Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Chapter 12

Insight into coronaviruses and natural products-based approach for COVID-19 treatment

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Chapter Outline

Introduction

In December 2019, Wuhan, a city in China's Hubei province, recorded several novel pneumonia cases caused by novel coronaviruses [1]. Thus Wuhan became the outbreak point of SARS-CoV-2 also referred to as HCoV-19 [2], which has now spread throughout the globe [3]. Corona viruses have caught global attention again after SARS-CoV in 2003 and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012. Corona virus-related respiratory disease is one of the fatal viral infections ever recorded with a

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2.2% mortality rate and a very high transmission rate [4]. Coronaviruses originated approximately >55 million years ago and coevolved with bats. The most recent common ancestor of coronaviruses has been estimated to exist since 8000 BCE [5]. The ideal hosts for the evolution and transmission of coronaviruses are bats (α-coronavirus and β-coronavirus) and birds (γ-coronavirus and δ-coronavirus) [6]. The infectious bronchitis virus (IBV) was the first coronavirus reported in the 1930s in chickens [7], while the first human coronavirus was reported in the 1960s [8]. After that, numerous human coronaviruses have been reported, including SARS-CoV (2003), NL63 (2004), HKU1 (2005), MERS-CoV (2012), and SARS-CoV-2 (2019). History indicates that coronavirus infection has a severe impact on human health. SARS-CoV (2002–2004) had infected ~8096 people in China, with a ~9.2% fatality rate [9] and MERS-CoV (2012) had infected ~2494 people in Saudi Arabia, with a ~37% fatality rate [10]. At present, SARS-CoV-2 (2019–2022) has infected more than ~186 million people globally with a ~2.2% fatality rate [11]. The SARS-CoV-2 infection has been declared a pandemic by the WHO on March 12, 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 been associated with milder symptoms [12]. The majority of corona viruses are responsible for extensive respiratory and gastrointestinal tract infections in humans and other animals [13,14].

Coronaviruses are responsible for causing lasting threats to human health, therefore it is necessary to understand their virology for controlling transmissions and to find effective treatments. The environment-dependent adaptability of coronaviruses through mutations and recombination provides them a wide host range and tissue tropism [15–17]. Due to the novelty of SARS-CoV-2, there is a lack of concrete data on the SARS-CoV-2 origin and COVID-19 treatments. The development of novel antiviral drugs for treating COVID-19 could take a long time, and the main concern could be biosafety because testing of drugs requires the culture of the deadly virus.

Akin to medicinal plants, bacteria, algae and fungi also synthesize various secondary metabolites for their defense, which target common biochemical pathways and cellular regulatory systems of pathogens [18]. Therefore, natural products can be a potent arsenal of broad-spectrum antiviral agents. One fundamental question could be why should one focus on natural products? Part of the answer to this is the fact that natural products unlike products of combinatorial chemistry are the outcomes of the evolution of millions of years during which these products have dabbled in the chemical diversity space to alter their structures in diverse ways so as to enable their hosts to be successful in a competitive world of biological warfare. Structural diversity is a fundamental prerequisite to hit a wide range of therapeutic targets, thus, natural products are considered as broad-spectrum agents for defense against pathogenic microbes [19]. Experimental evidences suggested that several natural

products have acted as anti-SARS-CoV agents including, glycyrrhizin (licorice/*Glycyrrhiza glabra*), lycorine (*Lycoris radiata*), and ginsenoside-Rb1 (*Panax ginseng*) [20,21]. Numerous scientific investigations have confirmed the use of honey as being effective in antimicrobial herbal formulations, which could be considered a natural alternative or combination with antiviral drugs [22].

Some questions are arising due to the current COVID-19 scenario like, why it's so lethal as compared to the other coronaviruses? Is it due to structural or genetic evolutions that improved their mode of action, sustainability, and resistance to the host? Is there any scope of natural products for the development of broad-spectrum antiviral drugs? To answer these questions, here we have reviewed the structural biochemistry and pathogenesis of coronavirus along with antiviral natural products and their herbal sources. Additionally, we have carried out the molecular docking study of these experimentally evaluated antiviral natural products to identify the anti-SARS-CoV-2 agents, which may be a foundation for discovering natural products-based drugs against COVID-19.

Coronavirus

Coronaviruses categorized under the family Coronaviridae are a group of (+) ss-RNA enveloped viruses [23]. The term "Corona" is derived from "crown or halo," which is due to the distinct appearance of virions resembling solar corona under an electron microscope, due to the surface embedded glycoprotein spike peplomers [24].

Morphology and genome

Coronaviruses are the largest RNA viruses with pleomorphic spherical form, projecting homotrimeric spike proteins (peplomers) surrounded by envelope proteins [25]. Envelop protein is implicated in the maturation and discharge of viruses, resulting in the progression of the infection. The diameter of the coronaviruses ranges from 120 to 160 nm [26]. The viral envelope, developed from the lipid bilayer where the trimeric spike proteins are anchored, interacts with host receptors to enable the virus entry [27]. β-Coronavirus has a 5–10 nm long globular projection of peplomers called hemagglutinin esterase (HE) [28]. Inside the core of virus, the transmembrane matrix proteins hold nucleocapsid associated with ss-RNA in a curved arrangement [29]. Peripheral lipid bilayer, envelope, matrix proteins, and nucleoproteins protect the genome when the virus is outside the host cell (Fig. 12.1) [30]. The genome size of coronaviruses is approximately 27–34 kb [23] which is protected by 3′ polyadenylation and 5′ methylation [26]. Genome organization of SARS-CoV-2 as 5′ UTR-[methylation]-(replicase/transcriptase)-(spike)-(envelope protein)-(matrix protein)-(nucleocapsid)-3′ UTR-[poly (A)] is slightly different in different strains of coronaviruses and they have good control of RNA

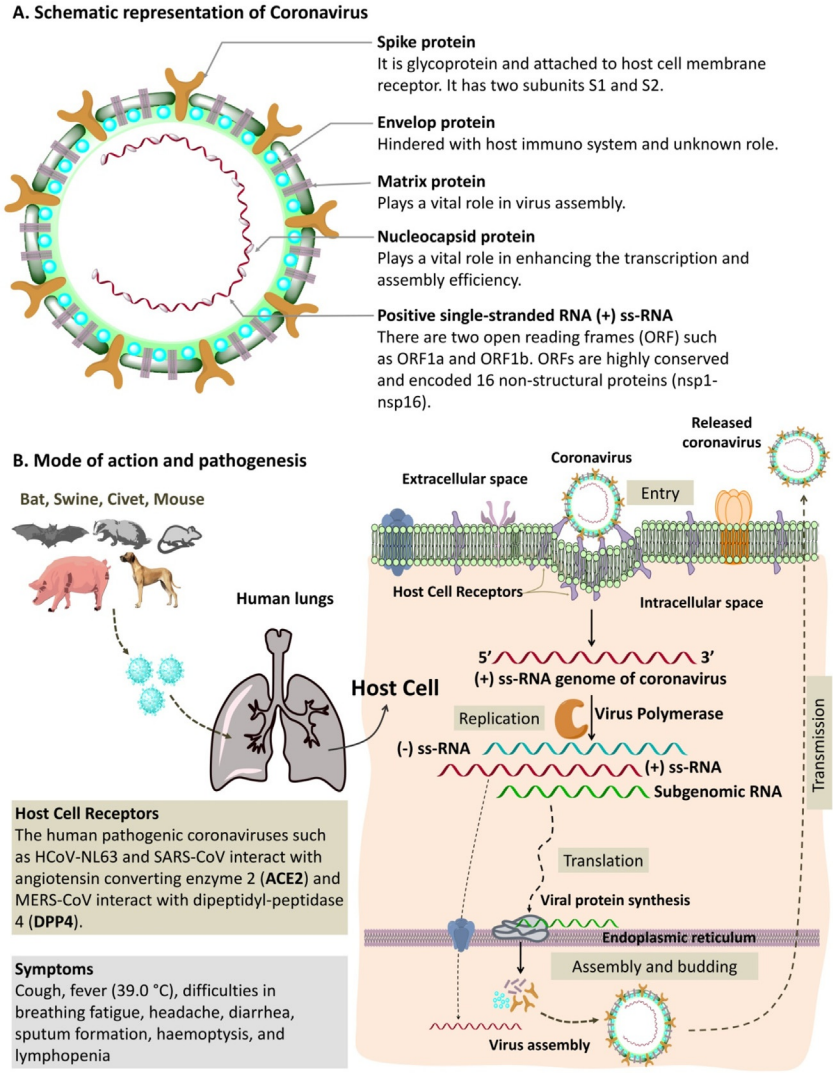

FIG. 12.1 (A) Diagrammatic representation of coronavirus which is composed of the structural proteins (spike protein, envelope protein, matrix protein, and nucleocapsid) and nonstructural proteins with positive single-stranded RNA (+) (ss-RNA) inside it. (B) Mode of action and pathogenesis of coronaviruses. The bats are the reservoir of several coronaviruses, while dogs, mice, swine, and civets are the transmission vectors for coronavirus contagion into 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 were carried out inside the endoplasmic reticulum and cytoplasm, following released by exocytosis from the host cell.

replication [26]. There are two open-reading frames (ORFs) in coronaviruses such as ORF1a and ORF1b, that cover the first two-third portion of the genome. The polyprotein ORF is highly conserved and encodes 16 nonstructural proteins (nsp1–nsp16) [26], including nsp1 which facilitates host mRNA lysis thereby inhibiting translation of host proteins [31–33]. Nsp13 is the RNA helicase/5′-triphosphatase [34,35] and nsp15 is endoribonuclease/NendoU [36,37]. The structural genes are common to all coronaviruses, while accessory genes are unique in number, organization, sequence and function encoded by specific coronaviruses. The translated product of the spike gene cleaved after synthesis into the N′ subunit is S1, which interacts with the host cell receptor. The C′ subunit is the S2 subunit, which facilitates membrane fusion [38,39]. The genome SARS-CoV-2 has shown ∼88% 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].

Mode of action and pathogenesis

Coronavirus enters into the host cell by the interaction between spike glycoprotein of the virus and its complementary receptor on the target cells. The conformation of receptor-binding domains (RBDs) situated on S1 region of spike glycoprotein is varied in different viruses. The RBD is present either at the N-terminus in mouse hepatitis virus (MHV) or at the C-terminus in SARS-CoV [42,43]. The interaction between spike glycoprotein and host receptor is the prime requisite for an infection and the tissue tropism of the coronaviruses. Most peptidases have been utilized as host entry gates by coronaviruses. Several α-coronaviruses used aminopeptidase N (APN) [44–47], HCoV-NL63 and SARS-CoV used angiotensin-converting enzyme 2 receptor (ACE2) [48,49], MERS-CoV used dipeptidyl-peptidase 4 (DPP4) [50], and MHV used CEACAM1 [51,52] for the entry into the host cells. Following virus–host interaction, spike glycoprotein is degraded by the host cell proteases *via* acid-dependent proteolysis to promote fusion of viral and host cell membranes. Generally, intracellularly viruses fuse with acidic endosomes, but few viruses like MHV can fuse with the host cell plasma membrane. Due to the cleavage of spike glycoprotein, which results in exposure of a fusion peptide that penetrates the membrane and facilitates the formation of the antiparallel hexa-helix bundle [53]. This hexa-helix bundle allows for the viral and host membranes amalgamation, which leads to the ejection of the viral genome into the host cells. The cluster of nonstructural proteins (nsps) forms a replica–transcriptase complex including RNA-dependent RNA

polymerase (RdRp),which is involved in replication and transcription of RNA by catalyzing the synthesis of (−)-RNA from the (+)-RNA, while other nsps are responsible for assisting this process [26]. The exoribonuclease which lacks the RdRp provides additional fidelity to replication by its proofreading activity [26]. Membrane or M protein executes the assembly of viruses by protein–protein interactions followed by its coupling with the nucleocapsid and viral genome, which leads to the release of virions by exocytosis from the host cell (Fig. 12.1) [26].

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

The SARS-CoV and MERS-CoV are biological agents that threaten human health. In SARS-CoV infection, the physiological symptoms appear after 5.2 days which is the incubation period of the virus [3]. In SARS-CoV-2 infection, the period from the beginning of symptoms to death is approximately 1–6 weeks with a 14 days median [58]. Moreover, this fatality period is also dependent on age, status of ongoing health issues, and immune system of the person infected. 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, hemoptysis, and lymphopenia (Fig. 12.1) [59,60].

COVID-19 patients generally have high leucocytes count with elevated levels of proinflammatory cytokines and chemokines including IL1-β, IL1RA, IL2, IL7, IL8, IL9, IL10, IFNγ, basic FGF2, GCSF, GMCSF, IP10, MCP1, MIP1α, MIP1β, PDGFB, TNFα, and VEGFA [59]. The sputum sample is 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 and RNAemia [59]. SARS-CoV-2 accesses host lung cells via the transmembrane ACE2 receptor, which is highly expressed in type II alveolar cells of the lungs. Therefore COVID-19 causes serious damage to lungs [62]. Together with the lungs, gastrointestinal tract is also targeted by SARS-CoV-2 due to the abundant expression of ACE2 receptors is in the enterocytes, glandular cells, and endothelial cells of the gastrointestinal tract [63,64].

Challenges and opportunity in COVID-19 treatment

The COVID-19 is a novel pneumonia-like severe disease, which is an unprecedented challenge and drastically affecting global health and the economy. Due to its novelty with a high rate of mutations in the causative virus, and unavailability of an effective anti-SARS-CoV-2 drug; the world is dealing with serious challenges for the treatment of COVID-19. Another important concern is the shortage of ICU facilities, including isolation beds, ventilators, fluid management, and oxygen supports, which are also barriers to treatments. Approximately 20% of COVID-19 patients have tended to develop acute respiratory distress syndrome, while approximately 26% of patients have required an ICU facility [65]. Several new strains of SARS-CoV-2 including P.1 and P.2 (Brazil); B.1.617, B.1.617.1, B.1.617.2, and B.1.617.3 (India); B.1.525 (Nigeria); P3 (Philippines); B.1.351 (South Africa); B.1.1.7 (UK); B.1.351, B.1.526, B.1.526.1, B.1.427, B.1.429, and P.1 (USA) have been identified in different nations [66,67]. Different viral strains have different transmissibility and pathogenicity. The B.1.525 and B.1.617.1 strains of SARS-CoV-2 have been designated as variants of interest and variants of concern respectively by the World Health Organization [66]. Although numerous vaccines such as RNA-based Moderna and Pfizer-BioNTech; inactivated virus-based BBIBP-CorV, CoronaVac, Covaxin, CoviVac, Minhai-Kangtai, QazVac, and WIBP-CorV; viral vector-based Convidecia, Johnson & Johnson, Oxford-AstraZeneca, Sputnik Light, and Sputnik V; and viral protein subunit-based EpiVacCorona and RBD-Dimer are currently in use for the vaccination [68]. The ever-evolving mutant strains, effective treatment strategy, and vaccination of $\sim$ 8-billion-strong humanity are the major challenges for medical professionals. Besides that, one new challenge, in the form of COVID-19 associated fungal infection (mucormycosis) in the ENT region has also been reported [69,70]. So along with the vaccine, it is important to find out effective treatment for COVID-19.

Antiviral, antimalarial, and herbal medicines have been alternative options for the treatment of COVID-19. Presently, >85% of COVID-19 patients have been treated by the antiviral agents, including Oseltamivir, Lopinavir/Ritonavir, Ganciclovir, and Remdesivir [65]. The critical condition of COVID-19 has been managed by the combination of corticosteroids and antiviral agents

[71]. The effective antimalarial drug chloroquine phosphate showed antiviral and antiinflammatory potential. Thereby it has been used for inhibiting the aggravated effects of pneumonia [72]. The most effective anti-SARS-CoV medicinal herbs include *Astragali radix* (rich in steroidal saponins and isoflavonoids), *Glycyrrhizae radix* rhizome (rich in flavonoids and triterpenoid saponins), *Saposhnikoviae radix* (rich in chromones and coumarin), *Atractylodis macrocephalae* rhizome (rich in atractylon and atractylenolides), *Lonicerae japonicae* (rich in flavonoids, iridoid glycosides, and flavonoids), and *Forsythiae fructus* (rich in phenylethanoid glycosides and lignans) [73,74].

Potential therapeutic targets for COVID-19 treatment

There are several potential therapeutic targets to control coronavirus infection, which are primarily associated with virus entry, genome replication, translation, assembly, and exocytosis [75]. Nsp's are functional proteins that are essential for executing the life cycle of coronaviruses. Among them, RdRp, PLpro, 3CLpro, and helicases are the key and most valid therapeutic targets for designing and developing novel anticoronaviral drugs. Viral proteases may prove as excellent targets since they are responsible for proteolysis of the large polyprotein into different functional proteins such as replicase and polymerase [76]. Since viral growth relies on both viral and host cell proteins, the development of effective drugs against COVID-19, may require selective dysfunctionalization of both viral and host proteins. The spike glycoprotein recognizes the host cell ACE2 receptor and CD147 as an alternative receptor. The spike protein then undergoes proteolysis by host cell proteases including by transmembrane serine protease 2 (TMPRSS2) and furin [77,78]. Besides that, a direct fusion of SARS-CoV-2 with the host cell membrane has also been postulated to facilitate viral penetration into the cell through endocytosis [77]. In this viral entry pathway, some key proteins are 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 [77]. The probable viral and host protein targets for the anticoronaviral drugs are summarized in Table 12.1 with their natural inhibitors.

Anticoronaviral natural products

Natural products have had a very impressive track record of offering amazing drug like specialized secondary metabolites against diverse diseases that continue to afflict mankind [105]. In the context of drugs against viral diseases, medicinal plants have proved to be promising sources of novel antiviral

TABLE 12.1 Corona Virus: Therapeutic Targets and Their Natural Inhibitors

Targets	Description	Natural Molecule Modulators	Ref.
Papain-like protease (PLpro)	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	[79–82]
Main protease (Mpro/3CL-protease)	For 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, β-sitosterol, indigo, hesperetin, sinigrin, quercetin, gallocatechin gallate, and epigallocatechin gallate	[83–85]
RNA-dependent RNA polymerase (RdRp/nsp12)	It is a supramolecular complex associated with processivity clamps (nsp7 and nsp8), exoribonuclease, RNA helicase, and 5′-triphosphatase. Catalyzes replication of the viral RNA and transcription of subgenomic RNA	Monoethyl ester of meconic acid, extract from *Fructus Ligustri Lucidi*, silibinin A, silibinin B, and aureusidin	[86–89]
Exoribonuclease (Exo/nsp14)	Function as 3′–5′ proofreading ribonuclease. Hammering ExoN activity results enhance the antiviral potency of Remdesivir	—	[90]
Angiotensin-converting enzyme 2 Receptor (ACE2R)	It is a transmembrane receptor with peptidase activity that cleaves angiotensin II and other peptide hormones. ACE2R interacts with the spike protein of SARS-CoV-2. Inhibition of ACE2-spike protein interaction is considered an ideal site for antiviral therapeutics	Quercetin, quercetin-3-glucoside, quercetin-3-galactoside, cyanidin-3-galactoside, acteoside, emodin, leucosceptoside A, martynoside, acteoside isomer, isomartynoside, gluco-aurantioobtusin	[91–94]

Continued

TABLE 12.1 Corona Virus: Therapeutic Targets and Their Natural Inhibitors—Cont'd

Targets	Description	Natural Molecule Modulators	Ref.
Transmembrane serine protease 2 (TMPRSS2)	TMPRSS2 is a protease involved in cleaves of ACE-2 and the spike protein. It assists in viral entry into the host lung cells and inhibition of TMPRSS2 impedes viral entry into host cells	Sunflower trypsin inhibitor (SFTI-1)	[78,95]
Furin	It is a protease that causes proteolysis of inactive proteins precursor into their active forms. Notably, it cleaves viral envelope proteins	Catechins, gallic acid, neoandrographolide, andrographolide, baicalein, quercetin, phlogantholide, and epigallocatechin gallate	[96,97]
CD147	It is an alternative receptor for the SARS-CoV-2	—	[97]
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 acid	[98–100]
Vacuolar-type H^+ ATPase (V-ATPase)	V-ATPase is a proton pump located in endosomes and lysosomes, which minimizes 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	[101–104]

agents [106]. Unavailability of antiviral drugs against emerging infections like COVID-19 has compelled us to repurpose existing drugs against it. However the less than satisfactory performance of several repurposed drugs like Remdesivir is the driving force for the discovery of new antiviral agents which may be potent, specific, nontoxic, and affordable. The primary approaches for developing new herbal antiviral agents are the classical methods which involve random screening, phytochemical factors, and serendipity approaches. Another approach has ethnopharmacological traditional knowledge as its base, which offers a good choice for discovering novel antiviral agents. It involves the study of medicinal plants with a history of traditional uses as a potential source of bioactive agents with significant pharmacological activities [107,108]. Herbal formulations have several advantages including good effectiveness with less side effects, easy availability, and relatively low cost [109]. Herein, in the hope of identifying novel leads against COVID-19, we are more focused on compiling natural agents that have been experimentally evaluated against SARS-CoV. Several research institutes across the world including India have been working on screening and clinical testing of potential antiviral small molecules. These 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 12.2 we have compiled experimentally screened natural products against coronaviruses.

Molecular docking and pharmacokinetic study: For support

The structures of selected natural products (Fig. 12.2) were drawn by ChemDraw. The stereoconformers of the natural products and standard ligands were prepared by using LigPrep, Schrodinger 2017-2 by utilizing OPLS_2005 force field. 3D templates of the main protease (PDB entry: 6LU7) and RdRp (PDB entry: 7BV2) were retrieved from the RCSB-PDB database. Both protein structures were preprocessed, optimized, and minimized with the help of protein preparation wizard in Maestro, Schrodinger. Internal ligand, *i.e.*, N3 inhibitor in the main protease, was utilized as reference ligand, whereas Remdesivir was used as the standard ligand for RdRp. The molecular docking and pharmacokinetic screening were performed by standard procedure [119–121].

Docking analysis (Table 12.3) has revealed hit molecules, namely tetra-*O*-galloyl-*β*-D-glucose (**2**) and juglanin (**25**) against main protease, and tetra-*O*-galloyl-*β*-D-glucose (**2**) and glycyrrhizin against RdRp. It is noteworthy that the standard inhibitors N3 against protease and Remdesivir against

TABLE 12.2 Experimentally Screened Anticoronavirus Natural Molecules/Extract

Molecules/Extract	Source	Targets	Activity	Ref.
Glycyrrhizin	Liquorice roots	Replication unit	The IC_{50} 316–625 μg/mL	[20]
Tetra-*O*-galloyl-β-D-glucose			EC_{50} 4.5 μM and SI 240	[110]
Quercetin			EC_{50} 83.4 μM	[110]
Lycoris radiata extract (lycorine)	*L. radiata*		EC_{50} 2.4 μg/mL	[111]
Isatis indigotica root extract	*Isatis indigotica*	3CL protease	IC_{50} 53.8 ± 4.2 μg/mL (by cell-free assay) and 191.6 ± 8.2 μg/mL (by cell-based assay)	[84]
Indigo	*Isatis indigotica*	3CL protease	IC_{50} 300 μM (by cell-free assay) and 752 μM (by cell-based assay)	[84]
Indirubin	*Isatis indigotica*	3CL protease	IC_{50} 293 μM (by cell-free assay)	[84]
Indican	*Isatis indigotica*	3CL protease	IC_{50} 112 μM (by cell-free assay)	[84]
Sinigrin	*Isatis indigotica*	3CL protease	IC_{50} 121 μM (by cell-free assay) and 217 μM (by cell-based assay)	[84]
β-Sitosterol	*Isatis indigotica*	3CL protease	IC_{50} 115 μM (by cell-free assay) and 1210 μM (by cell-based assay)	[84]
Aloe-emodin		3CL protease	IC_{50} 132 μM (by cell-free assay) and 366 μM (by cell-based assay)	[84]
Hesperetin		3CL protease	IC_{50} 60 μM (by cell-free assay) and 8.3 μM (by cell-based assay)	[84]
Daidzein		3CL protease	IC_{50} 105 μM (by cell-free assay).	[84]
Emodin	*Rheum officinale* and *Polygonum multiflorum*	S protein and ACE2R interaction	IC_{50} 200 μM	[94]

Chrysin	*R. officinale* and *P. multiflorum*	S protein and ACE2R interaction	IC_{50} 400 μM	[94]
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 R interaction	IC_{50} 1–10 μg/mL	[94]
Ferruginol			CC_{50} 80.4 μM, EC_{50} 1.39 μM, and SI 58.0	[112]
Dehydroabieta-7-one			CC_{50} 305.1 μM, EC_{50} 4.00 μM, and SI 76.3	[112]
6,7-Dehydroroyleanone			CC_{50} 89.7 μM, EC_{50} 5.55 μM, and SI 16.2	[112]
α-Cadino			CC_{50} 76.8 μM, EC_{50} 4.44 μM, and SI 17.3	[112]
Honokiol			$IC_{50} > 100$ μM, CC_{50} 88.9 μM, EC_{50} 6.50 μM and SI 13.7	[112]
Magnolol			CC_{50} 68.3 μM, EC_{50} 3.80 μM and SI 18.0	[112]
Niclosamide			CC_{50} 22.1 μM, $EC_{50} < 0.1$ μM and $SI > 221$	[112]
Valinomycin			CC_{50} 67.5 μM, EC_{50} 1.63 μM and SI 41.4	[112]
Betulinic acid		3CL protease	IC_{50} 10 μM	[112]
Betulonic acid		3CL protease	$IC_{50} > 100$ μM	[112]
Savinin		3CL protease	IC_{50} 25 μM	[112]
Curcumin		3CL protease	IC_{50} 40 μM	[112]
Niclosamide		3CL protease	IC_{50} 40 μM	[112]

Continued

TABLE 12.2 Experimentally Screened Anticoronavirus Natural Molecules/Extract—Cont'd

Molecules/Extract	Source	Targets	Activity	Ref.
Leptodactylone	*Boenninghausen iasessilicarpa*		Protective activity against Vero-E6 cells infected by SARS-CoV at 100 μg/mL	[113]
Water fraction of *Houttuynia cordata*	*H. 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	[114]
The fraction of *Cinnamomi cortex*	*Cinnamomi cortex*		*n*-Butanol fraction (IC_{50} 7.8 ± 0.3 μg/mL) Ethanol fraction (IC_{50} 10.7 ± 04 μg/mL)	[115]
Biflavone amentoflavone	*Torreya nucifera*	3CL protease	IC_{50} 8.3 μM	[116]
Myricetin	Chromadex	Nsp13	Inhibited the 90% of ATPase activity of nsP13 at 10 μM	[117]
Scutellarein	*Scutellaria baicalensis*	Nsp13	Inhibited the 90% of ATpase activity of nsp13 at 10 μM	[117]
Quercetin, epigallocatechin gallate, gallocatechin gallate	*Pichia pastoris*	3CL protease	Quercetin (IC_{50} 73 μM), epigallocatechin gallate (IC_{50} 73 μM), and gallocatechin gallate (IC_{50} 47 μM) with K_i 25 ± 1.7 μM	[85]
Tanshinone I	*Salvia miltiorrhiza*	3CLpro and PLpro	The good inhibitory activity even at 0.7 μM by a deubiquitinating mechanism with good selectivity	[118]
Rosmariquinone	*Salvia miltiorrhiza*	3CLpro and PLpro	It possesses different kinetic mechanisms as well as slow and reversible inhibition	[118]

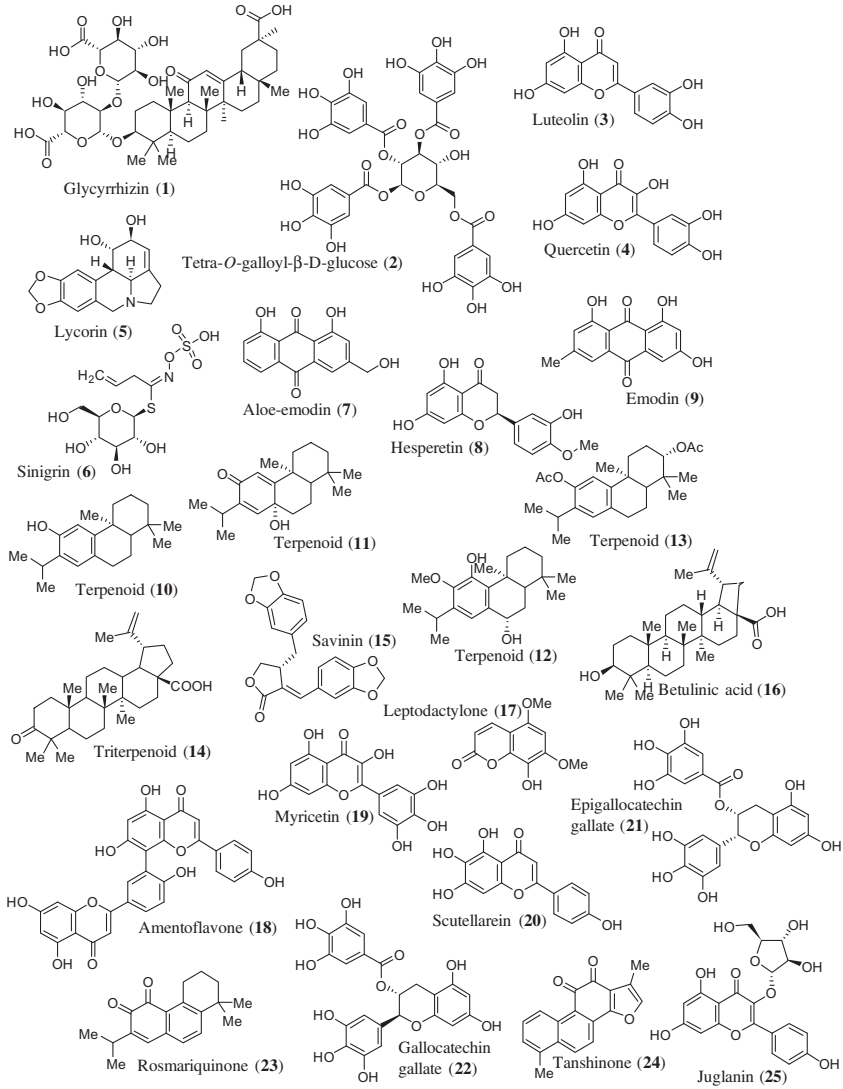

FIG. 12.2 Selected bioactive natural products against coronaviruses.

RdRP displayed lower binding energies at −8.178 and −4.23 kcal/mol, respectively, compared with selected natural compounds. 3D-interaction diagram for apex hit molecules have been presented in Fig. 12.3. *In silico* pharmacokinetic parameters were calculated with QikProp, Schrodinger. Pharmacokinetic analysis is a practical approach to minimize the failure rate during drug discovery. Pharmacokinetic parameter and their predicted values are mentioned in Table 12.3.

TABLE 12.3 Docking Score and ADME Parameters of Anti-SARS Candidates

Natural Candidate	Docking Score		Log Kp (−8.0 to 1.0)	PHOA (<25 poor, >80 good)	PCaco2 (<25 poor, >500 great)	Log Khsa (−1.5 to 1.5)	Log S (−6.5 to 0.5)	PMDCK (<25 poor, >500 great)	Rule of five	QPlog HERG (<5)	HB-acceptor (2.0–20.0)/ HB-donor (0.0–6.0)
	Mpro (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**

Bold text shows within recommended values.

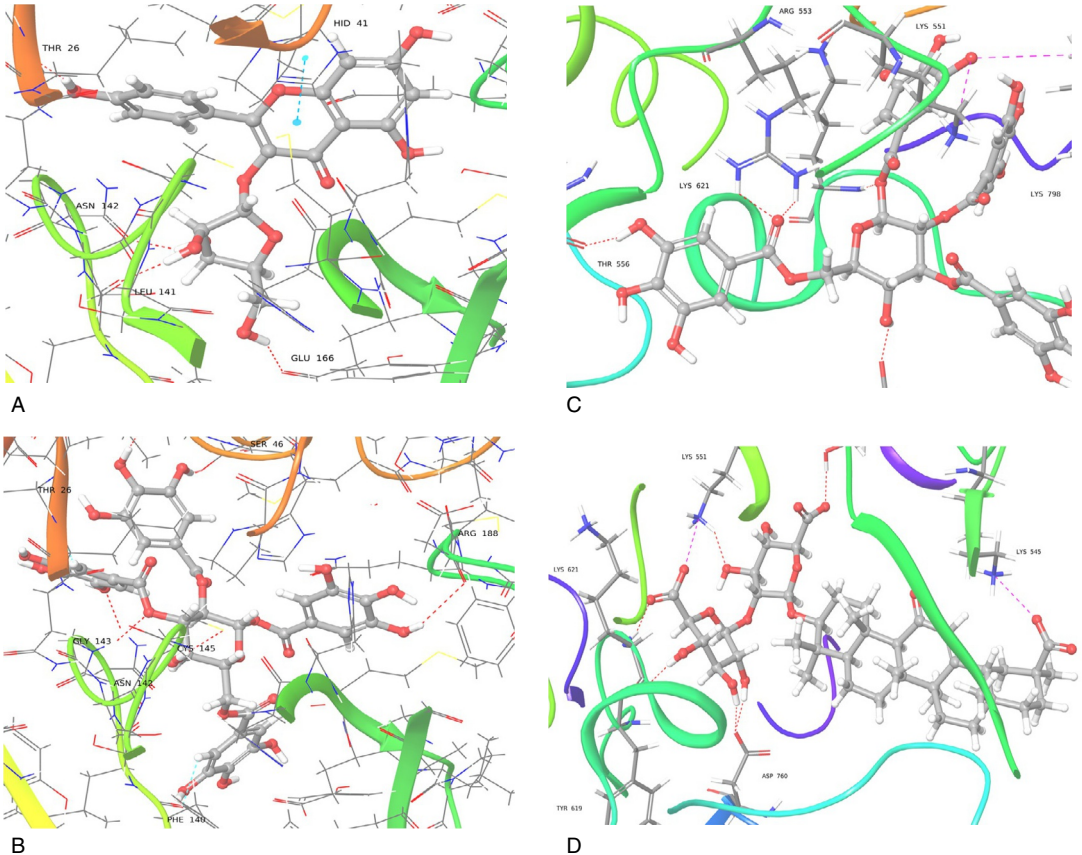

FIG. 12.3 Molecular interactions of (A) tetra-*O*-galloyl-β-D-glucose (−12.20 kcal/mol), and (B) juglanin (−8.96 kcal/mol) with main protease. (C) Tetra-*O*-galloyl-β-D-glucose (−11.74 kcal/mol), and (D) glycyrrhizin (−7.77 kcal/mol) with RdRp.

Conclusion

The contagion of the SARS-COV-2, their transmission rate, and lack of effective COVID-19 treatments are tough challenges 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 time, which can be seen through the publication of >1000 SARS-CoV-2 genomes till now. Several crystal structures of SARS-CoV-2 key proteins have been solved, 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. In this chapter, an outline of different coronaviruses is summarized, including SARS-CoV-2 and potential therapeutic targets for COVID-19 treatment. In addition, anticoronaviral natural molecules were experimentally evaluated with *in silico* studies against Mpro and RdRp proteases of SARS-CoV-2. The computational screening revealed three lead compounds, *i.e.*, glycyrrhizin (**1**), tetra-*O*-galloyl-β-D-glucose (**2**), and juglanin (**25**) that may be helpful for the management of COVID-19.

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Conflict of Interest

The authors declare no competing interests.

Abbreviations

ACE2	angiotensin-converting enzyme 2
APN	aminopeptidase N
CCoV	canine coronavirus
CTSL	cathepsin L
DPP4	dipeptidyl-peptidase 4
ECE	epizootic catarrhal enteritis
HE	hemagglutinin esterase
MERS-CoV	middle east respiratory syndrome-related coronavirus
MHV	mouse hepatitis virus
MRCA	most recent common ancestor
Nsps	nonstructural proteins
ORFs	open-reading frames
PIKfyve	phosphatidylinositol 3-phosphate 5-kinase

RBDs	receptor-binding domains
RdRp	RNA-dependent RNA polymerase
SARS-CoV	severe acute respiratory syndrome-related coronavirus
TMPRSS2	transmembrane serine protease 2
TPC2	two-pore segment channel 2
UTR	untranslated region
V-ATPase	vacuolar-type H^+ ATPase

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