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Investigation of Active Compounds in Propolis Structure Against Sars Cov-2 Main Protease by Molecular Docking Method: In Silico Study

Erkan ONER[1]✍, Ilter DEMIRHAN[2] , Ergul Belge KURUTAS[3] Serap YALIN[4]

[1]Adiyaman University Faculty of Pharmacy, Department of Biochemistry, Adiyaman-Türkiye, [2]Harran University, Vocational School of Health Services, Department of Electronic-Automation, Biomedical Device Technology Program, Sanlurfa-Türkiye, [3]Kahramanmaras Sutcu Imam University Faculty of Medicine, Department of Medical Biochemistry, Kahramanmaraş-Türkiye, [4]Mersin University Faculty of Pharmacy, Department of Biochemistry, Mersin-Türkiye

[1]https://orcid.org/0000-0002-6332-6484, [2]https://orcid.org/0000-0003-0054-7893, [3]http://orcid.org/0000-0002-6653-4801
[4]https://orcid.org/0000-0002-1286-2172
✉: erkanoner0803@gmail.com

ABSTRACT

It was aimed to investigate the active ingredients limonin, quercetin and kaempferol in propolis against SARS-CoV-2 main protease(MPro) using in silico methods. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) screening of ligands assists US to state their absorption properties, toxicity, and drug-likeness. Ligand molecules obtained from PubChem in smiles format were loaded on SWISSADME and PROTOX-II webservers for ADMET screening. The three compounds in propolis were obtained from the PubChem database. Compounds were located at the active site of the SARS-CoV-2 MPro receptor with PDB ID:6LU7. Molecular docking work was done with Autodock program. Molecular docking results were found as -8.7 kcal/mol in limonin, -7.5 kcal/mol in quercetin and -7.7 kcal/mol in kaempferol. In silico ADMET estimation showed they have a potential for antiviral therapy. In conclusion, we thought that propolis active components limonin, quercetin and kaempferol have the potential to be a SARS CoV-2 MPro inhibitor.

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Propolisin Aktif Bileşiklerinin Sars Cov-2 Ana Proteaz Yapısında Moleküler Yerleştirme Yöntemiyle Araştırılması: In Silico Çalışması

ÖZET

Propolisin aktif bileşikleri olan limonin, quercetin ve kaempferol'ü SARS-CoV-2 ana proteaza (MPro) karşı in silico yöntemlerle araştırması amaçlandı. Ligandların absorpsiyon, dağılım, metabolizma, atılım ve toksisite (ADMET) taraması, absorpsiyon özelliklerini, toksisitesini ve ilaca benzerliğini belirtmesine yardımcı olur. PubChem'den smiless formatında elde edilen ligand molekülleri, ADMET taraması için SWISSADME ve PROTOX-II web sunucularına yüklendi. Propolisteki üç bileşik, PubChem veritabanından elde edildi. Bileşikler, PDB ID:6LU7 ile SARS-CoV-2 MPro reseptörünün aktif bölgesine yerleştirildi. Autodock programı ile moleküler yerleştirme çalışması yapıldı. Moleküler yerleştirme sonuçları limoninde -8,7 kcal/mol, quercetin'de -7,5 kcal/mol ve kaempferol'de -7,7 kcal/mol olarak bulundu. In silico ADMET tahmini, antiviral tedavi potansiyeline sahip olduklarını gösterdi. Sonuç olarak, propolis aktif bileşenleri limonin, quercetin ve kaempferol'ün SARS CoV-2 MPro inhibitörü olma potansiyeline sahip olabileceği düşünülmektedir.

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INTRODUCTION

Coronaviruses (CoVs) are the etiological cause of serious infections in the respiratory tract as well as the digestive tract in both animals and humans. Previous

reviews of CoVs have indicated that from mammals to reptiles, and birds, a wide range of species have been affected by these viruses (Malik et al, 2020). COVID-19 was accepted as a pandemic disease by the WHO on January 30, 2020 (Rodriguez et al., 2020). Although various measures and effective treatment methods have been adopted by countries to reduce the course of the disease, prevention management strategies were limited for eradication. The SARS coronavirus main protease (Mpro) of the coronavirus consists of glycoprotein and it is required for virus replication (Hofmann et al., 2004).

The chemical composition of propolis differs depending on its source, and more than 300 components have been identified in raw propolis (Gulcin et al., 2010).

Many researchers report that propolis extract is effective in the prevention of viral infection on plants (such as cucumber mosaic, tobacco mottle, tobacco gangrene), animals (HSV-1, varicella-zoster, and influenza), and humans (human immunodeficiency-HIV, herpes simplex virus type 1 and 2, adenovirus type 2, pharyngitis virus, and poliovirus type 2 (Marcucci, 1995). Studies show that propolis has the potential to be used as an antiviral drug. (Silici et al., 2005). Propolis has a lethal effect against the influenza virus (type A) in vitro, while aqueous propolis extract greatly reduces the effect of the smallpox virus within 15 minutes (Hegazi et al., 2000).

The process of revealing the in silico structures of receptor-ligand complexes with various software is called molecular docking. The receptors consist of proteins, while the ligands may consist of another protein or small molecule. In drug discovery studies, the virtual screening process with the molecular docking method is becoming more and more important. Such a virtual scan is usually performed in three steps. First, the molecular insertion program predicts the optimal structure for the complex of a target protein and a compound from the screening libraries. Second, complexes are scored according to their binding energy strength. Finally, classification is made according to the placement scores, and the best grades are selected from the virtual scan results (Onodera et al., 2007).

The aim of this study was to investigate the propolis bioactive components limonin, quercetin and kaempferol compounds in SARS CoV-2 Mpro structure by molecular docking method and to conduct drug similarity studies of limonin, quercetin and kaempferol.

MATERIALS and METHODS

ADMET and toxicity prediction

The ADMET (absorption, distribution, metabolism, excretion and toxicity) screening helps determine the toxicity and drug-likeness of compounds. Ligand molecules and selected propolis active ligands (limonin, quercetin and kaempferol) obtained in smile format from PubChem (https://pubchem.ncbi.nlm.nih.gov) were uploaded to the SWISSADME and PROTOX-II web servers for ADMET screening. Investigating the pharmacokinetics and ADME properties of a molecule or compound is done on a server called SWISSADME. Lipophilicity, water solubility, drug similarity, pharmacokinetic properties of the molecule, blood-brain barrier (BBB) and intestinal permeability were estimated through this server. (Table 1). The analysis was carried out for each physicochemical property (Absorption, Distribution, Metabolism, Excretion, and Toxicity) by submitting a SMILE format of the query compounds taken from the PubChem database. PROTOX-II is a Rodent oral toxicity server that predicts LD50 value and toxicity class of query molecule. Toxicity values on the PROTOX-II web server are as follows: Class I: fatal if swallowed (LD50 ≤ 5 mg/kg), Class II: fatal if swallowed (5 mg/kg < LD50 ≤ 50 mg/kg), Class III: toxic if swallowed (50 mg/kg < LD50 ≤ 300 mg/kg), Class IV: harmful if swallowed (300 mg/kg < LD50 ≤ 2000 mg/kg), Class V: may be harmful if swallowed (2000 mg/kg < LD50 ≤ 5000 mg/kg) and Class VI: non-toxic (LD50 > 5000 mg/kg). (Banerjee P et al., 2018).

Table 1. Drug likeness rules and their properties
Çizelge 1. İlaç benzerlik kuralları ve özellikleri

Name of rule	Property	Rules
Lipinski's rule	Molecular weight	≤ 500
	Lipophilicity (logP)	≤ 5
	Hydrogen bond acceptor	≤ 10
	Hydrogen bond donors	≤ 5
Ghose's rule	Lipophilicity (logP)	− 5.6<logP< − 0.4
	Molecular weight	160 < MW <480
	Molar refractivity	40 < MR < 130
	Total number of atoms	20 < atoms < 70
Veber's rule	No. of rotatable bonds	≥ 10
	TPSA	≤ 140
	Hydrogen bond donor	≤ 12
	Hydrogen bond acceptor	≤ 12

Molecular Docking Method

Ligand System

Limonin, quercetin and kaempferol in propolis used in this study were taken from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). 3D structures of compounds were obtained in SDF format from PubChem. Compounds in SDF format were converted to PDB format from the Open Babel GUI program.

Protein Preparation

3D structure of the SARS-CoV-2 Mpro (PDB ID: 6LU7) was retrieved from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/). The resolution of the PDB ID: 6LU7 protein is 2.16 Å. Firstly, ligands and water molecules in the 6LU7 protein structure were removed from the receptor, after that, polar hydrogen and a

charge (colman charge) were added together with the receptor in the protein structure. All preparatory processes were carried out using AutoDock 4 software (Morris et al., 2009).

Validation Method

The N3 inhibitor (N-[(5-methylisoxazol-3-yl)carbonyl]alanyl-1-valyl-N~1~-((1R, 2Z)-4-(benzyloxy)-4-oxo-1-{[(3R)-2-oxopyrrolidin-3-yl] methyl } but-2-enyl)-1-leucinamide) was deconstructed using AutoDock 4 (Jin et al., 2020). N3 inhibitor, the natural ligand of SARS CoV-2 Mpro, was superimposed on the protein structure according to the insertion procedure. Also, the root mean square deviation (RMSD) value was checked using PyMOL software to validate. If the RMSD value is less than 2.0 Å, it indicates that the method is valid. (Bell & Zhang., 2019).

Molecular Docking

It was carried out by applying all the parameters valid for the simulation of molecular docking. SARS-CoV-2 Mpro structure active region coordinates and grid box dimensions were determined in Discovery Studio program. The active site coordinates of SARS-CoV-2 Mpro are x=-9.732, y=11.403 and z=68.925. Grid box sizes are 64 Å, 60 Å and 60 Å, respectively. 100 replicates were made for each active compound to ensure the accuracy of the binding energy and amino acid interactions. Molecular docking was done with AutoDock 4 (Laskowski, 1995).

RESULTS and DISCUSSION

ADMET and toxicity prediction

The SWISSADME analysis and toxicity estimation results are shown in Table 2. Limonin, quercetin, and kaempferol showed good human intestinal solubility (HIA), and the selected propolis active compounds all belong to the same class (Class-IV) in acute rat toxicity (LD50). These phytochemicals are inactive for cytotoxicity and hepatic toxicity.

The LD50 values of propolis active compounds are limonin: 244mg/kg, quercetin:159 mg/kg, and kaempferol:3919 mg/kg.

Drug likeness prediction

When both limonin, quercetin, and kaempferol molecules are evaluated based on the Lipinski, Ghose, and Veber rules, it has been observed that the molecules are compatible with these rules, that is, these molecules are within the limits that can be considered as drugs.

The radar image obtained from the SwissADME web server in Figure 1 indicates substances that can be considered drug-like in a pink area, based on 6 different physicochemical parameters. These parameters are lipophilic (LIPO), molecular size (SIZE), polarity (POLAR), solubility (INSOLU), flexibility (FLEX), and saturation (INSATU). The areas where these parameters are restricted specify certain value ranges for the candidate molecule.

Figure 1. The radar image of limonin, quercetin, and kaempferol molecule.
Şekil 1. Limonin, kersetin ve kaempferol molekülünün radar görüntüsü.

Firstly, when the radar images of limonin, quercetin, and kaempferol molecules are evaluated, it is seen that the only limonin is in the pink area in 6 different parameters, while the quercetin and kaempferol only deviate in terms of saturation.

Tophological Polar Surface Area (TPSA) is defined as the sum of areas on all polar atoms or molecules of a molecule, including primarily nitrogen and oxygen, and later hydrogen atoms. It is mostly used as an indicator for molecular transport through biological barriers, such as the blood-brain barrier (BBB), in the body. If this value is more than 140 Å2, molecular transport through cell membranes would be difficult. It has been shown that the TPSA values for candidate molecules targeted at central nervous systems should be less than 60-70 Å2 to overcome BBB. The TPSA values of limonin, quercetin, and kaempferol molecules were evaluated as 104.57Å2, 131.36Å2, and 111.13 Å2, respectively. Since the TPSA values obtained for these three molecules are greater than 60-70 Å2, they do not have the ability to cross the BBB (Figure 2, Table 2).

Figure 2. Boiled-Egg image of limonin, quercetin, and kaempferol molecule.
Şekil 2. Limonin, kuersetin ve kaempferol molekülünün haşlanmış yumurta görüntüsü.

Table 2. The results of the ADMET test with SwissADME
Çizelge 2. SwissADME ile ADMET testinin bulguları

Property	Limonin	Quercetin	Kaempferol
Molecular weight	470.51 g/mol	302.24 g/mol	286.24 g/mol
TPSA	104.57 Å²	131.36 Å²	111.13 Å²
iLOGP	2.87	1.63	1.7
XLOGP3	1.77	1.54	1.90
WLOGP	2.81	1.99	2.28
MLOGP	1.45	-0.56	-0.03
Silicos- IT LogP	3.83	1.54	2.03
Consensus Log P	2.55	1.23	1.58
ESOL Log S	-3.92	-3.16	-3.31
ESOL class	Soluble	Soluble	Soluble
Ali LogS	-3.40	3.60	-5.00
Ali class	Soluble	Soluble	Moderately soluble
Silicos- IT LogSw	-3.58	-3.91	-3.86
Silicos-IT class	Soluble	Soluble	Soluble
GI absorption	High	High	High
BBB perme- ant	Yes	Yes	Yes
Log Kp, cm/s (Skin penetration)	-4.87 cm/s	-4.74 cm/s	-5.93 cm/s
Lipinski violations	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Ghose violations	Yes	Yes	Yes
Veber violations	Yes	Yes	Yes
Egan violations	Yes	Yes	Yes
Muegge violations	Yes	Yes	Yes
Bioavailability score	0.55	0.55	0.55
PAINS alerts	0 alert	0 alert	0 alert
Brenk alerts	0 alert	0 alert	0 alert

Molecular weight is important to determining whether specific molecules can penetrate into particular types of barriers in the human body since large molecules can not pass through highly selective barriers. Since the molecular weight of limonin, quercetin and kaempferol is <500 g/mol, this value is within the limits of the molecule being a drug (Table 2).

Candidate drug molecules must have optimal hydrophilicity and lipophilicity (ClogP) values. CLogP values were calculated as 2.55, 1.23, and 1.58 for limonin, quercetin, and kaempferol, respectively (Table 2).

Validation Results

Revalidation was performed with the ligand N3 inhibitor to determine the strength of binding affinity. The result of the verification was shown in the Figure 3. The RMSD value of the ligand was found 1.5 Å and the binding energy was -6.9 kcal/mol.

Molecular Docking Results

The binding energies of propolis bioactive compounds after the insertion process are shown in Table 3. RMSD, and theoretically inhibitory concentration (Table 4) were calculated by molecular docking method in SARS-CoV-2 Mpro structure (PDB ID: 6LU7) for the active compounds N3 inhibitor, limonin, quercetin, and kaempferol compounds in propolis. Autodock vina results from the Molecular docking model were extracted with the 3D BIOVIA Discovery Studio 2020 program (Figure 4). In addition, the binding site estimates and bond structures of the bioactive compounds in the propolis structure in the SARS CoV-2 Mpro structure were determined (Figure 5-7).

The binding interactions of N3 inhibitor, which is an inhibitor of SARS CoV-2 Mpro receptor, and propolis active compounds were compared. According to the results, the molecular docking scores of the bioactive components limonin, quercetin, and kaempferol were determined as <-6.5 kcal/mol. Docking scores indicate good binding in the SARS CoV-2 Mpro structure. Since molecular docking study result was below 2 Å, it showed that docking study was accurate and successful.

The binding energy of N3 inhibitor was -6.9 kcal/mol, limonin -8.7 kcal/mol, quercetin -7.5 kcal/mol, and kaempferol -7.7 kcal/mol in SARS CoV-2 Mpro (PDB ID: 6LU7) structure and all results showed high binding energy. When we compared the binding energy of the N3 inhibitor with the binding energy of the active components of propolis, we saw that the N3 inhibitor had low binding affinity. Similar results were obtained when compared with other studies. In addition, inhibitor concentrations were found to be 41 µM in limonin, 85µM in quercetin, and 115 µM in kaempferol. ADMET results have shown that three compounds can meet the characteristics of being a drug.

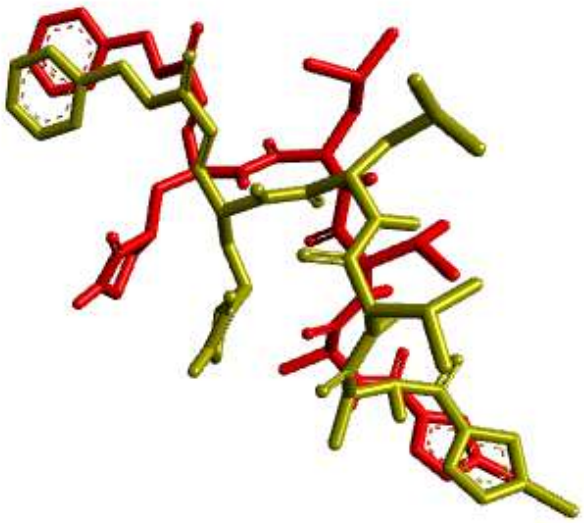
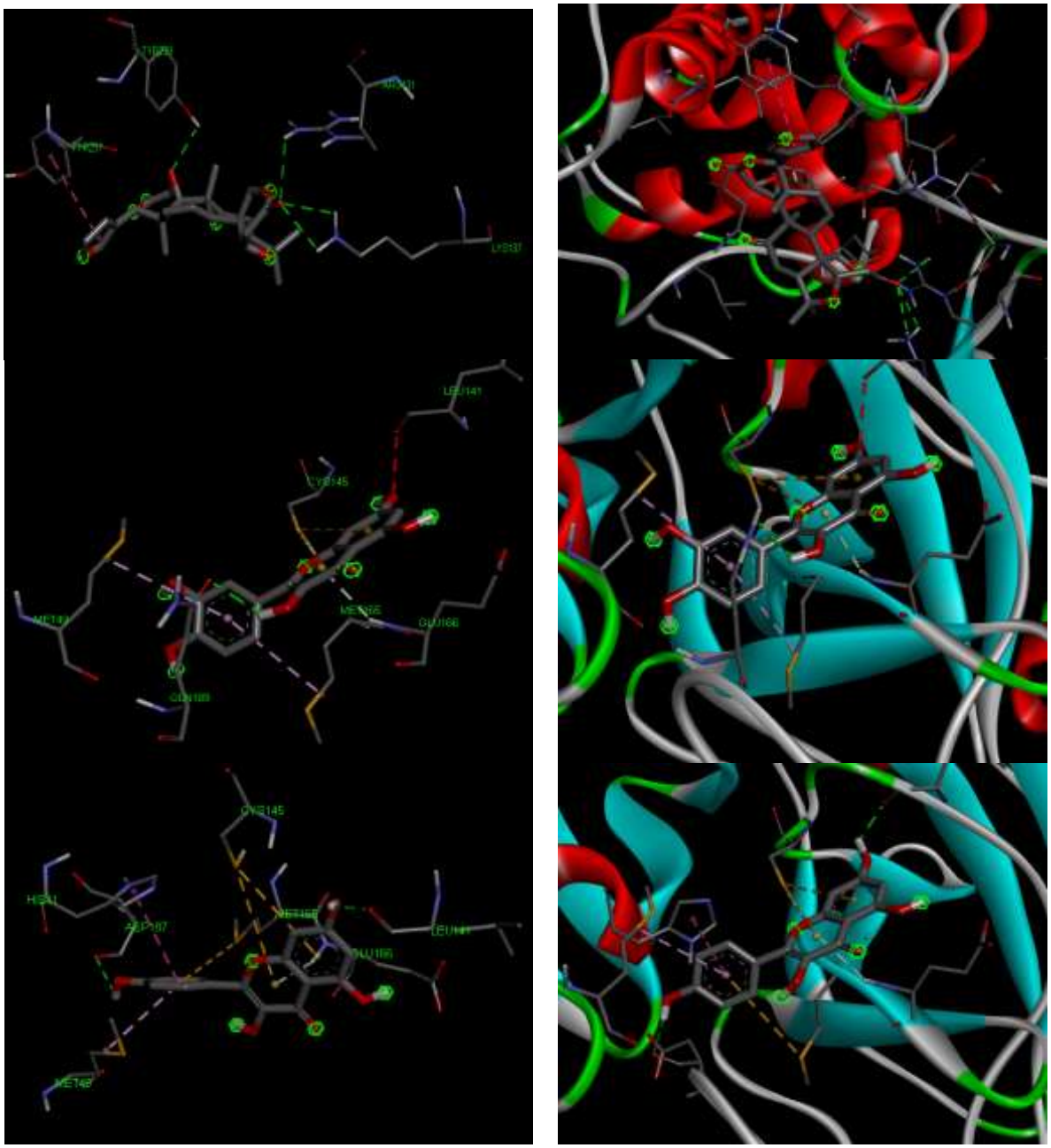


Figure 3. SARS-CoV-2 MPro receptor state before validation (red), state of the receptor after insertion (yellow), inhibitor model

Şekil 3. Doğrulama öncesi SARS-CoV-2 MPro reseptör durumu (kırmızı), yerleştirme sonrası reseptör durumu (sarı), inhibitör modeli

Table 3. Molecular docking results of propolis compounds in SARS CoV 2 Mpro structure
Çizelge 3. SARS CoV 2 Mpro yapısındaki propolis bileşiklerinin moleküler kenetlenme sonuçları

Analysis Program	Visualization Program	Protein	Ligand	Docking Score(kcal/mol)	Amino Acid	Residue
Autodock Vina	3 D BIOVIA Discovery Studio Visualizer	6LU7	N3 inhibitor	-6.9	VAL171, TYR199, LEU286, LEU287	ALA194, MET276,
Autodock Vina	3 D BIOVIA Discovery Studio Visualizer	6LU7	Limonin	-8.7	ARG131, TYR239, TYR237	LYS137,
Autodock Vina	3 D BIOVIA Discovery Studio Visualizer	6LU7	Quercetin	-7.5	MET49, CYS145, GLU166, GLN189	LEU141, MET165,
Autodock Vina	3 D BIOVIA Discovery Studio Visualizer	6LU7	Kaempferol	-7,7	HIS41, LEU141, MET165, ASP187	MET49, CYS145, GLU166,



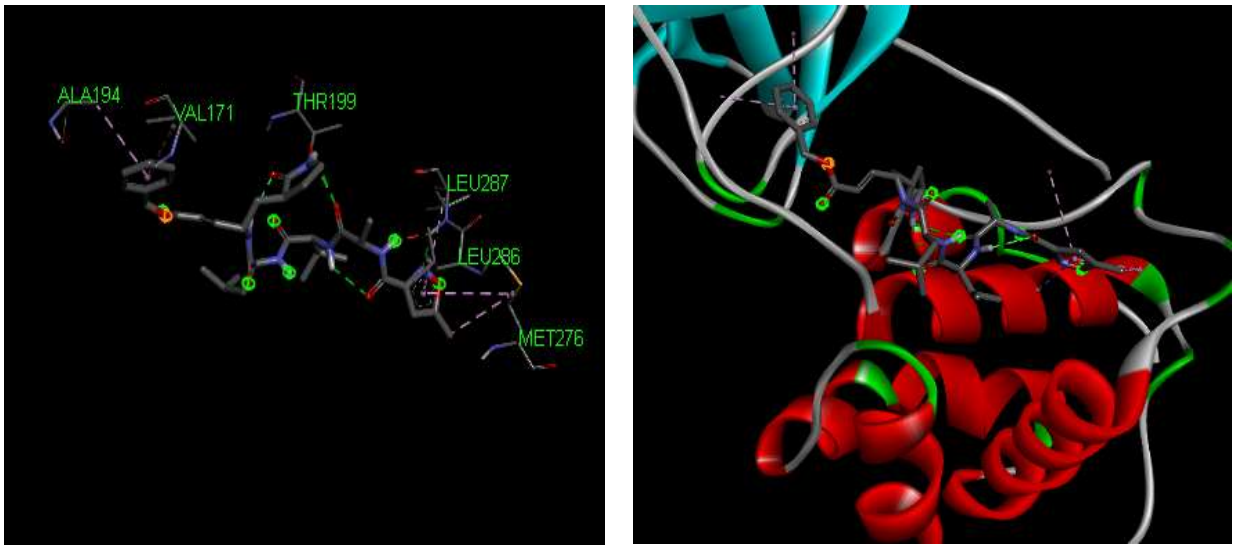


Figure 4. Molecular docking results of active compounds in the structure of SARS CoV 2 Mpro, N3 inhibitor and propolis
Şekil 4. SARS CoV 2 Mpro, N3 inhibitörü ve p[illegible]aktif bileşiklerin moleküler kenetlenme sonuçları

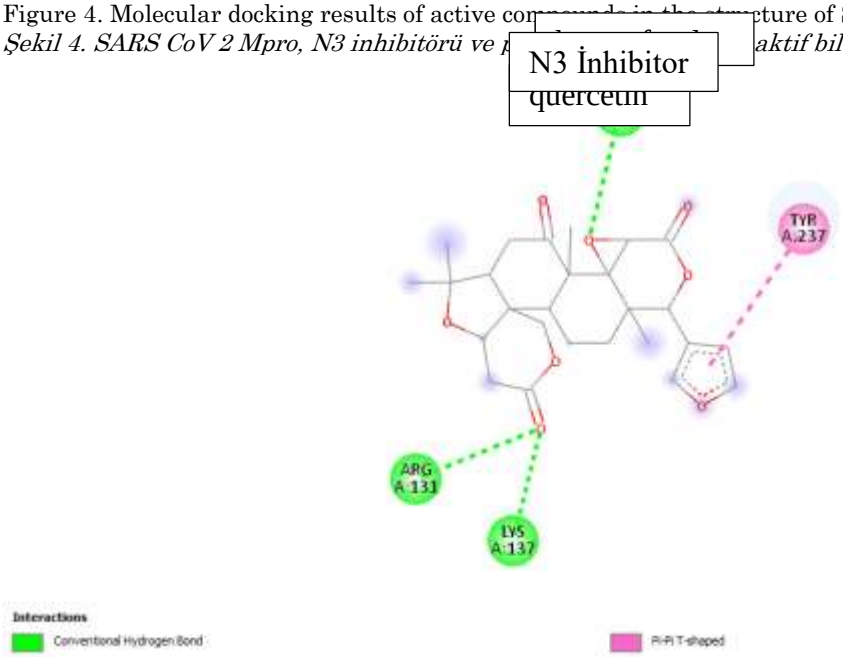


Figure 5. Bond structures in limonin SARS CoV-2 Mpro structure.
Şekil 5. Limonin SARS CoV-2 Mpro yapısındaki bağ yapıları.

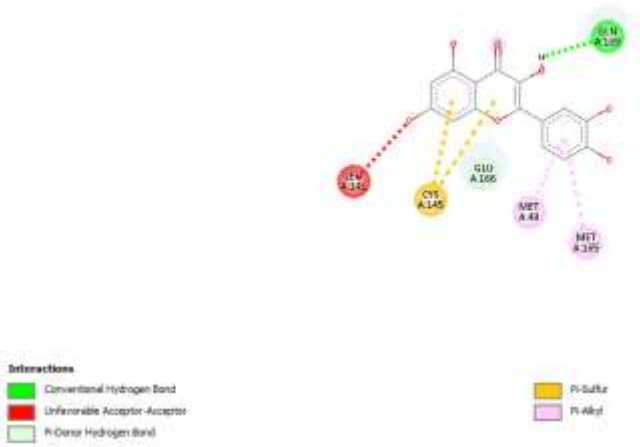


Figure 6. Bond structures in quercetin SARS CoV-2 Mpro structure.
Şekil 6. Quercetin'in SARS CoV-2 Mpro yapısındaki bağ yapıları

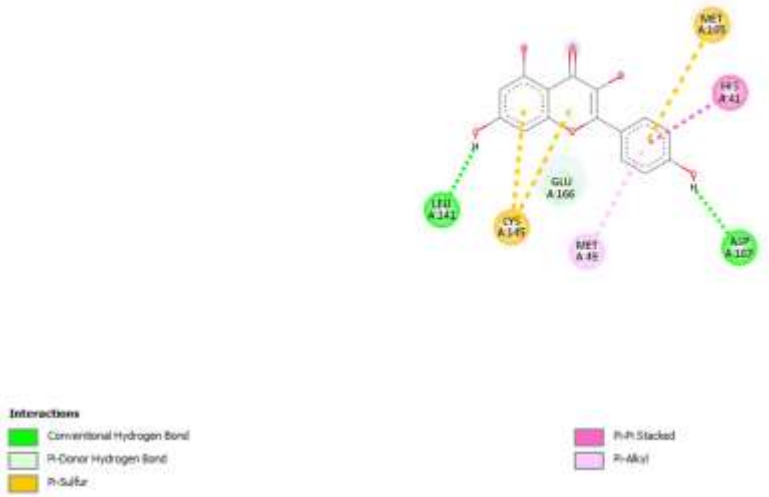


Figure 7. Bond structures in kaempferol SARS CoV-2 Mpro structure.
Şekil 7. Kaempferol'ün SARS CoV-2 Mpro yapısındaki bağ yapıları

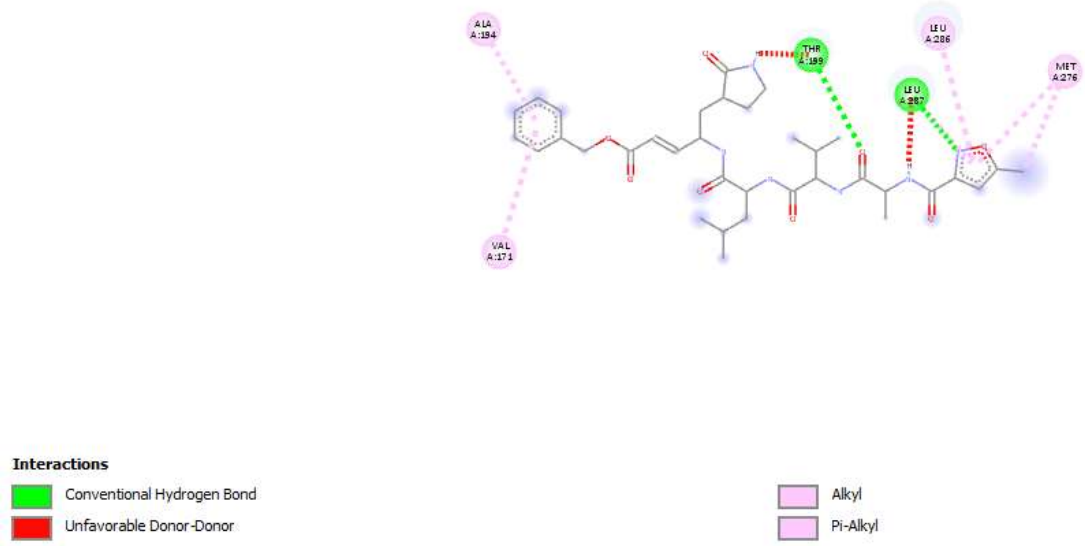


Figure 8. Bond structures in N3 inhibitor SARS CoV-2 Mpro structure.
Şekil 8. N3 inhibitörü'nün SARS CoV-2 Mpro yapısındaki bağ yapıları

Table 4. RMSD and Inhibition constant scores of limonin, quercetin, and kaempferol in SARS CoV 2 Mpro structure
Çizelge 4. SARS CoV 2 Mpro yapısında limonin, kersetin ve kaempferolün RMSD ve İnhibisyon konsantrasyonları

Analysis Program	Protein	Ligand	RMSD (Å)	Inhibition Constant
Autodock Grid	6LU7	Limonin	1.70	41 µM
Autodock Grid	6LU7	Quercetin	1.99	85 µM
Autodock Grid	6LU7	Kaempferol	1.82	115 µM

Many studies have reported that propolis and/or its components support strengthening the immune system and reducing inflammation due to their anti-inflammatory properties. These properties will help reduce the symptoms and harmful effects caused by COVID-19 (Vardeny et al., 2020).

Jin et al. found that the N3 inhibitor is promising in the SARS CoV-2 Mpro construct (Jin et al. 2020).

Vardhan et al. stated that limonin has a good binding affinity to the SARS CoV-2 Mpro (PDB ID: 6LU7) structure in their study. The binding affinity result was -8.7 kcal/mol and it was similar to this result (Vardhan et al., 2020).

In the study of Khan et al., the molecular docking score of the kaempferol compound was -6.4 kcal/mol and the inhibitory concentration was 116 micromolar in the SARS CoV-2 Mpro structure. (Khan et al., 2021). A close result was found when compared with this result. Yang et al. showed that kaempferol has a high binding

energy (-7.5 kcal/mol) at its major receptor (ACE2) for viral entry (Yang et al., 2018).

Arokiyaraj et al. determined that the binding affinities of quercetin were -6.49 kcal/mol and kaempferol was -7.76 kcal/mol in the SARS-CoV-2 Mpro structure (PDB ID: 6LU7) (Arokiyaraj et al., 2020). Their results were consistent with these findings. Luo et al. showed that 54 patients with novel coronavirus pneumonia improved their immune ability against COVID-19 after traditional Chinese medicine treatment and shortened patients' hospital stay. Compound quercetin, luteolin, kaempferol, acacetin etc., were all involved in the treatment of various disease stages on the compound level both in generality and individuality (Luo et al., 2020).

CONCLUSION

In summary, coronavirus has emerged as the deadliest disease the world has faced after the Spanish flu. It is important to find a solution to control this virus urgently. It is important to carry out studies on this virus with computer-aided drug design programs in terms of being fast and saving time. We conducted a computer-assisted drug discovery study against the protein involved in the action mechanism of SARS CoV-2. These results show that the bioactive compounds of propolis (limonin, quercetin, and kaempferol) have the ability to inhibit the target protein Mpro (PDB ID:6LU7) in SARS CoV-2 in the least energy conformation. We suggest that three compounds can prevent the coronavirus infection.

Author contributions

Concept – E.O., S.Y.; Design – İ.D., E.O.,; Supervision – E.B.K.; Resources – E.O., İ.D.; Materials – E.O.; Data Collection and/or Processing – E.O., İ.D.; Analysis and/or Interpretation – E.O., E.B.K., S.Y.; Literature Search – İ.D., E.O.; Writing – İD.,E.O.; Critical Reviews – E.B.K., İ.D.,S.Y.

Conflict of interest statement

The authors declared no conflict of interest in the manuscript.

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