# Inhibitory effect of phytochemicals towards SARS-CoV-2 papain like protease (PLpro) proteolytic and deubiquitinase activity

Anasha Kawall[1], Devin S. M. Lewis[2], Avini Sharma[1], Krishna Chavada[3], Rahul Deshmukh,[4] Srujana Rayalam[3], Vicky Mody[3], Shashidharamurthy Taval[3]

[1]Department of Biomedical Sciences, Philadelphia College of Osteopathic Medicine – Georgia Campus, Suwanee, GA, USA.

[2]Division of Research, Philadelphia College of Osteopathic Medicine – Georgia Campus, Suwanee, GA, USA.

[3]Department of Pharmaceutical Sciences, School of Pharmacy, Philadelphia College of Osteopathic Medicine – Georgia Campus, Suwanee, GA, USA.

[4]Department of Pharmaceutical Sciences, College of Pharmacy, Rosalind Franklin University – North Chicago, IL, USA.

**Running Title:** SARS-CoV-2 PLpro inhibition by phytochemicals

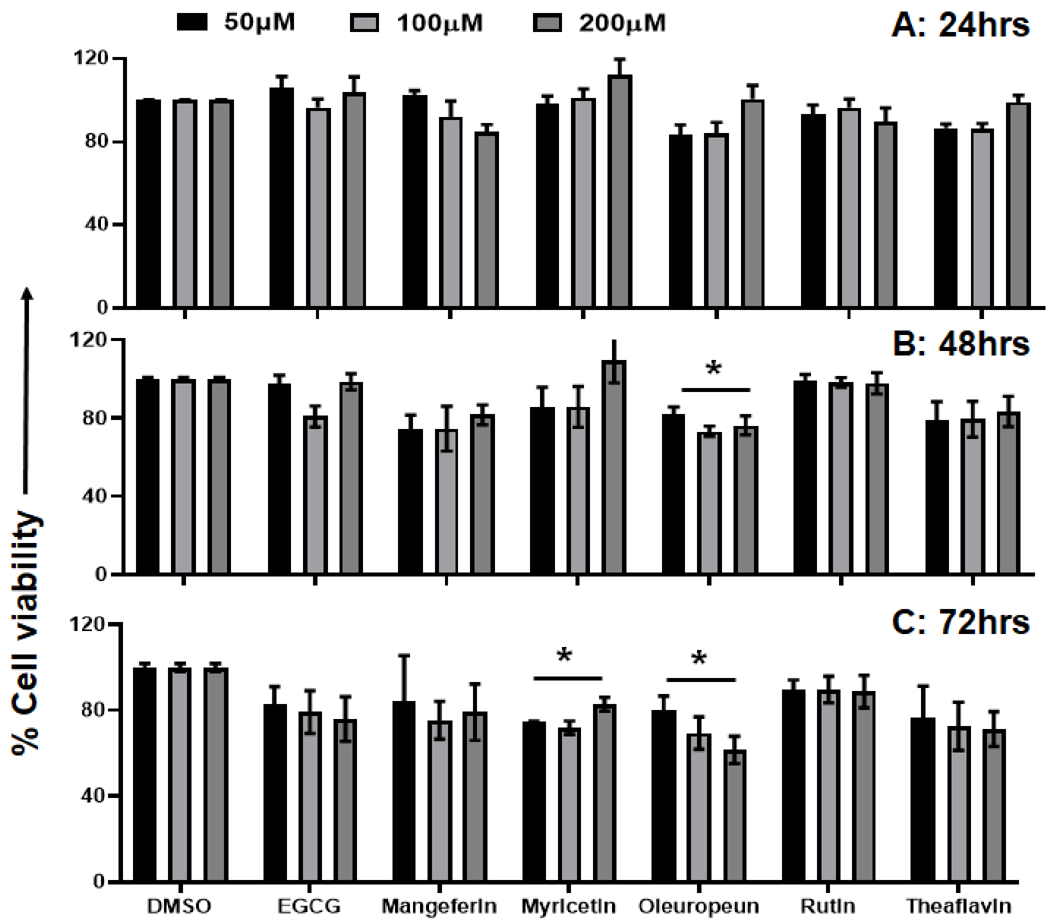
**Keywords:** SARS-CoV-2, replication, PLpro, phytochemicals, natural compounds

***Corresponding Authors:**

Shashidharamurthy Taval, Ph.D.,
Associate Professor, Department of Pharmaceutical Sciences,
Philadelphia College of Osteopathic Medicine, School of Pharmacy,
Room 3031, 625 Old Peachtree Road, Suwanee, GA-30024,
Tel: 678-407-7373, Fax: 678-407-7347, Email: rangaiahsh@pcom.edu

Vicky Mody, Ph.D.,
Associate Professor, Department of Pharmaceutical Sciences,
Philadelphia College of Osteopathic Medicine, School of Pharmacy,
Room 3031, 625 Old Peachtree Road, Suwanee, GA-30024,
Tel: 678-407-7386, Fax: 678-407-7347, Email: vickymo@pcom.edu

**Supplementary figure 1.**



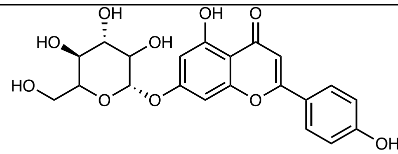
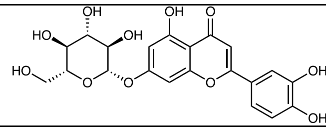
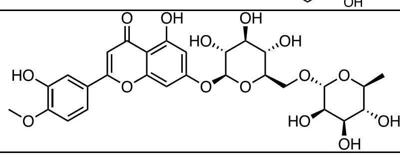
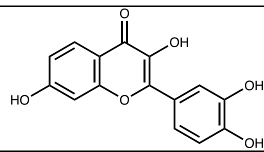
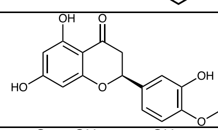
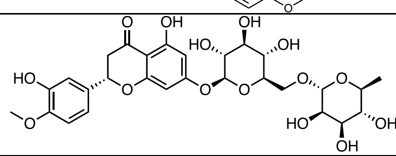
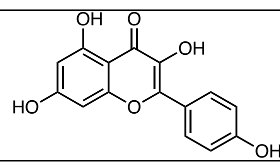
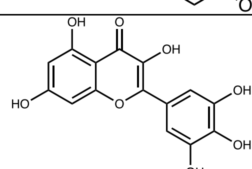
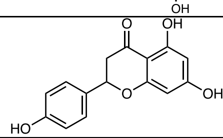
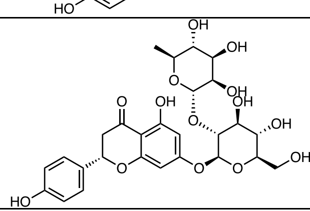

**Cytotoxicity of selected phytochemicals in African green monkey kidney epithelial cell line Vero-E6.** Cell viability assay was performed as described under materials and methods. EGCG, mangiferin, rutin, and theaflavin were not cytotoxic, but myricetin and oleuropein exhibited minimal cytotoxicity (20-30%) in Vero-E6 cells at the tested doses and time points. 0.1% DMSO treated cells were considered as positive control. Representative of two individual experiments (n=2) with triplicate values were presented graphically. Two-way ANOVA with Dunnett's Comparison post-test was used to calculate the statistical significance compared to the DMSO treated cells. <p.05 considered as statistically significant.

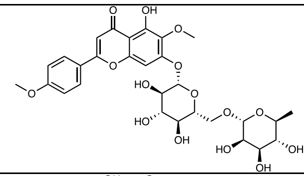
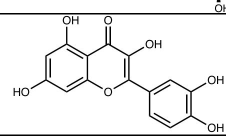
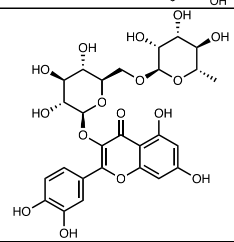
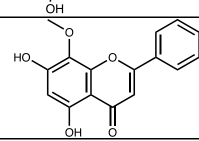
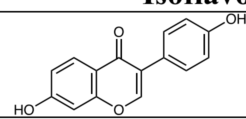
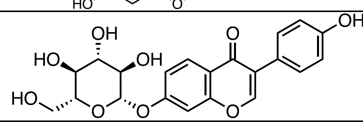
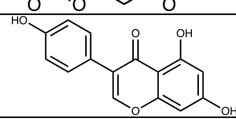
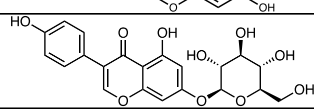
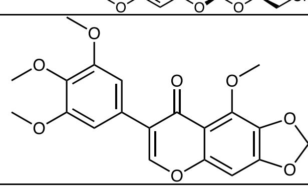
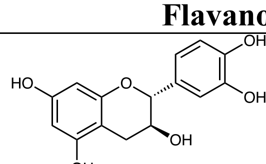
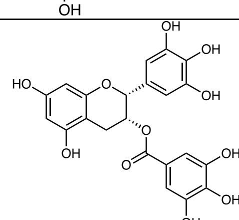
**Supplementary table 1: List of tested phytochemicals with catalog number and manufacturers**

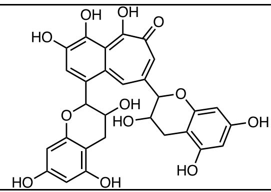
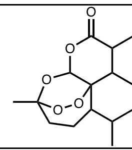
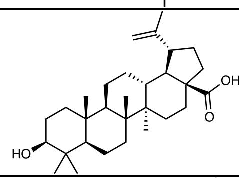
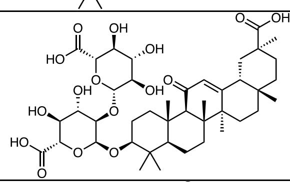
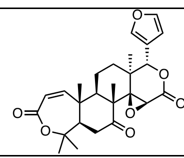
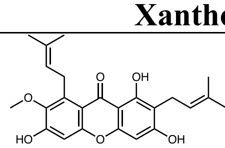
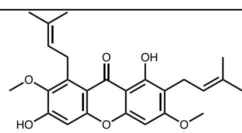
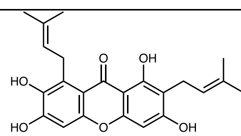
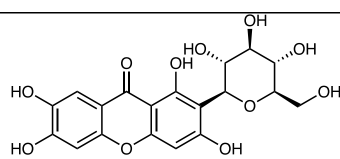
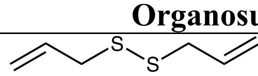
| Phytochemical | Manufacturer | Catalog number |
|---|---|---|
| **Flavonones** | | |
| Naringenin | Cayman Chemicals | 14173 |
| Naringin | Cayman Chemicals | 17923 |
| Hesperidin (Hesperetin 7-rutinoside) | MedChemExpress | HY-15337 |
| Hesperetin | MedChemExpress | HY-N0168 |
| Apigenin 7-glucoside | Cayman Chemicals | 26813 |
| Pectolinarin | MedChemExpress | HY-N0314 |
| Quercetin | Cayman Chemicals | 10005169 |
| Rutin | Cayman Chemicals | 19868 |
| Myricetin | Cayman Chemicals | 10012600 |
| Kaempferol | MedChemExpress | HY-14590 |
| Wogonin | Cayman Chemicals | 14248 |
| Fisetin | MedChemExpress | HY-N0182 |
| Cynaroside (Luteolin 7-glucoside) | MedChemExpress | HY-N0540 |
| Diosmin | MedChemExpress | HY-N0178 |
| **Isoflavones** | | |
| Daidzein | Cayman Chemicals | 10005166 |
| Daidzin | Cayman Chemicals | 13202 |
| Genistein | Cayman Chemicals | 10005167 |
| Genistin | MedChemExpress | HY-N0595 |
| Irisflorentin | MedChemExpress | HY-N0005 |
| **Flavonones** | | |
| Epigallocatechin gallate | MedChemExpress | HY-13653 |
| Catechin | MedChemExpress | HY-N0898 |
| Theaflavin | Cayman Chemicals | 25129 |
| **Terpenoids** | | |
| Artemisinin | Cayman Chemicals | 11816 |
| Glycyrrhizic acid | Cayman Chemicals | 11847 |
| Betulinic acid | Cayman Chemicals | 11686 |
| Obacunone | MedChemExpress | HY-N0428 |
| **Xanthones** | | |
| α-Mangostin | MedChemExpress | HY-N0328 |
| b-Mangostin | MedChemExpress | HY-N0941 |
| ɣ-Mangostin | MedChemExpress | HY-N1957 |
| Mangiferin | MedChemExpress | HY-N0290 |
| **Organosulfur** | | |
| Diallyl Disulfide | Cayman Chemicals | 10012582 |
| Diallyl Trisulfide | Cayman Chemicals | 10012577 |

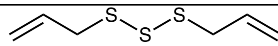
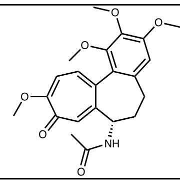
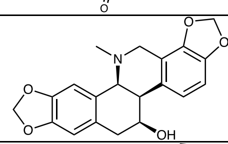
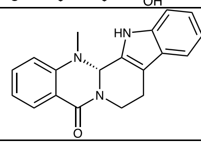
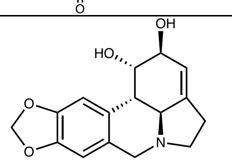
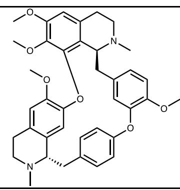
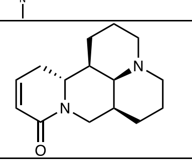
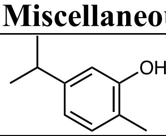
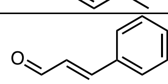
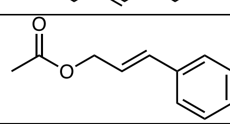
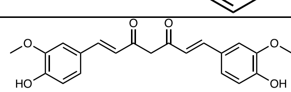
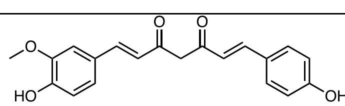
| | | |
|---|---|---|
| **Alkaloids** | | |
| Colchicine | MedChemExpress | HY-16569 |
| Chelidonine | MedChemExpress | HY-N2369 |
| Evodiamine | MedChemExpress | HY-N0114 |
| Lycorine | MedChemExpress | HY-N0288 |
| Tetrandrine | MedChemExpress | HY-13764 |
| Sophocarpine | Cayman Chemicals | 29598 |
| **Miscellaneous Flavonoids** | | |
| Withaferin A | Cayman Chemicals | 11352 |
| Guggulsterone | Cayman Chemicals | 10011296 |
| Geniposide | MedChemExpress | HY-N0009 |
| Rosmarinic Acid | Cayman Chemicals | 70900 |
| Resveratrol | Cayman Chemicals | 14942 |
| Trifolirhizin | MedChemExpress | HY-N0616 |
| Oleuropein | Cayman Chemicals | 21220 |
| Xanthohumol | Cayman Chemicals | 15399 |
| Geraniol | Cayman Chemicals | 23166 |
| Curcumin | MedChemExpress | HY-N0005 |
| Cinnamyl Acetate | Sigma-Aldrich | 166170 |
| Carvacrol | Cayman Chemicals | 33753 |
| Cinnamaldehyde | Sigma-Aldrich | W228613 |
| Demethoxycurcumin | Cayman Chemicals | 10961 |
| Honokiol | MedChemExpress | HY-N0003 |

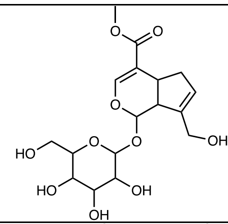
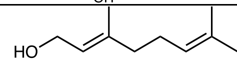
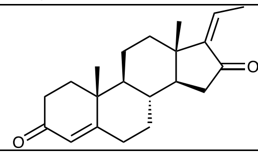
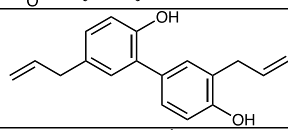
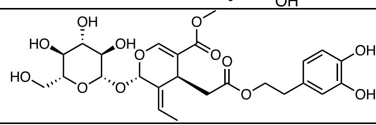
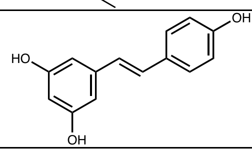
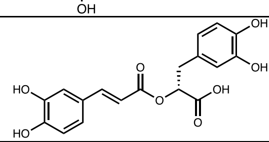
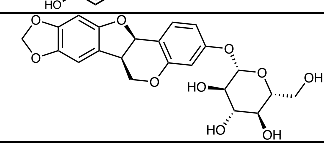
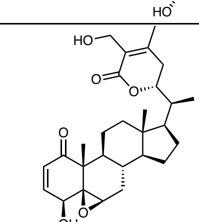
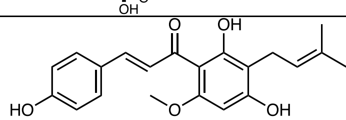
**Supplementary table 2: Chemical structures of literature reviewed for phytochemicals with potential anti-SARS-CoV-2 effects *in-vitro* and *in-silico* studies**

| Phytochemical | Structure | Type of Study | Reference |
|---|---|---|---|
| **Flavonones** | | | |
| Apigenin 7-glucoside |  | *In silico* | [1, 2] |
| Cynaroside (Luteolin 7-glucoside) |  | *In vitro* | [3] |
| Diosmin |  | *In silico* | [1] |
| Fisetin |  | *In silico* | [2] |
| Hesperetin |  | *In silico* | [2, 4] |
| Hesperidin |  | *In vitro* | [5] |
| Kaempferol |  | *In silico* | [1, 2] |
| Myricetin |  | *In silico* | [1, 2] |
| Naringenin |  | *In silico* | [1, 2] |
| Naringin |  | *In silico* | [1] |

| | | | |
|---|---|---|---|
| Pectolinarin |  | *In silico* | [1] |
| Quercetin |  | *In silico* | [1, 2] |
| Rutin |  | *In silico* | [1, 6] |
| Wogonin |  | *In silico* | [7] |
| **Isoflavones** | | | |
| Daidzein |  | *In silico* | [8] |
| Daidzin |  | *In silico* | [9] |
| Genistein |  | *In silico* | [6, 8] |
| Genistin |  | *In silico* | [10] |
| Irisflorentin |  | *In silico* | [11] |
| **Flavanols** | | | |
| Catechin |  | *In silico* | [6] |
| Epigallocatechin gallate |  | *In silico*<br>*In vitro* | [5, 6] |

| | | | |
|---|---|---|---|
| Theaflavin |  | *In silico* | [1] |
| **Terpenoids** | | | |
| Artemisinin |  | *In vitro* | [12] |
| Betulinic acid |  | *In vitro* | [13] |
| Glycyrrhizic acid |  | *In silico* | [14, 15] |
| Obacunone |  | *In silico* | [14] |
| **Xanthones** | | | |
| α-Mangostin |  | *In silico* | [16, 17] |
| b-Mangostin |  | *In silico* | [17] |
| ɣ-Mangostin |  | *In silico* | [17] |
| Mangiferin |  | *In silico* | [18] |
| **Organosulfur** | | | |
| Diallyl Disulfide |  | *In silico* | [19] |

| | | | |
|---|---|---|---|
| Diallyl Trisulfide |  | *In silico* | [19] |
| **Alkaloids** | | | |
| Colchicine |  | *In silico* | [20] |
| Chelidonine |  | *In silico* | [21] |
| Evodiamine |  | *In vitro* | [22] |
| Lycorine |  | *In silico and in vitro* | [6] |
| Tetrandrine |  | *In silico* | [6] |
| Sophocarpine |  | *In silico* | [6] |
| **Miscellaneous Flavonoids** | | | |
| Carvacrol |  | *In silico* | [8] |
| Cinnamaaldehyde |  | *In silico* | [8] |
| Cinnamyl Acetate |  | *In silico* | [23] |
| Curcumin |  | *In vitro* | [7] [24] |
| Demethoxycurcumin |  | *In silico* | [25] |

| | | | |
|---|---|---|---|
| Geniposide |  | *In vitro* | [26] |
| Geraniol |  | *In silico* | [26] |
| Guggulsterone |  | *In silico* | [27] |
| Honokiol |  | *In silico* | [7] |
| Oleuropein |  | *In vitro* | [5] |
| Resveratrol |  | *In silico* | [6, 7] |
| Rosmarinic Acid |  | *In silico* | [11] |
| Trifolirhizin |  | *In silico* | [28] |
| Withaferin A |  | *In silico* | [29] |
| Xanthohumol |  | *In vitro* | [30] |

References:


1. Murugesan, S., et al., *Targeting COVID-19 (SARS-CoV-2) main protease through active phytocompounds of ayurvedic medicinal plants - Emblica officinalis (Amla), Phyllanthus niruri Linn. (Bhumi Amla) and Tinospora cordifolia (Giloy) - A molecular docking and simulation study.* Comput Biol Med, 2021. **136**: p. 104683.
2. Vijayakumar, B.G., et al., *In silico pharmacokinetic and molecular docking studies of natural flavonoids and synthetic indole chalcones against essential proteins of SARS-CoV-2.* Eur J Pharmacol, 2020. **886**: p. 173448.
3. D. S. N. B. K. Prasanth, M.M., Vivek Chandramohanc, Gangadharappa Bhavyad, Atmakuri Lakshmana Raoe, Siva Prasad Pandaa, G. S. N. Koteswara Raof, Guntupalli Chakravarthia, Nayudu Tejag, Peddireddy Suguna Ranih, Gummadi Ashug, Chittiprolu Purnadurganjalie, Puvvala Akhile, Gorriputti Vedita Bhavanie, and Tirumalasetti Jaswitha, *In-silico strategies of some selected phytoconstituents from Melissa officinalis as SARS CoV-2 main protease and spike protein (COVID-19) inhibitors.* MOLECULAR SIMULATION, 2021. **46**(6): p. 457-470.
4. Cheng, F.J., et al., *Hesperidin Is a Potential Inhibitor against SARS-CoV-2 Infection.* Nutrients, 2021. **13**(8).
5. Aynur MK, C.M.A., Ihsan K, Ali Arslan, *Antiviral Effects of Some Flavonoids on SARS-CoV-2* Turkish Journal of Science and Health 2021. **2**(3): p. 24.34.
6. Gurung, A.B., et al., *Unravelling lead antiviral phytochemicals for the inhibition of SARS-CoV-2 M(pro) enzyme through in silico approach.* Life Sci, 2020. **255**: p. 117831.
7. Monjur AL, M.D., *Search for therapeutics against COVID 19 targeting SARS-CoV-2 papain-like protease: an in silico study.* Research Square, 2022.
8. Elfiky, A.A., *Natural products may interfere with SARS-CoV-2 attachment to the host cell.* J Biomol Struct Dyn, 2021. **39**(9): p. 3194-3203.
9. Huang C-W, H.H.-A., Tsai S-C, et al, *In Silico Target Analysis of Treatment for COVID-19 Using Huang-Lian-Shang-Qing-Wan, a Traditional Chinese Medicine Formula.* Natural Product Communications, 2021. **16**(10).
10. Harisna, A.H., et al., *In silico investigation of potential inhibitors to main protease and spike protein of SARS-CoV-2 in propolis.* Biochem Biophys Rep, 2021. **26**: p. 100969.
11. Yang, R., et al., *Chemical composition and pharmacological mechanism of Qingfei Paidu Decoction and Ma Xing Shi Gan Decoction against Coronavirus Disease 2019 (COVID-19): In silico and experimental study.* Pharmacol Res, 2020. **157**: p. 104820.
12. Cao, R., et al., *Anti-SARS-CoV-2 Potential of Artemisinins In Vitro.* ACS Infect Dis, 2020. **6**(9): p. 2524-2531.
13. Wen, C.C., et al., *Specific plant terpenoids and lignoids possess potent antiviral activities against severe acute respiratory syndrome coronavirus.* J Med Chem, 2007. **50**(17): p. 4087-95.
14. Vardhan, S. and S.K. Sahoo, *In silico ADMET and molecular docking study on searching potential inhibitors from limonoids and triterpenoids for COVID-19.* Comput Biol Med, 2020. **124**: p. 103936.
15. Patil, R., et al., *Computational and network pharmacology analysis of bioflavonoids as possible natural antiviral compounds in COVID-19.* Inform Med Unlocked, 2021. **22**: p. 100504.

16. Hidayat, S., et al., *The interaction of alpha-mangostin and its derivatives against main protease enzyme in COVID-19 using in silico methods.* J Adv Pharm Technol Res, 2021. **12**(3): p. 285-290.
17. ANM Ansori, V.K., AA Parikesit, FA Dian, RT Probojati, M Rebezov, P Scherbakov, P Burkov, G Zhdanova, A Mikhalev, Y Antonius, MRF Pratama, NI Sumantri, TH Sucipto, and R Zainul, *Bioactive Compounds from Mangosteen (Garcinia mangostana L.) as an Antiviral Agent via Dual Inhibitor Mechanism against SARSCoV- 2: An In Silico Approach.* Pharmacognosy Journal, 2022. **14**(1): p. 85-90.
18. Singh, R., et al., *Protease Inhibitory Effect of Natural Polyphenolic Compounds on SARS-CoV-2: An In Silico Study.* Molecules, 2020. **25**(20).
19. Aanouz, I., et al., *Moroccan Medicinal plants as inhibitors against SARS-CoV-2 main protease: Computational investigations.* J Biomol Struct Dyn, 2021. **39**(8): p. 2971-2979.
20. Ehab M. Mostafa, M.G., Mohammed M. Ghoneim, Shaimaa Hussein, Ahmed H. El-Ghorab, Mohamed A. Abdelgawad2, Arafa Musa., *Repurposing of FDA Approved Alkaloids as COVID 19 Inhibitors; in silico Studies.* Pharmacognosy Journal, 2021. **13**(1): p. 110-123.
21. Wang Z, W.D., Belecciu T, Eaves J, Bachmann M, *Phytochemical Drug Discovery for COVID-19 Using High-resolution Computational Docking and Machine Learning Assisted Binder Prediction.* ChemRxiv, 2022. **Version 2**.
22. Vidoni, C., et al., *Targeting autophagy with natural products to prevent SARS-CoV-2 infection.* J Tradit Complement Med, 2022. **12**(1): p. 55-68.
23. Kulkarni, S.A., et al., *Computational evaluation of major components from plant essential oils as potent inhibitors of SARS-CoV-2 spike protein.* J Mol Struct, 2020. **1221**: p. 128823.
24. Bormann, M., et al., *Turmeric Root and Its Bioactive Ingredient Curcumin Effectively Neutralize SARS-CoV-2 In Vitro.* Viruses, 2021. **13**(10).
25. Mulu, A., et al., *The impact of curcumin derived polyphenols on the structure and flexibility COVID-19 main protease binding pocket: a molecular dynamics simulation study.* PeerJ, 2021. **9**: p. e11590.
26. Umeoguaju, F.U., et al., *Plant-Derived Food Grade Substances (PDFGS) Active Against Respiratory Viruses: A Systematic Review of Non-clinical Studies.* Front Nutr, 2021. **8**: p. 606782.
27. Ali, F., et al., *Implication of in silico studies in the search for novel inhibitors against SARS-CoV-2.* Arch Pharm (Weinheim), 2022. **355**(5): p. e2100360.
28. ShuoWang, et al., *Chloroform extract from Sophora Tonkinensis Gagnep. inhibit proliferation, migration, invasion and promote apoptosis of nasopharyngeal carcinoma cells by silencing the PI3K/AKT/mTOR signaling pathway.* J Ethnopharmacol, 2021. **271**: p. 113879.
29. Kumar, V., et al., *Withanone and Withaferin-A are predicted to interact with transmembrane protease serine 2 (TMPRSS2) and block entry of SARS-CoV-2 into cells.* J Biomol Struct Dyn, 2022. **40**(1): p. 1-13.
30. Yuan, S., et al., *SARS-CoV-2 exploits host DGAT and ADRP for efficient replication.* Cell Discov, 2021. **7**(1): p. 100.