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MINI-REVIEW ARTICLE

Prospects of Antiviral Drugs Derived from Natural Products: Targeting SARS-CoV Entry and Replication

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Abstract: ***Abstract*****:** The recent coronavirus disease (COVID-19) pandemic outbreak caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its rapid spread from continent to continent pose a global health emergency. Researchers are making headway to combat the ongoing COVID-19 to prevent further losses. Many natural antiviral compounds have been explored for their potential application in treating viral infections, including those caused by SARS- and MERS-CoV. This review focuses on natural compounds that have been showing promising results against SARS-CoV, SARS-CoV-2 and MERS-CoV, along with their mechanism of action. The entry and replication of CoV are among the major mechanism for the spread of COVID-19. In this context, natural compounds inhibiting the proteins essential for SARS-CoV-2 entry and replication in the nanomolar (nicotianamine) and micromolar (baicalin, baicalein, scutellarein, dihydromyricetin, quercetagetin, myricetin, amentoflavone, herbacetin, isobavachalcone, quercetin 3-β-d-glucoside, helichrysetin, hirsutenone, hirsutanonol, oregonin, rubranol, rubranoside B, rubranoside A, tanshinones, emodin, and griffithsin) concentration could be potential sources of new anti-SARS-CoV-2 drugs.

Keywords: COVID-19, SARS-CoV-2, 2019-nCoV, SARS-CoV, MERS-CoV, natural products, antiviral drugs, traditional Chinese medicine.

1. INTRODUCTION

Coronaviruses (Cov) are non-segmented, positive-sense single-stranded RNA viruses belonging to the subfamily *Orthocoronavinae* in the family of *Coronaviridae* [1]. These viruses are classified into 4 genera (α−, β−, γ− and δ-CoV), which are widely detected in various animal species, including human [2]. α− and β−CoV genera commonly infect mammals, whilst γ− and δ-CoV tend to infect birds. Six CoVs that infect humans have been identified previously, two α−CoV (HCoV-229E and HCoV-NL63) and five β−-CoV (HCoV-HKU1, HCoV-OC43, Middle East respiratory syndrome (MERS)-CoV, and severe acute respiratory syndrome (SARS)-CoV. Among these CoVs, SARS- and MERS-CoV are highly pathogenic, first identified in 2002 and 2012, respectively, which caused major pandemic outbreaks of fatal respiratory tract infections [3]. Recently, a novel strain of β−CoV (SARS-CoV-2) closely related to SARS- and MERS-CoV is spreading rapidly from continent to continent, resulting in over 100,000 deaths worldwide as of April 14 2020 [4].

The World Health Organization (WHO) has since declared the CoV disease 2019 (COVID-19) outbreak a global pandemic in March 2020. Upon genome sequencing, SARS--CoV-2 showed 82% and 50% nucleotide sequence homology with SARS-CoV and MERS-CoV respectively, suggesting their similar mechanism in causing pathogenesis [5, 6]. Thus, previous reported antiviral drugs against SARS- and MERS-CoV could be useful to limit SARS-CoV-2. The CoV genome encodes a number of proteins, such as 3 chymotrypsin-like proteases (3CLpro), papain-like protease (PLpro) and spike (S) protein are among the most well-studied, as they are essential for viral entry and replication. These three proteins would be attractive drug targets of SARS--CoV-2.

The traditional use of medicinal plants has been widely practiced in Southeast Asia, including China, Pakistan, India, Thailand, Japan, Sri Lanka and Bangladesh since prehistoric times; classical example of the traditional antiviral agent is turmeric derived from the plant *C. longa* L.., which has been used to treat influenza [17]. Many active con-

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stituents in traditionally used medicinal plants have since been discovered for their potential pharmacological activities. For example, one of the active ingredients of turmeric, curcumin was reported to possess broad-spectrum biological and pharmacology properties, including antiviral property against human immunodeficiency virus (HIV) [18], H1N1, hepatitis B virus (HBV) [19], HCV [20], and herpes simplex virus (HSV) [21]. The importance of traditional medicines has been recognized by WHO in providing essential care [22]. Furthermore, many FDA-approved synthetic antiviral drugs were inspired by active constituents in medicinal plants such as Celgosivir [23], Oseltamivir [24], and Alisporivir [25] owing to their acceptability, safety and natural origin [17]. Thus, ethnopharmacological studies could serve as a powerful tool in the discovery of safer and affordable drugs against viral infection. Evidence from various worldwide studies reporting the antiviral activities of active constituents in medicinal plants towards CoVs is outlined along with their mechanism of action, as summarized in Table **1** and illustrated in Fig. **1**.

2. DRUG TARGETS

2.1. 3CLpro

β−CoV encodes two polyproteins, pp1a and pp1b, upon transcription using host ribosome. Various non-structural proteins (nsp) such as nsp13 that are vital for viral replication are then generated from each polyprotein through proteolytic processing by 3CLpro and PLpro [26]. Thus, inhibitors of these two main proteases may have an advantage in inhibiting the replication of SARS-CoV-2.

Su *et al*. identified two flavonoid compounds baicalin and baicalein, originally derived from the root of Scutellaria baicalensis Georgi, as potent inhibitors of SARS- CoV-2 3CLpro *in vitro* at 6.41 μM and 0.94 μM, respectively [7]. This is in agreement with findings of recent work by Liu *et al*., in which the inhibitory effect of baicalein to SARS-CoV--2 3CLpro was accessed (Liu *et al*., 2020). X- ray crystallographic analysis showed that baicalein uniquely bound to the core of 3CLpro substrate-binding pocket to prevent other substrates from interacting with the active site by acting as a "shield", thus inhibiting proteolytic activity [7]. The use of baicalein was also deemed safe and well- tolerated by healthy participants administered with a single oral dose of 100 – 2800 mg in a clinical trial (CTR20132944), where no signs of toxicity in the liver and kidney were observed [27]. Four other baicalein analogues, namely scutellarein (derived from *S. lateriflora* L.), dihydromyricetin, quercetagetin and myricetin (derived from *A. japonica* were found to inhibit SARS-CoV-2 3CLpro at 5.8 μM, 1.2 μM, 1.24 μM, and 2.86 μM, respectively [8]. Besides, scutellarein and myricetin were shown to inhibit the ATPase activity of SARS-CoV helicase *in vitro* (IC_{50}= 0.86 μM and 2.71 μM), but not the DNA unwinding activity [28]. It is interesting that both compounds did not inhibit the helicase activity of hepatitis C virus due to structural differences. Thus, helicase of SARS-CoV-2 is considered as a target for both scutellarein and myricetin to treat COVID- 19 by blocking SARS--CoV-2 from replication as it shares strikingly similar features of helicase with SARS-CoV [29].

Amentoflavone, a biflavonoid isolated from *T. nucifera* (L.) Siebold & Zucc, has been recognized to exhibit potent inhibitory effect against SARS-CoV 3CLpro, demonstrating an IC_{50} value of 8.3 μM [9]. Structure-activity relationship studies revealed non-competitive inhibition mode of 3CLpro inhibition. The 3CLpro of SARS-CoV and SARS-CoV-2 are about 96% identical with 12-point mutations (Val35Thr, Ser46Ala, Asn65Ser, Val86Leu, Lys88Arg, Ala94Ser, Phe134His, Asn180Lys, Val202Leu, Ser267Ala, Ser284Ala and Leu286Ala) [30], and none of them interact with amentoflavone. Therefore, the high level of sequence similarity of the two 3CLpro indicates the possibility of the same mechanism of action against SARS-CoV- 2 as SARS-CoV.

Recent findings using tryptophan-based assay indicated that four flavonoids, herbacetin, isobavachalcone, quercetin 3-β-d-glucoside and helichrysetin identified from a library of forty flavonoids significantly inhibited MERS- CoV 3CLpro with IC_{50} of 40.59 μM, 35.85 μM, 37.03 μM and 67.04 μM, respectively [12]. Sequence alignment studies showed that 3CLpro of SARS-CoV-2 shares 87% sequence identity with MERS-CoV [30]. Nonetheless, point mutations that were identified in MERS-CoV appear to interact with all four flavonoids. However, the inhibitory effect of herbacetin, isobavachalcone, quercetin 3-β-d-glucoside and helichrysetin on SARS-CoV-2 cannot be ruled out until experimental validations are done.

2.2. PLpro

In addition to processing viral polyprotein, PLpro aids CoVs in evading ubiquitin- and ISG15-dependent host innate immune responses during SARS-CoV infection [31]. Some natural products had been reported for their anti-SARS-CoV capacities *vial* inhibition of PLpro, including hirsutenone (a diphenylheptanoid) derived from the stem bark of *A. japonica* stem bark [11], geranylated flavonoids derived from *P. tomentosa* (Thunb.) fruits [32], and tanshinones from *S. miltiorrhiza* Bunge [13]. Owing to the low sequence similarity (29%) between MERS-CoV and SARS--CoV-2 PLpro due to highly divergent sequences as compared to SARS-CoV (80%) [33], natural anti-MERS-CoV agents are not discussed; however, this option shouldn't be totally ruled out.

Early reports on the selective noncompetitive inhibitory effect against SARS-CoV PLpro activity were found in hirsutenone with an IC_{50}value of 4.1 μM [11]. The study extended these observations by measuring the inhibition of PLpro deubiquitinase, revealing that hirsutenone inhibited PLpro deubiquitination activity at an IC_{50} value of 3 μM. Other diphenylheptanoids, including hirsutanonol, oregonin, rubranol, rubranoside B, and rubranoside A showed relatively weak inhibitory activity by at least 24-fold (IC_{50} ranging from 102 μM - 145 μM) [11]. Structure-activity relationship investigation revealed that the catechol and α,β−unsaturated carbonyl moiety markedly influenced the inhibition of

Table 1. List of natural compounds that displayed inhibitory activity of 3CLpro, PLpro, and S protein of CoVs and ACE2.

Coronaviruses	Compounds	Sources	Mechanism of Action	Refs.
SARS-CoV-2	Baicalin and baicalein	*Scutellaria baicalensis* Georgi	Inhibition of 3CLpro	[7]
SARS-CoV-2	scutellarein	*Scutellaria lateriflora* Linnaeus	Inhibition of 3CLpro and nsp13	[8]
SARS-CoV-2	Dihydromyricetin, quercetagetin, and myricetin*	*Ampelopsis japonica* (Thunberg) Makino	Inhibition of 3CLpro and nsp13*	[8]
SARS-CoV	Amentoflavone	*Torreya nucifera* Linnaeus Siebold & Zucc	Inhibition of 3CLpro	[9]
MERS-CoV	Herbacetin, rhoifolin and pectolinarin	Flavonoid library	Inhibition of 3CLpro	[10]
SARS-CoV	Hirsutenone	*Alnus japonica* Thunberg	Inhibition of PLpro and deubiquitination activity	[11]
SARS-CoV	Hirsutanonol, oregonin, rubranol, rubranoside B, and rubranoside	*Ampelopsis japonica* Thunbergs Makino	Inhibition of PLpro	[11]
SARS-CoV	Flavonoids	*Paulownia tomentosa* Thunberg	Inhibition of PLpro	[12]
SARS-CoV	Tanshinones	*Salvial miltiorrhiza* Bunge	Inhibition of PLpro and deubiquitination activity	[13]
SARS-CoV	Emodin	*Rheum officinale* Baill. and *Polygonum multiflorum* Thunberg Moldenke	Interuption of interaction between S protein and ACE2	[14]
SARS-CoV, MERS-CoV	Grifffithsin	*Griffithsia*	Inhibition of S protein	[15, 16]

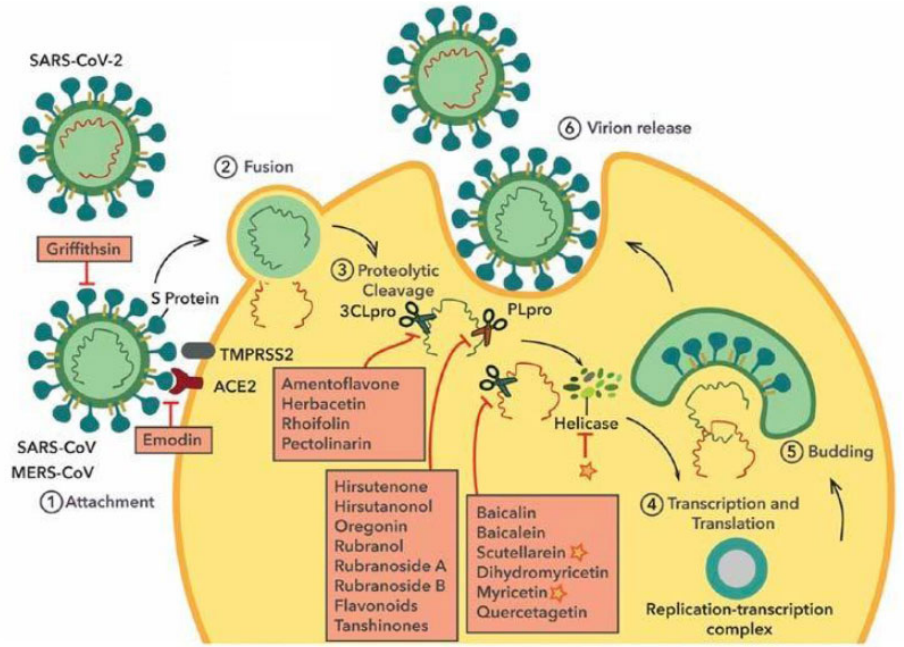

Fig. (1). Potential targets of SARS-CoV-2 for natural anti-CoV agents to prevent SARS-CoV-2 entry and replication. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

SARS-CoV PLpro through the formation of a covalent bond between the carbonyl group and the active site of PLpro. However, it is not sure whether hirsutenone can inhibit SARS-CoV-2 PLpro the same way as it did with SARS--CoV, as the interacting residues remain unknown.

Twelve flavonoids extracted from the fruits of a polyphenol-rich plant *P. tomentosa*, blocked PLpro of SARS--CoV in a dose dependent manner with IC_{50}values ranging between 5.0 μM to 14.4 μM [32]. Among these flavonoids, geranylated Tormentin A, Tormentin B, Tormention C, Tormentin D and Tormentin E containing unique dihydro-2H-pyran moiety were newly identified and these geranylated flavonoids showed greater inhibitory activity than their parental compounds that were derived from the cyclization of the geranyl group. Kinetic study showed that all twelve flavonoids blocked PLpro of SARS-CoV through mixed-type reversible inhibition by binding to the allosteric site of the enzyme, and compound 2 (tomentin B) showed the greatest inhibition among all (3.5 μM). Altogether, these studies will stimulate further modifications of investigations of naturally derived flavonoids.

Seven tanshinones derived from *S. miltiorrhiza* inhibited PLpro activity in a time-dependent manner with IC_{50}values ranging from 0.8 μM to 30 μM [13]. Except tanshinone 7 (rosmariquinone) showing mixed-type reversible slow-binding inhibition of the enzyme, all tanshinones inhibited PLpro non-competitively. The authors suggested that the introduction of three-ringed abietane could be associated with the different acting mechanisms of rosmariquinone. Among the seven tashinones, tanshinone 4 (cryptotanshinone) exhibited the greatest inhibition against SARS-CoV PLpro with an IC_{50}value of 0.8 μM; however, the inhibitory effect on deubiquitinase was moderate (IC_{50}= 87.6 μM). The rationale behind this apparent discrepancy is

2.3. S Protein/Angiotensin-converting Enzyme-2 (ACE2)

The enveloped-anchored S protein (glycoprotein) of CoV has an important role in the early steps of host cell attachment and entry [34]. Studies showed that the engagement of the S1 domain of S protein and host cell receptor ACE2 in the lung facilitated the entry. Following ACE2 binding, the S2 domain facilitated the viral-membrane fusion and transfer of SARS-CoV viral particles into the cell, subsequently stimulated inflammatory responses that resulted in respiratory distress [35-38]. Furthermore, the interaction between SARS viruses, including SARS-CoV-2 and ACE2, was suggested to be potentially associated with the increased susceptibility to SARS-CoV-2 infection [39].

An anthraquinone phytochemical from *R. officinale* Baill. and *P. multiflorum* (Thunb.) Moldenke named emodin was reported to inhibit SARS-CoV infectivity (IC_{50} = 200 μM), slightly weaker than the structurally similar promazine, an anti-psychotic drug, which showed a significant inhibitory effect on SARS-CoV replication [14, 40]. Furthermore, the study extended these observations by using a recombinant expression of ACE2 in Vero E6 cells, revealing that emodin (50 μM) abolished the interaction between SARS-CoV S protein and ACE2 [14].

Griffithsin (GRFT), a red algae-derived lectin, was observed to exhibit broad-spectrum antiviral activity including SARS-CoV [15], MERS-CoV [16] and HIV [41] by targeting S protein to prevent viral entry. In fact, recent studies showed that SARS-CoV-2 S protein and human ACE2 interaction was necessary for the entry of SARS-CoV- 2 into cells and the binding affinity was similar to that of SARS--CoV, thus allowing efficient human-to-human transmission [34, 42, 43]. SARS-CoV-2 shares 64% and 90% amino acid sequence identity with SARS-CoV S1 receptor-binding domain (RBD) and S2 fusion domain, respectively [44], and targeting the well-conserved RBD of SARS-CoV-2 could inhibit the entry of SARS-CoV into cells [45]. However, it is not clear whether GRFT inhibits SARS-CoV-2 S protein to prevent SARS-CoV-2 entry. Furthermore, cellular protease TMPRSS2 is essential for SARS-CoV-2 S protein priming to allow fusion of viral and cell membranes, as confirmed by a study by Hoffmann *et al.* [34]. However, naturally-derived TMPRSS2 inhibitor has not been identified yet, except synthetic serine protease inhibitor Camostat [34] and Nafamostat [46], which had been proven to block TMPRSS2 activity and approved for human use in Japan to treat pancreatitis [47].

Nicotianamine, a vitamin B3 derived from soybean, completely inhibited ACE2 at 900 nM, with an IC_{50} of 84 nM [48]. A recent molecular docking study showed that Nicotianamine is potentially bound to ACE2 with an estimated ΔG of -5.1 kcal/mol [49]. Depending on the progression of COVID-19, although inhibitors of ACE2 could potentially reduce the infectivity, downregulation of ACE2 (hallmark of COVID-19 progression) as observed among elderly male patients with chronic diseases after SARS-CoV-2 infection [50] renders them undesirable, which is in agreement with the vulnerability of this group of patients reported to date. At present, ACE2 inhibitors cannot be ruled out, and once infected with COVID-19, recombinant ACE2 replacement along with ACE2 inhibitors may possibly be favourable to attenuate lung injuries triggered by insufficient ACE2 [51].

3. CLINICAL EVIDENCE OF TRADITIONAL CHINESE MEDICINE IN TREATING COVID-19

As COVID-19 peaks in China, medical experts of COVID-19 from China have proved the worth of combination treatment of Traditional Chinese Medicine (TCM). By combining with modern medicine in COVID-19 treatment, the effectiveness was observed in 91.5% of patients (a total of 74,187), and more than 90% of the effective rate was demonstrated [52]. *Lian hua qing wen* (LH) capsule is known to have antiviral activity, including influenza, SARS and MERS [53, 54]. Glycyrrhizic acid, an extract of licorice found in LH capsules, has a potential inhibitory effect against SARS-CoV-2 3CLpro [55] and has been confirmed to inhibit SARS-CoV-2 replication *in vitro*, for which reason LH capsules were recommended by the National Health Commission (NHC) of the People's Republic of China as a drug candidate to treat COVID-19 according to the Diagnosis and Treatment Protocol for COVID-19 (Trial Version 7) [56]. In addition, three active ingredients of LH cap-

sule-rutin, forsythoside E, and hyperoside were screened out as potential 3CLpro inhibitor [57]. A multicenter randomized clinical trial (ChiCTR2000029434) confirmed that LH capsule was safe and effectively

improved the cardinal symptoms associated with COVID-19 (fever, fatigue, and cough), shortened the disease course, and improved the recovery of chest radiologic abnormalities within 14 days of treatment [58]. Specifically, the time to and rate of symptom recovery were significantly improved as compared with the control group. In addition, no adverse events were reported, confirming the favorable safety profile of LH capsule for COVID-19 treatment. Despite that, an extended study on the effect of LH capsules on viral shedding is needed concerning the transmissibility and infectivity.

Besides LH capsule, *Qing Fei Pai Du* decoction (QFPDD) was also strongly recommended by NHC, as published in the Diagnosis and Treatment Guideline of Novel Coronavirus Pneumonia (Trial Version 7) [56]. By docking and scoring of ingredients in QFPDD and SARS- CoV-2 proteins, nine of them presented a good docking score for both 3CLpro and Plpro [59]. As of February 29th, 1183 COVID-19 patients treated with QFPDD showed a great prognosis, with 640 patients discharged and 457 patients with improved symptoms [60]. A small sample of patients treated with QFPDD demonstrated that more than 60% of patients showed marked improvement in symptoms, whereas the illness of the remaining 30% of the patients was stabilized significantly. In addition, improvement of symptoms was noticed on day 3 with effective rates of more than 92% [61]. Despite the majority of studies showed positive outcomes upon TCM treatment, risks of bias associated with a small group of participants and vary treatment duration result in low credibility.

4. CONCLUDING REMARKS AND FUTURE DIRECTIONS

Fighting against the current pandemic provides an opportunity to understand the significance of natural active constituents as a source of therapeutic agents in treating emerging infectious diseases such as SARS-CoV. This review has highlighted numerous natural products in inhibiting CoVs entry and replication *vial* inhibition of 3CLpro, PLpro, S protein and ACE2. The striking genetic similarity among the β--CoVs and the understanding of virus-host interaction led to the discovery of natural compounds with a potential anti-SARS-CoV-2 activity that has been previously used to combat SARS-CoV. However, there is a lack of naturally derived compounds, and TCM using herbal formulae could be an alternative treatment of SARS-CoV-2. More than ten TCM are currently in clinical trials to assess their safety and effectiveness, including *Jin Yin Hua Tong* (ChiCTR2000029822), *Jing Yin* Granule (ChiCTR2000030255), *Xue Bi Jing* Injection (ChiCTR2000030388), and *Tan Re Qing* Capsule (ChiCTR2000029813). The reliability of these TCM should be evaluated in a larger sample size, multicentre, double-blinded and placebo-controlled clinical studies before incorporating these TCM in the treatment of COVID-19. Taken together, naturally occurring compounds hold a great promise in fighting against SARS-CoV-2 and we believe that these compounds will contribute to the development of anti- SARS-CoV-2 drugs.

AUTHOR CONTRIBUTIONS

The literature searches and data collection were performed by T.S.N. The manuscript was written by T.N.S and Y-Q.T The manuscript was critically reviewed and edited by C.L.Y., C.M.L, W.H.Y, B.H.G. and Y-Q. T. The project was conceptualized by B.H.G. and Y-Q. T.

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CONFLICT OF INTEREST

The authors have no conflicts of interest, financial or otherwise.

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