The Antiviral Activity of Bacterial, Fungal, and Algal Polysaccharides as Bioactive Ingredients: Potential Uses for Enhancing Immune Systems and Preventing Viruses

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Viral infections may cause serious human diseases. For instance, the recent appearance of the novel virus, SARS-CoV-2, causing COVID-19, has spread globally and is a serious public health concern. The consumption of healthy, proper, functional, and nutrient-rich foods has an important role in enhancing an individual's immune system and preventing viral infections. Several polysaccharides from natural sources such as algae, bacteria, and fungi have been considered as generally recognized as safe (GRAS) by the US Food and Drug Administration. They are safe, low-toxicity, biodegradable, and have biological activities. In this review, the bioactive polysaccharides derived from various microorganisms, including bacteria, fungi, and algae were evaluated. Antiviral mechanisms of these polysaccharides were discussed. Finally, the potential use of microbial and algal polysaccharides as an antiviral and immune boosting strategy was addressed. The microbial polysaccharides exhibited several bioactivities, including antioxidant, anti-inflammatory, antimicrobial, antitumor, and immunomodulatory activities. Some microbes are able to produce sulfated polysaccharides, which are well-known to exert a board spectrum of biological activities, especially antiviral properties. Microbial polysaccharide can inhibit various viruses using different mechanisms. Furthermore, these microbial polysaccharides are also able to modulate immune responses to prevent and/or inhibit virus infections. There are many molecular factors influencing their bioactivities, e.g., functional groups, conformations, compositions, and molecular weight. At this stage of development, microbial polysaccharides will be used as adjuvants, nutrient supplements, and for drug delivery to prevent several virus infections, especially SARS-CoV-2 infection.

Keywords: sulfated polysaccharides, immunomodulation, SARS-CoV-2, COVID-19, antiviral activity

INTRODUCTION

Viruses are the most numerous living organisms on the earth and can be found in terrestrial and aquatic environments. They are infectious agents containing a genetic material within a protein coat—requiring an appropriate host cell where they can replicate (called infection) often resulting in diseases. Viruses can infect all types of organisms: prokaryotes (archaea and bacteria), eukaryotes (animals, algae, plants, and protozoa), and giant viruses namely virophages (1). Like other viruses, human viruses are able to replicate and mutate. A new virus was discovered in December 2019 and characterized as a pandemic by World Health Organization (WHO) on March 11, 2020 (2). This virus was characterized as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes human infection called coronavirus disease 2019 (COVID-19). The disease has spread worldwide and caused over 200 million cases and 4 million deaths from its start until August 2021 (3).

Coronaviruses (CoV) are enveloped positive-sense single-stranded RNA (+ ssRNA) viruses with crown-like spikes on their spherical surface (4). Coronaviruses belong to the order *Nidovirales*, the suborder *Coronavirineae*, and the family *Caronaviridae*. The family was divided into the subfamilies of *Orthocoronavirinae* and *Letovirinae* by the International Committee on Taxonomy of Viruses (ICTV) in 2018 (5). The former sorts into 4 genera, including *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*, with α- and β-coronaviruses infect mammalian species, but γ-, and δ-coronaviruses infect avian species causing respiratory and enteric diseases both as acute and persistent infections (6, 7). The human coronavirus first emerged in patients with the common cold in the 1960s. Other human coronaviruses have emerged within the last two decades: SARS-CoV-1 (2003), Middle East respiratory syndrome (MERS-CoV, 2012), as well as SARS-CoV-2 (2019), which is the seventh human-infecting coronavirus identified (7).

SARS-CoV-2 belongs to the β-coronaviruses, which include SARS-CoV-1 and MERS-CoV. These viruses are highly pathogenic and have a high mortality rate (8). The RNA genome of SARS-CoV-2 is 25–32 kb and similar to SARS-CoV-1 (82% similarity) (4). The structural proteins of SARS-CoV-2 are the envelope (E), membrane (M), nucleocapsid (N), and spike (S) proteins (**Figure 1**). The spike of coronaviruses (S protein) is a glycoprotein associated with the pathogenesis because it is involved in virus adsorption and entry. Thus, the S protein is the virus' important virulence factor (5). The virus uses the S protein for cell binding and membrane fusion. The S protein binds to the angiotensin-converting enzyme 2 (ACE2), a host cell receptor, primed by the transmembrane protease serine protease 2 (TMPRSS2), with the interaction mediating the virus attachment and entry into a host cell (5, 6, 9). The ACE2 receptor is found in a range of human tissues and organs, including the small intestine, lungs, heart, testis, kidneys, blood vessels, muscle, adipose tissues, bladder, epithelia cells of the oral cavity, and the upper esophagus (10–12). Therefore, there are many targets for SARS-CoV-2 infection.

Four antiviral drugs were repurposed for COVID-19, including tocilizumab, remdesivir, favipiravir, and dexamethasone (13). Remdesivir, a drug approved by the US Food and Drug Administration (FDA) for COVID-19, showed a high efficacy as a COVID-19 treatment (14, 15). On the other hand, the WHO Solidarity Trial Consortium announced that remdesivir had little or no effect on hospitalized patients with COVID-19 (16). Various researchers have been evaluating and developing other antiviral agents, as well as vaccines, with high efficacy and low-toxicity (17). As of August 2021, people have been administered different Covid vaccines in many countries, but the pandemic still goes on. Nutrients in foods have an important role in stimulating human immunity and preventing viral infections. Several nutrients including polysaccharides, proteins, and lipids have been reported to have antiviral and immune-enhancing properties. In addition, micronutrients, such as vitamin A, C, D, and E, and few minerals, such as iron, selenium, and zinc have the potential to improve the immune system. Furthermore, natural extracts containing some non-nutrients, such as polyphenols, flavonoids, alkaloids, thiophenes, terpenoids, tannins, and lignins have also shown

Abbreviations: ACE2, Angiotensin-converting enzyme 2; ACV, Acyclovir; APS, Acidic polysaccharide; BoHV-1, Bovine herpesvirus type 1; BoHV-1, Bovine herpes virus type 1; CoV, Coronaviruses; COVID-19, Coronavirus disease 2019; Cox-B3, Coxsackie B virus type 3; CVB3, Coxsackie virus B3; CXCL10, C-X-C motif chemokine 10; ED$_{50}$, 50% effective dose; EMCV, Encephalomyocarditis virus; EPS, Exopolysaccharides; EV71, Enterovirus 71; FCoV, Feline coronavirus; FCV, Feline calicivirus; FDA, US Food and Drug Administration; FHV-1, Feline herpesvirus 1; FIV, Feline influenza; FPV, Feline panleukopenia; HAdV-5, Human adenovirus type 5; HAV, Hepatitis A virus; HCMV, Human cytomegalovirus; HHV-6, Human herpesvirus type 6; HIV, Human immunodeficiency virus; HSV, Herpes simplex virus; IC$_{50}$, 50% inhibitory concentration; ICP, Intracellular polysaccharides; ICTV, International Committee on Taxonomy of Viruses; IFN, Interferon; IHNV, Hematopoietic necrosis virus; IL, Interleukin; KHV, Koi herpesviruses; LAB, Lactic acid bacteria; MAPK, Mitogen-activated protein kinase; MERS-CoV, Middle East respiratory syndrome; MW, Molecular weight; NDV, Newcastle disease virus; NF-κB, Nuclear factor kappa B; NK, Natural killer cells; NO, Nitric oxide; PBMC, Peripheral blood mononuclear cells; PV-1, Poliovirus type 1; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SCFA, Short-chain fatty acids; SuHV-1, Suid herpesvirus type 1; TLR3, Toll-like receptor 3; TMPRSS2, Transmembrane protease serine protease 2; TMV, Tobacco mosaic virus; VSV, Vesicular stomatitis virus; VZV, Varicella zoster virus; WHO, World Health Organization.
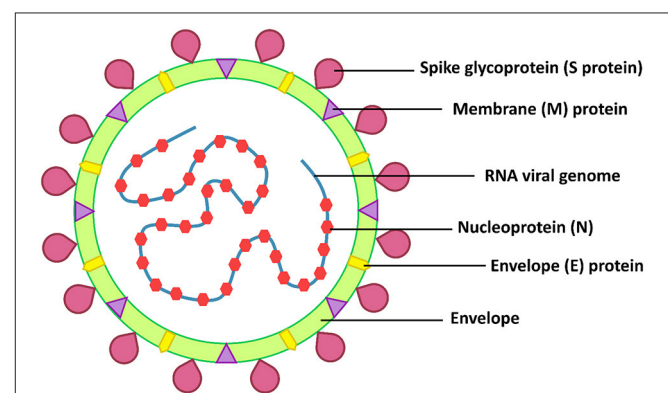

FIGURE 1 | SARS-CoV-2 structure.

biological activities (18). For instance, *Erodium glaucophyllum* extracts containing phenolic compounds such as gallic acid, quercetin 3-O-glucuronide, (+)-gallocatechin, and (+)-catechin exhibited antibacterial and antiviral activities (19). During the COVID-19 pandemic, healthy, nutrient-rich, and functional foods may be more important because their consumption may prevent and modulate the immune system (20). In addition, the development of bioactive ingredients, functional, and nutrient-rich foods that can moderate consumers' overall health will be more interested (21).

Polysaccharides are polymeric carbohydrates, defined as composed of more than 10 monomers that are linked by glycosidic linkages. Polysaccharides are grouped into 2 classes: homopolysaccharides (contain one type of monomer) and heteropolysaccharide (contain more than one type of monomer) (22). Polysaccharides from each sources have different branched chains, composition of monosaccharides, molecular weight (MW), and structural conformations (23). Polysaccharides are the most abundant biological macromolecules in nature and can be obtained from every living organisms including animals (24), plants (25), and microorganisms (26). In living cells, polysaccharides are involved in structure, storage, adhesion, and cell recognition (27). Microorganisms including archaea, bacteria, fungi, and microalgae produced diverse polysaccharides with different structures and functions. Moreover, microorganisms synthesize polysaccharides and secrete them to the outside, these are called exopolysaccharides (EPS). Their functions include cell adhesion, migration of bacteria in groundwater, protection from predators and white blood cells, protection from undesired environments (extreme environments), intercellular signal transduction, and molecular recognition (28, 29). Microbial polysaccharides are composed of not only monosaccharides, but also proteins, lipids, metal ions, extracellular DNA (eDNA), and other organic and inorganic compounds (30). Furthermore, polysaccharides derived from microorganisms, especially marine microorganisms may include sulfate groups, and are called sulfated polysaccharides (31). Sulfated polysaccharides are negatively-charged biopolymers found in the cell wall of marine algae (green, brown, and red algae). Sulfate groups are linked to the sugar structure's backbone to stabilize the structure in extreme environments, especially high salinity (32). Sulfated polysaccharides can be founded not only in marine microalgae and macroalgae, but also in marine animals, and marine bacteria (31). Microbial polysaccharides and sulfated polysaccharides show various biological activities such as immunomodulatory, antioxidant, antimicrobial, anticancer, and anti-inflammatory activities (30). In particular, the antiviral activity of microbial polysaccharides has been studied, showing in several cases an inhibitory effect against various animal, human, and plant pathogenic viruses (33–35). Many studies have reported that natural and modified polysaccharides could inhibit various virus infections (36). Some microbial polysaccharides had antiviral activity against various viruses including *Herpes simplex*, influenza, Newcastle disease (NDV), *Varicella zoster* (VZV), human immunodeficiency viruses (HIV), and human adenoviruses (37–45). According to their biological activities, the bioactive polysaccharides can

be applied as a bioactive ingredients to improve the immune system and reduce the damage caused by viruses (23). Among microbial polysaccharides, EPS produced by lactic acid bacteria (LAB) have been recognized as GRAS, which allows their use in food without the need for regulatory oversight in the USA (46). Although bioactive polysaccharides have been derived from plants, many researchers have investigated the characteristics, compositions, properties, biological activities of novel polysaccharides from various microorganisms (30). There are many advantages to using microbial polysaccharides compared to other polysaccharides. For example, the microbial polysaccharide production can be done using optimized conditions indoors. Microbes grow easily and fast with a high yield of polysaccharides. The recovery process of polysaccharides is simple. Moreover, microbial growth media are simple and non-toxic. If agricultural wastes are used as microbial growth media, the cost of the production is often decreased (32, 47). Microbial polysaccharides are biocompatible and biodegradable, and have no known toxic effects (23). As mentioned above, microbial polysaccharides show antioxidant, anti-inflammatory, antiviral, and immunomodulatory activities; therefore, microbial polysaccharides are attractive as antiviral agents or bioactive ingredients to treat viral infectious diseases, especially COVID-19. This review focuses on microbial polysaccharides with antiviral and immunomodulatory activities and their antiviral mechanisms, and provides the potential approach to use microbial polysaccharides as bioactive ingredients.

MICROBIAL POLYSACCHARIDES WITH ANTIVIRAL ACTIVITY

Antiviral Polysaccharides From Algae

Algae are eukaryotic photosynthetic organisms, often microorganisms. Some algae are unicellular, but some of them are multicellular organisms lacking of specialized tissues. Both micro- and macroalgae are good sources of biomedical compounds, especially polysaccharides (48). Algal polysaccharides are nontoxic, edible, biocompatible, biodegradable, and easily available; therefore, these biopolymers have been applied in many fields such as the food, pharmaceutical, and biomedical industries (49). Algal polysaccharides have several pharmaceutical properties, including anticancer (50), antioxidant (51), antimicrobial (52), anti-inflammatory (53), and immunomodulatory activities (54). Moreover, several algae, especially marine algae, can produced sulfate polysaccharides, which have different beneficial biological activities (50, 55–58). Different algal polysaccharides possess a variety of structures, composition, and conformations, which influence their properties (55). A summary of algal polysaccharides with antiviral potential are shown in **Table 1**.

Most of the algal polysaccharides have the ability to decrease viral infections by blocking the attachment of virus particles to host cell surfaces. In this line, three polysaccharides extracted from *Sargassum trichophyllum* (a brown alga) were characterized as laminaran, alginate and fucoidan, observing that only fucoidan showed an antiviral effect against herpes simplex virus type

TABLE 1 | The microbial polysaccharides with antiviral activity.

Source	Polysaccharide	Virus	Action	References
Algae				
Coccomyxa gloeobotrydiformi	Acidic polysaccharide (CmAPS)	Human influenza A virus: A/H1N1, A/H2N2, A/H3N2 and A/H1N1	Inhibited virus adsorption and virus-induced erythrocyte hemagglutination and hemolysis	(40)
Gracilaria lemaneiformis	Sulfated polysaccharide	Human influenza virus H1-364	Prevented virus adsorption and replication	(59)
Gyrodinium impudicum	Sulfated exopolysaccharide (p-KG03)	Ecephalomyocarditis virus (EMCV)	Inhibited EMCV infection in HeLa cells	(56)
Gyrodinium impudicum	Sulfated exopolysaccharide (p-KG03)	Influenza A virus	Inhibition of influenza virus replication	(60)
Himanthalia elongata	Polysaccharide	HSV-1	N/A	(61)
Hydroclathrus clathratus	Sulfated polysaccharide HC-b1	HSV-1, including acyclovir-resistant strain and clinical strain	Inhibited virus absorption and penetration, and inhibited replication of HSV-1 in host cells	(62)
Hydroclathrus clathratus	Sulfated polysaccharide	HSV-2	N/A	(63)
Laminaria japonica	Polysaccharide	Respiratory syncytial virus (RSV)	Inhibited RSV replication and induced IFN-α secretion	(64)
Laminaria japonica	LJ04 polysaccharide	Enterovirus 71 (EV71)	Inhibited viral proliferation, viral-induced apoptosis, and increased IFN-β expression	(65)
Laminaria japonica	Fucoidan	I-type influenza virus, adenovirus and parainfluenza virus I	N/A	(66)
Navicula directa	Naviculan	HSV-1, HSV-2, and Influenza A virus (H1N1)	Inhibited viral adsorption and penetration	(67)
Padina tetrastromatica	Polysaccharide	Herpes simplex virus type 1 and 2 (HSV-1 and HSV-2)	Inhibited virus adsorption	(37)
Porphyridium cruentum	i Polysaccharide	Varicella zoster virus (VZV)	i Polysaccharide may block different phases of viral replication cycle	(43)
Porphyridium sp.	Cell-wall sulfated polysaccharide	HSV-1, HSV-2, and VZV	Inhibited virus adsorption and/or production of new virions in host cells	(38)
Porphyridium sp., *P. aerugineum, and Rhodella reticulata,*	Polysaccharide	Murine leukemia virus (MuLV) and murine sarcoma virus (MuSV-124)	Inhibition of virus adsorption	(68)
Saccharina japonica,	Sulfated galactofucan (SJ-D-S-H) and glucuronomannan (Gn)	SARS-CoV-2	Binding SARS-Cov-2 spike glycoprotein	(69)
Sargassum fusiforme	Polysaccharide SFP	Avian leukosis virus subgroup J (ALV-J)	Inhibited on virus adsorption phase by binding to virions and showed inhibitory effects both *in vitro* and *in vivo*	(70)
Sargassum patens	Sulfated polysaccharide SP-2a	HSV-1	Inhibition of virus adsorption	(71)
Sargassum trichophyllum	Fucoidan	HSV-2	Inhibition of virus adsorption and/or virus penetration steps	(72)
Ulva lactuca	Sulfated polysaccharide	Japanese encephalitis virus (JEV)	Inhibited virus adsorption	(73)
Bacteria				
Arthrospira platensis	Spirulan-like molecules	Human cytomegalovirus, HSV-1, human herpesvirus type 6 and HIV-1	Inhibited the herpesviruses at an entry phase, but at a stage later than virus entry for HIV	(44)
Arthrospira platensis	Calcium spirulan (Ca-SP)	HSV-1, human cytomegalovirus, measles virus, mumps virus, influenza A virus, and HIV-1	Inhibited the penetration of virus into host cells	(74)
Arthrospira platensis	Exopolysaccharide	Koi herpesvirus (KHV)	Inhibited the viral replication	(75)
Bacillus licheniformis T14	Exopolysaccharide	HSV-2	Inhibited virus replication in human peripheral blood mononuclear cells (PBMC)	(76)
Lactobacillus delbrueckii OLL1073R-1	Exopolysaccharide	Intestinal viruses	Increased the expression of the antiviral factors MxA and RNase L	(77)

(Continued)

TABLE 1 | Continued

Source	Polysaccharide	Virus	Action	References
Lactobacillus plantarum strain N4(Lp)	Exopolysaccharide	Transmissible Gastroenteritis Virus (TGEV) - Coronavirus	Inhibition effect that co-incubation with TGEV "Coronavirus"	(78)
Lactobacillus spp.	Exopolysaccharide 26a	Human adenovirus type 5 (HAdV-5)	Suppressed the formation and release of HAdV-5 virions	(41)
Nostoc flagelliforme	Nostoflan	HSV-1, HSV-2,HCMV, and influenza A virus	Blocked virus adsorption and/or virus penetration steps	(79)
Pseudomonas sp. WAK-1	Extracellular glycosaminoglycan and sulfated polysaccharide	HSV-1, Influenza A virus	N/A	(80)
Fungi				
Auricularia auricula	Sulfated *Auricularia auricula* polysaccharide (AAPt)	Newcastle disease virus	Inhibit the cellular infectivity (in chicken embryo fibroblast, CEF) of NDV in three ways (pre-, post- and simultaneous-adding polysaccharide)	(42)
Fomes fomentarius	Polysaccharide BAS-F	Tobacco mosaic virus (TMV)	N/A	(34)
Inonotous obiquus	*Inonotus obliquus* polysaccharides (IOPs)	Feline calicivirus (FCV) strain F9, feline herpesvirus 1, feline influenza virus H3N2 and H5N6, feline panleukopenia virus and feline infectious peritonitis virus	Antiviral effects on virus particles through blocking viral binding/absorption	(35)
Porodaedalea pini (Brot.) Murrill (syn. *Phellinus pini*)	Polysaccharide EP-AV1 and EP-AV2	HSV-1, coxsackie virus B3 (CVB3)	N/A	(81)
Grifola frondosa	Heteropolysaccharide GFP1	Enterovirus 71	Inhibited EV71 replication and suppressed viral VP1 protein expression and genomic RNA synthesis	(82)
Lentinus edodes	Lentinan	Infectious hematopoietic necrosis virus (IHNV)	Inhibited viral replication	(83)
Cordyceps militaris	Acidic polysaccharide (APS)	Influenza A virus	Reduced virus titer the bronchoalveolar lavage fluid and the lung of mice infected with influenza A virus	(39)

N/A, Not available.

2 (HSV-2) (72). The 50% inhibitory concentration (IC_{50}) of the fucoidan was 18 $\mu g \cdot mL^{-1}$ when it was added during the viral infection, being lower than adding after the viral infection. Therefore, this polysaccharide might inhibit HSV-2 at the virus adsorption and/or penetration step(s) (72). *Sargassum henslowianum* produced antiviral fucoidans against both HSV-1 and HSV-2 (84). For instance, the authors observed how two fractions of the fucoidans (SHAP-1 and SHAP-2) could inhibit HSV-1 with IC_{50} of 0.89 and 0.82 $\mu g \cdot mL^{-1}$, respectively. Both SHAP-1 and SHAP-2 showed higher antiviral activity against HSV-2 with IC_{50} of 0.48 $\mu g \cdot mL^{-1}$. These fucoidans interfered with the virions' attachment to host cells (84). Moreover, low MW fucoidan fractions (LF1 and LF2) from *Laminaria japonica* could inhibit I-type influenza virus, adenovirus and parainfluenza virus I *in vitro*. The IC_{50} for LF1 were 0.3, 0.6, and 0.3 $mg \cdot mL^{-1}$, respectively, whereas The IC_{50} for LF2 were 0.6, 1.2, and 0.6 $mg \cdot mL^{-1}$, respectively (66). Fucoidan from *Cladosiphon okamuranus* also showed higher antiviral activity against NDV with lower cytotoxicity than Ribavirin, an antiviral drug, preventing this polysaccharide the viral infection at early steps by blocking the F protein (85). In addition, *Scytosiphon*

lomentaria, a brown seaweed, also produced fucoidans with antiviral activity, in particular, they had the ability to block HSV-1 and HSV-2 infections (86). Moreover, a fucoidan with high levels of sulfate groups also showed the highest antiviral activity against HSV-1 and HSV-2 (86).

Carrageenans are sulfated linear polysaccharides extracted from some red algae, such as *Chondrus*, *Gigartina*, *Hypnea*, and *Eucheuma* spp. (49). These polysaccharides showed an antiviral activity against several viruses. For instance, González et al. (87) reported a good inhibitory effect of carrageenan against some enveloped viruses, including HSV-1, HSV-2, Semliki Forest, vaccinia, and swine fever viruses, but they did not find any impact on vesicular stomatitis and measles viruses. Moreover, these authors also showed the antiviral activity of carrageenan against encephalomyocarditis virus (EMCV), a naked virus, but they did not observe significant effects on poliovirus or adenovirus. The carrageenan interfered with the viral protein synthesis. On the other hand, λ-carrageenan and moderately cyclized μ/ℓ-carrageenan extracted from a red seaweed (*Gigartina skottsbergii*) inhibited the viral attachment of HSV-1 and HSV-2 (88). Various types of carrageenans also have shown antiviral activities against

hepatitis A virus (HAV). The 50% effective dose (ED_{50}) for ι-carrageenan, λ-carrageenan, and κ-carrageenan against HAV were >400, >222, and >10 $\mu g \cdot mL^{-1}$, respectively [89]. λ-Carrageenan from *G. skottsbergii* showed an inhibitory effect on both bovine herpesvirus type 1 (BoHV-1) and Suid herpesvirus type 1 (SuHV-1). The IC_{50} of this polysaccharide was 0.52 and 10.4 $\mu g \cdot mL^{-1}$, respectively [90].

The red micro algae, *Porphyridium* spp., produce antiviral polysaccharides against many types of viruses, including HSV-1, HSV-2, and VZV. The algae inhibited viral entry and/or blocked virus replication in host cells [38, 43]. In this line, the sulfated polysaccharide SP-2a obtained from a brown alga, *Sargassum patens*, exhibited strong antiviral property against different strains of HSV-1. The EC_{50} of SP-2a against the standard, acyclovir (ACV)-sensitive and -resistant strains of HSV-1 were 5.5, 1.5, and 4.1 $\mu g \cdot mL^{-1}$, respectively. The SP-2a had a weak virucidal activity against the standard and ACV-sensitive strains of HSV-1, but not the ACV-resistant strain. During virus adsorption, the SP-2a showed ≥80% inhibition of adsorption against all strains of HSV-1 [71]. p-KG03 is a sulfated exopolysaccharide with an average MW of 1.87×10^7 Da extracted from a dinoflagellate, *Gyrodinium impudicum* strain KG03. The p-KG03 could inhibit EMCV in HeLa cells with an EC_{50} of 26.9 $\mu g \cdot mL^{-1}$, and influenza A at the virus adsorption step, but was ineffective against influenza B, HSV-1, HSV-2, human immunodeficiency virus type 1 (HIV-1), HIV-2, Coxsackie B virus type 3 (Cox-B3), and vesicular stomatitis virus (VSV). In addition, the p-KG03 also showed antiviral activity against influenza A virus at the virus adsorption step, but did not inhibit all influenza B virus isolates. The EC_{50} for p-KG03 against different strains of influenza A virus (H1N1: PR8 and Tw; H3N2: Se) ranged from 0.19 to 0.48 $\mu g \cdot mL^{-1}$ [56, 60]. Lee et al. [67] reported that *Navicula directa*, a diatom collected from deep-sea water in Toyama Bay (Japan), produced naviculan (a sulfated polysaccharide). The naviculan is a heteropolysaccharide consisting of fucose, xylose, galactose, mannose, rhamnose, and sulfate with an average MW of ~2.2 $\times 10^5$ Da. It is a broad-spectrum antiviral against HSV-1, HSV-2, and influenza A virus with IC_{50} of 14, 7.4, and 170 $\mu g \cdot mL^{-1}$, respectively, at the virus adsorption phase. Moreover, it could also interfere with the cell-cell fusion of HIV gp160- and CD4-expressing HeLa cells. Therefore, it might prevent HIV infections.

Antiviral Polysaccharides From Bacteria

Bacteria (including cyanobacteria or blue-green algae) have the ability to synthesize polysaccharides for various purposes such as storage, cell protection, and adhesion. Polysaccharides accumulated in cells are called intracellular polysaccharides (ICP). While those outside of cell are called extracellular polysaccharides or EPS. The latter are secreted by cells or produced extracellularly using cell wall-anchored enzymes [28]. Bacterial polysaccharides show biological (bioactive) activities, including anti-inflammatory, anticancer, antimicrobial, antioxidant, and immunomodulatory [91–97]. They showed an inhibitory effect against various viruses, both DNA and RNA viruses. The inhibitory effect is usually associated with

the viral adsorption and/or replication phases in host cells. For example, *Arthrospira platensis* (formerly *Spirulina platensis*) produced calcium spirulan, a sulfated polysaccharide, with antiviral activity against several enveloped viruses. The calcium spirulan composed of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, sulfate, and calcium. This polysaccharide showed antiviral activity against HSV-1, human cytomegalovirus (HCMV), measles, mumps, influenza A, and HIV-1 viruses by inhibiting virus penetration [74]. Spirulan-like substances extracted from *A. platensis* showed strong antiviral activity against HCMV, HSV-1, human herpesvirus type 6 (HHV-6), and HIV-1. Their mechanisms depended on the type of virus. For example, in the case of herpesviruses, spirulan-like substances inhibited virus adsorption and/or penetration steps, while HIV-1 was inhibited after viral entry. For HCMV, the inhibition occurred at intracellular steps, especially the viral protein synthesis step [44]. EPS from *A. platensis* also inhibited koi herpesviruses (KHV). Reichert et al. [75] reported that EPS from *A. platensis* between 18 and 36 $\mu g \cdot mL^{-1}$ suppressed KHV *in vitro* Furthermore, nostoflan, an acidic polysaccharide from *Nostoc flagelliforme* (a cyanobacterium), showed an interesting antiviral activity against enveloped viruses: HSV-1, HSV-2, HCMV, and influenza A viruses. The virus infections were blocked when nostoflan was added at the same time as viral infections. Therefore, nostoflan blocked the viruses at the virus adsorption stage. The IC_{50} values of nostoflan for HSV-1, HSV-2, HCMV, and influenza A viruses were 0.37, 2.9, 0.47, and 78 $\mu g \cdot mL^{-1}$, respectively [79]. In addition, other authors also observed how an EPS derived from *Bacillus licheniformis* strain T14 can prevent HSV-2 infection at 300 and 400 $\mu g \cdot mL^{-1}$ in human peripheral blood mononuclear cells (PBMC) [76]. EPS26a from *Lactobacillus* sp. could completely inhibit human adenovirus type 5 (HAdV-5) formation and release [41].

Bacterial polysaccharides also indirectly inhibited virus infections by modulation of the immune response. For instance, an EPS produced by *Lactobacillus delbrueckii* OLL1073R-1 activated the Toll-like receptor 3 (TLR3) and the expression of interferon (IFN)-α, IFN-β, MxA, and RNase L in porcine intestinal epithelial (PIE) cells, which was associated with the innate antiviral immune response [77]. Mizuno et al. [98] reported that an EPS from *Streptococcus thermophilus* ST538 can activate TLR3, thus promoting the expression of IFN-β, interleukin 6 (IL-6), and C-X-C motif chemokine 10 (CXCL10). Antiviral bacterial polysaccharides are also shown in **Table 1**.

Antiviral Polysaccharides From Fungi

Fungi are unicellular-to-multicellular eukaryotic microorganisms. They can produce a plethora of biologically active compounds, especially secondary metabolites. Similar to algae and bacteria, fungal polysaccharides (primary metabolites) also showed antiviral activity. Fungal polysaccharides can be derived from culture broth, mycelial culture and/or fruiting bodies [99]. Fungal polysaccharides, such as glucan, chitin, mannan, PSK or lentinan, showed antiviral potential

against animal, human, and plant viruses (100–103). Fungal polysaccharides with antiviral activity are summarized in **Table 1**. For example, BAS-F, a polysaccharide from *Fomes fomentarius*, at 2 $\mu g \cdot mL^{-1}$ can prevent tobacco mosaic virus (TMV) infection on the leaf surfaces (34). *Porodaedalea pini* (formerly known as *Phellinus pini*) produced two antiviral polysaccharides (EP-AV1 and EP-AV2) against HSV-1 and coxsackie virus B3 (CVB3) in Vero and HeLa cells, respectively. The EP-AV2 with a lower MW (∼100 kDa) showed more potent antiviral activity than EP-AV1 (∼1,010 kDa) against CVB3. It was observed how EP-AV1 and EP-AV2 polysaccharides inhibited the plaque formation caused by CVB3 in HeLa cells by 32 and 84% at 1 $mg \cdot mL^{-1}$, respectively. These polysaccharides specifically inhibited HSV-1 more than CVB3 as indicated by their EC_{50} values. The EC_{50} values of EP-AV1 and EP-AV2 for HSV-1 were 0.20 and 0.21 $\mu g \cdot mL^{-1}$, respectively, whereas CVB3 were 1 and 0.576 $mg \cdot mL^{-1}$, respectively (81). Furthermore, a polysaccharide extracted from the mycelium and fruiting body of *Lentides edodes* was able to inhibit poliovirus type 1 (PV-1) and bovine herpes virus type 1 (BoHV-1) with IC_{50} values of 0.19 and 0.1 $mg \cdot mL^{-1}$, respectively (101). In another study, *Grifola frondosa* mycelia were evaluated as a source of antiviral polysaccharides, observing that it had the antiviral polysaccharide, GFP1. This polysaccharide was a heteropolysaccharide containing glucose and fucose with a MW of ∼40.5 kDa. Zhao et al. (82) reported that GFP1 blocked enterovirus 71 (EV71) infection at the virus replication phase. The GFP1 suppressed the viral protein expression and viral RNA genome synthesis. Fungal polysaccharides also showed important antiviral properties against animal viruses. For example, a polysaccharide from *L. edodes*, called lentinan comprised of glucose, mannose, and galactose with MW of ∼3.79 × 10^5 Da showed antiviral activity against infectious hematopoietic necrosis virus (IHNV) infecting rainbow trout (*Oncorhynchus mykiss*) and several species of salmon. The LNT-I acted both direct inactivation and inhibition of viral replication with 62.3, and 82.4% inhibition, respectively (83). *Inonotus obliquus*, chaga mushroom, also produced broad-spectrum antiviral polysaccharides against feline viruses. The polysaccharides suppressed infections of feline calicivirus (FCV), feline herpesvirus 1 (FHV-1), feline panleukopenia (FPV), feline coronavirus (FCoV), and feline influenza (FIV, H3N2, and H5N6) viruses. These polysaccharides had low toxicity and blocked virus entry by affecting the virions and/or the receptor(s) on host cell surfaces (35).

Therefore, polysaccharides from different species show various biological activities with different levels of action. Sulfated polysaccharides derived from marine microalgae and seaweeds showed many different bioactive properties and were effective against viruses at low concentrations, compared to other polysaccharides. In addition, bacteria and fungi are easily grow on simple media or agricultural wastes. The production can be done using controllable conditions and they produce high amounts of polysaccharides. Therefore, it would also beneficial if the bioactivities and physicochemical properties of bacterial and fungal polysaccharides could be modified. The molecular modification of polysaccharides is an alternative approach to modulate their properties.

ANTIVIRAL MECHANISMS OF MICROBIAL POLYSACCHARIDES

In viral replication, there are 6 major steps during the infection: (1) virus attachment, (2) penetration, (3) uncoating, (4) genome replication and protein synthesis, (5) viral assembly, and (6) release of new virions (104). Different microbial polysaccharides have different antiviral mechanisms depending on virus types. The polysaccharides mostly prevented the initial steps of the virus life cycle. They interacted with virus particles and/or receptors on host cells to interfere with virus adsorption and invasion. However, some microbial polysaccharides could inhibit viral replication and protein translation. While others showed immune-enhancing activity, especially antiviral immune responses, which prevent virus infections and reduce disease severity (31, 105).
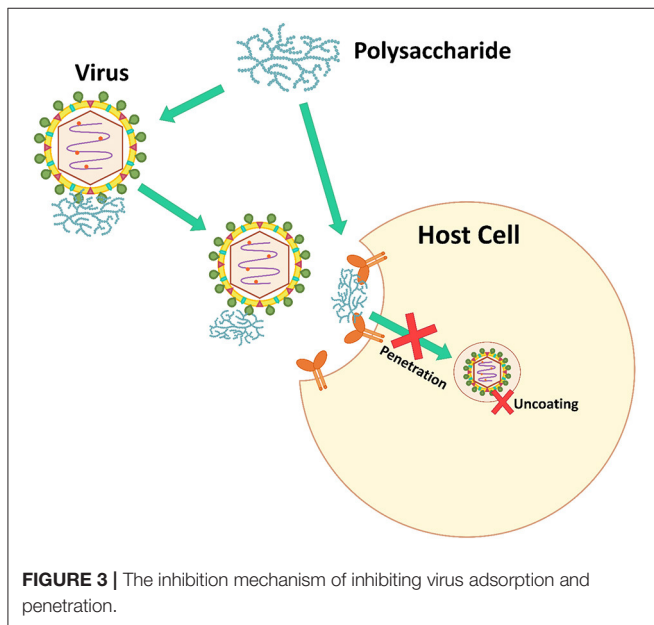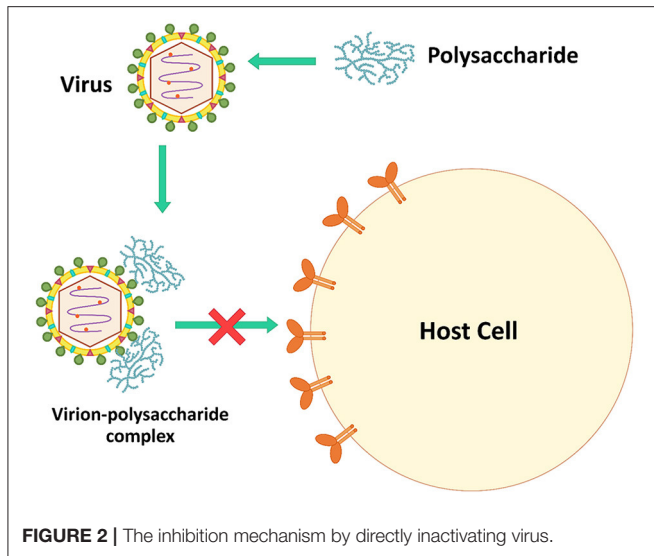
Inactivating Virus Particles Directly

Microbial polysaccharides, especially sulfated polysaccharides, have a negative charge that can interact directly with the viral surfaces. The virucidal activity of microbial polysaccharides is caused by theses interactions (106). The complexes interfere with the viral infection process, reducing viral proliferation in host cells (**Figure 2**). For example, polysaccharides extracted from *Auricularia auricular*, a basidiomycete mushroom, can inhibit NDV in CEF cells. During the process of adding polysaccharides and virus simultaneously, the virus inhibitory rates were higher than pre- and post-addition of the polysaccharides. These polysaccharides might be combined with virus particles to block virus attachment to host cells (42). *Inonotus obliquus* polysaccharides also directly blocked feline virus virions (FCV, FHV-1, FPV, feline coronavirus FCoV, and FIV). These polysaccharides were mixed with the viruses for 1 h before adding to the cell lines, decreasing significantly the viral infectivity compared to untreated viruses (35).

Inhibiting Virus Adsorption and Penetration

Viruses bind to a host cell surface using electrostatic interactions. Some microbial polysaccharides mimic virus particles. Microbial polysaccharides, especially sulfated polysaccharides, are strongly anionic and bind to the positively charged host cell receptors blocking virus attachment, which prevents virus infection (**Figure 3**) (7). Additionally, some microbial polysaccharides are able to prevent the allosteric process of viral protein formation and/or virus internalization and uncoating steps (106).
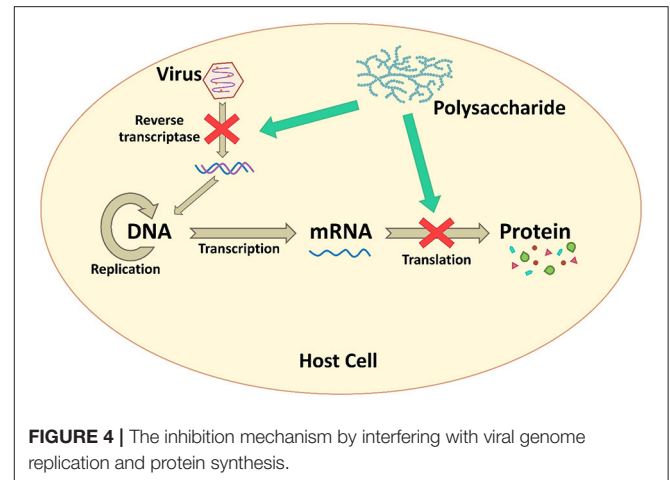
Many microbial polysaccharides act at this step. For example, a polysaccharide SP-2a from *S. patens* showed ≥80% inhibition against all strains of HSV-1 when added during virus adsorption (71). Fucoidans from a brown alga, *Padina tetrastromatica* showed the highest percentage (>70%) inhibition against HSV-1 during the virus adsorption period (37). Human influenza virus H1-364 was blocked by sulfated polysaccharides from *Gracilaria lemaneiformis*, a red alga, at virus adsorption and replication on host cells. The sulfated polysaccharides inhibited against the

FIGURE 2 | The inhibition mechanism by directly inactivating virus.

FIGURE 3 | The inhibition mechanism of inhibiting virus adsorption and penetration.

FIGURE 4 | The inhibition mechanism by interfering with viral genome replication and protein synthesis.
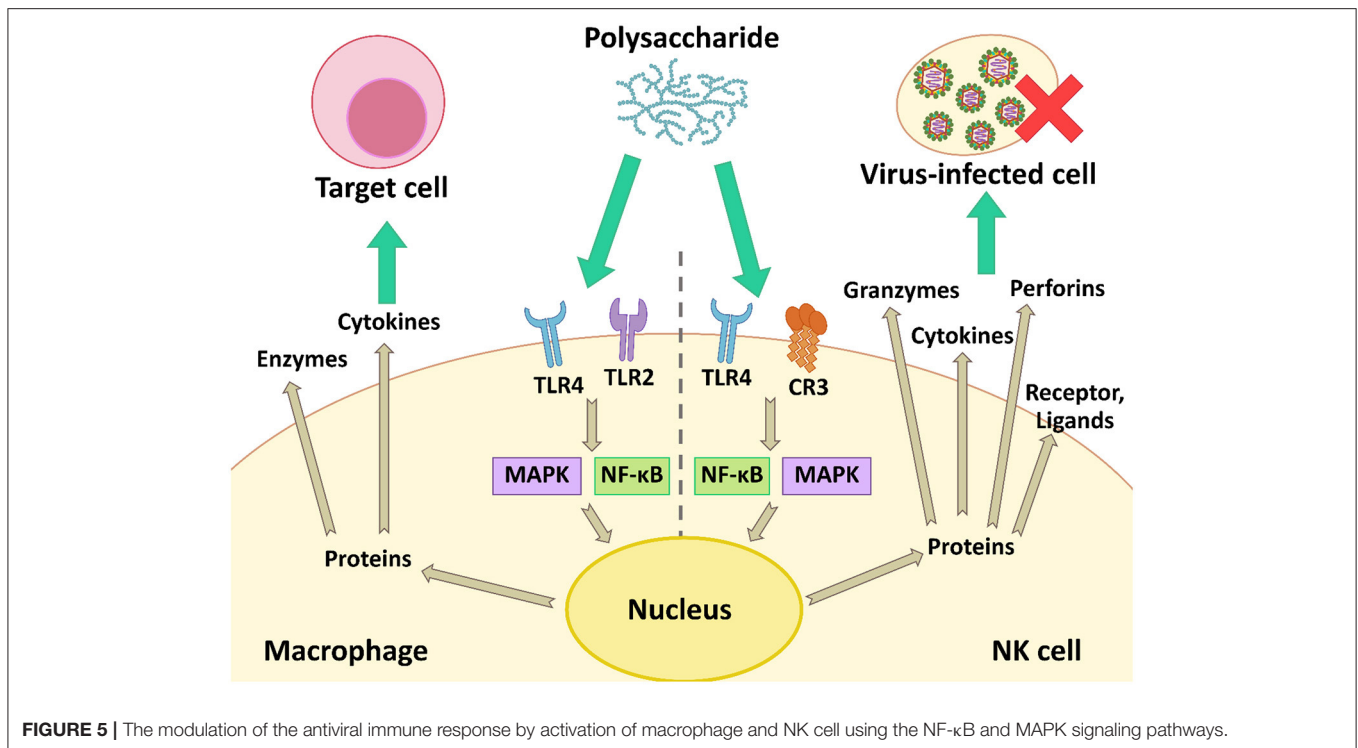
These polysaccharides show a broad-spectrum antiviral activity. González et al. (87) reported that carrageenan inhibited HSV-1 at viral protein synthesis. When carrageenan was added 1 h after HSV-1 infection, viral proteins were not detected, whereas when carrageenan was added immediately, viral proteins were detected. Furthermore, polysaccharide GFP1 from *G. frondosa*, which was composed of glucose and fucose with a MW of 40.5 kDa, acted on viral replication and protein synthesis against EV71. The GFP1 was effective in inhibiting EV71 when it was added before or shortly after the viral inoculation. The viral RNA synthesis and VP1 protein were suppressed in a dose-dependent manner (82).

Modulating Host Antiviral Immune Responses

During virus infection in animals, the body induces the immune responses to defend against viral infection. The responses regulate immune cells such as natural killer (NK) cells and macrophages, and increase the production of cytokines, i.e., the type I interferon system (IFN-α/β system) (36). The microbial polysaccharides interact with cell receptors on the macrophage and NK cell, and then activate the cells using the nuclear factor kappa B (NF-κB) and the mitogen-activated protein kinase (MAPK) signaling pathways. These proteins are inducible factors, which increases the gene expression of various cytokines, chemokines, enzymes, and other proteins involving both innate and adaptive immunity (27). The IFN secreted from activated immune cells triggers activation of other immune cells including NK cells, macrophages, and T-cell lymphocytes, which have important roles in the host immune system and antiviral responses. Meanwhile, microbial polysaccharides can activate NK cells that non-specifically kill virus-infected cells by secreting perforins and granzymes (**Figure 5**).

Several polysaccharides can enhance the antiviral immune responses, thus reducing the number of virus particles and the severity of diseases. For example, an EPS extracted from *S. thermophilus* ST538 was able to induce the expression of IFN-β, IL-6, and CXCL10 in response to TLR3 stimulation. These

virus at ≥60% during virus adsorption and replication, while these polysaccharides were not effective at the virus release step. The polysaccharides at 62.5 μg·mL^{-1} showed 83.5 and 83.0% inhibition against human influenza virus H1-364 at the virus adsorption and replication steps, respectively (59).

Inhibiting Viral Genome Replication and Protein Synthesis

Microbial polysaccharides, especially the low MW polysaccharides, show antiviral effects on infected host cells. They interfere directly with enzymes associated with the viral replication and inhibit other intracellular targets (107) as presented in **Figure 4**. Carrageenans are sulfated polysaccharide that are available from most of red seaweeds.

FIGURE 5 | The modulation of the antiviral immune response by activation of macrophage and NK cell using the NF-κB and MAPK signaling pathways.

immune factors are associated with antiviral immune responses, which induce the recruitment and activation of immune cells to struggle pathogens (98). Moreover, *L. delbrueckii* OLL1073R-1 produced immunomodulatory EPS. These EPS activated TLR3 and induced the expression of IFN-α, IFN-β, MxA, and RNase L. The latter two factors are known as antiviral factors (77). Polysaccharides also showed immune-enhancing activity. Ren et al. (83) reported that LNT-I from *L. edodes* mycelia could modulate the immune response by up-regulating the expression of IFN-1 and IFN-γ to prevent IHNV infection. In addition, an acidic polysaccharide (APS) from *Cordyceps militaris* enhanced TNF-α, IFN-γ, and nitric oxide (NO) production, and induced the expression of several cytokines: IL-1β, IL-6, IL-10, and TNF-α. These cytokines have the potential to prevent influenza A virus infection (39). Cao et al. (64) also reported a polysaccharide from *L. japonica* which could increase IFN-α secretion in a dose-dependent manner. The IFN-α level was 144 pg·mL^{-1} when cells were treated with 1000 μg·mL^{-1} of polysaccharide.

FACTORS INFLUENCING THE ANTIVIRAL ACTIVITY

Polysaccharides derived from different sources showed several unique characteristics, properties, and bioactivities at different levels. **Table 2** shows various microbial polysaccharides and/or polysaccharide fractions with different characteristics. Their MW, compositions, functional groups, and structural conformations including type of linkage and degree of branching associate with their biological properties, especially antiviral and immunomodulatory activities. Moreover, extraction and

purification methods affect the compositions of polysaccharides; therefore, these factors also influence biological activities of the polysaccharides (110).

Sulfate Content

Several studies have reported that sulfated polysaccharides could exhibit several biological activities (antiviral, anticancer, antioxidant, and immunomodulatory activities), so the sulfate contents could be an important factor affecting antiviral and other bioactivities. Sulfation has been used for enhancing various biological activities of polysaccharides (111, 112). For example, a marine *Pseudomonas* sp. WAK-1 produces extracellular glycosaminoglycan (A1) and sulfated polysaccharide (A2) with antiviral activity. Matsuda et al. (80) modified the polysaccharides by over-sulfation using a dicyclohexyl-carbodiimide-mediated reaction. The over-sulfated polysaccharides were called A1S and A2S, respectively. These 4 compounds showed antiviral activity against influenza A virus with EC$_{50}$ values of >100, >100, 11.0, and 2.9 μg·mL^{-1}, respectively. From the results, over-sulfated polysaccharides (A1S and A2S) showed higher antiviral activity against influenza A virus than the natural polysaccharides (A1 and A2). Moreover, a xylogalactofucan (sulfated polysaccharide) from a brown alga *Sphacelaria indica* also exhibited antiviral activity against HSV-1. The sulfate contents of the polysaccharide affected the antiviral property. The sulfate content of the purified polysaccharide was 4% (w/w). Bandyopadhyay et al. (113) chemically modified the xylogalactofucan produced derivatives with up to 7% (w/w). The IC$_{50}$ values of natural and artificially over-sulfated polysaccharides were 1.3 and 1.5 μg·mL^{-1}, respectively, while a desulfated derivative of the polysaccharide

TABLE 2 | Some microbial polysaccharides from various sources with different characteristics and bioactivities.

Name or fraction	Source	Average molecular weight (kDa)	Polysaccharide Characterisation	Observations	References
Acidic polysaccharide (APS)	*Cordyceps militaris*	576	Containing: D-galactose, L-arabinose, D-xylose, L-rhamnose, and D-galacturonic acid; Linkage: Ara$f$-(1 → , → 5)-Ara$f$-(1 → , → 4)-Gal$p$-(1 → and → 4)-GalAp-(1 → residues	Influenza A virus titres in the bronchoalveolar lavage fluid and the lung of mice were decreased. The APS increased the production of TNF-α, IFN-γ, and nitric oxide (NO)	(39)
EPS0142	*Lactobacillus plantarum* JLK0142	134	Containing: glucose and galactose in an approximate molar ratio of 2.13:1.06	*In vitro,* EPS0142 improved the phagocytic activity and induced NO secretion. *In vivo,* sIgA and cytokines (IL-2 and TNF-α) were increased	(108)
EPS-1	*Monascus purpureus*	69.7	Containing: mannose, glucose and galactose in the molar ratio of 8:1:11; Linkage: a backbone of 5-β-D-Gal$f$ and 2-β-D-Man$p$ with other sugar residues including 2,6-β-D-Gal$f$, T-α-D-Man$p$, 6-α-D-Man$p$, and 3,5-β-D-Gal$f$	EPS-1 induced cytokines secretion, including IL-6, TNF-α, and IL-10, and up-regulated the related mRNA expression levels.	(109)
Fucoidan ST-F	*Sargassum trichophyllum*	19.8	Containing: fucose (79.1 mol%) and galactose (19.9 mol%), and its sulfate content was 25.5%; Linkage: terminal, 1,4- and 1,3-linked fucose and terminal, 1,2- and 1,6-linked galactose residues	ST-F showed anti-HSV-2 activity with the IC_{50} of 18 µg·mL^{-1}	(72)
Fucoidans SHAP-1 and SHAP-2	*Sargassum henslowianum*	655 (SHAP-1) and 589 (SHAP-2)	Containing: fucose and galactose at a ratio of 3:1; sulfate content of 31.9%; Linkage: a backbone of α-(1→ 3)-linked L-Fuc$p$ and a side chain of terminally linked α-L-Fuc$p$ and α-D-Gal$p$ residues, and (1 → 2)-, (1 → 6)-, and (1→ 2,6)-linked β-D-Gal$p$ residues	Both SHAP-1 and SHAP-2 showed antiviral activity against HSV-1 and HSV-2. The IC_{50} values of SHAP-1 and SHAP-2 against HSV-1 were 0.89 and 0.82 µg·mL^{-1}, respectively, whereas both as low as 0.48 µg·mL^{-1} against HSV-2.	(84)
GFP1	*Grifola frondosa*	40.5	Containing: glucose and fucose; a backbone of 1,6-β-β-glucan and a single 1,3-α-β-fucopyranosyl side-branching unit	GFP1 could inhibit EV71 replication in Vero cells and suppress viral VP1 protein expression	(82)
Lentinan LNT-1	*Lentinus edodes*	379	Containing: glucose, mannose and galactose with the molar ratio of 19.26:1.20:1.00; Linkage: β-(1 → 3)-glucan backbone with -(1 → 6)-glucosyl side-branching units terminated by mannosyl and galactosyl residue	Lentinan directly inactivate IHNV and modulate immune responses by induce the expression of IFN-1 and IFN-γ, and reduce the expression of TNF-α, IL-2 and IL-11 in EPC cells	(83)
Naviculan	*Navicula directa*	222	Containing: fucose (26.6%), xylose (25.0%), galactose (20.7%), mannose (13.1%), and rhamnose (8.7%)	Naviculan had a broad antiviral spectrum against HSV-1, HSV-2, and Influenza A virus with the IC_{50} values of 14, 7.4, and 170 mg·mL^{-1}, respectively.	(67)
Nostoflan	*Nostoc flagelliforme*	211	→ 4)-β-D-Glc$p$-(1 → 4)-D-Xyl$p$-(1→ and → 4)-[β-D-GlcAp-(1 → 6)-]-β-D-Glc$p$-(1 → 4)-D-Gal$p$-(1 →	Nostoflan showed broad antiviral activity against HSV-1, HSV-2, HCMV, and Influenza A virus	(79)
Sulfated galactofucan (SJ-D-S-H) and glucuronomannan (Gn)	*Saccharina japonica*	13.7 (SJ-D-S-H) and 7.0 (Gn)	**SJ-D-S-H** Containing: 36% fucose, 10% galactose, and 21% sulfate **Gn** Containing: alternating 1, 4-linked β-D-GlcAp residues and 1, 2-linked α-D-Man$p$ residues	Both SJ-D-S-H and Gn showed good binding ability to SARS-CoV-2 spike glycoproteins. The IC_{50} values were 27 and 231 nM, respectively	(69)
Sulfated polysaccharide (CIF2)	*Cystoseira indica*	1,150	Containing: mainly fucose (24.4%), glucose (21.3%), mannose (20.6%), galactose (16.7%), rhamnose (11.3%), and xylose (5.8%); Linkage: (1 → 3)-fucopyranose, (1 → 3) and (1 → 6)-galactopyranose residues	*In vitro,* CIF2 increased the release of nitric oxide and inflammatory cytokines including TNF-α, IL-1β, IL-6, and IL-10 from RAW264.7 cells	(54)

had no effect on HSV-1. Furthermore, Ponce et al. (86) also reported that the level of sulfated esters in sulfated galactofucans extracted from a brown alga *Scytosiphon lomentaria* was an important factor influencing the antiviral activity. The whole extract (A) of *S. lomentaria* was fractionated to yield fractions A0, A5, A10, A20, A30, and A40, with different components, MW, and monosaccharide composition. A0 was soluble and the fraction A5 was an uronofucoidan. A10–A40 were galactofucans and showed antiviral activity against HSV-1 and HSV-2 with IC_{50} values in the range 0.76–10.0 $\mu g \cdot mL^{-1}$. Among the 4 galactofucan fractions, A30 (pure galactofucan) contained the highest sulfate content (29.5% SO_3Na) and the lowest uronate content (1.8%). A30 showed the strongest antiviral activity against HSV-1 and HSV-2 with IC_{50} values of 0.76 and 1.3 $\mu g \cdot mL^{-1}$, respectively. Therefore, the low content of uronic acids and the high content of sulfate was associated with the antiviral activity of these polysaccharides. In conclusion, sulfate content is an important factor influencing biological activities. Adding sulfate groups into polysaccharide structures led to enhance bioactivities, whereas desulfation decreased their bioactivities.

Molecular Weight

The MW of polysaccharides also influenced their biological properties. Polysaccharides with low MW could easily pass through target cells to act inside the cells. Moreover, the low MW polysaccharides might bind better to cell receptors to inactivate or activate the target cells (114). Some polysaccharides with lower MW showed high biological activities, but some polysaccharides with higher MW were better. For example, Surayot et al. (26) reported the effect of MW of an EPS from *Weisella confusa* on immunomodulatory activity. The EPS with low MW ($\leq 70 \times 10^3$ Da) could stimulate RAW264.7 cells to induce NO and production of various cytokines such as TNF-α, IL-1β, IL-6, and IL-10, but the native EPS (MW of $\sim 506 \times 10^6$ Da) had no immunomodulatory activity. In addition, Ponce et al. (86) reported that the galactofucan fraction A30 had the lowest MW ($\sim$8.5 kDa) and the low MW might be another factor influencing the antiherpes activity against HSV-1 and HSV-2. On the contrary, high molecular weight carrageenans from different rea algae (*Chondrus armatus*, *Kappaphycus alvarezii*, and *Tichocarpus crinitus*) had effective antiviral activity (115). The different carrageenans with molecular weight of 250, 390, and 400 kDa, respectively show antiviral activity with 88, 85, and 77%, respectively. While low molecular weight (LMW) derivatives (1.2–3.5 kDa) were obtained from different depolymerization methods. The LMW derivatives showed low antiviral properties (28–54%) compared to the native polysaccharides. Therefore, the antiviral activity of these polysaccharides depended on their molecular weight (115).

Enhancement of Bioactivities

To enhance the biological activities, natural microbial polysaccharides need molecular modification of their structure, size, and functional groups to optimize activity (114). The MW can be reduced using external energy and/or specific enzymes to break glucoside chains. Using ultrasonic disruption and microwave exposure to reduce the MW are "physical modification," whereas the enzymatic degradation is "biological modification" (114). For instance, Surayot et al. (26) hydrolyzed an EPS from *W. confusa* TISTR 1498 using HCl and heating in hot water or in a microwave oven. The low MW products could induce production of cytokines from RAW264.7 macrophage cells. Bioactivities were also enhanced by changing the functional substituents of polysaccharides, which is "chemical modification," such as alkylation, sulfation, sulfonation, phosphorylation, carboxymethylation, and selenization (114).

THE POTENTIAL USES OF MICROBIAL POLYSACCHARIDES TO PREVENT VIRAL DISEASES

Microbial polysaccharides showed various bioactivities, while almost always having any significant side-effects, yet are biodegradable, biocompatible, and cost-effective. Microbial and algal polysaccharides may be applied as drug resistance solutions. These polysaccharides can combine with other antiviral drugs for preventing drug-resistance strains (110). In addition to the prevention of viral infections, these polysaccharides also prevent recurrence of latent viruses. For example, calcium spirulan (Ca-SP) derived from *Spirulina platensis* was developed as microalgal cream, which effectively prevented the recurrence of HSV-1 (116). Therefore, the bioactive polysaccharides may be used to prevent viral diseases and reduce the risks of diseases, especially COVID-19.

SARS-CoV-2 has an S-protein on its envelope and the protein has an important role with binding to a host cell receptor (ACE2) (8). Heparin, heparan sulfates, and other sulfated polysaccharides can bind tightly to the S-protein *in vitro* (117). The binding inhibits viral infection. Other microbial polysaccharides showed immunomodulatory properties that stimulated the immune system to prevent SARS-CoV-2 infection. Several microbes can produce sulfated polysaccharides. Beneficial sulfated polysaccharides might be produced from natural microbial polysaccharides (114). Type I IFN, including IFN-α, -β, -ε, -κ, -ω, -δ, -ζ, and -τ, are essential cytokines for antiviral immune responses. Type I IFN can induce antiviral responses within infected and neighboring cells that block the spread of virus particles. They activate both innate and adaptive immune responses that promote NK cell functions and antibody production (118, 119). Hadjadj et al. (120) reported that most severe COVID-19 patients had a low type I IFN response (No IFN-β and low IFN-α production and activity). Many microbial and algal polysaccharides induced type I IFN production *in vivo*. Thus, these polysaccharides might be applied to modulate the immune system in both patients and healthy people. In severe COVID-19 cases, aggressive inflammatory responses were found and the inflammation caused tissue damage in many organs (121). Some microbial polysaccharides showed anti-inflammatory activity. These polysaccharides inhibited the production of pro-inflammatory cytokines including TNF-α, IL-1β, IL-6, and IL-8 (122–124).

ACE2 receptors are expressed by several tissues and organs as described above, especially the respiratory and gastrointestinal

tracts. Microbial polysaccharides with antiviral activity can be used as a nasal spray, metered dose inhaler, or delivered orally to prevent the binding of SARS-CoV-2 (117). Several natural polysaccharides have been designed as nanomaterials for drug delivery systems, such as antiviral agent. These nanomaterials may be not only used to treat the virus, but also to modulate the immune responses (7).

Algal, bacterial, and fungal polysaccharides and sulfated polysaccharides showed pharmaceutical properties due to their biological activities as mentioned above. These polysaccharides could be used as bioactive supplements in foods and could enrich nutritional quality. Indeed, some of these polysaccharides have been granted as GRAS status by the US FDA, so they can consume to enhance immune response and reduce the severity of viral diseases, especially COVID-19 (117). Some microbial polysaccharides have prebiotic properties, which enhance the proliferation of beneficial intestinal microflora, especially *Bifidobacterium* spp. (125). In addition, some algal polysaccharides (alginate and laminaran) could be fermented by gut microbiota and promoted the growth of *Bacteroides*, *Bifidobacterium*, and *Lactobacillus* species (126). When microbial polysaccharides were consumed, they could enhance the host's immune response and modulate the microbial community (microflora). The microbes degrade the polysaccharides into short-chain fatty acids (SCFA) such as acetic, propionic, and butyric acids. SCFA show benefit for the maintenance intestinal cells and modulating of the immune system (27). Microbial polysaccharides have the potential to be bioactive ingredients that can be added into foods or food products to enhance the nutritional quality of foods by modulating consumers' immune response. Therefore, the biological activities of foods supplemented with these polysaccharides should be investigated. The intake of foods with bioactive polysaccharides in patients and healthy people to prevent viruses and/or reduce the adverse symptoms needs further study.

CONCLUSION

Microorganisms produce various types of polysaccharides with unique characteristics and can be produced on a large scale with controllable conditions. Several microbial/algal polysaccharides show bioactivities, especially antiviral and immunomodulatory activities. They have strong antiviral effects by interfering with the life cycle of viruses and/or modulating host immune responses, which may benefit patients infected with COVID-19. Polysaccharides and sulfated polysaccharides from different microorganism and algae species have different characteristics and levels of bioactivities. Their constituents, structural conformations, MW, and functional groups significantly influence their bioactivities. To enhance their activities, physical, chemical, or biological modifications might be beneficial. The microbial polysaccharides have potential uses as adjuvants for antiviral vaccines and micro- and/or nano-particles for drug delivery systems. Some sulfated polysaccharides obtained from microbes and algae have been approved as GRAS, which may be used as bioactive ingredients adding in food products to prevent viruses. Many microbial polysaccharides are safe, biocompatible, biodegradable, and easily available. Therefore, the intake of proper dosage of the right polysaccharides may modulate physiological functions to prevent viral diseases and decrease their damage. They may be an alternative therapy to treat COVID-19 patients. In the future, the development of polysaccharides as functional food products should be explored. For foods supplemented with bioactive polysaccharides, more pharmaceutical investigations and clinical evidence are required to analyze their antiviral and immune-enhancing effects. The mechanisms that occur in the food products against viral infections should also be further investigated.

AUTHOR CONTRIBUTIONS

PS, TC, CT, and SY contributed to conception and supervised the project. WC, NL, KJ, and SS contributed in doing literature searches and wrote the manuscript draft. YP, PR, SW, JR, FB, and PS equally revised and approved the manuscript. All authors have read and approved the final draft manuscript.

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