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Research Article

Evaluation of phenolic acids of *Corylus avellana* L. as potential SARS CoV-2 Main protease inhibitors

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Abstract

The novel human coronavirus, called SARS-CoV-2, first appeared in late 2019 in Wuhan, causing a respiratory disease termed COVID-19 in China and has been declared a pandemic worldwide. Although many different vaccine development studies against SARS-CoV-2 have reached the final stage, the protection that these vaccines will provide to society is not known for now, and therefore, effective antiviral drugs should be developed. In this study, the effects of the phenolic acids found in *Corylus avellana* L. on SARS-CoV-2 Main protease (Mpro) were investigated by molecular docking analysis. Also, the pharmacophore properties, biological properties, pharmacokinetics and drug-likeness properties of the compounds examined in the study were evaluated. Molecular docking of Mpro and phenolic acids was done with Autodock Vina. Many of the phenolic acids investigated in the study have interacted with the active site and catalytic residues of the Mpro. Drug similarity of phenolic acids interacting with MPro and each of the interacting compounds were found to be potential target inhibitors against SARS-CoV-2 Mpro and it was determined that its use would have limited or no side effect on the body.

Keywords: SARS-CoV-2, molecular docking, bioactivity, pharmacophore features, *Corylus avellana* L.

Potansiyel SARS CoV-2 Ana proteaz inhibitörleri olarak *Corylus avellana* L.'nin fenolik asitlerinin değerlendirilmesi

Öz

SARS-CoV-2 adı verilen yeni insan koronavirüsü, ilk olarak 2019'un sonlarında Wuhan'da ortaya çıktığında Çin'de COVID-19 adlı bir solunum hastalığına neden oldu ve dünya çapında bir pandemi ilan edildi. SARS-CoV-2'ye karşı birçok farklı aşı geliştirme çalışması son aşamaya gelmiş olsa da bu aşıların topluma sağlayacağı koruma şimdilik bilinmemektedir ve bu nedenle etkili antiviral ilaçlar geliştirilmelidir. Bu çalışmada, *Corylus avellana* L.'de bulunan fenolik asitlerin SARS-CoV-2 ana proteaz (Mpro) üzerindeki etkileri moleküler yerleştirme analizi ile araştırılmıştır. Ayrıca çalışmada incelenen bileşiklerin farmakofor özellikleri, biyolojik özellikleri, farmakokinetikleri ve ilaca benzerlik özellikleri de değerlendirilmiştir. Mpro ve fenolik asitlerin moleküler yerleştirmesi Autodock Vina ile yapıldı. Çalışmada araştırılan fenolik asitlerin çoğu, Mpro'nun aktif bölgesi ve katalitik kalıntıları ile etkileşime girmiştir. MPro ile etkileşime giren fenolik asitlerin ilaç benzerliği ve etkileşen bileşiklerin her birinin SARS-CoV-2 Mpro'ya karşı potansiyel hedef inhibitörler olduğu bulundu ve kullanımının vücut üzerinde sınırlı veya hiçbir yan etkisi olmayacağı belirlendi.

Anahtar Kelimeler: SARS-CoV-2, moleküler yerleştirme, biyoaktivite, farmakofor özellikleri, *Corylus avellana* L.

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1. Introduction

The novel human coronavirus, called SARS-CoV-2, first appeared in late 2019 in Wuhan, causing a respiratory disease termed COVID-19 in China and has been declared a pandemic worldwide (Lu et al., 2020; Zhou et al., 2020). The global dissemination of COVID-19 disease caused by SARS CoV-2 is causing severe acute respiratory syndrome. This viral disease poses a major threat to global public health (Tang et al., 2020). Coronaviruses are classified into four subfamilies according to their form and host. Delta and gamma coronaviruses are thought to originate from pigs and birds, while the alpha-coronavirus and beta-coronavirus are thought to originate from bats (Banerjee et al., 2019; Paules et al., 2020). The RNA genome of this virus, called SARS-CoV-2, is approximately 82% similar to the SARS coronavirus (SARS-CoV) and both are beta-coronavirus viruses (Gorbalenya et al., 2020). SARS-CoV-2 is penetrating a human cell membrane using the angiotensin-converting enzyme 2 (ACE2) (Bourgonje et al., 2020). In a viral infection, the spike protein interacts with sensitive human cells. Once the coronavirus enters the cells, they adapt to the human host by facilitating the expression of the genes encoding the necessary proteins and encoding the genome. Genome change is done by coronavirus through gene change, recombination, gene insertion or deletion mechanisms (Graham and Baric, 2010; Belouzard et al., 2012). The SARS-CoV-2 incubation period varies between 7 and 14 days (Lai, et al., 2020). Some researchers concentrated on possible combinations of the lopinavir-ritonavir protease inhibitor, widely used in human immunodeficiency virus (HIV) patients, to treat COVID-19. However, this was not enough to stop the outbreak and treat all patients (Lu, 2020). The measures implemented are limited to avoiding complications and losses through preventive and supportive care (Rodríguez-Morales et al., 2020). Several studies were carried out to find proper Mpro inhibitors by re-investigating the effectiveness of drugs (Muralidharan et al., 2020; Kandeel et al., 2020). Some studies have shown that several well-known antiviral medicines (Kumar et al., 2020) and vitamins (Arun et al., 2020) have the possibility for SARS CoV-2 Mpro activity to be inhibited. Furthermore, various phytochemicals have been reported to be effective as major protease inhibitors.

One of the promising targets for the treatment of COVID-19 is the main protease. The 3C-like protease is referred to as 3CLpro and Mpro as it functions as the main protease enzyme. The 3CLpro enzyme of SARS-CoV-2 processes polyproteins to release a functional polypeptide by proteolytic action in the replicase enzyme (pp1a or pp1ab) (Lee et al., 2020). It divides into polyproteins and forms distinct proteins at various locations downstream. With extensive proteolytic processing, functional polypeptides are released from polyproteins primarily by a master protease (Mpro) of 33.8 kDa (Xia and Kang, 2011; Hegyi and Ziebuhr, 2002). The majority of the active site was found to contain residue 41, 140, 142-145, 161, 163, 166 and 172, and His41 and Cys145 play a significant catalytic role (Zhang et al., 2020).

Corylus avellana L. (hazelnut) is from the *Betulaceae* family and is a shrub that can grow up to 15 meters (Rushforth, 1999). It contains valuable nutrients such as plant proteins, vitamins of the B complex, vitamin E, essential minerals, and micronutrients such as plant sterols, tocopherols, unsaturated fatty acids and phytochemicals (Alasalvar et al., 2003; Kornsteiner et al., 2006). Several studies examine the curative impacts on the health of hazelnuts and their phytochemicals. Studies have reported that it improves memory and decreases neuroinflammation (Bahaeddin et al., 2017), reduces reduced low-density lipoprotein oxidation (Di Renzo et al., 2017), reduces metabolic syndrome in Alzheimer's type

neurodegeneration (Mollica et al., 2018), and increases the anti-inflammatory gene expression (Cappelli et al., 2018). The phenolic acid definition describes phenols of a functional group of carboxylic acids. The phenolic acids which naturally occur are classified into two groups, hydroxybenzoic acids and hydroxycinnamic acids (Masullo et al., 2015). The hazelnut tree kernel, called hazelnut, can be consumed raw or roasted. It is widely used as an ingredient in candies and cakes (chocolate, breakfast cereals, chopped nuts, pralines, nuts, nougat cookies and icecreams) or as a processed formulation, in the food industry (Platteau et al., 2011). There is no evidence in the literature that it is used pharmacologically or in drug development. This once again proves the importance of this study and its contribution to the field.

Molecular docking studies as *in silico* are a useful method for detecting and verifying the activities of chemical compounds. Researchers have used this method to investigate many different interactions. These methods were used by Shah et al. (2019) to determine the activity of mefenamic acid as a human cyclooxygenase-2 inhibitor, by Mohanty and Bhatnagar (2019) to investigate compound activities for adhesion kinase-growth factor receptor 2, and by Elfiky (2020) for determining effective drugs against SARS-CoV-2 virus.

Although many different vaccine development studies against SARS-CoV-2 have reached the final stage, the protection that these vaccines will provide to society is not known for now, and therefore, effective antiviral drugs should be developed. Therefore, to investigate potential and specific inhibitors of SARS-CoV-2 in this study, virtual screening was performed to determine the efficacy of phytochemicals found in *Corylus avellana* L. against SARS-CoV-2.

2. Material and Methods

Determination of phytochemical library

The phytochemicals found in *Corylus avellana* L. were determined by searching the literature. *Corylus avellana* L. has been reported to contain mainly gallic acid, salicylic acid, syringic acid, vanillic acid, p-hydroxybenzoic acid, 4-hydroxysalicylic acid and protocatechuic acid hydroxybenzoic acid derivatives, and derivatives of hydroxycinnamic acid as caffeic acid, ferulic acid, o-coumaric acid and sinapic acid (Prosperini et al., 2009; Pelvan et al., 2018).

Protein receptors preparation

3D structure of Mpro of SARS-CoV-2 main protease (PDB ID: 7BQY) (Jin et al., 2020) at 1.70 Å resolution was downloaded from Protein Data Bank. Protein was prepared using Biovia Discovery Studio 2020 Client for docking. The active site of the protein molecule was determined, then all ions, ligands and water molecules were extracted and the receptor molecule was loaded in with polar hydrogen atoms. Energy minimization of protein was performed with Gromos 43B1 using Swiss-PdbViewer v.4.1.0 (Guex and Peitsch, 2005) software and saved as PDB for analysis.

Ligand preparation

The 3-dimensional structures of gallic acid, salicylic acid, syringic acid, vanillic acid, p-hydroxybenzoic acid, 4-hydroxysalicylic acid, protocatechuic acid (hydroxybenzoic acid derivatives), caffeic acid, ferulic acid, o-coumaric acid and sinapic acid (hydroxycinnamic acid derivatives) used in the study were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov) in SDF format, and then uff-force field energy

minimization and conversion to PDB format were performed with Open Babel v.2.4.0 software (O'Boyle et al., 2011).

Molecular docking

MGL Tools (Morris et al., 2009) was carried out to create a grid box to include active areas, and the size of the grid box was determined and saved to cover the determined active areas. Molecular docking was done with Autodock Vina software (Trott & Olson, 2010). Confirmation with the lowest Vina score and lowermost root mean square deviation (RMSD) values were selected.

Visualization

Analysis of the interactions of the receptor-ligand structure of the complex was performed with the Biovia Discovery Studio 2020 Client software to identify interactions of an amino acid of a receptor with a ligand. Docking analysis, interactions, 2D and 3D visualizations were carried out with this program.

ADME and drug-likeness activity

Pharmacophore features and biological activity prediction

Drug affinity properties of phenolic acids were analyzed using the Molinspiration tool (Molinspiration, 2020). For this, the molecular properties, pharmacophore features and bioactivities of the compounds were estimated using the molinspiration interface. Pharmacophore features determined by logP, total polar surface area, number of atoms, molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, number of Lipinski's rule of five parameters violations and number of rotatable bonds. Biological activity prediction includes G-protein-coupled receptor (GPCR) ligands, ion channel modulator, nuclear receptor ligand, kinase and protease enzyme inhibitors.

Pharmacokinetics and drug-likeness properties

ADME (absorption, delivery, metabolism, elimination) provides an understanding of the drug-likeness properties of chemical compounds. The desired physicochemical properties of pharmacologically active drugs were specified in the Lipinski rules. Physicochemical properties and ADME specifications can be estimated as *in silico*. The SwissADME online server was used to predict the different pharmacokinetic properties of phytochemicals (Daina et al., 2017). Drug-likeness properties of these phytochemicals, such as absorption, distribution, metabolism and excretion (ADME) parameters, were mainly studied.

3. Research Findings

After successful docking of all the ligands used in these docking experiments, the results showed that the ligands bind significantly to the target protein. The lowest values of the binding affinities and the lowest RMSD values were preferred as the best docking pose. Molecular docking analyzes of hydroxybenzoic acid derivatives are given in Table 1. The docked complexes of seven ligands, which are hydroxybenzoic acid derivatives, with Mpro, show that hydrogen bonds (without vanillic acid) and hydrophobic interactions of these ligands with Mpro residues are located in the active site. Salicylic acid showed the best

binding affinity among hydroxybenzoic acid derivatives (-5.5 kcal/mol), the lowest binding affinity was observed in p-hydroxybenzoic acid (-4.6 kcal/mol).

Molecular docking of hydroxybenzoic acid derivatives with Mpro

Gallic acid formed four hydrogen bond interactions with Glu166, Leu14, Cys145 and Gly143, as well as hydrophobic interaction with Cys145 (Figure 1). It showed a binding affinity of 5.1 kcal/mol. Interaction with the catalytic residue Cys145, establishing both hydrogen bonding and hydrophobic interactions showed that gallic acid can be effective on Mpro.

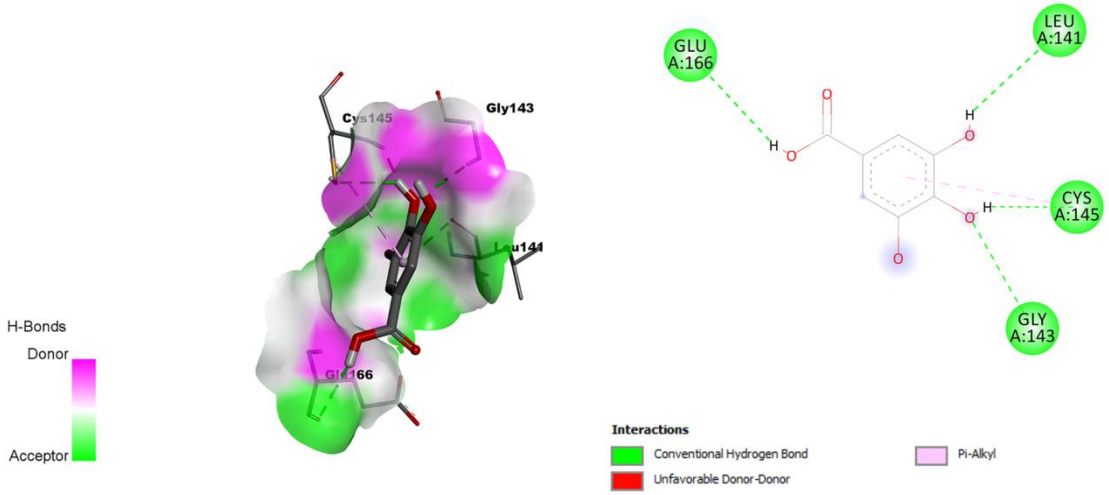

Figure-1. The docked pose of gallic acid with main protease (7BQY) and ligand interaction of gallic acid with 7BQY.

Salicylic acid had two hydrogen bond interactions with Glu166 and Gln1890, hydrophobic interactions with His41 (twice) and Met165 (Figure 2). The interaction showed a binding affinity of -5.5 kcal/mol. The interaction with the catalytic residue His 41 and Glu166 at the active site showed that this chemical compound can act on the main protease.

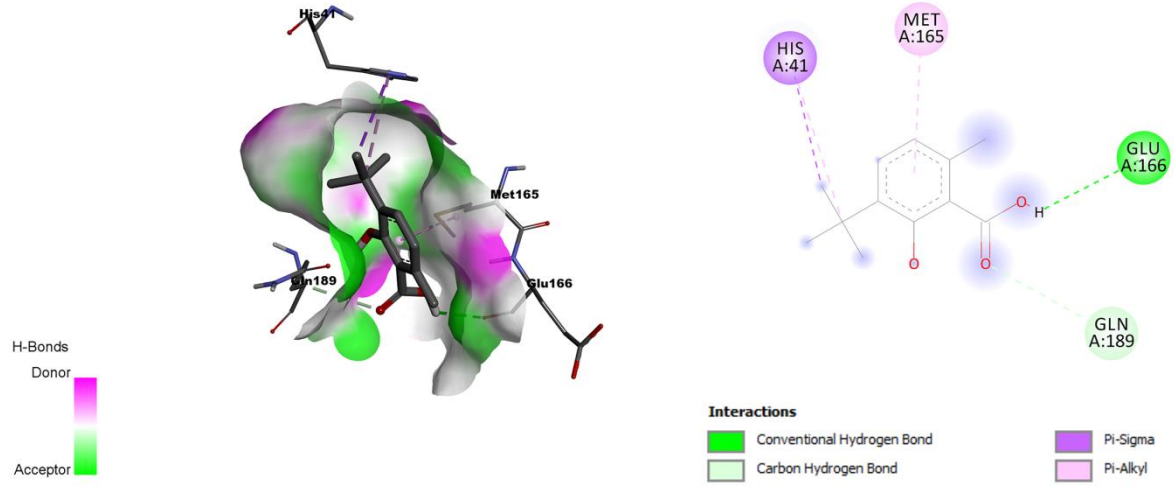

Figure-2. The docked pose of salicylic acid with main protease (7BQY) and ligand interaction of salicylic acid with 7BQY.

The syringic acid made hydrogen bond interactions with the catalytic residue Cys145 and with Gly143, Phe140, Glu166 in the active site. It also interacted with Gln189 by hydrogen bonding. It performed hydrophobic interactions with Asn142, Cys145 (twice), His41, and His163 at the active site (Figure 3). Docking showed a binding affinity of -5.1 kcal/mol. Hydrogen bonding and hydrophobic interactions with many residues in the active site showed that this compound may be the most effective on Mpro among the hydroxybenzoic acid derivatives studied.

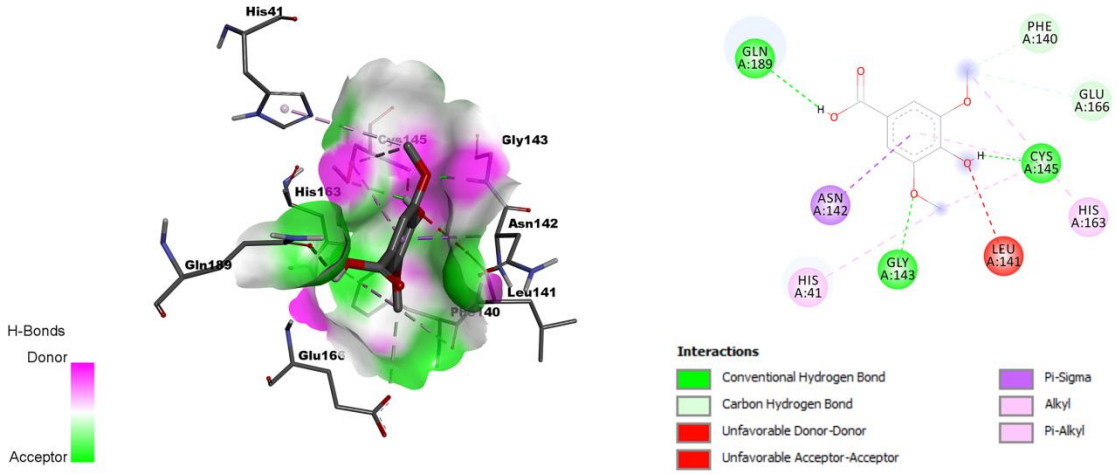

Figure-3. The docked pose of syringic acid with main protease (7BQY) and ligand interaction of syringic acid with 7BQY.

Vanillic acid did not perform any hydrogen bond interactions with Mpro residues, but hydrophobic interactions with the catalytic residues His41 (twice) and Cys145 (Figure 4). It also has a hydrophobic interaction with Met165. Docking showed a binding affinity of -4.9 kcal/mol. Although it shows weak hydrophobic interactions, its effect on catalytic residues indicates that this compound may be slightly effective.

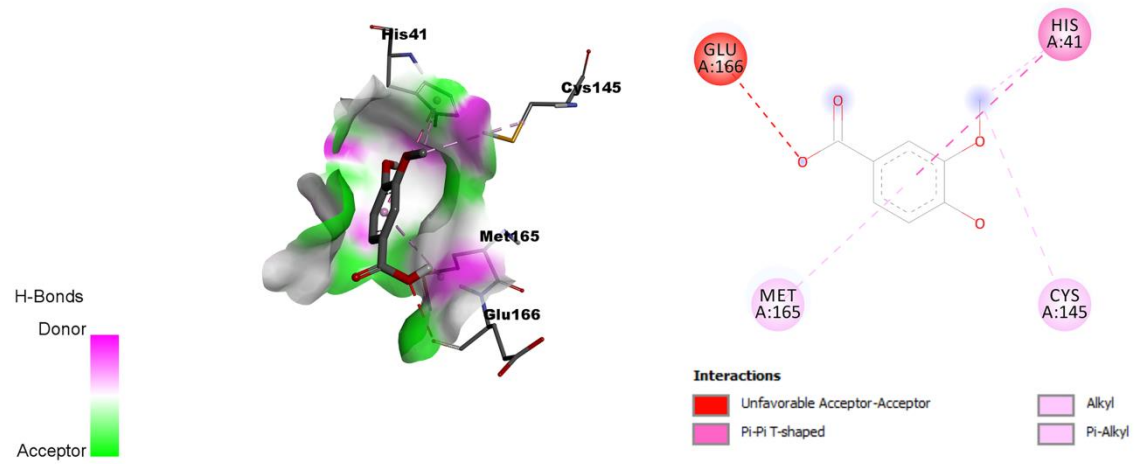

Figure-4. The docked pose vanillic acid with main protease (7BQY) and ligand interaction of vanillic acid with 7BQY.

P-hydroxybenzoic acid performed three hydrogen bond interactions with Met49, His164 and Cys44, had hydrophobic interaction with Met49 (Figure 5) and showed a binding affinity of -

4.6 kcal /mol. It did not interact with the catalytic residues His 41 and Cys145. No interaction occurred with the active site and the catalytic residues, indicating that its activity on Mpro is low.

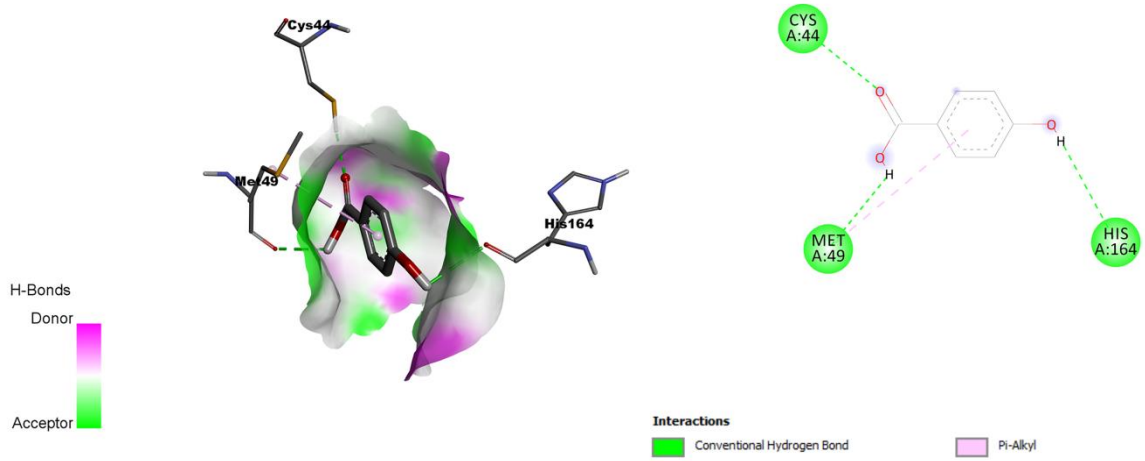

Figure-5. The docked pose of p-hydroxybenzoic acid with main protease (7BQY) and ligand interaction of p-hydroxybenzoic acid with 7BQY.

4-hydroxysalicylic acid has realized four hydrogen bond interactions with Ser144 (twice), His163 and Gly143 in the active site. The catalytic residue Cys145 had a hydrophobic interaction with the ligand (Figure 6). Docking showed a binding affinity of -5.0 kcal/mol. The hydrogen bonding and hydrophobic interactions with the residues in the active site showed that this compound can be effective on Mpro.

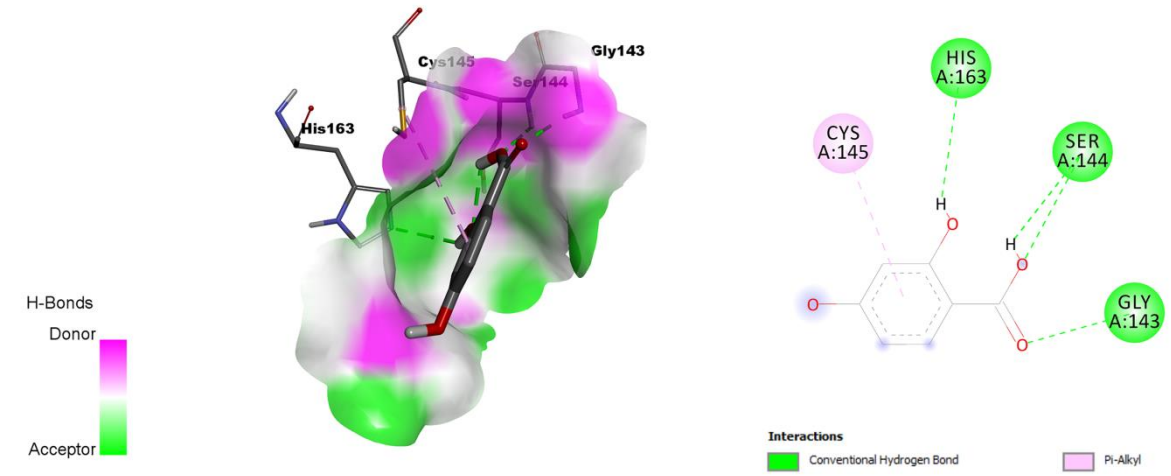

Figure-6. The docked pose of 4-hydroxysalicylic acid with main protease (7BQY) and ligand interaction of 4-hydroxysalicylic acid with 7BQY.

Protocatechuic acid has realized two hydrogen bond interactions with Glu166 and His163 located in the active site. The catalytic residue had a hydrophobic interaction with Cys145 (Figure 7). Docking showed a binding affinity of -4.9 kcal/mol. The hydrogen bonding and

hydrophobic interactions with the residues in the active site showed that this compound can be effective on Mpro.

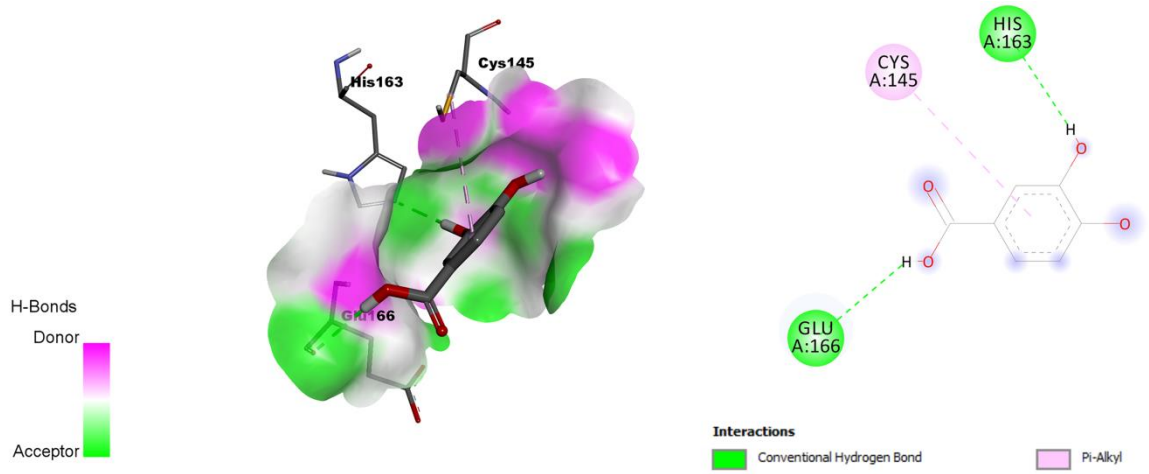

Figure-7. The docked pose of protocatechuic acid with main protease (7BQY) and ligand interaction of protocatechuic acid with 7BQY.

Molecular docking of hydroxycinnamic acid derivatives with Mpro

Caffeic acid made hydrogen bond interaction with Arg188 and no hydrophobic interaction occurred (Figure 8). Also, Met165 and pi-sulfur interaction have been realized. Docking showed a binding affinity of -5.5 kcal/mol. The absence of hydrogen bonding and hydrophobic interactions with the residues in the active site showed that this compound could not be effective on Mpro.

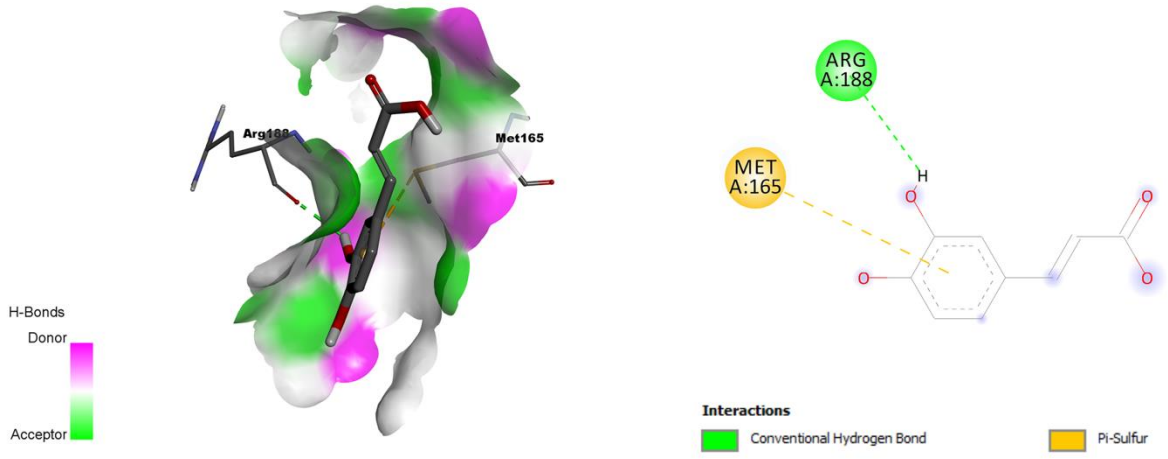

Figure-8. The docked pose of caffeic acid with main protease (7BQY) and ligand interaction of caffeic acid with 7BQY.

Ferulic acid carried out hydrogen bond interaction with the catalytic residue Cys145 and also with Arg188. Besides, hydrophobic interactions occurred with the catalytic residue His41 (twice) and also with Met165 (Figure 9). Docking showed a binding affinity of -5.7 kcal/mol.

The hydrogen bonding and hydrophobic interactions with catalytic residues showed that this compound could be effective on Mpro.

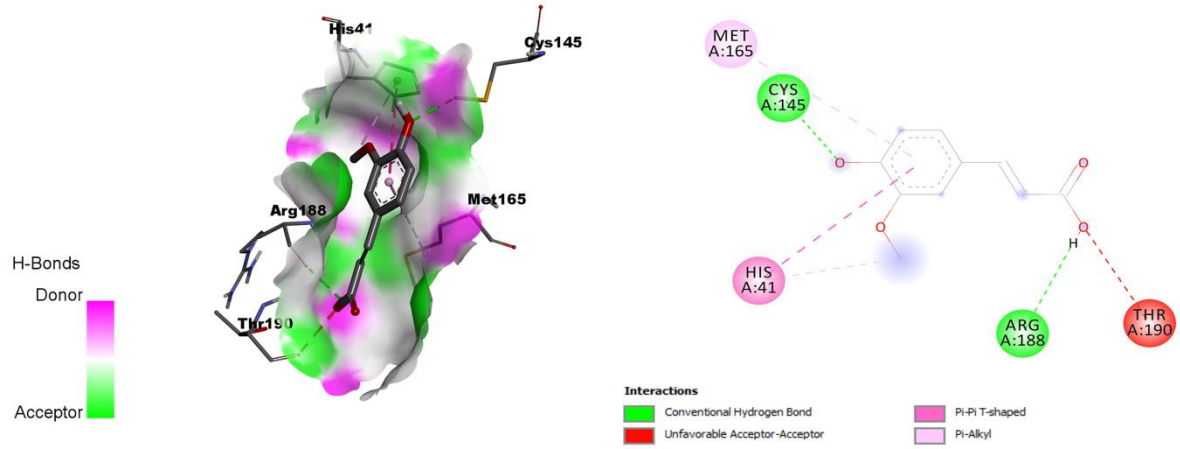

Figure-9. The docked pose of ferulic acid with main protease (7BQY) and ligand interaction of ferulic acid with 7BQY.

O-coumaric acid made hydrogen bonding interaction with Leu141 and hydrophobic interaction with Met165 (Figure 10). Also, the catalytic residue Cys145 and pi-sulfur interaction have been realized. Docking showed a binding affinity of -5.2 kcal/mol. The lack of hydrogen bonding and hydrophobic interactions with residues in the active site showed that this compound may be slightly effective on Mpro.

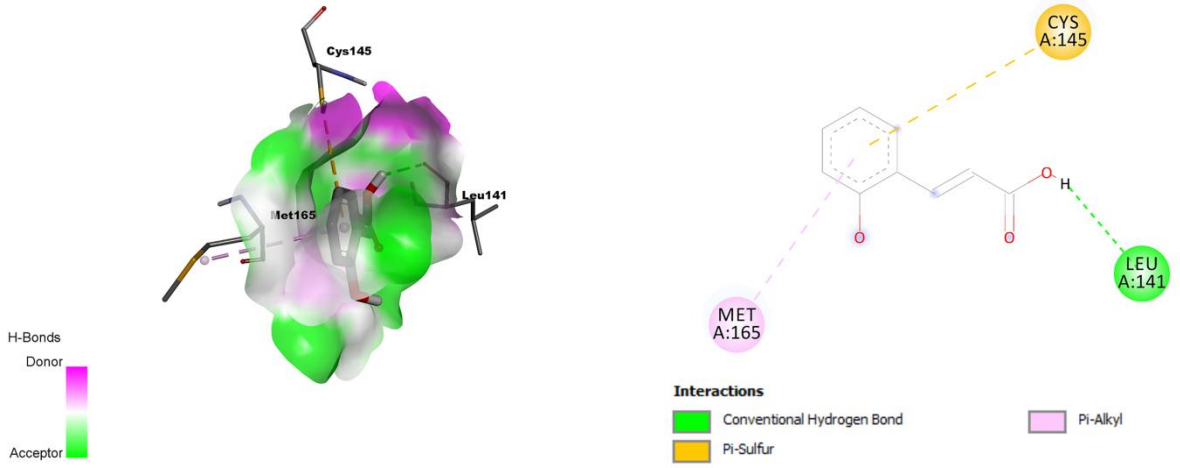

Figure-10. The docked pose of o-coumaric acid with main protease (7BQY) and ligand interaction of o-coumaric acid with 7BQY.

Sinapic acid made hydrogen bond interaction with His164 and Thr190 residues. Hydrophobic interactions occurred with catalytic residues His41 (twice) and Cys145, as well as with Met165 (Figure 11). Docking showed a binding affinity of -5.9 kcal/mol. The weak hydrophobic interactions with catalytic residues indicated that this compound may have some effect on Mpro.

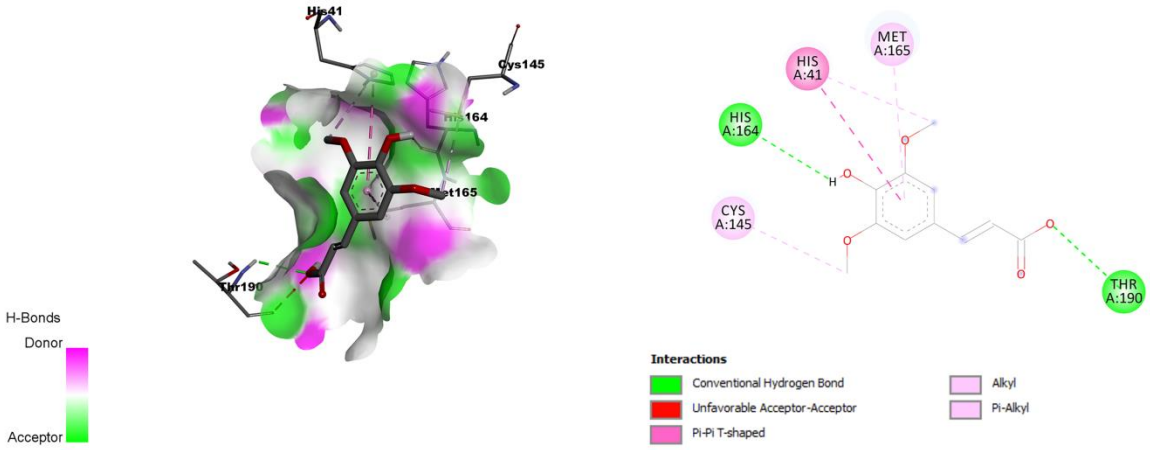

Figure-11. The docked pose of sinapic acid with main protease (7BQY) and ligand interaction of sinapic acid with 7BQY.

Table 1. Binding affinities and interactions of phenolic acids of *Corylus avellana* L. with SARS-CoV-2 Mpro

	Compound	Binding Affinity (kcal/mol)	Hydrogen binding interactions	Distance (Å)	Hydrophobic interactions	Distance (Å)
hydroxybenzoic acid derivatives	gallic acid	-5.1	GLU166 (N—H...O) LEU141 (N—H...O) CYS145 (N—H...O) GLY143 (HN...O)	2.65 2.71 2.76 2.67	CYS145	4.82
	salicylic acid	-5.5	GLU166 (N—H...O) GLN189 (HA...O)	2.47 2.45	HIS41 MET165 HIS41	3.49 4.81 4.45
	syringic acid	-5.1	GLN189 (N—H...OE1) CYS145 (HN...SG) GLY143 (HN...O) PHE140 (N—C...O) GLU166 (N—C...OE2)	2.45 2.63 1.95 3.52 3.78	ASN142 CYS145 CYS145 HIS41 HIS163	2.92 3.90 4.86 5.21 5.06
	vanillic acid	-4.9	-	-	HIS41 CYS145 MET165 HIS41	5.57 4.48 4.51 3.99
	p-hydroxybenzoic acid	-4.6	MET49 (N—H...O) HIS164 (N—H...O) CYS44 (HG...O)	2.37 2.71 2.37	MET49	4.80
	4-hydroxysalicylic acid	-5.0	SER144 (N—H...OG) HIS163 (N—H...NE2) GLY143 (HN...O) SER144 (HN...O)	2.39 2.62 1.98 2.06	CYS145	5.16
	protocatechuic acid	-4.9	GLU166 (N—H...O) HIS163(N—H...NE2)	2.32 2.14	CYS145	5.04
hydroxycinnamic acid derivatives	caffeic acid	-5.5	ARG188 (N—H...O)	2.23	-	-
	ferulic acid	-5.7	ARG188 (N—H...O CYS145 (HG...O)	2.35 2.46	HIS41 MET165 HIS41	5.58 5.09 4.07
	o-coumaric acid	-5.2	LEU141 (N—H...O)	2.32	MET165	5.15
	sinapic acid	-5.9	HIS164 (N—H...O) THR190 (HN...O)	2.86 2.79	HIS41 CYS145 MET165 HIS41	5.61 4.81 5.06 4.04

Pharmacophore features

Lipinski's rule of five states that, in general, an orally active drug has not more than 10 hydrogen bond acceptors (notably N and O), not more than 5 hydrogen bond donors (OH and NH groups), partition coefficient log P less than 5, molecular weight under 500 g/mol and number of violation less than 4 (Lipinski et al., 1997). All the compounds were found in compliance with Lipinski's rule of five and the results are reported in Table 2. The Log P calculation gives information on bioavailability regarding absorption, solubility and permeability. It must not be too hydrophilic to cross the gastrointestinal wall or too lipophilic to be absorbed in order to be able to act like a chemical drug. Low hydrophilicity and low LogP values can cause good permeability and absorption. The LogP (octanol/water partition coefficient) was calculated as the sum of part-based additives and correction factors using the methodology developed by Molinspiration. The Log*P* values must be less than 5.0 for the compounds to be likely to be absorbed. The method is reliable and can handle almost any organic molecule. Molecular Polar Surface Area (TPSA) is calculated as the sum of particle additives. Polar fragments with O and N centers are considered. PSA has proved to be a very strong drug-related descriptor, including intestinal uptake, blood-brain barrier penetration and bioavailability (Parvez et al., 2010). The pharmacophore properties of phenolic acids are given in Table 2. When the data were examined, Log P values of all phenolic acids, both hydroxybenzoic acid and hydroxycinnamic acid derivatives used in the study were less than 5; this shows that, in compliance with Lipinski's rules, the phenolic acids investigated can cross biomembranes. Molecules with a PSA value of less than 140 Å are expected to show better intestinal absorption. According to the data in Table 2, absorption of both hydroxybenzoic acid and hydroxycinnamic acid derivatives from the intestine is expected to be good.

Table 2. Pharmacophore features of phenolic acids

Compound		Log*P*	TPSA	natoms	MW	nON	nOHNH	nviol	nrotb	volume
hydroxybenzoic acid derivatives	gallic acid	0.59	97.98	12	170.12	5	4	0	1	135.10
	salicylic acid	3.3	57.53	15	208.26	3	2	0	2	201.81
	syringic acid	1.2	76.00	14	198.17	5	2	0	3	170.15
	vanillic acid	1.19	66.76	12	168.15	4	2	0	2	144.61
	p-hydroxybenzoic acid	1.37	57.53	10	138.12	3	2	0	1	119.06
	4-hydroxysalicylic acid	1.37	77.75	11	154.12	4	3	0	1	127.08
	protocatechuic acid	0.88	77.75	11	154.12	4	3	0	1	127.08
hydroxycinnamic acid derivatives	caffeic acid	0.94	77.75	13	180.16	4	3	0	2	154.50
	ferulic acid	1.25	66.76	14	194.19	4	2	0	3	172.03
	o-coumaric acid	1.67	57.53	12	164.16	3	2	0	2	146.48
	sinapic acid	1.26	76.00	16	224.21	5	2	0	4	197.57

TPSA: Total polar surface area (drug transport properties), natoms: Number of atoms, MW: Molecular weight (g/mol), nON: Number of hydrogen bond acceptors, nOHHN: Number of hydrogen bond donors, nviol: Number of Lipinski's rule of five parameters violations, nrotb: Number of Rotatable Bonds (molecular flexibility).

Biological activities

The biological activity of all hydroxybenzoic acid and hydroxycinnamic derivatives investigated in the study was analyzed under the criteria of G-protein-coupled receptor (GPCR) ligands, ion channel modulator, nuclear receptor ligand, kinase and protease enzyme inhibitors and are given in Table 3. The probability of bioactivity for organic molecules, bioactivity value is greater than 0.00 for the active, between -0.50 to 0.0 then moderately active, and inactive if less than -0.50. The data showed that almost all of the hydroxybenzoic acid and hydroxycinnamic derivatives were either inactive or moderately active in terms of the biological activities investigated.

Table 3. Biological activities of phenolic acids

	Compound	GPRC L	ICM	KI	NRL	PI	EI
hydroxybenzoic acid derivatives	gallic acid	-0.77	-0.26	-0.88	-0.52	-0.94	-0.17
	salicylic acid	-0.42	-0.12	-0.56	0.1	-0.74	0.04
	syringic acid	-0.65	-0.28	-0.69	-0.44	-0.82	-0.15
	vanillic acid	-0.85	-0.42	-0.99	-0.61	-1.12	-0.35
	p-hydroxybenzoic acid	-0.98	-0.39	-1.21	-0.62	-1.19	-0.41
	4-hydroxysalicylic acid	-0.81	-0.33	-0.99	-0.5	-1.02	-0.28
	protocatechuic acid	-0.88	-0.35	-1.1	-0.58	-1.09	-0.34
hydroxycinnamic acid derivatives	caffeic acid	-0.48	-0.23	-0.81	-0.1	-0.79	-0.09
	ferulic acid	-0.47	-0.3	-0.72	-0.14	-0.81	-0.12
	o-coumaric acid	-0.64	-0.37	-0.97	-0.25	-0.9	-0.21
	sinapic acid	-0.32	-0.2	-0.47	-0.03	-0.56	0.03

GPCR L: G-protein-coupled receptor ligands, ICM: ion channel modulator, KI: kinase inhibitor, NRL: nuclear receptor ligand, PI: protease inhibitor, EI: enzyme inhibitor.

Pharmacokinetics

Pharmacokinetic properties such as gastrointestinal absorption (GI), blood-brain barrier (BBB) permeation, and cytochrome P450 (CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4) inhibitors are crucial for any compound to be considered a drug candidate. Analysis of Table 4 showed that both hydroxybenzoic acid and hydroxycinnamic acid derivatives showed good gastrointestinal absorption (GI). Only p-hydroxybenzoic acid and salicylic acid from hydroxybenzoic acid derivatives, and only o-coumaric acid and ferulic acid from hydroxycinnamic acid derivatives showed blood-brain barrier (BBB) penetration properties. Among the hydroxybenzoic acid derivatives, only gallic acid, 4-hydroxysalicylic acid and protocatechuic acid showed cytochrome p450 3A4 inhibition properties, and none of the hydroxyicinamic acid derivatives showed cytochrome p450 enzyme inhibition.

Table 4. Pharmacokinetics and drug-likeness properties of phenolic acids

	Compound	GI	BBB	P-gp	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
hydroxybenzoic acid derivatives	gallic acid	High	-	-	-	-	-	-	+
	salicylic acid	High	+	-	-	-	-	-	-
	syringic acid	High	-	-	-	-	-	-	-
	vanillic acid	High	-	-	-	-	-	-	-
	p-hydroxybenzoic acid	High	+	-	-	-	-	-	-
	4-hydroxysalicylic acid	High	-	-	-	-	-	-	+
	protocatechuic acid	High	-	-	-	-	-	-	+
hydroxycinnamic acid derivatives	caffeic acid	High	-	-	-	-	-	-	-
	ferulic acid	High	+	-	-	-	-	-	-
	o-coumaric acid	High	+	-	-	-	-	-	-
	sinapic acid	High	-	-	-	-	-	-	-

(+: active, -:not active)

4. Results

In this study, the effects of hydroxybenzoic acid and hydroxycinnamic acid derivatives, which are phenolic acids contained in *Corylus avellana* L., on SARS-Cov-2 main protease were investigated by molecular docking, drug-likeness and biological activities. It was determined by molecular docking analysis that all hydroxybenzoic acid derivatives except vanillic acid and all of the hydroxycinnamic acid derivatives showed hydrogen binding interactions and hydrophobic interactions with the active site of MPro. By molecular docking analysis, it was determined that hydroxybenzoic acid derivatives gallic acid, salicylic acid, 4-hydroxysalicylic acid and protocatechuic acid and hydroxycinnamic acid derivatives ferulic acid and sinapic acid were effective on MPro. Different studies are reporting that natural herbal compounds can be effective in drug studies to be developed for SARS-CoV-2. Cherrak et al. (2020) reported that flavonoids interact with the SARS-CoV-2 main protease and the effect of flavonoids against the virus should be investigated *in vivo* and *in vitro*, which supports our findings. Vijayakumar et al. (2020) investigated the interactions of 23 natural flavonoids with SARS-CoV-2 and consequently reported that flavonoids could be reasonable agents against SARS-CoV-2 inhibition and that *in vitro* studies should be conducted to understand the anti-SARS-CoV-2 properties. Sampangi et al. (2020) reported that natural herbal compounds show binding affinities on the SARS-CoV-2 main protease and this may inhibit viral replication. Aanouz et al. (2020) reported that 67 natural compounds found in different medicinal plants establish different binding affinities and interactions with the main protease, especially digitoxigenin, crocin, and β-Eudesmol can inhibit SARS-CoV-2.

ADME, drug similarity and biological activity analysis showed that all hydroxybenzoic acid and hydroxycinnamic acid derivatives in the study obeyed the five rules of Lipinski, and the

values of GPCR ligand, ion channel modulator, nuclear receptor ligand, kinase and protease enzyme inhibitors were at normal values. Also, it was determined that all these chemical compounds will show gastrointestinal absorption and those other than p-hydroxybenzoic acid, salicylic acid, o-coumaric acid and ferulic acid will not show BBB permeability. This shows that many of these phenolic acids active on MPro will have no or limited side effects on the body when used.

Drug similarity of phenolic acids interacting with MPro and each of the interacting compounds were found to be potential target potent inhibitors against SARS-CoV-2 Mpro and it was determined that its side effects would be limited or would no side effects. Considering the molecular docking and pharmacokinetic activities, it has been determined that gallic acid, salicylic acid, 4-hydroxysalicylic acid, protocatechuic acid and sinapic acid can be used to design antiviral drugs effective against SARS-CoV-2. Although vaccination studies against Covid-19 have reached the final stage, considering the mutations that the virus may suffer and the problems that may occur during the vaccination process, the importance of developing antiviral drugs against this virus comes out. For future studies, it will be useful to examine the metabolic processes of these phenolic acids and their effects on SARS-CoV-2 *in vitro* and *in vivo*.

In addition, the evaluation of *Corylus avellana* L., which is especially used in the food industry, by the pharmaceutical industry, and the investigation of its phytochemicals considering that it may be pharmacologically effective, will provide important gains in combating SARS-CoV-2 and other diseases.

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