Potential of Plant Bioactive Compounds as SARS-CoV-2 Main Protease (M\textsuperscript{pro}) and Spike (S) Glycoprotein Inhibitors: A Molecular Docking Study

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Abstract

Since the outbreak of the COVID-19 (Coronavirus Disease 19) pandemic, researchers have been trying to investigate several active compounds found in plants that have the potential to inhibit the proliferation of SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2). The present study aimed to evaluate bioactive compounds found in plants by using a molecular docking approach to inhibit the Main Protease (M\textsuperscript{pro}) and Spike (S) glycoprotein of SARS-CoV-2. The evaluation was performed on the docking scores calculated using AutoDock Vina as a docking engine. A rule of five (RO5) was calculated to determine whether a compound meets the criteria as an active drug orally in humans. The determination of the docking score was done by selecting the best conformation of the protein-ligand complex that had the highest affinity (most negative Gibbs' free energy of binding / ΔG). As a comparison, nelfinavir (an antiretroviral drug), chloroquine and hydroxychloroquine sulfate (anti-malarial drugs recommended by the FDA as emergency drugs) were used. The results showed that hesperidin, nabiximols, pectolinarin, epigallocatechin gallate, and rhoifolin had better poses than nelfinavir, chloroquine, and hydroxychloroquine sulfate as spike glycoprotein inhibitors. Hesperidin, rhoifolin, pectolinarin, and nabiximols had about the same pose as nelfinavir, but were better than chloroquine and hydroxychloroquine sulfate as M\textsuperscript{pro} inhibitors. These plant compounds have the potential to be developed as specific therapeutic agents against COVID-19. Several natural compounds of plants evaluated in this study showed better binding free energy compared to nelfinavir, chloroquine, and hydroxychloroquine sulfate which so far are recommended in the treatment of COVID-19. As judged by the RO5 and previous study by others, the compounds kaempferol, herbacetin, eugenol, and 6-shogaol have good oral bioavailability, so they are also seen as promising candidates for the development lead compounds to treat infections caused by SARS-CoV-2.
Introduction

Coronavirus disease 2019 (COVID-19) is a disease caused by a new type of transmissible pathogenic human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of Betacoronaviruses (Beta-CoVs) [1, 2]. As of 11 March 2020, WHO has stated that COVID-19 has been characterized as a pandemic. The World Health Organization (2020), as of 3 April 2020, reported 932,166 confirmed cases and 46,764 deaths in 206 countries [3]. While in Indonesia, the death toll of COVID-19 reached 170 with the number of positive cases of 1,790 people as of 3 April 2020 [4].

COVID-19 infection is characterized by symptoms of acute respiratory distress such as fever 38.1oC - 39oC, dry cough, and shortness of breath with an incubation period of about 5 days (average 2-14 days) [5]. Until now, there is no specific therapy or vaccine available to treat and prevent COVID-19 [6, 7]. Therefore, there has been an increase in demand for the availability of medicines, vaccines, diagnostics and reagents, all related to COVID-19. This can lead to opportunities for irresponsible people to distribute falsified medical products.

Several agents are being used in clinical trials and protocols based on in vitro activity against SARS-CoV-2 or related viruses with limited clinical experience; however, the effectiveness of therapy for any type of drug has not been established [8]. Xu et al. [9] examined the effectiveness of tocilizumab (atilizumab, an immunosuppressive drug) in a retrospective analysis with the results such as reduced fever, oxygen demand, radiological features and decreased C-reactive protein (CRP). Bian et al. [10] in an open-labeled clinical trial (concurrent controlled add-on clinical trial) of meplazumab found a median virus clearance time, discharge time, and better repair time. A study based on molecular dynamics simulation (MDS) of a docked protein-ligand compound, nelfinavir, was predicted to be COVID-19 drug candidate as the best potential inhibitor against Main Protease (Mpro) [9]. On the other hand, despite little evidence on the effectivity of chloroquine and hydroxychloroquine, these two anti-malarial agents have been approved by the Food and Drug Administration (FDA) for emergency coronavirus treatment [6].

Because COVID-19 is a new disease with serious global health problems, research is still needed, including finding specific therapeutic regimens to overcome the morbidity and mortality it causes. Plant is one of the medicinal active compound sources that have been widely used to treat diseases caused by microbes [11–13]. There are many plant bioactive compounds reported to have activities as antifungal [14], antibacterial [15–17], and antiviral [18, 19]. The natural products that have been reported to have antiviral activity can be used as a starting point in finding potential bioactive compound candidates against SARS-CoV-2. Molecular docking can be used to predict how protein (receptor) interacts with bioactive compounds (ligands) [20, 21]. Several previous studies have been performed to investigate bioactive compounds in plants that have the potential to inhibit the proliferation of viruses [22–24].

Referring to the importance of early screening for the potential of bioactive compounds to find lead compound candidates or prevention of viral infections, this study aimed to evaluate several bioactive compounds found in several plants known by the community with a molecular docking approach. The results of the study are expected to be one of the references for further research in finding specific regimens to overcome COVID-19.

Materials and Methods

2.1. Determination of ligands
The selection of plant-derived compounds used as ligands in the docking process in this study was based on in silico and in vitro experiments that we and other researchers have previously conducted on the antiviral activity of these compounds. The information was obtained through digital library search. These compounds were quinine [25], nabiximols (a combination of cannabidiol [26] and tetrahydrocannabinol [27]), hesperidin [28, 29], rhoifolin [30], pectolinarin [30], morin [31], epigallocatechin gallate [32, 33], herbaacetin [30], ethyl cholate [34], kaempferol [35], tangeretin [36], chalcone [37], nobiletin [38], bis (3,5,5-trimethylhexyl) phthalate [34], 6-gingerol [39, 40], 6-shogaol [41], hydroxychloroquine sulfate [42], myristicin [43], and eugenol [44].

2.2. Determination of receptors

Two SARS-CoV-2 proteins were chosen as lead compound discovery targets: Main Protease (M\(^{\text{pro}}\)) (also called 3C-like protease - 3CL\(^{\text{pro}}\)) (PDB code: 6LU7) and Spike Glycoprotein (S) (PDB code: 6VXX).

2.3. Ligand and receptor preparation

Three-dimensional (3D) structures of M\(^{\text{pro}}\) of SARS-CoV-2 were retrieved from Protein Data Bank (http://www.rcsb.org/pdb) in .pdb formats. These proteins were served as receptors in the docking process. The files were opened using BIOVIA Discovery Studio Visualizer 2020. Water molecules and ligands that were still attached to the receptors were removed, and the receptors were stored in the .pdb format. Using Autodock Tools, polar hydrogen atoms were added to the receptors. Subsequently, the files were saved in .pdbqt format.

Ligand structures were obtained from the PubChem site (http://pubchem.ncbi.nlm.nih.gov). The search was done by entering the name of the ligand in the search option. Each ligand’s file was downloaded and saved. Files in the .sdf format were converted to .pdb using Open Babel. The .pdb format of the ligand was opened using the Autodock Tools tool. Torque adjustment was made by detecting root and adjusted as desired. The file was saved in .pdbqt format. Properties of active compounds were calculated using Lipinski’s rule of five calculated on SWISSADME predictor (http://www.swissadme.ch/) [45].

2.4. Active site determination

The location of the amino acids as active sites in the receptor region where the ligand was docked was determined using Autodock Tools. For this reason, a three-dimensional map of the grid box was made in the receptor region. The determination of this map was based on the type of docking used. A three-dimensional map was made as wide as the size of the receptor (Spike glycoprotein) itself so that the ligand was likely to be docked to all parts of the receptor (blind docking). In M\(^{\text{pro}}\)/3CL\(^{\text{pro}}\) docking, the three-dimensional map was only the size of the area to be docked (targeted docking).

2.5. Receptor-ligand docking

The docking was performed using Autodock Vina. Ligands and receptors that had been saved in the .pdbqt format were copied into the Vina folder. Then the vina configuration
file was typed into notepad, saved with the name ‘conf.txt’. Vina program was run through the command prompt.

2.6. Analysis and visualization

The results of the docking calculation were shown in the output in notepad format. Determination of the docking conformation of the ligand was done by selecting the pose with the highest affinity (most negative Gibbs' free energy of binding / ΔG).

Results

Lipinski's rule of five (RO5) of the docking compounds calculated on the SWISSADME predictor is shown in Table 1. The estimation of free energy of binding between potential inhibitors and receptors was calculated using a docking experiment. Table 2 and Figure 1 show the results of docking analysis between the selected compounds with M<sup>pro</sup> (3CL<sup>pro</sup>) and S protein. The docking results showed that some compounds from plants that had better binding positions with S protein compared to nelfinavir were hesperidin, nabiximols, pectolinarin, epigallocatechin gallate, and rhoifolin. Other compounds tended to be better positioned compared to chloroquine and hydroxychloroquine sulfate, except for 6-Shogaol. Binding poses to M<sup>pro</sup> that were better or equivalent to nelfinavir were hesperidin, rhoifolin, and pectolinarin. Some compounds showed better binding poses than chloroquine and hydroxychloroquine on M<sup>pro</sup>.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Molecular Formula</th>
<th>Properties</th>
<th>Meet RO5 criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(&lt;500 g/mol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>C&lt;sub&gt;32&lt;/sub&gt;H&lt;sub&gt;45&lt;/sub&gt;N&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;S</td>
<td>567.78</td>
<td>4.41</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;26&lt;/sub&gt;CN&lt;sub&gt;3&lt;/sub&gt;</td>
<td>319.87</td>
<td>4.15</td>
</tr>
<tr>
<td>Hydroxychloroquine sulfate</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;26&lt;/sub&gt;ClNO&lt;sub&gt;5&lt;/sub&gt;S</td>
<td>439.95</td>
<td>2.13</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>C&lt;sub&gt;28&lt;/sub&gt;H&lt;sub&gt;34&lt;/sub&gt;O&lt;sub&gt;15&lt;/sub&gt;</td>
<td>610.56</td>
<td>-1.06</td>
</tr>
<tr>
<td>Nabiximols</td>
<td>C&lt;sub&gt;42&lt;/sub&gt;H&lt;sub&gt;60&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>628.92</td>
<td>9.12</td>
</tr>
<tr>
<td>Pectolinarin</td>
<td>C&lt;sub&gt;29&lt;/sub&gt;H&lt;sub&gt;34&lt;/sub&gt;O&lt;sub&gt;15&lt;/sub&gt;</td>
<td>622.57</td>
<td>-0.09</td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>C&lt;sub&gt;22&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O&lt;sub&gt;11&lt;/sub&gt;</td>
<td>458.37</td>
<td>0.95</td>
</tr>
<tr>
<td>Rhoifolin</td>
<td>C&lt;sub&gt;27&lt;/sub&gt;H&lt;sub&gt;30&lt;/sub&gt;O&lt;sub&gt;14&lt;/sub&gt;</td>
<td>578.52</td>
<td>-0.81</td>
</tr>
<tr>
<td>Morin</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;</td>
<td>302.24</td>
<td>1.2</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;</td>
<td>286.24</td>
<td>1.58</td>
</tr>
<tr>
<td>Herbacetin</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;</td>
<td>302.24</td>
<td>1.33</td>
</tr>
<tr>
<td>Ethyl Cholate</td>
<td>C&lt;sub&gt;26&lt;/sub&gt;H&lt;sub&gt;44&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;</td>
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<td>3.5</td>
</tr>
<tr>
<td>Ligands</td>
<td>PubChem CID</td>
<td>Binding Free Energy</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>-------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6VXX</td>
<td>6LU7</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>64143</td>
<td>-8.8</td>
<td>-8.2</td>
</tr>
<tr>
<td>Hydroxychloroquine sulfate</td>
<td>12947</td>
<td>-7.3</td>
<td>-6.6</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>2719</td>
<td>-6.1</td>
<td>-5.3</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>10621</td>
<td>-10.4</td>
<td>-8.3</td>
</tr>
<tr>
<td>Nabiximols</td>
<td>9852188</td>
<td>-10.2</td>
<td>-8.2</td>
</tr>
<tr>
<td>Pectolinarin</td>
<td>168849</td>
<td>-9.8</td>
<td>-8.2</td>
</tr>
<tr>
<td>Epigallocatechin gallocate</td>
<td>65064</td>
<td>-9.8</td>
<td>-7.8</td>
</tr>
<tr>
<td>Rhoifolin</td>
<td>5282150</td>
<td>-9.5</td>
<td>-8.2</td>
</tr>
<tr>
<td>Morin</td>
<td>5281670</td>
<td>-8.8</td>
<td>-7.8</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>5280863</td>
<td>-8.5</td>
<td>-7.8</td>
</tr>
<tr>
<td>Herbacetin</td>
<td>5280544</td>
<td>-8.3</td>
<td>-7.2</td>
</tr>
<tr>
<td>Ethyl Cholate</td>
<td>6452096</td>
<td>-8.1</td>
<td>-6.7</td>
</tr>
<tr>
<td>Nobiletin</td>
<td>72344</td>
<td>-8.1</td>
<td>-6.4</td>
</tr>
<tr>
<td>Tangeretin</td>
<td>68077</td>
<td>-7.9</td>
<td>-6.5</td>
</tr>
<tr>
<td>Chalcone</td>
<td>637760</td>
<td>-7.5</td>
<td>-6.2</td>
</tr>
<tr>
<td>Quinine</td>
<td>3034034</td>
<td>-7.5</td>
<td>-6.9</td>
</tr>
<tr>
<td>6-Gingerol</td>
<td>442793</td>
<td>-6.3</td>
<td>-5.8</td>
</tr>
<tr>
<td>Bis(3,5,5-trimethylhexyl) phthalate</td>
<td>34277</td>
<td>-6.1</td>
<td>-5.6</td>
</tr>
<tr>
<td>Myristicin</td>
<td>4276</td>
<td>-6.1</td>
<td>-5.3</td>
</tr>
<tr>
<td>Eugenol</td>
<td>3314</td>
<td>-6.1</td>
<td>-5.4</td>
</tr>
<tr>
<td>6-Shogaol</td>
<td>5281794</td>
<td>-5.5</td>
<td>-5.8</td>
</tr>
</tbody>
</table>

Table 2. Molecular docking analysis of several plant compounds against S protein (6VXX) and M\textsuperscript{pro} (6LU7).
Figure 1: Histogram showing the binding energy value ΔG (-kcal/mol) of S protein and Mpro with several inhibitor compound candidates.

The list of plants that have active compounds used as ligands is presented in Table 3. The table shows that Citrus fruit has many active compounds, which are potential as anti-SARS-CoV-2, including hesperidin, rhoifolin, nobiletin, tangeretin, and chalcone. The table shows that only pectolinarin, epigallocatechin gallate, myristicin and eugenol have high bioavailability when administered orally.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Oral Bioavailability</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hesperidin</td>
<td>Low [46]</td>
<td>Citrus fruit (Citrus spp.), Peppermint (Mentha spp.), Yellow Toadflax (Linaria vulgaris)</td>
</tr>
<tr>
<td>Nabiximols</td>
<td>Low [47, 48]</td>
<td>Marijuana (Cannabis spp.)</td>
</tr>
<tr>
<td>Pectolinarin</td>
<td>Low to good [49, 50]</td>
<td>Plume thistles (Cirsium spp.), Yellow toadflax (Linaria vulgaris)</td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>Low [51, 52]</td>
<td>Tea (Camellia sinensis) (green tea), the skin of Apple (Malus domestica), Plum (Prunus domestica), Onion (Allium cepa), Hazelnut (Corylus avellana)</td>
</tr>
<tr>
<td>Rhoifolin</td>
<td>Low [53]</td>
<td>Rhus plant (Rhus succedanea), Bitter orange (Citrus aurantium), Bergamot (Citrus bergamia), Grapefruit (Citrus paradisi), Lemon (Citrus limon), Lablab beans (Lablab purpureus), Tomato (Lycopersicon esculentum), Artichoke (Cynara scolymus), Bananas (Musa spp.), Grape (Vitis vinifera)</td>
</tr>
<tr>
<td>Morin</td>
<td>Low [54]</td>
<td>Osage orange (Maclura pomifera), Almond (Prunus dulcis), Old fistic (Chlorophora tinctoria), Guava (Psidium guajava)</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>Low to good [55, 56]</td>
<td>Kale (Brassica oleracea var. sabellica), Beans (Phaseolus vulgaris), Tea (Camellia sinensis), Spinach (Spinacia oleracea), Broccoli (Brassica</td>
</tr>
</tbody>
</table>
oleracea var. Italica)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Activity</th>
<th>Source Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbacetin</td>
<td>Good [56]</td>
<td>Golden root (Rhodiola spp.), Gossypium (Gossypium hirsutum), Common horsetail (Equisetum arvense), Common boneset (Eupatorium perfoliatum)</td>
</tr>
<tr>
<td>Ethyl Cholate</td>
<td>N/A</td>
<td>Leaf of football fruit / keluak (Pangium edule)</td>
</tr>
<tr>
<td>Nobleitin</td>
<td>Low [57]</td>
<td>Citrus fruit (Citrus spp.)</td>
</tr>
<tr>
<td>Tangeretin</td>
<td>Low [58]</td>
<td>Citrus fruit (Citrus spp.)</td>
</tr>
<tr>
<td>Chalcone</td>
<td>Low [59]</td>
<td>Citrus fruit (Citrus spp.)</td>
</tr>
<tr>
<td>6-Gingerol</td>
<td>Low [60]</td>
<td>Fresh Ginger (Zingiber officinale)</td>
</tr>
<tr>
<td>Bis (3,5,5-trimethylhexyl) phthalate</td>
<td>N/A</td>
<td>Leaf of football fruit / keluak (Pangium edule)</td>
</tr>
<tr>
<td>Myristicin</td>
<td>N/A</td>
<td>Nutmeg (Myristica fragrans)</td>
</tr>
<tr>
<td>Eugenol</td>
<td>Good [61, 62]</td>
<td>Clove (Syzygium aromaticum)</td>
</tr>
<tr>
<td>6-Shogaol</td>
<td>Low to good [63, 64]</td>
<td>Ginger (Zingiber officinale)</td>
</tr>
</tbody>
</table>

Discussion

A number of different classes of bioactive molecules isolated from many plants have been shown to have antiviral activity [65, 66]. In determining that a compound has the potential as a drug, one of which is to follow the rule of five (RO5). According to this rule, orally active drugs must not have more than one violation of established criteria [67]. Therefore, each docking compound was checked whether it met the Lipinski's RO5. Some compounds that show violations towards RO5 are hesperidin (3), nabiximols (2), pectolinarin (3), epigallocatechin gallate (2), and rhoifolin (3) (Table 1). The rule is used for the evaluation of the drug-likeness, as well as a determination if any particular chemical compound possesses chemical and physical properties to be used as an active drug, which can be consumed orally in humans [45]. It also acts as a basis for the prediction of a high probability of success or failure of one compound with particular pharmacological or biological activity to be developed as a drug. This rule also suggests that if a compound shows two or more RO5 violations, then the compound shows low solubility or permeability [68].

Dozens of proteins are coded by a coronavirus, some of which are involved in viral replication and entry into cells. Main protease (M^{pro}/3CL^{pro}) is a key enzyme for coronavirus replication [69], and surface Spike (S) glycoprotein (S protein) is an essential binding protein for the fusion of the virus and cellular membrane via cellular receptor angiotensin-converting enzyme 2 (ACE2) [70]. SARS-Cov-2 is easily transmitted because the S protein on the surface of the virus binds very efficiently to ACE2 on the surfaces of human cells. Therefore, M^{pro} and S protein are ideal targets for drug design and development.

Efforts have been made globally to obtain vaccines or drugs for the prevention or treatment of COVID-19 infections. So far, remdesivir is the most promising COVID-19 drug, although the FDA has also approved the use of chloroquine and hydroxychloroquine. Coutard et al. [71] suggested finding an inhibitor for furin, because the S protein sequence has a specific furin-like cleavage. In addition, some researchers have targeted Main Protease (M^{pro}/3CL^{pro}) for treating coronaviral infection [24, 72].

The results of this study, which aimed at predicting the inhibition ability of compounds found in some plants against M^{pro} and S proteins, have revealed several results, showing that
these compounds have a better docking pose than nelfinavir, chloroquine, and hydroxychloroquine sulfate (Table 2 and Figure 1). If the results are juxtaposed, the potential candidates to become drugs targeting S protein and M\textsuperscript{pro} were hesperidin, nabiximols, rhoifolin, pectolinarin, morine, epigallocatechin gallate, and herbacetin. Some of the plants producing compounds, which are docked with the target protein can be seen in Table 3. Table 3 also contains information on the oral bioavailability of the compounds used as ligands in this analysis. However, only a few compounds have high bioavailability when administered orally based on studies that have been conducted by several other researchers, i.e., pectolinarin, kaempferol, herbacetin, eugenol and 6-shogaol. Of these, only pectolinarin does not meet RO5. The low oral bioavailability has become a common problem in drug design, since it may pose failure to a new drug in clinical trials, even though the compounds have high efficacy in the in vitro and/or in vivo tests [73]. This may incur a problem faced by scientists in the pharmaceutical industry [74]. Therefore, the oral bioavailability of a compound is essential to be taken into account when predicting the compound as a drug candidate. The oral availability of some compounds can be low if administered together with food. However, the oral availability of a compound can also be improved by various strategies [75, 76].

The major flavanone glycoside in the citrus peel is hesperidin [77]. Docking scores of this compound with S protein and M\textsuperscript{pro} were -10.4 and -8.3, respectively. Utomo et al. [78] have docked hesperidin against S protein (-9.6) and M\textsuperscript{pro} (-13.51). Chen et al [79] revealed that the best hesperidin position against SARS-CoV-2 3C-like protease (3CL\textsuperscript{pro}) was -10.1. Adem et al. [80] found that the ability of hesperidin was better than nelfinavir. Based on this finding, hesperidin has great potential to be a candidate for drugs, but its low oral bioavailability is a problem.

Cannabinoids are active compounds of Cannabis sativa and C. indica. The docking score of nabiximols (a combination of cannabidiol and tetrahydrocannabinol) against M\textsuperscript{pro} and S protein was -8 and -10.2, respectively. Besides being known as an anti-herpes simplex virus [27], this compound also has anti-inflammatory activity [81]. However, some researches show that this compound can increase the pathogenesis of the virus to the host [81–83].

The docking results using rhoifolin as ligand were -9.5 and -8.2 for S protein and M\textsuperscript{pro}, respectively. Rhoifolin is a flavone that was first discovered in the fresh leaves of Rhus succedanea in 1952 [84]. Besides, this compound was also found in Citrus grandis [85]. The results of rhoifolin docking on S protein were -9.5, and M\textsuperscript{pro} was -8.2. The rhoifolin binding score for SARS-CoV 3CL\textsuperscript{pro} shows a value of -9.565 [30].

The induced-fit docking result of pectolinarin against SARS-CoV 3CL\textsuperscript{pro} was -8.054 [30]. In this study, the best pose between pectolinarin and S protein was -9.8 and -8.2 with M\textsuperscript{pro}. Pectolinarin can be found in Plume thistles (Cirsium spp). Morin docking results by Jo et al. [30] against SARS-CoV 3CL\textsuperscript{pro} was -8.930. In this study, the best docking scores of morin against S protein and M\textsuperscript{pro} were -8.8 and -7.8, respectively. Almond, Old fustic, and Guava contain a high quantity of this compound.

Kaempferol can be found in spinach and kale. The best position of kaempferol against S protein was -8.5 and -7.8 against M\textsuperscript{pro}, while -8.526 was the best binding position of this compound against SARS-CoV 3CL\textsuperscript{pro} [30]. RO5 calculation results show that this compound has a high potential to be used as a drug, and some researchers have previously stated that its oral bioavailability varies from low to good. Besides having been reported to have the ability as an antiviral, this compound also shows immunomodulatory and anti-inflammatory activities [86, 87].

Epigallocatechin gallate is found in high quantity in tea (Camellia sinensis), especially in the form of green tea. The best binding position of this compound against S protein was -
9.8 and against M\textsuperscript{pro} was -7.8. It has been reported previously that this compound was able to inhibit the proteolytic activity of SARS-CoV 3CL\textsuperscript{pro} \cite{88}. Although it does not meet RO5, and its oral availability is low, it has immunomodulatory and anti-inflammatory activities \cite{89, 90}.

Herbacetin, which can be found \textit{Rhodiola} sp. (golden root), has antiviral activity against vesicular stomatitis virus (VSV), and a prototype of negative-strand RNA virus such as rabies and influenza viruses \cite{91}. The best binding pose of this compound against SARS-3CL\textsuperscript{pro} was -9.263, as reported by Jo et al. \cite{30}, while in this study, binding score of -8.3 against S protein and -7.2 against M\textsuperscript{pro} were obtained. They also stated that herbacetin might act as an MERS-CoV 3CL\textsuperscript{pro} inhibitor. Herbacetin is a very potential candidate as an anti-SARS-CoV-2 because it meets RO5 and has also been reported to have good oral bioavailability. Besides, this compound also has anti-inflammatory activity \cite{92}.

Two compounds found in Pangi leaves, bis(3,5,5-trimethylhexyl) phthalate and ethyl cholate, have the potential to be developed as anti-SARS-CoV-2 drugs, due to their good binding affinity with M\textsuperscript{pro} and S protein, and also because they meet RO5. Although there is no prior information about their oral availability, both compounds were reported to inhibit HIV-1 protease \textit{in silico}.

Other compounds such as nobiletin, tangeretin, chalcone, 6-gingerol, myristicin, eugenol, and 6-shogaol have fairly good binding affinity with M\textsuperscript{pro} and S protein, and meet RO5 criteria. These compounds, although their oral availability is low, they have immunomodulatory and anti-inflammatory activities \cite{39, 93–101}.

In a very limited time, we have been able to assess some potential M\textsuperscript{pro} and S protein of SARS-CoV-2 plant-derived inhibitors using molecular docking method. These results are only preliminary screening to facilitate subsequent tests starting from \textit{in vitro} and \textit{in vivo} (in animal models or human clinical trials).

Conclusions

Our study revealed that natural compounds hesperidin, nabiximols, pectolinarin, epigallocatechin gallate, and rhoifolin had better binding free energies with M\textsuperscript{pro} and S protein of SARS-CoV-2. Although the results of molecular docking of kaempferol, herbacetin, eugenol and 6-shogaol are not as good as those compounds, they have good oral availability and also meet RO5 criteria. These compounds have potential as antiviral phytochemicals that may inhibit the replication of the virus. However, further \textit{in vitro} and later \textit{in vivo} tests are needed to evaluate these potential inhibitors as clinical drugs.

Data Availability

The data related to this article are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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