



2021

In-silico efficacy of potential phytomolecules from Ayurvedic herbs as an adjuvant therapy in management of COVID-19

Follow this and additional works at: <https://www.jfda-online.com/journal>

 Part of the [Food Science Commons](#), [Medicinal Chemistry and Pharmaceutics Commons](#), [Pharmacology Commons](#), and the [Toxicology Commons](#)



This work is licensed under a [Creative Commons Attribution-Noncommercial-No Derivative Works 4.0 License](#).

Recommended Citation

Kumar, Bhanu; Misra, Ankita; Singh, Satyendra Pratap; Dhar, Yogeshwar Vikram; Rawat, Poonam; Chattopadhyay, Debprasad; Barik, Saroj Kanta; and Srivastava, Sharad (2021) "In-silico efficacy of potential phytomolecules from Ayurvedic herbs as an adjuvant therapy in management of COVID-19," *Journal of Food and Drug Analysis*: Vol. 29 : Iss. 4 , Article 3.
Available at: <https://doi.org/10.38212/2224-6614.3380>

This Original Article is brought to you for free and open access by Journal of Food and Drug Analysis. It has been accepted for inclusion in Journal of Food and Drug Analysis by an authorized editor of Journal of Food and Drug Analysis.

In-silico efficacy of potential phytomolecules from Ayurvedic herbs as an adjuvant therapy in management of COVID-19

Bhanu Kumar ^{a,1}, Ankita Misra ^{a,1}, Satyendra Pratap Singh ^{a,1}, Yogeshwar Vikram Dhar ^{b,1}, Poonam Rawat ^a, Debprasad Chattopadhyay ^c, Saroj Kanta Barik ^a, Sharad Srivastava ^{a,*}

^a Pharmacognosy Division, CSIR-National Botanical Research Institute, Lucknow, U.P. 226001, India

^b Bioinformatics Division, CSIR-National Botanical Research Institute, Lucknow, U.P. 226001, India

^c National Institute of Traditional Medicine, Belagavi, Karnataka 590010, India

Abstract

The recent COVID-19 outbreak caused by SARS-CoV-2 virus has sparked a new spectrum of investigations, research and studies in multifarious directions. Efforts are being made around the world for discovery of effective vaccines/drugs against COVID-19. In this context, *Ayurveda*, an alternative traditional system of medicine in India may work as an adjuvant therapy in compromised patients. We selected 40 herbal leads on the basis of their traditional applications. The phytomolecules from these leads were further screened through *in-silico* molecular docking against two main targets of SARS-CoV-2 i.e. the spike protein (S; structural protein) and the main protease (M^{PRO}; non-structural protein). Out of the selected 40, 12 phytomolecules were able to block or stabilize the major functional sites of the main protease and spike protein. Among these, Ginsenoside, Glycyrrhizic acid, Hesperidin and Tribulosin exhibited high binding energy with both main protease and spike protein. Etoposide showed good binding energy only with Spike protein and Teniposide had high binding energy only with main protease. The above phytocompounds showed promising binding efficiency with target proteins indicating their possible applications against SARS-CoV-2. However, these findings need to be validated through *in vitro* and *in vivo* experiments with above mentioned potential molecules as candidate drugs for the management of COVID-19. In addition, there is an opportunity for the development of formulations through different permutations and combinations of these phytomolecules to harness their synergistic potential.

Keywords: Ayurveda, COVID-19, Main protease, SARS-CoV-2, Spike-RBD protein

1. Introduction

The human coronavirus (CoV) is an enveloped, single stranded positive sense RNA virus of Coronaviridae family and *Nidovirales* order which is usually responsible for upper respiratory and digestive tract infections in humans [1]. Earlier, the Severe Acute Respiratory Syndrome-CoV (SARS-CoV) in 2002 and Middle East Respiratory Syndrome-CoV (MERS-CoV) in 2013 spread in several countries causing severe illnesses like pneumonia, bronchiolitis, meningitis etc. However, in the present scenario the fatality rate due to SARS-CoV-2 is ~2–3% [2,3]. It is believed that initially SARS-CoV-2 virus had spilled

over from animal reservoir to humans via an intermediate host and aggressively infected the humans with increased severity and then by human to human transmission leading to pandemic via global travel, as declared by the WHO on 11th March, 2020. The symptoms in COVID-19 patients typically resemble those of the SARS, which include dry cough, high fever and at later stages difficulty in breathing due to lower respiratory tract infection. The genome of CoV-2 (~30 kb) encodes four major structural proteins: Spike (S), Membrane (M), Envelope (E) and Nucleocapsid (N). Spike protein facilitates entry into the target cell with its short intracellular tail, transmembrane anchor, large ectodomain with receptor

Received 9 November 2020; revised 17 May 2021; accepted 26 August 2021.
Available online 15 December 2021.

* Corresponding author.

E-mail address: sharad_ks2003@yahoo.com (S. Srivastava).

¹ These authors contributed equally.

<https://doi.org/10.38212/2224-6614.3380>

2224-6614/© 2021 Taiwan Food and Drug Administration. This is an open access article under the CC-BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

binding (S-1) and membrane-fusing (S-2) subunits. Sequence of SARS-CoV-2 has 79.5% similarity with SARS-CoV. Receptor binding domain/motif (RBM) in Spike protein is conserved between CoV-1 and CoV-2, suggesting that Coronaviruses use the same Angiotensin-Converting Enzyme-2 (ACE2) receptor for entry to the host's receptors in alveolar epithelial cells of lungs [1].

Till date there is no effective remedy available for this virus and the current line of treatment includes use of combination of some antiviral agents and broad-spectrum antibiotics. Several inhibitors of HIV protease have been claimed to be efficacious against COVID-19; however, their results remain unclear in animal models [4]. Remdesivir has been found effective against SARS-CoV-2 both *in vitro* and *in vivo* [5]. The Indian Council of Medical Research (ICMR) has also recommended the use of Hydroxychloroquine (400 mg dose) for the treatment of confirmed or suspected COVID-19 patients [6]. The use of plasma therapy as an alternative line of treatment has been permitted in several countries but its safety and efficacy are under investigation [7]. Currently the management/treatment of COVID-19 has become quintessential and it is urgent to find out potential molecules either through modern medicine or from traditional systems of medicine to control the acuteness and casualties caused by COVID-19 infection.

As per *Ayurveda*, infectious diseases can be controlled by supporting the natural defense mechanisms and boosting the immune system of body that will be beneficial for eradication of the symptoms [8]. The concepts of epidemics and pandemics were known even at the time of *Ayurveda* as *Janapadodhwans*, which occur due to variations in *Vayu*, *Jala*, *Desh* and *Kala* [9]. One of the noted *Ayurvedacharya* of ancient India, Sushrut had classified eight different modes of communicable diseases in his book *Sushrut Samhita* [10]. It has been suggested that the *aupsargika roga* (communicable diseases) can be contained by practicing some physical precautions and taking some protective measures such as “*Dhupana*” (fumigation) with “*Rakshoghna Dravyas*” (antimicrobial agents).

Thus, an integrative Ayurvedic approach using poly-herbal combinations with medicinal plants having anti-inflammatory, immunity boosting, anti-asthmatic and anti-pneumonia activities with furin like- proprotein convertase enzyme inhibitor might help to combat COVID-19. The trimeric trans-membrane spike (S) glycoprotein of coronavirus is essential for the entry of virus into the cell via attachment, fusion and penetration. After attachment, the S glycoprotein must be cleaved by host

cell proteases to enable the exposure of fusion sequences for entry into the cell. As furin proteases are abundant in the human respiratory tract, it is possible that S-glycoproteins of SARS-CoV-2 is cleaved when it exits from epithelial cells and consequently infect other cells efficiently. Thus to inhibit the viral propagation, use of plant-based furin-like convertase inhibitors will be a key approach. However, systematic inhibition of furin like enzymes may result in toxicity due to its use in other cellular processes of human. So, the delivery of small molecules of plant origin can provide local relief against COVID-19. One most promising example is diterpenes and succinoyl esters of *Andrographis paniculata*, which exhibit prohormone/proprotein convertase (PC) inhibitory properties [11]. The flavonoids isolated from *Oroxylum indicum* were tested for inhibition of PC-enzymes including furin using *in vivo* fluorogenic peptide as substrate. The study interprets that “these flavonoids also efficiently blocked the PC4-mediated processing of a fluorogenic peptide derived from the processing site of its substrate, pro-Insulin Growth Factor-1 (proIGF-1)” and can have anticancer and antiviral activities [12]. In another study, chloroform soluble extract of *Morus alba* fruits, selected by proprotein convertase subtilisin-kexin type 9 (PCSK9) mRNA expression monitoring assay in HepG2 cells, led to the isolation of a new benzofuran isomoracin D and a naturally occurring N-(N-benzoyl-L-phenylalanyl)-L-phenylalaninol along with 13 known compounds. Out of those, moracin C was found to inhibit PCSK9 mRNA expression with an IC₅₀ of 16.8 μ M in HepG2 cells [13]. Besides this, various *in-silico* studies have been conducted on some compounds of natural origin like polyphenols, tannins, and polyacylated anthocyanins, exhibiting promising efficacy against SARS-CoV-2 [14–17].

The phytochemicals obtained from plants possess a wide spectrum of antimicrobial activity including potent antiviral activities against diverse groups of viruses [18]. This property of the plant extracts is due to the presence of a large number of phyto-constituents belonging to diverse groups such as phenols, flavonoids, alkaloids, saponins, terpenoids, glycosides, peptides etc. [15,19] with different modes of action in inhibiting the growth of viruses. The common mechanisms of actions include inactivation of viral particles [20], inhibition of viral adsorption and penetration [21] or entry, transcription [22], and viral protein synthesis [23]. The phenols or phenolic acids are important classes of phytochemicals displaying antiviral activity and the degree of activity depends on its number of hydroxyl groups [15,24]. Flavonoids obtained from ethanolic extract of *Ficus*

benjamina leaves were able to inhibit different strains of Herpes Simplex Virus (HSV) infection [25]. Berberine, a very potent member of protoberberine group of alkaloids inhibits HSV to enter into the host cells [26] while indole alkaloid harmelin inhibits HSV-1 and HSV-2 infection in mice by inhibiting immediate early transcription [27,28].

Apart from this, several plants have immunity boosting or immunomodulatory effects rendered by inducing the production of cytokines, interferons and interleukins [29,30]. *Artemisia annua*, a well-known anti-malarial plant is also reported to possess anti-viral potential against a number of viruses [31]. Although plants have immense potential for providing solutions to several emerging challenges and they can also have an important role as an adjuvant therapy in management of diseases until proper treatment is available. In order to select effective phytocompounds against COVID-19, in the present study, a number of herbal leads were selected on the basis of their traditional applications. The phytomolecules from these leads were further screened through *in-silico* molecular docking, against two major targets- the spike protein (S; structural protein) and the main protease (M^{PRO}; non-structural protein) of the SARS-CoV-2.

2. Material and methods

2.1. Screening and identification of phytomolecules

It is crucial to recognize the most effective therapeutic and functional herbal leads for the cure and prevention of SARS-CoV-2. Therefore, literature mining was performed and a number of plants and their phytomolecules (ligands) were selected from ancient Ayurvedic scriptures and *Samhitas* as well as recent scientific publications on the basis of their therapeutic applications. The protein structures of phytomolecules were retrieved from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and DrugBank (<https://drugbank.ca/>) for further analysis. Moreover, Remdesivir and Hydroxychloroquine were considered as standard ligands.

2.2. Characterization of selected phytomolecules

The selected phytomolecules (ligands) were further characterized on the basis of their applications, record of uses against respiratory disorders including SARS like symptoms, antiviral potential, their absorbance in the human body, half-life, toxicity, sustainability in humans with recommended dose and nature of solubility. Moreover, the most promising pharmacologically active

phytomolecules were further evaluated against human by using admetSAR prediction tool (<http://lmmd.ecust.edu.cn/admetSar2/>). To predict the essential pharmacokinetic nature of phytomolecules, properties such as absorption, permeability, distribution, metabolism, mechanism, excretion and toxicity were conducted to access their impact on the targets of human body along with safety assessments (Table S1).

2.3. Screening of SARS-CoV-2 targets

As per the previous studies, several targets including spike glycoprotein, papain like protease (PL^{PRO}), and main protease (M^{PRO}) like 3-chymotrypsin-like protease (3CL^{PRO}), Spike, RNA-dependent RNA polymerase (RdRp) were screened by computational approach to design the effective drugs against SARS-CoV-2 [32]. Out of different targets, main protease (M^{PRO}) was selected for molecular docking as it performs a decisive role in the processing of polyproteins that are translated from the viral mRNA [16,33]. Apart from that, Spike (S) protein was also selected to further validate the binding affinity of screened phytomolecules with other targets.

2.4. Molecular docking of phytocompounds with selected SARS-CoV-2 targets

To unravel the infallible curation efficacy against SARS-CoV-2, the screening of potential ligands have become a prime priority in current scenario. After the selection it remains important to recognize the binding affinity of potent phytomolecules at SARS-CoV-2 targets.

To find out this, the selected ligands were employed against the spike protein (structural protein) and main protease (non-structural protein) of SARS-CoV-2, with their corresponding structure in RCSB-PDB. These ligands were evaluated on the basis of two methods. Firstly, the structures were examined by using induced-fit docking to evaluate/analyze their active site blocking potential at structural level. The protein structures of SARS-CoV-2 main protease and spike-RBD with main protease were downloaded from RCSB-PDB database (<https://www.rcsb.org/>) using the ID, 6LU7, and 6LZG, respectively. These structures were further optimized in SPDBv tool (<https://spdbv.vital-it.ch/>) for gap and charge filling. A number of ligands were downloaded from PubChem and DrugBank databases to check against selected receptors proteins. The downloaded ligand structures were converted from SDF (structure data file) to PDB (protein data

bank) format using openbabel tool (<http://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html>).

The molecular interaction between protein and ligand were checked using two molecular docking methods. In the first step, the ligands were screened against receptor using autodock vina program (<http://vina.scripps.edu/>), ligands with higher dG were further selected for the induced-fit docking method based on the structural fit using Hex docking tool (<http://hex.loria.fr/>).

2.5. Ligand activity analysis

To refine the docking results and explore the interaction of ligand receptors with targeted site of SARS-CoV-2, the interactions of hydrogen bonds and hydrophobic contacts with residues of ligands were analyzed through schematic diagrams of protein-ligand interactions by using LIGPLOT v.4.5.3 [34]. Hydrogen bonds are indicated by dashed lines between the atoms involved, while hydrophobic contacts are represented by an arc with spokes radiating towards the ligand atoms they contact. The contacted atoms are shown with spokes radiating back.

3. Results

3.1. Screening of phytomolecules

Forty phytomolecules (ligands) were selected for the identification of potential candidates for higher binding/blocking affinity to suppress SARS-CoV-2. Out of these, 12 phytomolecules were screened on the basis of their bio-availability and therapeutic applications. The selected phytomolecules (Table 1) were reported as the major ingredients of different medicinal plants native to India and are easily available in the local markets in the respective phyto-geographical regions.

3.2. Characterization of selected ligands

The selected ligands were further characterized on the basis of their applications/record of uses against respiratory disorders including SARS-like symptoms, antiviral potential, absorbance in the human body, half-life, toxicity, sustainability in humans with recommended dose and nature of solubility (Table 1). The phytomolecules were reported earlier as non-toxic and recommended for human consumption. The biological half-life of the selected phytomolecules ranged from 16 min to 7 days which further explains the efficacy and digestion of the formulated drug after consumption in

dose dependent manner. Besides, the selected ligands also possessed antimicrobial activity against several human pathogens along with adjuvant beneficiary effects (Table 1).

3.3. Screening of SARS-CoV-2 targets

The main protease (M^{pro}) of SARS-CoV-2 was selected as target for molecular docking against this lethal virus responsible for COVID-19 respiratory illness. Out of its four structural proteins viz., spike (S), envelope (E), membrane (M) and nucleocapsid (N), the S, E and M proteins jointly participate in creating and maintaining the viral envelope. Apart from these, many other non-structural proteins (nsps) have also been identified in this virus [35], main protease being the most functionally important non-structural protein. The main protease processes the long polypeptide chain and proteolytically cleaves them, resulting in release of many other nsps, which also contribute to viral replication and other important functional processes [36]. This makes main protease an attractive target against SARS-CoV-2. The binding affinity of screened phytomolecules with spike protein of SARS-CoV-2 was also evaluated to analyze the binding affinity of selected ligands against structural protein.

3.4. Molecular docking of phytocompounds with selected SARS-CoV-2 targets

Remdesivir and Hydroxychloroquine were taken as standard ligand for comparison of both electrostatic potential as well as structural fit. The selected phytomolecules were evaluated against the binding affinity of standards. Out of 40 phytomolecules, 12 ligands viz., ginenoside, glycyrrhizic acid, etoposide, rutin, podophyllotoxin, colchicine, hesperidin, ampelopsin, meliampelopsin, berberine, teniposide and tribulosin were found promising against main protease and spike protein of SARS-CoV-2 (Table 2 and Table 3). Among these 12 promising ligands, nine showed higher blocking potential against main protease (Fig. 1) and spike protein (Fig. S1).

Hydroxychloroquine showed binding energy value (dG) of 537.1 and 225.9 in induced-fit method while electrostatic docking yielded 5.6 and 5.8 with main protease protein and spike protein respectively. The corresponding values in respect of Remdesivir following induced-fit method were 731.38 and 621.6 with main protease and spike protein, respectively. The values obtained following electrostatic docking were 9.4 and 7.4, with main protease and spike protein, respectively. The ligands were selected for further analysis on the basis of

Table 1. Half-life, toxicity, sustainability in human system and solubility of identified ligands.

Potential ligands	Previous reports	Half life	Toxicity	Sustainability in Human	Solubility
Ginsenoside (Rh2) A-Panaxadiol group	Anti-cancer Rh2 [74]; HIV protease inhibitor [75]; Anti-herpes virus [76]; protective effects against DOX-induced cardiotoxicity [61]; inhibits the growth of B16 melanoma cells [77]; promote apoptosis in leukemia cells [51]; protective role against lung cancer [60]; Decrease plasma glucose level in blood [59]; Strong activity in inhibition glioma cells A172 and T98G, breast cancer cells MCF7 and MDAMB-468, lung cancer cells H838, prostate cancer cells LNCap and PC3, pancreatic cancer cells HPAC and Panc-1, lung cancer cells A549 and H358 [78,79]. Largest absorption amount in jejunum and the fastest absorption rate in duodenum in rat experiment [78]. Inhibitory effects on hepatocyte apoptosis and liver fibrosis, reduce liver damage [84]; anti-inflammatory, anti-diabetic, antioxidant, anti-tumor, antimicrobial and anti-viral properties against flu, hepatitis and HIV [55]; Insulin resistance and reduce blood glucose level [56]; Anti-SARS-coronavirus [85], HIV, RSV, and Herpes simplex virus such as it reduces HMGB1 binding to DNA, and inhibit influenza virus polymerase activity; settle disturbed digestion; anti-inflammatory activity and reduce liver damage in Hepatitis B [54,86–88]	16 min. [80,81]	No toxic effect [82,83]	Vary in different reports (as per the mode of treatment and concentration)	DMSO and PBS buffer
Glycyrrhizic acid	Oral ingestion, partially digested by intestinal microflora (converts in to 18 β -glycyrrhetic acid) and complete absorption from the gut (18 β -glycyrrhetic acid is metabolized to 3 β -monoglucuronyl-18 β -glycyrrhetic acid in the liver)	3.5 h (in human)	Generally recognized as safe, non-lethal with mild chronic intoxication (mild hypertension, hypokalemia, edema, rhabdomyolysis or myoglobinuria [89,90]; Reduction of blood potassium level, which affects body fluid balance and nerves; increase body weight, muscle weakness, inhibition of cortisol metabolism within the kidney [91–93]	Very sustainable and digestible	Water
Rutin: Quercetin 3-rutinoside	Antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities [63]; Virucidal activity against enveloped viruses such as mengovirus, herpes simplex, para influenza type 3, pseudorabies, respiratory syncytial, and Sindbis viruses [94–97]; Targets, some non-structural proteins, including Nsp3b, Nsp3e, Nsp7_Nsp8 complex, Nsp9, Nsp10, Nsp14, Nsp15, and Nsp16, thereby affected the SARS-Cov2 virus RNA synthesis and replication [32]. Absorbed in small intestine and colon and rapidly metabolize in liver and circulate as methyl, glucuronide, and sulfate metabolites [98,99].	11–19 h [100,101]	Safe (Non-toxic) overdose cause some side effects including mild headache, flushing, rashes, or stomach upset	Oral intake inhibit platelets aggregation; poor absorption [102].	1:5 solution of DMF:PBS (pH 7.2)

(continued on next page)

Table 1. (continued)

Potential ligands	Previous reports	Half life	Toxicity	Sustainability in Human	Solubility
Meliacarpin: 122616-69-7	Anti-Inflammatory, insecticidal, rodenticidal, Anti-malarial, antibacterial, hepatoprotective, anti-oxidant, anti-diarrhoeal, deobstruent, diuretic, anti-diabetic, cathartic, emetic, anti-rheumatic, antihypertensive, anti-fertility, antelmintic, anti-pyretic and cytotoxic activities [103]; Reduce the load of Herpes simplex virus I and abolish ocular inflammation [104]; Pleiotropic agent that affects the replication of viral DNA and RNA and modulate the NFkB signaling pathway [105]; Inhibits HSV1 and HSV 2 replication [106]; Suppress H1N1, H3N2, and B influenza viruses replication [107]; Anti-SARS-coronavirus [108].	Not Determine	Gastrointestinal, Cardiovascular, Respiratory, or Neurological Effects, and high dose can be leads death in insects but human poisoning is rarely reported [109].	Not Determine	Partial soluble; Complete soluble in organic solvents
Berberine: 2086-83-1	Immunomodulatory, antioxidative, cardioprotective, hepatoprotective, and renoprotective effects [33,110–112]. Reducing fever, common cold, respiratory infections, and influenza [113]; Effective against metabolic diseases [114], diarrhea [115] and intestinal parasites [116]; repair mucous membranes of the upper respiratory tract and gastrointestinal system [117,118]; anti-influenza [119]; anti-Zika virus [120]; Reduces Chikungunya Virus-Induced Mitogen-Activated Protein Kinase Signaling [121]; Inhibit enterovirus-71 replication by modulating MEK/ERK signaling pathway and autophagy [40]; Suppress the infection of Middle east respiratory syndrome (MERS-CoV) coronavirus [122]; useful for the prevention and management of novel coronavirus (SARS-CoV-2) infection [123,124]	4–4.5 h [126]	Non-toxic with least side effects such as cramping, diarrhea, flatulence, constipation and stomach pain in the case of high dose without recommendation [127,128]	Very sustainable and useful in drug delivery system also	The salts are soluble in water, stable in acidic, and neutral media, while the base is soluble in organic solvents [129,130].
Hesperidin	Berberine is absorbed in Intestine of human body. Berberine accumulates within the intestine, where it may be metabolized by intestinal flora to improve its therapeutic effect on diabetes [125]. Useful for cardiovascular function, type II diabetes, and anti-inflammation, cutaneous functions, wound healing, UV protection, anti-inflammation, antimicrobial, antiskin cancer, and skin lighting [131]; reduce Rotavirus Infectivity [132]; anti-proliferative effect on human leukemic K562 cells [133]; reduce the replication of influenza virus [134,135]; inhibits enterovirus 71 replication [136]; protease inhibitor of SARS coronavirus [137]; potential 3CLpro inhibitors in SARS-CoV-2 [32] Multiple absorption (in blood stream, different tissues and intestine) [138,139].	6 h [140]	Non-toxic	Ameliorate gut microbiota and very sustainable [138]	Soluble in DMSO and dimethyl formamide

Etoposide: 33419-42-0	Effective against testicular cancer, lung cancer, lymphoma, leukemia, neuroblastoma and ovarian cancer because of topoisomerase inhibitor activity [62]; Suppress the Epstein–Barr virus (EBV), HSV-1, Paramyxoviruses and HIV infection [141–144]; Predicted drug which possesses Inhibitory effect against SARS-CoV-2 (2019-nCoV) [145] Directly absorb in blood stream of human body [62]	1.5 h (Half-life and terminal elimination half-life ranging from 4 to 11 h (FDA reference ID: 2919360)	High dose of Etoposide is relatively cell cycle specific, and it affects cells in the S and G2 phases of cell division. Myelosuppression, mild thrombocytopenia, mucositis, nausea, alopecia, and emesis. Low blood cell counts, vomiting, loss of appetite, diarrhea, hair loss, and fever [146]; FDA reference ID: 2919360)	Intravenous dose cause fetal harm during pregnancy; One of the World Health Organization's List of Essential Medicines, the safest and most effective medicines for health system [62].	Water soluble
Colchicine: 64-86-8	Under trial drug which possess Inhibitory effect against SARS-CoV-2 (2019-nCoV) [147–150]; Useful in cardiac disease, including coronary artery disease with anti-gout effect [67,68]; Effective against Chikungunya, Epstein–Barr and cytomegalovirus [151,152]; Prevent the inflammatory cardiac disorders, including stable coronary artery disease and postpericardiotomy syndrome [153,154] Pericardiotomy related issues in viral infections [155,156]; Suppress the infection of Influenza virus [157] Directly absorbed in the jejunum and ileum [68]. Use in the treatment of small cell lung cancer, brain tumors, Hodgkin's lymphoma, neuroblastoma, malignant lymphoma, reticulocyte sarcoma, acute leukaemia and acute lymphocytic leukemia which induce apoptosis by acting as topoisomerase II inhibitors [161,162]; Transcription factor MYB inhibitor which suppress the infection of avian myeloblastosis virus [163]; Inhibit the replication of Simian Virus 40 (SV40) [164]; Suppress adenovirus infection [165].	20–40- hrs [158]	Non-toxic; Higher dose may be cause Diarrhea, nausea, cramping, abdominal pain, and vomiting [148,159]	Oral administration is sustainable (but as per the prescribe limitation)	Soluble in DMSO (14 mg/ml), 100% ethanol (7 mg/ml), water (7 mg/ml) or dimethylformamide (12 mg/ml) [160].
Teniposide: VM26	Use for the treatment of genital warts and molluscum contagiosum during human papilloma virus infections [167,168]; Suppress testicular, breast, pancreatic, lung, stomach, and ovarian cancers [169]; Use as cathartic, purgative, vesicant, anti-helminthic and anti-tumor agent [170]; antineoplastic and antiviral activities [171]; inhibit the replication of measles and Herpes simplex type 1 viruses [172,173]; Inhibits the growth of P-388 murine leukemia and A-549 human lung carcinoma [174]	6–8 h [166]	Higher dose causes Gastrointestinal toxicity, hypersensitivity reactions and reversible alopecia [161]	Intravenous application burns if it leaks under the skin; Contraindicated during pregnancy and lactation, with severe liver or kidney impairment or severely impaired haematopoiesis [161].	Sparsely soluble in aqueous solution; 1:1 solution of DMSO:PBS (pH 7.2)
Podophyllotoxin: 518-28-5	Use for the treatment of genital warts and molluscum contagiosum during human papilloma virus infections [167,168]; Suppress testicular, breast, pancreatic, lung, stomach, and ovarian cancers [169]; Use as cathartic, purgative, vesicant, anti-helminthic and anti-tumor agent [170]; antineoplastic and antiviral activities [171]; inhibit the replication of measles and Herpes simplex type 1 viruses [172,173]; Inhibits the growth of P-388 murine leukemia and A-549 human lung carcinoma [174]	1.0–4.5 h [175]	Minimal side effects, which are typically limited to itching, swelling, irritation, burning and redness [176]; embryotoxic effect during pregnancy [177]; prohibited during breast feeding [178].	Safe for topical use; however, it can cause CNS depression as well as enteritis if ingested	Soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF) [179].

(continued on next page)

Table 1. (continued)

Potential ligands	Previous reports	Half life	Toxicity	Sustainability in Human	Solubility
Ampelopsin	Antioxidative, anti-inflammatory, anticancer, antimicrobial, cell death-mediating, and lipid and glucose metabolism –regulatory activities [180]; use in the treatment of nephritis, hepatitis, halitosis, and polyorexia [181]; Anti-aging and anti-neurodegenerative diseases [182,183]; exerts anti-inflammatory effects in fatty liver disease [184]; Protects against acute brain injury [185]; Induce growth inhibition and apoptosis in Breast Cancer [186]; Protect against Alzheimer disease, Parkinson's disease, depressive disorder, hypobaric hypoxia or fetal alcohol exposure (FAE) induced brain injury and behavioral deficits along with osteoporosis, asthma, kidney injury, nephrotoxicity [73]; anti-influenza and anti hepatitis activity [187,188]; Inhibit replication of infectious bronchitis virus [115]	Not-Determine	Mostly non-toxic; High dose leads Less headache, dizziness, nausea, and weakness [180]	Oral administration [185]	Soluble in DMSO to 100 mM and in ethanol to 100 mM [189]
Tribulosin: 79974-46-2	Protects myocardium against ischemia/reperfusion injury through PKC α activation [190]; Cytoprotective effects in heart [191]; Maintain the testosterone level; reduce the cholesterol and triglyceride levels in blood [192]. Multiple absorption (in blood stream, different tissues and intestine) [193]	6–8 Days [194]	No toxic effect [191]	Vary in different reports (as per the mode of treatment and concentration)	DMSO, Water, Methanol

greater dG values. In case of main protease, the binding affinity of selected ligands varied between 366.74 and 1312.5 (induced-fit method), and 6.1 and 9.4 (electrostatic docking). For spike protein, it ranged from 195.9 to 686.6 when ligands were analyzed following induced-fit method and 6.3 to 9.3 in electrostatic docking analysis. The findings of present investigation suggest that the selected ligands are not only binding effectively with the non-structural protein (i.e. main protease) but also attaching with the structural protein i.e. spike protein of SARS-CoV-2 (Table 2; Table 3).

3.4.1. Docking with main protease

The docking with main protease revealed that Ginsenoside, Glycyrrhizic acid, Hesperidin, Tribulosin and Teniposide showed high binding energy indicating their highly efficacy (Table 2). Ginsenoside (dG 1312.5), Glycyrrhizic acid (1250.6) and Teniposide (931.7) exhibited very high dG values when analyzed using induced-fit method. Glycyrrhizic acid (9.4), Hesperidin (9.1) and Tribulosin (9.0) had high binding energy when assessed through electrostatic interaction method. The selected ligands showed h-bonding with Gln189, His41, Ser144, Leu141, His164, His246, Leu242, Gln110, Asp153, Ser158, Pro108 and Thr196 amino acid residues, which directly or indirectly participate in active site and secondary hotspot formation, important for proteolytic cleavage activity of protease domain (Fig. 2).

3.4.2. Docking with spike protein

The docking of selected ligands with spike proteins revealed that Glycyrrhizic acid, Ginsenoside, Tribulosin, Etoposide and Hesperidin showed high binding energy (Table 3). Glycyrrhizic acid (686.6), Ginsenoside (686.3) and Tribulosin (612.7) exhibited high dG values when analyzed using induced-fit method. When analyzed using electrostatic interaction method Glycyrrhizic acid (9.3), Etoposide (8.7), Tribulosin (8.4) and Hesperidin (8.3) exhibited high binding energy with spike protein. The binding energy of these ligands also indicated that they are not only able to bind with non-structural targeted proteins but also are able to bind with structural proteins of SARS-CoV-2 (Fig. S1).

3.5. Positional binding affinity of selective ligands with main protease

The positional binding activity of ligands showed the selective preference of 'O' moiety (oxygen moiety) for binding against the various active site

Table 2. Binding energy of ligands against Main protease.

M ^{Pro} Human	Ligand	Binding energy dG (HEX) kcal mole ⁻¹	Binding energy dG (VINA) kcal mole ⁻¹
6lu7	Remdesivir (Control)	731.38	9.4
6lu7	Hydroxychloroquine (Control)	537.1	5.6
6lu7	Tribulosin	841.8	9
6lu7	Berberine	366.74	6.1
6lu7	Colchicine	701.4	7.1
6lu7	Etoposide	837.9	8.3
6lu7	Ginsenoside	1312.5	8.4
6lu7	Glycyrrhizic acid	1250.6	9.4
6lu7	Hesperidin	691.9	9.1
6lu7	Ampelopsin	476.2	7.3
6lu7	Meliacarpin	447.88	7.7
6lu7	Podophyllotoxin	720.1	8.3
6lu7	Rutin	744.2	8.9
6lu7	Teniposide	931.7	8.7

residues of main protease active site (Fig. 2). In case of ginsenoside, glycyrrhizic acid and hesperidin, it is evident that main chain 'O' tends towards the nucleophilic addition with the amino acid atoms (OC1, OE1, OE2). The preference of atom self-indicate towards more electronegative interaction, which makes the h-bonding stronger in case of these 3 ligands (Fig. 2C,D,J). While in the case of etoposide again the reactive 'O' moiety shows higher interaction affinity for the NE2 of His41, SG of Cys145, 'O' of Phe140 (Fig. 2E). This electronegative reaction pattern is also followed by podophyllotoxin (Fig. 2G). Teniposide, due to its flexible torsion flexibility involves a number of atoms of interacting amino acids and forms more strong bonds. Involvement of 'N' and 'O' moiety in teniposide makes it stronger nucleophile adductor to fuse the amino acids of main protease (Fig. 2F).

The preference for different amino acids of main protease active site also shows the ability of ligands to recognize suitable amino acid for binding (Fig. 2).

Again, the functional activities of these ligands are already known for their anti-inflammatory and anti-viral activity. Considering the mode of action of SARS-CoV-2, which may develop cytokine storm in patients, eventually increases the higher inflammatory condition in human body, thus making the immune system more susceptible and weak to combat the infection and effects of the virus. Our selected ligands will be highly useful not only due to their blocking and binding activity against main protease and spike protein, but also because of their anti-inflammatory potential and supporting ability of the human body by reducing the cytokine burst.

Another interesting observation was the binding of rutin, where it binds in a major cavity apart from the other ligands that showed more affinity towards a terminal site (Fig. 2H). As rutin is already known for its antiviral property, it could be a potent lead against this virus and as an ingredient in designing the effective formulation. The binding of these phytochemical leads were demonstrated by H-

Table 3. Binding energy of ligands against spike protein.

Spike protein	Ligand	Binding energy dG (HEX) kcal mole ⁻¹	Binding energy dG (VINA) kcal mole ⁻¹
6lzg	Remdesivir (Control)	621.6	7.4
6lzg	Hydroxychloroquine (Control)	225.9	5.8
6lzg	Tribulosin	612.7	8.4
6lzg	Berberine	195.9	6.8
6lzg	Colchicine	328.7	6.3
6lzg	Etoposide	327.3	8.7
6lzg	Ginsenoside	686.3	7.1
6lzg	Glycyrrhizic acid	686.6	9.3
6lzg	Hesperidin	416.2	8.3
6lzg	Ampelopsin	291	6.9
6lzg	Meliacarpin	226.7	6.9
6lzg	Podophyllotoxin	279.4	7.2
6lzg	Rutin	468.4	7.8
6lzg	Teniposide	482.2	7.6

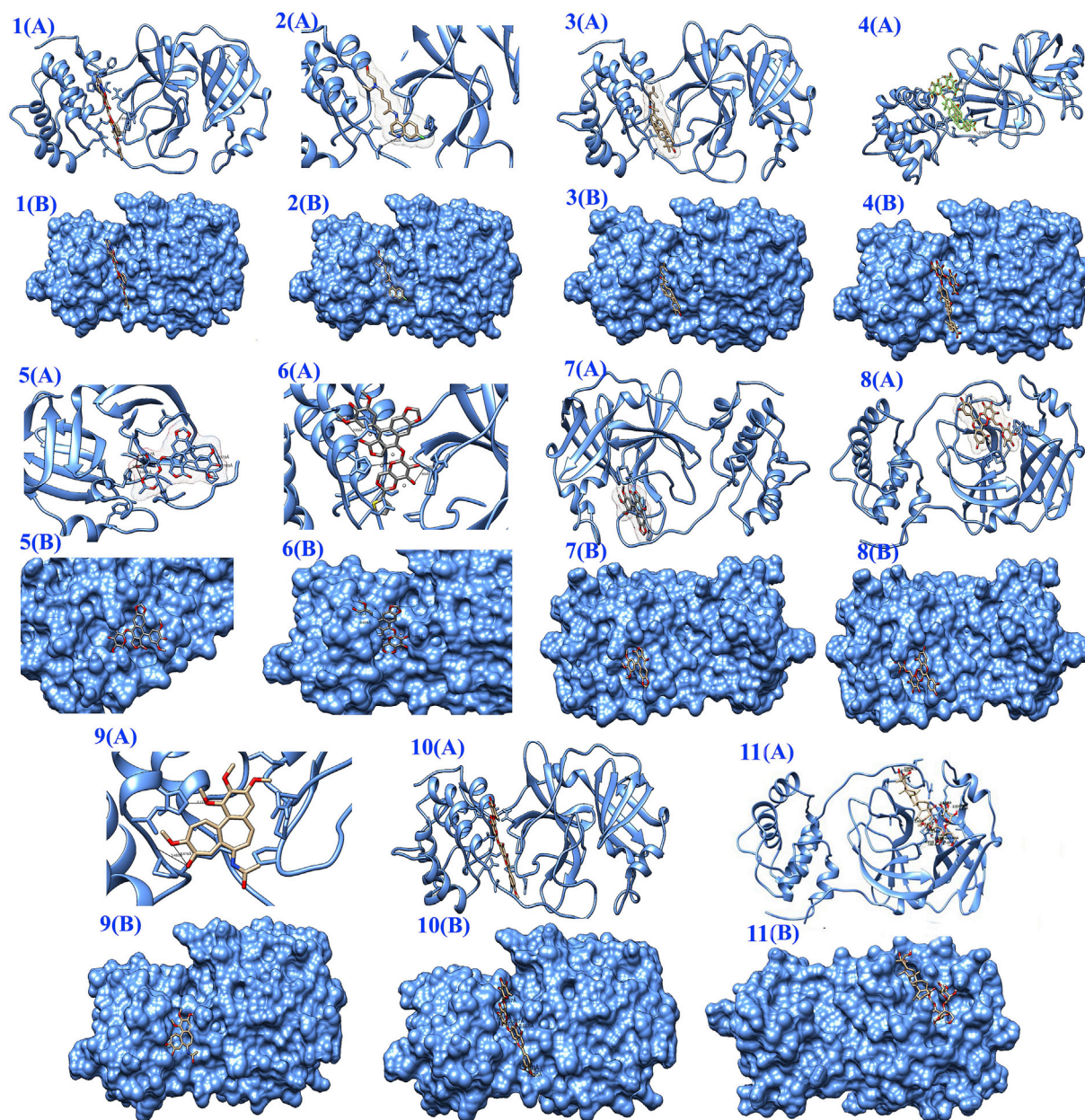


Fig. 1. Representation of molecular interaction between main protease of SARS-CoV-2 and phytochemicals based on molecular docking. (A) *h*-bonding and (B) cavity blocking by ligand in surface representation. In figure, 1 = Remdesivir; 2 = Hydroxychloroquine; 3 = Ginsenoside; 4 = Glycyrrhizic acid; 5 = Etoposide; 6 = Teniposide; 7 = Podophyllotoxin; 8 = Rutin; 9 = Colchicine; 10 = Hesperidin and 11 = Tribulosin.

bonding with major active site residues which suggest that the ligands with more than 1 torsion center are effectively targeting and binding with the functional cavities of both the proteins (Fig. 2).

4. Discussion

The infection of SARS-CoV-2 and its devastatingly spread all over the world through Wuhan city, Hubei province of China since its first report (December, 2019) has recorded a total of 15,18,03,822

confirmed cases of infection and 3,186,538 deaths in 213 countries including India which accounts for 1,95,57,457 infections and 2,15,542 fatalities till May 2nd, 2021 (<https://covid19.who.int/>). Its genome assembly is 29,881 bp in length (GenBank no. MN908947) encoding 9860 amino acids [37,38], shows 79% similarity with SARS coronavirus that belongs to the genus β -coronavirus [39]. The virus was therefore, named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on the Taxonomy of Viruses.

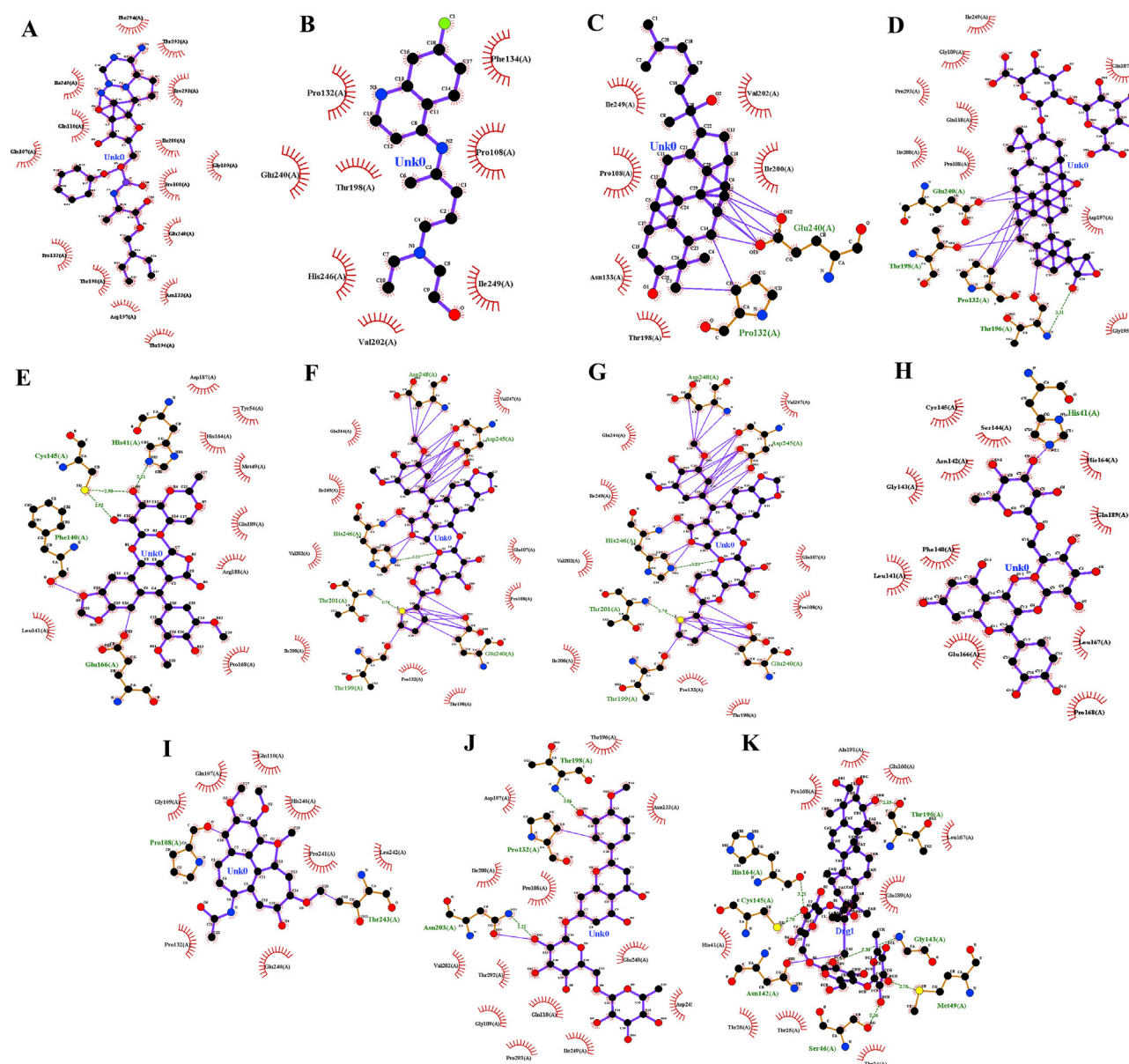


Fig. 2. Ligand activity plot of phytomolecules against the main protease active site residue. In figure, A = Remdesivir; B= Hydroxychloroquine; C = Ginsenoside; D = Glycyrrhizic acid; E = Etoposide; F= Teniposide; G = Podophyllotoxin; H = Rutin; I = Colchicine; J = Hesperidin and K = Tribulosin.

There are so many studies executed for in-depth knowledge about SARS-CoV-2 to develop effective drugs. But unfortunately, till date neither any authentic information nor any specific drug is available in the market for cure and prevention of COVID-19 caused by SARS-CoV-2. However, some previously used anti-viral drugs (against SARS and MERS) were screened by investigators, but only plant based Hydroxychloroquine and synthetic Remdesivir were reported to suppress the proliferation of SARS-CoV-2 in cell based *in-vitro* assay [40]. Now, it has been approved by the WHO for global

consumption against SARS-CoV-2 infections as preventive measures [7]. However, the mode of action of these antiviral drugs against SARS-CoV-2 in human system has not been reported yet. Further research is being carried out on various synthetic molecules e.g. favipiravir, ivermectin, ribavirin etc.

Apart from the synthetic molecules, several herbal molecules are also being tried for their anti-viral efficacy. A few such herbal claims with ability to cure fever, cough, fatigue and related illness hold promise for effective herbal formulations against SARS-CoV-2 [41]. However, development of an

effective herbal drug against SARS-CoV-2 based on quality screening and scientific validation is yet to be a reality. Therefore, the present investigation was carried out to assess the efficacy and binding affinities of the selected ligands/molecules with functional active sites in main protease (non-structural protein) and spike protein (structural protein) of SARS-CoV-2.

Traditional medicines and herbal products from different medicinal plants have immense potential to provide leads for therapeutically important compounds. Due to genetic and environmental diversity, a given medicinal plant species also varies in their chemical constituents [42,43]. Utilization of different herbal compounds/molecules as potent therapeutic agents against several ailments has gained momentum in recent years globally. Therefore, 40 herbal compounds were selected based on their previous applications/therapeutic uses for screening their ability to prevent COVID-19 on the basis of receptor-ligand docking on main active sites of SARS-CoV-2. Similar *in silico* screening of herbal compounds against SARS-CoV-2 has been undertaken recently by several workers [44–47].

SARS-CoV-2 contains several structural proteins including S, E, M, and N protein [48] along with non-structural proteins including papain-like protease, main protease as 3-chymotrypsin-like protease, and RNA-dependent RNA polymerase. Out of all the structural proteins, spike proteins initiate the host attachment and fusion of viral membrane during pathogenicity [32]. The main protease (M^{pro}) of previous SARS coronaviruses is the most studied protein, and also in SARS-CoV-2, it is responsible for the proteolytic cleavage of pp1a and pp1ab proteins for the initiation of viral replication and pathogenicity in the host [49]. Therefore, based on the structural description and inhibition mechanisms of the previously reported SARS-CoV, M^{pro} was made as the target for the suppression of SARS-CoV-2 infection [50]. This was the main reason for the selection of spike like proteins (structural protein) and main protease (non-structural protein) for the *in silico* screening of most potent herbal leads against SARS-CoV-2 [50–52].

The computational docking through Induced fit method and electrostatic binding, suggests that the screened herbal molecules/compounds including Glycyrrhizic acid, Ginsenoside, Tribulosin, Etoposide, Hesperidin and Rutin showed very promising binding affinity with spike protein. Similarly, a few compounds such as Ginsenoside, Glycyrrhizic acid, Teniposide, Tribulosin, Etoposide, Podophyllotoxin, Rutin, Cochicine and Hesperidin also showed higher value with main protease in induced fit and

secondary electrostatic screening, which indicates that selected ligands are not only targeting specific amino acids but also are able to block the reported main active site of the main protease. Many earlier workers have also shown that phytochemicals possess significant anti-viral activities [17,53]. For instance, Glycyrrhizic acid derivatives used for treating chronic hepatitis, not only suppress the replication of SARS-CoV [54] but also possess anti-oxidant, anti-inflammatory and anti-diabetic properties [55,56]. Ginsenoside derived from *Panax ginseng*, inhibits the glycoprotein activity of SARS-CoV [57,58]. Since lungs infection and elevated blood sugar level are directly associated with COVID-19 infection, Ginsenoside can reduce the plasma glucose level in blood and could be beneficial in controlling lung infection and cardiotoxicity [59–61].

Etoposide is a well-known antitumoral compound, also screened as protease (3CLpro) inhibitor of SARS-CoV-2 [38]. Because of its beneficiary role in reducing infections and enhancing immunity, WHO has included etoposide in its list of essential medicines [62]. Similarly, rutin (flavonoid), a well-known anti-oxidant and antimicrobial agent [63] was useful for the suppression of Murine CoV [64]. Rutin possibly initiates the suppression of functional protein assembly and host inflammation during the infection of SARS-CoV-2 [65]. It has been reported that colchicine, an anti-rheumatic drug efficiently suppresses the activation of nucleotide binding protein and leucine rich repeat protein 3 assemblies, and diminishes the level of cytokine storm during SARS-CoV-2 infection [66]. It is also very useful in cardiac disease and significantly possesses antioxidant along with several health beneficial properties [67,68]. Similarly, Hesperidin can prevent the host cell entry of the viron through interaction with ACE2 receptors and thereby suppressing the infection. Additionally, the anti-inflammatory and immunity boosting ability of Hesperidin might provide a better option for controlling cytokine storm in SARS-CoV-2 infection [69]. This is indicated in the present study with higher binding affinity of selected herbal compounds with spike protein as well as main protease. A few compounds viz., Tribulosin, Teniposide (FDA approved thiol-reacting drug) and Podophyllotoxin also showed promising binding efficiency with main protease and spike protein which is not yet explored for their anti-SARS-CoV-2 activities. However, these phytochemicals have such significant broad spectrum therapeutic applications as anti-oxidants, immune-stimulants, hormone balancers, and are used for treating cancer, liver, kidney and

cardiovascular disorders [70–73]. Although, fever, dry cough, dyspnea, fatigue and myalgia are the most commonly reported symptoms of COVID-19, insufficient immune response also leads to severe damage of cardiovascular system, gut, kidneys and brain. It enhances blood glucose level manifold in infected people and also leads to blood clot, vessel destruction, pulmonary embolism, loss of memory, and diarrhea. Besides controlling viral infection in COVID-19 patients, the selected herbal compounds also possess several other beneficiary properties which reduce the adverse effects of COVID-19. Although, a comprehensive wet lab validation and critical clinical trials are required to confirm the pharmacological activities of these compounds, yet the *in silico* study has provided important leads for the development of most potent herbal drug against SARS-CoV-2 in near future.

5. Conclusion

In the present investigation, 40 phytomolecules were analyzed, of which 12 phytomolecules were found potential, and can block or stabilize the major functional sites of main protease and spike-RBD protein. Ginsenoside, Glycyrrhizic acid, Hesperidin and Tribulosin exhibited high binding energy with both main protease and spike protein. Etoposide showed good binding energy only with Spike protein and Teniposide had high binding energy only with main protease. Our findings suggest that the identified phytomolecules could be promising leads against the infection of SARS-CoV-2 as the spike protein is the one that recognizes human ACE-2 receptors and is the known entry point for the virus into the host cell. The blocking response will reduce the initial recognition and infection of the virus in human body, while docking with the main protease indicates that our leads can play crucial role to

stopping the proteolytic cleavage done by the main protease, and could be a deciding step in decreasing the growth rate in later stage of viral life cycle.

Molecular docking shows that phytomolecules such as ginsenoside, glycyrrhizic acid and teniposide showed greater binding potential than the established drugs like Remdesvir and Hydroxychloroquine. Two ligands colchicine and rutin targeted the other hotspots of protein than the reported active site which makes them as promising targeted medicine. Phytomolecules such as tribulosin, ginsenoside and podophyllotoxin showed structural similarity with Remdesivir, which indicates that such structures has higher recognition for amino acids at the binding site. Apart from blocking the target proteins of SARS-CoV-2, these ligands have anti-inflammatory and immunomodulating activities thereby lowering the acute symptoms and fastening the recovery.

The findings of this study are useful to initiate *in vitro* and *in vivo* experimentation for finding potential drug molecules for the treatment of SARS-CoV-2 infections. In addition, different permutations and combinations of these phytomolecules may also be tried to achieve positive synergistic effects with enhanced bioavailability.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors are thankful to the Director, CSIR-National Botanical Research Institute, Lucknow for providing necessary facilities.

Appendix A.

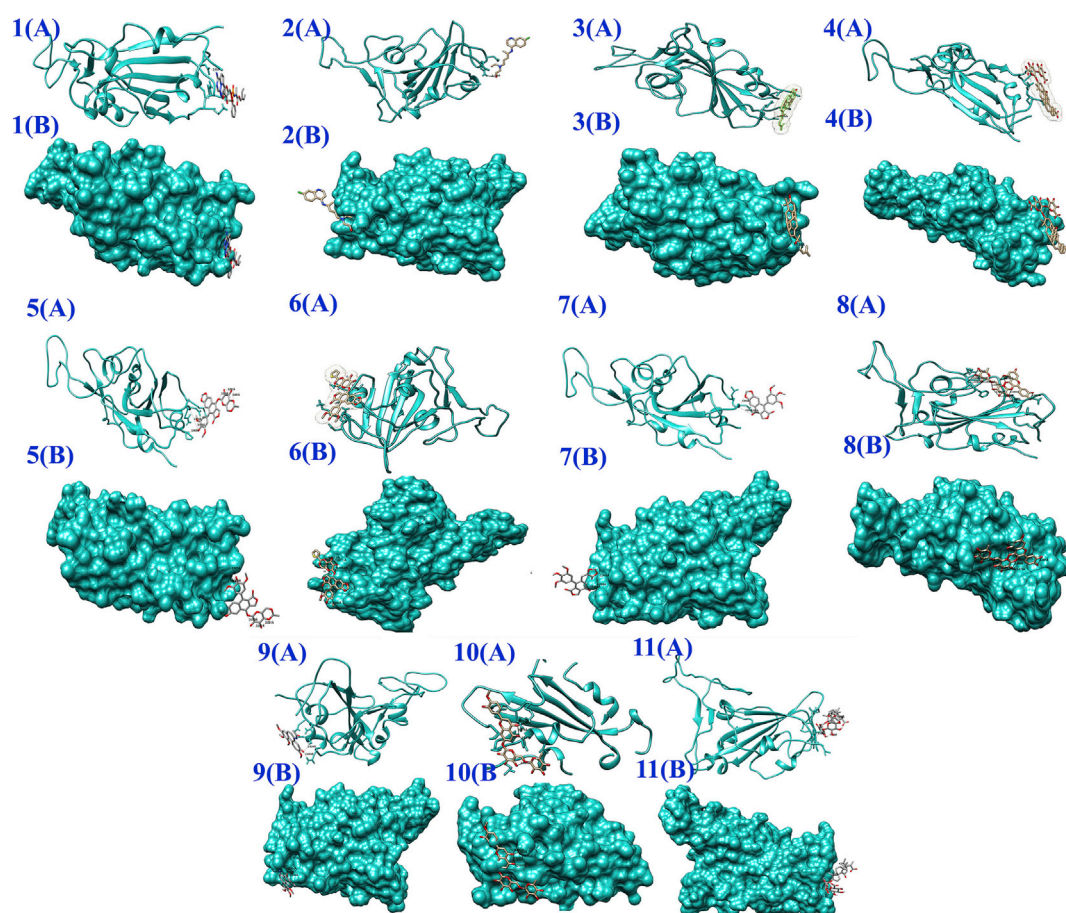


Fig. S1. Molecular interaction between spike protein of SARS-CoV-2 and potent phyto-molecules. Representation of molecular interaction between spike protein of SARS-CoV-2 and phytomolecules based on molecular docking. Figure (A) representing h-bonding and Figure (B) showing the cavity blocking by ligand in surface representation. In figure, 1=Remdesivir; 2= Hydroxycycloquine; 3= Ginsenoside; 4= Glycyrrhizic acid; 5= Etoposide; 6= Taniposide; 7= Podophyllotoxin; 8= Rutin; 9= Colchicine; 10= Hesperidin and 11= Tribulosin.

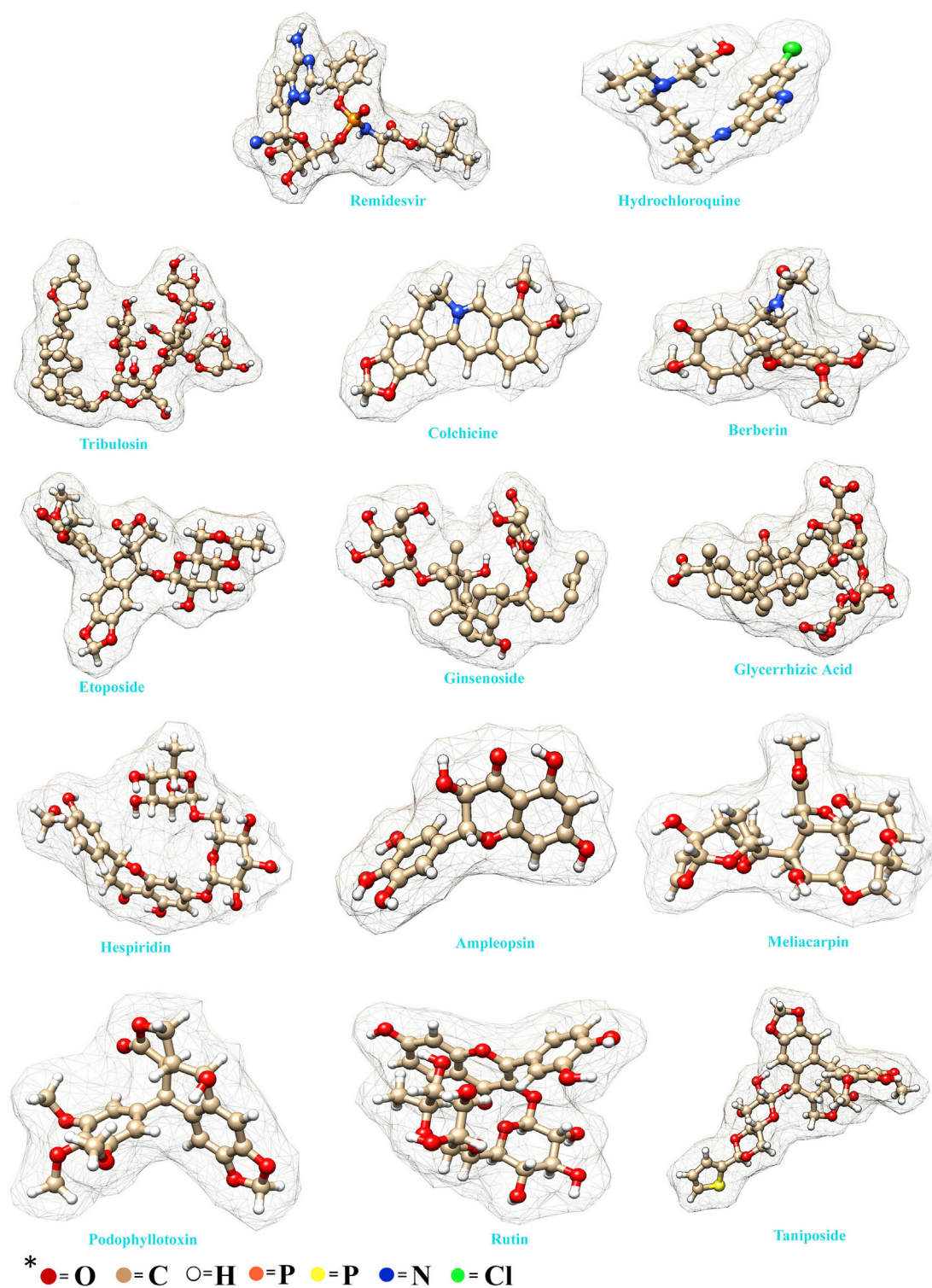


Fig. S2. Ligand leads with highlighted moieties.

Table S1. ADMET properties of most potential leads

Parameters	Ampelopsin	Berberine	Colchicine	Etoposide	Ginsenoside	Glycyrrhizic acid
Ames mutagenesis	—	—	—	—	—	—
Acute Oral Toxicity (c)	II	III	III	III	II	IV
Androgen receptor binding	+	+	+	+	+	+
Avian toxicity	—	—	—	—	—	—
Blood Brain Barrier	—	+	+	—	—	—
BRCP inhibitor	—	—	—	—	—	—
Biodegradation	—	—	—	—	—	—
Caco-2	—	+	+	—	—	—
Carcinogenicity (binary)	—	—	—	—	—	—
Crustacea aquatic toxicity	—	+	—	—	—	—
CYP1A2 inhibition	+	+	—	—	—	—
CYP2C19 inhibition	—	—	—	—	—	—
CYP2C9 inhibition	—	—	—	—	—	—
CYP2C9 substrate	—	—	—	—	—	—
CYP2D6 inhibition	—	+	—	—	—	—
CYP2D6 substrate	—	—	—	—	—	—
CYP3A4 inhibition	+	—	—	—	—	—
CYP3A4 substrate	—	+	+	+	+	+
Eye corrosion	—	—	—	—	—	—
Eye irritation	+	—	—	—	—	—
Hepatotoxicity	+	+	+	—	—	—
Human Intestinal Absorption	+	+	+	+	—	+
Human oral bioavailability	—	—	+	+	—	—
Acute Oral Toxicity	3.246833086	1.5449361	2.2246482	2.6902423	3.159646034	2.132831097
P-glycoprotein inhibitor	—	+	—	—	+	+
P-glycoprotein substrate	—	—	+	+	—	—
OATP1B1 inhibitor	+	+	+	+	+	—
OATP1B3 inhibitor	+	+	+	+	+	—
OATP2B1 inhibitor	—	—	—	—	—	—
OCT1 inhibitor	—	+	—	—	—	—
OCT2 inhibitor	—	—	—	—	—	+
P-glycoprotein inhibitor	—	+	—	—	+	+
P-glycoprotein substrate	—	—	+	+	—	—
Plasma protein binding	1.067124009	0.8344979	0.4847819	0.8784482	0.985907972	0.931964338
Subcellular localization	Mitochondria	Mitochondria	Nucleus	Mitochondria	Mitochondria	Mitochondria
Thyroid receptor binding	+	+	+	+	—	—
Water solubility	2.99937319	2.97369	2.560883	3.507494	4.333252	4.512803577

Hesperidin	Hydroxy-chloroquine	Meliacarpin	Podophyllotoxin	Remidesvir	Rutin	Taniposide	Tribulosin
–	+	+	–	–	–	+	–
III	III	I	III	III	III	III	II
–	–	+	+	+	+	+	+
–	–	–	–	–	–	–	–
–	+	+	+	+	–	+	–
+	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–
–	+	–	+	–	–	–	–
–	–	+	–	+	–	–	+
–	–	–	–	–	–	–	–
–	–	–	+	–	–	+	–
–	–	–	+	–	–	–	–
–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–
–	+	–	–	–	–	–	–
–	–	–	+	–	–	+	–
+	+	+	+	+	+	+	+
–	–	–	–	–	–	–	–
+	–	–	–	–	–	–	–
+	+	+	+	+	+	+	–
–	+	–	–	–	–	–	–
2.257097	2.664962053	4.12018585	1.972803831	3.42793131	2.593124	2.90450954	4.589214
–	–	–	–	+	–	–	+
–	+	+	–	+	–	+	–
+	+	+	+	+	+	–	+
+	+	+	+	+	+	–	+
–	–	–	–	–	–	–	–
–	+	–	–	–	–	–	–
–	–	–	–	–	–	–	+
–	–	–	–	+	–	–	+
–	+	+	–	+	–	+	–
1.0964364	0.756124914	0.94871801	0.936204255	1.18216193	0.982454	0.96763605	0.725269
Mitochondria	Lysosomes	Mitochondria	Mitochondria	Lysosomes	Mitochondria	Mitochondria	Mitochondria
+	+	+	+	+	+	+	–
2.648535	3.565742248	3.59128747	3.47339466	3.4735559	2.7724	3.198662	3.4138

References

- [1] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;579(7798):270–3.
- [2] De Wit E, Van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol* 2016;14(8):523.
- [3] Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *New Engl J Med* 2020; 382(13):1199–207.
- [4] Jordan PC, Stevens SK, Deval J. Nucleosides for the treatment of respiratory RNA virus infections. *Antiviral Chem Chemother* 2018;26. 2040206618764483.
- [5] Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, et al. First case of 2019 novel coronavirus in the United States. *New Engl J Med* 2020.
- [6] Rathi S, Ish P, Kalantri A, Kalantri S. Hydroxychloroquine prophylaxis for COVID-19 contacts in India. *Lancet Infect Dis* 2020;20(10):1118–9.
- [7] World Health Organization. Informal consultation on the potential role of chloroquine in the clinical management of COVID 19 infection. Geneva, Switzerland. 13th March 2020.
- [8] Bhinde S. Rasayana: a better alternative for disease prevention. *J Ayurveda Holistic Med* 2013;1:6–14.
- [9] Acharya YT, Samhita Charaka, editors. Varanasi: Chowkhamba Surbharati; 2000 (Ch. Vi. 3/6).
- [10] Samal J. Fundamental tenets of epidemiology in Ayurveda and their contemporary relevance. *IJHMS* 2016;9(1):20.
- [11] Basak A, Cooper S, Roberge AG, Banik UK, Chretien M, Seidah NG. Inhibition of proprotein convertases-1,-7 and furin by diterpines of *Andrographis paniculata* and their succinoyl esters. *Biochem J* 1999;338:107–13.
- [12] Majumdar S, Mohanta BC, Chowdhury DR, Banik R, Dinda B, Basak A. Proprotein convertase inhibitory activities of flavonoids isolated from *Oroxylum indicum*. *Curr Med Chem* 2010;17(19):2049–58.
- [13] Pel P, Chae HS, Nhoek P, Kim YM, Chin YW. Chemical constituents with proprotein convertase subtilisin/kexin type 9 mRNA expression inhibitory activity from dried immature *Morus alba* fruits. *J Agr Food Chem* 2017;65(26): 5316–21.
- [14] Muthuramalingam P, Jeyasri R, Valliammai A, Selvaraj A, Karthika C, Gowrishankar S, et al. Global multi-omics and systems pharmacological strategy unravel the multi-targeted therapeutic potential of natural bioactive molecules against COVID-19: An in silico approach *Genomics* 2020; 112(6):4486–504.

- [15] Mehany T, Khalifa I, Barakat H, Althwab SA, Alharbi YM, El-Sohaimy S. Polyphenols as promising biologically active substances for preventing SARS-CoV-2: a review with research evidence and underlying mechanisms. *Food Biosci* 2021;100891.
- [16] Khalifa I, Nawaz A, Sobhy R, Althwab SA, Barakat H. Pol-yacylated anthocyanins constructively network with catalytic dyad residues of 3CLpro of 2019-nCoV than monomeric anthocyanins: a structural-relationship activity study with 10 anthocyanins using in-silico approaches. *J Mol Graph Model* 2020;100:107690.
- [17] Khalifa I, Zhu W, Mohammed HH, Dutta K, Li C. Tannins inhibit SARS-CoV-2 through binding with catalytic dyad residues of 3CLpro: an in silico approach with 19 structural different hydrolysable tannins. *J Food Biochem* 2020;44(10): e13432.
- [18] Hassan ST, Masarcikova R, Berchova K. Bioactive natural products with anti-herpes simplex virus properties. *J Pharm Pharmacol* 2015;67(10):1325–36.
- [19] Mukherjee H, Ojha D, Bag P, Chandel HS, Bhattacharyya S, Chatterjee TK, et al. Anti-herpes virus activities of *Achyranthes aspera*: an Indian ethnomedicine, and its triterpene acid. *Microbiol Res* 2013;168(4):238–44.
- [20] Cheng HY, Yang CM, Lin TC, Lin LT, Chiang LC, Lin CC. Excoecarianin, isolated from *Phyllanthus urinaria* Linnaea, inhibits herpes simplex virus type 2 infection through inactivation of viral particles. *Evid-Based Complementary Altern Med* 2011;2011:259103.
- [21] Gescher K, Kühn J, Hafezi W, Louis A, Derksen A, Deters A, et al. Inhibition of viral adsorption and penetration by an aqueous extract from *Rhododendron ferrugineum* L. as antiviral principle against herpes simplex virus type-1. *Fito-terapia* 2011;82(3):408–13.
- [22] Lim YX, Ng YL, Tam JP, Liu DX. Human coronaviruses: a review of virus–host interactions. *Diseases* 2016;4(3):26.
- [23] Bertol JW, Rigotto C, de Padua RM, Kreis W, Barardi CRM, Braga FC, et al. Antiherpes activity of glucoevatomonoside, a cardenolide isolated from a Brazilian cultivar of *Digitalis lanata*. *Antiviral Res* 2011;92:73–80.
- [24] Ikeda K, Tsujimoto K, Uozaki M, Nishide M, Suzuki Y, Koyama AH, et al. Inhibition of multiplication of herpes simplex virus by caffeic acid. *Int J Mol Med* 2011;28(4):595–8.
- [25] Yarmolinsky L, Zaccai M, Ben-Shabat S, Mills D, Huleihel M. Antiviral activity of ethanol extracts of *Ficus Benjamina* and *Lilium candidum* in vitro. *N Biotechnol* 2009; 26(6):307–13.
- [26] Chin LW, Cheng YW, Lin SS, Lai YY, Lin LY, Chou MY, et al. Anti-herpes simplex virus effects of berberine from *Coptidis rhizoma*, a major component of a Chinese herbal medicine, Ching-Wei-San. *Arch Virol* 2010;155(12): 1933–41.
- [27] Bag P, Ojha D, Mukherjee H, Halder UC, Mondal S, Biswas A, et al. A dihydro-pyrido-indole potently inhibits HSV-1 infection by interfering the viral immediate early transcriptional events. *Antiviral Res* 2014;105:126–34.
- [28] Bag P, Ojha D, Mukherjee H, Halder UC, Mondal S, Chandra NS, et al. An indole alkaloid from a tribal folklore inhibits immediate early event in HSV-2 infected cells with therapeutic efficacy in vaginally infected mice. *PLoS One* 2013;8:77937.
- [29] Khanal P, Duyu T, Dey YN, Patil BM, Pasha I, Wanjari M. Network pharmacology of AYUSH recommended immune-boosting medicinal plants against COVID-19. 2020.
- [30] Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* 2003; 3(2):133–46.
- [31] D'Alessandro S, Scaccabarozzi D, Signorini L, Perego F, Ilboudo DP, Ferrante P, et al. The use of antimalarial drugs against viral infection. *Microorganisms* 2020;8(1):85.
- [32] Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharm Sin B* 2020;10(5):766–88.
- [33] Liu X, Wang XJ. Potential inhibitors against 2019-nCoV coronavirus M protease from clinically approved medicines. *J Gent Genomics* 2020;47(2):119.
- [34] Wallace AC, Laskowski RA, Thornton JM. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Eng Des Sel* 1995;8(2):127–34.
- [35] Gil C, Ginex T, Maestro I, Nozal V, Barrado-Gil L, Cuesta-Geijo MA, et al. COVID-19: drug targets and potential treatments. *J Med Chem* 2020;63(21):12359–86.
- [36] Narkhede RR, Pise AV, Cheke RS, Shinde SD. Recognition of natural products as potential inhibitors of COVID-19 main protease (Mpro): in-silico evidences. *Nat Prod Bio-prospect* 2020;10(5):297–306.
- [37] Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg Microbes Infect* 2020;9:221–36.
- [38] Chen L, Liu W, Zhang Q, Xu K, Ye G, Wu W, et al. RNA based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak. *Emerg Microbes Infect* 2020;9:313–9.
- [39] Lu Q, Shi Y. Coronavirus disease (COVID-19) and neonate: what neonatologist need to know. *J Med Virol* 2020;92(6): 564–7.
- [40] Wang H, Li K, Ma L, Wu S, Hu J, Yan H, et al. Berberine inhibits enterovirus 71 replication by downregulating the MEK/ERK signaling pathway and autophagy. *Virol J* 2017; 14(1):2.
- [41] Prasad A, Muthamilarasan M, Prasad M. Synergistic anti-viral effects against SARS-CoV-2 by plant-based molecules. *Plant cell rep* 2020;39(9):1109–14.
- [42] Misra A, Mishra P, Kumar B, Shukla PK, Kumar M, Singh SP, et al. Intra-specific molecular and chemical variabilities in natural populations of *Gloriosa superba* (L.) for identifying elite chemotypes for commercial utilization. *Fitoterapia* 2021;150:104831.
- [43] Misra A, Srivastava S, Kumar S, Shukla PK, Kumar M, Agrawal PK, et al. Chemotaxonomic studies on natural population of *Gloriosa superba* (L.) collected from Gangetic plain (India) and their in vitro antigout activity for the identification of elite germplasm(s). *J Ethnopharmacol* 2020; 249:112387.
- [44] Kar P, Sharma NR, Singh B, Sen A, Roy A. Natural compounds from *Clerodendrum* spp. as possible therapeutic candidates against SARS-CoV-2: an in-silico investigation. *J Biomol Struct Dyn* 2020;18:1–2.
- [45] Naik B, Gupta N, Ojha R, Singh S, Prajapati VK, Prusty D. High throughput virtual screening reveals SARS-CoV-2 multi-target binding natural compounds to lead instant therapy for COVID-19 treatment. *Int J Biol Macromol* 2020; 160:1–17.
- [46] Shawky E, Nada AA, Ibrahim RS. 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 Adv* 2020;10(47):27961–83.
- [47] Zhang DH, Wu KL, Zhang X, Deng SQ, Peng B. In silico screening of Chinese herbal medicines with the potential to directly inhibit 2019 novel coronavirus. *J Integr Med* 2020; 18(2):152–8.
- [48] Bosch BJ, van der Zee R, de Haan CA, Rottier PJ. The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. *J Virol* 2003;77:8801–11.
- [49] Du QS, Wang SQ, Zhu Y, Wei DQ, Guo H, Sirois S, et al. Polypeptide cleavage mechanism of SARS CoV Mpro and chemical modification of the octapeptide. *Peptides* 2004; 25(11):1857–64.
- [50] Ullrich S, Nitsche C. The SARS-CoV-2 main protease as drug target. *Bioorg Med Chem Lett* 2020;30(17):127377.
- [51] Huang J, Peng K, Wang L, Wen B, Zhou L, Luo T, et al. Ginsenoside Rh2 inhibits proliferation and induces

- apoptosis in human leukemia cells via TNF- α signaling pathway. *Acta Biochim Biophys Sin* 2016;48(8):750–5.
- [52] Joshi RS, Jagdale SS, Bansode SB, Shankar SS, Tellis MB, Pandya VK, et al. Discovery of potential multi-target-directed ligands by targeting host-specific SARS-CoV-2 structurally conserved main protease. *J Biomol Struct Dyn* 2020;5:1–6.
- [53] Islam MT, Sarkar C, El Kersh DM, Jamaddar S, Uddin SJ, Shilpi JA, et al. Natural products and their derivatives against coronavirus: a review of the non-clinical and pre-clinical data. *Phytoth Res* 2020;1–22.
- [54] Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. *The Lancet* 2003;361(9374):2045–6.
- [55] Ming LJ, Yin AC. Therapeutic effects of glycyrrhizic acid. *Nat Pro Commun* 2013;8(3). 1934578X1300800335.
- [56] Chia YY, Ton SH, Khalid BAK. Effects of glycyrrhizic acid on peroxime proliferator-activated receptor gamma (PPAR- γ), lipoprotein lipase (LPL), serum lipid and HOMA-IR in rats. *PPAR Res* 2009;6:125–41.
- [57] Li SY, Chen C, Zhang HQ, Guo HY, Wang H, Wang L, et al. Identification of natural compounds with antiviral activities against SARS-associated coronavirus. *Antivir Res* 2005;67(1):18–23.
- [58] Wu CY, Jan JT, Ma SH, Kuo CJ, Juan HF, Cheng YS. Small molecules targeting severe acute respiratory syndrome human coronavirus. *Proc Natl Acad Sci U S A* 2004;101:10012–7.
- [59] Lee WK, Kao ST, Liu IM, Cheng JT. Ginsenoside Rh2 is one of the active principles of *Panax ginseng* root to improve insulin sensitivity in fructose-rich chow-fed rats. *Hor Metab Res* 2007;39(5):347–54.
- [60] Mathiyalagan R, Wang C, Kim YJ, Castro-Aceituno V, Ahn S, Subramaniam S, et al. Preparation of polyethylene glycol-ginsenoside Rh1 and Rh2 conjugates and their efficacy against lung cancer and inflammation. *Molecules* 2019;24(23):4367.
- [61] Wang H, Yu P, Gou H, Zhang J, Zhu M, Wang ZH, et al. Cardioprotective effects of 20 (S)-ginsenoside Rh2 against doxorubicin-induced cardiotoxicity in vitro and in vivo. *Evid Based Complementary Altern Med* 2012;506214.
- [62] World Health Organization. World Health Organization model list of essential medicines: 21st list 2019 (No. WHO/MVP/EMP/IAU/2019.06).
- [63] Ganeshpurkar A, Saluja AK. The pharmacological potential of rutin. *Saudi Pharm J Saudi* 2017;25(2):149–64.
- [64] Chioh KH, Phoon MC, Putti T, Tan BK, Chow VT. Evaluation of antiviral activities of *Houttuynia cordata* Thunb. extract, quercetin, quercetrin and cinanserin on murine coronavirus and dengue virus infection. *Asian Pac J Trop Med* 2016;9(1):1–7.
- [65] Hu X, Cai X, Song X, Li C, Zhao J, Luo W, et al. Possible SARS-coronavirus 2 inhibitor revealed by simulated molecular docking to viral main protease and host toll-like receptor. *Future Virol* 2020.
- [66] Perricone C, Triggianese P, Bartoloni E, Cafaro G, Bonifacio AF, Bursi R, et al. The anti-viral facet of anti-rheumatic drugs: lessons from COVID-19. *J Autoimmun* 2020;102468.
- [67] Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 2006;440(7081):237–41.
- [68] Slobodnick A, Shah B, Krasnokutsky S, Pillinger MH. Update on colchicine, 2017. *Rheumatology* 2018;57(suppl_1):i4–11.
- [69] Haggag YA, El-Ashmawy NE, Okasha KM. Is hesperidin essential for prophylaxis and treatment of COVID-19 Infection? *Me Hypotheses*; 2020. p. 109957.
- [70] Semerdjieva IB, Zheljazkov VD. Chemical constituents, biological properties, and uses of *Tribulus terrestris*: a review. *Nat Prod Commun* 2019;14(8):1934578X19868394.
- [71] Kostova I, Dinchev D. Saponins in *Tribulus terrestris*—chemistry and bioactivity. *Phytochem Rev* 2005;4(2–3):111–37.
- [72] He X, Xiang N, Zhang J, Zhou J, Fu Y, Gong T, et al. Encapsulation of teniposide into albumin nanoparticles with greatly lowered toxicity and enhanced antitumor activity. *Int J Pharm* 2015;487(1–2):250–9.
- [73] Zhang J, Chen Y, Luo H, Sun L, Xu M, Yu J, et al. Recent update on the pharmacological effects and mechanisms of dihydromyricetin. *Front Pharmacol* 2018;9:1204.
- [74] Kim HS, Lee EH, Ko SR, Choi KJ, Park JH, Im DS. Effects of ginsenosides Rg3 and Rh2 on the proliferation of prostate cancer cells. *Arch Pharma Res* 2004;27(4):429.
- [75] Shi J, Cao B, Zha WB, Wu XL, Liu LS, Xiao WJ, et al. Pharmacokinetic interactions between 20 (S)-ginsenoside Rh2 and the HIV protease inhibitor ritonavir in vitro and in vivo. *Acta Pharmacol Sin* 2013;34(10):1349.
- [76] Kang S, Im K, Kim G, Min H. Antiviral activity of 20 (R)-ginsenoside Rh2 against murine gammaherpesvirus. *J Ginseng Res* 2017;41(4):496–502.
- [77] Nakata H, Kikuchi Y, Tode T, Hirata J, Kita T, Ishii K, et al. Inhibitory effects of ginsenoside Rh2 on tumor growth in nude mice bearing human ovarian cancer cells. *Jpn J Cancer Res* 1998;89(7):733–40.
- [78] Cao M, Zhang J, Zhao Y, Song X, Ma B. Advances in ginsenoside Rh2 and its derivatives. *World Sci Technol* 2012;14:2205–11.
- [79] Wang W, Zhao Y, Rayburn ER, Hill DL, Wang H, Zhang R. In vitro anti-cancer activity and structure–activity relationships of natural products isolated from fruits of *Panax ginseng*. *Cancer Chemother Pharmacol* 2007;59(5):589–601.
- [80] Qian T, Cai Z. Biotransformation of ginsenosides Rb1, Rg3 and Rh2 in rat gastrointestinal tracts. *Chi Med* 2010;5(1):19.
- [81] Qian T, Cai Z, Wong RN, Jiang ZH. Liquid chromatography/mass spectrometric analysis of rat samples for in vivo metabolism and pharmacokinetic studies of ginsenoside Rh2. *Rapid Commun Mass Spectr: Int J Dev Rapid Disseminat Res Mass Spectro* 2005;19(23):3549–54.
- [82] Carabin IG, Burdock GA, Chatzidakis C. Safety assessment of *Panax ginseng*. *Int J Toxicol* 2000;19:293–301.
- [83] Park SJ, Lim KH, Noh JH, Jeong EJ, Kim YS, Han BC, et al. Subacute oral toxicity study of Korean red ginseng extract in Sprague-Dawley rats. *Toxicol Res* 2013;29(4):285–92.
- [84] Li JY, Cao HY, Liu P, Cheng GH, Sun MY. Glycyrrhizic acid in the treatment of liver diseases: literature review. *BioMed Res Int* 2014;2014.
- [85] Hoeffer G, Baltina L, Michaelis M, Kondratenko R, Baltina L, Tolstikov GA, et al. Antiviral activity of glycyrrhizic acid derivatives against SARS-coronavirus. *J Med Chem* 2005;48:1256–9.
- [86] Fukuchi K, Okudaira N, Adachi K, Odai-Ide R, Watanabe S, Ohno H, et al. Antiviral and antitumor activity of licorice root extracts. *In Vivo* 2016;30(6):777–85.
- [87] Wang L, Yang R, Yuan B, Liu Y, Liu C. The antiviral and antimicrobial activities of licorice, a widely-used Chinese herb. *Acta Pharm Sin B* 2015;5(4):310–5.
- [88] Yeh CF, Wang KC, Chiang LC, Shieh DE, Yen MH, San Chang J. Water extract of licorice had anti-viral activity against human respiratory syncytial virus in human respiratory tract cell lines. *J Ethnopharmacol* 2018;148(2):466–73.
- [89] Van Gelderen CE, Bijlsma JA, Van Dokkum W, Savelkoul TJ. Glycyrrhizic acid: the assessment of a no effect level. *Hum Exp Toxicol* 2000;19(8):434–9.
- [90] Johns C. Glycyrrhizic acid toxicity caused by consumption of licorice candy cigars. *Can J Emer Med* 2009;11(1):94–6.
- [91] Black licorice: trick or treat? US Food and Drug Administration 30 October 2017. <https://www.fda.gov/consumers/consumer-updates/black-licorice-trick-or-treat>.
- [92] Ferrari P, Sansonnens A, Dick B, Frey FJ. In vivo 11 β -HSD-2 activity: variability, salt-sensitivity, and effect of licorice. *Hypertension* 2001;38(6):1330–6.

- [93] Penninkilampi R, Eslick EM, Eslick GD. The association between consistent licorice ingestion, hypertension and hypokalaemia: a systematic review and meta-analysis. *J Hum Hypertens* 2017;31(11):699–707.
- [94] Choi HJ, Kim JH, Lee CH, Ahn YJ, Song JH, Baek SH, et al. Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus. *Antivir Res* 2009;81(1):77–81.
- [95] Kaul TN, Middleton Jr E, Ogra PL. Antiviral effect of flavonoids on human viruses. *J Med Virol* 1985;15(1):71–9.
- [96] Vlietinck AJ, Vanden Berghe DA, Haemers A. Present status and prospects of flavonoids as anti-viral agents. *Prog Clin Biol Res* 1988;280:283–99.
- [97] Vrijsen R, Everaert L, Boeye A. Antiviral activity of flavones and potentiation by ascorbate. *J Gen Virol* 1988;69(7):1749–51.
- [98] Dabeek WM, Marra MV. Dietary quercetin and kaempferol: bioavailability and potential cardiovascular-related bioactivity in humans. *Nutrients* 2019;11(10):2288.
- [99] Hollman PC. Absorption, bioavailability, and metabolism of flavonoids. *Pharm Biol* 2004;42(sup1):74–83.
- [100] Graefe EU, Wittig J, Mueller S, Riethling AK, Uehleke B, Drewelow B, et al. Pharmacokinetics and bioavailability of quercetin glycosides in humans. *J Clin Pharm* 2001;41(5):492–9.
- [101] Olthof MR, Hollman PC, Vree TB, Katan MB. Bioavailabilities of quercetin-3-glucoside and quercetin-4'-glucoside do not differ in humans. *J Nutr* 2000;130(5):1200–3.
- [102] Flavonoids. Micronutrient information center. Corvallis, Oregon: Linus Pauling Institute, Oregon State University; November 2015.
- [103] Sultana S, Asif HM, Akhtar N, Waqas M, Rehman SU. Comprehensive review on ethanobotanical uses, phytochemistry and pharmacological properties of *Melia azedarach* linn. *AJPRHC* 2014;6(1).
- [104] Alche LE, Ferek GA, Meo M, Coto CE, Maier MS. An antiviral meliicarpin from leaves of *Melia azedarach* L. *Zeitschrift für Naturforschung C* 2003;58:215–9.
- [105] Barquero AA, Michelini FM, Alche LE. 1-Cinnamoyl-3, 11-dihydroxymeliicarpin is a natural bioactive compound with antiviral and nuclear factor- κ B modulating properties. *Biochem Biophys Res Commun* 2006;344:955–62.
- [106] Petrera E, Coto CE. The synergistic effect of IFN- α and IFN- γ against HSV-2 replication in Vero cells is not interfered by the plant antiviral 1-cinnamoyl-3, 11-dihydroxymeliicarpin. *Virol J* 2006;3(1):45.
- [107] Nerome K, Shimizu K, Zukeran S, Igarashi Y, Kuroda K, Sugita S, et al. Functional growth inhibition of influenza A and B viruses by liquid and powder components of leaves from the subtropical plant *Melia azedarach* L. *Arch Virol* 2018;163(8):2099–109.
- [108] Kim HY, Shin HS, Park H, Kim YC, Yun YG, Park S, et al. In vitro inhibition of coronavirus replications by the traditionally used medicinal herbal extracts, *Cimicifuga rhizoma*, *Melaleuca cortex*, *Coptidis rhizoma*, and *Phellodendron cortex*. *J Clin Virol* 2008;41(2):122–8.
- [109] Phua DH, Tsai WJ, Ger J, Deng JF, Yang CC. Human *Melia azedarach* poisoning. *Clin Toxicol* 2008;46(10):1067–70.
- [110] Dai P, Wang J, Lin L, Zhang Y, Wang Z. Renoprotective effects of berberine as adjuvant therapy for hypertensive patients with type 2 diabetes mellitus: evaluation via biochemical markers and color Doppler ultrasonography. *Exp Ther Med* 2015;10(3):869–76.
- [111] Domitrovic R, Jakovac H, Blagojevic G. Hepatoprotective activity of berberine is mediated by inhibition of TNF- α , COX-2, and iNOS expression in CCl₄-intoxicated mice. *Toxicol* 2011;280(1–2):33–43.
- [112] Neag MA, Mocan A, Echeverría J, Pop RM, Bocsan CI, Crişan G, et al. Berberine: Botanical occurrence, traditional uses, extraction methods, and relevance in cardiovascular, metabolic, hepatic, and renal disorders. *Front Pharmacol* 2018;9:557.
- [113] Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environ Health Perspec* 2001;109(suppl 1):69–75.
- [114] Feng X, Sureda A, Jafari S, Memariani Z, Tewari D, Annunziata G, et al. Berberine in cardiovascular and metabolic diseases: from mechanisms to therapeutics. *Theranostics* 2019;9(7):1923.
- [115] Berberine. PubChem, National Library of Medicine, US National Institutes of Health. March 9, 2020.
- [116] Singh IP, Mahajan S. Berberine and its derivatives: a patent review (2009–2012). *Expert Opin Ther Pat* 2013;23(2):215–31.
- [117] Chen C, Yu Z, Li Y, Fichna J, Storr M. Effects of berberine in the gastrointestinal tract- a review of actions and therapeutic implications. *Am J Chin Med* 2014;42:1053–70.
- [118] Yu C, Tan S, Zhou C, Zhu C, Kang X, Liu S, et al. Berberine reduces uremia-associated intestinal mucosal barrier damage. *Biol Pharm Bull* 2016;b16–280.
- [119] Enkhtaivan G, Muthuraman P, Kim DH, Mistry B. Discovery of berberine based derivatives as anti-influenza agent through blocking of neuraminidase. *Bioorg Med Chem* 2017;25(20):5185–93.
- [120] Batista MN, Braga ACS, Campos GRF, Souza MM, Matos RPAD, Lopes TZ, et al. Natural products isolated from oriental medicinal herbs inactivate Zika virus. *Viruses* 2019;11:49.
- [121] Varghese FS, Thaa B, Amrun SN, Simarmata D, Rausalu K, Nyman TA, et al. The antiviral alkaloid berberine reduces chikungunya virus-induced mitogen-activated protein kinase signaling. *J Virol* 2016;90(21):9743–57.
- [122] Rosa-Calatrava M, Terrier O, Proust A, Moules V. Centre National de la Recherche Scientifique CNRS, Université Claude Bernard Lyon 1 (UCBL) and Institut National de la Santé et de la Recherche Médicale INSERM, (2019). Anti-viral compositions for the treatment of infections linked to coronaviruses. US Patent Appl 16/340, 346.
- [123] Basha SH. Corona virus drugs—a brief overview of past, present and future. *J Peer Sci* 2020;2:1000013.
- [124] Maurya DK, Sharma D. Evaluation of traditional ayurvedic preparation for prevention and management of the novel Coronavirus (SARS-CoV-2) using molecular docking approach. 2020.
- [125] Cui HX, Hu YN, Li JW, Yuan K, Guo Y. Preparation and evaluation of antidiabetic agents of berberine organic acid salts for enhancing the bioavailability. *Molecules* 2019;24(1):103.
- [126] Gupta PK, Gurley BJ, Barone G, Hendrickson HP. Clinical pharmacokinetics and metabolism of berberine and hydrastine following an oral dose of goldenseal supplement. *Planta Med* 2010;76(5):P110.
- [127] Singh N, Sharma B. Toxicological effects of berberine and sanguinarine. *Front Mol Biosci* 2018;5:21.
- [128] Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. *Metabolism* 2008;57(5):712–7.
- [129] Grycova L, Dostal J, Marek R. Quaternary protoberberine alkaloids. *Phytochem* 2007;68(2):150–75.
- [130] Marek R, Seckarova P, Hulova D, Marek J, Dostal J, Sklenar V. Palmatine and berberine isolation artifacts. *J Nat Pro* 2003;66(4):481–6.
- [131] Man MQ, Yang B, Elias PM. Benefits of hesperidin for cutaneous functions. *Evid-Based Compl Alt* 2019;2019.
- [132] Lipson SM, Ozen FS, Louis S, Karthikeyan L. Comparison of α -glucosyl hesperidin of citrus fruits and epigallocatechin gallate of green tea on the Loss of Rotavirus Infectivity in Cell Culture. *Front Microbio* 2015;6:359.
- [133] Bino A, Vicentini CB, Vertuani S, Lampronti I, Gambari R, Durini E, et al. Novel lipidized derivatives of the bioflavonoid hesperidin: dermatological, cosmetic and chemopreventive applications. *Cosmetics* 2018;5(4):72.
- [134] Ding Z, Sun G, Zhu Z. Short communication Hesperidin attenuates influenza A virus (H1N1) induced lung injury in rats through its anti-inflammatory effect. *Antivir Ther* 2018;23:611–5.

- [135] Dong W, Wei X, Zhang F, Hao J, Huang F, Zhang C, et al. A dual character of flavonoids in influenza A virus replication and spread through modulating cell-autonomous immunity by MAPK signaling pathways. *Sci Rep* 2014;4:7237.
- [136] Tsai FJ, Lin CW, Lai CC, Lan YC, Lai CH, Hung CH, et al. Kaempferol inhibits enterovirus 71 replication and internal ribosome entry site (IRES) activity through FUBP and HNRP proteins. *Food Chem* 2011;128(2):312–22.
- [137] Lin CW, Tsai FJ, Tsai CH, Lai CC, Wan L, Ho TY, et al. Anti-SARS coronavirus 3C-like protease effects of *Isatis indigotica* root and plant-derived phenolic compounds. *Antiviral Res* 2005;68(1):36–42.
- [138] Estruel-Amades S, Massot-Cladera M, Perez-Cano FJ, Franch A, Castell M, Camps-Bossacoma M. Hesperidin effects on gut microbiota and gut-associated lymphoid tissue in healthy rats. *Nutrients* 2019;11(2):324.
- [139] Nectoux AM, Abe C, Huang SW, Ohno N, Tabata J, Miyata Y, et al. Absorption and metabolic behavior of hesperidin (rutinosylated hesperetin) after single oral administration to Sprague-Dawley rats. *J Agr Food Chem* 2019;67(35):9812–9.
- [140] Li YM, Li XM, Li GM, Du WC, Zhang J, Li WX, et al. In vivo pharmacokinetics of hesperidin are affected by treatment with glucosidase-like BglA protein isolated from yeasts. *J Agri Food Chem* 2008;56(14):5550–7.
- [141] Moro L, Vasconcelos AC, Martins AS. Etoposide-induced apoptosis in murine neuroblastoma (N2A) cells infected with Paramyxoviruses. *Arq Bras Med Vet Zoo* 2003;55(1):44–50.
- [142] Schindler M, Münch J, Kirchhoff F. Human immunodeficiency virus type 1 inhibits DNA damage-triggered apoptosis by a Nef-independent mechanism. *J Virol* 2005;79(9):5489–98.
- [143] Song Y, Wang Y, Wang Z. Requirement for etoposide in the initial treatment of Epstein-Barr virus-associated haemophagocytic lymphohistiocytosis. *Br J Haematol* 2019;186(5):717–23.
- [144] Volcy K, Fraser NW. DNA damage promotes herpes simplex virus-1 protein expression in a neuroblastoma cell line. *J Neurovirol* 2013;19(1):57–64.
- [145] Chen YW, Yiu C-PB, Wong KY. Prediction of the SARS-CoV-2 (2019-nCoV) 3C-like protease (3CLpro) structure: virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates. *F1000 Res* 2020;9:129.
- [146] Stuart MC, Kouimtzis M, Hill SR, editors. WHO model formulary 2008. World Health Organization; 2009.
- [147] Deftereos SG, Siasos G, Giannopoulos G, Vrachatis DA, Angelidis C, Giotaki SG, et al. The Greek study in the Effects of Colchicine in COvid-19 complications prevention (GRECCO-19 study): rationale and study design. *Hell J Cardiol* 2020.
- [148] Drugs and Lactation Database (LactMed). Bethesda (MD): National Library of Medicine (US); 2006. Colchicine.
- [149] Tursen U, Tursen B, Lotti T. Coronavirus-days in dermatology. *Dermol Ther* 2020.
- [150] US National Library of Medicine. Colchicine coronavirus SARS-CoV 2 trial (COLCORONA) (COVID-19). <https://clinicaltrials.gov/ct2/show/NCT04322682>.
- [151] Gultekin N, Kucukates E. Microtubule inhibition therapy by colchicine in severe myocarditis especially caused by Epstein-Barr and cytomegalovirus co-infection during a two-year period: a novel therapeutic approach. *J Pak Med Assoc* 2014;64:1420–3.
- [152] Redel H. A case of chikungunya virus induced arthralgia responsive to colchicine. *Open forum infectious diseases*, vol. 3. Oxford University Press; 2016. p. w114. No. 2.
- [153] Deftereos S, Giannopoulos G, Papoutsidakis N, Panagopoulou V, Kossyvakis C, Raisakis K, et al. Colchicine and the heart: pushing the envelope. *J Am Coll Cardiol* 2013;62(20):1817–25.
- [154] Imazio M, Brucato A, Ferrazzi P, Pullara A, Adler Y, Barosi A, et al. Colchicine for prevention of post-pericardiotomy syndrome and postoperative atrial fibrillation: the COPPS-2 randomized clinical trial. *Jama* 2014;312(10):1016–23.
- [155] Imazio M, Bobbio M, Cecchi E, Demarie D, Demichelis B, Pomari F, et al. Colchicine in addition to conventional therapy for acute pericarditis: results of the Colchicine for acute PERicarditis (COPE) trial. *Circulation* 2005;112(13):2012–6.
- [156] Imazio M, Brucato A, Cemin R, Ferrua S, Maggolini S, Beqaraj F, et al. A randomized trial of colchicine for acute pericarditis. *N Engl J Med* 2013;369(16):1522–8.
- [157] Watanabe T, Kawakami E, Shoemaker JE, Lopes TJ, Matsuoka Y, Tomita Y, et al. Influenza virus-host interaction screen as a platform for antiviral drug development. *Cell Host Microbe* 2014;16(6):795–805.
- [158] Slobodnick A, Shah B, Pillinger MH, Krasnokutsky S. Colchicine: old and new. *Am J Med* 2015;128(5):461–70.
- [159] Gürkan A, Oguz MM, Cengiz EB, Senel S. Dermatologic manifestations of colchicine intoxication. *Pediatr Emerg Care* 2018;34(7):e131–3.
- [160] Neil MJ. The Merck Index: an Encyclopedia of chemicals, drugs and biologicals. New Jersey: Whitehouse Station; 2006. Published by Merck Research Laboratories, Division of Merck and Co.
- [161] Jasek W. Austria-Codexvol. 1. Vienna: Österreichischer Apothekerverlag; 2007. p. 1731–4.
- [162] Tanoue LT, Detterbeck FC. Lung cancer: a practical approach to evidence-based clinical evaluation and management. Elsevier Health Sciences; 2018.
- [163] Yusenko M, Jakobs A, Klempnauer KH. A novel cell-based screening assay for small-molecule MYB inhibitors identifies podophyllotoxins teniposide and etoposide as inhibitors of MYB activity. *Sci Rep* 2018;8(1):1–11.
- [164] Richter A, Strausfeld U, Knippers R. Effects of VM26 (teniposide), a specific inhibitor of type II DNA topoisomerase, on SV40 DNA replication in vivo. *Nucleic Acids Res* 1987;15(8):3455–68.
- [165] Aneja R, Liu M, Yates C, Gao J, Dong X, Zhou B, et al. Multidrug resistance-associated protein-overexpressing teniposide-resistant human lymphomas undergo apoptosis by a tubulin-binding agent. *Can Res* 2008;68:1495–503.
- [166] Silberstein LE, Anastasi J. Hematology: basic principles and practice E-book. Elsevier Health Sciences; 2017.
- [167] Attia AM, Bakrey OA, Abd El-Motaleb SMF. The use of podophyllotoxin for treatment of genital warts. *Menoufia Med J* 2015;28:367.
- [168] Podofilox. The American Society of Health-System Pharmacists. Retrieved 8 December 2016.
- [169] Canel C, Moraes RM, Dayan FE, Ferreira D. Podophyllotoxin. *Phytochemistry* 2000;54(2):115–20.
- [170] Gordaliza M, Garc a PA, Del Corral JM, Castro MA, Gomez-Zurita MA. Podophyllotoxin: distribution, sources, applications and new cytotoxic derivatives. *Toxicon* 2004;44(4):441–59.
- [171] Ardalani H, Avan A, Ghayour-Mobarhan M. Podophyllotoxin: a novel potential natural anticancer agent. *Avicenna J Phytomed* 2017;7:285.
- [172] McKee TC, Bokesch HR, McCormick JL, Rashid MA, Spielvogel D, Gustafson KR, et al. Isolation and characterization of new anti-HIV and cytotoxic leads from plants, marine, and microbial organisms. *J Nat Prod* 1997;60(5):431–8.
- [173] Sudo K, Konno K, Shigeta S, Yokota T. Inhibitory effects of podophyllotoxin derivatives on herpes simplex virus replication. *Antivir Chem Chemother* 1998;9(3):263–7.
- [174] Liu YQ, Tian X. Synthesis of novel spin-labeled derivatives of podophyllotoxin as potential antineoplastic agents. *Synthetic Commun* 2005;35(21):2749–58.
- [175] World Health Organization. WHO model formulary. p. 307. ISBN 9789 241547659.
- [176] Bukowski JA. Review of the epidemiological evidence relating toluene to reproductive outcomes. *Regul Toxicol Pharmacol* 2001;33:147–56.

- [177] Sundharam JA. Is podophyllin safe for use in pregnancy? Arch Dermatol 1989;125(7):1000–1.
- [178] Podophyllotoxin. DermNet New Zealand". www.dermnetnz.org. Retrieved 2018-05-06.
- [179] Gan LS, Wang ZZ, Zhou CX. Solubility of podophyllotoxin in six organic solvents from (283.2 to 323.2) K. J Chem Eng Data 2009;54(1):160–1.
- [180] Li H, Li Q, Liu Z, Yang K, Chen Z, Cheng Q, et al. The versatile effects of dihydromyricetin in health. Evid-Based Compl Alt 2017;2017.
- [181] Murakami T, Miyakoshi M, Araho D, Mizutani K, Kambara T, Ikeda T, et al. Hepatoprotective activity of tocha, the stems and leaves of *Ampelopsis grossedentata*, and ampelopsin. Biofactors 2004;21(1–4):175–8.
- [182] Kou X, Liu X, Chen X, Li J, Yang X, Fan J, et al. Ampelopsin attenuates brain aging of D-gal-induced rats through miR-34a-mediated SIRT1/mTOR signal pathway. Oncotarget 2016;7(46):74484.
- [183] Martínez-Coria H, Mendoza-Rojas MX, Arrieta-Cruz I, Lopez-Valdes HE. Preclinical research of Dihydromyricetin for brain aging and neurodegenerative diseases. Front Pharmacol 2019;10:1334.
- [184] Chen S, Zhao X, Wan J, Li Ran, Qin Yu, Wang X, et al. Dihydromyricetin improves glucose and lipid metabolism and exerts anti-inflammatory effects in nonalcoholic fatty liver disease: a randomized controlled trial. Pharmacol Res 2015;99:74–81.
- [185] Ye XL, Lu LQ, Li W, Lou Q, Guo HG, Shi QJ. Oral administration of ampelopsin protects against acute brain injury in rats following focal cerebral ischemia. Exp Ther Med 2017;13(5):1725–34.
- [186] Zhou Y, Shu F, Liang X, Chang H, Shi L, Peng X, et al. Ampelopsin induces cell growth inhibition and apoptosis in breast cancer cells through ROS generation and endoplasmic reticulum stress pathway. PloS one 2014;9(2).
- [187] Chinese patent No: CN102772398A. Application of dihydromyricetin in preparation of drug preventing and treating influenza. <https://patents.google.com/patent/CN102772398A/en>.
- [188] Chinese patent No: CN108354923 Applications of dihydromyricetin in preparation of anti-influenza virus drugs.
- [189] Zhang P, Cai S, Song L, Zhang L, Fan H, Zhou L, et al. Solubility of dihydromyricetin in ethanol and water mixtures from 288.15 to 323.15 K. J Mol Liq 2015;211:197–202.
- [190] Zhang S, Li H, Yang SJ. Tribulosin protects rat hearts from ischemia/reperfusion injury. Acta Pharmacol Sin 2010;31:671–8.
- [191] Zhang S, Li H, Yang SJ. Tribulosin suppresses apoptosis via PKC epsilon and ERK1/2 signaling pathway during hypoxia/reoxygenation in neonatal rat ventricular cardiac myocytes. J Asian Nat Prod Res 2011;13(12):209–20.
- [192] Sharifi AM, Darabi R, Akbarloo N. Study of antihypertensive mechanism of *Tribulus terrestris* in 2K1C hypertensive rats: role of tissue ACE activity. Life Sci 2003;73(23):2963–71.
- [193] Zhu W, Du Y, Meng H, Dong Y, Li L. A review of traditional pharmacological uses, phytochemistry, and pharmacological activities of *Tribulus terrestris*. Chem Cent J 2017;11:60.
- [194] Rogerson S, Riches CJ, Jennings C, Weatherby RP, Meir RA, Marshall-Gradisnik SM. The effect of five weeks of *Tribulus terrestris* supplementation on muscle strength and body composition during preseason training in elite rugby league players. J Strength Cond Res 2007;21:348–53.