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Targeting CoV-2 Spike RBD: ACE-II Complex with Phenolic Compounds from Cistus (Cistus L.) Bee Pollen for COVID-19 Treatment by Molecular Docking Study

Moleküler Yerleştirme Çalışması ile COVID-19 Tedavisi için Cistus (Cistus L.) Arı Polenifenolik Bileşikleri ile CoV-2 Spike RBD: ACE-II Kompleksinin Hedeflenmesi

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Abstract

Spike glycoprotein on the surface of the SARS-CoV-2 is a class I fusion protein that plays a role in the initial attachment of the virus to the human ACE-II receptor. ACE-II has been implicated in the regulation of heart function and also as a functional receptor for the coronavirus that causes the severe acute respiratory syndrome (SARS). In the present study, a molecular docking study was performed using eleven flavonoids present in Cistus bee pollen against the CoV-2 Spike RBD/ACE-II complex and compared their affinity with the FDA approved drug hydroxychloroquine (HCQ).

Binding constants of eleven flavonoids, catechin, pinocembrin, chrysin, caffeic acid phenethyl ester, p-OH Benzoic acid, syringic acid, t-cinnamic acid, p-Coumaric acid, rutin, ferulic acid and gallic acid were measured using the AutoDock 4.2 molecular docking software. Also, these binding constants were then compared to the reference molecule, hydroxychloroquine.

According to docking analysis, the results showed us that catechin has the best inhibiton potential among the all analyzed molecules with the high binding energy (-7.77 kcal/mol) and the

Özet

SARS-CoV-2'nin yüzeyinde bulunan başak glikoproteini, virüsün ilk olarak insan ACE-II reseptörüne bağlanmasında rol oynayan sınıf I füzyon proteinidir. ACE-II, kalp fonksiyonunun düzenlenmesinde ve ayrıca şiddetli akut solunum sendromuna (SARS) neden olan koronavirüs için fonksiyonel bir reseptör olarak rol oynamaktadır. Bu çalışmada, CoV-2 Spike RBD/ACE-II kompleksinekarşı Cistus arıpolenindebulunan 11 flavonoid kullanılarak bir moleküler yerleştirme çalışması gerçekleştirilmiş ve FDA onaylı bir ilaç olan hidroksiklorokin (HCQ) ile afiniteleri karşılaştırılmıştır.

Kullanılan 11 flavonoid; kateşin, pinokembrin, krisin, kafeikasitfenetil ester, p-OH Benzoikasit, siringikasit, t-sinnamikasit, p-kumarikasit, rutin, ferulik asit ve gallik asidin bağlanma sabitleri AutoDock 4.2 moleküler program kullanılarak gerçekleştirilmiştir. Ayrıca, sonrasında bu bağlanma sabitleri hidroksiklorokin referans molekülü ile karşılaştırılmıştır.

Yerleştirme analiz sonuçları bize, analiz edilen tüm moleküller arasında kateşinin yüksek bir bağlanma enerjisine (-7,77 kcal/mol) ve en düşük K_i (2,03 uM) değeri ile en iyi inhibisyon potansiyeline sahip olduğunu göstermiştir. Bunu

lowest K_i (2.03 µM) and it is followed by pinocembrin, chrysin, caffeic acid phenethyl ester, respectively. Besides, the reference molecule hydroxychloroquine has binding energy of -7.53 kcal/mol and 3.04 µM. Consequently, high potential of flavonoids in extracts of Cistus bee pollen to interact with CoV-2 Spike RBD/ACE-II complex indicates that this natural product has high potential for Covid-19 treatment, but this needs to be supported by further studies.
Keywords: Covid-19, Cistus (*Cistus* L.) Bee Pollen, Flavonoids, CoV-2 Spike RBD/ACE-II, Molecular Docking

sırasıyla pinokembrin, krisin ve kafeikasit fenetil ester takip etmiştir. Ayrıca, referans molekül hidroksiklorokinin, -7,53 kcal/mol ve 3,04 uM'lik bağlanma enerjisine sahip olduğu sonucu elde edilmiştir. Sonuç olarak, Cistus arı poleni ekstraktlarındaki yüksek flavonoidlerin CoV-2 Spike RBD/ACE-II kompleksi ile etkileşime grime potansiyeli, bu doğal ürünün Covid-19 tedavisi için yüksek potansiyele sahip olduğunu göstermektedir ve bu çalışmanın daha fazla çalışma ile desteklenmesi gerekir.
Anahtar kelimeler: Covid-19, Cistus (*Cistus* L.) bal poleni, Flavonoidler, CoV-2 Spike RBD/ACE-II, Moleküler Yerleştirme

Abbreviations: HPLC, High performance liquid chromatography; RP-HPLC, Reversed phasehigh performance liquid chromatography

1. INTRODUCTION

Bee pollen is a natural source of protein, oil, vitamins, minerals and amino acids, which are gathered by the honey-bees from various flower species and necessary for the growth and development of bees (Ulusoy & Kolaylı, 2014). Pollens are the male reproductive cells, the primary food source of flowers and bees. Since ancient pollen was widely used for its medicinal properties, while in our days, is increasingly used as a health food and food supplement and is currently marketed throughout Europe and Asia as it shows antimicrobial, antioxidant, anti-inflammatory and gastroprotective activities (Ikeno et al., 2004; Koç et al., 2011; Komosinska-Vassev et al., 2015; Maruyama et al., 2010).

Bee pollen is known to contain lipids, sugars, proteins, amino acids, vitamins, carotenoids, polyphenols (mainly flavonoids) and carbohydrates (Human et al., 2006). In Turkey, the genus *Cistus* L. (Cisteceae) -commonly known as Laden, Pamukluk, Karağan- is generally represented by five species: *Laden C. creticus L., C. parviflorus Lam., C. laurifolius L., C. salviifolius L.* and *C. monspeliensis L.* (Sargın & Selvi, 2016). It spreads in the Mediterranean, Marmara and Black Sea regions. Traditionally, some species of Cistus have been used as herbal tea among people to improve digestive problems and colds. In studies with *C. creticus*, secondary metabolites, especially phenolic compounds, were detected to be found in leaf mesophilia and glandular trichomes. It is emphasized that secondary metabolites are antioxidant, anti-bacterial, anti-fungal, anti-inflammatory, and anti-cancer properties (Nikolaos et al., 2014; Papaefthimiou et al., 2014).

The world population is currently facing with a serious challenge to survive due to the rise of a global pandemic called Coronavirus Disease 2019 (COVID-19) (Kirchdoerfer et al., 2016). This pandemic, severe acute respiratory syndrome results from a positive RNA virus of the genus β-coronaviridae called Coronavirus 2 (SARS-CoV-2) (Fehr et al., 2015; Gorbalenya et al., 2020).

The newly discovered SARS-CoV-2 was characterized as a beta-coronavirus and considered the seventh discrete type of Coronavirus that can cause human disease. As per the reports provided by the World Health Organization (WHO), there is currently no effective treatment regime against SARS-CoV-2, including anti-virals or vaccines. The emerged global epidemic spread rapidly with 9.296.202 confirmed cases and 479.133 deaths across 213 countries, areas and territories (COVID-19 situation Report WHO, 26 June 2020).It is essential to find the drug for treatment by revealing the molecular characteristic of the virus. With the efforts of respected researchers, the crystal structure of spike glycoprotein, which plays an important role in virus infection, has been released (Chan et al., 2020; Chen & Du, 2020; Wrapp et al., 2020). This glycoprotein of SARS-CoV-2 exhibits little changes in the primary structure compared to the beta coronavirus, SARS-CoV, due to the mutation, providing a suitable target candidate of the new drugs (Xia et al., 2020). Spike glycoprotein of SARS-CoV-2 contains Receptor Binding Domain (RBD) that recognizes the target receptor leading to the splicing of the trimeric spike protein into s1 and s2. This domain facilitates membrane fusion and virus infection occurs through endocytosis (Yan et al., 2020).

According to the biochemical interaction analysis and crystal structure results, it was found that the SARS-CoV envelope-anchored spike protein has a strong binding affinity to human ACE-II enzyme (Li et al., 2005). It is also known that the residue 394 (Gln) in the SARS-CoV-2 receptor binding domain (RBD) corresponding to 479 residue in SARS-CoV is recognized by critical lysine 31 on the receptor of human ACE-II (Wu et al., 2012). Therefore, the RBD of spike glycoprotein is a preferable candidate for drug target to inhibit the initiation process of virus infection. Noted that there are many compounds have been treated to RBD spike glycoprotein (Li & De Clercq, 2020) or under *in silico* experiments as screening for drug candidates. However, the result showed a limited candidate molecule as a prospected drug due to the side effect threat (Senathilake et al., 2020; Smith & Smith, 2020; Wang, 2020).

So far, many studies have been carried out on COVID-19, but most researchers have focused primarily on clinical cases. However, this *in silico* study on the inhibition of Spike RBD-ACE-II complex, which is known to be associated with COVID-19, will encourage further research on candidate drug molecules of COVID-19.

2. MATERIAL AND METHODS

2.1. Chemicals

The chemical standards employed were all HPLC-grade pure. The common phenolic compounds used in this study were supplied by Sigma–Aldrich (Munich, Germany). 2,4,6-Tripyridyl-s-triazine (TPTZ), Folin–Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were

supplied by Sigma Chemical Co. (St Louis, MO, USA). Ethanol, methanol and acetonitrile were purchased from Sigma and methanol by Merck KGaA (Darmstandt, Germany). UV-VIS Spectrophotometer (Thermo Scientific Evolution TM 201, USA) was used in all absorbance measurements.

2.2. Samples and Preparation of Extracts

The pollen samples were obtained from the expert beekeepers of İstanbul Beykoz village, Turkey in 2018 flowering season. All the samples were stored at +4°C before analyses. Pollen samples were ground in a pestle. 5 g of sample was weighed and added to 100 mL methanol was added and shaken (HeidolphPromax 2020, Schwabach, Germany) for 24h. The suspension was then filtered twice with Whatman filter paper. The filtrate was sonicated for 3 h using a sonicator apparatus than the extract was used for HPLC analysis.

2.3. Analysis of Phenolic Compounds by HPLC

HPLC (Elite LaChrom Hitachi, Japan) analysis of phenolic compounds was performed on a reverse-phase C18 column (150 mm 4.6 mm, 5 mm; Fortis) using a gradient program with two solvent systems (A, 0.5% acetic acid in water and B, 2% acetonitrile in water). The sample injection volume was 25 µL, the column temperature was set at 30 °C, and the flow rate at 0.75 mL/min. The signals were detected at 280 nm by UV detection. The programmed solvent used began with a linear gradient held at 95% A for 3 min, decreasing to 80% A at 10 min, 60% A at 20 min, 20% A at 30 min and finally 95% A at 50 min (Çakır et al., 2018).

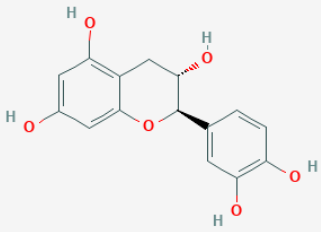
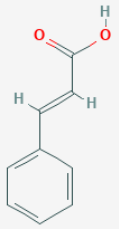
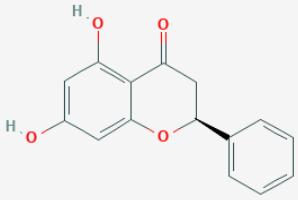
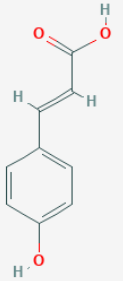
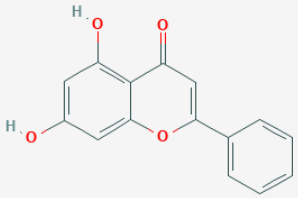
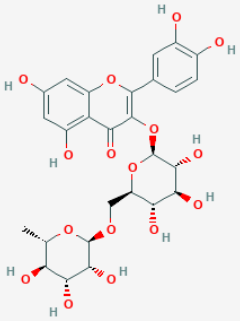
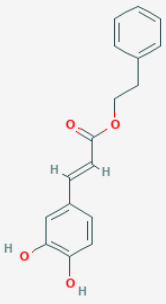
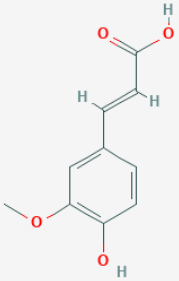
2.4. Molecular Docking

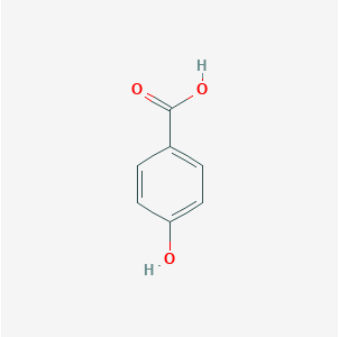
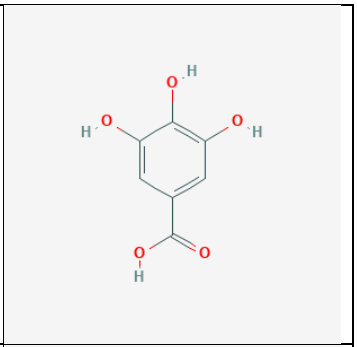
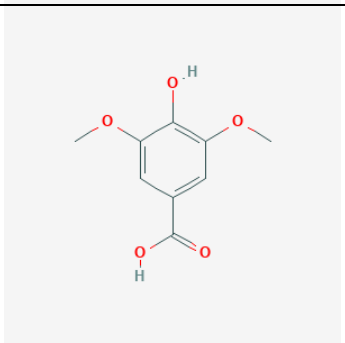
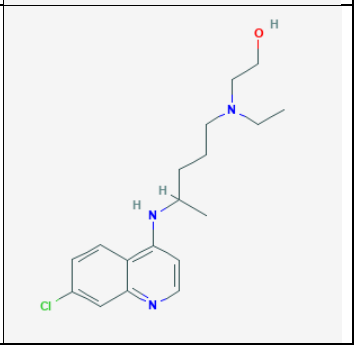
In this study, some flavonoids detected in the extracts of Cistus (Cistus L.) Bee pollen were used as ligand (Table 1) for CoV-2 Spike RBD/ACE-II complex. All the ligand structures were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and ChEMBL (https://www.ebi.ac.uk/chembl/) databases as SDF form and then converted to their proper readable file format (pdbqt) using AutoDock tools 1.5.6. The crystal structure of CoV-2 Spike RBD/ACE-II complex was retrieved from protein data bank web site (http://www.rcsb.org/pdb) (PDB ID: 6VW1: Resolution 2.68 Å). The prepared ligands and proteins were used as input files for AutoDock 4.2 software (Morris et al., 2009) and Lamarckian genetic algorithm was employed for all docking simulations. After energy minimization, the water molecules were deleted and the standard docking procedure was used for a rigid protein and a flexible ligand with torsion angles of 100 independent runs per ligand. All dockings experiments were performed as blind docking (blind docking refers to the use of a grid box which is large enough to encompass any possible ligand-receptor complex) using Autodock 4.2. A grid of 126, 126, and 126 points in x, y, and z directions was built with a grid spacing of 0.375 Å. The default settings were applied for all other parameters. Results of the molecular docking

described the affinity represented by docking score and binding interaction of each ligand on the interested protein target.

The final visualization of the docked structure was performed using BIOVIA Discovery Studio Visualizer 2018 (DassaultSystèmes BIOVIA, 2016.

Table 1. Compounds used for the molecular docking study.

Chemical compound	Formula	Chemical compound	Formula
Catechin ((2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol)		*t*-Cinnamic acid ((E)-3-phenylprop-2-enoic acid)	
Pinocembrin (5,7-Dihydroxy-2-phenyl-2,3-dihydro-4H-chromen-4-one)		*p*-Coumaric acid ((E)-3-(4-hydroxyphenyl)prop-2-enoic acid)	
Chrysin (5,7-dihydroxy-2-phenylchromen-4-one)		Rutin (Quercetin-3-rutinoside hydrate)	
Caffeic acid phenethyl ester (Caffeic acid 2-phenylethyl ester; β-Phenylethylcaffeate) (2-Phenylethyl (2E)-3-(3,4-dihydroxyphenyl)acrylate)		Ferulic acid ((E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid)	

p-OH Benzoic acid (4-hydroxybenzoic acid)		Gallic Acid (3,4,5-trihydroxybenzoic acid)	
Syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid)		Hydroxychloroquine (2-[4-[(7-chloroquinolin-4-yl)amino]pentyl-ethylamino]ethanol)*	

3. RESULTS AND DISCUSSION

3.1. Phenolic Composition of Propolis Extract

In this study, quantitative analyses of the phenolic compounds in extracts of cistus bee pollen were determined using of 19 phenolic compound standards (Figure 1). RP- HPLC chromatograms of standard phenolic compounds are summarized in Table 2.

According to the results of the study, protocatequic acid, epicatechin, caffeic acid, myrisetin, hesperetin, luteolin, daidzein and resveratrol could not be detected in *Cistus* L. pollen.

The highest amount of phenolic compounds in the bee pollen was found to be gallic acid (8528.00 µg/g), catechin (4691.70 µg/g) and rutin (4599.74 µg/g), respectively. These were followed by p-OH benzoic acid, ferulic acid, p-coumaric acid, chyrsin, CAPE, pinocembrin, syringic acid and *t*-cinnamic acid, respectively (Table 2). In Spanish Cistus sp. bee pollen, the most common phenolic compound was found as kaempferol. The difference in the phenolic compositions of pollen varies depending on geographical conditions, type of plant, harvesting time, and dominant pollen type (Kocot et al., 2018). In the present study, gallic acid was determined as to have the highest phenolic compound responsible for antioxidant properties.

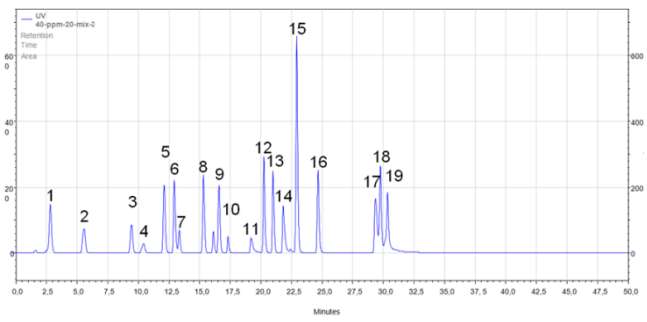


Figure 1. HPLC-UV chromatograms of phenolic standards. 1. Gallic acid, 2. Protocolatequic acid 3. p-OH Benzoic acid, 4. Catechin, 5. Caffeic acid, 6. Syringic acid, 7. Epicatechin, 8. p-Coumaric acid, 9. Ferulicacid, 10. Routine 11. Myrecetin, 12. Resveratrol, 13. Daidzein, 14. Luteolin, 15. t-Cinnamic acid, 16. Hesperetin, 17. Chrysin, 18. Pinocembrin, 19. CAPE

Table 2. Phenolic composition of *Cistus* L. bee polen.

Standards (µg/g)	Cistus Polen µg /100 g
Hydroxybenzoic acids	
Gallic acid	8528.00
Protocateuic acid	N.D
p-OH Benzoic acid	4207.50
Syringic acid	97.40
Catechin	
Catechin	4691.70
Epicatechin	N.D
Hydroxycinnamic acids	
Caffeic acid	N.D
p-Coumaric acid	2838.50
Ferulic acid	2844.90
t-Cinnamic acid	61.30
CAPE	851.80
Flavonols	
Rutin	4599.74
Myerecetin	N.D
Flavanones	
Hesperetin	N.D
Chyrsin	1426.12
Pinocembrin	106.44
Flavones	
Luteolin	N.D
Isoflavons	
Daidzein	N.D
Stilbands and Lignans	
Resveratrol	N.D

*N.D. not detected

3.2. Binding Affinity Analysis and Visualization of Docking Results

In the field of computer aided drug designing particularly for the identification of a candidate compound (Hughes et al., 2011; Raj et al., 2019), molecular docking is immensely employed to explore the various types of binding interaction of the prospective drug with the different domains or active sites on the target molecules. Among all different types of interactions such as H-bond, π-π, amide-π interactions *etc*., the binding efficacy of a ligand molecule with the active sites of a target have widely been explained by evaluating its hydrogen bonding pattern (Chen et al., 2016; Raj et al., 2019) and the nature of residues present at the active site. The binding energy (Kcal/mol) data allows us to study and compare the binding affinity of different ligands/compounds with their corresponding target receptor molecule. A lower binding energy indicates a higher affinity of the ligand for the receptor.

After successful docking of all the ligands employed in these docking experiments, the results showed significant binding of the ligands with the target proteins. After visualizing protein in Discovery Studio Visualizer it was found that CoV-2 Spike glycoprotein-Human ACE-II complex consist of A and B chains. The ligand is shown as yellow stick model whereas the protein is shown as solid ribbon.The amino acids taking part in the ligand-protein interaction are shown in bold to surround the ligand shown in yellow. The interaction shown by green dash lines refers to the hydrogen bonding interactions between the ligand and protein. Various amino acids involved

in the ligand protein interactions are shown as stick with different color and labeled by various colors. In order to achieve visibility of the docked ligands into the protein structure, ligands are shown as yellow color sticks in the binding pocket of the protein.

Hydroxychloroquine was docked with Cov-2 Spike glycoprotein-Human ACE-II complex as a reference molecule. The lowest energy conformations of all the ligand molecules were docked with protein. We focus here on Cistus L. bee pollen used by people to treat infections against CoV-2 Spike glycoprotein-Human ACE-II complex with molecular docking methods. For this purpose, we performed docking analysis with the compounds and found that catechin, pinocembrin, chrysin and caffeic acid phenethyl ester have a better affinity against CoV-2 Spike glycoprotein-Human ACE-II complex than hydroxychloroquine with low μM K_i values among the evaluated compounds (Table 3). After successful docking of all the ligands employed in these docking experiments, the results showed significant binding of the ligands with the target proteins.

Table 3. Summary of reference and compounds against CoV-2 Spike RBD/ACE-II with binding energy, K_i and interacted residues in the CoV-2 Spike RBD/ACE-II binding site.

No	Ligand Name	Binding Energy (kcal/mol)	K_i (uM)	CoV-2 Spike RBD/ACE-II residues interacting with ligands
1	Catechin ((2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol)	-7.77	2.03	Asp206, Lys562, Val212, Leu91, Ser563, Glu564, Pro565, Leu95, Glu208, Val209, Trp566
2	Pinocembrin (5,7-Dihydroxy-2-phenyl-2,3-dihydro-4H-chromen-4-one)	-7.75	2.09	Asn210, Leu95, Pro565, Ser563, Leu91, Val212, Val209
3	Chrysin (5,7-dihydroxy-2-phenylchromen-4-one)	-7.70	2.27	Asn210, Ser563, Pro565, Val212, Leu95, Leu91, Val209
4	Caffeic acid phenethyl ester (Caffeic acid 2-phenylethyl ester; β-Phenylethylcaffeate) (2-Phenylethyl (2E)-3-(3,4-dihydroxyphenyl)acrylate)	-7.68	2.34	Asn210, Trp566, Ala396, Pro565, Lys562, Val209, Val212, Leu95, Lys94
5	p-OH Benzoic acid (4-hydroxybenzoic acid)	-6.00	39.76	Pro500, Trp477, Leu456, Leu503, Lys481, Trp271, Arg169
6	Syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid)	-5.94	44.15	Phe347, Asn450, Tyr351
7	*t*-Cinnamic acid ((E)-3-phenylprop-2-enoic acid)	-5.90	47.29	Trp477, Leu503, Trp271, Trp165, Pro500

8	*p*-Coumaric acid ((E)-3-(4-hydroxyphenyl)prop-2-enoic acid)	-5.88	49.01	Trp165, Arg169, Pro500, Trp477, Leu456, Leu503, Lys481, Trp271
9	Rutin (Quercetin-3-rutinoside hydrate)	-5.86	50.39	Asp30, Gln96, Pro389, Gln388, Ala386, Arg393, His34, Tyr453, Val417, Ile418, Asp406, Arg408
10	Ferulic acid ((E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid)	-5.55	84.91	Lys441, Pro289, Ile291, Phe438
11	Gallic Acid (3,4,5-trihydroxybenzoic acid)	-5.33	124.37	Asn450, Tyr351, Phe347
12	Hydroxychloroquine (2-[4-[(7-chloroquinolin-4-yl)amino]pentyl-ethylamino]ethanol)*	-7.53	3.04	Arg393, Phe390, Leu391, His401, Ala348, Asp350

*Reference molecule

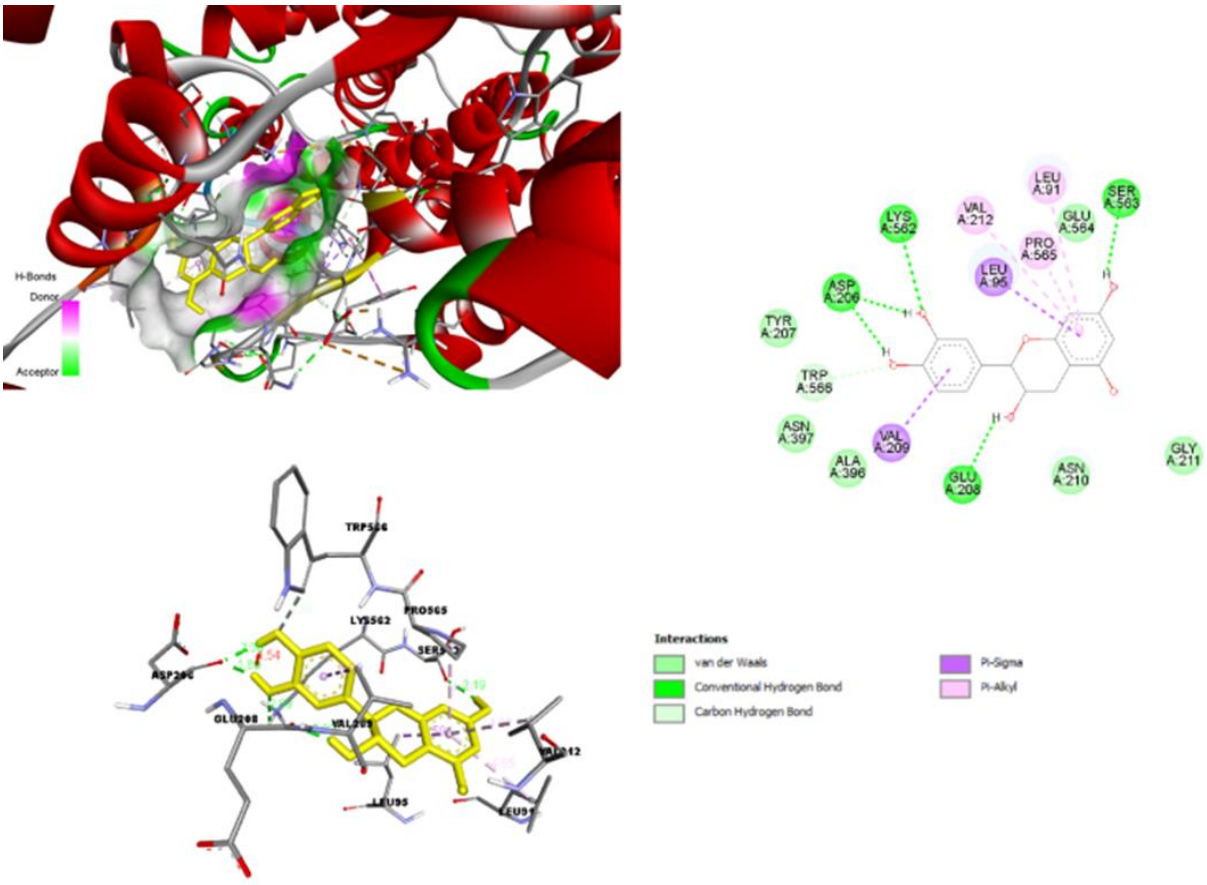


Figure 2. The two-dimension (2D) and three-dimension (3D) interaction analysis of Cov-2 Spike glycoprotein-Human ACE-II with cathechin.

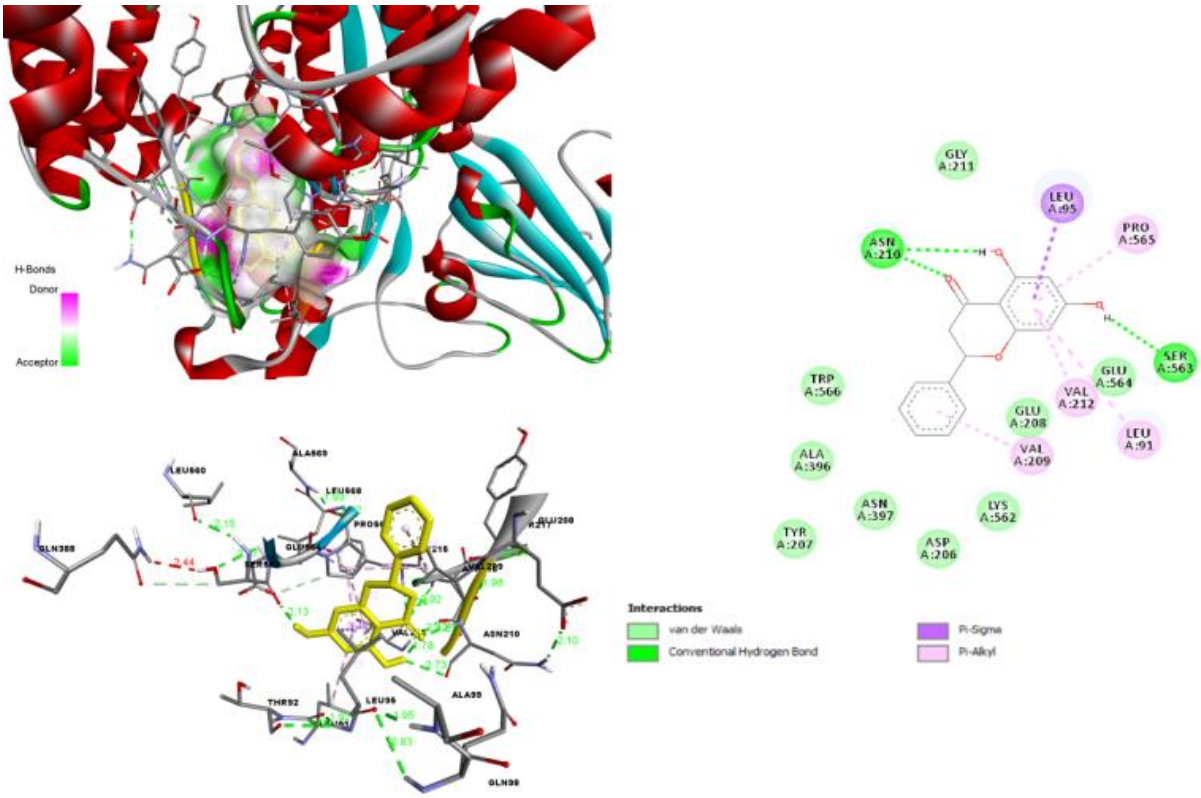


Figure 3. The two-dimension (2D) and three-dimension (3D) interaction analysis of Cov-2 Spike glycoprotein-Human ACE-II with pinocembrin

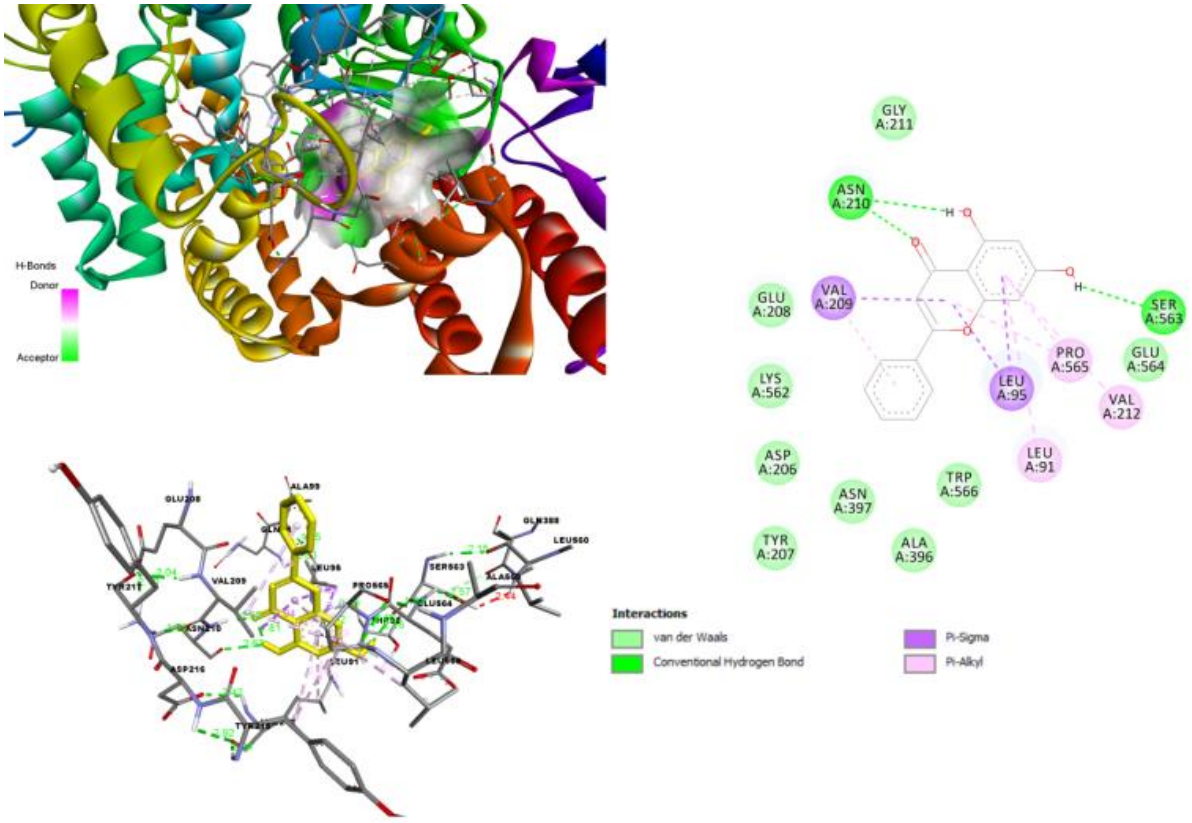


Figure 4. The two-dimension (2D) and three-dimension (3D) interaction analysis of Cov-2 Spike glycoprotein-Human ACE-II with chrysin.

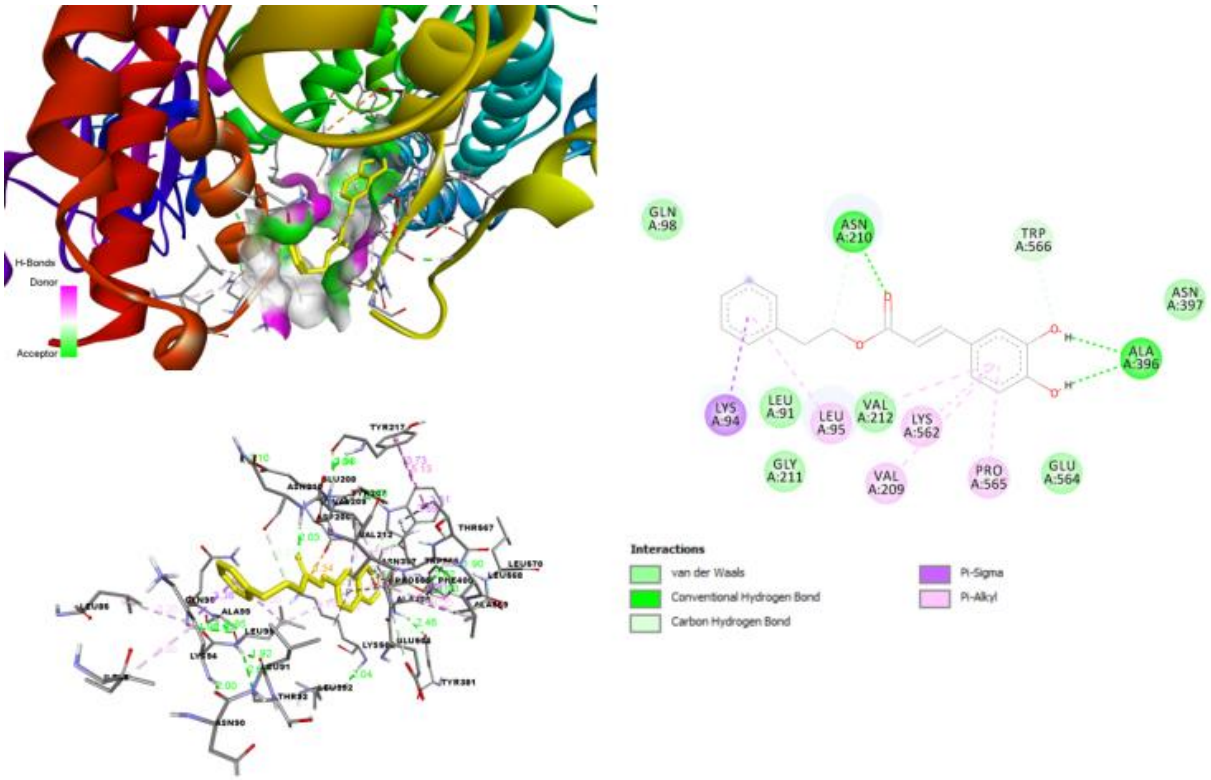


Figure 5. The two-dimension (2D) and three-dimension (3D) interaction analysis of Cov-2 Spike glycoprotein-Human ACE-II with CAPE.

Besides, these four compounds interacted with Val212, Pro565, Leu95 and Val209 in with Cov-2 Spike glycoprotein-Human ACE-II binding site. Especially, our docking study showed that, catechin has the best binding affinity to the Cov-2 Spike glycoprotein-Human ACE-II complex (Binding energy: -7.77kcal/mol, K_i 2.03 μM) whereas hydroxychloroquine has the lower binding affinity (Binding energy: -7.53kcal/mol, K_i 3.04 μM). The cathechin compound was observed to bind to the residues Asp206, Lys562, Val212, Leu91, Ser563, Glu564, Pro565, Leu95, Glu208, Val209, Trp566 of with Cov-2 Spike glycoprotein-Human ACE-II complex with the stronger hydrogen bond (Figure 2). It can be suggested that these residues can contribute to the enhancement of ligand affinity for with Cov-2 Spike glycoprotein-Human ACE-II complex. Furthermore, this molecule has the pi-sigma interaction with Leu95 and Val209, pi-alkyl interaction with Val212, Leu91 and Pro565 residues (Figure 2).

In recent years, flavonoids have gained a great amount of interest with regards to their potential for cardiovascular protection. In fact, many epidemiological studies associate an increased consumption of foods and beverages rich in flavonoids with a reduced risk of CVD death (Zhou et al., 2000; Joshipura et al., 2001; Kris-Etherton et al., 2002). Additionally, several of these flavonoids or their derivatives (i.e., diosmin, rutin and quercetin) are widely used as pharmaceutical agents for their vasoprotective properties (Muhammad et al., 2015).

Therefore, in this study, *in silico* effects of Cistus L. bee polen on Cov-2 Spike glycoprotein-Human ACE-II complex inhibition was

investigated with the eleven flavonoids as major substances. The results of this study showed that catechin, pinocembrin, chrysin and caffeic acid phenethyl ester compounds effectively inhibit the Cov-2 Spike glycoprotein-Human ACE-II complex. These compounds can be clinically tested and used for the treatment of Covid-19. Furthermore, Asp206, Lys562, Val212, Leu91, Ser563, Glu564, Pro565, Leu95, Glu208, Val209, Trp566 are the potential inhibitor targeting sites for the on Cov-2 Spike glycoprotein-Human ACE-II complex. Based on this information, we propose guidelines to develop novel and specific inhibitors that target on Cov-2 Spike glycoprotein-Human ACE-II complex.

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