Original Article

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Comparison of COVID-19 Virus Main Protease Inhibition Activities of Phenolic Acids By Molecular Docking

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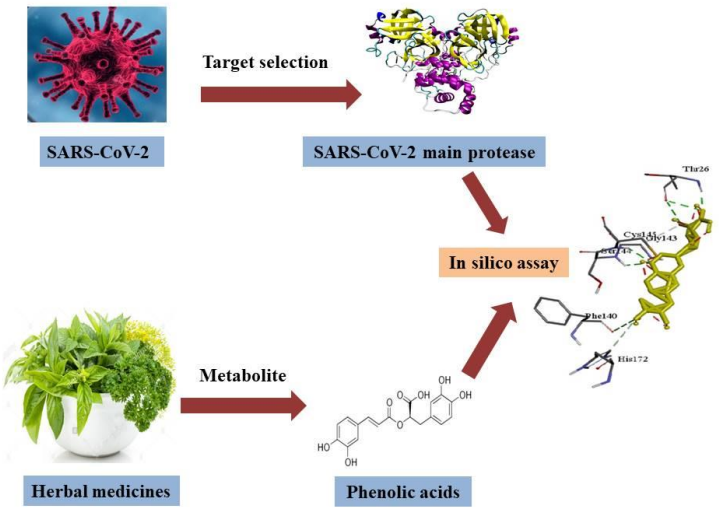
Graphical Abstract



Abstract

The Covid-19 pandemic is new challenge all around the world. This pandemic provides an emergency development of vaccines and drugs against this virus. As main proteases of CoV-19 have essential roles in the transmission and virulence of the virus. So, this enzyme has been considered as a critical target to inhibit COVID-19. Natural compounds are well known as rich sources of antiviral drugs due to their structural diversity and safety. In this study, we have screened thirteen phenolic acids (Cinnamic acid and Hydroxy benzoic derivatives) to compare the potential inhibitory activity of these molecules against COVID-19 protease. Systematic molecular docking simulation was done using AutoDock 4.2.6 to achieve the binding affinities and interactions between phenolic acids and protease. Cinnamic acids are better protease inhibitors in comparison with benzoic acid derivatives. Among Cinnamic acid derivatives, Rosmarinic acid exhibited the minimum Gibbs binding energy and highest docking scores. Also, Chorgenic > Ellagic > Ferulic acid could more effective respectively . According to obtained results, Rosmarinic acid has formed strong hydrogen bonding interaction with His163A, Ser144A, Cys145A, Gly143A and Thr26A, while

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the propyl cyclohexadione of Rosmarinic acid formed hydrophobic interaction with residue Gly 143A. It seems that Cinnamic acid derivatives of phenolic acid, particularly Rosmarinic acid could be efficient SARS-CoV 3CLpro inhibitors. In the next step, it is necessary to survey the effect of these natural products in inhibition of SARS-CoV replication through cell culture and in vitro assays. This study will improve preclinical knowledge about potential of natural compounds as SARS-CoV inhibitors.

Keywords: SARS-CoV-2, COVID-19, Phenolic acid, Main protease, Docking.

1. Introduction

Coronaviruses (CoVs) are characterized as single-stranded RNA viruses with large enveloped and positive senses. Different hosts such as avian, swine and humans can be infected by these viruses (1). Recently, this virus family is considered as a result of the emerging of two novel human coronaviruses from animal reservoirs. These two newborn viruses are severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV). They can be the cause of severe and life-threatening respiratory disease (2). The SARS-CoV genome encodes two large polyproteins functional proteins are formed by a proteolytic process of viral encoded cysteine proteases. These proteases are papain-like protease (PLpro) and the 3-chymotrypsin-like protease (3CLpro, main protease-Mpro). These proteases have essential roles in the transmission and virulence of the virus. As this function is critical, 3CLPro protease of SARS-CoV is a serious target for anti-SARS-CoV drug discovery and development (3, 4).

March 11, 2020, WHO has identified that Coronavirus disease 2019 (COVID-19) or (SARS-CoV-2) was the reason of pandemic outbreak. Consequently, the emergency demand for medicines, vaccines, diagnostics contributed to this virus raised (5). In this situation, as discovering a new drug is a time-consuming and costly process subsequently, repurposing is the proper strategy to determine potent molecules from the pre-existing molecules. One of the approaches is applying bioinformatics methods and molecular docking to screen existing drug or natural compounds against particular targets or cellular pathways (6).

During past decades, plants have been noticed as a source of therapeutic compounds and have been used in treatment protocols. Phenolic compounds, secondary metabolites of these plants, are responsible for various biological effects (7, 8).

They can be categorized as flavonoids, phenolic acids, tannins, stilbenes and lignans (8). They are famous antioxidant and anti-inflammation agents. Several researches revealed the potent antiviral activity of these bioactive compounds. Lin et al. reported anti-coronavirus activity of resveratrol (9). In addition, other studies showed that Forsythoside A (Forsythoside A) has antiviral effects against infectious bronchitisvirus (IBV), a virus belonging to the coronavirus family (10). Also, anti-IBV activity of the polyphenols of *Sambucus nigra* was reported by Chen *et al*. (11) it seems that this antiviral activity is related to synergistic effect of a large number of polyphenolic compounds against IBV (12).

Results from Tsai *et al*. investigation revealed that ethanol extract of *Sambucus formosana* Nakai and some phenolic acid constituents have potential antiviral against HCoVNL63. Phenolic acid constituents in extract showed antiviral activity in the following order Caffeic acid > Chlorogenic acid > Coumaric acid (13-16).

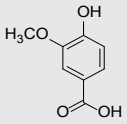
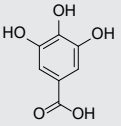
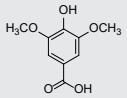
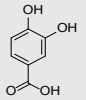
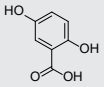
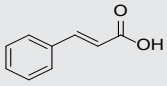
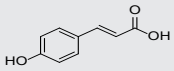
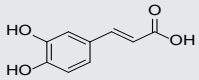
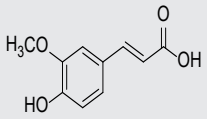
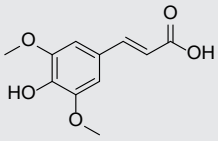
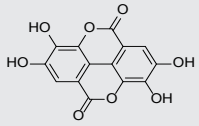
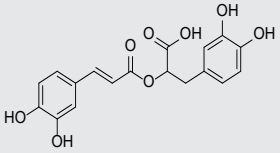
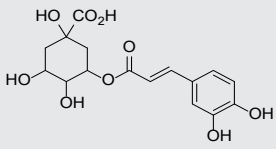
In this study, we studied Vanillic acid, Gallic acid, Syringic acid, Protocatechuic acid Gentisic acid, Cinnamic acid, P-cumaric acid, Caffeic acid, Ferulic acid, Sinapic acid, Ellagic acid, Rosmarinic acid and Chlorgenic acid as possible inhibitors for COVID-19 protease. The results would provide other researchers to consider novel candidate against COVID-19.

2. Materials and methods

2.1. Docking procedures

An in-house batch script (DOCK-FACE) for automatic running of AutoDock 4.2.6 was used to carry out the docking simulations (17) in a parallel mode (18). To prepare the receptor structure, the three-dimensional crystal structure of protease with an inhibitor N3 (PDB ID: 6LU7) (19) was

Table 1. Structure and characteristic of Cinnamic acid derivatives and Hydroxy benzoic acid derivatives.

Compound name	PubChem CID	Structure
Vanillic acid	72	
Gallic acid	370	
Syringic acid	3469	
Protocatechuic acid	8468	
Gentisic acid	10742	
Cinnamic acid	444539	
P-Cumaric acid	445858	
Caffeic acid	637542	
Ferulic acid	637775	
Sinapic acid	689043	
Ellagic acid	1794427	
Rosmarinic acid	5281792	
Chlorgenic acid	5281855	

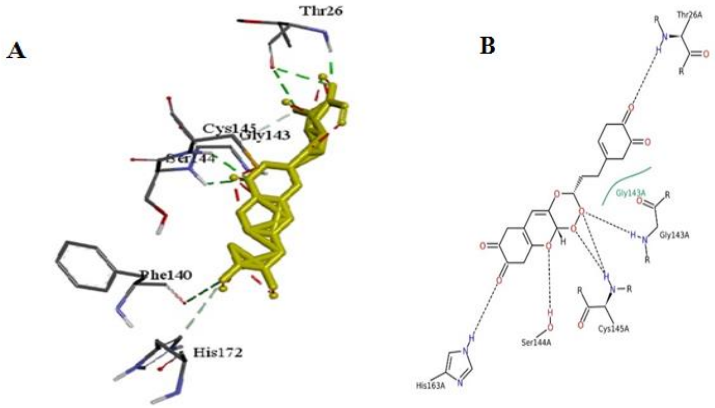


Figure 1. Rosmarinic acid in the COVID-19 main protease active site (left) and 2D interaction of Rosmarinic acid within binding site (right).

acquired from Protein Data Bank (PDB database; http://www.rcsb.org) (20) and water molecules and co-crystal ligand were removed from the structure. The PDB were then checked for missing atom types with the python script as implemented in MODELLER 9.17 (21). The ligand structures were made by HyperChem software package (Version 7, Hypercube Inc). For geometry optimization, Molecular Mechanic (MM+), followed by semi empirical AM1 method was performed. The prepared Ligands were given to 100 independent genetic algorithm (GA) runs. 150 population size, a maximum number of 2,500,000 energy evaluations and 27,000 maximum generations, maximum number of top individual that automatically survived 1, gene mutation rate 0.02 and crossover rate 0.8 were used for Lamarckian GA method. The grid points of 30, 30, and 30 in x-, y-, and z directions -11.993, 15.425, and 65.951 Å were used. Number of points in x, y and z was 65 respectively. All visualization of protein ligand interaction was evaluated using VMD software (22). Cluster analysis was performed on the docked results using a root mean square deviation (RMSD) tolerance of 2.4 Å.

2.1.1. In silico Drug likeness and pharmacokinetics calculation procedures

The physicochemical properties, drug-likeness, Absorbance Distribution, Metabolism

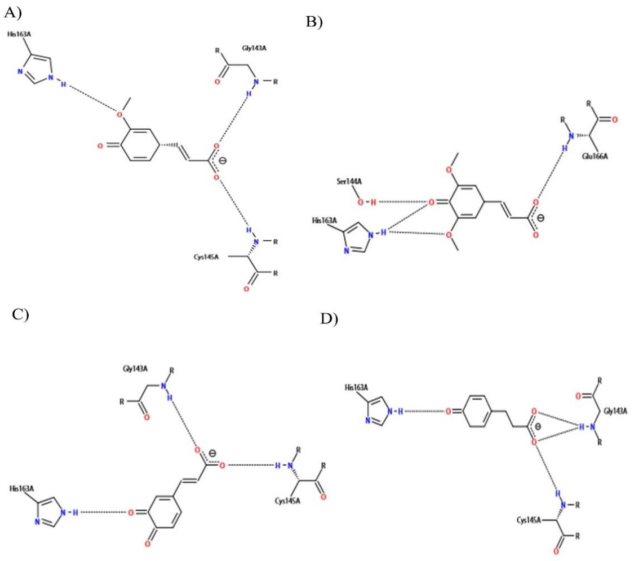


Figure 2. Computational binding modes of A) Ferulic acid, B) Sinapic acid, C) Caffeic acid, D) P-cumaric acid in the active site of COVID-19 protease (6LU7).

Table 2. Results of computational docking of Cinnamic acid derivatives and Hydroxy benzoic acid derivatives in active site of the COVID-19 main protease (PDB ID: 6LU7).

Compound name	ΔG binding (Kcal/mol)	Hydrogen bond
Vanillic acid	-3.85	His 163A, Gly143A, Cys145A
Gallic acid	-3.79	His163A, Gly143A, Cys145A
Syringic acid	-3.88	His163A, Cys145A, Gly143A
Protocatechuic acid	-4.45	His163A, Gly143A, Cys145A
Gentisic acid	-4.43	Ser144A, Gly143A
Cinnamic acid	-4.26	Cys145A, Gly143A
P-Cumaric acid	-4.47	His163A, Cys145A,
Caffeic acid	-4.23	His163A, Gly143A, Cys145A
Ferulic acid	-4.57	Cys145A, Gly143A, His163A
Sinapic acid	-4.26	His163A, Ser144A, Glu166A
Ellagic acid	-9.58	His163A, Cys145A
Rosmarinic acid	-10.48	His163A, Ser144A, Cys145A, Gly143A, Thr26A
Chlorgenic acid	-7.57	His163A, Ser144A, Glu166A, Arg188A

and Excretion (ADME) properties of Cinnamic acid derivatives and Hydroxy benzoic acid derivatives were derived from http://www.swissadme.ch/ the preADMET online server (http://preadmet.bmdrc. org/).

3. Results and Discussion

The main protease, 3CLpro of COVID-19, has been known as a therapeutic target (23) because of its important role in viral replication and infection. 3CLpro is a dimer protein with two promoters. The crystal structure of this protease showed that it contains three domains: domains I (residues 8−101) and II (residues 102−184) contain β-barrels and domain III (residues 201− 306) consists mainly of α-helices (4).

To understand and validate the antiviral activities of Cinnamic acid and Hydroxy benzoic derivatives toward COVID-19 virus, molecular docking studies were performed. The PDB ID: 6LU7 was selected as a docking template and the procedure was carried out by eliminating the structure of the co-crystallized ligand. All the docking protocols were performed on validated structures, with RMSD values 2 Å. The conformation with the lowest binding energies was considered as the

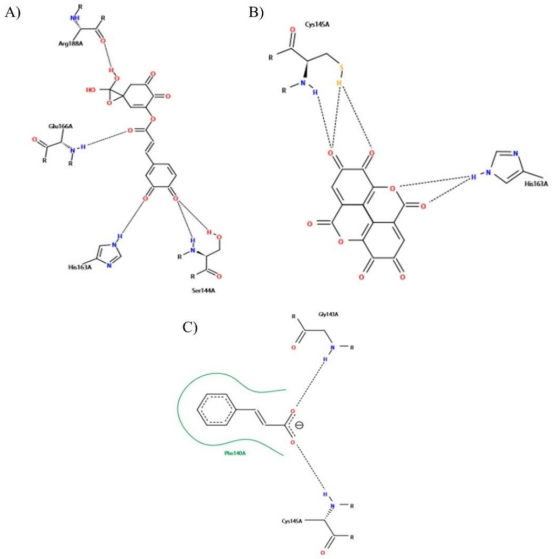


Figure 3. Interactions of A) Chlorogenic acid, B) Ellagic acid, C) Cinnamic acid in the binding site of receptor (6LU7).

Table 3. Physicochemical properties of Cinnamic acid derivatives and Hydroxy benzoic acid derivatives into the active site of the COVID-19 main protease.

Entry	MWa	LogPb	HBDc	HBAd	TPSA (A2) e	n-RBf	Lipinski violation
Vanillic Acid	168.15	1.08	2	2	66.76	2	0
Gallic Acid	170.12	0.42	4	5	97.99	1	0
Syringic Acid	198.17	0.95	2	5	75.99	3	0
Protocatehuic Acid	154.12	0.81	3	4	77.76	1	0
Gentisic Acid	154.12	0.81	3	4	77.76	1	0
Cinnamic Acid	148.16	1.93	1	1	37.3	2	0
P-Cumaric Acid	504.6	1.54	2	3	57.53	2	0
Caffeic Acid	180.16	1.15	3	4	77.76	2	0
Ferulic Acid	194.18	1.42	2	4	66.76	3	0
Sinapic Acid	224.21	1.29	2	5	75.99	4	0
Ellagic Acid	302.19	1.05	4	8	141.34	0	0
Rosmarinic Acid	360.31	2.07	5	8	144.52	7	0
Chlorgenic Acid	354.31	-0.75	6	9	164.75	5	1
Rule of Lipinski	≤ 500	≤ 5	≤ 5	≤ 10	≤ 140	≤ 10	≤ 1

a Molecular weight (MW). b Logarithm of partition coefficient between n-octanol and water (LogP). c Number of hydrogen bond donors (HBD). d Number of hydrogen bond acceptors (HBA). e Topological polar surface area(TPSA). f Number of rotatable bonds (nRB).

best docking result. The structure and properties of all studied molecules were presented in Table 1, also, the scores of docking results based on the free binding energies and hydrogen bond interactions involved in the binding mode were showed in Table 2. According to our result, Rosmarinic acid which belongs to the Cinnamic acid class, exhibited the minimum Gibbs binding energy and highest docking scores (ΔG bind=-10.74 kcal/mol) compared to co-crystal ligand of receptor (N3) ((ΔG bind N3=-6.08 kcal/mol) From the docking study parameters, it is understood that some of the Cinnamic acid derivatives, including Ellagic, Rosmarinic, Chorgenic and Ferulic acid might be effective protease inhibitors consequently, these structures could be helpful to manage the COVID-19 disease.

3D view of the Rosmarinic acid interactions (shown on the left side of Figure 1) indicated that it is involved with strong hydrogen bonding interaction with His163A, Ser144A, Cys145A, Gly143A and Thr26A, while the propyl cyclohexadione of Rosmarinic acid formed hydrophobic interaction with residue Gly 143A. Similarly, Ferulic acid, Caffeic acid and P-Cumaric acid interacted hydrogen bond interaction with His163A, Gly143A and Cys145A (Figure 2). As in order, Sinapic acid also binds to the COVID-19 active site via hydrogen bond interaction to His163A, Ser144A and Glu166A. As depicted in Figure 3, The binding mode of Chlorogenic acid can be summarized by hydrogen bond interaction with His163A, Ser144A, Glu166A and Arg188A. Ellagic acid also interacted to COVID-19 active site with residue His163A and Cys145A. It should be announced that phenyl propene of Cinnamic acid was aligned with the residue Phe 140A by hydrophobic interaction and also two hydrogen bond interaction was observed with Cys145A and Gly143A.

As shown in Figure 4, Vanillic acid, Syringic acid, Protocatechuic acid and Gallic acid of hydroxyl benzoic class were involved hydrogen bond interaction with His 163A, Gly143A and Cys145A. Additionally, Gentisic acid is interacted with Ser144A and Gly143A by hydrogen bond interaction. Based on the docking study, we suggested that Cinnamic acid class especially Rosmarinic acid identified for the treatment of novel SARS Coronavirus,

According to results of previous studies, several phenolic compounds were identified as inhibitors of SARS-CoV. For instance, a two-step screening method (frontal affinity chromatography-mass spectrometry coupled with a viral infec-

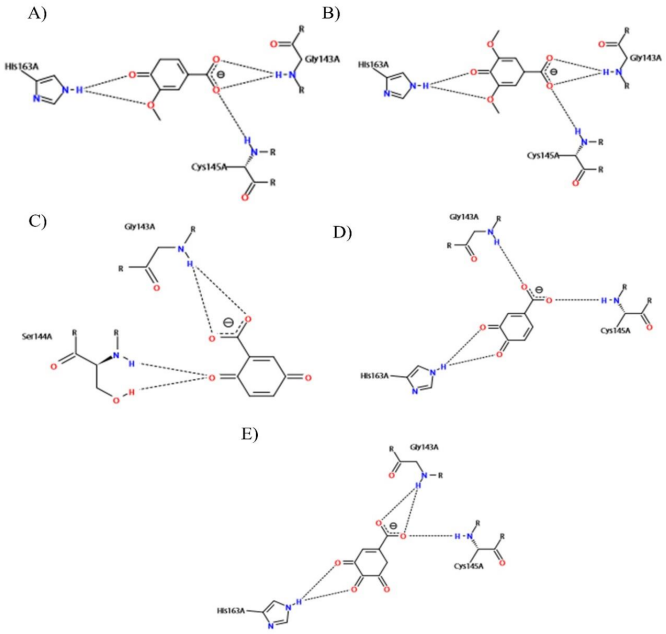


Figure 4. Interactions of A) Vanillic acid, B) Syringic acid, C) Gentisic acid, D) Protocatechuic acid and E) Gallic acid in the binding site of receptor (6LU7).

tion assay based on a human immunodeficiency virus (HIV)-luc/SARS pseudotyped virus) showed that luteolin has anti-SARS-CoV activities. This flavone inhibits virus entry by binding with the surface spike protein of this virus (24). Another docking study of Quercetin-3-β-Galactoside revealed that this compound was SARS-CoV 3CLpro inhibitor. The residue Gln189 (Q189) and hydroxy groups on the quercetin moiety are critical keys in the binding interaction (25). Also, Biflavone, Amentoflavone, which was isolated from ethanol extract of *T. nucifera* leaves, exhibited noncom-

Table 4. Insilico ADME of Cinnamic acid derivatives and Hydroxy benzoic acid derivatives into the active site of the COVID-19 main protease.

Entry	Absorption Distribution			
	% HIA [a]	Invitro Caco-2 cell permeability (nm s^{-1})	% Invitro plasma protein bonding	%BBB [b]
Vanillic Acid	85.37	19.93	52.1	0.62
Gallic Acid	53.7	13.85	68.35	0.35
Syringic Acid	82.02	18.83	69.77	0.54
Protocatehuic Acid	74.75	18.3	27.11	0.44
Gentisic Acid	74.75	18.3	69.61	0.44
Cinnamic Acid	97.84	21.03	60.85	1.86
P-Cumaric Acid	92.09	21.10	63.05	0.69
Caffeic Acid	82.3	21.1	40.29	0.49
Ferulic Acid	90.6	21.11	50.41	0.76
Sinapic Acid	88.55	19.85	47.5	0.72
Ellagic Acid	56.21	20.53	66.17	0.29
Rosmarinic Acid	62.48	20.72	86.24	0.10
Chlorgenic Acid	20.42	18.71	41.96	0.03

a Human Intestinal Absorption, b in vivo blood–brain barrier penetration

petitive inhibition effects on this enzyme. These activities were related to binding interactions with Val186 and Gln192 of target site (26).

3.1. Insilico Physicochemical parameters (ADME) prediction

The results of physicochemical properties of all studied compounds are revealed in Table 3. The molecular weight (MW) of the all compounds were in the range of 148-504. all of the compounds represented appropriate lipophilicity from The log P values, Most of the compounds except Chlorgenic Acid had acceptable limit of the hydrogen bond properties (as donors or acceptors). on the other hand, total polar surface area (TPSA) are within the acceptable limit Except, Ellagic Acid, Rosmarinic Acid and Chlorgenic Acid. Generally, the results demonstrate that all of the compounds conformances the Lipinski's rule and so, they can oral consumption.

Insilico ADME (adsorption, distribution, metabolism and excretion) properties of Cinnamic acid and Hydroxy benzoic acid derivatives are presented in Table 4. HIA analysis indicated that all compounds illustrated well absorbed compounds that caused easily absorbed from the intestine to the bloodstream. Besides, all of the compounds had Middle permeability parameters for penetration to biological membranes. Also, in vitro plasma protein binding (PPB) results presented that all of the compounds had chemical weakly bounds (less than 90%), so can easily diffuse or transport across to the cell membranes. finally, the percent of blood brain barrier parameters indicated that all of the studied compounds had moderate BBB percentage and in between, Chlorgenic Acid and Rosmarinic Acid had low blood-brain barrier percentage that they will not have neurotoxic effects.

4. Conclusion

Based on the free binding energies and hydrogen bond interactions involved in the binding mode, Cinnamic acid derivatives, the class of phenolic acid, especially Rosmarinic acid, could be efficient SARS-CoV 3CLpro inhibitors. These are supported by the inhibitory effects of some other phenolic compounds. In the next step, it is necessary to survey the effect of these natural products in the inhibition of SARS-CoV replication in cell culture. These types of studies improve preclinical knowledge about potential of natural compounds as SARS-CoV inhibitors. The physicochemical and drug-likeness properties of the all compounds illustrated that the compounds are followed to the Lipinski rules.

Acknowledgement

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Conflict of Interest

None declared.

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