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

COVID-19 Ana Proteazının Potansiyel İnhibitörü OlarakTıbbi Bitkilerden Elde Edilen Bazı Sekonder Metabolitlerin Moleküler Yerleştirme Çalışması

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ABSTRACT

Objectives: Coronaviruses (CoVs) are known to 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 (Mpro) in CoVs (PDB ID: 6LU7) that is used as a potential drug target to combat 2019-CoV. In the present study, total of 35 secondary metabolites from medical plants were selected and docked into the active site of 6LU7 by molecular docking studies to find out a potential inhibitory compound that may be used to inhibit the 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 was evaluated by using BIOVIA Dıscovery Studio Visualizer and and PyMOL (ver. 2.3.3, Schrodinger, LLC).
Results: Pycnamine, tetracannabinol, oleuropein, quercetin, primulic acid, kaempferol, dicannabidiol, lobelin, colchicine, piperidine, medicagenic acid, and narcotine are 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 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.
Keywords: COVID-19, molecolar docking, pycnamine, seconder metabolties.

ÖZ

Amaç: Koronavirüsün (CoV's) insanlarda ve hayvanlarda solunum yolları, karaciğer, merkezi sinir ve sindirim sistemlerini etkileyen enfeksiyonlara neden olduğu bilinmektedir. Bu çalışma, 2019-CoV ile mücadele için potansiyel bir ilaç hedefi olarak kullanılan koronavirüslerde (PDB ID: 6LU7) ana proteaz (Mpro) üzerine odaklanmıştır. Bu çalışmada, COVID-19'un neden olduğu enfeksiyonda kullanılabilecek potansiyel bir inhibitör bileşik bulmak için tıbbi bitkilerden toplam 35 sekonder metabolit seçilmiş ve bu bileşiklerin moleküler yerleştirme çalışmaları ile 6LU7'nin aktif bölgesine kenetlenmesi incelenmiştir.
Gereç ve Yöntemler: Ligandların kimyasal yapıları Drug Bank adlı elektronik bilgi bankasından temin edilmiştir (https://www.drugbank.ca/). Moleküler yerleştirme çalışmaları için AutoDockTools (ADT ver.1.5.6) programı kullanılmıştır. Moleküler yerleştirme sonuçları, BIOVIA Discovery Studio Visualizer ve PyMOL (ver. 2.3.3, Schrödinger, LLC) programları kullanılarak değerlendirilmiştir.
Bulgular: Piknamin, tetrakannabinol, oleuropein, kersetin, primulik asit, kemferol, dikannabidiol, lobelin, kolşisin, piperidin, medikagenik asit ve narkotin, COVID-19 M^{pro}'nun potansiyel inhibitörleri olarak bulunmuştur. Bu bileşikler arasında COVID-19'a karşı ilk kez değerlendirilen piknamin, diğer sekonder metabolitler ve referans ilaçlarla karşılaştırıldığında COVID-19 M^{pro}'ya yüksek afinite göstermiştir.
Sonuç: Moleküler yerleştirme çalışmalarından elde ettiğimiz sonuçlarımız, 2019-CoV ile mücadele için piknaminin *in vitro* olarak değerlendirilmesi gerektiğini göstermektedir. Buna ek olarak bu çalışma, piknaminin anti-CoV ilaçları için umut verici bir öncü bileşik olabileceğini gösterilmiştir.
Anahtar kelimeler: COVID-19, moleküler yerleştirme, piknamin, sekonder metabolitler

INTRODUCTION

Coronaviruses (CoVs) are known to 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 the 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, nelfinavir have been using to prevent further complications and organ damage caused by COVID-19.[4] Among all these drugs, nelfinavir, which has been using in clinics, was found as the most potential inhibitor drug against COVID-19 main protease based on its docking score according to the docking studies performed by Xu et al.[5] In docking studies, the main protease (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 drug development. Therefore, the use of secondary metabolites purified from medicinal plants in drug development against 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 inhibitor effects of alkaloids (atropine, caffeine, castanospermine, codeine, ephedroxan, hygrin, couscohygrin, colchicine, lobelin, tussulagin, punicalagin papaverine, pycnamine, piperidine, scopolamine, morphine, narcotine, pelletierin, ricinin, dicannabidiol and tetracannabinol), monoterpenes (citral A, thymol, oleuropein and harpagoside), sesquiterpene lactone (artemisinin), saponins (primulic acid and medicagenic acid), aryl alkene (aromatic ketone) (gingerol), and flavonoids (quercetin and kaempferol) structures against 2019-CoV M^{pro} were invastigated via molecular docking studies. We hope that the findings of this study will contribute to drug researches to combat COVID-19 and direct the researchers working in this field for further designs.

MATERIALS AND METHODS

Experımental *in silico* Part

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 to the proteins only polar hydrogen and Kollman charges were added. Finally, the pdbqt files of the proteins were saved.[26]

The chemical structures of the ligands were obtained from the Drug Bank (https://www.drugbank.ca/). The ligands not available 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. Torsions of the ligands were 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 by using BIOVIA Dıscovery Studio Visualizer (v20.1.0.19295). AutoDockTools (ADT ver.1.5.6) was used for molecular docking studies. Lamarckian genetic algorithm with local search (GALS) was used as a search engine, with a total of 10 runs. The active site of 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 and PyMOL (ver. 2.3.3, Schrodinger, LLC).[26]

RESULTS

Coronaviruses (CoVs) are known to cause infections that affect the respiratory tract, liver, central nervous and the digestive systems in humans and animals (27). 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. 6LU7 has been structured in PDB and has been publicly available since early February 2020. To date, this main protease (6LU7) has been studied by different groups to find inhibitors that can stop this enzyme activity and thus the replication of the CoVs.[8,27,28]

Nelfinavir, lopinavir, indinavir, and ritonaviprotease inhibitor drugs, of which ritonavir and lopinavir are proposed for the treatment of SARS and MERS.[29] In an *in vitro* study by Yamomoto et al. (2004), Nelfinavir was reported to strongly inhibit the replication of SARS-CoV in Vero E6 cells.[30] On the other hand, in an *in silico* study by Xu et al. (2020), nelfinavir was identified as the most potent inhibitor against COVID- 19 with its binding free energy score.[5] In our study, nelfinavir, lopinavir, indinavir, and ritonavir were used as standard drugs for comparison.

In the present study, a total of 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, tetracannabinol, oleuropein, quercetin, primulic acid, kaempferol, dicannabidiol, lobelin, colchicine, piperidine, medicagenic acid, and narcotine as potential inhibitors of the COVID-19 M^{pro} due 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.

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
Tetracannabinol	-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
Dicannabidiol	-8.52	Tussulagin	-5.91
Lobelin	-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
Reticulin	-7.29	İvermektin	-4.13
Papaverine	-7.16	Native Ligand	-7.96
Codeine	-7.07	Nelfinavir*	-10.70
Artemisinin	-7.03	Lopinavir*	-8.95
Scopolamine	-6.97	İndinavir*	-8.73
Morphine	-6.88	Ritonavir*	-7.81

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

Docking results analyzes and interactions with 6LU7 of these compounds are presented in Table 2 and Table 3. Table 2 shows the analysis of molecular docking results (binding energy/Gibbs Energy, ligand efficiency, inhibition constant, intermolecular energy, and van der Waals (VDW)-H Bond desolvation energy) for the compounds with binding energy less than -7.60 kcal/mol which is similar to binding free energy of ritonavir.

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

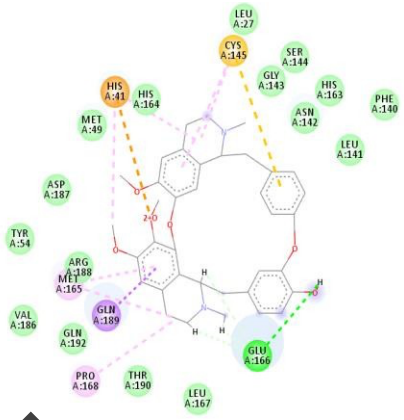
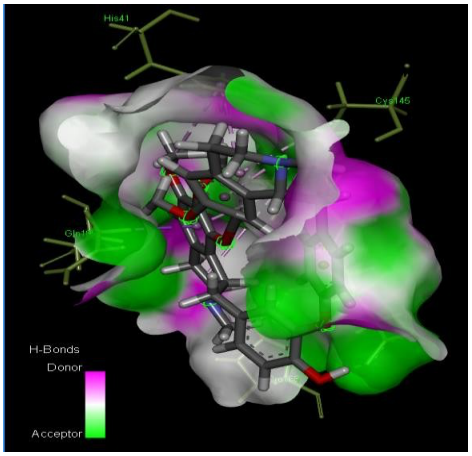
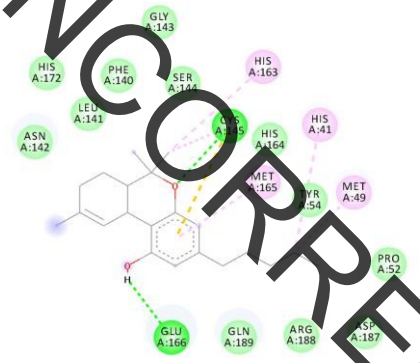
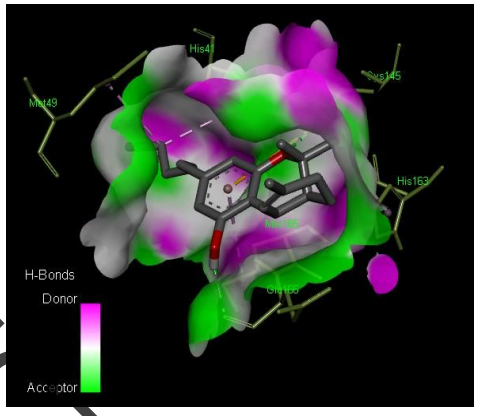
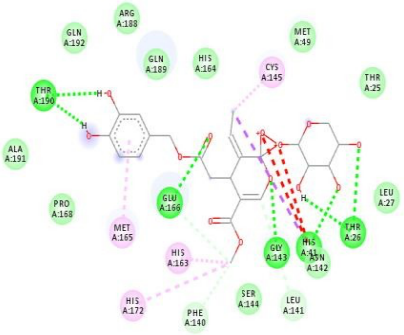
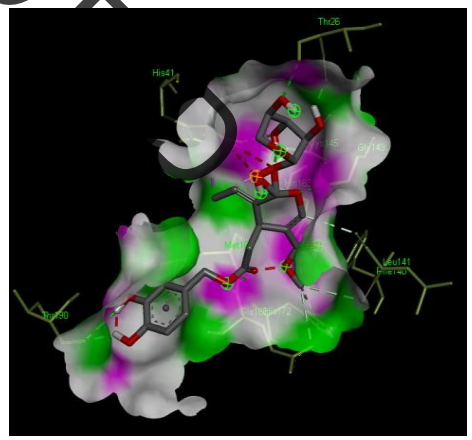
Compounds	Binding energy (kcal/mol)	Ligand Efficiency	Inhibition Constant	Intermolecular Energy	VDW-H Bond Desolvation Energy
Pycnamine	-11.30	-0.25	5.21 nM	-12.20	-11.85
Tetracannabinol	-9.10	-0.40	214.2 nM	-10.59	-10.58

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
Dicannabidiol	-8.52	-0.37	570.21 nM	-10.01	-9.97
Lobelin	-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

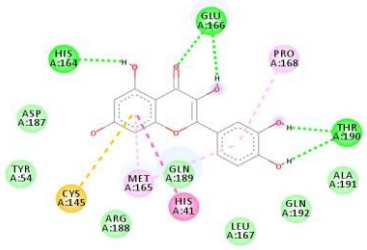
Table 3 shows 2D and 3D visualization of interactions between 6LU7 and the compounds presented in Table 2. According to Table 3, which shows interactions between compounds and 6LU7, nelfinavir forms H-bonds with the amino acids Gly143, His163, Thr190, Gln189 of 6LU7. Lopinavir forms H-bonds with the amino acids His41, Cys145, Gln189, and Glu166. Indinavir realizes H-bonds with the amino acid Asn142 while ritonavir, the last 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 Glu166. Tetracannabinol forms H-bonds with the amino acids Glu166, Cys145. Oleuropein realizes H-bonds with the 6LU7 amino acids His41, Thr26, Gly143, Glu166, Thr190. Quercetin realizes H-bonds with the 6LU7 amino acids Glu166, Thr190, His164.

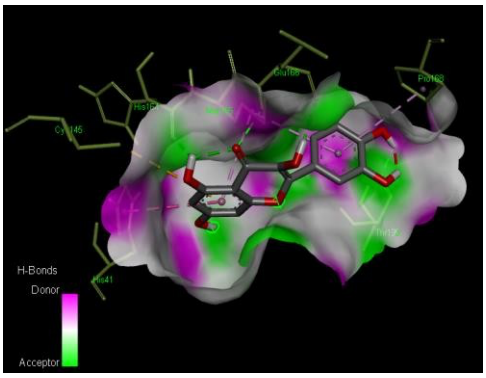
Primulic acid forms H-bonds with the amino acids Ser46, Ser144, Glu166, Gln189, Asn142, Cys145, Leu141. Kaempferol forms H-bonds with the 6LU7 amino acids Tyr54, Glu166, Gln192. Dicannabidiol realizes H-bond with the 6LU7 amino acid Glu166. Lobelin realizes H-bonds with the amino acids Gly143 and Glu166. Colchicine realizes H-bonds with the amino acids Gly143, Thr190, Gln189, Gln192. Piperidine forms H-bonds with the amino acids Gly143 and Asn142. Medicagenic acid forms H- bonds with the amino acids Thr26, Cys145, Glu166, His164. Narcotine realizes H- bonds with the 6LU7 amino acids Cys145 and Glu169. The results presented in Table 3 suggest that the M^{pro} amino acid Glu166 plays a crucial role in drug interactions. Besides, the other amino acids Asn142, Gln189, Cys145, and Thr26 are also predicted to play roles in drug interactions, as reported previous studies.[8,27] According to the results in Table 1 and Table 2, the most impressive compound of our study is pycnamine with a score of -11.30 kcal/mol which is higher than 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 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 the amino acid Glu166. In addition, pycnamine forms π-cation, π-sulfur, π-sigma and several hydrophobic interactions with the active site of 6LU7 as shown in Table 3.

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

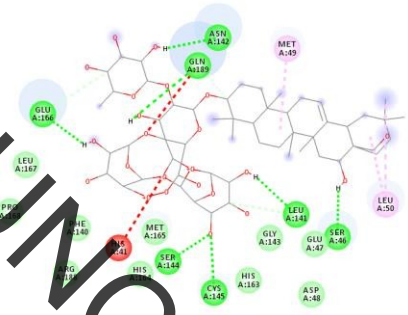
No	Compound	2D Representation of Compounds-6LU7 Interactions	3D Representation of Compounds-6LU7 Interactions
1	Pycnamine		
2	Tetracannabinol		
3	Oleuropein		

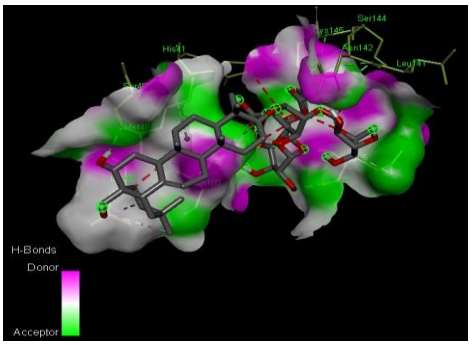
4 Quercetin



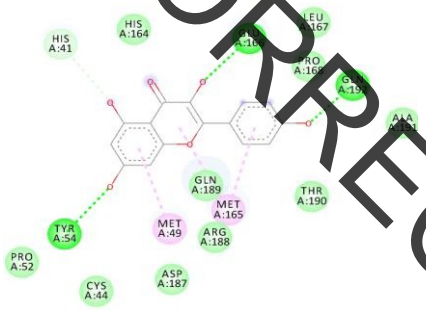


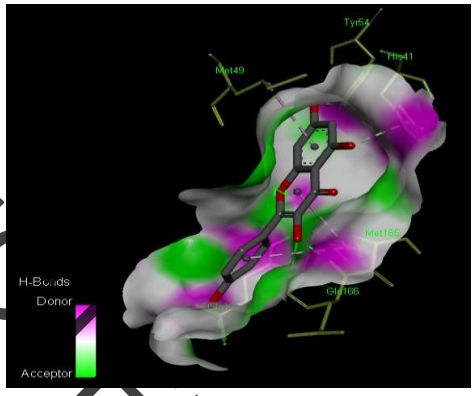
5 Primulic Acid



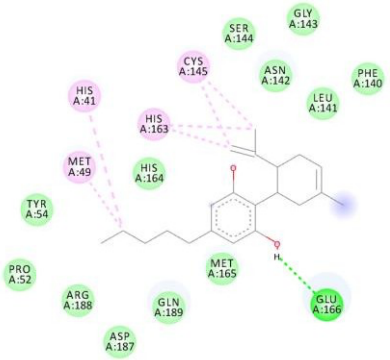


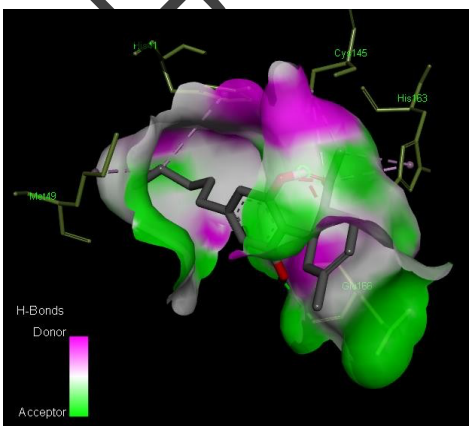
6 Kaempferol



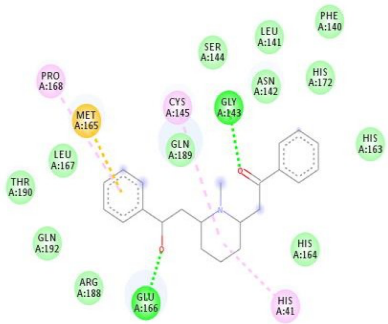


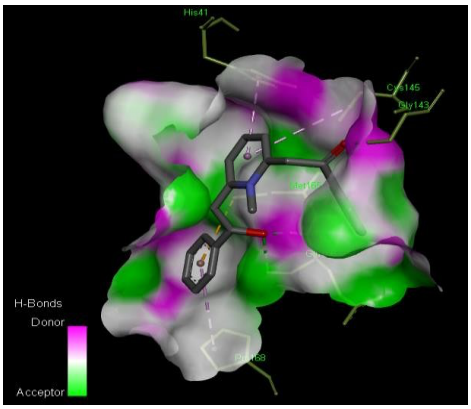
7 Dicannabidiol



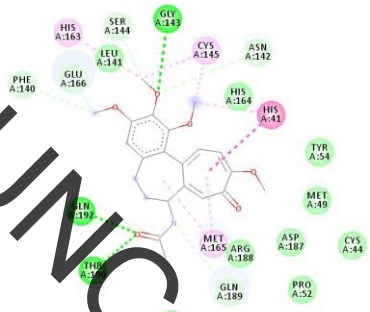


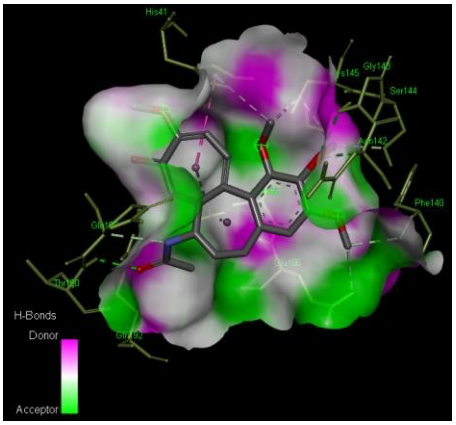
8 Lobelin



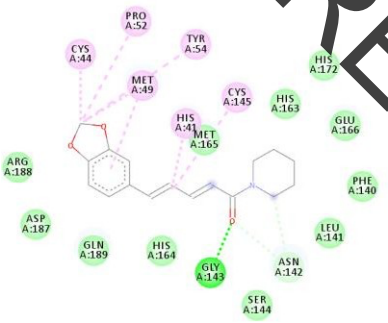


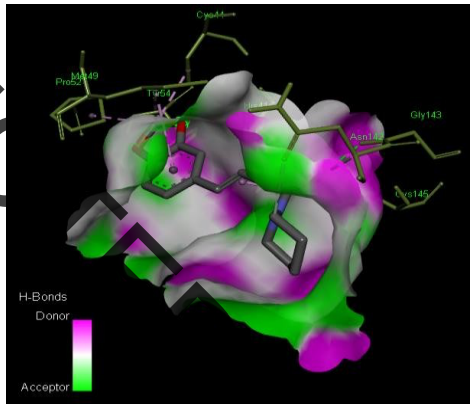
9 Colchicine



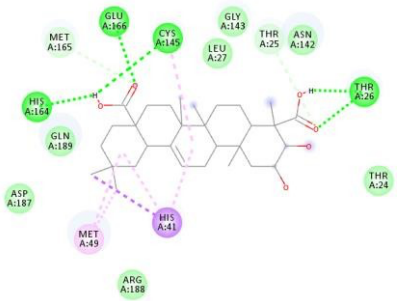


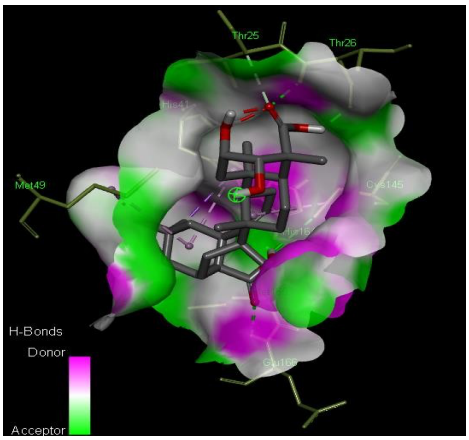
10 Piperidine



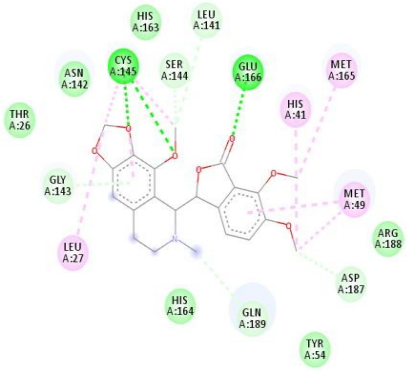


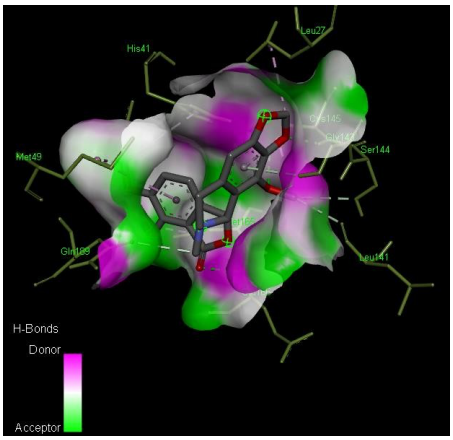
11 Medicagenic Acid



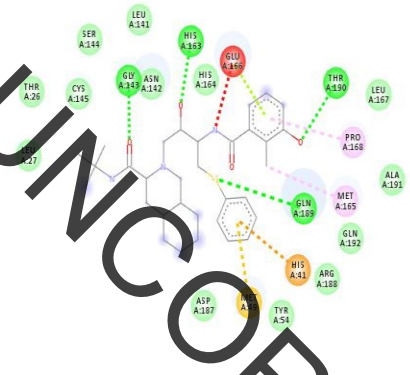


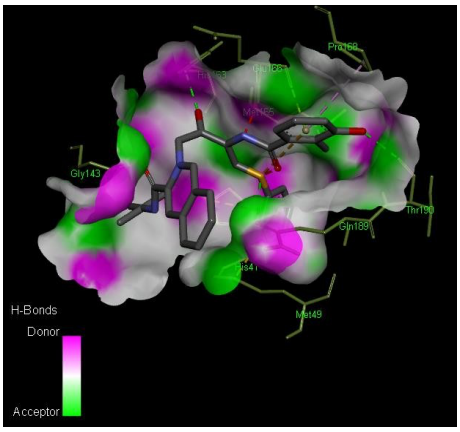
12 Narcotine



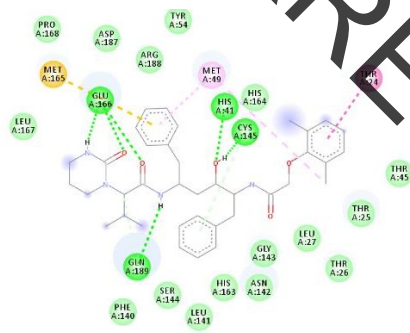


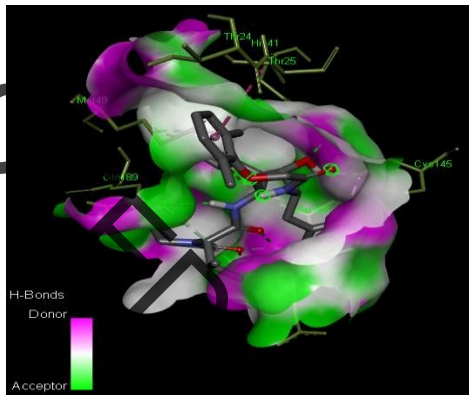
13 Nelfinavir



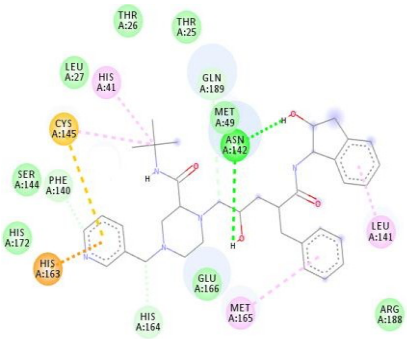


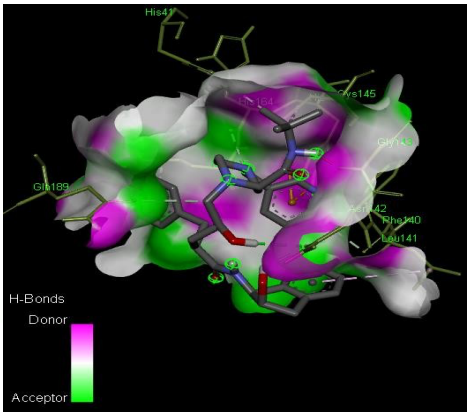
14 Lopinavir



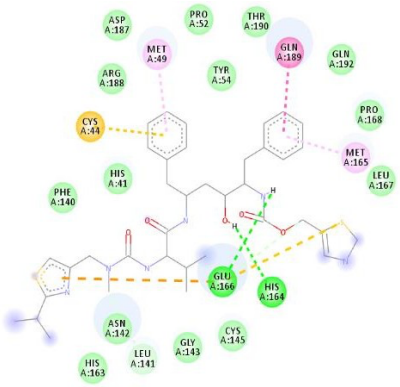


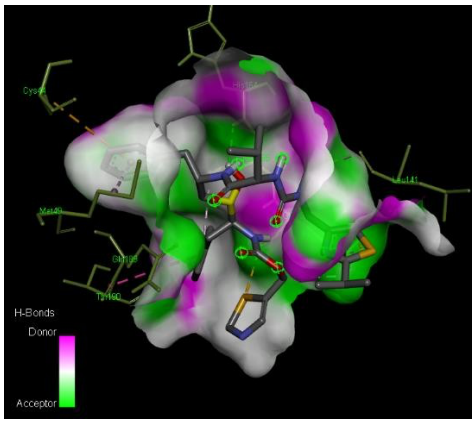
15 Indinavir





16 Ritonavir





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, antimicrobial in previous studies.[36-40] It was evaluated against COVID-19 for the first time by this study.

Tetrahydrocannabinol, which has the second lowest binding free energy score (-9.10 kcal/mol) in this study, is an alkaloid 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 the binding free energy score of -9.06 kcal/mol. It has been reported to have antiviral activity against mononucleosis herpes, hepatitis, rota, bovine, parvo, HIV, leukemia, respiratory syncytial, parainfluenza-3, salmonid rhabdoviruses.[42-46] In hepatitis B virus infected ducks, oleuropein reduces the virus entering the bloodstream.[47]

Quercetin, a flavonoid, found abundantly in fruits and vegetables, including onions, broccoli, buckwheat, peppers, Brassica vegetables, 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 (RSV), influenza virüs strain, parainfluenza virus type 3 (Pf3), sindbis virüs, rhinovirus, echovirus (type 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 attenuated virus- induced airway cholinergic hyperresponsiveness.[55]

Kaempferol is another flavonoid, found in most edible plants such as tea, fruits and vegetables consisting of *Allium cepa , Camellia sinensis, Citrus paradisi, Fragaria vesca, Lactuca sativa* as well as 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), 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 (2011).[59]

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

CONCLUSION

Currently, there is no antiviral drug for specific treatment of COVID-19 which is still a threat to global health. The main protease (M^{pro}) is used as a potential drug target to combat 2019-CoV. In this study, we evaluated several seconder metabolites obtained from medicinal plants against the COVID-19 M^{pro} by molecular docking studies to find out a potential inhibitory compound that may be used to inhibit the COVID-19 infection pathway. According to the results, pycnamine, tetracannabinol, oleuropein, quercetin, primulic acid, kaempferol, dicannabidiol, lobelin, colchicine, piperidine, medicagenic acid, and narcotine are 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 high affinity to the 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 which has been using 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 the COVID-19 M^{pro}. 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.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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