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RESEARCH ARTICLE

Can Antimalarial Phytochemicals be a Possible Cure for COVID-19? Molecular Docking Studies of Some Phytochemicals to SARS-CoV-2 3C-like Protease

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Abstract: ***Objective:*** To evaluate the efficacy of reported anti-malarial phytochemicals as lead compounds for possible drug development against COVID-19.

Methods: An *in silico* approach was used in this study to determine through molecular docking the binding affinities and site of binding of these phytochemicals to the 3C-like protease of COVID-19 which is considered as the main protease of the virus.

Results: A number of anti-malarial phytochemicals like apigenin-7-*O*-glucoside, decurvisine, luteolin-7-*O*-glucoside, sargabolide J, and shizukaols A, B, F, and G showed predicted high binding energies with ΔG values of -8.0 kcal/mol or higher. Shizukaols F and B demonstrated the best binding energies of -9.5 and -9.8, respectively. The acridone alkaloid 5-hydroxynoracronycine also gave a predicted high binding energy of -7.9 kcal/mol.

Conclusion: This is for the first time that decursivine and several shizukaols were reported as potential anti-viral agents. These compounds merit further studies to determine whether they can be effective drug candidates against COVID-19.

ARTICLE HISTORY

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1. INTRODUCTION

The current viral pandemic that is sweeping the world is caused by a Coronaviridae family virus termed COVID-19 or SARS-CoV-2. Two previous coronaviruses, Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), caused a stir among the world's population due to their contagiousness and lack of therapeutics to control these viruses. All three viruses are zoonotic. SARS originated in southern China in November 2002, and through nosocomial transmission, it rapidly spread to more than 30 countries across five continents, spreading infections among more than 8,000 people [1]. The clinical symptoms of SARS included fever, chills, myalgia, coughs, breathlessness, runny nose, sore throat, headache, and diarrhea. MERS infected 2,206 people but had a higher mortality rate. The clinical characteristics of MERS included fever, chills, generalized myalgia, drowsiness, cough, dyspnea, breathlessness, abdominal pain, nausea, vomiting, and diarrhea [2]. The latest in these coronavirus diseases (COVID-19 caused by SARS-CoV-2) was first reported from Wuhan, China, in late December of 2019. The common clinical characteristics of this latest coronavirus disease include fever, cough, dyspnea, diarrhea, and fatigue [3,4]. Consistent with the clinical characteristics, SARS-CoV-2 shares 79% sequence identity with the Severe Acute Respiratory Syndrome coronavirus and 50% identity with the Middle Eastern Respiratory Syndrome coronavirus [5].

The viral genome of SARS-CoV-2 reportedly encodes more than 20 proteins, among which are two proteases, the papain-like protease PLpro and chymotrypsin-like protease CLpro. These two proteases cleave two polyproteins of the virus; PP1A and PP1AB into 16 non-structural proteins

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(NSPs), which are essential for viral replication. The CLpro, otherwise also known as the main protease Mpro or 3C-like protease, is considered a promising drug target to block replication of the virus [6]. The 3C-like proteases of SARS and SARS-CoV-2 are highly similar, with only 12 variants in the amino acid residues (residue numbers in SARS-CoV-2 being 35, 46, 65, 86, 88, 94, 130, 180, 202, 267, 285, and 286) [6]. The catalytic residue numbers are the same, 41 and 145 [7]. The substrate-binding residue numbers are also the same, being 41, 49, 143, 144, 163-167, and 187-192 [8,9]. The SARS-CoV-2 3C-like protease is a dimer; the monomer reportedly contains three domains. Domain 1 contains residues 8-100; domain 2 contains residues 101-183, and domain 3 comprises residues 200-303. The N-terminal residues 1-7 play a role in dimerization and formation of the active site; the substrate-binding site is situated in a cleft between domains 1 and 2. The sequence identity of the SARS and SARS-CoV-2 C3-like proteases is 96.08%, and both proteases have formed a target for potential anti-viral phytochemicals and traditional Chinese medicinal compounds [10].

Two major factors regarding COVID-19 (among others) are that any therapeutic to the virus in the form of drugs is yet to be discovered. More than 100 vaccine manufacturers in various countries are rushing to develop one, and 26 candidate vaccines being on trial, and thus far, only three vaccines in the Western countries have obtained 'emergency approval' [11]. If a therapeutic is not quickly found, the actual life of humans and the economic stabilities of even richer countries may spin out of control. This is because the three vaccines developed by the USA and the European Union as well as two vaccines developed, one each by Russia and China, need two doses for proper vaccination. Considering that the world population is nearing 8 billion, that calls for the manufacture and timely administration of 16 billion doses of vaccine(s), which can prove difficult. According to the Congressional Research Service, "The economic fallout from the pandemic raises the risks of a global economic recession with levels of unemployment not experienced since the Great Depression of the 1930s. The human costs in terms of lives lost will permanently affect global economic growth in addition to the cost of rising levels of poverty, lives upended, careers derailed, and increased social unrest" [12]. It is to be noted that COVID-19 has caused 103,920,917 infections and 2,247,202 deaths as of February 2, 2021, throughout the various countries of the world (https://www.worldometers.info/coronavirus/).

One line of approach for drug discovery has been the 'repurposing' of the information that has been gathered during previous drug discovery efforts for SARS and MERS [13]. This approach has mainly focused on compounds effective against other viruses, preferably RNA viruses (COVID-19 being an RNA virus), and targeting potential entry mechanism(s) of the SARS-CoV-2 virus into host cells (reviewed in [13]). The other line of approach is to use plant extracts or selective phytochemicals to determine their anti-COVID-19 viral efficacies. Plants like *Clitoria ternatea*, *Leucas aspera*, and *Cassia alata* have been reported for anti-Corona virus (not COVID-19) activity [14]. *Clerodendrum inerme* has been mentioned as a potential herb in curing SARS-CoV-2 [15]. Extracts of *L. aspera* reportedly have anti-malarial activity [16]. Quinones isolated from *C. alata* also showed anti-malarial activity [17]. Thus, it is not uncommon for anti-malarial plants to possess also anti-viral activity. Since phytochemicals present in plants are responsible for their pharmacological activities, it was of interest to screen through *in silico* approach (molecular docking) a number of reported anti-malarial phytochemicals for their ability to bind to the 3C-like protease of SARS-CoV-2.

2. MATERIAL AND METHODS

2.1. Three-Dimensional Structure of COVID-19 Major Protease (3C-like Protease)

The pdb file (6LU7) of the main protease of SARS-CoV-2 (3C-like protease) was used in the present study [18]. The inhibitor (known as N3) was removed from the pdb file before using the protein's structure in our molecular docking studies. The interacting residues of N3 with the protease are His41, Met49, Phe140, Leu141, Asn142, Gly143, His163, His164, Glu166, Leu167, Pro168, Gln189, Thr190, and Ala191. The active residues at the catalytic site of the 3C-like protease of SARS-CoV-2 are His41 and Cys145. The monomeric form of the protease was used for molecular docking [19].

2.2. Compounds Used in Docking Studies

Anti-malarial phytochemicals present in different plant species belonging to a number of families were selected from a published review paper by Pan and others [20]. The criteria for selection was based on the phytochemical structures with the possibility of binding capacity to the 3C-like protease of COVID-19 taking into account that phytochemicals from a number of groups like flavonoids, alkaloids, and terpenoids were included. Ligand molecules were downloaded from Pubchem [21] in sdf format. They were optimized with the force field type MMFF94 using Openbable software and saved as pdbqt format. The structures of the compounds used in molecular docking studies with the 3C-like protease are shown in Fig. (**1**). Several current and out of use anti-malarial drugs were also chosen for binding studies to the 3C-like protease as controls.

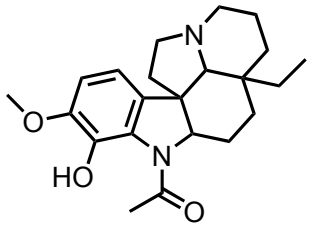
2.3. Ligand Molecular Docking Studies

We have conducted molecular docking (blind) using AutoDock Vina [22]. We report ΔG values as an average of the top values from the docking program. In our figures, we show the pose of some phytochemicals bound to SARS-CoV-2 main protease, as obtained from PyMOL and displayed in Discovery Studio [23].

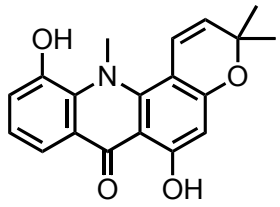
3. RESULTS

Molecular docking has become an important and powerful tool in computer-assisted drug discovery. This tool can be utilized to characterize the binding interaction between a small molecule and a protein at the atomic level. We have used blind docking, which makes no assumption on the binding site [24]. Altogether 35 anti-malarial phytochemicals (approved drugs against malaria and reportedly new anti-malarial phytochemicals) were evaluated *in silico* for their binding affinities (in molecular docking studies) to the 3C-like protease of SARS-CoV-2.

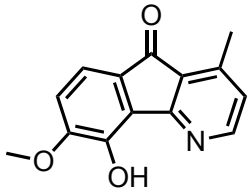
The structures of phytochemicals are shown in Fig. (**1**). A total of 8 anti-malarial phytochemicals like apigenin-7-*O*-glucoside, decurvisine, luteolin-7-*O*-glucoside, sargabolide J, and shizukaols A, B, F, and G showed predicted high binding energies with ΔG values of -8.0 kcal/mol or higher. Shizukaols F and B demonstrated the best predicted binding energies of -9.5 and -9.8 kcal/mol, respectively, followed by decursivine with predicted binding energy of -9.1 kcal/mol. The results are shown in Table **1**. As a group, shizukaols predicted good binding energies; the lowest predicted binding energy among the shizukaols of -6.9 kcal/mol was shown by shizukaol D. In comparison, some anti-malarial drugs like quinine, chloroquine, and artemisinin showed predicted binding energies of only -6.7, -4.6, and -7.0 kcal/mol, respectively to the 3C-like protease of SARS-CoV-2. However, the anti-malarial drug, mefloquine, showed a high predicted binding energy of -7.9 kcal/mol.



Aspidocarpine



5-hydroxynoracronycine



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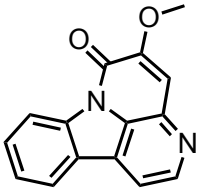
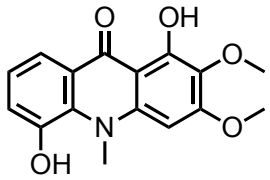
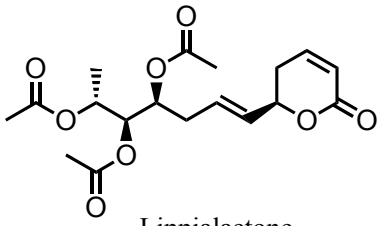


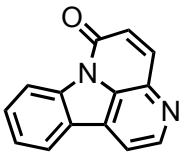
5-methoxycanthin-6-one



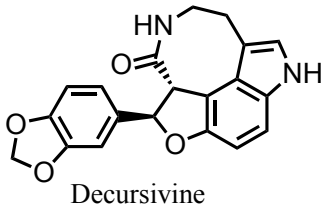
1,5-dihydroxy-2,3-dimethoxy -10-methyl-9-acridone



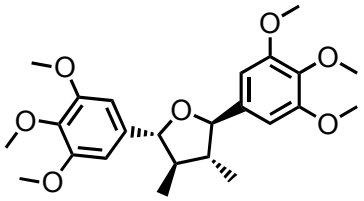
Lippialactone



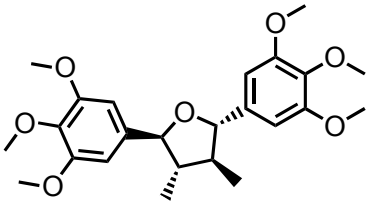
Canthin-6-one



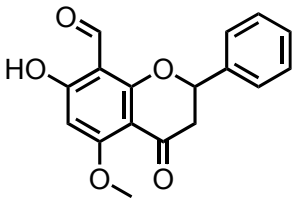
Decursivine



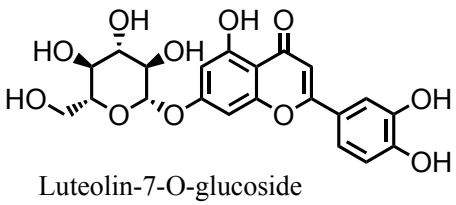
Epigrandisin



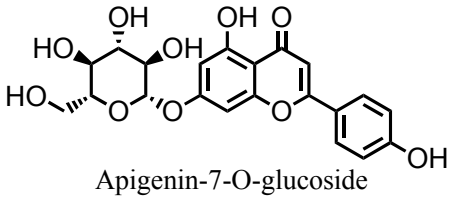
Grandisin



8-formyl-7-hydroxy-5-methoxyflavanone

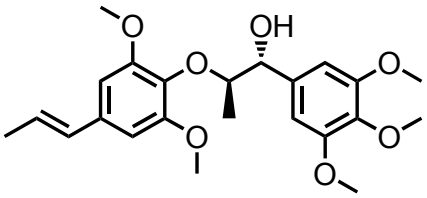


Luteolin-7-O-glucoside

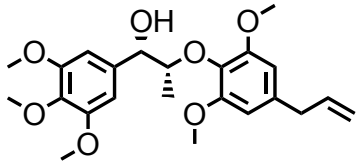


Apigenin-7-O-glucoside

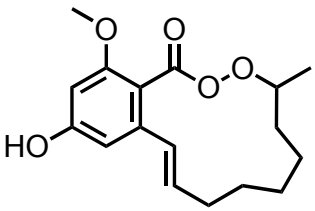
Fig. (1) contd….



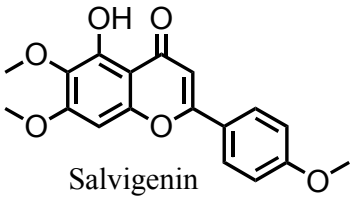
Polysyphorin



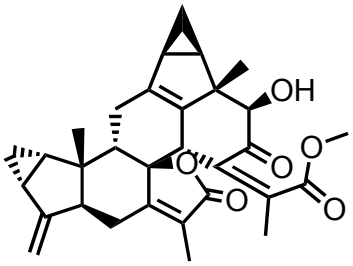
Rhaphidecursinol B



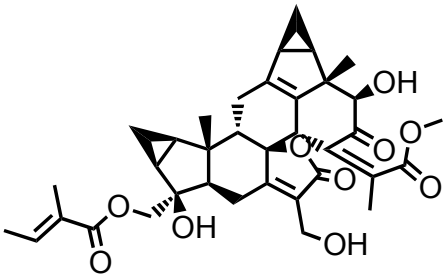
Rhaphidecurperoxin



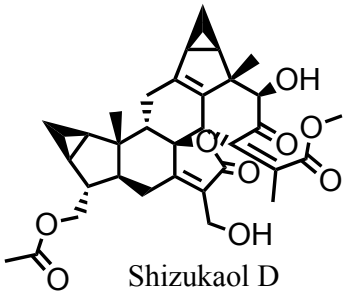
Salvigenin



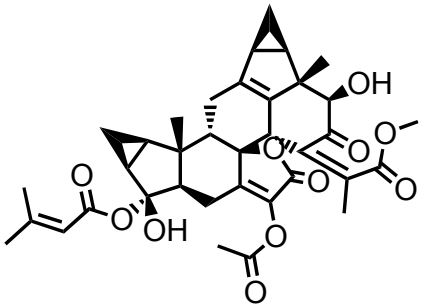
Shizukaol A



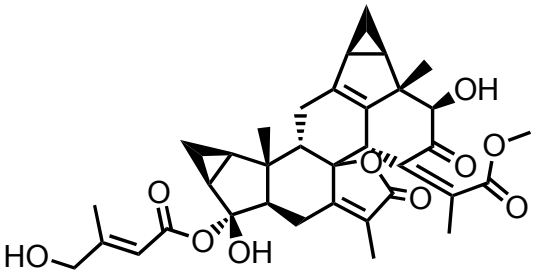
Shizukaol C



Shizukaol D



Shizukaol K



Shizukaol M

Fig. (1) contd….

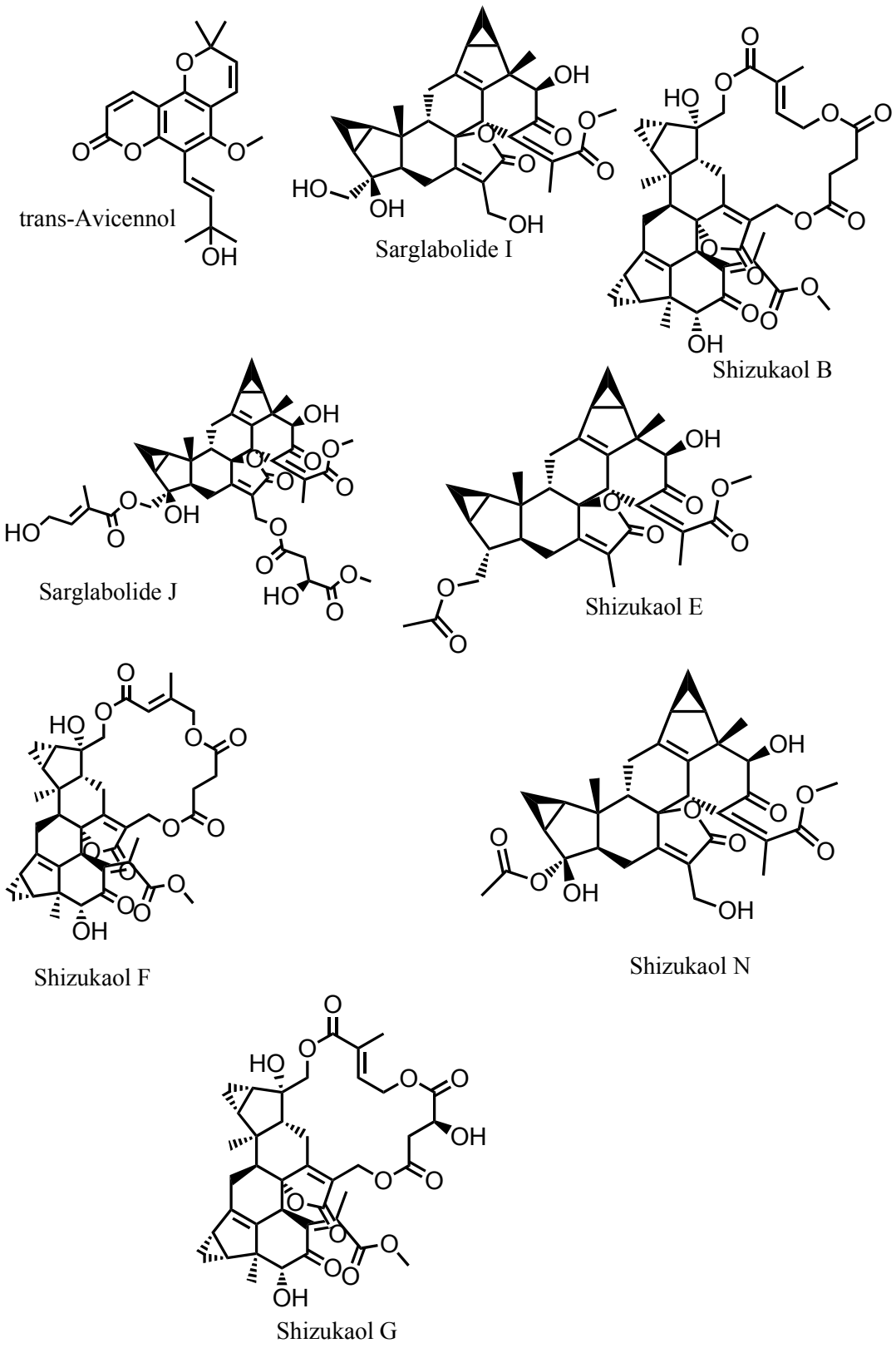


Fig. (1). Structure of phytochemicals.

Table 1. Binding energies of some reported anti-malarial phytochemicals to SARS-CoV-2 $3CL^{pro}/M^{pro}$ protease.

Phytochemical	Binding Energy (ΔG, kcal/mol) SARS-CoV-2
Shizukaol B	-9.8
Shizukaol F	-9.5
Decursivine	-9.1
Shizukaol G	-8.9
Luteolin-7-*O*-glucoside	-8.2
Apigenin-7-*O*-glucoside	-8.0
Shizukaol A	-8.0
Sarglabolide J	-8.0
5-Hydroxynoracronycine	-7.9
trans-Avicennol	-7.9
Mefloquine	-7.9
Shizukaol M	-7.8
Shizukaol E	-7.5
Sarglabolide I	-7.3
Shizukaol N	-7.1
Shizukaol C	-7.0
Shizukaol K	-7.0
Artemisinin	-7.0
Shizukaol D	-6.9
Salvigenin	-6.9
5-Methoxycanthin-6-one	-6.8
1,5-Dihydroxy-2,3-dimethoxy-10-methyl-9-acridone	-6.8
Quinine	-6.7
Rhaphidecurperoxin	-6.7
Aspidocarpine	-6.6
Canthin-6-one	-6.6
8-Formyl-7-Hydroxy-5-methoxyflavanone	-6.6
Lippialactone	-6.4
5-Hydroxy-6-methoxyonychine	-6.3
Epigrandisin	-6.2
Hydroxychloroquine	-6.0
Grandisin	-6.0
Polysyphorin	-5.7
Rhaphidecursinol B	-5.6
Chloroquine	-4.6

The binding of 5-hydroxynoracronycine to SARS-CoV-2 3C-like protease is shown in Fig. (**2**). 5-Hydroxynoracronycine interacts with residues His41, Leu141, Gly143, Ser144, Cys145, and His163, that is more with domain 2 amino acids. The compound reacts with both amino residues in the catalytic site; His 41 and Cys145. Of the substrate binding residues in SARS-CoV-2 3C-like protease (41, 49, 143, 144, 163-167, and 187-192), the compound interacts with His41, Gly143, Ser144, and His163, resulting in predicted binding energy of -7.9 kcal/mol.

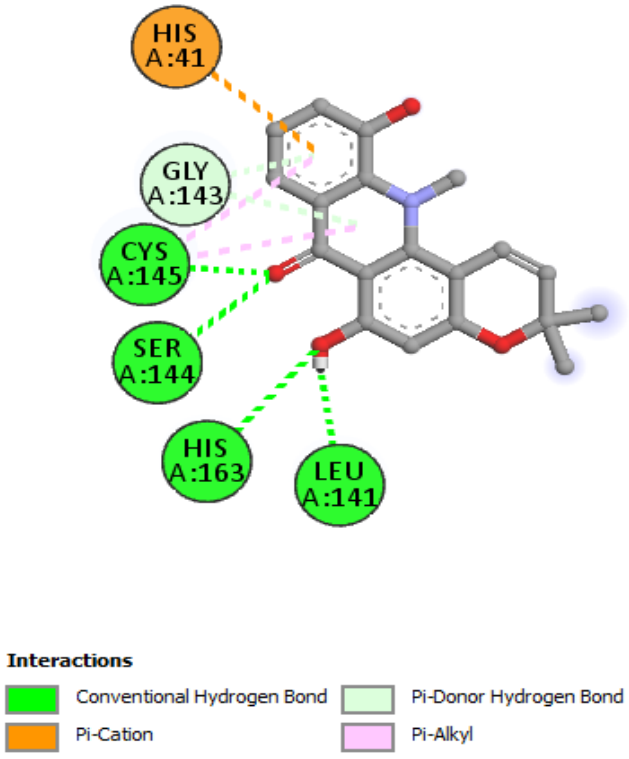


Fig. (2). Interaction of 5-hydroxynoracronycine to SARS-CoV-2 3C-like protease. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The binding of decursivine to SARS-CoV-2 3C-like protease is shown in Fig. (**3**). Decursivine interacts with amino acid residues His41, Met49, Cys145, Met165, Arg188, and Gln189, creating a strong binding with the active site as well as the first two domains of the SARS-CoV-2 3C-like protease (predicted binding energy = -9.1 kcal/mol).

The interaction of apigenin-7-*O*-glucoside with SARS-CoV-2 3C-like protease is shown in Fig. (**4**). The flavonoid derivative interacts with Thr26, Ser144, Cys145, His163, Met165, and Arg188. The compound reacts with one amino acid residue in the catalytic site, namely Cys145, and a number of residues in the substrate-binding site, namely Ser144, His163, Met165, and Arg188, resulting in predicted binding energy of -8.0 kcal/mol.

Comparison of luteolin-7-*O*-glucoside (another flavonoid derivative) interaction with the protease shows that the compound interacts with Thr25, Thr26, His41, Ser46, Leu141, Cys145, and Met165 (Fig. **5**). Thus, the compound reacts with both amino acid residues in the catalytic site, which is different from apigenin-7-*O*-glucoside. Other common interacting residues of SARS-CoV-2 3C-like protease with apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside are Thr26 and Met165. The binding energy of luteolin-7-*O*-glucoside (-8.2 kcal/mol) is also close to that of apigenin-7-*O*-glucoside.

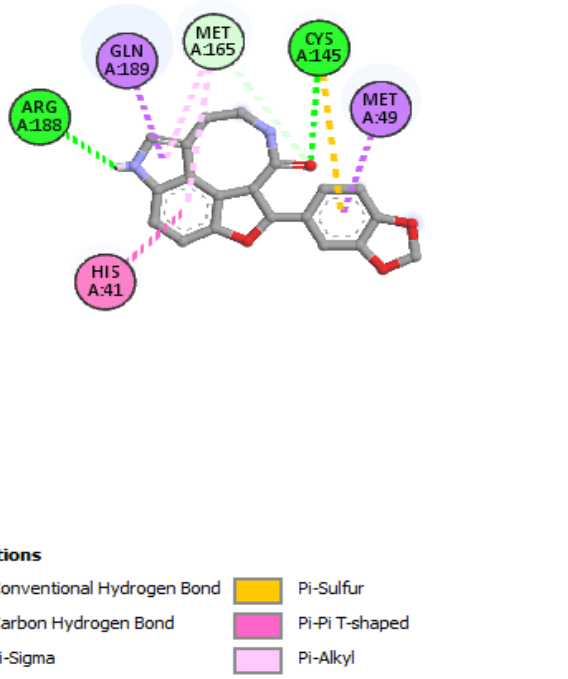


Fig. (3). Interaction of decursivine to SARS-CoV-2 3C-like protease. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

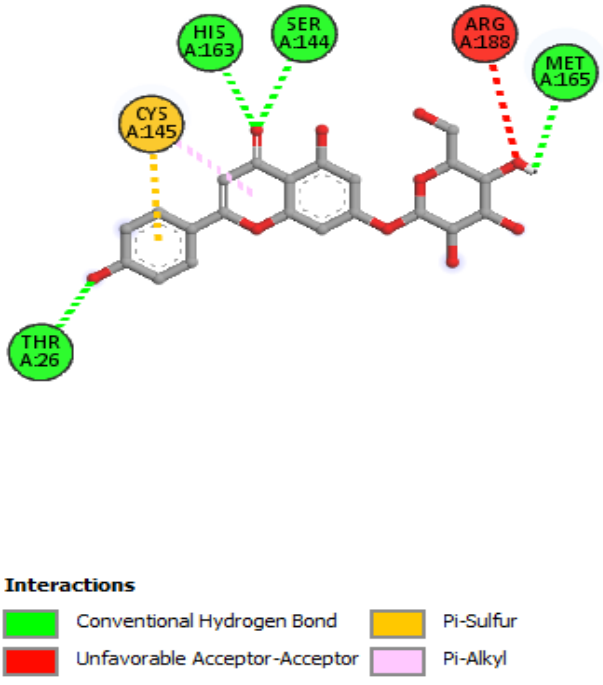


Fig. (4). Interaction of apigenin-7-*O*-glucoside to SARS-CoV-2 3C-like protease. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

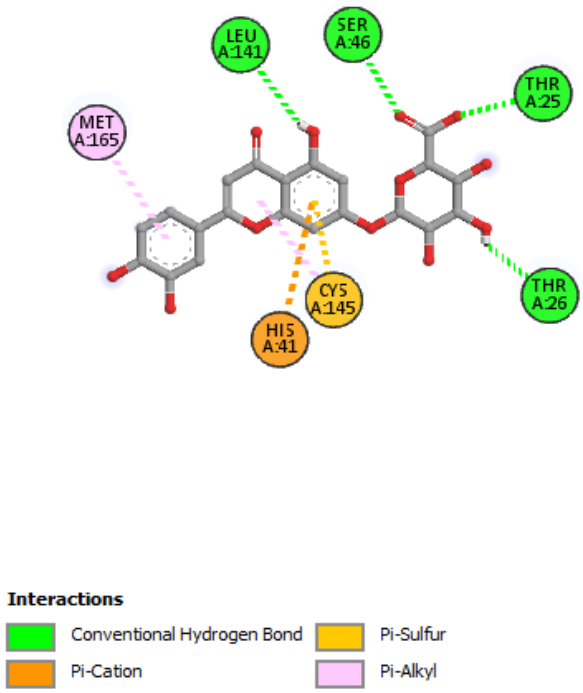


Fig. (5). Interaction of luteolin-7-*O*-glucoside to SARS-CoV-2 3C-like protease. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The interaction of shizukaol B (a lindenane-type dimeric sesquiterpene) with SARS-CoV-2 3C-like protease is shown in Fig. (**6**). The compound was found in the molecular docking studies to interact with amino acid residues Gln107, Asn151, Ile249, and Phe294. Shizukaol B interacts with domains 2 and 3 of the 3C-like protease with a binding energy of -9.8 kcal/mol.

4. DISCUSSION

Shizukaols are compounds possessing a common heptacyclic framework containing more than ten contiguous stereocenters. The Chloranthaceae family is a good source of shizukaols [25]. Apigenin and luteolin are structurally related flavonoids with anti-cancer [26,27], anti-viral (against Enterovirus 71, Chikungunya virus, Japanese encephalitis virus) [28-30], and anti-inflammatory activities [31]. Decursivine is an indole alkaloid. To our knowledge, this is the first report of decursivine and several shizukaols as potential anti-viral agents. These phytochemicals might act as lead compounds for new drugs against COVID-19, as may be inferred from their high binding energies (decursivine, shizukaols B and F). 5-Hydroxynoracronycine is an acridone alkaloid, which has been previously reported for anti-viral activity against the Epstein-Barr virus [32,33]. Acridone alkaloids may be another class of compounds, which need attention as anti-COVID-19 agents because of previously reported antiviral activity of 5-hydroxynoracronycine [32, 33], as well as the fairly strong binding of the compound to the 3C-like protease of SARS-CoV-2, as observed in the present study (to be noted that the binding energy of 5-hydroxynoracronycine and the anti-malarial drug mefloquine was the same at -7.9 kcal/mol). Sarglabolides, like shizukaols, are dimeric lindenane sesquiterpenoids and need further studies for their anti-COVID-19 activities.

An important question is why anti-malarial drugs do act as anti-virals? This is not a new finding and certainly not arising from the recent controversy surrounding the use of the two anti-malarial drugs, chloroquine, and hydroxychloroquine, for COVID-19 therapy [34]. It is possible that most anti-malarial drugs have complex structures, which enables them to have other activities besides anti-malarial activity. D'Alessandro and her group have reviewed the anti-viral activity of a number of anti-malarial drugs [35]. Among the various categories of anti-malarial drugs reviewed by the authors were artemisinin derivatives, aryl-aminoalcohols, aminoquinolines, and 'other anti-malarial drugs. The anti-viral effects of artemisinin (ART) derivatives against human cytomegalovirus (HCMV) *in vitro* have been quite extensively studied and reviewed [36]. ART and artesunate (AS) reportedly inhibited hepatitis B virus (HBV); ART also inhibited replication of hepatitis C virus (HCV) [37] and down-regulated the oncogenic human papilloma virus-39 (HPV-39) proteins E6 and E7 in an *in vitro* model of cervical carcinoma [38].

Various aryl-aminoalcohols have been reported to demonstrate anti-viral activity; quinine sulfate was active against dengue virus strains in different cell lines [39]; a number of reports reviewed by D'Alessandro [35] suggest that mefloquine may be effective against human JC polyomavirus (JCPyV)) and Zika virus (ZKV). Among aminoquinolines, chloroquine (CQ) may be effective against chikungunya virus (CHIKV) and amodiaquine against ZKV [40,41]. CQ also inhibited entry and replication of enterovirus (EV)-A71 in cell-based results [42], while amodiaquine reportedly showed inhibitory effects against dengue and Ebola virus [43,44]. Among other anti-malarial drugs are atovaquone and doxycycline, to name only two. Atovaquone is a naphthoquinone anti-malarial drug, which showed anti-viral activity *in vitro* against CHIKV [45]. Doxycycline (DOX), a semi-synthetic tetracycline antibiotic, is widely used alone or in combination with quinine against chloroquine-resistant *Plasmodium falciparum* cases of malaria [46]. DOX reportedly inhibited proliferation of a panel of human papillomavirus (HPV)-positive cervical cancer cell lines [47] and inhibited DENV propagation [48].

The present study opens up two future lines of research. The first is that if the compounds evaluated in the present study prove in practice to be ineffective against SARS-CoV-2, derivatives, and analogues of these compounds may be prepared and evaluated for SARS-CoV-2 virucidal activity. For instance, enteroviruses (polio, Coxsackie, echoviruses) are common infection-causing agents in humans, particularly the Coxsackie virus, which causes the common cold. Chloroquine has shown promising results against Coxsackievirus B-3. However, because of the chemical instability of the oxazoline ring in chloroquine, two analogues, namely 1-H-pyrazolo[3,4-b]pyridine (1) and quinaldine (2) analogues have been synthesized, with anti-viral activities against the Coxsackie virus [49]. The second is that future research interest can focus on more new anti-malarial phytochemicals, many of them being reviewed by Batista and

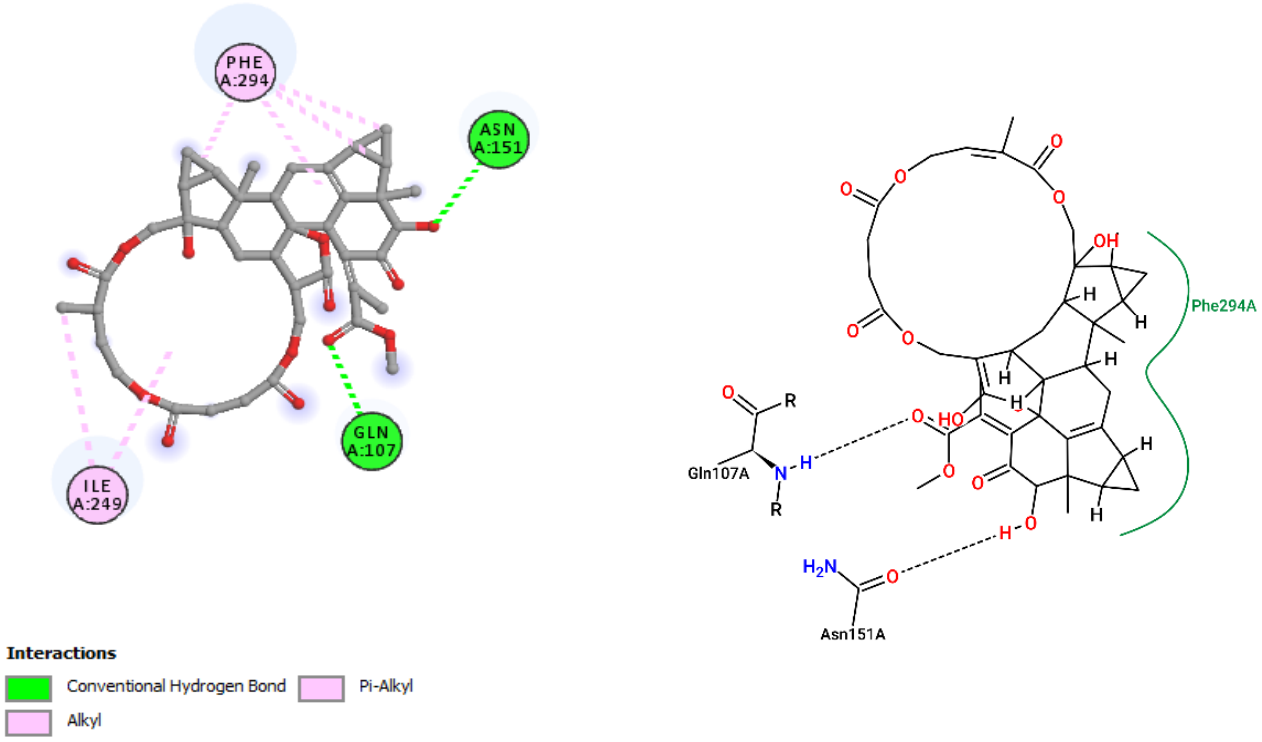


Fig. (6). Interaction of shizukaol B with SARS-CoV-2 3C-like protease. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

group [50]. To cite another example, Aloe extracts and Aloe phytochemical(s) have shown anti-plasmodial activity *in vitro* [51] and SARS-CoV-2 main protease binding *in silico*[52]. Such extracts can be promising sources of anti-SARS-CoV-2 drugs as well as drugs that can act against both COVID-19 and malaria. A drug that can be used against both malaria and COVID-19 will have an advantage in malaria-prone countries among individuals who have contracted both malaria and COVID-19 and can now be treated with a single drug with presumably lesser or no adverse effects.

CONCLUSION

A number of reported anti-malarial phytochemicals were assessed *in silico* for their binding affinities to the 3C-like protease of SARS-CoV-2 (COVID-19). Among the phytochemicals, flavonoid derivatives (apigenin-7-*O*-glucoside and luteolin 7-*O*-glucoside), acridone alkaloid (5-hydroxynoracronycine), sesquiterpenoids (like shizukaols A, B, F, and G), pyranocoumarin compound (trans-avicennol), and an indole alkaloid (decursivine) showed predicted high binding affinities to the 3C-like protease and merit potential for *in vivo* anti-viral studies against SARS- CoV-2. The major finding of the present study is that, at the very least several antimalarial compounds may prove to be of therapeutic value as anti-COVID-19 drugs.

AUTHOR CONTRIBUTIONS

MR coordinated the work and wrote the first draft of the manuscript. AH, RJ, KJ, TAB, and MSH did the molecular docking studies. MLP, VN, and CW supervised the molecular docking studies and edited the manuscript. All authors saw, contributed, and agreed with the final form of the manuscript, which is submitted.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Not applicable since no human or animals were involved.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

All authors give their consent to publication.

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STATEMENT OF AVAILABILITY OF DATA AND MATERIALS

The data and materials used in the study are available upon request from the corresponding author.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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