CHEMISTRY & BIODIVERSITY

Accepted Article

Title: The Efficacy of Traditional Medicinal Plants in Modulating the Main Protease of SARS-CoV-2 and Cytokine Storm

Authors: Jomin Choe, Phaik Har Yong, and Zhi Xiang Ng

To be cited as: *Chem. Biodiversity* **2022**, e202200655

Link to VoR: https://doi.org/10.1002/cbdv.202200655

10.1002/cbdv.202200655

Chem. Biodiversity

Review

The Efficacy of Traditional Medicinal Plants in Modulating the Main Protease of SARS-CoV-2 and Cytokine Storm

Jomin Choe,[a] Phaik Har Yong,[b] and Zhi Xiang Ng*,[a]

[a] School of Biosciences, Faculty of Science and Engineering, University of Nottingham Malaysia, 43500 Selangor, Malaysia,
e-mail: zhixiang.ng@nottingham.edu.my

[b] School of Bioscience, Faculty of Medicine, Bioscience and Nursing, MAHSA University, 42610 Selangor, Malaysia

Selected traditional medicinal plants exhibit therapeutic effects in coronavirus disease (Covid-19) patients. This review aims to identify the phytochemicals from five traditional medicinal plants (*Glycyrrhiza glabra, Nigella sativa, Curcuma longa, Tinospora cordifolia* and *Withania somnifera*) with high potential in modulating the main protease (Mpro) activity and cytokine storm in Covid-19 infection. The Mpro binding affinity of 13 plant phytochemicals were in the following order: Withanoside II > withanoside IV > withaferin A > α-hederin > withanoside V > sitoindoside IX > glabridin > liquiritigenin, nigellidine > curcumin > glycyrrhizin > tinocordiside > berberine. Among these phytochemicals, glycyrrhizin, withaferin A, curcumin, nigellidine and cordifolioside A suppressed SARS-CoV-2 replication and showed stronger anti-inflammatory activities than standard Covid-19 drugs. Both preclinical and clinical evidences supported the development of plant bioactive compounds as Mpro inhibitors.

Keywords: anti-inflammation • anti-viral • Covid-19 • herbs • phytochemical

1. Introduction

Coronavirus disease (Covid-19) is a human respiratory complication caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. SARS-CoV-2 is an airborne virus that infects humans through the nose, eyes, or mouth.[2] Long term SARS-CoV-2 infection may result in myocardial inflammation, acute respiratory disease syndrome (ARDS), pneumonia, organ failure and death.[3] The cysteine protease of SARS-CoV-2 virus is commonly known as the main protease (Mpro) (PBD ID: 6LU7) or 3-chymotrypsin-like cysteine protease that regulates viral maturation, replication, and gene transcription.[4] The viral activity could be suppressed by inhibiting the Mpro action, leading to the suppression of virions released from human host cells.[5] Hyperinflammation is a common complication of SARS-CoV-2 infection due to excessive human immune response against the virus via the expression of pro-inflammatory factors such as interleukins (IL)-2, 6, 8, 10, 1β, tumor necrosis factors (TNF), and interferons (IFN).[6] The overproduction of these pro-inflammatory cytokines and chemokines during hyperinflammation may ultimately leads to cytokine storm.[2]

The current Covid-19 treatment including the use of antiviral, anti-inflammatory, and immunomodulatory drugs. However, none of them can effectively halt the viral infection.[7] For instance, nelfinavir and lopinavir are typical standard drugs for Mpro 6LU7 while N3 is a computer designed native ligand with high stability and binding affinity (-8.37 kcal/mol) towards 6LU7 of Mpro.[8,9] However, the Mpro docking value of N3 is lower than nelfinavir and lopinavir.[8,10] Anti-inflammatory drugs like tocilizumab is mainly used on ARDS patients to inhibit the production of pro-inflammatory cytokine IL-6.[11,12]

The therapeutic role of medicinal plants is mostly attributed to their rich source of bioactive components including the polyphenols, saponins and alkaloids with pharmacological effects.[13,14] Selected bioactive compounds such as flavonoids, proanthocyanidins, alkaloids, and terpenoids with antiviral and immunomodulatory effects are believed to play a potential role in combating Covid-19.[13,15] Although several medicinal plants such as *Glycyrrhiza glabra, Nigella sativa, Curcuma longa, Tinospora cordifolia and Withania somnifera* have been previously demonstrated to attenuate Covid-19 infection, their interaction with Mpro of SARS-CoV-2 remains to be elucidated.[16] Besides that, scientific evidences on the role of natural bioactive compounds in combating cytokine storm are still lacking. Hence, this study aimed to review and explore the bioactive profile of *G. glabra, N. sativa, C. longa, T. cordifolia and W. somnifera* to determine their inhibitory potential against Mpro protease and cytokine storm in Covid-19 patients.

Jomin Choe is an emerging researcher with Biotechnology Honours degree from University of Nottingham Malaysia. Her research interest falls in the medical and pharmaceutical biotechnology field. She works in the research and development sector, in particular with CryoCord Sdn Bhd (Malaysia) on stem cell research for the treatment of multiple human diseases.

Chem. Biodiversity

Dr Phaik-Har Yong is a lecturer cum researcher in the School of Bioscience, Faculty of Medicine, Bioscience and Nursing, MAHSA University Malaysia. Her research interest relates to the screening of phyto-constituents for various enzyme inhibition and anti-oxidant activities in traditional medicinal plants.

Dr Zhi-Xiang Ng is an Assistant Professor in the School of Biosciences, University of Nottingham Malaysia. He is a Fellow of Advanced Higher Education (FHEA, UK), a member of Malaysian Scientific Association (MSA, Malaysia), Society of Chemical Industry (SCI, London, UK) and International Society of Global Health (ISoGH, Edinburgh, UK). His research interest relates to both human nutrition and health that fall into three broad areas: food sciences, molecular nutrition, metabolic diseases risk factors and biomarkers.

2. Methodology

Literature review was performed with original research articles published between June 2003 and June 2022 in four electronic databases, namely the Scopus, Ovid Medline, Web of Science and Google scholar. Specific keywords were used in the literature search including "*Curcuma longa"* OR "Turmeric", "*Glycyrrhiza Glabra"*, "*Nigella Sativa",* "*Withania somnifera*", "*Tinospora cordifolia"*, "pharmacology", "covid", "Covid-19" OR "Sars-Cov-2", "Mpro" OR "main protease", "phytochemicals", and "cytokine storm". The articles were screened for their titles and abstracts with the following inclusion criteria: Medicinal plants with phytochemicals and pharmacological activities related to Mpro, cytokine storm and Covid-19 as well as articles published in English language. Out of 315 shortlisted articles, 95 articles were included in this review.

3. Results and Discussion

3.1. Pathogenesis of SARS-CoV-2 and current treatment for Covid-19 Patients

Mpro of SARS-CoV-2 is a key enzyme that regulates viral replication and transcription.[8] It cleaves the polyproteins which are translated from the viral ribonucleic acid (RNA) of SARS-CoV-2 in order to form active viral proteins.[17] Due to its rate-limiting characteristic, the enzyme has been recognized as an attractive drug target site for Covid-19 patients.[10]

Cytokine storm occurs when both the innate and adaptive human immunity systems are triggered by SARS-CoV-2.[18] A sudden surge of pro-inflammatory cytokines overwhelms the human body, leading to organ failure, ARDS and death.[19] Cytokine storm is the major factor that contributes to the high mortality rate in Covid-19 patients.[18] Therefore, early detection of Covid-19 infection may prevent the escalation of cytokine storm via common immunological interventions involving the use of IL-6 antagonists, immunosuppressive drugs, and corticosteroids.[18]

The replication and maturation of SARS-CoV-2 could be prevented by inhibiting the Mpro action.[4] Several phytochemicals could bind to Mpro and destabilize the protein with incorrect protein folding, leading to the stoppage of viral replication.[20] The inhibition of Mpro also prevents the upscaling of cytokine storm. It has been shown that a reduction of viral replication may result in a lower secretion of uncontrolled pro-inflammatory cytokines in Covid-19 patients.[21] The suppression of inflammatory events during cytokine storm may reduce the Covid-19 patients' risk of ARDS development, organ failure and death.[3] Therefore, the prognosis of Covid-19 patients could be modulated by managing the outcome of Mpro action and cytokine storm.

3.2 *G. glabra*

3.2.1. *Geographical distribution, traditional application, and toxicity*

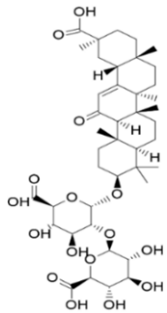
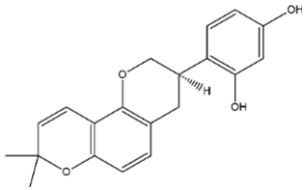
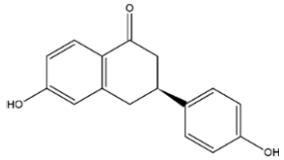
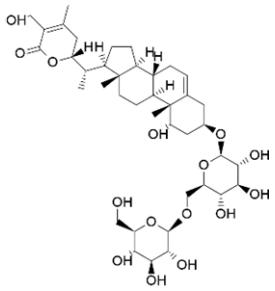
G. glabra is native to Mediterranean region and Asia.[22] It belongs to Fabaceae family and is commonly known as licorice or mulaithi. In folk medicines, *G. glabra* is considered as a prophylaxis for duodenal ulcers, gastric disorders, and dyspepsia while its essential oil is commonly used to manage hemorrhage, rheumatism, and diarrhea.[23] Due to the natural origin of *G. glabra,* it shows low toxicity and is considered relatively safer than most antiviral drugs.[21] However, there were cases where patients experienced mild gastrointestinal reaction, hypertension, hypokalemia, or fluid retention after consuming high doses of *G. glabra* extract.[21,23] *In silico* analysis revealed that *G. glabra* had good absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties, suggesting the plant's potential for further investigation as SARS-CoV-2 inhibitors.[24]

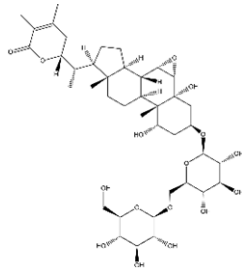
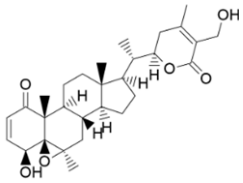
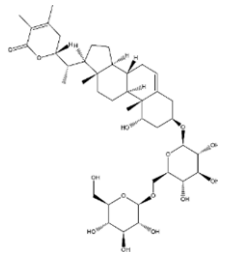
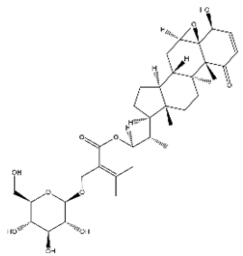
3.2.2. *Phytochemicals*

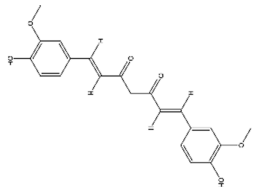
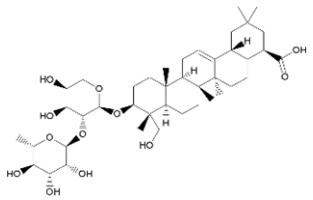
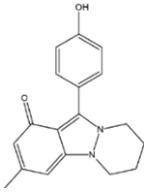
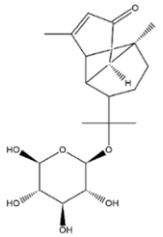
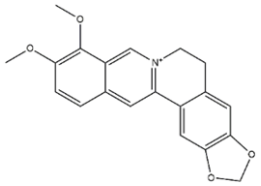
G. glabra composed of flavonoids, saponins, anthocyanin, tannin terpenoids, alkaloids, phlobatannin, anthraquinones, glycosides, and sterols.[25] The presence of alkaloids, saponin and flavonoids confers anti-bacterial and antioxidant activities in the plant.[22] Among the phytochemicals, three major bioactive compounds, namely the glycyrrhizin, glabridin and liquiritigenin (Table 1a-c) showed Mpro inhibitory and cytokine storm modulatory effect.

Chem. Biodiversity

Table 1. Selected plant phytochemicals with anti-viral potential

Structure	Phytochemical	PubChem CID	Sources	Characteristics
	(a) Glycyrrhizin	14982	Rhizome of *Glycyrrhiza glabra*[26]	• Anti-inflammation [27] • Binded to SARS-CoV-2 Mpro through hydrogen bonding and hydrophobic interactions[28] • Blocked SARS-CoV-2 replication by inhibiting Mpro at high concentration [29] • Better bioactive profile for drug candidate when compared to glabridin and liquiritigenin[29]
	(b) Glabridin	124052	Rhizome of *Glycyrrhiza glabra*[26]	• Formed strong hydrogen bonding at the active site of Mpro[26] • Boosted immunity in human host[26] • Evidence of anti-viral activity against SARS-CoV-2[26]
	(c) Liquiritigenin	114829	Rhizome of *Glycyrrhiza glabra*[26]	• Inhibited Mpro catalytic activity through molecular interaction[26] • Boosted immunity in human host[26] • Evidence of anti-viral activity against SARS-CoV-2[26]
	(d) Withanoside IV	71312551	Roots of *Withania somifera*[30]	• Interacted with amino acid residues in the active site of Mpro[31] • Strong binding affinity towards Mpro[31]

	(e) Withanoside II	101168811	Roots of *Withania somifera*[30]	• Interacted with amino acid residues in the active site of Mpro[31] • Strong binding affinity towards Mpro[31]
	(f) Withaferin A	265237	Leaves of *Withania somnifera*[30]	• Blocked SARS-CoV-2 viral entry into human host cells and reduced viral activity with computational docking analysis[32] • Exhibited antiviral effect by demonstrating strong binding affinity towards the active site of Mpro[33]
	(g) Withanoside V	10700345	Roots of *Withania somnifera*[30]	• Formed highly stable hydrogen-bonding at the active site of Mpro[15] • Shared similar function as native ligand N3 inhibitor[15]
	(h) Sitoindoside IX.	189586	Roots of *Withania somnifera*[30]	• Strong binding affinity towards Mpro[34] • Interacted with amino acid residues in Mpro[34]

	(i) Curcumin	969516	Rhizomes of *Curcuma longa*[35]	• Good chemical absorption, distribution, metabolism, excretion, and toxicity profile for the development of Covid-19 drug[36] • Possessed high Mpro inhibitory effect via covalent bonding with the Mpro protein residue[36]
	(j) α-hederin	73296	Seeds of *Nigella sativa*[37]	• Strongly interacted with SARS-CoV-2 Mpro via four hydrogen bondings[38] • Showed superior Mpro binding affinity than synthetic drugs[37]
	(k) Nigellidine	136828302	Seeds of *Nigella sativa*[39]	• Interacted with the amino acid residues in Mpro via three hydrogen bondings[37] • Strong binding affinity towards Mpro[37]
	(l) Tinocordiside	177384	Stem of *Tinospora cordifolia*[40]	• Strongly interacted with Mpro[41] • Control viral entry and replication in the host cell[41] • Well-known for its immunomodulatory effect[41]
	(m) Berberine	2353	Stem of *Tinospora cordifolia*[40]	• Docked significantly to Mpro[41] • Regulated Mpro activity and inhibited SARS-CoV-2 viral activity[42]

Mpro: main protease, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

Other phytochemicals in *G. glabra* such as β glycyrrhetinic acid, isoliquiritigenin, and ursolic acid have also been demonstrated to suppress the expression of pro-inflammatory cytokines in humans.[43]

3.2.3. *Regulation of Mpro and cytokine storm*

The Mpro binding affinity of glycyrrhizin or glycyrrhizic acid (-7.3 kcal/mol) was comparable to the standard drug nelfinavir (-10.72 kcal/mol) and lopinavir (−9.41 kcal/mol) (Figure 1).[9,26] Glabridin and liquiritigenin bound to Mpro at -8.0 kcal/mol and -7.7 kcal/mol, respectively with AutoDock 4.2 model (Figure 1).[26] Apart from binding to Mpro, *G. glabra* also inhibited SARS-related virus replication by blocking the virus entry into the human host cells.[44] It has been demonstrated that 1.6 mg/mL of glycyrrhizin completely suppressed the Mpro that regulated the adsorption, penetration, and replication of SARS-CoV-2.[29] The antiviral effect of glycyrrhizin against SARS infection has been confirmed via the reduction of SARS-CoV-2 RNA expression in Vero E6 cells treated with glycyrrhizin.[29,45] In addition, glycyrrhizin could relieve Covid-19 symptoms with its liver-protective effect and the suppression of airway mucus hypersecretion.[46] Although *in silico* simulation model is commonly used to evaluate the docking value of newly designed compounds,[47] *in vivo* and clinical experimental models are still required to confirm the antiviral potential of *G. glabra*.[47]

The pharmacological activities of *G. glabra* including anti-oxidation, anti-inflammatory, anti-tussive, anti-cancer, anti-viral, cyto-protection and immunomodulation.[28,48] The anti-inflammatory action of *G. glabra* is mainly attributed to its ability to suppress oedema in inflammatory events associated with myocardial and lung diseases.[23,27] A recent study has reported that β glycyrrhetinic acid, isoliquiritigenin, and ursolic acid from *G. glabra* inhibited gene expression of an inflammatory factor, TNF-α, in mice with cytokine storm complication.[43] Glycyrrhizin from *G. glabra* also attenuated the production of inflammatory cytokines such as TNFα, IL-1β and IL-6 by inhibiting the toll-like receptor 4/nuclear factor kappa light chain (TLR-4/NF-κB) signaling pathway, leading to a reduction in viral replication activity.[46,49] The anti-inflammatory role of *G. glabra* has been proven to be effective in managing inflammatory diseases with *in vitro* and *in vivo* models.[50] For instance, Fiore et al. [51] have showed that SARS-CoV-2 patients who are treated with *G. glabra* displayed lower viral load and higher survival rate when compared to that of placebo group. The above evidence supports the potential anti-inflammatory role of *G. glabra* in attenuating the progression of Covid-19 infection.

3.3. *W. somnifera*

3.3.1. *Geographical distribution, traditional application, and toxicity*

W. somnifera or Ashwagandha is a medicinal plant commonly known as winter cherry or Indian Ginseng under the Solanaceae family which grows in sub-tropical regions.[30] This medicinal plant has long been used in the Ayurvedic and Sidha medicinal system to manage chronic diseases such as tuberculosis and upper respiratory diseases.[30,52] *W. somnifera* is traditionally used to improve human physical and psychological well-being.[53] The plant has been recently determined to be non-toxic and safe for human consumption.[15] Since Verma and Kumar[53] have previously reported that *W. somnifera* could improve the effectiveness of modern drugs with fewer side effects, the medicinal plant is viewed as a repurposed drug for managing Covid-19 patients.[15]

3.3.2. *Phytochemicals*

The main phytochemical constituents of *W. somnifera* include alkaloids, steroidal lactones, tannin, saponins and flavonoids.[52] Among these phytochemicals, withanone, withanoside, sitoindoside and withaferin (Table 1d-h) have been proposed as potential inhibitors for Mpro in SARS-CoV-2.[31] The phytochemicals of *W. somnifera* showed a strong binding affinity towards Mpro active site and inhibited the viral activity in the host cell.[31] Nevertheless, the effectiveness of *W. somnifera* as Mpro SARS-CoV-2 inhibitor could be enhanced by combining all the above phytochemicals in drug formulation.[32]. The phytochemicals from *W. somnifera* have a non-toxic and safe ADMET profile as well as drug-like quality, suggesting that they could serve as Covid-19 medication.[15]

3.3.3. *Regulation of Mpro and cytokine storm*

The molecular docking scores of five phytochemicals isolated from *W. somnifera* against Mpro, namely the withanoside II, withanoside IV, withaferin A, withanoside V, and sitoindoside IX (Table 1d-h) have shown decreased in the following order: -11.30 kcal/mol > -11.02 kcal/mol > -9.83 kcal/mol > -8.96 kcal/mol > -8.37 kcal/mol (Figure 1).[31,33] The docking scores were performed by using molecular dynamics simulation model which showed the types and the number of interactions between the phytochemicals and Mpro.[31]

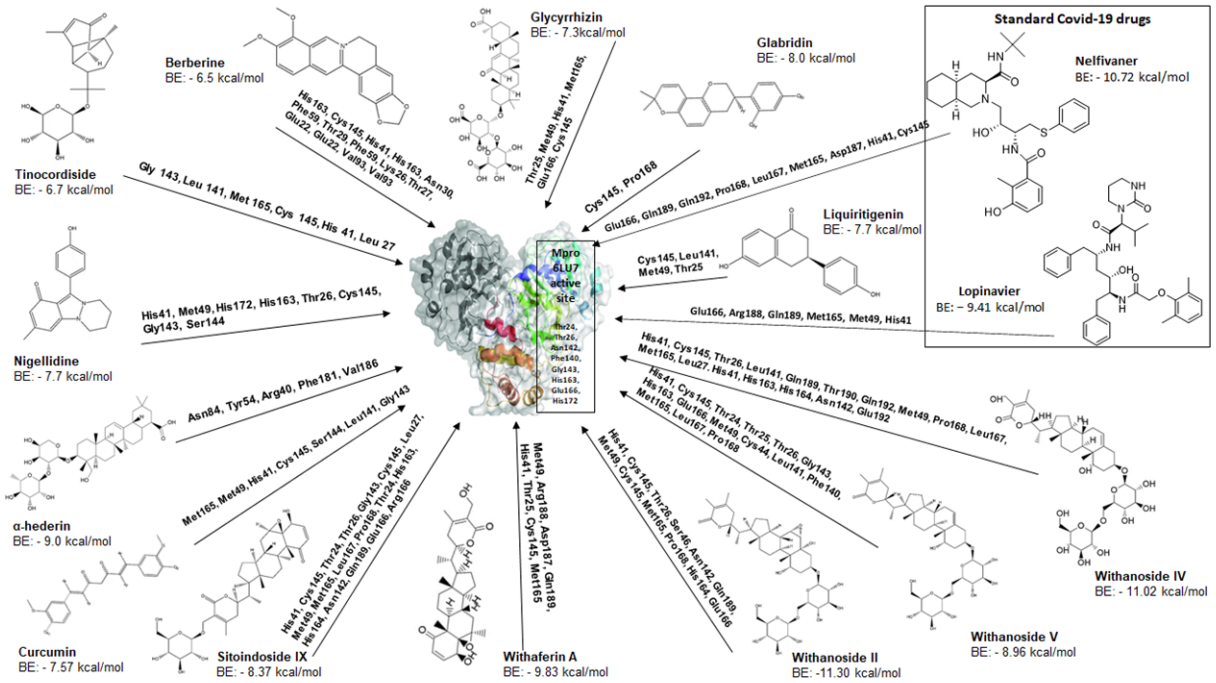


Figure 1: Selected phytochemicals and standard covid-19 drugs that interact with the amino acid residues in Mpro active site. Arg: arginine, Asn: asparagine, Asp: aspartate, BE: binding energy, Cys: cysteine, Glu: glutamate, Gln: glutamine, Gly: glycine, His: histidine, Leu: leucine, Lys: lysine, Met: methionine, Mpro: main protease, Phe: phenylalanine, Pro: proline, Ser: serine, Thr: threonine, Ytr: tyrosine, Val: valine

Table 2: *In silico*, *in vivo* and *in vitro* findings of phytochemical interaction with Mpro of SARS-CoV-2.

Phytochemicals	*In silico* study	*In vitro* study	*In vivo* study
Glycyrrhiza glabra			
Glycyrrhizin	A docking analyses on the inhibitory potential of licorice against viral Mpro showed that the key residues in Mpro active site strongly interacted with the glycyrrhizin compounds.[47]	Glycyrrhizin had antiviral effect on SARS associated viruses' replication in Vero cells.[45] Antiviral activity assay with *in vitro* Vero E6 cells effectively prevented SARS-CoV-2 cellular proliferation at a concentration of 0.5 mg/mL.[29]	-
Glabridin	Autodock 4.2 method showed that glabridin, when bounded to 6LU7 protease site, could obstruct viral replication. [26]	-	-
Liquiritigenin	Autodock 4.2 method showed that when liquiritigenin bounded to 6LU7 protease site, it could likely obstruct viral replication.[26]	-	-
Withania somnifera			
Withanoside IV	Docking study showed that the binding energy of withanoside IV was significantly higher than those of standard drugs.[31]	-	-
Withanoside V	Docking study found that withanoside V could serve as powerful Mpro inhibitor with its antiviral properties.[31]	-	-
Withanoside II	Docking study revealed that the binding energy of withanoside II was	-	-

	higher than those of standard drugs, but withanoside IV has stronger binding energy to the active site of Mpro.[31]		
Withaferin A	Molecular docking tool showed that withaferin A exhibited antiviral activity by inhibiting the Mpro or via the blocking of host cell receptor GRP78.[33]	Attenuation of side effects in pulmonary fibrosis by reducing the inflammatory cytokine responses which had been proven to be useful for Covid-19 treatment.[54] -	Improved side effects of pulmonary fibrosis by reducing inflammatory cytokine responses which had been proven useful for Covid-19 treatment.[54] Through *in vivo* studies, withaferin A was shown to possess anti-inflammatory ability by reducing the inflammation in rats.[55]
Sitoindoside IX	Molecular dynamics simulation study revealed a high docking energy towards Mpro.[31]	An Ayurvedic formulation, NOQ19 which contained *W. somnifera* herb displayed potent antiviral activity against SARS-CoV-2 in Vero E6 cell line.[56]	-
Curcuma longa			
Curcumin	Molecular docking study on Mpro receptor showed that curcumin could act as a covalent inhibitor, suggesting its potential to be used in Covid-19 treatment.[36]	*C. longa* could modulate the NF-kB/MAPK and RIG-1/STAT-1/2 signaling pathways and suppress the expression of inflammatory cytokines.[57] Curcumin effectively neutralized SARS-CoV-2 action in Vero E6 and Calu-3 cells.[58]	Covid-19 treatment with curcumin has been proven to be beneficial due to its antiviral, antiemetic antinociceptive, anti-inflammatory, antiapoptotic, antifibrotic, and antipyretic effects in animal models such as rats and mice. Apart from inhibiting cytokines and chemokines, curcumin also reduced myalgia and fatigue in mice.[35] Curcumin attenuated inflammation which may halt Covid-19 progression in animal models.[58]
Nigella sativa			
α-hederin	Molecular docking study suggested α-hederin showed high binding affinity towards Mpro.[37]	-	-
Nigellidine	Molecular docking study showed that nigellidine exhibited high binding affinity towards Mpro. [37]	-	-
Tinospora cordifolia			
Tinocordiside	Numerous computational analyses suggested that tinocordiside closely interacted with viral Mpro.[15,33]	-	-
Berberine	Berberine could suppress Mpro protein's activity and prevented viral replication.[59]	-	Recent research has shown that berberine and its derivatives showed therapeutic potential, particularly against viral entrance and reproduction, while preventing virally induced inflammatory reactions.[60]

Mpro: Main protease, NF-kB/MAPK: Nuclear factor kappa B/Mitogen-activated protein kinase, RIG-1/STAT: retinoic acid-inducible gene I/signal transducer and activator of transcription, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

Interestingly, the Mpro binding affinity of withanoside II and IV were stronger than standard drugs nelfinavir (-10.72 kcal/mol) and lopinavir (−9.41 kcal/mol).[9] When these plant bioactive compounds were combined with other drugs like dexamethasone, they could potentially improve the effect of Covid-19 drug treatment.[34] *In vivo* and *in vitro* studies (Table 2) on the binding affinity of *W. somnifera* towards Mpro are currently unavailable for further analysis.[34]

Apart from regulating cell apoptosis and homeostasis of human immune system, *W. somnifera* also exhibited anti-inflammation, anticancer, anti-tumorigenic, anti-angiogenic, anti-invasive, antiviral, antioxidant, and immunomodulatory activities.[34] The anti-inflammatory effect of *W. somnifera* has been demonstrated by its ability to reduce the secretion of cytokines such as IL-2, IL-6, TNF-α, interferon-gamma (IFN-γ), IFN- γ protein 10 (IP-10) in Covid-19 patients through the confirmation by *in vivo* and *in vitro* models.[42] On the contrary, long-term administration of withaferin A suppressed the pro-inflammatory factor NF-κB which subsequently resulted in the immunosuppression of human islets.[61] Several experimental models have demonstrated that withaferin A suppressed IL-1β and IL-6 while alleviating cytokine storm symptoms.[32] Since *W. somnifera* could alleviate oxidative stress caused by papain-like proteases during lung fibrosis, this study suggests that the medicinal plant may play a significant role in alleviating the cytokine storm in Covid-19 patients due to its prophylactic action against inflammatory diseases.[32,62]

3.4. *C. longa*

3.4.1. *Geographical distribution, traditional application, and toxicity*

C. longa is a member of the Zingiberaceae family which is native to South Asia.[63] The plant is widely known as turmeric with multiple health benefits, including the roles as antacid, spice, food ingredient and color dye.[64] *C. longa* also possesses neuroprotective effect against Parkinson's and Alzheimer's diseases.[65] The plant has been traditionally used to manage biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis.[63] Since most bioactive compounds derived from *C. longa* are non-hepatotoxic, non-mutagenic and non-carcinogenic, *C. longa* is considered safe which was supported by the absence of side effects in humans who receiving a high dosage of *C. longa* extract and its phytochemical curcumin (Table 1i).[64,65]

3.4.2. *Phytochemicals*

C. longa contains phytochemicals with functional activity such as flavonoids, coumarins, steroids, saponins, tannin and phenols.[66] It has been speculated that *C. longa* may play a role in Covid-19 management due to the action of polyphenolic compounds in boosting human immune system while inhibiting the proliferation of SARS-CoV-2.[67] Among these phytochemicals, curcumin (Table 1i) which is mainly found in the rhizomes, may serve as a natural ingredient for Covid-19 treatment due to its strong interaction with Mpro of SARS-CoV-2.[35] The ADMET characteristics of curcumins have further confirmed that they may be deemed potential as drug-like compounds.[68]

3.4.3. *Regulation of Mpro and cytokine storm*

The past molecular docking studies have demonstrated that the Mpro binding affinity of *C. longa* compounds ranged from -7.04 kcal/mol to -9.08 kcal/mol.[69] Five types of *C. longa* constituents have been discovered to bind tightly to Mpro of SARS-CoV-2.[69] Garg et al.[20] have reported that the Mpro binding score of curcumin was -7.57 kcal/mol (Figure 1). The Autodock 4.2 model was used to obtain the molecular docking scores.[20] The interaction of curcumin with Mpro via covalent and hydrogen bonding showed increased stability over time through CovDock.[36] Curcumin also displayed Van der Waals forces, hydrophobic and pi-based interaction with other proteins at the active site of Mpro.[70] Although the binding of curcumin to Mpro was not as strong as those of standard drugs nelfinavir (-10.72 kcal/mol) and lopinavir (−9.41 kcal/mol), its fairly good ADMET parameters makes it as a potential Covid-19 treatment agent.[9,36] More *in vitro* and *in vivo* studies are required to confirm the docking results,.

The current notable pharmacological effect of *C. longa* against Covid-19 symptoms include anti-nociceptive, anti-emetic, anti-pyretic, anti-fatigue, anti-apoptotic, anti-fibrotic, anti-inflammatory and antioxidant activities.[35] Clinical studies have demonstrated that *C. longa* inhibited the expression of toll-like receptors, NF-κB, inflammatory cytokines, chemokines, and bradykinin, which indirectly reduced oxidative stress and other Covid-19 symptoms.[71] Curcumin from *C. longa* has been demonstrated to suppress inflammation in mice infected with *Staphylococcus aureus* by downregulating macrophage inflammatory proteins 2, TNF-α, transforming growth factor beta (TGF-β), and IL-6 (Table 2).[35] Another *in vitro* study found that curcumin suppressed the production of proinflammatory cytokines, including TNF-α, IFN-α,

interferon-gamma-induced protein 10 and IL-6 in influenza A virus-infected human macrophages via NF-κB pathway.[54] As the ability of curcumin in regulating the NF-κB signaling pathway has been validated via both *in vitro* and *in vivo* studies, this phytochemical shows great potential as an alternative ingredient to manage cytokine storm in Covid-19 patients.[72]

3.5. *N. sativa*

3.5.1. *Geographical distribution, traditional application, and toxicity*

N. sativa is an indigenous Mediterranean plant native to Southeast Asia.[39] The medicinal plant belongs to the family of Ranunculaceae and it is commonly known as black seed or black cumin. The effectiveness of *N. sativa* seed extract has been previously reported against asthma, allergies, arthritis, and inflammatory diseases.[73] Since *N. sativa* is traditionally used to manage respiratory diseases and endothelial dysfunction, the plant has been suggested to play a therapeutic role in Covid-19 patients with similar lung complications.[39] Along with its excellent solubility, gut absorption, and drug-likeness profile, this plant is a promising source for future structural optimization to create more powerful derivatives for Covid-19 drugs.[74] Due to its immunomodulatory effect and low toxicity, *N. sativa* could be a good alternative source to manage inflammatory diseases. [38,73]

3.5.2. *Phytochemicals*

The major phytochemicals found in *N. sativa* including terpenoids, saponins, flavonoids, and alkaloids.[75] The role of *N. sativa* as herbal medicine is mainly attributed to the wide range of phytochemicals with antioxidant, anti-inflammatory, anti-hypertensive, anti-cancer, immunomodulatory, anti-bacterial and hepatoprotective effects.[73] Among the various phytochemicals, nigellidine and α-hederin (Table 1j-k), have been previously identified as potential alternative sources for Covid-19 treatment due to their strong Mpro binding affinities.[37]

3.5.3. *Regulation of Mpro and cytokine storm*

The binding affinities of α-hederin and nigellidine towards the active site of Mpro were -9.0 kcal/mol and -7.7 kcal/mol, respectively (Figure 1).[37] These results were obtained using Autodock vina 1.1.2 in PyRx 0.8 system.[37] Although standard Covid 19 drugs lopinavir (-10.72 kcal/mol) and nelfinavir (-9.41 kcal/mol) displayed higher Mpro binding affinities than these phytochemicals, α-hederin is a likely Mpro inhibitor candidate for Covid-19 treatment due to its close interaction with Mpro enzyme in clinical trial.[38] Further *in vivo* studies with different experimental models are essential to validate the antiviral effect and support the relatively high binding strength of both phytochemicals to Mpro.[37]

In vivo and *in vitro* models showed that α-hederin exhibited antihistaminic, anti-eosinophilic, antileukotrienes, anti-immunoglobulin characteristics by reducing numerous pro-inflammatory cytokines (Table 2).[76] *N. sativa* and its metabolites could induce antioxidation, anti-inflammation and immunomodulation which may play crucial roles in COVID-19 pathophysiology treatment. Many COVID-19 comorbidities, inclusive of diabetes, cardiovascular diseases, rheumatoid arthritis, and a range of bacterial and viral infections, responded well to supplementation with *N. sativa* seed and/or oil.[76] Molecular docking studies have demonstrated that nigellidine could bind to receptor IL1R at -6.23 kcal/mol and attenuated inflammatory cytokines as well as SARS-CoV-2 viral activity.[73] Nigellidine also attached to the active site of inflammatory cytokines (IL-6 and IL-1) and reduced the pathogenesis of H9N2 virus.[73] A clinical trial has reported that *N. sativa* could stabilize a patient's cytokine profile throughout Covid-19 treatment.[50] Besides, *N. sativa* could act as a bronchodilator and oxidative stress reliever to alleviate Covid-19 symptoms among the patients.[77] Further clinical studies on the bioavailability of *N. sativa* extract may ascertain the effectiveness of *N. sativa* in Covid-19 treatment.

3.6. *T. cordifolia*

3.6.1. *Geographical distribution, traditional application, and toxicity*

T. cordifolia is a plant species under Menispermaceae family.[40] This medicinal plant is commonly found in Asia where it is widely known as Guduchi, Giloy or heart-shaped moonseed.[78] *T. cordifolia* plays an important part in Ayurvedic folk and tribal medicines where the whole plant has been documented to manage asthma, fever, diabetes, stomach ache, jaundice, rheumatism, urinary problems, cough, allergies, inflammation, prolonged diarrhea, anemia, and dysentery.[40,79] The medicinal plant also showed low toxicity and has been claimed to be safe for repurposing into Covid-19 therapeutic agent.[15]

3.6.2. *Phytochemicals*

The major phytochemicals detected in *T. cordifolia* mainly comprise glycosides, steroids, alkaloids, lignans, phenolics,

aliphatic compounds, sesquiterpenoid and diterpenoid lactones.[80] The plant is well-known for its immunomodulatory effect due to the presence of tinocordioside, cordifolioside A, magnoflorine, and syringin.[41] It was interesting to find out that berberine, iocolumbin, magnoflorine and tinocordiside from *T. cordifolia* could bind to SARS-CoV-2 proteins and prevented the virus attachment and replication in human host cells.[55] Besides, magnoflorine also displayed anti-cytokine storm potential.[81] Based on the above finding, it is pertinent to suggest *T. cordifolia* with immunomodulatory activities as a likely candidate to prevent oxidative damage and increase immune homeostasis response in Covid-19 patients.[82] The phytochemicals from *T. cordifolia* which exhibited drug-like effect is considered safe for human consumption.[15]

3.6.3. *Regulation of Mpro and cytokine storm*

The pharmacological activities of *T. cordifolia* against Covid-19 include immunomodulation, antioxidant, anti-hyperglycemia, anti-inflammation and anti-hepatotoxicity.[15] Previous molecular docking and *in silico* simulation studies have demonstrated that the Mpro binding affinity of tinocordiside and berberine from *T. cordifolia,* (Table 1l-m) were -6.7 kcal/mol, -6.5 kcal/mol, respectively (Figure 1).[77] The docking protocol was based on the autodock vina.[82] Although the Mpro binding affinity of these phytochemicals were weaker than the standard antiviral drugs nelfinavir (-10.72 kcal/mol) and lopinavir (−9.41 kcal/mol), their reliable origin and drug-like properties made them a likely drug candidate to combat Covid-19 disease.[9,42] However, more *in vivo* and *in vitro* studies are needed to validate the molecular docking results.

T. cordifolia extract has been previously reported to suppress pro-inflammatory cytokines (IL-1β, IL-6, IL-17, TNF-α) in macrophages, arthritis and neuroinflammatory animal models.[42] Berberine could inhibit several signaling pathways in different parts of the virus cycle, leading to the inhibition of pro-inflammatory cytokines secretion.[56] In addition, it has been proven to inhibit viral replication of various types of viruses including the influenza virus.[56] Among the various phytochemicals, the immunomodulatory effect of cordifolioside A was stronger than berberine due to its interaction with TGF-β and TNF-α receptors.[81] Although its binding affinity to Mpro was quite low, cordifolioside A has high potential in modulating the cytokine storm event in Covid-19 patients.[82] The discovery of anti-inflammatory role in *T. cordifolia* extract via the angiogenic animal model provides further evidence for the anti-inflammatory and anti-angiogenic potential of *T. Cordifolia* (Table 2).[83]

3.7 Interaction of phytochemicals with Mpro

Figure 1 depicts the amino acid residues from the active site of Mpro that interacted with plant phytochemicals. Standard drugs such as nelfinavir and lopinavir bound strongly to the active site of Mpro. Three of the amino acid residues from the active site of Mpro formed hydrogen bonds with the standard drugs. However, the binding score of nelfinavir was higher than lopinavir due to the additional hydrophobic interaction among the amino acid residues.[9] Out of all the phytochemicals in *G. glabra*, glycyrrhizin displayed the most abundant interacting residues with Mpro.[26] However, glabridin with the lowest interaction showed higher docking value than other phytochemicals and standard drugs.[26] For *W. somnifera,* sitoindoside IX displayed the most interacting residues with the active site of Mpro.[31] Although the interaction of withanoside II was not as many as sitoindoside IX, its high docking score surpassed those of standard drugs.[31] Curcumin had three hydrogen bonds that could react with Mpro but it was one of the phytochemicals with the lowest docking value.[26] Among the *N. sativa* phytochemicals, α-hederin with high binding energy towards Mpro showed lesser residue interaction than nigellidine.[37] Both tinocordiside and berberine from *T. cordifolia* collectively exhibited the lowest Mpro binding energy.[15]

3.8 *In silico*, *in vitro* and *in vivo* findings on the effect of phytochemical on Mpro of SARS-CoV-2

Table 2 summarized the *in silico*, *in vitro* and *in vivo* finding of the interaction of selected phytochemicals with Mpro of SARS-CoV-2. A number of *in silico studies* have investigated the docking values of phytochemicals from *G. glabra* at the active site of SARS-CoV-2. Among the phytochemicals, glycyrrhizin was identified as the most likely bioactive compound to inhibit Mpro with a binding energy of -7.3 kcal/mol.[47] *In vitro* study with Vero E6 cell lines has demonstrated that the antiviral potential of glycyrrhizin was due to its action in suppressing SARS-CoV-2 multiplication.[29, 45] Majority of *in silico* studies have utilized AutoDock 4.2 docking tool to predict the binding affinity of glabridin and liquiritigenin.[26] Unfortunately, there was a lack of *in vitro* and *in vivo* finding to confirm the interaction of glabridin and liquiritigenin with Mpro.

Nevertheless, *G. glabra* displayed anti-inflammatory potential in several animal models by inhibiting the pro-inflammatory cytokines.[84,85,86] Clinical trials of glycyrrhizin were mostly targeting diseases such as SARS-CoV-2. Patients who were infected with SARS-CoV-2, when treated with glycyrrhizin, showed early sign of recovery.[87] Another clinical trial involving the glycyrrhizin treatment led to a lower prevalence of hepatic impairment and a rise in lymphocytes among the experimental subjects.[88] Glycyrrhizin also shorten the average duration to achieve 50% improvement from severe pulmonary lesions among the SARS-CoV-2 patients.[89]

Majority of the phytochemicals in *W. somnifera* showed high docking scores for Mpro.[31,33] The docking scores for withanoside II, withanoside IV, withaferin A, withanoside V, and sitoindoside IX were -11.30 kcal/mol, -11.02 kcal/mol, -9.83 kcal/mol, -8.96 kcal/mol, -8.37 kcal/mol, respectively. [31,33] Among these phytochemicals, withaferin A and sitoindoside IX have been investigated further with *in vitro* models. Nevertheless, *in vitro* and *in vivo* evidences of the anti-inflammatory effect of withaferin A supported the possible pharmacological role of *W. somnifera* in Covid-19 patients.[57,58,59] Interestingly, *W. somnifera* also showed its synergistic anti-viral potential when combined with Ayurvedic drugs. The polyphenols presented in *W. somnifera* could potentially prevent SARS-CoV-2 infection in Vero E6 cell lines.[90] It is noteworthy to highlight that most phytochemicals in *W. somnifera* are yet to be investigated in-depth with *in vivo* and *in vitro* methods. In addition, clinical trial on *W. somnifera* against Covid-19 infection is currently unavailable.

C. longa is one of the medicinal plants which has been explored with *in silico*, *in vitro* and *in vivo* methods (Table 2). Although *in silico* finding revealed that the Mpro binding energy of curcumin was weaker than other phytochemicals, since *C. longa* has been in the market for a long time.[64], curcumin is still favorable by most studies with its high potential as an immune booster for Covid-19 patients [35,36,54]. The anti-viral effect of curcumin has been evaluated *in vitro* with Vero E6 and Calu-3 cells.[60] Two registered clinical trials (IRCT20121216011763N46 and IRCT20200611047735N1) which evaluate the mechanism of curcumin in treating Covid-19 patients are currently ongoing.[60]

Several *in silico* investigation on the phytochemicals of *N. sativa* revealed that both nigellidine and α-hederin interacted significantly with the active site of Mpro. Previous *in vitro* and *in vivo* findings suggested that *N. sativa* extracts exhibited anti-histaminic, anti-eosinophilic, anti-leukotrienes, anti-immunoglobulin, and anti-inflammatory effect.[76] Since *N. sativa* seeds possessed immuno-potentiating effects,[76] it was widely believed that *N. sativa* seeds, when taken orally together with honey three times daily, could potentially alleviate Covid-19 symptoms.[37] To date, no clinical trial has been performed to evaluate the efficacy of *N. sativa* in Covid-19 treatment.[37]

T. cordifolia with anti-inflammatory effect could suppress the pro-inflammatory cytokines secretion via *in vitro* model.[42] Several computational studies have collectively suggested that tinocordiside and berberine from *T. cordifolia* may interact with Mpro to inhibit viral replication.[15,33,55] Another *in vivo* study has demonstrated that the anti-inflammatory characteristic of berberine may inhibit viral entry into living cells.[56] In general, there is still limited evidence-based *in vitro* and *in vivo* studies to support the specific roles of tinocordiside and berberine in managing COVID-19 infection.

3.9 Why are traditional medicines preferred over standard drugs?

Standard drug lopinavir has previously failed for ADMET parameters, therefore it was not suitable for long-term Covid-19 patients treatment.[91] Although nelfinavir has the highest docking value among the standard drugs discussed in this review, there was a violation within the ADMET parameters of nelfinavir that led to possible toxicity in human subject.[92,93] On the contrary, phytochemicals extracted from traditional medicinal plants such as glycyrrhizin scored better in ADMET than glabridin and liquiritigenin.[24] Besides, glycyrrhizin is a non-carcinogenic, and non-tumorigenic alternative source that can bind effectively to the active site of Mpro. Hence, glycyrrhizin is considered a better drug candidate when compared to other phytochemicals in *G. glabra*.[24] Phytochemicals from Ayurvedic medicinal plants such as *W. somnifera* and *T. cordifolia*'s with favorable ADMET parameters have been scientifically proven safe to use against Covid-19.[15] As curcumin has demonstrated low toxicity in humans from several clinical investigations,[35] the phytochemical is suitable to be repurposed as drug. On the other hand, nigellidine met all the drug-likeness criteria in ADMET profile while its binding affinity towards Mpro was better than that of N3 natural ligand of Mpro.[94] For *N. sativa*, its phytochemicals have passed the ADMET profiling.[95] As the seeds and oils of *N. sativa* did not show any side effect,

the medicinal plant is a strong candidate source for Covid-19 treatment.[95]

4. Future Perspective

The computational approach may hold some promise for the development of novel antiviral medications with fewer side effects and a stronger focus on the intended target. As the majority of *in vivo* and *in vitro* investigations were centered on viruses like HIV and SARS-CoV, the next step is to confirm *in silico* results with clinical trial investigations involving animal models and Covid-19 patients.[24] With the current limited *in vitro* and *in vivo* evidences, the safety dose, toxicity, lower limit of tolerance, ADMET parameters, and the possible interaction with other drugs requires further validation. The exploitation of natural compounds from medicinal plants for Covid-19 treatment ought to be further explored due to their low toxicity and reliable origin when compared to that of the synthetic antiviral drugs. Validation on the therapeutic mechanism of phytochemicals via *in vitro* and *in vivo* studies shall offer more insight into their safety and efficacy in living mammalian cells and tissues. Although the five medicinal plants exhibited great potential to reduce Covid-19 symptoms, in-depth clinical trials with Covid-19 patients may offer a better understanding of the bio-accessibility and bioavailability of plant phytochemicals in living subjects. The identification of antiviral mechanisms in specific plant bioactive compounds offers another opportunity to exploit the polyherbal formulation of medicinal plants in Covid-19 treatment.

5. Conclusion

The current review has compared the Mpro binding affinity and immunomodulatory effect of phytochemicals from the five selected traditional medicinal plants with standard drugs which currently used in Covid-19 treatment. The five medicinal plants exhibited similar pharmacological profiles, including antioxidant, anti-viral and immunomodulatory activities. Medicinal plant extracts that interact and inhibit Mpro could lower the risk of cytokine storm while alleviating Covid-19 symptoms. Pre-clinical evidences have suggested that *G. glabra*, *N. sativa*, *T. cordifolia*, *C. longa* and *W. somnifera* are worthy alternative natural sources to combat Covid-19 infection. The isolated plant phytochemicals such as withanoside II, withanoside IV, withaferin A, α-hederin, withanoside V, sitoindoside IX, glabridin, liquiritigenin, nigellidine, curcumin, glycyrrhizin, tinocordiside, and berberine could bind and inhibit Mpro of SARS-CoV-2. Among these phytochemicals, glycyrrhizin, withaferin A, curcumin, nigellidine and cordifolioside A have shown strong anti-inflammatory effects in Covid-19 patients.

Conflict of Interests

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgements

This study was supported by the School of Biosciences, University of Nottingham Malaysia.

Compliance with ethical standard

Ethics approval: Not applicable
Consent to participate: Not applicable
Consent to publish: Not applicable.
Material/Code availability: Other data used to support the findings of this study are available from the corresponding author upon request.

Author Contribution Statement

Zhi Xiang Ng and Jomin Choe designed the study. Jomin Choe extracted data and wrote the original draft. Zhi Xiang Ng and Phaik Har Yong reviewed and edited the manuscript.

References

[1] K. Roedl, G. Söffker, D. Fischer, J. Müller, D. Westermann, M. Issleib, S. Kluge and D. Jarczak, 'Effects of COVID-19 on in-hospital cardiac arrest: incidence, causes, and outcome – a retrospective cohort study', *Scand. J. Trauma, Resusc. Emerg. Med.* **2021**, *29*, 1-11.

[2] V. Kumar, K. U. Doshi, W. H. Khan and A. S. Rathore, 'COVID-19 pandemic: mechanism, diagnosis, and treatment', *J. Chem. Technol. Biotechnol.* **2021**, *96*, 299-308.

[3] F. Coperchini, L. Chiovato, L. Croce, F. Magri and M. Rotondi, 'The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system', *Cytokine Growth Factor Rev.* **2020**, *53*, 25-32.

[4] W. Dai, B. Zhang, X. M. Jiang, H. Su, J. Li, Y. Zhao, X. Xie, Z. Jin, J. Peng, F. Liu, C. Li, Y. Li, F. Bai, H. Wang, X. Cheng, X. Cen, S. Hu, X.

Yang, J. Wang, X. Liu, G. Xiao, H. Jiang, Z. Rao, L. K. Zhang, Y. Xu, H. Yang and H. Liu, 'Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease', *Science* **2020**, *368*, 1331-1335.

[5] H. M. Mengist, X. Fan and T. Jin, 'Designing of improved drugs for COVID-19: Crystal structure of SARS-CoV-2 main protease Mpro', *Signal Transduction Targeted Ther.* **2020**, *5*, 1-2.

[6] A. A. Rabaan, S. H. Al-Ahmed, J. Muhammad, A. Khan, A. A. Sule, R. Tirupathi, A. A. Mutair, S. Alhumaid, A. Al-Omari and M. Dhawan, 'Role of inflammatory cytokines in COVID-19 patients: A review on molecular mechanisms, immune functions, immunopathology and immunomodulatory drugs to counter cytokine storm', *Vaccines* **2021**, *9*, 436.

[7] R. L. Kruse, 'Therapeutic strategies in an outbreak scenario to treat the novel coronavirus originating in Wuhan, China', *F1000Research* **2020**, *9*.

[8] Z. Jin, X. Du, Y. Xu, Y. Deng, M. Liu, Y. Zhao, B. Zhang, X. Li, L. Zhang, C. Peng, Y. Duan, J. Yu, L. Wang, K. Yang, F. Liu, R. Jiang, X. Yang, T. You, X. Liu, X. Yang, F. Bai, H. Liu, X. Liu, L. W. Guddat, W. Xu, G. Xiao, C. Qin, Z. Shi, H. Jiang, Z. Rao and H. Yang, 'Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors', *Nature* **2020**, *582*, 289-293.

[9] G. N. Trivedi, J. Karlekar, H. Dhameliya and H. Panchal, 'A review on the novel coronavirus disease based on In-silico analysis of various drugs and target proteins', *J. Pure Appl. Microbiol.* **2020**, *14*, 849-860.

[10] S. Bharadwaj, E. I. Azhar, M. A. Kamal, L. H. Bajrai, A. Dubey, K. Jha, U. Yadava, S. G. Kang and V. D. Dwivedi, 'SARS-CoV-2 Mpro inhibitors: identification of anti-SARS-CoV-2 Mpro compounds from FDA approved drugs', *J. Biomol. Struct. Dyn.* **2020**, 1-16.

[11] G. Magro, 'SARS-CoV-2 and COVID-19: Is interleukin-6 (IL-6) the 'culprit lesion'of ARDS onset? What is there besides Tocilizumab? SGP130Fc', *Cytokine: X* **2020**, *2*, 100029.

[12] L. Zhang, D. Lin, X. Sun, U. Curth, C. Drosten, L. Sauerhering, S. Becker, K. Rox and R. Hilgenfeld, 'Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved a-ketoamide inhibitors', *Science* **2020**, *368*, 409-412.

[13] B. Adhikari, B. P. Marasini, B. Rayamajhee, B. R. Bhattarai, G. Lamichhane, K. Khadayat, A. Adhikari, S. Khanal and N. Parajuli, 'Potential roles of medicinal plants for the treatment of viral diseases focusing on COVID-19: A review', *Phytother. Res.* **2021**, *35*, 1298-1312.

[14] T. U. Singh, S. Parida, M. C. Lingaraju, M. Kesavan, D. Kumar and R. K. Singh, 'Drug repurposing approach to fight COVID-19', *Pharmacol. Rep.* **2020**, *72*, 1-30.

[15] P. Shree, P. Mishra, C. Selvaraj, S. K. Singh, R. Chaube, N. Garg and Y. B. Tripathi, 'Targeting COVID-19 (SARS-CoV-2) main protease through active phytochemicals of ayurvedic medicinal plants–*Withania somnifera* (Ashwagandha), *Tinospora cordifolia* (Giloy) and *Ocimum sanctum* (Tulsi)–a molecular docking study', *J. Biomol. Struct. Dyn.* **2020**, *40*, 1-14.

[16] M. Boozari and H. Hosseinzadeh, 'Natural products for COVID-19 prevention and treatment regarding to previous coronavirus infections and novel studies', *Phytother. Res.* **2021**, *35*, 864-876.

[17] T. Mohammad, A. Shamsi, S. Anwar, M. Umair, A. Hussain, M. T. Rehman, M. F. AlAjmi, A. Islam and M. I. Hassan, 'Identification of high-affinity inhibitors of SARS-CoV-2 main protease: Towards the development of effective COVID-19 therapy', *Virus Res.* **2020**, *288*, 198102.

[18] M. Soy, G. Keser, P. Atagündüz, F. Tabak, I. Atagündüz and S. Kayhan, 'Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment', *Clin. Rheumatol.* **2020**, *39*, 2085-2094.

[19] X. Li, Q. Qiu, M. Li, H. Lin, S. Cao, Q. Wang, Z. Chen, W. Jiang, W. Zhang, Y. Huang, H. Luo and L. Luo, 'Chemical composition and pharmacological mechanism of ephedra-glycyrrhiza drug pair against coronavirus disease 2019 (COVID-19)', *Aging* **2021**, *13*, 4811-4830.

[20] S. Garg, A. Anand, Y. Lamba and A. Roy, 'Molecular docking analysis of selected phytochemicals against SARS-CoV-2 Mpro receptor', *Vegetos* **2020**, *33*, 766-781.

[21] G. Pastorino, L. Cornara, S. Soares, F. Rodrigues and M. B. P. Oliveira, 'Liquorice (*Glycyrrhiza glabra*): A phytochemical and pharmacological review', *Phytother. Res.* **2018**, *32*, 2323-2339.

[22] M. Parvaiz, K. Hussain, S. Khalid, N. Hussnain, N. Iram, Z. Hussain and M. A. Ali, 'A review: Medicinal importance of *Glycyrrhiza glabra* L.(Fabaceae family)', *Global J. Pharmacol.* **2014**, *8*, 8-13.

[23] M. Jiang, S. Zhao, S. Yang, X. Lin, X. He, X. Wei, Q. Song, R. Li, C. Fu and J. Zhang, 'An "essential herbal medicine"—Licorice: A review of phytochemicals and its effects in combination preparations', *J. Ethnopharmacol.* **2020**, *249*, 112439.

[24] V. Srivastava, A. Yadav and P. Sarkar, 'Molecular docking and ADMET study of bioactive compounds of *Glycyrrhiza glabra* against main protease of SARS-CoV2', *Mater. Today: Proc.* **2020**., *49*, 2999-3007.

[25] J. H. Chandra and H. Gunasekaran, 'Screening of phytochemical, antimicrobial and antioxidant activity of glycyrrhiz glabra root extract', *J. Environ. Biol.* **2017**, *38*, 161-165.

[26] M. H. Sampangi-Ramaiah, R. Vishwakarma and R. U. Shaanker, 'Molecular docking analysis of selected natural products from plants for inhibition of SARS-CoV-2 main protease', *Curr. Sci.* **2020**, *118*, 1087-1092.

[27] D. Armanini, C. Fiore, J. Bielenberg, C. Sabbadin and L. Bordin, 'Coronavirus-19: Possible Therapeutic Implications of Spironolactone and Dry Extract of *Glycyrrhiza glabra* L. (Licorice)', *Front. Pharmacol.* **2020**, *11*, 558418.

[28] I. I. Hejazi, M. A. Beg, M. A. Imam, F. Athar and A. Islam, 'Glossary of phytoconstituents: Can these be repurposed against SARS CoV-2? A quick *in silico* screening of various phytoconstituents

from plant *Glycyrrhiza glabra* with SARS CoV-2 main protease', *Food Chem. Toxicol.* **2021**, *150*, 112057.

[29] L. van de Sand, M. Bormann, M. Alt, L. Schipper, C. S. Heilingloh, E. Steinmann, D. Todt, U. Dittmer, C. Elsner and O. Witzke, 'Glycyrrhizin Effectively Inhibits SARS-CoV-2 Replication by Inhibiting the Viral Main Protease', *Viruses* **2021**, *13*, 609.

[30] M. Umadevi, R. Rajeswari, C. S. Rahale, S. Selvavenkadesh, R. Pushpa, K. S. Kumar and D. Bhowmik, 'Traditional and medicinal uses of *Withania somnifera*', *The Pharm. Innov.* **2012**, *1*, 102-110.

[31] M. K. Tripathi, P. Singh, S. Sharma, T. P. Singh, A. Ethayathulla and P. Kaur, 'Identification of bioactive molecule from *Withania somnifera* (Ashwagandha) as SARS-CoV-2 main protease inhibitor', *J. Biomol. Struct. Dyn.* **2021**, *39*, 5668-5681.

[32] A. Saggam, K. Limgaokar, S. Borse, P. Chavan-Gautam, S. Dixit, G. Tillu and B. Patwardhan, '*Withania somnifera* (L.) Dunal: Opportunity for Clinical Repurposing in COVID-19 Management', *Front. Pharmacol.* **2021**, *12*, 623795

[33] H. Sudeep, K. Gouthamchandra and K. Shyamprasad, 'Molecular docking analysis of Withaferin A from *Withania somnifera* with the Glucose regulated protein 78 (GRP78) receptor and the SARS-CoV-2 main protease', *Bioinformation* **2020**, *16*, 411-417.

[34] M. Dhawan, M. Parmar, K. Sharun, R. Tiwari, M. Bilal and K. Dhama, 'Medicinal and therapeutic potential of withanolides from *Withania somnifera* against COVID-19', *J. Appl. Pharm. Sci.* **2021**, *11*, 6-13.

[35] F. Babaei, M. Nassiri-Asl and H. Hosseinzadeh, 'Curcumin (a constituent of turmeric): New treatment option against COVID-19', *Food Sci. Nutr.* **2020**, *8*, 5215-5227.

[36] D. M. Teli, M. B. Shah and M. T. Chhabria, '*In silico* Screening of Natural Compounds as Potential Inhibitors of SARS-CoV-2 Main Protease and Spike RBD: Targets for COVID-19', *Front. Mol. Biosci.* **2021**, *7*, 599079.

[37] S. Khan, F. Siddiqui, S. Jain and G. Sonwane, 'Discovery of potential inhibitors of SARS-CoV-2 (COVID-19) Main Protease (Mpro) from *Nigella Sativa* (black seed) by molecular docking study', *Coronaviruses* **2021**, *2*, 384-402.

[38] P. Halder, U. Pal, P. Paladhi, S. Dutta, P. Paul, S. Pal, D. Das, A. Ganguly, I. Dutta and S. Mandal, 'Evaluation of potency of the selected bioactive molecules from Indian medicinal plants with MPro of SARS-CoV-2 through *in silico* analysis', *J. Ayurveda and Integr. Med.* **2022**, *13*, 100449.

[39] N. Sharma, D. Ahirwar, D. Jhade and S. Gupta, 'Medicinal and phamacological potential of *Nigella sativa*: a review', *Ethnobot. leafl.* **2009**, *2009*, 11.

[40] N. Choudhary, M. Siddiqui, S. Azmat and S. Khatoon, 'Tinospora cordifolia: ethnobotany, phytopharmacology and phytochemistry aspects', *Int. J. Pharm. Sci. Res.* **2013**, *4*, 891-899.

[41] A. Balkrishna, S. K. Solleti, S. Verma and A. Varshney, 'Application of humanized zebrafish model in the suppression of SARS-CoV-2 spike protein induced pathology by tri-herbal medicine coronil via cytokine modulation', *Molecules* **2020**, *25*, 5091.

[42] L. Sapra, A. Bhardwaj, Z. Azam, D. Madhry, B. Verma, S. Rathore and R. K. Srivastava, 'Phytotherapy for treatment of cytokine storm in COVID-19', *Front. Biosci. (Landmark Ed)* **2021**, *26*, 51-57.

[43] J.-X. Zhou and M. Wink, 'Evidence for anti-inflammatory activity of isoliquiritigenin, 18β glycyrrhetinic acid, ursolic acid, and the traditional chinese medicine plants *Glycyrrhiza glabra* and *Eriobotrya japonica*, at the molecular level', *Medicines* **2019**, *6*, 55.

[44] J. Cinatl, B. Morgenstern, G. Bauer, P. Chandra, H. Rabenau and H. Doerr, 'Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus', *Lancet* **2003**, *361*, 2045-2046.

[45] H. Murck, 'Symptomatic protective action of glycyrrhizin (licorice) in COVID-19 infection?', *Front. Immunol.* **2020**, *11*, 1239.

[46] P. Luo, D. Liu and J. Li, 'Pharmacological perspective: glycyrrhizin may be an efficacious therapeutic agent for COVID-19', *Int. J. Antimicrob. Agents* **2020**, *55*, 105995.

[47] A. M. Tolah, L. M. Altayeb, T. A. Alandijany, V. D. Dwivedi, S. A. El-Kafrawy and E. I. Azhar, 'Computational and *In Vitro* Experimental Investigations Reveal Anti-Viral Activity of Licorice and Glycyrrhizin against Severe Acute Respiratory Syndrome Coronavirus 2', *Pharmaceuticals* **2021**, *14*, 1216.

[48] Z. X. Ng, U. R. Kuppusamy, I. Tajunisah, K. C. S. Fong, and K. H. Chua, Investigation of SLC2A1 26177A/G gene polymorphism via high resolution melting curve analysis in Malaysian patients with diabetic retinopathy, *J. Diabetes Complicat.* **2012**, *26,* 388-392.

[49] P. Gowda, S. Patrick, S. D. Joshi, R. K. Kumawat and E. Sen, 'Glycyrrhizin prevents SARS-CoV-2 S1 and Orf3a induced high mobility group box 1 (HMGB1) release and inhibits viral replication', *Cytokine* **2021**, *142*, 155496.

[50] E. Shawky, A. A. Nada and R. S. Ibrahim, 'Potential role of medicinal plants and their constituents in the mitigation of SARS-CoV-2: identifying related therapeutic targets using network pharmacology and molecular docking analyses', *Rsc Advances* **2020**, *10*, 27961-27983.

[51] C. Fiore, M. Eisenhut, E. Ragazzi, G. Zanchin and D. Armanini, 'A history of the therapeutic use of liquorice in Europe', *J. Ethnopharmacol.* **2005**, *99*, 317-324.

[52] B. A. Mir, J. Khazir, N. A. Mir, T.-u. Hasan and S. Koul, Botanical, chemical and pharmacological review of *Withania somnifera* (Indian ginseng): an ayurvedic medicinal plant', *Indian J. Drugs Dis.* **2012**, *1*, 147-160.

[53] S. K. Verma and A. Kumar, 'Therapeutic uses of *Withania somnifera* (Ashwagandha) with a note on withanolides and its pharmacological actions', *Asian J. Pharm. Clin. Res.* **2011**, *4*, 1-14.

[54] A. Balkrishna, S. Haldar, H. Singh, P. Roy and A. Varshney, 'Coronil, a tri-herbal formulation, attenuates spike-protein-mediated SARS-CoV-2 viral entry into human alveolar epithelial cells and pro-inflammatory cytokines production by inhibiting spike

protein-ACE-2 interaction,' *J. Inflammation Res.* **2021**, *14*, 869-884.

[55] M. A. Morsy, S. S. Patel, A. A. El-Sheikh, J. K. Savjani, A. B. Nair, J. N. Shah and K. N. Venugopala, 'Computational and biological comparisons of plant steroids as modulators of inflammation through interacting with glucocorticoid receptor', *Mediators Inflammation* **2019**, *2019*, 3041438.

[56] D. Kanchibhotla, S. Subramanian, R. M. R. Kumar, K. V. Hari and M. Pathania, 'An In-vitro evaluation of a polyherbal formulation, against SARS-CoV-2,' *J. Ayurveda Integr. Med.* **2022**, *13*, 100581.

[57] H. Ti, Z. Mai, Z. Wang, W. Zhang, M. Xiao, Z. Yang and P. Shaw, 'Bisabolane-type sesquiterpenoids from *Curcuma longa* L. exert anti-influenza and anti-inflammatory activities through NF-κB/MAPK and RIG-1/STAT1/2 signaling pathways', *Food Funct.* **2021**, *12*, 6697-6711.

[58] M. Bormann, M. Alt, L. Schipper, L. van de Sand, V. T. K. Le-Trilling, L. Rink, N. Heinen, R. J. Madel, M. Otte and K. Wuensch, 'Turmeric root and its bioactive ingredient curcumin effectively neutralize SARS-CoV-2 *in vitro*', *Viruses* **2021**, *13*, 1914.

[59] V. Sagar and A. H. Kumar, 'Efficacy of natural compounds from *Tinospora cordifolia* against SARS-CoV-2 protease, surface glycoprotein and RNA polymerase', *Virology* **2020**, 1-10.

[60] A. Warowicka, R. Nawrot and A. Goździcka-Józefiak, 'Antiviral activity of berberine', *Arch. Virol.* **2020**, *165*, 1935-1945.

[61] K. Kumano, M. A. Kanak, P. B. Saravanan, J. P. Blanck, Y. Liu, S. Vasu, M. Lawrence and B. Naziruddin, 'Withaferin A inhibits lymphocyte proliferation, dendritic cell maturation *in vitro* and prolongs islet allograft survival', *Sci. Rep.* **2021**, *11*, 10661.

[62] D. S. Mandlik and A. G. Namdeo, 'Pharmacological evaluation of Ashwagandha highlighting its healthcare claims, safety, and toxicity aspects', *J. Diet. Suppl.* **2021**, *18*, 183-226.

[63] R. P. Yadav, G. Tarun, C. Roshan and P. Yadav, 'Versatility of turmeric: A review the golden spice of life', *J. Pharmacogn. Phytochem.* **2017**, *6*, 41-46.

[64] S. Balaji and B. Chempakam, 'Toxicity prediction of compounds from turmeric (*Curcuma longa* L)', *Food Chem. Toxicol.* **2010**, *48*, 2951-2959.

[65] R. S. Ahmad, M. B. Hussain, M. T. Sultan, M. S. Arshad, M. Waheed, M. A. Shariati, S. Plygun and M. H. Hashempur, 'Biochemistry, safety, pharmacological activities, and clinical applications of turmeric: a mechanistic review', *J. Evidence-Based Complementary Altern. Med.* **2020**, *2020*, 7656919.

[66] S. Irshad, A. Muazzam, Z. Shahid and M. B. Dalrymple, '*Curcuma longa* (Turmeric): An auspicious spice for antibacterial, phytochemical and antioxidant activities', *Pak. J. Pharm. Sci.* **2018**, *31*, 2689-2696.

[67] C. M. Mbadiko, C. L. Inkoto, B. Z. Gbolo, E. M. Lengbiye, J. T. Kilembe, A. Matondo, D. T. Mwanangombo, E. M. Ngoyi, G. N. Bongo and C. M. Falanga, 'A mini review on the phytochemistry, toxicology and antiviral activity of some medically interesting Zingiberaceae species', *J. Complement. Altern. Med. Res*. **2020**, *9*, 44-56.

[68] M. Sankar, B. Ramachandran, B. Pandi, N. Mutharasappan, V. Ramasamy, P. G. Prabu, G. Shanmugaraj, Y. Wang, B. Muniyandai and S. Rathinasamy, '*In silico* Screening of Natural Phytocompounds Towards Identification of Potential Lead Compounds to Treat COVID-19', *Front. Mol. Biosci.* **2021**, *8*.

[69] S. Gupta, A. K. Singh, P. P. Kushwaha, K. S. Prajapati, M. Shuaib, S. Senapati and S. Kumar, 'Identification of potential natural inhibitors of SARS-CoV2 main protease by molecular docking and simulation studies', *J. Biomol. Struct. Dyn.* **2021**, *39*, 4334-4345.

[70] M. A. A. Ibrahim, A. H. M. Abdelrahman, T. A. Hussien, E. A. A. Badr, T. A. Mohamed, H. R. El-Seedi, P. W. Pare, T. Efferth and M. E. F. Hegazy, '*In silico* drug discovery of major metabolites from spices as SARS-CoV-2 main protease inhibitors', *Comput. Biol. Med.* **2020**, *126*, 104046.

[71] G. F. Parisi, G. Carota, C. Castruccio Castracani, M. Spampinato, S. Manti, M. Papale, M. Di Rosa, I. Barbagallo and S. Leonardi, 'Nutraceuticals in the Prevention of Viral Infections, including COVID-19, among the Pediatric Population: A Review of the Literature', *Int. J. Mol. Sci.* **2021**, *22*, 2465.

[72] S. Han, J. Xu, X. Guo and M. Huang, 'Curcumin ameliorates severe influenza pneumonia via attenuating lung injury and regulating macrophage cytokines production', *Clin. Exp. Pharmacol. Physiol.* **2018**, *45*, 84-93.

[73] S. Maiti, A. Banerjee, A. Nazmeen, M. Kanwar and S. Das, 'Active-site Molecular docking of Nigellidine with nucleocapsid-NSP2-MPro of COVID-19 and to human IL1R-IL6R and strong antioxidant role of Nigella-sativa in experimental rats', *J. Drug Targeting* **2020**, *30*, 1-23.

[74] S. Ahmad, H. W. Abbasi, S. Shahid, S. Gul and S. W. Abbasi, 'Molecular docking, simulation and MM-PBSA studies of nigella sativa compounds: a computational quest to identify potential natural antiviral for COVID-19 treatment', *J. Biomol. Struct. Dyn.* **2021**, *39*, 4225-4233.

[75] M. A. Farag, H. A. Gad, A. G. Heiss and L. A. Wessjohann, 'Metabolomics driven analysis of six Nigella species seeds via UPLC-qTOF-MS and GC–MS coupled to chemometrics', *Food Chem.* **2014**, *151*, 333-342.

[76] M. N. Islam, K. S. Hossain, P. P. Sarker, J. Ferdous, M. A. Hannan, M. M. Rahman, D. T. Chu and M. J. Uddin, 'Revisiting pharmacological potentials of *Nigella sativa* seed: a promising option for COVID-19 prevention and cure', *Phytother. Res.* **2021**, *35*, 1329-1344.

[77] E. M. F. Barakat, L. M. El Wakeel and R. S. Hagag, 'Effects of *Nigella sativa* on outcome of hepatitis C in Egypt', *World J. Gastroenterol.* **2013**, *19*, 2529-2536.

[78] D. K. Verma, P. Kumar and M. El-Shazly, 'Unmasking the many faces of Giloy (*Tinospora cordifolia* L.): a fresh look on its

phytochemical and medicinal properties', *Curr. Pharm. Des.* **2021**, *27*, 2571-2581.

[79] T. S. Panchabhai, U. P. Kulkarni and N. N. Rege, 'Validation of therapeutic claims of *Tinospora cordifolia*: A review', *Phytother. Res.* **2008**, *22*, 425-441.

[80] N. Choudhary, M. Siddiqui and S. Khatoon, 'Pharmacognostic evaluation of *Tinospora cordifolia* (Willd.) Miers and identification of biomarkers', *Indian J. Tradit. Knowl.* **2014**, 543-550

[81] M. Manne, G. Goudar, S. R. Varikasuvu, M. C. Khetagoudar, H. Kanipakam, P. Natarajan, M. D. Ummiti, V. A. Yenagi, S. Chinthakindi and P. Dharani, 'Cordifolioside: potent inhibitor against M pro of SARS-CoV-2 and immunomodulatory through human TGF-β and TNF-α', *3 Biotech* **2021**, *11*, 1-25.

[82] S. Murugesan, S. Kottekad, I. Crasta, S. Sreevathsan, D. Usharani, M. K. Perumal and S. N. Mudliar, '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*, 104683.

[83] P. Leyon and G. Kuttan, 'Effect of *Tinospora cordifolia* on the cytokine profile of angiogenesis-induced animals', *Int. Immunopharmacol.* **2004**, *4*, 1569-1575.

[84] Y. Fu, J. Chen, Y.-J. Li, Y.-F. Zheng and P. Li, 'Antioxidant and anti-inflammatory activities of six flavonoids separated from licorice', *Food Chem.* **2013**, *141*, 1063-1071.

[85] X.-r. Wang, H.-g. Hao and L. Chu, 'Glycyrrhizin inhibits LPS-induced inflammatory mediator production in endometrial epithelial cells', *Microb. Pathog.* **2017**, *109*, 110-113.

[86] Y. Xiao, J. Xu, C. Mao, M. Jin, Q. Wu, J. Zou, Q. Gu, Y. Zhang and Y. Zhang, '18β-glycyrrhetinic acid ameliorates acute Propionibacterium acnes-induced liver injury through inhibition of macrophage inflammatory protein-1α', *J. Biol. Chem.* **2010**, *285*, 1128-1137.

[87] H. Lu, N. Huo, G. Wang, H. Li, L. Nie and X. Xu, 'Clinical observation of therapeutic effect of compound glycyrrhizin on SARS', *China Pharmacy* 2001, *10*, 521436.

[88] W. H. Organization, 'SARS: clinical trials on treatment using a combination of traditional Chinese medicine and Western medicine: report of the WHO International Expert Meeting to review and analyse clinical reports on combination treatment for SARS, 8-10 October 2003, Beijing, People's Republic of China', **2004**.

[89] C. Wu, X. Xu, H. Lu, X. Lin, F. Hou, Y. Yu, G. Wang and L. Nie, 'Analysis of the chest X-ray manifestations in SARS patients treated with compound glycyrrhizin', *China Pharmacy* **1991,** *12*, 521972.

[90] G. Annunziata, M. Sanduzzi Zamparelli, C. Santoro, R. Ciampaglia, M. Stornaiuolo, G. C. Tenore, A. Sanduzzi and E. Novellino, 'May polyphenols have a role against coronavirus infection? An overview of *in vitro* evidence', *Frontiers in Medicine* **2020**, *7*, 240.

[91] V. S. Shaikh, Y. I. Shaikh and K. Ahmed, 'A molecular docking study of lopinavir towards SARS-COV-2 target protein', *Eng. Sci.* **2020**, *12*, 113-116.

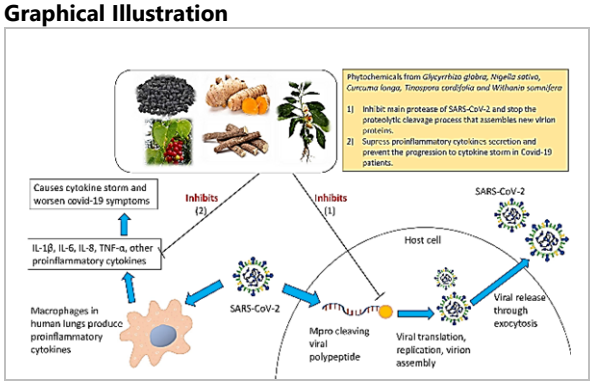
[92] D. Kumar, V. Chandel, S. Raj and B. Rathi, '*In silico* identification of potent FDA approved drugs against Coronavirus COVID-19 main protease: A drug repurposing approach', *Chem. Biol. Lett.* **2020**, *7*, 166-175.

[93] M. W. El-Saadi, T. Williams-Hart, B. A. Salvatore and E. Mahdavian, 'Use of in-silico assays to characterize the ADMET profile and identify potential therapeutic targets of fusarochromanone, a novel anti-cancer agent', *In silico pharmacology* **2015**, *3*, 1-20.

[94] T. Sumaryada and C. A. Pramudita, 'Molecular docking evaluation of some Indonesian's popular herbals for a possible COVID-19 treatment', *Biointerface Res. Appl. Chem.* **2021**, *11*, 9827-9835.

[95] Y. I. Shaikh, V. S. Shaikh, K. Ahmed, G. M. Nazeruddin and H. M. Pathan, 'The revelation of various compounds found in Nigella sativa L.(Black Cumin) and their possibility to inhibit COVID-19 infection based on the molecular docking and physical properties', *Eng. Sci.* **2020**, *11*, 31-35.

Graphical Illustration



Twitter Text

This review paper focuses on the potential drug-like properties of selected medicinal plants as SARS-CoV-2 virus inhibitors. To better understand the roles of plant bioactive compounds in combating Covid-19 infection, data involving their protein binding affinity, absorption, distribution, metabolism, and toxicity characteristics from selected five medicinal plants are compared and discussed. This paper shall shed more insight to the public and scientific community in identifying plant bioactive compounds with high therapeutic potential to develop as anti-covid 19 drugs. The official twitter account for the University of Nottingham Malaysia and the School of Bioscience are @UoNMalaysia and @UNMBiosciences, respectively.