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Identification of Honey Flavonoids as Potential Inhibitors of SARS-CoV-2 RNA-Dependent RNA Polymerase and Main Protease: An *In silico* Analysis

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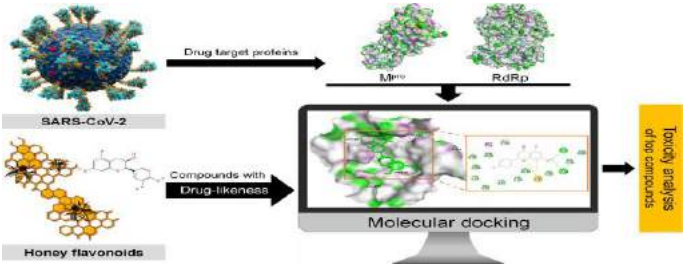
Abstract

The worldwide spread of COVID-19 caused by SARS-CoV-2 is a serious health crisis which requires a safe and efficacious treatment to combat the disease. RNA-dependent RNA polymerase (RdRp) and main protease (M^{pro}) are vital enzymes in the life cycle of SARS-CoV-2, considered as effective drug targets. In the current investigation, fourteen (14) honey flavonoids were analyzed for their potential to inhibit RdRp and M^{pro} using the computational approach. Firstly, flavonoids were screened based on their drug-likeness which determined all the compounds, except epigallocatechin gallate, as orally bioavailable drugs with easy absorbance and high permeability. Secondly, the screened thirteen (13) flavonoids were subjected to molecular docking analysis in order to identify the potent inhibitors of SARS-CoV-2 target proteins (RdRp and M^{pro}). The analysis revealed significant binding affinity of all compounds with both target proteins. Luteolin showed the most stable binding interaction (−7.6 kcal/mol) with RdRp, while apigenin and kaempferol displayed the binding energy of −7.8 kcal/mol with M^{pro}. Low binding energies and stable interactions indicated these compounds' potential inhibition of target proteins. Toxicity analysis depicted these top compounds as safe drugs, which further showed their significant probability to bind the target proteins in human body as a result of target prediction analysis. The above findings predict the anti-COVID-19 potential of honey flavonoids as safe drugs in which top inhibitor compounds exhibit good pharmacodynamic properties and target accuracy. For the future, wet lab experiments involving the *in vitro* and *in vivo* assays are recommended to investigate the effectiveness of honey flavonoids in the treatment of COVID-19.



1. Background

During late December 2019, a viral infection emerged in Wuhan, China and soon its spread took the form of a pandemic causing millions of deaths, worldwide [1, 2]. The infection was characterized by fever, diarrhea, cough, and pneumonia and referred to as COVID-19 [3, 4]. The causative agent was labeled as SARS-CoV-2 and identified as a member of the positive-sense single-stranded RNA coronavirus family [5]. Initially, COVID-19 cases were limited to Wuhan. However, human-to-human efficient transmission caused exponential growth in the number of reported cases and millions of deaths (worldometers.info/coronavirus/) have been reported worldwide since then. Although several vaccines have been developed, there remains a dire need to develop potent drugs against COVID-19 to combat its deadliest variants.

Understanding the SARS-CoV-2 life cycle is crucial for targeting viral proteins in order to ensure drug discovery. The virus enters into the host cell through the human ACE2 (angiotensin-converting enzyme 2) receptor by binding it with spike protein, followed by uncoating of the virus and polypeptides biosynthesis using the host cell machinery [6–8]. Afterwards, RNA is synthesized with the viral RNA-dependent RNA polymerase (RdRp) enzyme [9]. Numerous viral proteins necessary for the SARS-CoV-2 replication and catalyzed by main protease (M^{pro}) and papain-like protease (PLpro) are executed through mRNA to enable viral multiplication [10]. In view of their significance in the viral life cycle, all of these proteins act as potential drug targets and their inhibition would cause the blockage of the viral life cycle.

Natural products without harmful side effects are vital for drug synthesis needed to treat numerous diseases [11]. Flavonoids found in natural honey reportedly have anti-inflammatory, antineoplastic, antiulcer, and antiviral effects and are beneficial against several chronic diseases [12]. They are also effective against several viral diseases, such as HIV [13, 14], genital and labial herpes [15, 16], herpes simplex viruses, adenoviruses, [17] and hepatitis B [18]. The current investigation was conducted to assess the anti-COVID-19 effect of honey flavonoids inhibiting the RdRp and M^{pro} enzymes of SARS-CoV-2 with the application of computational and bioinformatic tools.

2. Methods

2.1. Data Set

The 3D coordinates of fourteen (14) flavonoids (Figure 1) present in different types of honey [19] were retrieved as ligands (.SDF format) from the PubChem database [20]. Crystal structure (3D) of target proteins, RdRp (6M71), and M^{pro} (6LU7) were acquired from the Protein Data Bank [21] in .PDB format.

2.2. Drug-likeness (ADME) Analysis

Physicochemical properties of a drug molecule influence its efficacy, metabolism and safety and are considered as essential in drug discovery. These properties were estimated by Lipinski's rule of five [22]. For this purpose, molecular weight (Da), hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), and LogP values for each compound were noted. These parameters were analyzed via the SwissADME tool [23] using the PubChem SMILES of compounds as input. Flavonoids showing drug-likeness (obeying Lipinski's rule of five) were considered for further analysis.

2.3. Ligand Preparation

The ligand (flavonoids) structures were imported to the OpenBabel tool in PyRx 0.8 software [24] and using the universal force field (UFF), their energies were minimized. The numbers of steps and the number of steps for the update were 2000 and 1, respectively. Minimization was stopped at the energy difference of < 0.01 kcal/mol. After energy minimization, ligands were transformed to the .PDBQT format.

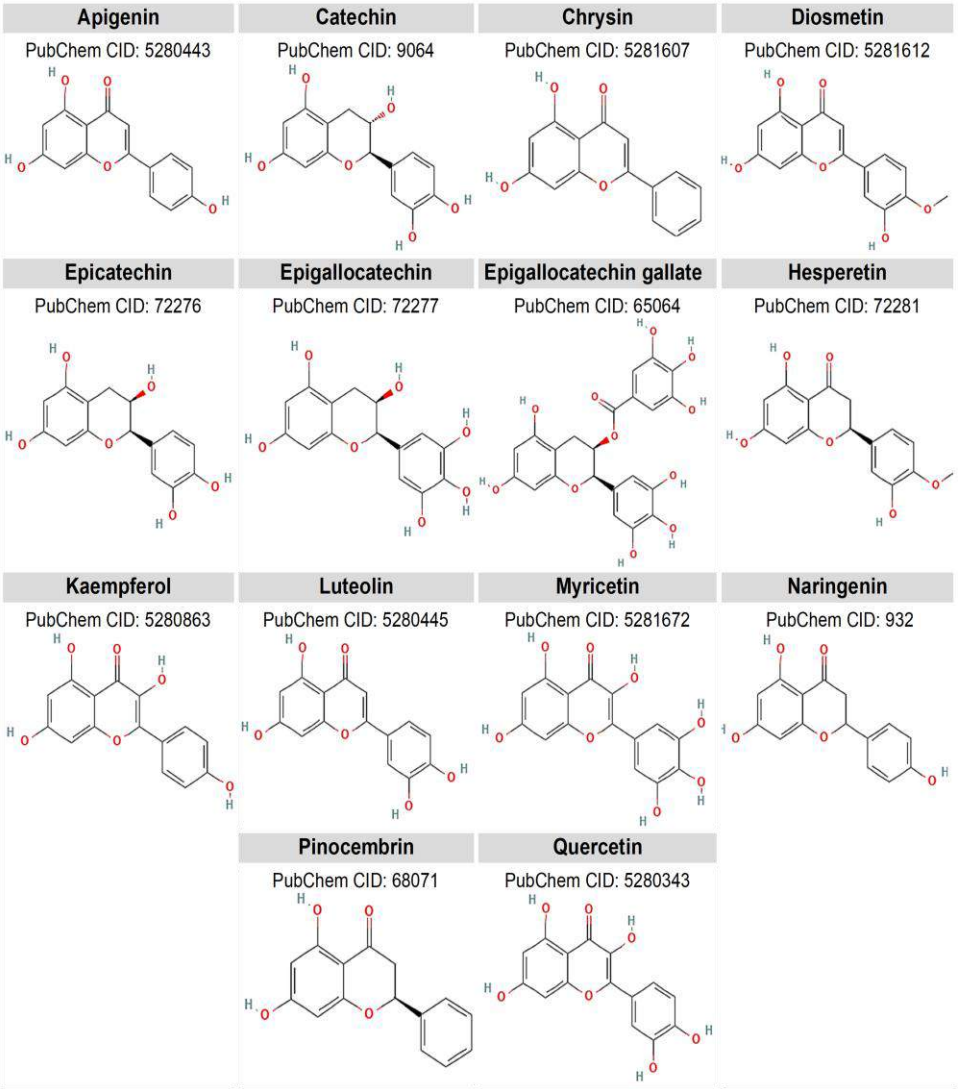


Figure 1. Set of Fourteen (14) Honey Flavonoids along with PubChem CIDs.

2.4. Protein Preparation

Using BIOVIA Discovery Studio 2021 [25], the attached inhibitors and water molecules were removed from each receptor protein. To compute Kollman charges for the protein and to add polar hydrogen, the AutoDock Tools (ADT) graphical user interface was employed. The protein structures were then imported in PyRx 8.0 [24], in order to convert them to .PDBQT format.

2.5. Molecular Docking Analysis

Molecular docking analysis of honey flavonoids and target proteins (RdRp and M^{pro}) was conducted with the application of AutoDock Vina incorporated in the PyRx 0.8 software [24]. For docking analysis, a three-dimensional grid box was mapped at maximum on 3D protein to allow the binding of ligands on all parts of receptor proteins. The visualization of ligand-protein interaction was performed using BIOVIA Discovery Studio 2021 [25].

2.6. Toxicity Prediction

Toxicology prediction indicates the number of small molecules that human and animal models could tolerate. Online tool pkCSM [26] was used to predict the toxicology of ligands using PubChem SMILES. The values of Minnow toxicity, *Tetrahymena pyriformis* toxicity, oral rat chronic toxicity (LOAEL), and oral rat acute toxicity (LD50), as well as their maximum tolerated dose for human beings, were obtained for each ligand. Moreover, the parameters of AMES toxicity, hERG I and hERG II inhibitors, hepatotoxicity, and skin sensitization were also determined.

2.7. Target Prediction

Studying the molecular target is important for assessing its potential cross-reactivity or phenotypical side effects due to small biomolecule actions [27]. SwissTargetPrediction database [28], was used to predict the targets of honey flavonoids in *Homo sapiens* using the PubChem SMILES as input. SwissTargetPrediction is a tool of molecular similarity match in 2D and 3D, containing 376,342 active compounds on 3,068 target macromolecules [28].

3. Results

3.1. Drug-likeness (ADME) Analysis

ADME analysis depends on Lipinski's rule of five, a thumb rule used to assess drug-likeness. It evaluates pharmacokinetic parameters for designing and developing a drug. According to this rule, a small molecule assigned as a drug should follow the sequence: molecular weight ≤ 500 Da, number of hydrogen bond donors (HBD) ≤ 5, number of hydrogen bond acceptors (HBA) ≤ 10, and LogP (lipophilicity) ≤ 5 [22], as observed for ninety percent (90%) orally available drugs which have acquired Phase II clinical status. These characters govern the first step of oral bioavailability [29].

The SwissADME server was used to assess the molecular characterstics of all ligands in order to evaluate their potential against therapeutic targets [30]. Figure 2 depicts the results of physicochemical properties. The molecular weight of honey flavonoids ranged from 254.24 Da (chrysin) to 458.37 Da (epigallocatechin gallate), indicating that all compounds had a molecular weight ≤ 500. As far as the number of H-Bond donors (HBD) is concerned, epigallocatechin, myricetin, and epigallocatechin gallate violated the HBD ≤ 5 with HBD values of 6, 6, and 8, respectively. The number of HBR was 4 for chrysin and 11 for epigallocatechin

gallate, whereas epigallocatechin gallate violated Lipinski's rule (HBR ≤ 10). The value of LogP ranged from 0.42 (epigallocatechin) to 2.55 (chrysin), showing no violation. Overall, epigallocatechin and myricetin demonstrated one violation of Lipinski's rule but showed sufficient drug-likeness to be used as drugs. On the other hand, epigallocatechin gallate violated two parameters and showed no drug-likeness at all. Hence, it would not be safe to use it as a drug, so it was not included in molecular docking analysis. Compounds obeying Lipinski's rule would become orally bioavailable drugs, easily absorbed and highly permeable [22, 28].

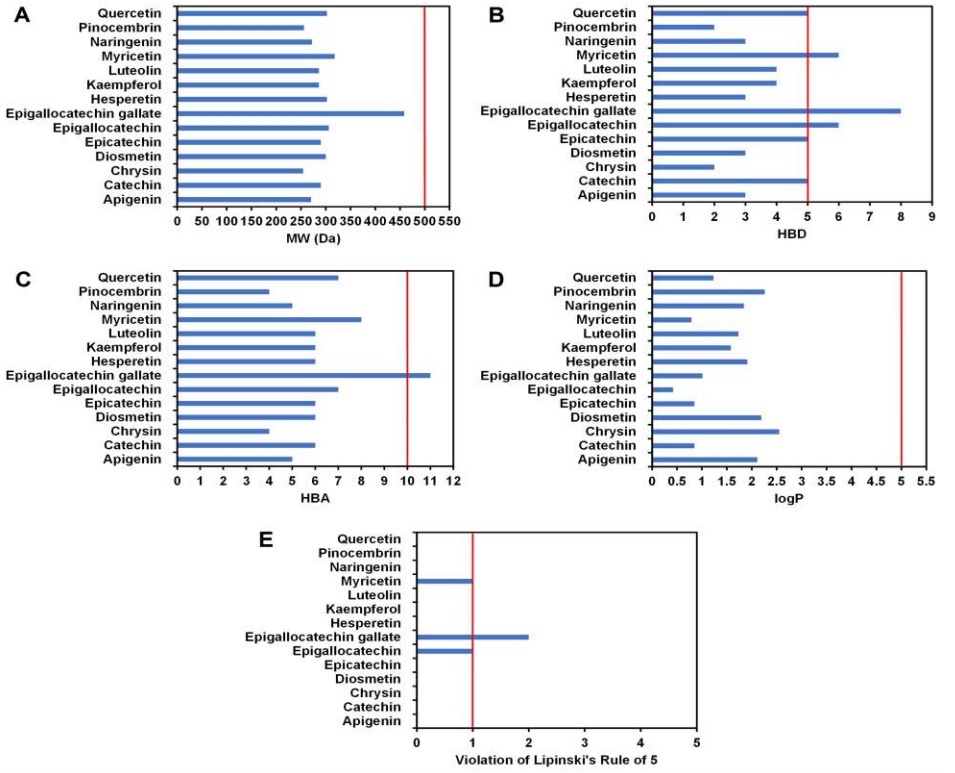


Figure 2. ADME Analysis Showing the Parameters of Lipinski's rule for Fourteen (14) Honey FlavonoidsA: All flavonoid compounds have molecular weight less than 500 Da (red line). B: Myricetin, epigallocatechin gallate and epigallocatechin violate the Lipinski's rule by having H−Bond donor (HBD) more than 5 (red line). C: All compounds have H−Bond acceptor (HBA) ≤ 10 except epigallocatechin gallate with HBD more than 10 (red line). D: All flavonoids contain logP less than 5 (red line). E: Myricetin and epigallocatechin depict one and epigallocatechin gallate show two violations of Lipinski's rule of five.

3.2. Molecular Docking Analysis

In modern drug discovery, computer-aided drug design has become one of the most important techniques as it minimizes the labor and cost incurred in the drug discovery process. It allows the researchers to reduce synthetic and biological testing efforts, accelerating the drug development process [31].

This method has proven to be efficient, especially for screening antiviral synthetic or natural compounds through computational approaches such as docking, thus saving time and money [32]. In the current study, molecular docking was performed between thirteen (13) screened honey flavonoids (except epigallocatechin gallate) and two SARS-CoV-2 target proteins, namely RdRp and M^{pro}. Their binding energies and interactions are depicted in figures 3, 4 and 5. The results demonstrated that all ligands showed significant binding affinities with both target proteins (binding energies below –6.0 kcal/mol cut−off value) [33].

The results of molecular docking with RdRp revealed that luteolin potentially inhibited the viral protein the most with the lowest value of binding energy obtained as −7.6 kcal/mol, followed by epigallocatechin and hesperetin with −7.5 kcal/mol, apigenin, chrysin, and myricetin with −7.4 kcal/mol, diosmetin and quercetin with −7.3 kcal/mol, naringenin with −7.2 kcal/mol, kaempferol and pinocembrin with −7.1 kcal/mol, catechin with −7.0 kcal/mol, and epicatechin with −6.8 kcal/mol binding energy (Figure 3).

The lowest binding energy of luteolin indicates its strongest binding affinity with the target protein, leading to the most stable inhibition. Molecular docking with M^{pro} ranked the honey flavonoids based on binding energies in the following sequence: apigenin and kaempferol (−7.8 kcal/mol), naringenin (−7.7 kcal/mol), diosmetin, luteolin and quercetin (−7.4 kcal/mol), myricetin (−7.3 kcal/mol), catechin, chrysin, hesperetin and pinocembrin (−7.2 kcal/mol), and epicatechin and epigallocatechin (−7.1 kcal/mol) (Figure 3). Apigenin and kaempferol were found to be the most potent inhibitors of SARS-CoV-2 M^{pro} with the lowest energy score (−7.8 kcal/mol), indicating their inhibition with high stability. Another potent molecule was naringenin with a slight difference of energy (−7.7 kcal/mol).

The 3D and 2D interactions of the top three ligands with SARS-CoV-2 target proteins RdRp and M^{pro} are shown in figures 4 and 5. These figures depict that the conventional H-Bond, van der Waals, pi-donor H-Bond, pi-sulfur, pi-pi stacked, pi-alkyl, and pi-cation forces mainly hold honey flavonoids (ligands) in the active sites of target proteins.

3.3. Toxicity Prediction

Figure 6 depicts the summary of pkCSM predictions [26] for top inhibitors of RdRp and M^{pro}. All flavonoids were found to have the value of log LC50 more than −0.3, thus showing no toxicity for Minnow fish. While, the values of *T. pyriformis* toxicity were found to be 0.326, 0.312, and 0.38 log µg/L for luteolin, kaempferol and apigenin, respectively.

The values of oral rat chronic toxicity (LOAEL) were evaluated as 2.409 for luteolin, 2.298 for apigenin, and 2.505 log mg/kg_bw/day for kaempferol. Whereas, lethal dosage (LD50) was observed as 2.455, 2.45 and 2.449 mol/kg for luteolin, apigenin, and kaempferol, respectively.

The value of the maximum tolerated dose (MTD) for human beings was 0.531 mg/kg/day for luteolin, while the values for

apigenin and kaempferol were 0.328 and 0.499 mg/kg/day, respectively. These outcomes revealed that all compounds showed no AMES toxicity. None of the compounds inhibited the human ether-a-go gene (hERG) I and II or caused hepatotoxicity or skin sensitization

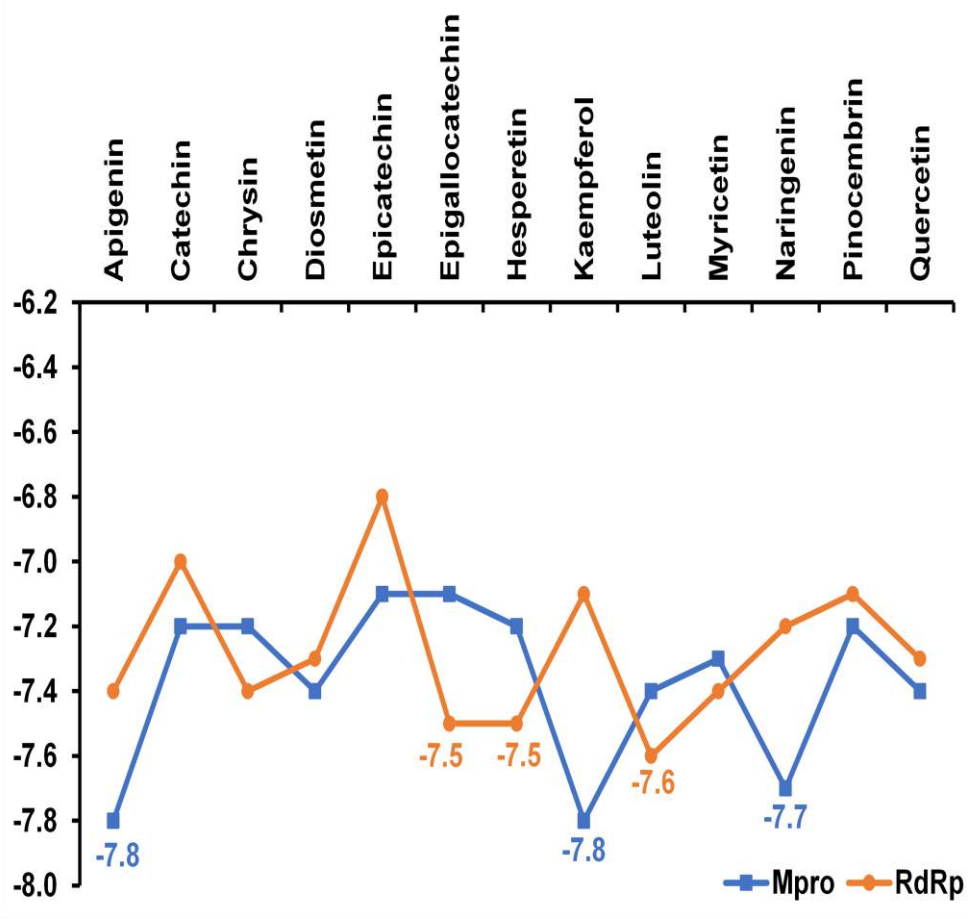


Figure 3. Binding Energies of Honey Flavonoids obtained from the Molecular Docking Analysis with SARS-CoV-2 RdRp (6M71) and M^{pro} (6LU7) Enzymes.

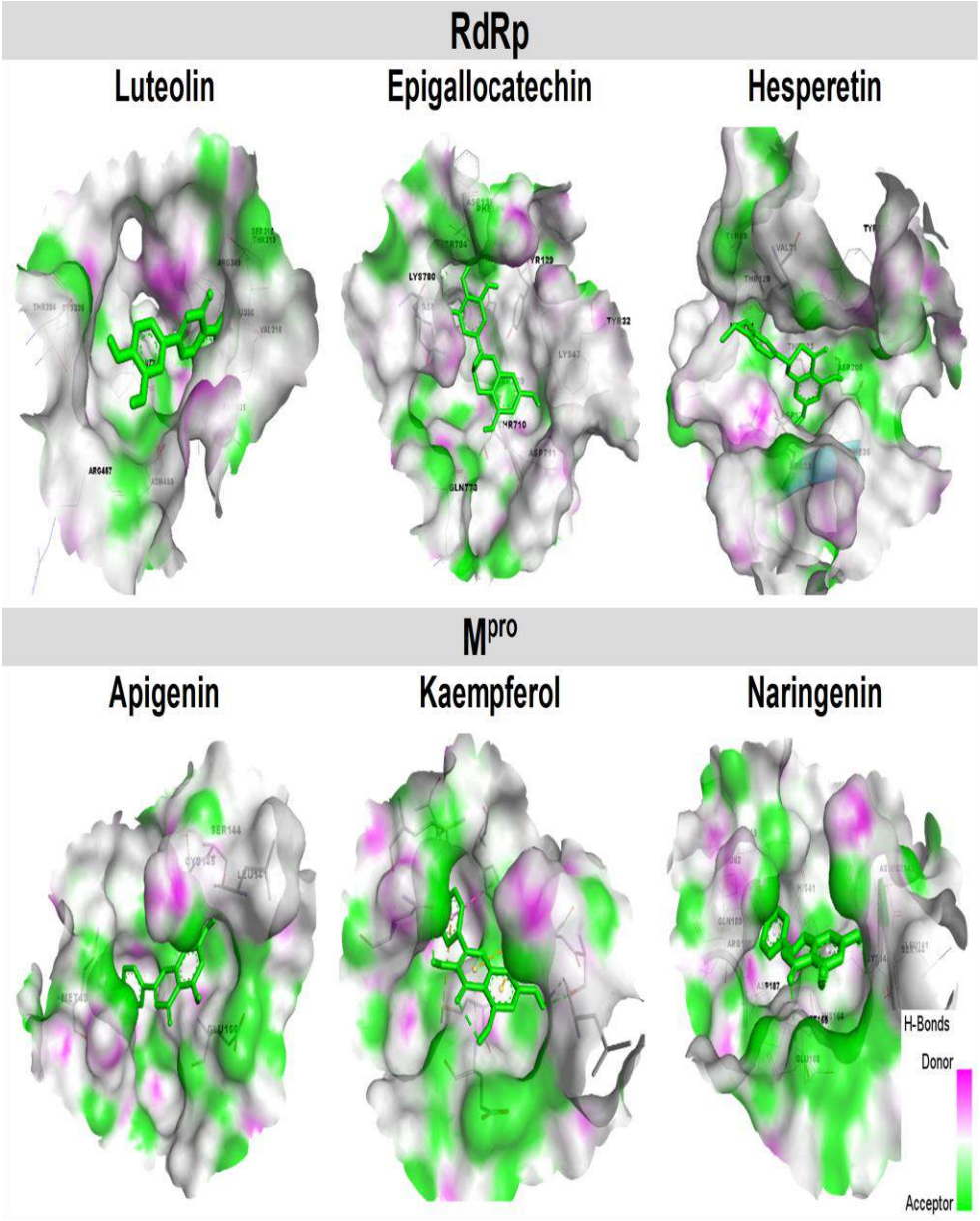


Figure 4. 3D Binding Conformation of Top Three Honey Flavonoid Inhibitors of SARS-CoV-2 RdRp (6M71) and M^{pro} (6LU7) Active Sites (Hydrogen Bond Interaction).

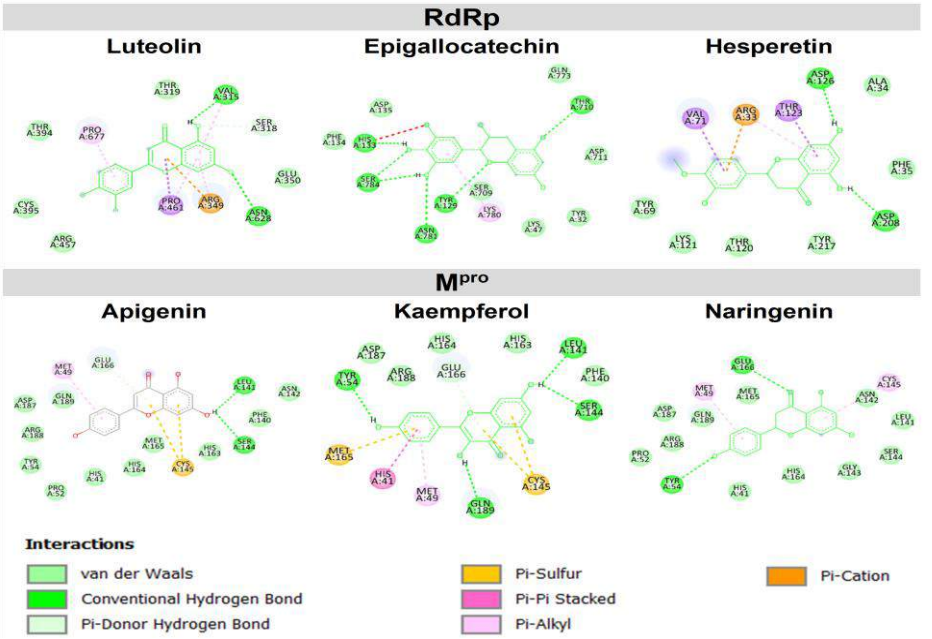


Figure 5. 2D Presentation of Non-Bond Interactions of Top Three Honey Flavonoids with the Amino acid Residues at SARS-CoV-2 M^{pro} (6LU7) and RdRp (6M71) Active Site.

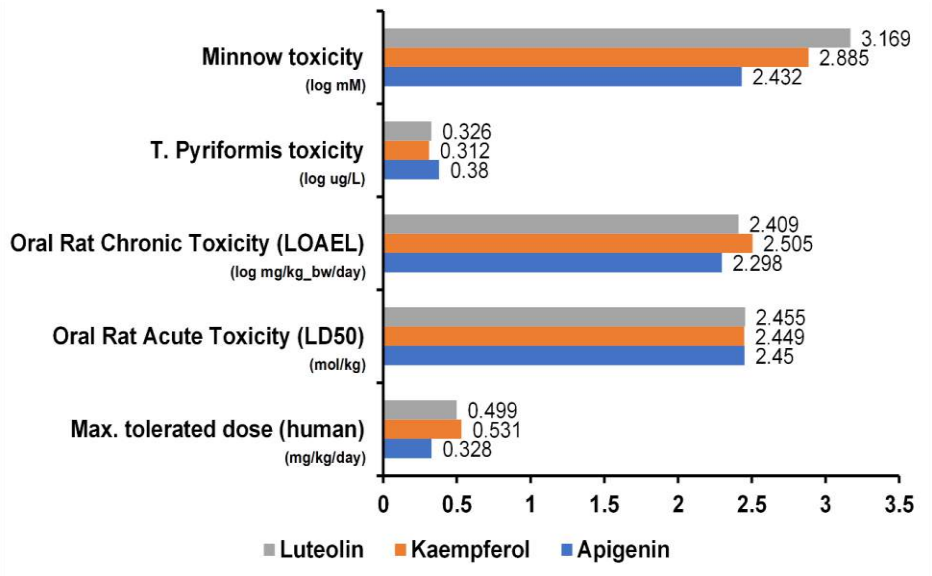


Figure 6. Toxicity Parameters for Top Honey Flavonoids used as SARS-CoV-2 RdRp and M^{pro} Inhibitors.

3.4. Target Prediction

The target prediction analysis for top ligands (based on binding energy), that is, luteolin for RdRp and apigenin and kaempferol for M^{pro} inhibition, was performed via SwissTargetPrediction software. Figusre 7 displays the top twenty-five observations as pie-charts. Luteolin was found to efficiently target the enzyme (20.0%), kinase (16.0%), oxidoreductase (12.0%), lyase (16.0%), other cytosolic proteins (4.0%), family A G protein−coupled receptor (4.0%), membrane receptor (4.0%), secreted protein (4.0%), protease (4.0%), cytochrome P450 (4.0%), and primary active transporter (4.0%). For apigenin, the pie-chart predicted 16.0% of the enzyme, 24.0% of kinase, 2.0% of oxidoreductase, 8.0% of cytochrome P450, 8.0% of the nuclear receptor, 4.0% of other cytosolic proteins, 4.0% of hydrolase, 8.0% of family A G protein-coupled receptor, 8.0% of primary active transporter, and 4.0% of secreted proteins as well as other ion channels as a target. The analysis predicted that kaempferol targeted the enzyme (20.0%), oxidoreductase (16.0%), kinase (12.0%), lyase (16.0%), primary active transporter (12,0%), transcription factor (4.0%), nuclear receptor (4.0%), cytochrome P450 (4.0%), family A G protein−coupled receptor (4.0%), hydrolase (4.0%) and protease (4.0%). The average probability score for luteolin was found to be 0.532, while for apigenin and kaempferol it was 0.4389 and 0.535, respectively.

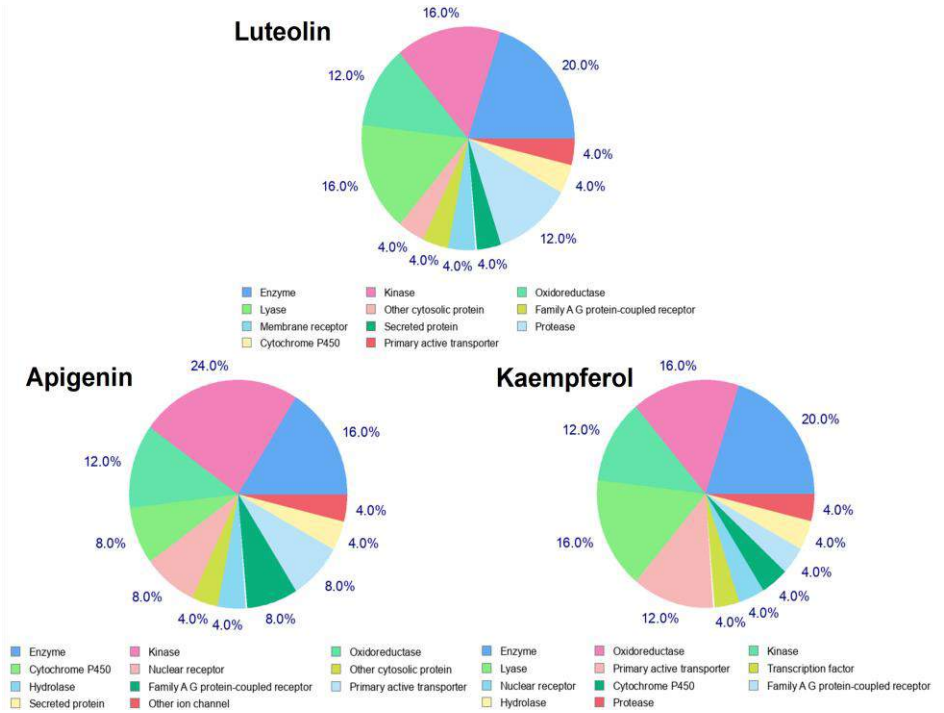


Figure 7. Top Twenty-Five (25) targets predicted by SwissTargetPrediction Database for Top Honey Flavonoid Inhibitors of SARS-CoV-2 RdRp and M^{pro}.

4. Discussion

Honey is well-known as an antimicrobial agent and it has been proved to show antiviral properties against several lethal viruses. The current study evaluated flavonoid compounds from the honey source for their anti-COVID-19 potential. Firstly, flavonoids were screened based on Lipinski's rule of five for drug-likeness. Then, molecular docking was used to analyze the binding affinities of compounds that inhibited the two target enzymes of SARS-CoV-2. Potent inhibitors were then assessed for their toxicity and target prediction.

Lipinski's rule of five is the key parameter used to assess the drug-likeness of potent medicines and chemical compounds. According to the rule, a chemical compound can be utilized as pharmaceutical drugs if it follows the rule. In the current study, it was found that most of the flavonoids followed the rule with molecular weight ≤ 500 Da, number of H-bond donors (HBD) ≤ 5, number of H-bond acceptors (HBA) ≤ 10, and LogP (lipophilicity) ≤ 5. Although, epigallocatechin and myricetin violated one parameter of Lipinski's rule of five but showed overall drug-likeness. On the contrary, epigallocatechin gallate showed no drug-likeness by violating two parameters. Among the parameters of Lipinski's rule of five, low MW indicates that a molecule is light and can easily cross the cell membrane. Molecules with ≤ 500 Da are the most suitable for oral absorption [22]. A heteroatom lacking a formal positive charge saves pyrrole nitrogen, heteroaromatic oxygen, sulfur, halogens, and higher oxidation states of sulfur, phosphorus, and nitrogen, as well as the oxygen connected to them, and is regarded as an HBD. While HBA is referred to as a heteroatom with at least one bound hydrogen and the sum of these heteroatoms (O and N atoms) should be ≤ 10 [22]. Both HBD and HBA are critical as they synergize between macromolecules, such as target proteins and chemical-like drug molecules, and remain important for oral absorption [22]. LogP is n-octanol/water partition coefficient and plays an important role in the absorption of medication in the mouth [22]. It also facilitates the interaction of a drug molecule with its target [34]. Having both lipophilic and hydrophilic qualities, n-octanol is a superb mimic of the characters of phospholipid membrane [34]. Compounds with logP ≤ 5 exhibit great oral qualities.

Molecular docking analysis of honey flavonoids (showing drug-likeness) was performed with two target proteins of SARS-CoV-2 virus, that is, RdRp and M^{pro}. Potent inhibitors of target proteins were identified based on their binding energies. All compounds with binding energies less than the cutoff value of –6.0 kcal/mol significantly inhibited both viral proteins [33]. Binding energies of flavonoids with RdRp ranged from −7.6 to −6.8 kcal/mol. Luteolin was found to be the most potent RdRp inhibitor with the binding energy of −7.6 kcal/mol, indicating its strong and stable interaction with target proteins. In the current study, honey flavonoids showed better results than compounds from *Nigella sativa*, where 1,2-dimethylcyclopentan-1-olinhibit showed the least energy (−4.6 kcal/mol) for docking against SARS-CoV-2 RdRp [35].

Similarly, the binding energies of remdesivir and galidesivir with SARS-CoV-2 RdRp were observed as −6.6 and −6.2 kcal/mol, respectively [36]. Single-stranded RNA viruses utilize RdRp for gene transcription and genome replication.

Therefore, RdRp is considered an important target for antiviral drugs and several pharmaceutical firms focus on it to develop the RdRP inhibitors of RNA viruses [37]. Favipiravir targets the RdRp of influenza viruses and has been approved against the influenza viruses in Japan [38, 39]. Remdesivir is used to treat human coronaviruses and filoviruses, including Marburg virus and Ebola virus [40].

Molecular docking with M^{pro} exhibited that apigenin and kaempferol (−7.8 kcal/mol) inhibited the target protein most potentially, while the lowest binding affinity was shown by epigallocatechin (−7.1 kcal/mol). The current investigation showed better results than the application of a combination of three drugs namely ritonavir, ostelmivir, and lopinavir against SARS-CoV-2 M^{pro}, which reported binding energies as −5.11, −4.65, and −4.1 kcal/mol, respectively [41]. Binding energies for ritonavir, oseltamivir, remdesivir, favipiravir, ribavirin, hydroxychloroquine, and chloroquine were −7.3, −4.7, −6.5, −5.4, −5.6, −5.3, −5.1 kcal/mol, respectively against SARS-CoV-2 M^{pro} [42]. Another *in silico* investigation of honeybees' products (caffeic acid, chrysin, galangin, lumichrome, caffeic acid, phenethyl ester, and 3−phenyllactic acid) showed that binding energies ranged from −6.383 to −4.387 kcal/mol [43]. For SARS-CoV-2, M^{pro} enzyme is an essential enzyme which cleaves the polyproteins to produce several active enzymes, including exo-ribonuclease, endo-ribonuclease, and RNA polymerase [44]. Protease enzyme is regarded as an important target for several viruses and many drugs targeting the viral protease have been developed. Nelfinavir, ritonavir, atazanavir, indinavir, saquinavir, lopinavir, amprenavir, darunavir, and tipranavir have been proved to show antiviral effects against human immunodeficiency virus or HIV (type 1) by targeting the protease [45]. Similarly, Sofobuvir, voxilaprevir, glecaprevir, grazoprevir, paritaprevir, asunaprevir, ritonavir, telaprevir, and boceprevir are known to target the hepatitis C virus (HCV) protease [46]. Thus, the development of drugs targeting SARS-CoV-2 RdRp and M^{pro} has clinical applications.

Computational and bioinformatics tools also predict the harmful effects of candidate drug molecules. Unsuitable molecules can be removed during drug screening because of their toxicity. *In silico* analysis indicated that top inhibitors of RdRp and M^{pro} are safe drug candidates without any toxicity. Lethal concentration (LC50) is the concentration of molecules that causes fifty percent (50%) mortality in the Fathead Minnows fish test group. LC50 with a value lower than 0.5 mM (log LC50 < -0.3) shows acute toxicity [26]. None of the flavonoid compounds exhibited Minnow toxicity in toxicity analysis. For *T. pyriformis* (a protozoan) toxicity, a compound with pIGC50 > -0.5 log μg/L is regarded as toxic [26]. During treatment, applying low-moderate drugs for a long time is a serious concern. Oral rat chronic toxicity (LOAEL) describes the toxicity induced by the lowest dose administered to rats via oral administration [26]. The values of LOAEL were evaluated as 2.409 for luteolin, 2.298 for apigenin, and 2.505 log mg/kg_bw/day for kaempferol. Oral rat acute toxicity indicates the administration of the lethal dosage (LD50) (mol/kg), which is the quantity of a single dose of the selected compound that causes fifty percent (50%) of deaths in a test animal group [26]. Its values were obtained as 2.455, 2.45 and 2.449 mol/kg for luteolin, apigenin and kaempferol, respectively. The maximum tolerated dose (MTD) for human beings (log mg/kg/day) indicates the threshold of

toxic chemicals for them. It is the maximum dose that is recommended as the starting dose during clinical trials (Phase I). It is regarded as low when its value is ≤ 0.477 log mg/kg/day, while > 0.477 log mg/kg/day is taken as high [26]. The value of MTD for luteolin was 0.531 mg/kg/day, while the values of apigenin and kaempferol were 0.328 and 0.499 mg/kg/day, respectively. The outcome revealed that all compounds showed no AMES toxicity. A compound with AMES toxicity could be mutagenic and carcinogenic [26]. The inhibition of human ether-a-go-go gene (hERG) I and II was not predicted for any compound. The inhibition of K+ ion channels is encoded by hERG and causes the development of long QT syndrome or torsade de pointes, resulting in fatal ventricular arrhythmia [47, 48]. hERG channels inhibition toxicity and it has caused the removal of several drugs from the market [26]. None of the compounds was found positive for hepatotoxicity. Hepatotoxicity prediction (measured on the bases of the side effects of 531 compounds associated with the liver) classifies a compound as hepatotoxic based on the physiological or pathological events that disrupt the functions of normal liver [26]. Similarly, skin sensitization depicts the serious effects of a compound when applied to the skin [26]. In the current report, no compound showed skin sensitization.

In case of target prediction, the average probability score for luteolin was 0.532, while for apigenin and kaempferol it was 0.4389 and 0.535, respectively. Previous studies showed that a greater than zero probability value depicts a reasonable drug-ligand interaction [49, 50]. The above scores precisely demonstrate the probability of targeting a given protein through a bioactive molecule [23]. This makes an inference that the small compound may have high target attraction towards the specific binding site it is directed to.

From a biological and pharmacological perspective, all honey flavonoids were discovered to have anti-COVID-19 potential. Luteolin proved to be the best inhibitor of SARS-CoV-2 RdRp, while apigenin and kaempferol of M^{pro}. These flavonoid compounds also showed antiviral activities against several other viruses. Luteolin exhibited inhibitory effects against the SARS-CoV virus [51]. Besides, luteolin was observed to have antiviral potential against the respiratory syncytial virus, human immunodeficiency virus or HIV (type 1), Epstein-Barr virus, Japanese encephalitis virus, enterovirus 71, and coxsackievirus A16 [52–56].

Similarly, apigenin also reportedly showed SARS-CoV M^{pro} proteolytic activity [57]. Further, apigenin was documented to depict antiviral effects against the influenza virus, human immunodeficiency virus (HIV), herpes simplex viruses, hepatitis B and C, African swine fever virus, enterovirus 71, Epstein-Barr virus, and foot-and-mouth disease virus [14, 18, 58–64]. Kaempferol previously showed antiviral potential against the SARS-CoV [65], herpes simplex viruses, human immunodeficiency virus or HIV (type 1), and pseudorabies virus [66–68]. It is noteworthy that honey also exhibits anti-inflammatory and immunomodulatory activities. So, it is proposed that drugs based on honey components could be used to attenuate the expression of proinflammatory factors and receptors likely to cause acute respiratory distress, a major mortality cause associated with the patients of COVID-19, while boosting the immune system.

5. Conclusion

At present, the COVID-19 pandemic is a major challenge to the health sector of all the countries. The discovery of vaccines effective against SARS-CoV-2 is a major breakthrough. Still, there is a need to develop efficient drugs to combat its deadliest variants. Various synthetic compounds are currently investigated to treat the disease. However, due to their side effects, the use of natural compounds against COVID-19 is encouraged and their potential in this regard is being assessed. Keeping in view that honey has been reported to show antiviral activities, the current study investigated honey flavonoids as inhibitors of two important enzymes of SARS-CoV-2, that is, RdRp and M^{pro} employing *in silico* tools. Luteolin showed the most stable inhibition of RdRp with the lowest energy, while apigenin and kaempferol were the most efficient honey flavonoids that inhibited M^{pro}. Thus, these compounds could be used to block the SARS-CoV-2 spread by blocking these enzymes. Future studies may examine whether these compounds are safe for oral use without any toxicity and have good target accuracy in the human body.

6. Limitations and Future Perspective

To further validate the anti-COVID-19 potential of honey flavonoids, computational simulations of molecular dynamics are required to predict how atoms move over time in protein structure, depending on a general model of physics regulating interatomic interactions [69]. The potential for the emergence of novel coronaviruses in the future as well as their evolving nature necessitate the development of broad-spectrum antivirals. Future research should focus on the development of RNA-dependent RNA polymerase and main protease inhibitor antiviral drugs inhibiting the virus cell cycle. Further, the application of honey flavonoids to SARS-CoV-2 *in vitro* and *in vivo* before the clinical assay should be assessed.

List of abbreviations

COVID-19: Coronavirus disease 2019
Da: Dalton
HBA: Number of hydrogen bond acceptors
HBD: Number of hydrogen bond donors
hERG: the human Ether-à-go-go-Related Gene
Kcal/mol: Kilocalorie per mole
LD50: Lethal Dose 50
LOAEL: Oral rat chronic toxicity
M^{pro}: Main protease
MTD: Maximum tolerated dose
RdRp: RNA-dependent RNA polymerase
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

Ethical approval and consent to participate

Not applicable

Competing interests

The author has no competing interests.

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