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Herbal Compounds from *Syzygium aromaticum* and *Cassia acutifolia* as a Shield against SARS-CoV-2 M^{pro}: a Molecular Docking Approach

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Abstract: Novel coronavirus (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) was first identified in China in December 2019. Currently, the novel coronavirus disease 2019 (COVID-19) is the most infectious disease worldwide. In the absence of a vaccine or drug, herbal compounds may be used to treat or control this disease. To explore novel potent inhibitors that suppress this virus's growth, we performed molecular docking studies on SARS-CoV-2 M^{pro} using 17 effective herbal compounds, along with three reference drugs. Docking results showed that crategolic acid from *Syzygium aromaticum* (clove) had the highest binding affinity with SARS-CoV-2 M^{pro} protease, followed by sennoside (A, B, C, and D) compounds from *Cassia acutifolia* (Sana Makki). Crategolic acid and sennoside (A, B, C, and D) contain amino acid residues and hydrogen bonds involved in the protein-ligand interaction. The present study confirms that crategolic acid and sennoside represent the strongest potential inhibitors of SARS-CoV-2 M^{pro}. This study's results may help in vivo studies validate the usefulness of compounds from clove and Sana Makki in preparing herbal medicine for the treatment of COVID-19. This analysis supports the production of new drugs for the treatment and control of COVID-19.

Keywords: SARS-CoV-2; COVID-19; M^{pro} protease; molecular docking; herbal medicine; Sana Makki; clove.

1. Introduction

Novel coronavirus disease 2019 (COVID-19) is currently spreading worldwide. It is a respiratory disease with various symptoms, including headaches, a runny nose, a high fever, and breathing difficulties [1]. COVID-19 is more dangerous for older people and those with a weak immune system [2]. Elderly subjects suffering from heart problems, diabetes, and breathing issues are likely to develop multiple organ failure [3, 4]. On the 11th of March 2020, the World Health Organization (WHO) classified COVID-19 as a pandemic. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused 1,382,106 deaths worldwide as of the 23rd of November 2020 [5]. Herbal medicine is used extensively worldwide for the treatment and control of various diseases. According to the WHO, approximately 80% of the global population depends on herbal medicine to treat diseases. Herbal compounds are also used as prophylactics for the prevention and control of COVID-19 [6]. Herbal medicines can positively affect the treatment and control of MERS-CoV and SARS-CoVs [6, 7]. Kim *et al.*

screened 502 natural compounds against MERS-CoV and found that dihydrotanshinone extracted from *Salvia miltiorrhiza* root, which is commonly used in traditional Chinese medicine, exhibits antiviral activities [8]. Ouabain, a cardiotonic drug obtained from *Strophanthus gratus* seeds, may also be suitable for anti-MERS-CoV therapy [9]. Yu and colleagues screened 64 purified natural compounds and found that scutellarein, obtained from *Scutellaria lateriflora*, is a potent inhibitor of SARS-CoV helicase protein and could be used to tackle SARS outbreaks [10]. A study conducted by Van Doremalen *et al.* found that tea-derived supplements (tannic acid and theaflavin-3-gallate from black tea) might prevent SARS-CoV infection [11].

In the current COVID-19 pandemic, many herbs and natural compounds have been tested via molecular docking and molecular dynamics simulations for their activity against SARS-CoV-2. For example, within one derived from ashwagandha leaves (*Withania somnifera*) and other compounds from ginger (*Zingiber officinale*) have been suggested as potential inhibitors of SARS-CoV-2 [12, 13]. Other potent herbal inhibitors for MERS-CoV, SARS-CoV, and SARS-CoV-2 include griffithsin, silvestrol, emodin, escin, daidzin, 10-hydroxyusambarensine, quercetin, naringenin, oleuropein, resveratrol, ellagic acid, benzoic acid, and gallic acid [6, 7]. However, further *in vitro* and *in vivo* studies are needed to validate these herbs' efficiency and their natural compounds against SARS-CoV-2. Since the COVID-19 situation is continuously deteriorating, more studies are needed to develop new prophylactics against SARS-CoV-2. The National Health Commission of China has announced that herbal medicine could be used in combination with Western medicine to treat COVID-19 [14]. Herbal medicines include herbs, herbal foodstuffs (teas), and herb extracts, including those from clove (*Syzygium aromaticum*), Sana Makki (*Cassia acutifolia*), black seed (*Nigella sativa*), chamomile (*Anthemis hyalina*), ginger (*Z. officinale*), and garlic (*Allium sativum*). These herbs are used as food ingredients in many Asian countries, including India, Pakistan, and China. Herbal compounds have many advantages over commercial medicines, such as low toxicity and low allergenic potency. Therefore, we selected several potent herbs (clove, Sana Makki, black seed, and chamomile) for this study. These herbs have medicinal properties for different diseases [4, 15-17]. *N. sativa* possesses antiviral, anti-inflammatory, and anticancer properties [18, 19]. *C. acutifolia* is used for the treatment of constipation, gonorrhea, bronchial congestion, wounds, diarrhea, intestinal gas, skin diseases, dyspepsia, fever, and hemorrhoids. *S. aromaticum* compounds possess antioxidant, anti-inflammatory, antimicrobial, and antiparasitic properties [20-23]. *A. hyalina* may terminate the replication of coronaviruses [24]. Molecular docking analysis is used to determine the interaction between molecules and identify the location where a ligand binds to a target protein. Bioinformatics analyses are used to determine the appropriate binding energy between proteins and ligands. A combination of these techniques, such as virtual screening, structure-based drug design, and molecular docking, can be used to predict ligands in drug discovery for specific diseases [25, 26]. In the present study, we compared the molecular docking of 17 herbal compounds with the SARS-CoV-2 M^{pro} protease. We also considered the amino acid residues and types of hydrogen bonds involved in the interactions between proteins and ligands. This study may assist with the formulation of new dietary supplements and the discovery of effective drugs for the treatment and control of COVID-19.

2. Materials and Methods

2.1. Receptor preparation.

The 3D structure of SARS-CoV-2 M^{pro} (PDB ID: 6Y2F) was downloaded from the protein data bank (http://www.rscb.org) with a resolution of 1.95 Å in PDB format (Figure 1) and converted to the PDBQT format using AutoDock tool 1.5.6.0 (http://mgltools.scripps.edu) [27]. During the preparation of the receptor, water molecules were removed, and polar hydrogen was added. The receptor grid box was generated with dimensions of 40 × 40 × 40 Å.

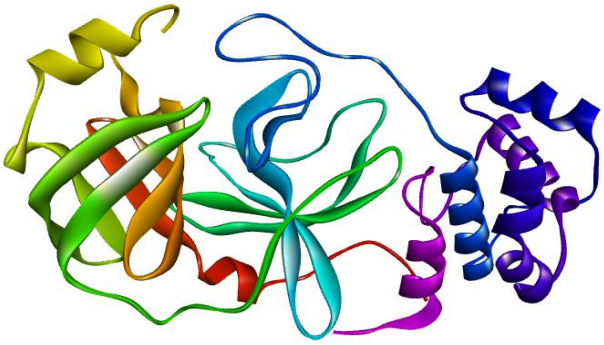


Figure 1. 3D Structure of SARS-CoV-2 M^{pro} (PDB ID: 6Y2F).

2.2. Ligand preparation.

The selection of herbal compounds was based on the use of the herbs and their extracts in traditional medicine to prevent COVID-19 and as recommended in previous studies. Details of the herbal compounds and their medicinal uses are provided in Table 1. In total, 17 herbal compounds and three reference drugs (hydroxychloroquine, azithromycin, and remdesivir) were selected for molecular docking studies. The structures of the selected herbal compounds and drugs were downloaded from the ChemSpider database in MDL Molfile (MOL) format and converted to the PDBQT format using Open Babel Server 2.4.1. The PDBQT format data were used as the input for the AutoDock Vina software for molecular docking analyses. The output file of Autodock Vina was visualized using Discovery Studio Visualizer 2.0.

Table 1. Details of herbal compounds.

Herb	Active compound	Chem Spider ID	Medicinal use	Reference
Black Seed (*Nigella sativa*)	Nigellicine	9577233	Antiviral, enhance immunity, hepatitis-C, diabetes mellitus, hypertension, hyperlipidemia, asthma, cardiac dysfunction, anti-inflammatory	[28-33]
	Nigellidine	30777529		
	Nigellimine,	19514		
	Thymoquinone	9861		
	Thymohydro-quinone	86459		
Sana Makki **(***Cassia acutifolia***)**	Sennoside A	65892	Gonorrhea, bronchial congestion, wounds, diarrhea, intestinal gas, skin diseases, dyspepsia, fever, hemorrhoids, chronic constipation	[34, 35]
	Sennoside B	82569		
	Sennoside C	28428662		
	Sennoside D	28428663		
Clove (*Syzygium aromaticum*)	Acetyl eugenol	6869	Antioxidant, anticancer, antimicrobial, antibacterial, osteoporosis, stomach ulcers, antiviral effects	[38-39]
	Beta-caryophyllene	4444848		
	Vanillin	13860434		
	Crategolic acid	66312		
	Kaempferol	4444395		
	Rhamnetin	4445008		
Chamomile (*Anthemis hyalina*)	Carvacrol	21105867	Antiviral, Antimalarial, Anti-inflammatory	[24, 28]
	α-pinene	6402		

2.3. Molecular docking.

Molecular docking is used to identify amino acid interactions between proteins and ligands for structure-based drug design [40]. The docking was performed using AutoDock Vina 1.1.2 (http://vina.scripps.edu) [41]. This version has greater accuracy in predicting ligand-protein interactions and runs faster than its previous version, AutoDock 4.2. AutoDock Tool 1.5.6 (http://mgltools.scripps.edu) was used to prepare the protease PDBQT format and Open Babel (http://openbabel.org) was used to prepare the ligand PDBQT format. The molecular docking procedure was performed using AutoDock Vina software. Grid spacing was adjusted to 0.375 Å, and the exhaustiveness value was set to eight. The conformational docked poses were assessed by computing the root mean square deviation (RMSD) values. The best docking positions were those in conformations with the lowest binding energy and RMSD values [41]. Autodock Vina's outputs were stored in PDBQT files with nine poses, and outputs with different poses were analyzed using Discovery Studio Visualizer (https://www.3dsbiovia.com) [42].

3. Results and Discussion

The 3D structure of SARS-CoV-2 M^{pro} obtained from the protein databank was optimized, and 20 compounds, including the test and reference compounds, were selected to bind with the receptor protein. All compounds were docked with SARS-CoV-2 M^{pro} and ranked based on their binding affinities. In Autodock Vina, the binding energy defines which ligand has a stable complex interaction with a protein, and a more negative binding affinity represents a more stable ligand-receptor interaction [41]. Compounds with binding affinities of −6.0 or less were considered more suitable agents for combatting COVID-19. A relative analysis of reference drugs and herbal compounds is presented in Table 2, representing the list of active compounds obtained after docking studies.

Table 2. Docking Score of Drugs and herbal compounds with SARS-CoV-2 Mpro (PDB ID: 6Y2F).

SL. No	Herbal compound / drugs	Binding Affinity (Kcal/mol)	H-Bond Length (Å)	Hydrogen Bond Name	Hydrophobic residue
Drugs					
1	Hydroxychloroquine	-5.9	2.47821 2.17638 2.25876	A: Lys102:Hz1 - :Unk0: O A:Gln110:He22 - :Unk0:N A: Ser158: Hg - :Unk0: O	Phe294, Val104, Pro293
2	Azithromycin	-8.4	2.35051 2.83838 2.80474 2.74874 2.78285 3.39074 3.59192 3.22475 2.64441 3.62061	A: Gln107:He21 - :Unk0: O A: Gln107:He21 - :Unk0: O A: Gln107:He22 - :Unk0: O A: Gln107:He22 - :Unk0: O A: Asn180:Hd22 - :Unk0: O :Unk0:O - A:Glu178:Oe1 A: Arg105: Cd - :Unk0: O A: Arg105: Cd - :Unk0: O : Unk0: C - :Unk0: O : Unk0: C - A: Arg105: O	Arg105
3	Remdesivir	-8.4	1.97095 2.11076 2.31365 1.92092 3.05913 3.03252 3.27642 3.37354	A: Lys5:Hz2 - :Unk0: O A: Thr199:Hg1 - :Unk0: O A: Asn238:Hd22 - :Unk0: O A: Asp289: Hn - :Unk0: O A: Glu290: Hn - :Unk0: O :Unk0:O - A:Glu288:Oe2 :Unk0:O - A:Asp289:Od1 : Unk0: O - A: Asn238: O	Lys137, Leu286

SL. No	Herbal compound / drugs	Binding Affinity (Kcal/mol)	H-Bond Length (Å)	Hydrogen Bond Name	Hydrophobic residue
			3.65697 3.55265	A: Lys137: Ce - :Unk0: O : Unk0: C - A: Gln127: O	
Herbal compounds					
4	Nigellicine	-6.9	2.87867 2.84422 3.39912	A: Asn151:Hd21 - :Unk0: O A: Thr292:Hg1 - :Unk0: O :Unk0:C - A:Asn151:Od1	Phe294
5	Nigellidine	-6.8	2.09645 2.18539	A: Phe294: Hn - :Unk0: O : Unk0: H - A: Gly109: O	Ile249, Val202, Pro293
6	Nigellimine	-5.4	2.62321 1.97156 3.39494 2.46254	A:lys102:hz1 - :unk0:n A: gln110:he21 - :unk0: O : Unk0: C - A: Arg105: O A:Ser158:Hg - :Unk0	Val104, Ile106
7	Thymoquinone	-5.4	2.09633 2.11835 3.57748	A: Lys5: Hn - :Unk0: O A: Trp207:He1 - :Unk0: O A: Ser284: Cb - :Unk0: O	Phe291
8	Thymohydroquinone	-5.3	2.66366 2.24953	A: Gln110:He22 - :Unk0: O : Unk0: H - A: Ile152: O	Phe294
9	Sennoside A	-10.6	2.44966 3.06338 2.6553 2.70032 2.77418 1.9447 2.89035 3.32685 3.04473 3.49849	A: Lys5:Hz2 - :Unl1: O A: Arg131:Hh12 - :Unl1: O A: Arg131:Hh22 - :Unl1: O A: Ser139: Hg - :Unl1: O A: Phe140: Hn - :Unl1: O A: Phe140: Hn - :Unl1: O A: His172:He2 - :Unl1: O : Unl1: O - A: Gln127: O :Unl1:O - A:Asp289:Od1 A: His172:Ce1 - :Unl1: O	Lys137, Leu286
10	Sennoside B	-10.8	2.41445 3.02611 2.70415 2.10349 2.76671 2.7052 1.92094 2.86391 2.99741 2.79155 3.26535 3.48448	A: Lys5:Hz2 - :Unl1: O A: Arg131:Hh12 - :Unl1: O A: Arg131:Hh22 - :Unl1: O A: Lys137:Hz3 - :Unl1: O A: Ser139: Hg - :Unl1: O A: Phe140: Hn - :Unl1: O A: Phe140: Hn - :Unl1: O A: His172:He2 - :Unl1: O : Unl1: O - :Unl1: O :Unl1:O - A:Thr199:Og1 : Unl1: O - A: Gly170: O A: His172:Ce1 - :Unl1: O	Lys137, Leu286
11	Sennoside C	-10.8	2.50709 2.2163 2.38838 2.62954 2.70406 2.42161 3.36129 3.06806 2.77459	A: Lys5:Hz2 - :Unl1: O A: Lys5:Hz3 - :Unl1: O A: Lys5:Hz3 - :Unl1: O A: Lys5:Hz3 - :Unl1: O A: Ser123: Hg - :Unl1: O A: Ser139: Hg - :Unl1: O :Unl1:O - A:Glu288:Oe1 A: Ser284: Cb - :Unl1: O : Unl1: C - A: Lys137: O	Tyr126, Lys5, Ala116
12	Sennoside D	-10.6	2.55465 3.06179 2.72155 2.27521 2.77583 2.73468 1.88708 2.85506 2.66043 3.00935 3.09808	A: Lys5:Hz2 - :Unl1: O A: Arg131:Hh12 - :Unl1: O A: Arg131:Hh22 - :Unl1: O A: Lys137:Hz3 - :Unl1: O A: Ser139: Hg - :Unl1: O A: Phe140: Hn - :Unl1: O A: Phe140: Hn - :Unl1: O A: His172:He2 - :Unl1: O A: Leu287: Hn - :Unl1: O : Unl1: O - :Unl1: O : Unl1: O - A: Phe140: O	Lys137, Leu286

SL. No	Herbal compound / drugs	Binding Affinity (Kcal/mol)	H-Bond Length (Å)	Hydrogen Bond Name	Hydrophobic residue
			2.73331 3.49087	:Unl1:O - A:Thr199:Og1 A: His172:Ce1 - :Unl1: O	
13	Acetyl eugenol	-5.9	2.02266 2.54619 2.8421 3.44491 3.50397	A: Gln110:He22 - :Unl1: O A: Gln110:He22 - :Unl1: O A: Thr292:Hg1 - :Unl1: O : Unl1: C - A: Thr111: O :Unl1:C - A:Thr111:Og1	Phe294
14	Beta-caryophyllene	-7.7		No Hydrogen bond	Lys5, Leu282, Phe3, Trp207, Phe291
15	Vanillin	-4.7	2.57948 3.35104 3.01478 3.01682 3.23635	A: Gln110:He22 - :Unl1: O : Unl1: O - A: Thr111: O :Unl1:O - A:Thr111:Og1 :Unl1:O - A:Asn151:Od1 :Unl1:O - A:Asp295:Od1	Phe29
16	Crategolic acid	-13.5	2.32865 2.36503 3.15769	A: Lys5:Hz2 - :Unl1: O A: Lys5:Hz3 - :Unl1: O :Unl1:O - A:Glu288:Oe2	Lys13
17	Kaempferol	-7.0	3.03232 3.19361 2.92212 2.89559 3.28826 2.83604 3.95235	A: His246:Hd1 - :Unl1: O : Unl1: O - A: Thr111: O :Unl1:O - A:Thr111:Og1 :Unl1:O - A:Asn151:Od1 :Unl1:O - A:Asp295:Od1 A:Gln110:He22 - :Unl1 :Unl1:O - A:Phe294	Phe29, Ile249, Pro293
18	Rhamnetin	-7.3	2.48944 2.38062 3.06544 2.71467 1.99755 3.03566 3.37976 3.0958 3.00554 3.0293 3.31878 3.70092	A: Lys5:Hz2 - :Unl1: O A: Lys5:Hz2 - :Unl1: O A: Lys5:Hz3 - :Unl1: O A: Arg131:Hh22 - :Unl1: O A: Asp289: Hn - :Unl1: O :Unl1:O - A:Glu288:Oe2 :Unl1:O - A:Glu288:Oe2 :Unl1:O - A:Asp289:Od1 :Unl1:O - A:Thr199:Og1 :Unl1:O - A:Thr199:Og1 :Unl1:O - A:Asp289:Od2 : Unl1: C - A: Lys137: O	Lys137, Leu286
19	Carvacrol	-5.7	2.13065 3.3317 3.62797	A: Trp207:He1 - :Unl1: O : Unl1: O - A: Leu282: O A: Ser284: Cb - :Unl1: O	Phe291
20	α-Pinene	-5.9	-	No Hydrogen Bond	Phe294

3.1. Docking scores of reference drugs.

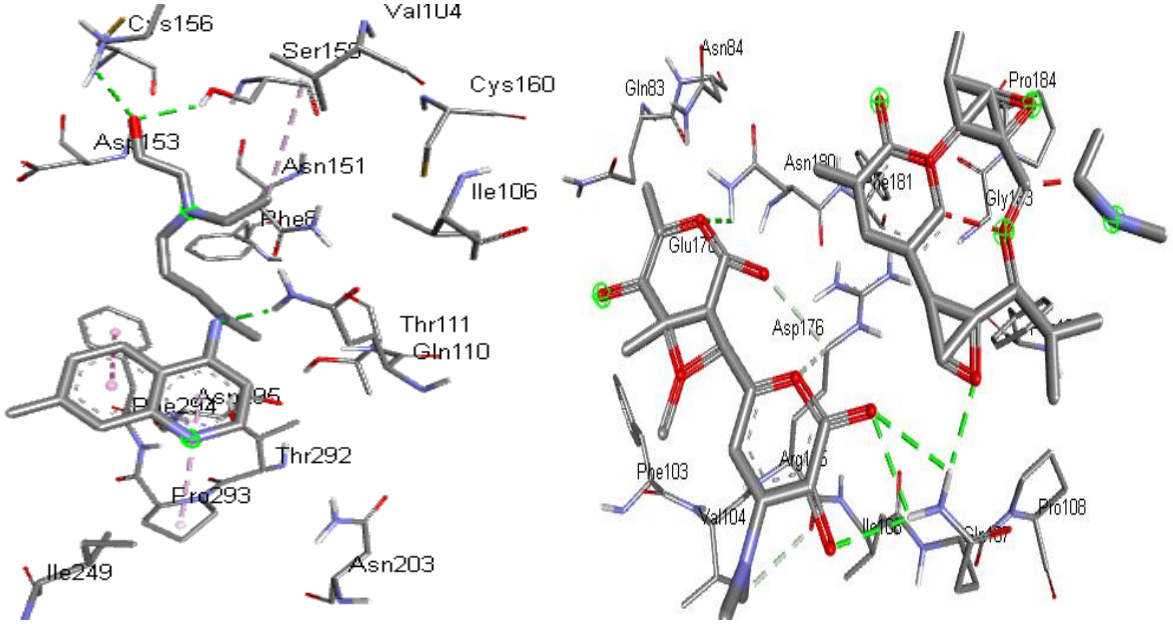
The binding energies of the reference drugs hydroxychloroquine, azithromycin, and remdesivir against SARS-CoV-2 M^{pro} were −5.9, −8.4, and −8.4 kcal/mol, respectively (Table 2). These drugs are effective against SARS-CoV-2 and are used to treat COVID-19 in many countries [43-45]. Therefore, these three medicines were used as reference molecules in the present study.

3.2. Docking scores of herbal compounds.

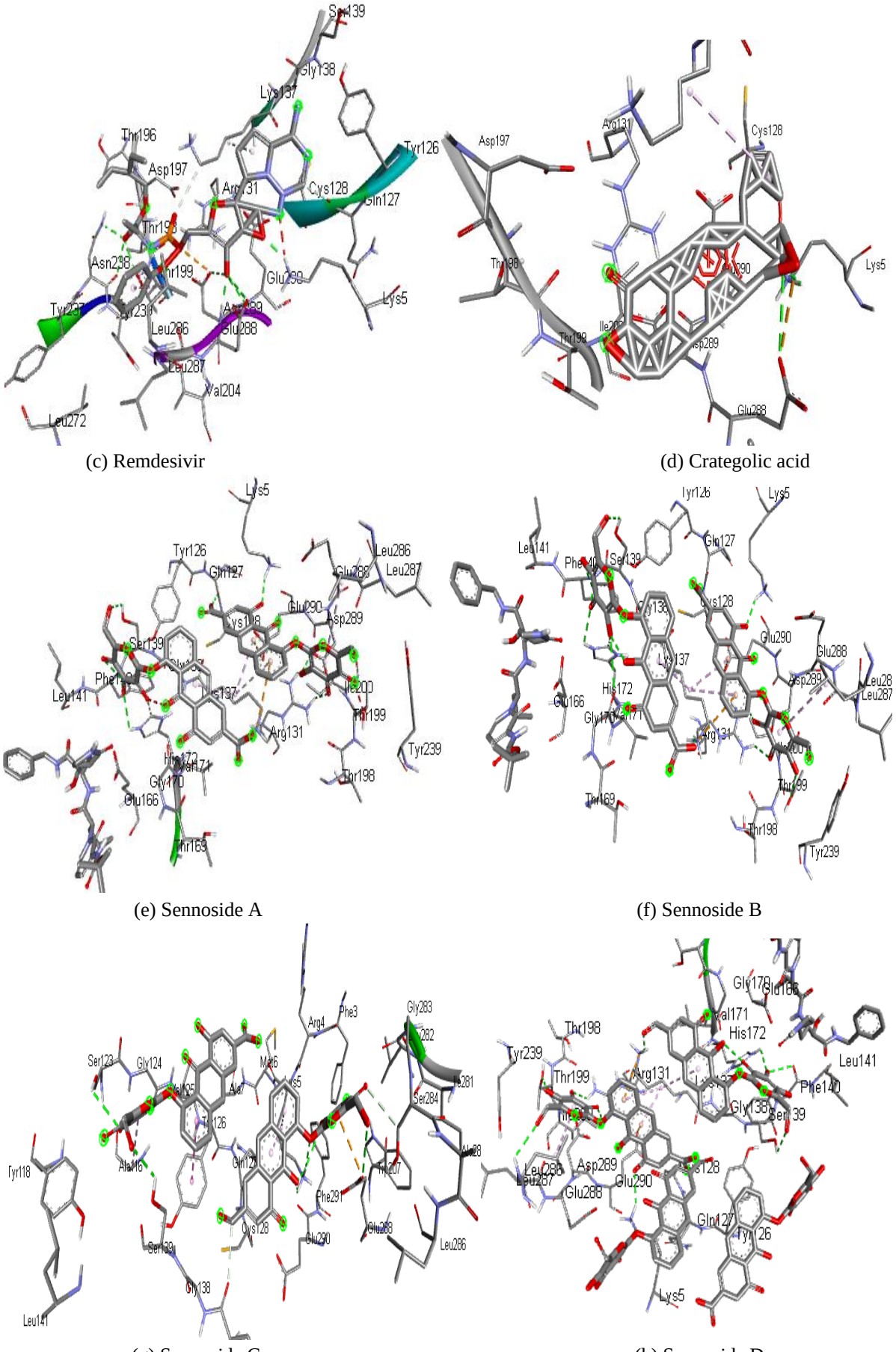
Among the 17 tested herbal compounds, 10 had a binding affinity of less than −6.0 kcal/mol, including nigellicine (−6.9), nigellidine (−6.8), sennoside A (−10.6), sennoside B (−10.8), sennoside C (−10.8), sennoside D (−10.6), beta-caryophyllene (−7.7), crategolic acid (−13.5), kaempferol (−7.0), and rhamnetin (−7.3). Crategolic acid had the lowest binding

affinity with M^{pro} protease (−13.5 kcal/mol), followed by sennoside B, sennoside C, sennoside A, and sennoside D (−10.8, −10.8, −10.6, and −10.6 kcal/mol, respectively). Beta-caryophyllene, rhamnetin, kaempferol, nigellicine, and nigellidine had a moderate binding affinity with SARS-CoV-2 M^{pro} protease (Table 2). The hydrogen bond length and hydrophobic residue involved in docking are presented in Table 2.

This study used three reference drugs and 17 herbal compounds for docking studies (Table 2). Of the 17 tested compounds, the five most prominent are discussed in detail. During hydroxychloroquine interaction with SARS-CoV-2 M^{pro} protease, three hydrogen bonds were formed (A:Lys102:Hz1 - :Unk0:O, A:Gln110:He22 - :Unk0:N, and A:Ser158:Hg - :Unk0:O) with a distance of 2.47821, 2.17638, and 2.25876 Å, respectively. Hydroxychloroquine also had hydrophobic interactions with Phe294, Val104, and Pro293 (Figure 2a). Azithromycin formed 10 hydrogen bonds (A:Gln107:He21 - :Unk0:O, A:Gln107:He21 - :Unk0:O, A:Gln107:He22 - :Unk0:O, A:Gln107:He22 - :Unk0:O, A:Asn180:Hd22 - :Unk0:O, :Unk0:O - A:Glu178:Oe1, A:Arg105:Cd - :Unk0:O, A:Arg105:Cd - :Unk0:O, :Unk0:C - :Unk0:O, and :Unk0:C - A:Arg105:O) with a distance of 2.35051, 2.83838, 2.80474, 2.74874, 2.78285, 3.39074, 3.59192, 3.22475, 2.64441, and 3.62061 Å, respectively. The hydrophobic interactions with Arg105 are shown in Figure 2b. Remdesivir formed 10 hydrogen bonds (A:Lys5:Hz2 - :Unk0:O, A:Thr199:Hg1 - :Unk0:O, A:Asn238:Hd22 - :Unk0:O, A:Asp289:Hn - :Unk0:O, A:Glu290:Hn - :Unk0:O, :Unk0:O - A:Glu288:Oe2, :Unk0:O - A:Asp289:Od, :Unk0:O - A:Asn238:O, A:Lys137:Ce - :Unk0:O, and :Unk0:C - A:Gln127:O), at a distance of 1.97095, 2.11076, 2.31365, 1.92092, 3.05913, 3.03252, 3.27642, 3.37354, 3.65697, and 3.55265 Å, respectively. The hydrophobic interactions with Lys137 and Leu286 are presented in Figure 2c. With more negative binding energy and more hydrogen bonds, azithromycin and remdesivir were found to be better agents than hydroxychloroquine molecules. Crategolic acid interacted with M^{pro} protease (PBD ID: 6Y2F) with a binding energy of −13.5 kcal/mol and three hydrogen bonds (A:Lys5:Hz2 - :Unl1:O, A:Lys5:Hz3 - :Unl1:O, and :Unl1:O - A:Glu288:Oe2) at a distance of 2.32865, 2.36503, and 3.15769 Å, respectively. The amino acid residue involved in the hydrophobic interactions was Lys13 (Figure 2d). Sennosides A, B, C, and D had binding energies of −10.6, −10.8, −10.8, and −10.6 kcal/mol, respectively, and more than 10 hydrogen bonds (Figure 2e, 2f, 2g, and 2h). Hydrogen bonds are essential for maintaining the structural stability of the protein-ligand complex.



(a) Hydroxychloroquine (b) Azithromycin



(c) Remdesivir (d) Crategolic acid

(e) Sennoside A (f) Sennoside B

(g) Sennoside C (h) Sennoside D

Figure 2. Interaction of reference molecule (**a**) Hydroxychloroquine; (**b**) Azithromycin; (**c**) Remdesivir; and herbal compounds (**d**) Crategolic acid; (**e**) Sennoside A; (**f**) Sennoside B; (**g**) Sennoside C; (**h**) Sennoside D with SARS-CoV-2 M^{pro}.

COVID-19 is spreading worldwide, with nearly 58,229,138 confirmed cases and 1,382,106 deaths as of the 23rd of November 2020 [5]. At present, there are no available drugs to combat COVID-19 with 100% inhibition of SARS-CoV-2. Accordingly, there is an urgent need to identify new compounds with anti-SARS-CoV-2 activity to treat COVID-19. Therefore, this study aimed to explore other herbs and herbal compounds commonly used in foods and as traditional medicines that may be effective against SARS-CoV-2. The mortality rate of COVID-19 appears to be lower in Asian countries than in European and American countries, which may be due to dietary habits, among other factors. Some Asian foods, including Indian foods, contain many herbs and herbal ingredients compared to American and European foods. Over the last decade, considerable efforts have been made to identify important medicinal herbs from traditional medicine and anti-SARS-CoV activity compounds. As per an FAO report, more than 25% of drugs currently present in the pharmacopeia are derived from plants or their products [46]. Many plants (herbs, shrubs, and trees) have antiviral activity, especially against HIV, H1N1, HSV-1, Zika virus, poliovirus, dengue virus, influenza virus, herpes simplex virus, hepatitis viruses, and coronaviruses [46], and their modes of action have been determined. Some important herbs used in traditional Chinese medicine for the treatment of SARS-CoV infection include *Armeniaca sibirica*, *Astragalus membranaceus*, *Atractylodes macrocephala*, *Dryopteris crassirhizoma*, *Ephedra sinica*, *Ephedrae herba*, *Forsythia suspense*, *Houttuynia cordata*, *Isatis indigotica*, *Lonicera japonica*, *Mentha haplocalyx*, *Pogostemon cablin*, *Rheum palmatum*, *Rhodiola rosea*, and *Scutellaria baicalensis* [6]. In addition to the whole herbs, their compounds, such as glycyrrhizin from *Radix glycyrrhizae* and baicalin from *S. baicalensis*, can also be used to inhibit coronavirus activity [6]. Divya *et al.* also identified more than 15 South Indian herbs traditionally used to treat various viral infections and may have the ability to combat SARS-CoV-2 [47]. Combining two phytonutrients, curcumin, glycyrrhizic acid, and vitamin C may have the ability to combat novel coronavirus infections [48].

Molecular docking studies conducted by Chikhale *et al.* showed that withanoside X and quercetin glucoside from Indian ginseng (*W. somnifera*) may have antiviral activities against SARS-CoV-2 and could be used in the treatment of COVID-19 [49]. Another molecular docking study by Ghosh *et al.* recommended eight polyphenols from green tea as potential inhibitors against SARS-CoV-2 M^{pro} [50]. Two potent herbal compounds from *Curcuma longa* L. have also been reported as SARS-CoV-2 M^{pro} inhibitors through *in silico* approaches [51]. By targeting the main protease (SARS-CoV-2 M^{pro}) and angiotensin-converting enzyme 2, 318 phytochemicals from 11 plants with antiviral activity were screened, and 10 compounds were found to be significant against the targets [52]. The compound tinosponone from the Indian medicinal plant *Tinospora cordifolia* (Guduchi) may also be a potent inhibitor of SARS-CoV-2 protease [53]. A previous *in silico* approach (AutoDock Vina) showed that epigallocatechin gallate, a principal constituent of *Camellia sinensis* (green tea), has the highest binding affinity with spike (S) protein of SARS-CoV-2 and may help to prevent COVID-19 [4]. Some flavonoids have antiviral properties that can inhibit the entry of SARS-CoV-2 into the host cells [54]. In the present study, the docking scores of crategolic acid and sennoside (A, B, C, and D) were better than those of the reference drugs hydroxychloroquine, azithromycin, and remdesivir. These results revealed that crategolic acid and sennoside (A, B, C, and D) bound effectively to the M^{pro} protease (PBD ID: 6Y2F) and hydrogen bonds, as well as hydrophobic interactions, were involved (Table 2 and Figure 2). These herbal compounds possess antioxidant, anti-inflammatory, antimicrobial, and antiparasitic properties [44-49]. Therefore,

these compounds may be used to control and treat COVID-19 and increase immunity to prevent this viral disease.

4. Conclusions

This molecular docking study assisted in determining the possible binding approaches of 20 ligands with the SARS-CoV-2 M^{pro} protease. Among the tested compounds, crategolic acid (the active ingredient of clove) had the highest binding affinity to this protease, likely due to it having the greatest number of hydrogen bonds, followed by sennoside A, B, C, and D (the active ingredients of Sana Makki). Therefore, including clove and Sana Makki as dietary supplements in the diet may decrease the incidence rate of COVID-19. These herbs and their potent antiviral compounds may be used to formulate new dietary supplements or be added to existing formulations against SARS-CoV-2. Many other traditional herbal medicines are currently used for the treatment of patients with COVID-19 [6]. However, *in vivo* and *in vitro* studies are required to examine and confirm the antiviral effects of these herbs (clove and Sana Makki) and their compounds (crategolic acid and sennoside) against SARS-CoV-2 and to discover effective drugs for the treatment and control of COVID-19.

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Conflicts of Interest

The authors declare no conflict of interest.

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