	Plants	Class of phytochemicals present	Identified phytochemicals with antiviral activity	Indications	Country
1	*Achyranthes aspera* L. (Amaranthaceae)	Flavonoids, alkaloids, terpenoids Goyal et al. (2007)	Oleanolic acid **(168)** Mukherjee et al. (2013)	HSV-1 HSV-2 HIV- Mukherjee et al. (2013)	Africa, south Afrcia
2	*Adansonia digitata* L. (Malvaceae)	Phenolics	Nil	HSV-1	Nigeria Senegal Sulaiman et al. (2011)
3	*Andrographis paniculata* (Burm.f.) Nees (Acanthaceae)	Diterpenoids, flavonoids, polyphenols Pongtuluran and Rofaani (2015)	Andrographolide **(63)** **(**Pongtuluran and Rofaani (2015)	HSV-1 SRV EBV Wiart et al. (2005) DV Panraksa et al. (2017)	Nigeria Hamidi et al. (1996)
4	*Aspalathus linearis* (Burm.f.) R.Dahlgren (Fabaceae)	Phenolics Rahmasari et al. (2017)	Aspalathin **(105),** nothofagin **(106**), isoorientin **(104**), orientin **(103**), quercetin **(73**), luteolin **(170)** Rahmasari et al. (2017)	HIV Influenza Rahmasari et al. (2017)	South Africa
5	*Azadirachta indica* A. Juss. (Meliaceae)	Carbohydrates	Polysaccharides P1 and P2	PV-1 Faccin-Galhardi et al. (2012)	African countries
6	*Bulbine frutescens* (L.) Willd. (Xanthorrhoeaceae)	Phenolics, alkaloids, flavonoids Shikalepo et al. (2018)	Myricitin **(32)**, xanthohumol **(96**), scutellarin **(95),** methoxyflavone **(169)** Shikalepo et al. (2018)	HIV-1 Shikalepo et al. (2018)	South Africa
7	*Canavalia ensiformis (L.) DC.* (Fabaceae)	Protein	Lectins (Concanavalin A) **Figure 6**	HSV Marchetti et al. (1995)	Nigeria Africa
8	*Cocos nucifera* L. (Arecaceae)	Phenolics Esquenazi et al. (2002) Tannins Lima et al. (2015) Flavonoids Vlietinck et al. (1997)	Catechins **(133)** Esquenazi et al. (2002), myricetin **(136)** Vlietinck et al. (1997)	EBV CMV VV Lima et al. (2015) HIV-1 Vlietinck et al. (1997)	Kenya
9	[a]*Combretum micranthum* G.Don (Combretaceae)	Phenolics, tannins Ferrea et al. (1993), Flavonoids Welch (2010)	Catechin **(133)**, catechinic acid Ferrea et al. (1993) cinnamtanins **(98),** pavetanins **(97),** AOCA(Alkaline auto-oxidized catechins) Vlietinck et al. (1997), Apigenin **(156)** Welch (2010)	HSV-1 HSV-2 Ferrea et al. (1993) HIV-1 Vlietinck et al. (1997)	Nigeria
10	*Echinacea purpurea* (L.) Moench (Compositae)	Phenolics, Alkamides Vimalanathan et al. (2005)	Cichoric acid **(108)** Vimalanathan et al. (2005) Iwu (2014)	HIV Awortwe et al. (2013) HSV Influenza Barnes et al. (2005)	South Africa Zimbabwe
11	*Glycyrrhiza glabra* L. (Fabaceae)	Triterpenes (saponins), flavonoids Vlietinck et al. (1997)	Glycyrrihizin and its derivatives **(107)**, liochalchone, isolicoflavonol, glycocoumarin, glycyrrhizoflavone, licopyranocoumarin	HSV-1 HIV Vlietinck et al. (1997)	South Africa
12	*Macaranga barteri* Müll. Arg. (Euphorbiaceae)	Phenolics (stilbenes) Ogbole et al. (2018), Segun et al. (2019)	Vedehanin **(110)**, schwenfurthin, mappai Ogbole et al. (2018), Segun et al. (2019)	EV[b] Ogbole et al. (2018), Segun et al. (2019)	Nigeria
13	*Musa acuminata* L. Musa spp (Musaceae)	Protein	Lectins Peumans et al. (2000) **Figure 7**	Anti-HIV Swanson et al. (2010)	Nigeria, tropical Africa
14	*Oldenlandia affinis* (Roem. and schult.) DC. (Rubiaceae)	Peptides	Cyclotides (KB1, KB8) Ireland et al. (2008) **Figure 5**	HIV Daly et al. (2004)	Dr. Congo
15	*Papaver somniferum* L. (Papaveraceae)	Alkaloids Vlietinck et al. (1997)	Papaverine **(99)** Vlietinck et al. (1997)	HIV-1 Vlietinck et al. (1997)	Nigeria
16	*Rapanea melanophloeos* (L.) Mez (Primulaceae)	Flavonoids Mehrbod et al. (2018a)	Quercetin **(73)** Mehrbod et al. (2018a)	Influenza A Mehrbod et al. (2018a)	South Africa
17	*Zingiber officinale* Roscoe (Zingiberaceae)	Terpenoids Chrubasik et al. (2005), Iwu (2014)	Beta sesquiphellandrene **(109)** Chrubasik et al. (2005), Iwu (2014)	RhV RSV Chrubasik et al. (2005), Iwu (2014)	Nigeria

HIV–Human Immunodeficiency Virus; HSV 1–Human Simplex Virus one; HSV 2–Human Simplex Virus two; RhV–Rhinovirus; RSV–Respiratory Syncytial Virus; EBV–Epstein-Barr Virus; CMV–Cytomegalovirus; VV–Visna Virus; DV–Dengue Virus; SRV–Simian Retrovirus; PV-1 -Poliovirus type 1.

[a]*As a part of the seven Keys preparation, it is used to treat small-pox, chicken pox and measles. Welch (2010).*

[b]*It is only effective against serotypes E7 and E19. (Segun, et al., 2019); (Ogbole et al., 2018).*

target viral membrane while steroids and flavonoids target viral RNA synthesis (**Figure 3**). For instance, the interaction between the spike glycoproteins of SARS COV-2 and the host cell angiotensin converting enzyme 2 (ACE2) receptors which leads to viral attachment and entry, culminating in COVID-19 could be prevented or blocked effectively [**Level V**] by

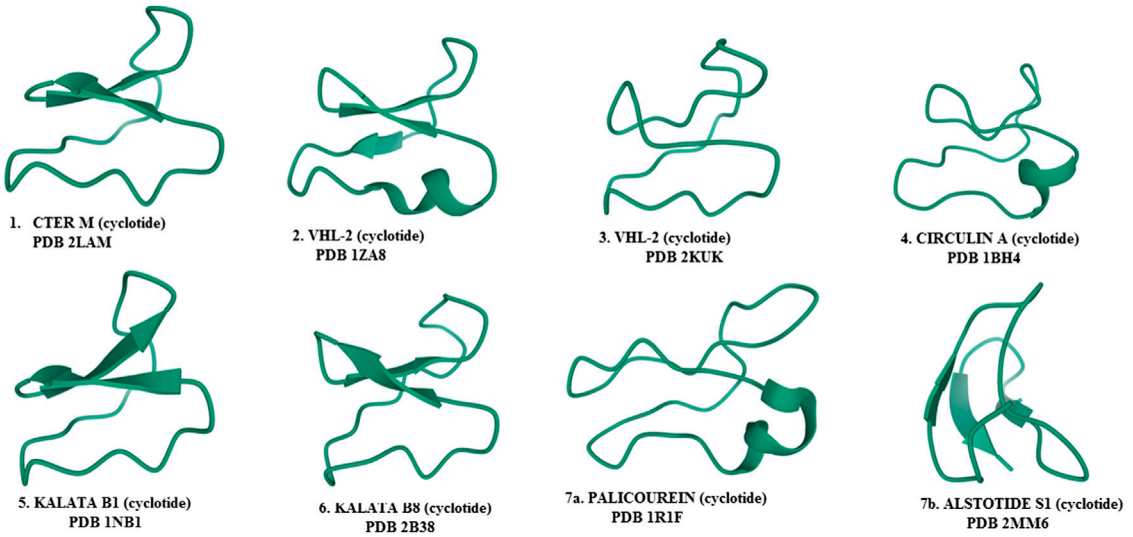


FIGURE 5 | Antiviral cyclotides (cysteine-rich peptides) isolated from plants.

antiadhesive phytocompounds such as phenolics, tannins and polysaccharides (Jassim and Naji, 2003; Hensel et al., 2020) reported in some African antiviral plants including *Adansonia digitata* L., *Andrographis paniculata* (Burm.f.) Nees, *Combretum micranthum* G.Don, *Macaranga barteri* Müll. Arg., *Azadirachta indica* A. Juss. (**Table 1**). These antiviral metabolites accumulate in high amounts in several plant families used in TAM including the Lamiaceae, Meliaceae, Asteraceae, Arecaceae, Acanthaceae, Combretaceae, Zingiberaceae, Euphorbiaceae and Malvaceae (**Figure 4**). However, further mechanistic studies, safety investigation as well as clinical studies are required for their clinical applications.

Plant-derived cysteine knot peptides including antimicrobial peptides and defensins whose bioactivities like other types of defensins are able to block viral infection by clustering the viral particles and blocking receptor binding (Nguyen et al., 2016; Weber, 2020). These hormone-like disulphide-stabilized peptides have been described to mediate in the inhibition of viral entry, viral particle disruption, interference with essential cell signaling or viral gene expression, or by other poorly-understood mechanisms. Furthermore, in addition to the direct antiviral activities outlined above, antimicrobial peptides and defensins modulate adaptive immune responses following their ability to attract immune cells (Weber, 2020). Cystine knot polypeptides (**Figure 5**) are well distributed in tropical African flora within the Apocynaceae, Rubiaceae, Violaceae, Curcubitaceae, Leguminoseae, Poaceae and Fabaceae plant families (**Figure 4**). Molecular studies have shown that these suites of peptides bind to viral spike and membrane proteins (Nguyen et al., 2016) and may therefore be early acting in preventing viral attachment and entry into the host cell. As some of the most exploited plant families in TAM (with the exception of Violaceae), plants species from them could help in COVID-19 treatment [**Level V**] and therefore deserve further anti-SARS CoV-2 molecular studies. Interestingly, knottin peptidyl therapeutics are stable to extreme conditions and easily extracted under aqueous mediums commonly used in TAM. Unfortunately, despite their emerging therapeutic potentials, research in cysteine knotted polypeptides has not received adequate scientific attention as less than 1% of African flora has been screened for peptide drug discovery (Attah et al., 2016a). Carbohydrate-binding lectin proteins from African Musa species and *Canavalia ensiformis (L.) DC.* Fabaceae have shown interesting broad spectrum antiviral. However, the clinical application of lectin proteins will require further in-depth research to circumvent inherent limitations including toxicity, stability and bioavailability in order to ensure that their druggable targets will offer a therapeutic benefit (Mitchell et al., 2017).

Therapeutic Convergence in the Use of Antimalarial Plants Against Viral Infections in Africa

Antimalarial drugs derived from medicinal plants used in Traditional African Medicine have been found useful as repurposed drugs in managing other diseases including viral infections such as HIV, Ebola, and other viral hemorrhagic diseases due to lack of effective therapeutic agents. The active constituents of these plants have various mechanisms of action which are often not fully elucidated against malaria parasites. The complexity of these constituents sometimes lead to side effects that have been studied for repurposing them for the treatment of other conditions such as non-malaria infectious diseases (Das, 2015; Haładyj et al., 2018; Wolf et al., 2006). The geographical distribution between malaria and viral diseases where malaria endemic regions of the world such as Africa and Asia appear to experience relatively low cases of COVID-19-related mortalities led to the consideration of a possible therapeutic convergence between antimalarial plants (which have continued to be used against malaria in Africa) and viral pathogens including the

dreaded SARS CoV-2. One possible explanation attributable to this unresolved therapeutic convergence is the mechanism of activity of these medicinal plants; several antimalarial phytomedicines which tend to produce more bioactivity as antioxidants, anti-inflammatory and immunomodulatory may function both as antimalarials and antiviral since the underlying mechanism of activity is not directly targeting the pathogen but rather boosting the immunity of the host, effective and efficient resolution of early inflammatory/anti-inflammatory cytokines (Afolayan et al., 2020) and scavenging of generated lethal free radicals (Iheagwam et al., 2020). This school of thought has been put forward to explain why many widely used African phytomedicines have gained more anecdotal claims of efficacy yet they do not easily kill the malaria parasite *in vitro* but produce good *in vivo* activity. For instance, Adebayo et al. (2017) demonstrated the poor *in vitro* but potent *in vivo* antimalarial activity of disulphide-rich peptide fraction of *Morinda lucida* (Adebayo et al., 2017). These antimicrobial peptides have been reported to possess immunostimulating and antioxidant activities (Nguyen et al., 2016) as well as antiviral property (Boas et al., 2019). Apparently, the lethal COVID-19 is reported to be induced by the invasion of SARS CoV-2 into a human host and has been associated with cytokine storm (Jose and Manuel, 2020) and neutrophil-induced oxidative stress (Laforge et al., 2020) which often result in mortality. So, it is reasonable to assume that antimalarial plants widely used in TAM with well documented *in vivo* antioxidant, anti-inflammatory and immunomodulatory potentials might offer some therapeutic benefits in COVID-19 management. A treatize of antimalarial plants used in TAM with documented antioxidant, anti-inflammatory and immunomodulatory activities as well as level of documented evidence has been presented in **Supplementary Table S1**. However, the authorization of the repurposed use of these botanical antimalarials should be evidence-informed with impressive clinical data and supported by the best evidence. Considering repurposing antimalarial African traditional phytomedicines for COVID-19 management, endemic and naturalized African plants which have shown therapeutic promise as antimalarials following clinical studies should be considered and these include *Vernonia amygdalina*, *Nuclea pobeguinii* (Pobéguin ex Pellegr.) Petit, *Argemone mexicana* L., *Artemisia annua* L., *Citrus aurantiifolia* (Christm.) Swingle (Aracil and Green, 2019) and *Morinda lucida* Benth (Rubiaceae). Interestingly, available evidence indicates that these promising antimalarial plants additionally have the potential to tackle oxidative stress, regulate inflammatory response and stimulate the immune system to overcome complications observed in COVID-19 [**Level III**] (Haudecoeur et al., 2018; Asante et al., 2019; Madzuki et al., 2019; Afolayan et al., 2020; Jain et al., 2020; Zibaee et al., 2020). Meanwhile, some of these reports lack quality and will require validation. Bioactive compounds identified in the plants include; for *V. amygdalina* - vernolide **(116),** vernodalin **(117),** hydroxyvernolide **(120)** and vernodalol **(123),** vernoniosides B1-B3 and vernoniosides A1-A4 **(124)**; for *N. pobeguinii* - strictosamide **(138),** 19-O-methylangustoline, angustoline **(139)**, *A. Mexicana* - berberine **(140),** tetrahydroberberine, protopine **(141),** benzophenanthridines, 8-acetonyl dihydrosanguiranine, 8-methoxy dihydrosanguiranine **(142),** *pancorine* **(144),** *O-methylzanthoxyline* **(145),** *nor-chelerythrine* **(125),** *arnottianamide* **(146)** *cryptopine* **(147),** *muramine* **(148),** *argemexicaine A, argemexicaine B* **(149)***;* for *A. annua* - artemisinin **(157)***; C. aurantiifolia* - apigenin **(156)** and *Morinda lucida* - Morindin **(154),** oruwal **(152),** oruwalol **(155),** oruwacin **(150),** molucidin **(151),** Damnacanthal **(153),** Ursolic acid **(17),** polypeptides (Kraft et al., 2003; Challand and Willcox, 2009; Brahmachari et al., 2013; Haidara et al., 2016; Haudecoeur et al., 2018; Divneet Kaur, 2019). Overwhelming evidence supports the standardization of the leaf and seed of *M. oleifera* for a possible clinical application [**Level III**] as it has demonstrated broad range of antiviral activity in various studies (Biswas D. et al., 2020) while the disulphide-stabilized miniproteins (Morintides), lectins, hevein-like peptides, protein hydrolysates and glucosinolates/isothiocynates isolated from the plant have shown impressive effects, including as antiadhesives, anti-inflammatory, antioxidants and immunomodulatory compounds (Kini et al., 2017; Moura et al., 2017; Coriolano et al., 2018; Fahey et al., 2019; Liang et al., 2019; Sousa et al., 2020). Aside immunomodulation and free radical scavenging, one mechanism of activity of these lectins and stable polypeptides involve the competitive inhibition of adhesion of pathogen proteins to host polysaccharide receptors [**Level III, V**] (Sharon, 1986; Boas et al., 2019). Further *in vivo* and clinical evaluations will be required to assess the specific significance of these reports and in particular the possible role of Moringa-derived products in COVID-19 management.

Traditional African Medicines of the D. R. Congo and Nigeria have developed *N. pobeguinii* and *N. latifolia* for clinical application in malaria therapy which may form a starting point for herbal repurposing for COVID-19 management. For instance, a diherbal preparation containing *N. latifolia* and *Cassia occidentalis* (Manalaria®), was authorized for malaria treatment in D.R. Congo which later formed part of the Congolese List of Essential Drugs (Pousset et al., 2006; Memvanga et al., 2015; Haudecoeur et al., 2018). While in Nigeria, aqueous extracts of *N. pobeguinii* (codenamed PR 259 CT1) was successfully taken through preclinical investigation and phase 1 of clinical trials [**Level I**, for malaria] for the treatment of uncomplicated malaria (Mesia et al., 2011; Mesia et al., 2012a; Mesia et al., 2012b) and could offer hope in COVID-19 management after requisite investigative screening and standardization. Furthermore, the aqueous root extract of *N. latifolia* otherwise known as NIPRD AM1®, has been clinically studied in uncomplicated malaria and found to be therapeutically helpful as an antimalarial (Gamaniel, 2009) and should therefore be given attention for investigative management of COVID-19 [**Level I**, for malaria]. Nevertheless, such investigation should follow after these chemically complex herbal mixtures have been taken through extensive acute, subacute and chronic toxicity studies as well as the metabolite profiling using modern analytical methods.

MAMA Powder and *MAMA* Decoction are authorized indigenous polyherbal antimalarials which have been scientifically formulated by Prof Elujoba, the Head of the

Village Chemist located within Obafemi Awolowo University, Ife, Nigeria. *MAMA* Powder contains stem bark of *Alstonia boonei* De Wild (Apocynaceae) and seed of *Picralima nitida* Stapf (Apocynaceae) while *MAMA* Decoction is made up of the leaves of *Mangifera indica* L. (Anacardiaceae), *Alstonia boonei* De Wild (Apocynaceae), *M. lucida* and *Azadirachta indica* A. Juss (Meliaceae) (Odediran et al., 2014). In an *in vivo* experiment using rodents (Adepiti et al., 2014), *MAMA* Decoction showed antimalarial activity at 240 mg/kg without any observable toxic effect when administered up to 2 g/kg body weight. Human observational study has further reinforced the *in vivo* activity while the efficacy claims by treated patients on *MAMA* herbal remedy has multiplied malarial patients' demand for the herbal medicine. An elaborate preclinical study with superior scientific quality, documentation of chemical fingerprint as well as clinical trial and a possible repurposing for COVID-19 management is encouraged.

Azadirachta indica A. Juss. (Neem) (Dongoyaro, Margosa) Meliaceae, is a medicinal plant with more than 140 chemically active compounds isolated from the different parts including its flowers, leaves, seeds, roots, fruits, and bark and had been employed in managing many diseases. The active compounds have been identified as anti-inflammatory, anti-ulcer, anti-hyperglycaemic, immune-modulator, anti-mutagenic, anti-oxidant, antiviral and anti-carcinogenic drugs. The earliest three active constituents to be characterized namely nimbin **(81)**, nimbidin **(126)** and nimbinene **(127)** were described in 1942. Since then several compounds have been isolated and characterized and were shown to be chemically similar and biogenetically derivable from a tetracyclicterpenes. The neem kernel accumulates liminoids responsible for the bitterness and also found in other plant species such as Rutaceae and Simaroubaceae. Their biological activities include pesticides, antifeedants and cytotoxic properties. The leaves yielded quercetin **(73)** and nimbosterol as well as liminoids (nimbin and its derivatives). Quercetin **(73)** and Beta-sitosterol **(85)** were the first flavonoid and phytosterol purified from the fresh leaves of neem and were known to have antifungal and antibacterial activities (Fabricant and Farnsworth, 2001). Although the mechanism of action has not been fully elucidated, it is speculated that the observed therapeutic role of *Azadirachta indica* is due to the rich source of antioxidant and other valuable active compounds which include azadirachtin **(84)**, nimbolinin **(87)**, nimbin **(81)**, nimbidin **(126)**, nimidol **(89)**, salannin **(83)** and quercetin **(73)**. An earlier study reported the virucidal activity of the leaf extract of *A. indica* against Coxsackievirus B-4 whose mechanism was proposed to be via interference with an early stage of the virus replication cycle (Badam et al., 1999). In a recent study, the *in vivo* intraperitoneal administration of methanol extracts of *A. indica* at a dose of 25 mg/kg body weight to murine hepatitis virus infected mice significantly reduced the expression of viral Nucleocapsid protein at the acute stage of infection. Since the murine hepatitis virus represents a prototype coronavirus, the therapeutic potential of the flavonoid, phytosterol and terpenoid-rich extracts of *A indica* has been reinforced [**Level V**]. *In vitro*, Neuro-2A cell-line treated with 200 μg/ml methanol extracts of *A. indica* inhibited virus-induced cell-to-cell fusion (Sarkar et al., 2020). More recently, a computational prediction of SARS-CoV-2 structural protein inhibitors from *A.* indica indicated their potential to inhibit the functionality of membrane and envelope proteins [**Level IV***] (Borkotoky and Banerjee, 2020). The free radical scavenging activity has been linked to the presence of nimbolide **(88)** and azadirachtin **(84)** while the anti-inflammatory activity is thought to be via the regulation of proinflammatory enzyme activities including cyclooxygenase (COX) and lipoxygenase (LOX) enzyme (Biswas K. et al., 2020) [**Level IV**]. This plant, although a component of some polyherbal antimalarial remedies including *MAMA* Decoction, has not been extensively validated preclinically, clinically and standardized as an anti-infective remedy and therefore deserves further scientific attention especially as a potential herbal remedy in COVID-19 treatment.

Therefore, application of Neem in health management includes the use of its leaf, flower and stem bark in disease prevention because of its strong antioxidant potential (Sithisarn et al., 2005; Priyadarsini et al., 2009). The anti-inflammatory activity has been related to suppression of the functions of macrophages and neutrophils relevant to inflammation by nimbidin. Other findings revealed immunomodulator and anti-inflammatory effect of the stem bark and leave extracts, and antipyretic activities of the seed oil. The antimicrobial activities of Neem include inhibition of growth of organisms such as viruses, bacteria and pathogenic fungi (Ghonmode et al., 2013). The antimalarial activity of extracts using *Plasmodium berghei* revealed reduced level of parasitaemia with the limonoids being the active ingredients (Akin-Osanaiya et al., 2013). Another study using *P. falciparum* also showed significant reduction in both gametocytes and asexual forms of the parasite (Udeinya et al., 2008). Few of these studies lack depth and will require further work to make this plant an interesting candidate for clinical evaluation.

There are several compounds from various African plants that have been proven to have antimalarial properties which may provide researchers with starting points for antiviral drug discovery. Indoles with antimalarial properties have been derived from two plants species growing in Cameroon such as *Penianthus longifolius* Miers (Menispermaceae) and *Glossocalyx longicuspis* Benth (Siparunaceae). The compounds include Palmitine **(130)** from *P. longifolius* Miers, Linodenine from *G. brevipes* Benth. Also from Nigeria plant, there is Fagaronine **(128)** from *Fagara zanthoxyloides* Lam. (Rutaceae) and Alstonine **(129)** from *Picralima nitida* (Stapf) T. Durand and H. Durand (family Apocynaceae). *Triphyophyllum peltatum* (Hutch. and Dalziel) Airy Shaw (Dioncophyllacea) is a tropical African plant from which a potent antimalarial alkaloid, Habropetaline A **(131)** was isolated. The compound showed good effect against *P. falciparum*, without cytotoxicity, with respective IC_{50} values 5.0 and 2.3 ng ml^{-1} for the strains K1 (Chloroquine and pyrimethamine resistant) and NF54 (sensitive to all known drugs). It was found to be almost as active as artemisinin and one of the most potent natural products used against *P. falciparum* (Bringmann et al., 2003). There are several

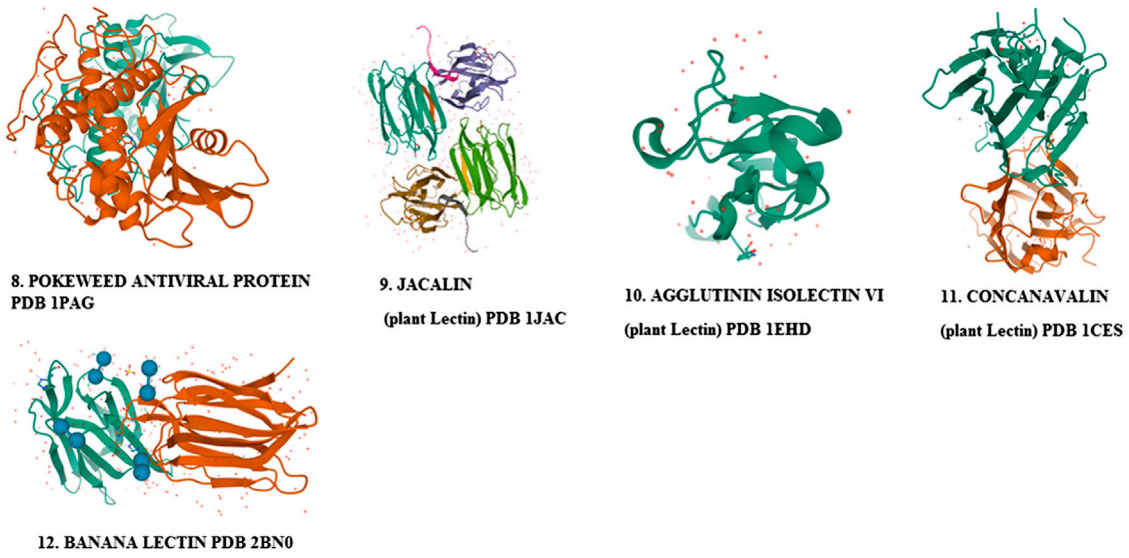


FIGURE 6 | Antiviral proteins isolated from plants.

observations that point to the fact that naphthoisoquinoline alkaloids are promising lead compounds for the development of anti-malarial drugs which of course could be tried against viral pathogens. Cryptolepines **(36)** from *Sida acuta* Burm.f. (Malvaceae), a plant growing in Ivory coast showed a good antimalarial activity (Banzouzi et al., 2004). *Cryptolepis sanguinolenta* (Lindl.) Schltr. of the family Periplocaceae growing in diverse regions in Africa, have also exhibited potent anti-malarial properties (Ablordeppey et al., 1990; Cimanga et al., 1999; Barku et al., 2012). Following a recently reported *in silico* experiments, several of these antimalarial alkaloids from African plants have shown interesting predicted inhibition of SARS CoV-2 viral proteins [**Level IV**] (Li et al., 2005; Borquaye et al., 2020) and this support the need for further *in vitro*, *in vivo* and clinical investigation on their therapeutic potential for COVID-19 treatment.

Bisnorterpenes, purified from the roots of *Salacia madagascariensis* Lam. DC. of the family Celastraceae, a shrub found in East Africa whose roots are used in the treatment of malaria fever and menorrhagia specifically in Tanzania for its potent antiprotozoal activity (Murata et al., 2008). Recent *in silico* studies supports the anti-SARS CoV-2 activity [**Level IV**] of bisnorterpenes such as 22-Hydroxyhopan-3-one and 6-Oxoisoiguesterin which have been isolated from endemic African plants with impressive binding affinities for the $3CL^{pro}$ of coronaviruses of −8.6 and 9.1 kcal mol^{-1} respectively (Gyebi et al., 2020). *Aframomum exscapum* (Sims) Hepper (Zingiberaceae) synthesizes acyclic triterpenes compounds such as S-nerolidol **(157)** isolated from the seeds and represents an important constituent of essential oils used in the treatment of malaria. This compound is also found in *Artemisia herba alba* Asso and in *Cymbopogon citratus* (DC.) Stapf. (Poaceae), and is able to arrest development of the intraerythrocytic stages of malaria (Titanji et al., 2008) and as such may be considered in future search for anti-viral agents including SARS-CoV-2. *Hyptis suaveolens* (L.) Poit. from Nigeria has also yielded abietane-type diterpenoid endoperoxide, a molecule with high anti-plasmodial activity (Chukwujekwu et al., 2005). Sesquiterpenes and sesquiterpene lactones **(51)** derived from *Vernonia* spp. are known to have interesting anti-plasmodial activities. Vernodalin **(132)** is the most active compound in bitter leaf. The plant has many uses in Traditional African Medicine, the leaves of *V. amygdalina* Del. are used in the treatment of various diseases including malaria and viral infections. Recent *in silico* anti-SARS-CoV-2 investigation reported promising activity of terpenes, iridoids and lignans which are able to effectively interact with the host enzyme transmembrane protease serine 2 (TMPRSS2) [**Level IV**]. This enzyme facilitates viral particle entry into host cells, and its inhibition blocks virus fusion with angiotensin-converting enzyme 2 (ACE2). The structural complexity of these plant metabolites and the presence of hydroxyl moieties and aromatic rings significantly improves the inhibition of their molecular target (Rahman et al., 2020). Traditional African Medicine knowledge could be very useful in drug discovery efforts from African medicinal plants, but the quality and reproducibility of such investigation is key. Chinedu and colleagues in a review of plants used in malarial treatment, reported over one hundred indigenous plants which have been employed traditionally in the management of malaria infection in six African Countries namely Nigeria, Ghana, Ethopia, Benin, Cameroon and Togo (Chinedu et al., 2014). Komlaga and colleagues have also evaluated some of the plants employed in the traditional management of malaria in Ghana, namely *Persea americana* Mill (Lauraceae), *Theobroma cacao* L. (Malvaceae) and *Tridax procumbens* (L.) L. (Compositae) and found that they

TABLE 2 | African Plants with evidence-based *in silico* therapeutic potentials against SARS-CoV-2 [Level IV].

African plant	Country	Plant organ	Bioactive compound tested *In silico*	Viral protein targeted	Binding affinity (Kcal/mol)	References
Amaranthus tricolor L. (Amaranthaceae)	Nigeria (Benin), Kenya and Tanzania	Seedlings	Amaranthin **(134)**	[b]SARS-CoV-2 3CLpro	−18.14	Kaur et al. (2006), National Center for Biotechnology Information (2020); ul Qamar et al. (2020), Wu et al. (2006), Xin et al. (2011)
Camellia sinensis L. Kuntze (Theaceae)	East Africa (Kenya, etc)	Root barks	Myricetin 3-O-beta-D-glucopyranoside **(136)**	[b]SARS-CoV-2 3CLpro	−18.42	Xu et al. (2008)
Fraxinus Sieboldiana blume (Oleaceae)	Sub-saharan Africa	Stem bark	Calceolarioside B **(135)**	[b]SARS-CoV-2 3CLpro	−19.87	Kim et al. (2002), Lin et al. (2008), Lin et al. (2007), National Center for Biotechnology Information (2020)
Glycyrrhiza uralensis Fisch. ex DC. (Fabaceae)	South Africa, Libya	Leaves	Licoleafol **(137)**	[b]SARS-CoV-2 3CLpro	−19.64	Das et al. (2020), National Center for Biotechnology Information (2020), ul Qamar et al. (2020)
Hyptis atrorubens Poit (Lamiaceae)	Nigeria, sub-saharan Africa	Leaves and stem	Methyl rosmarinate **(31)**	[b]SARS-CoV-2 3CLpro	−20.62	Abedini et al. (2013), National Center for Biotechnology Information (2020), ul Qamar et al. (2020); Woo and Piao (2004)
Myrica *Cerifera* L. (Myricaceae)	Nigeria	Root bark	Myricitrin **(32)**	[b]SARS-CoV-2 3CLpro	−22.13	National Center for Biotechnology Information (2020), Paul et al. (1974), ul Qamar et al. (2020)
Nigella sativa L. (Ranunculaceae)	Algeria	Seed oil	A-terpineol **(21)**	[a]SARS-CoV-2:ACE2 interface	−5.8	Ahmad et al. (2020), Ali and Blunden (2003)
Nigella sativa L. (Ranunculaceae)	Algeria	Seed oil	P-cymene **(22)**	[a]SARS-CoV-2:ACE2 interface	−5.8	Ali and Blunden (2003), Malik et al. (1995)
Nigella sativa L. (Ranunculaceae)	Algeria	Seed oil	T-anethole **(23)**	[a]SARS-CoV-2:ACE2 interface	−6.2	Ali, B. H. and Blunden (2003), Malik and Zaman (1992)
Nigella sativa L. (Ranunculaceae)	Algeria	Seed oil	Carvacrol **(24)**	[a]SARS-CoV-2: ACE2 interface	−7.0	(Arunasree (2010), Azizi et al. (2012), Lima et al. (2013), Landa et al. (2009), Li et al. (2016), Yin et al. (2012)
Nigella sativa L. (Ranunculaceae)	Algeria	Seed	Thyhydromoquinone **(25)**	[a]SARS-CoV-2:ACE2 interface	−6.1	Khan et al. (2011), Salim. (2020), Worthen et al. (1998)
Nigella sativa L. (Ranunculaceae)	Algeria	Seed oil	Thymol **(26)**	[a]SARS-CoV-2:ACE2 interface	−6.1	Bulugaha and Arachchige. (2012), Islam et al. (2004), Salem (2005)
Nigella sativa L. (Ranunculaceae)	Algeria	Seed oil	Thymoquinone (TQ) **(27)**	[a]SARS-CoV-2:ACE2 interface	−6.7	Badary et al. (2003), Bulugahapitiya and Arachchige (2012), Houghton et al. (1995), Kacem and Meraihi (2006), Randhawa, (2008), Salem (2005), Salim (2020)
Nigella sativa L. (Ranunculaceae)	Algeria	Seed	Dithymoquinone (nigellone) **(28)**	[a]SARS-CoV-2:ACE2 interface	−8.6	El-Dakhakhny (1965), Mahmoud et al. (2002), Randhawa (2008), Salem (2005)
Nigella sativa L. (Ranunculaceae)	Algeria	Seed	Carone	[a]SARS-CoV-2:ACE2 interface	−6.5	Salem (2005), Salim. (2020)
Phaseolus vulgaris L. (Fabaceae)	Nigeria, sub-saharan Africa	Root	3,5,7,3′,4′,5′-hexahydroxy flavanone-3-Obeta-D-glucopyranoside **(33)**	[b]SARS-CoV-2 3CLpro	−19.10	National Center for Biotechnology Information (2020), Rao, (1990), ul Qamar et al. (2020)
Phyllanthus Emblica L. (Phyllanthaceae)	Nigeria, Ghana, North Africa	Leaves and branches	(2S)- eriodictyol 7-O-(6″ O'galloyl)-beta-dglucopyranoside **(33)**	[b]SARS-CoV-2 3CLpro	−19.47	National Center for Biotechnology Information (2020), ul Qamar et al. (2020)
Psorothamnus fremontii (Torr. ex A.Gray) barneby (Fabaceae)	Uganda, South Africa	Roots	5,7,3′,4′-tetrahydroxy-2'-(3,3-dimethylallyl) isoflavone **(30)**	[b]SARS-CoV-2 3CLpro	−29.57	National Center for Biotechnology Information (2020), ul Qamar et al. (2020)

[a]*SARS-CoV-2:ACE2 interface: Binding affinities of docked compounds were obtained using Autodock/vina with Chloroquine as reference standard scoring a binding energy of -7.2; Dithymoquinone (nigellone) (28) demonstrated the most promising binding energy lower than the reference standard (Ahmad et al., 2020).*

[b]*SARS-CoV-2 3CLpro, Molecular Operating Environment (MOE) was used for molecular docking, ligand-protein interaction and drug likeness analyses while the antiviral drug, nelfinavir was used as the standard drug which produced a binding energy of −17.31. All compounds reported showed a lower binding energy than the reference compound used.*

have good antiplasmodial activities to justify their employment in such treatment (Komlaga et al., 2015). African Medicinal plants used in treating malaria may therefore represent promising areas to investigate for their potential in treating viral infections including the novel coronavirus (COVID-19) and HIV. However, since their findings are only preliminary, there is still a long path to clinical application as these remedies must be well standardized, authorized for use and administered by qualified medical personnel to African populations.

Beyond Claims: Identifying Key COVID-19 Potential Phytotherapies in Africa

Medicinal plants have continued to play an important role in providing primary healthcare needs across the African region particularly during sudden outbreak of deadly diseases like COVID-19. Emerging technologies, including the mining of plant-derived chemical libraries and application of computational techniques including ligand docking and other methods in computer-aided drug design (CADD), are increasingly deployed in rapidly selecting candidate screening compounds for a fast-tracked drug discovery process particularly during emergency situations like the ongoing COVID-19 pandemic. *In silico* analysis reduces the investigational time-line to identify "hits" and the analysis of their suitability in combating pathogenic diseases and thus shortens the drug discovery pipeline (Terstappen and Reggiani, 2001; Pascolutti and Quinn, 2014; Ubani et al., 2020). Documented hits compounds which have demonstrated interesting *in silico* activities against SARS-CoV-2 and isolated from African plants (**Figure 7**) include amaranthin **(134)** (*Amaranthus tricolor* L.- Amaranthaceae), myricitrin **(32)** (*Myrica cerifera* (L.) Small - Myricaceae), isoflavones **(30)** (*Psorothamnus arborescens* (A.Gray) Barneby - Fabaceae), nigellicine **(21)**, nigellidine **(22)**, nigellone **(28)**, carvacrol **(24)**, hederin **(25)**, thymol **(26)**, thymoquinone **(27)**, thymohyroquinone **(29)** (*Nigella sativa* L.), Calceolarioside B **(135)** (*Fraxinus sieboldiana* Blume - Oleaceae), Licoleafol **(137)** (*Glycyrrhiza uralensis* Fisch. ex DC - Fabaceae), methyl rosmarinate **(31)** (*H. atrorubens* Poit), myricetin 3-O-beta-D glucopyranoside **(136)** (*Camellia sinensis* L. Kuntze - Theaceae). **Table 2** presents a full list of these compounds and the plants producing them while **Figure 6** presents the chemical structures of the compounds. These *in silico* findings with limited evidence should form the basis for future in-depth *in vitro*, *in vivo* and clinical studies rather than indiscriminate application of preliminary data which could constitute a public health concern.

Attempts are at present being fast-tracked to discover, repurpose or otherwise develop preventive and treatment options for COVID-19 from the wealth of indigenous knowledge on the use of plants sourced from African plant biodiversity in combating infectious diseases. However, for a phytomedicine to be officially approved and authorized for use, it needs to be scientifically investigated and taken through accelerated clinical trials. The African media, especially the social media, internet, television and radio has been populated with anecdotal claims on COVID-19 herbal vaccines, symptomatic treatment and even cure. Several of these claims are coming from important personalities in the society including religious leaders, traditional/community leaders, Traditional Medical Practitioners (TMPs), research institutions or from establishments producing herbal remedies. Many of these yet-to-be validated claims have originated from Eastern Africa (Madagascar), West Africa (Nigeria) and Central Africa (Cameroon). In fact, Madagascar was the foremost African country to authorize the use of an indigenous herbal remedy known as COVID Organics (CVO) for the prevention and cure of COVID-19. The World Health Organization (WHO) carefully discouraged the official positioning of CVO as a magic bullet for the cure of the disease and emphasized that only evidence-based claims with satisfactory efficacy and safety margins via clinical trials could justify the claims of the government of Madagascar. As a result, the WHO and African CDC are cooperating with and supporting the government to design and conduct clinical trials to validate the efficacy and possible adverse effects of CVO polyherbal formulation. This may involve multi-centre clinical trials involving countries in Africa such as Tanzania, Equatorial Guinea and Congo-Brazzaville that had received the herbal remedy (WHO, 2020).

In Nigeria, the social media, television, internet and radio media have been flooded with claims of symptomatic treatment, cure or prevention of COVID-19. Many of these anecdotal claims flying over the virtual space have provided African researchers starting points for a plant-derived drug discovery studies against COVID-19; many of the claims have originated from eminent Nigerians such as the traditional leader of the Yoruba nation, religious prophets and Priests, Botanists, Biochemists and a host of Nigerian scientists in academia; several of these claims are currently under scientific investigation for adverse effects and efficacy. Officially, the Nigerian government has not approved or authorized the use of any indigenous phytomedicine to combat COVID-19, reason being that no herbal remedy currently claimed to prevent, manage or cure the infectious disease has been taken through a rigorous scientific investigation via clinical trials. Meanwhile, the Nigerian government through the National Agency for Food and Drug Administration and Control (NAFDAC) is now processing not less than 21 herbal formulations for "safe use" under listing status. These polyherbal formulations according to NAFDAC, have been claimed to boost immunity with a parallel anti-infective activity capable of providing relief to symptoms associated with COVID-19. More so, a documented evidence of clinical trial which is required to support efficacy claims is lacking until the time of this writing. However, the Bioresources Development Group (BDG), Abuja, Nigeria; International Center for Ethnomedicine and Drug Development (InterCEDD) Nsukka, Nigeria, has submitted the previously NAFDAC listed IHP Detox tea for clinical trials which is titled: "Efficacy and safety of IHP Detox Tea (a special blend of *Andrographis paniculata* (Burm.f.) Nees (Acanthaceae), *Garcinia kola* Heckel (Clusiaceae) and *Psidium guajava* L. (Myrtaceae)) for treatment of COVID-19): a pilot placebo-controlled randomized trial". The clinical trial is to be undertaken at the Nigeria Center for disease Control

TABLE 3 | African Plants which are less widely applied in TAM with *in vivo* and *in vitro* evidence-based antiviral potentials [Level V].

African plant	Country	Plant organ	Bioactive compound isolated	Viral protein targeted	References
Alangium chinense (Lour.) harms (Cornaceae)	Cameroon, Ethiopia, tropical Africa.	Roots	Sesquiterpenoids and alkaloids	Coxsackie B3	Zhang et al. (2013)
Azadirachta indica A. Juss (Meliaceae)	Ghana	Bark	Bark extract	HSV-1	Martins et al. (2009)
Azadirachta indica A. Juss (Meliaceae)	Ghana	NP	Polysaccharides	Poliovirus	Faccin-Galhardi et al. (2012)
Calophyllum L. (Calophyllaceae)	Kenya, Madagascar	NP	Coumarin and xanthone	HIV RT[≠]	
Camellia sinensis (L.) kuntze (Theaceae)	South Africa Kenya Malawi Rwanda Nigeria	Green tea	Epigallocatechin **(171),** lucidone **(172)**	HBV	Xu et al. (2008)
Cryptopleura ramosa (hudson) L. Newton (Delesseriaceae)	South Africa	NP	Sulfated galactans	HSV-1 and HSV-2 replication in vero	Carlucci et al. (1997)
Ferula narthex Boiss. (Apiaceae)	North Africa	NP	Sesquiterpenecoumarins **(51)**	Influenza	Lee et al. (2009)
Glycine max (L.) Merr. (Fabaceae)	Zambia, Zimbabwe and South Africa	NP	Rhamnogalacturonan	CMV[≠] cytotoxicity	Steinmassl and Anderer (1996), Huisman et al. (2001)
Glycyrrhiza glabra L. (Fabaceae)	North Africa	Leaflets	Chalones **(52)**	Influenza	Dao et al. (2011)
Griffithsia (wrangeliaceae)	South Africa	NP	Griffithsin	HIV clade C	Danaher et al. (2011)
Hypericum perforatum L. (hypericaceae)	South Africa	Stem and petals	Hypercin **(47)**	HCV[≠]	Jacobson et al. (2001)
Ligustrum lucidum W.T.Aiton (Oleaceae)	South Africa Algeria		Oleanolic acid **(168)** and ursolic acid **(17)**	HCV	Kong et al. (2013)
Marrubium peregrinum L. (Lamiaceae)	Northern Africa	NP	Ladanein **(173)** (BJ486K), a flavonoid	All HCV genotypes	Haid et al. (2012)
Momordica charantia L. (Cucurbitaceae)	Nigeria	NP	Recombinant MAP 30	HIV	
Phyllanthus niruri L. (Phyllanthaceae)	West Africa	Leaf	[b]Niruriside **(48)**	HIV	Dharmaratne et al. (2002), Lee-Huang et al. (1995), Qian-Cutrone et al. (1996)
Piper longum L. (Piperaceae)	Madagascar		Longumosides and amide alkaloids	HBV	Jiang et al. (2013)
Punica granatum L. (*Lythraceae*)	North Africa		Punicagalin	Enterovirus 71	Mouhajir et al. (2001), Yang et al. (2012)
Punica granatum L. (Lythraceae)	South Africa	NP	[a]Polyphenols	Enveloped viruses, Food borne surrogate viruses	Kotwal (2008), Neurath et al. (2004), Neurath et al. (2005), Su et al. (2010), Sundararajan et al. (2010)
Reynoutria japonica houtt. (Polygonaceae)	South Africa	Leaves	[c]Resveratrol+	HIV, EBV, HCV	De Leo et al. (2012), Heredia et al. (2000)
Rubus fruticosus L. (Rosaceae)	South Africa	NP	Extract	HSV-1[≠]	Danaher et al. (2011)
Salvia rosmarinus spenn. (Lamiaceae)	North Africa Ethiopia	Np	Carnosic **(49)**	RSV	Shin et al. (2013)
Sambucus nigra L. (Adoxaceae)	Northern Africa	NP	Liquid extract	Influenza	Krawitz et al. (2011)
Swietenia macrophylla king (Meliaceae)	West Africa	Stem	[d]3-hydroxy caruilignan (3-HCL-C)	HCV	Wu et al. (2012)
Woodfordia fruticosa (L.) kurz (Lythraceae)	Tanzania, Madagascar	Flowers	Gallic acid **(54)**	Enterovirus HCV	Choi et al. (2010)

[NP]*Not Provided.* [≠]*Only* in vitro *activitiy reported;*

[a]*HIV-1 entry inhibitors from pomegranate juice adsorbed onto corn starch. The resulting complex blocks virus binding to CD4 and CXCR4/CCR5 and inhibits infection by primary virus clades A to G and group O; the antiviral effects of pomegranate polyphenols are mediated in different ways depending on the nature of the virus. In the case of influenza virus, elimination of infectivity by pomegranate polyphenols is primarily a consequence of damage to virion integrity, rather than simply a coating of viral particles.*

[b]*inhibitory activity against protein binding to RNA.*

[c]*protein synthesis inhibition, decreases reactive oxygen species (ROS) levels, and suppressession of the EBV-induced activation of the redox-sensitive transcription factors NF–kB and AP-1.*

[d]*3-HCL-C interfered with HCV replication by inducing IFN-stimulated response element transcription and IFN-dependent anti-viral gene expression. HIV–Human Immunodeficiency Virus; HSV 1–Human Simplex Virus one; HSV 2–Human Simplex Virus two; EBV–Epstein-Barr Virus; CMV–Cytomegalovirus; HBV–Hepatitis B Virus; RSV–Respiratory Syncytial Virus; HCV–Hepatitis C Virus.*

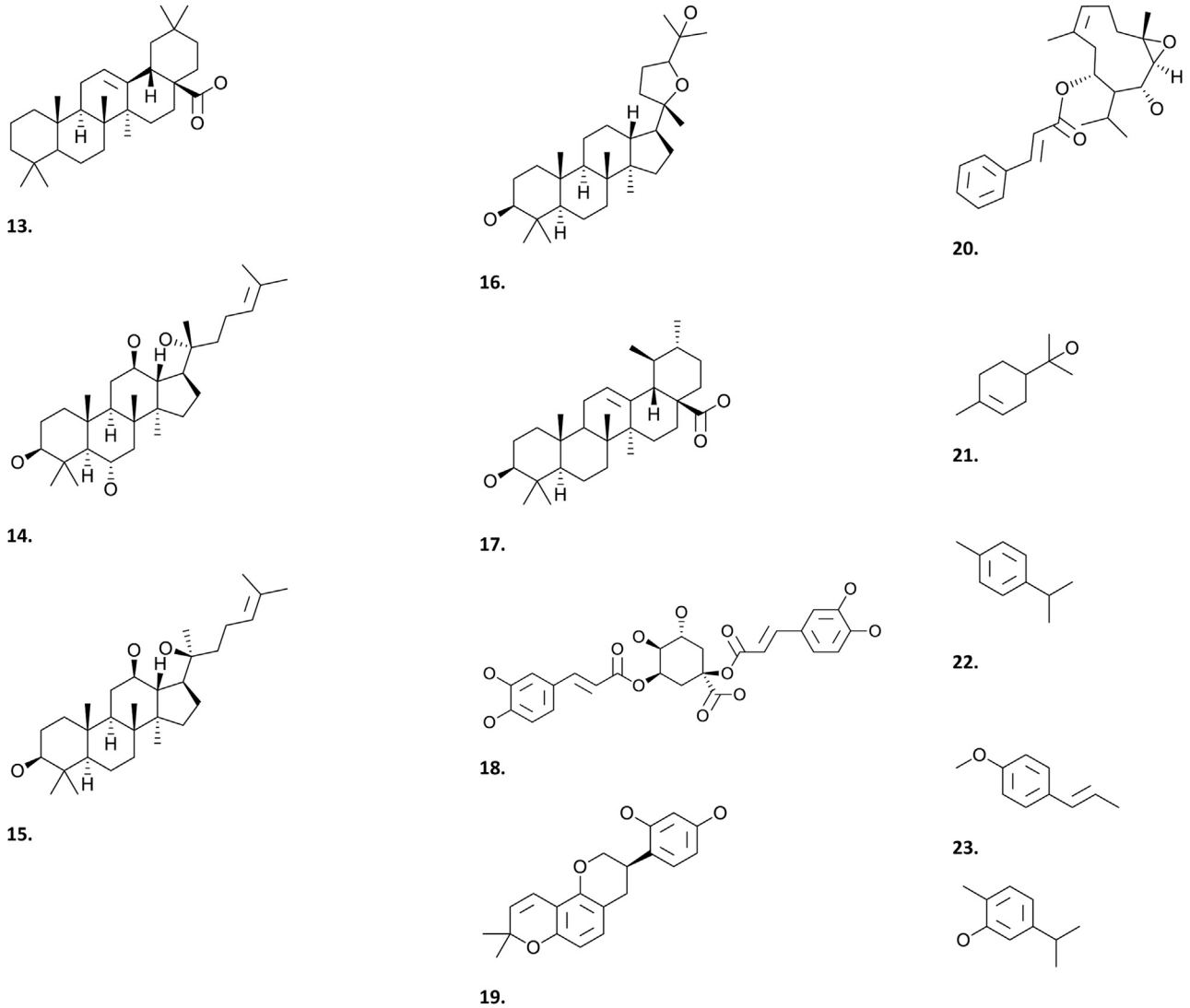


FIGURE 7 | Structures of some plant-derived secondary metabolites with antiviral activities.

(NCDC) COVID-19 isolation site in Lagos, Nigeria and has been registered with the Pan African Clinical Trials Registry: at www.pactr.org with registration number of PACTR202004761408382. The identified main bioactive phytoconstituents of the *Andrographis paniculata* is andrographolide **(61)** while kolaviron, Garcinia biflavonoids **(59–60)** has been reported in *Garcinia kola* (Lin et al., 2009; Buba et al., 2016).

Other indigenous anti-COVID-19 herbal remedies and polyherbal formulations listed by the Nigerian NAFDAC but still lack clinical trial data and not yet authorized for use by the government but available in the market space include: IHP Garcinia, IHP Detox, IHP Immunovit (products of (InterCEDD, Nigeria), CUGZIN capsule, 290 mg (produced by PaxHerbal, Nigeria) and VIVE active (Rx Agroprocessing, Nigeria). The Nigerian Federal Ministry of Health in collaboration with NAFDAC is supporting three foremost and promising remedies for funding considerations to enable clinical trials in a bid to champion an evidence-informed use of indigenous phytomedicines in Nigeria.

Cameroon is another country located in central Africa whose anti-COVID-19 herbal claims has attracted much attention and use of unauthorized herbal remedies is widespread despite serious concerns expressed by the WHO regarding such uninvestigated anecdotal claims which could place African populations in great risk, create false confidence and discourage them from adherence to recommended global preventive measures. For instance, two phytomedical remedies (Elixir COVID and Adsak COVID) which have been developed from undisclosed indigenous plants have been claimed to reverse the effect of COVID-19, clear the virus from patients' body fluid while

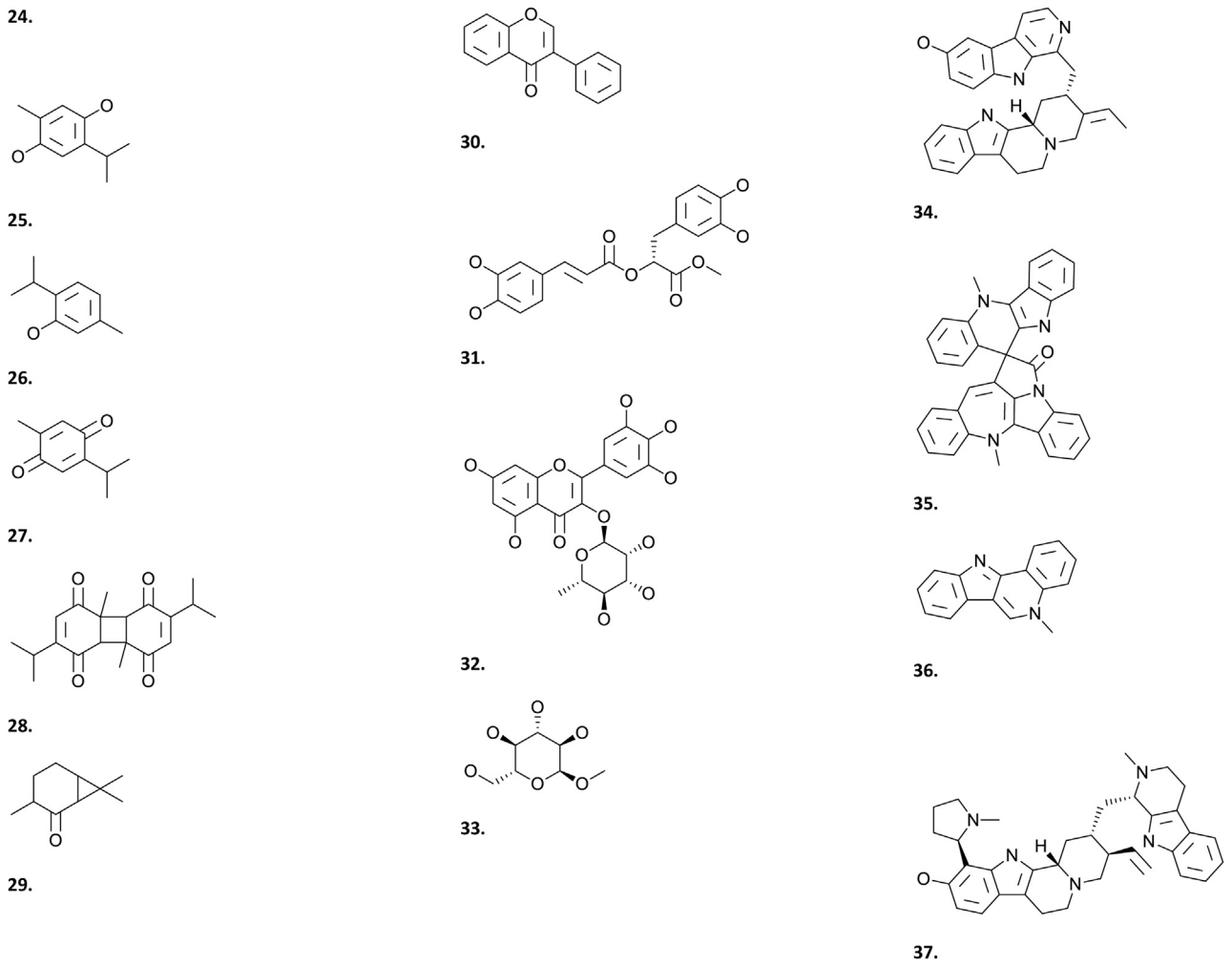


FIGURE 7 | (Continued).

essential oils have been claimed to cure at least 1500 COVID-19 patients. These remedies lack scientific evidence (Africa CDC, 2020; WHO, 2020) and should be holistically validated for a possible clinical application.

Perspectives on the Therapeutic Potentials of African Plants

Africans may lack access to western repurposed drugs that are now used to manage COVID-19 in developed countries, but they have unlimited access to medicinal plants which can be standardized for effective and safe use. These tropical plants accumulate both primary and secondary metabolites with a broad range of *in silico*, *in vitro* and *in vivo* activities including antiviral properties (**Tables 1–3**). Many of the antiviral primary metabolites such as polysaccharides and antiviral proteins (**Figures 5**, **6**) accumulated in African plants reported in this review have not attracted much research attention and exploitation in antiviral drug discovery. Even of more scientific interest are the highly stable low molecular weight peptides known as cysteine-knot peptides among which, cyclotides (**Figure 5**; 1–7) are most stable due to their continuous circular configuration, low molecular weight, abundance, sequence variability, oral bioavailability, target specificity, low *in vivo* toxicity and wide distribution in plants families including Violaceae, Rubiaceae, Fabaceae, Curcubitaceae and Solanaceae (Gründemann et al., 2013; Attah et al., 2016b; de Veer et al., 2019). Reported antiviral cyclotides include Cter M **(1)**, vhl-2 **(2)**, cyclotide vhl-1 **(3)**, CIRCULIN A **(4)**, kalata B1**(5)**, kalata B8 **(6)**, Cyclotide Palicourein **(7)** and Alstotide S1 **(8)** (Daly et al., 1999; Daly et al., 2004; Chen et al., 2005; Poth et al., 2011; Wang et al., 2017). The hydrophobic nature of these interesting peptides appear to be very important for their activity against enveloped viruses (Badani et al., 2014; Wang et al., 2017). Antiviral Kalata B1 and B8 have been isolated from an indigenous plant *Oldenlandia affinis* (Roem. and Schult.) DC. (Rubiaceae) used in Traditional African Medicine to aid delivery in Central Africa (Gran et al., 2000) and as an antimalarial herb in

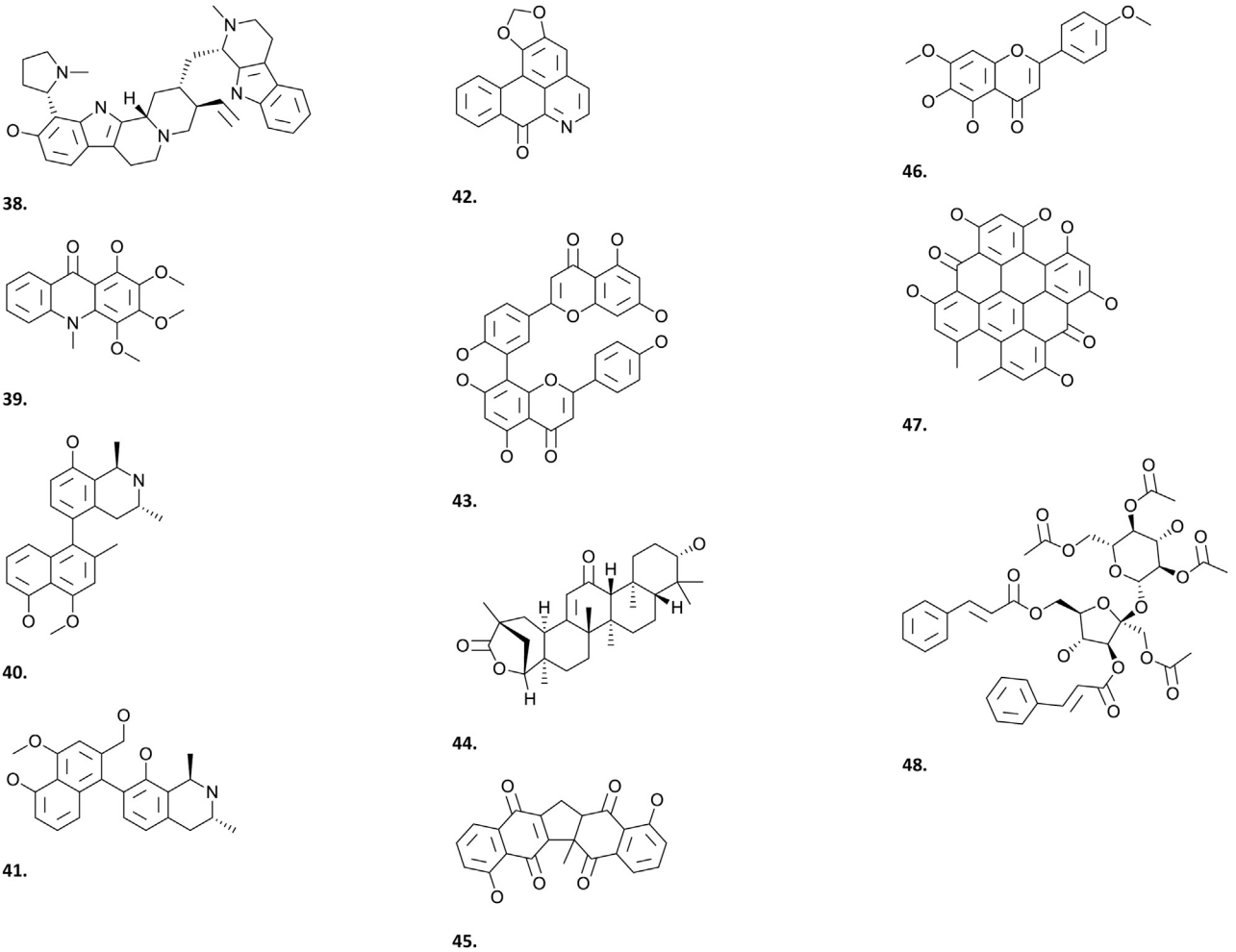


FIGURE 7 | (Continued).

Nigeria (Nworu et al., 2017); cyclotide-rich aqueous extract of *Oldenlandia affinis* DC. represent a potential multitarget peptide drug candidate that awaits scientific investigation against COVID-19. However, phytomedicines containing antiviral Kalata B1 may be contraindicated in pregnancy (Saether et al., 1995) and more useful during the late stage of hyper-inflammation observed in COVID-19 owing to the immunosuppressant activity of Kalata B1 (Gründemann et al., 2013). Meanwhile, the therapeutic potentials of these peptides still lacks clinical evidence to support the interesting *in vitro* and *in vivo* findings.

Exploring and exploiting medicinal plants for antiviral activity should be premised on the demonstration of prophylactic and/or therapeutic efficacy at an optimal amount in metabolic fluid. Similarly, plants and their bioactive metabolites have been shown to modulate immunological activities making them suitable candidates for biological response modifiers with the potential to alleviate symptoms and prevent death associated with infectious viral outbreak (Kurokawa et al., 2010). Therefore, the Africa Centers for disease Control and Prevention (Africa CDC) has provided standard guidelines for Member States when herbal remedies or medicines are proclaimed or developed in their countries (Africa CDC, 2020).

Since the global R and D community is relentlessly working on getting an effective treatment to stop the COVID-19 pandemic, symptomatic management of the viral symptoms and Prevention of infection through divergent approaches should be encouraged. For instance, evidence-based and documented scientific publications on the antiviral and immunomodulatory potentials of African plants could provide some clues on prevention and management of COVID-19. Examples of such African plants widely used in traditional medicine across the region which have received *in silico* anti-COVID19 screening (Rowaiye et al., 2020) for bioactivity include *M. indica* L., *Manihot esculenta* Crantz. (Euphorbiaceae), *A. occidentale* L.,

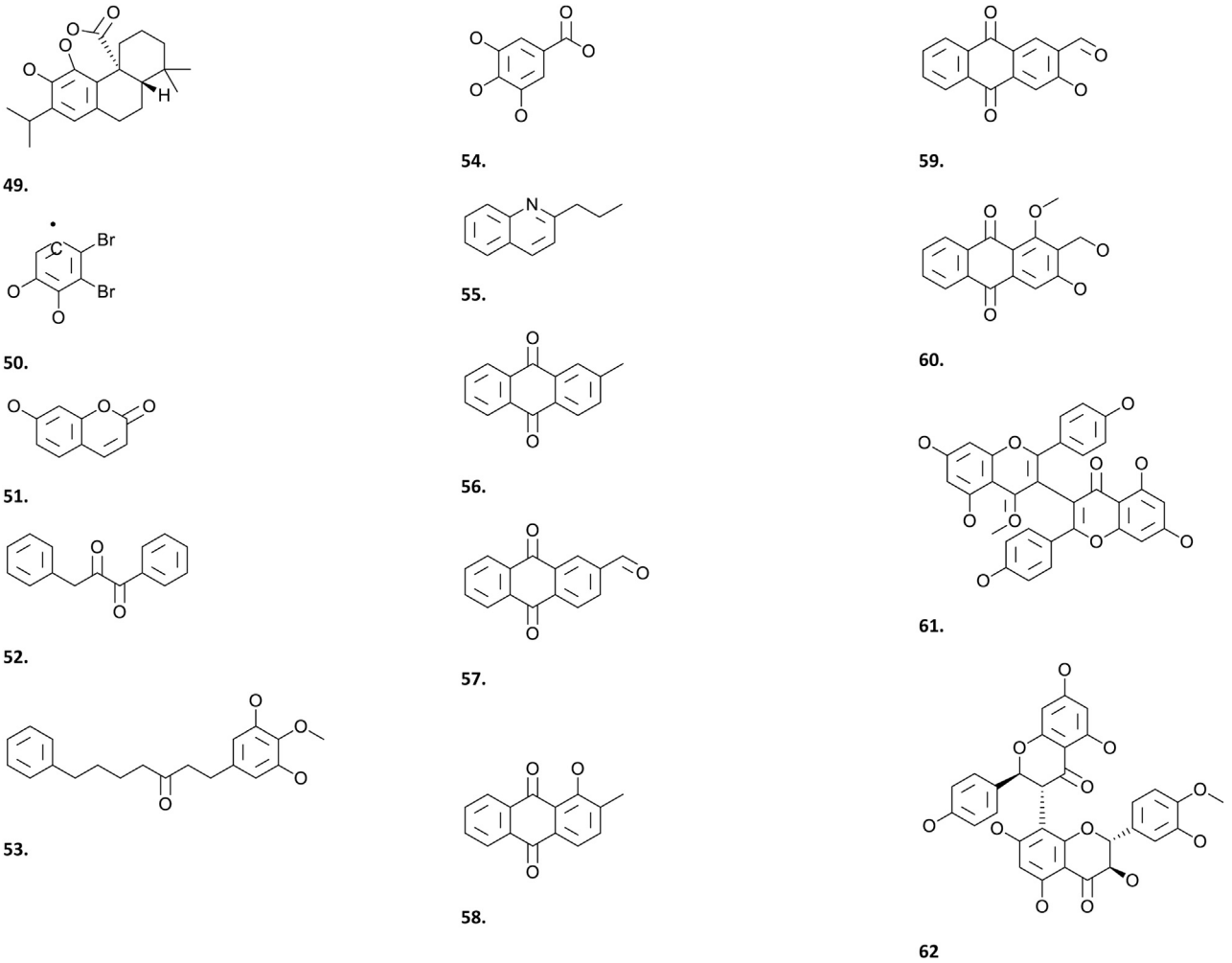
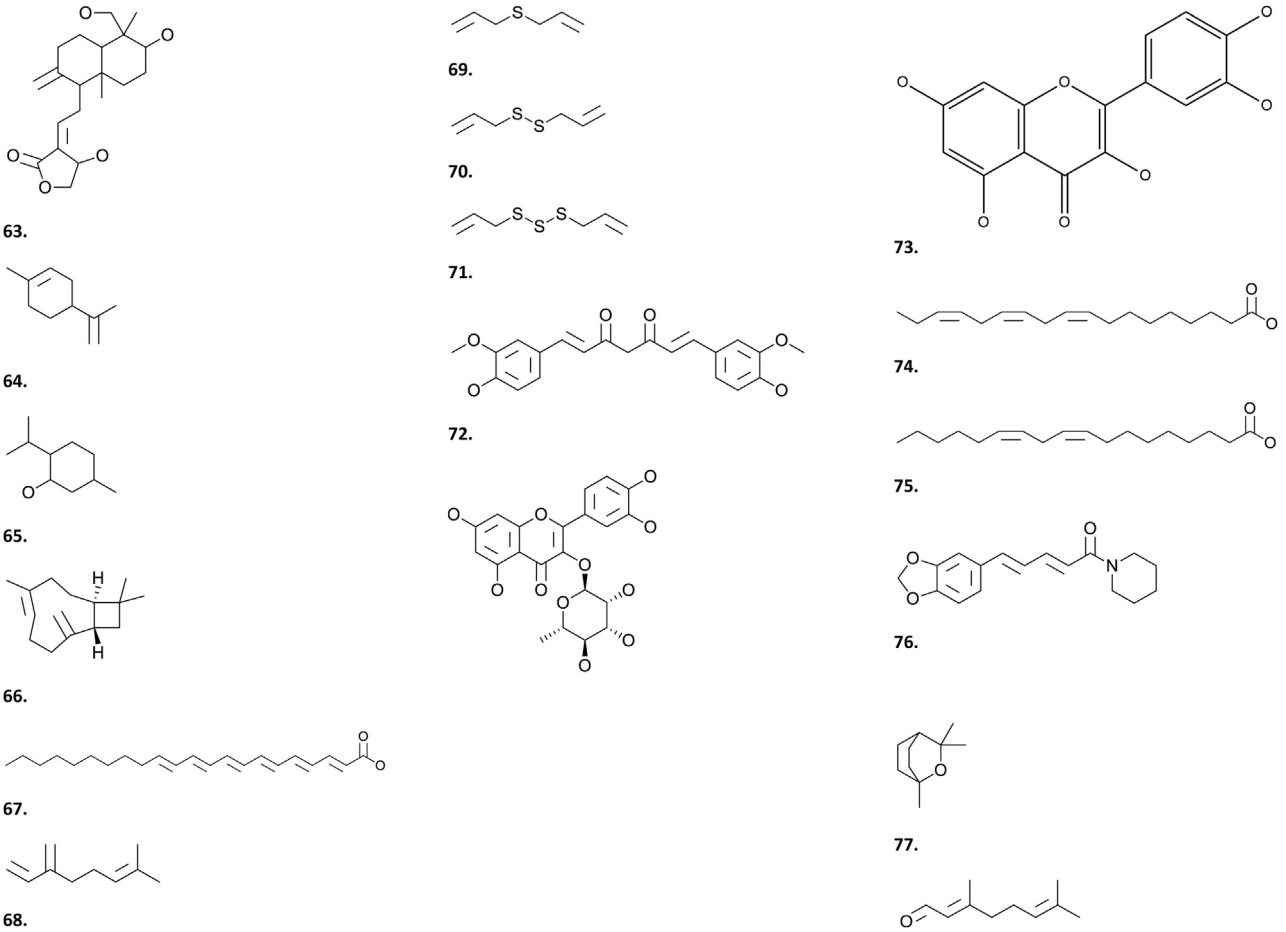


FIGURE 7 | (Continued).

Uraria picta (Jacq.) Desv. (Fabaceae) *and Corchorus olitorius* L.(Malvaceae) Others are simply immune boosters including *V. amygdalina* Delile., *M. oleifera* Lam, *Telfairia occidentalis* Hook.f. (Cucurbitaceae), among others. Findings from this preliminary study have limited evidence until indepth preclinical and clinical studies are done. Some commonly used Nigerian medicinal plants that may have potentials for the symptomatic management of COVID-19 include *Capsicum L* (Solanaceae), *Z. officinale* Roscoe, *Xylopia ethiopica* (Dunal) A.Rich. (Annonaceae), *C. papaya* L., *A. cepa* L., *G. kola* Heckel, *A. sativum* L. Several other antiviral plants (**Table 1**) used in Nigerian ethnomedicine such as *Senna siamea* (Lam.) H.S.Irwin and Barneby (Fabaceae) and *Zephyranthes candida* (Lindl.) Herb (Amaryllidaceae) (Ogbole et al., 2013) could also be of scientific interest for further research. Tannins and glucosinolates **(94)** with broad anti-infective activities (Chodur et al., 2018; Hensel et al., 2020; Nie et al., 2020) from seeds of *M. oleifera* Lam., a popular and widely used tropical plant may equally be of research interest as potential prophylactic and anti-COVID-19 herbal supplement. *B. ferruginea* Benth is another tropical plant for future investigation against COVID-19; it is popular in African ethnomedicine to fight difficult infectious diseases as well as a prophylactic in some anti-infective remedies [**Level V**] (Ozerov et al., 1994; Cimanga et al., 1999). *P. guajava* L. has shown interesting broad spectrum antimicrobial activities, good antiviral property and polyphenolic compounds (catechin-**133**, quercetin-**73** and gallic acid - **54**) derived from the stem bark and leaves have been linked with the reported bioactivity (Sriwilaijaroen et al., 2012; Naseer et al., 2018; Trujillo-Correa et al., 2019). Howbeit, this study lacks *in vivo* and clinical evidence as only preliminary in silico and a more elaborate *in vitro* data has been documented.

A recent CADD-directed fluorogenic enzyme inhibition assay reported corilagin and rhoifolin, two natural products of African origin, with micromolar range inhibitory activity against the main protease (3CLpro) enzyme of the SARS-CoV2 (Loschwitz et al., 2020). Interestingly, the investigation which had commenced



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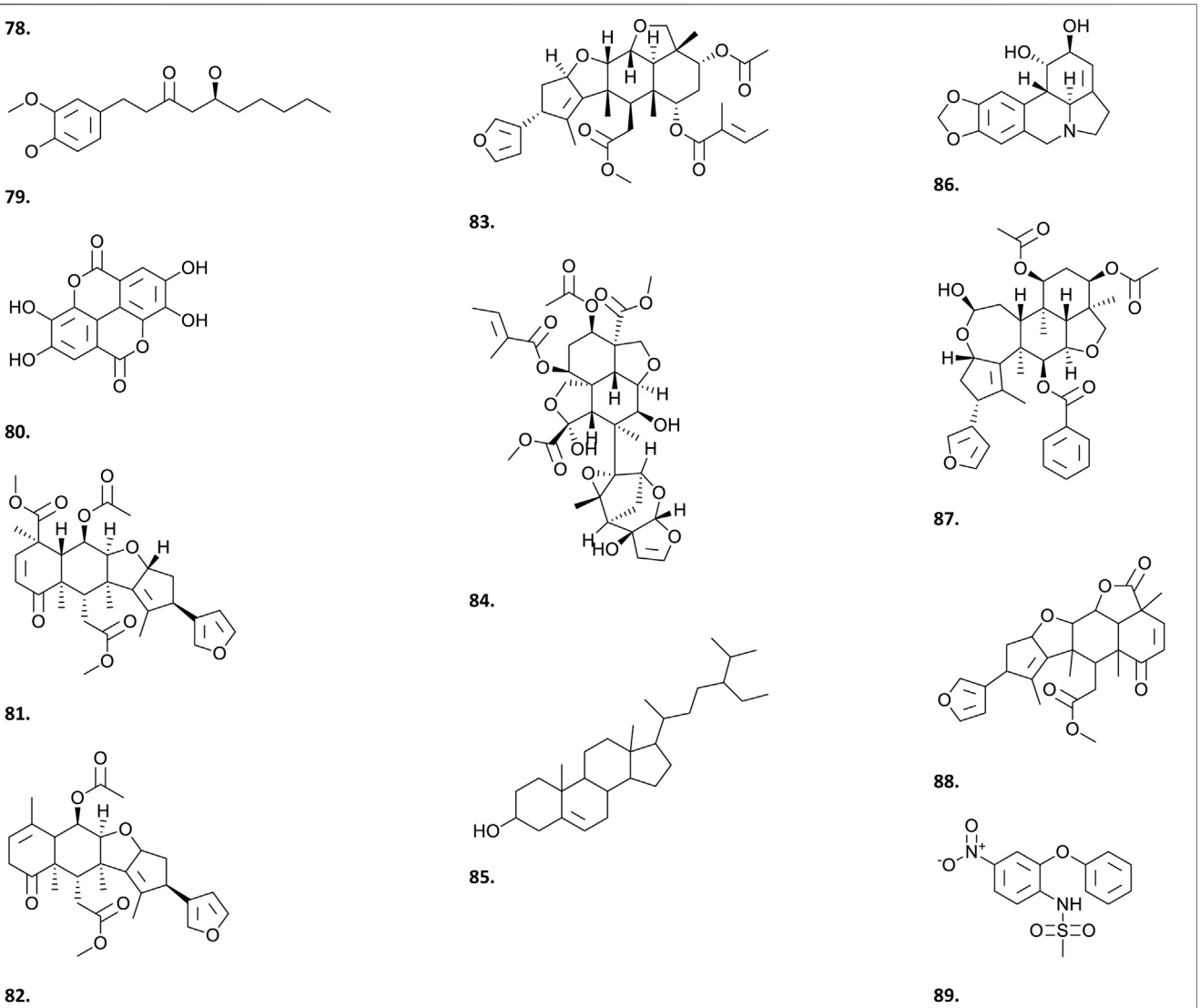
with the screening of an over 1.2 million virtual compound library (Olubiyi et al., 2020) identified corilagin and rhoifolin in the top eight compounds with respect to main protease inhibitory activity. Corilagin, an ellagitannin, is widely distributed in several African plants and is known to be present in the plant families including Euphorbiaceae (e.g. *Acalypha wilkesiania* Muell Arg and *Acalypha australis* L., *Euphorbia longana* Lam., *Phyllanthus emblica* L., *P. urinaria* L., *P. tenellus* Roxb., *P. niruri* L., etc), Geraniaceae (*Geranium sibiricum* L.), Combretaceae (*Terminalia catappa* L.), to mention a few. Rhoifolin on the other hand is a tri-substituted flavone and has been reported in *Uraria picta* (Jacq.) DC, a perennial tropical plant with distribution extending through most parts of Sub-Saharan Africa. With *in vitro* inhibitory activities of both natural products in the micromolar range, it is yet to be determined if the reported potencies will extend to *in vivo* situations. But the establishment of the SARS-CoV2 3CLpro inhibitory activities for both natural products further support the potential of African plants to potentially furnish herb-based remedies and lead compounds that can be developed into clinically useful treatment for COVID-19 [**Level III**].

Coadministration of Phytomedicines and Western Medicines in COVID-19 Management: Drawback of Herb-Drug Interaction

The use of phytomedicines as adjuvants in the therapeutic treatment of diseases has received a drawback due to the occurrence of deleterious herb-drug interactions (Rivera and Loya, 2013). A typical medicinal plant is a biological laboratory of hundreds of bioactive metabolites with significant influence on the pharmacokinetics and pharmacodynamics of drugs when co-administered or used as adjuvants. The co-administration of orthodox drugs alongside herbal medicines may bring about pharmacodynamic interactions that may result in synergistic, additive or antagonistic pharmacological end-points. More so, an important consequence of herb-drug interactions is the pharmacokinetic dimension that alters the level of the drug in systemic circulation. This may result from the activity of the herbs leading to elevation or inhibition of the function of certain drug metabolizing enzymes or efflux transporters. As a rule of the thumb, the bioavailabilities of bioactive compounds are enhanced



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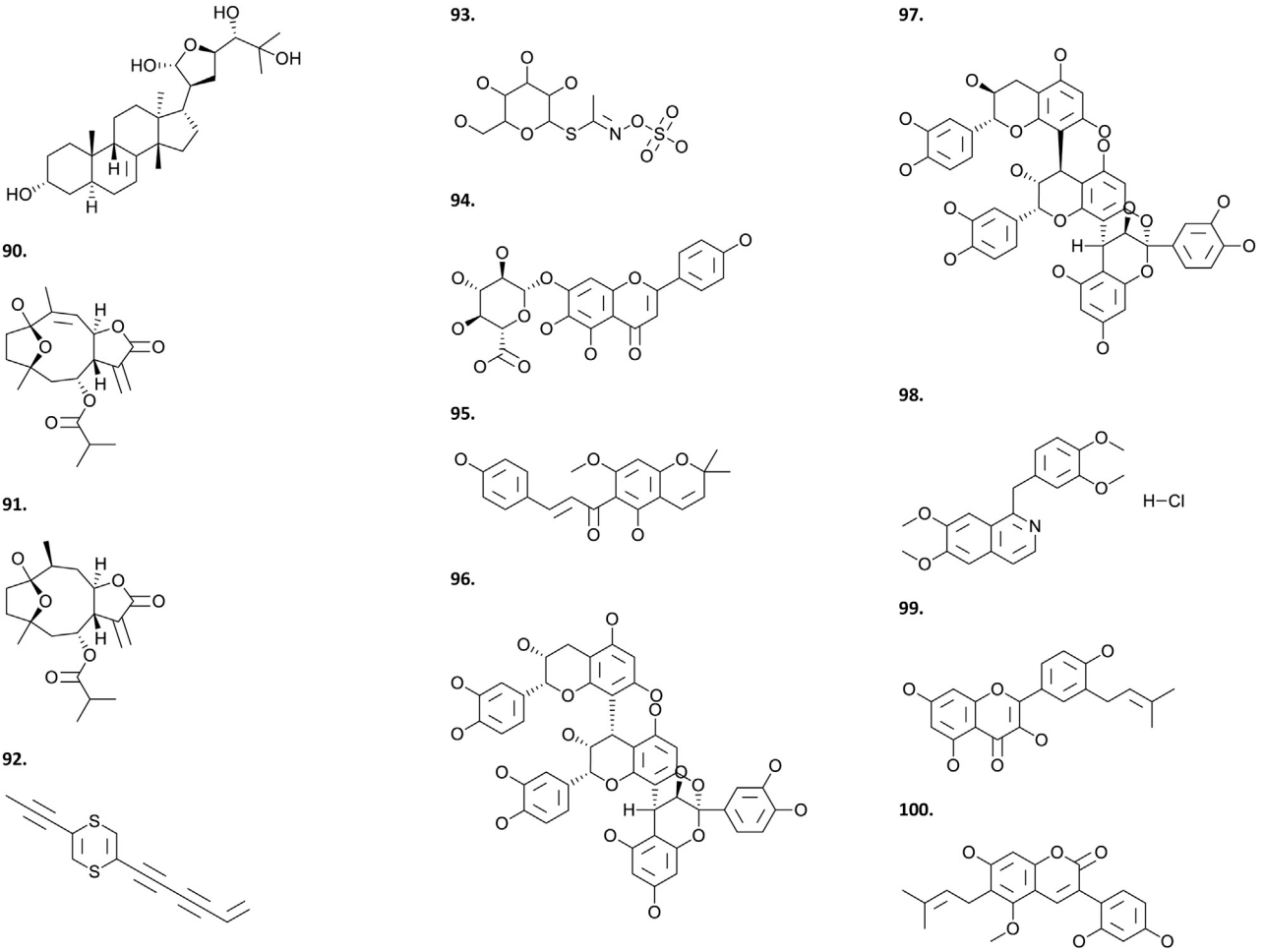
by the inhibition of drug metabolizing enzymes or efflux transporters; in contrast, induction of drug metabolizing enzymes or efflux transporters reduces drug bioavailability (Patil et al., 2014). Hence, precautionary measures and adequate monitoring (pharmacovigilance) are essential when co-administering drugs with narrow therapeutic window or safety margin with herbs as any variation in plasma concentrations can result in adverse events or treatment failure (McFadden and Peterson, 2011).

Generally, most of the drugs used in humans are metabolized by a class of enzymes known as cytochrome P450 (CYP) (Thomford et al., 2016). The CYP enzyme comprises diverse isoenzymes whose function can be altered by phytochemicals present in phytomedicines. In herb-drug interaction, the herbal drug may induce or inhibit the same isoenzyme that is responsible for the metabolism of the synthetic drug. If the co-administered herbal drug inhibits the isoenzyme, the synthetic drug will not be metabolized; this will lead to high levels of the drug in physiological fluid which consequently results in toxicity. In contrast, if the herbal drug induces the isoenzyme, this could result in rapid metabolism of the drug whose optimal therapeutic concentration in systemic circulation may not be reached leading to treatment failure, and possibly development of resistance (Zhou et al., 2003). Herb-drug interaction can also occur if the same isoenzyme is responsible for the metabolism of both the herbal drug and synthetic drug. For instance, *in vitro* studies have shown that CYP3A4, 3A5 and CYP19 enzymes are inhibited by hypoxoside, an active component of *Hypoxis hemerocallidea* Fisch., C.A. Mey. and Avé-Lall. (Hypoxidaceae); *Hyptis suaveolens* (L.). Poit. (Lamiaceae), *Boerhavia diffusa* L (Nyctaginacea), *Launaea taraxacifolia* (Willd.) Amin ex C. Jeffrey (Asteraceae) and *Myrothamnus flabellifolia* Welw.



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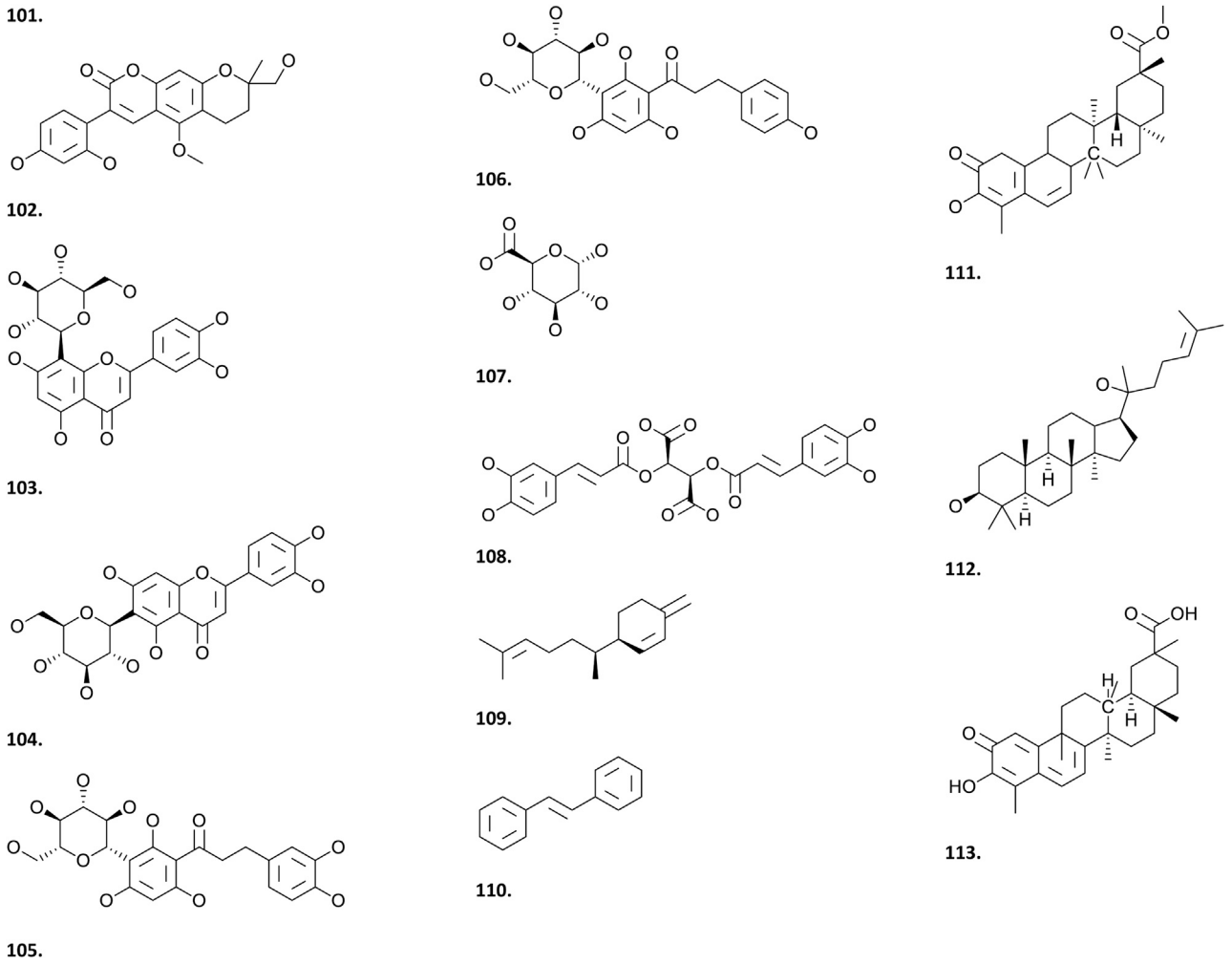
(Myrothamnaceae) inhibit CYP2B6 activity in a concentration -dependent manner. *Sutherlandia frutescens* (L.) R.Br. (Fabaceae) was shown to inhibit CYP3A4. In other words, the drugs metabolized by these enzymes will become toxic if administered concurrently with these plants commonly used in African Traditional medicine. On cultured cells, *Agarista salicifolia* (Lam.) G.Don (Ericacea), *Turraea holstii* Gürke (Meliaceae) and *Sterculia africana* (Lour.) Fiori (Malvacea) causes more than two-fold induction of CYP3A4 mRNA (Mills et al., 2005). Unfortunately, only limited data exist in Africa regarding *in vivo* herb-drug interaction since most patients do not report intake of herbal medicines to health practitioners during treatment. Yet one common practice in Sub Saharan Africa is the prescription of antimicrobials and the parallel consumption of widely used traditional medicines which are potentially harmful to the liver (Riebensahm et al., 2019).

Western drugs which have so far attracted attention as potential treatment options for COVID-19 include chloroquine **(158)**, hydroxychloroquine **(159)**, azithromycin **(160)**, ceftriaxone **(161)** (for patients with pneumonia), remdesivir **(162)**, favipiravir **(163)**, ribavirin **(164)**, lopinavir–ritonavir **(165)** (used in combination) and of recent dexamethasone that have been shown to reduce mortality rate of COVID- 19 patients. The CYP enzymes such as CYP2C8, CYP3A4, CYP2D6 and CYP1A1 can metabolize chloroquine (Spaldin et al., 1994; Projean, 2003a; Kim et al., 2003; Gil and Gil Berglund, 2007) and catalyzes the dealkylation of chloroquine **(158)** and hydroxychloroquine **(159)** to pharmacologically active compound (McChesney, 1983; Spaldin et al., 1994; Furst, 1996; Projean, 2003b) CYP3A4 is responsible for the metabolism of Dexamethasone (DEX) **(166)** to 6-hydroxyDEX (6OH-DEX) **(167)** (Tomlinson et al., 1997). Remdesivir **(162)** metabolism is mediated by hydrolases, however, it has been shown to exert weak inhibitory effects on CYP3A4, OATP1B1, OATP1B3, bile acid export pump, multidrug resistance-associated protein (Sciences, 2020), and sodium-taurocholate cotransporter protein. It is also established that remdesivir **(162)** is a substrate of CYP2C8, CYP2D6, CYP3A4, OATP1B1,



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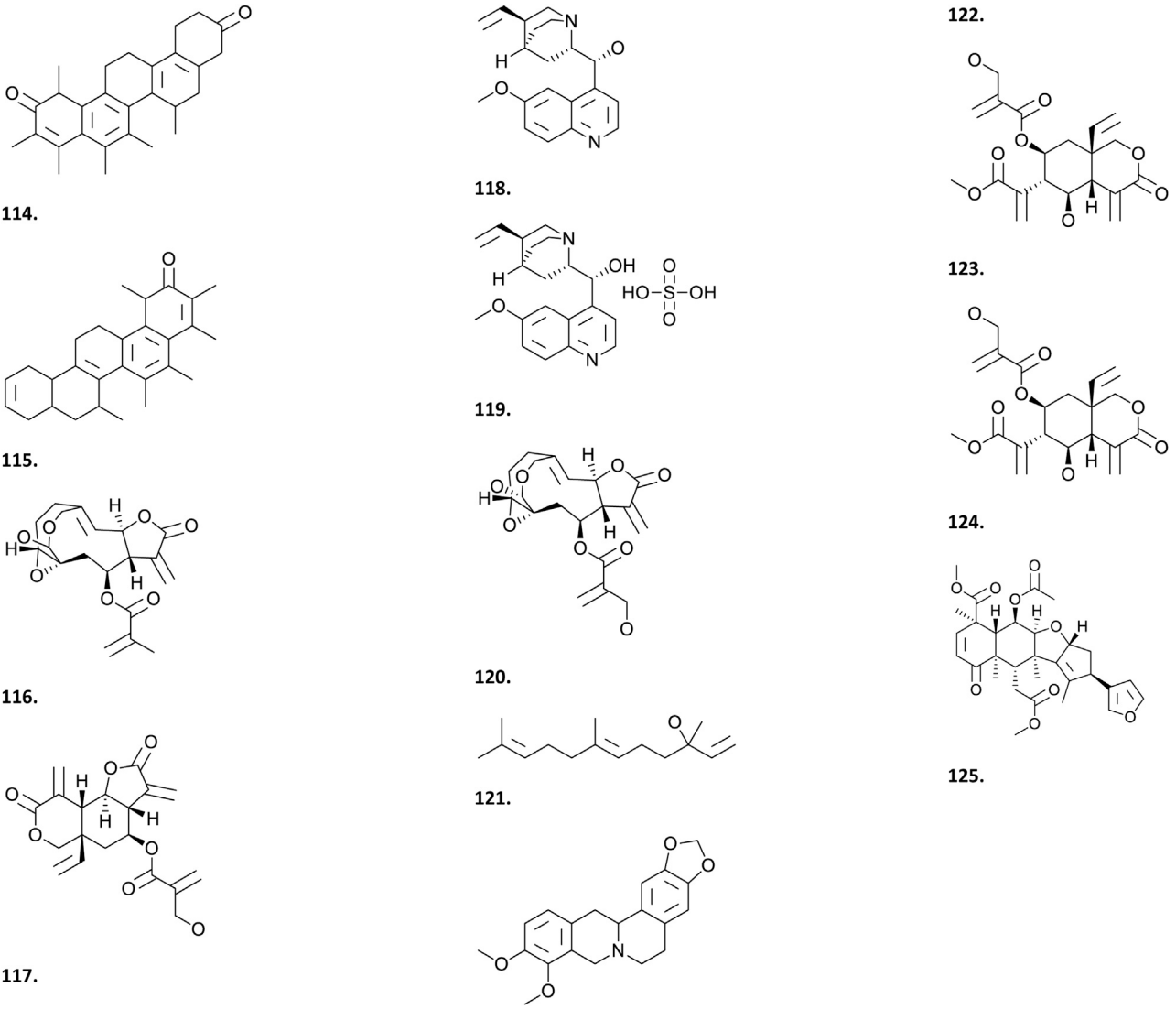
OATP1B3, bile acid export pump, multidrug resistance-associated protein (European Medicines Agency, 2020), and sodium-taurocholate cotransporter protein. Lopinavir and ritonavir **(165)** therapy strongly induces CYP2C19 activity and mildly induces CYP1A2 and CYP2C9. Both intestinal and hepatic CYP3A are inhibited by lopinavir and ritonavir therapy, the former being greatly affected (Yeh et al., 2006). The metabolism of favipiravir **(163)** is mediated by aldehyde oxidase (AO) and xanthine oxidase in the hepatocyte cytosol, and not by CYP450 enzymes. But there are reports which support favipiravir **(163)** as an inhibitor of CYP2C8 (Madelain et al., 2016).

Several widely distributed African medicinal plants with long history in therapeutic use, are employed in the management of different infectious diseases **(Tables 1–3)** and many of them are being repurposed for COVID-19 by herbal firms and local healthcare providers in Africa. Some of such plants e.g *Artemisia* plant species including *Artemisia abrotanum* L. (Asteracea), *Artemisia caruifolia* Buch. -Ham. ex Roxb (Asteracea), *Artemisia pontica L.* (Asteracea), *Artemisia herba-alba* Asso (Compositae), *Artemisia absinthium* L. (Asteracea), *Artemisia afra* Jacq (Asteraceae) significantly inhibits CYP3A4 (Lazaridi, 2014). Aqueous infusions (3.3 mg/L) of *Artemisia annua* displays significant reduction in the CYP3A4 activity (Lazaridi, 2014). Many of the plants applied in TAM accumulate bioactive polyunsaturated fatty acids (PUFA) such as linoleic **(75)**, linolenic **(74)**, docosahexaenic acid **(67)** which have been reported to have profound inhibitory effect on CYP3A4. Linoleic **(75)** and linolenic acid **(74)** are the most common acids found in plants of the *Artemisia* family. *Artemisia annua* is the main active component of the claimed anti-COVID-19 herbal formulation popularly called COVID organics by Madagascar. In another study, 75, 52 and 5% of CYP3A4 were respectively inhibited by100 μg/mL grapefruit oil, Eucalyptol **(77)** and menthol **(65)** (Zhang and Lim, 2008). Curcumin **(72)** (40 μM), 6-gingerol **(79)** (100 μM), citral **(78)** (250 μM), d-limonene **(64)** (400 μM), β-caryophyllene **(66)** (500 μM), 1,8-cineole **(77)** (1 mM), myrcene (1 mM) shows



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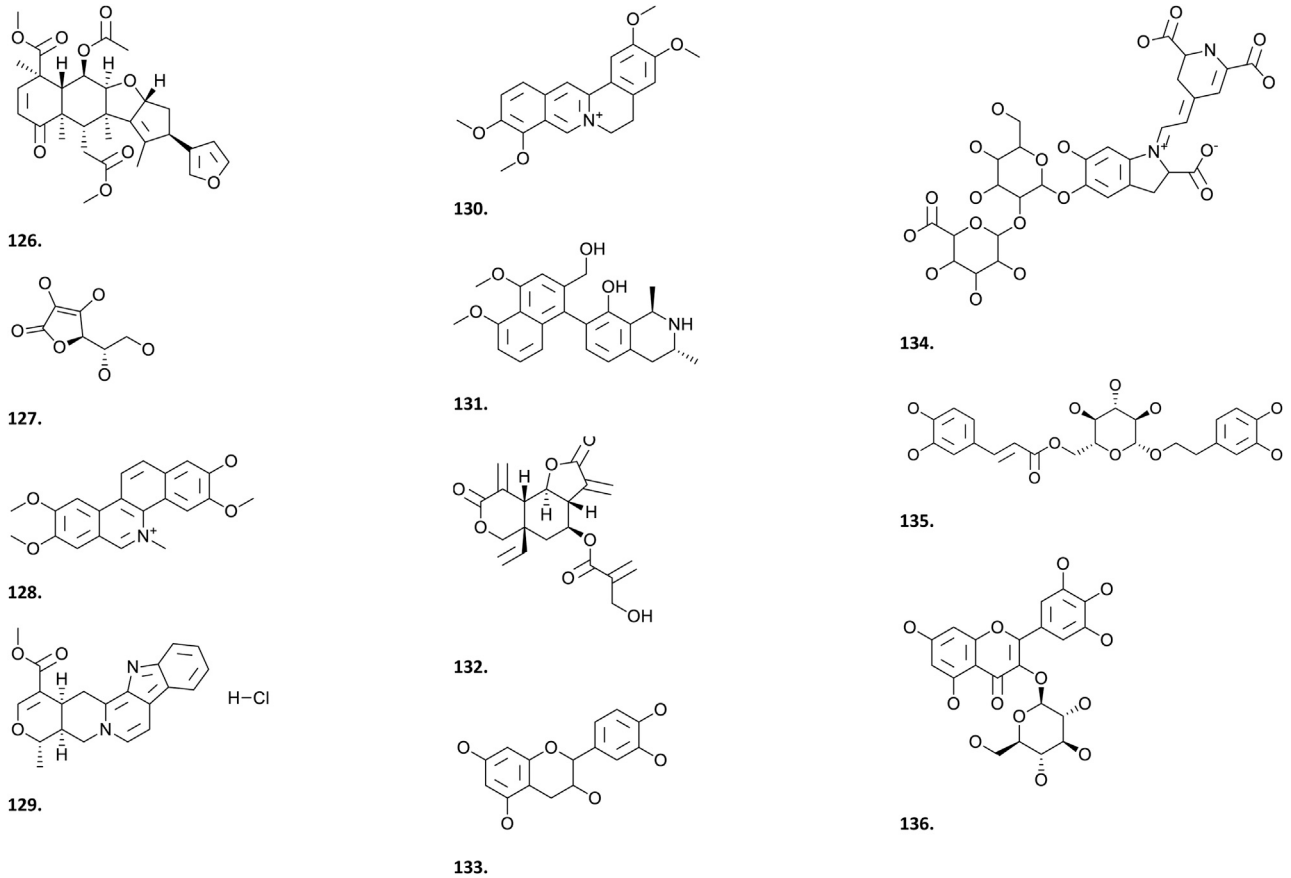
inhibitory effect on CYP3A4; piperine **(76)** has been shown to enhance bioavailability (Lutgen, 2016). These bioactive plant metabolites which have demonstrated antiviral properties have been reported in several plants used in TAM for the symptomatic treatment of COVID-19 in Nigeria (**Table 1** and **Figure 2**). This means the co-administration of these herbal medicines with orthodox drugs used in COVID-19 management could result in herb-drug interaction that potentially may be hepatotoxic. However, this area of study has not received adequate scientific attention, particularly by *in vivo* experiments and should be of research interest in future.

Garcinia kola (bitter kola) is widely used in TAM, the West African sub-region in particular, for a host of conditions including infectious diseases and management of upper respiratory infections (Buba et al., 2016). GARCINIA-IHP, used for symptoms of cold and sore throat, pains, cough, nasal congestion, viral infections and inflammation, is one of the herbal health products marketed by InterceDD Health Products in Nigeria. This herbal formulation is currently under consideration as a repurposed remedy in COVID-19 management after successful clinical trials. As a result of the radical media promotion of claims on *Garcinia kola* in COVID-19 prevention and management which lacks scientific evidence, the seed of the plant has become one of the many other local herbal recipes consumed without any control by a majority of people in West Africa. The correct doses of bitter kola to achieve the best therapeutic effects and without any adverse side effects are often not followed. However, scientific report suggests that *Garcinia kola* induces CYP3A4 transcription by a multiple of 3.7-factors in HepG2 cells at 90 μmol/L (Nwankwo et al., 2000)



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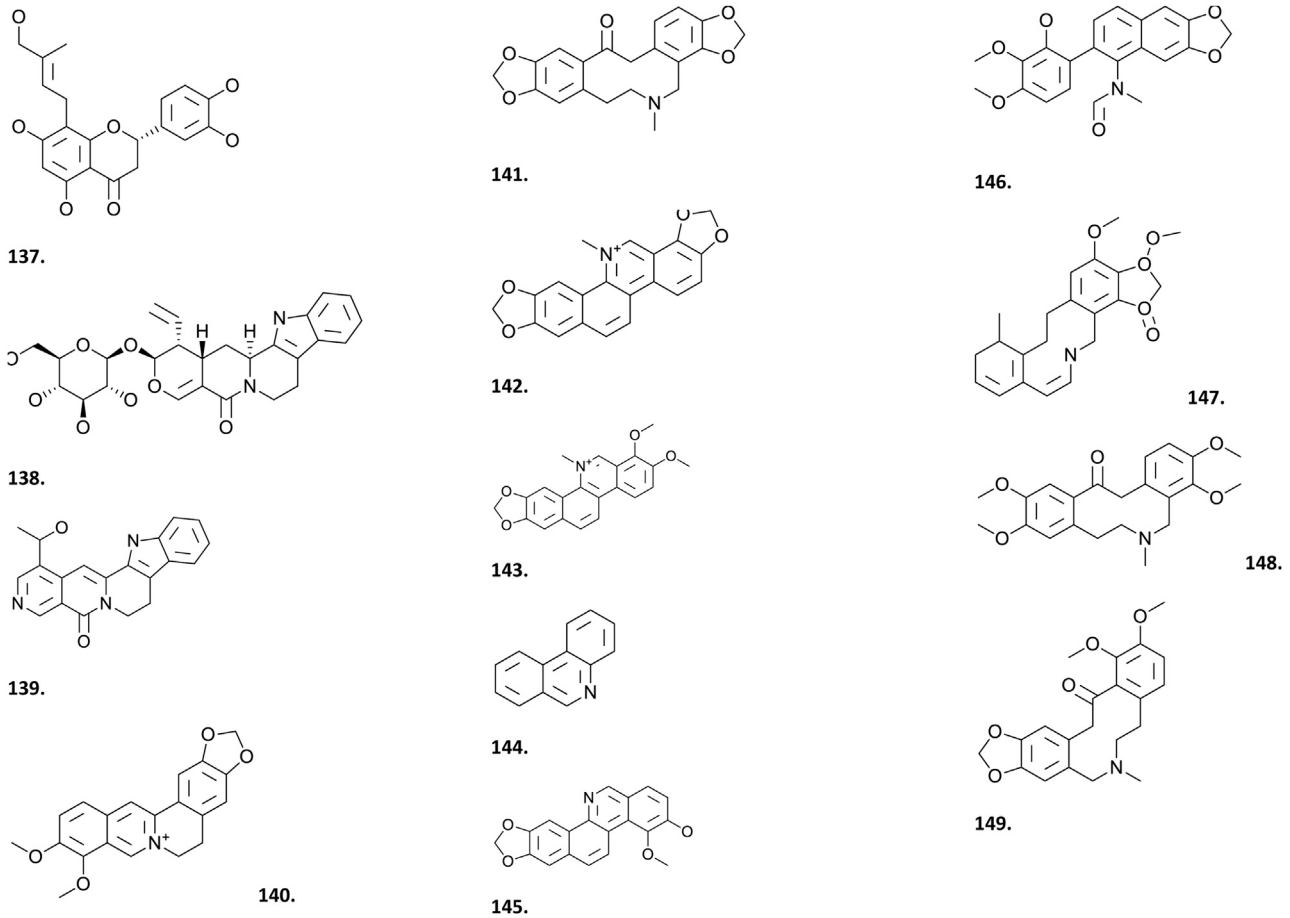
increasing the possibility for herb-drug interaction. Future investigations will therefore focus on the extensive *in vitro* and *in vivo* anti-SARS CoV-2 activities of extracts of *Garcinia kola*, in depth toxicity studies and a holistic investigation of a possible herb-drug interaction.

Allium species (*Allium cepa* and *Allium sativum*) are widely used in Traditional African medicines for the management of infectious diseases and has since been regularly canvassed by some of the COVID-19 infected users in West Africa for the prevention and symptomatic management of COVID-19. These species are among the recipes recommended by the traditional leadership of the Yoruba ethnic nationality in Nigeria and are claimed to be efficacious in COVID-19 prevention and "cure". Garlic oil, obtained from *A. sativum* bulb contains sulfur-compounds such as diallyl sulfide **(69)** (DAS), diallyl disulfide **(70)** (DADS), and diallyl trisulfide **(71)** (DATS) which induce CYP2B and NAD(P)H quinone oxidoreductase 1 (NQO1). DAS **(69)** facilitate the induction of CYP2B10 mRNA and also activate human CYP2B6 and NQO1 promoters which are primarily regulated by constitutive androstane receptor (CAR) and nuclear factor E2-related factor 2 (Nrf2) transcription factors, respectively (Fisher et al., 2007). Further investigation is therefore needed to unveil the mechanisms of possible herb-drug interaction.

Pharmacokinetic Considerations in Developing Potential anti-COVID-19 Herbal Medicines

The popular school of thought tends toward the discovery of a single metabolite specific for one macromolecular target. However, modern medicine via the formulation of multi-component medications containing two, three, or more active components is increasingly accepting the limits of the single-molecule hypothesis. Such multi-component medications are of special importance in anti-infective therapies, and in fact have become the obligatory standard of management in malaria, a protozoan infection, tuberculosis a bacterial infection, and acquired immune deficiency syndrome a viral infection. The overarching aim is to employ the combined drug compounds to target multiple macromolecular targets.

Such multi-component systems natively form a core aspect of plant-derived preparations which range from crude extracts, to carefully designed fractional combinations, and to a lower extent



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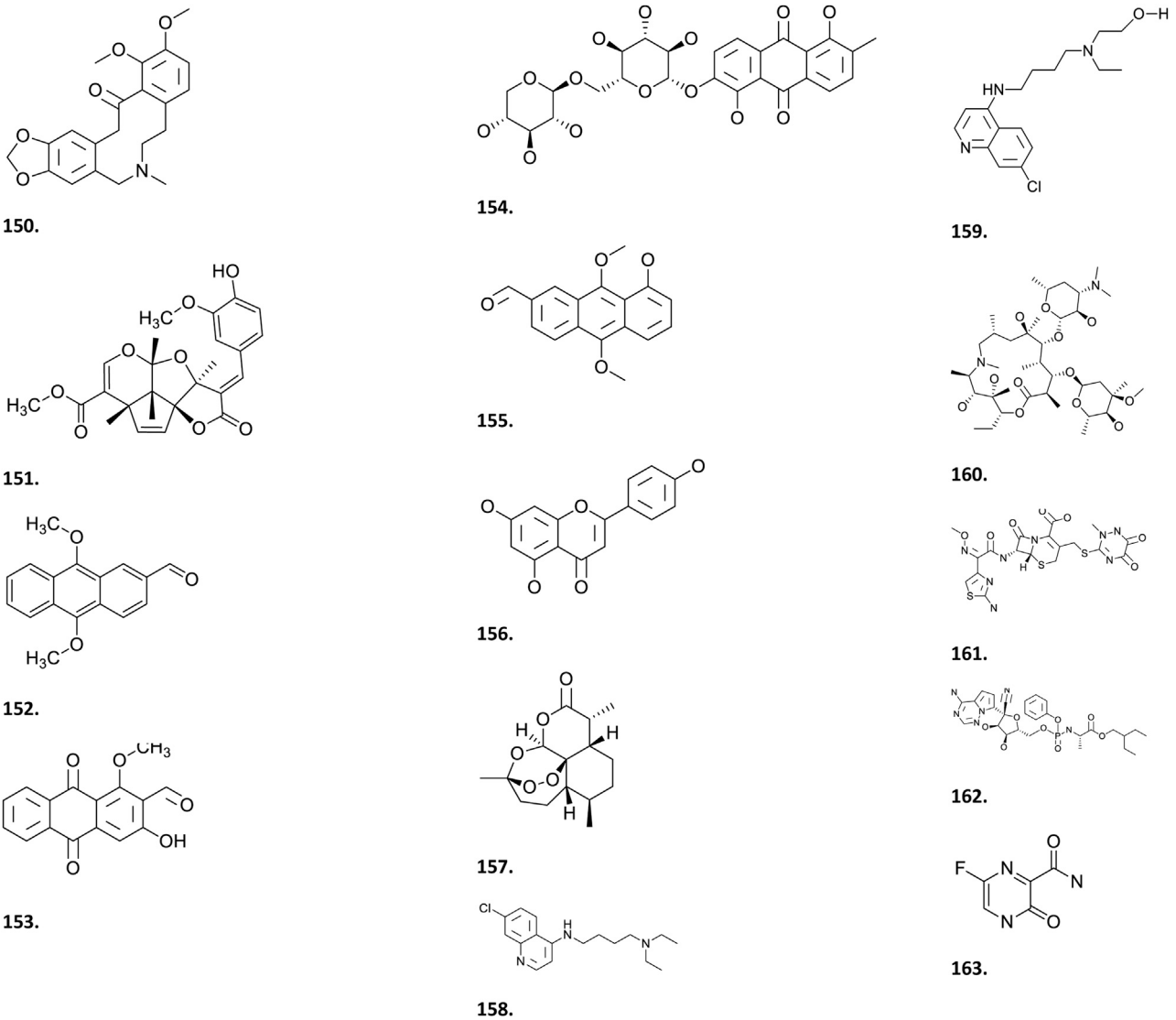
pure natural products. Since most component active principles exist in lower amounts than found in mainstream pharmaceuticals, toxicities from these preparations are generally rarer especially when prepared using properly validated quality assurance processes or following some local preparation methods. Additionally benefit results from the presence of multiple natural products capable of modulating multiple aspects of the biochemical process of interest, a property that is of special interest in antiviral and antimalarial management. Interestingly, some of these botanicals have been suggested to produce strong biological effects even at the low concentrations at which they are present in herbal preparations (Johanna et al., 2012). Together with their beneficial ability to prevent resistance development, herb-based preparations should preferably form a core component of the search for treatment of the current COVID-19 pandemic.

Since the pharmacodynamic effects resulting from herb-based preparations eventually depend on the component principles interacting with biological macromolecules, their successful use also depends critically on pharmacokinetics. With respect to the pharmacokinetics of herbal products, the simultaneous presence of multiple structurally distinct natural products in the same preparation presents a definite layer of challenge not seen with single compound pharmaceuticals but that is often of clinical significance (Fugh-Berman, 2000). Each natural product present in such multi-component preparations possess physicochemical attributes that are often divergent such that it is hardly realistic to describe the overall pharmacokinetics based on a single natural product however significant such compound may be. Instead, it is sometimes more prudent to define these parameters for the bulk product based on an all-or-none basis rather than merely trying to extrapolate the properties from data derived for individual natural products. This was aptly demonstrated in a 2013 study showing the strong CYP3A4-inhibiting activity of an African herbal preparation NIPRD-AM1 with antimalarial activities (Bulus Adzu et al., 2013). NIPRD-AM1 was developed in Nigeria and found to be responsible for diminishing by half the enzymatic activity of the cytochrome P450 enzyme, but following co-administration with metronidazole it was reported to exert no effect on the metabolic disposition of the drug (Obodozie et al., 2011). It is certainly not inconceivable to expect to find present individual natural products with varied effects on the hepatic enzymes in such herbal formulations as NIPRD-AM1; it is however the overall effect of the mixture that is



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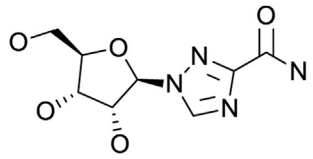
of practical importance for quality assurance as well as for clinical applicability especially involving the analysis of interference with different metabolizing enzymes and with the absorption process of drugs that are likely to be co-administered.

Absorption and bioavailability profiling is challenging for herbal mixtures, partly because existing mathematical models can only suitably describe single drug molecules. The AUC and bioavailability for example, are referenced against measured systemic concentrations of individual drugs and can only describe one drug substance at a time. This however is not to downplay the importance of equivalent means of profiling herb-based preparations. In fact, many natural products are associated with deficient molecular properties compromising solubility, membrane permeation and thus absorption and bioavailability. This is however not surprising since the evolutionary process that emerged the natural products inside plants is isolated from processes within the human biological system where a fine balance of molecular features is often required for receptor interaction. Natural products present in plants have no such evolutionary imperative beyond the functions natively played in their host plants. Therefore, a good number of natural products with good *in vitro* biological profiles have been reported with suboptimal physicochemical properties. Interestingly both the pharmacological potency and poor absorption properties of the component natural products are closely tied with their complex chemical structures. Polyphenols are a class of phytochemicals whose immunomodulatory properties can be useful in herbal preparations for COVID19. They are however often characterized by poor absorption and deactivation by gastric conditions (D'Archivio et al., 2010). In the same category is the group of curcuminoids whose antiviral, anti-inflammatory and antioxidant properties should ordinarily qualify as viable



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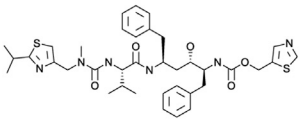

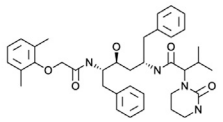

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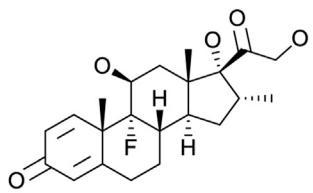

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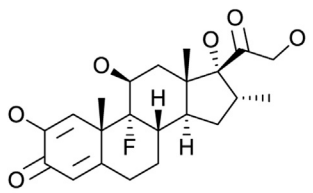

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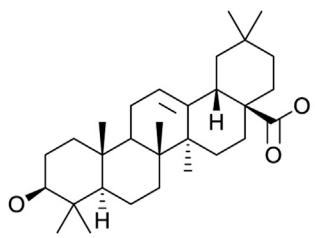

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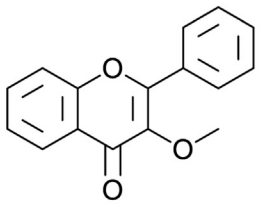

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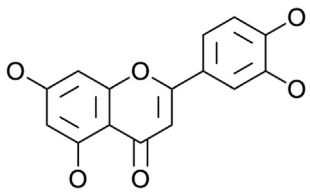

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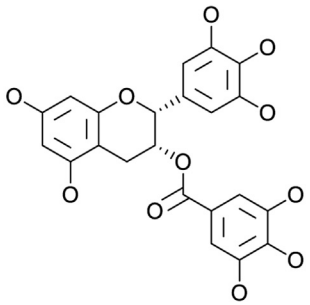

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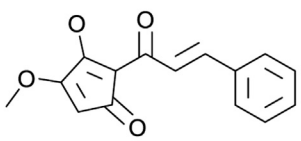

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components of anti-COVID19 treatment. However, curcuminoids (**72**) are associated with high logP values that are generally above 3.0 for the monomeric forms like curcumin and turmeric, and logP values higher than 7.0 for the dimer forms: this together with the high molecular weight associated with dimerized curcuminoids (**72**) associate these

compounds with low bioavailability. In spite of its established diverse pharmacological activities (Sreejayan and Rao, 1996; Goel et al., 2001; Chainani-Wu, 2003; Xu et al., 2008; Mathy-Hartert et al., 2009; Henrotin et al., 2010; Shakibaei et al., 2011; Zhang et al., 2016), only trace amounts of curcumin **(72)** were found in the systemic circulation following oral administration (Bisht et al., 2007). This has expectedly limited clinical applications. And by extension, poor pharmacokinetics *in vivo* may hide otherwise potent anti-SARS-CoV2 preparations and such considerations should be factored in designing anti-SARS-CoV2 activity exploration experiments.

A positive aspect of the use of herb-based preparations in pharmacotherapy is the observation that the derivable pharmacological benefits often emanate from multiple constituent natural products that are sufficiently structurally diverse and yet related to permit the modulation of varied and yet related biochemical processes. It is crucial to point out that the anti-coronavirus activities identified for these natural products were obtained by *in vitro* experiments often involving enzyme-based inhibition assays against isolated viral enzymes. In the absence of biological membranes and the complexity associated within vivo systems, pharmacokinetics is thus unlikely to constitute any real challenge. On the other hand, had these natural products or their multi-component herbal preparations been tested in *in vivo* situations, they might have been thought to lack the anti-coronavirus activities with which they have been credited. Pharmacokinetic factors and experimental design are thus crucial considerations in the identification of potent anti-SARS-CoV2 herb-based products, and it is recommended that testing should in the first stages employ enzyme-based assays while physicochemical/pharmacokinetic liabilities can later be remedied by employing appropriate formulation techniques.

CONCLUSION

In this review, several in silico, *in vitro* and few *in vivo* studies have revealed the therapeutic potential of plant-derived bioactive compounds for the treatment of viral infections in TAM, but the data are too preliminary to be adopted in clinical settings. We have shown clearly that available data are not sufficient to encourage the clinical use of TAM against viral infections, COVID-19 in this special case. Despite the huge potential of TAM, one big and notable gap in knowledge of antiviral application of TAM is the lack of well documented human studies with comparative data. This calls for more research effort geared toward the clinical application of TAM during viral epidemics. Among the primary and secondary metabolites documented, polysaccharides, lectins, cyclotides, alkaloids, flavonoids, tannins and terpenes have been widely exploited and studied for the treatment of several viral diseases and virtually tested for efficacy against SARS-CoV-2. However, only a few of these identified compounds such as KB1, KB8 and andrographolide have good scientific evidence; others require a more elaborate evidence while quite a few have evidence that are rather inconclusive or outright poor quality evidence. For instance, the cyclotide Kalata B1 and B8 from *O. affinis* have been supported by a good evidence for antiviral (anti-HIV) as well as immunostimulating activities in addition to their well-documented oral bioavailability, target specificity, low toxicity, desirable stability in body fluid, mechanism of activity and the uterotonic potential of KB1. Homologues of these knottin peptide therapeutics have recently been identified in some endemic tropical African plants including *Rinorea dentata and Rinorea oblongifolia,* thus expanding their natural sources *in planta*.

The emergence of the deadly COVID-19 pandemic has demonstrated the extent of (over 85%) dependence of African populations on medicinal plants for primary healthcare needs. Traditional Chinese Medicine (TCM), which is currently on a progressive pre-clinical and clinical standardization path, has proved to be effective during the outbreak of SARS-CoV and now SARS-CoV-2 as TCM has been well integrated to western medicines with outcomes supporting their continued use and integration. Africa can follow this same path to evidence-based application of indigenous phytomedicines in the prevention and treatment of viral diseases including COVID-19. Findings from this review indicates that extensive clinical studies are urgently needed to evaluate their therapeutic efficacy and adverse effects especially herb-drug interaction. Importantly, the identification of commercially available herbal medicines used in TAM as documented in this review, which have received an initial regulatory approval for use in humans, and active against viruses, might accelerate their repurposed considerations, observational applications, clinical trials and eventual clinical use, particularly during sudden outbreaks of highly pathogenic viruses like the SARS COV-2. However, future research should equally investigate their mechanism of activity in order to improve their formulation, antiviral activity and to reduce the risk of side effects.

While few of the anti-COVID 19 herbal claims are now undergoing scientific investigation for efficacy and safety, many of the claims are content-secretive making it difficult to validate scientifically destroying the prospect for their clinical application. Although no indigenous phytomedicine has been scientifically validated and authorized for use to prevent or treat COVID- 19, yet there is a growing interest in traditional medicines as potential remedies for COVID-19 in Africa. As a follow up, the World Health Organization (WHO) and the Africa Centers for disease Control and Prevention (Africa CDC) launched a 25-member Regional Expert Committee on Traditional Medicine for COVID-19 to support countries in a collaborative effort to conduct clinical trials on traditional medicines in compliance with international standards.

Phytomedicines used in Traditional African Medicines are most often sourced from the wild and less often cultivated, serves over 85% of the populations within the region, yet have not received utmost respect for good clinical practice (GCP) during the production process. Plants used in Traditional African Medicine are, until now substantially and unsustainably collected from the wild. The tropical forests, including the Sub-Saharan tropical African flora, which covers up to half of the world's angiospermic plants have been described to be in danger of a continuous decline at an estimated 16.8 million ha/

annum (Purvis et al., 2019). This riotous and unabated competition for the unsustainable depletion of plant resource in Africa which are most often collected for medicinal and non-medicinal purposes constitutes a threat to plant biodiversity and their availability to future generations. Thus, the sustainable use of medicinal plant resource in Africa should encourage their purpose-driven cultivation for use in African ethnomedicine as well as for production of evidence-led phytomedicines. In addition, the establishment of requisite programs for medicinal plant resource utilization and conservation of endemic African plants are opportunities for future studies. This has become urgent as plant cultivation offers pharmacological advantages over collection from the wild due to variation in quality and composition resulting from environmental and genomic mutations (WHO et al., 1993). Furthermore, cultivated plants reduces the possibility for variation in addition to the unlikely event of getting the therapeutic benefit. More so, the sustainable use of plants via cultivation has the potential of reducing the likelihood of adulteration and wrong identification. The efficacy and toxicity assessment of phytomedicines used in Africa for the prevention and treatment of COVID-19 is urgently needed to justify or discourage their local uses. According to WHO Africa, "*even if therapies are derived from traditional practice and natural, establishing their efficacy and safety through rigorous clinical trials is critical*". Therefore, future research should focus on an indepth scientific investigation to demystify the bioactive component and establish chemical fingerprint for such complex herbal phytomedicines and mechanistically study them for an evidence-rooted and verifiable plant-derived medicines with potential for COVID 19 management. Consequently, the integration of Traditional African Medicine into the western-based national healthcare structure and clinical study of potential herb-drug interaction are research outlook for consideration by research institutions in Africa and the government of African countries.

AUTHOR CONTRIBUTIONS

AFA: Conceptualization, data mining, draft preparation. AAF: Data mining, some chemical structures and writing. OO: Data mining and writing. HD-A: Data mining and writing. AO: Data mining and table preparation. AE: Reviewing and Editing. CB: Conceptualization, reviewing and editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.596855/full#supplementary-material.

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REVIEW
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Withania somnifera (L.) Dunal: Opportunity for Clinical Repurposing in COVID-19 Management

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As the COVID-19 pandemic is progressing, the therapeutic gaps in conventional management have highlighted the need for the integration of traditional knowledge systems with modern medicine. Ayurvedic medicines, especially Ashwagandha (*Withania somnifera* (L.) Dunal, WS), may be beneficial in the management of COVID-19. WS is a widely prescribed Ayurvedic botanical known as an immunomodulatory, antiviral, anti-inflammatory, and adaptogenic agent. The chemical profile and pharmacological activities of WS have been extensively reported. Several clinical studies have reported its safety for use in humans. This review presents a research synthesis of *in silico*, *in vitro*, *in vivo*, and clinical studies on *Withania somnifera* (L.) Dunal (WS) and discusses its potential for prophylaxis and management of COVID-19. We have collated the data from studies on WS that focused on viral infections (HIV, HSV, H1N1 influenza, etc.) and noncommunicable diseases (hypertension, diabetes, cancer, etc.). The experimental literature indicates that WS has the potential for 1) maintaining immune homeostasis, 2) regulating inflammation, 3) suppressing pro-inflammatory cytokines, 4) organ protection (nervous system, heart, lung, liver, and kidney), and 5) anti-stress, antihypertensive, and antidiabetic activities. Using these trends, the review presents a triangulation of Ayurveda wisdom, pharmacological properties, and COVID-19 pathophysiology ranging from viral entry to end-stage acute respiratory distress syndrome (ARDS). The review proposes WS as a potential therapeutic adjuvant for various stages of COVID-19 management. WS may also have beneficial effects on comorbidities associated with the COVID-19. However, systematic studies are needed to realize the potential of WS for improving clinical outcome of patients with COVID-19.

Keywords: Ashwagandha, Ayurveda, *Rasayana*, Immunomodulation, Inflammation, Cytokine, Adjuvant

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INTRODUCTION

The COVID-19 or coronavirus disease 2019 is a contagious disease caused by SARS-CoV-2. The rapidly spreading disease is considered as one of the causes of mortality globally (Zhou P. et al., 2020) ("WHO Announces COVID-19 Outbreak a Pandemic" 2020) ("WHO Coronavirus Disease (COVID-19) Dashboard" 2020).

Understanding the pathophysiology of this disease is rapidly advancing with the availability of new research data. Current evidence suggests that most individuals are asymptomatic or are suffering from mild symptoms. The patients who progress to severity develop pneumonia and ARDS and

require hospitalization. The SARS-CoV-2 virus uses the angiotensin-converting enzyme 2 (ACE2) receptor for cell entry and the transmembrane serine protease 2 (TMPRSS2) for spike protein priming (Hoffmann et al., 2020). Upon host cell entry, +ssRNA is released which then takes over the cellular ribosomal machinery to synthesize structural proteins and enzymes essential for viral replication (Du et al., 2009) (Alanagreh et al., 2020). The infected cells recruit immune cells for viral clearance, which release cytokines combatively that induce hyperinflammation leading to organ damage (Mortaz et al., 2020). Thus, COVID-19 is characterized by collapsed immune balance, hyperinflammation, cytokine storm, and multiorgan failure. The common clinical symptoms of COVID-19 observed so far are fever, cough, breathlessness, and fatigue. The pathological observations revealed ground-glass opacities, pneumonia, and hematological abnormalities (Fu et al., 2020). Patients with comorbidities such as diabetes, hypertension, cancer, tuberculosis, etc., are at an increased risk of complications (Sanyaolu et al., 2020).

The extent of COVID-19 has highlighted challenges for healthcare systems. The advancing clinical observations are rapidly changing the management protocols. The present empirical pharmacotherapeutics involves antivirals (e.g., remdesivir), antimalarials (e.g., chloroquine and hydroxychloroquine), antibiotics (e.g., azithromycin), and in few cases immunomodulators (e.g., tocilizumab) ("Clinical Management of Severe Acute Respiratory Infection When COVID-19 Is Suspected" 2020) ("Revised Advisory on the Use of Hydroxychloroquine (HCQ) as Prophylaxis for SARS-CoV-2 Infection" 2020) ("Clinical Management Protocol", 2020). However, the adverse effects of these drugs remained a concern. An approach of convalescent plasma therapy is also being explored (Rajendran et al., 2020). The accelerated vaccine development has undergone clinical trials and obtained the provisional license for emergency use in a pandemic. The rapid outbreak of the disease necessitates an integration of traditional knowledge systems with modern medicine. Ayurveda, an ancient Indian medicine system, can provide probable candidates for natural product drug discovery even for emerging diseases (Patwardhan et al., 2004). The traditional wisdom of Ayurvedic medicine has a lot to offer for the management of COVID-19.

Withania somnifera (L.) Dunal (Ashwagandha/WS) is one of the extensively prescribed botanicals in Ayurveda practice for its multimodal effects (Vaidya, 2000). The diverse pharmacological activities including immunomodulatory, anti-inflammatory, antioxidant, anti-stress, antihypertensive, and antidiabetic along with organ-protective effects have been studied extensively by researchers (Mishra et al., 2000). The scientific evidence supports the prophylactic effect of WS to maintain immune homeostasis in inflammatory and infectious diseases (Minhas et al., 2011) (Teixeira et al., 2006).

The chemical profile of several extracts and formulations of WS has been well documented in previous studies. Briefly, withanolides (steroidal lactones), the main phytochemical of WS, play a central role in exhibiting multimodal effects synergistically. These are a group of C_{28}-steroidal lactone triterpenoids, which majorly include withaferin A, withanolide A, B, and D, withanoside IV and V, withasomniferin A, withanone, sitoindoside IX and X, 12-deoxywithastramonolide, etc. Moreover, other polyphenols including catechin, naringenin, syringic acid, and p-coumaric acid were also found in significant quantities in WS extracts. A combination of such versatile phytochemicals potentiates WS as a strong therapeutic agent (Kalra and Kaushik, 2017) (Alam et al., 2011).

This is a narrative review based on reported scientific literature of experimental studies preferably indexed in PubMed database. The collected properties are represented in compliance with traditional use of WS as per Ayurveda literature. Using the search terms such as immunity, cytokine modulation, inflammation, and organ protection, the review analyses the literature for several pharmacological activities of WS. These search terms were chosen in the context of pathophysiological aspects of COVID-19.

This review collates the biochemical actions of WS on several viral infections and diseases based on available literature. These actions are mapped, with the background of advancing pathophysiological insights of COVID-19. Depending upon the available scientific evidence, the review advocates WS as an adjuvant to current pharmacotherapeutics, underlining an integrative approach in COVID-19. The review also suggests use of WS in the management of comorbidities. Collectively, the review presents a research synthesis of reported *in silico*, *in vitro*, *in vivo*, and clinical studies that offer therapeutic benefits of WS for prophylaxis and clinical management of COVID-19.

PROBABLE ROLE OF *WITHANIA SOMNIFERA* IN COVID-19 PATHOPHYSIOLOGY

Especially in symptomatic patients, COVID-19 exhibits pathophysiological milestones such as viral entry followed by a variety of clinical manifestations. A few patients progress to immune response with cytokine storm and hyperinflammation followed by multi-organ failure. WS is reported to mitigate prior pathophysiological aspects in disease progression and protect vital organs (**Figure 1**). This section of the review maps the pharmacological properties supported by molecular mechanisms of WS to pathophysiological milestones of COVID-19.

Viral Entry and Load

SARS-CoV-2 preferentially attacks pneumocytes for their multiplication. This occurs by the binding of viral spike protein to cellular ACE2 receptor followed by viral endocytosis (Du et al., 2009). The virus utilizes ribosomal machinery for mRNA translation into viral proteins with simultaneous mRNA replication using RNA-dependent RNA polymerase (RdRp) enzyme. The viral copies are exocytosed out of infected cells for further encroachments (Alanagreh et al., 2020).

The antiviral properties of WS may interfere with viral entry and subsequent life cycle. The metabolites of WS were explored for their antiviral potential against SARS-CoV-2 proteins using a

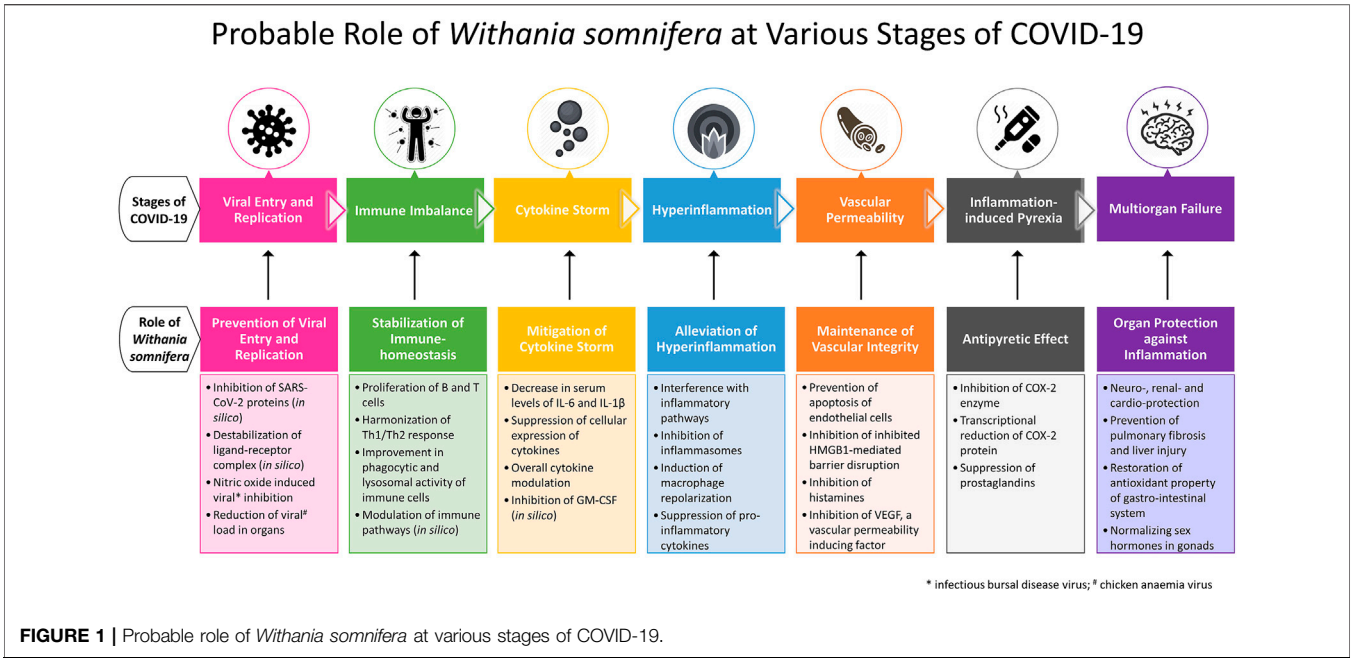

FIGURE 1 | Probable role of *Withania somnifera* at various stages of COVID-19.

computational molecular docking tool. It is indicated that withanone may disrupt host–virus interaction by destabilizing the complex of ACE2 and receptor-binding domain of spike protein (Balkrishna et al., 2021). Withaferin A and withanone are predicted to block main protease (Mpro) and TMPRSS2 enzyme to interfere with viral entry and replication (Kumar et al., 2020) ("Ashwagandha Takes Lead in IIT-Delhi Study to Be COVID-19 Warrior", 2020) (Sharma. S. and Deep, 2020). This was also supported by an *in vitro* study that revealed withanone to downregulate mRNA of TMPRSS2 in MCF7 cells (Kumar et al., 2020). A study carried out by our team predicted the potential of withacoagin and withanolide B to block viral spike protein and RdRp enzyme with a high binding affinity (Borse et al., 2020). Moreover, many recent virtual screening studies reported the potential of withanolides to inhibit SARS-CoV-2 proteins with a high binding affinity (Khanal et al., 2021) (Srivastava et al., 2020) (Parida et al., 2020) (Shree et al., 2020) (Tripathi. M. K. et al., 2020) (Chikhale et al., 2020). One of the molecular docking analysis revealed that withaferin A inhibited host receptor glucose-regulated protein 78 (GRP78) found to be upregulated in patients with COVID-19 (Sudeep et al., 2020) (Sabirli et al., 2021). The pharmacophore of withanolides is found to be associated with the induction of cytoprotective heat-shock response (Wijeratne et al., 2014) and inhibition of HSP90 (Gu et al., 2014). A recent report shows the dependency of SARS-CoV-2 on host HSP90 (Li C. et al., 2020) (Kasperkiewicz, 2021). Therefore, detailed pharmaco-mechanistic studies may help to decipher the potential of withanolides in SARS-CoV-2 viral inhibition.

The overarching potential of WS in several other viral diseases is quite evident. A molecular docking and simulation study revealed a high binding affinity of withaferin A with neuraminidase of H1N1 influenza virus (Cai et al., 2015) and DNA polymerase of herpes simplex virus (Grover et al., 2011) at the functional site of enzymes. Administration of WS extract (1%) through feed reduced viral load of lymphoid organs such as thymus and spleen in anemia virus-infected chicks (Latheef et al., 2017). Chick models of infectious bursal disease (IBD) also showed a reduced viral persistence accompanied with lymphocyte stimulation on feeding with a dietary supplement of 1% root powder of WS (Ganguly et al., 2020). The same group of researchers predicted stimulation of NO production by WS extract to inhibit the IBD virus *in vitro* (Ganguly et al., 2018). An *ex vivo* experiment carried out on PBMCs of HIV patients suggested that WS aqueous extract reduces the expression of disease progression marker CD38 on $CD8^+$ T lymphocytes. This necessitates the probable anti-HIV potential of WS (Maurya et al., 2019).

A recent overview of *in vitro* studies indicated the importance of polyphenols against SARS-CoV-2 infection (Annunziata et al., 2020). A group of catechin derivatives screened against SARS-CoV nucleocapsid protein showed a significant inhibitory effect (Roh, 2012). Therefore, the potential of methanolic extract of WS containing high polyphenol concentrations (catechin, naringenin, syringic acid, p-coumaric acid, etc.) to inhibit SARS-CoV-2 infection is scientifically intuitive (Alam et al., 2011).

Computational approaches also suggest possible molecular mechanisms regarding the ability of WS to inhibit viral entry. However, experimental and clinical data are limited. This highlights the need for the investigation of WS metabolites as antiviral agents in COVID-19 with systematic experiments.

Immune Homeostasis

An infectious agent triggers a pathological immune response orchestrated by the several types of immune cells along with secreted biomolecules. An immediate immune response to infections includes antigen recognition and phagocytosis. It is

followed by the delayed response of inflammation (Tosi, 2005). In SARS-CoV-2 infection, a sudden elevation of immune response brings the biological architecture at high risk due to hypersecretion of inflammatory molecules (Tufan et al., 2020). This infection also triggers gross pathological alterations in immune cells leading to immune imbalance (Gustine and Jones, 2020). Under such circumstances an intervention is needed, which can balance the immune response in a way that is host tolerable and adverse to viral replication. Interestingly, the collected scientific reports on immunomodulatory effects indicate that WS maintains immune homeostasis rather than unidirectional stimulation or suppression (Patwardhan et al., 2020).

An observational clinical study on isolated PBMCs of patients with COVID-19 showed elevated innate immunity (macrophages and neutrophils) and lowered cell-mediated immunity (B and T cells) (Sadanandam et al., 2020). The T-cell response is highlighted to protect against viral infections including COVID-19 (Luo et al., 2020). An aqueous and hydroalcoholic extract of WS and withanolide A significantly improved cell-mediated immune response. It promoted proliferation of B and T cells along with Th1 response in healthy, chronically stressed, ovalbumin antigen immunized and immunocompromised mice (Khan. B.et al., 2006) (Malik et al., 2007) (Kour et al., 2009) (Khan. S. et al., 2009) (Bani et al., 2006; Sun, 2019). Different chemotypes of WS hydroethanolic extract significantly modulated the proliferation of B and T cells with special reference to balanced Th1/Th2 response in healthy mice (Susheela Kushwaha et al., 2012a). WS hydromethanolic and aqueous root extract administered in healthy mice showed enhanced leukocyte and platelet counts (Davis and Kuttan, 2000) (Agarwal et al., 1999). The immunomodulatory potential of WS extract in viral diseases was also tested in chicks. WS depicted significant cell-mediated immune response confirmed by an increased count of $CD4^+$ and $CD8^+$ T cells compared with control (Latheef et al., 2017) (Davis and Kuttan, 2002). Similarly, aqueous and ethanolic extract of WS roots and leaves normalized the leukocyte count in *E. coli* infected guinea pigs that were proportionate to control (El-Boshy et al., 2013). Additionally, several fractions of WS hydroethanolic extract and withaferin A significantly modulated the Th1/Th2 response in parasite-infected mouse model (Kushwaha S. et al., 2012b). Dietary supplementation of WS root powder to Rohu fishes improved the phagocytic and lysosomal activity of immune cells over a month. This was accompanied by a significant increase in the survival rate of fishes even after bacterial infection (Arun Sharma et al., 2010). Phagocytic activity of peritoneal macrophages was also enhanced in healthy mice administered with WS hydromethanolic extract (Davis and Kuttan, 2000). An *in silico* network ethnopharmacological investigation by our group revealed the ability of bioactive compounds of WS to modulate immune pathways (Chandran and Patwardhan, 2016). However, it needs to be combined with experimental pharmacology using suitable models. A clinical study revealed an immune-activating effect of the WS root extract administered with *anupana* (i.e., whole milk as a vehicle) as was indicated by a significant activation of T cells and NK cells after 4 days of twice daily consumption (Mikolai et al., 2009).

A recent clinical study of patients with COVID-19 showed increased serum levels of GM-CSF (Zhou Y. et al., 2020). GM-CSF binds to its receptor on immune cells to stimulate the expression of cytokines. Therefore, few therapeutic approaches are suggesting to target GM-CSF to mitigate cytokine storm (Ayisha Sharma and Vaidya, 2020). In this background, withanolide A might be beneficial as it is found to have a substantial binding affinity with GM-CSF receptor in a molecular docking study (Posa et al., 2016).

The property of WS in optimizing immune response is being explored in the management of COVID-19. The Ministry of AYUSH, Govt. of India, has initiated multicentric clinical studies in India. These studies have been aimed to understand WS applications in the management of COVID-19 (CTRI/2020/05/025429).

Cytokine Storm

Viral infections such as SARS-CoV-2 trigger excessive generation of pro-inflammatory cytokines known as cytokine storm (Tang et al., 2020). Cytokines mediate the cross-talk between immune cells and infected cells to clear infectious agents (Mehta et al., 2020). With this background, **Table 1** shows the summary of the role of WS to reduce inflammatory cytokines.

The characterization of COVID-19 pathophysiology highlighted the decisive role of IL-1β and IL-6. It has been found that increased level of these cytokines is associated with the severe stage of COVID-19 (Aziz et al., 2020). WHO released an informal consultation to explore the potential role of available antagonists of IL-1β and IL-6 in the disease management. Tocilizumab, a monoclonal antibody and an IL-6 receptor inhibitor, is being considered to treat patients with COVID-19. This blueprint also stated the variability of IL-6 concentrations in patients with COVID-19. Therefore, it was recommended to restrict the tocilizumab treatment to patients with high IL-6 levels ("WHO R&D Blueprint COVID-19 Informal Consultation on the Potential Role of IL-6/IL-1 Antagonists in the Clinical Management of COVID 19 Infection" 2020) (Michot et al., 2020).

In such a scenario, WS may also be helpful to suppress IL-1β and IL-6 as evident in several models. WS aqueous extract along with fatty acids and withaferin A inhibited IL-6 and IL-1β released by monocytes, macrophages, and keratinocytes *in vitro* (Balkrishna et al., 2020) (Dubey et al., 2018) (Neog et al, 2018) (Sikandan et al., 2018). Withaferin A and WS aqueous extract significantly reduced serum levels of IL-1β and IL-6 secreted by bone marrow-derived dendritic cells and macrophages (Morsy et al., 2019) (Sultana etal., 2017) (Noh et al., 2016) (Kim et al., 2015) (Khan et al., 2018). Withaferin A also suppressed IL-1β expression of the fibrotic lung of a mouse (Bale et al., 2018a). WS root powder significantly reduced IL-6 secretion in ascitic fluid and serum of lupus erythematic mouse model (Minhas et al., 2012). Clinically, WS ethanolic extract attenuated IL-1β expression in PBMCs of healthy individuals and arthritic patients (Singh D. et al., 2007).

TABLE 1 | Suppression of inflammatory cytokines by withanolides and *Withania somnifera* extracts in several study models.

No	Intervention	Model	Cytokines	References
1	Withaferin A	Macrophages (RAW 264.7)	IL-1β, IL-6, IL-23, and TNF-α	Neog, Sultana, and Rasool (2018)
2	Withaferin A	Monocytes (THP-1)	IL-1α, IL-1β, IL-2, IL-3, IL-5, IL-10, IL-12p70, IL-18 B Pα, IL-13, IL-23, IL-33, IL-34, IP-10, GM-CSF, PDFG-AA, CCL2/mcp-1, CCL17/TARC, SDF1α/CXCL12, CCL20/mip-3α, KLK3, angiopoientin-1, IGFBP-2, TFF3, BAFF, BDNF, FLT3LG, IGFBP-3, ACRP30/adiponectin, GH, leptin, LIF, SHBG, aggrecan, angiogenin, HGF, NGAL, TSP-1, and CST3	Dubey et al. (2018)
3	Withaferin A	Murine bone marrow-derived macrophages	IL-6 and TNF-α	Noh et al. (2016)
4	Withaferin A	Murine bone marrow-derived dendritic cells	IL-1β	Kim et al. (2015)
5	WS root aqueous extract	Serum of collagen-induced arthritic rat	IL-1β, IL-6, and TNF-α	M. A. Khan et al. (2018)
6	Withaferin A	Synovial macrophages of arthritic rat	IL-1β, IL-6, MCP-1, TNF-α, and VEGF	Sultana, Neog, and Rasool (2017)
7	WS extract as dietary supplement	LPS and Con-A induced spleen lymphocytes of rats	TNF-α	Yamada et al. (2011)
8	Withaferin A	Lung tissues of mouse model of pulmonary fibrosis	IL-1β and TNF-α	Bale et al. (2018b)
9	WS root powder	Serum and ascitic fluid of pristane-induced mouse model of systemic lupus erythematosus	IL-6, NO, ROS, and TNF-α	Minhas et al. (2012)
10	WS root hydro-methanolic extract	Healthy mice	TNF-α	L Davis and Kuttan (1999)
11	WS root and leaves aqueous and ethanolic extract	Guinea pigs infected with *E. coli*	TNF-α	El-Boshy et al. (2013)
12	WS root ethanolic extract	PBMCs of healthy individuals and patients with rheumatoid arthritis	IL-1β, IL-12, and TNF-α	Singh D. et al. (2007)
13	WS root ethanolic extract	Macrophages (RAW 264.7)	NO	Singh D. et al. (2007)

All these activities from several models demonstrate WS activity to suppress IL-1β and IL-6. Thus, WS can be considered for the investigation to mitigate cytokine storm in patients with COVID-19, with special reference to IL-1β and IL-6.

Hyperinflammation

An aspect of hyperinflammation highlights COVID-19 as a chronic disease. It is characterized by alterations in cytokine milieu and inflammatory pathways (Tufan et al., 2020) (Gustine and Jones, 2020). The inflammatory pathways are mediated through several factors including cytokines, receptor proteins, inflammasomes, and nuclear factors, etc. Withanolides have been reported to regulate the inflammatory pathways such as NF-κB, JAK/STAT, Nrf2, and HIF-1. This favors withanolides in clinical application to manage chronic diseases associated with inflammation (White et al., 2016). The anti-inflammatory action of WS through suppression of inflammatory cytokines has been already shown in **Table 1**. This section highlights other mechanistic studies of WS that show interference in inflammatory pathways in the background of hyperinflammation in COVID-19.

Immune cells facilitate inflammatory signaling through several immune factors and receptor proteins. Peroxiredoxins (e.g., Prx1) expressed by alveolar macrophages are immune factors capable of inducing acute inflammation in the lungs (Knoops et al., 2016) (Kinnula et al., 2002) (Diet et al., 2007). Prx1 acts as DAMPs to bind TLR4 and stimulates the secretion of pro-inflammatory cytokines (Riddell et al., 2010). A piece of strong mechanistic evidence revealed that withaferin A inhibits the chaperone activity of Prx1 and reduces the infection-induced TLR4 expression in macrophages (Noh et al., 2016) (Zhao Q. et al., 2015). Furthermore, cytoplasmic glucocorticoid receptors (GRs) contribute to the anti-inflammatory effect upon binding of glucocorticoids (class of steroids) through distinct molecular mechanisms (Baschant and Tuckermann, 2010). Molecular docking of withanolide D targets 3D structural model of GPCR (expressed on inflammatory cells), which suggested curbing of inflammation (Sun and Ye, 2012) (Chinthakunta et al., 2018). Withaferin A showed the agonistic effect on GR with strong binding affinity comparable to that of fluticasone, a synthetic GR agonist. An anti-inflammatory effect was validated using *in vivo* analysis wherein withaferin A reduced granuloma (collection of macrophages that induces inflammation) in rats (Morsy et al., 2019).

Inflammasomes (intracellular multiprotein complexes) and macrophage polarization enables the regulation of inflammation in chronic diseases (Parisi et al., 2018) (Guo et al., 2015). AIM2 and NLRP3 inflammasomes represented by M1 macrophages and monocytes are responsible for the secretion of pro-inflammatory cytokines (Wang Z. et al., 2020). Withaferin A is found to modulate AIM2 inflammasome accompanied with a reduction in M1 macrophage-mediated pro-inflammatory cytokines and STAT3-induced macrophage repolarization (Neog et al., 2018) (Ngoungoure and Owona, 2019). Macrophage repolarization by withaferin A was also significant in arthritic rats, ameliorating the inflammatory response

(Sultana et al., 2017). The severe cases of COVID-19 exhibited dysregulation of NLRP3 inflammasome, which is emerging as a potential therapeutic target (van den Berg and Te Velde, 2020) (Shah, 2020). Withaferin A also suppressed the expression of NLRP3 inflammasome in monocytes (Dubey et al., 2018), dendritic cells (Kim et al., 2015), and in lung tissues (H. M. Zhao et al., 2019). Additionally, administration of withaferin A normalized inflammation-induced FoxO3a gene expression in diseased (scleroderma) mouse model proportionate to that of a control group (Peng, 2010) (Bale et al., 2018b). A few oxidative enzymes such as lipoxygenases contribute to inflammation by inducing the production of leukotrienes (Rådmark and Samuelsson, 2009). An *in vitro* and *in silico* analysis of aqueous and hydroethanolic extracts of WS with 5-lipoxygenase (isolated from human polymorphonuclear leukocytes) showed a significant inhibition better than conventional anti-inflammatory agents (Madhusudan et al., 2016). WS aqueous extract also significantly reduced the lymphocyte proliferation in the inflammatory arthritic rat model (Rasool and Varalakshmi, 2006).

An activation of the NF-κB pathway is a decisive mechanism in inducing inflammation (Liu et al., 2017). It potentiates cytokine secretion and leukocyte recruitment to contribute to the inflammatory response. Therefore, it has been considered as a therapeutic target in inflammatory diseases (Lawrence, 2009). WS has emerged as a potential modulator of the NF-κB pathway with withaferin A as an inhibitor of NF-κB activation (Heyninck et al., 2014). Similarly, withaferin A also inhibited bacterial infection-induced NF-κB activation in macrophages and dendritic cells of mice (Noh et al., 2016). Withaferin A (Neog et al., 2018) (Bale et al., 2018a) and fatty acids of WS seeds (Balkrishna et al., 2020) reduced NF-κB expression in monocytes, macrophages, and fibrotic lung tissues of mice. WS aqueous extract was found to inhibit NF-κB activation in collagen-induced arthritic rats (Khan et al., 2018). The mechanistic studies revealed that NF-κB inhibition occurs due to the binding of withaferin A to the thiol group of cysteine residues (Heyninck et al., 2014) (Gambhir et al., 2015). Withaferin A found to block nuclear translocation of NF-κB in lymphocytes along with a significant reduction in cytokine release by monocytes (Gambhir et al., 2015) (Dubey et al., 2018). The ethanolic extract and withaferin A were found to inhibit nuclear translocation of NF-κB in PBMCs of healthy individuals and arthritic patients (Singh D. et al., 2007).

The role of WS in inflammatory diseases is explored by several researchers. Mazzio et al. explored several natural products for their anti-inflammatory activity in the context of the acute systemic inflammatory response to prevent sepsis. The study revealed explicitly that WS has better anti-inflammatory potential than all other natural products accompanied with negligible toxicity (Mazzio et al., 2016). Similarly, Minhas et al. investigated the prophylactic and therapeutic effects of WS in systemic lupus erythematosus. WS showed protective and anti-inflammatory effects before and after the disease induction, respectively. These effects were validated by attenuation of pro-inflammatory cytokines on the administration of WS root powder (Minhas et al., 2011) (Minhas et al., 2012). A rectal application of WS extract gel formulation also exhibited remedial effect against inflammatory bowel disease to combat inflammation (Pawar et al., 2011).

The collected mechanistic studies suggest that WS and withanolides restrict inflammatory response through regulating cytokine expression, modulating inflammatory receptor proteins, and inhibiting the NF-κB pathway. This encourages the investigation of WS in COVID-19 hyperinflammation.

Vascular Integrity and Alveolar Consolidation

The effect of inflammatory mediators on blood vessels that are carrying them is obvious. Patients with COVID-19 showed that immune cells, inflammatory cytokines, and vasoactive molecules increase gaps between endothelial cells that line blood vessels. This leads to vascular leakage that causes infiltration of inflammatory cells (Teuwen et al., 2020). As a result, immune cells and cytokines enter previously occupied alveolar space, leading to consolidation that is visible through lung imaging (Jacobi et al., 2020).

Withaferin A significantly suppressed apoptosis of endothelial cells to pacify disruption of the blood–brain barrier in a mouse model of traumatic brain injury (Zhou Z. et al., 2020). An individual vascular permeability study revealed that withaferin A protects endothelial barrier *in vitro* and *in vivo*. Withaferin A significantly inhibited HMGB1-mediated barrier disruption, acetic acid-induced hyperpermeability, and restricted transendothelial migration of leukocytes (Lee et al., 2012) (Ahmad and Dar, 2017). WS aqueous extract may prevent histamine-mediated contraction of endothelial cells, which leads to avoid venular intercellular gaps (Joris et al., 1972) (Sahni and Srivastava, 1993).

Patients with COVID-19 showed elevated levels of vascular endothelial growth factor (VEGF), a vascular permeability inducing factor, in the blood (Senger et al., 1993) (Huang et al., 2020). A clinical trial (NCT04275414) to explore bevacizumab, an anti-VEGF, in patients with COVID-19 has been registered with clear pathological rationale ("Bevacizumab in Severe or Critical Patients With COVID-19 Pneumonia (BEST-CP)" 2020). Molecular docking studies revealed that withaferin A is a potent inhibitor of VEGF (Saha et al., 2013). Withaferin A suppressed VEGF expression in macrophages of arthritic rats (Sultana et al., 2017) and lung tissues of pulmonary fibrotic mice (Bale et al., 2018b).

These studies support the protective and preventive effects of WS in vascular permeability-induced alveolar consolidation. Thereby, WS may help reduce breathlessness in patients with COVID-19. Therefore, systematic clinical studies are required to validate this effect of WS.

Inflammation-Induced Organ Failure

The clinical complications of COVID-19 have been demonstrated to influence multiple vital organs (Zheng K. I. et al., 2020). The systemic inflammatory response coupled with direct viral association with several organs through the ACE2 receptor amplifies the complications. Multi-organ failure including liver damage, renal failure, and cardiovascular impairment contributes

to death (Zaim et al., 2020). The systemic inflammation is a plausible cause of consecutive organ failure. The inflammatory mediators released by immune cells circulate via blood and induce inflammation of several organs leading to damage (Cao, 2020).

The clinically evident holistic approach of Ayurveda designates health as the central point of concern. Ayurveda interventions are aimed at strengthening homeostatic mechanisms and optimizing adaptation limits. Unlike the organ-specific approach of modern biomedicine, Ayurveda focuses on systemic revitalization to protect organs thereby avoiding failure (Payyappallimana and Venkatasubramanian, 2016). WS has emerged as a widely acclaimed organ-protective drug capable of potentiating several organs to fight against infections and inflammation (Baliga et al., 2015). This section of the review highlights the probable role of WS in the biological consequences of inflammation-induced organ failure in COVID-19.

Brain

The neurological deformations are associated with COVID-19 pathophysiology. The major clinical observations of patients incorporate encephalitis, necrotic hemorrhage, and epileptic seizures (Carod-Artal, 2020). A systematic review of neurological manifestations in COVID-19 describes headache and anosmia as the most common symptoms (Whittaker et al., 2020).

Neuroinflammation triggered by immune cells is a key mechanism to induce neurological complications (Vito et al., 2017) (Vito et al., 2017). WS leaf aqueous extract ameliorated inflammatory cytokine-induced neuroinflammation by inhibiting the NF-κB pathway in the rat model (Gupta and Kaur, 2018). The same extract also showed a neuroprotective effect by normalizing MAP2 expression in microglial cells (Gupta and Kaur, 2019). Therefore, this extract can be a promising candidate to prevent neuroinflammation. Withanolide A significantly inhibited cerebral ischemia-induced apoptosis and necrotic cell death, which emphasizes its ability to maintain neural network and cognitive function (Mukherjee et al., 2020) (Kuboyama et al., 2005). Withanolide A also found to prevent hypoxia-induced neurodegeneration through modulation of glutathione biosynthesis (Baitharu et al., 2014). WS hydroalcoholic extract is also reported to be an anti-stress agent in an animal model of depression (Tripathi et al., 1998). Several withanolides can cross the blood–brain barrier, which promote their usage in developing a therapeutic and preventive drug for neurological disorders (Vareed et al., 2014).

Heart

The COVID-19 patients also showed heart-related complications such as cardiomyopathy and myocarditis. The pathological observations revealed elevated levels of cardiac troponin I, IL-6, and ACE2 receptors (Adão and Guzik, 2020).

Myocarditis and further heart dysfunction are coupled with systemic inflammation. The alcoholic extract of WS leaf normalized troponin I release in the blood and thereby preserved structural and functional integrity of contractile myocardium in a rat model (Khalil et al., 2015). WS root powder has shown strong inhibition of proinflammatory cytokine IL-6 in a mouse model of systemic inflammation (Ingawale et al., 2020). These evidences suggest the probable cardioprotective effect of WS in patients with COVID-19.

Liver

Patients with COVID-19 with severe categories showed liver damage, which was evident by abnormal ranges of liver enzymes and other markers. There was a significant rate of mortality due to liver failure on SARS-CoV-2 infection (Bangash et al., 2020). Hepatic inflammation can also be considered as a cause of damage.

Withanolide-rich fraction isolated from WS root methanolic extract found to restore the marker enzyme levels in drug-induced hepatic cytotoxicity in rat models of cytokines (Devkar et al., 2016). Pre-administration of withaferin A also showed the hepatoprotective effect on bromobenzene-induced injury in a mouse model evidenced by normalized functional enzymes (Vedi and Sabina, 2016). WS root powder normalized carbendazim-induced histopathological alterations in rat liver (Akbarsha et al., 2000).

Lungs

The nasal tract, nasopharyngeal cavity, and lungs are the main sites for SARS-CoV-2 infection. Pulmonary inflammation, anosmia, respiratory distress, and lung endothelial dysfunction with endothelitis are the major symptoms associated with COVID-19 (Gengler et al., 2020) (Varga et al., 2020). Reports also highlighted that patients with pulmonary fibrosis are more prone to SARS-CoV-2 infection (George et al., 2020).

Endothelial dysfunction due to inflammation results in pulmonary hypertension. WS root powder attenuated endothelitis in a rat model by limiting expression of proliferating cell nuclear antigen (PCNA). It also suppressed the level of inflammatory markers IL-10 and TNF-α by regulating the NF-κB transcription factor (Kaur et al., 2015b), while polysaccharide arabinogalactan from WS has a distinct antitussive activity through the action of the mu-opioid receptor pathways (Nosálová et al., 2015). Withaferin A from WS has shown protective effects against pulmonary fibrosis and lung damage by suppressing the expression of growth factor b1 in animal studies (H. M. Zhao et al., 2019).

Anosmia is neuroinflammatory dysfunction of olfactory cells due to entry of COVID-19 through the nasal tract. WS has a neuroprotective activity by regulating Sema3a factor in olfactory cells in a mouse model (Raghavan and Shah, 2015).

Gastrointestinal Tract

GI tract dysfunction is also found to be associated with COVID-19. A population study of COVID-19 patients reported common symptoms as nausea, diarrhea, abdominal pain, anorexia, and vomiting (Yang and Tu, 2020). Pathological findings indicated a high quantity of ACE2 expression in proximal and distal enterocytes of the small intestine (Tian et al., 2020).

The immune response-induced inflammation against COVID-19 is the main cause of GI tract dysfunction. WS

root aqueous extract expressed antidiarrheal activity and maintained gastrointestinal mobility in the rat model by inhibiting cyclooxygenase and regulating inflammatory markers induced by NF-κB transcription factor (Pawar et al., 2011). Withaferin A from WS also has shown anti-inflammatory potential in the pancreas and liver by upregulating Nrf2 transcription factor and restoring antioxidant mechanism in experimental mice (Tiruveedi et al., 2018).

Kidney

The renal system is also found to be a target of COVID-19. Pathological findings reported direct entry of COVID-19 in kidney cells through increased ACE-2 receptor, while the inflammatory response to infection shows proteinuria and increased levels of serum creatinine and urea nitrogen (Valizadeh et al., 2020).

The inflammatory mechanism is responsible for renal dysfunction. WS root powder was found to show a nephroprotective effect by stabilizing urea nitrogen and creatine level and restoring antioxidant enzyme expression in animal models (Jeyanthi and Subramanian, 2010) (Vedi et al., 2014). This is followed by normalizing histopathological changes in the kidney (Vasavan et al., 2020). Withaferin A balances apoptotic markers to prevent cytotoxicity of bromobenzene in the kidney cells (Vedi and Sabina, 2016).

Muscles

Rhabdomyolysis, myalgia, and muscle weakness are symptoms found in COVID-19 patients (De Giorgio et al., 2020). They persist after recovery from viral infection as a post-COVID-19 complication (Huang et al., 2021).

Several clinical trials involving the administration of WS aqueous extract found an increase in the overall muscle strength (Wankhede et al., 2015) (Ziegenfuss et al., 2018). Rhabdomyolysis is associated with altered levels of serum urea and creatinine (Walid, 2008). The WS root extract also found to reduce elevated levels of serum urea and creatinine in rats (Shimmi et al., 1970).

Pancreas

The complications of COVID-19 have also found to induce acute pancreatitis (de-Madaria and Capurso, 2021). The biochemical reports of COVID-19 patients revealed increased levels of pancreatic enzymes including lipase and amylase (de-Madaria et al., 2020).

Withaferin A (Tiruveedi et al., 2018) and aqueous extract of WS roots (Anwer et al., 2012) showed protective effects against acute pancreatitis through enzymatic modulation of oxidative stress and inflammation in animal model. Additionally, withaferin A also reduced the serum levels of lipase and amylase enzymes elevated in a mouse model of acute pancreatitis (Tiruveedi et al., 2018).

Gonads

COVID-19 is associated with gonadal inflammation. Primary pathological symptoms include damage to spermatozoan, abnormal secretion of sex hormones, and increased level of ACE-2 in Leydig cells in testes (Li R. et al., 2020).

Inflammation-induced cytokines are the main molecules for the dysfunction of the testes. Administration of methanolic extract of WS root has shown regulation of gonadotropic hormone by controlling GABA neurons in a mouse model (Bhattarai et al., 2010). This same extract also restored prolactin and estrogen level by inhibiting aromatase in a fish model (Nasimi Doost Azgomi et al., 2018).

POSSIBLE ROLE OF *WITHANIA SOMNIFERA* IN COVID-19 MANAGEMENT

An intervention provided in addition to mainstream administration is considered as an adjuvant. The primary aim of the adjuvant is to enhance the beneficial effects and reduce the adverse effects of mainstream administration. This section of the review discusses the potential of WS as an adjuvant to current pharmacotherapeutics and vaccine development in COVID-19.

Therapeutic Adjuvant

The current therapeutic approaches in the management of COVID-19 involves the use of hydroxychloroquine, chloroquine, azithromycin, and emergency antiviral drug remdesivir ("Revised Advisory on the Use of Hydroxychloroquine (HCQ) as Prophylaxis for SARS-CoV-2 Infection" 2020) ("Clinical Management Protocol" 2020). The beneficial effects of these drugs in COVID-19 are being explored extensively through several clinical trials. The rationale behind using these drugs is empirical, and the clinical data for use in COVID-19 are inconclusive. However, wide use of these drugs is premature and might expose patients to rare but serious harms (Ferner et al., 2020).

The major concern with the use of hydroxychloroquine and chloroquine is cardiac arrhythmia (Borba et al., 2020) (Blignaut et al., 2019). A combination with azithromycin was found to worsen this condition by increasing the risk of cardiovascular mortality, angina, and heart failure (Lane et al., 2020). The arrhythmic complication was also observed in patients with COVID-19 treated with hydroxychloroquine and azithromycin (Chorin et al., 2020). Similarly, remdesivir was found to deteriorate the condition of COVID-19 patients by triggering cardiopulmonary (5%) failure and respiratory failure or ARDS (10%) (Wang Y. et al., 2020). In this background, WS is reported to show cardiorespiratory protection. The pharmacological effects of WS in several cardiovascular diseases have been explored (Ojha and Arya, 2009). It was found to augment endogenous myocardial antioxidant enzymes (Mohanty et al., 2008) and restore altered hemodynamic parameters (Mohanty et al., 2004). An exploration of WS in a clinical trial was found to improve cardiorespiratory endurance (Shenoy et al., 2012).

A few cases reported hydroxychloroquine-associated hepatic failure leading to death. The histopathological observations showed necrosis of liver parenchymal cells and moderately percolated inflammatory cells (Makin et al., 1994). WS is also a hepatoprotective agent capable of normalizing drug-induced altered liver enzymes (Al-Awthan et al., 2014). Withanolides were

found to reduce inflammatory mediators of rat liver induced by acetaminophen overdose. The histological alterations such as liver necrosis were also restored by withanolides in a dose-dependent manner (Devkar et al., 2016). Moreover, WS is a safe drug and there have been no observations of hepatotoxicity by WS in clinical trials (Paramadhas and Alagirisamy, 2016).

An approach of convalescent plasma therapy is also showing promising results in COVID-19 management. The beneficial effects of convalescent plasma therapy have been demonstrated by increased neutralizing antibody titer accompanied by reduced SARS-CoV-2 RNA in patients (Rajendran et al., 2020). WS is also investigated for its effects on humoral immune response. WS root powder and extract significantly increased circulating antibody titer in healthy and immunocompromised mice (Davis and Kuttan, 2000) (Bani et al., 2006). WS extract is also capable of enhancing antibody-dependent cellular cytotoxicity wherein circulating antibody recognizes infected target cell and recruits NK cells to induce apoptosis (Davis and Kuttan, 2002). A hydroalcoholic extract of WS augmented IgG and IgM titers to reach peak value and serum levels of IgG2a over IgG1in ship RBC immunized mice (Malik et al., 2007). 2,3-Dihydro-3-sulfonile withanone isolated from WS leaf extract also triggered IgG2a secretion by LPS-induced splenocytes (Khan S. et al., 2009). Interestingly, the expression of IgG2a is correlated with the clearance of influenza virus (Huber et al., 2006). This suggests a probable protective effect of WS against SARS-CoV-2 through antibody regulation.

Overall, the preceding studies suggest that WS may show benefits to decrease adverse effects of drugs and adds up to antibody response in COVID-19 management. The multimodal activities of WS described in the previous section may exhibit the probable synergistic effects. Therefore, WS can be a promising candidate to be considered as a therapeutic adjuvant in COVID-19 management.

Vaccine Adjuvant

The COVID-19 pandemic has accelerated vaccine development. Several research institutes and industries have paced up the vaccine studies. Amid the rapidly spreading virus, there is an urgent need for an effective vaccine (Kaur and Gupta, 2020). Our team had previously proposed a combination of vaccines and herbal immunostimulants as an innovative approach to increase vaccine efficacy (Gautam et al., 2008). Gautam et al. have explored the potential of WS as a vaccine adjuvant to boost vaccine immunogenicity. Oral feeding of WS aqueous extract to DPT vaccine-immunized mice significantly increased antibody titer on challenge with live *B. pertussis* cells. This was accompanied by improved overall health status and reduced mortality (Gautam et al., 2004). Our team also patented an innovative method to obtain withanolide-rich WS fraction in the context of vaccine adjuvant (Jadhav et al., 2010).

In the background of viral diseases, the immune responses to influenza and SARS-CoV-2 infection were found to have similar aspects (Zheng and Perlman, 2018). A recent study demonstrated the key role of NK cells in an adaptive immune response against influenza infection (Mooney et al., 2020). WS is known to enhance NK cell activity of cell lysis and antibody-dependent cellular cytotoxicity in healthy mice (Davis and Kuttan, 2002). Considering all the previously mentioned leads, it is indicative to explore WS in COVID-19 vaccine development as an adjuvant.

Box 1 | Ayurveda Perception of Organotropism: Implication to COVID-19 Management

Ayurveda is a health-promoting system that includes herbal medicine, therapeutic procedures, diet modulation, and lifestyle management. The herbal interventions are aimed at maintaining homeostasis. The concept of *Dhatu-gamitva*, that is, the affinity of any substance (in this case herbal formulations) toward a particular organ or tissue, can be referred to as organotropism.

The physiological understanding of Ayurveda involves the concept of *Dhatu* that can be referred to as tissue structures. There are seven stand-alone but interconnected *Dhatu* in their order of optimizing nourishment. These are namely *Rasa* (~body fluid), *Rakta* (~liver, spleen, and lungs, etc.), *Mamsa* (~muscles), *Meda* (~adipose tissue), *Asthi* (~bones), *Majja* (~neurons), and *Shukra* (~gametes). The organs are developed from specific combinations of *Dhatu*, which collectively show specific physiological functions. The status of *Dhatu* plays a major role in the prognosis of any disease. The herbal intervention is determined by considering its capability to nourish weak *Dhatu*. This approach strengthens host defense to eradicate the root cause of disease along with its management.

Ayurveda would characterize COVID-19 as intensified *Shotha* (inflammation) with vitiated *Doshas* (entities that regulate pathophysiological mechanisms, namely, *Vata*, *Pitta*, and *Kapha*). There are several interventions mentioned in Ayurveda such as *Pippali* (*Piper longum*), *Yashtimadhu* (*Glycyrrhiza glabra*), *Adrak* (*Zingiber officinale*), *Bibhitaki* (*Terminalia bellirica*), and *Amla* (*Phyllanthus emblica*), which are indicated in COVID-19-like illness. However, Ayurveda being a whole system yet personalized medicine considers the suitability of intervention to individual body constitution. WS shows potential of augmenting all aforementioned organs. Moreover, WS is also prescribed for its property to alleviate inflammation and *Dosha* vitiation. Therefore, WS is one of the drugs of choice for organ protection and symptom management in COVID-19.

Box 2 | *Rasayana* Effect of *Withania somnifera*: Implication to COVID-19 Management

Rasayana is one of the therapeutic approaches of Ayurveda. The *Rasayana* effect (rejuvenating and adaptogenic property) is considered as an ability to optimize physiological mechanisms and normalize the stress alterations without influencing normal physiological functions (Rege et al., 1999). The *Rasayana* drug along with a suitable dietary regimen provides health benefits including cognizance, immunity, longevity, intellect, youthfulness, and physical strength (Balasubramani et al., 2011). WS is well known for its diverse pharmacological activities (Singh et al., 2011). These activities of WS play a crucial role in safeguarding and promoting health through immunomodulation (Tiwari et al., 2014).

The clinical observations of COVID-19 involve cytokine storm and hyperinflammation followed by multi-organ failure (Tufan et al., 2020). Comorbidities like hypertension, diabetes, cancer, and tuberculosis, etc., along with immunocompromised state add up to the complications (Sanyaolu et al., 2020). WS, being a *Rasayana* botanical, shows immune homeostasis through cytokine modulation (Rasool and Varalakshmi, 2006), anti-inflammatory (Ritchie and Singanayagam, 2020), and organ-protective effects. There is well-explored scientific evidence of clinical benefits of WS in diabetes (Durg et al., 2020), hypertension (Shalini Kushwaha et al., 2012c), and cancer (Biswal et al., 2013). Therefore, the *Rasayana* effect of WS may be beneficial in the multifactorial pathophysiology of COVID-19.

PROBABLE ROLE OF *WITHANIA SOMNIFERA* IN THE CLINICAL MANAGEMENT OF COVID-19

The multimodal effects of WS discussed in this review point toward its use in the clinical management of COVID-19. Depending upon the clinical observations, COVID-19 has been classified into three levels of severity such as mild, moderate, and severe ("Clinical Management Protocol", 2020) (Siddiqi and Mehra, 2020). This section highlights the probable effects of WS in the background of the severity of COVID-19.

Mild

An early infection phase with primary nonspecific symptoms such as malaise, fever, and dry cough is considered as a mild level. The treatment strategies involve the use of antipyretic and antiviral drugs with a focus on symptomatic relief ("Clinical Management Protocol" 2020) (Siddiqi and Mehra, 2020). WS is prescribed in Ayurveda practice to manage these primary symptoms. The fever is mediated by COX-2 enzyme and prostaglandin molecules (Li et al., 2001) (Kita et al., 2015). The antipyretic effect of WS is evident through COX-2 inhibition (Yu and Kim, 2013) (Devkar et al., 2016) (Prabhakaran et al., 2012) and prostaglandin suppression (Min et al., 2011). WS needs to be investigated for its efficacy in viral fever and other symptoms. The antiviral properties of WS mentioned in this review seem promising and need to be tested clinically in patients with mild symptoms.

Moderate

The second moderate level considers pulmonary involvement with or without hypoxia. This stage is characterized by the recruitment of immune cells at the infected site, i.e., alveoli followed by pulmonary inflammation, alveolar consolidation, and vascular permeability. At this stage, a rational administration of anti-inflammatory therapy such as methylprednisolone is suggested ("Clinical Management Protocol" 2020) (Siddiqi and Mehra, 2020). WS is well known for its anti-inflammatory action in several inflammatory diseases. It normalizes the inflammatory signals to restore normal physiological functioning of immune cells in disease conditions (Gupta and Singh 2014) (Khan et al., 2019) (Kaur et al., 2015a). Some COVID-19 patients experience a secondary bacterial infection. WS may act as a drug of choice in such cases ("Clinical Management Protocol" 2020) (Rawat and Bisht, 2014) (Owais et al., 2005). However, this activity needs to be tested in the COVID-19 scenario. The multimodal effects of WS may be useful for the speedy recovery of patients and may prevent from progressing to a severe disease.

Severe

A severe stage of systemic hyperinflammation is caused by immune cells-induced cytokine storm. The dysfunction of cell-mediated immunity due to decreased T cell count and increased secretion of inflammatory mediators is observed in the severe stage (Siddiqi and Mehra, 2020). An immunomodulatory intervention is recommended for physiological management of the severe condition. WS is a promising immunomodulatory agent to maintain physiological immune harmonizing. It helps in balancing Th1/Th2 response to stimulate cell-mediated immunity in mice (Khan B. et al., 2006) (Malik et al., 2007) (Kour et al., 2009) (Khan S. et al., 2009) (Bani et al., 2006) (Susheela Kushwaha et al., 2012a). Furthermore, the antibodies produced by the humoral response of WS may be beneficial (Malik et al., 2007) (Khan S. et al., 2009) (Bani et al., 2006) (Davis and Kuttan, 2000). The systemic inflammation may lead to multi-organ failure. The reported anti-inflammatory and organ-protective effect of WS (Tiwari et al., 2014) may be useful in reducing the severity of inflammation-induced organ damage. As discussed above, WS may be beneficial for patients at different stages of the infection and may prevent progression to the severe stage.

The post-discharge detection of viral titer in recovered patients is another major issue. There is a significantly noticeable number of relapse cases in recovered patients. However, the mechanism of relapse remains unknown (An et al., 2020). The patients are recovering based on their physiological resistance when subjected to empirical treatments (Shin, 2020). Therefore, there is a need for a relatively safe prophylactic agent capable of sustaining physiological resistance through immune balance. WS can be a promising option to execute prophylaxis against relapse. It is a relatively safe drug even for long-term administration (Raut et al., 2012). Moreover, pre-existing comorbidities add up to the complications. Patients with COVID-19 suffering from comorbidities often need to undergo disease-specific poly-pharmacy regimens. WS being a safe drug can be used as one of the complementary medicines to mitigate COVID-19 symptoms. The adaptogenic effects of WS might re-establish physiological balance in the background of the stressed conditions of comorbidities (Singh et al., 1982).

The clinical safety profile of WS aqueous extract has already been studied. A dosage of 625 mg twice a day was well tolerated by almost all the healthy volunteers without any toxicities. Organ function tests were found normal even after a month of administration (Raut et al., 2012). A systematic review also advocated the WS root to be safe and clinically effective in several disorders including schizophrenia, rheumatoid arthritis, diabetes, and infertility. It was also found safe in preclinical toxicity studies carried out for 8 months (Tandon and Yadav, 2020). However, systematic toxicity studies on WS are needed in the COVID-19 scenario.

DISCUSSION

The experimental trends generated from healthy and disease models indicate that WS has a potential for 1) maintaining immune homeostasis, 2) regulating inflammation, 3) suppressing pro-inflammatory cytokines, 4) organ protection (in the nervous system, heart, lung, liver, and kidney), and 5)

anti-stress, antihypertensive, and antidiabetic activities. The dose of WS extracts ranged from 10–300 mg/kg in various preclinical experimental models have been discussed in this review. However, it was found that the concentration range of 50–200 mg/kg of different extracts of WS and 2 mg/kg of withaferin A tested in animal models significantly showed anti-inflammatory, cytokine regulatory, and immunomodulatory effects. Moreover, 10–50 μg/ml of withaferin A stimulated cytokine expression in monocyte and macrophage cell lines (**Supplementary Material** provides details on the type of extract/metabolite, experimental model, activity, and dose range).

The possible role of WS in regulating various biochemical processes in COVID-19 pathophysiology can be extrapolated from reported studies. The current understanding of the pharmacotherapeutic treatment of COVID-19 relies on immunological phenomena associated with viral infections. It has been proposed that the therapeutic strategies to improve immune profile could be beneficial in clinical outcomes. For example, an antiviral drug remdesivir with the immunomodulator tocilizumab has been recognized to refine pharmacological efficacy (Michot et al., 2020). In such a scenario, WS being a potent immunomodulator can be considered as an excellent alternative given its proven safety and efficacy profile.

The next phase of COVID-19 vaccine development would be an association with synthetic adjuvant. WS, having proven attributes such as affordability, efficacy, and safety, can be evaluated as a vaccine adjuvant. Systematically and ethically designed studies are the need of the hour. These studies with rational measurable outcomes (e.g., antibody titer, and cytokine profile, etc.) would throw light on the fundamental benefits of WS.

The post-COVID-19 complications also deserve a serious attention to achieve quality of life (Cortinovis et al., 2021). Several clinical follow-up studies on recovered COVID-19 patients reported persisted fatigue, muscle weakness, and organ dysfunction (Huang et al., 2021) (Zhao et al., 2020). This warrants implication of muscle-strengthening (Ziegenfuss et al., 2018) and organ-protective (Kaur et al., 2015b) action of WS.

The study of literature suggests that WS has important molecular and pharmacological characteristics to act as a therapeutic adjuvant for prophylaxis and treatment of COVID-19. Therefore, WS may synergistically improve the clinical outcomes of COVID-19 pharmacotherapeutics in current use. WS may be explored in healthcare workers undergoing COVID-19 vaccination for modulation in antibody titer to understand the real-world adjuvant potential. Besides, WS may have a salutary effect on other comorbidities associated with the COVID-19 disease. Our recently published review has addressed this possibility in cancer (Saggam et al., 2020). To realize the potential of WS in COVID-19, additional evidence needs to be generated by experimental validation of *in silico* observations followed by clinical studies. The focus on evaluating pharmacokinetic–pharmacodynamic profile and drug interactions along with safety and efficacy in healthy volunteers and patients with COVID-19 for prophylactic and therapeutic use is required. This review attempts mapping of phytochemical diversity and reported pharmacological activities of WS with COVID-19 pathophysiology. The review proposes potential pharmacological activities of WS extracts and metabolites in the context of COVID-19, which are based on rational extrapolations from available literature leading to testable hypotheses. However, there is a need for systematic studies with appropriate controls to validate these hypotheses in suitable experimental models. Moreover, the activity of any botanical depends on a number of variables including geographical variation of species, cultivation and collection methods, extraction process, and administration (route, dose, and duration). The extract should be prepared from the authentic and standardized raw material with a robust protocol to ensure batch-to-batch reproducibility, potency, safety, and efficacy. These sources of variability need to be accounted during experimental designs for exploring WS effects.

The multidimensional research on WS with a deeper understanding of biological mechanisms is a promising area for future exploration as a therapeutic adjuvant. Given the recent observations that COVID-19 pandemic will have a long-term trajectory, there will be a significant need for research and development of COVID-19 therapeutics and adjuvants (Lurie et al., 2021). In this context, the current research synthesis about WS indicates a potential role in COVID-19 management and may serve as a rational strategy for exploration of other *Rasayana* botanicals.

AUTHOR CONTRIBUTIONS

AS: conceptualization, research synthesis, biochemistry, molecular mechanisms, data analysis, illustrations, and manuscript writing. KL: research synthesis, biochemistry, physiology, and manuscript writing. GT: clinical practice of Ayurveda, ethnopharmacology, research synthesis, and manuscript writing. SD: clinical pathophysiology and pharmacotherapeutics, molecular mechanisms, and manuscript writing. PC-G: molecular mechanisms and manuscript writing. SB: pharmacotherapeutics, pharmacology, and manuscript writing. BP: conceptualization, therapeutic strategy, and manuscript writing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.623795/full#supplementary-material

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β-Caryophyllene, A Natural Dietary CB2 Receptor Selective Cannabinoid can be a Candidate to Target the Trinity of Infection, Immunity, and Inflammation in COVID-19

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Coronavirus disease (COVID-19), caused by novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an ongoing pandemic and presents a public health emergency. It has affected millions of people and continues to affect more, despite tremendous social preventive measures. Identifying candidate drugs for the prevention and treatment of COVID-19 is crucial. The pathogenesis and the complications with advanced infection mainly involve an immune-inflammatory cascade. Therefore, therapeutic strategy relies on suppressing infectivity and inflammation, along with immune modulation. One of the most promising therapeutic targets for the modulation of immune-inflammatory responses is the endocannabinoid system, particularly the activation of cannabinoid type 2 receptors (CB2R), a G-protein coupled receptor which mediates the anti-inflammatory properties by modulating numerous signaling pathways. To pharmacologically activate the CB2 receptors, a naturally occurring cannabinoid ligand, beta-caryophyllene (BCP), received attention due to its potent anti-inflammatory, antiviral, and immunomodulatory properties. BCP is recognized as a full selective functional agonist on CB2 receptors and produces therapeutic effects by activating CB2 and the nuclear receptors, peroxisome proliferator-activated receptors (PPARs). BCP is regarded as the first dietary cannabinoid with abundant presence across cannabis and non-cannabis plants, including spices and other edible plants. BCP showed tissue protective properties and favorably modulates numerous signaling pathways and inhibits inflammatory mediators, including cytokines, chemokines, adhesion molecules, prostanoids, and eicosanoids. Based on its pharmacological properties, molecular mechanisms, and the therapeutic potential of BCP as an immunomodulator, anti-inflammatory, organ-protective, and antiviral, we hypothesize that BCP could be a promising therapeutic and/or preventive candidate to target the triad of infection, immunity, and inflammation in

COVID-19. In line with numerous studies that proposed the potential of cannabinoids in COVID-19, BCP may be a novel candidate compound for pharmaceutical and nutraceutical development due to its unique functional receptor selectivity, wide availability and accessibility, dietary bioavailability, nonpsychoactivity, and negligible toxicity along with druggable properties, including favorable pharmacokinetic and physicochemical properties. Based on reasonable pharmacological mechanisms and therapeutic properties, we speculate that BCP has potential to be investigated against COVID-19 and will inspire further preclinical and clinical studies.

Keywords: COVID-19, SARS-CoV-2, beta-caryophyllene, immunomodulators, natural products

INTRODUCTION

COVID-19, a public health emergency and pandemic, has affected millions of people worldwide and continues to do so, despite numerous preventive measures, and this situation will continue until a vaccine is developed (Huang et al., 2020). The severity of infection varies from patients being asymptomatic to pre-symptomatic to symptomatic with different stages of illness, ranging from mild, moderate, to severe (Yang et al., 2020). The symptoms include fever, dry cough, sore throat, diarrhea, rashes on the skin, face, or toes, shortness of breath, loss of smell, anorexia, fatigue, headache, myalgia, anosmia, and ageusia, identified as the clinical criteria for diagnosis of COVID-19 (Jin et al., 2020). The majority of deaths are happening due to complications, such as severe pneumonia, acute respiratory distress syndrome (ARDS), shock, sepsis, and resultant multi-organ failure (Huang et al., 2020; Yang et al., 2020). The pathogenesis of COVID-19 emerges as a multifaceted, multi-system, multi-organ disorder, including viremia to overt the activation of immune responses and inflammatory processes that result in a dysregulated immune pattern, manifested by a massive rise in the levels of pro-inflammatory cytokines, chemokines, and adhesion molecules (Dhama et al., 2020). This causes the onset of a "cytokine storm" or "cytokine release syndrome", which mainly causes ARDS and further leads to pathogenic effects through a quite ubiquitous target at a multiple-organ level (Dhama et al., 2020; Tang et al., 2020; Vinciguerra et al., 2020).

At present, many drugs are being repurposed for supportive management in COVID-19 based on docking studies, pharmacological rationale, and clinical experiences (Jean and Hsueh, 2020; Kandeel and Al-Nazawi, 2020; Rabaan et al., 2020; Singh et al., 2020). The pathogenesis and the complications developed with the infection mainly involve an immune-inflammatory cascade; therefore, the therapeutic strategies focus on reducing inflammation and immune modulation of this cascade (Dhama et al., 2020; García, 2020; Scavone et al., 2020; Zhou et al., 2020). Despite recent availability of vaccine for prophylaxis, massive efforts are ongoing for the discovery of novel drugs for the treatment and prevention of COVID-19 (Jean and Hsueh, 2020; Kandeel and Al-Nazawi, 2020; Rabaan et al., 2020; Singh et al., 2020). In parallel with repurposing modern medicines, there are numerous attempts to explore natural products with potential to target the interplay of viral infection and immune-inflammatory axis (Bahramsoltani and Rahimi, 2020; Basu et al., 2020; Benarba and Pandiella, 2020; Hensel et al., 2020; Mondal et al., 2020; Narkhede et al., 2020). Over the past few months it has been suggested that natural products hold great promise in the management of COVID-19 due to their antiviral, anti-inflammatory, and immunomodulator activities (Bahramsoltani and Rahimi, 2020; Basu et al., 2020; Benarba and Pandiella, 2020; Mondal et al., 2020; Narkhede et al., 2020). Thus, identifying candidate compounds which have selectivity against viral components as well as prevent viral entry, enhance immunity and attenuate inflammatory factors in host could be important in context to COVID-19.

Many propositions have been made on the possible therapeutic potential of essential oils-derived phytochemicals, including many terpenes or terpeno-alcoholic compounds, in COVID-19 (Asif et al., 2020; Boukhatem and Setzer, 2020; da Silva et al., 2020; Diniz et al., 2021). Many of the terpene components present in cannabis are widely consumed in food and used in traditional medicine (Anil et al., 2021). Some of these compounds showed potential to modulate the endocannabinoid system, which represents one of the newest therapeutic targets in regard to regulation of innate and adaptive immunity and immunomodulatory and anti-inflammatory properties. The endocannabinoid system is targeted by plant-derived compounds, termed phytocannabinoids, which have gained attention for therapeutic modulation of cannabinoid type-1 receptors (CB1R) and type-2 (CB2R), the components of endocannabinoid system (Oláh et al., 2017). The latest therapeutic strategy in targeting the endocannabinoid system is to activate the CB2R, a G-protein coupled receptor which appears to regulate immunity, inflammation, and pain. The activation of CB2R has been shown to exert potent anti-inflammatory, immunomodulatory, and organ-protective properties with no psychotropic effects, which are commonly observed with CB1R. Over the past few months, it has been suggested that modulation of the endocannabinoid system by cannabinoids, including cannabidiol, could be useful in prophylaxis and treatment of COVID-19 and may improve prognosis (Costiniuk and Jenabian, 2020; Esposito et al., 2020). Recently, extract of *Cannabis sativa* containing phytocannabinoids and terpenes were shown to modulate the inflammatory mediators in alveolar epithelial cells (A549) in COVID-19-associated inflammation and suggested that the phytocannabinoid mix formulation exerted better activity in

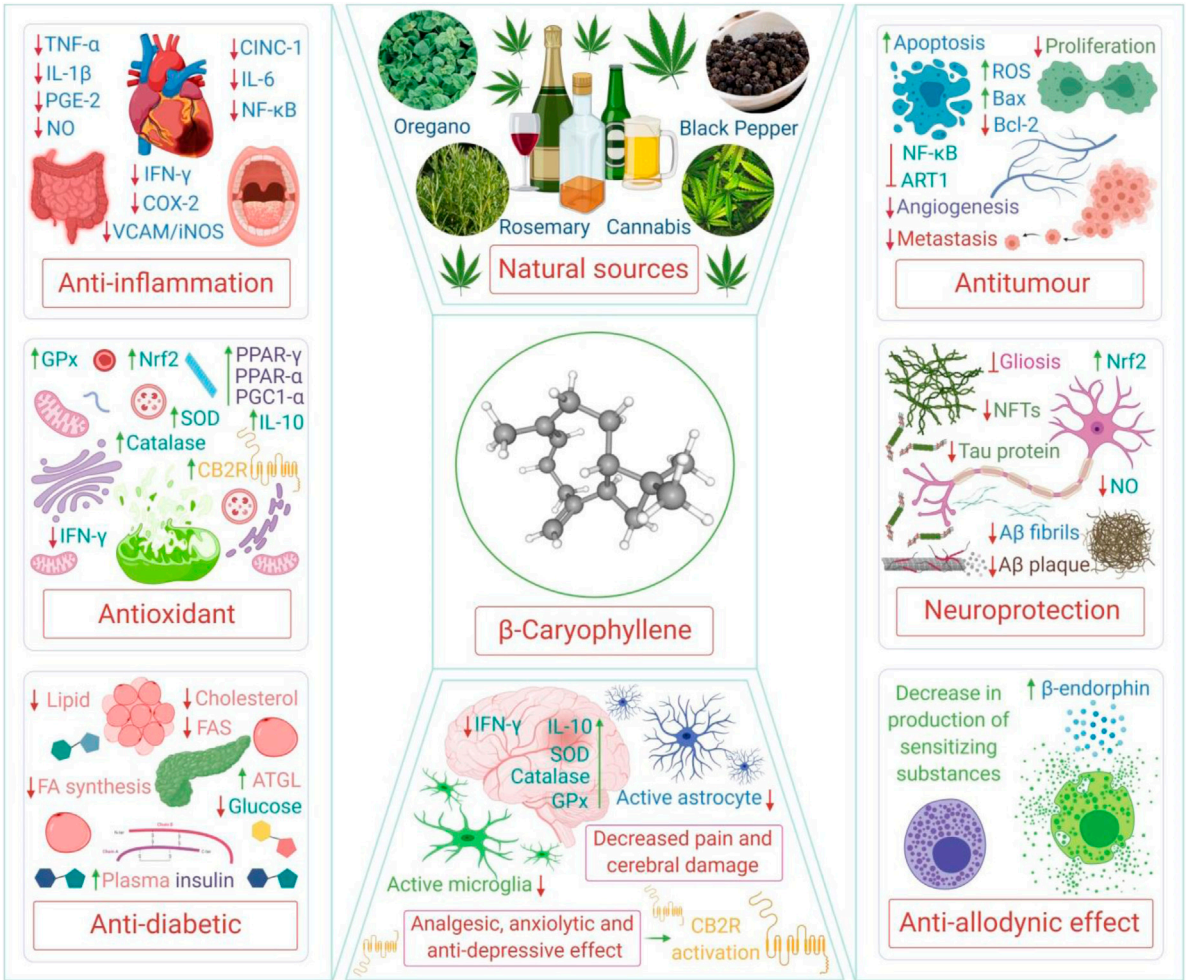

FIGURE 1 | The structure and various polypharmacological properties and therapeutic potential of BCP. TNF-α, tumor necrosis factor alpha; IL, interleukin; PGE-2, prostaglandin E2; NO, nitric oxide; CINC-1, cytokine-induced neutrophil chemoattractant 1; NF-κB, nuclear factor kappa B; IFN-γ, interferon gamma; COX-2, cyclooxygenase-2; VCAM, vascular cell adhesion protein; iNOS, inducible nitric oxide synthase; GPx, glutathione peroxidase; SOD, superoxide dismutase; PPAR-γ, peroxisome proliferator-activated receptor gamma; PGC1-α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; Nrf2, nuclear factor erythroid 2–related factor 2; FAS, fatty acid synthase; ATGL, adipose triglyceride lipase; ROS, reactive oxygen species; Bax, Bcl-2 associated X protein; Bcl-2, B-cell lymphoma 2; ART1, arginine ADP-ribosyltransferase 1; NFTs, neurofibrillary tangles; Aβ, amyloid beta; CB2R, cannabinoid receptor type 2.

comparison with individual fractions from cannabis (Anil et al., 2021). Many cannabinoids, including cannabidiol, have been suggested for their possible potential as preventive agents or therapeutic adjuvants with other agents in targeting the trinity of infection, inflammation, and immunity in COVID-19 (Byrareddy and Mohan, 2020; Costiniuk and Jenabian, 2020; Esposito et al., 2020; Nagarkatti et al., 2020; Sexton, 2020; Raj et al., 2021).

Among numerous cannabinoids, beta-caryophyllene (β-Caryophyllene; BCP), a naturally occurring terpene, has received enormous attention in the past few years due to its recognition as a full functional agonist on CB2R which imparts its therapeutic potential by mediating anti-inflammatory and immunomodulatory properties (Gertsch et al., 2008). BCP, chemically known as trans-(1R,9S)-8-Methylene-4,11,1 is the first dietary cannabinoid of natural origin, with an abundant presence in a variety of spice blends and citrus flavors, as an additive or preservative, and for aroma in food products and beverages (Gertsch, 2008; Gertsch et al., 2008). BCP is one of the constituents of commonly consumed edible plants, such as cinnamon (Cinnamomum spp.), basil (Ocimum spp.), pepper (Piper spp.), breakfast mint [*Perilla frutescens* (L.) Britton], coriander (*Coriandrum sativum* L.), chestnut (*Aesculus hippocastanum* L.), sage (*Salvia officinalis L.*), cubeb pepper (*Piper cubeba* L.f.), thyme (*Thymus vulgaris* L.), myrrh [*Myrrhis odorata* (L.) Scop.], curry leaves [*Murraya koenigii* (L.) Spreng.], hops (*Humulus lupulus* L.), cloves [*Syzygium aromaticum* (L.) Merr. & L.M. Perry], hemp (*Cannabis sativa* L.), lavender (*Lavandula angustifolia* Mill.), oregano (*Origanum vulgare* L.), and rosemary (*Rosmarinus officinalis* L.), among others. Recently, a majority of the plant derived compounds showed potential in COVID-19 due to their antioxidant and anti-inflammatory properties and are of limited occurrence in certain

genera and species or to a specific individual plant that may limit supply and demand. However, BCP is unique in terms of wide dietary availability and accessibility across numerous plant genera and species (Sharma et al., 2016). Till date, the presence of BCP has been confirmed in more than two thousand plants, including edible, medicinal, and ornamental plants. BCP is mainly synthesized by plants as a defense mechanism against insects and aphids, and plays a role in pollination. It is usually localized in the aerial parts of the plants including leaves, flowers, spate, inflorescence, and buds, with a low presence in the stem, roots, and rhizomes (Sharma et al., 2016). The structure and various polypharmacological properties and therapeutic potential of BCP are depicted in **Figure 1**.

In a recent molecular docking study, 171 components, including BCP, present in the essential oils of numerous plants were analyzed against SARS-CoV-2 main protease (SARS-CoV-2 M^{pro}), SARS-CoV-2 endoribonucleoase (SARS-CoV-2 Nsp15/NendoU), SARS-CoV-2 ADP-ribose-1″-phosphatase (SARS-CoV-2 ADRP), SARS-CoV-2 RNA-dependent RNA polymerase (SARS-CoV-2 RdRp), the binding domain of the SARS-CoV-2 spike protein (SARS-CoV-2 rS), and human angiotensin–converting enzyme (hACE2) (da Silva et al., 2020). Very recently in an *in silico* study, BCP was shown to target M^{pro} ($3CL^{pro}$), the main protease in SARS-CoV-2 involved in the processing of translating the viral RNA into the viral polyproteins (Narkhede et al., 2020). BCP interacted with the amino acid residues of SARS-CoV-2 via pie-alkyl interactions and showed good affinity along with druggable properties (Narkhede et al., 2020). In another recent study, Muthuramalingam et al., 2020 (Muthuramalingam et al., 2020) carried out a cheminformatics and interactome study using *in silico* approaches and found that BCP is one of the potential compounds among 259 phytochemicals screened for targeting thirteen COVID-19 immune genes regulating numerous signaling pathways. The study unveiled that 154 compounds interact with COVID-19-associated immune genes. BCP and its derivative, β-caryophyllene oxide, was found to target immune genes, and was suggested useful for designing and developing as a potential agent against COVID-19 (Muthuramalingam et al., 2020).

The present review scientifically contemplates the therapeutic prospects of BCP in COVID-19. The possibilities of BCP as a candidate in COVID-19 have been discussed based on reported findings, particularly immunomodulatory, anti-inflammatory, and antiviral properties. Additionally, CB2R activation has been suggested as a possible therapeutic target in COVID-19. Based on the role of CB2R in immune-inflammatory mechanisms, we hypothesized that BCP endowed with CB2R agonist properties may potentially limit the severity and progression of COVID-19 by modulating infection, immunity, and inflammation. The potent anti-inflammatory activity mediating multiple pathways and mediators of inflammation, including the inhibition of pro-inflammatory cytokines, chemokines, and adhesion molecules, along with the suppression of macrophage infiltration and neutrophil-endothelial cell interaction, might constitute a promising pharmacological and nutritional approach to inhibit the cytokine storm, which is a major reason for death in COVID-19. The potential of BCP in improving host cellular immunity against infection and its good antiviral and antibacterial activity, along with the antioxidant effects, may further help in controlling the symptoms and the worsening of the disease, secondary infections, complications, progression, and resultant death. BCP has potential to protect from the risk factors, prevent the entry of the virus, and ameliorate organ damage and the pathological manifestation of SARS-CoV-2 on the different organ systems. A scheme of the effect of BCP mediating CB2R activation has been proposed in context of infection, inflammation, and immunity in COVID-19 (**Figure 2**).

The literature reviewed herein indicates that BCP may be a promising candidate as a preventive and therapeutic agent or adjuvant for COVID-19 given its pharmacological and molecular mechanisms, including its CB2R agonist property, integrating with its antiviral, anti-inflammatory, and immunomodulatory properties in numerous experimental studies (Sharma et al., 2016). However, no study has yet directly demonstrated the efficacy of BCP against SARS-CoV-2 infections. But, based on pharmacological properties, a logical approach has been presented on the therapeutic potential of BCP in COVID-19.

BCP AS A FUNCTIONAL CB2 RECEPTOR AGONIST

Gertsch and colleagues first recognized BCP as a functional CB2R agonist using numerous model systems, including *in silico*, *in vitro*, and *in vivo* studies (Gertsch et al., 2008). In the molecular docking studies, BCP was observed to interact with CB2R on the same binding sites as that of CP55, 940, a CB2R agonist. It binds well in a hydrophobic sac involving lipophilic amino acid residues and it was suggested that the double bond with conformation E of BCP is vital for the receptor binding (Gertsch et al., 2008). Accumulating experimental studies have demonstrated the CB2R activation mediated effects of BCP in attenuating inflammation, oxidative stress, apoptosis, fibrosis, and immune modulation. The CB2R-dependent anti-inflammatory mechanism of BCP has been demonstrated in oral mucositis (Picciolo et al., 2020), glioblastoma (Irrera et al., 2020), neuropathic pain (Klauke et al., 2014; Aly et al., 2019), bipolar disorders (Hwang et al., 2020), wound healing (Koyama et al., 2019), interstitial cystitis (Berger et al., 2019), autoimmune encephalomyelitis/multiple sclerosis (Alberti et al., 2017; Askari et al., 2019), neurocognitive disorders (Lindsey et al., 2019; Chávez-Hurtado et al., 2020), arthritis (Irrera et al., 2019), metabolic and neurobehavioral alterations (Youssef et al., 2019), insulin resistance and vascular inflammation (Youssef et al., 2019), hyperglycemia (Basha and Sankaranarayanan, 2016), peripheral neuropathy (Segat et al., 2017), atherosclerosis (Zhang et al., 2017), cardiotoxicity (Meeran et al., 2019), osteoporosis (Shan et al., 2017), vascular dementia (Lou et al., 2017), dopaminergic neurodegeneration/Parkinson's disease (Javed et al., 2016), Alzheimer's disease (Cheng et al., 2014), cerebral ischemia-reperfusion (Poddighe et al., 2018), liver fibrosis (Mahmoud et al., 2014), pulmonary

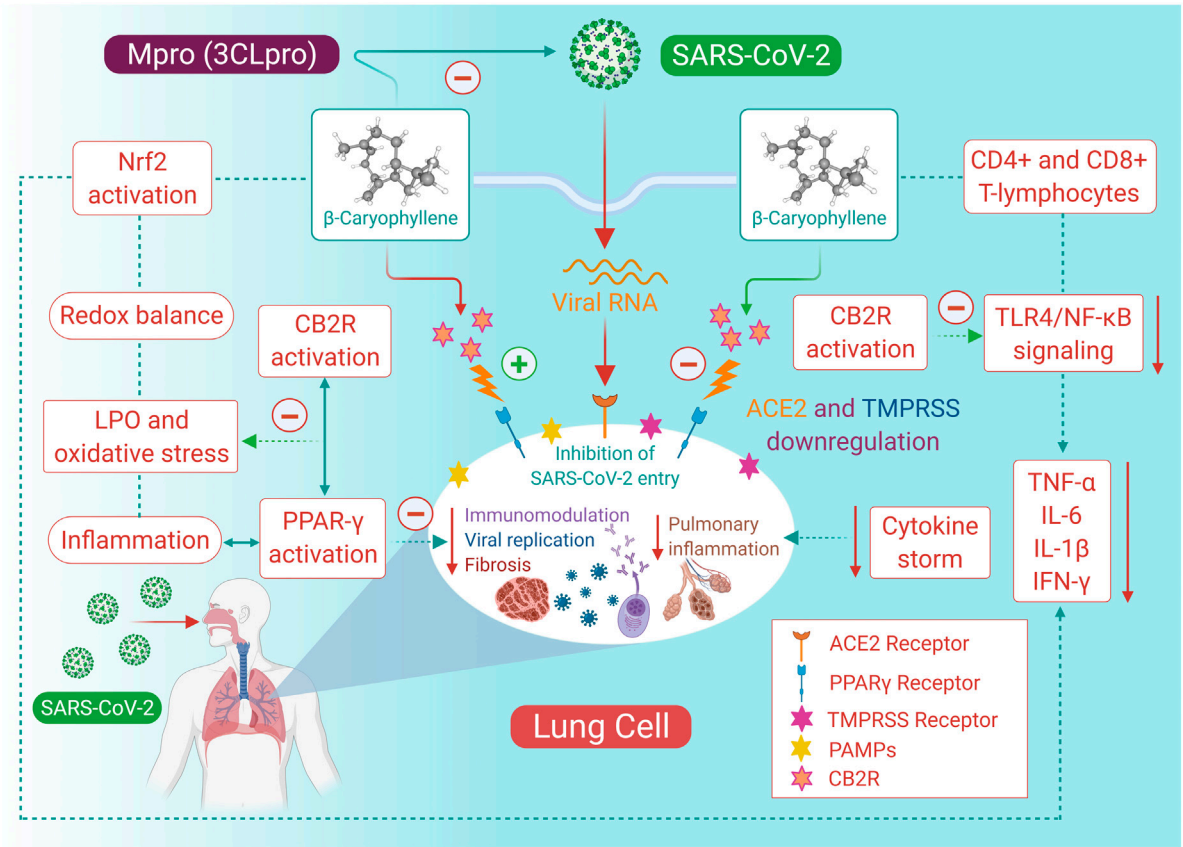

FIGURE 2 | The proposed possible mechanisms and potential of BCP in COVID-19.

inflammation (Andrade-Silva et al., 2016), intestinal inflammation (Bento et al., 2011), acute myocardial infarction (Younis and Mohamed, 2019), acute renal injury (Horváth et al., 2012), diabetic nephropathy (Li et al., 2020), and lipid disorders (Youssef et al., 2019).

In the majority of the experimental models involving inflammatory states similar to those of human diseases, the principal pharmacological and molecular mechanism observed is the inhibition of pro-inflammatory cytokines, NF-κB, adhesion molecules, and chemokines and the subsequent modulation of signaling pathways, mainly involving toll-like receptors, opioid receptors, SIRT1/PGC-1α, AMPK/CREB, MAPK/ERK, Nrf2/Keap1/HO-1, and the activation of nuclear peroxisome proliferator-activated receptors (PPARs). Cannabinoids are known to interact or crosstalk with a family of PPARs, including three subtypes: PPAR-α, PPAR-β/δ, and PPAR-γ. These subtypes are encoded by distinct genes and are regulated by steroids and lipid metabolites and mainly control lipid and glucose homeostasis and inflammatory responses (O'Sullivan, 2016). PPAR-γ agonists, pharmacologically known as thiazolidinediones, are clinically available drugs for use as insulin sensitizers in insulin resistance/type 2 diabetes mellitus. Recently, thiazolidinedione has been suggested for repurposing in COVID-19 due to its potential to attenuate cytokine storms (Ciavarella et al., 2020). PPAR-γ agonists were shown to inhibit the replication of numerous viruses, including human immunodeficiency virus, respiratory syncytial virus, hepatitis B, and hepatitis C viruses (Skolnik et al., 2002; Du et al., 2017). Further, PPAR-γ agonists have been shown to reduce morbidity and mortality in influenza A virus infections (Bassaganya-Riera et al., 2010). The activation of PPAR-γ in resident alveolar macrophages was reported to significantly ameliorate pulmonary inflammation and enhance host recovery following respiratory viral infections (Huang et al., 2019). Following amelioration of the tissue damage, PPAR-γ activation also controls the overproduction of cytokines. Thus, BCP may pause the onset of the cytokine storm from resident macrophages.

In addition to activation of CB2R, BCP also activates PPAR-α which favorably modulates the lipid metabolism by increasing the ability of hormone nuclear receptors PPAR-α and estrogen-related receptor α (ERRα) to drive the transcription of fatty acid oxidation enzymes by increasing the levels of peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α), as well as stimulating sirtuin 1 (SIRT1) deacetylase activity (Zheng et al., 2013; Wu et al., 2014). The role of sirtuin in the transcription and replication of viruses is well known and the activation of PPAR-α and lipolysis showed to reduce hepatitis C virus genotype-associated lipid metabolic disorder in liver

diseases (Patra et al., 2019). PPAR-α activation was also shown to beneficially influence inflammation in alveolar epithelial cells, suggesting a potentially beneficial role of PPAR-α in ARDS (Hecker et al., 2015). Thiazolidinediones have shown numerous adverse effects, such as weight gain, osteoporosis, heart failure, stroke, and an increased risk of urinary cancer. Since BCP is natural, non-toxic, and devoid of the adverse effects of synthetic cannabinoids, it could be a safer alternative over synthetics. Together, the role of BCP as a PPAR-γ, as well as a PPAR-α agonist, seems promising in the regulation of the lipid and glucose metabolism, along with additional regulatory roles on cell proliferation and differentiation, vascular homeostasis, and inflammation, and the immune systems. Thus, BCP may be possibly useful to control the orchestrated immune-inflammatory events in COVID-19.

IMMUNOMODULATORY PROPERTIES OF BCP

SARS-CoV-2 enters the host cells by binding to ACE2 receptors and the pathogen associated molecular patterns (PAMPs) on the virus alert innate immune cells, the anti-viral effectors, such as T CD8+ cells, NK cells, neutrophils, monocytes, and macrophages about the presence of the invading virus. The innate immune cells, which express pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs), detect PAMPs to achieve a suitable immune response against the invading pathogen (Keam et al., 2020). PRR and PAMP interaction triggers phagocytosis and stimulates the synthesis of pro-inflammatory cytokines, such as type I interferon, IFNα/β and type II, IFN-γ, and chemokines, such as CXCL-10 and CCL-2, to onset an antiviral environment (Allegra et al., 2020).

In the case of severe infection, the viruses are sensed by monocytes, tissue macrophages, and resident dendritic cells, resulting in an uncontrolled pro-inflammatory cytokines (IFN, TNF-α, IL-1β, and IL-6) production, leading to a phenomenon called a "cytokine storm", which damages the respiratory epithelial cells of the host (Allegra et al., 2020). The immune responses are critical for the eradication of the virus and the resolution of the active disease. CB2R represents an important receptor target for immune regulation and is predominantly expressed by immune cells of the immune system, such as B cells, T cells, CD8+ lymphocytes, CD4+ lymphocytes, NK cells, neutrophils, macrophages, basophils, eosinophils, platelets, mast cells, dendritic cells, microglia, and astrocytes (Howlett and Abood, 2017). The CB2R are well expressed in several organs, including the liver, spleen, thymus, brain, lungs, kidneys, tonsils, nasal epithelium, and PBMC, which are present in the pancreas, uterus, and reproductive tissues (Cabral et al., 2015). Both cannabinoid receptors, CB1R and CB2R, play an important role in the modulation of the immune system, potentially inducing immunosuppression (Cabral et al., 2015; Hernández-Cervantes et al., 2017). The therapeutic targeting of CB2R has received enormous attention, since these novel therapeutic agents would have no psychotropic effects, as is the case with CB1R.

Human CB2R were first cloned in 1993 from the promyelocytic leukemia cell line HL-60 (Munro et al., 1993) and the first CB2R-deficient mouse was generated in 2000 (Buckley et al., 2000); therefore, CB2R represent the relatively newest therapeutic targets. Mice deficient in CB2R showed an increased susceptibility and vulnerability to influenza infection, demonstrating that CB2R are important in immunoregulation in respiratory viral infections (Kapellos et al., 2019). The activation of CB2R exerted potent immunomodulation, mediating cell death induction, cytokine suppression, and inhibition of cell proliferation, along with the stimulation of regulatory T cell induction and anti-inflammatory cytokines (Rieder et al., 2010; Karmaus et al., 2012). However, few studies notably demonstrate that CB2R may modulate susceptibility to the experimental cerebral malaria through a CCL17-dependent mechanism (Alferink et al., 2016).

Since the recognition of BCP as an agonist of CB2R, numerous studies have demonstrated the therapeutic benefits of BCP by suppressing immune-inflammatory cascade when CB2R are activated. The activation of CB2R was reported to suppress lung pathology in infants infected with acute respiratory syncytial virus by reducing the levels of cytokines and chemokines (Tahamtan et al., 2018). In HIV patients, the activation of CB2R was shown to impair a productive infection and viral transmission involving a crosstalk/interaction between CB2R (Costantino et al., 2012) and to inhibit the replication of the virus in monocytes and macrophages (Ramirez et al., 2013). Recently, BCP was shown to modulate systemic and local immunity in an experimental autoimmune encephalomyelitis model (Askari et al., 2019) and the immunomodulatory effect has been attributed to the ability of BCP to inhibit CD4+ and CD8+ T lymphocytes and pro-inflammatory cytokines (Alberti et al., 2017). The immunomodulatory activity of BCP was also explained by an enhanced phagocytic capability, following an increased lysosomal activity and nitric oxide production in macrophages (Carvalho et al., 2017).

Further, BCP exerted a potent immunomodulatory effect by simultaneously inhibiting both Th1 cytokines, including IL-2 and IFN-γ, and Th2 cytokines, including IL-4, IL-5, and IL-10, in primary splenocytes (Ku and Lin, 2013). Also, BCP is present in many plants, such as *Chrysanthemum indicum* L. (Hwang and Kim, 2013), *Pterodonem arginatus* Vogel (Alberti et al., 2014), *Myracrodruon urundeuva* Allemão (Carvalho et al., 2017), *Schizonepeta tenuifolia* (Benth.) Briq. (Ng et al., 2018), and copaiba oil (Urasaki et al., 2020), in which it exerts immunomodulatory activity. Taken together, the studies demonstrate that CB2R play a key role in balancing the immune response and BCP by activating CB2R, holding promise in the therapeutic modulation of immune-inflammatory changes in patients with SARS-CoV-2 infection.

In COVID-19, the use of immunomodulators is receiving attention and being regarded as a "sub-etiological treatment" in the absence of an effective antiviral drug. Additionally, BCP

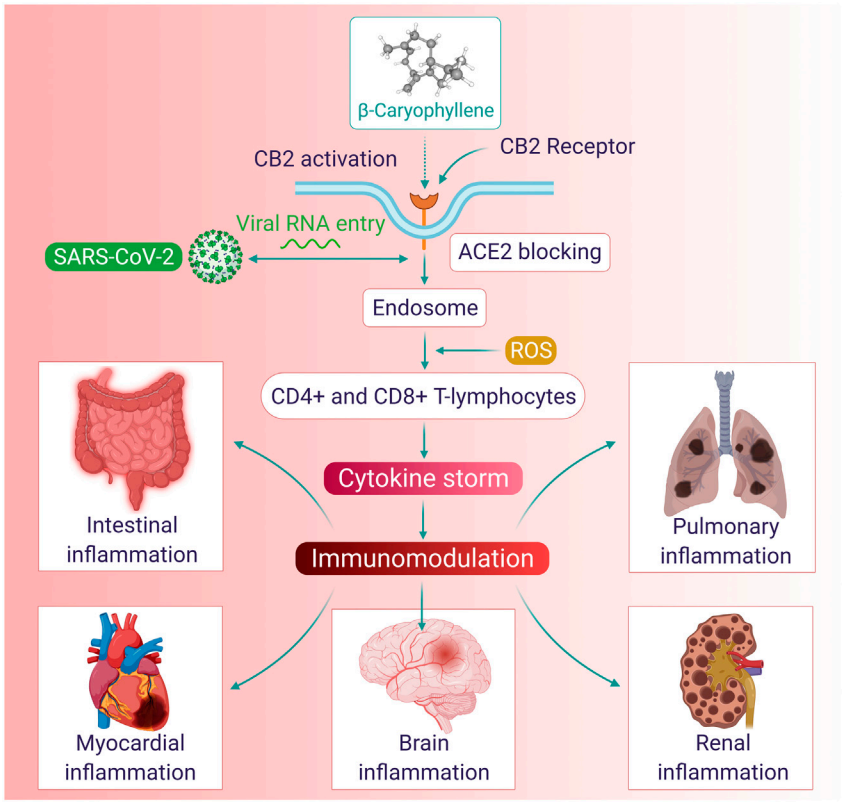

FIGURE 3 | The immunomodulatory mechanisms and organ-protective effects of BCP.

was shown to reduce ACE activity, which may be useful as ACE2 receptors, as the gateways of the virus entry, play a role from the entry of the virus to viremia and from respiratory distress to sepsis (Adefegha et al., 2017; Ajiboye et al., 2019). The immunomodulatory role of BCP is represented in **Figure 3**. Given the role of immunomodulators on the modulation of the hyperimmune-inflammatory response in COVID-19 patients, BCP may be a potential candidate for modulating immunity in patients at risk of infection and chronic metabolic and/or degenerative diseases, as well as in preventing the development and reducing the severity of COVID-19.

ANTI-INFLAMMATORY PROPERTIES OF BCP

Cytokines regulate both inflammation and the immunopathology of viral infection. The massive production of proinflammatory cytokines is the key element that leads to an acute systemic hyperinflammatory state, to a cytokine storm syndrome, determining the intensity and severity of symptoms, eliciting the onset of acute respiratory distress, involving the extrapulmonary system, and increasing the risk of multiple organ failure and mortality during SARS-CoV-2 infection (Allegra et al., 2020). Mounting evidence demonstrates that BCP exerts potent anti-inflammatory properties in all body organs, including the liver, kidneys, brain, heart, pancreas, and blood, and suppresses systemic inflammation by inhibiting proinflammatory cytokines in macrophages and other inflammatory mediators, as well as signaling pathways (Yamaguchi and Levy, 2020). BCP was shown to exhibit a CB2R-dependent anti-inflammatory property by inhibiting lipopolysaccharide/endotoxin (LPS)-induced phosphorylation of kinases ERK1/2 and JNK1/2 in macrophages, since it is recognized as a CB2R agonist and a dietary cannabinoid (Gertsch et al., 2008). Macrophages in the lungs express CB2R, which, upon further activation by a CB2R agonist, reduced the release of pro-inflammatory cytokines (such as IL-6) and angiogenic factors (Staiano et al., 2016).

Many pathways were shown to be responsible for the anti-inflammatory activity in macrophages, including inhibiting the Ras-MAPK pathway, JNK pathway, TNF-α translation, and the inhibition of proinflammatory cytokines, including TNF-α (Gertsch et al., 2008; Rajesh et al., 2008). TNF-α triggers the activation of Ras, p38 MAPK, ERK1/2, SAPK/JNK, HMGB1/TLR4, and Akt pathways, and ultimately, the expression of proinflammatory cytokines, cellular proliferation, and migration (Gertsch et al., 2008; Rajesh et al., 2008). Additionally, numerous studies have also demonstrated the

anti-inflammatory effects of BCP by activating CB2R and their subsequent pathways (Youssef et al., 2019). The CB2R-dependent anti-inflammatory effect of BCP has been demonstrated in inflammatory states of the heart (Meeran et al., 2019), liver (Cho et al., 2015; Arizuka et al., 2017; Varga et al., 2018), intestines (Cho et al., 2015), kidneys (Horváth et al., 2012; Hammad et al., 2018), lungs (Andrade-Silva et al., 2016), brain (Fontes et al., 2017; Yang et al., 2017; Askari et al., 2019; Askari and Shafiee-Nick, 2019), pancreas (Basha and Sankaranarayanan, 2016), urinary bladder (Berger et al., 2019), joints (Rufino et al., 2015; Irrera et al., 2019; D'Ascola et al., 2019), skin (Koyama et al., 2019), oral cavity, and blood (Brito et al., 2019). BCP also showed anti-inflammatory effects mediating histaminergic and arachidonic acid pathways (Oliveira-Tintino et al., 2018). Though, evidence supports that CB2R activation has anti-inflammatory effects, it has yet to be targeted to treat human disease.

BCP is present in many plants, such as *Campomanesia phaea* (O.Berg) Landrum (Lorençoni et al., 2020), *Pterodon pubescens* (Benth.) Benth. (Basting et al., 2019), *Ocimumm icranthum* Willd. (de Pinho et al., 2012), *Mosla dianthera* (Buch.-Ham. ex Roxb.) Maxim. (Wu et al., 2012), *Cordia verbenacea* A. DC. (Basting et al., 2019), *Duguetia furfuracea* (A.St.-Hil.) Saff. (Saldanha et al., 2019), *Cinnamomum osmophloeum* Kaneh. (Tung et al., 2008), *Croton campestris* A.St.-Hil., A. Juss. & Cambess. (Oliveira-Tintino et al., 2018), *Pinus spp.*(Basholli-Salihu et al., 2017), and *Copaiba oil* (Ames-Sibin et al., 2018), and has been considered responsible for their anti-inflammatory effects by suppressing proinflammatory cytokines and other inflammatory mediators.

ANTIVIRAL PROPERTIES OF BCP

The role of plant-based natural products are well explored for their antiviral properties and are gaining attention for their therapeutic potential in COVID-19 (Mahmud et al., 2020; ul Qamar et al., 2020). The antiviral role of plant-derived compounds in inhibiting replication and blocking entry of viruses, including coronaviruses, in the host cells has been well reviewed elsewhere (Wen et al., 2007; Dhama et al., 2018; Hensel et al., 2020). The antiviral potential of many plant-derived compounds against SARS-CoV-2 has been recently demonstrated in *in silico*, *in vitro,* and *in vivo* studies (Bahramsoltani and Rahimi, 2020; Basu et al., 2020; Benarba and Pandiella, 2020; Mondal et al., 2020; ul Qamar et al., 2020). Many of the compounds showed targeting of SARS-CoV-2 using bioinformatic tools such as *in silico* analysis, molecular docking, or molecular farming to enhance the production of recombinant proteins including vaccines and antibodies (Rosales-Mendoza et al., 2020). In search of antiviral compounds, a library of plant-derived constituents containing 32,297 phytochemicals have been screened in molecular docking and results displayed that nine compounds, including myricitrin, methyl rosamarinate, licoleafol, and amaranthin, may curb the activity of $3CL^{pro}$ enzymes in SARS-CoV-2 (ul Qamar et al., 2020). The inhibitory activity on the proteases and other molecular targets should be assessed for specificity, affinity, dose-response, and kinetics in experimental studies. The binding of these compounds limits the availability of the substrate, modifies configuration of active sites, and prevents dimerization, viral entry, and/or viral replication. The role of cannabinoids against virus replication, maturation, transmission, and entry in particular has been demonstrated in *in silico* and *in vitro* studies (Khodadadi et al., 2020; Mohammed et al., 2020; Salles et al., 2020; Wang et al., 2020; Raj et al., 2021). It has become apparent that agents which have antiviral properties corroborated with anti-inflammatory and immunomodulatory properties are important to target the trinity of infection, inflammation, and immunity in context of COVID-19. To tackle SARS-CoV-2, the identification of viral protease appears as a striking therapeutic target to limit the replication of SARS-CoV-2 and many of the compounds are being investigated for their potential to target replication by inhibiting viral components such as M^{pro} ($3CL^{pro}$), PL^{pro} and spike proteins. The protease of SARS-CoV-2 emerged as an attractive target to inhibit the replication of the virus. Recently, BCP was shown to target SARS-CoV-2 virus via pie-alkyl interactions to PHE 294 of SARS-CoV-2 with an affinity of -7.2 in an *in silico* docking study (Narkhede et al., 2020).

The antiviral properties of BCP or BCP-containing plants have been summarized in **Table 1**. The antiviral properties against herpes simplex virus type 1 (HSV-1) from the essential oils of many plants have been attributed to their chemical constituents, including BCP (Astani et al., 2011). The IC_{50} and TC_{50} for BCP were found to be 0.25 and 35 μg/ml, respectively (Astani et al., 2011). In plaque reduction assays, BCP exerted a concentration-dependent antiviral effect, with a selectivity index (ratio of TC_{50}/IC_{50}) of 140. BCP showed 98% reduction in infectivity, comparable to acyclovir, a standard antiviral drug. The authors suggested that BCP has potential to inactivate the herpes virus and may affect the structure of the virion envelope, which is essential for adsorption or entry into the host cells (Astani et al., 2011). BCP was shown to inhibit both Herpes Simplex Virus-2 (HSV-2) and acyclovir-resistant strain infections with a similar or lower selectivity index, compared to the BCP-rich essential oil of *Salvia desoleana* Atzei & V. Picci. However, BCP was not found to inhibit HSV-1 infection (Loizzo et al., 2008; Cagno et al., 2017). The selectivity index value >1 suggests that the compound has inhibitory action on viral replication and has low cytotoxicity on the host cells, thus a high selectivity index demonstrates better action of the compound. In the absence of guidelines on acceptability or appropriateness of selectivity index, values greater than 10 are considered better candidates for antiviral actions. The extracts rich in BCP displayed a very high selectivity index that indicates potent antiviral activity with negligible cytotoxicity on the host cells. The therapeutic efficacy and safety may also have different implications and should be taken in account considering the severity of viral infections and its onset, whether acute or chronic. BCP was tested in cell-based assays and showed a selectivity index of 71.1 in inhibiting the replication of the dengue virus (DENV-2). BCP acts as a viricidal by interfering with the very early steps of the viral replication cycle and *in silico* data showed that BCP

TABLE 1 | The antiviral activities of β-Caryophyllene (BCP) or BCP containing plants.

Sources	BCP (%)	Viral targets	References
Mosla dianthera (Buch. -Ham. ex Roxb.) Maxim	14.49	Influenza virus A (IVA)	Wu et al. (2012)
Glechon spathulata Benth	14.2	Human Herpes Virus Type 1 (HSV-1)	Venturi et al. (2015)
Glechon marifolia Benth	32.2	HSV-1	Venturi et al. (2015)
Illicium verum Hook.f	-	HSV-1	Astani et al. (2011)
Buddleja cordobensis Griseb	16.5	DENV-2, JUNV and HSV-1	Duschatzky et al. (2005)
Cinnamomum zeylanicum Blume	0.5–6.7	Influenza type A (H1N1)	Setzer (2016)
Eupatorium patens D. Don ex Hook. and Arn	14.1	HSV-1	García et al. (2003)
Gaillardia megapotamica (Spreng.) Baker	6.7	DENV-2, JUNV and HSV-1	Duschatzky et al. (2005)
Hyptis mutabilis (Rich.) Briq	10.9	Human Herpes Virus Type 2 (HSV-2)	Brand et al. (2015)
Jungia polita Griseb.	8.1	DENV-2, JUNV and HSV-1	Duschatzky et al. (2005)
Lavandula angustifolia Mill	5.1	H1N1	Setzer (2016)
Lepechinia vulcanicola J.R.I. Wood	8.7	HSV-1, HSV-2	Brand et al. (2015)
Lippia turbinata Griseb.	6.4	HSV-1	García et al. (2003)
Melissa officinalis L	14.2	HSV-2	Allahverdiyev et al. (2004)
Ocimum campechianum Mill	13.0	HSV-2	Brand et al. (2015)
Thymus capitatus (L.) Hoffmanns. and Link	2.9	Cytopathogenic murine norovirus	Moussaoui et al. (2013)
Thymus vulgaris L	7.0	HSV-1	Schnitzler et al. (2007)
Zataria multiflora Boiss.	3.0	Real time PCR (H9N2 subtype of AIV)	Shayeganmehr et al. (2018)

specifically targets the dengue virus proteins. BCP was also found useful in Epstein-Barr virus-associated diseases. BCP has been recognized in the essential oil of *Waldheimia glabra* (Decne.) Regel, popularly known as 'Ghaan-Poe', is used for influenza in Tibetan medicine (Manzo et al., 2016; De et al., 2017). The essential oil showed antiviral activity against influenza virus H3N2 in an *in vitro* assay and was found comparable to ribavirin, a standard antiviral drug (Manzo et al., 2016).

Further, the anti-inflammatory activity of essential oil was evidenced by the inhibition of NO production in LPS-stimulated macrophages and was found to be more potent than the standard drug dexamethasone. BCP was found in the essential oil of *Teucrium pseudochamaepitys* Georgi, an important Tunisian flora element that is used in traditional medicine for its antiviral activity against an enterovirus, Coxsackie 4 (CV-B4), known for causing myocarditis and CNS pathologies (Hammami et al., 2015). The essential oil showed potent antioxidant properties. The BCP-containing essential oil of *Glechon spathulata* Spreng. and *Glechon marifolia* Benth. are traditionally used in viral infections for their viricidal activity against HSV-1 strain KOS, VR733 (ATCC), or 29-R (ACV^{res}) (Venturi et al., 2015). The essential oil of *Glechon spathulata* Spreng. exhibited activity against all strains and *Glrchon marifolia* Benth. was found to be active against two strains, KOS and VR733. HSV-1 was more susceptible to the oil of *Glechon spathulata* Spreng. than that of *Glechon marifolia* Benth. The viral titer was reduced by up to 2 $\log_{10}$ for KOS and VR-733 strains. BCP-containing essential oil of *Mosla dianthera* (Buch.-Ham. ex Roxb.) Maxim., a herb popularly used in respiratory illnesses, showed antiviral activity in mice infected with influenza virus A (Wu et al., 2012). It exerted potent antioxidant, anti-inflammatory, and antiviral effects, as evidenced by the reduced serum levels of IFN-γ and IL-4, viral titer in the lungs, amelioration of pneumonia, and an increased endogenous antioxidant level in the lung tissues. The findings were suggestive of its possible use in influenza and viral pneumonia (Wu et al., 2012). The essential oil obtained from *Fortunella margarita* (Lour.) Swingle, commonly known as Kumquats, which belongs to the citrus family, contained BCP and was shown effective against avian influenza-A virus (H5N1). BCP-containing essential oil of *Schizonepeta tenuifolia* (Benth.) Briq. was shown to inhibit norovirus replication through the induction of antiviral interferon production during virus replication by inducing the expression of both type I and type II interferons and increasing the transcription of interferon-β in infected RAW 264.7 cells via an increased phosphorylation of interferon regulatory factor 3, a critical transcription regulator for type I interferon production (Ng et al., 2018). Very recently, BCP on oral supplementation showed antiviral and immunomodulatory potential in an *in vivo* viral model of Newcastle disease virus (Hassanin et al., 2020).

In COVID-19 patients, the prevalence of coinfections has been reported and the co-pathogens may be bacteria, such as *Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae, Mycoplasma pneumoniae, Chlamydia pneumonia, Legionella pneumophila*, and *Acinetobacter baumannii*, fungi, such as *Candida* species and *Aspergillus flavus*, or viruses, such as influenza, coronavirus, rhinovirus/enterovirus, parainfluenza, metapneumovirus, influenza B virus, and human immunodeficiency virus (Lai et al., 2020). Additionally, the antibacterial and antifungal effects of BCP have been reported in a number of studies (Schmidt et al., 2010; Rather et al., 2012; Dahham et al., 2015; Nieto-Bobadilla et al., 2015; Yang et al., 2015; Okoh et al., 2019;). Taken together, the antiviral and antibacterial activities, BCP may be a promising agent for secondary infections, as well as the viral infections.

ANTIOXIDANT PROPERTIES OF BCP

Besides the immune-inflammatory changes, macrophages and neutrophils can produce numerous reactive oxygen species (ROS), including H_2O_2, (O_2^-), (•OH), which further activates many signaling pathways and the onset of inflammation and cell

death in many organs, including the lungs (Imai et al., 2008). Oxidative stress and the subsequent activation of NF-kB-toll-like receptor signaling pathways, triggered by viral pathogens such as SARS-CoV-2, are believed to amplify the host inflammatory response that results in acute lung injury (Saleh et al., 2020). Additionally, the hyper inflammatory/oxidative state may lead to the dysfunction of mitochondria, the hub of cellular oxidative homeostasis, and cause platelet damage, which, upon interaction with coagulation cascades, aggravates the clotting events and thrombus formation.

Mitochondrial oxidative stress may contribute to microbiota dysbiosis, altering the coagulation pathways and fueling the inflammatory/oxidative response, leading to a vicious cycle of events (Saleh et al., 2020). Oxidative stress further primes endothelial cells to acquire a pro-thrombotic and pro-inflammatory phenotype, predisposing patients to thromboembolic and vasculitic events and disseminated intravascular coagulopathy (Panfoli, 2020). Nrf2, a transcription factor which regulates the redox balance and the expression of genes involved in immunity and inflammation, is believed to defend against SARS-CoV-2 (McCord et al., 2020). The suppressed redox status of a cell enhances its susceptibility to oxidative stress, which may lead to cell death and viral release (Khomich et al., 2018). SARS-CoV-2 infections can lead to alterations of the redox balance in infected cells through the modulation of NAD^+ biosynthesis and PARP function, along with altering the proteasome and mitochondrial function in cells, thereby leading to enhanced cell stress responses that further exacerbate inflammation. ROS production can increase IL-6 production and lipid peroxidation, resulting in cell damage (Nasi et al., 2020). Virus-induced inflammation and oxidative stress could be the common mechanisms responsible for the cardiovascular, pulmonary, renal, and neurological symptoms in COVID-19 patients (Nuzzo and Picone, 2020). BCP was found to exert protective effects in renal cells by suppressing ROS generation, NADPH oxidase 2/4 expression, and by controlling cell proliferation and inflammation by inhibiting proinflammatory cytokines, Nrf2/HO-1 and NF-κB/Nrf2 signaling pathways (Li et al., 2020).

BCP is present in *Ocimum sanctum* L. (Kamyab and Eshraghian, 2013), *Pinus spp.*(Xie et al., 2015), *Salvia officinalis* L. (El-Hosseiny et al., 2016), *Citrus limoni* (L.) Osbeck (Oboh et al., 2014), *Stachys pilifera* Benth. (Sadeghi et al., 2020), *Pistacia lentiscus* L. (Mohamed et al., 2018), *Eplingiella fruticose* (Salzm. ex Benth.) Harley & J.F.B. Pastore (Beserra-Filho et al., 2019), *Lantana montevidensis* (Spreng.) Briq. (de Oliveira et al., 2019), *Azadirachta indica* A. Juss. (Okoh et al., 2019), *Rosmarinus officinalis* L. (Mohamed et al., 2016), *Aquilaria crassna* Pierre ex Lecomte (Dahham et al., 2015), and *Copaiaba oil* (Ames-Sibin et al., 2018) and has been shown to augment the levels of endogenous antioxidants, exerting ferric reducing properties, a Fe^{2+} chelation, and radicals scavenging activity in DPPH, FRAP, ORAC, ABTS, •OH, and NO assays (Oboh et al., 2014; Pant et al., 2014). BCP also enhances tolerance against stress, augments chaperons, and improves the antioxidant power (Srivastava et al., 2016). BCP mitigates the oxidative stress by counteracting ROS generation, inhibiting lipid peroxidation and glutathione depletion, free radical scavenging, and augmenting the endogenous antioxidant defense in the tissues of different organs, such as the heart (Ojha et al., 2016; Baldissera et al., 2017; Meeran et al., 2019), brain (Choi et al., 2013; Ojha et al., 2016; Tian et al., 2016), intestine (Bento et al., 2011), liver (Arizuka et al., 2017; Baldissera et al., 2017; Varga et al., 2018), stomach (Tambe et al., 1996), kidneys (Horváth et al., 2012; Hammad et al., 2018), pancreas (Basha and Sankaranarayanan, 2016), and blood (Youssef et al., 2019), which may aid the protective, as well as the adaptative, responses against viral infections and drugs.

BCP has been shown superior to probucol, α-humulene, α-tocopherol (Calleja et al., 2013), and synthetic CB2R agonist, JWH133 (Klauke et al., 2014). Also, BCP was shown to correct neurobehavior (anxiety, depression, and memory deficit), and neurochemical (oxidative, inflammatory, and neurotrophic factor) alterations in diet-induced obese rats (Youssef et al., 2019). Taken together, it is evident that BCP attenuated the oxidative stress and subsequent inflammation in organ dysfunction and metabolic disorders, favorably modulated redox signaling pathways (Baldissera et al., 2017; Varga et al., 2018), which are akin to the pathophysiology of SARS-CoV-2 infection.

BCP MAY BE PROSPECTIVE IN COVID-19 ASSOCIATED SEPSIS

SARS-CoV-2 infections may lead to sepsis and to subsequent multi-organ failure. Sepsis involves both the inflammatory response and immune suppression in response to an infection (Mira et al., 2017). CB2R plays a vital role in neutrophil/leukocyte recruitment, thereby suppressing infection and inflammation during sepsis (He et al., 2019). However, CB2R was also shown to contribute to septic immune dysfunction and mortality (Csóka et al., 2009). In a recent review the role of CB2R as a therapeutic target has been suggested based on the reports from preclinical animal models or *in vitro* cultured cells (He et al., 2019). The authors suggested that due to the lack of clinical evidence and the ambiguous underlying mechanisms, the clinical application of CB2R stimulation in sepsis is yet to be confirmed further (He et al., 2019). In many recent studies specific CB2R synthetic agonists, including HU-308 (Liu et al., 2020), GW405833 (Zhou et al., 2020), JWH133 (Çakır et al., 2020), and natural agonist, BCP (Brito et al., 2019), have been shown to ameliorate lung tissue damage, inhibiting oxidative stress, release of inflammatory mediators, recruitment of leucocytes and bacteremia, and improve survival in different preclinical models of sepsis. CB2R agonists were reported to ameliorate leukocyte adhesion to the endothelium, oxidative stress, systemic inflammatory mediators, microcirculatory dysfunction, bacteremia, and lung injury, along with an improvement in survival in experimental models of sepsis (Sardinha et al., 2014; Toguri et al., 2015). CB2R activation specifically mitigated septic lung injury by suppressing inflammatory mediators and augmenting autophagy (Liu et al., 2014; Liu et al., 2020). In an experimental model of polymicrobial

sepsis, CB2R activation decreased the histopathological damage in the brain, heart, lungs, and liver by reducing the levels of caspase-3, p-NF-κB, TNF-α, IL-1β, and IL-6 in these tissues, as well as in the serum, and improved the anti-inflammatory cytokine IL-10 levels (Çakır et al., 2020).

To model sepsis, many of the experimental models rely on LPS-induced macrophages, which involve the activation and release of inflammatory mediators, including cytokines (Brito et al., 2019). BCP was reported to reduce the level of leukocytes, cytokines TNF-α, IL-6, IL-12, and IFN-γ, and increase the levels of IL-4 and IL-5 (Brito et al., 2019). BCP was shown to suppress inflammatory mediators and exert inhibitory effects on macrophages (Tung et al., 2008; Yamaguchi and Levy, 2019; Yamaguchi and Levy, 2020). Although the role of CB2R in sepsis has mixed reports, BCP has been shown to be beneficial in sepsis via CB2R activation and the off target effects cannot be excluded (Meza and Lehmann, 2018). Additionally, BCP is known to have a better safety profile over synthetic cannabinoids. Given the association of SARS-CoV-2 infections and sepsis-induced life-threatening organ dysfunction, BCP may be a promising candidate for COVID-19 associated sepsis.

BCP also attenuated neuronal necrosis, receptor-interaction protein kinase-1 (RIPK1), receptor-interaction protein kinase-3 (RIPK3) expression, and mixed lineage kinase domain-like protein (MLKL) phosphorylation in cerebral ischemia by inhibiting high-mobility group box 1 (HMGB1)-toll-like receptor 4 (TLR4) signaling pathways and proinflammatory cytokines. HMGB1, which is released by macrophages and monocytes in response to high levels of proinflammatory cytokines, plays a critical role in allowing innate immune cells to respond to both infection and injury. After its release, HMGB1 binds to its receptor for an advanced glycation of the end-products, which further activates MAPK and NF-κB, resulting in an overgeneration of various cytokines, causing a massive neutrophil infiltration into the lungs, and subsequent acute lung injury. The agents that target the release of HMGB1 are suggested to be useful in reducing mortality by preventing the progression from respiratory distress to sepsis (Wyganowska-Świątkowska et al., 2020). Given the protective role of BCP on redox homeostasis and on the immune-inflammatory cascade in acute cerebrovascular disorders, it holds therapeutic promise for neurological manifestations of SARS-CoV-2.

BCP MAY BE PROSPECTIVE IN COVID-19 ASSOCIATED NEUROLOGICAL MANIFESTATIONS

SARS-CoV-2 is considered to be neurovirulent and neuroinvasive, in parallel with adherence to endothelial cells and cardiomyocytes (Sweid et al., 2020). Ischemic stroke, venous thrombosis, and intracerebral hemorrhage are the reported neurological manifestations of SARS-CoV-2 infection (Jiménez-Ruiz et al., 2020). The pathophysiology of ischemic stroke or cerebral hemorrhage includes an increased level of inflammatory cytokines in the brain, subsequent to the activation of microglia, astrocytes, and adhesion molecules, along with leukocyte recruitment and an impaired blood brain barrier. CB2R are upregulated during the inflammatory activation and CB2R agonists have been shown to be effective in acute ischemia and hemorrhagic stroke (Capettini et al., 2012).

BCP has been shown to exert a protective role on neurological deficit and neuroinflammation in experimental models, including middle cerebral artery occlusion induced-cerebral ischemia by suppressing the oxidative stress, inflammatory mediators, apoptosis, and reduction in brain edema, as well as preservation of tight junction proteins and repair of blood brain barrier (Zhang et al., 2017; Tian et al., 2019). BCP exerted its protective effects mediating CB2R activation (Choi et al., 2013) and its associated mechanisms, including the downregulation of TLR4 pathways to suppress inflammation and polarizing microglial phenotype from M1 to M2 (Tian et al., 2019), PI3K/Akt signaling pathway to suppress apoptosis (Zhang et al., 2017), an upregulation of the modulation of AMPK/CREB signaling (Choi et al., 2013), and the upregulation of Nrf2/HO-1 pathway to suppress oxidative stress and apoptosis (Lou et al., 2016).

BCP MAY BE PROSPECTIVE IN COVID-19 ASSOCIATED CARDIOVASCULAR CONDITIONS

SARS-CoV-2 infection has been reported to increase the susceptibility of patients affected by coronary artery disease and risk factors of atherosclerotic cardiovascular disease to develop adverse outcomes and lead to death (Vinciguerra et al., 2020). SARS-CoV-2 mediating ACE2 receptors infect endothelial cells, which regulate inflammation, vasomotor tone, and hemostatic balance. Pathological conditions associated with atherosclerotic progression, such as heart failure, coronary heart disease, hypertension, and diabetes mellitus, are the predictive factors for severity and susceptibility during SARS-CoV-2 infection (Vinciguerra et al., 2020). The pathogenesis involves endothelial dysfunction, altered vasopermeability, and formation of pulmonary microthrombi subsequent to inflammation, hypoxia, oxidative stress, mitochondrial dysfunction, and DNA damage. Patients with preexisting pulmonary vascular diseases also appear to have an increased risk of morbidity and mortality (Potus et al., 2020).

Atherosclerosis is considered as an ideal pathogenetic substrate for high viral replication ability, leading to adverse outcomes, as found in patients with cardiovascular factors. SARS-CoV-2 may aggravate atherosclerosis due to an excessive and aberrant plasmatic concentration of cytokines (Vinciguerra et al., 2020). Atherosclerosis involves vascular inflammation, characterized by a narrowed vascular lumen in the entire tunica intima and a reduced elasticity of the arterial walls. CB2R activation mitigated endothelial cell activation, transendothelial migration of monocytes, and monocyte/neutrophil-endothelial adhesion, and suppressed the proliferation and migration of human coronary vascular smooth muscle cells induced by TNF-α (Rajesh et al., 2007). A

pneumonia causing pathogen, *Chlamydia pneumoniae*, provokes atheroma lesions by releasing heat shock proteins, which, by activating Hsp60 on endothelial cells, increase vascular smooth muscle cell proliferation. BCP was found to inhibit Hsp60-induced vascular smooth muscle cell proliferation and its potential in atherosclerosis has been suggested (Fukuoka et al., 2004). BCP also ameliorated acute myocardial injury by improving cardiac function, reducing infarct, restoring myocyte enzymes, and suppressing inflammation by inhibiting HSP-60/TLR/MyD88/NFκB signaling pathways (Younis and Mohamed, 2019). BCP was found to counteract drug-induced cardiomyopathy by attenuating inflammation, oxidative stress, and apoptosis by activating CB2R (Meeran et al., 2019). BCP mitigated hypercholesterolemia, dyslipidemia, and vascular inflammation, reduced atherogenic and coronary risk index, and corrected lipid metabolism by inhibiting proatherogenic vascular cell adhesion molecule 1 (VCAM-1) and restoring vascular eNOS/iNOS expression by maintaining the NO levels, mediating the activation of CB2 and PPAR-γ receptors in a high-fat diet and fructose-induced obesity (Baldissera et al., 2017; Harb et al., 2018; Youssef et al., 2019).

Furthermore, in addition to correcting the lipid profile, BCP-mediating CB2R-dependent mechanism inhibited leukocyte-endothelial attachment, neutrophil recruitment, and macrophage infiltration, inducing VCAM-1 to mediate the JAK2/STAT1/IRF-1 pathway (Zhang et al., 2017). BCP is one of the most important components of Copaiba oil, popularly used in Brazil for respiratory and cardiovascular illnesses. The nanocapsules of copaiba oil were shown to attenuate monocrotaline-induced pulmonary arterial hypertension in rats by counteracting the oxidative stress and inflammation, and by improving the cardiac function (Campos et al., 2017). One of the major clinical features and reasons for death in COVID-19 patients is respiratory distress syndrome, that also leads to acute cardiac injury (Huang et al., 2020). The potential of BCP on pulmonary vasculature is also promising and can be useful in reducing the risk of cardiopulmonary complications. The available studies are clearly suggestive of the therapeutic benefits of BCP in atherosclerosis, acute myocardial infarction, dyslipidemia, obesity, and fatty liver and could be important in preventing the worsening of the condition in COVID-19 patients.

BCP MAY BE PROSPECTIVE IN COVID-19 ASSOCIATED INTESTINAL INFLAMMATION

BCP was found to reduce the number of enterobacteria in the luminal and mucosal components, improving the clinical course of an intestinal inflammation in the mice model of colitis (Nieto-Bobadilla et al., 2015). BCP ameliorated intestinal inflammation in the animal models by mediating the activation of CB2 and the PPAR-γ pathway (Cho et al., 2007; Bento et al., 2011). It suppressed MPO activity and reduced the serum levels of protein and mRNA of IL-6 by 55% (Cho et al., 2007). IL-6 signaling pathway appears as one of the potential therapeutic targets for COVID-19. BCP also suppressed N-acetylglucosaminidase activity and the levels of mRNA expression of TNF-α, IL-1β, IFN-γ, chemokines, and the activation of extracellular signal-regulated kinase 1/2, NF-κB, IκB-kinase α/β, cAMP response element binding, and the expression of caspase-3 and Ki-67. BCP increased IL-4 levels and forkhead box P3 mRNA expression in the colon (Cho et al., 2007; Bento et al., 2011). In macrophages challenged with LPS, BCP reduced the levels of cytokines, such as TNF-α, keratinocyte-derived chemokines, and MIP-2.

Recently, in patients infected with SARS-CoV-2, an inflammatory response in the gut is evidenced by diarrhea and increased IL-6 and fecal calprotectin levels, showing the activation of neutrophils (Effenberger et al., 2020). Additionally, diarrhea appears as one of the most frequent symptoms in patients infected with SARS-CoV-2 (D'Amico et al., 2020). Given the role of BCP in suppressing intestinal inflammation (Cho et al., 2007; Bento et al., 2011) and diarrhea (Nieto-Bobadilla et al., 2015), BCP may hold great therapeutic promise for COVID-19.

BCP MAY BE PROSPECTIVE IN COVID-19 ASSOCIATED AIRWAY INFLAMMATION

In many reports, vaccination of *Bacillus Calmette-Guérin* (BCG), a live attenuated vaccine of *Mycobacterium bovis* strain, is believed to provide protection against SARS-CoV-2 infection. BCG vaccination is believed to be associated with the induction of trained immunity, a kind of epigenetic reprogramming of innate immune cell types (Goodridge et al., 2016). In vaccinated individuals, monocytes and/or natural killer cells exhibit an upregulation of surface markers of activation and synthesis of cytokines, such as IL-1β, IL-6, IFN-γ, and TNF-α, in response to infection compared to non-vaccinated individuals; this helps in the faster clearance of pathogens, including influenza (Arts et al., 2018). BCP was found to ameliorate pulmonary inflammation in a mice model of *Mycobacterium Bovis* BCG-induced pulmonary inflammation by suppressing neutrophil accumulation, suppressing CXCL1/KC, LTB_4, IL-12, and NO production, and mediating the CB2R activation (Andrade-Silva et al., 2016).

Additionally, BCP was also found to exert spasmolytic effects on the tracheal smooth muscle in the isolated organs (Pinho-da-Silva et al., 2012). BCP produced antispasmodic effects on the isolated tracheal smooth muscle of rats by inhibiting voltage-dependent L-type Ca^{2+} channels. BCP did not affect Ca^{2+} release from the intracellular storage. Further, the inhibitory effect on epithelial COX and a balance between relaxant and constrictor prostanoids exerted by BCP suggested that it may be useful in asthma-like conditions (Pinho-da-Silva et al., 2012). BCP containing essential oil of *Croton sonderianus* Müll. Arg. was found to exert myorelaxant activity in rat airway smooth muscles, which is suggestive of its potential in bronchospasm (Pinho-da-Silva et al., 2010). During viral infections, the activation of selective CB2R by agonists was shown to suppress leukocyte migration into the site of inflammation (Tahamtan et al., 2018). CB2 agonists in HIV-1 infection also reduced infection in

primary $CD4^+$ T cells, as well as CXCR4-activation-mediated G-protein activity and the phosphorylation of MAPK (Costantino et al., 2012). The CB2 selective property of BCP is reasonably speculated as a basis for its potential to inhibit virus replication, bacterial growth, and to regulate neutrophil recruitment, thus regulating inflammation.

The acute viral respiratory infections may increase the chances of secondary bacterial infections due to a compromised host immune response and thereby worsen the condition. SARS-CoV-2 was also reported to cause secondary bacterial infection (Dong et al., 2020). BCP is present in many plants, such as *Artemisia capillaris* Thunb. (Yang et al., 2015), *Juniperus rigida* var. hibernica Pshenn. (Meng et al., 2016), *Lavandula coronopifolia* Poir. (Ait Said et al., 2015), *Juglans regia* L. (Rather et al., 2012), *Mosla dianthera* (Buch.-Ham. Ex Roxb.) Maxim. (Wu et al., 2012), *Thymbra spicata* L. (Saidi et al., 2012), and *Lantana camara* subsp. *glandulosissima* (Hayek) R.W. Sanders (Tesch et al., 2011), which have been shown to exert inhibitory activity against respiratory pathogens and many virus, fungi, bacteria, and parasites in experimental studies and in human isolates.

BCP MAY BE PROSPECTIVE IN COVID-19 ASSOCIATED LIVER DYSFUNCTION

Liver impairment has been reported in patients with SARS-CoV-2 infection (Feng et al., 2020; Sun et al., 2020) and it is believed to be due to systemic inflammation caused by a cytokine storm or pneumonia-associated hypoxia, and the drug regimens containing acetaminophen (Zhang et al., 2020). ACE2 receptors in the bile duct epithelial cells are expressed twenty times more than in hepatocytes and this plausibly explains that SARS-CoV-2 infection may cause bile duct epithelial cell damage (Lee et al., 2020). BCP was reported to ameliorate liver fibrosis in a bile duct ligation induced model, suppressing inflammation and apoptotic cell death by mediating the activation of CB2R (Mahmoud et al., 2014). BCP has also been reported to ameliorate drug induced liver injuries, such as ketoprofen-induced liver injury (Kelany and Abdallah, 2016), carbon tetrachloride-induced liver injury (Calleja et al., 2013), and D-galactosamine and lipopolysaccharide-induced liver failure by suppressing inflammation and mediating TLR4 and RAGE signaling pathways (Cho et al., 2015). BCP was partially attributed to the hepatoprotective effects of many plants, such as *Ocimum sanctum* L. (holy basil) (Kamyab and Eshraghian, 2013).

SARS-CoV-2 infection also increases vulnerability in patients with non-alcoholic fatty liver disease (NAFLD), a chronic liver disease characterized by hepatic steatosis (fatty liver), inflammation and hepatocyte damage (steatohepatitis), and lipotoxicity (Prins and Olinga, 2020). The expression of ACE2 is increased in cholangiocytes and hepatocytes during chronic liver damage and was increased in a diet-induced experimental model of NAFLD (Prins and Olinga, 2020). Metabolic perturbations, such as obesity, insulin resistance, hyperglycemia, dyslipidemia, and systemic hypertension, which constitute metabolic syndrome, are one of the risk factors of NAFLD (Friedman et al., 2018; Prins and Olinga, 2020). BCP showed a cholesterol-lowering effect by inhibiting the activity of hepatic hydroxy-methylglutaryl coenzyme A reductase in experimental models of hypercholesterolemia (Arizuka et al., 2017; Baldissera et al., 2017; Harb et al., 2018). Besides correcting the lipid metabolism, BCP also increased high density lipoprotein and attenuated liver injury and fibrosis, restored liver function enzymes and improved antioxidants (Harb et al., 2018).

BCP also attenuated chronic and binge alcohol-induced liver injury and inflammation by attenuating the pro-inflammatory phenotypic `M1` switch of Kupffer cells and by decreasing the expression of vascular adhesion molecules intercellular adhesion molecule 1, E-selectin, and P-selectin, as well as the neutrophil infiltration, and corrected hepatic metabolic dysregulation (Varga et al., 2018). BCP inhibited palmitate-inducible lipid accumulation in human HepG2 hepatocytes by activating AMPK mediating CB2R-dependent Ca^{2+} signaling pathway (Kamikubo et al., 2016). Mechanistically, BCP regulated hepatic lipid and glucose metabolism by modulating adenosine monophosphate (AMP)-activated protein kinase (AMPK), the main cellular energy sensor (Xu et al., 2018). Considering its hepatoprotective roles, BCP could be promising in conditions of liver injury associated with SARS-CoV-2 infection.

BCP MAY BE PROSPECTIVE IN COVID-19 ASSOCIATED RENAL INJURIES

Acute kidney injury is one of the major complications in patients with SARS-CoV-2 infection (Cheng et al., 2020). ACE2 receptors located on the apical membrane and tubular cells facilitate viral entry and the infection elicits inflammatory responses that cause acute kidney injury (Fanelli et al., 2020; Soleimani, 2020). BCP ameliorated acute kidney injury in experimental models by attenuating renal impairment and tubular injury, suppressing renal inflammatory mediators, oxidative stress, apoptotic cell death, and preserving renal morphology via activation of CB2R (Horváth et al., 2012; Hammad et al., 2018).

BCP is present in many plants, such as *Stachys pilifera* Benth. (Sadeghi et al., 2020), *Salvia officinalis* L. (Koubaa et al., 2019), *Rosmarinus officinalis* L. (Mohamed et al., 2016), and *Pluchea indica* (L.) Less. (Sirichaiwetchakoon et al., 2020), and has been shown to be responsible for the renoprotective effects against drug induced-acute kidney injury, as well as diabetic and chronic kidney diseases, by restoring the renal function and suppressing oxidative stress, inflammation, and apoptosis. BCP also attenuated renal inflammation and oxidative stress by regulating NF-κB/Nrf2 signaling pathways in diabetic kidney diseases (Li et al., 2020). Given the increased risk of renal dysfunction in COVID-19 and the worsening of conditions in patients with chronic kidney or diabetic kidney disease, BCP may be a valuable candidate in preventing renal dysfunction in patients with COVID-19.

TISSUE PROTECTIVE EFFECTS OF BCP MAY BE PROSPECTIVE IN COVID-19 ASSOCIATED ORGAN INJURIES

Besides the lungs, the main site of virus entry and injury, SARS-CoV-2 infection may also affect other organs or organ systems, including the hepatic, renal, neurological, cardiovascular, musculoskeletal, gastrointestinal, hematological, olfactory, gustatory, ophthalmic, and cutaneous systems (Lai et al., 2020). Cardiac manifestations of SARS-CoV-2 involve endothelial damage, an altered lipid profile, endotoxemia, catecholamine, hypoperfusion, unstable hemodynamics, and drug-induced toxicity. BCP showed protective effects against catecholamine-induced myocardial injury and drug-induced cardiotoxicity by improving hemodynamics and alleviating endotoxemia by suppressing inflammation, oxidative stress, and apoptosis via activation of CB2R (Meeran et al., 2019).

The clinical manifestations of COVID-19 range from mild to severe with extensive involvement of the lungs, from pneumonia to ARDS, acute liver injury, acute cardiac injury, and neurological manifestations that may lead to multi-organ failure with a poor prognosis (Wang et al., 2020; Zhu et al., 2020). Severe lung disease with extensive alveolar damage and progressive respiratory failure leads to deadly outcomes (Yang et al., 2020). The fatalities are higher in older people with cardiometabolic diseases, cancer, immunocompromised patients, or patients with comorbidities. BCP was found to ameliorate renal dysfunction in acute and chronic kidney injury and diabetic kidneys.

BCP was found to be effective in liver failure by suppressing liver necrosis, fibrosis, and restoring liver function, mediating CB2R activation. BCP has been shown to be neuroprotective in models of cerebral ischemia, dopaminergic neurodegeneration, seizures, dementia, neurocognitive disorders, depression, anxiety, and encephalitis. BCP improved systemic inflammation and oxidative status with no hepatotoxicity, as with nonsteroidal anti-inflammatory drugs (Ames-Sibin et al., 2018). It also reduced nausea, epigastric pain, and diarrhea, and improved gastrointestinal activity (Patra et al., 2010). BCP was also found to promote wound healing by modulating numerous signaling pathways (Parisotto-Peterle et al., 2020). Hematological abnormalities, including lymphopenia and leukopenia, have been reported in COVID-19 patients (Ding et al., 2020). The occurrence of leukopenia induced by chemotherapeutic drugs in an experimental model has been shown to be prevented by BCP (Campos et al., 2015).

Upon oral administration, BCP was found to be bioavailable in almost every organ, including the liver, kidneys, heart, lungs, and blood (Pant et al., 2019). BCP was shown to modulate stress-related genes, provide resistance against stress, improve life span, reduce ageing, and was considered one of the best adaptogenic compounds to enhance the tolerance against stress. The interactions between phytocannabinoids and terpenoids have been suggested to exert synergy for the therapeutic benefits in pain, inflammation, depression, anxiety, addiction, epilepsy, cancer, and microbial infections (Russo, 2011). Given the impact of COVID-19 on organ functions and considering the organ-protective effect of BCP, it is reasonable to hypothesize that the organ-protective activity of BCP will be beneficial in COVID-19.

SAFETY AND TOXICITY OF BCP

The United States Food and Drug Administration (USFDA) included BCP in the list of compounds regarded as Generally Recognized as Safe (GRAS) for its use as an additive and preservative in food products and beverages. BCP was shown to modulate the expression of drug metabolizing enzymes (phase I and II) in cell lines, rodents, and human liver microsomes, which may influence the bioavailability and efficacy of concomitantly administered drugs (Ambrož et al., 2019). BCP was shown to have a chemopreventive effect and is free from genotoxicity (Álvarez-González et al., 2014), mutagenicity (Di Giacomo et al., 2016), and clastogenicity (Di Sotto et al., 2010).

BCP exerted synergistic and/or additive actions with many drugs including azithromycin (Zhang et al., 2020), atovaquone (Zhang et al., 2020), metaxolone (Yamaguchi and Levy, 2020), imipramine (Askari et al., 2019), fluoxetine (Askari and Shafiee-Nick, 2019), docosahexaenoic acid (Brito et al., 2019), curcumin (Srivastava et al., 2016; D'Ascola et al., 2019), baicalein (Yamaguchi and Levy, 2016), catechin (Yamaguchi and Levy, 2016) and vitamins, which are suggested to be useful for repurposing for COVID-19. In many experimental studies, BCP was found to be better than the standard modern drugs such as phenylbutazone (Basile et al., 1988), probucol (Calleja et al., 2013), tocopherol (Calleja et al., 2013), ribavirin (Wu et al., 2012), atorvastatin (Campos et al., 2015), glibenclamide (Basha and Sankaranarayanan, 2016), and pioglitazone (Youssef et al., 2019). BCP delivered by inhalation was found to be bioavailable in the saliva and appears safe and tolerable (Tarumi and Shinohara, 2020). BCP was convincingly shown to mitigate drugs or xenobiotics-induced organ injuries; for example, it was found to improve the therapeutic efficacy of immunosuppressive drugs and reduce their side effects, such as myelosuppression and hepatotoxicity in experimental arthritis (El-Sheikh et al., 2019). BCP studied at the therapeutic doses was found devoid of organ toxicity in the experimental studies.

CLINICAL EFFICACY AND SAFETY OF BCP

BCP administered orally at a dose of 126 mg/day was evaluated in patients with peptic ulcer in a randomized double-blind, placebo-controlled trial (Shim et al., 2019). BCP improved dyspepsia symptoms by reducing *Helicobacter pylori* infections, improving nausea and epigastric pain, and mediating the inhibition of proinflammatory cytokines (Shim et al., 2019). BCP (3%) was evaluated in nineteen women for 20 min using an odor exposure device and was found to improve the libido and vaginal sensation during intercourse in women by improving the salivary

testosterone concentrations with no effect on estrogen (Tarumi and Shinohara, 2020).

BCP was administered to diabetes patients with diabetes-related complications; painful distal symmetric polyneuropathy was found to relieve polyneuropathy with an increased amplitude and a reduction of pain, with good tolerance and no adverse effects (Semprini et al., 2018). Recently, in a placebo-controlled clinical study, patients with hand arthritis applied BCP-containing copaiba oil topically and BCP was found to be safe, well tolerated, and beneficial in reducing pain and inflammation (Bahr et al., 2018).

DOSAGE FORMS AND PHARMACEUTICAL DEVELOPMENT OF BCP

Many formulations containing BCP have been developed, including Amukkara Choornam (Patra et al., 2010), CIN-102, a coated pellets and matrix mini-tablet (Nieto-Bobadilla et al., 2015), and PipeNig®-FL, a high standardized content of BCP (Geddo et al., 2019). BCP is highly lipophilic, less soluble in water and, upon exposure to air, it easily oxidizes. To overcome its low bioavailability, many novel drug delivery systems have been developed. Various kinds of formulations, such as liposomes, nanoemulsions, nanofibers, microemulsions, nanoparticles, micelles, phospholipid complexes, nanocarriers, nanocomposites, hydrogels, and matrix formulations using cyclodextrin, have been developed to enhance the solubility, stability, and release pattern of BCP (Santos et al., 2018). Novel formulations will pave the way for the pharmaceutical development of BCP and may aid in improving its clinical usage.

LIMITATIONS

Since the emergence of COVID-19, a significant number of natural products, including plant extracts and phytochemicals, have been proposed for their possible use as a preventive agent or as an adjuvant in COVID-19 (Asif et al., 2020; Boukhatem and Setzer, 2020; da Silva et al., 2020; Diniz et al., 2021). Though, given their pleiotropic and immunomodulatory nature, the role of phytocannabinoids are reasonably suggested useful, but caution should be exercised. The potential application of cannabinoids in COVID-19 management can't be overlooked until proof-of-concept studies become available (Pastor et al., 2020; Anil et al., 2021). The cannabinoids shown to possess potent immunomodulatory, anti-inflammatory, and antimicrobial properties are proposed for their use in COVID-19. However, few of the phytocannabinoids have been screened in molecular docking studies for their potential activity against viral targets using the *in-silico* tools. The role of phytocannabinoids is believed to be delicate given their action on inflammation and immune modulation and the possibility of the unfavorable effects in acute infection due to risk of immunosuppression (Sexton, 2020). Among numerous cannabinoids, BCP has been shown to be more convincing in terms of its immunomodulatory, anti-inflammatory, and antiviral effects. BCP is one of the main compounds identified in a large number of dietary plants and is widely accessible and well-studied for its therapeutic benefits. However, the safety and efficacy of BCP still needs to be established in preclinical and clinical trials for its evidence-based use and application in humans. BCP is a functional agonist of CB2R and devoid of psychotropic effects, which makes BCP a fascinating candidate molecule for further investigation.

A majority of the experimental research carried out on the therapeutic benefits of phytochemicals are based on ethnopharmacological usage of the particular plant rich in these. Many have also been evaluated for their antiviral properties in addition to their anti-inflammatory and immunoregulatory roles in numerous immune-related disease models. Since the emergence of COVID-19, the repurposing of drugs began first with target identification and continues to be used in the screening of druggable agents against viral infections. It is noteworthy to state that until recent years, the antiviral potential of natural products were shown to be effective in *in vitro* studies, whereas the SARS-CoV emerged in 2003 (Kim et al., 2014; Park et al., 2017). But none of them have been evaluated meticulously enough to translate their effects to humans despite their potential efficacy in preclinical studies. This is due to many reasons, including the lack of an integrated approach. A recent report suggested that if an integrated and rigorous approach could have been followed since the emergence of SARS-CoV, we may have progressed to clinical studies and developed some useful agents in the process of drug discovery and development, which involves the testing of druggable compounds from laboratory to clinics (Pandey et al., 2020). It can be proposed that the phytochemicals should be investigated and validated in the preclinical models of COVID-19 despite strong evidence for their anti-inflammatory, immunomodulatory, and antiviral properties.

In the present manuscript, the possible role of BCP in COVID-19 has been proposed based on the previously reported potent pharmacological activity of BCP against infection, inflammation, and immunity in experimental models of human diseases other than SARS-CoV-2. Many authors proposed the hypotheses that CB2R, an important constituent in endocannabinoid system, may play a role in targeting the trinity of infection, inflammation, and immune dysregulation (Nagoor Meeran et al., 2020). Given the role of CB2R activation in attenuating inflammation, viral replication, and favorable modulation of immune systems, BCP endowed with the CB2R selective agonist property has been pharmacologically reasoned to be a candidate for its possible use as preventive agent or therapeutic adjunct in COVID-19. There are reports of long-term complications in some patients even after recovery from COVID-19. Thus, given the tissue protective effects and effect on numerous tissues remodeling effects, BCP could be a candidate to be investigated for possible use in improving prognosis and combating the long-term complications in COVID-19. Taking into consideration the safety of BCP in humans, dietary use, and efficacy of BCP in various disease models in experimental studies, BCP may be a valuable agent to be investigated further for COVID-19.

The available reports clearly demonstrate that the progression and complications of COVID-19 involves cytokine storm, therefore, cannabinoids activating CB2R may inhibit cytokine storm due to their additional organ-protective effects. However, until now there has been no clear evidence available on the antiviral activity of BCP on SARS-CoV-2. There is no preclinical or clinical data available on whether BCP can protect against COVID-19 or may be useful in treatment of COVID-19. The recent availability of animal models could be important in evaluating its preclinical efficacy. There is a lack of clinical data and rigorous pharmacokinetics in humans. Therefore, preclinical evaluation, including duration of use and dose, is suggested. The safety and interaction with concomitant drugs, as well as the heterogeneity of the target population, should also be considered before the possible use of BCP whether in prevention or as adjunct treatment. Nonetheless, there is an opportunity for further investigation to investigate the possible use against COVID-19. Considering the safety evidenced in numerous experimental and few clinical studies, further studies are encouraged to recommend the clinical usage and pharmaceutical development of BCP.

CONCLUSION

BCP is a unique molecule in various ways, such as being dietary, devoid of psychotropic effects, possessing negligible toxicity, wide availability in plants, oral bioavailability, a druggable property, and functional receptor selectivity. BCP interacts or binds to different receptors, including CB2, PPAR-α, and PPAR-γ, opioid, histaminergic, TRPV, and TLR, and has enzyme inhibitory activities, including amylase, lipase, α-glucosidase, HMG-CoA reductase, acetylcholinesterase, secretase, cyclooxygenase, and nitric oxide synthase. Taken together, the receptor selectivity made it a distinctive candidate with a pharmacological rationale for pharmaceutical development, more than an antioxidant molecule, which is common with natural products-based nutraceuticals. Integrating the potent anti-inflammatory, immunomodulator, and antiviral properties of BCP and its potential benefits in pathological features of cardiovascular, neurological, gastrointestinal, hematological, renal, ocular, and cutaneous systems, which are the common accompaniments of SARS-CoV-2 infection, the benefits of BCP and plants rich in BCP may be important for COVID-19. The candidature of BCP in COVID-19 treatment may appear somewhat speculative but cannot be overlooked as it possesses favorable physiochemical and druggable properties with dietary use. However, the suggestion on the possible use of BCP in COVID-19 remains inconclusive until the *in-silico* observations could be confirmed in the experimental studies and further proof of the concept studies.

The polypharmacological properties, including receptor selectivity provide rationale and its drug-like properties, provide more realism for its future in drug discovery and development. Additionally, the antioxidant, anti-stress, and longevity potential provide a nutritional basis of its use to boost the immunity and suppress overt oxidative stress and subsequent hyperinflammatory states. Considering the recognition of safety status by USFDA and its favorable pharmacokinetic and physicochemical properties, BCP itself or plants containing a high amount of BCP may be important for nutritional or dietary usage. BCP and the plants containing BCP as a major ingredient may be candidates for developing novel antiviral and immunomodulator therapies for coronaviruses. However, further research is needed to address novel drug discovery employing the chemical scaffold or pharmacophores of BCP. The natural dietary availability and CB2 receptors mediated functional properties and selectivity are suggestive of developing BCP-based nutraceuticals and pharmaceuticals as candidate compounds for COVID-19 and other coronavirus diseases. The opinion of the authors on possible candidature for possible use of BCP in COVID-19 is solely based on available literature on the effects of BCP against infection, immunity, and inflammation in corroboration with the *in-silico* studies. The authors do not promote the use of BCP in any form for COVID-19 until clear evidence becomes available from proof of the concept studies.

AUTHOR CONTRIBUTIONS

SO conceptualized the study and hypothesis. NJ, CS, HH, and HJ performed literature search. NJ draw the schemes and drafted the artwork. SA drafted the tables. NJ, CS, HJ, CP, SG, and SO contributed significantly in editing the manuscript. All authors read, edited and approved the manuscript.

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REVIEW
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Anti–SARS-CoV-2 Natural Products as Potentially Therapeutic Agents

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Severe acute respiratory syndrome–related coronavirus-2 (SARS-CoV-2), a β-coronavirus, is the cause of the recently emerged pandemic and worldwide outbreak of respiratory disease. Researchers exchange information on COVID-19 to enable collaborative searches. Although there is as yet no effective antiviral agent, like tamiflu against influenza, to block SARS-CoV-2 infection to its host cells, various candidates to mitigate or treat the disease are currently being investigated. Several drugs are being screened for the ability to block virus entry on cell surfaces and/or block intracellular replication in host cells. Vaccine development is being pursued, invoking a better elucidation of the life cycle of the virus. SARS-CoV-2 recognizes O-acetylated neuraminic acids and also several membrane proteins, such as ACE2, as the result of evolutionary switches of O-Ac SA recognition specificities. To provide information related to the current development of possible anti–SARS-COV-2 viral agents, the current review deals with the known inhibitory compounds with low molecular weight. The molecules are mainly derived from natural products of plant sources by screening or chemical synthesis via molecular simulations. Artificial intelligence–based computational simulation for drug designation and large-scale inhibitor screening have recently been performed. Structure–activity relationship of the anti–SARS-CoV-2 natural compounds is discussed.

Keywords: SARS-CoV-2, ACE2 inhibitor, natural products, replication inhibitor, virus entry blocker

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INTRODUCTION

General Virology of Coronaviruses

The coronaviruses (CoVs) target humans and animals with exchangeable infectivity, causing a zoonotic outbreak. SARS-CoV-2 or 2019-nCoV spreads and causes the human life crisis of COVID-19 by infecting the human respiratory tract and causing pneumonia (Zhou et al., 2020). In addition, SARS-CoV-2 spreads by easy transmission among people, and COVID-19 patients exhibit flu-like symptoms such as fever and cough. Enveloped CoVs contain positive ssRNA genomes with relatively small RNAs (approximately 30 kb). They are classified into the *Riboviria–Nidovirales–Cornidovirineae–Coronaviridae–Orthocoronavirinae*–CoV genus (α-, β-, γ-, and δ-CoV). Most mammals are infected by α-CoV and β-CoV only, while avians and some mammals are infected by δ-CoV and γ-CoV. SARS-CoV-2, belonging to the β-CoV genus, and bat SARS-like CoV-ZXC-21 are similar in their RNA genomes. The COVID-19–causing CoV isolates exhibit 79% identity with the previously named SARS-CoV and 50% identity with the Middle East respiratory syndrome (MERS) virus (Chan et al., 2020).

SARS-CoV-2 viral proteins include RNA-dependent RNA polymerase (RdRp) and hemagglutinin-esterase (HE) enzymes as well as proteins including spike (S), envelope (E), membrane (M), and nucleocapsid (N) (**Figure 1**) (Tu et al., 2020). Nonstructural protein 3 (Nsp3), Nsp5, Nsp9, and Nsp12 RdRp are enzymes. The E-, S-, and M-proteins are embedded

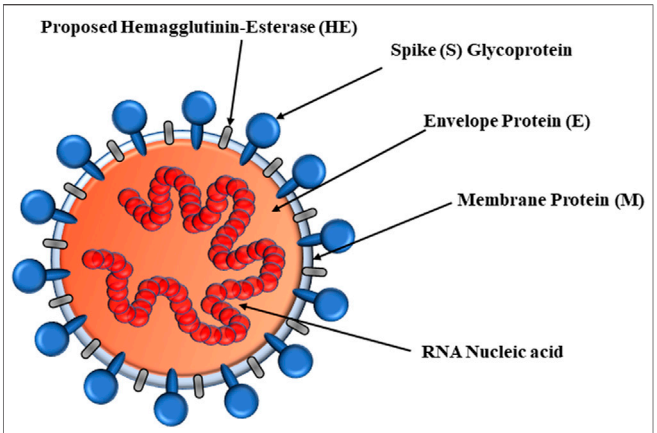

FIGURE 1 | Structure of enveloped coronavirus virion.

into the endoplasmic reticulum (ER) membrane and translocated to the ER–Golgi intermediate compartment (ERGIC). As the first step, the S-glycoprotein of SARS-CoV-2 binds to surfaced O-acetyl (Ac)-neuraminic acids of host cells. The neuraminic acid-O-Ac-esterase of HE evolved from the influenza C virus, nidoviruses, and salmon anemia virus (teleost orthomyxovirus). Fusion of S-glycoprotein and HE is important for CoV attachment to neuraminic acid–bearing host receptors (Tortorici et al., 2019). Therefore, the HE found in the β-CoV genus mediates viral attachment to O-Ac-neuraminic acids. The glycoproteins of the HA, HE, S, and HA-esterase-fusion protein (HEF) bind to the host receptor. However, certain α-CoV and γ-CoV are deficient of the neuraminic acid-O-Ac-esterases but bind to Ac-neuraminic acids or glycolyl-neuraminic acids. Murine CoVs esterize the C4-O-Ac (Smits et al., 2005). The SARS-CoV-2 S-glycoprotein N-terminal domain recognizes the surface entry site, binding to 9-O-Ac-neuraminic acid in a similar manner to CoV HEs as well as influenza C and D HEFs. Bovine CoV (BCoV) and human CoV (HCoV)-OC43 can recognize the 5,9-Ac2-neuraminic acids (Vlasak et al., 1988) and bear neuraminic acid 9-O-Ac-esterase. Most β-CoVs bind to the 9-O-Ac-neuraminic acids, but mutant strains target 4-O-Ac-neuraminic acids. Specifically, the HEs of β-CoVs recognize the 9-O-Ac-neuraminic acids, although certain species bind to the 4-O-Ac forms (Kim, 2020). In fact, 9-O-Ac-neuraminic acid is the recognition site for HCoV-OC43, β1-CoV, and SARS-CoV-2 S-glycoproteins, but MERS-CoV recognizes the α2,3-linkage. The CoV glycoproteins, BCoV HEs, and influenza virus HEFs are specific for 9-O-Ac-neuraminic acid (Mani et al., 2020; McKee et al., 2020), but the influenza HA is specific for glycolylneuraminic acids (Vogel et al., 2006). Upon interaction with neuraminic acid, host furin proteases cleave the S-glycoprotein to potentiate entry into host cells (Oliveira et al., 2017).

The current global COVID-19 pandemic is threatening the daily lives of human beings. The disease biology is a topic of interest. To overcome the disease, the academic society urgently needs to exchange the pandemic CoV-controlling drugs, but no truly effective agent has yet been discovered. In this review, antiviral candidate agents and the availability of natural compounds are discussed.

NATURAL PRODUCTS TO TARGET AND INHIBIT INFECTION OF CORONAVIRUSES

Recently, natural phytochemicals that exhibit anti-CoV activity have been extensively summarized (Li et al., 2005). LMW molecules exhibit antiviral activity. Recently, development of anti-CoV drugs has also been applied for molecular docking via simulation approaches. Computer-based artificial intelligence technology contributes to the development of anti-CoV agents. Human angiotensin-converting enzyme (ACE)-2, papain-like protease (PLpro), main 3C-like protease (3CLpro), RdRp, helicase, N7 methyltransferase, human DDP4, receptor-binding domain (RBD), cathepsin L, type II transmembrane (TM) Ser-protease, or transmembrane protease serine (TMPRSS)-2 is mainly targeted. CoV 3CLpro and PLpro are polyprotein-specific viral proteases. RdRp is a complementary RNA strand synthetic replicase. Remdesivir inhibits RdRp in the ssRNA genome of CoV, where the RdRp mediates RNA replication and remdesivir acts as an ATP analog and thus inhibits RdRp.

Currently, effective anti-CoV agents are not available, although several drugs have been prescribed and some natural compounds exhibit antiviral activity. Natural resources are a tremendous treasure trove of chemical compounds that are applicable for various viral infections. Natural products are produced by the metabolic pathways of a given organism, but humans utilize them for their benefits from the modern view of pharmacology. Therefore, phytochemicals have been screened to test their effectiveness against viruses, and some natural products inhibit the infection and amplification of viruses with a broad antiviral spectrum (Pour et al., 2019). Naturally occurring compounds such as artemisinin, baicalin, curcumin, rutin, glycyrrhizic acid, hesperidin, hesperetin, and quercetin have been examined for their anti-CoV activity by various assay based on the viral life cycle. However, none of the natural compounds have direct antiviral activity against CoVs or other RNA viruses. Only GA has been frequently described to be the most active component in several previous articles. Indeed, the molecular action mechanisms of the natural products are not specific because current candidates of natural antiviral agents are mainly examined by using *in vitro* cell-based assays or computer modeling through docking simulation before application to animal and clinical studies (Li et al., 2005; Packer and Cadenas, 2011). Conventional approaches to natural products were a mix of chemical analysis and structure–function relationship analysis. Recent AI-aided approaches combined with *in silico* computational simulation is cost-effective for the prediction of chemical compounds (Müller et al., 2018). Currently, new concepts of AI-aided *in silico* computational approaches have evolved for drug prediction based on drug candidate–ligand/receptor interaction. These approaches utilize known structures of the molecules to predict *in silico* docking molecules. Fundamental limitations of these AI *in silico*

approaches have also been identified. In fact, in blocking or inhibiting viral proteases, S-glycoprotein, and the entry of SARS-CoV-2, several plant compounds have been suggested through computer simulation techniques (Li et al., 2005).

Natural compounds including chemoenzymatic modified molecules can be used in ethnopharmacology to modulate SARS-CoV-2/nCoV-19 infections due to current limited therapeutic options. The efficacy of natural products depends on the CoV strains. Several natural products inhibit viral replication (Müller et al., 2020), implying antiviral properties. (Kalhori et al., 2021) For example, several compounds exhibit promising prospects for CoV treatment in human patients, as described above for lycorine, scutellarein, silvestrol, tryptanthrin, saikosaponin B2, and polyphenolic compounds such as caffeic acid, isobavachalcone, myricetin, psoralidin, and quercetin, as well as lectins such as griffithsin. For example, *Lycoris radiata* (L'Hér.) Herb lycorine shows cytopathogenic and antiviral activities against SARS-associated CoV (Yu et al., 2012). Currently known natural products that are pharmacologically effective for SARS-CoV-2 inhibition are shown in **Figure 2**, with the synthetic compounds previously utilized for other targets in humans.

Natural products that show viral inhibitory activity are promising candidates as anti-CoV agents. Natural products to combat the CoVs are reviewed in this article, focusing on the general properties of CoVs and suggesting applicable drugs and natural compounds effective against several CoV species. Viral proteins such as 3CLpro, PLpro, N, S, and ACE2 have been targeted for antiviral replication or anti-infection. Some limited antiviral agents as inhibitors specific for proteases and RNA synthases are known to block viral replication (Häkkinen et al., 1999). CoV bioactive natural products can also enhance and strengthen host immunity. Vitamins A and C lower susceptibility to infections and help in the prevention of viral infections through host immune function (Häkkinen et al., 1999).

Inhibition of Ribonucleic Acid Helicase eIF4A and Protein Expression

SARS-CoV helicase, a virus replication enzyme, is involved in the unwinding of RNA. Helicases in protein sequences are commonly conserved during evolution in CoVs and other related nidoviruses. CoV helicase is an important therapeutic target because it hydrolyzes all deoxyribonucleotide and ribonucleotide triphosphates in SARS-CoV. Therefore, SARS-CoV-2 helicase has been targeted to screen for inhibitors. The 420-amino acid–long helicase is phylogenetically homologous to the helicases of other CoVs. Favipiravir or hydroxychloroquine, described later in further detail, recognizes SARS-CoV-2 helicase with weak affinities.

Aglaia sp. silvestrol inhibits the replication of MERS types with an EC50 value of 1.3 nM, acting as an inhibitor of RNA helicase eIF4A and protein expression via blocking replication/transcription complex formation (Miean and Mohamed, 2001). Silvestrol inhibits HcoV-229E protein synthesis with an EC50 of 3 nM. Silvestrol also inhibits HCoV-229E *ex vivo* in bronchial epithelial cells via RNA helicase eIF4A inhibition (Lau et al., 2008). The polyphenolic compounds myricetin and scutellarein inhibit the helicase activity of SARS-CoVs. Phenolic compounds including myricetin and scutellarein of *Isatis indigotica* Fort. and *Torreya nucifera* L. inhibit SARS-CoV helicases including nsP13 helicase (Cho et al., 2013). *Scutellaria baicalensis* Georgi (Scutellaria radix) myricetin and scutellarein inhibit the ATPase activity of the SARS-CoV helicase Nsp-13 (Yu et al., 2012). Myricetin is enriched in fruits such as cranberry *Vaccinium oxycoccos* L. (Mikulic-Petkovsek et al., 2012) and in vegetables such as *Calamus scipionum* Lam. and garlic (Qing et al., 2016). Scutellarein from *S. baicalensis* is a strong inhibitor of SARS-CoV helicase because it inhibits SARS-CoV helicase Nsp13 via ATPase activity inhibition but not via direct inhibition of helicase activity. The flavonoid quercetin is structurally similar to other polyphenolics such as myricetin and scutellarein and shows similar inhibitory activity of SARS-CoV helicase (Yang et al., 2010). In addition, naturally occurring tomentins of *Paulownia tomentosa* (Thunb.) Steud., belonging to Scrophulariaceae, reversibly and allosterically inhibit the PLpro activity of SARS-CoV (Lung et al., 2020).

Inhibition of Ribonucleic Acid Genome Synthesis and Replication

RdRp, also known as nsp12, synthesizes a complementary RNA strand by using the original virus RNA genome as template. Inhibition of the SARS-CoV-2 RdRp enzyme is a potential therapeutic option for COVID-19 patients. Tryptanthrin inhibits viral RNA synthesis and PLpro-2 enzyme activity, important for the early, late, and post-entry step of HCoV replication. *Strobilanthes cusia* (Nees) Kuntze tryptanthrin, an indoloquinazoline moiety–carrying alkaloid, and the indigodole B (5aR-ethyltryptanthrin) alkaloid (Wen et al., 2007) have anti–HCoV-NL63 activity. *S. cusia* tryptanthrin and indigodole B also block RNA synthesis and PLpro-2 enzyme activity. Tryptanthrin is effective against SARS-CoV-2 and other HCoVs. The antiviral EC50 values were 1.52 μM for tryptanthrin and 2.60 μM for indigodole B. Tryptanthrin also has multiple pharmacological activities (Chen et al., 2008). In addition, tryptanthrin and indigodole B have direct antiviral activities to HCoV-NL63. *Houttuynia cordata* Thunb. aqueous extracts inhibit the enzyme activities of the viral 3CL protease and viral RdRp of SARS-CoV (Hoever et al., 2005). In computer modeling, *Camellia sinensis* (L.) Kuntze theaflavin (TF), 3,4,5-trihydroxy-1,8-bis [(2R,3R)-3,5,7-trihydroxy-2-chromanyl]-6-benzo (Kim, 2020) annulenone ($C_{29}H_{24}O_{12}$), has been demonstrated to interact with the SARS-CoV-2 RdRp (Zhang et al., 2020). The TFs, known as antioxidant polyphenols, are formed from the precursor flavan-3-ols, which occur in green tea leaves, via condensation and enzymatic oxidation. Several derivatives, including TF-3′-gallate, TF-3-gallate, and TF-3–3′-di-gallate, are known. The TFs belong to the thearubigins, polymeric polyphenols, which show a red color, with a tropolone moiety.

Similarly, redwood *Sequoia sempervirens* (D.Don) Endl. natural phenol ferruginol compounds have various terpenoid substructures, such as betulonic acid [$C_{30}H_{46}O_3$; 3-oxolup-20

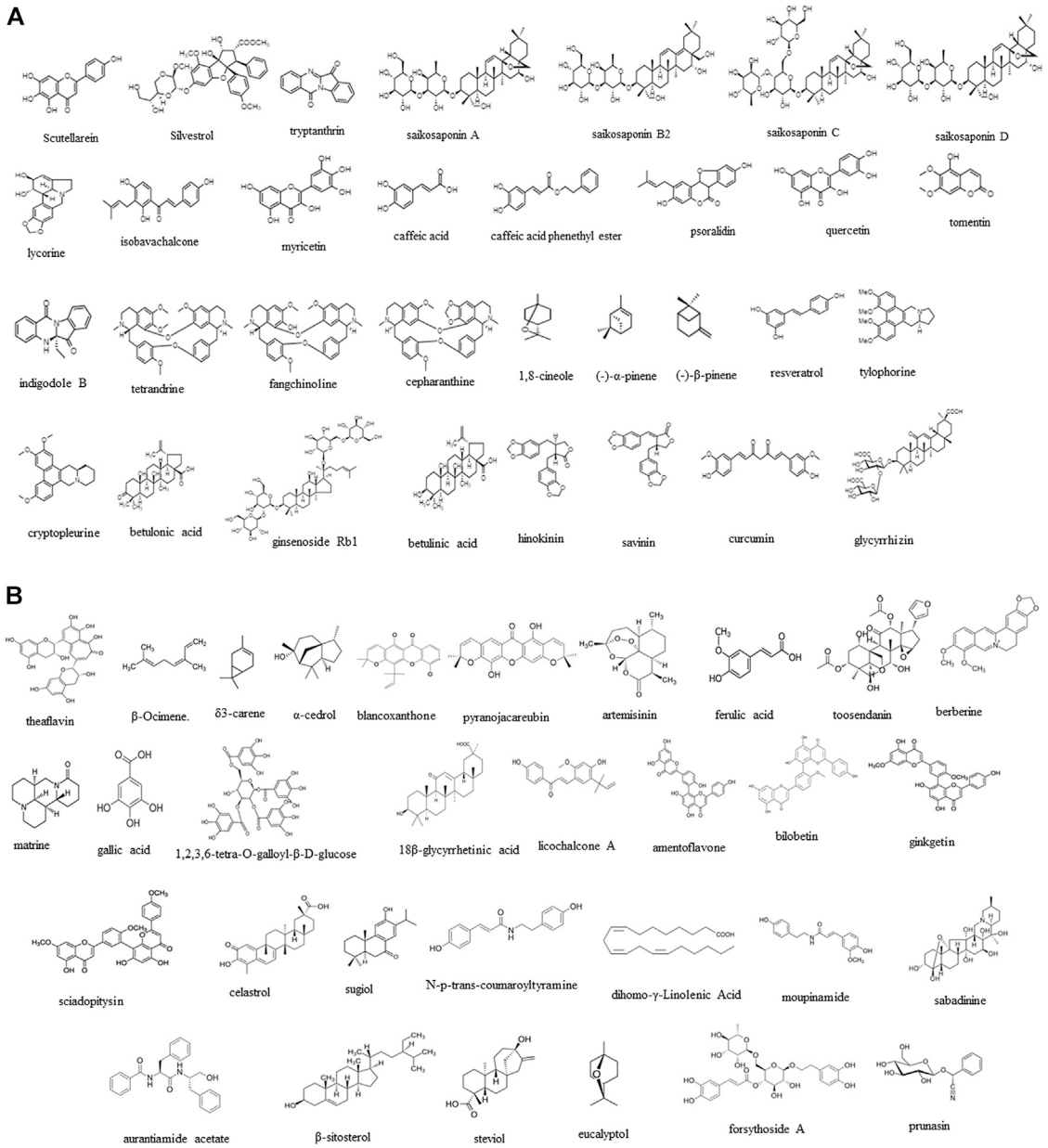

FIGURE 2 | (A–E) Molecular structures experimentally effective for SARS-CoV therapeutic target. Scutellarein (CAS No: 529–53-3), silvestrol (CAS No: 697235-38-4), tryptanthrin (CAS No: 13220-57-0), saikosaponin A (CAS No: 20736-09-8), saikosaponin B2 (CAS No: 58316-41-9), saikosaponin C (CAS No: 20736-08-7), saikosaponin D (CAS No: 20874-52-6), lycorine (CAS No: 476-28-8), isobavachalcone (CAS No: 20784-50-3), myricetin (CAS No: 529-44-2), caffeic acid (CAS No: 331-39-5), caffeic acid phenethyl ester (CAS No: 104594-70-9), psoralidin (CAS No: 18642-23-4), quercetin (CAS No: 117-39-5), tomentin (CAS No: 28449-62-9), indigodole B, theaflavin (CAS No: 4,670–05-7), β-ocimene (CAS No: 3,338–55-4), δ3-carene (CAS No: 13,466–78-9), α-cedrol (CAS No: 77–53-2), blancoxanthone (PubChem CID: 11703574), pyranojacareubin (CAS No:78,343–62-1), artemisinin (CAS No: 63,968–64-9), ferulic acid (CAS No: 1,135–24-6), toosendanin (CAS No: 58,812–37-6), berberine (CAS No: 633–65-8), matrine (CAS No: 519–02-8), gallic acid (CAS No:149–91-7), 1.2,3,6-tetra-O-galloyl-β-D-glucose (CAS No: 79,886–50-3), 18β-glycyrrhetinic acid (CAS No: 471–53-4), licochalcone A (CAS No: 58,749–22-7), amentoflavone (CAS No:1,617–53-4), bilobetin (CAS No: 521–32-4), ginkgetin (CAS No: 481–46-9), sciadopitysin (CAS No: 521–34-6), celastrol (CAS No: 34,157–83-0), sugiol (CAS No: 511–05-7), N-*p*-trans-coumaroyltyramine (CAS No: 36,417–86-4), dihomo-γ-linolenic acid (CAS No: 1783–84-2), moupinamide (CAS No: 66,648–43-9), sabadinine (CAS No: 5,876–23-3), aurantiamide acetate (CAS No: 56,121–42-7), β-sitosterol (CAS No: 83–46-5), steviol (CAS No: 471–80-7), eucalyptol (CAS No: 470–82-6), forsythoside A (CAS No: 79,916–77-1), prunasin (CAS No: 99–18-3), tetrandrine (CAS No: 518–34-3), fangchinoline (CAS No: 436–77-1), cepharanthine (CAS No: 481–49-2), 1,8-cineole (CAS No: 470–82-6) (-)-α-pinene (CAS No: 7,785–70-8), (-)-β-pinene (CAS No: 18,172–67-3), ginsenoside Rb1 (CAS No: 41,753–43-9), resveratrol (*trans*-3,5,4′-trihydroxystilbene), homoharringtonine (CAS No: 501–36-0), tylophorine (CAS No: 482–20-2), cryptopleurine (CAS No: 482–22-4), betulonic acid (CAS No: 4,481–62-3), betulinic acid (CAS No: 472–15-1), hinokinin (CAS No: 26,543–89-5), savinin (CAS No: 493–95-8), curcumin (CAS No: 458–37-7), glycyrrhizin (CAS No: 1,405–86-3), neohesperidin (CAS No: 13,241–33-3), hesperidin (CAS No: 520–26-3), emodin (6-methyl-1,3,8-trihydroxyanthraquinone) (CAS No: 518–82-1), neoandrographolide (CAS No: 27,215–14-1), kouitchenside I (CAS No: 1444411-79-3), isobavachalcone (CAS No: 20,784–50-3), luteolin (CAS No: 491–70-3), 7-methylluteolin, apigenin (CAS No: 520–36-5), kaempferol (CAS No:

(*Continued*)

FIGURE 2 | 520–18-3), baicalin (CAS No: 491–67-8), wogonoside (CAS No: 51,059–44-0), ebselen (CAS No: 60,940–34-3), afzelin (CAS No: 482–39-3), biorobin (CAS No: 17,297–56-2), δ-viniferin (CAS No: 253,435–07-3), taiwanhomoflavone A (CAS No: 265,120–00-1), lactucopicrin 15-oxalate (CAS No: 303,130–75-8), nympholide A (CAS No: 604,004–58-2), phyllaemblicin B (CAS No: 307,504–07-0), tanshinone I (CAS No: 568–73-0), cryptotanshinone (CAS No: 35,825–57-1), dihydrotanshinone I (CAS No: 87,205–99-0), rosmariquinone (CAS No: 27,210–57-7), tannic acid (CAS No: 1,401–55-4), 3-isotheaflavin-3-gallate (CAS No: 30,462–34-1), theaflavin-3,3′-digallate (CAS No: 33,377–72-9), sinigrin (CAS No: 3,952–98-5), hesperetin (CAS No: 520–33-2), β-sitosterol (CAS No: 83–46-5), bavachalcone (CAS No: 28,448–85-30), broussochalcone A (CAS No: 99,217–68-2), broussochalcone B (CAS No: 28,448–85-3), 4-hydroxyisolonchocarpin (CAS No: 41,743–38-8), papyriflavonol A (CAS No: 363,134–28-5), 3′-(3-methylbut-2-enyl)-3′,4,7-trihydroxyflavane, kazinol A (CAS No: 99,624–28-9), kazinol B (CAS No: 99,624–27-8), kazinol F (CAS No: 104,494–35-1), kazinol J (CAS No: 104,778–05-4), broussoflavan A (CAS No: 99,217–69-3), tomentin A/B/C/D/E (CAS No: 36,034–36-3), 3′-O-methyldiplacol, 4′-O-methyldiplacol, 3′-O-methyldiplacone (CID No: 14,539,951), 4′-O-methyldiplacone (CID No: 24,854,122), mimulone (CAS No: 97,126–57-3), diplacone (CAS No: 73,676–38-7), bavachinin (CAS No: 19,879–30-2), neobavaisoflavone (CAS No: 41,060–15-5), isobavachalcone (CAS No: 20,784–50-3), 4′-O-methylbavachalcone (CAS No: 0,784–60-5), psoralidin (CAS No: 18,642–23-4), corylifol A (CAS No: 775,351–88-7), stictic acid (CAS No: 549–06-4), pristimerin (CAS No: 1,258–84-0), tingenone (CAS No: 50,802–21-6), iguesterin (CAS No: 53,527–47-2), silymarin (silibinin) (CAS No: 22,888–70-6), daidzein (CAS No: 486–66-8), genistein (CAS No: 446–72-0), formononetin (CAS No: 485–72-3), biochanin A (CAS No: 491–80-5), linolenic acid (CAS No: 463–40-1), palmitic acid (CAS No: 57–10-3), hydroxytyrosol (CAS No: 10,597–60-1), *cis-p*-coumaric acid (CAS No: 501–98-4), cinnamaldehyde (CAS No: 14,371–10-9), thymoquinone (CAS No: 490–91-5), hydroxytyrosol (CAS No: 10,597–60-1), eckol (CAS No: 88,798–74-7), 7-phloroeckol, dieckol (CAS No: 88,095–77-6), phlorofucofuroeckoln (CAS No: 128,129–56-6), juglanin (CAS No: 5,041–67-8), catechin gallate (CAS No: 1,257–08-5), (–)-gallocatechin gallate (CAS No: 5,127–64-0), rutin (CAS No: 153–18-4), cinanserin (CAS No: 1,166–34-3), sivestrol (CAS No: 697,235–38-4), ouabain (CAS No: 630–60-4), tetrandrine (CAS No: 518–34-3), fangchinoline (CAS No: 33,889–68-8), cepharanthine (CAS No: 481–49-2), diterpene (CAS No: 28,957–10-0), diterpene aldehyde, sesquiterpene (CAS No: 72,826–63-2), triterpene (CAS No: 125,343–14-8), lignan (CAS No: 6,549–68-4), halitunal (CAS No: 133,076–08-1), antibacterial agents such as pivampicillin (CAS No: 33,817–20-8), hetacillin (CAS No: 3,511–16-8), cefoperazone (CAS No: 62,893–19-0), clindamycin (CAS No: 18,323–44-9), antidiabetic drug troglitazone (CAS No: 97,322–87-7), antihypertensive drug losartan (CAS No: 114,798–26-4), analgesia drug ergotamine (CAS No: 113–15-5), antibacterial drug cefmenoxime (CAS No: 65,085–01-0), and hepatoprotective drug silybin (CAS No: 22,888–70-6).

(29)-en-28-oic acid; CAS No: 4481-62-3; CID 122844] and betulinic acid [$C_{30}H_{48}O_3$; (3β)-3-hydroxy-lup-20 (29)-en-28-oic acid; CAS No: 472-15-1; CID 64971]; 8β-hydroxyabieta-9 (11),13-dien-12-one; 3β,12-diacetoxyabieta-6,8,11,13-tetraene; curcumin; hinokinin; and savinin. These compounds inhibit the replication of SARS-CoV (Loizzo et al., 2008). *Toona sinensis* (Juss.) M.Roem. extracts inhibit SARS-CoV replication (Jeong et al., 2014). Glycyrrhizin inhibits the replication of SARS-CoV after viral entrance into Vero cells, inhibiting virus attachment and entry (Shen et al., 2005). Several glycyrrhizin-derived compounds inhibit SARS-CoV replication more effectively (EC50 of 5–50 μM), but they are highly cytotoxic to Vero cells (Hoever et al., 2005). Lignin, betulinic acid, and desmethoxyreserpine inhibit viral replication as well as 3CLpro (Cheng et al., 2006). Especially desmethoxyreserpine blocks virus entry. *Laurus nobilis* L. and *Thuja orientalis* (L.) Franco essential oils are also inhibitors of viral replication. For example, *L. nobilis* β-ocimene (3,7-dimethyl-1,3,6-octatriene; CAS 502-99-8), 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo-2.2.2-octane; CAS No: 470-82-6), *α*-pinene, and *β*-pinene, as well as *T. orientalis α*-pinene, δ3-carene, and *α*-cedrol inhibit viral replication (McDonagh et al., 2014).

β-Ocimene acts as a plant defense and antifungal agent and is derived from the plant genus *Ocimum*. Pinene ($C_{10}H_{16}$) is a bicyclic monoterpene. 1,8-Cineole oil, known as eucalyptol, is a cyclic ether and a monoterpenoid. Its synonyms are cajeputol; 1,8-epoxy-*p*-menthane; 1,8-oxido-*p*-menthane; and 1,3,3-trimethyl-2-oxabicyclo octane (Chan et al., 2020; Chan et al., 2020; Chan et al., 2020). *a*-Pinene is also a major constituent of the essential oil of *Rosmarinus officinalis* L (rosemary). Two enantiomers (1S,5S)- or (–)-α-pinene and (1R,5R)- or (+)-α-isomer are present. δ3-Carene or 3-carene is also a bicyclic monoterpene and has a pungent odor. *α*-Cedrol is a sesquiterpene alcohol and an essential oil component. It is an antioxidant with antiseptic, anti-inflammatory, anti-spasmodic, tonic, astringent, diuretic, sedative, insecticidal, and antifungal activities (Kim et al., 2008) and has been used in traditional medicine. *Calophyllum blancoi* Planch. & Triana pyranoxanthones such as blancoxanthone ($C_{23}H_{22}O_5$; 5,10-dihydroxy-2,2-dimethyl-12-(2-methylbut-3-en-2-yl)-2H,6H-pyrano[3,2-b]xanthen-6-one) and pyranojacareubin inhibit HCoV-229E–infected host cell growth (Kim et al., 2010). The pyranoxanthones tested against HCoV-229E reverse *in vitro* virus-induced cytopathic effects. Blancoxanthone exhibits viral inhibition at 3 ug/ml in MRC-5 cells. *Bupleurum* sp., *Heteromorpha* sp., and *Scrophularia scorodonia* L. saikosaponin compounds interfere with the early replication step and entrance of HCoV-229E (Ulasli et al., 2014).

For animal CoVs such as feline CoVs, LMW molecules including artemisinin, baicalin, curcumin, quercetin, rutin, glycyrrhizic acid, hesperidin, and hesperitin inhibit replication of feline viruses (FCoVs) such as feline infectious peritonitis virus (FIPV)-1146 and FECV1683 via cytotoxicity of the virus-infected cells (O'Flaherty et al., 2019). For murine hepatitis virus (MHV) CoVs, ferulic acid, isoferulic acid, toosendanin, berberine, protoberberine alkaloids, matrine, oxymatrine, sophoranone, and sophocarpine isolated from *Cimicifuga racemosa* L., *Melia* sp., *Coptis* sp., *Phellodendron* sp., and *Sophora subprostrata* Chun & T. Chen. (Fabaceae) inhibit the replication of the murine hepatitis virus (MHV)-A59 strain (Song et al., 2014; Jin et al., 2019; Sun et al., 2019). Methanol extracts from the plants of *Sophorae* sp., *Acanthopanacis* sp., *Sanguisorbae* sp., and *Torilis* sp. possibly inhibit RdRp or other protease activity of MHV-A59. *Nigella sativa* L. and *Citrus sinensis* L. ethanol extract inhibits viral replication of MHV-A59 via an undetermined mechanism (Jin et al., 2019).

Artemisinin, isolated from *Artemisia annua* L. in 1972 by Dr. Tu Youyou, co-recipient of the 2015 Nobel Prize in Medicine, is an anti–*Plasmodium falciparum* malaria drug. It is a sesquiterpene lactone with an endoperoxide 1,2,4-trioxane ring, which is necessary to exert its activity. Artemisinin is a potential therapeutic candidate for certain RNA viruses (de Vries et al., 1997). Ferulic acid, as a hydroxycinnamic acid and a component of lignin, is a major metabolite of chlorogenic acid

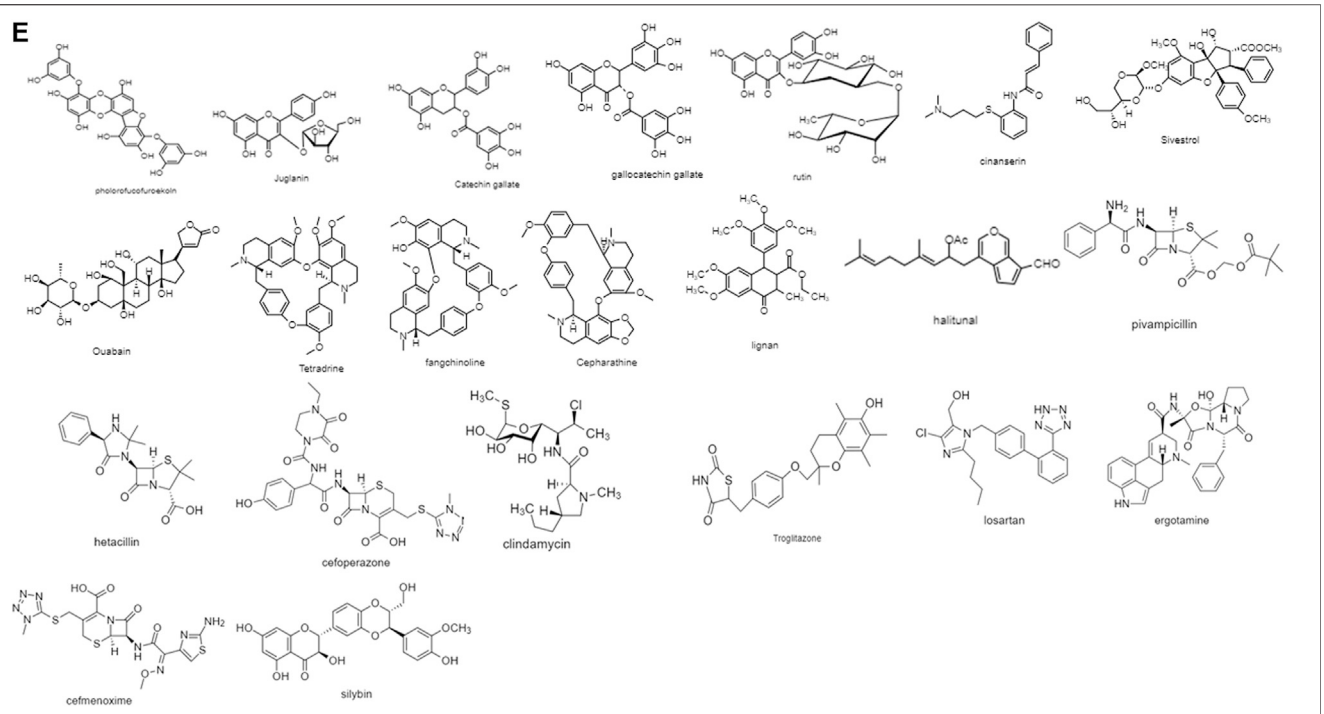

FIGURE 2 | (Continued).

along with caffeic acid (CA) and isoferulic acid. Ferulic acid and its derivatives, including caffeoyltyramine, feruloyltyramine, and feruloyloctopamine, also inhibit SARS-CoV PLpro activity (Li, 2015). Recently, Adem et al. (2020) described that CA derivatives such as khainaosides, 6-O-caffeoyl-arbutin, and vitexfolin have been suggested to be inhibitory candidates with higher binding activities than that of nelfinavir against SARS CoV-2 S-protein as well as Nsp15 and Mpro enzymes by using molecular docking simulation via Web engines named Toxtree and www.swissadme.ch (Adem et al., 2021). Toosendanin ($C_{30}H_{38}O_{11}$), a triterpenoid isolated from the bark of *Melia azedarach* L., has analgesic, insecticidal, anti-botulinum, antimicrobial, and anti-inflammatory activities, and antiviral RNA polymerase complex activities (Simmons et al., 2013). Matrine, an alkaloid of *Sophora flavescens* Aiton, inhibits IL-1β expression and MyD88/NF-κB and NLRP3 inflammasome in the inflammatory response in porcine respiratory syndrome virus–infected pigs (Hulswit et al., 2019). Using a structure- and activity-based computational approach, natural products have been analyzed for the Nsp-9 (PDB ID-6W4B) enzyme inhibition of SARS-CoV-2 RNA replication and S-protein binding (Chandel et al., 2020). Baicalin exhibits binding affinity to both S-protein and Nsp9 enzyme.

Inhibition of N-Protein and S-Glycoprotein Synthesis and Replication

Modulation of S-Glycoprotein

S-glycoprotein recognizes the host cell receptor to enter the cells through endosomal fusion, after which the S-glycoprotein is cleaved, endosomal membranes released, and RNA liberated into the cytosol (Langereis et al., 2012). S-glycoprotein interacts with its receptors via its RBD and plays a role in host tropism and pathogenicity, and in proposing some therapeutic clues (Xiong et al., 2013). Therefore, modulation of the S-glycoprotein is a potential target to control SARS-CoV-2 propagation. Tetrandrine, fangchinoline, and cepharanthine as bis-benzylisoquinoline alkaloids from *Stephania tetrandra* var. *glabra* Maxim. protect cells from virus-induced cell death. In addition, they inhibit viral replication, as well as CoV S-glycoprotein and N-protein synthesis. Also, they induce the virus-induced host response by the p38MAPK pathway. Terpenoid compounds such as *α*- and *β*-pinene as well as cineole interact with the infectious bronchitis virus (IBV) N-protein to inhibit the N-protein–RNA interaction and block IBV replication. The terpenoids bind to the N-terminal active site of the N-protein. The active site is composed of five amino acid residues (Yang et al., 2011; Müller et al., 2020). These conserved amino acids in the active sites are commonly located in various IBVs. CA from *Sambucus javanica* subsp. *chinensis* Fukuoka (elderberry) extract inhibits the HCoV strain HCoV-NL63 (Weng et al., 2019). CA inhibits HCoV S-glycoprotein attachment to host cells. Chlorogenic acid and gallic acid (3,4,5-trihydroxybenzoic acid) also exhibit the same activities as CA. Gallic acid is a trihydroxybenzoic acid and forms dimeric ellagic acid. Tannins are hydrolyzed to glucose and gallic acid (gallotannin), or glucose and ellagic acid (ellagitannin). CA also inhibits the hepatitis B virus (Wang et al., 2009). *Sambucus nigra* L. extract (black elderberry) has been used for

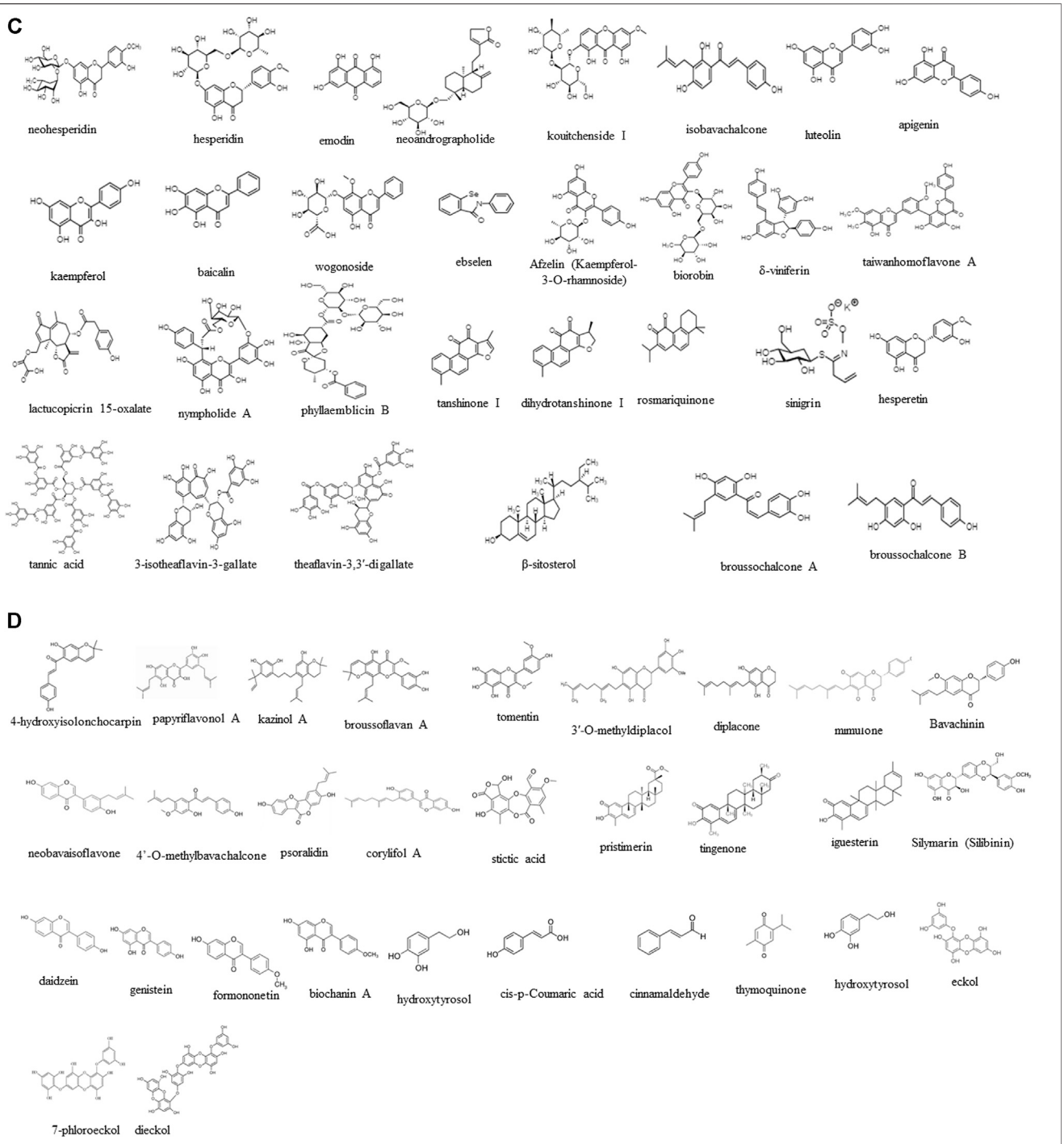

FIGURE 2 | (Continued).

treating cold and flu symptoms. The adsorption, bioavailability, metabolism, and delivery mechanism of the extracts are documented for therapeutic plasma concentrations (Wittemer et al., 2005).

Four saikosaponins inhibit human CoV-229E infectivity. These saikosaponin pentacyclic triterpenoid glycoside derivatives purified from *Bupleurum* spp., *Heteromorpha* spp., and *S. scorodonia* L. also have anti-HIV and anti-HCoV-22E9 activities *in vitro* (Ushio and Abe, 1992; Chiang et al., 2003; Ulasli et al., 2014). Saikosaponins show anti-CoV activity, and saikosaponin B2 also shows the highest potency with an EC50 of 1.7 μM and inhibits the early stage of CoV viral attachment to host receptors via S-glycoprotein and penetration into the cells. The *Streptomyces parvulus* actinomycin D antibiotic also inhibits

CoV attachment and penetration stages (O'Flaherty et al., 2019). *Panax ginseng* (T.Nees) C.A. Mey. ginsenoside Rb1 (gynosaponin C) as steroidal glycosides and triterpene saponins exhibit antiviral activity (Wu et al., 2004). *Stephania tetrandra* var. *glabra* bis-benzylisoquinoline alkaloid compounds such as tetrandrine, fangchinoline, and cepharanthine exhibit antiviral activity on HCoV-OC43 (Kim et al., 2019). They inhibit virus-induced cell death via blocking of virus replication and S-glycoprotein and N-protein synthesis with the host response. Resveratrol (CAS number: 501–36-0), a stilbenoid, inhibits MERS-CoV replication and infection in a cell-based system by inhibition of MERS-CoV N-protein expression and MERS-CoV–induced host cell death (Lin et al., 2017). Resveratrol inhibits SARS-CoV-2 infection.

The NIH clinical collection of 727 tested antiviral compounds showed that the alkaloid omacetaxine (homoharringtonine) shows a nonomolar IC50 level (Cao et al., 2015). Two alkaloids of *Tylophora indica* (Burm. f.) Merr., tylophorine and 7-methoxycryptopleurine, inhibit transmissible gastroenteritis CoV replication (Yang et al., 2010). The *T. indica* alkaloids tylophorine and 7-methoxycryptopleurine block replication in CoV-infected cells of swine testicular tissues (Cho et al., 2006). 7-Methoxycryptopleurine (IC50 of 20 nM) is rather more efficient than tylophorine (IC50 of 58 nM). Tylophorine also blocks virus RNA replication and NF-κB activation mediated by cellular JAK phosphorylation in CoV (Yang et al., 2017). Tylophorine and 7-methoxycryptopleurine inhibit N- and S-glycoprotein activity. Dihydrotanshinone recognizes the S-glycoprotein of SARS-CoV-2 to block its entry (Zhang et al., 2020). *Rhus chinensis* Mill. luteolin and tetra-O-galloyl-β-D-glucose (TGG) specifically recognize the S2 subunit and prevent viral entry of SARS-CoV (Yi et al., 2004). Luteolin also binds to the S2 protein to exert its antiviral capacity by interfering with virus–cell attachment and consequent fusion. TGG and luteolin exhibit anti–SARS-CoV activities. Therefore, LMW natural products, which bind to the SARS-CoV S-glycoprotein, can block virus infection in its host cells.

For animal CoVs such as avian IBV, *Alstonia scholaris* (L.) R. Br. alstotide-1 and -3 interfere with membrane proteins and S-glycoproteins but not the nucleocapsid proteins of avian IBV (Nguyen et al., 2015). These peptide-derived drugs are potentially applicable for therapeutic characteristics, although they are poor in oral bioavailability. However, alstotides are suggested to be permeable to cells, stable, and nontoxic with anti-IBV activities. Alstotide-1 interacts with the IBV M-protein during the assembly and budding of virus particles. M-protein is a glycosylated and membrane-spanning protein. The alstotide-1 and M-protein interaction implicates that alstotide-1 inhibits the assembly and budding of virus particles. *Punica granatum* L. polyphenols also interact with the surface S-glycoprotein of murine CoV, MHV-A59 (Sundararajan et al., 2010).

Inhibition of Interaction of S-Glycoprotein With ACE2

The β-CoV SARS-CoV recognizes ACE2 in respiratory epithelial or type I and II alveolar epithelial cells of the lung in membrane-bound and soluble forms (Alifano et al., 2020). ACE2 is a type I membrane-anchored carboxypeptidase with an N-terminal signal peptide. The host SARS-CoV-2 receptor ACE2 in the renin–angiotensin system (RAS) plays a role in lung infection through removal of the barrier. The SARS-CoV-2 S-glycoprotein recognizes ACE2. ACE2 is necessary for a virus receptor. The receptor-binding motif (RBM) recognizes human ACE2 (Li, 2015; Gheblawi et al., 2020). The α-CoV HCoV-NL63 and the lineage B β-CoV SARS-CoV S-glycoproteins are well known to bind to ACE2, but β-CoV MERS virus is not specific for the ACE2 recognition, while the α-CoV HCoV-NL63 is specific for the ACE2 recognition. Thus, the S viral protein drives the first attachment step on respiratory cell surfaces. This is a therapeutic target. Host ACE2 is the known host site for the S-glycoprotein RBD. The RBD sequence of the SARS-CoV-2 S-glycoprotein is homologous to the RBD of the SARS-CoV S-glycoprotein. ACE2 is also a SARS-CoV-2 drug target. To date, the ACE2 protein can be recognized by the antidiabetic troglitazone, antihypertensive losartan, anti-analgesic ergotamine, antibacterial cefmenoxime, and hepatic-protective silybin. *Phyllanthus emblica* L. phyllaemblicin G7, the genus *Swertia*, *Citrus aurantium* L. xanthones, neohesperidin, and hesperidin bind to the ACE2 protein, but not to the ACE2–S-protein RBD interface. A flavonoid hesperidin isolated from citrus peel interacts with the SARS-CoV-2 receptors (Meneguzzo et al., 2020). In molecular docking analysis, flavonoids and anthraquinones exhibit binding capacities to ACE2. Their binding sites of ACE2 protein are different from that of the viral S-protein. For example, the flavone chrysin (CID: 5281607) isolated from the medicinal plant *Oroxylum indicum* binds to the ACE2 protein *in silico* (Basu et al., 2020).

The S-glycoprotein cleavage TMPRSS2 enzyme potentiates SARS- and MERS-CoVs infections. Several antibacterial agents such as pivampicillin, hetacillin, cefoperazone, and clindamycin, and antiviral kouitchenside I potentially inhibit the TMPRSS2 enzyme (Wu et al., 2020). The anthraquinone emodin (CAS No: 518-82-1) blocks the SARS-CoV S-glycoprotein and ACE2 interaction (Ho et al., 2007). Glycyrrhizin-modified compounds such as 18β-glycyrrhetinic acid are known to be anti–SARS-CoV agents due to their cytopathogenic effects (Haiying et al., 2003; Hoever et al., 2005). 18β-Glycyrrhetinic acid is a glycyrrhizin metabolite that is converted by intestinal microbes in humans and is an inhibitor of the complement cascade. 18β-Glycyrrhetinic acid inhibits DNA polymerases and suppresses TNF-α expression. Glycoside chain modification of glycyrrhizin with 2-acetamido-β-D-glucopyranosylamine increased its antiviral activity by 10-fold, through increased interaction with the S-glycoprotein. Glycyrrhizin and its derivatives, such as 18β-glycyrrhetinic acid and licochalcone A, are constituents of *Glycyrrhiza uralensis* Fisch. (licorice), *G. glabra* L., or *G. inflata* Bat. (Fu et al., 2016).

In addition, 18β-glycyrrhetinic acid and licochalcone A bind to the ssRNA virus nucleoprotein (NP), a target candidate for therapeutic development, because these natural ligands influence the RNA-binding property of NP. The two agents specifically recognize the RNA-binding groove of NP (PDB code 4Z9P) and disrupt the NP–viral ssRNA interaction through a

conformational shift of NP oligomers to impair ssRNA assembly. Glycyrrhizin and glycyrrhetinic acid also block SARS-CoV replication (Cinatl et al., 2003). In addition, glycyrrhizin inhibits H5N1 influenza A virus replication (Michaelis et al., 2011). Glycyrrhizin and glycyrrhetinic acid are also anti-inflammatory, antiviral, and anti-allergic agents. Licochalcone A is a natural phenolic chalconoid found in the *Glycyrrhiza* species and exhibits antimalarial and antiviral activities. It inhibits influenza neuraminidases (NAs) of influenza subtypes such as H1N1, H9N2, and oseltamivir-resistant novel H1N1strains (Chen et al., 1994; Dao et al., 2011).

Inhibition of NLRP3 Inflammasome Signaling

SARS-CoV protein domains modulate NLRP3 inflammasome–triggered pulmonary inflammation via chemokines. Therefore, the NLRP3 inflammasome is a potential candidate for therapeutic agents against CoV-mediated inflammatory diseases. Natural products such as flavonoids interfere with signaling mediated by the NLRP3 inflammasome. Respiratory inflammatory SARS-CoVs induce the NLRP3 inflammasome in macrophages and Th1 cells. Several flavonoids inhibit NLRP3 inflammasome–related inflammatory response to SARS-CoVs. Such flavonoids include isobavachalcone, saikosaponin B2, silvestrol, tryptanthrin, CA, quercetin, myricetin, psoralidin, scutellarein, luteolin, apigenin, kaempferol, baicalin, and wogonoside (Sun et al., 2015; Fu et al., 2016; Choe and Kim, 2017; Lim et al., 2018; Zhang et al., 2018; Chen et al., 2019; Chen et al., 2019; Yamagata et al., 2019).

Inhibition of SARS-CoV Mpro, PLpro, 3CLpro, and Related Proteases

The CoV genomes encode a polypeptide which contains a protease region. Two cysteine proteases, PLpro and 3CLpro, are directly associated with RNA virus replication. PLpro and 3CLpro cleave the viral polyprotein and produce nonstructural proteins for viral replication at the commonly conserved 11 substrate-recognition sites. Structure-based information on PLpro from SARS-CoV or other CoVs is limited. 3CLpro is also called the CoV main protease (MPro) (MW 34 kDa). Thus, Mpro is used as a target for anti-CoV drugs. Mpro controls overall RNA replication and transcription. Therefore, it is a target protease, and computational *in silico* simulation enables the discovery of SARS-CoV-2 Mpro-specific inhibitors (Jin et al., 2020). 3CLpro has 100% identity with other SARS-CoV genomic RNA sequences. The 3CLpro of bat and human SARS-CoV-2 exhibits 99.02% amino acid sequence homology. The SARS-CoV-2 3CLpro protein is homologous with the known SARS-CoV, HCoV, MERS-CoV, and BCoV.

Among the virus targets, CoV Mpro and ACE2 are the main targets to screen. An Mpro inhibitor N3 was designed by AI-driven drug simulation and docking. Michael acceptor inhibitor or N3 inhibits the SARS- and MERS-CoV Mpros through an inhibitory mechanism of irreversible covalent bond formation with Mpro (Jin et al., 2020). Additionally, another SARS-CoV-2 Mpro inhibitor, ebselen, was designed, with N3 and ebselen exhibiting antiviral activity against SARS-CoV-2. Also, multiple natural compounds including afzelin, biorobin, hesperidin, δ-viniferin, myricitrin, taiwanhomoflavone A, lactucopicrin 15-oxalate, nympholide A, and phyllaemblicin B recognize SARS-CoV-2 Mpro with additional binding activities to hACE-2 and RdRp (Rasool et al., 2020). In the molecular interaction of natural products with the Mpro docking pocket, *Psorothamnus arborescens* var. *simplifolius* (Parish) Barneby 5,7,3′,4′-tetrahydroxy-2'-(3,3-dimethylallyl) isoflavone (PubChem 11610052) showed a high binding affinity via rigid hydrogen bonds to the catalytic dyad amino acid residues, and binding to the RBD at the amino acid residues. Similarly, *Myrica cerifera* L. myricitrin (PubChem 5281673) and methyl rosmarinate showed RBD to and receptor binding affinities to SARS-CoV-2 Mpro in a stability assay of ligand–protein complex (Joshi et al., 2020). Withanolide derivatives such as withaferin isolated from *Ashwagandha* species and the CA derivative CA phenethyl ester (CAPE) exhibit binding capacities to Mpro enzyme. CAPE and withaferin recognize the SARS-CoV-2 Mpro SBD with equivalent potentials to the known N3 protease inhibitor, as analyzed using dynamic simulation (Kumar et al., 2020). As potential COVID-19 Mpro inhibitors, flavonoid derivatives including curcumin derivatives, apigenin derivatives, oleuropein, catechin derivatives, and kaempferol have been suggested as candidates in the docking analysis (Khaerunnisa et al., 2020). In a recent report using the docking analysis (Erlina et al., 2020), ermanin compound known as kaempferol-di-O-methyl ether, myricetin glycosides, peonidin arabinosides, quercetin rhamnosides, rhamnetin mannosyllsides, and hesperidin have also been suggested to exhibit SARS-CoV-2 protease inhibitory activities. A cyclic ether and monoterpenoid component, jensenone, which is found in eucalyptus plant oil, potentially inhibits Mpro enzyme activity (Sharma and Kaur, 2020). Similarly, phytochemicals such as *Allium cepa* L. oleanolic acids, *Cocos nucifera* L. α-tocotrienols, *Psidium guajava* L. asiatic acids, and *Eucalyptus globulus* culinosides exhibit anti–SARS-CoV-2 activity in molecular dynamic docking analysis. Oleanolic acid specifically binds to the Mpro enzyme (Fitriani et al., 2020).

The SARS-CoV PLpro cleaves junctions spanning Nsp1–Nsp4. PLpro also deubiquitinates proteins and helps the virus to evade the innate immune response (Ratia et al., 2008). Therefore, PLpro is a target for drug development against disease-associated deubiquitinating enzymes (Ghosh et al., 2010). Cinnamic amides isolated from *Tribulus terrestris* L. fruits inhibit PLpro activity (Song et al., 2014). Some plants including *Cassia tora* L., *Cibotium barometz* (L.) J. Sm., *Dioscorea polystachya* Turcz., *Gentiana scabra* Bunge, and *Taxillus chinensis* (DC.) Danser showed SARS-CoV 3CLpro enzyme activity (Wen et al., 2011). Natural compounds such as lignins, tannins, and coumarins have also been found to exhibit CoV inhibitory activities (Islam et al., 2020). The diterpenoid 8β-hydroxyabieta-9 (11),13-dien-12-one and a lignin compound savinin inhibited SARS-CoV 3CLpro activity. *Salvia miltiorrhiza* Bunge tanshinone I, IIA, IIB; dihydrotanshinone; methyl tanshinonate; cryptotanshinone; and rosmariquinone

inhibited 3CLpro and PLpro with anti-infection and anti-replication activities, where tanshinone I and dihydrotanshinone I are strong 3CLpro and PLpro inhibitors (Park et al., 2012). The above *S. miltiorrhiza* Bunge tanshinone derivatives are noncompetitive inhibitors of protease enzyme isomerization. Especially, rosmariquinone reversibly inhibits the slow binding during cysteine protease isomerization (Park et al., 2012).

Black tea phenolic components such as tannic acid, 3-isotheaflavin-3-gallate, and theaflavin-3,3′-digallate also exhibited 3CLpro inhibition (Chen et al., 2005). *Isatis indigotica* Fortune ex Lindl. phenolic compounds including sinigrin, indigo, emodin, hesperetin, and β-sitosterol inhibited 3CLpro activity (Lin et al., 2005). *Torreya nucifera* (L.) Siebold & Zucc. flavones, biflavones, amentoflavone, apigenin, luteolin, bilobetin, ginkgetin, sciadopitysin, and quercetin also inhibit SARS-CoV 3CLpro (Ryu et al., 2010). Amentoflavone, bilobetin, ginkgetin, and sciadopitysin biflavonoids are constituents of *Ginkgo biloba* L. Other plants such as *Chamaecyparis obtusa* (Siebold & Zucc.) Endl. and *Hypericum perforatum* L. are also known to contain these compounds. They inhibit the cathepsin B inhibitor and influenza virus NA. Amentoflavone, bilobetin, ginkgetin, and sciadopitysin are noncompetitive inhibitors of CoV 3CLpro (Ryu et al., 2010). Myricetin and scutellarein inhibit SARS-CoV 3CLpro activity (Cho et al., 2013). *Broussonetia papyrifera* (L.) L'Hér. ex Vent. broussochalcone A/B, kazinol A/B/F/J, broussoflavan A, 4-hydroxyisolonchocarpin, papyriflavonol A, and 3′-(3-methylbut-2-enyl)-3′,4,7-trihydroxyflavane inhibit the 3CLpro and PLpro enzymes, where papyriflavonol A is the best inhibitor of the PLpro enzyme (Ryu et al., 2010). *B. papyrifera* 3′-(3-methylbut-2-enyl) 3′,4,7-trihydroxyflavane noncompetitively inhibits PLpro activity (Park et al., 2017). However, these compounds do not inhibit PLpro of MERs-CoV, indicating strain dependence. Other polyphenolic compounds such as kazinol F and broussochalcone A of the same *B. papyrifera* inhibit MERS-CoV PLpro.

Paulownia tomentosa tomentins 3′-O-/4′-O-methyldiplacol, 3′-O-/4′-O-methyldiplacone, mimulone, diplacone, and 6-geranyl-4′,5,7-trihydroxy-3′,5′-dimethoxyflavone inhibit PLpro of SARS-CoV (Lung et al., 2020). *Psoralea corylifolia* L. polyphenolics including bavachinin, neobavaisoflavone, isobavachalcone, 4′-O-methylbavachalcone, psoralidin, and corylifol A inhibit PLpro activity. Among these, isobavachalcone and psoralidin exhibit the highest antiviral activity with reversible inhibitory activity against PLpro via a type I mechanism (Kim et al., 2014). Quercetin inhibits PLpro and 3CLpro proteases (Ryu et al., 2010). For porcine epidemic diarrhea virus (PEDV), quercetin-7-rhamnoside, a disaccharide glucoside, inhibits viral activity rather than quercetin alone. In a computer-based simulation for a protease 3CLpro inhibitor of feline CoVs, naturally occurring compounds such as 7-methylluteolin, stictic acid, and quercetin-7-rhamnoside showed inhibition. However, only stictic acid prevented virus-induced death and virus attachment to the host cells, while 7-benzyl luteolin and steviol showed no inhibitory effects (Theerawatanasirikul et al., 2020). Similarly, quercetin-7-rhamnoside exhibits higher antiviral activity against animal CoVs than quercetin (Choi et al., 2009). *Psoralea corylifolia* bavachinin, neobavaisoflavone, isobavachalcone, 4′-O-methylbavachalcone, psoralidin, and corylifol inhibited PLpro of SARS-CoV (Lin et al., 2005). Interestingly, psoralidin strongly inhibited the protease activity of SARS-CoV. Using an *in vitro* cell-based assay of Vero E6 cells, terpenoids and lignoids were shown to block 50% cell growth of Vero E6 cells infected with SARS-CoV. Betulinic acid and savinin competitively inhibit SARS-CoV 3CLpro with a Ki of 8.2 and 9.1 μM, respectively (Loizzo et al., 2008). Quinone-methide triterpenes such as celastrol (tripterine), pristimerin, tingenone, and iguesterin of *Triterygium regelii* Sprague & Takeda inhibit the 3CLpro activity (IC50 = 5.5, 9.9, and 2.6 μM) as competitive inhibitors (Ryu et al., 2010). Celastrol as a pentacyclic triterpenoid in quinone methides also inhibits the RNA of hepatitis C and dengue viruses (Tseng et al., 2017; Yu et al., 2017). *Torreya nucifera* ethanolic extract contains SARS-CoV 3CLpro inhibitors. Biflavone amentoflavone was identified as a potent 3CLpro inhibitor via molecular docking (Ryu et al., 2010). Geranylated flavonoids are the strongest inhibitors of PLpro activity (Lung et al., 2020).

Sugiol, coumaroyltyramine, N-cis-feruloyltyramine, kaempferol, quercetin, cryptotanshinone, and tanshinone IIA inhibit PLpro and 3CLpro. Dihomo-γ-linolenic acid and moupinamide (feruloyltyramine) inhibit 3CLpro and PLpro, respectively (Cheng et al., 2006). Through computer docking modeling of SARS-CoV 3CLpro, *Veratrum sabadilla* Retz. sabadinine inhibits CoV protease (Toney et al., 2004). *Artemisia annua* aurantiamide acetate inhibits the active pocket of the CoV cathepsin-L protease (Wang et al., 2007). *Isatis indigotica* sinigrin, indigo, β-sitosterol, aloe-emodin, and hesperetin (Islam et al., 2020) as well as *Rheum palmatum* L. anthraquinones inhibit 3CLpro (Luo et al., 2009). *Houttuynia cordata* Thunb. water extract inhibits 3CLpro protease (Yang et al., 2010). For animal CoVs, stictic acid, 7-methylluteolin, quercetin-7-rhamnoside, 7-benzyl luteolin, and steviol, which exist in plants such as lichen, inhibit the 3CLpro protease activity of FIPV1146 (FCoV) (Theerawatanasirikul et al., 2020). *Uncaria tomentosa* (Willd. ex Schult.) DC, known as cat's claw, exhibits 3CLPro inhibitory activity as found by molecular docking analysis (Yepes-Pérez et al., 2020). Isolated phytochemicals such as cadambine, speciophylline, and proanthocyanidin of *Uncaria tomentosa* effectively interacted with 3CLpro.

As described above, CoV proteases are considered antiviral targets for the reduction of virus replication and host pathogenicity. However, nM affinity–leveled compounds are not developed for the targets. Apart from the conventional discovery from natural products, computer-aided methods to design drugs have been applied by using chemical databases for the inhibitor screening of SARS-CoV 3CLpro activity. Moreover, the known crystal structure of HCoV-229E 3CLpro facilitates design of inhibitors (Anand et al., 2003). Currently, SARS-CoV

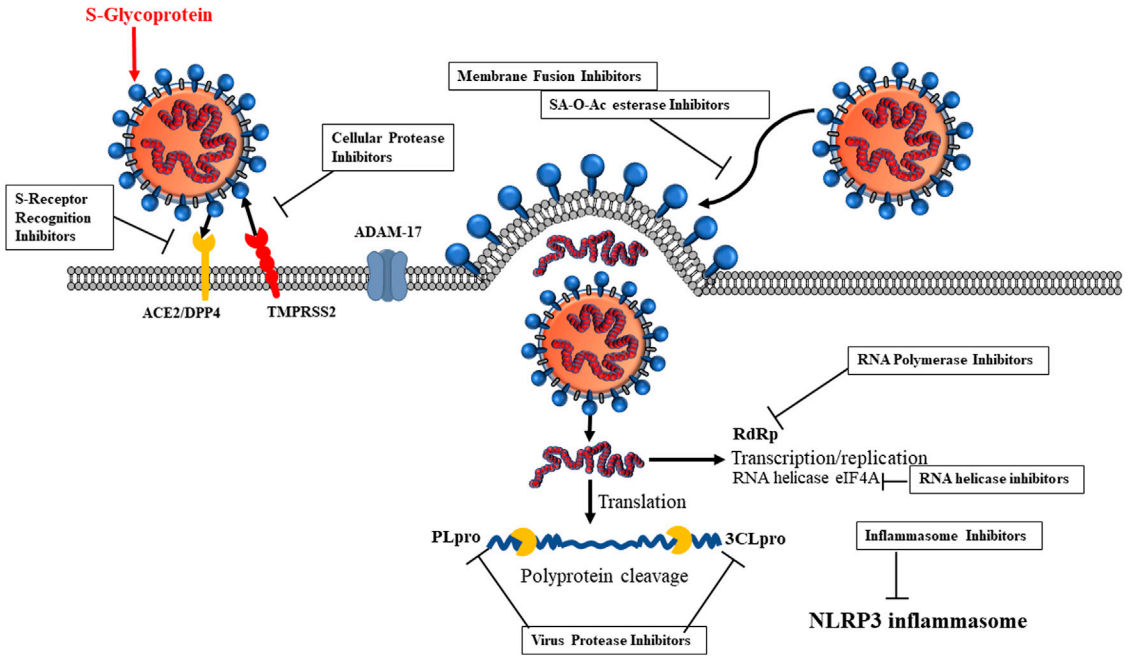

FIGURE 3 | Inhibitor candidates for SARS-CoV-2 life cycle. 1) S-glycoprotein receptor recognition, 2) cellular protease, 3) virus proteases, 4) RNA polymerase, 5) inflammasome, 6) RNA helicase, 7) SA-O-Ac-esterase.

3CLpro (PDB: 1Q2W and 1UK4) and SARS-CoV 3CLpro are elucidated for their 3D structures (Yang et al., 2003).

ADAM17 and TMPRSS2 Serine Protease Inhibitors

SARS-CoV-2 also utilizes TMPRSS2 for infection. A disintegrin and metallopeptidase domain (ADAM) family comprises Zn-metalloproteinases, and ADAM17 is a specific TNF-α–converting enzyme (TACE), thus also named TNF-α sheddase. ADAMs include ADAM-9, -10, and -12. ADAM17 sheds the ACE2 enzyme and catalyzes the formation of the soluble ACE2 N-terminal carboxypeptidase domain from ACE2 (Tipnis et al., 2000) and also converts pro-TNF-α to soluble TNF-α. Thus, ADAM17 is an anti-inflammatory target. Interaction of SARS S-glycoprotein and ACE2 cleaves ACE2 via ADAM17/TACE, facilitating its shedding and virus entry (Chen et al., 2008). TMPRSS2, human airway trypsin-like protease (HAT), TM protease, serine 13, serine protease DESC1, furin, factor Xa, and endosomal cathepsin L/B can cleave the SARS-CoV S-protein, facilitating SARS-CoV infection (Heurich et al., 2014). However, only TMPRSS2 allows SARS-CoV infection (Haga et al., 2010; Park et al., 2016; Zuniga et al., 2020). ACE2 interaction and TMPRSS2 activation potentiate the viral attachment to host cells. Thus, TMPRSS2 is a target for therapeutic agents (**Figure 3**).

SARS-CoV S-cellular TNF-α–converting enzyme activation facilitates virus entry, and thus this enzyme is an antiviral target. The inhibitor TAPI-2 inhibits virus entry of SARS-CoV into host cells. TAPI-2 inhibits SARS S-glycoprotein–mediated ACE2 shedding and TNF-α synthesis in the lung (Haga et al., 2010). ADAM17 inhibitors are widely beneficial for various diseases related to tumor immunosurveillance, cancer, and inflammatory diseases. As described previously, ADAM17 inhibitors reduce TNF-α–induced proinflammatory diseases and are attractive target candidates for the inflammatory diseases involved in SARS-CoVs. For example, a dual and selective small molecular inhibitor of ADAM17 and ADAM10, named INCB7839, is currently under combined usage with rituximab for B-cell non-Hodgkin lymphoma therapy (Witters et al., 2008). Although ADAM17 inhibitors such as matrix metalloproteinase (MMP) inhibitors marimastat and prinomastat inhibit ADAM17 activity (Packer and Cadenas, 2011), they are not clinically applicable due to ADAM17 sequence homology with the MMP enzymes and physiological problems. In this context, naturally occurring molecules have been used to attempt to develop selective ADAM17 inhibitors by using *in silico* approaches toward ligands and targets. Through the binding of ADAM17 to ligands, silymarin has been purified as an ADAM17-specific inhibitor that binds to the active amino acid residues in the ADAM17 protein. The inhibiting capacity has been compared with a previously known inhibitor, IK682. Silymarin is found in *Silybum marianum* (L.) Gaertn., known as milk thistle; and *Cynara cardunculus* L., known as wild artichokes; *Curcuma longa* L. turmeric rhizome; and *Coriandrum sativum* L. coriander seeds (Borah et al., 2016).

Cryptotanshinone, a natural compound isolated from *S. miltiorrhiza*, modulates androgen receptor (AR) transcriptional

regulation and downregulates TMPRSS2 gene expression as an AR target gene in androgen-responsive tumor cells. Interestingly, cryptotanshinone selectively inhibits the AR gene and thus has potential as anti-AR or SARS-CoV therapy (Xu et al., 2012).

Inhibition of GRP78 (HSPA5) Interaction *in Silico*

MERS-CoV spikes also recognize a 78-kDa glucose-regulated protein (GRP78) known as Byun1, heat shock 70-kDa protein 5 (HSPA5), and binding immunoglobulin protein (BiP). HSP5A is an ER-resident unfolded protein response (UPR) protein and acts as an alternative entry site via S-protein interaction for human viruses including papillomavirus, Ebola virus, Zika virus, and HCoVs, as well as the fungus *Rhizopus oryzae* (Pujhari et al., 2019; Elfiky, 2020; Elfiky, 2020; Ibrahim et al., 2020). Viral infection increases HSPA5 translocation to the plasma membrane (PM) and forms a membrane protein complex. In addition, GRP78 regulates MERS-CoV entry in the presence of DPP4. Lineage D β-CoV and bat CoV HKU also recognize the GRP78 (Ho et al., 2007; Yang et al., 2010), as simulated by molecular modeling and docking (Rao et al., 2002). Other ER molecules such as activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and protein kinase RNA (PKR)–like ER kinase (PERK) (Ibrahim et al., 2019) are involved. GRP78 releases IRE1, ATF6, and PERK activation, contributing to translation and refolding. GRP78 translocates to the membrane and recognizes the virus by its substrate-binding domain β (SBDβ), which is bound by the RBD. The binding region is molecularly targeted for COVID-19–specific drugs. Therefore, natural products can inhibit the HSPA5 binding to the S-glycoprotein. Small natural products prevent the S-glycoprotein–HSPA5 SBDβ interaction *in silico*. The effects of natural products that cause HSPA5 SDBβ dysfunction prevent SARS-CoV-2 S recognition because the HSPA5 SBDβ is the binding site for the SARS-CoV-2 S-glycoprotein. During viral infection, the HSPA5 (GRP78) translocated to the cell PM recognizes the SARS-CoV-2 S-protein. In *in silico* AI computer-aided simulation, several natural products recognize HSPA5 SBDβ. HSPA5 SBDβ-binding natural products can block virus attachment to the host cells if they are stressed. Thus, anti–COVID-19 agents specific for HSPA5 SBDβ recognition can be beneficial for elderly humans with cell stress. Therefore, approaches using AI computer-based molecular docking simulation yielded some natural products that bind to HSPA5 SBDβ (Elfiky, 2020). Four *Cicer arietinum* L. phytoestrogens, daidzein, genistein, formononetin, and biochanin A, recognize HSPA5 SBDβ. In addition, other natural compounds such as chlorogenic acid, linolenic acid, palmitic acid, CA, CA-phenethyl ester (CAPE), hydroxytyrosol, *cis-p*-coumaric acid, cinnamaldehyde, and thymoquinone showed moderate binding affinities to HSPA5 SBDβ. Phytoestrogens bear the same recognition affinity to HSPA5 SBDβ. Estrogenic hormones such as estrogens, progesterone, testosterone, and cholesterol have also binding affinities to HSPA5 SBDβ. From the binding affinity, phytoestrogens and estrogens are found to be the most feasible ligands to bind to HSPA5. Phytoestrogens such as daidzein, genistein, formononetin, and biochanin A also bind to estrogen receptors (ER) of humans and murines *in silico* and act like estrogen-like molecules (Sayed and Elfiky, 2018). Olive leaf hydroxytyrosol has moderate binding affinity to HSPA5 SBDβ. CA and *p*-coumaric acid also have average binding affinities to surface HSPA5 SBDβ and compete for recognition by the S-glycoprotein. The CAPE has a medium binding affinity to HSPA5 SBDβ. Cinnamaldehyde and thymoquinone have average binding affinity to HSPA5 SBDβ.

Inhibition of SARS-CoV by Plant Lectins

Plant lectins are potent inhibitors of CoV infection of host cells. Plant lectin-like protein interacts with virus surface proteins. Agglutinins, including mannose-specific lectin, inhibit the attachment and replication of SARS-CoV. Lectins can inhibit SARS-CoV-2 infection. Lectins such as griffithsin exert anti-CoV activity by multiple mechanisms (Moghaddam et al., 2014; Dai et al., 2019). Of the 33 plant lectins screened for the inhibition of SARS-CoV, *L. radiata* agglutinin was found to be effective (Keyaerts et al., 2007). GlcNAc-specific lectin, (GlcNAc)n-specific lectin, Gal-specific lectin, Man/Glc-specific lectin, Gal/GalNAc-specific lectin, GalNAcα(1.3)Gal > GalNAc > Gal-specific lectin, and Man/GalNAc-specific lectins inhibit the viral attachment to host cells and replication in host cells (Keyaerts et al., 2007). For example, *Urtica dioica* L. agglutinin inhibits viral replication in the penetration stages and binds to the S-glycoprotein and GlcNAc-like residues on the envelope glycan (Kumaki et al., 2011). Lectins from *Allium porrum* L., *Nicotiana tabacum* L., and *U. dioica* inhibit the virus propagation (EC50) (Yonesi and Rezazadeh, 2020).

Plant lectins are promising antiviral agents against influenza, herpes simplex virus (Hwang et al., 2020), and Ebola (Michelow et al., 2011; Covés-Datson et al., 2019). *Galanthus nivalis* L. agglutinin recognizes the S-glycoprotein and membrane proteins of feline CoV. Red alga *Griffithsia* sp. griffithsin directly interacts with S-glycoprotein (O'Keefe et al., 2010) and MERS-CoV (Millet et al., 2016). Griffithsin lectin purified from the *Griffithsia* sp. has three identical glycan-binding domains (GBDs) (O'Keefe et al., 2010). Different inhibition spectrums of griffithsin against different strains may be caused by genomic differences of the S-glycoproteins between SARS-CoV strains, potentiating different binding to the GBDs and affinity to the S-glycoproteins. Griffithsin lectin is relatively a small molecule and classified to be a MERS-CoV and HCoV inhibitor (EC50 of 0.0032–0.33 μM) (Millet et al., 2016). The three carbohydrate-binding domains specific for S-glycoprotein glycans inhibit MERS-CoV viral attachment to host cells (EC50 of 0.125 μM) (O'Keefe et al., 2010). Griffithsin has low toxicity and is a candidate agent against SARS-CoV-2. Griffithsin was effective for the SARS-CoV Urbani/Tor-II strains and not effective for the Frank strain. Human mannose-binding lectin (MBL) protects mice from fatal Ebola infections (Michelow et al., 2011). The legume Jack bean, *Canavalia ensiformis* (L.) DC., lectin concanavalin A (Con-A) is a phytagglutinin that hemagglutinates the hemagglutinating encephalomyelitis CoV, via binding to glycoconjugates (Greig and Bouillant, 1977). The therapeutic utility of Con-A is limited due to its hepatotoxic side

effects. Leguminous *Dioclea lasiocarpa* Mart. ex Benth. lectin DLasiL inhibits feline CoV at an EC50 of 5 nM. Interestingly, *Galanthus nivalis* L. lectin, agglutinin, recognizes the S-glycoprotein and feline coronavirus (FCoV) NTU156 (Hsieh et al., 2010). *Griffithsia* sp. griffithsin blocks PEDV (NJPEDV) attachment to host cells (Li et al., 2019).

Virus Entry Inhibitors via Nonspecific Inhibition

Lycorine, emetine, berbamine, and mycophenolate mofetil are known to inhibit several CoV strains including MHV-A59, HCoV-OC43/-NL63, and MERS-CoV (Shen et al., 2019). Additionally, mycophenolate mofetil showed immunosuppressive activity on the related virus-infected cells. Similarly, marine brown alga species *Ecklonia cava* Kjellman eckols, 7-phloroeckol, phlorofucofuroeckoln, and dieckol, blocked virus binding to porcine epidemic cells (Kwon et al., 2013). *Cinnamomum cassia* (L.) J. Presl. cortex procyanidin A2/B1 and cinnamtannin B1 inhibited SARS-CoV infection (Zhuang et al., 2009). Among these, procyanidin A2 inhibits the early stage of virus entry by blocking the clathrin-dependent endocytosis pathway. As virus entry inhibitors, tetra-O-galloyl-beta-D-glucose and luteolin prevent SARS-CoV entry into host cells (Yi et al., 2004). Upon interaction with ACE2, SARS-CoVs are incorporated into vesicle forms to facilitate entry into the cells. Juglanin inhibits SARS-CoV channel 3a (Schwarz et al., 2014). (−)-Catechin gallate and (−)-gallocatechin gallate block the nanoparticle-based RNA oligomer of SARS-CoV (Roh, 2012). *Houttuynia cordata* Thunb. quercetin, quercetrin, rutin, and cinanserin inhibit murine CoV (Chiow et al., 2016).

Aglaia foveolata Pannell sivestrol blocks Cap-dependent translation of HCoV-229E mRNA genome (Müller et al., 2018). Ouabain reduces the viral titers, yields, and viral RNA copy numbers (Yang et al., 2018). Its carboxylic amide derivatives exhibit specific SARS-CoV antiviral activity (Kim et al., 2019). Plant alkaloids such as cepharanthine, tetrandrine, and fangchinoline protected HCoV-OC43–infected human lung MRC-5 cells from cell death (Kim et al., 2019; Majnooni et al., 2020). Cepharanthine also blocks the SARS-CoV protease enzyme (Zhang et al., 2005). Some diterpenes, sesquiterpenes, triterpenes, lignans, and curcumin also exhibited antiviral activities against SARS-CoV (Yamagata et al., 2019). The marine algae *Halimeda tuna* (Ulvophyceae, Chlorophyta) diterpene aldehyde, halituna, shows antiviral activity against murine CoV A59 (Koehn et al., 1991). Some compounds inhibit SARS-CoV S-protein RBD interaction with ACE2. For example, the cathepsin L inhibitor inhibits fusion of viral membrane with host cell PM, blocking virus entry (Adedeji et al., 2013). On the other hand, *Aglaia* sp. silvestrol specifically inhibits the RNA helicase eIF4A of MERS-CoV (Müller et al., 2018). The *Boenninghausenia sessilicarpa* H. Lév. bioactive coumarin, leptodactylone, exhibits cytopathogenic effects on SARS-CoV–infected cells (Yang et al., 2007). *Pelargonium sidoides* DC. 11% ethanol extract interferes with the virus surface and causes inactivation of respiratory viruses (Michaelis et al., 2011).

For animal CoVs, *Sambucus nigra* L. lectins and flavonols also disrupt virion structure, compromising virus membrane integrity of avian IBV (Chen et al., 2014). *Mentha piperita* L., *Thymus vulgaris* L., and *Desmodium canadense* (L.) DC. 40% ethanol extracts directly inactivate the virus envelope structure of avian IBV (Lelesius et al., 2019). *Houttuynia cordata* essential oils and methyl-nonyl-ketone inhibit the release of avian IBV (Yin et al., 2011). Plant eucalyptol blocks the interaction of RNA with the nucleocapsid protein of avian IBV (Yang et al., 2010), and α-/β-pinene suppresses the N-protein function, hindering the interaction of avian IBV RNA and N-protein (Yang et al., 2011). *Forsythia suspensa* (Thunb.) Vahl forsythoside A affects cell signaling of avian IBV-infected avian cells (Li et al., 2011). For bovine CoVs, *Rosa nutkana* C. Presl and *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem. prunasin exhibits cytotoxicity against BCoV (McCutcheon et al., 1995). *Ziziphus jujuba* Mill. jubanine G and H as well as nummularine B exhibit cytotoxicity in PEDV-infected cells (Kang et al., 2015). *Ginkgo biloba* polysaccharides dose-dependently inhibit viral attachment and the entry steps of PEDV CoV-777 (Lee et al., 2015). *Houttuynia cordata* quercetin 7-rhamnoside interacts directly with PEDV (Song et al., 2011). *H. cordata* quercetin 7-rhamnoside, quercetin, apigenin, and luteolin exhibit cytotoxicity in PEDV-infected host cells (Choi et al., 2009). *Prunus serrulata* var. *spontanea* (Maxim.) E.H. Wilson polyphenols exhibit cytotoxicity in PEDV (KPEDV9)-infected host cells (Yook et al., 2010).

Carcinoembryonic Antigen Cell Adhesion Molecule Receptor

The N-terminal domain of S1 recognizes CEACAM1. S-glycoprotein–CEACAM receptor binding leads to S-glycoprotein–mediated fusion of membrane. For example, MHV recognizes the CEACAM expressed on BHK cell cultures (Heino et al., 2000). In MERS-CoV, CEACAM5 isoforms are associated with attachment (Naskalska et al., 2019). Therefore, MERS-CoV recognizes CEACAM5 as the attachment and entry site (Chan et al., 2016). In the structural aspect, the S1 N-terminal domain exhibits an identical tertiary structure compared with human galectins which recognize Gal-residues. The S1 N-terminal domain of the MHV recognizes mouse CEACAM1a and that of BCoV recognizes carbohydrate residues (Peng et al., 2011; Peng et al., 2012; Walls et al., 2016). Because CEACAM1a mRNA is alternatively spliced, HCoVs have been suggested to be evolutionarily recombinant between the host galectin and the S1-glycoprotein genes. However, the BCoV S1-glycoprotein gene is not subjected to such recombination but bears the glycan-binding lectin activity. MHV S1-glycoprotein has also been suggested to acquire mouse CEACAM1a-binding capacity (Peng et al., 2017), suggesting that CoVs receive evolutionary pressure to acquire the interaction capacity with host receptors over cross-species (Li, 2015; Li, 2016). Moreover, soluble forms of CEACAM directly involve in S-glycoprotein–mediated PM fusion, inducing conformational shifts (Matsuyama and Taguchi, 2002; Taguchi and

Matsuyama, 2002). On the host side, host organisms have also evolved to escape the lethal pressure from coronavirus infections. The acquired geno- and phenotypes of such hosts are expressed for SA-recognizing proteins. For example, Siglecs are representatively expressed to utilize the innate responses of host immune cells.

Major Histocompatibility Complex Class I (MHC-I) C and DC-SIGN (CD209) for Coronavirus Attachment Site

CoV-HKU1 spikes additionally bind to MHC-I C (Chan et al., 2009). HCoV-HKU1 S-glycoprotein also binds to MHC-IC known as HLA-C (Song et al., 2011). SARS-CoV utilizes dendritic cell (DC)–specific intercellular adhesion molecule (ICAM)-3–grabbing nonintegrin (DC-SIGN) (Marzi et al., 2004). SARS-CoV also uses the C-type lectins of DC-SIGN and DC-L-SIGN. DC/L-SIGN recognizes the S-glycoprotein glycans, where seven N-glycan sites are known to enable DC/L-SIGN–mediated infection (Marzi et al., 2004; Han et al., 2007).

Dipeptidyl Peptidase-4, Aminopeptidase N, and Tetraspanin CD9

Ser exopeptidase dipeptidyl peptidase-4 (DPP-4)/human CD26 is the MERS-CoV receptor. DPP4 is a ubiquitous membrane-type aminopeptidase in the PM. The MERS-CoV S1 N-terminal domain binds to DPP4 (Raj et al., 2013; Gheblawi et al., 2020; Letko et al., 2020). The CD9 tetraspanin, but not the CD81 tetraspanin, interacts with DPP4 and TMPRSS2 (Earnest et al., 2017). These CD9–DPP4–TMPRSS2 receptors and proteases permit entrance of the MERS-CoV pseudovirus into the host cells. Tetraspanin CD9 binds to the DPP4–TMPRSS2 complex, and this triggers the S-glycoprotein. The α-CoV HCoV-229E S-glycoprotein binds to human aminopeptidase N (hAPN) (Yeager et al., 1992). hAPN (CD13) is a TM alanyl aminopeptidase and Zn-dependent metalloprotease (EC 3.4.11.2) with a MW of 150 kDa and made up of 967 amino acids. The C-terminal domain has zinc–MMP-related pentapeptides. The S1 C-terminal region is the APN-binding domain (Deng et al., 2016). Porcine APN (pAPN) and hAPN exhibit about 80% protein similarity. PEDV can bind to hAPN and neuraminic acid as its co-receptors, as human cytomegalovirus, pCoV, FIPV, feline enteric virus (FeCV), and canine CoVs recognize them (Delmas et al., 1992; Söderberg et al., 1993; Tresnan et al., 1996; Nomura et al., 2004). APN is the functional receptor for HCoV-229E (Yeager et al., 1992; Zhu et al., 2018). Bestatin, an APN inhibitor, binds to its catalytic site (Milewska et al., 2014).

Heparan Sulfate as Human Coronavirus Entry Site

MHV and HCoV-NL63 are known to interact with heparan sulfate (HS) (Watanabe et al., 2007; Milewska et al., 2014). The HS proteoglycans (HSPGs) are recognized by M-protein in the absence of the S-glycoprotein in the HCoV-NL63 entry into host cells. Then, the M-protein and S-glycoprotein enhance virus entry into the host cells (Milewska et al., 2014; Naskalska et al., 2019). In general, ACE2, APN, HSPA5, furin, O-Ac-neuraminic acid, and HSPGs are the CoV-binding molecules. Apart from the precise targeting of the molecules, several medicinal plant resources also exhibit antiviral activities against respiratory and influenza virus diseases. For example, *Panax ginseng* can prevent viral respiratory diseases and influenza virus diseases (Cheng et al., 2020). *Pelargonium sidoides* also prevents respiratory viral infections (Im et al., 2015). *Astragalus mongholicus* Bunge can treat common cold and upper respiratory infections and also prevent influenza virus infections (Kolodziej, 2011; Liang et al., 2019). Compounds and extracts with anti-CoV activities are summarized in **Table 1**.

Relationship Between Structures and Activities of Natural Products

The anti–SARS-CoV-2 natural compounds have been subjected to screening for understanding their structure–activity relationships (SARs). A possible approach to understand the SARs and inhibitory mechanism(s) is to resolve the inhibitor–target complex by using analytic tools. For example, crystallized complexes of the natural products and target proteins such as enzymes, surface proteins, and host receptors can be instrumentally analyzed. However, information on the successful SARs and the inhibitory mechanism(s) are currently limited. Instead, using molecular *in silico* ducking simulation and computational analysis, the SAR results have been reported. Using molecular modeling and docking techniques, potential binding abilities of the compounds to the pocket sites, interface sites, or catalytic sites of targets including proteases and the ACE2–S-glycoprotein complex have been suggested. The functional groups of the binding pocket interact with targets in van der Waals, hydrophilic, hydrophobic, and H-bond interactions.

As regards natural anthraquinones, rings and substituted glycosides differentially inhibit SARS-CoV-2 targets (Li and Jiang, 2018). For example, dihydroxyanthraquinone with C1 and C2–OH groups differently inhibit SARS-CoV-2 infection (Li and Jiang, 2018). Anthocyanins interact with the active site pockets of Mpro and human ACE2, where the active site of Mpro is polar in its chemical property, having affordable binding energies. Delphinidin, an anthocyanin derivative, forms H-bond in the binding site and π stacking with the hydrophobic pocket. A diglycosidic anthocyanin, delphinidin 3,5-diglucoside binds to the Mpro and ACE2 (Sharma and Shanavas, 2020), where it recognizes the flavylium nucleus ring and the Mpro catalytic site. In addition, the −OH groups of the phenyl ring recognize the Mpro S1 catalytic site through H-bonds (Sharma and Shanavas, 2020). The benzene ring and the Hie41 of the hydrophobic Mpro S2 domain form the p–π interaction. The −OH group of the flavylium nucleus binds to the Mpro S4, while the −OH groups of the benzoyl moiety of non-glycosidic 3,5-di-O-galloylshikimic acid form H-bonds with the Mpro cavity site. The OH- group and oxygen atoms of the benzoyl groups bind to the Mpro cavity site (Sharma and

TABLE 1 | Summary of anti-CoV compounds and extracts.

Names of compounds and extracts	Target specificity	Plant	References
Lycorine	Cytopathogenic	*Lycoris radiata* (L'Hér.)	Yu et al. (2012)
Silvestrol	RNA helicase eif4a	*Aglaia* sp.	Miean and Mohamed, (2001), Lau et al. (2008)
Tryptanthrin	RdRp, PLpro 2	*S. cusia* (Nees) Kuntze	Wen et al. (2007)
Saikosaponin	Replication/entrance, S-glycoprotein	*Bupleurum* sp., *Heteromorpha* sp., *Scrophularia scorodonia* L.	Ulasli et al. (2014), Ushio and Abe, (1992), Chiang et al. (2003)
Cafffeic acid (chlorogenic acid, isoferulic acid), CAPE	Mpro, GRP78 (HSPA5)	*Sambucus javanica* subsp. *chinensis* Fukuoka (elderberry)	Li. (2015), Weng et al. (2019), Wang et al. (2009), Kumar et al. (2020), Sayed and Elfiky (2018)
Linolenic acid, palmitic acid, oleanolic acid	3CLpro, Mpro	*Allium cepa* L.	Cheng et al. (2006), Sharma and Kaur (2020), Fitriani et al. (2020)
Isobavachalcone, broussochalcone A/B	3CLpro, PLpro	*Broussonetia papyrifera* (L.) L'Hér. Ex vent	Ryu et al. (2010)
Myricetin	Helicase nsp13, NLRP3 inflammasome, 3CLpro	*Isatis indigotica* Fort., *Torreya nucifera*, *Vaccinium oxycoccos* L., *Calamus scipionum* Lam.	Cho et al. (2013), Mikulic-Petkovsek et al. (2012), Qing et al. (2016), Yang et al. (2010), Chen et al. (2019)
Psoralidin	PLpro	*Psoralea corylifolia* L.	Kim et al. (2014)
Quercetin	Plpro, 3CLpro, NLRP3	*Psoralea corylifolia* L.	Ryu et al. (2010), Kim et al. (2014)
Scutellarein	Helicase nsp13, ATPase, 3CLpro	*Isatis indigotica* Fort., *Torreya nucifera*, *S. baicalensis*	Cho et al. (2013), Yang et al. (2010)
Silvestrol	NLRP3 inflammasome, RNA helicase eIF4A	*Aglaia* sp.	Lim et al. (2018), Müller et al. (2018)
Baicalin	NLRP3 inflammasome, S-glycoprotein, Nsp9, replication	*Scutellaria baicalensis*, *S. lateriflora*	O'Flaherty et al. (2019), Chandel et al. (2020), Lim et al. (2018), Fu et al. (2016)
Wogonoside	NLRP3 inflammasome	*Scutellaria baicalensis*	Lim et al. (2018), Sun et al. (2015)
Kaempferol	NLRP3 inflammasome, PLpro, 3CLpro, 3a channel protein	*Rosmarinus officinalis*, *Sambucus nigra*, *Viola odorata* L.	Lim et al. (2018), Khaerunnisa et al. (2020), Schwarz et al. (2014)
Apigenin, flavones, biflavones, amentoflavone, bilobetin, ginkgetin, sciadopitysin	NLRP3 inflammasome, 3CLpro	*Torreya nucifera* (L.) Siebold & Zucc., *Ginkgo biloba* L., *Chamaecyparis obtusa* (Siebold & Zucc.) Endl., *Hypericum perforatum* L.	Lim et al. (2018), Yamagata et al. (2019), Ryu et al. (2010)
Tomentin (3′-O-/4′-O-methyldiplacol, 3′-O-/4′-O-methyldiplacone, mimulone, diplacone, 6-geranyl-4′,5.7-trihydroxy-3′,5′-dimethoxyflavanone)	PLpro	*Paulownia tomentosa* (Thunb.) Steud.	Lung et al. (2020)
Tryptanthrin	PLpro-2	*Strobilanthes cusia* (Nees) Kuntze	Wen et al. (2007), Chen et al. (2008)
Indigodole B	PLpro-2	*Strobilanthes cusia* (Nees) Kuntze	Wen et al. (2007)
Theaflavin	RdRp	*Camellia sinensis* (L.) Kuntze	Zhang et al. (2020)
3,4,5-Trihydroxy-1,8-bis [(2 R,3 R)-3,5,7-trihydroxy-2-chromanyl]-6-benzo() annulenone	RdRp	*Camellia sinensis* (L.) Kuntze	Zhang et al. (2020)
Betulonic acid	Replication, 3CLpro	*Sequoia sempervirens* (D.Don) Endl.	Loizzo et al. (2008)
Betulinic acid	Replication, 3CLpro	*Sequoia sempervirens* (D.Don) Endl.	Loizzo et al. (2008).Cheng et al. (2006)
8β-hydroxyabieta-9 (11),13-dien-12-one, 3β,12-diacetoxyabieta-6,8,11,13-tetraene	Replication	*Sequoia sempervirens* (D.Don) Endl.	Loizzo et al. (2008)
Curcumin	Replication, Mpro	*Curcuma longa*	Loizzo et al. (2008), Yamagata et al. (2019), Khaerunnisa et al. (2020)
Hinokinin	Replication	*Sequoia sempervirens* (D.Don) Endl.	Loizzo et al. (2008)
Savinin	Replication, 3CLpro	*Sequoia sempervirens* (D.Don) Endl.	Loizzo et al. (2008), Islam et al. (2020)
Glycyrrhizin, 18β-glycyrrhetinic acid, licochalcone	S-glycoprotein, N-protein, attachment/entry	*Glycyrrhiza uralensis* Fisch. (licorice), *G. glabra* L., *G. inflata* Bat.	Hoever et al. (2005), Shen et al. (2019), Haiying et al. (2003), Fu et al. (2016), Cinatl et al. (2003), Michaelis et al. (2011)
Vitamins A and C	Host immunity	Various	Häkkinen et al. (1999)
Lignin	Replication, 3CLpro	*Sequoia sempervirens* (D.Don) Endl.	Cheng et al. (2006), Yamagata et al. (2019), Islam et al. (2020), Park et al. (2012)
Desmethoxyreserpine	Replication, 3CLpro	*Rauvolfia canescens*	Cheng et al. (2006)
B-Ocimene	Replication	*Laurus nobilis* L.	McDonagh et al. (2014)
1,8-Cineole	Replication, 3CLpro, N-protein	*Laurus nobilis* L.	Müller et al. (2020), McDonagh et al. (2014), Yang et al. (2011), Yang et al. (2010)
α-Pinene	Replication, N-protein	*Laurus nobilis* L., *Rosmarinus officinalis* L. (rosemary)	Müller et al. (2020), McDonagh et al. (2014), Yang et al. (2011)

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TABLE 1 | (*Continued*) Summary of anti-CoV compounds and extracts.

Names of compounds and extracts	Target specificity	Plant	References
β-Pinene	Replication, N-protein	*Laurus nobilis* L.	Müller et al. (2020), McDonagh et al. (2014), Yang et al. (2011)
δ3-Carene	Replication	*Laurus nobilis* L.	McDonagh et al. (2014)
α-Cedrol	Replication	*Laurus nobilis* L.	McDonagh et al. (2014), Kim et al. (2008)
Blancoxanthone	Cytopathic effects	*Calophyllum blancoi* Planch. & Triana	Kim et al. (2010)
Pyranojacareubin	Cytopathic effects	*Calophyllum blancoi* Planch. & Triana	Kim et al. (2010)
Gallic acid	S-glycoprotein	Tea leaves, oak bark	Wang et al. (2009)
Artemisinin	Replication	*Artemisia annua* L.	O'Flaherty et al. (2019), de Vries et al. (1997)
Quercetin, quercetin 7-rhamnoside, quercetrin	Replication, NLRP3 inflammasome, 3CLpro, PLpro	*Torreya nucifera* (L.) Siebold & Zucc., *Houttuynia cordata* Thunb.	O'Flaherty et al. (2019), Choe and Kim, (2017), Ryu et al. (2010), Theerawatanasirikul et al. (2020), Chiow et al. (2016), Lee et al. (2015), Song et al. (2011)
Rutin	Replication	*Houttuynia cordata* Thunb.	Li et al. (2005), Packer and Cadenas, (2011), O'Flaherty et al. (2019)
Hesperidin, hesperitin	Replication Mpro, ACE2, RdRp	*Citrus aurantium* L.	O'Flaherty et al. (2019), Rasool et al. (2020)
Glycyrrhizic acid, glycyrrhetinic acid, glycyrrhizin	Replication, neucleoprotein, S-glycoprotein	*Glycyrrhiza uralensis* Fisch. (licorice), *G. glabra* L., *G. inflata* Bat.	Li et al. (2005), Packer and Cadenas (2011), O'Flaherty et al. (2019), Fu et al. (2016)
Ferulic acid (caffeoyltyramine, feruloyltyramine, feruloyloctopamine)	Replication, PLpro	*Cimicifuga racemosa* L., *Melia* sp., *Coptis* sp., *Phellodendron* sp., *Sophora subprostrata* Chun & T.Chen. (Fabaceae)	Song et al. (2014), Jin et al. (2019), Sun et al. (2019), Li. (2015)
Isoferulic acid	Replication, PLpro	*Cimicifuga racemosa* L., *Melia* sp., *Coptis* sp., *Phellodendron* sp., *Sophora subprostrata* Chun & T.Chen. (Fabaceae)	Song et al. (2014),Jin et al. (2019),Sun et al. (2019)
Toosendanin	Replication, RdRp	*Cimicifuga racemosa* L., *Melia* sp., *Coptis* sp., *Phellodendron* sp., *Sophora subprostrata* Chun & T.Chen. (Fabaceae)	Song et al. (2014), Jin et al. (2019), Sun et al. (2019)
Berberine	Replication, RdRp	*Cimicifuga racemosa* L., *Melia* sp., *Coptis* sp., *Phellodendron* sp., *Sophora subprostrata* Chun & T.Chen. (Fabaceae)	Song et al. (2014), Jin et al. (2019), Sun et al. (2019)
Matrine	Replication, RdRp	*Cimicifuga racemosa* L., *Melia* sp., *Coptis* sp., *Phellodendron* sp., *Sophora subprostrata* Chun & T.Chen. (Fabaceae)	Song et al. (2014),Jin et al. (2019),Sun et al. (2019)
Oxymatrine	Replication, RdRp	*Cimicifuga racemosa* L., *Melia* sp., *Coptis* sp., *Phellodendron* sp., *Sophora subprostrata* Chun & T.Chen. (Fabaceae)	Song et al. (2014), Jin et al. (2019), Sun et al. (2019)
Sophoranone	Replication, RdRp	*Cimicifuga racemosa* L., *Melia* sp., *Coptis* sp., *Phellodendron* sp., *Sophora subprostrata* Chun & T.Chen. (Fabaceae)	Song et al. (2014), Jin et al. (2019), Sun et al. (2019)
Sophocarpine	Replication, RdRp	*Cimicifuga racemosa* L., *Melia* sp., *Coptis* sp., *Phellodendron* sp., *Sophora subprostrata* Chun & T.Chen. (Fabaceae)	Song et al. (2014), Jin et al. (2019), Sun et al. (2019)
Khainaosides	S-glycoprotein, Nsp15, Mpro	*Vitex glabrata*	Adem et al. (2021)
6-O-caffeoylarbutin	S-glycoprotein, Nsp15, Mpro	*Vaccinium dunalianumas*	Adem et al. (2021)
Vitexfolin	S-glycoprotein, Nsp15, Mpro	*Vitex rotundifolia*	Adem et al. (2021)
Toosendanin	RNA polymerase complex	*Melia azedarach* L.	Simmons et al. (2013)
Matrine	Myd88/NF-κB, NLRP3 inflammasome	*Sophora flavescens* Aiton	Hulswit et al. (2019)
Tetrandrine	Replication, S-glycoprotein, N-protein	*Stephania tetrandra* var. *glabra*	Kim et al. (2019)
Fangchinoline	Replication, S-glycoprotein, N-protein	*Stephania tetrandra* var. *glabra*	Kim et al. (2019)
Cepharanthine	Replication, S-glycoprotein, N-protein	*Stephania tetrandra* var. *glabra*	Kim et al. (2019)
Actinomycin D	Cell attachment	*Streptomyces parvulus*	O'Flaherty et al. (2019)
Ginsenoside (gynosaponin)	Cell attachment, S-glycoprotein	*Panax ginseng* (T.Nees) C.A.Mey	Wu et al. (2020)
Resveratrol	Replication, N-protein	*Vitis vinifera*, *Polygonum cuspidatum*, *Vaccinium macrocarpon*	Lin et al. (2005)

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TABLE 1 | (*Continued*) Summary of anti-CoV compounds and extracts.

Names of compounds and extracts	Target specificity	Plant	References
Omacetaxine (homoharringtonine)	Replication, N-protein, S-glycoprotein	*Tylophora indica* (Burm.f.) Merr.	Cao et al. (2015)
Tylophorine, 7-methoxycryptopleurine	Replication, N- and S-glycoprotein, NF-κB, JAK	*T. indica*	Yang et al. (2010), Cho et al. (2006), Yang et al. (2017)
Dihydrotanshinone, tanshinone, cryptotanshinone, rosmariquinone	S-glycoprotein, 3CLpro, PLpro	*Salvia miltiorrhiza* Bunge	Zhang et al. (2020), Park et al. (2012)
Tetra-O-galloyl-β-D-glucose	S2-protein	*Rhus chinensis* Mill.	Yi et al. (2004)
Luteolin, 7-methylluteolin, 7-benzyl luteolin	S-glycoprotein, NLRP3 inflammasome, 3CLpro, cytotoxicity	*Rhus chinensis* Mill., *Torreya nucifera* (L.) Siebold & Zucc., *Houttuynia cordata*	Yi et al. (2004), Zhang et al. (2018), Ryu et al. (2010), Theerawatanasirikul et al. (2020), Song et al. (2011), Choi et al. (2009)
Alstotide	M-protein, S-glycoprotein	*Alstonia scholaris* (L.) R.Br.	Nguyen et al. (2015)
Polyphenols	S-glycoprotein	*Punica granatum* L.	Sundararajan et al. (2010)
Steviol	3CLpro	*Stevia rebaudiana*	Theerawatanasirikul et al. (2020), Theerawatanasirikul et al. (2020)
Phyllaemblicin	ACE2	*Phyllanthus emblica* L.	Meneguzzo et al. (2020)
Neohesperidin, hesperidin	ACE2	*Citrus aurantium* L.	Meneguzzo et al. (2020)
Chrysin	ACE2	*Oroxylum indicum*	Basu et al. (2020)
Emodin	S-glycoprotein, 3CLpro	*Isatis indigotica* Fortune ex Lindl.	Ho et al. (2007), Islam et al. (2020), Lin et al. (2005)
Hesperetin	3CLpro	*Isatis indigotica*	Islam et al. (2020)
Anthraquinone	3CLpro	*Rheum palmatum* L.	Luo et al. (2009)
Cadambine	3CLpro	*Uncaria tomentosa* (Willd. ex Schult.) DC.	Yepes-Pérez et al. (2020)
Speciophylline	3CLpro	*Uncaria tomentosa* (Willd. ex Schult.) DC.	Yepes-Pérez et al. (2020)
Proanthocyanidin	3CLpro	*Uncaria tomentosa* (Willd. ex Schult.) DC.	Yepes-Pérez et al. (2020)
Silymarin	Adam17	*Silybum marianum* (L.) Gaertn., *Cynara cardunculus* L., *Curcuma longa* L.	Borah et al. (2016)
Lycorine, berbamine	Viral entry, RNA, DNA, and protein synthesis	*Lycoris radiata* (Amaryllidaceae), *Berberis amurensis*	Shen et al. (2019)
Mycophenolate	Immunosuppression	*Penicillium stoloniferum*	Shen et al. (2019)
Eckol, 7-phloroeckol, phlorofucofuroeckoln, dieckol	Attachment, entry, replication, S-glycoprotein	Brown alga *Ecklonia cava* Kjellman	Kwon et al. (2013)
Procyanidin A2/B1, cinnamtannin B1	Virus entry, transferrin receptor	*Cinnamomum cassia* (L.) J. Presl	Zhuang et al. (2009)
Tetra-O-galloyl-beta-D-glucose, luteolin	Virus entry, S-glycoprotein	*Galla chinensis*, *Veronica linariifolia* Pall.	Yi et al. (2004)
Juglanin	Channel 3a	*Quercus ilex* L., Viola odorata L.	Schwarz et al. (2014)
Catechin gallate, gallocatechin gallate	N-protein, RNA oligomer	Green tea, buckwheat, *Dianthus caryophyllus*	Roh. (2012)
Cinanserin	Virus entry, replication	*Houttuynia cordata* Thunb.	Chiow et al. (2016)
Sivestrol	Cap-dependent translation	*Aglaia foveolata* Pannell	Müller et al. (2018)
Ouabain	Replication, cell membrane sodium/potassium pump, na^+/k^+-ATPase	*Acokanthera schimperi, Strophanthus gratus*	Yang et al. (2018)
Cepharanthine	Protease, cytotoxicity, S- and N-protein expression	*Stephania tetrandra*, Menispermaceae species	Kim et al. (2019), Zhang et al. (2005)
Fangchinoline	Cytotoxicity, S- and N-protein expression	*Stephania tetrandra*, Menispermaceae species	Kim et al. (2019)
Tetrandrine	Cytotocixity, S- and N-protein expression	*Stephania tetrandra*, Menispermaceae species	Kim et al. (2019)
Diterpene aldehyde, halituna	Cytotoxicity	Marine algae *Halimeda tuna* (Ulvophyceae, Chlorophyta)	Yamagata et al. (2019), Koehn et al. (1991)
Coumarin	Cytotoxicity	*Boenninghausenia sessilicarpa* H.Lév.	Yang et al. (2007)
Leptodactylone	Cytotoxicity	*Boenninghausenia sessilicarpa* H.Lév.	Yang et al. (2007)
Methyl-nonyl-ketone	Virus release	*Houttuynia cordata*	Yin et al. (2011)
Eucalyptol	RNA with N-protein	*Eucalyptus globulus* oil	Yang et al. (2010)
α-/β-Pinene	N-protein	*Sideritis* spp., *Salvia* spp., *Cannabis*	Yang et al. (2011)
Forsythoside A	Signaling, replication	*Forsythia suspensa* (Thunb.) Vahl	Li et al. (2011)
Prunasin	Cytotoxicity	*Rosa nutkana* C. Presl, *Amelanchier alnifolia* (Nutt.) Nutt. ex M.Roem.	McCutcheon et al. (1995)
Jubanine G/H	Cytotoxicity	*Ziziphus jujuba* Mill.	Kang et al. (2015)
Nummularine B	Cytotoxicity	*Ziziphus jujuba* Mill.	Kang et al. (2015)
Polysaccharide	Viral attachment	*Ginkgo biloba*	Lee et al. (2015)
Polyphenol	Cytotoxicity	*Prunus serrulata* var. *spontanea* (Maxim.) E.H. Wilson	Yook et al. (2010)
Afzelin (kaempferol rhamnoside)	Mpro, ACE2, RdRp	*Nymphaea odorata*	Rasool et al. (2020)

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TABLE 1 | (*Continued*) Summary of anti-CoV compounds and extracts.

Names of compounds and extracts	Target specificity	Plant	References
Biorobin	Mpro, ACE2, RdRp	*Acalypha indica*	Rasool et al. (2020)
δ-Viniferin (resveratrol dehydrodimer)	Mpro, ACE2, RdRp	*Vitis vinifera*	Rasool et al. (2020)
Taiwanhomoflavone A	Mpro, ACE2, RdRp	*Cephalotaxus wilsoniana*	Rasool et al. (2020)
Lactucopicrin 15-oxalate	Mpro, ACE2, RdRp	Asteraceae	Rasool et al. (2020)
Nympholide A	Mpro, ACE2, RdRp	*Nymphaea lotus* Linn.	Rasool et al. (2020)
Phyllaemblicin B	Mpro, ACE2, RdRp	*Phyllanthus emblica*	Rasool et al. (2020)
5,7,3′,4′-Tetrahydroxy-2'-(3,3-dimethylallyl) isoflavone	Mpro	*Psorothamnus arborescens* var. *simplifolius* (Parish) Barneby	Joshi et al. (2020)
Myricitrin	Mpro	*Myrica cerifera* L.	Joshi et al. (2020)
Methyl rosmarinate	Mpro	*Myrica cerifera* L.	Joshi et al. (2020)
Withaferin	Mpro	*Ashwagandha* species	Kumar et al. (2020)
Oleuropein	Mpro	*Olea europaea*	Khaerunnisa et al. (2020)
Ermanin	Mpro	*Tanacetum microphyllum*	Erlina et al. (2020)
Jensenone	Mpro	*Eucalyptus jensenii*	Sharma and Kaur, (2020)
Oleanolic acid	Mpro	*Allium cepa* L.	Fitriani et al. (2020)
α-Tocotrienol	Mpro	*Cocos nucifera* L.	Fitriani et al. (2020)
Asiatic acid	Mpro	*Psidium guajava* L.	Fitriani et al. (2020)
Culinoside	Mpro	*Eucalyptus globulus*	Fitriani et al. (2020)
Cinnamic amide	PLpro	*Tribulus terrestris* L.	Song et al. (2014)
Tannin, tannic acid	3CLpro	*Rhus semialata*, *Quercus infectoria*, *Rhus coriaria*	Islam et al. (2020), Chen et al., (2005)
Coumarin	3CLpro	*Cinnamomum cassia*, *Dipteryx odorata*	Islam et al. (2020)
8β-Hydroxyabieta-9 (11),13-dien-12-one	3CLpro	*Thuja standishii* (Cupressaceae)	Islam et al. (2020)
Stictic acid	3CLpro	*Rheum palmatum* L.	Theerawatanasirikul et al. (2020)
Coumaroyltyramine	3CLpro, PLpro	*Allium fistulosum*	Cheng et al. (2006)
Moupinamide (feruloyltyramine)	3CLpro, PLpro	*Piper nigrum*	Cheng et al. (2006)
Dihomo-c-linolenic acid	3CLpro, PLpro	*Asphodelus tenuifolius*, *Aizoon canariense*, *Emex spinosus*	Cheng et al. (2006)
Sugiol	3CLpro, PLpro	*Calocedrus formosana* Florin (Cupressaceae)	Cheng et al. (2006)
Tanshinone, dihydrotanshinone, methyl tanshinonate, cryptotanshinone, rosmariquinone, N-cis-feruloyltyramine	3CLpro, PLpro, androgen receptor	*Salvia miltiorrhiza* Bunge	Cheng et al. (2006), Park et al. (2012), Xu et al. (2012)
3-Isotheaflavin-3-gallate	3CLpro	Green tea, black tea, puer tea	Chen et al. (2005)
Theaflavin-3,3′-digallate	3CLpro	Green tea, black tea, puer tea	Chen et al. (2005)
Sinigrin	3CLpro	*Isatis indigotica* Fortune ex Lindl.	Islam et al. (2020), Lin et al. (2005)
Indigo	3CLpro	*Isatis indigotica* Fortune ex Lindl.	Islam et al. (2020), Lin et al. (2005)
Hesperetin	3CLpro	*Isatis indigotica* Fortune ex Lindl.	Lin et al. (2005)
B-sitosterol	3CLpro	*Isatis indigotica* Fortune ex Lindl.	Islam et al. (2020), Lin et al. (2005)
Kazinol A/B/F/J	3CLpro, PLpro	*B. papyrifera*	Ryu et al. (2010)
Broussoflavan A	3CLpro, PLpro	*B. papyrifera*	Ryu et al. (2010)
4-Hydroxyisolonchocarpin	3CLpro, PLpro	*B. papyrifera*	Ryu et al. (2010)
Papyriflavonol	3CLpro, PLpro	*B. papyrifera*	Ryu et al. (2010)
3′-(3-Methylbut-2-enyl)-3′,4.7-trihydroxyflavane	3CLpro, PLpro	*B. papyrifera*	Ryu et al. (2010)
Papyriflavonol A	PLpro	*B. papyrifera*	Ryu et al. (2010)
Bavachinin	PLpro	*Psoralea corylifolia* L.	Lin et al. (2005), Kim et al. (2014)
Neobavaisoflavone	PLpro	*Psoralea corylifolia* L.	Lin et al. (2005), Kim et al. (2014)
Isobavachalcone	PLpro	*Psoralea corylifolia* L.	Lin et al. (2005), Kim et al. (2014)
4′-O-methylbavachalcone	PLpro	*Psoralea corylifolia* L.	Lin et al. (2005), Kim et al. (2014)
Corylifol A	PLpro	*Psoralea corylifolia* L.	Lin et al. (2005), Kim et al. (2014)
Psoralidin	PLpro	*Psoralea corylifolia* L.	Lin et al. (2005), Kim et al. (2014)
Celastrol	3CLpro	*Triterygium regelii* Sprague & Takeda	Ryu et al. (2010)
Pristimerin	3CLpro	*Triterygium regelii* Sprague & Takeda	Ryu et al. (2010)
Tingenone	3CLpro	*Triterygium regelii* Sprague & Takeda	Ryu et al. (2010)
Iguesterin	3CLpro	*Triterygium regelii* Sprague & Takeda	Ryu et al. (2010)
Sabadinine	3CLpro	*Veratrum sabadilla* Retz.	Toney et al. (2004)
Aurantiamide	Cathepsin-L	*Artemisia annua*	Wang et al. (2007)
Daidzein	GRP78 (HSPA5), estrogen receptor	*Cicer arietinum* L.	Sayed and Elfiky (2018)
Genistein	GRP78 (HSPA5), estrogen receptor	*Cicer arietinum* L.	Sayed and Elfiky (2018)
Formononetin	GRP78 (HSPA5), estrogen receptor	*Cicer arietinum* L.	Sayed and Elfiky (2018)

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TABLE 1 | (*Continued*) Summary of anti-CoV compounds and extracts.

Names of compounds and extracts	Target specificity	Plant	References
Biochanin A	GRP78 (HSPA5), estrogen receptor	*Cicer arietinum* L.	Sayed and Elfiky (2018)
Hydroxytyrosol	GRP78 (HSPA5)	Olive leaf	Sayed and Elfiky (2018)
Cis-p-coumaric acid	GRP78 (HSPA5)	*Gnetum cleistostachyum*	Sayed and Elfiky (2018)
Cinnamaldehyde	GRP78 (HSPA5)	*Cinnamomum*	Sayed and Elfiky (2018)
Thymoquinone	GRP78 (HSPA5)	*Nigella sativa*, *Monarda fistulosa*	Sayed and Elfiky (2018)
Griffithsin	Attachment, viral entry	Red algae *Griffithsia*	Dai et al. (2019), Moghaddam et al. (2014)
Agglutinin	Attachment, viral entry	*L. radiata*	Keyaerts et al. (2007)
GlcNAc-specific lectin	Attachment, viral entry	*Polyporus squamosus*	Keyaerts et al. (2007)
(GlcNAc)n-specific lectin	Attachment, viral entry	*Psathyrella velutina*	Keyaerts et al. (2007)
Gal-specific lectin	Attachment, viral entry	Mistletoe	Keyaerts et al. (2007)
Man/Glc-specific lectin	Attachment, viral entry	*Canavalia ensiformis*	Keyaerts et al. (2007)
Gal/GalNAc-specific lectin	Attachment, viral entry	*Erythrina corallodendron*	Keyaerts et al. (2007)
GalNAcα(1.3)Gal > GalNAc > Gal-specific lectin	Attachment, viral entry	*Artocarpus lakoocha*	Keyaerts et al. (2007)
Man/GalNAc-specific lectin	Attachment, viral entry	*Chlorophyllum molybdites*	Keyaerts et al. (2007)
Agglutinin	Replication, S-glycoprotein, GlcNAc	*Urtica dioica* L., *Galanthus nivalis* L	Kumaki et al. (2011)
Lectin	Propagation	*Allium porrum* L., *Nicotiana tabacum* L., *U. dioica*	Yonesi and Rezazadeh (2020)
Griffithsin	S-glycoprotein	*Griffithsia* sp.	O'Keefe et al. (2010), Millet et al. (2016), Li et al. (2019)
Concanavalin A	Hemagglutinate	*Canavalia ensiformis* (L.) DC.	Greig and Bouillant (1977)
DLasiL	Attachment, viral entry	*Dioclea lasiocarpa* Mart. ex Benth.	Hsieh et al. (2010)
Agglutinin	Hemagglutinate	*Galanthus nivalis* L.	Hsieh et al. (2010)
Lectin	Virion membrane	*Sambucus nigra* L.	Chen et al. (2014)
Aqueous extracts	3CLpro, RdRp	*Houttuynia cordata* Thunb.	Yang et al. (2010), Hoever et al. (2005)
Aqueous extracts	Replication	*Toona sinensis* (Juss.) M.Roem.	Jeong et al. (2014)
Methanol extracts	RdRp, protease	*Sophora* sp., *Acanthopanacis* sp., *Sanguisorbae* sp., *Torilis* sp.	Jin et al. (2019)
Ethanol extracts	Replication	*Nigella sativa* L., *Citrus sinensis* L.	Jin et al. (2019)
Aqueous extracts	Attachment, viral entry	*Sambucus nigra* L. extract (black elderberry)	Wittemer et al. (2005)
Ethanolic extract	3CLpro	*Torreya nucifera*	Ryu et al. (2010)
Ethanol extracts	Viral attachment	*Pelargonium*	Michaelis et al. (2011)
Ethanol extracts	Virus envelope	*Mentha piperita* L., *Thymus vulgaris* L., *Desmodium canadense* (L.) DC.	Lelesius et al. (2019)

Shanavas, 2020). In contrast, for ACE2, oxygen of the COOH of 5-di-O-galloylshikimic acid binds to the Mpro cavity site via H-bonds, and non-covalent and ionic interactions. The −OH groups of the benzoyl moiety form H-bonds with the Mpro cavity site, where the side chain groups bind to benzoyl rings via the p–π stacking interaction. Therefore, the −OH groups are crucial for the SAR.

Flavones such as apigenin and quercetin inhibit 3CLpro activity, which coincides with the enzyme-inhibitory data. The 3CLpro inhibitory potential of biflavone with apigenin residue at the flavone C-3′ is enhanced, indicating that the 3CLpro inhibitory activity is upregulated by the additional apigenin residue at C-3′. In fact, the biflavonoid amentoflavone inhibits the 3CLpro activity. Quercetin recognizes the S-glycoprotein–ACE2 interface site (Smith and Smith, 2020; Williamson and Kerimi, 2020). A quercetin derivative, avicularin (Fukunaga et al., 1989) has also the Mpro-binding affinity. A similar scutellarein glucoside has the Mpro- and ACE2-binding affinities, where the −OH groups of glycoside form the H-bonds with the Mpro catalytic site. Another −OH group of the phenyl ring also forms H-bond with the Mpro. The phenyl ring also forms the π–π stacking interaction. The carbonyl oxygen and −OH group of the chromone nucleus form the H-bonds (Sharma and Shanavas, 2020). Similar to delphinidin diglucoside and scutellarein glucoside, L-arabinoside of avicularin binds to the catalytic site through H-bonds. The benzene ring involves in the π–π stacking with the hydrophobic subsite. Also, the −OH group of the chromone nucleus and benzene ring recognize the active site through H-bonds. The −OH groups of the arabinoside and phenyl ring recognize Mpro domain 1. Multiple π–π stacking interactions are formed between the chromone nucleus and the Mpro domain. The carbonyl group of the main nucleus forms the H-bonds with the Mpro. Likely, a flavanone glycoside, hesperidin, forms multiple H-bonds with the Mpro.

The flavonoid myricetin binds to both nsp13 and anti-3CLpro (Ananda Silva et al., 2020) as well as the TMPRSS2 active pocket through the 3 H-bonds, van der Waals forces, and π-anion (Pooja et al., 2021). Similarly, baicalein interacts with TMPRSS2 via 3 H-bonds, and van der Waals and π-stacking interactions. Aesculitannin B (Pooja et al., 2021) and proanthocyanidin bind to the TMPRSS2 active site via 5 H-bonds, and van der

Waals and amide–π stacking interaction. Hydrocinnamic caffeic acid and ferulic acid recognize the Mpro active site via the H-bonds. Caffeic acid forms the H-bonds with both E- and N-proteins (Bhowmik et al., 2020). A bioflavonoid rutin also forms H-bonds with M- and N-proteins. Theaflavin interacts with the catalytic pocket groove near the RdRp active site through H-bonds and π–cation interaction, resulting in low docking score (Lung et al., 2020). Membrane binding of the alkyl gallates depends on alkyl chain lengths (Stefaniu et al., 2020) by high polarity–triggered reactivity. Therefore, the position of the –OH groups on the benzoic acid ring seems to be essential, compared with the number or type of ester, –OH, and methoxy groups.

For the SAR of glycyrrhizin and glycyrrhetinic acid, the free –OH (C-3), carbonyl (C-11), and COOH (C-30) groups influence the antiviral activity, while esterification of the –OH group on C-3 or the COOH group on C-30 decreases the activity. In addition, the dual esterification in the C-3 and C-30 decreases the activity, while substitution of the C-30 increases the activity (Wang et al., 2012). Betulonic acid, a triterpenoid, has an anti–SARS-CoV activity through the ketoxime backbone (Kazakova et al., 2011). The betulonic acid has a –OH and a COOH with a double bond at position C-20, 3-OH and 28-COOH groups (Regueiro-Ren et al., 2018), and C-3 and C-17 positions are crucial for the activity. Polyphenolic tannins show different binding capacities to the 3CLpro due to their SAR activities. The tannins recognize the receptor-binding spot and putative catalytic dyad of the 3CLpro. Tannic acid is a specific polyphenolic form of tannin with weak acidic properties due to the grouped phenols, where –OH groups, ketone groups (=O), and phenolic rings involve in binding to the 3CLpro through H-bonds and other forces (Khalifa et al., 2020). For example, hydrolyzable tannins including pedunculagin directly recognize the catalytic dyads and 3CLpro receptor–binding site with 5 H-bonds. Similarly, castalin and tercatain recognize the 3CLpro receptor–binding site via H-bonds and arene–arene interactions, influencing the catalytic dyad residues. Other hydrolyzable tannins including punicalin and isoterchebin secondarily recognize the catalytic dyad residues of the 3CLpro. Thymoquinone also interacts with the catalytic site of the 3CLpro via multiple H-bonds and π–H interactions (Kadil. et al., 2020). The –OH and carbonyl groups interact with the targets via H-bonds. For example, the –OH group binds to the Mpro, Nsp15, and S-glycoprotein (Kodchakorn et al., 2020).

CONCLUSION

The COVID-19 outbreak is a global pandemic health problem. The SARS-CoV-2 RNA sequence has been known to be highly homologous with those of the CoVs. For the present crisis of pandemic SARS-CoV-2 infections, therapeutic and preventive approaches are simultaneously required to overcome the current life-threatening disease. Because development of blockers and inhibitors of viral entry and replication is urgent, computational AI has been incorporated to accelerate drug designation. Natural resources contain promising ligands for the development of therapeutic targets. Naturally occurring compounds are potentially promising resources for their antiviral properties. SARS-targeting agents can be effective against related CoV strains due to their similar life cycles. LMW compounds can be generated, discovered, and simulated with AI assistance for target molecules. Chemical derivative modification of known structures by AI-based technologies can enhance such drug activities. Thus, natural products may be useful for use in medical therapy of SARS-CoV-2 infections.

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SUPPLEMENTARY MATERIAL

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GLOSSARY

COVID-19 coronavirus disease 2019
SARS-CoV severe acute respiratory syndrome-related coronavirus
MERS-V Middle East respiratory syndrome virus
HCoV human CoV
HE hemagglutinin-esterase
RdRp RNA-dependent RNA polymerase
E envelope
N nucleocapsid
S spike glycoprotein
M membrane matrix glycoprotein
ER endoplasmic reticulum
ERGIC endoplasmic reticulum–Golgi intermediate compartment
Nsp nonstructural protein
TM transmembrane
PLpro Papain-like protease
RBD receptor-binding domain
ssRNA single-stranded RNA
NA neuraminidase
Neu5Ac N-acetylneuraminic acid
Neu5,9Ac2 9-O-acetyl-N-acetylneuraminic acid
Neu5Ac9NAc 9-acetamido-9-deoxy-N-acetylneuraminic acid
HEF HE fusion protein
BCoV Bovine CoV
MHV murine hepatitis virus
CEACAM carcinoembryonic antigen cell adhesion molecule
9-O-Ac-SA 9-O-acetylated SA
IBV infectious bronchitis virus
pAPN porcine aminopeptidase N
GRP78 membrane-associated 78-kDa glucose-regulated protein
ACE2 angiotensin-converting enzyme 2
DPP4 dipeptidyl peptidase-4dipeptidyl peptidase-4
hAPN human APN
MHC-I major histocompatibility complex class I
DC-SIGN dendritic cell–specific intercellular adhesion molecule-3-grabbing nonintegrin
HSPG heparan sulfate proteoglycan
HSPA5 heat shock protein A5
RAS renin–angiotensin system
ADAM A disintegrin and metallopeptidase domain
TACE TNF-α–converting enzyme
TMPRSS transmembrane protease serine
HAT human airway trypsin-like protease
MSPL serine 13
DPP4 dipeptidyl peptidase-4dipeptidyl peptidase-4
BiP immunoglobulin protein
UPR unfolded protein response
ATF6 activating transcription factor 6
IRE1 inositol-requiring enzyme 1
PERK protein kinase RNA (PKR)–like ER kinase
SBD substrate-binding domain
PEDV porcine epidemic diarrhea coronavirus virus
FIPV feline infectious peritonitis virus
FeCV feline enteric virus
GAG glycosaminoglycan
RBM receptor-binding motif
3CLpro 3-chymotrypsin–like protease

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REVIEW
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Naturally Occurring Bioactives as Antivirals: Emphasis on Coronavirus Infection

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The current coronavirus disease (COVID-19) outbreak is a significant threat to human health and the worldwide economy. Coronaviruses cause a variety of diseases, such as pneumonia-like upper respiratory tract illnesses, gastroenteritis, encephalitis, multiple organ failure involving lungs and kidneys which might cause death. Since the pandemic started there have been more than 107 million COVID-19 infections caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and ~2.4 million deaths globally. SARS-CoV-2 is easily transmitted from person-to-person and has spread quickly across all continents. With the continued increase in morbidity and mortality caused by COVID-19, and the damage to the global economy, there is an urgent need for effective prevention and treatment strategies. The advent of safe and effective vaccines has been a significant step forward in the battle against COVID-19, however treatment of the

Abbreviations: 3CLpro, chymotrypsin 3C-like protease; BCoV, bovine coronavirus; CCV, canine coronavirus; CEP, cepharanthine; COVID-19, coronavirus disease; COX-2, cyclooxygenase-2; CoV, Coronavirus; DPP4, Dipeptidyl-peptidase-4; ERK, extracellular-signal regulated kinase; FAN, fangchinoline; FCoV, feline coronavirus; IBV, infectious bronchitis virus; JAK-STAT, Janus kinase-signal transducer and activator of transcription; MDA5, Melanoma differentiation associated protein-5; MERS CoV, Middle East respiratory syndrome coronavirus; MHV, Murine hepatitis virus; NF-κB, Nuclear factor-κB; PEDV, porcine epidemic diarrhea virus; PLpro, Papain like protease; PRCoV, Porcine respiratory coronavirus; RDRP, RNA dependent RNA polymerase; SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; SARS-CoV, severe acute respiratory syndrome coronavirus; TCM, traditional Chinese medicine; TET, tetrandrine; TGEV, transmissible gastroenteritis virus; TLR, Toll like receptor; UPR, unfolded protein response; hACE-2, human angiotensin converting enzyme-2; hCoVs, human coronaviruses.

symptoms associated with the disease still requires new anti-viral and anti-inflammatory drug therapies. To this end, scientists have been investigating available natural products that may be effective against SARS-CoV-2, with some products showing promise in fighting several viral infections. Since many natural products are dietary components or are prepared as dietary supplements people tend to consider them safer than synthetic drugs. For example, Traditional Chinese Medicines have been effectively utilized to treat SARS-CoV-2 infected patients with promising results. In this review, we summarize the current knowledge of COVID-19 therapies and the therapeutic potential of medicinal plant extracts and natural compounds for the treatment of several viral infections, with special emphasis on SARS-CoV-2 infection. Realistic strategies that can be employed for the effective use of bioactive compounds for anti-SARS-CoV-2 research are also provided.

Keywords: Coronavirus, SARS-CoV-2, bioactive compounds, natural compounds, COVID-19

INTRODUCTION

Coronavirus disease (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that has endangered the whole world and its pandemic is of global public health concern (Rothan and Byrareddy, 2020). Regardless of rigorous control in many countries and quarantine efforts, the spread of COVID-19 is still ongoing. Coronaviruses (CoV) were initially thought to be associated with mild respiratory illnesses in humans and not fatal, until the recent outbreak. However, the current CoV infection, SARS-CoV-2, has proven to be one of the most pathogenic and contagious viral infections, presenting clinically as severe atypical pneumonia and severe acute respiratory diseases (Perlman and Netland, 2009; Lauber et al., 2012; Coronavirus disease, 2019). Briefly, SARS-CoV-2 infection is characterized by sore throat, high fever, chills, cough, breathlessness, severe pneumonia, and death due to multi-organ failure involving kidneys and lungs often leading to death (Coronavirus disease, 2019; WHO Novel Coronavirus–China).

Currently, COVID-19 is thought to be a zoonosis, with the virus being transmitted from animals to humans, then mutating to promote human-to-human transmission (Coronavirus disease, 2019). SARS-CoV-2 is similar to SARS-CoV (also a zoonotic CoV) that first appeared in 2002 in Southern China, then spread throughout the world (Coronavirus disease, 2019; Hui and Zumla, 2019). In 2012, another novel strain of CoV emerged, also causing a SARS-like disease epidemy, although it did not develop into a pandemic. MERS (MERS-CoV, another zoonotic CoV) was endemic to the Middle East, with a particularly high fatality rate (around 35%) (Ajlan et al., 2014; Azhar et al., 2019), and was thought to be caused by contact with camels or camel-based products during the 2012–2013 outbreak (Azhar et al., 2019). Interestingly, birds and other mammals also suffer from a variety of CoV infections, which are mostly fatal, such as the infectious bronchitis virus (IBV) in poultry, transmissible gastroenteritis virus (TGEV) in pigs and bovine coronavirus (BCoV) in cattle, all associated with huge economic losses(Pyrc et al., 2008).

In terms of treatment of COVID-19 infections, the current focus has been to test already approved antiviral medications, molecules that bind to the virus, such as antibodies, siRNA, ribozymes, and many natural products, including Traditional Chinese Medicines (TCM) (Gu and Korteweg, 2007; Kumar et al., 2013). In fact the use of natural products and traditional plant-based medicines for the treatment of COVID-19 is common in many developing countries, and investigations of naturally derived bioactive substances have shown promising anti-viral effects against a number of viruses through multiple mechanisms (Suwannarach et al., 2020; Mukhtar et al., 2008). Additionally, naturally occurring bioactive substances from plants have significant anti-inflammatory, antifungal, antibacterial, and immunomodulatory effects (Suwannarach et al., 2020; Mukhtar et al., 2008). Thus, considering that natural plant derivatives are already reported to have antiviral effects, they may be good options to be explored for the research and development of novel anti-SARS-CoV-2 therapeutics and value added approaches to current therapies. The aim of this review was to provide an overview of SARS-CoV-2, and current therapies with a focus on the effects of naturally occurring medicinal plant extracts and bioactive compounds on CoVs, to outline their possible development for the prevention and treatment of SARS-CoV-2 infection.

CORONAVIRUSES: A BRIEF OVERVIEW

Coronaviruses (CoVs) are characteristically enveloped, with a positive-sense single-stranded RNA genome, a lipid membrane originating from the host cell, and club-like spikes on their surfaces (**Figure 1**). (Song et al., 2004) Proteins protruding from viral membrane gives the virus its characteristic halo-like appearance, which is the reason for the name "corona" (Ludwig and Zarbock, 2020). CoVs belong to the order Nidovirales, that includes the families: arteriviridae, Coronoviridae, Mesoviridae and the Roniviridae. The Coronoviridae is comprised of the Coronavirinae and Torovirinae subfamilies. Coronavirinae subfamily is further divided into four genera: alpha (α), beta (β), gamma (γ), and delta (δ) CoVs (Cui et al., 2019). Among

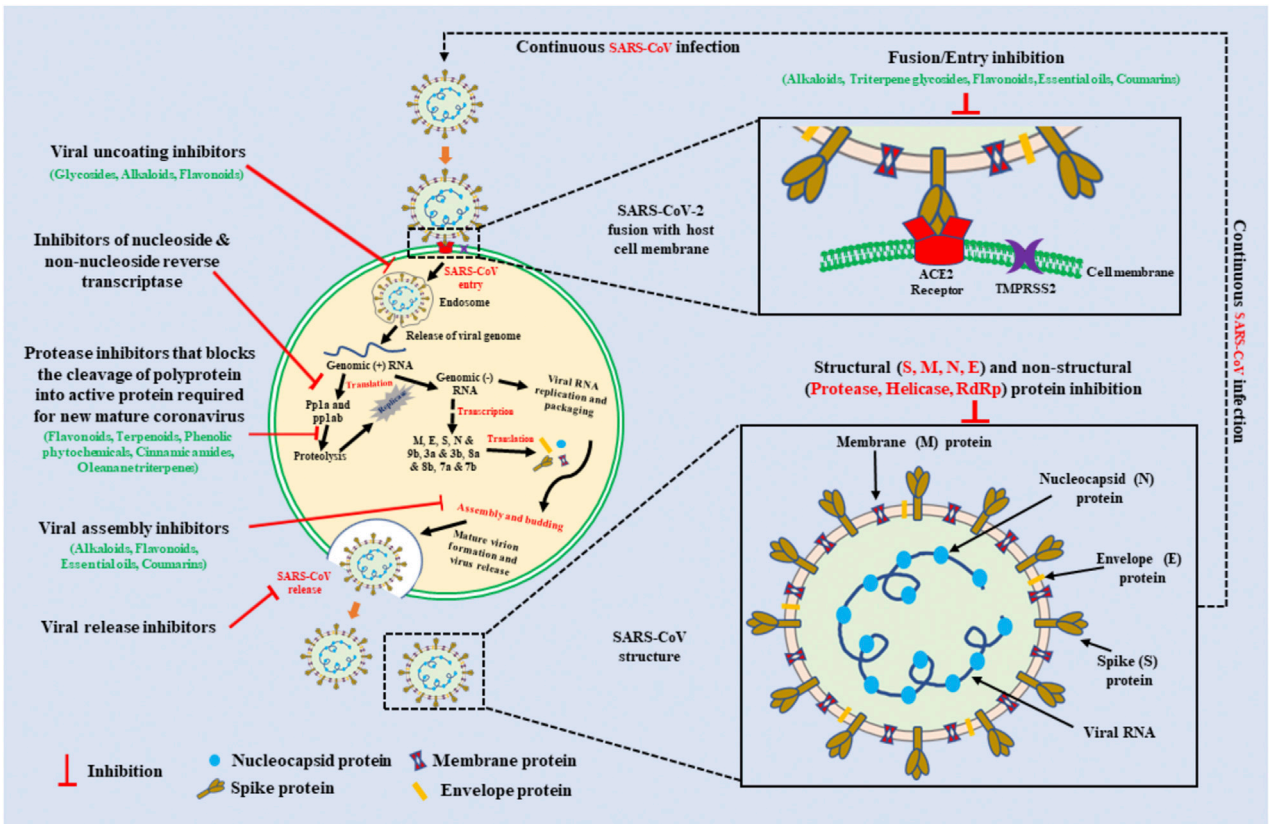

FIGURE 1 | Schematic representation of a Coronavirus replication cycle, SARS-CoV virus structure, and the molecular targets for naturally occurring plant extracts and their active compounds against on viral infection. For attachment with host cells, SARS-CoV S protein use the cellular attachment factor Angiotensin-converting enzyme 2 (ACE2) and the cellular protease Transmembrane protease serine 2 (TMPRSS2) for their activation. SARS-CoV enters into host cells via endocytosis and release its RNA genome inside the cell. Further early and late protein synthesis occurs. Finally, viral assembly and then release outside the cells via exocytosis. The natural compounds that can target at different stages of viral replication may inhibit SARS-CoV infection. Inhibitors derived from plant sources such as alkaloids, glycosides, phenolic phytochemicals, essential oils, coumarins, cinnamic amides, and oleanane triterpenes may provide great treatment. Schematic diagram of SARS-CoV virus structure represents the single stranded positive-sense viral RNA, Spike (S), Nucleocapsid (N), Membrane (M), and Envelope (E) protein. Bioactive compounds that can inhibit structural and non-structural proteins of coronaviruses can be used with inhibitors of viral replication cycle.

these, α- and β-CoV can infect mammals, whereas γ- and δ-CoV mainly infect birds, however CoVs are found in humans and several animal species (Ludwig and Zarbock, 2020), and have high mutation rates thereby enabling CoVs to infect different species (Duffy et al., 2008). Most recently, a novel member of the human CoV emerged and is now formally named as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) (Ludwig and Zarbock, 2020). This unique strain of CoV was previously reported in humans, and is able to cause disease in humans, masked palm civets, mice, dogs, cats, camels, pigs, chickens, and bats. SARS-CoV-2 has been observed to cause severe respiratory and gastrointestinal sickness in animals and humans and may be transmitted through aerosols and direct/indirect contact.

Some specific structural proteins have been identified in the virus that play significant roles in its pathogenesis and development of severe infection. These protein molecules are encoded by the SARS-CoV genome and have been identified as possible targets for new anti-CoV agents. These proteins include: the spike (S) protein that allows for the entry of the virus into cells; the SARS-CoV chymotrypsin 3C-like protease ($3CL^{pro}$) that is required for the viral life cycle; the ntpase/helicase, RNA-dependent RNA polymerase (RDRP); the membrane (M) protein that is required for viral budding; the envelope (E) protein that plays a role in CoV assembly; and the nucleocapsid (N) phosphoprotein that is related to viral RNA inside the virion and possibly other viral protein-mediated processes. By binding to and interacting with these critical proteins, drugs and natural compounds may prevent infection or alter viral replication and spread. Thus, a better understanding of these proteins, and other important components of the SARS-CoV, may increase investigations of novel anti-SARS-CoV agents using a targeted approach against these specific proteins.

Characteristics of Coronavirus Proteins

CoVs have a spherical structure with ~125 nm diameter (Fehr and Perlman, 2015). The genome consists of a positive single-stranded RNA with a 5′ cap and a 3′ poly-A tail (**Figure 1**). Around 20 kb of the genome at the 5' end consists of a replicase gene, which encodes about 16 non-structural proteins (Snijder et al., 2016). The structural spike (S), nucleocapsid (N), envelope

(E), and membrane (M) and the accessory proteins are encoded by the rest of the genome (Nga et al., 2011). The replicase gene encodes for open reading frames (ORFs), rep1a and rep1b, which express respectively the polyproteins pp1a and pp1ab. Translation of both polyproteins from the same ORF is mediated by a slippery sequence and ribosomal frameshifting caused by an RNA pseudoknot (Brierley et al., 1989; Baranov et al., 2005). The nsps 1–11 occur in pp1a whereas nsps 1–16 occur in pp1ab (Ziebuhr et al., 2000).

The spike glycoprotein (~150 kDa) is a type-1 transmembrane protein, with around 20–25 N-glycosylation sites and is present as a homotrimer on viral surface. The protein has two domains, an N-terminal S1 domain, and a C-terminal S2 domain. The S1 domain has the receptor binding sites and the S2 domain contains two heptad repeats that mediate viral fusion with the host cell. The S protein attaches to different receptors on the host cell based on the type of coronavirus. For example, α-CoV interact with aminopeptidase N, whereas SARS-CoV and HCoV-NL63 interact with angiotensin-converting enzyme 2 (ACE2) on the host cell surface. Other known receptors include CEACAM1 and dipeptidyl-peptidase 4, respectively, used by MHV and MERS-CoV to enter the host cells (Kubo et al., 1994; Bosch et al., 2003; Belouzard et al., 2009).

The membrane glycoprotein (~30 kDa) is responsible for giving the viral shape. It is a dimer which promotes the membrane curvature and binds to nucleocapsid-RNA complex during virus packaging. The M protein has three domains: an N-terminal ectodomain, followed by three transmembrane domains, and a C-terminal endodomain. The ectodomain is either O- or N-linked, glycosylated depending on the type of the virus and is susceptible to proteolytic cleaving (Nal et al., 2005; Neuman et al., 2011).

The envelope protein (~8–12 kDa) is present at limited quantities in the virus. The protein sequence varies among distinct viruses, although structurally similar. It is a transmembrane protein with ion channel activity. Its main role is in the assembly and release of viral particles (Nieto-Torres et al., 2014).

The N protein (~45–50 kDa) is the protein component of the helical nucleocapsid. Both the N- and C-terminal domains of the protein bind to RNA in a phosphorylation-dependent, beads-on-string manner (Chang et al., 2006). The N protein interacts with transcription regulating sequences, regions of 3' UTR on viral RNA, and genomic RNA packaging signals (Cologna et al., 2000; Chen et al., 2005). Nuclear localization of N protein in some viruses has been detected, although its role in viral replication is not yet known. N protein helps in packaging the viral RNA into the virus, besides to interacts with NS3 and M protein (Sturman et al., 1980; McBride et al., 2014).

A subset of β-CoVs have an additional hemagglutinin-esterase structural protein. There is some speculation that this protein may enhance viral entry and spread through interactions with sialic acids on the surface glycoproteins, acting as hemagglutinin and acetyl-esterase (Klausegger et al., 1999). Apart from the replicase and structural genes, CoVs have additional ORFs interspersed between and/or overlapping with these genes that encode for accessory proteins. CoVs can have one to nine accessory proteins (ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9, ORF10, and ORF14) depending on the virus type (Nga et al., 2011). Most are nonstructural proteins, but sometimes they can be a part of the virus structure, for instance the SARS-CoV 3a protein (Fischer et al., 1997). In the most studied animal CoV, murine hepatitis virus, accessory proteins have been found unnecessary for viral replication in tissue culture, although they seemed to enhance the *in vivo* virulence (Narayanan et al., 2008).

The non-structural CoVs proteins play distinct roles in virus-mediated infection. For example, Nsp1 helps in blocking host cell translation and immune response, conferring a favorable environment for virus propagation (Huang et al., 2011; Tanaka et al., 2012). Nsp3 is a papain-like protease that cleaves the nsp1/nsp2, nsp2/nsp3, and nsp3/nsp4 boundaries (Ziebuhr et al., 2000). It also prevents host cell degradation, which is required for proper host proteome functioning. Nsp4 and Nsp6 are transmembrane proteins that may act as a basis for the double membraned vesicles where virus replication and assembly take place (Oostra et al., 2008; Gadlage et al., 2010). Nsp5, also known as Mpro, is a serine-like protease that catalyzes the remaining 11 cleavage events of the replicase gene product (Ziebuhr and Siddell, 1999; Ziebuhr et al., 2000; Ziebuhr, 2005). Nsps7 and eight act as processivity clamps for the polymerase, Nsp12 (Zhai et al., 2005). Nsp10 is a cofactor for Nsp16, which protects viral RNA from MDA5 recognition and viral RNA from host antiviral mechanisms (Bouvet et al., 2010; Decroly et al., 2011). Nsp12 is a RNA-dependent RNA polymerase. Nsp13 is a RNA helicase with a 5′-triphosphatase activity (Ivanov et al., 2004; Ivanov and Ziebuhr, 2004; Minskaia et al., 2006), and Nsp14 is a methyltransferase (mtase) that adds 5′ cap to viral RNA, also having a 3′-5′ exonuclease activity required for viral genome proofreading (Chen et al., 2009). Nsp15 is an endoribonuclease that cleaves extra viral RNA as a defensive measure from host attacks. The functions of other Nsps are not yet clear.

Mechanisms of Infection and Targeted Tissues

Coronaviruses are highly contagious, and may be spread by inhalation or ingestion of virus-containing droplets, leading to clinical symptoms, such as coughing and sneezing among others (Boopathi et al., 2020). Viral N protein allows the virus to hijack human cell mechanisms to create viral factories (Boopathi et al., 2020). For penetration into the host cell, CoVs depend on envelope fusion with the host cell membrane, and the S protein facilitates the CoVs entry into host cells by binding with host hACE2 receptors (Belouzard et al., 2012; Ou et al., 2020). After the interaction with the hACE2 receptors, the S protein undergoes acid-dependent cleavage by a host protease at two sites. The first cleavage separates the receptor-binding site and the fusion domain on the S protein, while the second cleavage exposes the fusion peptide S2', that mediates viral fusion with host membranes. Translation of the replicase gene from the viral genome occurs and both genomic and sub-genomic viral RNAs are synthesized by negative-strand intermediates. Sub-genomic

RNAs code for structural and accessory proteins, which are translated and inserted into the endoplasmic reticulum (ER) and move along the ER-Golgi intermediate compartment where virus assembly takes place (Fehr and Perlman, 2015). The N protein with bound viral RNA forms budding structures at the compartment membrane and M protein incorporate other proteins into the virus structure by interacting with them and the nucleocapsid. Finally, the assembled virus particles are released from the host cell by exocytosis (Tooze et al., 1984; Krijnse-Locker et al., 1994).

In humans, CoV infections were linked only to mild respiratory illnesses until the advent of the 2002/2003 SARS-CoV outbreak, then the 2012/2013 MERS-CoV outbreak, and finally the 2019/2020 SARS-CoV-2 pandemic. These outbreaks were associated with severe pneumonia-like respiratory illness and death due to multi-organ failure. Human CoVs primarily infect immunocompromized individuals, such as the elderly, and patients suffering from other chronic diseases including diabetes, hypertension, obesity and heart disease, with most fatalities reported in patients over the age of 50 (Pene et al., 2003; Gorse et al., 2009). CoVs start by infecting the lung epithelium, then enter macrophages and dendritic cells producing significant proinflammatory cytokines and chemokine secretion called "cytokine storm". This cytokine storm, led by interleukin (IL)-6, is a severe response to viral infection, and initiate molecular events that are the basis of multi-organ failure and death associated with COVID-19 infections (Gu and Korteweg, 2007; Van Der Hoek et al., 2005). CoVs further use host systems for their propagation, and by inhibiting host translation, CoVs convert host translation mechanisms to viral translation (Fung et al., 2016). Viral protein synthesis induces ER stress, which in turn induces unfolded protein response (UPR) that inhibits host translation by protein kinase RNA-like ER kinase (PERK)-induced phosphorylation of the translation initiating factor eIF2α. Also, Nsp1 inhibits the initial steps of translation, such as the conversion of 48 initiation complex into 80s complex, which could be seen in SARS-CoV (Lokugamage et al., 2012). Moreover, CoVs have developed mechanisms to escape the host innate immune system. MDA-5 and toll-like receptors (TLRs) are host factors that detect viral RNA and proteins (Bruns and Horvath, 2014; Totura et al., 2015). Activation of such "sensors" leads to the activation of interferon (IFN) signaling and nuclear factor-κB (NF-κB) pathway and induces IFN-stimulated genes by the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) (JAK-STAT) signaling cascade (Schoggins and Rice, 2011). However, different CoVs have evolved different strategies of immune system evasion. As an example, the Nsp1 protein of SARS-CoV and the N protein of MHV suppress IFN signaling, and accessory proteins, such as ORF3b and ORF6 of SARS-CoV, ORF4a, 4b, and five of MERS-CoV have also been reported to inhibit IFN signaling (Kopecky-Bromberg et al., 2007; Siu et al., 2014). Subsequently, the Nsp3 overexpression suppresses IFN signaling that causes interference in both host immune signaling factors processing and function (Matthews et al., 2014; Li et al., 2016). Moreover, Nsp16 mediates 2′-O methylation of viral RNAs masking it from MDA5 (Menachery et al., 2014). Thus, taken together, such immune suppression strategies help to enhance the pathogenicity of CoV infections.

Minor changes in the S protein have been observed in CoV, that may alter the infectivity of the virus in different hosts. For example, hSARS-CoV can infect both palm civets and humans, whereas palm civet SARS-CoV cannot infect humans. For hSARS-CoV, two-point mutations in the S protein were identified, that allow the S protein to bind to the hACE-2 receptor (Li et al., 2005). Similarly, transmissible gastroenteritis coronavirus (TGEV) and porcine respiratory coronavirus (PRCoV), both bind to porcine aminopeptidase N (APN). Due to a deletion in the N-terminal domain of the S protein, TGEV infects the epithelial cells of both respiratory tract and small intestine, while PRCoV can only infect the pulmonary epithelium (Rasschaert et al., 1990; Schultze et al., 1996). Some CoVs also interact with C-type lectins on host cell surfaces. For example, DC-SIGN is a receptor on macrophages and dendritic cells, while L-SIGN is a receptor in liver and lung endothelial cells. Such receptors usually recognize and bind glycosylated viral antigens. In this way, SARS-COV, human CoV 229E (HCoV-229E), infectious bronchitis virus (IBV), and feline CoV (FCoV) can infect these cells by interacting with these receptors by their highly glycosylated S proteins (Jeffers et al., 2004; Jeffers et al., 2006). For example, some IBV strains cause urinogenital and reproductive tract infection in chicken, but also respiratory disease (Perlman and Netland, 2009), while mouse hepatitis virus (or murine CoV, MHV), strain A59 may cause hepatic and enteric infections, and strain JHMV causes neurological disorders similar to multiple sclerosis in mice (Lampert et al., 1973; Houtman and Fleming, 1996). Overall, CoVs have a wide range of tissue tropism from lungs, gut, liver, kidneys, reproductive tract to nervous system.

ANIMAL AND HUMAN INFECTIONS BY CORONAVIRUS

Animal Infections by Coronavirus

There are some indications that SARS-CoV-2 is a zoonosis, a disease originating from animals and transmitted to humans. Overall, CoVs are known to cause multiple health implications, including enteritis, hepatitis, respiratory illnesses, encephalitis, demyelinating disease, urinary and reproductive tract infections, with symptoms such as diarrhea, cough, wasting, decreased milk or egg production being also present, in both mammals and birds. Below, are briefly described some examples:

- Avian infectious bronchitis virus, first discovered in the 1930s, and often causes fatal respiratory illnesses in young chickens and a decrease in eggs production. It spreads by oral-fecal route and air. Approximately eight viral serotypes have been characterized. The primary target is trachea, although it can also infect the bronchia, kidneys, and reproductive tract, including ovaries and oviducts. Antiviral antibodies can be detected three days after the infection (Wege et al., 1982; Jackwood, 2012).

- Turkey CoV was first detected in the 1950s. Infection triggers transmissible enteritis with symptoms like diarrhea, weight loss, and depression in turkeys with low mortality rates. Its primary target is the gut, leading to loss of microvilli, hemorrhage, and loss of goblet cells in the small intestine. These symptoms can be found 1 day after the infection (Wege et al., 1982).
- Bovine CoV is transmitted by the oral route and causes bovine viral diarrhea in young calves. It can also affect different ruminant, such as camels, elks, and deer. The primary target is the intestinal absorptive epithelium, with consequent extensive loss of water and ions. These symptoms can be seen within 20–30 h post-infection. It has been suggested that offspring-transmitted maternal antibodies can induce a certain degree of protection against the infection (Wege et al., 1982; Saif, 2010).
- Canine CoV was first detected in military dogs in the 1970s, with symptoms including mild gastroenteritis (vomiting and diarrhea). It is transmitted orally and the symptoms are observed within a week after the infection. Target tissues include small and large intestine and lymph nodes (Wege et al., 1982).
- Hemagglutinating encephalomyelitis virus causes vomiting and neurological symptoms in pigs, and often leads to death. It is transmitted by the oronasal route, primarily infecting the respiratory tract, tonsils, and then the small intestine from where it moves to peripheral ganglia through nerves. Symptoms appear 4 days after the infection. Neuronal tissue infection causes small intestine paralysis, leading to starvation and even death (Wege et al., 1982).
- Transmissible gastroenteritis virus causes vomiting and diarrhea in pigs, often causing death. It is transmitted orally and it infects the absorptive small intestine cells, despite respiratory disease can also occurs. Antibodies can be transferred from mother to suckling pigs.
- Porcine epidemic diarrhea virus causes severe gastroenteritis in piglets and is associated with high mortality.
- Porcine hemagglutinating encephalomyelitis virus causes vomiting, decay, encephalitis and enteritis in pigs. Different vaccines have been developed for transmissible gastroenteritis virus (TEGV) and porcine epidemic diarrhea virus (PEDV) (Perlman and Netland, 2009; Langel et al., 2016; Gerdts and Zakhartchouk, 2017; Pascual-Iglesias et al., 2019; Xue et al., 2019).
- Rat CoV is transmitted nasally and causes fatal respiratory disease in rats. The primary targets of infection are nasal epithelium and lungs. Another strain, the sialodacryoadenitis virus, infects the salivary and lacrimal glands. Infection spreads through the respiratory tract to the lymph nodes and eventually affects the salivary glands causing rhinitis and necrosis of the gland duct epithelium (Wege et al., 1982).
- Feline infectious peritonitis virus (FIPV) affects wild and domestic cats. It infects a wide range of tissues, such as trachea, intestine, liver, kidneys, and reticuloendothelial system. It causes loss of appetite, depression, fever, neurological symptoms, pleuritis, peritonitis, fibrin deposition in abdominal organs, proteinuria, and anemia. Data have shown that a high level of antibodies cannot prevent the infection, suggesting that this could be an immunopathological disease. Live, attenuated FIPV that contains a deletion of the group-specific gene, the 3abc cluster, was reported to protect cats from the lethal homologous challenge (Haijema et al., 2004).
- Murine hepatitis virus (MHV) strain-JHM-was first isolated in the 1940s from spontaneously paralyzed mice. It can be transmitted by urine, feces, intrauterine, and by respiratory route. It is responsible for diseases, like hepatitis, encephalomyelitis, and enteritis. JHM is especially neurotropic, targeting oligodendritic cells, and triggering a demyelinating disease; plaques can be found in the white matter of the central nervous system (CNS). Other strains of MHV, such as MHV-2, MHV-3, and MHV-A59 often cause fatal disease by destroying the parenchymal and Kupffer cells in the liver (Gombold et al., 1995).

Human Infections by Coronavirus

CoVs were initially thought to cause mild respiratory infections in humans until the 2002–2003 SARS-CoV outbreak, 2012–2013 MERS-CoV outbreak, and the current 2019–2020 SARS-CoV-2 pandemic, which has infected >107 million people and caused ~2.3 million deaths globally, but numbers are still rising. The first human CoVs were discovered in the 1960s by studying fluids from people with respiratory infections. The first detected hCoV was named B814, isolated from a boy suffering from a common cold. Later, hCoV-229E (α-CoVs) was isolated together with five other strains, and showed similarity to B814, IBV, and MHV. The infection was similar to the common cold, with symptoms such as sneezing, sore throat, headache, and runny nose. The other strains, OCH38 and OCH43 (β-CoV, lineage A) caused symptoms similar to 229E. NL63 (α-CoV) was identified in 2004 in two separate cases, one from a 7-month-old child suffering from febrile bronchiolitis, fever, conjunctivitis, coryza, and the other from an 8-month-old child with pneumonia. NL63 also causes acute laryngotracheitis. Finally, HKU1 was isolated from a 71-year-old man suffering from pneumonia, with infection symptoms being similar to common cold. These viruses cause 15–30% of the commonly occurring respiratory tract diseases. There is no direct antiviral therapy for these viruses, they naturally end their course and are most often not fatal (Su et al., 2016; Cui et al., 2019).

However, in 2002, during the SARS outbreak in Guangdong province, China, 8,273 cases were reported with 775 deaths. Interestingly, SARS-CoV (β-CoVs lineage B)-like viruses were identified in palm civets and raccoon dogs, commonly found in game-animal markets in China, suggesting that they may serve as intermediate virus reservoirs. Later, the identification of two novel bat CoVs that were more similar to SARS-CoV than any other virus showed horseshoe bats to be the natural SARS-CoV reservoirs, given that they use ACE2 receptors to infect the host. SARS-CoV-infected patients have symptoms such as myalgia and diarrhea, and other typical common cold symptoms. The virus mainly affected the respiratory and gastrointestinal tracts, liver, kidney, and brain. High pro-inflammatory cytokines and chemokines levels were also detected in infected patients, thus

TABLE 1 | Overview of the ten vaccines that have been approved by one or more regulatory agencies worldwide.

Vaccine	Type	Company	#Shots	Efficacy %	Storage
BNT162	mRNA	Pfizer/BioNTech	2	95	−80
mRNA-1273	mRNA	Moderna	2	95	−80
BBIBP-CorV	Inactivated virus	Beijing institute of biological products/sinopharm	2	79–86	2–8
WIBP	Inactivated virus	Wuhan institute of biological products	NS	NS	NS
CoronaVac	Inactivated virus	Sinopharm	2	50.4–91.25	
BBV152	Inactivated virus	Bharat biotech/Indian medical research council	2	60–70	2–8
Sputnik V	Viral vector	Gamaleya	2	91.6	2–8
ADZ1222	Viral vector	AstraZeneca/Oxford	2	63	2–8
Ad5-nCoV	Viral vector	CanSino biologics	2	92.5	2–8
EpiVacCorona	Peptide	VECTOR	NS	NS	NS

suggesting that deaths may result of immunopathological complications (Wang and Eaton, 2007).

In 2012, MERS-CoV (β-CoV lineage c) was identified from a 60-year-old person in Saudi Arabia who died from severe respiratory disease. Between 2012 and 2014, 855 cases with 333 deaths were reported. Symptoms begin with a sore throat, fever, cough, myalgia, and progress to severe pneumonia, septic shock, and death from kidney failure. Anti-MERS-CoV antibodies and similar MERS-CoV particles were detected in dromedary camels that live in close association with humans in Saudi Arabia. Moreover, studies have shown that a bat CoVs HKU4 is phylogenetically similar to MERS-COV, where it uses DPP4 as a receptor to infect hosts (e.g. bats, humans, camels, horses and rabbits) (Memish et al., 2013).

Finally, the current COVID-19 caused by SARS-CoV-2 (Situation reports) was first detected in a wet market in Wuhan, China. Characteristically, it is very similar to bat CoVs and similar viral particles have been detected in pangolin, with such animals being sold in China's wet markets for food purposes, and as components of TCM. Such findings raised speculations that the virus may have originated from bats, while the pangolins may have acted as intermediate hosts. Clinically, it is a rapidly spreading infection and it is transmitted from person-to-person (Zhang et al., 2020), with infected individuals presenting typical symptoms of pneumonia, sore throat, fever, chills and shortness of breath. Death occurs as a consequence of multi-organ failure, mostly involving lungs and kidneys.

COVID-19 INFECTION PREVENTION AND THERAPEUTIC INTERVENTIONS: A GENERAL OVERVIEW

Early efforts to prevent the spread of SARS-CoV-2 included early diagnosis, the isolation of infected people, frequent hand washing, wearing of masks and maintaining physical distancing have continued in most countries. Thankfully the development of vaccines for COVID-19 has been swift, and it is estimated that there are ~66 vaccine candidates being clinically developed, with as many as 170 in pre-clinical development (Belete, 2021; Funk et al., 2020; World Health Organization; Centers for disease control, 2021; Food and Drug Administration). At least ten vaccines have been approved by at least one national regulatory authority for public use: two mRNA vaccines (Pfizer/BioNTech and Moderna vaccines), four inactivated virus vaccines: BBIBP-CorV (Beijing Institute of Biological Products/Sinopharm, China), WIBP (Wuhan Institute of Biological Products, China), and CoronaVac (Sinopharm, China), BBV152 (Bharat Biotech, India), three non-replicating viral vector vaccines: Sputnik V (Gamaleya Research Institute, Russia), the AstraZeneca/Oxford vaccine (Oxford, United Kingdom), and Ad5-nCoV (CanSino Biologics, China), and one peptide vaccine EpiVacCorona (State Research Center of Virology and Biotechnology-VECTOR, Russia) (**Table 1**).

In the United States and other countries, two mRNA vaccines have been authorized for general use to prevent COVID-19: the Pfizer-BioNTech COVID-19 (also approved by the World Health Organization, WHO) and Moderna COVID-19 vaccines (FDA). Both of these vaccines require two shots (Pfizer- 21 days apart and Moderna- 28 days a part) but are 95% effective (FDA, **Table 1**). In addition, three new vaccines are currently in phase three clinical trials including: the AstraZeneca-Oxford's COVID-19 vaccine (now approved by the WHO), the Janssen COVID-19 vaccine, and the Novavax COVID-19 vaccine (Funk et al., 2020; Belete, 2021; World Health Organization; Centers for disease control, 2021; Food and Drug Administration). In China, India, and Russia, approved COVID-19 vaccines include Sputnik V (Gamelaya, Russia), BBV152 (Bharat Biotech, India) and CoronaVac (Sinovac, China). Information from clinical trials suggest that CoronaVac is anywhere from 50.4 to 91.25% effective depending on the country sponsoring the study (Funk et al., 2020; Belete, 2021; World Health Organization; Centers for disease control, 2021; Food and Drug Administration). CoronaVac is an inactivated virus vaccine that uses traditional technology similar to BBIBP-CorV and BBV152, other inactivated-virus vaccines for COVID-19 in phase III trials. CoronaVac does not need to be frozen, and both the vaccine and raw material for formulating the new doses could be transported and refrigerated at 2–8°C (36–46°F), temperatures at which flu vaccines are kept (**Table 1**). Reportedly, CoronaVac may be stable for up to three years in storage, that potentially offers significant advantages in vaccine distribution to regions where cold storage is an issue. The Sputnik V vaccine was developed by the Gamaleya Research Institute of Epidemiology and Microbiology and is an adenovirus viral vector

vaccine (**Table 1**). Analysis of the clinical trial published in The Lancet, suggests that this vaccine was 91.6% efficacy without unusual side effects (Logunov et al., 2020; Logunov et al.,2021).

Interestingly, the first two FDA (United States) approved vaccines from Pfizer and Moderna were novel mRNA vaccines that differ from conventional vaccines which trigger an immune response by injecting weakened or attenuated viruses into the body. The mRNA vaccines induce the body to produce the "S" or spike protein that triggers the immune response to produce antibodies against the spike protein, thereby protecting patients from severe infections even if they become infected with SARS-CoV-2 (Funk et al., 2020; Belete, 2021; World Health Organization; Centers for disease control, 2021; Food and Drug Administration). Unlike conventional vaccines, the mRNA vaccines do not use live or even attenuated virus that causes COVID-19 thus, patients cannot get COVID-19 from the vaccine. In addition, mRNA vaccines do not impact DNA, as mRNA does not enter the cell nucleus. A major disadvantage of this type of vaccine is that it requires storage at −20°C (Moderna) and −70°C (Pfizer). Interestingly, the AstraZeneca-Oxford vaccine is made from a genetically engineered virus that causes the common cold with the intention that it will train the immune system to respond to SARS-CoV-2 infections (**Table 1**). It is ~66% effective and can be stored in a fridge. The vaccines that only require refrigeration may be easier to distribute, and thus a lot more useful to developing countries that may not be able to store large amounts of vaccine at low temperatures. Many other vaccines are under development and some are in clinical trials, it is beyond the scope of this review to discuss all of the vaccine candidates, and some excellent reviews have already been published (Funk et al., 2020; Belete, 2021).

While vaccines are critical for the fight against COVID-19, they are not 100% effective, and it will take an extended period of time to vaccinate the global population, thus the development of interventional drugs to manage symptoms, reduce viral load, reduce cytokine storm, inflammation and the other symptoms of COVID-19 that cause significant morbidity and mortality should be a significant focus of scientific and medical research.

Currently, only one anti-viral drug, remdesivir, has been approved by the U.S. FDA to treat COVID-19 in adults and children over the age of 12 years of age (Food and Drug Administration). Other anti-viral drugs, such as favipiravir and merimepodib are also currently being tested. The use of the corticosteroid dexamethasone (DEXA) to reduce inflammation and treat or prevent organ dysfunction and lung injury from COVID-19 is also recommended (Food and Drug Administration). Reports have suggested that DEXA use may reduce the risk of COVID-19 related death by as much as 30% for patients on ventilators and by ~ 20% for COVID-19 patients who require supplemental oxygen (Food and Drug Administration). In addition, the FDA has granted an emergency use authorization for baricitinib (Olumiant), a drug normally used for arthritis to treat COVID-19 in specific cases (Food and Drug Administration). Baricitinib has both anti-inflammatory and anti-viral activities and appears to reduce inflammation due to COVID-19. This opens the door for the research and development of other drugs with similar activities, including natural products that have potent anti-inflammatory and anti-viral effects. Other treatments include immune-based therapies including convalescent plasma. The FDA has also granted emergency use authorization for convalescent plasma therapy to treat COVID-19 (Food and Drug Administration). Convalescent plasma is obtained from blood donated by patients that have recovered from COVID-19 and is given by intravenous administration to other COVID-19 patients. Anti-malaria drugs, such as hydroxychloroquine and chloroquine were at one point thought to potentially be useful, however they since have been found to have death rate twice as high as patients who have not received it. Thus, it is clear that other drugs with efficacy against COVID-19 are urgently needed, and natural products can offer a value-added approach to treatment.

Since the previous SARS and MERS outbreaks, researchers have actively started looking for other investigational new drugs for the treatment and prevention strategies CoV infections. A number of small molecules and natural products are in pre-clinical tests, with studies focus being mainly viral proteins, including proteases, polymerase and the entry protein (S protein) as novel antiviral targets, since these proteins mediate the most important functions of the virus life cycles, as described above. For example, lopinavir, a protease inhibitor used to treat human immunodeficiency virus (HIV), is reported to have *in vitro* and *in vivo* activities against SARS and MERS (Chen et al., 2004; Chong et al., 2015). Nucleoside analogs are widely used for several viral diseases, such as HIV and flaviviruses. As CoVs replicate their RNA through negative-strand intermediates, nucleoside analogs can get incorporated into RNA intermediates causing chain termination and interfering with polymerase activity. For example, ribavirin, and mizoribine have been studied for their anti-CoV activity, but have only marginal effects (Warren et al., 2014). BCX4430, an adenosine analog, inhibits the polymerase activity in a wide range of RNA viruses, including CoVs (Warren et al., 2014). Micromolar concentrations of acyclic fleximer nucleoside analogs inhibited MERS-CoV and HCoV-NL63 *in vitro* (Peters et al., 2015). Viral helicases catalyze the unwinding of dsRNA intermediates in an ATP-dependent manner. Furthermore, *in vitro* studies have demonstrated the inhibitory effect of bananins and 5-hydroxychromone derivates on the unwinding and atpase activities of SARS-CoV helicase (Tanner et al., 2005; Kim et al., 2011). SSYA10–001 is a triazole that only inhibits the unwinding helicase activity of many CoVs (Kim et al., 2011). Anti-spike antibodies also have been studied *in vivo*; briefly, they bind to the ACE-2 receptor, thus preventing interaction with S protein. The S2 domain of S protein has two heptads repeating regions, HR1 and HR2, which are needed to associate into a 6-helix bundle to mediate membrane fusion. Synthetic peptides that bind to HR1 and HR2 have shown to inhibit SARS-CoV and HCoV-NL63 replication *in vitro*. Exogenous INFs have been used as antiviral agents for animal CoVs and their efficacy has been reported against HCoV-229E. A synergistic effect has been observed to IFN-β and ribavirin against SARs-CoV. In addition, antibiotics, such as actinomycin D were able to inhibit HCoV-229E replication (Kennedy and Johnson-Lussenburg, 1979), while eremomycin, vancomycin, and valinomycin D also have anti-SARS-CoV effects (Adedeji

TABLE 2 | Effects of medicinal herbal extracts on coronavirus.

Plant species	Extraction solvent	Anti-CoV	Target	Ref
Actaea racemosa [*syn: Cimicifuga racemosa*]	Methanol	0.0044 ± 0.0029 4.7 ± 1.2 12.2 ± 3.6	Mouse hepatitis virus A59 (MHV-A59) porcine epidemic diarrhea virus (PEDV) vesicular stomatitis virus (VSV)	106
Houttuynia cordata	Water	Inhibit the activity of SARS-CoV 3CLpro to 50% of control at the highest testing dose (1,000 g/ml)	SARS-CoV 3C-like protease (3CLpro) and RNA-dependent RNA polymerase (RdRp)	107
Melia azedarach	Methanol	0.0198 ± 0.0195 6.7 ± 0.4 20.5 ± 10.5	MHV-A59 PEDV VSV	106
Coptidis chinensis	Methanol	<0.0000 5.1 ± 1.5 29.9 ± 24.4	MHV-A59 PEDV VSV	106
Phellodendron amurense	Methanol	0.0024 ± 0.0012 5.9 ± 0.4 40.0 ± 3.8	MHV-A59 PEDV VSV	106
Paeonia suffruticosa	Methanol	19.5 ± 3.5 79.2 ± 0.9 64.5 ± 15.0	MHV-A59 PEDV VSV	106
Sophora subprostrata	Methanol	4.9 ± 2.2 7.8 ± 0.6 10.8 ± 7.2	MHV-A59 PEDV VSV	106
Torreya nucifera	Ethanol	62% at 100 μg/ml	SARS 3C-like protease (3CLpro)	108

et al., 2014). However, so far, none of these drugs have been approved for COVID-19 treatment.

Medicinal Plants as Anti-Coronavirus Agents

For over a hundred years, medicinal plants and natural products have played an important role as novel sources for drug development, including antiviral agents (Lin et al., 2014). In fact, numerous medicinal plant extracts and compounds have shown antiviral effects, *in vitro* or *in vivo*, against a wide range of viruses. Thus, it is not surprising that since the COVID-19, SARS, and MERS outbreaks, many researchers have focused on natural products activity, particularly TCM, for both prevention and treatment of novel CoVs infections. In addition, along with their antiviral activities, many natural products are known to increase the immune function, acts as anti-inflammatory and antioxidant agents, at same time that contributes to a balanced healthy status, among other effects.

Natural products, in general, and medicinal plants, in particular, have been widely used globally against CoV infections, with some of these medicinal plant extracts and compounds showing experimental effectiveness in inhibiting CoV growth. For example, it is widely known that the S protein of SARS-CoV-2 commonly interacts with lectin-like receptors in host cells. Thus, researchers have been testing plant-derived lectins for their ability to inhibit the interaction between viral S protein and host receptors (Keyaerts et al., 2007). Lectins derived from Common snowdrop, *Amaryllis*, and Leek revealed to be able to inhibit SARS-CoV replication (Keyaerts et al., 2007). Moreover, specific plant-derived metabolites have also been explored for the development of anti-CoV agents. For example, glycyrrhizin, a bioactive substance extracted from licorice root, is an approved intravenous drug with anti-SARS-CoV activity, although an exact mechanism of action is not entirely clear. Similarly, baicalin derived from *Scutellaria baicalensis*, escin from horse chestnut and reserpine from members of the *Rauwolfia* genus have also revealed promissory anti-SARS-CoV activity (Therapeutic Options for COVID-19 Patients | CDC,; Kim et al., 2008). Traditional Chinese Medicines have been widely used in China and are reported to be effective in SARS-CoV-2 infected individuals (interestingly, in around 90% of Chinese patients), with excellent outcomes (Kim et al., 2008).

While many medicinal plant extracts have been investigated, only eight medicinal plants have demonstrated good inhibitory effects against CoV (**Table 2**). An aqueous extract of *Houttuynia cordata* Thunb. inhibited the activity of SARS-CoV 3C-like protease (3CLpro) and RNA-dependent RNA polymerase (RdRp) to 50% of control at 1,000 g/mL[107].

Natural Products for the Research and Development of Anti-CoV Agents

Investigations of TCM products for antiviral purposes began around 2003, when the SARS CoV first appeared in China in 2002. TCM herbs used for COVID-19 prevention include: *Atractylodes macrocephala, Glycyrrhiza uralensis*, *Astragalus membranaceus*, *Saposhnikoviae divaricate*, *Forsythia suspensa*, *Platycodon grandiflorum*, *Lonicera japonica*, *Atractylodes chinensis*, *Agastache rugosa*, *Cyrtomium fortunei*, *Scrophularia ningpoensis*, *Eupatorium fortunei*, *Ophiopogon japonicus*, *Phragmites communis*, *Dendrobium nobile*, and *Adenophora stricta*, and are the most commonly used herbs in different regions of China (Luo et al., 2020; Yang et al., 2020). For example, in one study, participants who received Yupinfeng powder, consisting of *A. membranaceous*, *Glycyrrhiza glabra*, *S. divaricate*, *A. macrocephala*, *L. japonica*, and *F. suspensa*, did not get infected by SARS-CoV (Luo et al., 2020; Yang et al., 2020). In another clinical trial, it was observed that

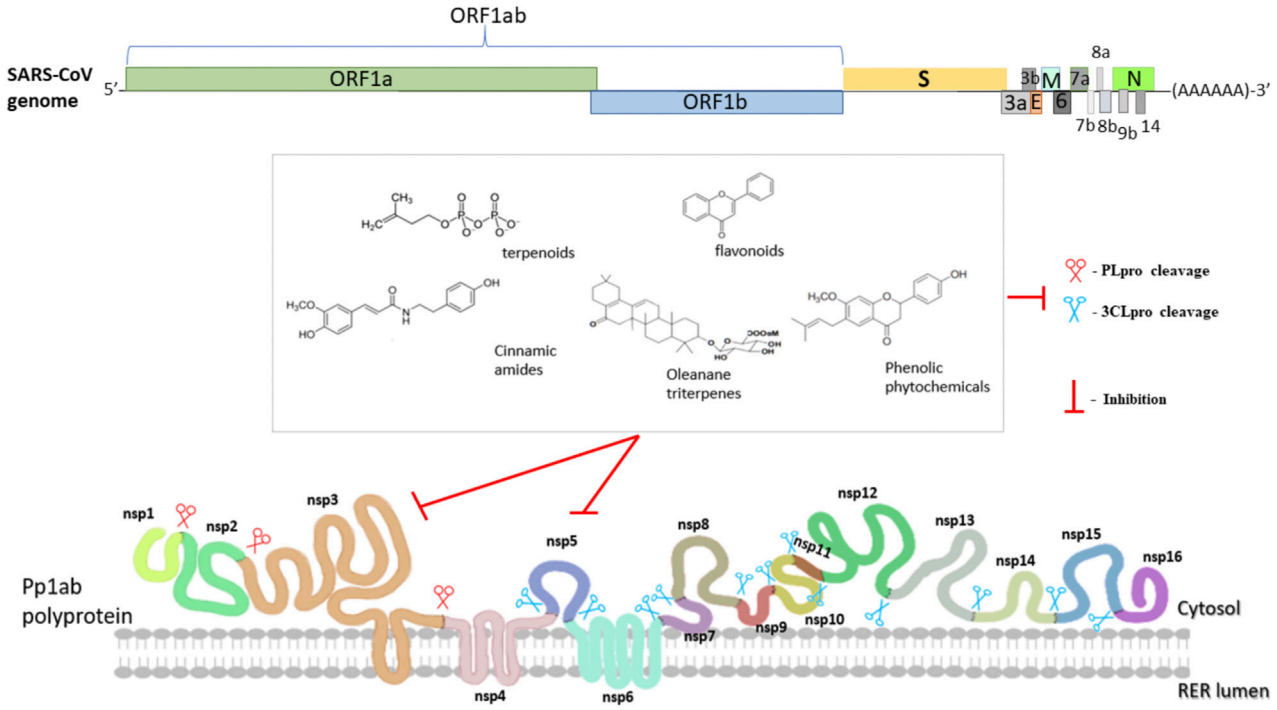

FIGURE 2 | Schematic diagram of SARS-CoV-2 virus genome organization and viral proteases inhibition by natural compounds. The complete genome sequence is ~30 kb long. Two viral proteases (PLPro and 3CLPro) are responsible for cleaving polyproteins into the functional individual Nsps such as helicase, RDRP and methylases. Nsp3 also known as PLPro is a papain like protease that cleaves Nsp1/Nsp2, Nsp2/Nsp3 and Nsp3/Nsp4 boundaries whereas Nsp5 also known as Mpro or 3CLPro is a serine like protease catalyzing the rest 11 cleavage reactions. **Table 3** shows the natural compounds that are known to inhibit these viral proteases.

participants who take Kangdu bu fei decoction, composed of *A. membranaceous*, *Aster tataricus*, *Morus alba*, Hedyotic diffusa, *Duchesnea indica* and Scutellaria barbata extracts did not get infected by SARS.110.

Over the years, the anti-CoV activities of naturally occurring compounds derived from commonly used herbal extracts in TCM have been characterized. Briefly, kaempferol derivatives are able to inhibit the 3a ion channel of SARS-CoV (Schwarz et al., 2014). Tetra-O-galloyl-β-d-glucose and luteolin obtained from *Galla chinensis* and *Veronica lina riifolia*, respectively, bind to the surface spike protein of SARS-CoV (Yi et al., 2004). Quercetin and TSL-1 from *Toona sinensis* also inhibited the cell entry of SARS-CoV (Chen et al., 2008). Wang et al. (Wang et al., 2007), used MD simulations and discovered that MOL376, a compound derived from a Chinese medicinal plant inhibited cathepsin L, a target for SARS treatment and suggesting that this compound may be developed as an effective SARS therapy (Wang et al., 2007). In addition, to *Nigella sativa* potent anti-SARS-CoV effects have been reported (Idrees et al., 2020). Other researchers also found that the phytochemical bonducellpin D exhibited broad-spectrum inhibitory effects on SARS-CoV M^{pro} enzyme through an *in silico* approach (Gurung et al., 2020). Similarly, there have been several reports highlighting that glycyrrhizin, an active component isolated from licorice, reduced the replication of two clinical strains of SARS (Cinatl et al., 2003; Hoever et al., 2005). Later in 2005, the same group of researchers described that the semi-synthesis of 15 glycyrrhizic acid (GA) derivatives increased the antiviral activities of such substances against SARS (Cinatl et al., 2003; Hoever et al., 2005). The addition of a 2-acetamido-β-D-glucopyranosylamine to the GA glucoside increased the antiviral activity by 10-fold when compared with GA alone. Furthermore, the addition of an amide or a free 30-COOH function to GA, increased the antiviral activity by ~70-fold (Hoever et al., 2005), suggesting that GA may be a good starting point for the development of novel anti-CoV drugs.

During the same period, reports of the anti-SARS effects of TCM and isolated substances also started to appear in the literature. The activity and mode of action of some of these bioactive molecules with anti-SARS effects is briefly presented in **Figure 2**, and discussed below.

In 2004, Wu *et al.*, reported that extracts of *Eucalyptus* spp., *Lonicera japonica*, and the purified compound ginsenoside-Rb1 (isolated from *Panax ginseng*) reduced the SARS-CoV replication *in vitro*, although only at high concentrations (100 μM) (Wu et al., 2004). Other TCM preparations, composed of *artemisia annua, Lycoris radiata, Pyrrosia lingua*, and *Lindera aggregata* extracts also reduced the *in vitro* replication of SARS-CoV (Li et al., 2005). These four plant extracts inhibited viral replication in Vero cell-based assays, with median effective concentrations (EC_{50}) ranging from 2.4 to 88.2 μg/ml, with *L. radiata* extract being the most active. Interestingly, bioassay-guided fractionation of *L. radiata* extract led to the isolation and identification of lycorine as the most active anti-SARS-CoV agent, with EC_{50} concentrations for lycorine ranging from 15.7

to 48.8 nM (Li et al., 2005). Similarly, in 2005, Lin et al. reported that extracts and phenolic compounds from *Isatis indigotica* root inhibited the SARS chymotrypsin CoV 3C-like protease ($3CL^{pro}$) (Lin et al., 2005). $3CL^{pro}$ is the viral protease responsible for proteolytic processing of replicase polyproteins in CoVs, necessary for viral life cycle (Lin et al., 2005). Therefore, this protease is considered to be an important molecular target for the development of new anti-CoV drugs.

When screening 720 naturally occurring substances, Chen et al. found that compounds from *Camellia sinensis* (tea leaves) inhibited the $3CL^{Pro}$ activities (Inhibition of SARS-CoV 3C-like Protease Activity by Theaflavin-3,3'-digallate (TF3)). Both tannic acid and 3-isotheaflavin-3-gallate had a median inhibitory concentration in the range of 3–7 μM. Furthermore, screening of tea extracts showed that extracts from green, oolong, Puer, and black teas were also active, with Puer and black teas being the most active *in vitro*. Another tea substance with $3CL^{pro}$ inhibitory activity was identified as theaflavin-3,3′-digallate (Inhibition of SARS-CoV 3C-like Protease Activity by Theaflavin-3,3'-digallate (TF3)). In 2006, Cheng et al. reported that specific triterpene glycosides isolated from *Bupleurum*, *Hetermorpha* and *Scrophularia* species, namely saikosaponins A, B2, C and D, inhibited the human HCoV-229E replication *in vitro* (Cheng et al., 2006). These compounds also reduced early stage HCoV-22E infections, meaning that they may have possible preventative effects by suppressing both attachment and penetration of CoV into cells (Cheng et al., 2006). Ho *et al,* also tested three TCM-derived herbal extracts from roots of *Rheum officinale*, and roots and vine of *Polygonum multiflorum*, and obtained median inhibitory concentrations ranging from 1 to 10 μg/ml (Ho et al., 2007). Mechanistically, the extracts inhibited the SARS-CoV S protein binding. Moreover, using bioassay-guided fractionation, the authors isolated the anthraquinone compound emodin from *Rheum* and *Polygonum* genus, with the observed effects being attributed its presence in the extracts; briefly, this compound inhibited the virus replication by inhibition of the interaction between CoV S protein and hACE2 receptor (Schwarz et al., 2011). Emodin was also shown to inhibit the 3a protein ion channel, which forms a cation-selective channel that allows the viral release into the infected cells. Thus, taken together, substances that can inhibit the 3a protein and the virus release are also interesting for the development of anti-SARS agents (Ho et al., 2007; Schwarz et al., 2011).

In another study, Wen et al., investigated the anti-SARS-CoV activity of 221 natural compounds (Wen et al., 2007). As main outcomes, the authors demonstrated that many of the compounds reduced virus replication, particularly eight abietane-type diterpenes, two labdane-type diterpenes, two sesquiterpenes, and two triterpenes. Also, four lignan compounds (including savinin, honokiol, and magnolol), as well as curcumin, strongly inhibited SARS-CoV activity at concentrations of 3.3–20 μM (Wen et al., 2007). α-cadinol and hinokinin also significantly inhibited viral replication at 1 μM, suggesting that abietane-type diterpenoids and lignans may be interesting sources for the development of novel anti-SARS CoV agents. Kim et al. (Kim et al., 2008), investigated the effects of 22 Chinese herbs extracts on the replication of two CoVs, namely mouse hepatitis virus A59 (MHV-A59) and porcine epidemic diarrhea virus (PEDV). Among the studied extracts, *C. racemosa*, *Melia azedarach*, *Coptidis chinensis*, *P. amurense*, and *S. subprostrata* reduced the MHV-A59 replication with an EC_{50} ranging from 2.0 to 27.5 μg/ml, with both RNA and protein expression being also changed (Kim et al., 2008). In 2008, Lau et al., investigated the antiviral and immune effects of *Houttuynia cordata*, a TCM plant used to treat pneumonia (Lau et al., 2008). The aqueous extract increased mouse spleen lymphocytes proliferation *in vitro*, and increased IL-2 and IL-10 production. Treatment with HC aqueous extract also increased the proportion of $CD4^+$ and $CD8^+$ T cells, besides to be able to inhibit the SARS-CoV $3CL^{pro}$ and suppress RNA-dependent RNA polymerase activity (Lau et al., 2008). Zhuang et al., investigated the anti-SARS activities of a butanol fraction of Cinnamon bark, and its isolated chemical constituents. The butanol fraction of *Cinnamomum verum* weakly inhibited the replication of wild-type SARS-CoV and HIV/SARS-CoV S pseudovirus, with an IC_{50} of 7.8–43.1 and 149.5–283.4 μg/ml, respectively (Zhuang et al., 2009). Two substances, namely procyanidin A2 and B1, exerted anti-SARS-CoV activity with median inhibitory concentrations of 29.9–41.3 μM and 15.69–37.35 μM, respectively (Zhuang et al., 2009). Around the same time, several Chinese patents were filed, showing that naturally occurring diterpenes were able to co-crystallize with $3CL^{pro}$ of the SARS virus (Rao et al., 2007; Method for separating SARS coronavirus main proteinase inhibitor from traditional Chinese medicine, 2009; Method for screening SARS corona virus major protease inhibitor from traditional Chinese medicine and screened SARS corona virus major protease inhibitor, 2010; Kumar et al., 2013). In this report, pseurata A, B and C, leukamenin E, glaucocalyxin B and D, liangshanin A, all inhibited $3CL^{pro}$, meaning that all may be considered potential candidates for new anti-SARS CoV agents formulation. Moreover, other naturally occurring compounds, such as scutellarein, quercetagetin, myricetin, and robinetin were reported to inhibit SARS $3CL^{pro}$ (IC_{50} = 25 μM) (Song et al., 2009).

In 2010, Kim et al., assessed 19 herbal extracts used in TCM and assessed their effects against CoV MHV-A59. Among the studied extracts, that derived from *Sanguisorba officinalis*, *Acanthopanax gracilistylus* and *Torilis japonica* markedly reduced the MHV-A59 replication, as well as the viral RNA and protein levels (Song et al., 2009). These extracts were also able to reduce viral replication in the JHM strain of MHV, porcine epidemic diarrhea virus, and vesicular stomatitis virus. Median effective concentrations of such extracts were in the range of 0.8–3.7 μg/ml. *A. gracilistylus* and *T. japonica* extracts also displayed anti-inflammatory activities and reduced COX-2 activity in MHV-A59-infected cells. They also activated the extracellular signal-related kinase (ERK) and p38 or ERK alone (Kim et al., 2010). Moreover, in 2010, Rhy et al. (Ryu et al., 2010), assessed the anti-SARS-CoV effects of four quinone-methide triterpenes, celastrol, pristimerin, tingenone and iguesterin, isolated and identified from *Triterygium regelii*. These triterpenes inhibited the SARS-CoV $3CL^{pro}$ activity with

median inhibitory concentrations of 10.3, 5.5, 9.9, and 2.6 μM, respectively (Ryu et al., 2010). In 2011, a *Celastrus orbiculatus*-derived extract and its fractions inhibited the $3CL^{pro}$ activity with an IC_{50} of 17.8–38.9 μg/ml (Kumar et al., 2013). An ethanol extract and ethyl acetate fraction of brown seaweed line tree inhibited the SARS CoV with an IC_{50} of 14.7 and 8.5 μg/ml, respectively (Kumar et al., 2013). The isolated active substances revealed significant anti-CoV effects, with celastrol, pristimerin, tingenone and iguesterin having IC_{50} values ranging from 2.6 to 10.3 nM. Other natural compounds that have been identified as inhibitors of SARS-CoV helicase nsP13 and $3CL^{pro}$, include the phenolic compounds myricetin and scutellarein isolated from *Torreya nucifera* (Ryu et al., 2010; Yu et al., 2012). Wen et al., in 2011, screened over 200 Chinese herbal extracts for their anti-SARS-CoV activities in cultured Vero E6 cells. As main findings, the authors stated that six herbal extracts, namely from *Gentiana scabra*, *Dioscorea batatas*, *Cassia tora* and *Taxillus chinensis* and two *Cibotium barometz* extracts inhibited the SARS-CoV replication at concentrations between 25 and 200 μg/ml (Wen et al., 2011). Moreover, *D. batatas* and *C. barometz* methanol extracts significantly inhibited the SARS-CoV $3CL^{pro}$ activity, with a median inhibitory concentration of 39–44 μg/ml, respectively (Wen et al., 2011). Also, Chang et al. reported the anti-human CoV activity of *Euphorbia neriifolia* and its derived triterpenes, with 3β-friedelanol revealing to be more effective than the control actinomycin D, thus suggesting the importance of the friedelane skeleton as a basis for drug development (Chang et al., 2012).

More recently, rocaglamide and silvestrol, two naturally occurring compounds that were isolated from *Aglaia* species, have also proved to have excellent antiviral activity (Schulz et al., 2020). Furthermore, a recent study by Muller et al. (Müller et al., 2018), reported that silvestrol, significantly inhibited cap-dependent viral mRNA translation in CoV-infected human embryonic lung fibroblast cells. The median effective concentrations were of 1.3–3 nM, with significant effects being stated both on MERS-CoV and HCoV-229E. The activity of silvestrol was also investigated in the highly pathogenic MERS-CoV, using peripheral blood mononuclear cells. Mechanistically, silvestrol inhibited the CoV structural and nonstructural protein (N, nsp8) expression, as well as viral replication and transcription complexes formation (Song et al., 2009). Furthermore, *Stephania tetrandra* extracts and isolated alkaloids, tetrandrine (TET), fangchinoline (FAN), and cepharanthine (CEP) were investigated in HCoV-OC43-infected MRC-5 human lung cells. These substances significantly inhibited the virus replication and increased cell death with an IC_{50} range of 0.33–1.01 μM. Moreover, TET, FAN, and CEP inhibited the S and N proteins expression (Kim et al., 2019).

Traditional Chinese Herbal Medicine and Western Medicine: Clinical Approach

Chinese herbal medicines used in combination with Western medicines (antibiotics and corticosteroids) vs. Western medicines alone have been increasingly assessed for their safety and efficacy, and its impact in patients infected with SARS-CoV is not an exception (Liu et al., 2012; Wang et al., 2020).

A systematic review identified a quasi-randomized controlled trial (RCT) and 22 RCTs of Chinese herbal medicines in combination with Western medicines for the treatment of SARS-CoV that met the inclusion criteria (Liu et al., 2012). However, 10 RCTs were not included in the final analyses due to lack of outcome measures and randomization. Twelve RCTs and one quasi-RCT were finally evaluated involving a total of 640 SARS-CoV-infected patients. A total of 12 Chinese herbal combinations were taken into consideration. The primary outcome was mortality, while secondary outcomes included symptoms' reduction, pulmonary infiltration, quality of life, duration of hospitalization, corticosteroids' use, and occurrence of adverse events. Data obtained in this systematic review suggested that the combination of Chinese herbal medicines with Western medicines were not more effective than Western medicines alone in reducing mortality (Liu et al., 2012). However, the combination of both medicines was effective for reducing SARS-CoV symptoms, including fever, cough, breathing difficulties, and serious sequelae, when compared with Western medicines alone. Also, both medicines, used in combination seemed to be effective in reducing lung infiltration and the corticosteroids use, and in improving the quality of life of SARS-CoV-infected patients, although did not reduce the number of days in the hospital. All these Chinese herbal treatments were combination prescriptions, that have been personalized for patients. Also worth of note is that a recently published study revealed that four COVID-19 patients receiving treatment with Shufeng Jiedu Capsule, a TCM, in combination with lopinavir/ritonavir and arbidol had an improvement in respiratory symptoms (Wang et al., 2020).

Thus, the combination of TCM with Western medicine in SARS-CoV-infected patients seems to be promising. However, despite the recent advances, more in-depth clinical trials are needed toward a clearer understanding on the safety and effectiveness of Chinese herbal medicines used in combination with Western drugs, as well as of detailed studies reporting the occurrence of adverse events.

ANTI-CORONAVIRUS EFFECTS OF NATURALLY OCCURRING BIOACTIVE COMPOUNDS

Many natural products have been reported to inhibit CoV growth or the activity of targeted enzymes, such as SARS-CoV PLpro, with the most promissory ones belonging to the terpenes and flavonoids classes (Hamill et al., 2006; Park et al., 2012; Cho et al., 2013; Kim et al., 2014; Yang et al., 2015; Jo et al., 2019; Jo et al., 2020). Most of tested natural products have inhibitory activity on CoV (**Table 3**) with IC_{50} values ranging from 0.86 to 283.5 μM on SARS-Cov 3CLPro and PLPro and from 1.7 to 19.9 μM on hCoV 229E. Scutellarein, hirtutenone, cryptotanshinone, myrcetin, rosmariquinone and tanshinone IIA have shown the highest inhibitory effect with IC_{50} values below 5 μM (Park et al., 2012; Cho et al., 2013; Yang et al., 2015).

However, in general, although medicinal plants and natural products have anti-CoV activity, most of the results are still preliminary. Only a few studies assessed the effectiveness of

TABLE 3 | Effects of natural products on coronavirus.

Compound	Source	Anti-Cov	Target/Strains	Ref
18-Hydroxyferruginol	*Tripterygium regelii*	220.8 ± 10.4	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
18-Oxofer- ruginol	*Tripterygium regelii*	163.2 ± 13.8	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
30-O-Methyldiplacol	*Paulownia tomentosa*	9.5 ± 0.10	SARS-Cov Plpro	Cho et al. (2013)
30-O-Methyldiplacone	*Paulownia tomentosa*	13.2 ± 0.14	SARS-Cov Plpro	Cho et al. (2013)
4′-O-Methylbavachalcone	*Psoralea corylifolia*	10.1 ± 1.2	SARS-Cov) papain-like protease (Plpro)	Kim et al. (2014)
40-O-Methyldiplacol	*Paulownia tomentosa*	9.2 ± 0.13	SARS-Cov Plpro	Cho et al. (2013)
40-O-Methyldiplacone	*Paulownia tomentosa*	12.7 ± 0.19	SARS-Cov Plpro	Cho et al. (2013)
6-Geranyl-40,5,7-Trihydroxy-30,50-Dimethoxyflavanone	*Paulownia tomentosa*	13.9 ± 0.18	SARS-Cov Plpro	Cho et al. (2013)
Abietic acid	*Pinus* spp.	189.1 ± 15.5	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Amentoflavone	*Tripterygium regelii*	8.3 ± 1.2	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Apigenin	—	280.8 ± 21.4	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Bavachinin	*Psoralea corylifolia*	38.4 ± 2.4	SARS-Cov) papain-like protease (Plpro)	Kim et al. (2014)
Bilobetin	*Tripterygium regelii*	72.3 ± 4.5	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Corylifol A	*Psoralea corylifolia*	32.3 ± 3.2	SARS-Cov) papain-like protease (Plpro)	Kim et al. (2014)
Cryptotanshinone	*Salvia miltiorrhiza*	226.7 ± 6.2; 0.8 ± 0.2	SARS-Cov 3CL (Pro) and PL (Pro)	Park et al. (2012)
Curcumin	*Curcuma longa*	5.7 ± 0.3	SARS-Cov Plpro	Yang et al. (2015)
Dihydrotanshinone I	*Salvia miltiorrhiza*	38.7 ± 8.2; 8.8 ± 0.4	SARS-Cov 3CL (Pro) and PL (Pro)	Park et al. (2012)
Diplacone	*Paulownia tomentosa*	10.4 ± 0.16	SARS-Cov Plpro	Cho et al. (2013)
Esculetin-4-Carboxylic acid ethyl Ester	*Axinella corrugata*	46	SARS-Cov 3clpro	Hamill et al. (2006)
Ferruginol	*Tripterygium regelii*	49.6 ± 1.5	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Ginkgetin	*Tripterygium regelii*	32.0 ± 1.7	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Helichrysetin	*Helichrysum odaratissimum*	67.04 μm	MERS-Cov 3C-Like protease (3clpro)	Jo et al. (2019)
Herbacetin	—	33.17	SARS 3C-Like protease (3clpro)	Jo et al. (2020)
Herbacetin	—	40.59	MERS-Cov 3C-Like protease (3clpro)	Jo et al. (2019)
Hinokiol	*Tripterygium regelii*	233.4 ± 22.2	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Hirsutanonol	*Alnus japonica*	7.8 ± 1.7	SARS-Cov Plpro	Yang et al. (2015)
Hirsutenone	*Alnus japonica*	4.1 ± 0.3	SARS-Cov Plpro	Yang et al. (2015)
Isobavachalcone	*Psoralea corylifolia*	35.85	MERS-Cov 3C-Like protease (3clpro)	Jo et al. (2019)
Isobavachalcone	*Psoralea corylifolia*	7.3 ± 0.8	SARS-Cov) papain-like protease (Plpro)	Kim et al. (2014)
Isopimaric acid	*Tripterygium regelii*	283.5 ± 18.4	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Kayadiol	*Tripterygium regelii*	137.7 ± 12.5	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Luteolin	*Reseda luteola*	20.0 ± 2.2	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Methyl Dehydroabi- Etate	*Tripterygium regelii*	207.0 ± 14.3	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Methyl Tanshinonate	*Salvia miltiorrhiza*	21.1 ± 0.8; 9.2 ± 2.8	SARS-Cov 3CL (Pro) and PL (Pro)	Park et al. (2012)
Mimulone	*Paulownia tomentosa*	14.4 ± 0.27	SARS-Cov Plpro	Cho et al. (2013)
Myricetin	—	2.71 ± 0.19	—	Yu et al. (2012)
Neobavaisoflavone	*Psoralea corylifolia*	18.3 ± 1.1	SARS-Cov) papain-like protease (Plpro)	Kim et al. (2014)
N-Trans-Caffeoyltyramine	*Tribulus terrestris*	44.4 ± 0.6	SARS-Cov Plpro	Song et al. (2014)

(Continued on following page)

TABLE 3 | (*Continued*) Effects of natural products on coronavirus.

Compound	Source	Anti-Cov	Target/Strains	Ref
N-Trans-Coumaroyltyramine	*Tribulus terrestris*	38.8 ± 0.4	SARS-Cov Plpro	Song et al. (2014)
N-Trans-Feruloyloctopamine	*Tribulus terrestris*	26.6 ± 0.5	SARS-Cov Plpro	Song et al. (2014)
N-Trans-Feruloyltyramine	*Tribulus terrestris*	70.1 ± 0.7	SARS-Cov Plpro	Song et al. (2014)
O-acetyl-18-Hydroxyferruginol	*Tripterygium regelii*	128.9 ± 25.2	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Oregonin	*Alnus japonica*	20.1 ± 2.2	SARS-Cov Plpro	Yang et al. (2015)
Pectolinarin	*Cirsium* spp.	37.78	SARS 3C-Like protease (3clpro)	Jo et al. (2020)
Platyphyllone	*Alnus japonica*	>200	SARS-Cov Plpro	Yang et al. (2015)
Platyphyllonol-5-Xylo- Pyranoside	*Alnus japonica*	>200	SARS-Cov Plpro	Yang et al. (2015)
Psoralidin	*Psoralea corylifolia*	4.2 ± 1.0	SARS-Cov) papain-like protease (Plpro)	Kim et al. (2014)
Quercetin	*Toona sinensis*	23.8 ± 1.9	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Quercetin 3-B-D-Glucoside	—	37.03	MERS-Cov 3C-Like protease (3clpro)	Jo et al. (2019)
Rhoifolin	*Rhus saccedanea*	27.45	SARS 3C-Like protease (3clpro)	Jo et al. (2020)
Rosmariquinone	*Salvia miltiorrhiza*	14.4 ± 0.7; 4.9 ± 1.2	SARS-Cov 3CL (Pro) and PL (Pro) assay	Park et al. (2012)
Rubranol	*Alnus japonica*	12.3 ± 0.9	SARS-Cov Plpro	Yang et al. (2015)
Rubranoside A	*Alnus japonica*	9.1 ± 1.0	SARS-Cov Plpro	Yang et al. (2015)
Rubranoside B	*Alnus japonica*	8.0 ± 0.2	SARS-Cov Plpro	Yang et al. (2015)
Saikosaponin A	*Bupleurum* spp., *heteromorpha* spp. *And scrophularia scorodonia*	8.6 ± 0.3	Human Coronavirus 229E	Cheng et al. (2006)
Saikosaponin B2	*Bupleurum* spp., *heteromorpha* spp. *And scrophularia scorodonia*	1.7 ± 0.1	Human Coronavirus 229E	Cheng et al. (2006)
Saikosaponin C	*Bupleurum* spp., *heteromorpha* spp. *And scrophularia scorodonia*	19.9 ± 0.1	Human Coronavirus 229E	Cheng et al. (2006)
Saikosaponin D	*Bupleurum* spp., *heteromorpha* spp. *And scrophularia scorodonia*	13.2 ± 0.3	Human Coronavirus 229E	Cheng et al. (2006)
Sciadopitysin	*Tripterygium regelii*	38.4 ± 0.2	SARS 3C-Like protease (3clpro)	Ryu et al. (2010)
Scutellarein	*Scutellaria* spp.	0.86 ± 0.48	Colorimetry-based ATP Hydrolysis	Yu et al. (2012)
Tanshinone	*Salvia miltiorrhiza*	38.7 ± 8.2; 8.8 ± 0.4	SARS-Cov 3CL (Pro) and PL (Pro)	Park et al. (2012)
Tanshinone IIA	*Salvia miltiorrhiza*	89.1 ± 5.2; 1.6 ± 0.5	SARS-Cov 3CL (Pro) and PL (Pro)	Park et al. (2012)
Tanshinone IIB	*Salvia miltiorrhiza*	24.8 ± 0.8; 10.7 ± 1.7	SARS-Cov 3CL (Pro) and PL (Pro)	Park et al. (2012)
Terrestriamide	*Tribulus terrestris*	21.5 ± 0.5	SARS-Cov Plpro	Song et al. (2014)
Terrestrimine	*Tribulus terrestris*	15.8 ± 0.6	SARS-Cov Plpro	Song et al. (2014)
Tomentin A	*Paulownia tomentosa*	6.2 ± 0.04	SARS-Cov Plpro	Cho et al. (2013)
Tomentin B	*Paulownia tomentosa*	6.1 ± 0.02	SARS-Cov Plpro	Cho et al. (2013)
Tomentin C	*Paulownia tomentosa*	11.6 ± 0.13	SARS-Cov Plpro	Cho et al. (2013)
Tomentin D	*Paulownia tomentosa*	12.5 ± 0.22	SARS-Cov Plpro	Cho et al. (2013)
Tomentin E	*Paulownia tomentosa*	5.0 ± 0.06	SARS-Cov Plpro	Cho et al. (2013)

natural products directly on the virus because most of them targeted CoV proteases. Therefore, the reported effects of these substances on the whole virus should be confirmed and then experiments should be performed on animal models of CoV infections before starting clinical trials.

Paving the Way for Clinical Applications Against SARS-CoV-2 in Humans

The emergence of SARS-CoV-2 as a cause of the COVID-19 worldwide pandemic has prompted an urgent need to research and develop new vaccines and drugs to tackle its pathogenesis. Even with the advent of effective vaccines for SARS-CoV-2, there are currently few direct acting antiviral and other drugs to treat the symptoms of COVID-19. There is an urgent need for the development of such therapies as vaccines are not 100% effective and some patients may still have symptoms, albeit less severe, the vaccines may require annual shots, and a high percentage of the global population need to be vaccinated before the vaccines can offer maximum protection.

Natural products, with their diversity of chemical classes and structures, and preliminary data suggesting that some of these compounds may be active against CoVs offer an

excellent starting point for drug discovery in this field. Fortunately, researchers have been able to identify and sequence proteins that are crucial elements for viral infection, replication, and virus-host interactions that can be used as tools for screening novel antiviral substances, including natural products. In fact, preliminary data suggest that naturally occurring bioactive compounds that can target: 1) SARS-CoV-2 entry/fusion; 2) virus uncoating inside the host cell, 3) nucleoside and non-nucleoside reverse transcriptase, 4) viral protease, 5) and viral release. In addition, data suggest that natural products may: 1) block SARS-CoV-2 entry by inhibiting its attachment and fusion to host cells, 2) stop viral replication by inhibiting viral protease and viral nucleic acid as well as protein synthesis, 3) block viral survival in host cells, and 4) boost the host immune response. The effects of natural products on the immune system.

As briefly stated above, SARS-CoV-2 infection activates the host immune cells that cause cytokine storm, and this activation is directly linked to disease severity and poor prognosis (Huang et al., 2020). There are many reports of natural products that have been shown to modulate the host's immune responses (Khanna et al., 2020; Silveira et al., 2020). Medicinal plant extracts and natural compounds have the potential to be used alone or as adjunct therapies in combination with current antiviral treatments and other drugs. In fact, TCM therapies, have been suggested to be effective in human studies in combination with conventional treatments. Thus, medicinal plant extracts and natural compounds make excellent candidates for testing as novel antiviral, anti-inflammatory and immune enhancing therapies, alone or in combination with other drugs to develop more effective, and safer clinical interventions and promissory outcomes.

CONCLUSION AND FUTURE PERSPECTIVES

Previous CoVs, and the new CoV, named SARS-CoV-2, have markedly compromised the global public health. The approval of recent vaccines has significantly improved the long-term outcomes for COVID-19 infections however, vaccines are not 100% effective and some serious symptoms still persist. In addition, it will take a significant period of time to vaccinate everyone globally, thus there is still a critical need to research and develop safe and effective drug therapies.

Natural products have been successfully used for centuries for treating a plethora of diseases, including infectious diseases. To date, the published data suggest that natural products make excellent candidates to serve as a starting point for the search and development of novel treatments and preventative agents for CoVs infections. However, so far, much of this research is *in vitro*, and few experiments have been performed in animal models. Furthermore, new randomized clinical trials need to be performed, as some of the trials of TCMs have shown a reduction in both symptoms and sequelae, but not in mortality rates (Huang et al., 2020). Moreover, the application of new methods/technologies, such as high throughput screening and molecular biology must also be employed to isolate bioactive compounds from plant extracts. Thus, future studies should focus on these aspects of natural products research to more effectively research and develop these compounds as effective treatment strategies for SARS-CoV-2 infection. Taken together, data discussed here highlight the need for a concerted global effort to test natural products for their ability to fight the emerging CoVs strains, particularly in combination with conventional drugs toward to provide more effective, safer and targeted therapies.

AUTHOR CONTRIBUTIONS

JS-R, SAA, YT, NC-M: conceptualization. CR, PT, GM, HA, SK, KG, RG, NG, RS, DR, CR, ZR, SAA, NC-M, JS-R, and YT: validation investigation. KG, RS, DR, CR, ZR: resources. PT, GM, HA, SK, KG, RG, NG, RS, DR, SAA, CR, NC-M,YT, ZR, and JS-R: data curation. ZR, NC-M, GM, CR, and JS-R: review and editing. All authors: writing. All authors read and approved the final version and contributed equally to the manuscript.

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REVIEW
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Potentials of Antitussive Traditional Persian Functional Foods for COVID-19 Therapy†

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Coronavirus disease 2019 is a worldwide pandemic resulting in a severe acute respiratory syndrome. Remdesivir is the only FDA-approved drug for hospitalized patients older than age 12. It shows the necessity of finding new therapeutic strategies. Functional foods (FFs) could have co-therapeutic and protective effects against COVID-19 infection. Traditional Persian medicine (TPM), one of the safest and most popular schools of medicine for hundreds of years, has recommended potential FF candidates to manage such a global pandemic. To reveal the potential of TPM in terms of antitussive FFs, traditional Persian pharmacopoeia "Qarabadin-e-Salehi" was searched using the keywords "*Soaal*" and "*Sorfeh*." Also, a search of MEDLINE, PubMed Central, Google Scholar, and Science Direct was performed for the relevant literature published from the inception up to March 2021. A combination of search terms including "cough, antitussive, antioxidant, anti-inflammation, antiviral, COVID-19, mucoactive, mucolytic, expectorant, and mucoregulatory" was also applied. The potential mechanism of action in SARS-CoV-2 infection was discussed. Twelve TPM FFs were found including Laooqs, Morabbas, a Saviq, a soup, and a syrup. They are combinations of two to seven ingredients. Natural compounds of mentioned formulations have the main pharmacological mechanisms including antiviral, anti-inflammatory, antioxidant, antihistamine, bronchodilator, immunomodulatory, and mucoactive effects as well as central or peripheral antitussive activities. FFs are cost-effective, easily accessible, and safe options for both treatment and prevention of COVID-19. They might have positive psychological effects along with their pharmacological effects and nutritional virtues. They could also manage persistent respiratory discomforts after recovery from COVID-19.

Keywords: antitussive, functional foods, traditional Persian medicine, phytochemical, COVID-19

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a worldwide pandemic resulting in a sudden increase in hospitalizations due to pneumonia and damages to different organs (Wiersinga et al., 2020). This viral pulmonary infection occurs when respiratory mucosa cells are infected by the SARS-CoV-2 virus. The epithelium of the respiratory tract is composed of various cells including goblet cells producing mucus as the first barrier of the incoming viruses, ciliated cells, non-ciliated cells, and club cells producing proteases. After the attachment of S spike protein of the virus to the ACE2 receptor of

host cells, the virus penetrates into the cells (Azer, 2020; Subbarao and Mahanty, 2020; Wiersinga et al., 2020). Then, pro-inflammatory cytokines and type I interferons are expressed to induce an antiviral state. The cytokine storm condition is seen after immune response dysregulation in severe SARS-CoV-2 cases (Subbarao and Mahanty, 2020). Also, lung edema and necrotic changes can be seen as the results of proinflammatory cytokines (Azer, 2020). Most of the infected patients get a mild to moderate pulmonary illness, whereas others experience severe pneumonia. Common mild COVID-19 symptoms are sore throat, cough (usually dry cough, but in some cases phlegmatic cough), headache, fever, myalgia, fatigue, anosmia, anorexia, and diarrhea. Severe COVID-19 is associated with pneumonia, dyspnea, confusion, pain in chest, fever, and anorexia (Clemency et al., 2020; Donma and Donma, 2020; Struyf et al., 2020). The most common symptom of COVID-19 is dry cough (Li and Ma, 2020). As coughing is one of the main modes of viral transmission among individuals, it is supposed that viruses have developed cough mechanisms to enhance their transmission. For instance, a virus can induce coughing by selective changes in neural signaling. In addition, stimulated mucus production by a virus can induce coughing, at least by irritation sense in the airway tract (Footitt and Johnston, 2009). Epidemiologic studies reveal that droplet expulsion during coughing is one of the most common routes of COVID-19 transmission (Wiersinga et al., 2020). In fact, although cough is a defensive reflex of lungs to clear the respiratory system, excessive cough can trigger a wide range of complications including respiratory, cardiovascular, GI, neurologic, constitutional, genitourinary, musculoskeletal, ophthalmologic, dermatological, and psychosocial problems (Nosalova et al., 2006; Irwin et al., 2020). Several adverse events such as headache, laryngeal trauma, pulmonary interstitial emphysema, and brady- or tachyarrhythmias are reported as possible complications induced by excessive cough in COVID-19 patients (Jalali et al., 2020). Also, throat pain, throat scratching, and difficulty in swallowing are common complications associated with coughing (Chiru et al., 2020). Dry cough is a common symptom prevalent in 60–86% of hospitalized cases (Carfì et al., 2020). Persistent cough could decrease life quality by interfering with normal activities and sleep (Weinberger and Lockshin, 2017). Chronic cough could occur in months after recovery from COVID-19, and it may lead to substantial community morbidity (Fraser, 2020). Hence, this concerning situation would require an optimal management for future public health. Many antiviral drugs including favipiravir, lopinavir-ritonavir, ribavirin, and hydroxychloroquine have shown poor efficacy in the treatment of COVID-19 (Martinez, 2020). In October 2020, remdesivir (the potent antiviral agent inhibiting RNA-dependent RNA polymerase) received FDA approval for hospitalized patients older than age 12 (Gordon et al., 2020; U.S. Food and Drug Administration, 2020). Besides, several COVID-19 vaccines are developed and currently evaluated in human trials (Lazarus et al., 2021). Although discovering a safe and effective vaccine is the best solution to manage coronavirus disease 2019 (Matteo et al., 2020), other therapeutic strategies such as using traditional medicine prescriptions could be a solution for local people, before a safe and effective vaccine or/and drug is available.

Functional foods (FFs) have possible co-therapeutic and protective effects against the COVID-19 virus (Matteo et al., 2020). These food and drink traditional formulations are natural that are taken as part of one's daily diet yielding physiological benefits that may help to enhance body health and well-being (Roberfroid, 2002; Krystallis et al., 2008). Since many people of the world are confined to their homes in these quarantine days, inclusion of available natural foods in their daily diet could be a rational suggestion to enhance the immunity of their body against COVID-19. This might decrease the risk of the infection in healthy people and also increase the rapid recovery of patients after SARS-CoV-2 infection (Yang et al., 2020).

Traditional Persian medicine (TPM) is a famous medical doctrine based on humors which are special bodily fluids required for the physiological functioning of each organism (Sirasi, 1990; Hamedi et al., 2013). General health status in TPM is regulated by the equilibrium of four humors including blood, phlegm, bile, and black bile (Alam et al., 2020). According to TPM, diet is a very important factor because food can be converted into the bodily humors. Each food has its particular qualities, and its excessive consumption can induce extreme quantities of one special humor (Jackson, 2001). Food intake for medical purposes has a long history in TPM deliberating foods essential not only for energy providing, but also as a factor to affect the humoral balance of the body. In medieval Persia, the great physicians such as Rhazes (854–921 A.D.) and Avicenna (980–1037 A.D.) wrote the first manuscripts about diet, nutrition, and health regimes. They considered nutrition as an independent and highly developed medical science (Nikaein et al., 2012). In fact, TPM has a rich cuisine presenting diverse recipes for different kinds of FFs (Gharibzahedi, 2018). According to the TPM point of view, if a disease can be treated with food, medicine should not be administered. Furthermore, there are many FFs in TPM which are recommended to accompany the medications (Amiri Ardekani et al., 2020). Actually, TPM has categorized foods, drugs, and their intermediate formations into five general groups including *Ghaza-e-Motlaq* (absolute aliment), *Ghaza-e-Davai* (FF), *Dave-e-Ghazai* (pharmaconutrient), *Dava-e-Motlaq* (absolute medicament or drug), and *Sam-e-Motlaq* (poison). This classification is comparable to that of modern medicine (i.e., nutrients, FFs, nutraceuticals, and poisons) (Soleymani and Zargaran, 2018). Indeed, medicine and food are shaded into each other as recorded by TPM. Avicenna asserted a distinction between food and medicine, indicating that food is a substance assimilated by the body, while medicine assimilates the body to itself. But both medicine and food can affect the body of the person who consumed them (Sirasi, 1990).

TPM Qarabadinic manuscripts are traditional pharmacopoeias containing many multi-ingredient formulations some of which are FFs. For instance, Qarabadin-e-Salehi (Amale Saleh) written by Mohammad Saleh Ghaeni Heravi in 1766 A.D. is a complete and comprehensive Persian language pharmacopoeia on TPM formulations. It could be defined as an example of Persian literature which is written prior to the replacement of TPM by Western medicine in Iran

and it has a unique place among traditional pharmacy manuscripts (Zarshenas et al., 2013; Badr et al., 2014; Farjadmand et al., 2017). TPM has recommended numerous natural formulations to manage such respiratory discomforts. TPM antitussive formulations are categorized into two major classes. One could modify the major cause (*Ezaleh-sabab*) of disease such as infective humors or local inflammations and the other could relieve cough symptomatically. In addition, TPM believes that using antitussive agents is necessary when cough occurs during fever, if not, it may result in persistent fever in patients (Avicenna, 1025). So, antitussive formulations mentioned in TPM manuscripts are recommended for both prevention and treatment of cough and its relevant discomforts. In this regard, the present study introduces traditional Persian antitussive FFs with a review on their potential healing effects against COVID-19 through the recent evidence-based published articles.

METHODS

In this study, the research was done in two steps presented as follows:

A. The potential antitussive FFs recommended in TPM.
B. Efficacy and pharmacological mechanisms related to FF ingredients for antitussive properties in COVID-19.

Section A

In the first step, traditional FFs for the treatment or prevention of dry cough recommended in TPM were introduced. For this purpose, the literature in Qarabadin-e-Salehi, one of the most complete and recent books on TPM compound remedies, was searched using the keywords of cough ("*Soaal*" and "*Sorfeh*" in Persian). Twelve recommended FFs were found and the traditional names of plants were matched with the current scientific plant names using a book providing the scientific names of TPM plants in accordance with their morphological descriptions (Ghahraman and Okhovvat, 2004). In the next step, scientific names were validated according to The Plant List website (The Plant List, 2013). The traditional temperaments of natural ingredients were defined according to "Makhzan-al-Adviah" book (Aghili Khorasani et al., 1771).

Section B

Pharmacological studies related to 22 natural ingredients of the selected FFs were gathered through search of MEDLINE, PubMed Central, Google Scholar, and Science Direct by the combination of the scientific names or common names of each ingredient with "cough, antitussive, antioxidant, anti-inflammation, antiviral, COVID-19, mucoactive, mucolytic, expectorant, and mucoregulatory." Also, the relevant studies about the isolated chemical compounds of each ingredient were included. All data gathering and literature research were done from the inception until March 2021. Articles published in English were only considered.

RESULTS

TPM has described particular FFs for respiratory disorders. In this article, five types of antitussive Persian FFs including Laooqs, Morabbas, Savighs, soups, and syrups have been discussed. These FFs have different textures and processing methods. The antitussive mechanisms of the mentioned TPM FFs in this article could be summarized to the following four aspects. i) Mucoactive functions by mucolytic properties (stimulation of ciliary beating, decreasing the viscosity of mucus, and viral adhesiveness), expectorant effects (increasing mucus secretion, gastro-pulmonary reflex, hydration of the airway mucus), and mucoregulatory activities (normalizing mucus, emptying mucus glands, and enhancing ciliary transport). ii) Central antitussives effects through brainstem sensory afferents. iii) Peripheral antitussive properties through C-fiber sensory afferents. iv) Other antitussive mechanisms including anti-inflammatory, antioxidant, antiviral, antihistamine, bronchodilator, and immunomodulatory effects (Footitt and Johnston, 2009; Zanasi et al., 2020). More details about the mentioned TPM antitussive FFs listed alphabetically are described as follows:

Laooq

Laooq, a semisolid traditional formulation, includes powdered medicinal plants in honey or a viscous syrup. Being similar to linctus, Laooq can be considered a dosage form specifically prescribed for the respiratory system. It has been used orally through licking, and its high viscosity leads to longer transit time through esophagus. The ingredients are often demulcents and antitussive agents (Zarshenas et al., 2013).

Garlic Laooq

Garlic Laooq is made of *Allium sativum* L. (Amaryllidaceae) cooked bulb (hot and dry temperament), tallow (hot and dry temperament), and honey (hot and dry temperament). It has been traditionally recommended for removing phlegm from lungs (Ghaeni Heravi, 1766). Garlic contains enzymes comprising allinase, peroxidases, and myrosinase. Also, garlic has sulfur-containing compounds including alliin, allicin, allylpropyl disulfide, diallyl disulfide, diallyl trisulfide, ajoene, and vinyldithiines. Its terpenes are α- and β-phellandrene, citral, geraniol, and linalool. Other constituents of garlic include proteins such as glutamyl peptides, amino acids such as arginine and glutamic acid, volatile oils, minerals, lipids, prostaglandins (A2, D2, E2, F1a, F2) trace elements, and vitamins (Barnes et al., 2013). One of the main indications of garlic has been for pulmonary diseases and coughs (Papu et al., 2014). Garlic has shown antiviral properties, specifically against influenza virus, parainfluenza virus type 3, herpes simplex viruses, vaccinia virus, and vesicular stomatitis virus. Its main virocide constituents are ajoene, allicin, allyl methyl thiosulfinate, and methyl allyl thiosulfinate. Garlic supplements have prevented common cold viruses (Singh and Singh, 2008). Its phytochemicals have anti-inflammatory and antioxidant activities. They inhibit the production of free radicals, increase cellular antioxidant enzymes, support endogenous radical scavenging activities, and suppress the activity of NF-kB. *In vitro*, the extract of aged garlic

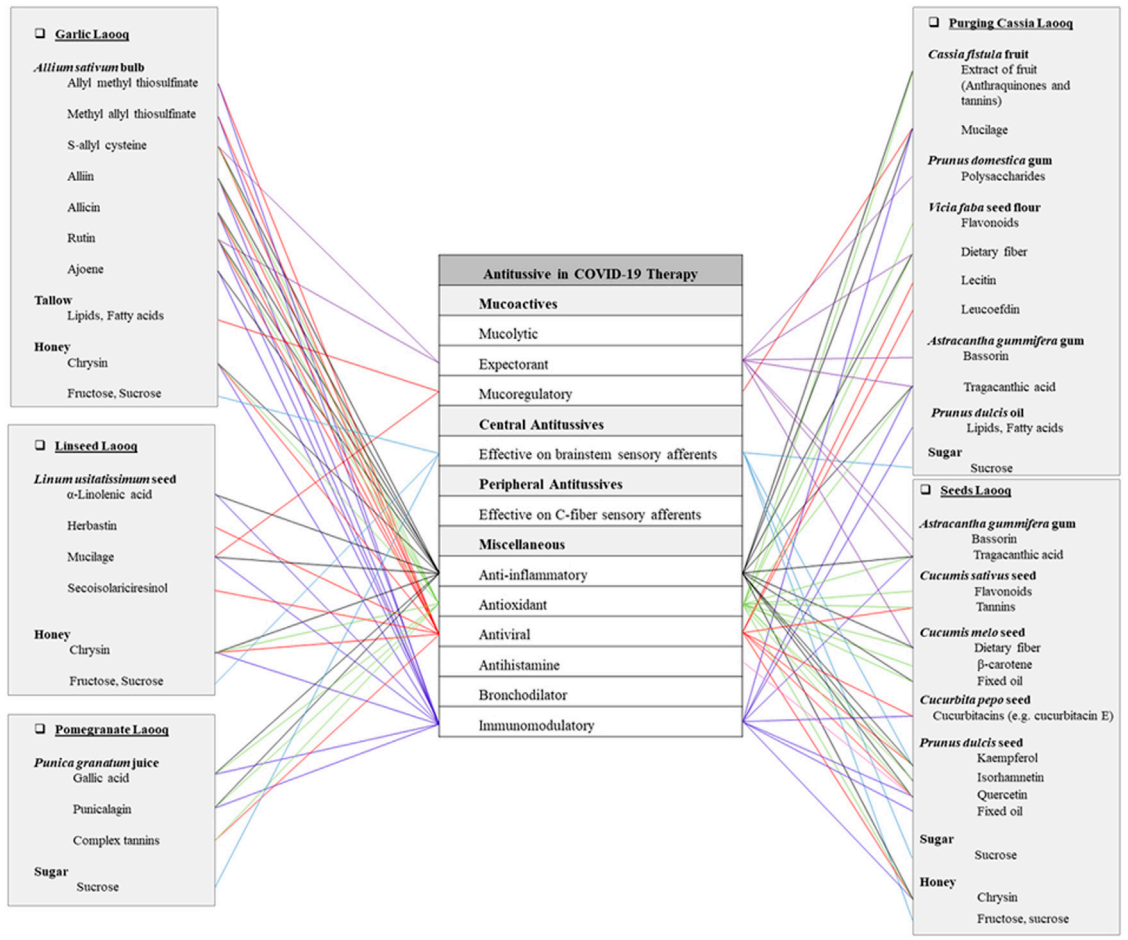

FIGURE 1 | Potentials of antitussive Persian Laooqs in COVID-19 therapy.

and s-allylcysteine blocked the oxidation of low-density lipoprotein and it could protect endothelial cells of pulmonary artery against injury induced by oxidized low-density lipoprotein (Barnes et al., 2013). Also, s-allylcysteine increases the mucus secretion (Park et al., 2014). *In vitro* and *in vivo* studies have shown that garlic has several immune-boosting effects including macrophage phagocytosis induction and lymphocyte proliferation, stimulation of lymphocyte- and macrophage-infiltration into transplanted tumors, induction of the release of interferon-γ, and increase in natural killer cell activity and interleukin-2 production. Ajoene has shown *in vitro* inhibitory effects on the release of lipopolysaccharide-induced PGE2 in macrophages due to the inhibition of COX-2 activity (Barnes et al., 2013). Also, it is reported that the production of COX-2 and PGE2 is prevented by NF-κB inactivation (El-Saber Batiha et al., 2020). Moreover, emigration of neutrophilic granulocytes into epithelia is inhibited by garlic extracts (El-Saber Batiha et al., 2020). Garlic phytochemicals such as S-allyl cysteine, alliin, and allicin have shown antiviral, antifibrotic, antioxidant, anti-inflammatory, and immunomodulatory properties in recent studies. Allicin has shown dual S-thioallylation of SARS-CoV-2 M pro in a recent *In silico* study (Shekh et al., 2020). Garlic stimulates natural killer cell activity and keeps the immune homeostasis by its sulfur-containing compounds. Furthermore, allilin has shown the positive effects to prevent intra-alveolar edema and decrease inflammatory cytokines as well as neutrophils infiltration into the alveolar region. Additionally, sucrose methyl 3-formyl-4-methylpentanoate, another phytochemical in garlic, has shown inhibitory effects on the alveolar damage, lung infection, and thrombotic lesions. Generally, these preclinical studies demonstrate the efficacy of garlic in respiratory infections, alveolar edema, sepsis, pulmonary fibrosis, and acute lung injury, all of which are common symptoms in advanced COVID-19 patients (Thota et al., 2020; Oladele et al., 2020). In addition, garlic enhances the immune system through the reduction of leptin levels which has proinflammatory characteristic (Donma and Donma, 2020). Rutin has shown stimulatory effects on mucus secretion in recent studies (Jeong, 2009). Rutin isolated from garlic has a binding affinity toward COVID-19 main protease (Majumder and Mandal, 2020). Furthermore, a molecular docking analysis highlighted that garlic organosulfur essential oil components could have strong interactions with the main protease PDB6LU7 of coronavirus 2 and ACE2 amino acids. These

findings reveal that garlic could contribute to block the coronavirus invasion in the body (**Figure 1**) (Thuy et al., 2020). The other ingredient of garlic Laooq is tallow. Chemical components of tallow are lipids and fatty acids comprising oleic, palmitic, stearic, myristic, and linoleic acids. These constituents are responsible for emollient properties (Leung, 2015; Kelm and Wickett, 2017).

Linseed Laooq

Linseed laooq is made of *Linum usitatissimum* L. (Linaceae) seed (hot and dry temperament) and honey (hot and dry temperament). It has been traditionally recommended for dry cough (Ghaeni Heravi, 1766). Linseed contains cyanogenic glycosides comprising diglucosides linustatin, neolinustatin, and some linseed samples have linamarine in trace amounts. Fixed oil in linseed is identified as α-linolenic acid (45–50%), linoleic acid (16–20%), oleic acid (18–24%), palmitic acid (5–7%), and stearic acid (0.25–5%). Furthermore, minor constituents of flavonoids such as herbacetin and kaempferol derivatives have been detected in linseed. Besides, polysaccharides including arabinoxylane and rhamnogalacturonan, and other constituents for example nonprotein aminoacids, protein, sterols, tocopherols, and several phenolic compounds such as p-coumaric and caffeic acids have been reported in linseed (Barnes et al., 2013). Linseed has various types of lignans such as (+)-pinoresinol, (+)-lariciresinol, and (−)-matairesinol, as well as secoisolariciresinol diglucoside that has shown antiviral properties in recent studies (Barnes et al., 2013; Chhillar et al., 2020). The seeds contain both mucilage and oil with laxative effects (Uden et al., 1994). Mucilage is a large hydrophilic polysaccharide with a highly branched structure that is able to trap water to form a gel in bronchial mucosa. The muco-adhesive properties of these polysaccharides are responsible for treating cough indirectly by the modulation of the sensitivity of cough receptors to protect them from local irritations as well as their local soothing actions and demulcent properties (Nosalova et al., 2013). Mucilage has immunomodulatory and anti-inflammatory effects (Bokov et al., 2020). Indeed, mucilage can prepare polysaccharide layers on inflamed epithelial tissue to protect and rehydrate it. In a recent study, oral administration of rhamnogalacturonan has shown significant effects in reduction of cough frequency and intensity (Mahboubi, 2020). In one *in vivo* study, rhamnogalacturonan promoted the expectoration and reduced the intensity and frequency of cough attacks (Nosál'ova et al., 1992). Also, arabinoxylan polysaccharide in linseed stimulates the immune responses. The pectin of linseed extracted in the acidic fraction of seed has a rhamnogalacturonan basis with lubricating properties suitable for using in demulcent and emollient substance (van Dam et al., 2017). Linseed tea is a suitable drink to relieve cough, cold symptoms, and bronchitis (Uden et al., 1994). Plants rich in polysaccharides such as *L. usitatissimum* show antioxidant properties (Kardošová and Machová, 2006; Kaithwas and Majumdar, 2012). Linseed oil has shown inhibitory effects on leukotriene-, histamine-, PGE2-, and bradykinin-induced inflammation. It blocks local vasodilatation, capillary permeability, leucocytes migration, and exudation during inflammation. Also, linseed decreases the expression of COX-1 and COX-2 significantly (Akbar, 2020). The results from an *in vivo* study have shown immunomodulatory effect of α-linolenic acid that increased INF-γ, and the ratio of INF-γ/IL4 as well as index of Th1/Th2 decreased IL-4, and preventive effect on tracheal responsiveness and inflammatory markers comparable to dexamethasone (Kaveh et al., 2019). In a FRET-based screening method, it was suggested that herbacetin (3,4′,5,7,8-pentahydroxyflavone) isolated from linseed may have proteolytic activity when tested against 3CL protease of coronavirus 2 (**Figure 1**) (Solnier and Fladerer, 2020).

Pomegranate Laooq

This Laooq is made of *Punica granatum* L. (Lythraceae) concentrated juice (cold and wet temperament) and sugar (hot and dry temperament). It has been traditionally recommended for coughs associated with hot distemprament (Ghaeni Heravi, 1766). Pomegranate has anthocyanins comprising delphinidin 3,5-diglucoside, cyanidin 3,5-diglucoside, pelargonidin 3,5-diglucoside, delphinidin 3-glucoside, a cyanidin–pentoside–hexoside, cyanidin 3-glucoside, cyanidin 3-rutinoside, pelargonidin 3-glucoside, and a cyanidin-pentoside. Its gallotannins include monogalloyl-hexoside and digalloylhexoside. Its ellagitannins include ellagic acid and its derivatives, galloyl-HHDP-hexoside, pedunculagin I and II, casuaricitin, valoneic acid, pomegranate gallagic acid, and gallagyl esters include punicalin and punicalgin. Hydroxybenzoic acids of pomegranate include gallic acid and protocatechuic acid, while its hydroxycinnamic acids contain caffeoyl hexoside, chlorogenic acid, *p*-coumaric acid, and their derivatives. Also, a dihydroflavonol named dihydrokaempferol-hexoside has been isolated from pomegranate juice (Fischer et al., 2011). Pomegranate phytochemicals have shown positive effects in the management of pulmonary inflammation (Shekhar et al., 2017). Pomegranate has shown inhibitory effects on inflammatory pathways such as NF-k B pathway. Indeed, pomegranate juice is rich in polyphenols having high antioxidant and anti-inflammatory properties *in vitro* and *in vivo*. Pomegranate juice has various effects such as increasing serum antioxidant capacity, reducing inflammation, and decreasing the activity of angiotensin-converting enzyme. According to a recent study, the antioxidant activity of total pomegranate juice was superior to its purified polyphenols. Therefore, it represents the chemical synergy and multifactorial effects of pomegranate whole extract compared to its single active ingredients. Gallic acid and punicalagin in pomegranate juice stimulate the expression of macrophage PON2 and the activation of PAPR gamma and AP-1 transcription factors. Daily consumption of pomegranate juice increases the antioxidant and antimicrobial capacities in the immune system (Reddy et al., 2007; Charles, 2013). Punicalagin, which is a polyphenolic compound isolated from pomegranate, has shown considerable positive *in vivo* effects to inhibit lung edema, inflammatory cell infiltration, and pro-inflammatory cytokines (IL-6, TNF-α, and IL-1β) discharge (He et al., 2020). Pomegranate has numerous applications for asthma, bronchitis, fever, cough, and inflammation (Reddy et al., 2007; Lansky and Newman, 2007).

Punica granatum is used as a main component in local medications against cold, cough, and fever (Ballabh and Chaurasia, 2007). Pomegranate fruit is a rich source of antioxidants (Syed et al., 2013). On the other hand, pomegranate peel extract could inhibit myeloperoxidase production to reduce lungs inflammation. Another study on pomegranate fractions revealed reduction of neutrophils recruitment in the lung area and inhibition of changes in vascular pulmonary permeability. Furthermore, tannins available in pomegranate possess antioxidant and antimicrobial secretion activities (**Figure 1**) (Reddy et al., 2007; Shekhar et al., 2017).

Purging Cassia Laooq

This Laooq is made of *Cassia fistula* L. (Leguminosae) fruit (hot and wet temperament), *Prunus domestica* L. (Rosaceae) bark gum (hot and dry temperament), *Vicia faba* L. (Leguminosae) seed flour (cold and dry temperament), *Astracantha gummifera* (Labill.) Podlech (Leguminosae) gum (moderate and wet temperament), and *Prunus dulcis* (Mill.) D.A.Webb (Rosaceae) seed oil (moderate and wet temperament). It has been traditionally recommended for cough and pulmonary infections (Ghaeni Heravi, 1766). Methanol extract of *C. fistula* fruit pulp consists of flavonoids, saponins, steroids, triterpenoids, glycosides, anthraquinones, tannins, gums, amino acids, and mucilage. This extract demonstrated significant antioxidant activity. Pulp also contains antifungal constituents, betulinic acid, b-sitosterol, stigmasterol, ergosterol, fucosterol, lupeol, α-amyrin, and friedelin (Akbar, 2020). According to recent studies, *C. fistula* has laxative, antimicrobial, antioxidant, anti-inflammatory, and anti-pyretic properties. Also, it can control nasal infections and coughs (Tanveer et al., 2019; Pawar and Killedar, 2017; Nikhat and Fazil, 2020). Methanol extract of *Cassia fistula* showed significant antitussive activity (Bhakta et al., 1998). Stigmasterol has shown suppressing effects on allergen-induced asthma (Antwi et al., 2017). On the other hand, *Prunus dulcis* gum is a mixture of high-molecular polysaccharides such as hemicelluloses compounds having antitussive properties (Bouaziz et al., 2017; Bouaziz et al., 2015). *Vicia faba* L. is another ingredient of purging cassia Laooq. Seeds of *V. faba* are rich in proteins (globulins, albumins, and glutelins), carbohydrates, vitamins, folic acid, niacin, dietary fiber, and macro and micro nutrients. According to a recent report, dietary fiber has potential beneficial effects on lungs such as reducing inflammation and enhancing the antioxidant processes. It has been suggested that a high-fiber diet might reduce the occurrence of chronic cough symptoms (Butler et al., 2004). Fatty acids, α-tocopherol, phytosterol, stigmasterol, and campesterol are other constituents of the seed (Pasricha et al., 2014). Faba bean lectin protein has shown binding affinity to HIV-1carbohydrates (François and Balzarini, 2012). Traditionally, cooked faba beans have been applied against cough and inflammation (Prabhu and Rajeswari, 2018). Owing to rich content of phenolic compounds, the seeds have antioxidant property (Pasricha et al., 2014). A study revealed that Leucoefdin found in *Vicia faba* has the potential to inhibit M^{Pro} protease, which is responsible for the formation of functional viral polyprotein (Singh and Mishra, 2020). It is suggested that *Vicia faba* may help to fight better against coronavirus 2 infection (**Figure 1**) (Khalil et al., 2020). The two other ingredients of purging cassia laooq including *A. gummifera* gum and *P. dulcis* oil are discussed in seeds Laooq and almond Morabba sections, respectively.

Seeds Laooq

Seeds Laooq is made of *Astracantha gummifera* (Labill.) Podlech (Leguminosae) gum (moderate and wet temperament), *Cucumis sativus* L. (Cucurbitaceae) seed (cold and wet temperament), *Cucumis melo* L. (Cucurbitaceae) seed (hot and wet temperament), *Cucurbita pepo* L. (Cucurbitaceae) seed (cold and wet temperament), *Prunus dulcis* (Mill.) D.A.Webb (Rosaceae) fruit (hot and wet temperament), sugar (hot and dry temperament), and honey (hot and dry temperament). It has traditionally been recommended for dry cough (Ghaeni Heravi, 1766). Gum tragacanth (*Astracantha gummifera* (Labill.) Podlech) as an adhesive agent and a thickener has a wide range of usage in food and pharmaceutical industries. It is a complex mixture of various polysaccharides acting as laxative and antitussive (Noreen et al., 2019; Fattahi et al., 2013). A water-swellable polysaccharide (bassorin) and a pectic polymer (tragacanthic acid) are available in gum tragacanth (Nayeb morad, et al., 2018; Stephen and Phillips, 2006). Inhalation of *A. gummifera* 2.5% w/v and 5%w/v decreased significantly the number of coughs induced by chemicals in animals (Saadat et al., 2018). *Cucumis melo* L. seeds, possessing dietary fibers, minerals, and antioxidants such as β-carotene, are a valuable source of nutrients with different medicinal indications such as digestive, antitussive, and demulcent (Ibrahim et al., 2019). Melon seed oil containing linoleic acid, lecithin, and cephalin acts as an antimicrobial, antioxidant, and anti-inflammatory agent (Simona et al., 2009). Seeds of *C. sativus* have been traditionally used against fevers and burning sensations (Seliya and Patel, 2009). Ethanolic extract of *C. sativus* seed contains flavonoids, phenols, carbohydrates, terpenoids, and tannins (Begum et al., 2019). *Cucurbita pepo* seed contains amino acids, phenolic compounds, phytosterols, tocopherols, cucurbitacins, minerals, and unsaturated fatty acids such as oleic and linoleic acids (Dotto and Chacha, 2020). Cucurbitacin E isolated from pumpkin seed has shown anti-inflammatory characteristics (Jang et al., 2008). *Prunus* gums are hydrophilic carbohydrates with high molecular weights. They are composed of monosaccharide units linked by glucosidic bonds (Bouaziz et al., 2016). Owing to low-toxicity, stability, and availability, the gums are applied in pharmaceutical industries as an emulsifying agent, disintegrant, suspending agent, and binder (**Figure 1**) (Rahim et al., 2018).

Morabba

Morabba has been a popular FF in TPM and the word "Morabba" means treated or trained in Persian. The general meaning of "Morabba" is that ingredients should be treated in the process of jam preparation (Dehkhoda, 1999; Ghaeni Heravi, 1766). In other

words, Morabba is a traditional FF similar to jam in which the chopped or sliced natural ingredients are treated in a base of honey, grape juice (*doushab*), or syrup (Ghaeni Heravi, 1766).

Natural honey is a common base of cough jams in TPM (Ghaeni Heravi, 1766). It is a natural sweet food material made from nectar of flowers. Honey is composed mainly of glucose and fructose, and containing amino acids, proteins, enzymes, minerals, vitamins, and other minor compounds (Burlando and Cornara 2013). Its phenolic constituents specifically chrysin modulate the oxidative stress and inflammatory conditions. Current biomedical findings have proved the immunomodulatory and respiratory protective effects of honey. Honey and chrysin have shown beneficial effects through affecting total inflammatory cells, eosinophils, macrophages, lymphocytes, neutrophils, p-Akt, IFN-γ level, serum total IL-4, IgE, and IL-13, α-Smooth muscle protein expression, and ERK1/2 pathways. In addition, chrysin has shown therapeutic effects in the lung injury model through the regulation of TNF-α, NAD-dependent deacetylase (SIRT1)/Nrf2, and IL-1β levels, β-glucuronidasem and myeloperoxidase levels, HO-1, MDA, GSH, VCAM-1, ICAM-1, and NF-κBp65 pathways (Talebi et al., 2020). Recently, chrysin is identified as a COVID-19 main protease inhibitor according to *in silico* studies (Lima et al., 2020). Moreover, chrysin has shown *in vitro* inhibitory effects on herpes-virus intracellular replication (Berretta et al., 2020). Other relevant investigations expressed that honey might have antitussive properties with no side effects (Mulholland and Chang, 2009; Werner and Laccourreye, 2011). In a recent study on upper respiratory tract infections, honey has shown higher therapeutic effects than usual care (Abuelgasim et al., 2021). Besides, a Cochrane systematic review has suggested that honey may have better effects than diphenhydramine on suppression of children's cough (Oduwole et al., 2018). The efficacy of honey in reducing the COVID-19 symptoms in humans is being studied (Matteo et al., 2020). An *in silico* analysis indicates that M pro may be the anti-covid-19 target of flavon, flavonols, and phenolic esters content of honey (Matteo et al., 2020).

Doushab is another sweet base of TPM cough jams. It is a viscous dark brown liquid obtained from *Vitis vinifera* L. juice (Vitaceae) when the whole grape (*V. vinifera*) fruit is cooked, filtered (to separate its seeds and peel), and then concentrated to get doushab (Gharibzahedi, 2018). This concentrated grape juice contains high amounts of glucose and fructose, essential minerals, and polyphenols (Bozkurt et al., 1999; Haas et al., 2018). Flavonoids such as quercetin, isorhamnetin, and kaempferol derivatives are identified in grape fruits (Georgiev et al., 2014). The protective effects of flavonoids on lungs may be due to their antioxidant and anti-inflammatory properties. Their antioxidant activity involves inhibition of nitric oxide synthase and xanthine oxidase as well as direct free-radical scavenging activity. Besides, the mechanisms of action related to anti-inflammatory properties of flavonoids could be described as inhibitory effects on the 5-lipoxygenase and cyclooxygenase pathways in the metabolism of arachidonic acid (Butler et al., 2004). Quercetin has shown important biological activities including anti-inflammatory, antioxidative, and antihistamine actions as well as protective and preventive effects in controlling asthma complications (Cesarone et al., 2019; Derosa et al., 2021). Quercetin as an antiasthmatic, immunomodulatory, and bronchodilatory agent has induced a relaxation effect in tracheal rings and reduced the inflammatory cytokines and eosinophil peroxidase in the lungs according to one *in vivo* study in a murine model of asthma (Oliveira et al., 2015). Also, quercetin has shown protective effects on COVID-19-induced acute kidney injury (Gu et al., 2021). Furthermore, kaempferol has shown central antitussive activities (Huang, et al., 2020a; Huang et al., 2020b). Gallic acid, as one of the important secondary metabolites present in *Vitis vinifera* L. fruits, has exhibited various biological characteristics such as anti-inflammatory, antimicrobial, and antioxidant properties (Arora et al., 2016). A hot water extract of grape peel has presented antiviral (influenza virus) activity in former studies (Bekhit et al., 2011). Procyanidins in grape extract is identified as a potent antiviral agent (Dai et al., 2012). Also, procyanidins revealed potential therapeutic properties against COVID-19 in molecular docking studies (Maroli et al., 2020). Moreover, resveratrol is a flavonol component of grape that has the ability to bind with the ACE2 target site of COVID-19 according to recent *in silico* studies (Matteo et al., 2020). Resveratrol has antioxidant and immune-stimulatory properties (Ramdani and Bachari, 2020; Santos et al., 2021). Also, by virtue of its anti-inflammatory and anti-thrombotic properties, resveratrol is expected to lower the mortality rate of COVID-19 disease (Giordo et al., 2021).

Based on recent clinical trials on cough formulations, researchers have proposed that there should be one or more characteristics having some physiological effects in the base of cough formulations. For instance, most liquid antitussive preparations are very sweet. Thus, the researchers have suggested that sweet taste may be able to modulate cough sensitivity. According to current evidence-based studies, a close anatomical relation in the brainstem is found between the mechanisms involved in the cough reflex and those processing taste signals. Therefore, primary taste afferents might be responsible for modulation of activity patterns in the brainstem networks controlling airway protective behaviors (Wise et al., 2014). Using honey as a cough remedy throughout history is accordant with this idea. Evidence-based studies have shown that mouth rinsing with the solution of sweet sucrose could increase the cough thresholds. The basic mechanisms related to cough suppression by the sweet taste are not found yet. However, surviving data indicate the potential effects of taste on modulation of cough sensitivity would be a promising issue for further investigations (Wise et al., 2014).

Almond Morabba

Almond Morabba is made of *Prunus dulcis* (Mill.) D.A.Webb (Rosaceae) kernel (hot and wet temperament), Dushab (*Vitis vinifera* L.) (Vitaceae) (hot and wet temperament), and honey (hot and dry temperament). It has been traditionally recommended for dry cough. To prepare this formulation, ripe almond kernel is peeled and boiled with the mixture of water and Dushab (grape juice). Then almond is macerated in that liquid for

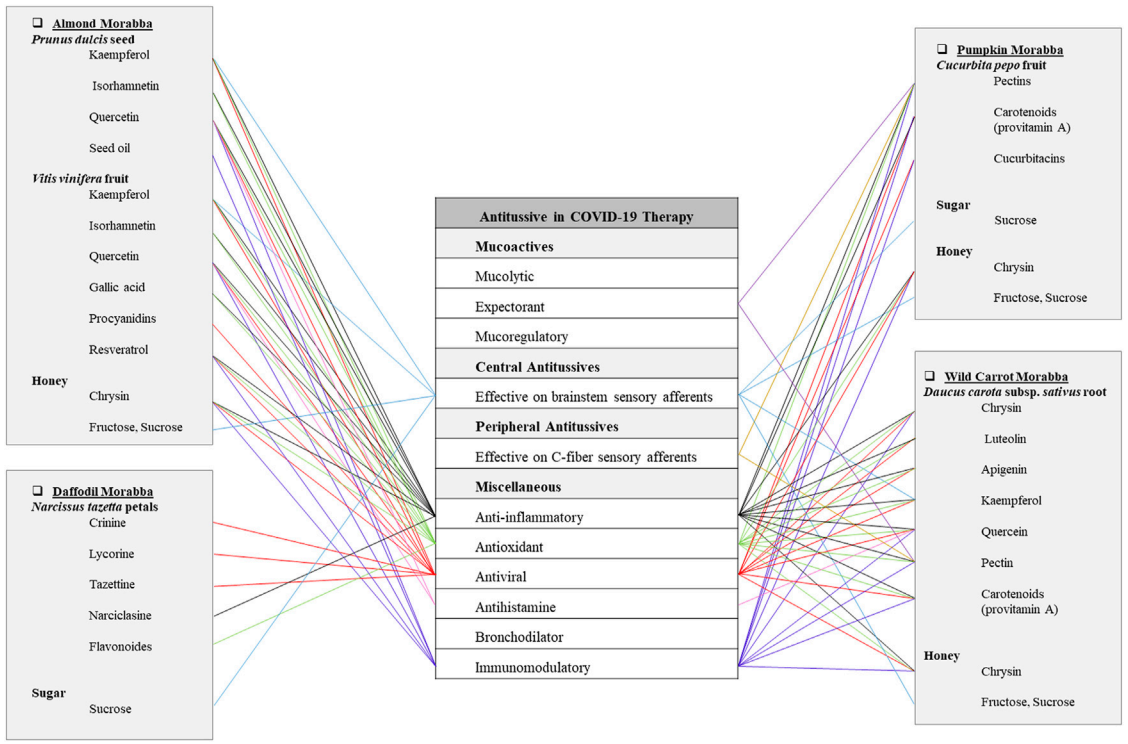

FIGURE 2 | Potentials of antitussive Persian Morabbas in COVID-19 therapy.

three days. On the third day, the macerated almond is put in honey and boiled till the mixture gets consolidated. It is kept for 40 days and then becomes ready for taking. It is considered to be suitable for cough and wheezing in TPM (Ghaeni Heravi, 1766). Sweet almond is rich in fatty acids, carbohydrates, proteins, vitamins (vitamin E, B, etc.), minerals, and various bioactive ingredients (polyphenols, phytosterols, etc.) which are consumed as natural anti-inflammatory, antioxidant, antimicrobial, and antiviral agents (Barreca et al., 2020). Different classes of flavonoids including anthocyanidins (cyanidin), flavonols, flavanones, and flavan-3-ols are reported in almond. Among the flavan-3-ols, dihydrokaempferol, (−)-epicatechin, and (+)- catechin are the most abundant compounds but gallocatechin gallate, dihydroquercetin, epicatechin gallate, and epicatechin glycoside are also reported. Additionally, the most abundant flavonoid group in almond are flavonols including kaempferol, isorhamnetin, quercetin, isorhamnetin and their rutinosides, 3-O-glucosides, and galactosides. Also, the main stilbene compound in almond is identified as resveratrol-3-O-glucoside (Barreca et al., 2020). Actually, almond is beneficial to improve the immune system (Ali and Alharbi 2020). The phenolic content of the nuts may decrease or even prevent the processes of oxidative stress-related disorders (Isfahlan et al., 2010; Alkhatib, 2020; Subhashinee et al., 2006). Also, Almond oil can be helpful for the improvement of the immune system and the prevention of many degenerative diseases (Kostadinović Veličkovska et al., 2018). On the other hand, almond kernel has shown prebiotic properties (Gibson and Roberfroid, 1995) while probiotics are supposed to be useful in the management of COVID-19 infection (**Figure 2**) (Giannoni et al., 2020).

Daffodil Morabba

This Morabba is made of *Narcissus tazetta* L. (Amaryllidaceae) petals (hot and dry temperament) and sugar (hot and dry temperament). It has been traditionally recommended for cough and dyspnea. To prepare this TPM formulation, daffodil petals and sugar are mixed and made into jam. According to Persian medicine, it is considered to be suitable for special respiratory disorders. Daffodil contains alkaloids such as crinine, lycorine, and tazettine that presented *in vitro* antiviral and antimalarial activities (Kornienko and Evidente, 2008). Lycorine is a broad-spectrum antiviral substance against coronavirus infection and it is possible to have therapeutic effects in COVID-19 infection (Choudhry et al., 2020; Khalifa et al., 2020). Narciclasine is another alkaloid isolated from the bulbs of different varieties of *Narcissus*. It has significantly reversed the gene expression changes in a moexipril-treated HCC515 cell line which is a suggested model for human lung injury in COVID-19. Also, narciclasine has shown *in vivo* anti-inflammatory effects and lung injury reduction (Kornienko and Evidente, 2008; He and Garmire, 2020). Moreover, some flavan derivatives, β-coumaranone and phenylpropanoid structures with potent *in vitro* antioxidant activity, and a mannose-binding lectin with potent antiviral activity have been isolated

from the bulbs of *N. tazetta* (Ooi et al., 2010; Fu et al., 2016). These findings indicate that daffodil may have beneficial effects in COVID-19 (**Figure 2**).

Pumpkin Morabba

Pumpkin Morabba is made of *Cucurbita pepo* L. (Cucurbitaceae) fruit (cold and wet temperament), honey (hot and dry temperament), and sugar (hot and dry temperament). It has been traditionally recommended as a lung-protective FF. The peeled pumpkin is chopped and boiled in water till it becomes semi-cooked, then honey and sugar are added, and the mixture is boiled more till it gets consolidated. Pumpkin contains minerals as calcium, phosphorous, iron, sodium, and potassium. Also, it contains vitamins such as vitamin A, thiamin, riboflavin, niacin, and ascorbic acid (Fernández-López et al., 2020). Pumpkin fruit contains various water-soluble pectins with antitussive efficacy equal to and even more than codeine. Furthermore, pectins did not show any adverse effects in one *in vivo* study. So, it declares that they are safer than conventional opioid cough suppressants (Nosáľová et al., 2011). Pectic polysaccharides are rich in galactopyranosyluronic acid (GalpA), and galactans are polysaccharides with high proportion of galactose (Ferreira et al., 2015). After oral administration of pectins, they can cover the mucus terminals in the epipharyngeal nerve and decrease the sensitivity of cough receptors to irritations which leads to cough suppression. Also, it is considered that these kinds of polysaccharides can increase the saliva production contributed to antitussive properties by the activation of the swallow reflex that is competing with cough reflex in the central level (Nosalova et al., 2013). Pectins have immunomodulating, anti-inflammatory, and antitussive properties. In a recent study, in which cough reflex was induced by citric acid in guinea pigs, pectins from *C. pepo* inhibited the frequency and intensity of coughing attacks. In another study, pectins from the *Althaea officinalis* L., stimulated the activity of airway mucus and the peristalsis of respiratory bronchioles collaborated with the enhanced bronchial glands secretion (Zaitseva et al., 2020). In addition to antitussive properties, pectin fractions of pumpkin have shown antioxidant effects (Torkova et al., 2018). Furthermore, pumpkin has some triterpenes such as cucurbitacins, and some tetraterpenes such as carotenoids. Carotenoids have pro-vitamin A and immunomodulatory activity. Also, pumpkin has antioxidant substances such as β-carotene, lutein, and zeaxanthin that can improve the immune system function (Montesano et al., 2018; Swamy, 2020). According to a recent study, a heat treatment on raw pumpkin could significantly increase the bioaccessibility of its β-carotene compounds (Thakur et al., 2020). On the other hand, it is notable that in one *in silico* study, cucurbitacins (including cucurbitacin E and B, and isocucurbitacin B) represented strong binding affinity to the main protease of COVID-19 that leads to blockage of the COVID-19 viral replication. On the other hand, in recent studies, cucurbitacin B and E showed immune-enhancing activities against HSV-1 and BVDV/HIV, respectively, and they did not show any side effects (**Figure 2**) (Alagu Lakshmi et al., 2020).

Wild Carrot Morabba

Wild Carrot Morabba is made of *Daucus carota* subsp. *sativus* (Hoffm.) Arcang. (Apiaceae) root (hot and wet temperament) and honey (hot and dry temperament). It has been traditionally recommended for antitussive purposes. To prepare this jam, wild carrot is boiled in water containing honey. Then it is placed in another pot and boiled with honey gently until it loses its water. Wild carrot contains flavonoids from flavones group such as apigenin, chrysin, and luteolin, flavonols group such as kaempferol and quercetin, and various glycosides. In addition, the carrot plant has furanocoumarins of 8-methoxypsoralen and 5-methoxypsoralen. Other constituents are choline, daucine (alkaloid), fatty acids (butyric, palmitic), pectins, and coumarins (Barnes et al., 2013; Jafari et al., 2017). Cholinergic actions have been reported from *in vitro* studies, indicating the spasmodic properties of wild carrot in both smooth and skeletal muscle. This cholinergic activity has been attributed to its choline content (Barnes et al., 2013). On the other hand, chrysin is reported to have inhibitory effects on COVID-19 main protease and herpes-virus intracellular replication (Lima et al., 2020; Berretta et al., 2020). While luteolin has the potential to bind to Spike-2 protein, PLpro, M pro/3CLro, and ACE2 (Fuzimoto and Isidoro, 2020; Shawan et al., 2021), apigenin can bind to active residues of ACE2 which intercede host viral interface (Khanna et al., 2021). According to previous studies, *D. carota* herb is a rich source of provitamin A. It is notable that the preparation method of carrot affects its carotenoid content. Water cooking of carrot without any pressure (the same as Persian wild carrot jam recipe) is reported to be the best method for reducing its carotenoids loss (Sant'Ana et al., 1998). Indeed, it is reported that thermal treatment of carrot can have a positive effect on the micellarization of its carotenes as well as disruption of protein-carotenoid complexes in its food matrix and softening its cell wall. This process would significantly improve the bioaccessibility of carotenoids in carrots (Thakur et al., 2020). Furthermore, Bioinformatics findings suggest pharmacological mechanisms for vitamin A against COVID-19 through immunomodulation, anti-inflammatory reaction, and antioxidant properties. Seven core targets of vitamin A against COVID-19, including CAT, EGFR, ICAM1, IL10, MAPK1, MAPK14, and PRKCB, have been detected (**Figure 2**) (Li R. et al., 2020).

Saviqs

Saviq is the flour made of roast grains or fruits. According to TPM, Saviq is prepared by a brief roasting process "to the extent that the odor of roasted flour is smelled." This traditional description may indicate the shortness of heating process to induce a number of modifications such as destruction of microstructures responsible for releasing of the bound phytonutrients (Qi et al., 2018; Thakur et al., 2020). But, the potential nutritional aspects of the flour would not have considerable changes after a short heat treatment (Schnorr et al., 2016; Qi et al., 2018). It is evident that roasted fruits have more antioxidant effects than the raw ones according to recent studies (Navajas-Porras et al., 2020). Saviq is a popular

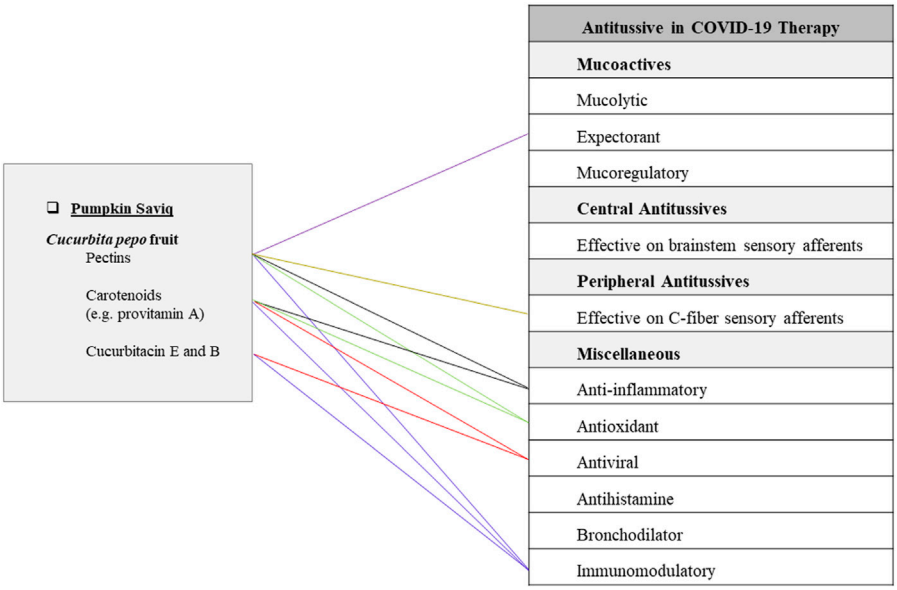

FIGURE 3 | Potentials of pumpkin Saviq as an antitussive Persian functional food in COVID-19 therapy.

snack in Iran. It has beneficial effects related to its ingredient materials with more astringent characteristics than its raw materials (Shafiee et al., 2019).

Pumpkin Saviq

It is made of *Cucurbita pepo* L. (Cucurbitaceae) roast fruit (cold and wet temperament). It has been traditionally recommended as a very effective FF for coughs associated with hot distemperament. For preparing pumpkin Saviq, pumpkin is peeled, chopped, dried, and after roasting, grinded to become a fine powder. It is considered to be helpful as an antitussive agent (Ghaeni Heravi, 1766). The current investigations related to pumpkin potential for the treatment of COVID-19 are described in the part pumpkin jam (**Figure 3**).

Soups (Shorbas)

Soup is a popular nutritious and flavorful watery food in TPM. It is used in winter and cold weathers especially for the prevention or treatment of common cold and influenza. For Iranians, Shorba is a kind of folk soup which is sometimes salty and spicy (Ghaeni Heravi, 1766). Consumption of a bowl of hot soup and breathing its warm vapors is considered to have mucolytic effects (Kirkpatrick, 1996).

Rooster Soup

This soup is made of *Gallus gallus domesticus* (Phasianidae) meat (hot and dry temperament), *Polypodium vulgare* L. (Polypodiaceae) rhizome (hot and dry temperament), *Anethum graveolens* L. (Apiaceae) aerial part (hot and dry temperament), and *Apium graveolens* L. (Apiaceae) aerial part (hot and dry temperament). It has been traditionally recommended for the treatment of phlegmatic cough, pulmonary infection, and dyspnea. According to TPM manuscripts, rooster soup contains a potent active ingredient to cure the respiratory infections, phlegmatic coughs, and dyspnea (Gharashi, 1288). The recipe of this traditional soup is that the rooster is killed and its feathers and visceral content are removed. Then it is stuffed with polypody, dill, and celery and further it is boiled till the meat of rooster crushes and it is filtered to prepare a soup (Ghaeni Heravi, 1766). It is known that soft connective tissue and the comb of rooster are good sources of hyaluronic acid (HA). Indeed, the rooster comb has the highest concentrations of HA among animal tissues (Boeriu et al., 2013). Thus, an important active ingredient of this traditional soup seems to be HA, which is an extracellular matrix polysaccharide with the ability of trapping water to produce a hydrogel substance (Swann, 1968; Garvin et al., 2020). In fact, HA is a carbohydrate compound with repeating disaccharide units of glucosamine and N-acetylglucosamine (Ferreira et al., 2015). HA, as a lubricant at the epithelium surface of airway, can increase the intercellular function of adhesion molecules in airway mucus by modulating its surface properties and improving the surface activities of the respiratory tract surfactants. Moreover, HA strengthens the bronchial epithelial barrier, stimulates the cellular host defense mechanisms, and increases the ciliary beating in mucosal host defense. Besides, HA is associated with protective mechanisms against cell death including the interactions of HLA-Toll-like receptor, basal activation of NF-κB, and specific interactions with several cell surface receptors such as CD44. Generally, HA is considered a protective factor in various cell aggressions against the airway epithelium and the epithelial integrity homeostasis. Also, it is a lubricant agent supporting the good ciliary and cough clearance in the airway mucus (Zahm et al., 2011). HA has relevant interactions with immune cells. In a normal lung, alveolar macrophages are surrounded by a HA layer. In acute lung infection, HA levels are immediately increased suggesting a

potential role for HA in the promotion of leukocyte accessibility to the lung injury site. Additionally, HA can produce a provisional matrix to promote tissue repair (Johnson et al., 2018). Notably, it is evident that in lungs of COVID-19 patients, the production of HA is increased and its degrading enzyme is greatly decreased. In addition, the levels of ACE2 and bradykinin in their lung cells are increased. These changes trigger leakage of fluid into the patient's lungs and the produced HA combines to that fluid. It results in a hydrogel formation that prevents gas exchange in the lungs and it leads to a drastic condition in severe COVID-19 patients (Garvin et al., 2020). According to recent investigations, alveolar HA level is usually elevated in lung injuries, while aerosolized HA has shown healing effects in lung diseases associated with elastic fiber injury. It is reported that the larger HA molecules possess anti-inflammatory properties and smaller molecules have pro-inflammatory activities (Noble et al., 2011; Esposito et al., 2017). Thus, there is a size-effect relationship regarding hyaluronan molecules. Those with a molecular weight of 1,050–1,338 kDa have shown stimulatory activity on various immune cells in recent studies. Also, those with the molecular weight of 45.2–145 kDa have shown stronger immunostimulatory activity after the process of hydrolysis (Ferreira et al., 2015). A recent study suggested that the administration of exogenous HA by aerosol could have therapeutic effects on diseases in which their exacerbation alters the surface properties of the mucus and the mucucilliary clearance functions. Another study revealed that HA 40 kDa protected the airway epithelium against the injury during bacterial infection (Zahm et al., 2011). Moreover, HA has antioxidant activity and it regulates inflammatory cell recruitment, inflammatory cytokines release, and stem cell migration in inflammation and tissue injuries (Noble et al., 2011; Hafsa et al., 2017). These findings provide a new insight on the probability of using HA molecule to manage COVID-19 respiratory symptoms. On the other hand, rooster meat, as another ingredient of this soup, is a rich source of immunomodulatory peptides, vitamins, and minerals that can increase the immunity of body against coronavirus by enhancing of macrophages and monocytes functions (Alkhatib, 2020).

Polypody (*Polypodium vulgare* L.) is another ingredient of the traditional rooster soup. Polipody contains flavonoids such as kaempferol and quercetin derivatives, as well as hydroxycinnamic acids including caffeic acid derivatives and chlorogenic acid. Also, it has phytoecdysteroids such as 20-hydroxyecdysone and polypodin B, steroidal saponins such as osladin and polypodosaponins. Furthermore, numerous triterpenoids comprising cuphan, cycloartane, dammaran, and phernan, and some other phytochemicals such as cycloartanyl acetate, cycloaudenyl acetate, linoleic acid esters, and phytosterols are reported in polypody extracts. Pharmacological and clinical studies on *P. vulgare* are rare (Barnes et al., 2013), but recent studies on *P. leucotomos* Poir. phenolic compounds (e.g., chlorogenic, coumaric, vanillic, caffeic, and ferulic acids) have demonstrated antioxidant properties through *in vitro*, *in vivo*, and human studies (Berman et al., 2016). In a recent clinical trial, *P. leucotomos* extract prevented the infection processes in athletes by enhancing their immune system. Besides, *in vitro* studies on polypody extract have demonstrated its pleiotropic effect on different cytokines of the immune system. In fact, *P. leucotomos* has displayed both humoral and cellular immunomodulatory activities through *in vitro* studies (Solivellas and Martin, 2012; Sánchez-Rodríguez et al., 2018). On the other hand, polypody expressed healing properties in the treatment of tyrosine kinase-induced phototoxicity in a case report study (Korman et al., 2019). It is noteworthy that a recent bioinformatics analysis has suggested that any herb with the anti-tyrosine kinase activity could be a good drug candidate for treating COVID-19 infection (Sriwijitalai and Wiwanitkit, 2020).

Dill (*Anethum graveolens* L.) is another ingredient of TPM rooster soup. It has two major flavonoids including isorhamnetin 3-O-β-D-glucuronide and quercetin 3-O-β-D-glucuronide, as well as other minor components including 3-glucosides, 3-galactosides, and 3-rhamnoglucosides of quercetin and isorhamnetin. Volatile components of dill include carvone, limonene, α-phellandrene, dill ether (anethofuran), coumarins, myristicin, flavonoids, steroids, and phenolic acids. 8-hydroxygeraniol, β-D-glucopyranosides, and p-menth-2-ene-1,6-diol have also been isolated from the dill herb. A furanocoumarin, several coumarin derivatives, phenolic acids such as caffeic, ferulic, and chlorogenic acids are detected in dill seeds. Major constituents of hydro distilled essential oil from aerial parts of Persian dill are limonene, α-phellandrene, dill ether, and sabinene. Limonene and sabinene have been identified as its main antioxidant compounds (Akbar, 2020). Dill also contains carotenoids, ascorbic acid, and minerals (Naidu et al., 2016). Oral indication of dill (aqueous extract), in one *in vivo* study, showed potential antioxidant properties (Oshaghi et al., 2016). Generally, vegetables are known as FFs that can prevent and control viral infections by inducing antioxidant and anti-inflammation activities to modulate the immune system (Alkhatib, 2020). Also, coumarins have shown strong antioxidant activities (Shekhar et al., 2017). It is believed that the water-soluble antioxidants can protect lipid-soluble antioxidants via a polar paradox (Nayak et al., 2015). Interactions between the matrices of soup vegetables and the lipid fractions during cooking are also notable.

Celery (*Apium graveolens* L.), another ingredient of rooster soup, possesses flavonoids including apigenin, apiin, quercetin, isoquercitrin, kaempferol, and luteolin, coumarins including apigravin, apiumoside, apiumetin, bergapten, celereoside, celerin, isoimperatorin, isopimpinellin, osthenol, umbelliferone, rutaretin, seselin, and 8-hydroxy-5-methoxypsoralen, and fatty acids, caffeic, p-coumaric, and ferulic acids (Barnes et al., 2013; Kooti and Daraei, 2017; Chonpathompikunlert et al., 2018; Li M. Y. et al., 2020). It has powerful antioxidant properties due to its phytochemical compounds such as caffeic, p-coumaric, and ferulic acids, apigenin, kaempferol, luteolin, quercetin, saponin, and tannin. In particular, celery has more apigenin content compared with other plants (Kooti and Daraei, 2017; Chonpathompikunlert et al., 2018; Li M. Y. et al., 2020). Additionally, celery plant extracts have shown anti-inflammatory activities *in vitro* and *in vivo* (Barnes et al., 2013; Akbar, 2020).

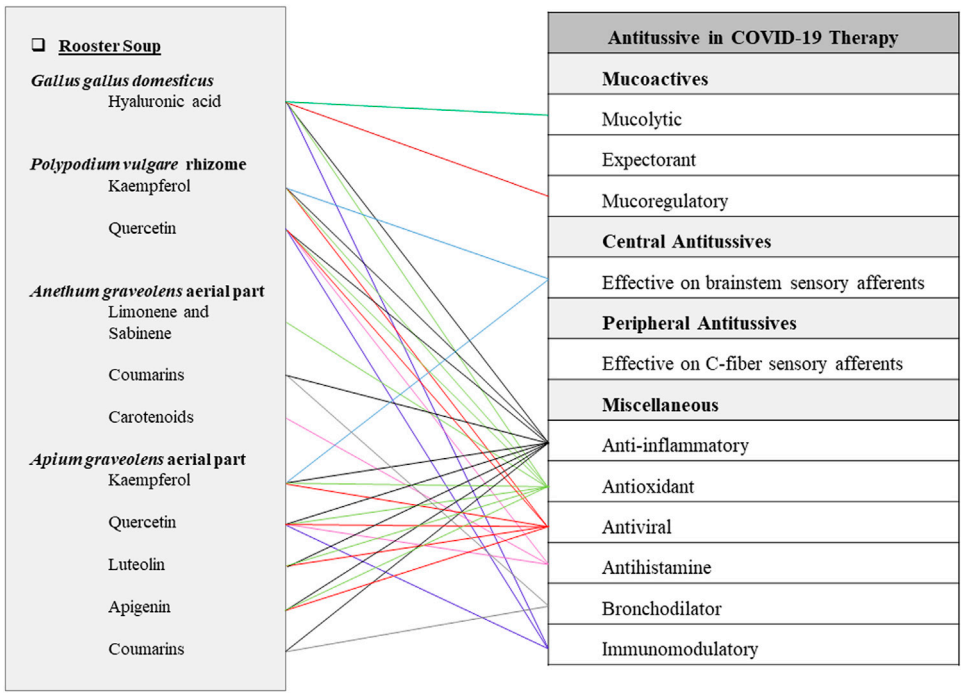

FIGURE 4 | Potentials of rooster soup as an antitussive Persian functional food in COVID-19 therapy.

Besides, one teaspoon of celery seeds mixed with foods, taken three times a day, has shown beneficial effects in chest pain, asthma, and bronchitis in recent investigations. Its coumarins are supposed to have the muscle relaxant activities, as well as its antispasmodic property that is pertained to the essential oil in seeds (Peter, 2012). Apigenin, luteolin, kaempferol, and quercetin have antioxidant and anti-inflammatory activities (Tian et al., 2021). Furthermore, flavonoid compounds such as apigenin, kaempferol, and quercetin showed activity against COVID-19 through suppression of M pro enzymes. Also, the target for apigenin is considered to be spike protein, 6LU7, and 6Y2E proteases (Bhuiyan et al., 2020; Matteo et al., 2020). In addition, luteolin can bind to Spike-2 protein of SARS-CoV without any cytotoxic effects (**Figure 4**) (Fuzimoto and Isidoro, 2020).

Syrups

Syrup is a well-known liquid dosage form in both traditional and modern medicine. Sweet violet (*Viola odorata* L.) syrup has been one of the most popular TPM drinks recommended for antitussive properties (Ghaeni Heravi, 1766). According to clinical and *in vivo* studies, the syrupy vehicle of antitussive syrups possesses a local demulcent effect on the mucosa of respiratory tract and relieves the irritation of cough in the mucosa. Also, drinking syrup might have little expectorant property when the bulk of syrup enters into the stomach. These experiments provide a logical reason for traditional application of antitussives in a vehicle of syrup, and also justify the persist use of this form of medication (Boyd, 1946). This syrup has been recommended for the prevention or management of epidemic situations such as COVID-19 in the ancient times (Ansari et al., 2020).

Sweet Violet Syrup

This syrup is made of *Viola odorata* L. (Violaceae) flower (cold and wet temperament) and sugar (hot and dry temperament). It has been traditionally recommended for dry cough, respiratory infections, and fever. To prepare this Persian FF, sweet violet flower is boiled in water till two-thirds of the water evaporates. The residue of petals should be strained and filtered. Then sugar is added to the liquid and the syrup is boiled to be more concentrated (Ghaeni Heravi, 1766). Sweet violet flower contains mucilage, anthocyanins, flavonoids, and methyl salicylate (Tobyn et al., 2011). Aqueous-methanol leaves extract possesses alkaloids, coumarins, tannins, phenolics, flavonoids, and saponins. Aqueous extract contains vitexin while the ethanol extract contains vitexin, isovitexin, rutin, and kaempferol-6-glucoside (Akbar, 2020). *V. odorata* is a rich source of antioxidants (Mousavi et al., 2016). Vitexin has shown decreasing effects in lung edema and alveolar protein content. An *in vivo* study revealed that vitexin could suppress neutrophil recruitment and proinflammatory cytokine levels, but increase Nrf2 and HO-1 activity. These findings expressed that vitexin could suppress LPS-induced acute lung injury by controlling the Nrf2 pathway (Lu et al., 2018). On the other hand, vitexin has shown spasmolytic effects on rat-isolated duodenums by increasing cGMP and activating K+-channels (Ragone et al., 2007). According to *in-silico* virtual studies, vitexin has potential inhibitory effects on spike protein and 3CLpro or M pro of COVID-19 virus (Naik et al., 2021). Furthermore, rutin has

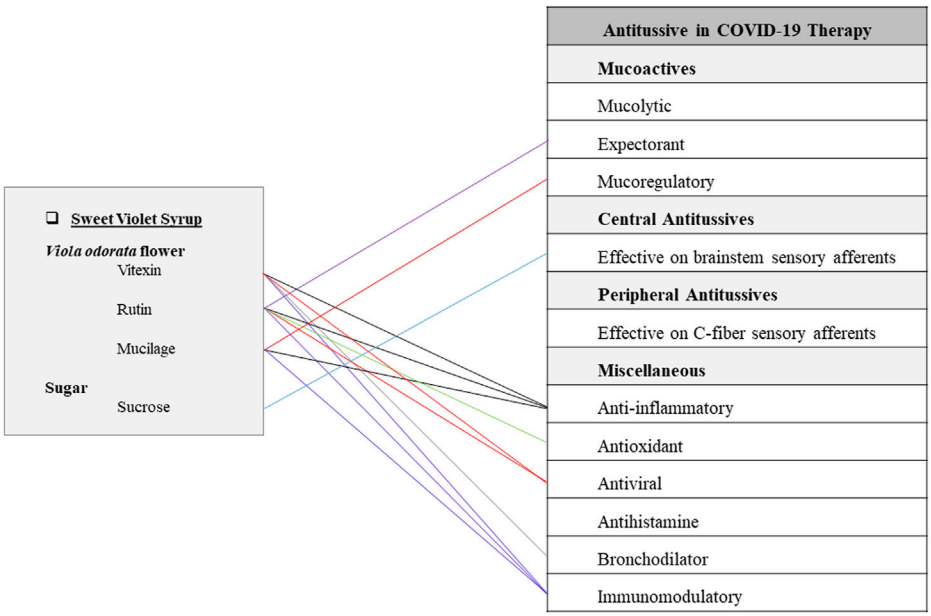

FIGURE 5 | Potentials of sweet violet syrup as an antitussive Persian functional food in COVID-19 therapy.

shown *in vivo* antiasthmatic activity by decreasing eosinophils and neutrophils in lung (Ganeshpurkar and Saluja, 2017). Evidence from current studies has shown that sweet violet successfully has treated the children's cough (Qasemzadeh et al., 2015). Also, sweet violet has shown anti-inflammatory, anti-asthmatic, analgesic, anti-microbial, antipyretic, and lung tissue protecting characteristics in current studies (Yazdi et al., 2020; Arsley et al., 2018; Muhammad et al., 2012). Demulcent effect of mucilage compounds in this herb could be beneficial for healing oral and pharyngeal irritations or other related complications such as throat pain, throat scratching, dry cough, and difficulty in swallowing (**Figure 5**) (Kheterpal et al., 1989; Brinckmann et al., 2003).

DISCUSSION AND CONCLUSION

TPM that is the heritage of prominent traditional Persian physicians has been long and widely used in the prevention and treatment of various difficult miscellaneous diseases by virtue of its abundant sources, diverse structures, and novel activities. Also, local traditional herbs are the source of clues or inspirations for the scientists in the field of drug discovery. TPM has classified practical medicine into hygiene and therapeutic medicine branches. It has had focused specially on FFs benefits to human health. The significance of FFs in TPM view is so much that the great Iranian scientist, Rhazes, said: "When you can use foods for treatment of diseases, avoid medicaments." On the other hand, the memory background and nutrient nature of FFs might evoke better compliance in patients and better therapeutic outcomes (Amiri Ardekani et al., 2020). Therefore, TPM FFs would be beneficial natural supplements and potential candidates for COVID-19 therapy. Cough, as a pathological reflex, is a common symptom of SARS-CoV-2 infection. In the present study, a review has been done on possible protective and therapeutic pharmacological mechanisms of antitussive TPM FFs against COVID-19 with the notice that elimination of cough is an imperative issue to decrease transmission of the SARS-CoV-2 pathogen. TPM FFs are made of safe edible natural compounds which are included in the daily diet of people all around the world. In this regard, FFs would be favorable medical agents to combat the current global pandemic. Moreover, FFs could be consumed as protective agents by healthy people and ones who are prone to get COVID-19. An advantage of providing TPM FF formulations is that their preparation method and other required information about their indication and dose of administration are cited in TPM manuscripts based on traditional experiments. In this study, 12 TPM FF formulations were introduced which include 5 Laooqs (garlic, linseed, pomegranate, purging cassia, seeds Laooqs), 4 Morabbas (almond, daffodil, pumpkin, and wild carrot Morabbas), 1 Saviq (pumpkin Saviq), 1 soup (rooster soup), and 1 syrup (sweet violet syrup) formulation. They are combinations of 2–7 ingredients. The various FF types affect the bioavailability of their phytonutrients through changing the microstructural elements such as cell walls, starch granules, and proteins as well as the physical state of their raw materials (Parada and Aguilera, 2007; Marze, 2017). For instance, syrups and Morabbas usually undergo a cooking process in which the bioaccessiblility of the nutrients is increased because of the structural changes responsible for releasing the bound compounds in the food matrices (Thakur et al., 2020). Some phytochemicals such as soluble antioxidants may be destroyed in heating treatment but not those that are bound in the matrices

(Parada and Aguilera, 2007). In this context, it is suggested that the complex mixtures of phytochemicals in FFs are causing strong health benefits because of their synergistic and/or additive effects (Nayak et al., 2015).

This article provides a phytochemical approach for using traditional Persian antitussive FFs to combat COVID-19. The most prevalent secondary metabolites among 22 natural ingredients of mentioned TPM FFs are quercetin and kaempferol which are present in 5 plant sources, while apigenin, isorhamnetin, luteolin, and rutin are prevalent in 2 plant sources. It is worth nothing that both kaempferol and quercetin have shown potential direct inhibitory effects on 3CLpro and PLpro, two enzymes that are required for the replication of SARS-CoV-2 virus according to recent studies (Zhang et al., 2020). Some of the chemical compounds contained in the mentioned FFs are found to be multifunction (**Figures 1–5**). For instance, rutin has anti-inflammatory, antioxidant, antiviral, immunomodulatory, and expectorant characteristics. Also, quercetin has anti-inflammatory, antioxidant, antihistamine, antiviral, and immunomodulatory properties (**Figure 1**). In addition, pectins are anti-inflammatory, antioxidant, immunomodulatory, expectorant, and peripheral antitussive substances (**Figure 2**). Furthermore, HA has anti-inflammatory, antioxidant, immunomodulatory, mucolytic, and mucoregulatory properties (**Figure 4**). Therefore, the main ingredients of the mentioned TPM FFs are predicted to have potential pharmacological benefits against COVID-19. Some of the mentioned components are prevalent in several plants. For example, flavonoids such as apigenin, kaempferol, luteolin, and quercetin are found in many plant species. Some other molecules are more infrequently found in the nature: for instance, vitexin in sweet violet flower which has decreasing effects in lung edema and alveolar protein content (Lu et al., 2018), or lycorine in daffodil which is a broad-spectrum antiviral substance against human coronaviruses infection (Choudhry et al., 2020; Khalifa et al., 2020). Generally, natural constituents of the mentioned formulations have main pharmacological mechanisms including mucoactive functions by expectorant, mucolytic, and mucoregulatory activities as well as central and peripheral antitussives effects, anti-inflammatory, antioxidant, antiviral, antihistamine, bronchodilator, and immunomodulatory effects.

Laooq, made of powdered natural plants in a viscous syrup or honey, is a popular TPM formulation with local demulcent and antitussive effects. High viscosity of Laooq during licking leads to its longer transit time through esophagus (Iwu et al., 2009; Zarshenas et al., 2013). Sugars used in Laooqs, Morabbas, and syrups have sweetening and texturizing effects (Bayarri et al., 2004). TPM has described special mechanisms of action for each compound formulation based on humoral doctrine. For instance, sugar is considered to have a hot and dry nature with muco-protective and emollient properties on the respiratory tract and lungs according to TPM. Also, it could help clear away pulmonary toxins and infections (Gharashi, 1288). This traditional point of view is in consent with modern investigations. Syrup-based formulations and oral solution of sucrose have shown demulcent effects on the irritated mucosa of pharynx and cough suppressive effects in evidence-based studies (Boyd, 1946; Wise et al., 2014). On the other hand, chrysin present in honey has antioxidant, anti-inflammatory, antiviral, immunomodulatory, and respiratory protective effects through regulating total inflammatory cells, eosinophils, macrophages, lymphocytes, neutrophils, p-Akt, IgE, serum total IL-4, IL-13, IFN-γ level, α-Smooth muscle protein expression, TNF-α, NAD-dependent deacetylase (SIRT1)/Nrf2, IL-1β levels, HO-1, MDA, GSH, VCAM-1, ICAM-1, and ERK1/2, β-glucuronidasem and myeloperoxidase levels, and NF-κBp65 pathways (Talebi et al., 2020). Recently, chrysin is identified as a COVID-19 main protease inhibitor according to *in silico* studies (Lima et al., 2020). Moreover, flavon, flavonols, and phenolic esters content of honey have shown *in silico* inhibitory effects on M pro of COVID-19 (Matteo et al., 2020). Honey is considered a hot and dry agent with potent antitussive and emollient characteristics responsible for clearing away toxins in the respiratory tract according to TPM (Gharashi, 1288). Another traditional mechanism of action in Laooqs is seen in *P. dulcis* which is a hot and wet substance suppressing cough and eliminating dampness in the lungs. Additionally, fatty acids in oily seeds such as *L. usitatissimum*, *C. sativus*, *C. melo*, *C. pepo*, and *P. dulcis* are demulcent agents that form a soothing film over mucus membranes. Through mucoprotection, the existing inflammation in the respiratory system decreases. Cold-temperament materia medica can balance the heat resulting from fever and inflammation. Seeds of *C. sativus* and *C. melo*, and flower of *V. odorata*, have an important role in controlling high body temperature. Based on TPM, dry cough and fever are related ailments to bile with hot and dry quality. Therefore, fruits and herbs that remove extra bile from the body can improve the condition. Fruits of *P. granatum*, and *C. fistula* are two ingredients effective on bilious disorders (Badr and Sardari, 2019). According to our results, garlic Laooq, traditionally recommended as a phlegm remover from lungs in TPM, has remarkable anti-inflammatory, antiviral, and immunomodulatory properties. Linseed Laooq, as a TPM FF for dry cough, has notable mucoregulatory and immunomodulatory effects. Pomegranate Laooq, traditionally recommended for cough associated with hot dis-temperament (infection), has notable anti-inflammatory and immunomodulatory properties. Purging cassia Laooq, as traditional FF for pulmonary infection and cough, has remarkable expectorant and anti-inflammatory effects. Furthermore, seeds Laooq, traditionally known as a dry cough remedy, has significant anti-inflammatory, immunomodulatory, and expectorant properties (**Figure 1**).

Morabba, another popular food in Iran, is made of the treated chopped fruits, flowers, or other natural ingredients in the base of honey, Doushab (grape juice), or sugar. Doushab is considered to possess hot and wet temperament with lower hotness and higher wetness properties. The nature of Doushab is responsible for its muco-protective and emollient features (Ghaeni Heravi, 1766). It exhibits anti-inflammatory, anti-microbial, and antioxidant properties in current investigations (Arora et al., 2016). Furthermore, procyanidins in Doushab is a potent antiviral agent that revealed possible therapeutic effects against COVID-

19 in molecular docking studies (Dai et al., 2012; Maroli et al., 2020). Additionally, resveratrol in Doushab could bind to the ACE2 target site of coronavirus-2 according to recent *in silico* studies (Matteo et al., 2020). Almond Morabba, traditionally indicated as dry cough remedy, has remarkable central antitussive effects as well as anti-inflammatory, immunomodulatory, antiviral, and antihistamine effects. Daffodil Morabba, recommended for cough and dyspnea in TPM, has notable antiviral effects. Pumkin Morraba, as a lung protective FF, has notable anti-inflammatory and immunomodulatory as well as expectorant and peripheral antitussive activities. Wild carrot Morabba, traditionally known as an antitussive FF, has noticeable anti-inflammatory, antiviral, immunomodulatory, as well as expectorant and peripheral antitussive effects (**Figure 2**).

Saviq, as a popular snack in Iran, possesses various beneficial effects related to its ingredient materials (Shafiee et al., 2019). Pumpkin Saviq is considered a potent antitussive FF in TPM because of its cold and wet nature to provide anti-inflammatory and demulcent properties (Gharashi, 1288). It has been traditionally indicated for hot coughs. According to evidence-based studies, pumpkin Saviq can have anti-inflammatory, antiviral, and immunomodulatory as well as expectorant and peripheral antitussive activities (**Figure 3**).

Soup, as a well-known nutritious FF in TPM, is prepared by a cooking process that the duration of heating can affect the bioavailability of its nutrients. A recent study demonstrated that high-intensity cooking promotes heat degradation of meet proteins in chicken soup. As the result, water-soluble degradation substances can be released in the soup liquid, triggering an increase in its protein content (Qi et al., 2018). Eating a bowl of hot soup is considered to be very useful for cough and respiratory discomforts due to its mucolytic effects by breathing its warm vapors (Kirkpatrick, 1996). According to the TPM literature, rooster soup is responsible for eliminating dampness and infections in the lung and clearing away the residues. Therefore, it has been recommended for phlegmatic cough, pulmonary infection, and dyspnea. Besides, our study revealed that rooster soup can have remarkable anti-inflammatory, antiviral, antihistamine, and immunomodulatory activities as well as mucoactive and bronchodilator properties according to recent investigations (**Figure 4**). On the other hand, rooster soup contains HA from which pharmaceutical supplements could be extracted. In the past, HA supplements were extracted directly from rooster comb presented in the TPM rooster soup (Swann, 1968). It is noteworthy that the alveolar HA level is elevated in lung injuries, as well as in COVID-19 infection (Esposito et al., 2017; Garvin et al., 2020). Concurrently, aerosolized HA has shown preventive effects on lung diseases accompanied with elastic fiber injuries (Noble et al., 2011). Taken together, according to the traditional indications and recent studies, we hope that the TPM rooster soup containing HA may bring new insights to treat COVID-19 and its associated pulmonary edema.

Syrup is another popular functional food mentioned in this article. Sweet violet flower that is a cold and wet substance is supposed to induce a moderate coldness along with its wetting properties. This special nature has made *V. odorata* a potent herbal medicine for hot pulmonary disorders (such as infections) and dry respiratory discomforts. Sweet violet syrup, traditionally known as a remedy for dry cough, respiratory infection, and fever, has notable anti-inflammatory, immunomodulatory, and mucoactive properties (**Figure 5**).

In conclusion, FFs can have beneficial effects against the present viral pandemic. Eating FFs containing flowers, fruits, vegetables, and other edible natural substances, instead of consuming chemical drug dosage forms, might have positive psychological effects on COVID-19 patients. Indeed, organoleptic characteristics of FFs including their appearance, aroma, and taste do not induce the unpleasant feelings of using chemical drug dosage forms. Moreover, FFs could have pharmacological effects along with their high nutritional virtues. TPM FFs can reinforce the body power and enhance the immunity system. Also, FFs are cost-effective, easily accessible, and almost safe formulations for both treatment and prevention of the disease. Acquaintance of people and healthcare providers with Persian medicine FFs can be helpful in this global epidemic and may provide better treatment and prevention options. In addition, since the new concerns have grown about persistent respiratory complications after recovery from COVID-19 and its subsequent community morbidity, substantial management for future public health is required. This review recommends TPM FFs to manage such persistent respiratory discomforts after recovery from COVID-19. On the whole, we can conclude that FFs have co-therapeutic and protective effects against COVID-19 infection. TPM has recommended specific antitussive FFs that are safe even in high doses. The correlation of pharmacological mechanisms of action and the molecules found in the current study revealed that cough symptom, as a common pathological reflex in COVID-19 patients, can be alleviated by TPM FFs. Though the scientific or academic evidences are weak, the knowledge and application of traditional local medicine/FF is really a treasure for the health of local people, especially for the emergency epidemic situation such as COVID-19. And the intention is worth encouraging to find a solution during difficult times to deal with the epidemic. Further studies are suggested to focus on the antitussive mechanism of action and bioaccessibility of nutritional compounds in traditional Persian FFs.

AUTHOR CONTRIBUTIONS

GM: conceptualization, methodology, writing—original draft, investigation. PB: methodology, writing—review and editing, investigation. MZ: writing—original draft and investigation. AM: conceptualization, methodology, writing—review and editing, supervision, project administration. All authors approved the submitted version.

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SYSTEMATIC REVIEW
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Efficacy and Safety of Traditional Chinese Medicine in Coronavirus Disease 2019 (COVID-19): A Systematic Review and Meta-Analysis

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Introduction: Until now, there is no clinically approved specific medicine to treat COVID-19. Prior systematic reviews (SRs) have shown that traditional Chinese medicine (TCM) reduces the number of patients with severe disease and time to fever clearance, promotes clinical effectiveness, and improves chest images and the negativity rate of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acid test. Few SRs arrived at a definitive conclusion, and more randomized controlled trials (RCTs) were published. We conducted this study to summarize the latest evidence of TCM in COVID-19.

Methods: Eight online databases were searched from December 2019 to July 2020, updated to March 2021. Only RCTs evaluating the clinical efficacy and safety of TCM in the treatment of COVID-19 were included. Primary outcomes were clinical cure and the negativity of the SARS-CoV-2 nucleic acid test. Secondary outcomes included clinical deterioration, ARDS, mechanical ventilation, death, time to fever clearance, duration of hospitalization, and chest imaging improvement. Safety outcomes included adverse events and serious adverse events during treatment. Two reviewers selected the included articles, assessed the risk of bias, and extracted data independently and in duplicate.

Results: A total of 25 RCTs involving 2222 participants were selected in the systematic review, and seven RCTs were included in the meta-analysis. The results showed that TCM plus routine treatment was significantly better than routine treatment alone in clinical cure (risk ratio [RR] = 1.20, 95% confidence interval (CI) [1.04, 1.38], $P = 0.01$) and chest image improvement (RR = 1.22, 95% CI [1.07, 1.39], $P = 0.01$) and could reduce clinical deterioration (RR = 0.39, 95% CI [0.18, 0.86], $P = 0.02$), ARDS (RR = 0.28, 95% CI [0.11, 0.69], $P = 0.01$), mechanical ventilation (RR = 0.30, 95% CI [0.12, 0.77], $P = 0.01$), or death rate (RR = 0.28, 95% CI [0.09, 0.84], $P = 0.02$). No significant difference between TCM and routine treatment in the negativity of SARS-CoV-2 nucleic acid test (RR = 1.08, 95% CI [0.94, 1.23], $P = 0.29$) was observed. Finally, there was no overall significant difference in

the incidence of adverse events between the two groups. The summary of evidence showed moderate confidence of a benefit of 11.8% in clinical cure and 14.0% in chest image improvement and a reduction of 5.9% in clinical deterioration, 25.4% in ARDS, 18.3% in mechanical ventilation, and 4.5% in death with TCM plus routine treatment compared to routine treatment alone in patients with COVID-19. A low confidence of a benefit of 5.4% in the negativity of SARS-CoV-2 nucleic acid test was also observed.

Conclusions: Synethized evidence of 21 outcomes in 8 RCTs showed moderate certainty that TCM treatment plus routine treatment may promote a clinical cure and chest image improvement compared to routine treatment alone while reducing clinical deterioration, development of ARDS, use of mechanical ventilation, and death in patients with COVID-19. TCM treatment plus routine treatment may not promote the negativity of the SARS-CoV-2 nucleic acid test compared to routine treatment alone. TCM treatment was found to be safe for patients with COVID-19.

Keywords: traditional Chinese medicine, COVID-19, randomized controlled trial, systematic review, meta-analysis, SARS-CoV-2

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a new acute respiratory infectious disease, and the global epidemic is still spreading since the outbreak in December 2019, becoming a major global public health event. Through active prevention, control, and treatment, the epidemic situation in China has been basically controlled, with only minor local outbreaks and a few imported cases from abroad in individual areas, whereas the epidemic situation in other countries remains difficult. There are still no effective clinical therapeutic drugs that can cure the disease.

Traditional Chinese medicine (TCM) has been used in the whole process of the novel coronavirus disease treatment in China, and the "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia Trial Version 3" clearly stated that 91.5% (or 74,187) of COVID-19 patients were treated with Chinese herbal medicine (CHM) (National Health Commission of the People's Republic of China, 2020a; The State Council Information Office of the People's Republic of China, 2020). A large number of clinical studies have shown that early intervention with CHM and integrated traditional Chinese and western medicine can reduce clinical symptoms, shorten the course of the disease, prevent severe forms of the disease, improve the cure rate, and reduce mortality (Gao et al., 2020; Ren et al., 2020; Yang Y. et al., 2020).

Although more than 20 systematic reviews (SRs) were conducted to evaluate the clinical efficacy of TCM on the treatment of COVID-19 (Ang et al., 2020; Cai et al., 2020; Fan et al., 2020; Jin L. et al., 2020; Liang et al., 2020; Liu et al., 2020; Luo et al., 2020; Pang et al., 2020; Qi et al., 2020; Sun C.-Y. et al., 2020; Wang S. X. et al., 2020; Wu et al., 2020; Xiong X. et al., 2020; Zeng et al., 2020; Zhang H. Y. et al., 2020; Zhang W. B. et al., 2020; Zhou Z. et al., 2020; Gao et al., 2021; Liu M. et al., 2021; Ouyang et al., 2021; Zhou et al., 2021), most of them did not assess the quality of evidence and did not arrived at a definite conclusion (Ang et al., 2020; Cai et al., 2020; Fan et al., 2020; Jin L. et al., 2020; Liu et al., 2020; Qi et al., 2020; Sun C.-Y. et al., 2020; Wang S. X. et al., 2020; Xiong X. et al., 2020; Zeng et al., 2020; Zhang H. Y. et al., 2020; Zhang W. B. et al., 2020; Gao et al., 2021; Liu M. et al., 2021; Ouyang et al., 2021; Zhou et al., 2021). What is more, in 12 previously published SRs (Ang et al., 2020; Jin L. et al., 2020; Luo et al., 2020; Pang et al., 2020; Qi et al., 2020; Sun C.-Y. et al., 2020; Wang S. X. et al., 2020; Xiong X. et al., 2020; Zeng et al., 2020; Zhang H. Y. et al., 2020; Zhang W. B. et al., 2020; Liu M. et al., 2021), the authors did not evaluate the eligibility and quality of the included trials, retrospective observational studies were mistakenly regarded as randomized controlled trials (RCTs), and these SRs included synthesized data of observational studies with RCTs in the meta-analysis. One prior SR included a trial of suspected cases of COVID-19 (Fan et al., 2020). In addition, RCTs of TCM published recently were not included in previous SRs. For example, a rigorous double-blinded RCT was not included in all the previously published SRs; this study demonstrated that Xuebijing injection might suppress the cytokine storm in severe cases of COVID-19 patients (Luo et al., 2021). The current study was guided by the following questions. Can TCM treatment 1) promote clinical cure, 2) accelerate the clearance of SARS-CoV-2, and/or 3) prevent unfavorable clinical outcomes (e.g., health deterioration, ARDS, use of mechanical ventilation, or death) when integrated with western medicine? 4) How confident are we of the answers obtained? In addition, 5) is TCM treatment safe for COVID-19 patients?

The objective of this study was to perform a SR and meta-analysis of low risk of bias RCTs to evaluate the available evidence on clinical efficacy and safety of TCM in the treatment of COVID-19.

MATERIALS AND METHODS

This SR was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement

and checklist (Moher et al., 2009) (Additional File 1). This study was registered on PROSPERO (No. CRD42020171564). We updated the PROSPERO record on April 21, 2020. This study also followed an unpublished written protocol.

Eligibility Criteria

Type of Studies

This SR included RCTs and excluded observational and animal studies because evidence obtained from RCTs is more convincing (Balshem et al., 2011). The meta-analysis only included outcomes assessed as low risk of bias.

Types of Participants

This SR included participants diagnosed with COVID-19 through etiological or serological tests. Mild, ordinary, severe, and critical cases were included, and clinical classifications followed the Diagnosis and Treatment Protocol of COVID-19 (National Health Commission of the People's Republic of China, 2020b).

Types of Intervention and Control

Randomized studies of Chinese medicine interventions as the sole treatment or combined with other treatments were included in this study. Chinese medicine interventions include Chinese medicine formulas (e.g., Qingfei Paidu decoction, Huashi Baidu formula, and Xuanfei Baidu formula), Chinese patented medicine (e.g., Jinhua Qinggan granule and Lianhua Qingwen capsule), and Chinese medicine injections (e.g., Xuebijing and Xiyanping injections). Non-pharmacological studies were excluded. Placebo, standard medication treatment, and usual care were included as control groups. Usual care recommended by NHS's protocol includes rest in bed, support therapy, ensuring sufficient caloric intake, monitoring water and electrolyte balance, monitoring vital signs, and oxygen saturation; standard medication treatment recommended by NHS's protocol includes antiviral treatment (alpha interferon, lopinavir/ritonavir, ribavirin, chloroquine phosphate, and Arbidol) and antibiotic drug treatment (National Health Commission of the People's Republic of China, 2020b).

Types of Outcomes

Randomized studies reporting outcomes related to clinical efficacy and safety of TCM in COVID-19 treatment were included in this study.

Search Strategy

We searched PubMed, EMBASE, CENTRAL, Web of Science, the Chinese Biomedical Literature Database (CBM), the China National Knowledge Infrastructure (CNKI), the Wanfang database, and the Chinese Scientific Journals Database (VIP database). Initial database searches were performed from December 2019 to July 2020 and were updated in March 2021. The language was restricted to English and Chinese. We also searched the Chinese Clinical Trial Registry (ChiCTR) and ClinicalTrials.gov to identify ongoing and completed trials. RCTs included in previously published SRs and meta-analysis were additional records in our comprehensive search.

The search strategy was a combination of controlled vocabulary (MeSH terms and Emtree terms) and free-text terms. The search strategy for PubMed is shown in Additional File 2. Modifications to the search strategy were used with other databases.

Screening and Selection

Search results were imported to EndNote X8. Two authors reviewed the titles and abstracts in the database search results after duplicates were removed. The full text was then reviewed and assessed for its eligibility. Screening and selection were independently processed in duplicate by the two reviewers. RCTs that met the inclusion criteria were included. The process is summarized using a PRISMA flow diagram.

Data Extraction

The following data were extracted from the included studies: 1) identification information (first author and year of publication); 2) general information (study setting, sample size, and duration); 3) participants (clinical classification of COVID-19, age, and sex); 4) intervention details (type of Chinese medicine intervention, routes of delivery, name of Chinese patented medicine or formula, dose, frequency, and duration); 5) comparison details (name, dose, frequency, and duration of treatment); 6) outcomes details. Authors of the trials were contacted for any missing or incomplete data. The composition of formulation and patented drugs will be reported in botanical scientific names, not the Latin drug names used in pharmacopeia to avoid confusion (Rivera et al., 2014).

Outcome Justification and Prioritization

Because the specific outcomes reported in the included studies were somewhat inconsistent with our outcome of interest, we made some minor amendments to our registered record and written protocol. The selection of outcomes was based on the two Core Outcome Sets of COVID-19 (Jin X. et al., 2020; Qiu R. et al., 2020) and advice of doctors participating in the treatment of COVID-19 in Wuhan.

Primary Outcomes

The primary outcomes of this study were improved clinical cure and the negativity of the SARS-CoV-2 nucleic acid test.

Clinical cure was defined according to the following criteria: recovery of body temperature for more than 3 days, symptom recovery, marked improvement in chest CT images, and two consecutive negative SARS-CoV-2 nucleic acid tests (at least 1 day apart) (National Health Commission of the People's Republic of China, 2020b).

Secondary Outcomes

Secondary outcomes of this study included the following: 1) clinical deterioration, 2) incidence of unfavorable clinical events of acute respiratory distress syndrome (ARDS), mechanical ventilation, and intensive care unit (ICU) admission, 3) death, 4) time to fever clearance, 5) duration of hospitalization, and 6) chest imaging improvement. Clinical deterioration was defined as the progression of clinical

classification (from the status at randomization), which includes ① from a mild case to moderate, severe, or critical case; ② from a moderate case to a severe or critical case;③ from a severe case to a critical case. The definition of clinical classification was defined by NHS's protocol (National Health Commission of the People's Republic of China, 2020b), as follows: ① mild cases: mild clinical symptoms without signs of pneumonia on imaging; ② moderate cases: fever and respiratory symptoms with radiological findings of pneumonia; ③ severe cases: respiratory distress (≧30 breaths/min), oxygen saturation ≤93% at rest, arterial partial pressure of oxygen (PaO2)/fraction of inspired oxygen (FiO2) ≦ 300 mmHg (l mmHg = 0.133 kPa), lesion progression within 24–48 h > 50% on chest imaging; ④ critical cases: respiratory failure requiring mechanical ventilation and shock, with other organ failures that require ICU care.

Safety Outcomes

Safety outcomes included adverse events (AEs) and serious AEs, defined by the International Conference on Harmonization-Good Clinical Practice (ICH-GCP) guidelines (International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use, 2015), that occurred during treatment. The terminologies and severity of AEs according to the Common Terminology Criteria for Adverse Events (CTCAE) (U.S. Department of Health and Human Services, 2017) and any other criterion will be included.

Quality Assessment

The Risk of Bias 2 Tool was used to assess the methodological quality of the included studies (Sterne et al., 2019). We evaluated outcomes of the included studies of the risk of bias of the randomization process, deviation from intended intervention, missing outcome data, outcome measurement, and selection of the reported result. A low risk of bias in all five domains will lead to a low risk of overall bias. The RCTs of low risk of overall bias will be included in the meta-analysis; RCTs of unclear and high risk of overall bias will be included in the descriptive analysis.

Evidence Synthesis for Randomized Controlled Trials

Meta-analysis was carried out when adequate data of primary and secondary outcomes were obtained, the results among the studies were homogeneous, and forest plots were presented. The mean differences (MD) for continuous data and risk ratio (RR) for dichotomous data with 95% confidence intervals (CIs) were evaluated. The random-effects model was used when synthesizing data for the meta-analysis. We quantified inconsistency by applying the I^2 statistic; a value of $I^2 > 50\%$ was considered substantial heterogeneity (Higgins et al., 2019). Subgroup and sensitivity analyses were performed to explore the source of heterogeneity if substantial heterogeneity existed. Stata 16 was used in data synthesis to perform a meta-analysis. Meta-analysis was precluded in some conditions (limited evidence for comparison or different effect measures) (Higgins et al., 2019), and descriptive analysis was used in these conditions.

Publication Bias

Publication bias of the cumulative evidence among individual studies was evaluated using a graphical method of funnel plot and the Egger test (Egger et al., 1997) if at least ten studies were included for the synthesized outcome.

Quality of Evidence

The quality of the cumulative evidence was evaluated using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system (Guyatt et al., 2008). The risk of bias, inconsistency, indirectness, imprecision, and publication bias were evaluated. Quality of evidence was classified as high, moderate, low, or very low (Guyatt et al., 2008). We presented our findings in a Summary of Finding (SoF) table. Risk difference (RD) was used to interpret the effect of TCM treatment (Poole et al., 2015; Zhang et al., 2018).

RESULTS

Included Studies

The process of study selection is shown in **Figure 1**. A total of 25 RCTs (Ai et al., 2020; Chen et al., 2020; Ding et al., 2020; Fu et al., 2020; Hu et al., 2020; Li and Zhang, 2020; Lin et al., 2020; Qiu M. et al., 2020; Sun H. M. et al., 2020; Wang J.-b. et al., 2020; Wen et al., 2020; Ye and CHAMPS Collaborative Group, 2020; Xiong W.-z. et al., 2020; Yu et al., 2020; Zhang C. T. et al., 2020; Zhang Y. L. et al., 2020; Zhao et al., 2020a; Zheng et al., 2020; Zhou W. M. et al., 2020; Chen et al., 2021; Duan et al., 2021; He and Zhang, 2021a; Liu W. et al., 2021; Luo et al., 2021; Wang et al., 2021) with 2,222 participants were selected in our SR and seven trials were included in quantitative synthesis (Fu et al., 2020; Hu et al., 2020; Wang J.-b. et al., 2020; Wen et al., 2020; Yu et al., 2020; Zheng et al., 2020; Luo et al., 2021). Of the included trials, 24 were open-labeled RCTs, and one trial was a double-blinded RCT (Luo et al., 2021). All of the trials were conducted in mainland China, 19 of which were published in Chinese and six in English (Hu et al., 2020; Wang J.-b. et al., 2020; Xiong W.-z. et al., 2020; Ye and CHAMPS Collaborative Group, 2020; Zhao et al., 2020a; Luo et al., 2021). There were four multi-center RCTs (Hu et al., 2020; Li and Zhang, 2020; Sun H. M. et al., 2020; Zheng et al., 2020) and 20 single-center RCTs and one trial that did not mention the location of the trials (Zhang Y. L. et al., 2020). Five RCTs were registered in the Chinese Clinical Trial Registry (Hu et al., 2020; Wen et al., 2020; Xiong W.-z. et al., 2020; Ye and CHAMPS Collaborative Group, 2020; Luo et al., 2021) and one in ClinicalTrials.gov (Wang J.-b. et al., 2020). We searched the ChiCTR and ClinicalTrials.gov but found no additional records.

Details of selected RCTs are shown in **Tables 1**, **2**. The composition of formulation and patented drugs are shown in **Table 3**. The course of treatment was 5–21 days, and the follow-up time was 5–29 days. The intervention groups of all 25 trials received TCM treatment plus routine treatment. The efficacy of the TCM formula was evaluated in 16 trials, six trials evaluated oral Chinese patented drugs (Hu et al., 2020; Sun H. M. et al., 2020; Yu et al., 2020; Zhang Y. L. et al., 2020; Chen et al., 2021; Duan et al., 2021), three trials evaluated Chinese

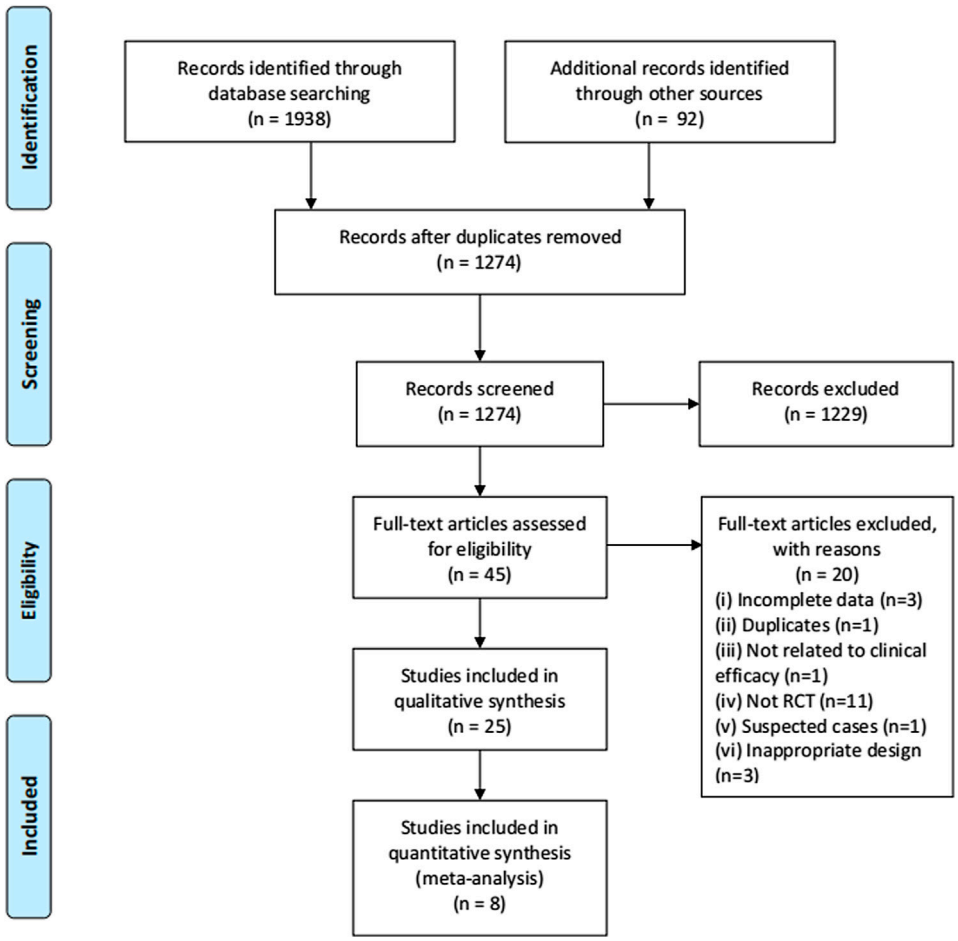

FIGURE 1 | Flowchart of study selection.

medicine injection of Xuebijing (Chen et al., 2020; Wen et al., 2020; Luo et al., 2021), one trial evaluated Chinese medicine extracts (Zhou W. M. et al., 2020), and one trial evaluated the clinical efficacy of the TCM formula and oral Chinese patented drugs (Liu W. et al., 2021). Control groups received routine treatment recommended by the Diagnosis and Treatment Protocol of Coronavirus Disease 2019, which includes antiviral treatment (alpha interferon inhalation, lopinavir/ritonavir, ribavirin, and Arbidol), antibacterial treatment, oxygen therapy, and supportive treatment (National Health Commission of the People's Republic of China, 2020b).

Risk of Bias of Selected Studies

We assessed the risk of bias of 58 outcomes in 25 RCTs: 21 outcomes in eight RCTs were assessed as "low risk" and were included in the meta-analysis, 30 as "some concerns," and 7 as "'high risk." Five trials did not report allocation sequence concealment, and 19 outcomes in these trials were assessed as "some concerns" in the randomization process (Fu et al., 2020; Li and Zhang, 2020; Qiu M. et al., 2020; Wen et al., 2020; Zhao et al., 2020a). One trial used patients' hospitalization number to grouping and was assessed as "high risk" in the randomization process; the trial allocated odd-numbered patients to group A and allocated even-numbered patients to group B (Xiong W.-z. et al., 2020). Five trials (Qiu M. et al., 2020; Wang J.-b. et al., 2020; Xiong W.-z. et al., 2020; Zhang Y. L. et al., 2020; Duan et al., 2021) had deviations from the intended intervention and did not use an appropriate analysis (e.g., intention-to-treat [ITT] analysis); thus, nine outcomes in these trials were assessed as "some concerns" in deviations from intended intervention. One trial had imbalanced deviations between groups, and two outcomes were assessed as "high risk" (Sun H. M. et al., 2020). Four trials (Sun H. M. et al., 2020; Ye and CHAMPS Collaborative Group, 2020; Chen et al., 2021; Duan et al., 2021) did not report all the outcome data for nearly all participants randomized, and six outcomes in these trials were assessed as "high risk" in missing outcome data. Two objective outcomes (death and negativity of SARS-CoV-2 nucleic acid test), in which that assessment of the outcome cannot be influenced by knowledge of intervention received, were assessed as "low risk" in outcome measurement. Four studies (Fu et al., 2020; Hu et al., 2020; Wang J.-b. et al., 2020; Ye and CHAMPS Collaborative Group, 2020) were conducted in a blinded fashion to study allocation for outcome assessors, one trial (Luo et al., 2021) was a double-blinded RCT, and one trial (Yu et al., 2020) assessed the outcome with two independent assessors; eleven

TABLE 1 | Study design, population's details, and outcome of selected studies.

Study	Study design	Sample size		Age		Sex (male/female)		Clinical classification (mild/moderate/severe/critical)		Outcome
		TCM + RT	RT	TCM + RT	RT	TCM + RT	RT	TCM + RT	RT	
Ding et al. (2020)	Single-center	51	49	54.7 ± 21.3	50.8 ± 23.5	39/12	39/10	10/36/5/0	11/34/4/0	⑥⑩
Duan et al. (2021)	Single-center	82	41	51.99 ± 13.88	50.29 ± 13.17	39/43	23/18	82/0/0/0	41/0/0/0	③⑩
Fu et al. (2020)	Single-center	37	36	45.26 ± 7.25	44.68 ± 7.45	19/18	19/17	0/37/0/0	0/36/0/0	①③⑩
Li and Zhang (2020)	Multi-center	6	6	52.00 ± 6.56	50.00 ± 10.00	2/4	3/3	0/0/6/0	0/0/6/0	①⑧⑩
Qiu et al. (2020b)	Single-center	25	25	53.35 ± 18.35	51.32 ± 14.62	13/12	14/11	0/25/0/0	0/25/0/0	③⑥⑨
Sun et al. (2020b)	Multi-center	32	25	45.4 ± 14.1	42.0 ± 11.7	17/15	11/14	4/28/0/0	3/22/0/0	③⑥
Wen et al. (2020)	Single-center	20	20	47.1 ± 5.2	47.7 ± 5.7	12/8	9/11	0/0/20/0	0/0/20/0	②③⑩
Yu et al. (2020)	Single-center	147	148	48.27 ± 9.56	47.25 ± 8.67	82/65	89/59	14/133/0/0	13/135/0/0	③⑥⑦⑩
Zheng et al. (2020)	Multi-center	65	65	17–84	18–85	42/23	44/21	0/0/59/6	0/0/60/5	①⑦
Zhou et al. (2020b)	Single-center	52	52	52.47 ± 10.99	51.11 ± 9.87	32/20	28/24	0/52/0/0	0/52/0/0	①③⑩
Hu et al. (2020)	Multi-center	142	142	50.4 ± 15.2	51.8 ± 14.8	79/63	71/71	—	—	①②③⑥⑩
Wang et al. (2020b)	Single-center	24	23	46.8 ± 14.4	51.4 ± 17.6	14/10	12/11	—	—	②④⑤⑥⑦⑨⑩
Ye and CHAMPS Collaborative Group, (2020)	Single-center	28	14	65 (53.5–69)	59 (47–67)	2/25	4/10	—	—	①③⑤⑥⑦
Zhao et al. (2020a)	Single-center	15	24	—	—	8/7	14/10	0/0/15/0	0/0/24/0	①⑧⑨
Ai et al. (2020)	Single-center	55	43	43.98 ± 12.6	45.95 ± 18.3	24/31	17/26	8/40/7/0	6/33/4/0	⑧⑩
Chen et al. (2021)	Single-center	30	30	50.16 ± 5.11	49.52 ± 5.06	17/13	18/12	—	—	③⑨⑩
Chen et al. (2020)	Single-center	15	15	42.6 ± 3.5	43.1 ± 3.2	8/7	9/6	—	—	①⑩
He and Zhang, (2021a)	Single-center	34	30	—	—	—	—	—	—	②⑥
Lin et al. (2020)	Single-center	41	41	46.02 ± 12.09	43.80 ± 12.34	15/26	23/18	0/41/0/0	0/41/0/0	③⑥⑧⑩
Liu et al. (2021b)	Single-center	44	44	48.51 ± 4.56	48.43 ± 4.52	16/28	15/29	44/0/0/0	44/0/0/0	①⑩
Wang et al. (2021)	Single-center	70	70	48.0 ± 13.2	49.4 ± 13.3	35/35	36/34	0/70/0/0	0/70/0/0	⑥⑧⑩
Zhang et al. (2020c)	Single-center	22	23	53.7 ± 3.5	55.6 ± 4.2	9/13	10/13	0/22/0/0	0/23/0/0	⑥⑩
Zhang et al. (2020d)	—	80	40	53.4 ± 13.7	52.0 ± 14.1	50/30	23/17	0/80/0/0	0/40/0/0	③⑩
Luo et al. (2021)	Single-center	29	28	60.26 ± 15.62	56.35 ± 18.28	—	—	0/0/29/0	0/0/28/0	③④⑤⑦⑨
Xiong et al. (2020b)	Single-center	22	20	57.10 ± 14.00	62.40 ± 12.30	—	—	—	—	⑩

① *Clinical cure,* ② *negativity of SARS-CoV-2 nucleic acid test,* ③ *clinical deterioration,* ④ *ARDS,* ⑤ *mechanical ventilation,* ⑥ *chest image improvement,* ⑦ *death,* ⑧ *duration of hospitalization,* ⑨ *time to fever clearance, and* ⑩ *adverse events.*
TCM, traditional Chinese medicine; RT, routine treatment.

outcomes in these six trials were assessed as "low risk" in the outcome measurement. Eleven outcomes in nine trials (Ding et al., 2020; Lin et al., 2020; Qiu M. et al., 2020; Sun H. M. et al., 2020; Yu et al., 2020; Zhang C. T. et al., 2020; Zhao et al., 2020a; Chen et al., 2021; He and Zhang, 2021a) did not report measurements of outcomes and were assessed as "some concerns." A summary of the risk of bias is shown in **Figure 2**.

Clinical Cure

Clinical cure was reported in nine RCTs; five trials used the TCM formula as the TCM intervention (Fu et al., 2020; Li and Zhang, 2020; Ye and CHAMPS Collaborative Group, 2020; Zhao et al., 2020a; Zheng et al., 2020), one trial used an oral Chinese patented drug (Hu et al., 2020), one trial used a Chinese medicine injection of Xuebijing (Chen et al., 2020), one trial used Chinese medicine extracts (Zhou W. M. et al., 2020), and one trial used the TCM formula and oral Chinese patented drugs (Liu W. et al., 2021). Three of the trials assessed as low risk of bias (Fu et al., 2020; Hu et al., 2020; Ye and CHAMPS Collaborative Group, 2020) and two trials that had a similar time point of outcome measurement were included in the meta-analysis (Fu et al., 2020; Hu et al., 2020). The result showed that TCM plus routine treatment could increase clinical cure better than routine treatment alone at 14–15 days (RR = 1.20, 95% CI [1.04, 1.38], p = 0.01) (**Figure 3**). An I^2 = 0% indicated that there was no heterogeneity between the two RCTs. A forest plot of the clinical cure is shown in **Figure 3**. Another study reported that no patients in either the TCM plus routine treatment group or routine treatment group were clinically cured at 7 days (Ye and CHAMPS Collaborative Group, 2020).

Negativity of SARS-CoV-2 Nucleic Acid Test

The negativity status of the SARS-CoV-2 nucleic acid test was reported in 3 RCTs: one trial used the TCM formula as the TCM intervention (He and Zhang, 2021a), one trial used an oral Chinese patented drug of Lianhua Qingwen Capsules (Hu et al., 2020), and one trial used a Chinese medicine injection of Xuebijing (Wen et al., 2020). Two trials assessed as low risk of bias were included in the meta-analysis (Hu et al., 2020; Wen et al., 2020). The time point of the nucleic acid test was 7 days (Wen et al., 2020) and 14 days (Hu et al., 2020). No significant difference between TCM plus routine treatment and routine treatment alone was observed (RR = 1.08, 95% CI [0.94, 1.23], p = 0.29) (**Figure 4**). An I^2 = 0% indicated that there was no heterogeneity between the two RCTs. A forest plot of negativity of the SARS-CoV-2 nucleic acid test is shown in **Figure 4**. Another trial (Wang J.-b. et al., 2020) assessed as low risk of bias reported no significant difference in the time to the negativity of the nucleic acid test between the two groups (p = 0.263).

Clinical Deterioration

Clinical deterioration was reported in 13 RCTs: four trials used the TCM formula as the TCM intervention (Fu et al., 2020; Lin

TABLE 2 | Intervention details of selected studies.

Study	Intervention		Course of Treatment
	Routine treatment	TCM plus routine treatment	
Ding et al. (2020)	Antivirus treatment (alpha interferon inhalation, 50 μg twice daily; ribavirin 0.5 g intravenously twice daily), antibacterial treatment, and oxygen therapy for severe cases	The same treatments as in the control group and Qingfei Touxie Fuzheng Recipe 150 ml orally twice daily	10 days
Duan et al. (2021)	Antivirus treatment and antibacterial treatment	The same treatments as in the control group and Jinhua Qinggan Granule 10 g thrice daily	5 days
Fu et al. (2020)	Antiviral treatment (Aribidol 0.2 g orally thrice daily), and Ambroxol hydrochloride 30 mg orally thrice daily	The same treatments as in the control group and Toujie Quwen granules one portion twice daily	15 days
Li and Zhang (2020)	Supportive treatments, antiviral treatment (alpha interferon inhalation and ribavirin), and antibacterial treatment	The same treatments as in the control group and Qingfei Paidu Decoction, one unit of decoction divided into two portions, one portion orally twice daily	6 days
Qiu et al. (2020b)	Antiviral treatment: alpha interferon inhalation, 50 μg twice daily; and lopinavir/ritonavir, 400 mg/100 mg twice daily	Antiviral treatment and Maxing Xuanfei Jiedu decoction, 150 ml thrice daily	10 days
Sun et al. (2020b)	Supportive treatments and antiviral treatment (alpha interferon inhalation and lopinavir/ritonavir)	The same treatments as in the control group and Lianhua Qingke granule one unit thrice daily	14 days
Wen et al. (2020)	Routine treatment recommended by the COVID-19 Diagnosis and Treatment Protocol	Routine treatment as in the control group and Xuebijing injection 100 ml intravenously, twice daily	7 days
Yu et al. (2020)	Antiviral treatment (Aribidol 0.2 g orally thrice daily), antibacterial treatment (moxifloxacin 0.4 g orally once daily), and Ambroxol hydrochloride 30 mg orally thrice daily	The same treatments as in the control group and Lianhua Qingwen Capsules, 6 g thrice daily	7 days
Zheng et al. (2020)	Supportive treatments, antiviral treatment (alpha interferon inhalation, lopinavir/ritonavir, and Aribidol), antibacterial treatment (moxifloxacin), and methylprednisolone	The same treatments as in the control group and TCM formula, one unit of formula yielded 300 ml, divided into three portions, one portion orally thrice daily	14 days
Zhou et al. (2020b)	Supportive treatments and antiviral treatment (lopinavir/ ritonavir, 500 mg twice daily)	The same treatments as in the control group and diammonium glycyrrhizinate capsules (three capsules thrice daily)	2 weeks
Hu et al. (2020)	Supportive treatment such as oxygen therapy, antiviral medications and symptomatic therapies	Supportive treatment and Lianhua Qingwen Capsules (four capsules thrice daily)	14 days
Wang et al. (2020b)	Supportive treatments and antiviral treatment (alpha interferon inhalation, 50 μg twice daily; and lopinavir/ritonavir, 400 and 100 mg twice daily, respectively)	The same treatments as in the control group and Keguan-1 19.4 g twice daily	14 days
Ye and CHAMPS Collaborative Group, (2020)	Standard care: supplementary oxygen, intravenous fluids, and routine pharmaceutical medications. Ribavirin/Arbidole was part of the standard care in China	Standard care as in the control group and TCM formula, one unit of formula yielded 400 ml of decoction, divided into two portions, one portion orally twice daily	7 days
Zhao et al. (2020a)	General treatment: bed rest and supportive treatments; ensuring sufficient calories and water intake; maintaining water-electrolyte balance and homeostasis	General treatment as in the control group and TCM prescription orally	2 weeks
Ai et al. (2020)	Antiviral therapy such as abidol, lopinavir, tolonavir or chloroquine, and symptomatic treatment such as oxygen therapy, anti-inflammatory, and expectorant treatment	The same treatments as in the control group and TCM granules of "Pneumonia No.1 Prescription," 100 ml orally twice daily	12 days
Chen et al. (2021)	General treatment such as bed rest, supportive treatments, ensuring sufficient calories and water intake. Antiviral treatment (alpha interferon aerosol inhalation, 5 million IU with 2 ml sterile water, inhalation twice daily; lopinavir/ritonavir, two tablets orally twice daily)	The same treatments as in the control group and Lianhua Qingwen capsule, four capsules, orally thrice daily	10 days
Chen et al. (2020)	Supportive treatment and antiviral treatment such as alpha interferon aerosol inhalation and lopinavir/ritonavir orally	The same treatments as in the control group and 100 ml of Xuebijing injection with 250 ml NS, intravenous drip, twice daily	2 weeks
He and Zhang (2021a)	Symptomatic supportive treatment and antiviral treatment recommended by 6th edition protocol	The same treatments as in the control group and Shengmai San, orally twice daily, modified according to syndrome differentiation	7 days
Lin et al. (2020)	General treatment such as bed rest, supportive treatments, ensuring sufficient calories and water intake. Antiviral treatment (alpha interferon aerosol inhalation, 5 million IU with 2 ml sterile water, inhalation twice daily; lopinavir/ritonavir, two tablets orally twice daily)	The same treatments as in the control group and Xuanfei Qingre recipe, 150 ml orally twice daily	14 days
Liu et al. (2020)	Antiviral treatment (Aribidol 0.2 g orally thrice daily, Oseltamivir 15 mg orally twice daily) and supportive treatment	The same treatments as in the control group and Lianhua Qingwen capsule, 1.4 g, orally thrice daily. "Pneumonia No.2 Prescription," one unit of decoction divided into two portions, one portion twice daily	21 days
Wang et al. (2021)	Supportive treatments, antiviral treatment (Aribidol 0.2 g orally thrice daily), antibacterial treatment (moxifloxacin 0.4 g orally once daily)	The same treatments as in the control group and TCM granules of Qingfei Paidu Decoction, 100 ml orally twice daily	10 days

(Continued on following page)

TABLE 2 | (*Continued*) Intervention details of selected studies.

Study	Intervention		Course of Treatment
	Routine treatment	TCM plus routine treatment	
Zhang et al. (2020c)	Supportive treatment and antiviral treatment recommended by 4th edition protocol	The same treatments as in the control group and TCM granules of Dayuan Decoction, one unit of decoction divided into two portions, one portion twice daily	7 days
Zhang et al. (2020d)	Supportive treatment and treatment (alpha interferon inhalation, 5 million U with 2 ml sterile water, inhalation twice daily; lopinavir/ritonavir, two tablets orally twice daily)	The same treatments as in the control group and Jinyinhua Oral liquid, 60 ml, thrice daily	10 days
Luo et al. (2021)	Supportive treatment, antiviral treatment (alpha interferon inhalation), antibiotic agents, noninvasive and invasive ventilation if necessary. 150 ml NS, intravenous drip, every 12 h	The same treatments as in the control group and 50 ml XBJ injection diluted with 100 ml NS, intravenous drip, every 12 h	14 days
Xiong et al. (2020b)	Routine treatment recommended by the COVID-19 Diagnosis and Treatment Protocol	Routine treatment and Xuanfei Baidu decoction 200 ml, orally twice daily	1 week

et al., 2020; Qiu M. et al., 2020; Ye and CHAMPS Collaborative Group, 2020), six trials used oral Chinese patented drugs (Chen et al., 2020; Hu et al., 2020; Sun H. M. et al., 2020; Yu et al., 2020; Zhang Y. L. et al., 2020; Duan et al., 2021), two trials used a Chinese medicine injection of Xuebijing (Luo et al., 2021; Wen et al., 2020), and one trial used Chinese medicine extracts (Zhou W. M. et al., 2020). Four trials (Fu et al., 2020; Hu et al., 2020; Ye and CHAMPS Collaborative Group, 2020; Luo et al., 2021) were assessed as low risk of bias; three trials that had similar time points of outcome measurement were included in the meta-analysis (Fu et al., 2020; Hu et al., 2020; Luo et al., 2021). The meta-analysis showed that TCM plus routine treatment could prevent clinical deterioration better than routine treatment alone at 14–15 days (RR = 0.39, 95% CI [0.18, 0.86], p = 0.02) (**Figure 5**). An I^2 = 0% indicated that there was no heterogeneity between the three RCTs. A forest plot of clinical deterioration is shown in **Figure 5**. Another trial of low risk of bias (Ye and CHAMPS Collaborative Group, 2020) reported no difference in clinical deterioration rate between two groups of severe cases at 7 days (7.14 vs. 7.14%).

Incidence of Unfavorable Clinical Events

Incidence of ARDS was reported in 2 RCTs (Wang J.-b. et al., 2020; Luo et al., 2021): one trial used the TCM formula as the TCM intervention (Wang J.-b. et al., 2020) and one trial used Chinese medicine injection of Xuebijing (Luo et al., 2021). Both two trials were assessed as low risk of bias and were included in the meta-analysis. The result showed that TCM plus routine treatment could decrease the incidence of ARDS compared to routine treatment alone (RR = 0.28, 95% CI [0.11, 0.69], p = 0.01). An I^2 = 0% indicated that there was no significant heterogeneity between the two RCTs. A forest plot of chest image improvement is shown in **Figure 6**.

Incidence of mechanical ventilation was reported in 3 RCTs (Wang J.-b. et al., 2020; Ye and CHAMPS Collaborative Group, 2020; Luo et al., 2021): two trials used the TCM formula as the TCM intervention (Wang J.-b. et al., 2020) and one trial used Xuebijing injection (Luo et al., 2021). All three trials were assessed as low risk of bias and were included in the meta-analysis. The result showed that TCM plus routine treatment could decrease the incidence of mechanical ventilation compared to routine treatment alone (RR = 0.30, 95% CI [0.12, 0.77], p = 0.01). An I^2 = 0% indicated that there was no significant heterogeneity between the three RCTs. A forest plot of chest image improvement is shown in **Figure 7**.

The incidence of ICU admission was not reported as an outcome in the included trials, and thus meta-analysis was not conducted.

Chest Image Improvement

Chest image improvement was reported in 11 RCTs: eight trials used the TCM formula as the TCM intervention (Ding et al., 2020; Lin et al., 2020; Qiu M. et al., 2020; Wang J.-b. et al., 2020; Ye and CHAMPS Collaborative Group, 2020; Zhang C. T. et al., 2020; He and Zhang, 2021a; Wang et al., 2021) and three trials used oral Chinese patented drugs (Hu et al., 2020; Sun H. M. et al., 2020; Yu et al., 2020). Three trials assessed as low risk of bias were included in the meta-analysis (Hu et al., 2020; Wang J.-b. et al., 2020; Yu et al., 2020). The time point of chest image assessment was 7 days (Yu et al., 2020) and 14 days (Hu et al., 2020; Wang J.-b. et al., 2020). The result showed that TCM plus routine treatment was better than routine treatment alone (RR = 1.22, 95% CI [1.07, 1.39], p = 0.01). An I^2 = 30.87% indicated that there was no significant heterogeneity between the three RCTs. A forest plot of chest image improvement is shown in **Figure 8**. Subgroup analysis showed no significant difference between oral TCM patented drugs and the TCM formula (p = 0.65).

Death

Cases of death were reported in five RCTs: three trials used the TCM formula as the TCM intervention (Wang J.-b. et al., 2020; Ye and CHAMPS Collaborative Group, 2020; Zheng et al., 2020), one trial used an oral Chinese patented drug (Yu et al., 2020), and one trial used Xuebijing injection (Luo et al., 2021). Three trials assessed as low risk of bias were included in the meta-analysis (Yu et al., 2020; Zheng et al., 2020; Luo et al., 2021). The result showed that TCM plus routine treatment could decrease death compared to routine treatment alone (RR = 0.28, 95% CI [0.09, 0.84], p = 0.02) (**Figure 7**). An I^2 = 0 indicated no significant heterogeneity between the three RCTs. A forest plot of death is shown in **Figure 9**.

TABLE 3 | Composition of formulation and patented drugs.

Study	Formulation or patented drugs	Source	Composition	Quality control reported?	Chemical analysis reported?
Ding et al. (2020)	Qingfei Touxie Fuzheng Recipe	—	*Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba praeparata cum melle] 6 g, Gypsum Fibrosum 20 g, *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum] 10 g, Lonicera japonica Thunb. [Caprifoliaceae; *Lonicerae japonicae* flos] 30 g, *Forsythia suspensa* (Thunb.) Vahl [Oleaceae; Forsythiae fructus] 15 g, *Phragmites australis* (Cav.) Trin. ex Steud. [Poaceae; Phragmitis rhizoma] 30 g, *Coix lacryma-jobi var. ma-yuen* (Rom.Caill.) Stapf [Poaceae; Coicis semen] 30 g, body of sick *Bombyx mori* Linnaeus [Bombycidae; Bombyx batryticatus] 10 g, *Cryptotympana pustulata* Fabricius [Cicadidae; Cicadae periostracum] 10 g, *Reynoutria japonica* Houtt. [Polygonaceae; Polygoni cuspidati rhizoma et radix] 15 g, *Curcuma longa* L. [Zingiberaceae; Curcumae longae rhizoma] 10 g, *Paeonia lactiflora* Pall. [Paeoniaceae; Paeoniae radix alba] 10 g, *Pseudostellaria heterophylla* (Miq.) Pax [Caryophyllaceae; Pseudostellariae radix] 20 g, *Glycyrrhiza uralensis* Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma] 15 g	N	N
Duan et al. (2021)	Jinhua Qinggan granule	Beijing Juxiechang Pharmaceutical	*Lonicera japonica* Thunb. [Caprifoliaceae; Lonicerae japonicae flos], Gypsum Fibrosum, *Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba praeparata cum melle], *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum], *Scutellaria baicalensis* Georgi [Lamiaceae; Scutellariae radix], *Forsythia suspensa* (Thunb.) Vahl [Oleaceae; Forsythiae fructus], *Fritillaria thunbergii* Miq. [Liliaceae; Fritillariae thunbergii bulbus], *Anemarrhena asphodeloides* Bunge [Asparagaceae; Anemarrhenae rhizoma], *Arctium lappa* L. [Asteraceae; Arctii fructus], *Artemisia annua* L. [Asteraceae; Artemisiae annuae herba], *Mentha canadensis* L. [Lamiaceae; Menthae haplocalycis herba], *Glycyrrhiza uralensis* Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma]	N	N
Fu et al. (2020)	Toujie Quwen granules	Guangdong E-fong Pharmaceutical	*Forsythia suspensa* (Thunb.) Vahl [Oleaceae; Forsythiae fructus] 30 g, *Cremastra appendiculata* (D.Don) Makino [Orchidaceae; Cremastrae pseudobulbus pleiones pseudobulbus] 20 g, *Lonicera japonica* Thunb. [Caprifoliaceae; Lonicerae japonicae flos] 15 g, *Scutellaria baicalensis* Georgi [Lamiaceae; Scutellariae radix] 10 g, *Isatis tinctoria* L. [Brassicaceae; Isatidis folium] 10 g, *Bupleurum chinense* DC. [Apiaceae; Bupleuri radix] 5 g, *Artemisia annua* L. [Asteraceae; Artemisiae annuae herba] 10 g, *Cryptotympana pustulata* Fabricius [Cicadidae; Cicadae periostracum] 10 g, *Kitagawia praeruptora* (Dunn) Pimenov [Apiaceae; Peucedani radix] 5 g, *Fritillaria cirrhosa* D.Don [Liliaceae; Fritillariae cirrhosae bulbus] 10 g, *Fritillaria thunbergii* Miq. [Liliaceae; Fritillariae thunbergii bulbus] 10 g, *Prunus mume* (Siebold) Siebold and Zucc. [Rosaceae; Mume fructus] 30 g, *Scrophularia ningpoensis* Hemsl. [Scrophulariaceae; Scrophulariae radix] 10 g, *Astragalus mongholicus* Bunge [Fabaceae; Astragali radix] 45 g, *Poria cocos* (Schw.) Wolf [Polyporaceae; Poria] 30 g, *Pseudostellaria heterophylla* (Miq.) Pax [Caryophyllaceae; Pseudostellariae radix] 15 g	N	N
Li and Zhang (2020)	Qingfei Paidu Decoction	-	*Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba] 9 g, *Glycyrrhiza uralensis* Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma praeparata cum melle] 6 g, *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum] 9 g, Gypsum Fibrosum 15–30 g, *Cinnamomum cassia* (L.) J.Presl. [Lauraceae; Cinnamomi ramulus] 9 g, *Alisma plantago-aquatica* L. [Alismataceae; Alismatis rhizoma] 9 g, *Polyporus umbellatus* (Pers.) Fries [Polyporaceae; Polyporus] 9 g, *Atractylodes macrocephala* Koidz. [Asteraceae; Atractylodis macrocephalae rhizoma] 9 g, *Poria cocos* (Schw.) Wolf [Polyporaceae; Poria] 15 g, *Bupleurum chinense* DC. [Apiaceae; Bupleuri radix] 16 g, *Scutellaria baicalensis* Georgi [Lamiaceae; Scutellariae radix] 6 g, *Pinellia ternata* (Thunb.) Makino [Araceae; Pinelliae rhizoma praeparatum cum zingibere et alumine] 9 g, *Zingiber officinale* Roscoe [Zingiberaceae; Zingiberis rhizoma recens] 9 g, *Aster tataricus* L.f. [Asteraceae; Asteris radix et rhizoma] 9 g, *Tussilago farfara* L. [Asteraceae; Farfarae flos] 9 g, *Iris domestica* (L.) Goldblatt and Mabb. [Iridaceae; Belamcandae rhizoma] 9 g, *Asarum sieboldii* Miq. [Aristolochiaceae; Asari radix et rhizoma] 6 g, *Dioscorea oppositifolia* L. [Dioscoreaceae; Dioscoreae rhizoma] 12 g, *Citrus* × *aurantium* L. [Rutaceae; Aurantii fructus immaturus] 6 g, *Citrus reticulata* Blanco [Rutaceae; Citri reticulatae pericarpium] 6 g, *Pogostemon cablin* (Blanco) Benth. [Lamiaceae; Pogostemonis herba] 9 g	N	N
Qiu et al. (2020b)	Maxing Xuanfei Jiedu Decoction	Pharmacy of Chongqing Traditional Chinese Medicine Hospital	*Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba] 9 g, *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum] 12 g, Gypsum Fibrosum 15–30 g, *Fritillaria thunbergii* Miq. [Liliaceae; Fritillariae thunbergii bulbus] 12 g, *Cryptotympana pustulata* Fabricius [Cicadidae; Cicadae periostracum] 10 g, body of sick *Bombyx mori* Linnaeus [Bombycidae; Bombyx batryticatus] 15 g, *Curcuma longa* L. [Zingiberaceae; Curcumae longae rhizoma] 12 g, *Platycodon grandiflorus* (Jacq.) A.DC. [Campanulaceae; Platycodonis radix] 12 g, *Citrus* × *aurantium* L. [Rutaceae; Aurantii fructus] 12 g, *Amomum tsao-ko* Crevost and Lemarié [Zingiberaceae; Tsaoko fructus] 9 g, *Amomum kravanh* Pierre ex Gagnep. [Zingiberaceae; Amomi fructus rotundus] 12 g	N	N
Sun et al. (2020b)	Lianhua Qingke granule	Shijiazhuang Yiling Pharmaceutical	*Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba], Gypsum Fibrosum, *Forsythia suspensa* (Thunb.) Vahl [Oleaceae; Forsythiae fructus], *Scutellaria baicalensis* Georgi [Lamiaceae; Scutellariae radix], *Morus alba* L. [Moraceae; Mori cortex], *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum], *Kitagawia praeruptora* (Dunn) Pimenov [Apiaceae; Peucedani radix], *Pinellia ternata* (Thunb.) Makino [Araceae; Pinelliae rhizoma praeparatum cum alumine], *Citrus reticulata* Blanco [Rutaceae; Citri reticulatae pericarpium], *Fritillaria thunbergii* Miq. [Liliaceae; Fritillariae thunbergii bulbus], *Arctium lappa* L. [Asteraceae; Arctii fructus], *Lonicera confusa* DC. [Caprifoliaceae; Lonicerae flos], *Rheum palmatum* L. [Polygonaceae;	N	N

(Continued on following page)

TABLE 3 | (*Continued*) Composition of formulation and patented drugs.

Study	Formulation or patented drugs	Source	Composition	Quality control reported?	Chemical analysis reported?
			Rhei radix et rhizoma], *Platycodon grandiflorus* (Jacq.) A.DC. [Campanulaceae; Platycodonis radix], *Glycyrrhiza uralensis* Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma]		
Wen et al. (2020)	Xuebijing injection	Tianjin Chase Sun Pharmaceutical	*Carthamus tinctorius* L. [Asteraceae; Carthami flos], *Paeonia lactiflora* Pall. [Paeoniaceae; Paeoniae radix rubra], *Ligusticum striatum* DC. [Apiaceae; Chuanxiong rhizoma], *Salvia miltiorrhiza* Bunge [Lamiaceae; Salviae miltiorrhizae radix et rhizoma], *Angelica sinensis* (Oliv.) Diels [Apiaceae; Angelicae sinensis radix]	N	N
Yu et al. (2020)	Lianhua Qingwen granule	Beijing Yiling Pharmaceutical	*Forsythia suspensa* (Thunb.) Vahl [Oleaceae; Forsythiae fructus], *Lonicera japonica* Thunb. [Caprifoliaceae; Lonicerae japonicae flos], *Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba praeparata cum melle], *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum], Gypsum Fibrosum, *Isatis tinctoria* L. [Brassicaceae; Isatidis radix], *Dryopteris crassirhizoma* Nakai [Polypodiaceae; Dryopteridis crassirhizomatis rhizoma], *Houttuynia cordata* Thunb. [Saururaceae; Houttuyniae herba], *Pogostemon cablin* (Blanco) Benth. [Lamiaceae; Pogostemonis herba], *Rheum palmatum* L. [Polygonaceae; Rhei radix et rhizoma], *Rhodiola crenulata* (Hook.f. and Thomson) H.Ohba [Crassulaceae; Rhodiolae crenulatae radix et rhizoma], 1-menthol, Glycyrrhiza uralensis Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma]	N	N
Zheng et al. (2020)	Xiaochaihu Decoction and Maxing Shigan Decoction	—	Xiaochaihu Decoction and Maxing Shigan Decoction: *Bupleurum chinense* DC. [Apiaceae; Bupleuri radix] 20 g, *Scutellaria baicalensis* Georgi [Lamiaceae; Scutellariae radix] 12 g, *Pinellia ternata* (Thunb.) Makino [Araceae; Pinelliae rhizoma praeparatum] 12 g, *Codonopsis pilosula* (Franch.) Nannf. [Campanulaceae; Codonopsis radix] 15 g, *Zingiber officinale* Roscoe [Zingiberaceae; Zingiberis rhizoma] 10 g, *Ziziphus jujuba* Mill. [Rhamnaceae; Jujubae fructus] 12 g, *Glycyrrhiza uralensis* Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma praeparata cum melle] 10 g, *Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba] 10 g, *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum] 12 g, Gypsum Fibrosum 30 g, *Phragmites australis* (Cav.) Trin. ex Steud. [Poaceae; Phragmitis rhizoma] 30 g, *Aster tataricus* L.f. [Asteraceae; Asteris radix et rhizoma] 15 g, *Tussilago farfara* L. [Asteraceae; Farfarae flos] 15 g, *Cryptotympana pustulata* Fabricius [Cicadidae; Cicadae periostracum] 10 g, *Coix lacryma-jobi var. ma-yuen* (Rom.Caill.) Stapf [Poaceae; Coicis semen] 20 g, *Hordeum vulgare* L. [Poaceae; Hordei fructus germinatus] 20 g	N	N
	Modified Sanren Decoction		— Modified Sanren Decoction: *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum] 10 g, *Amomum kravanh* Pierre ex Gagnep. [Zingiberaceae; Amomi fructus rotundus] 10 g, *Coix lacryma-jobi var. ma-yuen* (Rom.Caill.) Stapf [Poaceae; Coicis semen] 30 g, *Magnolia officinalis* Rehder and E.H.Wilson [Magnoliaceae; Magnoliae officinalis cortex] 10 g, *Pinellia ternata* (Thunb.) Makino [Araceae; Pinelliae rhizoma praeparatum] 10 g, *Tetrapanax papyrifer* (Hook.) K.Koch [Araliaceae; Tetrapanacis medulla] 10 g, *Glycyrrhiza uralensis* Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma] 10 g, Talci pulvis 10 g, *Anemarrhena asphodeloides* Bunge [Asparagaceae; Anemarrhenae rhizoma] 10 g, *Scutellaria baicalensis* Georgi [Lamiaceae; Scutellariae radix] 10 g, *Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba] 8 g, *Poria cocos* (Schw.) Wolf [Polyporaceae; Poria] 10 g, *Bupleurum chinense* DC. [Apiaceae; Bupleuri radix] 15 g, *Lophatherum gracile* Brongn. [Poaceae; Lophatheri herba] 10 g		
Zhou et al. (2020b)	Diammonium Glycyrrhizinate Capsules	Chia Tai TianQing Pharmaceutical	Diammonium glycyrrhizinate 50 mg	N	N
Hu et al. (2020)	Lianhua Qingwen Capsules	Shijiazhuang Yiling Pharmaceutical	*Forsythia suspensa* (Thunb.) Vahl [Oleaceae; Forsythiae fructus], *Lonicera japonica* Thunb. [Caprifoliaceae; Lonicerae japonicae flos], *Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba praeparata cum melle], *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum], Gypsum Fibrosum, *Isatis tinctoria* L. [Brassicaceae; Isatidis radix], *Dryopteris crassirhizoma* Nakai [Polypodiaceae; Dryopteridis crassirhizomatis rhizoma], *Houttuynia cordata* Thunb. [Saururaceae; Houttuyniae herba], *Pogostemon cablin* (Blanco) Benth. [Lamiaceae; Pogostemonis herba], *Rheum palmatum* L. [Polygonaceae; Rhei radix et rhizoma], *Rhodiola crenulata* (Hook.f. and Thomson) H.Ohba [Crassulaceae; Rhodiolae crenulatae radix et rhizoma], 1-menthol, *Glycyrrhiza uralensis* Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma]	Y, prepared according to *The Pharmacopeia of People's Republic of China*	N
Wang et al. (2020b)	Keguan-1	Beijing Tcmages Pharmaceutical	*Lonicera japonica* Thunb. [Caprifoliaceae; Lonicerae japonicae flos] 30 g, *Forsythia suspensa* (Thunb.) Vahl [Oleaceae; Forsythiae fructus] 30 g, *Morus alba* L. [Moraceae; Mori folium] 15 g, *Chrysanthemum* × *morifolium* (Ramat.) Hemsl. [Asteraceae; Chrysanthemi flos] 10 g, *Coix lacryma-jobi var. ma-yuen* (Rom.Caill.) Stapf [Poaceae; Coicis semen] 30 g, *Fritillaria thunbergii* Miq. [Liliaceae; Fritillariae thunbergii bulbus] 15 g, *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum] 9 g	N	Y, HPLC
Ye and CHAMPS Collaborative Group, (2020)	Modified Maxingshigan Formula;	Jiangyin Tianjiang Pharmaceutical	Modified Maxingshigan Formula: *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum] 10 g, Gypsum Fibrosum 30 g, *Trichosanthes kirilowii* Maxim. [Cucurbitaceae; Trichosanthis fructus] 30 g, *Rheum palmatum* L. [Polygonaceae; Rhei radix et rhizoma] 6 g (added at the end of decoction preparation), *Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba] 6 g, *Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba praeparata cum melle] 6 g, *Descurainia sophia* (L.) Webb ex Prantl [Brassicaceae; Descurainiae semen] 10 g, *Prunus persica* (L.) Batsch [Rosaceae; Persicae semen] 10 g, *Amomum tsao-ko* Crevost and Lemarié [Zingiberaceae; Tsaoko fructus] 6 g, *Areca catechu* L. [Arecaceae; Arecae semen] 10 g, *Atractylodes lancea* (Thunb.) DC. [Asteraceae; Atractylodis rhizoma] 10 g;	Y, prepared according to 2015 Chinese Pharmacopoeia	N

(Continued on following page)

TABLE 3 | (*Continued*) Composition of formulation and patented drugs.

Study	Formulation or patented drugs	Source	Composition	Quality control reported?	Chemical analysis reported?
	Modified Shenfutang formula		Modified Shenfutang Formula: *Panax ginseng* C.A.Mey. [Araliaceae; Ginseng Radix et Rhizoma] 15 g, *Aconitum carmichaelii* Debeaux [Ranunculaceae; Aconiti lateralis radix praeparata] 10 g (cook prior to mixture with other herbs); *Cornus officinalis* Siebold and Zucc. [Cornaceae; Corni fructus] 15 g		
Zhao et al. (2020a)	Yidu-toxicity Blocking Lung Decoction	Guangdong E-fong Pharmaceutical	*Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum] 10 g, Gypsum Fibrosum 30 g, *Trichosanthes kirilowii* Maxim. [Cucurbitaceae; Trichosanthis fructus] 30 g, *Rheum palmatum* L. [Polygonaceae; Rhei radix et rhizoma] 6 g, *Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba] 6 g, *Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba praeparata cum melle] 6 g, *Descurainia sophia* (L.) Webb ex Prantl [Brassicaceae; Descurainiae semen] 10 g, *Prunus persica* (L.) Batsch [Rosaceae; Persicae semen] 10 g, *Amomum tsao-ko* Crevost and Lemarié [Zingiberaceae; Tsaoko fructus] 6 g, *Areca catechu* L. [Arecaceae; Arecae semen] 10 g, *Atractylodes lancea* (Thunb.) DC. [Asteraceae; Atractylodis rhizoma] 10 g	N	N
Ai et al. (2020)	Pneumonia No.1 Formula	-	*Artemisia annua* L. [Asteraceae; Artemisiae annuae herba] 10 g, *Astragalus mongholicus* Bunge [Fabaceae; Astragali radix] 45 g, *Cremastra appendiculata* (D.Don) Makino [Orchidaceae; Cremastrae pseudobulbus pleiones pseudobulbus] 20 g, *Forsythia suspensa* (Thunb.) Vahl [Oleaceae; Forsythiae fructus] 30 g, *Scutellaria baicalensis* Georgi [Lamiaceae; Scutellariae radix] 10 g, *Lonicera japonica* Thunb. [Caprifoliaceae; Lonicerae japonicae flos] 15 g, *Isatis tinctoria* L. [Brassicaceae; Isatidis folium] 10 g, *Bupleurum chinense* DC. [Apiaceae; Bupleuri radix] 5 g, *Cryptotympana pustulata* Fabricius [Cicadidae; Cicadae periostracum] 10 g, *Kitagawia praeruptora* (Dunn) Pimenov [Apiaceae; Peucedani radix] 5 g, *Fritillaria cirrhosa* D.Don [Liliaceae; Fritillariae cirrhosae bulbus] 10 g, *Fritillaria thunbergii* Miq. [Liliaceae; Fritillariae thunbergii bulbus] 10 g, *Prunus mume* (Siebold) Siebold and Zucc. [Rosaceae; Mume fructus] 30 g, *Scrophularia ningpoensis* Hemsl. [Scrophulariaceae; Scrophulariae radix] 10 g, *Poria cocos* (Schw.) Wolf [Polyporaceae; Poria] 30 g, *Pseudostellaria heterophylla* (Miq.) Pax [Caryophyllaceae; Pseudostellariae radix] 15 g	N	N
Chen et al. (2021)	Lianhua Qingwen Capsules	Shijiazhuang Yiling Pharmaceutical	*Forsythia suspensa* (Thunb.) Vahl [Oleaceae; Forsythiae fructus], *Lonicera japonica* Thunb. [Caprifoliaceae; Lonicerae japonicae flos], *Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba praeparata cum melle], *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum], Gypsum Fibrosum, *Isatis tinctoria* L. [Brassicaceae; Isatidis radix], *Dryopteris crassirhizoma* Nakai [Polypodiaceae; Dryopteris crassirhizomatis rhizoma], *Houttuynia cordata* Thunb. [Saururaceae; Houttuyniae herba], *Pogostemon cablin* (Blanco) Benth. [Lamiaceae; Pogostemonis herba], *Rheum palmatum* L. [Polygonaceae; Rhei radix et rhizoma], *Rhodiola crenulata* (Hook.f. and Thomson) H.Ohba [Crassulaceae; Rhodiolae crenulatae radix et rhizoma], 1-menthol, *Glycyrrhiza uralensis* Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma]	–	–
Chen et al. (2020)	Xuebijing Injection	Tianjin Chase Sun Pharmaceutical	*Carthamus tinctorius* L. [Asteraceae; Carthami flos], *Paeonia lactiflora* Pall. [Paeoniaceae; Paeoniae radix rubra], *Ligusticum striatum* DC. [Apiaceae; Chuanxiong rhizoma], *Salvia miltiorrhiza* Bunge [Lamiaceae; Salviae miltiorrhizae radix et rhizoma], *Angelica sinensis* (Oliv.) Diels [Apiaceae; Angelicae sinensis radix]	N	N
He and Zhang, (2021a)	Modified Shengmaisan Formula	–	–	N	N
Lin et al. (2020)	Xuanfei Qingre Formula	–	*Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba] 9 g, *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum] 12 g, Gypsum Fibrosum 30 g (cook prior to mixture with other herbs), *Glycyrrhiza uralensis* Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma] 6 g, *Prunus persica* (L.) Batsch [Rosaceae; Persicae semen] 12 g, *Benincasa hispida* (Thunb.) Cogn. [Cucurbitaceae; Benincasae semen] 30 g, *Phragmites australis* (Cav.) Trin. ex Steud. [Poaceae; Phragmitis rhizoma] 30 g, *Coix lacryma-jobi var. ma-yuen* (Rom.Caill.) Stapf [Poaceae; Coicis semen] 30 g, *Platycodon grandiflorus* (Jacq.) A.DC. [Campanulaceae; Platycodonis radix] 9 g, *Pinellia ternata* (Thunb.) Makino [Araceae; Pinelliae rhizoma praeparatum cum zingibere et alumine] 12 g, *Allium chinense* G.Don [Amaryllidaceae; Allii macrostemonis bulbus] 12 g, *Amomum tsao-ko* Crevost and Lemarié [Zingiberaceae; Tsaoko fructus] 6 g, *Pogostemon cablin* (Blanco) Benth. [Lamiaceae; Pogostemonis herba] 10 g	N	N
Liu et al. (2020)	Lianhua Qingwen Capsules Pneumonia No.2 Formula	Lianhua Qingwen Capsules (Shijiazhuang Yiling Pharmaceutical) Pneumonia No.2 Formula (-)	Pneumonia No.2 Formula: *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum], *Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba], *Ginkgo biloba* L. [Ginkgoaceae; Ginkgo semen], *Pheretima aspergillum* (E.Perrier) [Megascolecidae; Pheretima], *Descurainia sophia* (L.) Webb ex Prantl [Brassicaceae; Descurainiae semen], *Schisandra chinensis* (Turcz.) Baill. [Schisandraceae; Schisandrae chinensis fructus], *Pinellia ternata* (Thunb.) Makino [Araceae; Pinelliae rhizoma], *Glycyrrhiza uralensis* Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma], *Perilla frutescens* (L.) Britton [Lamiaceae; Perillae fructus], *Morus alba* L. [Moraceae; Mori cortex], *Tussilago farfara* L. [Asteraceae; Farfarae flos]	N	N
Wang et al. (2021)	Qingfei Paidu Decoction	Hefei CR Sanjiu Medical and Pharmaceutical	*Glycyrrhiza uralensis* Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma praeparata cum melle] 6 g, *Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba] 9 g, Gypsum Fibrosum 15–30 g, *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum] 9 g, *Polyporus umbellatus* (Pers.) Fries [Polyporaceae; Polyporus] 9 g, *Cinnamomum cassia* (L.) J.Presl. [Lauraceae; Cinnamomi ramulus] 9 g, *Atractylodes macrocephala* Koidz. [Asteraceae; Atractylodis macrocephalae rhizoma] 9 g, *Alisma plantago-aquatica* L. [Alismataceae; Alismatis rhizoma] 9 g, *Bupleurum chinense* DC. [Apiaceae; Bupleuri radix] 16 g, *Poria cocos* (Schw.) Wolf [Polyporaceae; Poria] 15 g, *Scutellaria baicalensis* Georgi [Lamiaceae; Scutellariae radix] 6 g, *Iris domestica* (L.) Goldblatt and Mabb. [Iridaceae; Belamcandae rhizoma] 9 g, *Pinellia ternata*	N	N

(Continued on following page)

TABLE 3 | (*Continued*) Composition of formulation and patented drugs.

Study	Formulation or patented drugs	Source	Composition	Quality control reported?	Chemical analysis reported?
			(Thunb.) Makino [Araceae; Pinelliae rhizoma praeparatum cum zingibere et alumine] 9 g, *Aster tataricus* L.f. [Asteraceae; Asteris radix et rhizoma] 9 g, *Zingiber officinale* Roscoe [Zingiberaceae; Zingiberis rhizoma recens] 9 g, *Pogostemon cablin* (Blanco) Benth. [Lamiaceae; Pogostemonis herba] 9 g, *Citrus* × *aurantium* L. [Rutaceae; Aurantii fructus immaturus] 6 g, *Citrus reticulata* Blanco [Rutaceae; Citri reticulatae pericarpium] 6 g, *Asarum sieboldii* Miq. [Aristolochiaceae; Asari radix et rhizoma] 6 g, *Dioscorea oppositifolia* L. [Dioscoreaceae; Dioscoreae rhizoma] 12 g, *Tussilago farfara* L. [Asteraceae; Farfarae flos] 9 g		
Zhang et al. (2020c)	Modified Dayuan Formula	Sichuan Neo-Green Pharmaceutical	*Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba praeparata cum melle] 10 g, *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum] 15 g, Gypsum Fibrosum 20 g, *Trichosanthes kirilowii* Maxim. [Cucurbitaceae; Trichosanthis pericarpium] 20 g, *Rheum palmatum* L. [Polygonaceae; Rhei radix et rhizoma] 6 g, *Descurainia sophia* (L.) Webb ex Prantl [Brassicaceae; Descurainiae semen] 10 g, *Prunus persica* (L.) Batsch [Rosaceae; Persicae semen] 10 g, *Amomum tsao-ko* Crevost and Lemarié [Zingiberaceae; Tsaoko fructus] 6 g, *Areca catechu* L. [Arecaceae; Arecae semen] 10 g, *Atractylodes lancea* (Thunb.) DC. [Asteraceae; Atractylodis rhizoma] 10 g	N	N
Zhang et al. (2020d)	Jinyinhua Oral Liquid	Zhenao Honeysuckle Pharmaceutical	*Lonicera japonica* Thunb. [Caprifoliaceae; Lonicerae japonicae flos]	N	N
Luo et al. (2021)	Xuebijing Injection	Tianjin Chase Sun Pharmaceutical	*Carthamus tinctorius* L. [Asteraceae; Carthami flos], *Paeonia lactiflora* Pall. [Paeoniaceae; Paeoniae radix rubra], *Ligusticum striatum* DC. [Apiaceae; Chuanxiong rhizoma], *Salvia miltiorrhiza* Bunge [Lamiaceae; Salviae miltiorrhizae radix et rhizoma], *Angelica sinensis* (Oliv.) Diels [Apiaceae; Angelicae sinensis radix]	N	N
Xiong et al. (2020b)	Xuanfei Baidu decoction	-	*Ephedra sinica* Stapf [Ephedraceae; Ephedrae herba] 8 g, *Prunus armeniaca* L. [Rosaceae; Armeniacae semen amarum] 15 g, Gypsum Fibrosum 30 g, *Atractylodes lancea* (Thunb.) DC. [Asteraceae; Atractylodis rhizoma] 10 g, *Coix lacryma-jobi var. ma-yuen* (Rom.Caill.) Stapf [Poaceae; Coicis semen] 30 g, *Pogostemon cablin* (Blanco) Benth. [Lamiaceae; Pogostemonis herba] 15 g, *Reynoutria japonica* Houtt. [Polygonaceae; Polygoni cuspidati rhizoma et radix] 20 g, *Descurainia sophia* (L.) Webb ex Prantl [Brassicaceae; Descurainiae semen] 15 g, *Verbena officinalis* L. [Verbenaceae; Verbenae herba] 30 g, *Phragmites australis* (Cav.) Trin. ex Steud. [Poaceae; Phragmitis rhizoma] 30 g, *Artemisia annua* L. [Asteraceae; Artemisiae annuae herba] 25 g, *Citrus reticulata* Blanco [Rutaceae; Citri exocarpium rubrum] 20 g, *Glycyrrhiza uralensis* Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma] 10 g	N	N

Time to Fever Clearance

Time to fever clearance as the outcome was reported as an outcome in five trials: three trials used the TCM formula as the TCM intervention (Qiu M. et al., 2020; Wang J.-b. et al., 2020; Zhao et al., 2020a) and two trials used a Xuebijing injection (Chen et al., 2020; Luo et al., 2021). Only one trial (Luo et al., 2021) was assessed as low risk of bias; therefore, meta-analysis was not conducted for this outcome. The trial of low risk of bias (Luo et al., 2021) reported that the duration of fever in the Xuebijing injection group was shorter than that for the control group (5.54 ± 2.32 days vs. 7.34 ± 2.42 days, $p = 0.018$).

Duration of Hospitalization

Duration of hospitalization was reported as an outcome in five trials, and all five trials used the TCM formula as the TCM intervention (Ai et al., 2020; Li and Zhang, 2020; Lin et al., 2020; Zhao et al., 2020a; Wang et al., 2021). All five trials were assessed as "some concerns"; thus, meta-analysis was not conducted. A significant reduction in the duration of hospitalization in TCM groups compared to routine treatment groups was reported in four trials (Ai et al., 2020; Li and Zhang, 2020; Lin et al., 2020; Wang et al., 2021), whereas another trial (Zhao et al., 2020a) reported no significant difference between the two groups.

Adverse Events

Nineteen studies reported AEs as an outcome: seven trials (Chen et al., 2020; Ding et al., 2020; Hu et al., 2020; Wang J.-b. et al., 2020; Zhang Y. L. et al., 2020; Chen et al., 2021; Luo et al., 2021) reported that there was no obvious difference in the incidence of AEs between the TCM plus routine treatment group and routine group, five trials (Ai et al., 2020; Fu et al., 2020; Lin et al., 2020; Yu et al., 2020; Zhang C. T. et al., 2020) reported no treatment-related AEs in both groups, two trials (Wen et al., 2020; Xiong W.-z. et al., 2020) reported no TCM treatment-related AEs, three trials (Zhou W. M. et al., 2020; Liu W. et al., 2021; Wang et al., 2021) reported that TCM plus routine treatment could decrease the incidence of AEs more than routine treatment, and only one trial reported one serious AE in the routine treatment group and no serious AEs in the TCM plus routine treatment group (Wang J.-b. et al., 2020). One trial reported one allergic reaction in the TCM plus routine treatment group and no AEs in the routine treatment group (Li and Zhang, 2020). Another trial reported 27 AEs of diarrhea in the TCM plus routine treatment group, with eight patients stopping the medication on their own because of intolerance to diarrhea, and no AEs in the routine treatment group (Duan et al., 2021).

We synthesized the overall incidence of AEs reported in the 17 RCTs; two trials did not report AEs in the control groups and were not included in the meta-analysis (Wen et al., 2020; Xiong W.-z. et al., 2020). The result showed no significant differences in the overall incidence of AEs between the two groups ($p = 0.10$). The forest plot of incidence of AEs is shown in **Figure 10**.

Subgroup Analysis

Because the number of studies included in the meta-analysis was small, subgroup analysis was only conducted for the chest image improvement outcome.

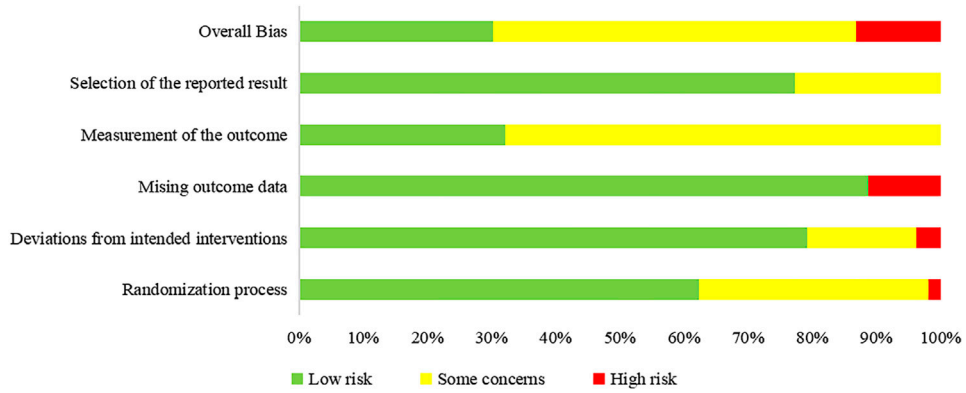

FIGURE 2 | Summary of risk of bias.

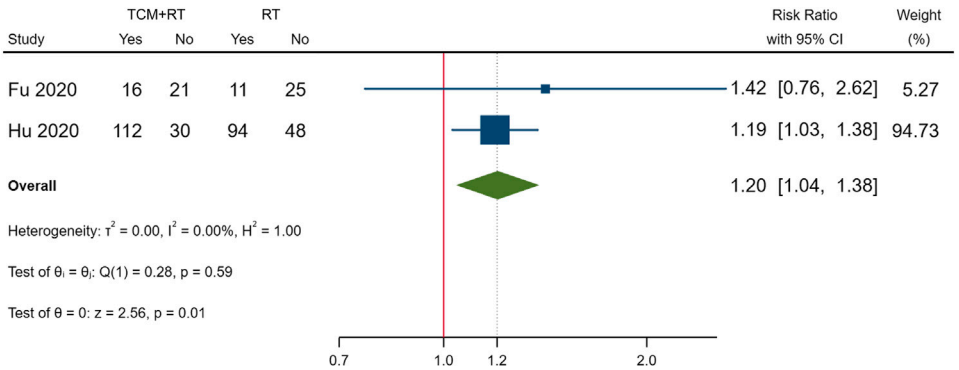

FIGURE 3 | Forest plot of clinical cure.

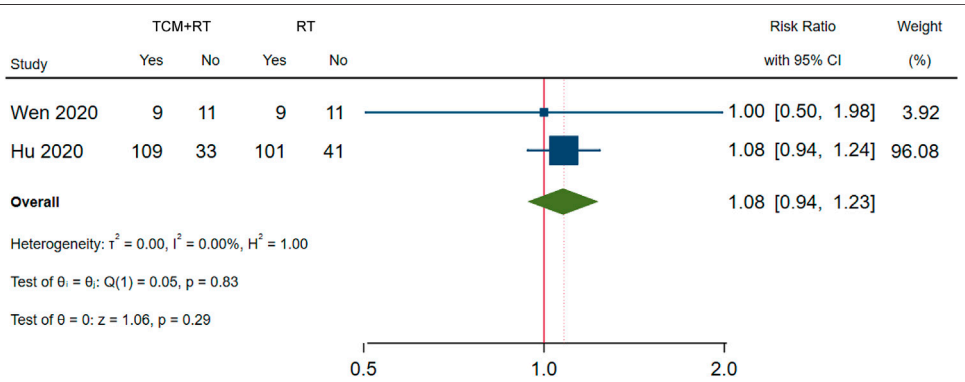

FIGURE 4 | Forest plot of the negativity of the SARS-CoV-2 nucleic acid test.

Publication Bias

Owing to the limited number of studies included in the meta-analysis, a funnel plot and Egger's test were not employed to assess the publication bias. Some publication bias was probably present since unpublished RCTs were not included in this SR.

Quality of Evidence

The GRADE system was used to assess the quality of evidence. Evidence was assessed as moderate for clinical cure, clinical deterioration, ARDS, mechanical ventilation, death, and chest image improvement outcomes. For the negativity of the SARS-

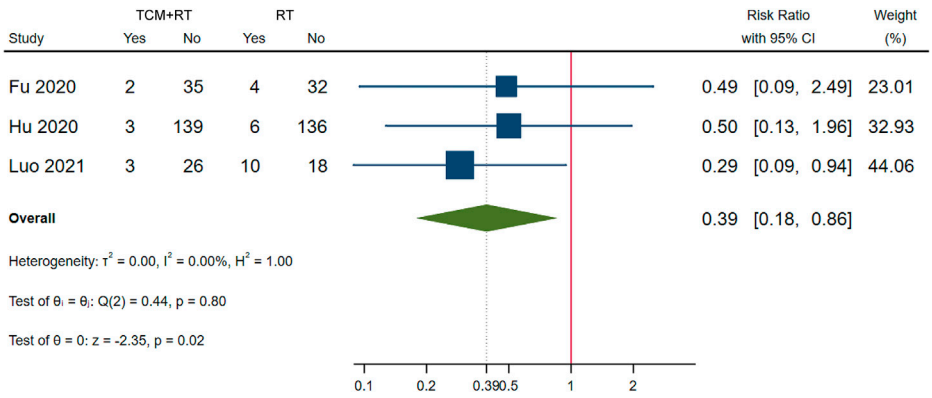

FIGURE 5 | Forest plot of clinical deterioration.

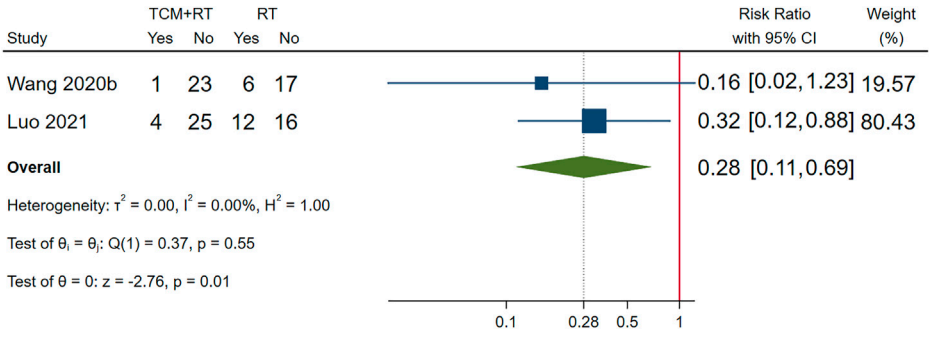

FIGURE 6 | Forest plot of ARDS.

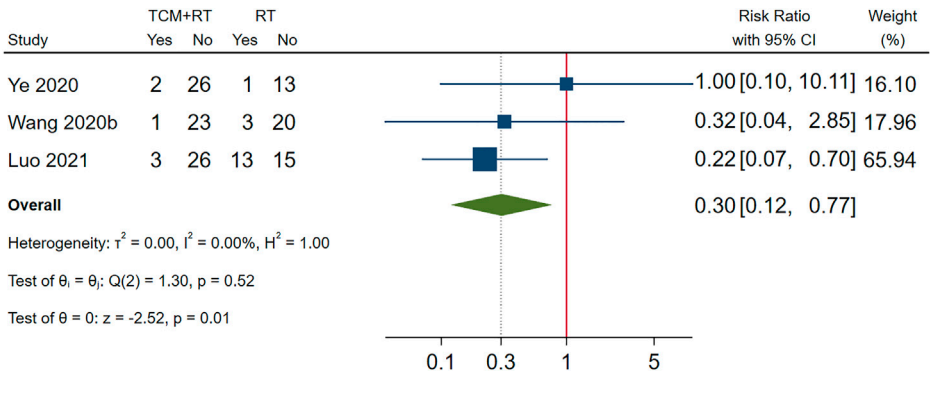

FIGURE 7 | Forest plot of mechanical ventilation.

CoV-2 nucleic acid test, the quality was very low. A summary of findings is shown in **Table 4**.

Summary of Evidence

With the RD calculated in **Table 4** and the quality of evidence, we present our summary of evidence. The synthesized evidence showed moderate confidence of a benefit of 11.8% in clinical cure and 14.0% in chest image improvement and a reduction of 5.9% in clinical deterioration, 25.4% in ARDS, 18.3% in mechanical ventilation, and 4.5% in death with TCM treatment plus routine treatment compared to routine treatment alone in patients with COVID-19 (**Figures 3**, **5–9**; **Table 4**). Low confidence of a benefit of 5.4% in the negativity of the SARS-CoV-2 nucleic acid test was also observed (**Figure 4**;

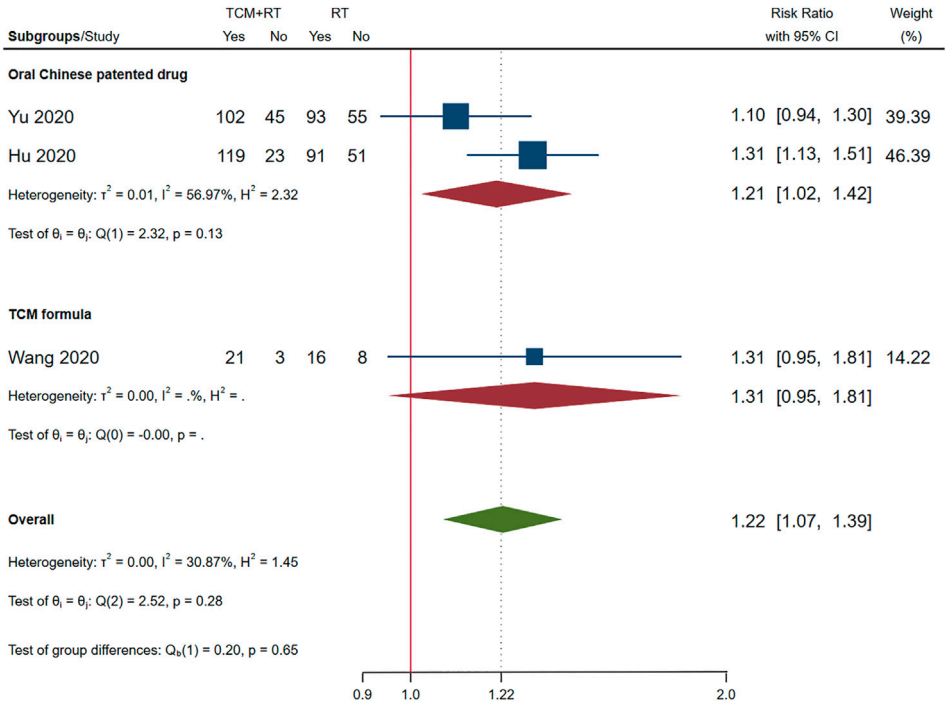

FIGURE 8 | Forest plot of chest image improvements.

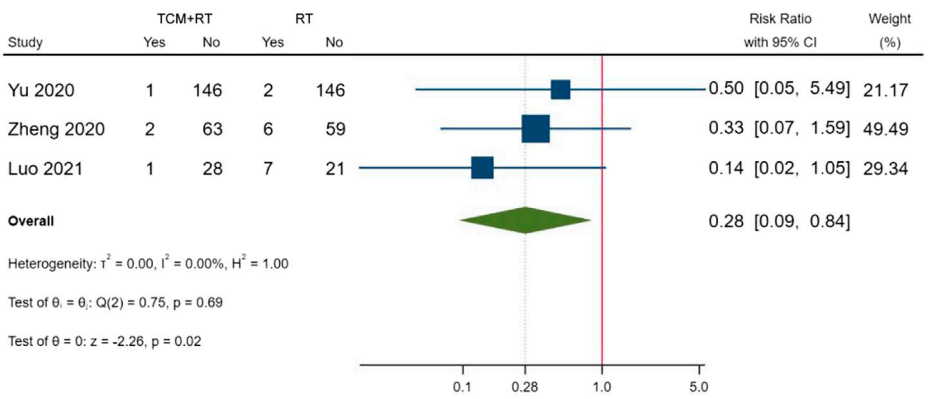

FIGURE 9 | Forest plot of death.

Table 4). There were no significant differences in the overall incidence of AEs between the TCM plus routine treatment group and routine treatment group (**Figure 10**).

DISCUSSION

Our findings showed moderate confidence that TCM treatment of Toujie Quwen granules and Lianhua Qingwen Capsules plus routine treatment could promote a clinical cure, TCM treatment of Keguan-1 and Lianhua Qingwen Capsules plus routine treatment could promote chest image improvement, TCM treatment of Toujie Quwen granules, Lianhua Qingwen Capsules, and Xuebijing injection plus routine treatment could reduce clinical deterioration, TCM treatment of Keguan-1 and Xuebijing injection could reduce the development of ARDS, TCM treatment of Keguan-1, syndrome differentiation decoction, and Xuebijing injection could reduce the use of mechanical ventilation, and TCM treatment of syndrome differentiation decoction, Lianhua Qingwen Capsules, and Xuebijing injection plus routine treatment could reduce death compared to routine treatment alone in patients with COVID-19 (**Figures 3**, **5–9**; **Table 4**). In addition, our findings showed that TCM treatment plus routine treatment may not promote the negativity of SARS-

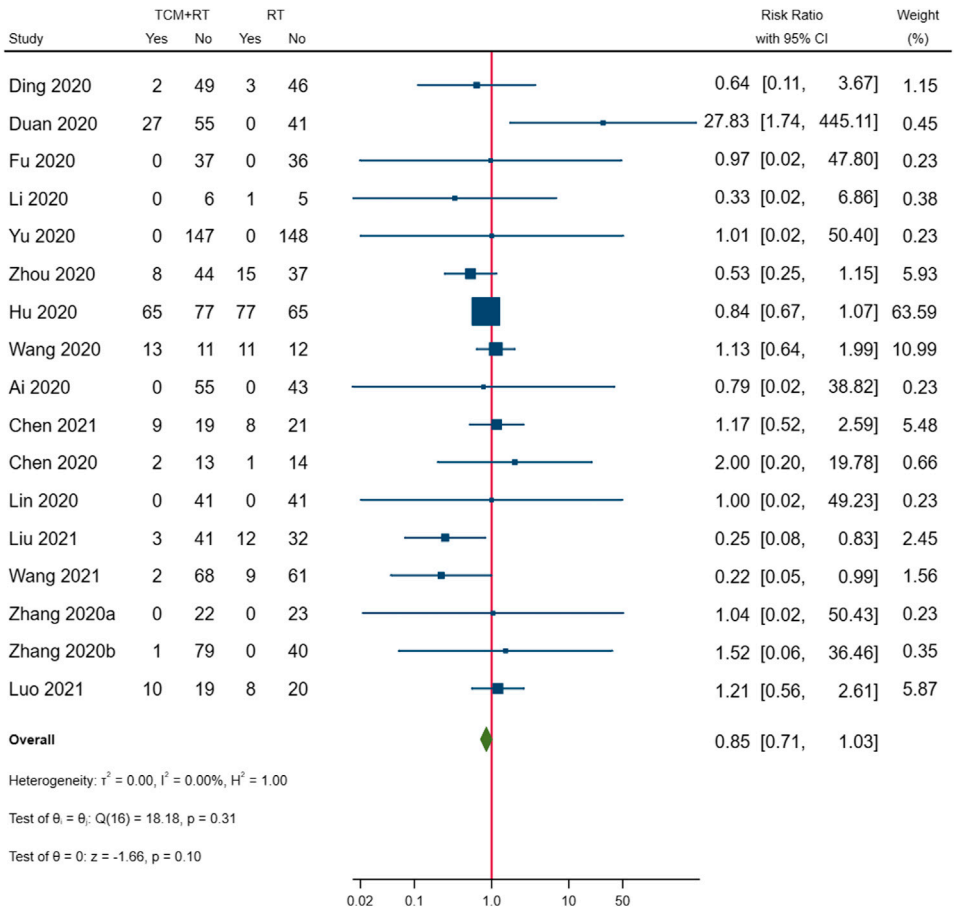

FIGURE 10 | Forest plot of incidence of adverse events.

CoV-2 nucleic acid test compared to routine treatment alone (**Figure 4**; **Table 4**), and no significant differences were observed in the overall incidence of AEs between TCM plus routine treatment group and routine treatment group (**Figure 10**).

About 7.4–41.8% of COVID-19 patients developed ARDS (Huang et al., 2020; Rubin et al., 2020; Wu et al., 2020), and the mortality rate of COVID-19 patients with ARDS was 30.4–52.4% (Huang et al., 2020; Schlesinger et al., 2020; Wu et al., 2020). Pathoanatomy confirmed that COVID-19 is accompanied by a significant lymphocyte-predominant mononuclear inflammatory infiltrate (Tian et al., 2020). The nature of ARDS was an excessive and uncontrolled inflammatory response, forming a cytokine storm (Guan et al., 2020). TCM could promote immune balance and eliminate inflammation through cytokines-related pathways such as TLR and TNF (Peng et al., 2020). Ma Xing Shi Gan component inhibited the inflammatory response by interfering with TLR4/NF-κB/MAPK signaling pathway and reducing the release of inflammatory factors IL-1β, IL-6, and TNF-α (Yang R. et al., 2020). In addition, previous studies had found that a variety of phytochemical components contained in TCM such as flavonoids, alkaloids, terpenoids, polyphenols, and quinones can intervene in the occurrence, progression, and outcome of ALI/ARDS through a variety of mechanisms (He et al., 2021b). A double-blinded randomized controlled trial demonstrated that Xuebijing injection may suppress the cytokine storm and prevent the progression to ARDS in severe COVID-19 patients by regulating the secretion of pro-inflammatory cytokine IL-6, IL-8, and TNF-α (Luo et al., 2021). Another trial showed that Keguan-1 significantly improved the time to fever resolution and reduced the development of ARDS (Wang J.-b. et al., 2020). A retrospective single-center study found that TCM treatment of Shenhuang Granule significantly reduced the occurrence of ARDS (36.3 vs. 63.5%, p = 0.012) and the likelihood of receiving mechanical ventilation (66.7% vs. 72 84.7%, p = 0.028) and shortened the time from ICU admission to discharge (32 [20–73] days vs. 76 [63–79] days, p = 0.0074) (Feng et al., 2021). In addition, a retrospective study also found that in COVID-19, the mortality rate of cases that received TCM treatment was lower than that of cases that did not receive TCM treatment, whether in all cases or severe cases (6.2 vs. 35% for all cases; 22.1 vs. 77.7% for severe cases) (Shu et al., 2020). This synthesized evidence in this SR showed that the intervention of TCM treatment plus routine treatment could

TABLE 4 | Summary of findings.

TCM plus routine treatment compared to standard treatment for COVID-19

Patient or population: COVID-19
Setting: RCT
Intervention: TCM plus routine treatment
Comparison: Routine treatment

Outcome № of participants (studies)	Relative effect (95% CI)	Anticipated absolute effects (95% CI)			Certainty
		Risk without TCM treatment	Risk with TCM treatment[a]	Risk Difference	
Clinical cure	**RR 1.20**	59.0%	**70.8%**	**11.8% more**	⊕⊕⊕O
№ of participants: 357 (2 RCTs)	(1.04 to 1.38)		(61.3 to 81.4)	(2.4 more to 22.4 more)	MODERATE[b]
The negativity of SARS-CoV-2 nucleic acid test	**RR 1.08**	67.9%	**73.3%**	**5.4% more**	⊕OOO
	(0.94 to 1.23)		(63.8 to 83.5)	(4.1 fewer to 15.6 more)	VERY LOW[b,c,d]
№ of participants: 324 (2 RCTs)					
Clinical deterioration	**RR 0.39**	9.7%	**3.8%**	**5.9% fewer**	⊕⊕⊕O
№ of participants: 414 (3 RCTs)	(0.18 to 0.86)		(1.7 to 8.3)	(8 fewer to 1.4 fewer)	MODERATE[b]
ARDS	**RR 0.28**	35.3%	**9.9%**	**25.4% fewer**	⊕⊕⊕O
№ of participants: 104 (2 RCTs)	(0.11 to 0.69)		(3.9 to 24.4)	(31.4 fewer to 10.9 fewer)	MODERATE[b]
Mechanical ventilation	**RR 0.30**	26.2%	**7.8%**	**18.3% fewer**	⊕⊕⊕O
№ of participants: 146 (3 RCTs)	(0.12 to 0.77)		(3.1 to 20.1)	(23 fewer to 6 fewer)	MODERATE[b]
Chest image improvement	**RR 1.22**	63.7%	**77.7%**	**14.0% more**	⊕⊕⊕O
№ of participants: 627 (3 RCTs)	(1.07 to 1.39)		(68.2 to 88.5)	(4.5 more to 24.8 more)	MODERATE[d]
Death	**RR 0.28**	6.2%	**1.7%**	**4.5% fewer**	⊕⊕⊕O
№ of participants: 482 (3 RCTs)	(0.09 to 0.84)		(0.6 to 5.2)	(5.7 fewer to 1 fewer)	MODERATE[b]

[a]*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). CI: Confidence interval; RR: Risk ratio*

Explanations:

[b]*Small sample size, the optimal information size criterion is not met.*

[c]*95% CI overlaps no effect (RR of 1.0).*

[d]*The clinical heterogeneity between the trials exists, so we rate down for this outcome.*

The bold was provided by GRADE to highlight the effect

reduce the incidence of unfavorable clinical events of clinical deterioration, ARDS, mechanical ventilation, and death in patients with COVID-19. This evidence demonstrated that TCM treatment in the early stages may suppress the cytokine storm, prevent the progression to ARDS, decrease the use of mechanical ventilation, and eventually reduce the mortality of COVID-19 patients.

Our study had several strengths. We employed explicit eligibility criteria, conducted a comprehensive search of eight online databases, assessed eligibility and risk of bias critically, addressed important clinical efficacy-related outcomes, and assessed the quality of evidence using the GRADE system. Unlike 12 prior SRs (Ang et al., 2020; Jin L. et al., 2020; Luo et al., 2020; Pang et al., 2020; Qi et al., 2020; Sun C.-Y. et al., 2020; Wang S. X. et al., 2020; Xiong X. et al., 2020; Zeng et al., 2020; Zhang H. Y. et al., 2020; Zhang C. T. et al., 2020; Liu M. et al., 2021) that synthesized the data of both RCTs and observational studies in the same meta-analysis, this review excluded observational studies and updated the RCTs to summarize the latest evidence. We included ten newly published RCTs in this SR (Chen et al., 2020; Lin et al., 2020; Wen et al., 2020; Xiong W.-z. et al., 2020; Zhao et al., 2020a; Zheng et al., 2020; Chen et al., 2021; He and Zhang, 2021a; Luo et al., 2021; Wang et al., 2021) and a double-blinded RCT in the meta-analysis of the outcomes of clinical deterioration and death to synthesize new evidence (Luo et al., 2021). Furthermore, unlike other SRs that included both confirmed and suspected cases, this study excluded trials containing suspected cases. This study assessed the risk of bias of individual outcomes in the included RCTs with Risk of Bias Tool 2 but did not assess the risk of bias of individual studies. Unlike prior SRs that included both the low risk of bias studies and studies with "some concerns" or high risk of bias in a quantitative synthesis, this review only included outcomes with low risk of bias in the meta-analysis. We also assessed the quality of evidence critically using the GRADE system to a degree of confidence in the evidence.

There were several limitations in this SR. First, publication bias was probably present, as unpublished RCTs were not included in this systematic review. Second, only six of the 25 included studies were registered in the ChiCTR or in ClinicalTrials.gov, and selective reporting bias was not assessed rigorously. Third, only one trial was a double-blinded RCT, and only four trials used allocation concealment for outcome assessors. Finally, the evaluated treatments contained several different interventions and different courses of treatment in both TCM and routine treatments, thus leading to clinical heterogeneity among trials.

The time points of nucleic acid tests were baseline after randomization and at 14 days (Hu et al., 2020). In the early stages of the epidemic, nucleic acid tests were insufficient, which led to the negativity of the SARS-CoV-2 nucleic acid test being

rarely reported as a primary outcome. It was reported that honeysuckle decoction inhibits SARS-CoV-2 replication and accelerates the negative conversion of infected patients (Zhou L. K. et al., 2020). However, we failed to conclude whether TCM accelerates negative conversion owing to limited evidence.

The risk of bias of included studies was critically evaluated, with only 30.2% (16/53) of outcomes being assessed as "low risk" in overall bias. The poor quality of clinical trials was a reason for the low quality of evidence in prior SRs (Ang et al., 2020; Xiong X. et al., 2020). Several reasons lead to the poor quality of included trials, but the leading cause was the absence of a blinded method, putting aside the huge number of patients and the shortage of human resources in the early stage of pandemic. The absence of a blinded method to outcome assessors caused poor performance in the measurement of outcome domain in RoB 2. Missing data and deviations from intended intervention may have also lead to poor quality. Finally, inappropriate analysis (e.g., per-protocol analysis) used to estimate the effect of the intervention may be another possible cause of the poor quality of the included trials.

Three of 25 included studies reported quality control of herbs or patented drugs (Hu et al., 2020; Wang J.-b. et al., 2020; Ye and CHAMPS Collaborative Group, 2020); the quality was in accordance with *The Pharmacopeia of People's Republic of China*. Only one trial reported chemical analysis based on the analysis of the relative amounts of the standard compounds in components of Keguan-1 by high-performance liquid chromatography (HPLC) (Wang J.-b. et al., 2020). The standard compounds include chlorogenic acid, galuteolin, amygdalin, forsythoside A, forsythin, rutin, 3,5-dicaffeoylquinic acid, peimine, peiminine, and glyceryl trioleate (Wang J.-b. et al., 2020).

The results of this SR showed a moderate grade of confidence that TCM plus routine treatment promotes a clinical cure of COVID-19 patients compared to routine treatment alone. Our findings indicated a potential benefit of TCM integrated with western medicine in the treatment of COVID-19. The reason for the downgrade of the clinical cure is that the small sample size was below the optimal information size. We will update this study and the evidence when more rigorous RCTs with larger sample sizes are published in the future.

As the epidemic is mostly controlled in mainland China at this time, there are very few ongoing clinical trials of TCM on COVID-19 in the country. Some multi-center RCTs conducted in mainland China are in the process of publication. We searched ClinicalTrials.gov for clinical trials conducted overseas, and there was one ongoing trial in Singapore (Zhao, 2020b). Further RCTs of TCM and COVID-19 are still needed in countries where TCM treatment is legal and may be administered to patients.

CONCLUSION

Synethized evidence of 21 outcomes in eight RCTs showed moderate certainty that TCM plus routine treatment could promote a clinical cure and chest image improvement compared to routine treatment alone while reducing clinical deterioration, development of ARDS, use of mechanical ventilation, and death in patients with COVID-19. TCM treatment plus routine treatment may not promote the negativity of the SARS-CoV-2 nucleic acid test compared to routine treatment alone. TCM treatment was found to be safe for patients with COVID-19.

AUTHOR CONTRIBUTIONS

HW conceived this study. HW and BX registered the protocol and performed the search, screen, inclusion, and quality assessment of the included trials. HW, BX, and YZ performed the evidence synthesis. HW, BX, and YD drafted the first version of this manuscript. BX, YZ, HH, and XL provided critical revisions and edited the manuscript. JL and RG revised the manuscript. All authors read and approved the final manuscript for submission.

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SUPPLEMENTARY MATERIAL

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The reviewer RQ declared a shared affiliation, with no collaboration, with two authors, BX and HH, to the handling editor at the time of the review.

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