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ORIGINAL ARTICLE

Molecular Docking Study of Several Seconder Metabolites from Medicinal Plants as Potential Inhibitors of COVID-19 Main Protease

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ABSTRACT

Objectives: Coronaviruses (CoVs) cause infections that affect the respiratory tract, liver, central nervous, and the digestive systems in humans and animals. This study focused on the main protease (M^{pro}) in CoVs (PDB ID: 6LU7) that is used as a potential drug target to combat 2019-CoV. In this study, a total of 35 secondary metabolites from medical plants was selected and docked into the active site of 6LU7 by molecular docking studies to find a potential inhibitory compound that may be used to inhibit Coronavirus Disease-2019 (COVID-19) infection pathway.

Materials and Methods: The chemical structures of the ligands were obtained from the Drug Bank (https://www.drugbank.ca/). AutoDockTools (ADT ver. 1.5.6) was used for molecular docking studies. The docking results were evaluated using BIOVIA Discovery Studio Visualizer and PyMOL (ver. 2.3.3, Schrodinger, LLC).

Results: Pycnamine, tetrahydrocannabinol, oleuropein, quercetin, primulic acid, kaempferol, dicannabidiol, lobelin, colchicine, piperidine, medicagenic acid, and narcotine is found to be potential inhibitors of the COVID-19 M^{pro}. Among these compounds, pycnamine, which was evaluated against COVID-19 for the first time, showed a high affinity to the COVID-19 M^{pro} compared with other seconder metabolites and reference drugs.

Conclusion: Our results obtained from docking studies suggest that pycnamine should be examined *in vitro* to combat 2019-CoV. Moreover, pycnamine might be a promising lead compound for anti-CoV drugs.

Key words: COVID-19, molecular docking, pycnamine, seconder metabolites

INTRODUCTION

Coronaviruses (CoVs) cause disorders in both the respiratory tract and the digestive system in humans and animals.[1] During an epidemic in Wuhan, China at the end of 2019, the new CoV strain was identified and named 2019-nCoV. In a very short time, this newly emerging virus spread to almost all countries and the disease is officially named as Coronavirus Disease-2019 (COVID-19) by World Health Organization (WHO).[2] According to WHO's COVID-19 Weekly Epidemiological Update Report released on May 11, 2021, the number of confirmed cases reached 157,362,408 including 3,277,834 deaths in the world as of May 9, 2021.[3]

Currently, there are several vaccines for COVID-19, but no antiviral drugs are available for specific treatment of COVID-19. However, some antiviral drugs such as lopinavir, ritonavir, remdesivir, and nelfinavir have been using to prevent further complications and organ damage caused by COVID-19.[4] Among all these drugs, nelfinavir, which has been used in clinics, was found as the most potential inhibitor drug against COVID-19 main protease (M^{pro}) based on its docking score according to the docking studies conducted by Xu et al.[5] In docking studies, M^{pro} is used as a potential drug target to combat 2019-CoV.[6-8]

Secondary metabolites obtained from medicinal plants and their semi-synthetic derivatives have been widely used in new

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drug development. Therefore, the use of secondary metabolites purified from medicinal plants in drug development against severe acute respiratory syndrome (SARS)-CoV becomes important.[9] There are many studies reporting the antiviral effects of many compounds with alkaloid,[10-12] flavonoid,[13,14] monoterpene,[15-19] sesquiterpene lactone,[20,21] saponoside,[22,23] and aryl alkene[24,25] structures.

In this study, the potential innhibitory effects of alkaloids (atropine, caffeine, castanospermine, codeine, ephedroxane, hygrine, cuscohygrine, colchicine, lobeline, tussilagine, punicalagin, papaverine, pycnamine, piperidine, scopolamine, morphine, narcotine, pelletierine, ricinine), cannabinoids (cannabidiol and tetrahydrocannabinol), monoterpenes (citral A, thymol, oleuropein, and harpagoside), sesquiterpene lactone, *e.g.* artemisinin, saponins (primulic acid and medicagenic acid), aryl alkene (aromatic ketone), *e.g.* gingerol, and flavonoids (quercetin and kaempferol) were investigated on 2019-CoV Pro *via* molecular docking studies. We hope that the findings of this study will contribute to drug research to combat COVID-19 and direct the researchers working in this field to further designs.

MATERIALS AND METHODS

Experimental in silico part

The 2019-CoV M^{pro} (PDB ID: 6LU7) structure was obtained from The Protein Data Bank (PDB, https://www.rcsb.org/). The pdb file of the 6LU7 protein was prepared using chain A and transferred to AutoDockTools (ADT ver. 1.5.6). Water molecules of the structures were removed and only polar hydrogen and Kollman charges were added to the proteins. Finally, the pdbqt files of the proteins were saved.[26]

Chemical structures of the ligands were obtained from the Drug Bank (https://www.drugbank.ca/). The ligands unavailable in the Drug Bank were drawn in ChemDraw (Professional, version 19.0.1.28), passed to ChemDraw 3D (professional, version 19.0.1.28) and minimized. Torsion of the ligands was examined and then the files of the ligands were saved as pdbqt format by AutodockTools (ADT ver. 1.5.6).

The active site of the 6LU7 was defined using BIOVIA Discovery Studio Visualizer (v20.1.0.19295). AutoDockTools (ADT ver. 1.5.6) was used for molecular docking studies. Lamarckian genetic algorithm with local search was used as a search engine, with 10 runs. The active site of the protein was defined by a grid box of 60 x 60 x 60 points. Ten conformers of the ligands were considered to evaluate the docking results. Finally, the conformer with the lowest binding free energy was evaluated by BIOVIA Discovery Studio Visualizer and PyMOL (ver. 2.3.3, Schrodinger, LLC).[26]

Statistical analysis

No statistical analysis was used in this study.

RESULTS

CoVs cause infections that affect the respiratory tract, liver, central nervous and the digestive systems in humans and animals.[27] This study focused on the M^{pro} in CoVs (PDB ID: 6LU7) that is used as a potential drug target to combat 2019-CoV. 6LU7 has been structured in PDB and has been publicly available since early February, 2020. To date, this M^{pro} (6LU7) has been studied by different groups to find potential inhibitors that can stop this enzyme activity and, thus, the replication of CoVs.[8,27,28]

Nelfinavir, lopinavir, indinavir, and ritonaviprotease inhibitory drugs, of which ritonavir and lopinavir is proposed for treating SARS and MERS.[29] In an *in vitro* study by Yamamoto et al.[29], nelfinavir was reported to strongly inhibit the replication of SARS-CoV in Vero E6 cells.[30] However, in an *in silico* study by Xu et al.[5], nelfinavir was identified as the most potent inhibitor against COVID-19 with a binding free energy score. In our study, nelfinavir, lopinavir, indinavir, and ritonavir were used as standard drugs for comparison.

In this study, 35 secondary metabolites from medical plants were selected and docked into the active site of 6LU7. Docking studies were performed by AutoDockTools (ADT ver. 1.5.6). Table 1 shows the binding free energy scores of all selected molecules. The native ligand for 6LU7 is *n*-[(5-methylisoxazol-3-yl) carbonyl]alanyl-l-valyl-*n*~1~-(1r,2z)-4-(benzyloxy)-4-oxo-1-[[(3r)-2-oxopyrrolidin-3-yl] methyl]but-2-enyl)-l-leucinamide. According to the results presented in Table 1, the binding free energy scores of the compounds were between -11.30 kcal/mol and -4.13 kcal/mol. We investigated pycnamine, tetrahydrocannabinol, oleuropein, quercetin, primulic acid, kaempferol, cannabidiol, lobeline, colchicine, piperidine, medicagenic acid, and narcotine as potential inhibitors of the COVID-19 M^{pro} because to the binding free energy scores of -11.30, -9.10, -9.06, -8.94, -8.94, -8.70, -8.52, -8.30, -8.28, -7.74, -7.71, and -7.60 kcal/mol, respectively.

Analysis of docking results and interactions with six of these compounds are presented in Tables 2 and 3. Table 2 shows the analysis of molecular docking results (binding energy/Gibbs Energy, ligand efficiency, inhibition constant, intermolecular energy, and Van der Waals-H Bond desolvation energy) for the compounds with binding energies less than -7.60 kcal/mol, which is similar to the binding free energy of ritonavir.

Table 3 shows 2D and 3D visualizations of interactions between 6LU7 and the compounds presented in Table 2. According to Table 3, which shows interactions between compounds and 6LU7, nelfinavir forms H-bonds with the amino acids Gly143, His163, Thr190, Gln189 of 6LU7. Lopinavir forms H-bonds with the amino acids His41, Cys145, Gln189, and Glu166. Indinavir realizes H-bonds with the amino acid, *i.e.* Asn142, while ritonavir, the latest standard drug, forms H-bonds with the amino acids His164 and Glu166. When the interactions of the seconder metabolites in Table 3 are evaluated, the following results are seen: pycnamine forms H-bond with the amino acid, *i.e.* Glu166. Tetracannabinol forms H-bonds with the amino acids Glu166, Cys145. Oleuropein realizes H-bonds with the 6LU7 amino acids, *e.g.* His41, Thr26, Gly143, Glu166, and Thr190. Quercetin realizes H-bonds with 6LU7 amino acids, *e.g.* Glu166, Thr190, and His164.

Table 1. Binding free energy scores of the compounds

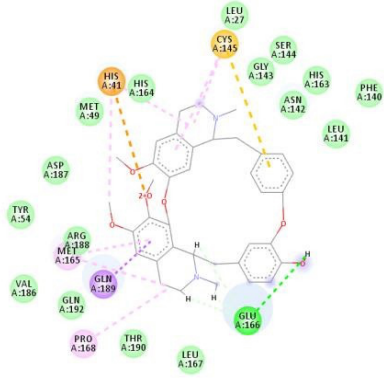
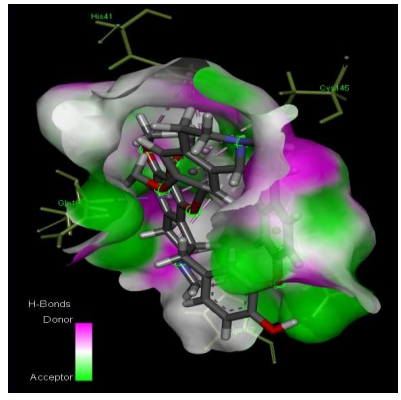
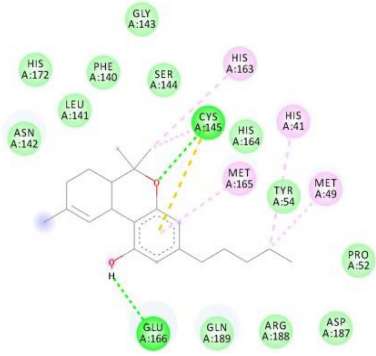
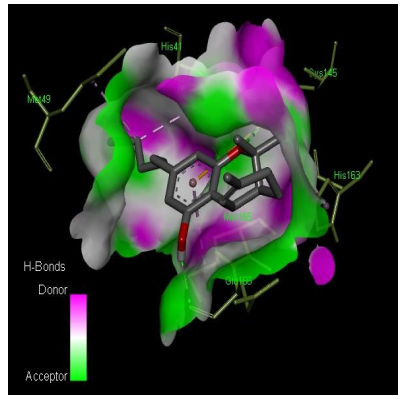
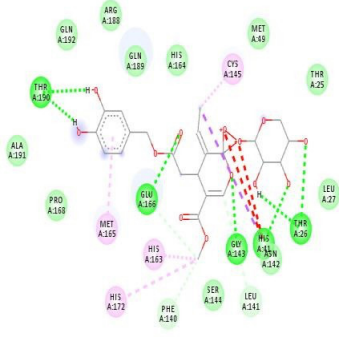
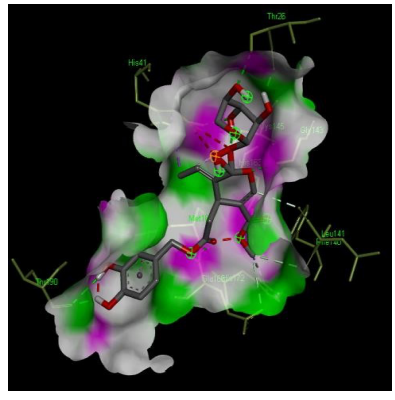
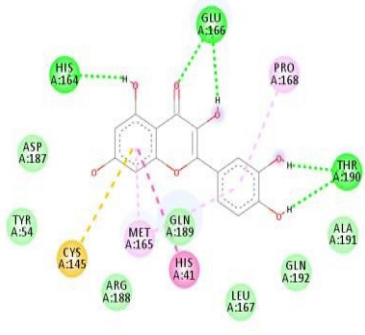
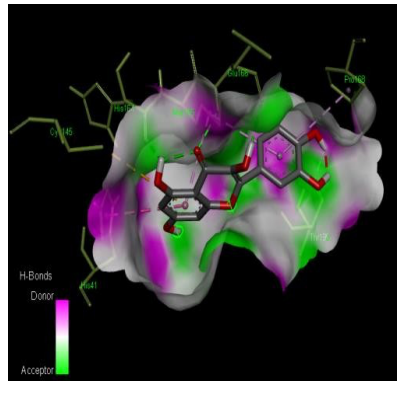
Compounds	Binding free energy (kcal/mol)	Compounds	Binding free energy (kcal/mol)
Pycnamine	-11.30	Harpagoside	-6.82
Tetrahydrocannabinol	-9.10	Atropine	-6.70
Oleuropein	-9.06	Punicalagin	-6.63
Quercetin	-8.94	Couscohygrin	-6.41
Primulic acid	-8.94	Gingerol	-6.27
Kaempferol	-8.70	Ephedroxan	-5.96
Cannabidiol	-8.52	Tussulagin	-5.91
Lobeline	-8.30	Castanospermine	-5.90
Colchicine	-8.28	Pelletierin	-5.30
Piperidine	-7.74	Citral-A	-4.98
Medicagenic acid	-7.71	Thymol	-4.95
Narcotine	-7.60	Caffeine	-4.64
Butylscopolamine	-7.42	Hygrin	-4.55
Hyoscyamine	-7.39	Ricinin	-4.51
Reticuline	-7.29	Ivermektin	-4.13
Papaverine	-7.16	Native ligand	-7.96
Codeine	-7.07	Nelfinavir*	-10.70
Artemisinin	-7.03	Lopinavir*	-8.95
Scopolamine	-6.97	Indinavir*	-8.73
Morphine	-6.88	Ritonavir*	-7.81

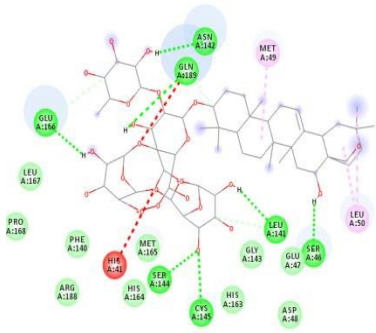
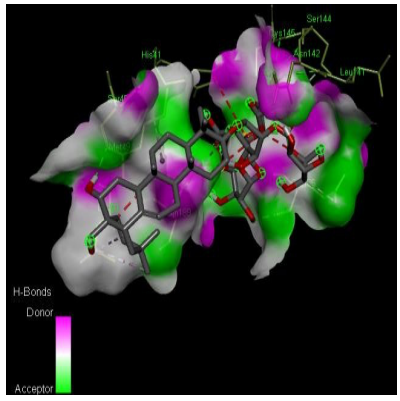
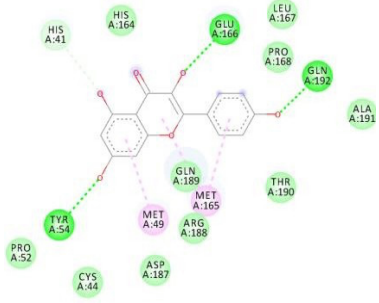
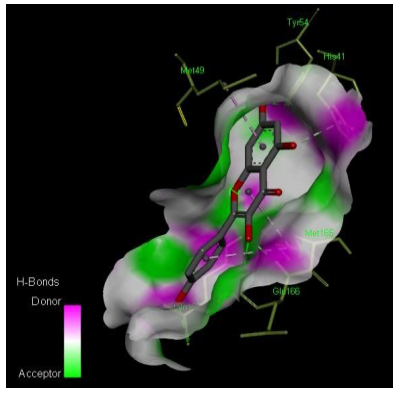
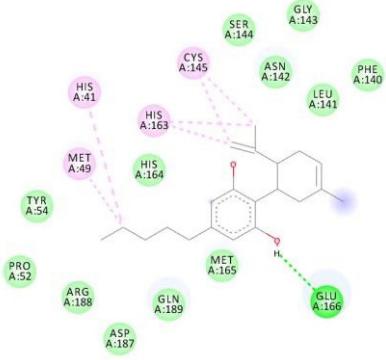
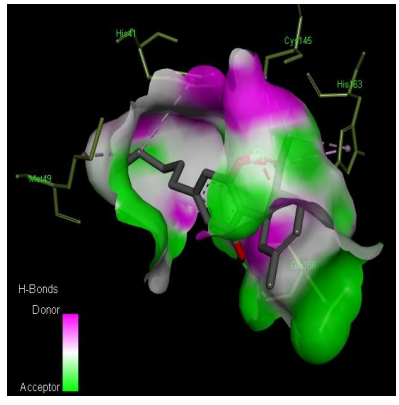
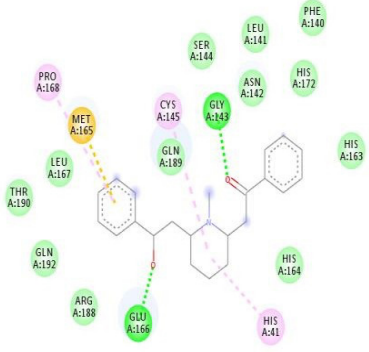
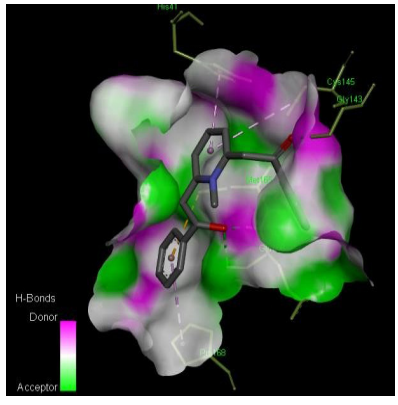
*Nelfinavir, lopinavir, indinavir, and ritonavir are HIV protease inhibitor drugs

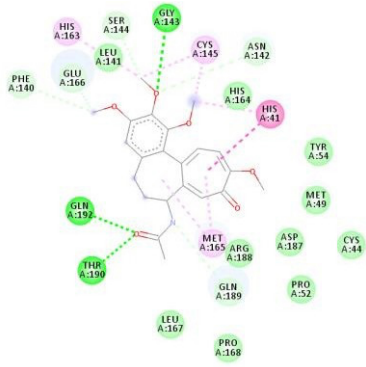
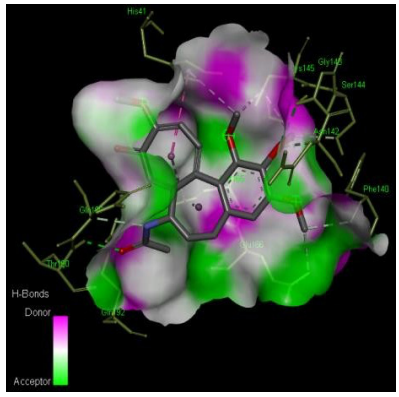
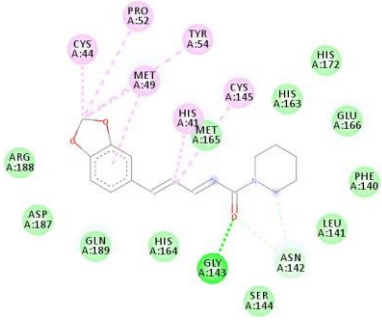
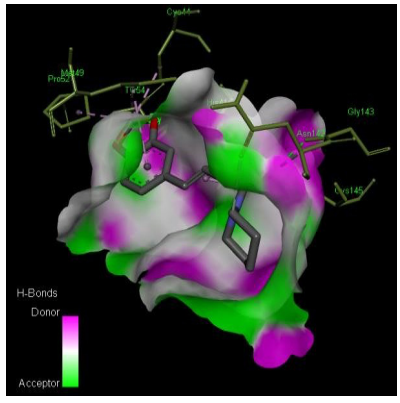
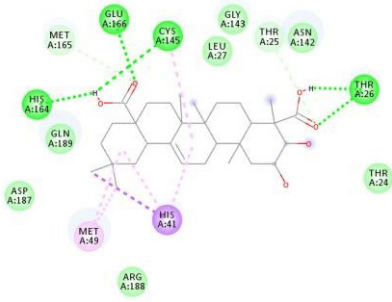
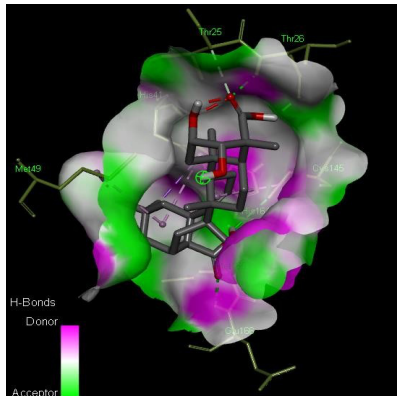
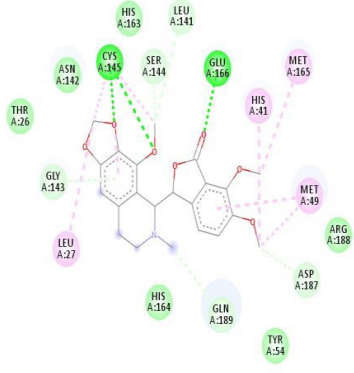
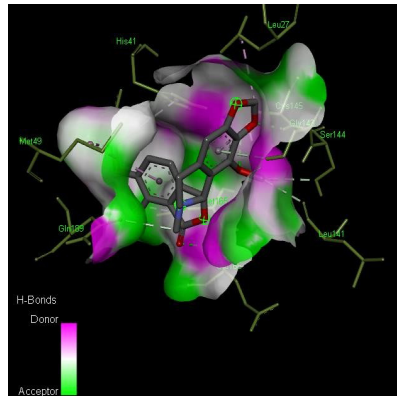
Table 2. Molecular docking results analysis of compounds with low binding energy scores and the drugs used in clinic

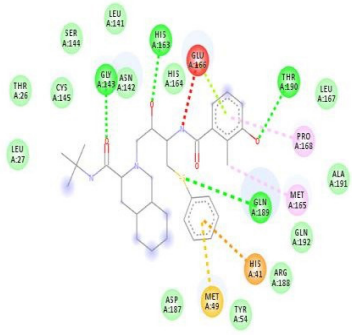
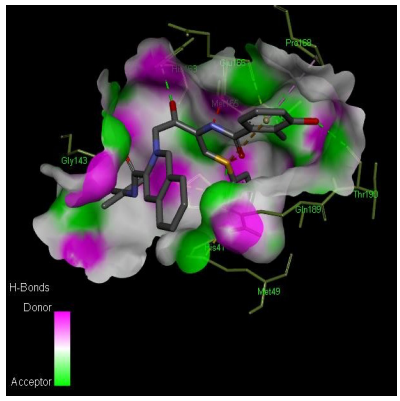
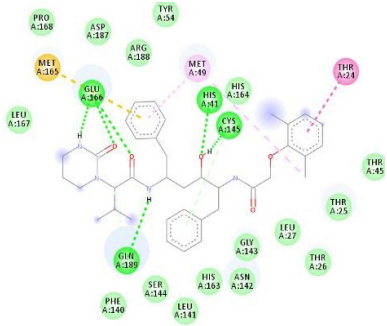
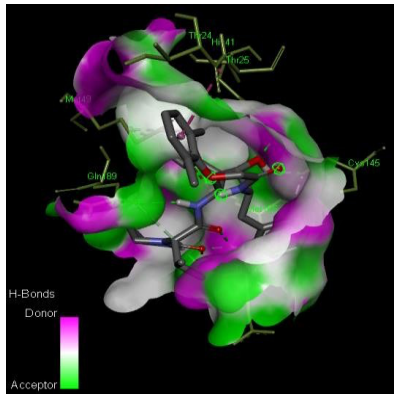
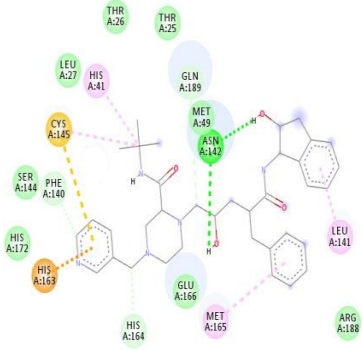
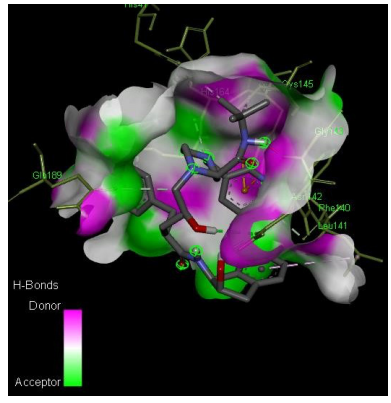
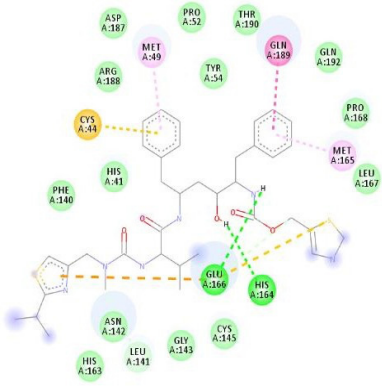
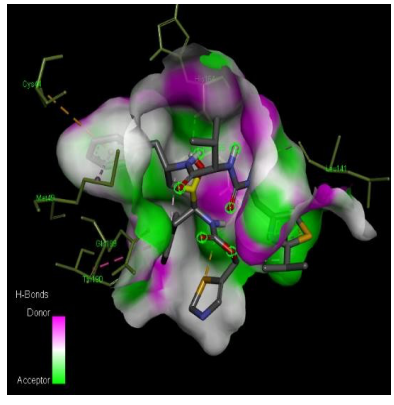
Compounds	Binding energy (kcal/mol)	Ligand efficiency	Inhibition constant	Intermolecular energy	Van der Waals-H Bond desolvation energy
Pycnamine	-11.30	-0.25	5.21 nM	-12.20	-11.85
Tetrahydrocannabinol	-9.10	-0.40	214.2 nM	-10.59	-10.58
Oleuropein	-9.06	-0.25	229.87 nM	-13.23	-12.96
Quercetin	-8.94	-0.41	277.84 nM	-9.24	-9.13
Primulic acid	-8.94	-0.12	279.96 nM	-14.01	-13.70
Kaempferol	-8.70	-0.41	422.9 nM	-8.99	-8.88
Cannabidiol	-8.52	-0.37	570.21 nM	-10.01	-9.97
Lobeline	-8.30	-0.33	821.52 nM	-10.09	-9.98
Colchicine	-8.28	-0.29	-856.99 nM	-9.77	-9.66
Piperidine	-7.74	-0.37	2.11 μM	-8.64	-8.58
Medicagenic acid	-7.71	-0.21	2.21 μM	-9.50	-9.66
Narcotine	-7.60	-0.25	2.69 μM	-8.79	-8.42
Nelfinavir	-10.70	-0.27	14.45 nM	-12.78	-12.72
Lopinavir	-8.95	-0.19	275.32 nM	-13.72	-13.55
Indinavir	-8.73	-0.19	400.34 nM	-12.90	-12.33
Ritonavir	-7.81	-0.16	1.9 μM	-13.47	-13.39

Table 3. Two-dimensional and three-dimesnsional interaction diagrams for several compounds

No	Compound	2D representation of compounds-6LU7 interactions	3D representation of compounds-6LU7 interactions
1	Pycnamine		
2	Tetrahydrocannabinol		
3	Oleuropein		
4	Quercetin		

5	Primulic acid		
6	Kaempferol		
7	Cannabidiol		
8	Lobeline		

9	Colchicine		
10	Piperidine		
11	Medicagenic acid		
12	Narcotine		

13	Nelfinavir		
14	Lopinavir		
15	Indinavir		
16	Ritonavir		

Primulic acid forms H-bonds with the amino acids, *e.g.* Ser46, Ser144, Glu166, Gln189, Asn142, Cys145, and Leu141. Kaempferol forms H-bonds with the 6LU7 amino acids, *e.g.* Tyr54, Glu166, and Gln192. Dicannabidiol realizes H-bond with the 6LU7 amino acid, *i.e.* Glu166. Lobelin realizes H-bonds with the amino acids, *e.g.* Gly143 and Glu166. Colchicine realizes H-bonds with the amino acids, *e.g.* Gly143, Thr190, Gln189, and Gln192. Piperidine forms H-bonds with the amino acids, *e.g.* Gly143 and Asn142. Medicagenic acid forms H- bonds with the amino acids, *e.g.* Thr26, Cys145, Glu166, and His164. Narcotine realizes H- bonds with the 6LU7 amino acids, *e.g.* Cys145 and Glu169. The results presented in Table 3 suggest that the M^{pro} Glu166 plays a crucial role in drug interactions. Besides, the other amino acids, *e.g.* Asn142, Gln189, Cys145, and Thr26 are also predicted to play roles in drug interactions, as reported in previous studies.[8,27]

According to the results in Tables 1 and 2, the most impressive compound of our study is pycnamine with a score of -11.30 kcal/mol, which is higher than that of nelfinavir. When the results in Table 2 are evaluated, it is seen that pycnamine has a predicted inhibition constant value (5.21 nM) approximately 3 times lower than that of nelfinavir (14.45 nM). According to pycnamine-6LU7 complex presented in Table 3, hydroxy moiety of pycnamine forms a hydrogen bond with the side chain of Glu166. Additionally, pycnamine forms *π*-cation, *π*-sulfur, *π*-sigma, and several hydrophobic interactions with the active site of 6LU7, as shown in Table 3.

DISCUSSION

Pycnamine is an alkaloid found in some species of Menispermaceae (*Triclisia patens* Oliv., *T. dictyophylla* Diels, *Pycnarrhena manillensis* Vidal, *P. ozantha* Diels) and Ranunculaceae families (*Thalictrum cultratum* Wall., *Isopyrum thalictroides* L.).[31-36] Pycnamine was reported to be a potential antimalarial, antiplasmodial, antiamoebic, and antimicrobial in previous studies.[36-40] It was evaluated against COVID-19 for the first time in this study.

Tetrahydrocannabinol, which has the second lowest binding free energy score (-9.10 kcal/mol) in this study, purified from *Cannabis sativa* L. was reported to inhibit macrophage extrinsic antiherpesvirus activity.[41]

Oleuropein, a secoiridoid monoterpene and the main component of *Olea europaea* L., is a potential inhibitor of the COVID-19 M^{pro} due to it is binding free energy score of -9.06 kcal/mol. It has antiviral activity against mononucleosis herpes, hepatitis, rota, bovine, parvo, HIV, leukemia, respiratory syncytial, parainfluenza-3, and salmonid rhabdoviruses.[42-46] In hepatitis B virus infected ducks, oleuropein reduced the virus entering the bloodstream.[47]

Quercetin, a flavonoid, is found abundantly in fruits and vegetables including onions, broccoli, buckwheat, peppers, *Brassica* species, apples, grapes, berries, tea, and wine as well as many nuts, seeds, barks, flowers, leaves, and spices.[48]

Quercetin also demonstrated a dose-dependent antiviral activity against poliovirus type 1, *Herpes simplex* virus (HSV-1, HSV-2), and respiratory syncytial virus, influenza virus strain, parainfluenza virus type-3, sindbis virus, rhinovirus, echovirus (types-7, -11, -12, and -19), coxsackievirus (A21 and B1), poliovirus (type-1 Sabin) and grouper iridovirus in cell cultures.[49-53] Early *in vivo* studies showed that oral treatment with quercetin-protected mice from lethal Mengo virus.[54] In mice infected with rhinovirus, quercetin treatment decreased viral replication and attenuates virus- induced airway cholinergic hyperresponsiveness.[55]

Kaempferol is another flavonoid derivative found in most edible plants such as tea, fruits, and vegetables consisting of *Allium cepa* L., *Camellia sinensis* (L.) Kuntze, *Citrus paradisi* Macfad., *Fragaria vesca* L., *Lactuca sativa* L., and in medicinal plants such as *Tilia tomentosa* Moench., *Aloe vera* L., *Crocus sativus* L., *Vitis vinifera* L., *Ginkgo biloba* L., *Hypericum perforatum* L., *Phyllanthus acidus* L., *Ribes nigrum* L., *Rosmarinus officinalis* L., *Hippophae rhamnoides* L., and *Sambucus nigra* L.[56] Antiviral activity of kaempferol on the influenza viruses (H1N1 and H9N2), HIV-1, flavivirus, two RNA viruses (murine norovirus and feline calicivirus), and human cytomegalovirus were mentioned.[14,48,51,57,58]

Primulic acid is a saponin found in some species of Primulaceae [*Primula officinalis* L., *P. elatior* (L.) Hill, *P. veris* L.] and Poaceae (*Panicum repens* L.),[59-63] and was reported to have antiviral activity by Helal and Melzig.[58]

Finally, cannabidiol, the potential inhibitor of COVID-19 M^{pro}, purified from the *C. sativa* L.,[64,65] and was reported to show high efficacy against viral hepatitis in previous studies.[64]

CONCLUSION

At currently, there is no antiviral drug for specific treatment of COVID-19, which is still a threat to global health. M^{pro} was used as a potential drug target to combat 2019-CoV. In this study, we evaluated several secondary metabolites obtained from medicinal plants against COVID-19 M^{pro} by molecular docking studies to identify a potential inhibitory compound that may be used to inhibit COVID-19 infection pathway. According to the results, pycnamine, tetrahydrocannabinol, oleuropein, quercetin, primulic acid, kaempferol, cannabidiol, lobeline, colchicine, piperidine, medicagenic acid, and narcotine are found to be potential inhibitors of COVID-19 M^{pro}. Among these compounds, pycnamine, which was evaluated against COVID-19 for the first time, showed high affinity to COVID-19 M^{pro} compared with other seconder metabolites and reference drugs. According to the results in this study, pycnamine has a binding free energy score of -11.30 kcal/mol, which is higher than nelfinavir used in clinics as the most potent inhibitor drug against COVID-19 M^{pro}. As a conclusion, this study has clearly shown that pycnamine may strongly inhibit COVID-19 M^{pro}. Our results obtained from the docking studies suggest that pycnamine should be examined *in vitro* to combat 2019-CoV. Moreover, pycnamine might be a promising lead compound for anti-CoV drugs.

Ethics

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.G., S.B., Design: S.B., Data Collection or Processing: S.B., S.G., Z.G., Analysis or Interpretation: S.B., S.G., Literature Search: S.B., S.G., Z.G., Writing: S.B., S.G., Z.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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