Identification of Flavonoids of *Kalanchoe Pinnata* as Candidate Drugs for COVID-19 Gamma-Variant Treatment

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**Abstract** Treatment of COVID-19 that is based on plants could be a more cost-effective therapy against the disease. Flavonoids, a group of compounds that have been observed to have various effects, including antiviral activity, were chosen as the candidate molecule for treatment of COVID-19. *Kalanchoe Pinnata* is one of the plants containing flavonoids that has been demonstrated to have antiviral activity. The structure of ACE2 and various flavonoids were retrieved and cleaned from unnecessary residues. The ACE2 structure was subjected to molecular docking in order to analyze the binding affinity. Following that, the ADME properties of each flavonoid were analyzed accordingly. The QSAR analysis was also performed for each type of flavonoid. Lastly, molecular dynamics simulation was conducted. All of the tested compounds were able to bind to human ACE2 and SARS-CoV-2 Spike protein, but were unable to compete with them as the binding affinity of the compounds to the protein were lower compared to ACE2-Spike interaction. The ADME and toxicity analysis showed that most of the ligands were able to be absorbed by the GI tract, but have low bioavailability. The compounds also cause no major toxicity effects and were able to be sufficiently distributed to the body. Molecular dynamics analysis also revealed that among the compounds, quercetin and rutin were able to interact with ACE2 and Spike protein stably. The QSAR analysis showed that friedelin, kaempferol, quercetin, and rutin are mostly non-toxic, but the high Cramer values indicate that there are no initial safety impressions for these molecules and could cause toxicity. In conclusion, quercetin and rutin have potential to be a candidate for COVID-19 drug development based on the in-silico predictions results obtained. Friedelin and Narcissin whose affinity to the proteins were relatively stronger but had unstable interactions from molecular dynamics simulation results, may also be a potential COVID-19 treatment with further investigation. However, further research is required to assess the effectiveness and also specially to measure the toxicity of the compounds.

**Keywords**: Flavonoids, SARS-CoV-2 gamma variant, Spike protein, ACE2 receptor.

**Introduction**

COVID-19 or Coronavirus disease-19, previously named 2019 nCoV, is a disease that emerged from a seafood wet market in Wuhan, China in late December 2019 [1, 2]. The disease outbreak spreads quickly to other countries and has been declared as a pandemic by the world health organization (WHO) since 11 March 2020 [1, 3]. The virus causes the COVID-19 disease and is found to be Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which can be
transmitted through respiratory droplets and close contact [2]. The mortality rate of COVID-19 was relatively low to moderate, with mortality rate of 2-5% and an estimated R0 (Average number of people infected by 1 person) around 2-3 [1, 4]. However, as per 27 September 2021, there have already been nearly 232 million cases of COVID-19 globally, with more than 4.7 million deaths reported to the WHO [5].

The virus causing COVID-19 is genetically related to the coronavirus that caused the SARS outbreak in 2003 and therefore named SARS-CoV-2 [6, 7]. The viral structure of coronavirus was focused on the spike protein, which will mediate SARS-CoV-2 the entry of SARS-CoV-2 into the cells [8]. To fulfill its function, SARS-CoV-2 spike binds to its receptor human ACE2 (hACE2) through its receptor-binding domain (RBD) and is proteolytically activated by human proteases [8].

To accommodate patients’ condition with the current pandemic situation, therapeutic managements are available provided by hospitals, however treatments are still very limited since this is a novel virus. Antiviral therapies that are allowed to be used after authorization are Remdesivir, Hydroxychloroquine, and Lopinavir. The Conventional Oxygen Therapy is one of the options to alleviate patients in severe conditions to the point of difficulty in breathing [9].

Currently, researchers are still developing treatments for COVID-19, along with the nature of the SARS-CoV-2, including the experiment for drug repurposing and also proposing other therapies to combat this virus. However, due to the high cost of development, plant-based treatment would be a cheaper beneficial alternative as an antiviral therapy [10].

Flavonoids are a group of natural substances that are commonly found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. As a natural product, flavonoids are well known for their beneficial effects on health, such as their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function [11]. Flavonoids have been studied against a wide range of DNA and RNA viruses [12]. A wide range of flavonoids like Apigenin, Luteolin, Vitexin, Apigenin, Isoquercetin, Quercetin, Catechin, Naringenin and many others are used in a wide range of viruses. Their mechanism of actions vary from one flavonoid to another, including inhibiting viral replication, translation of viral proteins, virion assembly, entry of virus into host cells, glycosylation of viral proteins, envelope protein synthesis and some even have virucidal activity [13].

Herbs has been a widely chosen source of medicine worldwide and has proven effective in treating a wide variety of diseases, for instance, Echinacea plant that has been used to produce drugs like methotrexate, amiodarone, ketoconazole and has been proven effective in treating hepatotoxicity [14]. Kalanchoe Pinnata is a plant originating from Madagascar, Africa that has been proven to have compounds contributing towards antiviral effects, such as quercetin, which has demonstrated anti-hepatitis C virus activity [15]. There is also evidence that another compound named bufadienolides contributes to the antiviral activity of K.Pinnata [16]. These observations suggest the use of flavonoids as a treatment for diseases caused by viruses to be of high potential and very promising and therefore, COVID-19 might be treated in this fashion as well.

Materials and Methods

The workflow of the study includes retrieval and processing of the structures, binding affinity analysis, ADME and toxicity analysis, QSAR analysis, and also molecular dynamics simulation, as seen on Figure 1.
Figure 1. The workflow of the study that was done, starting from structure retrieval to molecular dynamics simulations.

Protein and Flavonoid Structure
The spike (S) protein of SARS-CoV-2 Gamma variant (Pango lineage: P.1) (PDB ID: 7NXC) and human ACE2 receptor (PDB ID: 6M1D) were obtained from RCSB PDB. The flavonoids of Kalanchoe pinnata are obtained from PubChem in the form of SDF files. The list of flavonoids used in the study can be found in Table 1.

Table 1. The flavonoids in Kalanchoe pinnata that were used in the study.

<table>
<thead>
<tr>
<th>PubChem CID</th>
<th>Compound Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>5282102</td>
<td>Astragalin</td>
</tr>
<tr>
<td>91472</td>
<td>Friedelin</td>
</tr>
<tr>
<td>5280445</td>
<td>Luteolin</td>
</tr>
<tr>
<td>5280805</td>
<td>Rutin</td>
</tr>
<tr>
<td>5280863</td>
<td>Kaempferol</td>
</tr>
<tr>
<td>5280343</td>
<td>Quercetin</td>
</tr>
<tr>
<td>128861</td>
<td>Cyanidin</td>
</tr>
<tr>
<td>439533</td>
<td>Taxifolin (Dihydroquercetin)</td>
</tr>
<tr>
<td>5481663</td>
<td>Narcissin (Isorhamnetin-3-rutinoside)</td>
</tr>
</tbody>
</table>

Preliminary Processing
The structures of SARS-CoV-2 S protein (PDB ID: 7NXC) and human ACE2 receptor (PDB ID: 6M1D) were retrieved from RCSB PDB while the flavonoids were retrieved from PubChem. The structures were cleaned off unnecessary residues, including water and protein complexes included in the retrieved PDB file using PyMol. The cleaned structures were assessed through the values in the Ramachandran plot obtained from the SWISS-MODEL server (https://swissmodel.expasy.org).

Molecular Docking and Binding Affinity Analysis
The interactions between the observed molecules were observed with protein-ligand docking and protein-protein docking. The protein-ligand docking runs to dock the flavonoids from Kalanchoe
pinnata with S protein and ACE2 receptor with PyRx-Phyton Prescription 0.8 and Discovery Studio Visualizer 2021 v21.1.0.20298 [17, 18]. As for the protein-protein docking with S proteins to the ACE2 receptor, the molecular docking was done with the ClusPro server (https://cluspro.bu.edu/home.php) [19, 20, 21, 22]. The binding affinities of the protein-flavonoids interaction and S-ACE2 receptor interaction were compared for observation of the competitiveness of flavonoids. The binding affinities of protein-protein interaction was done through PRODIGY service (https://wenmr.science.uu.nl/prodigy/) which is a service that is included in Haddock server [23, 24]. PRODIGY, also known as protein binding energy prediction, is a collection of web services focused on the prediction of binding affinity in biological complexes as well as the identification of biological interfaces from crystallographic one [24]. To use any of the PRODIGY tools, 3D structures of the users’ complex or complexes in PDB or mmCIF format was required. If the structure was unavailable, users only need to provide the ID of its PDB entry. The binding affinity of protein-ligand interaction was done with PyRx.

**ADME and Toxicity Prediction**

The adsorption, distribution, metabolism, excretion (ADME), and toxicity of the flavonoids were observed with the ADMETLab 2.0 server (https://admetmesh.scbdd.com/service/evaluation/index) for its safety towards the body. The ADMETLab 2.0 server allows to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, druglike nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery. The ADMETLab 2.0 server employs a multi-task graph attention (MGA) framework with a database of models generated from over entries of 0.25M that spans 53 endpoints [25]. The server provides more accurate results compared to its predecessor, ADMETLab, whose dataset consists of only 288,967 entries with only 31 endpoints and many limitations [25, 26]. Toxicity was observed via the ADMETLab 2.0, and also PASS server. The Prediction of Activity Spectra for Substances or PASS server is a bioinformatics tool allowing prediction of the compound’s biochemical mechanisms and pharmacological effects [27]. The PASS server uses multi-level-neighborhoods-of-atoms (MNA) structure descriptors to represent the chemical structure to predict the possibilities of the compound’s biological activities [27].

**QSAR Analysis**

The Quantitative Structure-Activity Relationship (QSAR) plays an important role in detecting the correlation between the chemical structure with biological activity or chemical reactivity quantitatively. It takes into account a lot of factors like number of atoms, number and type of chemical bonds, molecular connectivity, ionization constant, molecular mass, lipophilic parameters and many others depending on the software used [28]. QSAR analysis of the flavonoids were done with Toxtree software (https://apps.ideaconsult.net/data/ui/toxtree), which is a web based QSAR tool that focuses on the molecular analysis with additional toxicity analysis towards the organs, different specific endpoints, pathways and targets [29].

**Molecular Dynamics Simulation**

The selected flavonoids and the protein it docks to were observed for molecular dynamics in order to analyze any conformational changes and stability during binding with the CabsFlex 2.0 server. The server was based on a low computational cost modeling server, CABS-flex, that employs coarse-grained protein modeling that can predict the dynamics of protein as an indication to their stability based on the root mean square fluctuation (RMSF) plot [30]. The CabsFlex 2.0 offered some advantages compared to its predecessor, including its ability to input larger proteins, customization of parameters for simulations and contact maps [31]. The molecular dynamics simulation parameters were set to the default setting during the simulation.

**Results and Discussion**

**Molecular Docking of Ligands to Human Ace2 Protein and Sars-Cov-2 Spike Protein**

The molecular docking revealed that all tested ligands could interact with both ACE2 receptor and Spike protein of gamma variant (pango lineage: P.1). All of the ligands were able to bind with human ACE2 and SARS-CoV-2 P.1 Spike protein. However, none of the ligands have stronger
binding affinity to the proteins compared to the affinity between human ACE2 and SARS-CoV-2 spike protein and thus may not be able to compete with them to prevent virus binding to ACE2 receptors. As shown in Table 2 that none of the flavonoids could compete with the proteins as binding partners, as the binding affinities of the ligands to the target proteins, no stronger than -9.1 kcal/mol, are lower compared to the Spike-ACE2 complex whose binding affinity was -17.0 kcal/mol. Unexpectedly, quercetin and kaempferol binding affinities to the proteins are lower compared to other ligands. Friedelin, however, had higher binding affinities to both proteins compared to other ligands, followed by Rutin and Narcissin. Cyanidin seems to be the ligand with the worst binding affinity towards the proteins.

Based on the results, although these flavonoids compounds were able to bind to the ACE2 or Spike protein, they may not be able to compete competitively with the SARS-CoV-2 spike protein to inhibit the SARS-CoV-2 infection. However, their structures might be able to be optimized in further research to compete with the binding of Spike protein.

When compared to some present drugs, the binding affinity value is not far from the mentioned ligands in Table 2. From Duru et al. (2021), there are various drugs that are assessed for binding affinity with ACE2 and spike glycoprotein [32]. The drugs that have potential highest binding affinity are lopinavir, ritonavir, nafamostat, ivermectin, and camostat. For binding affinity with ACE2, lopinavir has binding affinity of -10.1 kcal/mol, ritonavir was -8.9 kcal/mol, nafamostat was -8.7 kcal/mol, ivermectin was -7.7 kcal/mol, and camostat was -7.4 kcal/mol. Additionally binding affinity of drugs with spike glycoprotein include ivermectin (-9.0 kcal/mol), nafamostat (-7.8 kcal/mol), and camostat (-7.4 kcal/mol), lopinavir (-7.3 kcal/mol), and ritonavir (-6.9 kcal/mol). According to the study, nafamostat has good potential as an inhibitor compound due to the dual bridging characteristic with both ACE2 and spike protein [32]. These drugs binding affinity, when compared against Spike-ACE2 complex binding affinity, is still lower. When compared to the ligands, friedelin is the highest one that has comparable value with the other drugs; therefore, flavonoids might be able to be used as COVID-19 treatment. However, it is important to note that there may be differences in the parameters or protein structures used in the molecular docking study of Duru et al. (2021) [32] and this study. Therefore, a study to compare the binding affinity and interactions between the compounds (flavonoids and the mentioned drugs) and the human ACE2 and SARS-CoV-2 Spike protein can be done.

Table 2. The binding affinity of tested molecules. The binding affinity of Spike-ACE2 was obtained with the PRODIGY server while the ligand-proteins were determined with PyRx.

<table>
<thead>
<tr>
<th>Interactor 1</th>
<th>Interactor 2</th>
<th>Binding Affinity (kcal mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE2</td>
<td>Spike</td>
<td>-17.0</td>
</tr>
<tr>
<td>Friedelin</td>
<td>ACE2</td>
<td>-9.1</td>
</tr>
<tr>
<td></td>
<td>Spike</td>
<td>-8.0</td>
</tr>
<tr>
<td>Luteolin</td>
<td>ACE2</td>
<td>-8.1</td>
</tr>
<tr>
<td></td>
<td>Spike</td>
<td>-6.9</td>
</tr>
<tr>
<td>Rutin</td>
<td>ACE2</td>
<td>-9.0</td>
</tr>
<tr>
<td></td>
<td>Spike</td>
<td>-7.6</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>ACE2</td>
<td>-7.7</td>
</tr>
<tr>
<td></td>
<td>Spike</td>
<td>-6.8</td>
</tr>
<tr>
<td>Quercetin</td>
<td>ACE2</td>
<td>-8.1</td>
</tr>
<tr>
<td></td>
<td>Spike</td>
<td>-6.7</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>ACE2</td>
<td>-7.9</td>
</tr>
<tr>
<td></td>
<td>Spike</td>
<td>-6.7</td>
</tr>
<tr>
<td>Taxifolin</td>
<td>ACE2</td>
<td>-8.2</td>
</tr>
<tr>
<td></td>
<td>Spike</td>
<td>-6.8</td>
</tr>
<tr>
<td>Astragalin</td>
<td>ACE2</td>
<td>-8.7</td>
</tr>
<tr>
<td></td>
<td>Spike</td>
<td>-6.6</td>
</tr>
<tr>
<td>Narcissin</td>
<td>ACE2</td>
<td>-8.7</td>
</tr>
<tr>
<td></td>
<td>Spike</td>
<td>-7.2</td>
</tr>
</tbody>
</table>
**Antiviral Compounds of K.Pinnata with the Adme-Tox Analysis**

The ADME analysis of the compounds showed that most of the compounds have a desirable characteristic for a good drug (Figure 2). The ADME data shows that most of the antiviral compounds are able to be absorbed via gastrointestinal tract, however the bioavailability of the compound was low. In addition, no acute oral toxicity is observed and thus the compounds are safe to be consumed orally. The volume of distribution is also sufficient in the body and the compounds mostly are able to penetrate the blood brain barrier. Most of the compounds are not bound to blood plasma, making the distribution of the drugs more effective; however due to this characteristic the bioavailability of the compound is relatively low as the drugs will distribute in the body quickly. The compound was easily excreted via the renal clearance by the body and there are no major toxicity effects from the compound. The ADME data also shows a low risk of hepatotoxicity and carcinogenicity, however there is a high to moderate risk of drug induced liver injury and this needs to be further investigated to assess the drugs effective concentration and to assess the toxic concentration of the compound. However, from the results (Figure 2), it could be deduced that luteolin, quercetin, and cyanidin may have better absorption and distribution with potentially less toxicity; however, they have poor bioavailability and therefore may need further investigation for suitable dosage to exert their therapeutic effect. Friedelin have relatively good absorption and relatively non-toxic, but have poor bioavailability and distribution; while rutin have relatively good distribution and bioavailability but are more toxic and harder to absorb.

![Figure 2. ADME analysis of the antiviral compound in K.Pinnata. Green color represents desirable properties and Red color represents non-desirable properties. Plasma protein binding (PPB); volume of distribution (Vd); blood brain barrier permeability (BBB), fraction unbound (Fu).](image_url)

**Molecular Dynamics Results**

Molecular dynamics simulations had to be done in order to calculate the motion and equilibrium of each atom or molecule. Much information can be collected through molecular dynamics, such as determining whether a biomolecular system will respond to some perturbation from the environment and solvent, making this simulation crucial in analyzing the biomolecule interactions. Proper management of its ligands was one of the most challenging parts of molecular modeling. Much available software for estimating molecular dynamics was not very effective since they are complex and have parameters with different and uncertain force fields [30]. Due to this matter, it was pointed out as a primary reason for a scientist to generate automated tools that were highly favored. Cabs-flex 2.0 (http://biocomp.chem.uw.edu.pl/CABSflex2), in which the online simulation of molecular dynamics was performed for covers in this step as it provides a rapid modeling approach for simulating the stability of biomolecules movements. It was based on the CABS model, a well-known coarse-grained protein modeling tool that can produce protein dynamics at a low computational cost and is eligible to cover the indication of the protein stability based on the root mean square fluctuation (RMSF) plot. Its parameters, such as distance restraint generator, additional distance restraints, and advanced simulation, were set to the default values throughout the simulations. The results were mainly focused on the stability of each molecule which will be generated based on the RMSF plot that differs only between 1 to 3 Å [30]. The more stable the atomic movement in each biomolecule was, the better the prediction and results.

The molecular dynamics results show an overall stable result from each flavonoid and the protein receptors. However, among the ACE2 and Ligand, the compound ACE2 and Quercetin were shown stable movement results, as the RMSF plot shown almost 90% of its atoms movement...
were below 3 Å (Figure 3A, B). For the SpikeP1 and Ligand, the compound SpikeP1 and Rutin were shown stable movement results (Figure 3C, D). The stable interaction indicates that these compounds may be a good candidate for alternative medicine for COVID-19. The rest of the compounds showed unstable interactions with the proteins (Figure 4, 5).

Figure 3. Molecular dynamics simulation of ligands and its interactors. The molecular model structure of ACE2 and Quercetin (A). The RMSF plot of ACE2 and Quercetin shows a stable interaction of ACE2 and Quercetin (B). The molecular model structure of SpikeP1 and Rutin (C). The RMSF plot of SpikeP1 and Rutin showing a stable interaction (D).
Figure 4. The RMSF plot of ligands and its interactors from molecular dynamics simulations. The compounds’ interactions with Human ACE2 receptor (left) and SARS-CoV-2 P.1 Spike protein (right) showed in the form of RMSF plot. Interaction with Astragalin (A, B), Cyanidin (C, D), Friedelin (E, F), and Kaempferol (G, H) were considered unstable.
Figure 5. The RMSF plot of ligands and its interactors from molecular dynamics simulations. The compounds’ interactions with Human ACE2 receptor (left) and SARS-CoV-2 P.1 Spike protein (right) showed in the form of RMSF plot. Interaction with Luteolin (A, B), Narcissin (C, D), Quercetin (E, F), Rutin (G, H), and Taxifolin were observed. Only ACE2-Quercetin (E) and Spike-Rutin (H) were considered stable.
**QSAR Analysis**

The QSAR analysis from Toxtree shows only the results of four out of the total of nine selected compounds based on the Cramer rules (general toxicity), Verhaar scheme, eye and skin irritation, DNA and protein binding alerts and many others (Table 3). Due to unknown reasons, the QSAR software only generated four flavonoid compounds out of nine.

**Table 3. The QSAR analysis results of the 4 out of 9 selected compounds**

<table>
<thead>
<tr>
<th>Molecule Name</th>
<th>Friedelin</th>
<th>Kaempferol</th>
<th>Quercetin</th>
<th>Rutin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cramer Rules</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Verhaar Scheme</td>
<td>Class 5</td>
<td>Class 1</td>
<td>Class 1</td>
<td>Class 5</td>
</tr>
<tr>
<td>Eye Irritation</td>
<td>No</td>
<td>Unknown</td>
<td>Unknown</td>
<td>No</td>
</tr>
<tr>
<td>Skin Irritation</td>
<td>No</td>
<td>Corrosive to skin</td>
<td>Corrosive</td>
<td>No</td>
</tr>
<tr>
<td>Structure Alerts for the in vivo micronucleus assay in rodents</td>
<td>No</td>
<td>Class 1</td>
<td>Class 1</td>
<td>N/A</td>
</tr>
<tr>
<td>Skin sensitisation alerts (M. Cronin)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>DNA binding alerts</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Protein binding alerts</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Michael acceptors</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Benigni/Bossa rules for carcinogenicity and mutagenicity</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>In vitro mutagenicity (Ames test) alerts by ISS</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>ILSI/Kroes decision tree for TTC</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

The QSAR analysis showed all of the molecules showed high Cramer results meaning that they have no initial safety impression and may even have significant toxicity even in low dosage. The classes of the Verhaar Scheme mean different things: Class 1 is inert chemicals, class 2 is less inert, class 3 is reactive chemicals, chemicals acting by a specific mechanism and class 5 meaning not classified into the four classes. The micronucleus assay is basically going to assess the genotoxic properties of the molecules in vivo and in vitro. Michael receptors are actually electrophilic and therefore, very reactive that may induce adverse reactions like binding to DNA and proteins. Other possible toxic mechanisms include binding towards the cysteine thiols that is commonly associated with atherosclerosis and oxidative stress induction. However, some studies also suggest that binding towards cysteine thiols may also prevent carcinogenesis. Quercetin for instance, shows high toxicity in low dose in humans above recommended dose (<1.5 μg/day), however, it does not show toxicity when tested in vitro, positive for Michael’s acceptor, unreactive and only causes skin irritation therefore, the safety cannot be assessed only from QSAR but also from ADMETox to come with a more concrete solution of the safety in the body as well as in the target site as they are still lots of gray area in the results for safety.

**Flavonoids of K. Pinnata as Covid-19 Treatment**

There have been some studies on several flavonoids compounds as treatment for COVID-19. On rutin, for example, In silico and in vitro studies has indicated that it is able to inhibit Mpro activity of SARS-CoV-2 [33,34]. Rutin, as observed in this study, although not competitive, could bind to ACE2 and Spike protein, was considered relatively safe through ADMETox and QSAR analysis, and were able to interact stably with the SARS-CoV-2 gamma variant’s spike protein; therefore, it could be a potential COVID-19 treatment. Other in silico study on Narcissin has also identified it as a strong SARS-CoV-2 protein [35]. Several in silico studies have found friedelin to be able to bind not only to SARS-CoV-2 spike protein, but also to the virus main protease Mpro and RNA-dependent RNA polymerase (RdRp) [36,37]. However, the RMSF plot from the Molecular Dynamics simulation results of both these compounds are less than desirable, showing unstable
interactions (Figure 4E,F; Figure 5C,D). Although in this study both Narcissin and Friedelin have relatively stronger affinity to the Spike protein and ACE2 receptor, other studies on their interactions have thus far mainly been observed in In silico setting and with Spike protein and therefore may need further investigation in vitro and in vivo to ascertain their effect. In addition, the binding affinity of both compounds in this study have been reportedly higher in their interaction with the human ACE2 receptor compared to SARS-CoV-2 spike protein. Therefore, further research on them can be done in vitro with observation on their possible mechanism of action in inhibiting the Spike-ACE2 interactions, and on these two compounds’ interaction with human ACE2 receptors to further observe their possibility of being a COVID-19 treatment.

In silico studies on kaempferol and quercetin also reported that they were able to bind to SARS-CoV-2 Spike protein and might be able to be used as a COVID-19 treatment [38,39]. In addition, in vitro study has found that kaempferol and quercetin, whose binding affinity to both the Spike protein and human ACE2 receptor are lower compared to friedelin, rutin, and narcissin in this study, were reported to be able to inhibit the 3C-like protease of SARS-CoV-2, a protein important for viral replication [40]. Therefore, both quercetin and kaempferol might be able to be used as a treatment for COVID-19, but might not function mainly as Spike protein- or ACE2-inhibitor.

Other compounds might also be able to be used as a COVID-19 treatment. Astragalin, for example, was reported to be able to bind with SARS-CoV-2 Mpro and Spike protein in an in silico study [36]. Some in silico studies also found luteolin to be able to bind with SARS-CoV-2 Mpro and papain-like protease (PLpro) and also human ACE2 [41], and able to block SARS-CoV-2 viral fusion [42]. Taxifolin is also currently hypothesized as a candidate of SARS-CoV-2 replication inhibitor, however in vivo studies on this topic have yet to be done and therefore its effect towards SARS-CoV-2 infection remains inconclusive [43]. However, in vitro, in vivo, and human studies still have to be done to prove the reliability of these compounds in inhibiting SARS-CoV-2 activity.

Conclusions
All of the ligands were able to interact with ACE2 and Spike protein of SARS-CoV-2 Gamma variant (pango lineage: P.1), have desirable ADME and are largely non-toxic. The ACE2 and Quercetin alongside the SPIKEP1 and Rutin shows a stable interaction of its molecular movement based on its RMSF plot, which will indicate that it will be a good candidate for alternative medicine for COVID-19. However, further research is needed for the mentioned flavonoids to be considered as effective drug compounds. In vitro and in vivo research are crucial in order to determine the effectiveness and dosage. These compounds have the potential to cause liver toxicity and exert AMES toxicity, this is quite detrimental to patients. Due to this reason, proper dosage must be established first.

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

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