An Overview of Coronaviruses: *In-Silico* Approach to Decipher Anti-SARS-CoV-2 Natural Products

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Abstract

In current study, we have focused on the outline of different coronaviruses including COVID-19, along with potential therapeutic targets of SARC-CoV-2. Moreover, experimentally evident anti-coronavirus natural molecules were subjected for *in silico* screening against M^{pro} and RdRp of COVID-19, in order to predict effective cure agent for same. The chemical structures of all selected molecules and standard ligands were drawn by ChemDraw for molecular docking and pharmacokinetic analysis. All ligands were prepared using OPLS_2005 force field of LigPrep tool, Schrodinger suite 2017-4 keeping default setting for generation of ionization and tautomeric state as well as low energy 3D-conformer. The template of main protease (6LU7) and RdRp (7BV2), were retrieved from the RCSB-PDB database. Both protein's structure was pre-processed, and minimized by utilizing default setting as integrated into the software package Maestro, Schrodinger. Receptor grid was generated by specifying around centroid of internal merged ligand atom i.e. N3 in case of M^{pro}, while best suited docking site was used in case of RdRp protein, predicted by Sitemap analysis tool, Schrodinger package. Molecular docking of selected bioactive natural products against target proteins was performed by using Glide module of Schrodinger package. Few water and avoidable molecules were removed, which amalgamated with docking site of the proteins template. The extra-precision (XP) and flexible molecular docking algorithm were employed to investigate free binding energy. *In silico* pharmacokinetic parameter was calculated with QikProp module of Schrodinger was utilized to predict *in silico* pharmacokinetic parameter of top ranked natural molecules. Docking analysis have revealed hit molecules namely tetra-*O*-galloyl-*β*-D-glucose (**2**) and juglanin (**25**) against main protease, in reference of N3 molecule (docking score = -5.95 Kcal/mol), while glycyrrhizin (**1**) and tetra-*O*-galloyl-β-D-glucose (**2**) are good against RdRp in reference of Remdesivir (docking score = -4.23 Kcal/mol). *In-silico* parameter revealed three lead compounds i.e. glycyrrhizin (**1**), tetra-*O*-galloyl-β-D-glucose

(**2**) and juglanin (**25**) which can be seen as hopeful molecule for COVID-19 treatment in upcoming time. Overall review lesson is to develop a specific and effective drug molecule against the current crisis i.e. COVID-19 derived from natural source.

Keywords*: Coronavirus, SARS-CoV-2, COVID-19, Natural products, In-silico screening*.

1. Introduction

In December 2019, a modern city Wuhan, Hubei province of China, which have recorded several novel pneumonia cases, caused by novel coronavirus-infected pneumonia (NCIP) [1]. Wuhan is the central point of a pandemic upsurge of SARS-CoV-2 also referred to as HCoV-19 [2] causative agent of COVID-19 which subsequently expanded [3] and it has caught global attention again after SARS-CoV in 2003 and the Middle East respiratory syndrome-related coronavirus (MERS-CoV), in 2012 outbreak. It is the deadliest coronavirus infection ever recorded with a mortality rate of 4-5% and a very high rate of transmission [4]. The coronavirus was originated more than 55 million years ago which was co-evolved with bats, and its most recent common ancestor (MRCA) has been estimated to have existed as recently as 8000 BCE [5]. The evolution of MRCA's of the different genera of Coronaviridae family such as the α-coronavirus (2400 BCE), the β-coronavirus (3300 BCE), the γ-coronavirus (2800 BCE), and the δ-coronavirus (3000 BCE) has been estimated [5]. The ideal hosts for the evolution and transmission of coronaviruses are that bats (α-coronavirus and β-coronavirus) and birds (γ-coronavirus and δ-coronavirus) [6]. The infectious bronchitis virus (IBV) was the first coronaviruses reported in the 1930s in chickens[7], while the first human coronaviruses were reported in the 1960s [8]. After that numerous human coronaviruses have been reported, including SARS-CoV (2003), HCoV NL63 (2004), HKU1 (2005), MERS-CoV (2012), and SARS-CoV-2 (2019). History indicated that coronavirus outbreaks have a severe impact on human health. SARS-CoV (2002-2004) had infected~ 8,096 people in China, with a~9.2 % fatality rate [9]. MERS-CoV (2012) has infected~2494 people in Saudi Arabia, with a~ 37 % fatality rate [10]. Currently, SARS-CoV-2 (2019-2020) had infected more than~19 million people globally with a~3.8 % fatality [11]. A SARS-CoV-2 infection has been declared as a pandemic by the WHO on the 12th of March 2020. It was the seventh

human coronavirus strain identified, which is associated with a severe respiratory infection like SARS-CoV and MERS-CoV, whereas NL63, HKU1, 229E, and OC43 have associated with mild sign [12]. The majority of the coronaviruses are responsible for extensive respiratory and gastrointestinal tract infections in humans and other animals [13][14].

Coronaviruses have stable and lasting threats for human health therefore we necessitate understanding their virology for controlling the transmission of coronavirus to stabilize public health and global economies. Due to the environment-dependent adaptability of coronaviruses through mutations and recombination, it provides them wide host range and tissue tropism [15][16][17]. Due to novelty of SARS-CoV-2 virus, there is lack of concrete data on the SARS-CoV-2 origin and COVID-19 treatments. The complete development of novel antiviral drugs for treating COVID-19 could be protracted, and the main concern could be a bio-safety. Therfore, it seems idealistic to investigate clinically tested molecules within a restricted time when the infection is spreading in exponential manner. Animals depend on their innate and adaptive immune system in order to defend against pathogenic microbes. Similarly, bacteria, algae, fungi, and plants, synthesize a variety of secondary metabolites for their defense mechanism. These natural products often target common biochemical pathways and cellular regulatory systems, which can be hijacked by viruses for their proliferation [18]. Therefore, natural products can be a potent arsenal of broad-spectrum antiviral agents. Why natural products? Because they are the outcome of million years of evolution, biologically compatible, and exhibited diverse stereochemistry which remains unrelated to existing drugs skeleton. Structural diversity is a fundamental prerequisite to hits a wide-range of therapeutic targets thus, natural products are considered as broad-spectrum agents for the defense purpose [19]. Traditional Chinese and Indian Ayurveda Medicine System have been utilized for thousands of years. Experimental evidence suggested that several natural products have acted as anti-SARS-CoV agents including, Glycyrrhizin (licorice/*Glycyrrhiza glabra*),

lycorine (*Lycoris radiate*), and Ginsenoside-Rb1 (*Panax ginseng*) [20][21]. Numerous scientific investigations were confirmed honey has been as an effective antimicrobial natural formulation which could be considered an excellent alternative or combination for antiviral drugs [22].

Some questions are arising due to the current scenario like, why it's so lethal as compared to the other coronaviruses? Is it any structural or genetic evolutions which improved their mode of action, sustainability, and resistibility to the host? Is there any scope of natural products for the development of broad-spectrum antiviral drugs? In order to answer these questions, here we have reviewed the biochemistry, pathogenesis of coronavirus and antiviral medicinal plants, and natural products as cure agent. Intentionally, we have directed our attention on the identification of the anti-SARS-CoV-2 agents through docking study of experimentally evident natural products against SARS-CoV, which may act as foundation for the discovery of natural products based drugs.

2. Coronavirus

Coronaviruses are a group of (+) ssRNA enveloped viruses [23] that cause respiratory or gastrointestinal infection in birds and mammals. In humans, it has showed mild symptoms such as common cold, similar to rhinoviruses infections in some cases, while in other cases such as SARS, MERS, and COVID-19, it can be lethal. Coronaviruses categorized under family Coronaviridae, which meaning "crown" or "halo", which refers to the distinctive appearances of virions resembling solar corona under an electron microscope, due to the surface embedded glycoprotein spike peplomers [24].

2.1. Morphology and genome

Coronaviruses are the largest RNA viruses with the pleomorphic spherical form with projecting homotrimeric spike-proteins (peplomers) surrounded by envelope proteins [25].

Envelop protein has implicated the maturation and discharge of viruses, results in the progression of the infection. The diameter of the coronaviruses is ranging around from 120-160 nm [26]. The viral envelope developed by a lipid bilayer where the trimeric spike proteins are anchored which interacts with host receptors to enable the virus entery [27]. β-coronavirus has a 5-10-nm long shorter projection of peplomers called hemagglutinin esterase (HE) [28]. Inside the core of transmembrane proteins, which hold nucleocapsid associated with ss-RNA in a curvature arrangement [29]. Peripheral lipid bilayer, envelop, matrix proteins, and nucleo-proteins protect the genome when the virus is exterior the host cell (**Fig. 1**) [30].

The genome size of coronaviruses is approximately 27-34 kb [23] which has protected 3′ by polyadenylation and a 5′ by methylation [26]. Genome organization of SARS-CoV-2 as 5′ UTR-[methylation]-(replicase/transcriptase)-(spike)-(envelope protein)-(matrix protein)-(nucleocapsid)-3′ UTR-[poly (A)] which is slightly different in different strains of coronaviruses [26]. There are two open reading frames (ORF) in coronaviruses such as ORF1a and ORF1b, that covered the first 2/3 portion of the genome. ORF is highly conserved and encoded 16 non-structural proteins (nsp1-nsp16) [26], including nsp1 which facilitate host cellular mRNA lysis and obstruct translation, consequently, impede innate immune response [31][32][33], nsp13 is the RNA helicase and 5′ triphosphatase [34] [35], and nsp15 is endoribonuclease and NendoU [36] [37]. The structural genes are common to all coronaviruses, while accessory genes are unique in number, organization, sequence, and function that encoded by specific coronaviruses. The translated product of the spike gene is cleaved after synthesis into the N' subunit is S1, which interacts with host cell receptor; and the C' subunit is S2 subunit, which facilitate membrane fusion [38][39]. The genome SARS-CoV-2 has shown 87.99 % sequence identity with bat-SL-CoVZC45, 87.23% with bat-SL-CoVZXC21, and 79% with SARS-CoV [40]. SARS-CoV-2 has classified into two types

based on population genetic analysis, such as L-type (~ 70%) and S-type (~ 30%). The L-type strains are more aggressive and infectious, which are evolutionarily developed from S-type strains [41].

2.2. Mode of Action and Pathogenesis

Coronavirus entere into the host cell by the interaction between spike-glycoprotein and its complementary receptor. The S1 region of spike glycoprotein position of receptor binding domains (RBD) is varied in different viruses, either at the N-terminus (MHV) or at the C-terminus (SARS- CoV) [42][43]. The binding between the spike-glycoprotein and host receptor is the prime requisite for an infection and the tissue tropism of the coronaviruses. Mostly peptidases have utilized as host entry gate by coronaviruses. Several α-coronaviruses interact with aminopeptidase N (APN) [44][45][46][47], while HCoV-NL63 and SARS-CoV interact with angiotensin-converting enzyme 2 (ACE-2) [48] [49], MERS-CoV utilize dipeptidyl-peptidase 4 (DPP4) [50], and MHV interact with CEACAM1 [51] [52]. Following virus-host interaction, spike glycoprotein has depredated by acid-dependent proteolysis by the host cell proteases, to promotes fusion of viral and host cell membranes. Generally, viruses fuse intracellularly with acidic endosomes, but few viruses like MHV, can fuse with host cell plasma membrane. Due to the cleavage of spike-glycoprotein, which results exposure to a fusion peptide that penetrates the membrane and endorse the formation of the antiparallel Hexa-helix bundle [53]. This Hexa-helix bundle allows for the viral and host membranes amalgamation, which leads to ejection of the viral genome into the host cells. The cluster of non-structural proteins (nsp's) forms a replicase-transcriptase complex including RNA-dependent RNA polymerase (RdRp) which involved in replication and transcription of RNA by catalyzing the synthesis of (-)-RNA from the (+)-RNA, while other nsp's are responsible for assisting this process [26]. The exoribonuclease provides additional fidelity to

the replication by its proof reading activity which is lack in RdRp enzyme [26]. Membrane or M protein execute the assembly of viruses by protein-protein interactions followed by its coupling with the nucleocapsid and viral genome, which leads to release of virions by exocytosis from the host cell (**Figure 1**) [26].

Coronaviruses cause a severe upper respiratory and gastrointestinal tract infection in mammals and birds including livestock; therefore, it can be a serious threat to the farming industry. Coronavirus target the respiratory and urogenital tract in IBV infection, but it also spreads throughout the body of chicken [54]. Porcine and bovine coronavirus causes diarrhea in young animals and both are considered as economically significant viruses. Feline enteric coronavirus showed minor clinical symptoms, but the mutated form of the same virus is responsible for feline infectious peritonitis (FIP), which causes a high fatality. Similarly, ferret enteric coronavirus infects a ferret that causes epizootic catarrhal enteritis (ECE), which is gastrointestinal and deadlier one [55]. Canine coronavirus (CCoV) has also two forms, first one is mild form which causes gastrointestinal symptoms while other is severe form which causes respiratory symptoms. Coronaviruses in rodents is mouse hepatitis virus (MHV) is responsible for a worldwide murine contagion with a high fatality, particularly in laboratory mice [56]. Pigs are also the target of swine acute diarrhoea syndrome coronavirus (SARS-CoV) which shows symptoms like diarrhea [57].

Previous outbreaks of coronavirus like as SARS-CoV and MERS-CoV which is biological agents that threaten human health. In the case of SARS-CoV infection, the physiological symptoms appear after 5.2 days which is incubation period [3]. In SARS-CoV-2 infection, the period from the beginning of symptoms to death is approximately 1-6 weeks' days with a 14 days median [58]. Moreover, this fatality period is also dependent on the age, status of ongoing health issues, and immune system. If infected patients are > 70 years old, those are on higher risk for fatality [58]. The symptoms of COVID-19 are cough with fever (body

temperature of 39.0 °C), difficulties in breathing fatigue, headache, diarrhea, sputum formation, haemoptysis, and lymphopenia (**Figure 1**) [59] [60].

COVID-19 patients generally have high leucocytes count with elevated levels of pro-inflammatory cytokines and chemokines including IL1-*β*, IL1RA, IL2 IL7, IL8, IL9, IL10, IFN*γ*, basic FGF2, GCSF, GMCSF, IP10, MCP1, MIP1α, MIP1β, PDGFB, TNF*α*, and VEGFA [59]. The sputum sample has taken for confirmation of COVID-19 infection by real-time polymerase chain reaction [61]. Moreover, the C-reactive protein level in blood is around 16.16 mg/L which is higher than the basal range (0–10 mg/L) [61]. The important pathophysiogenesis of COVID-19 are severe pneumonia, acute cardiac injury, RNAaemia [59]. SARS-CoV-2 accesses host lung cells via transmembrane ACE2 receptor which is highly expressed in type II alveolar cells of the lungs, therefore lungs are most distress organ by COVID-19 [62]. Together with lungs, a gastrointestinal tract also targeted by SARS-CoV-2 due to abundant expression of ACE2 is in the enterocytes, glandular cells, and endothelial cells of the gastrointestinal tract [63][64].

2.3. Challenges and Opportunity in COVID-19 Treatment

The COVID-19 is a novel pneumonia-like severe disease, which is an unprecedented challenge and drastically affects global healths and the economy. Due to its novelty of this virus, there is lack of vaccine, effective anti-SARS-CoV-2 drug, therefore, we confront several challenges for the definitive treatment of COVID-19. Another important concern is the shortage of ICU facilities including isolation beds, ventilators, fluid management, and essential medicines like Hydroxychloroquine, which are also the barrier for treatments. Approximately 20.1% of COVID-19 patients were developed SARS, while 25.9% of patients required ICU facility for treatment [65].

The antiviral, anti-malarial, and herbal medicines have been alternative options for the treatment of COVID-19. Presently > 85% of COVID-19 patients have been treated by the anti-viral agents, including Oseltamivir, Lopinavir/Ritonavir, and Ganciclovir, while Remdesivir at present under clinical trials [65]. The critical condition of COVID-19 has been managed by the combination of corticosteroids and anti-viral agents along with atomized inhalation of IFNγ [66]. The effective anti-malarial drug Chloroquine phosphate showed anti-viral and anti-inflammatory potential, thereby it has been exploiting for the inhibiting the aggravated effects of pneumonia [67]. Some traditional Chinese herbal formulation was used for the treatment of SARS-COV infection, and also for COVID-19 management. The most effective anti-SARS-COV medicinal herbs, include *Astragali radix* (steroidal saponins and isoflavonoids), *Glycyrrhizae radix* Rhizome (flavonoids and triterpenoid saponins), *Saposhnikoviae radix* (chromones and coumarin), *Atractylodis macrocephalae* Rhizome (atractylon and atractylenolides), and *Lonicerae japonicae* (flavonoids, iridoid glycosides, and flavonoids), *Forsythiae fructus* (phenylethanoid glycosides, lignans) [68][69]. Convalescent plasma (immuno-globulins of recovered patients) has also been useful for selective and potential approach obliging for immediate and short-term treatment of COVID-19 [70]. Earlier convalescent plasma therapy has been utilized for the recovery of the patients of H5N1, avian influenza, SARS, Ebola, and influenza A (H1N1 pdm09) infections [71][72].

2.4. Potential therapeutic targets for COVID-19 treatment

There are several potential targets in order to restrain coronavirus infection, which primarily associated with virus entry, viral genome replication, translation, assembly, and exocytosis [73]. Nsp's are functional proteins which are essential for executing the life cycle of coronaviruses. Among them, RdRp, PL^{pro}, $3CL^{pro}$, and helicase are the key and most valid molecular targets for designing and development of an anti-coronaviral drug owing to their

vital biological role. Viral proteases may prove as remarkable targets responsible for proteolysis of large polyprotein chain into different functional proteins such as replicase and polymerase [74]. For the development of an effective drugs against COVID-19, it is essential to hamper viral as well as host protein targets. The spike glycoprotein recognize the host cell receptor proteins ACE2 and CD147 as an alternative receptor, then spike protein proteolyzed by host proteases including transmembrane serine protease 2 **(**TMPRSS2) and furin [75][76]. Besides that, direct fusion of SARS-CoV-2 with the host cell membrane, it has also been postulated to penetrate through endocytosis [75]. In this pathway, some key proteins including, Vacuolar-type H^+ ATPase (V-ATPase), Cathepsin L (CTSL), two pore segment channel 2 (TPC2), and Phosphatidylinositol 3-phosphate 5-kinase (PIKfyve), which assist the formation of endosomes [75]. Above all mentioned viral and host proteins are considered as a probable target for the anti-coronaviral drugs and which are summarized in **Table 1** with its natural inhibitors.

3. Anti-coronaviral natural products

Natural products always remain a crucial platform, in order to search a bioactive drug molecule, and play a vital role in the drug discovery process [77]. Medicinal plants have the potential for promising sources of novel antiviral prototypes [78]. Several compounds from extracts of diverse species of higher plants have shown antiviral activity such as tannins, flavones and alkaloids, which displayed *in vitro* activity against numerous viruses. Limited availability of currently available antiviral drugs is the driving force for the discovery of new antiviral agents. The primary approaches for the discovery of new herbal agent is the classical method involves random screening, phytochemical factors and serendipity approaches. The secondary approach is traditional knowledge and practices on ethnopharmacology, which may prove as one good choice for the discovery of antiviral agents, and it involves the study

of medicinal plants with a history of traditional use as a potential source of substances with significant pharmacological activities [79] [80]. Herbal based therapeutic agent has several advantages too such as high effectiveness, less side effect, easy availability, and relatively low cost. Herein, we are more focused on compiling natural agents against SARS-CoV, which will anchor in the discovery of new antiviral agent especially against the current disaster started from Wuhan city of China.

At present, there is unavailablity of any potential existing or newly developed antiviral drug which can successfully treat COVID-19. Nevertheless, several research institutes are working on screening and clinical testing of potential antiviral small-molecules. The small molecules ligands can be categorized into two groups based on their therapeutic target - molecules of the first group are acting on the protein targets of coronaviruses, while molecules of the second group interact with host proteins to modulate the host immune system. In **Table 2** we have compiled experimentally screened natural products against coronaviruses.

4. Molecular docking screening & pharmacokinetic study of natural inhibitor's

The structures of all selected natural products (Fig. 2) were drawn by ChemDraw for molecular docking and ADME analysis against SARS-CoV-2 therapeutic targeted proteins. The stereochemical conformers of the natural products and standard ligands were prepared by using LigPrep tool, Schrodinger suite 2017-2, by utilizing OPLS_2005 force field. 3D template of main protease (PDB entry: 6LU7) & RdRp (PDB entry: 7BV2), were retrieved from the RCSB-PDB database. Both selected protein's structure was pre-processed, optimized and minimized with the help of protein preparation wizard in Maestro software, Schrodinger by using default setting. We have generated receptor grid around the best suited docking site which was analysed by sitemap wizard, in case of RdRp protein (7BV2), while internal merged ligand i.e. N3 atom were selected in order to generate receptor grid in case of

main protease (6LU7). Molecular docking of selected proteins with bioactive natural products was executed by using Glide, Schrodinger. Few water and avoidable molecules were removed, which amalgamated with docking site of the proteins template. The extra-precision (XP) algorithm with flexible molecular docking setting was employed to investigate binding affinity of ligands toward protein. Internal ligand i.e. N3 inhibitor [(Benzyl (3S,6S,9S,12R,Z)-9-isobutyl-6-isopropyl-3-methyl-1-(5-methylisoxazol-3-yl)-1,4,7,10-tetra oxo-12-(((R)-2-oxopyrrolidin-3-yl)methyl)-2,5,8,11-tetraazapentadec-13-en-15-oate)] in main protease crystal structure was utilized as reference ligand, whereas Remdesivir was used as reference in case of RdRp enzyme, in order to validate apex hit molecules against COVID-19.

Docking analysis (**Table. 3**) has revealed hit molecules namely tetra-*O*-galloyl-β-D-glucose (**2**), & juglanin (**25)** against protease**,** and same molecule i.e. tetra-*O*-galloyl-β-D-glucose (**2**) and glycyrrhizin against RNA replicase in reference of N3 inhibitor (docking score = -5.95 Kcal/mol) & Remdesivir (docking score = -4.23 Kcal/mol). 3D-interaction diagram for apex hit molecules have shown in fig. 3. *In silico* pharmacokinetic parameters were calculated with QikProp module of Schrodinger suit. The pharmacokinetic profile study is one strong point to minimize drug failure rate during drug discovery process. Recommended pharmacokinetic parameter and their corresponding value is mentioned in below **Table. 3**.

5. Conclusion

The pandemic contagion of the SARS-COV-2, their transmission rate and availability of no effective COVID-19 treatment is one of tough challenge for the medical and pharmaceutical fraternities. In an unprecedented display of effort and collaboration, the scientific community has made great strides in such a short amount of time which can be seen through publication of >1,000 SARS-CoV-2 genomes till now. Several crystal structures of key proteins of SARS-CoV-2 have been solved now, including the spike glycoprotein, RNA replication machinery proteins, and viral proteases. The life cycle of SARS-CoV-2 is now reasonably well understood, owing to years of study on related coronaviruses. Here we have discussed what researchers have now learned about the viral infection pathway of COVID-19 with an emphasis on the emerging targets for new drugs and vaccines. We warmly believe that 'for every illness in the living organisms, somewhere in world there exist plants which are cure'. Hence, Nature makes available an easy way out for any complex difficulty. This encourages us to perform *in-Silico* screening of experimentally validated anti-coronaviral natural compounds to forecast the biocompatible inhibitors against coronavirus. *In-silico* parameter revealed three lead compounds i.e. tetra-*O*-galloyl-β-D-glucose (2), juglanin (25) & glycyrrhizin (1) which can be seen as hopeful molecule for COVID-19 treatment in upcoming time. Therefore, our

broad study from experimental to *in silico* stage, generates a high impact to produce safe natural therapeutics against current threat i.e. COVID-19.

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Author Contribution

Patil Shivprasad Suresh and Shiv Shankar Gupta contributed to data collection, *in silico* screening, investigation, writing, designing, and editing of initial draft of manuscript. Anmol contributed to the investigation, data collection writing and editing of initial draft of manuscript. Upendra Sharma contributed to the conceptualization, Supervision, manuscript design and editing of manuscript.

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Conflicts of interest

The authors declare no competing interests.

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Figures and Tables

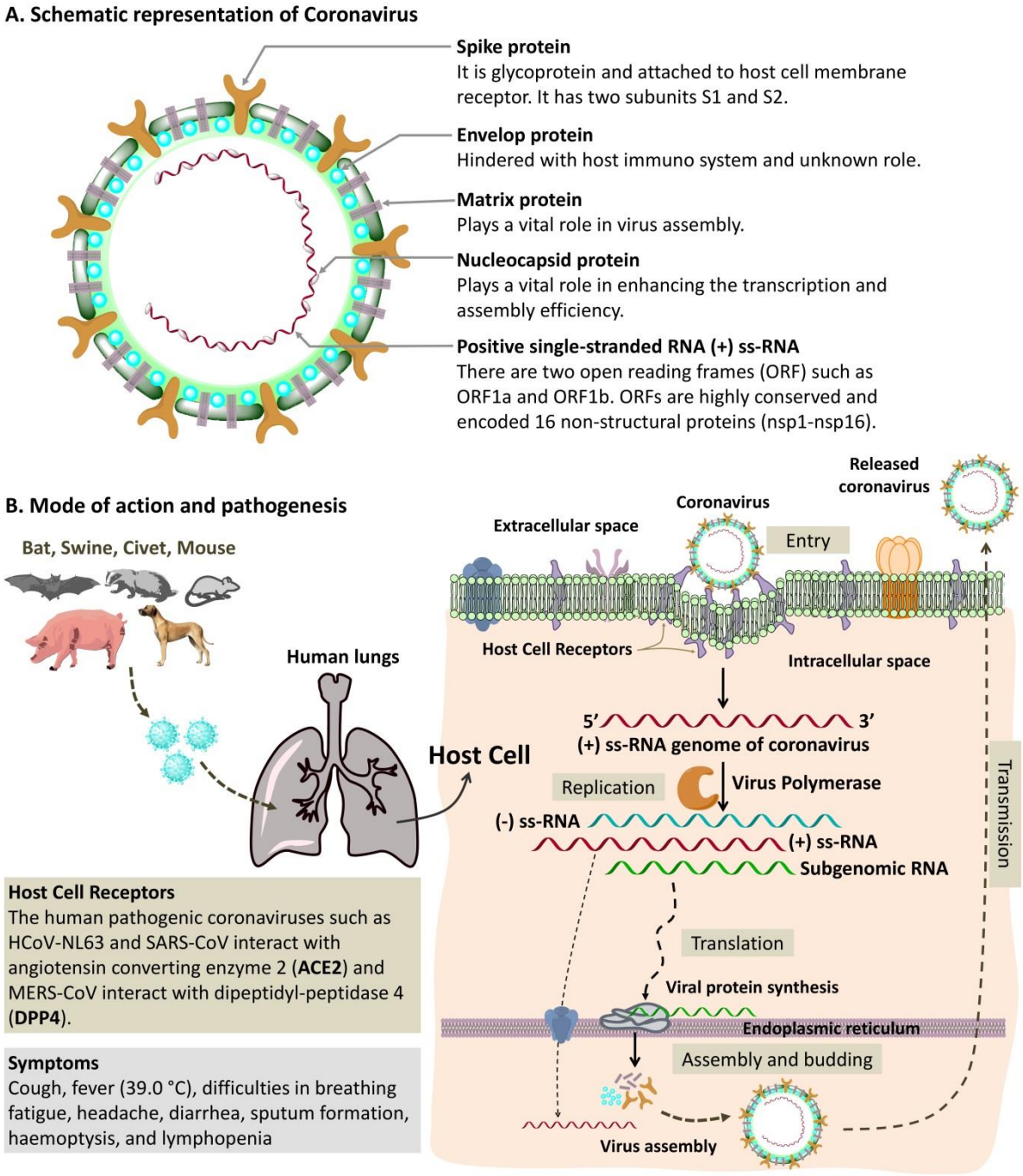


Figure 1. **A**) Diagrammatic representation of coronavirus, which is composed of the structural proteins, including spike protein, envelope protein, matrix protein, and nucleo-capsid, and inside the capsule, it has positive single-stranded RNA (+) ss-RNA). **B**) Mode of action and pathogenesis of coronaviruses. The bats are the reservoir of several types of coronaviruses and dog, mouse, swine, and civet are the transmission vectors for coronavirus contagion into the humans. Coronaviruses infection in humans causes respiratory syndrome such as SARS,

MERS, and COVID-19. Coronaviruses primarily act on the respiratory system and gastrointestinal tract, interact with host cells by the membrane enzymes mostly peptidases, which is a critical phase for virus entry. After that replication, translation, and assembly of viral proteins and genome has carried out inside the endoplasmic reticulum and cytoplasm following released by exocytosis from the host cell.

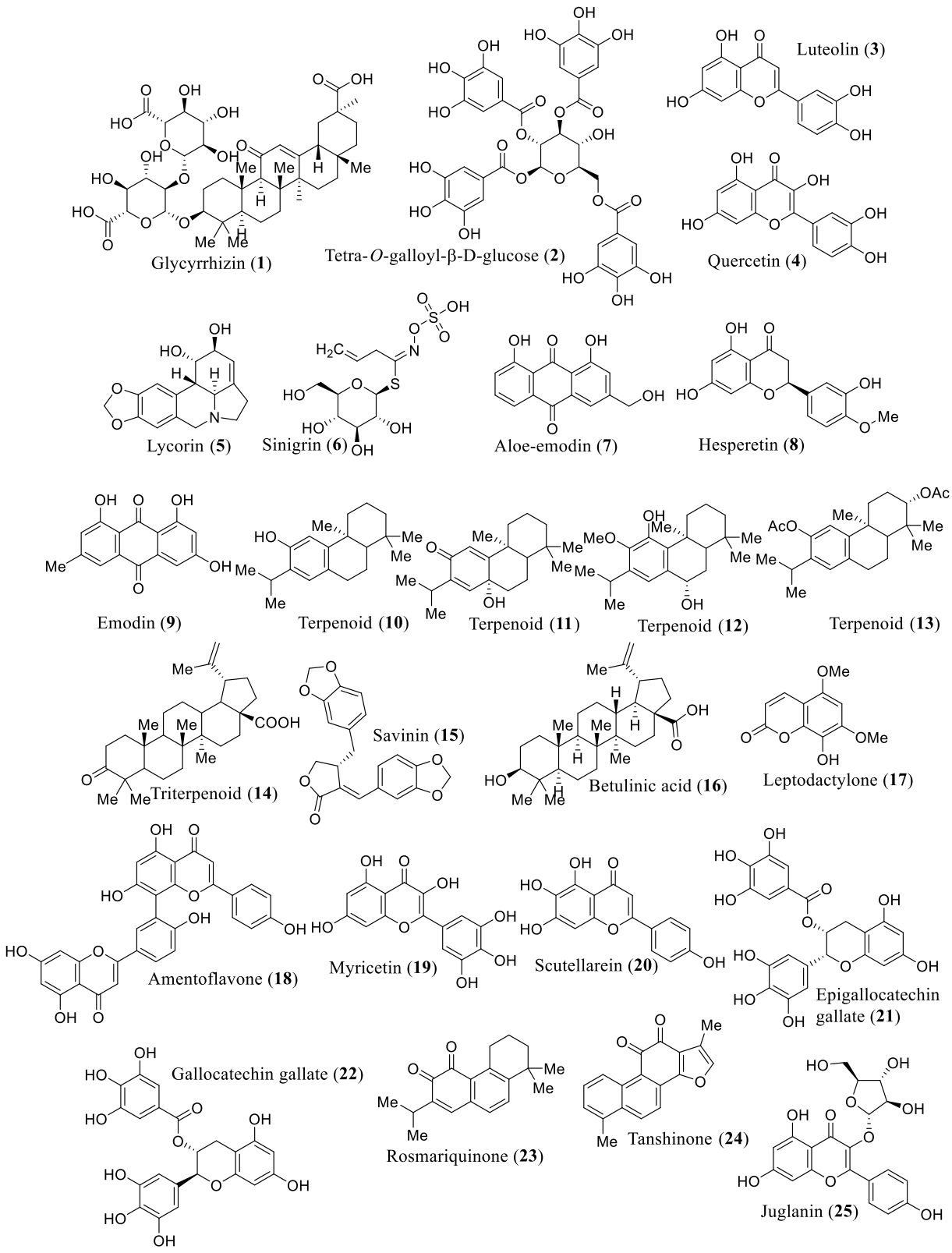
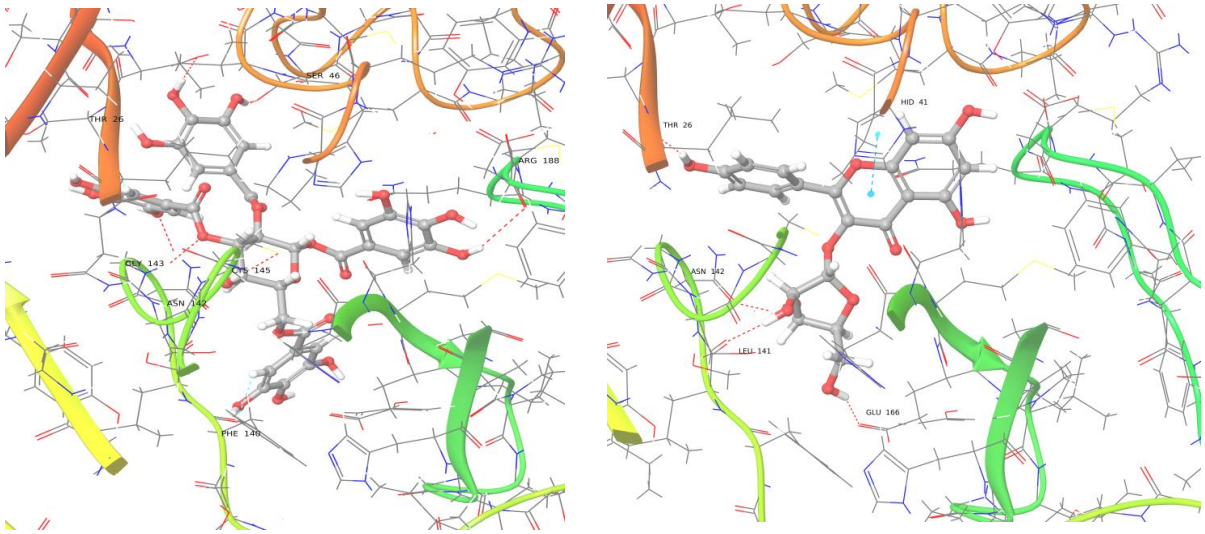


Figure 2. Selected bioactive natural products against coronaviruses.

A



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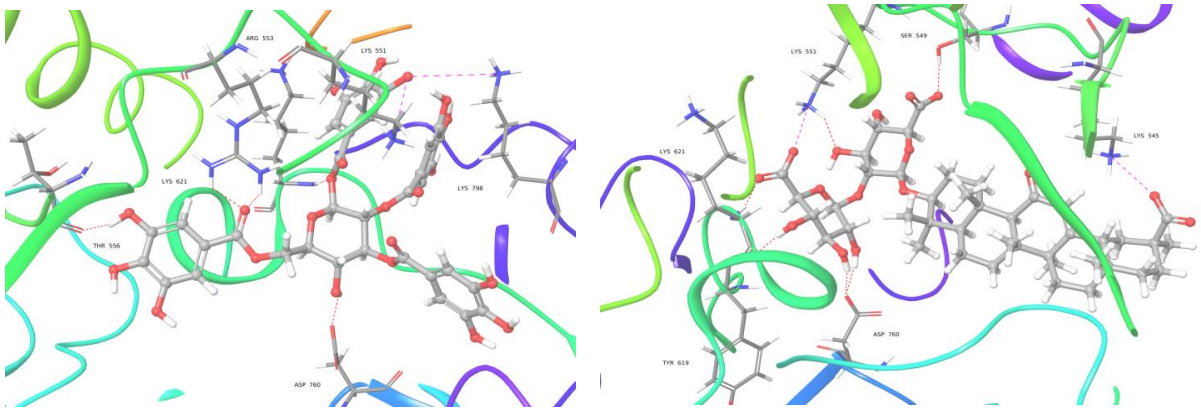

Figure 3. A) 3D-interaction of Tetra-O-galloyl-*β*-D-glucose (-12.20 Kcal/mol) and juglanin (-8.96 Kcal/mol) against main protease Enzyme (6lu7); **B**) 3D-interaction of Tetra-O-galloyl-*β*-D-glucose (-11.74 Kcal/mol) and glycyrrhizin (-7.77 Kcal/mol) against RdRp enzyme (7BV2).

Table 1

Therapeutic target and its natural inhibitor

Targets	Description	Natural molecule modulators	Ref.
Papain-like protease (PL^{pro})	Essential for CoV replication, and involved in the proteolytic processing of Nsp1-3	Cinnamic amides, ferulic acid, tomentin A, tomentin B, tomentin C, tomentin D, tomentin E, bavachinin, neobavaisoflavone, isobavachalcone, 4′-O-methyl-bavachalcone, psoralidin, and corylifol A.	[81] [82] [83] [84]
Main protease (M^{pro}/ 3CL-Protease)	Function as proteolytic processing of Nsp4-16 including RdRp and replicase-transcriptase complex (RTC).	Esculetin-4-carboxylic acid methyl ester, esculetin-4-carboxylic acid ethyl ester, aloe-emodin, beta-sitosterol, indigo, hesperetin, sinigrin, quercetin, gallocatechin gallate, and epigallocatechin gallate.	[85] [86] [87]
RNA-dependent RNA polymerase (RdRp/ nsp12)	It is a supra-molecular complex associated with processivity clamps (nsp7 and nsp8), exoribonuclease, RNA helicase, and 5′-triphosphatase. Replication of the viral RNA and transcription of sub-genomic RNA.	Monoethyl ester of meconic acid, extract from *Fructus Ligustri Lucidi,* Silibinin A, silibinin B, and aureusidin.	[88] [89] [90] [91]
Exoribonuclease (Exo/nsp14)	Function as 3′-5′ proofreading ribonuclease. Hammering ExoN activity results enhance the antiviral potency of remdesivir.	NA	[92]
Angiotensin-converting enzyme 2 (ACE2)	It is a transmembrane receptor with peptidase activity to cleave angiotensin II and other peptide hormones. ACE2 is interacting with the spike protein of SARS-CoV-2. To prevent ACE2-spike protein coupling is considered an ideal model for antiviral therapeutics.	Quercetin, quercetin-3-glucoside, quercetin-3-galactoside, cyanidin-3-galactoside, acteoside, Emodin, leucosceptoside A, martynoside, acteoside isomer, isomartynoside, gluco-aurantioobtusin.	[93] [94] [95] [96]
Transmembrane serine protease 2 (TMPRSS2)	TMPRSS2 is a protease involved in cleaves of ACE2 and the spike protein. It assists in viral entry into the host lung cells. Inhibition of TMPRSS2 results impedes viral entry into host cells.	Sunflower trypsin inhibitor (SFTI-1).	[76] [97]
Furin	It is a protease that proteolyzed inactive proteins precursor into their active form. Notably, it cleaving viral envelope proteins.	Catechins, gallic acid, neoandrographolide, Andrographolide, baicalein, quercetin, phlogantholide, and epigallocatechin gallate.	[98] [99]
CD147	It is an alternative receptor for the SARS-CoV-2.	NA	[99]
Cathepsin L (CTSL)	Cathepsin L is a pH-dependent protease localized in the lysosome that mediates the entry of the virus via endosomes.	Gallinamide A gathisflavone, tetrahydro-robustaflavone, 3-oxo-urs-12-en-28-oic acid, 3- epiursolic acid, 3-(hydroxyimino) oleanolic acid, and 3-(hydroxyl-imino) masticadienoic	[100] [101] [102]

		acid.	
Vacuolar-type H^+ ATPase (V-ATPase)	V-ATPase is a proton pump located into endosomes and lysosomes, which minimized the pH. At acidic pH cathepsins required for the endocytosis of SARS-CoV-2.	Destruxins, Archazolid A, Archazolid B, concanamycin A, bafilomycin A1, 11-deoxy-apicularen, Apicularen B, Open apicularen, Apicularen A, salicylihalamide A, lobatamide A, apicularen A, cruentaren, Benzolactone enamides, oximidine I.	[103] [104] [105] [106]

Table 2

Experimentally screened anti-coronavirus natural molecules/extract

Molecules/ extract	Source	Targets	Activity	Ref.
Glycyrrhizin	liquorice roots	Replication unit	The IC_{50} value is 316–625 mg/L	[20]
Tetra-*O*-galloyl-β-D-glucose			An EC_{50} value is 4.5 μM and a selective index is 240.	[107]
Quercetin			The EC_{50} is 83.4 μM	[107]
Lycoris radiata extract (lycorine)	*Lycoris radiata*,		The EC_{50} value of 2.4±0.2 μg/ml.	[108]
Isatis indigotica root extract	*Isatis indigotica*	3CL protease	The IC_{50} value is 53.8 ± 4.2 μg/ml by the cell-free assay and 191.6 ± 8.2 μg/ml by the cell-based assay.	[86]
Indigo	*Isatis indigotica*	3CL protease	The IC_{50} value is 300 μM by the cell-free assay and 752 μM by the cell-based assay.	[86]
Indirubin	*Isatis indigotica*	3CL protease	The IC_{50} value is 293 μM by the cell-free assay.	[86]
Indican	*Isatis indigotica*	3CL protease	The IC_{50} value is 112 μM by the cell-free assay.	[86]
Sinigrin	*Isatis indigotica*	3CL protease	The IC_{50} value is 121 μM by the cell-free assay and 217 μM by the cell-based assay	[86]
β-sitosterol	*Isatis indigotica*	3CL protease	The IC_{50} value is 115 μM by the cell-free assay and 1210 μM by the cell-based assay	[86]
Aloe-emodin		3CL protease	The IC_{50} value is 132 μM by the cell-free assay and 366 μM by the cell-based assay	[86]
Hesperetin		3CL protease	The IC_{50} value is 60 μM by the cell-free assay and 8.3 μM by the cell-based assay	[86]
Daidzein		3CL protease	The IC_{50} value is 105 μM by the cell-free assay.	[86]
Emodin	*Rheum officinale* and *Polygonum multiflorum*	S protein and ACE2 interaction	The IC_{50} value is 200 μM	[96]
Chrysin	*Rheum officinale* and *Polygonum multiflorum*	S protein and ACE2 interaction	The IC_{50} value is 400 μM	[96]
Radix et Rhizoma Rhei, Radix Polygoni multiflori, and *Caulis Polygoni multiflori* extract	*Radix et Rhizoma Rhei, Radix Polygoni multiflori, and Caulis Polygoni multiflori*	S protein and ACE2 interaction	The IC_{50} value ranged from 1 to 10 μg/ml.	[96]
Ferruginol			The CC_{50} 80.4 μM, EC_{50} 1.39 μM, and selective index are 58.0.	[109]
Dehydroabieta-7-one			A CC_{50} 305.1 μM, EC_{50} 4.00 μM, and selective index are 76.3.	[109]
6,7-dehydroroyleanone			The CC_{50} 89.7 μM, EC_{50} 5.55 μM, and selective index are 16.2.	[109]

α-cadino			The CC_{50} 76.8 µM, EC_{50} 4.44 µM, and selective index are 17.3.	[109]
Honokiol			The IC_{50} value is >100 µM, CC_{50} 88.9 µM, EC_{50} 6.$_{50}$ µM and selective index is 13.7.	[109]
Magnolol			The CC_{50} 68.3 µM, EC_{50} 3.80 µM and selective index is 18.0.	[109]
Niclosamide			The CC_{50} 22.1 µM, EC_{50} <0.1 µM and selective index is >221.	[109]
Valinomycin			The CC_{50} 67.5 µM, EC_{50} 1.63 µM and selective index is 41.4	[109]
Betulinic acid		3CL Protease	The IC_{50} value is 10 µM	[109]
Betulonic acid		3CL Protease	The IC_{50} value is >100 µM	[109]
Savinin		3CL Protease	The IC_{50} value is 25 µM	[109]
Curcumin		3CL Protease	The IC_{50} value is 40 µM	[109]
Niclosamide		3CL Protease	The IC_{50} value is 40 µM	[109]
Leptodactylone	*Boenninghausenia sessilicarpa*		Protective activity against Vero-E6 cells infected by SARS-CoV at a concentration of 100 µg/ml	[110]
water fraction of *Houttuynia cordata*	*Houttuynia cordata*	3CL protease and RdRp	It has shown biphasic action i.e. it reduces viral replication as well as helps in activating immunity to prevent viral infection.	[111]
The fraction of *Cinnamomi Cortex*	*Cinnamomi Cortex*		The IC_{50} value of *n*-Butanol fraction (7.8±0.3 µg/ml) The IC_{50} value of Ethanol fraction (10.7±04 µg/ml)	[112]
Biflavoneamentoflavone	*Torreya nucifera*	3CL Protease	The IC_{50} value is 8.3 µM	[113]
Myricetin	Chromadex	Nsp13	Inhibited the 90% of ATPase activity of nsP13 at a 10 µM concentration.	[114]
Scutellarein	*Scutettaria baicalensis*	Nsp13	Inhibited the 90% of ATPase activity of nsP13 at a 10 µM concentration.	[114]
Quercetin, epigallocatechin gallate, gallocatechin gallate	*Pichia pastoris*	3CL Protease	The IC_{50} values of quercetin (73 µM), epigallocatechin gallate, (73 µM) and gallocatechin gallate (47 µM) with K*i* value of 25 ± 1.7 µM.	[87]
Tanshinone I	*Salvia miltiorrhiza*	$3CL^{pro}$ and PL^{pro}	The good inhibitory activity even at 0.7 µM concentration by a deubiquitinating mechanism with good selectivity.	[115]
Rosmariquinone	*Salvia miltiorrhiza*	$3CL^{pro}$ and PL^{pro}	It possesses different kinetic mechanisms as well as slow & reversible inhibition.	[115]

Table 3

Docking score & ADME parameters of Anti-SARS candidates (Bold text shows recommended values)

Natural candidate	Docking Score		LogKp (–8.0 - 1.0)	PHOA (<25 poor, >80 good)	PCaco2 (<25 poor, >500 great)	Log Khsa (–1.5 - 1.5)	logS (–6.5 - 0.5)	PMDCK (<25poor, >500 great)	Rule of five	QPlogHERG (<5)	HB-accept (2.0 -20.0) HB-donate (0.0 - 6.0)
	M^{pro} (6lu7)	RdRp (7bv2)									
Tetra-*O*-galloyl-β-D-glucose	**-12.20**	**-11.74**	-12.19	0.0	0.001	**-1.24**	**-4.53**	0	**3**	**-7.823**	20.4/13
Juglanin	**-8.96**	**-4.97**	**-5.72**	29.02	9.41	**-0.65**	**-2.78**	3.2	1	**-5.23**	**11.3/5**
Epigallocatechin gallate	**-8.35**	**-7.13**	**-7.54**	0.0	1.03	**-0.44**	**-3.49**	0.3	2	**-5.62**	**8.8**/8
Myricetin	**-7.33**	**-5.67**	**-6.38**	27.43	6.97	**-0.49**	**-2.64**	2.3	1	**-4.97**	**6/5**
Scutellarein	**-7.18**	-3.94	**-4.68**	63.31	50.80	**-0.2**	**-3.03**	19.8	0	**-5.02**	**4.5/3**
Quercetin	**-6.69**	**-4.83**	**-5.49**	52.20	19.29	**-0.34**	**-2.88**	6.9	0	**-5.07**	**5.3/4**
Luteolin	**-6.38**	-4.14	**-4.86**	61.49	42.00	**-0.20**	**-3.06**	16.1	0	**-5.02**	**4.5/3**
Aloe-emodin	**-6.05**	-3.52	**-4.49**	66.29	79.02	**-0.31**	**-2.59**	31.9	0	**-4.51**	**5.2/1**
Gallocatechin gallate	-5.89	**-7.08**	**-7.3**	2.01	1.31	**-0.43**	**-3.35**	0.4	2	**-7.30**	**8.8**/8
Hesperetin	-5.67	-4.11	**-4.07**	75.40	132.07	**0.02**	**-3.73**	55.5	0	**-4.94**	**4.8/2**
Sinigrin	-5.41	**-6.14**	**-6.27**	21.29	1.89	**-1.47**	**-0.94**	1.05	0	**-1.95**	**14/5**
Emodin	-5.25	-2.05	**-4.71**	68.32	79.84	**-0.10**	**-3.05**	32.2	0	**-4.33**	**4.3/1**
Amentoflavone	-4.51	**-5.16**	**-6.33**	24.46	2.46	**0.68**	**-6.79**	0.8	2	**-7.27**	**7.5/4**
Laptodactylone	-4.23	-2.96	**-3.11**	**83.17**	**645.66**	**-0.46**	**-2.09**	308.3	0	**-3.94**	**4.8/1**
Savinin	-4.11	-1.40	**-1.66**	**100**	**2491.65**	**-0.53**	**-1.06**	**1327.1**	0	**-2.51**	**6/0**
Terpenoid (13)	-4.08	0.0	**-2.50**	**100**	**1911.12**	**1.03**	**-6.44**	**996.3**	1	**-4.45**	**4.5/0**
Terpenoid (11)	-3.92	-2.14	**-2.61**	**100**	**2029.96**	**0.81**	**-4.97**	**1063.4**	0	**-3.30**	**2.8/6**
Terpenoid (12)	-3.86	-2.58	**-2.24**	**100**	**2568.42**	**0.73**	**-4.83**	**1371.4**	0	**-3.42**	**3.2/2**
Terpenoid (10)	-3.78	0.0	**-2.04**	**100**	**3810.83**	**1.15**	**-5.75**	**2100.7**	1	**-3.59**	0.8/**1**
Rosmariquinone	-3.72	-1.53	**-2.62**	**100**	**1720.64**	**0.35**	**-4.43**	**889.4**	0	**-4.13**	**4/0**
Tanshinone	-3.62	-1.87	**-2.21**	**100**	**1485.61**	**-0.07**	**-3.49**	**758.9**	0	**-4.94**	**4.5/0**
Lycorin	-3.58	**-6.56**	**-4.58**	77.15	363.96	**-0.34**	**-1.45**	183.6	0	**-4.09**	**6.9/2**
Glycyrrhizin	-3.41	**-7.77**	-8.64	0	0.01	**-0.71**	**-5.12**	0.0	**3**	**-0.46**	21.3/**6**
Triterpenoid (14)	-2.62	-2.99	**-3.14**	**93.89**	268.41	**1.41**	**-7.15**	151.8	1	**-1.97**	**4/1**
Betulinic acid	-2.35	0.0	**-2.96**	**94.63**	296.40	**1.36**	-6.94	169.0	1	**-1.95**	**3.7/2**