Medicinal plant compounds as promising inhibitors of coronavirus (COVID-19) main protease: an in silico study

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Medicinal Plant Compounds as Promising Inhibitors of Coronavirus (COVID-19) Main protease. An *in-silico* study

Abstract

Background: The novel Coronavirus (COVID-19) has spread rapidly across the globe and has involved more than 213 countries and territories. Due to a lack of effective therapy or vaccine, urgent and concerted efforts are needed to identify therapeutic targets and medications. COVID-19 main protease represents a major target for drug treatment to inhibit viral function.

Objectives: The present study sought to evaluate medicinal plant compounds as potential inhibitors of the COVID-19 main protease using molecular docking and molecular dynamic analysis.

Methods: The PDB files of COVID-19 main protease and some medicinal plant compounds were retrieved from the Protein Data Bank (http://www.rcsb.org) and Pubchem server, respectively. The Gromacs software was used for simulation studies, and molecular docking analysis was done using Autodock 4.2. The COVID-19 main protease simulation, compared with some phytochemicals docked to the COVID-19 main protease, were analyzed.

Results: Glabridin, catechin, and fisetin had the greatest tendency to interact with the COVID-19 main protease by hydrogen and hydrophobic bonds. Docking of these phytochemicals to COVID-19 main protease led to an increase in the radius of gyration (Rg), decrease in the Root mean square fluctuation (RMSF), and induced variation in COVID-19 main protease secondary structure.

Conclusion: The high tendency interaction of glabridin, catechin, and fisetin to COVID-19 main protease induced conformational changes on this enzyme. These interactions can lead to enzyme inhibition. This simulated study indicates that these phytochemicals may be considered as potent inhibitors of the viral protease; however, more investigations are required to explore their potential medicinal use.

Keywords: COVID-19 main protease, Medicinal Plant Compounds, Inhibitors, Molecular docking

1. Introduction

At the end of December 2019, a novel Coronavirus was identified in Wuhan, China. Since then, this virus has spread rapidly throughout China, and the world, primarily via human-to-human transmission [1]. On March 11, 2020, the World Health Organization (WHO) declared the novel coronavirus (COVID-19) outbreak as a pandemic, and by August 16th, 2020, a total of 21,294,845 confirmed cases and 761,779 deaths had been reported in 213 countries and territories [2]. COVID-19 [now known as SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2)] is a zoonotic virus and etiologic agent of severe respiratory diseases. It has been reported that the novel virus not only infects respiratory tracts but can also cause damage in the liver, kidney, heart, and digestive tract [3].

On January 11th, 2020, scientists isolated and sequenced the novel coronavirus genome [4], and identified two proteases, 3C-like protease (3Clpro) and papain-like protease (Plpro), which are essential for its survival [5]. Polyprotein1 a/b are encoded by SARS-CoV-2, and following proteolytic cleavage by 3Clpro and Plpro, the resulting proteins are involved in viral infection, transcription, and replication [6]. The 3C-like protease (also called the main protease) is a vital protein for the proteolytic processing and maturation of the virus. In addition, Liu et al. showed the crystallized structure of the COVID-19 main protease and found that targeting and inhibition of the main protease may be a potential treatment against SARS-CoV-2 infection [7]. Furthermore, Xu et al., who performed molecular dynamic simulation studies on nelfinavir, praziquantel, pitavastatin, and perampanel, to predict their inhibitory effects against the COVID-19 main protease, found that nelfinavir has the greatest inhibitory potential among these 4 drugs against the COVID-19 main protease [8].

Despite promising recent advances in the development of COVID-19 vaccines, based on previous modeling studies, several major limitations, including; the time-consuming process of the approvement and global mass production, the potentially high price of the vaccine during initial release, the determination of vaccination strategies, such as location-specific ring-vaccination, age-specific vaccination, and assessing long-term risks and benefits of dengue vaccination, are faced by health care providers and government officials, particularly in less-

developed countries. Therefore, due to the deteriorating, global, situation, urgent and concerted efforts are needed to identify more readily available therapeutic targets and medications [9, 10].

Medicinal plants have gained increasing prominence for the treatment of a wide variety of infectious and non-infectious diseases; indeed, it is estimated that ~25% of routine drugs are derived from plant compounds. In contemporary practice, due to significant progress in separation techniques and the spread of a number of emerging infectious diseases, plants and their bioactive compounds could be promising candidates for drug discovery [11]. A recent study showed that oral administration of black raspberry seed, and its gallic acid, to influenza virus infected-mice significantly increased the survival rate, and decreased the viral titers in the lungs [12]. Moreover, monoterpenes thymol, carvacrol, and p-cymene are reported to have antiviral activity and can inhibit replication of herpes simplex virus type, probably via interaction with the viral envelope [13].

In-silico based screening is one of the most useful approaches to discover new antiviral drugs. Computational screening of natural compounds and phytochemicals using molecular docking study reduces time, money, and possible errors corresponding to the clinical trials [14, 15]. In the preceding decade, a large number of active phytochemicals with therapeutic activity have been discovered, and it has been explicated that they exert antiviral effects through scavenging capacities, antioxidant activities, or the inhibition of RNA/DNA replication. Moreover, these compounds present low cytotoxicity and high bioavailability, which makes them desirable candidates for antiviral drug development [16]. Therefore, the present study screened bioactive compounds from medicinal plants, including; carvacrol, catechin, cinnamaldehyde, coumarin, cyanidin, delphinidin, fisetin, genistein, glabridin, isorhamnetin, liquiritigenin, nelfinavir, p-cymene, piperine, pterostilbene, silibinin, and thymoquinone, against the COVID-19 main protease, using molecular docking and molecular dynamic simulation.

2. Material and Methods

2.1 PDB files Preparation

The protein data bank (PDB) file of COVID-19 main protease (PDB ID: 6LU7) was obtained from the protein data bank server (www.rcsb.org). Subsequently, the water and inhibitor molecules were removed. In the next step, the medicinal plant compound files, including

carvacrol (CID; 10364), catechin (CID; 9064.), cinnamaldehyde (CID; 637511), coumarin (CID; 323), cyanidin (CID; 128861), delphinidin (CID; 128853), fisetin (CID; 5281614), genistein (CID; 5280961), glabridin (CID; 124052), isorhamnetin (CID; 5281654), liquiritigenin (CID;114829), nelfinavir (CID; 64143), p-cymene (CID; 7463), piperine (CID; 638024), pterostilbene (CID; 5281727), silibinin (CID; 31553), and thymoquinone (CID; 10281) were obtained from Pubchem server. Ultimately, the mentioned files were optimized and converted to PDB files using Avogadro software.

2.2. Molecular Dynamic (MD) Simulation in water

The MD simulation of the 3CLpro structure was studied in pure water with the forcefield of G43A1 and the SPC216 model using the GROMACS 2018 simulation package. The grid boxes with a dimension of 60×120×90 nm^3 (x×y×z) were designed for protein. Through estimation of the electrical charge of each protein, appropriate amounts of chloride and sodium ions were used to neutralize the molecules to prepare 140 mM concentration of Na^+ and Cl^- in water for protein [18]. The energy of the systems was minimized for 50000 steps by steepest descent method and equilibrated for 2 ns in the NVT ensemble. The time of simulation was 50 nanoseconds (ns) and the temperature was maintained at 300ºK for all the simulation intervals. Finally**,** the output PDB file was used as an input receptor file for molecular docking.

2.3. Molecular Docking

The molecular docking study was performed using Autodock software version 4.2. All the above-mentioned ligands were docked on the simulated COVID-19 main protease to find the best binding sites for the ligand-receptor and to determine the most stable free energy state of ligand-receptor. In the current study, a grid box with dimensions of 60×120×90 nm^3 (x×y×z) was created for the COVID-19 main protease protein and ligands docked to it. The PDBQ and PDBQT files were created for each of the mentioned molecules and defined as ligand and for the COVID-19 main protease molecule was defined as a receptor. Autogrid4 –p n.gpf –l n.gle Linux command was used to generate the n.gle text file. After 200 runs of molecular docking on ligands, the Genetic Algorithm and Lamarckian GA parameters were used. The *autodock4–p n.dpf–l n.dlg* Linux command was used to generate the n.dlg text file. The obtained data from the n.dlg file was analyzed [19].

In this study, LigPlot plus v.2.1 software was used to specify the number of hydrophobic and hydrogen bonds between the COVID-19 main protease with each of the ligands. Furthermore, the type and number of existed amino acids in the binding site were determined [20].

2.4.MD simulation of 3CLpro with ligands

Phytochemicals with the most affinity for binding to the CLpro 3 enzyme that have been determined in docking, such as Catechin, Fisetin, and Glabridin, were selected to evaluate their inhibitory impact on the CLpro 3 enzyme using molecular dynamics simulation

Therefore, the molecular dynamics simulation of the 3CLpro, as a receptor, was carried out with Catechin, Fisetin, and Glabridin as ligands, and parameterization was performed using the PRODRG server. The MD simulation was performed at 140 mM concentration of Na^+ and Cl^- in water according to the above-mentioned procedure. As previously mentioned, the paths stored in the simulation were applied to analyze the structural parameters of the complex. The simulation results of the 3CLpro molecule, with and without ligands, were comparatively analyzed using the Grapher version 15 software [21].

3. Results

3.1. Molecular Docking

Fig. 1 depicts the three-dimensional (3D) structure of Glabridin, Catechin, and Fisetin docked to the COVID-19 main protease and amino acid residues at the binding site. According to Table 1, Catechin is attached to the protease by hydrogen bonds associated with the Tyr57, Glu166, Asp187, and Gln192 amino acid residues and hydrophobic bonds that involved the His41, His164, Met165, Pro168, Arg188, Gln189, and Thr190 amino acid residues. Accordingly, Fisetin and Glabridin had a high tendency for binding to the COVID-19 main protease at the binding site, by interaction with amino acid residues via hydrogen bonds (four amino acid residues for Fiestin including Tyr54, Glu166, Asp187, and Gln192 and three amino acid residues including Gly143, Glu166, Arg188) and hydrophobic bonds (including His41, His164, Met165, Leu167, Pro168, Arg188, Gln189, Thr190, and Ala191 for Fiestin and Thr26, Leu27, His41, Glu139, Leu141, Asn142, Cys145, His164, Met165 for Glabridin).

In addition, Table 1 details the molecular interaction of different plant compounds with the COVID-19 main protease. Interestingly, the interaction of Catechin, Fiestin, and Glabridin with the COVID-19 main protease released a high binding energy (BE) of -8.12, -8.11, and -8.25 Kcal/mol, respectively. Moreover, Table 1 demonstrates the final intermolecular energy (FIE)

(kcal/mol), estimated inhibition constant (EIC) (μM), and hydrogen and hydrophobic bonds of all ligands at the binding site.

3.2. MD Simulation

Root Mean-Square Deviation (RMSD)

Fig. 2 depicts the changes in the Root Mean-Square Deviation (RMSD) in the simulation of 3CLpro, alone (black graph), and its complexes with Catechin, Fisetin, and Glabridin (red graph), at 50 ns simulation time. Although an oscillation and instability was observed at the beginning of the simulation time, the system reached a steady-state after 30 ns, and the amount of RMSD fluctuations decreased. As shown in Fig. 2, during simulation time, mean RMSD decreased for the protein in complex with catechin and increased for the protein in combination with Glabridin and Fisetin.

Total Energy (TE)

Fig. 3 demonstrates the alterations in Total Energy (TE) in the simulation of the COVID-19 main protease protein alone (black graph) and its complexes with Catechin, Fisetin, and Glabridin (red graphs) at 50 ns simulation time. Binding of ligands to protein results in the release of energy, which facilitates the effect of this compound on the receptor protein.

The radius of gyration (Rg)

Fig. 4 describes the rate of radius of gyration (Rg) alterations of protease protein alone (black graph) and protease protein binding to Catechin, Fisetin, and Glabridin (red graph) throughout 50 nanoseconds from the simulation time. The Fig. 4 reveals an oscillation in the Rg rate during the simulation time. However, the mean rotational radius for the duct proteins with all three compounds Catechin, Fisetin, and Glabridin increased during the simulation time. Interestingly, Fig. 4 shows that Fisetin and Glabridin, when bound to protease proteins, increase Rg levels more than Catechin.

Hydrogen bonds (H-bound)

Fig. 5 demonstrates the rate of alterations in hydrogen bonds of protease protein alone (black graph), and protease protein binding to Catechin, Fisetin, and Glabridin (red graph), over 50 nanoseconds of the simulation time. Accordingly, Fig. 5 shows that during the simulation time, a

slight increase in hydrogen bonds was induced in the docked protein to all three compounds Catechin, Fisetin, and Glabridin compared to the protein alone.

Root mean square fluctuation (RMSF)

Figure 6 shows the rate of Root mean square fluctuation (RMSF)changes for each of the amino acid residues of the protease protein alone (black graph) and the protease protein docked with the Catechin, Fisetin, and Glabridin (red graph) throughout 50 nanoseconds. As the figure 6 describes, the greatest reduction in RMSF fluctuations occurred in Catechin-docked protein. The inhibitory effects of Catechin, Fisetin, Glabridin on the fluctuations of amino acid residues of protease proteins, that were evaluated using the RMSF parameter, demonstrated that these compounds could inhibit amino acid residues of 120 to 146, as well as 180-200 amino acid residues in the protease protein.

Secondary structure

Figure 7 demonstrates the rate of alterations in the secondary structures of the protease protein, alone (black graph), and the protease protein docked with Catechin, Fisetin, and Glabridin (red diagram), after 50 nanoseconds of the simulation time. Docking all three (Catechin, Fisetin, and Glabridin) with protein causes a severe reduction in the Coil and Bend structure. Furthermore, docking these compounds with protein increases the β-sheet structure.

4. Discussion

Currently, there is a limited arsenal available to help combat coronaviruses, as no drug or vaccine has yet been approved. Although several options including vaccines, peptides, interferon therapies, and monoclonal antibodies could be developed to control or prevent emerging infections of COVID-19, it requires months to years for ultimate approval [5, 10]. Interestingly, over 1800 years ago, traditional Chinese medicine was considered to be the mainstay of infectious diseases treatment [22], indeed, in addition to antiviral properties, this historical disease management strategy has been considered by researchers in recent years for managing and/or treating cancer, inflammatory and cardiovascular diseases, diabetes, infertility, and other pathological conditions [23-25]. Generally, phytochemicals, particularly polyphenols, possess antiviral activities against several viral components and actions [26]. Herbal compounds could be

applied in a variety of formats, including diet, coating on masks, using as an air-disinfectant, and as a surface sanitizing agent to combat novel COVID-19 [27].

Currently, computer-aided drug design is considered a pivotal approach in modern drug discovery due to its beneficial properties, such as minimizing costs, accelerating drug development process, and non-requirement of specialist operators [17].

Molecular docking reliably predicts the prevailing binding trends between a ligand and a protein, and thereby indicates how the ligand might inhibit the protein. The findings of the present molecular docking study revealed that some phytochemical compounds, such as Catechin, Fisetin, and Glabridin, may be potential inhibitors for the COVID-19 main protease. Glabridin, with three hydrogen bonds and 9 hydrophobic bonds in the binding site, had the highest binding affinity to the protease among the studied compounds (Fig. 1 and Table 1). The binding free energy between the Glabridin and COVID-19 main protease was -8.25 kcal/mol, which is extremely close to nelfinavir (-9.68 kcal/mol) as a potent inhibitor of the protease (Table 1). More importantly, the minimum effective concentration of Glabridin for this binding is 0.90 μM, which is too low to elicit toxic effects on the human body cells. Glabridin is a prenylated isoflavonoid and main component in the root of Glycyrrhiza glabra L. (commonly known as licorice). Several studies have reported that Glabridin possesses a various range of biological properties, such as anti-inflammatory, anti-oxidant, anti-atherogenic, anti-tumorigenic, anti-microbial, and antibacterial activities [28-30]. It has also been reported that Glabridin possesses potent anti-hepatitis C virus (HCV) activity, with an IC_{50} value of 6.2 μg/mL, suggesting that Glabridin could be considered an appropriate candidate for developing an anti-HCV drug [31]. Importantly, the minimum effective concentration for Glabridin binding to the viral main protease is much lower than its toxic concentrations for normal cells.

Catechin was the second most effective compound among the studied natural products, with a binding energy value of -8.12 kcal/mol. Catechin is the main polyphenol present in green tea and many fruits, including grapes, cacao, and apples [32]. Green tea catechins have been shown, both in vivo and in vitro, to possess anti-viral activities against the influenza virus. Chang et al. reported that catechin can inhibit the growth of the influenza A (H1N1) virus with EC_{50} of 18.4 μg/mL in the MDCK cell line; further delineating that catechin could be considered a potent inhibitor of virus mRNA replication and neuraminidase activity of influenza virus [33]. Furthermore, the clinical trial by Matsumoto et al. showed that the incidence of influenza

infection among the participants who had consumed catechin/theanine for 5 months was significantly lower than the placebo group, suggesting that green tea catechins might be effective prophylactic agents for influenza infection [34]. In accord with the present study, a recent molecular docking investigation showed that catechin can bind to the spike protein of COVID-19 (S-Protein) and its cellular receptor (ACE2), with a great affinity (-10.5 Kcal/mol and -8.9 Kcal/mol), and it can be considered as a potential molecule for developing a new drug against viral infection [35]

Among the analyzed compounds, Fisetin had the highest affinity for the COVID-19 main protease, after Glabridin and Catechin, with a binding energy value of -8.1Kcal/mol. Fisetin is a naturally occurring flavonoid found in many fruits and vegetables, such as kiwis, grapes, persimmons, apples, strawberries, cucumbers, and onions [36]. It has also been stated that Fisetin has several anti-inflammatory, anti-tumoral, and antioxidant activities [37]. Indeed, Zandi et al. revealed that Fisetin has anti-viral activity against dengue virus and exerts its activity by blocking the replication of the virus [38]. In addition, it has been documented that Fisetin significantly inhibits the enzymatic activity and replication of enterovirus A71, in a dose-dependent pattern [39]. Furthermore, in Rane et al., it was demonstrated that Fisetin interacts strongly with the spike protein of the COVID-19 with binding energies of -8.5 Kcal/mol; thereby suggesting that this bioactive compound has the potential ability to be considered as a novel drug candidate [40]. Also, other studied chemicals in the current investigation demonstrated a high tendency to bind to the binding site of the viral main protease.

Previous studies have identified the two amino acid residues, His41 and Cys145, as catalytic residues in the binding site of the COVID-19 main protease [17]. The results of the present study showed that Catechin and Fisetin interact with His41 through hydrophobic bonding. Interestingly, Glabridin interacts with both catalytic amino acid residues and bears the strongest resemblance to Nelfanivir. Indeed, these phytochemicals appear to be promising main protease inhibitors, as they bond to catalyzing residues of the protease binding site.

The present simulation study revealed that binding of the functional Catechin, Fisetin, and Glabridin compounds yields a remarkable impact on the protein protease 3CLpro of COVID-19. The simulated systems in the current study stabilized in terms of the RMSD changes rate after 30 nanoseconds, as the changes in RMSD per time were minimized from 30 nanoseconds onwards

(Fig. 2). However, the present findings demonstrated a sharp decrease in the mean RMSD in the protein docked with Glabridin, and a slight increase in the RMSD in the protein docked with Catechin and Fisetin. These changes in RMSD markedly affected the structural fluctuations of the 3CLpro protein [41].

The current findings demonstrated that the high affinity of the binding of all three active compounds (Catechin, Fisetin, and Glabridin) to the 3CLpro protease protein releases energy, and subsequently facilitates interaction between the receptor-ligand. This high tendency to bind causes the rotational radius of the protein to be drastically affected. Indeed, binding of all three studied compounds to the protein, particularly Galibridin, resulted in an increase in the rotational radius of the protein (Fig. 4), which indicates the rigorous alterations induced by these compounds on the spatial structure of the protein [42].

The inhibitory effects of Catechin, Fisetin, Glabridin on the fluctuations of amino acid residues of protease proteins, that were evaluated using the RMSF parameter (Fig. 6), demonstrated that these compounds could inhibit amino acid residues of 120 to 146, as well as 180-200 amino acid residues in the protease protein. In particular, Catechin and Fisetin decreased the Cys 145 residue fluctuation of the COVID-19 main protease from 0.12 nm to 0.10 nm, while Glabridin decreased the Cys 145 residue fluctuation of theCOVID-19 main protease RMSF from 0.12 nm to 0.90 nm. Indeed, such reductions in RMSF can induce the inhibitory effects of these phytochemical compounds on the COVID-19 main protease.

In the present study, we noted that the binding of Catechin, Fisetin, Glabridin elicited drastic changes in the secondary structure of the protein (Figure 7). These alterations include a sharp decrease in the secondary structure of the coil, and a sharp increase in the secondary structure of the β-sheet. Since the coil regions are considered as the functional regions of the protein, decreasing the amount of these secondary structures greatly reduces the flexibility of the protein, and increasing the β-sheet regions severely limits the flexibility of the protein; therefore, the studied chemicals appear to be able to reduce the activity of this enzyme dramatically.

The current findings indicated that a number of herbal compounds could directly affect the structure of the main protease protein of the Coronavirus through their high tendency to bind and interact with this enzyme. These interactions elicit fundamental changes in the protein second and third structures, subsequently disrupting the function of this enzyme. This simulated study

indicates that the induced alterations in enzyme structure could lead to inhibition of its activity. However, the present findings reveal the necessity to conduct further *in vitro* and *in vivo* studies for confirmation of the inhibitory role of these known compounds.

Conclusion

The results of the present study showed that Glabridin, Catechin, and Fisetin have a high tendency to bind to the COVID-19 main protease, and this binding can cause changes in protein structure, leading to inhibition of the virus function and subsequent limitation of the virus activity. Moreover, Glabridin, Catechin, and Fisetin may be potent inhibitors of the virus protease and considered as attractive candidates for drug discovery; however, further *in vivo* and *in vitro* instigations are needed to confirm the veracity of these *in-silico* findings.

Declaration of Conflicting Interests

The authors declare no conflicts of interest.

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Figure legends:

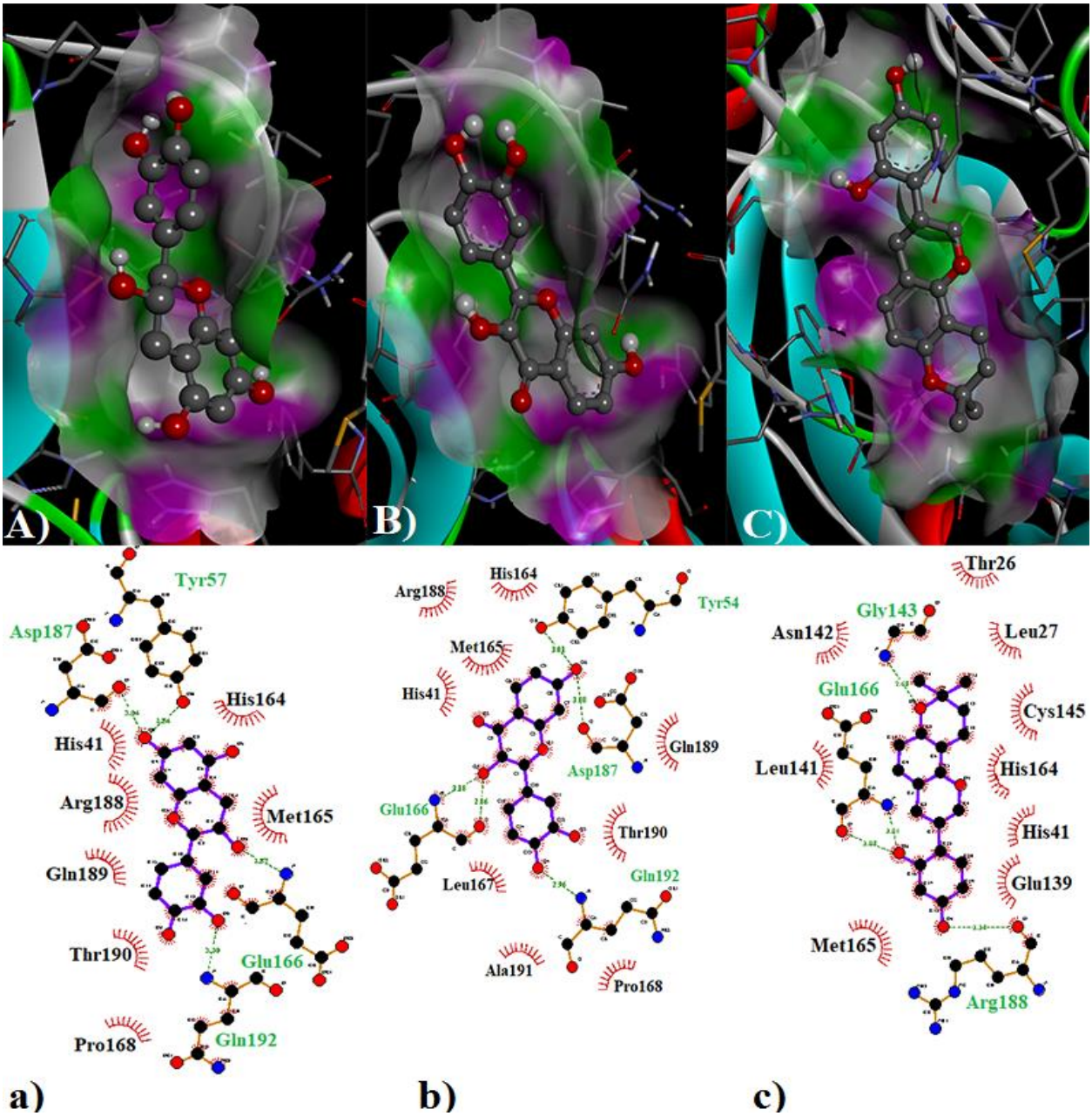

Figure 1. The three-dimensional structures and binding sites of (A); Catechin, (B); Fisetin, and C); Glabridin in COVID-19 main protease (above) and amino acid residues of protease interacted with (a); Catechin, (b); Fisetin, and (c); Glabridin in the binding site.

Figure 2. Changes in the Root mean square deviation (RMSD) during 50 nanoseconds simulation time. (A); Catechin, (B); Fisetin, and C); Glabridin. The black lines correspond to the RMSD of protein alone and the red lines correspond to the RMSD of protein docked with the ligands.

Figure 3. Changes in the Total Energy (TE) during 50 nanoseconds simulation time. (A); Catechin, (B); Fisetin, and C); Glabridin. The black lines correspond to the TE of protein alone and the red lines correspond to the TE of protein docked with the ligands.

Figure 4. Changes in the Radius of gyration (Rg) during 50 nanoseconds simulation time. (A); Catechin, (B); Fisetin, and C); Glabridin. The black lines correspond to Rg of protein alone and the red lines correspond to the Rg of protein docked with the ligands.

Figure 5. Changes in the number of hydrogen bonds during 50 nanoseconds simulation time. (A); Catechin, (B); Fisetin, and C); Glabridin. The black lines correspond to the number of hydrogen bonds of protein alone and the red lines correspond to the number of hydrogen bonds of a protein docked with ligands.

Figure 6. Changes in Root mean square fluctuation (RMSF) during 50 nanoseconds simulation time. (A); Catechin, (B); Fisetin, and C); Glabridin. The black lines correspond to the RMSF of the protein alone and the red lines correspond to the RMSF of the protein docked with ligands.

Figure 7. Changes in the secondary structures during 50 nanoseconds simulation time.

Table 1: Molecular interaction of different herbal plant compounds with COVID-19 main protease (PDB; 6LU7)

Ligand-receptor	BE kcal/mol	FIE kcal/mol	EIC µM	Interaction bonds	
				Hydrogen Bonding	Hydrophobic Bonding
Carvacrol	-5.11	-5.70	180.80	Glu166	Arg188, Gln189, His164, His41, Met165
Catechin	-8.12	-9.90	1.13	Gln192, Glu166, Tyr57, Asp187	His164, Met165, Pro168, Thr190, Gln189, Arg188, His41,
Cinnamaldehyde	-4.56	-5.16	454.11	Glu166	His41, Asp187, Tyr54, Arg188, His164, Gln189, Met165
Coumarin	-5.26	-5.26	139.43	Val68, Val77,	Asn63, His64, Phe66, Gln74, Leu75, Leu67, Arg76
Cyanidin	-7.89	-9.68	1.64	Asp187, Tyr54, Glu166, Gln192	His41, Cys145, Met165, His164, Thr190, Ala191, Pro168, Gln189, Arg188
Delphinidin	-7.69	-9.78	2.31	Tyr54, Asp187, Glu166, Gln192	Arg188, Gln189, Ala191, Thr190, Pro168, His164, Met165, His41
Fisetin	-8.11	-9.60	1.14	Tyr54, Asp187, Gln192, Glu166	Gln189, Thr190, Pro168, Ala191, Leu167, His41, Met165, Arg188, His164
Genistein	-6.80	-7.99	10.36	His41, Gln192, Thr190	Cys145, His164, Met165, Ala191, Leu167, Pro168, Glu166, Arg188, Gln189
Glabridin	-8.25	-9.14	0.90	Glu166, Arg188, Gly143	Thr26, Leu27, Cys145, His164, His41, Glu139, Met165, Leu141, Asn142
Isorhamnetin	-7.61	-9.40	2.63	Tyr54, Gln192, Glu166, Asp187	His164, Arg188, Gln189, Pro168, Ala191, Thr190, Leu167, Met165, His41,
Liquiritigenin	-7.58	-8.48	2.77	Tyr54, Gln192,	Arg188, Gln189, Thr190, Ala191, Leu167, Pro168, Glu166, Met165, Asp187, His41, His164
Nelfinavir	-9.68	-13.26	0.08	Gln192, Thr190, Gln189, Glu166	Ala191, Arg188, His41, Met49, His164, Cys145, Met165, Asn142, His163, Ser144, Leu141, Gly170, Leu167, Pro168
p-cymene	-4.79	-5.09	306.35	----------	Arg217, Thr257, Ile213, Val303, Gln256, Thr304, Gln306, Phe305, Val212
Piperine	-7.89	-8.78	1.65	Gly143	Pro52, Tyr54, Met49, Cys44, His164, His41, Cys145, Met165, Asn142, Phe140, His163, Glu166, Ser144, Leu141, Arg188, Gln189, Asp187,
Pterostilbene	-7.62	-9.11	2.60	Gly143, His167	Glu166, Ser144, Cys145, Asn142, His41, Asp187, Met49, Gln189, Arg188, Met165, His164, Leu141, Phe140,
Silibinin	-7.55	-10.24	2.91	Glu166, Asp187, Tyr54	Gln189, Met165, Arg188, Pro168, Thr190, Ala191, Cys145, His164, His41
Thymoquinone	-5.49	-5.79	94.45	Lys5, Trp207	Phe3, Arg4, Leu282, Glu288, Phe291

Abbreviations: BE; Estimated Free Energy of binding (kcal/mol), FIE; final intermolecular energy (kcal/mol), EIC; estimated inhibition constant (µM).