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Research Article

IN Silico Approach of Some Selected Honey Constituents as SARS-CoV-2 Main Protease (COVID-19) Inhibitors

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Abstract

Objectives: The emergence and spread of severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19), led researchers around the world to study the crystal structure of the main protease (Mpro), 3-chymotrypsin-like cysteine protease, which is an essential enzyme for processing polyproteins. Inhibition of this key activity in the life cycle of the virus is a target in the scientific search for a drug to overcome this disease. Honeybee products have demonstrated antiviral and other beneficial properties that could prove useful in this effort.

Methods: A molecular modeling approach was used to evaluate the activity of 6 active honeybee product compounds for the ability to inhibit the SARS-CoV-2 Mpro using Schrödinger Maestro v10.1 software (Schrödinger LLC, New York, NY, USA).

Results: All 6 of the ligands demonstrated good binding affinity with the receptor in different ways. Four compounds had strong binding affinity with a good glide score and may inhibit the SARS-CoV-2 Mpro and replication of the virus.

Conclusion: Honeybee product constituents may provide an effective ligand for SARS-CoV-2 Mpro inhibition and may be valuable in the search for COVID-19 therapeutic drugs.

Keywords: Honeybee, molecular docking, Mpro inhibition, propolis, SARS-CoV-2, structure-activity relationship

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The crystal structure of the severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2) main protease (M^{pro}) is a subject of study by medicinal chemists around all the world in an effort to develop an antiviral drug for the novel coronavirus 2019 (COVID-19), now a global pandemic. The virus causing COVID-19 does not produce many proteins and there are not many targets for inhibition. The 3-dimensional (3D) structure of the SARS-CoV-2 M^{pro} is similar to that of the SARS-CoV M^{pro}; the RNA genome has 82% nucleotide identity with SARS-CoV, which belongs to the *Betacoronavirus* genus.[1, 2] The principal drug target is the M^{pro} (3-chymotrypsin-like cysteine protease), which is an essential enzyme for polyprotein processing.[3–5]

The M^{pro} operates at 11 locations of the large polyprotein, 790 kDa, which is key to viral reproduction.[6] The inhibition of these active enzyme sites is a primary target for producing an anti-COVID-19 drug. Since there is no known similar cleavage specificity among human proteases, it is unlikely such inhibitors would be toxic.[7]

Natural products have a role in the treatment and production of drugs for several diseases with the appeal of not contributing unknown harmful side effects.[8] Bee products have been used in medicine for tumor treatment and immune-related diseases, among others.[9, 10] Honey and propolis, a resinous compound produced by bees, have several biological properties that have anti-inflammatory, antibiotic, antifungal, antiviral, antioxidant, anti-cancer, immunomodulatory, and hepatoprotective effects.[11–13]

The general composition of propolis is about 50% resins, 30% waxes, 10% essential oils, 5% pollen, and 5% various

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organic compounds, including polyphenols and flavonoids. More than 200 components have been identified. [14–17] The chemical composition of honey depends mainly on the plant sources and collection environment. Manuka honey, a dark honey derived from the manuka tree, has attracted attention for its biological properties, and particularly antiviral activity. Several studies have shown that there are many flavonoids and polyphenolic compounds in manuka honey [18–21] that may be the source of its antimicrobial, antiviral, and antioxidant effects.[22–25]

Given the demonstrated biological effects of honey and propolis, 6 compounds that have previously drawn attention [23, 26] were selected to study potential anti-COVID-19 potency. An in silico approach (molecular docking), was used to examine the properties of 3-phenyllactic acid, caffeic acid phenethyl ester (CAPE), lumichrome, galangin, chrysin, and caffeic acid, with the hope that this study might illuminate a potential role for honeybee product constituents in an anti-COVID-19 drug.

Methods

Preparation of Honeybee Product Chemical Compounds (Ligands)

The 2-dimensional (2D) structure of the 6 selected chemical compounds of honey and propolis were obtained from the PubChem database in the structure-data file format. The Schrödinger Maestro v10.1 LigPrep tools (Schrödinger LLC., New York, NY, USA) were used to perform the conversion to 3D structures using ionization variation, stereochemical correction, energy minimization, and optimization of geometry. The simulation was completed using the Optimized Potentials for Liquid Simulation-2005 (OPLS_2005) force field and the Epik module to determine ionization states at pH 7.0 +/- 2.0, and the options for generating tautomers, desalting, and varying chiral centers to generate a single low-energy ring confirmation per ligand. The optimized ligands were then used for docking. The 2D structure of the selected compounds and their PubChem compound identifiers are shown in Figure 1.

COVID-19 Main Protease Identification and Preparation

The 3D crystal structure of the COVID-19 M^{pro} in complex with N-(2-phenylethyl) methanesulfonamide (PDB ID: 5R7Y) was downloaded from the Protein Data Bank (PDB; https://www.rcsb.org) in 1.65 Å resolution. The protein structure was refined by assigning bond orders, adding missing hydrogen atoms and disulfide bonds, and removing water molecules within 5 Å of the heteroatom. The properties of the side chain hydroxyl groups of asparagine, glutamine,

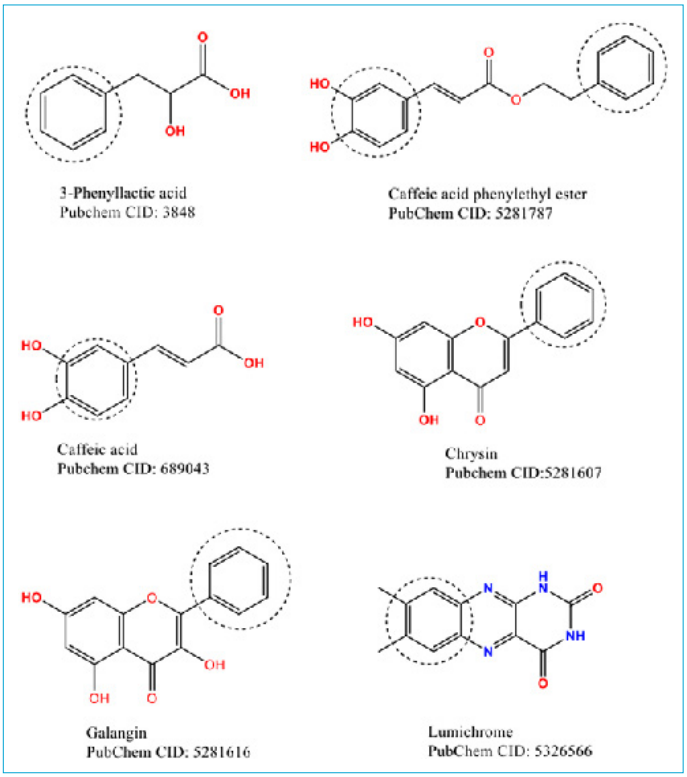

Figure 1. Two-dimensional structures of 6 honeybee product chemical compounds Caffeic acid phenethyl ester.

and histidine were optimized using the OPLS_2005 force field. The minimization was restrained to the input protein coordinates by a predefined root mean square deviation (RMSD) tolerance of 0.3 Å.

Receptor Grid Generation

The interaction between the prepared ligands and receptor proteins was studied by creating a receptor grid. An already bound ligand was excluded from the grid generation and the site of a docked ligand was confined to an enclosing box, centroid of the docked pose, and similar in size to the workspace ligand.

Molecular Docking (Glide Docking)

The prepared ligands and protein were docked using the Schrödinger Maestro software with the standard precision flexible ligand mode and 10 poses per ligand. Glide score was used to perform the final scoring of energy-minimized poses. The lowest glide scores (the best docked pose) for each ligand were recorded, and the RMSD value of the difference between the observed X-ray crystallography of the protein (native structure) and the predicted confirmation of input ligand geometry was calculated. The 3D structure of the docked protein and the binding interaction distance of each ligand with the receptor was analyzed using the PyMOL program.

Results

Several studies have reported on honeybee product constituents as antiviral compounds,[23-26] and computational methods may have an important role in the design of drug to meet the critical need for response to COVID-19 with the potential of minimal harmful side effects.

Docking simulation of the 6 selected honeybee products with the COVID-19 M^{pro} revealed very interesting results. The details of all docking scores, glide scores, potential energy, and the RMSD for the ligands to the selected protein are summarized in Table 1. All of the ligands demonstrated good binding affinity to the receptor in different ways. The glide score of 3 of the compounds (numbers 2, 4, and 5) reflected significant electrostatic attraction as well as more than 1 hydrogen bond between ligand hydroxyl groups and amino acid residues. The binding affinity of ligands 1-6 with the receptor is illustrated in Figure 2.

Electrostatic interactions between the phenyl ring and the aromatic ring of HIE-41 of the receptor with a separation of 4.2 Å (pi-pi stacking) led to stabilization of CAPE residue within the receptor, and the terminal residues of amino acids THR-24 and THR-26 displayed a strong hydrogen bond with the 2 hydroxyl groups of the CAPE ligand (Fig. 2).

Discussion

Chrysin demonstrated binding with ER-46, THR-24, and THR-26 through a hydrogen bond at 2.4, 2.6, 2.1 Å, respectively, as well as strong electrostatic interaction of the phenyl ring with HIE-41 at 3.8 Å.

Galangin interacted with the receptor with a glide score -6.307 Kcal/mol through a hydrogen bond with 2 amino acid residues, SER-46 and THR-24, as well as pi-pi interaction with HIE-41. In contrast, caffeic acid interacted with high affinity to the COVID-19 M^{pro} through a hydrogen bond of its hydroxyl groups with 2 other amino acid residues, GLN-189 and HIE-164, at 2.0 and 2.8 Å, respectively, in addition to another pi-pi interaction with HIE-41 at 4.1 Å (Fig. 2).

The lumichrome and 3-phenyllactic acid ligands revealed moderate binding affinity with the receptor with glide scores of -5.205 and -5.867 Kcal/mol, respectively. Lumichrome and 3-phenyllactic acid binding within the pocket as a result of pi-pi stacking with HIE-41 was observed, while 3-phenyllactic acid demonstrated a strong hydrogen bond with only 1 amino acid residue, GLN-189 (Fig. 2).

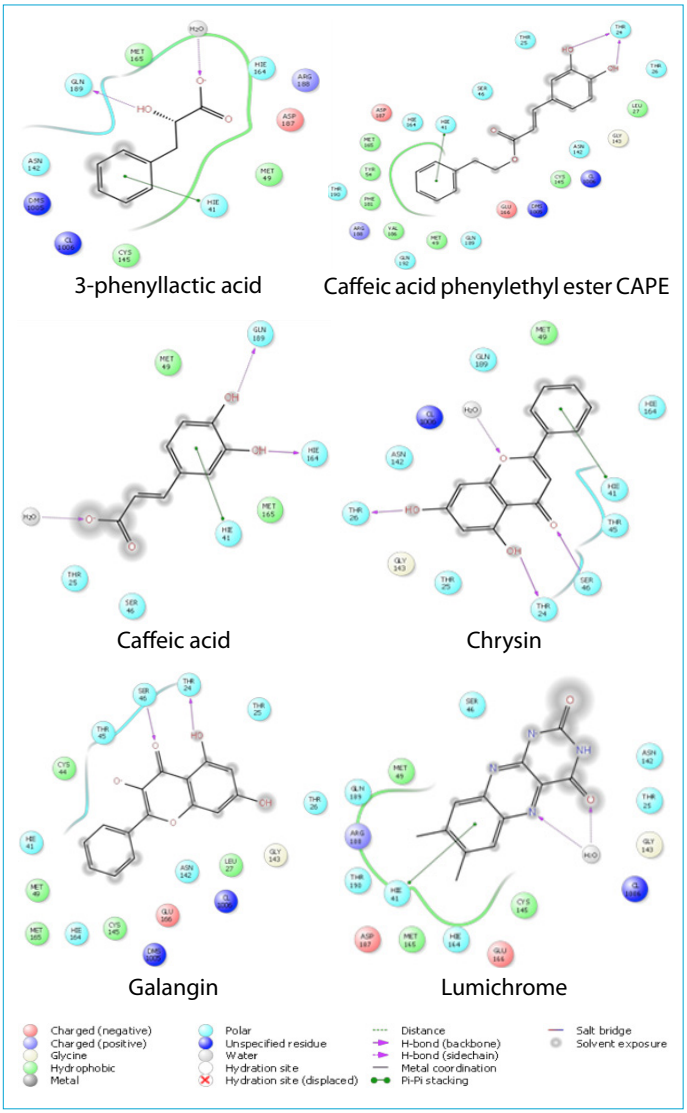

Figure 2. Ligand interaction of 6 honeybee product contituents with the COVID-19 main protease. Caffeic acid phenethyl ester.

Table 1. Docking results of 6 ligands with the COVID-19 main protease.

Number	Ligands	Potential energy	Docking score	Glide score	RMSD
1	3-phenyllactic acid	34.546	-5.867	-5.868	0.049
2	Caffeic acid phenethyl ester (CAPE)	46.07	-6.383	-6.386	0.048
3	Caffeic acid	14.22	-4.387	-4.387	0.035
4	Chrysin	63.126	-6.097	-6.103	0.047
5	Galangin	74.258	-6.295	-6.307	0.044
6	Lumichrome	94.141	-5.205	-5.205	0.040

RMSD: Root mean square deviation.

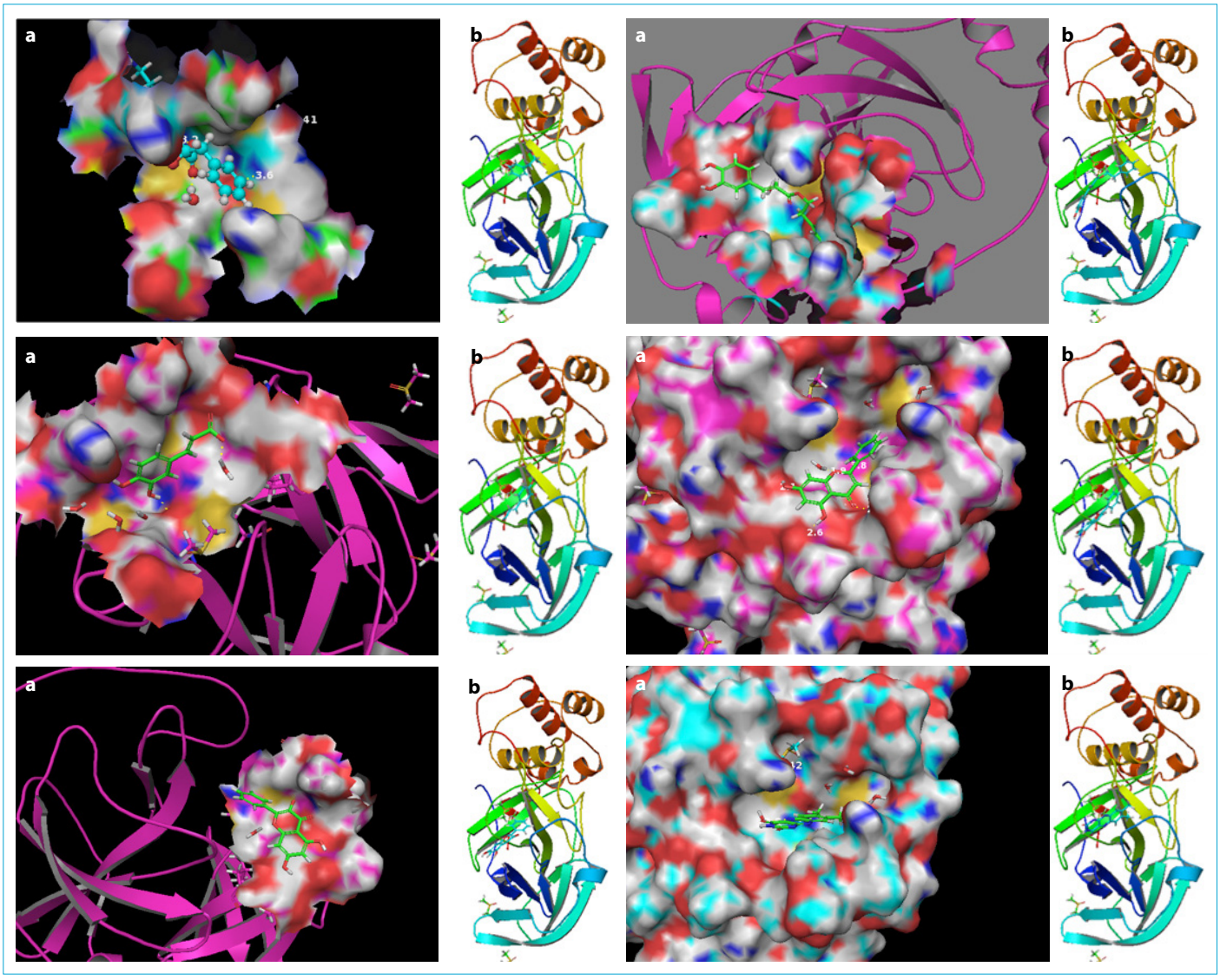

Figure 3. (a) Three-dimensional interaction diagrams of 6 ligands docked in the active site of the COVID-19 main protease. Red coloring reflects a high polar area, blue indicates a mild polar area, and gray illustrates a hydrophobic area. **(b)** Crystal structure of the COVID-19 main protease in complex with the 6 ligands studied.

The 3D crystal structures of the 6 docked ligands with the COVID-19 M^{pro} are presented in Figure 3.

Conclusion

Honeybee products are known to contain a wide range of flavonoid compounds with several interesting biological properties. This was an in silico study of the biological activity of 6 compounds present in honey and propolis as antiviral agents against the COVID-19 M^{pro}. Our results revealed that 4 compounds had strong binding affinity with a good glide score and may inhibit the COVID-19 M^{pro} and replication of the virus. In vivo follow-up research is needed, but these are encouraging early findings for a natural anti-COVID-19 drug with the potential of few harmful side effects.

Disclosures

Ethics Committee Approval: The study was approved by the Local Ethics Committee.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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