In silico analysis and identification of honey flavonoids as potential inhibitors of SARS-CoV-2 RNA-dependent RNA polymerase and main protease

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Abstract:
COVID-19 caused by SARS-CoV-2 is a serious health crisis worldwide and requires a safe and efficacious treatment to combat the disease. RNA-dependent RNA polymerase (RdRp) and main protease (Mpro) are vital enzymes in the SARS-CoV-2 life cycle and are considered effective drug targets. In the current investigation, fourteen (14) flavonoids from honey were assessed to analyze their potential for RdRp and Mpro inhibition using the computational approach. First, flavonoids were screened based on drug-likeness, which determined all the compounds except epigallocatechin gallate as orally bioavailable drugs with easy absorbance and high permeability. Screened thirteen (13) flavonoids were subjected to molecular docking analysis to identify the potent inhibitors of SARS-CoV-2 target proteins (RdRp and Mpro). The analysis revealed the significant binding affinities of all compounds with both target proteins. Luteolin showed the most stable binding interactions (−7.6 kcal/mol) with the RdRp while apigenin and kaempferol displayed the binding energy of −7.8 kcal/mol with Mpro. Low binding energies and stable interactions indicate these compounds' potential inhibition of target proteins. Toxicity analysis depicted these top compounds as safe drugs while target prediction showed their significant probability of target accuracy in the human body. The findings predict the anti-COVID-19 potential of honey flavonoids as safe drugs where top inhibitor compounds exhibit good pharmacodynamics properties and target accuracy. Further wet-lab experiments involving the in vitro and in vivo assays are recommended to investigate the effectiveness of honey flavonoids to cure the COVID-19.

Keywords: SARS-CoV-2, honey flavonoids, in silico analysis, molecular docking, ADME

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

Understanding the SARS-CoV-2 life cycle is crucial for targeting the viral proteins for 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 the virus and polypeptides biosynthesis using host cell machinery [6–8]. Later, RNA is synthesized by the viral RNA-dependent RNA polymerase (RdRp) enzyme [9]. Numerous viral proteins necessary for the SARS-CoV-2 replication, catalyzed by main protease (Mpro) and papain-like protease (PLpro) will be executed through the mRNA to enable viral multiplication [10]. In the view of significance in the viral life cycle, all these proteins could be taken as drug targets and inhibition of these proteins would cause the blockage of the viral life cycle.

Natural products without harmful side effects are vital for drug synthesis to treat numerous diseases [11]. Flavonoids in natural honey have been reported to have anti-inflammatory, antineoplastic, antiulcer and antiviral effects and are beneficial against several chronic diseases [12]. Honey flavonoids have been 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 Mpro enzymes of SARS-CoV-2 with the applications of computational and bioinformatic tools.

2 Methods

2.1 Data set

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

![Figure 1. Set of fourteen (14) honey flavonoids along with PubChem CIDs.](image)

2.2 Drug-likeness (ADME) analysis

The physicochemical properties influence the efficacy, metabolism and safety and are considered very important in drug discovery. These properties were estimated by Lipinski’s rule of five [22]. For that, molecular weight (Da), hydrogen bond donor (HBD), hydrogen bond acceptor (HBA) and LogP values for each compound were noted. These parameters were analyzed by the SwissADME tool [23] by inputting the PubChem SMILES of compounds. Flavonoids showing the drug-likeness (obeying Lipinski’s rule) were considered for further analysis.
2.3 Ligand Preparation
The ligands (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 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.

2.4 Protein preparation
Using BIOVIA Discovery Studio 2021 [25], 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 proteins structures were then imported in PyRx 8.0 [24] to convert them in .PDBQT format.

2.5 Molecular docking analysis
Molecular docking analysis of honey flavonoids and target proteins (RdRp and Mpro) was conducted with the application of AutoDock Vina incorporated in the PyRx 0.8 software [24]. For docking analysis, three–dimensional grid box was mapped at maximum on 3D protein to allow the binding of ligands on all parts of receptor proteins. Visualization of ligand-protein interactions 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] predicted the toxicology of ligands using PubChem SMILES. The values of Minnow toxicity, Tetrahymena pyriformis toxicity, oral rat chronic toxicity (LOAEL), oral rat acute toxicity (LD50) and maximum tolerated dose for humans 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 the potential cross-reactivity or phenotypical side effects due to small biomolecule action [27]. SwissTargetPrediction database [23] predicted 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 the 376,342 active compounds on 3,068 target macromolecules [23].

3 Results
3.1 Drug-likeliness (ADME) analysis
ADME analysis depends on Lipinski’s rule of five, a thumb rule to assess the drug-likeness. This evaluates the pharmacokinetic parameters for designing and developing a drug. According to this rule, a small molecule being assigned as a drug should follow: molecular weight ≤ 500 Da, number of hydrogen bond donors (HBD) ≤ 5, number of hydrogen bond acceptors (HBA) ≤ 10, LogP (lipophilicity) ≤ 5 [22, 28], as observed for ninety percent (90%) orally available drugs which have acquired phase II clinical status. These characters govern the 1st step of oral bioavailability [29].
The SwissADME server assessed the molecular characters of all ligands to evaluate the potential of ligands against therapeutic targets [30]. The results of physicochemical properties have been explained in Figure 2. The molecular weight of honey flavonoids ranged from 254.24 Da (chrysin) to 458.37 Da (epigallocatechin gallate), indicating that all compounds have molecular weight ≤ 500. In the case of the number of H−Bond donors (HBD), epigallocatechin, myricetin and epigallocatechin gallate violated the HBD ≤ 5 by having the HBD values of 6, 6 and 8, respectively. The number of HBR ranged as 4 for chrysin and 11 for epigallocatechin gallate where epigallocatechin gallate violated Lipinski’s rule (HBR ≤ 10). The value of LogP varied from 0.42 (epigallocatechin) to 2.55 (chrysin) showing no violation. Overall, epigallocatechin and myricetin showed one violation of Lipinski’s rule but showed drug-likeness and can be used as a drug. While epigallocatechin gallate violated two parameters and showed no drug-likeness. Hence, epigallocatechin gallate would not be safe as a drug so it was not included in molecular docking analysis. Compounds obeying Lipinski’s rule would be 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 flavonoids. A: 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 engaged in the drug discovery process. It allows the researchers to reduce the synthetic and biological testing efforts leading to the acceleration in the drug development process [31]. This method has proven an efficient tool, especially for screening antiviral synthetic or natural compounds by computational approaches such as docking, saving time and money resources [32]. In the present study, molecular docking was performed between thirteen (13) screened honey flavonoids (except epigallocatechin gallate) and two SARS-CoV-2 target proteins i.e., RdRp and Mpro. Their binding energies and interactions have been depicted in Figures 3, 4 & 5. Our results demonstrated that all ligands had significant binding affinities with both target proteins (binding energies below −6.0 kcal/mol cut-off value) [33].

Results of molecular docking with the RdRp revealed that luteolin inhibited the viral protein most potentially with the lowest value of binding energy i.e., −7.6 kcal/mol followed by the 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 Mpro ranked the honey flavonoids based on binding energies as
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), epicatechin and epigallocatechin (−7.1 kcal/mol) (Figure 3). Apigenin and kaempferol were the most potent inhibitors of SARS-CoV-2 M\textsuperscript{pro} with the lowest energy score (−7.8 kcal/mol), indicating their inhibition with high stability. Another potent molecule was the naringenin with a slight difference of energy (−7.7 kcal/mol).

Figure 3. Binding energies of honey flavonoids obtained from the molecular docking analysis with SARS-CoV-2 RdRp (6M71) and M\textsuperscript{pro} (6LU7) enzymes.

The 3D and 2D interactions of the top three ligands with SARS-CoV-2 target proteins RdRp and M\textsuperscript{pro} have been shown in Figures 4 & 5. The conventional H-bond, van der Waals, π-donor H–bond, π–sulfur, π–π stacked, π–alkyl, π–cation forces mainly held the honey flavonoids (ligands) in the active sites of target proteins.

Figure 4. 3D binding conformation of top three honey flavonoid inhibitors of SARS-CoV-2 RdRp (6M71) and M\textsuperscript{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\textsuperscript{pro} (6LU7) and RdRp (6M71) active site.

3.3 Toxicity prediction
Summary of pkCSM predictions [26] for top inhibitors of RdRp and M\textsuperscript{pro} have been displayed in Figure 6. All flavonoids depicted the value of log LC50 more than −0.3 showing no toxicity for Minnow fish, while the values of T. pyriformis toxicity were found as 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. 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 humans was 0.531 mg/kg/day for luteolin while apigenin and kaempferol depicted 0.328 and 0.499 mg/kg/day, respectively. The outcome 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 6. Toxicity parameters for top honey flavonoids used as SARS-CoV-2 RdRp and M\textsuperscript{pro} inhibitor.

3.4 Target prediction
The target prediction analysis for top ligands (based on binding energy) i.e., luteolin for RdRp and apigenin and kaempferol for M\textsuperscript{pro} inhibition, was performed by SwissTargetPrediction
software and the top twenty-five observations were displayed as pie-charts in Figure 7. 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. 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 as 0.532, while for apigenin and kaempferol it was 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\textsuperscript{pro}.

### 4 Discussion

Honey has been known as an antimicrobial agents science ancient times and it has been proved to show antiviral properties against several lethal viruses. The present study evaluated flavonoid compounds from the honey source for their anti-COVID-19 potential. First, flavonoids were screened based on Lipinski rule-of-five parameters for drug-likeness then molecular docking analyzed the binding affinities of these compounds to inhibit the two important target enzymes of SARS-CoV-2. Potent inhibitors were then assessed for their toxicity and target prediction.

The Lipinski rule-of-five is a key parameter used to assess the drug-likeness of potent medicines and chemical compounds. According to the rule, chemical compounds can be utilized as pharmaceutical if it follows the rule. In the present study, most of the flavonoids followed the rule as molecular weight ≤ 500 Da, number of hydrogen bond donors (HBD) ≤ 5, number of hydrogen bond acceptors (HBA) ≤ 10, LogP (lipophilicity) ≤ 5. However, epigallocatechin and myricetin violated one parameter of Lipinski’s rule but showed overall drug-likeness. Epigallocatechin gallate by violating two parameters showed no drug-likeness. Among parameters of Lipinski rule-of-five, low MW indicates that a molecule is light and can easily cross the cell membrane. Molecules with ≤ 500 Da are favored for oral absorption [28]. A heteroatom lacking a formal positive charge saves pyrrole nitrogen, heteroaromatic, oxygen, sulfur, halogens, and higher oxidation states of sulfur, phosphorus, and nitrogen, including the oxygens connected to them is considered as H-bond donor (HBD). While hydrogen bond acceptor (HBA) is referred to as a heteroatom with one bound hydrogen at least and the sum of these heteroatoms (O and N atoms) should be ≤ 10 [22, 28]. Both HBD and HBA are considered critical as they synergize between macromolecule such as target protein and chemicals like drug molecules as well as they are important for oral absorption [22, 28]. LogP
is n-octanol/water partition coefficient and plays an important role in the absorption of medication in the mouth [28]. It also facilitates the interactions of a drug molecule with its target [34]. Because of the possession of both lipophilic and hydrophilic qualities, n-octanol was considered a superb mimic of the characters of phospholipid membrane [34]. Compounds with logP ≤ 5 exhibit great oral qualities.

The molecular docking analysis of honey flavonoids (showing drug-likeness) was performed with two target proteins of SARS-CoV-2 virus *i.e.*, RdRp and M^{pro} and potent inhibitors of target proteins were identified based on the binding energies. All compounds significantly inhibited both viral proteins with binding energies less than the cutoff value of −6.0 kcal/mol [33]. Binding energies of flavonoids with RdRp ranged from −7.6 to −6.8 kcal/mol. Luteolin was found as the most potent RdRp inhibitor with the binding energy of −7.6 kcal/mol indicating its strong and stable interactions with target proteins. Honey flavonoids in the current study showed better results than different compounds from *Nigella sativa* where 1,2-dimethylcyclopentan-1-ol inhibit 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 groups have considered it to develop the RdRPs 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 combination of 3 drugs consisting of ritonavir, oseltamivir and lopinavir against SARS-CoV-2 M^{pro} which reported the binding energies as −5.11, −4.65 and −4.1 kcal/mol, respectively [41]. The binding energies for ritonavir, osetlamivir, 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 on honeybee’s product (caffeic acid, chrysin, galangin, lumichrome, caffeic acid, phenethyl ester and 3−phenyllactic acid) showed the binding energies varied from −6.383 to −4.387 kcal/mol [43]. Reports indicate that M^{pro} enzyme is essential for SARS-CoV-2. This enzyme cleaves the polyproteins to produce several active enzymes, including the exo-ribonuclease, endo-ribonuclease and RNA polymerase [44]. Protease enzyme is considered 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 evaluated to show antiviral effects against human immunodeficiency virus type 1 by targeting the protease [45]. Sofobuvir, voxilaprevir, glecaprevir, grazoprevir, paritaprevir, asunaprevir, ritonavir, telaprevir and boceprevir targeted the hepatitis C virus
protease [46]. Thus, the development of drugs targeting SARS-CoV-2 RdRp and M\textsuperscript{pro} have clinical applications.

Computational and bioinformatics tools also predict the harmful effects of candidate drug molecules. The unsuitable molecules could be removed during the drug screening due to their toxicity. In silico analysis indicated that top inhibitors of RdRp and M\textsuperscript{pro} are safe drug candidates without any toxicity. The lethal concentration (LC\textsubscript{50}) is the concentration of molecules causing 50% mortality in the Fathead Minnows fish test group. LC\textsubscript{50} having values lower than 0.5 mM (log LC\textsubscript{50} < −0.3) is considered to show acute toxicity [26]. None of the flavonoid compounds was found to exhibit the Minnow toxicity due to toxicity analysis. For T. pyriformis (a protozoan) toxicity, a compound with pIC\textsubscript{50} > −0.5 log μg/L is regarded as toxic [26]. During the 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 to rats on 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 lethal dosage (LD\textsubscript{50}) (mol/kg), which is the quantity of a single dose of the compound that causes 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 humans (log mg/kg/day) indicates the threshold of chemicals for humans. It is the maximum dose that is recommended as 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]. Values of MTD for luteolin was 0.531 mg/kg/day while apigenin and kaempferol depicted 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]. Inhibition of human ether-a-go-go gene (hERG) I and II were not predicted for any compound. 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 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 base of 531 compounds side effects associated with the liver) classifies a compound as hepatotoxic based on physiological or pathological events that disrupt the functions of the normal liver [26]. While skin sensitization depicts the serious effects of a compound when applied to the skin [26]. In the present report, no compound showed skin sensitization.

In the case of target prediction, the average probability score for luteolin was found as 0.532 while for apigenin and kaempferol it was 4389 and 0.535, respectively. Previous studies have shown that a more than zero probability value depicts a reasonable drug-ligand interaction [49, 50]. These scores precisely demonstrate the probability of targeting a given protein by a bioactive molecule [23]. The probability score of analyzed ligands indicated that they have a better attraction towards the target of the specific binding site that is directed to.

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

Similarly, apigenin was also reported to have SARS-CoV Mpro proteolytic activity [57]. Further, apigenin was documented to depict the antiviral effects against the influenza virus, human immunodeficiency virus, 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 the antiviral potential against the SARS-CoV [65], herpes simplex viruses, human immunodeficiency virus 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 attenuate the expression of proinflammatory factors and receptors likely to cause acute respiratory distress, a major mortality cause associated with patients of COVID-19 while boosting the immune system.

5 Conclusions

At present, the COVID-19 pandemic is a major challenge to the health sector of the entire world. The discovery of the vaccine against the SARS-CoV-2 is a breakthrough. However, there is still a need to develop efficient drugs to combat the deadliest virus. Several scientists are investigating various synthetic compounds to treat the disease, however, due to their side effects, natural compounds are being encouraged to assess their potential against COVID-19. Knowing that honey has been reported to show antiviral activities, the current study investigated the honey flavonoids as inhibitors of two important enzymes of SARS-CoV-2, i.e., RdRp and Mpro employing the 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 to inhibit the Mpro. Thus, these compounds could be used to block the SARS-CoV-2 spread by blocking these enzymes. Further studies indicated that these compounds are safe for oral use without any toxicity and have good target accuracy in the human body.

6 Limitation and future perspectives

To further validate the anti-COVID-19 effects of honey flavonoids, this study lacks computational molecular dynamics (MD) simulations and are required to performed to predict how atoms in the protein structure move over time depending on a general model of the physics regulating interatomic interactions [69]. The potential for novel coronaviruses to arise in the future, as well as the evolving nature of coronaviruses, need 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. Future study should be undertaken on the application of honey flavonoids to SARS-CoV-2 using in vitro and in vivo studies before the clinical assay.

List of abbreviations

COVID-19: Coronavirus disease of 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
Mpro: Main protease
MTD: Maximum tolerated dose
RdRp: RNA-dependent RNA polymerase
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

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Not applicable

Competing interests
The author has no competing interests

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7 References


17. Lyu SY, Rhim JY, Park WB. Antiviral activities of flavonoids against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in vitro. *Arch Pharm Res.* 2005;28(11):1293-


30. Tsaion K, Bottlaender M, Mabondzo A. ADDME - Avoiding Drug Development


55. Fan W, Qian S, Qian P, Li X. Antiviral activity of luteolin against Japanese encephalitis


68. Yarmolinsky L, Huleihel M, Zaccai M, Ben-Shabat S. Potent antiviral flavone

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Figure 8. Set of fourteen (14) honey flavonoids along with PubChem CID.
Figure 9. ADME analysis showing the parameters of Lipinski’s rule for fourteen (14) honey flavonoids. A: 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.
Figure 10. Binding energies of honey flavonoids obtained from the molecular docking analysis with SARS-CoV-2 RdRp (6M71) and Mpro (6LU7) enzymes.
Figure 11. 3D binding conformation of top three honey flavonoid inhibitors of SARS-CoV-2 RdRp (6M71) and Mpro (6LU7) active sites (hydrogen bond interaction).
Figure 12. 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 13. Toxicity parameters for top honey flavonoids used as SARS-CoV-2 RdRp and M\textsuperscript{pro} inhibitor.

Figure 14. Top twenty-five (25) targets predicted by SwissTargetPrediction database for top honey flavonoid inhibitors of SARS-CoV-2 RdRp and M\textsuperscript{pro}.