

# COMPUTATIONAL EXAMINATION OF FLAVONOID COMPOUNDS: UTILIZATION OF MOLECULAR SIMULATION TO DISCOVER DRUG CANDIDATES FOR COVID-19

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## ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the novel coronavirus disease 19 (COVID19) pandemic. Despite drugs had been deployed through intense research, none has been proven to cure the infection. Flavonoids, a natural substance, have been reported to exhibit various pharmaceutical and medical properties. The aim of this research is to discover lead compounds for covid-19 from flavonoid compounds through *in silico* study. Through computational examination, the potential of flavonoid compounds against SARS-CoV-2 has been examined and it shows that some flavonoids have potential against SARS-CoV-2.

**Keywords:** 3CLpro, SARS-CoV-2, Flavonoid Compounds, Molecular Docking, Molecular Dynamics.

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## INTRODUCTION

SARS-CoV-2, a beta-coronavirus, is responsible for the pandemic disease that is happening worldwide, called Covid-19, and infecting almost 500 million people in the world, according to World Health Organization (WHO) per March 2022. In Indonesia, according to the data from the ministry of health in March 2022, SARS-CoV-2 caused more than 5 million positive cases with a 2.6% mortality rate. The disease primarily affects the respiratory tract; however, it can also affect the other organs, such as the gastrointestinal tract, depending on the severity of the cases.<sup>1,2</sup> Several drugs had been deployed in clinical trials, but none has been proven to cure coronavirus infection.<sup>3</sup> Flavonoids are a group of natural substances belonging to a class of plant secondary metabolites consisting of various phenolic structures found in various plant types.<sup>4</sup> Flavonoids have been reported to exhibit various pharmaceutical and medical properties such as antioxidant, antibacterial, anti-carcinogenic, antiviral, and antiinflammation.<sup>5</sup> Papain-like protease (PLpro) and 3-chymotrypsin-like cysteine protease (3CLpro) are two main proteases which are essential for viral propagation.<sup>6</sup> SARS-CoV-2 and SARS-CoV's 3CLpro and PLpro share 96% and 83% similarity, respectively.<sup>7,8</sup> The rapid increase of technology in structural information across protein families has resulted in *in silico study* as the basis for screening potential candidate compounds against many types of diseases.<sup>9</sup> Through a computational approach, the interaction between two molecule structures can be identified in a process called molecular docking.<sup>9,10</sup> The results from molecular docking can be further analyzed through molecular dynamics, which is a simulation of prediction on how every atom in a protein or a molecule will move over time.<sup>11,12</sup> The aim of this research is to discover lead compounds for covid-19 from flavonoid compounds through *in silico* study.

## EXPERIMENTAL

### Hardware and Software

A personal computer with the specification of 16GB RAM, GTX 1060 6GB, Intel(R) Core I7-8750H 2.2 GHz (12CPU), Windows 10 Home 64-bit, was used in this experiment and used the following software for the *In-silico* study:

1. Pyrx 0.8 (<https://pyrx.sourceforge.io/>) and Autodock Vina. (<http://vina.scripps.edu/download.html>) for molecular docking simulation.

2. Gromacs (<http://www.gromacs.org/>) in ubuntu environment for molecular dynamics simulation.
3. Pymol 2.0 (<https://pymol.org/2/>) for ligand preparation.
4. Ligplus (<https://www.ebi.ac.uk/thornton-srv/software/LigPlus/>) for visualization of the ligand-protein interactions.
5. SwissADME (<http://www.swissadme.ch/>) and Toxtree (<http://toxtree.sourceforge.net/>) for toxicity analysis of potential compounds.

### Literature Review for Potential Flavonoid Compound

A literature review with the keywords of flavonoid compounds against RSV, common cold, influenza, SARS, and MERS was conducted to screen the compound that has the potential against SARS-CoV-2.

### PASS Online Screening

Prediction of Activity Spectra for Substance (PASS) online is a software application (<http://www.pharmaexpert.ru/passonline/>) that was used for further screening. General antiviral activities, antiviral activity against influenza, and 3CLpro inhibitor were used as the parameters.

### Ligand and Receptor Preparation

The crystal three-dimensional (3D) structure of SARS-CoV-2 3CLpro was obtained in the format of PDB from the RCSB protein data bank database (<https://www.rcsb.org/>) (PDB ID:6LU7). The flavonoid compounds that passed through the screening process were obtained in the 3D SDF file from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>).

### Molecular Docking Simulation

The molecular docking was conducted using the Autodock Vina in Pyrx 0.8. Grid box or also known as area of docking within the macromolecule was set to center of X = -9.50, Y=11.45, Z=70.56 with dimension of X = 13, Y= 20, Z=13.

### Visualization

Visualization of the complexes was conducted to illustrate the chemical interaction between the ligands and macromolecules in two-dimensional (2D) using the ligplus v2.2 (<https://www.ebi.ac.uk/thornton-srv/software/LigPlus/>).

### Toxicity Prediction

The toxicity assay was conducted using both the Toxtree (<http://toxtree.sourceforge.net/>) and SwissADME (<http://www.swissadme.ch/>) which were available on the online platform.

### Molecular Dynamic Simulation

A molecular dynamic simulation was deployed using GROMACS. GROMACS has been installed in the Ubuntu (2004.2021.825.0) environment. The forcefield that was used in this study is charmm36 (<https://www.charmm.org/charmm/resources/charmm-force-fields/>) force field due to its widely used in molecular modeling and simulation. The simulation was conducted for 1 nanosecond (ns) runtime.

## RESULTS AND DISCUSSION

### Pass Online Screening

Based on the screening with PASS online server, 20 compounds have the potential to act as an inhibitor for SARS-CoV-2 3CLpro, which is archived in Zenodo.<sup>13</sup> The summarized Pa scored of potential compounds can be seen in Fig.-1 below. The score indicates the probability of the compound being active for the parameter activity.

### Molecular Docking Simulation and Visualization

Autodock Vina can predict 9 different binding conformations between the ligand and the receptor using their algorithm.<sup>14</sup> The results show the best binding conformation of each potential ligand with the receptor, which can be seen in Zenodo.<sup>15</sup> The result of molecular docking simulation showed that all of the possible compounds exceeding the score of -6 kcal/mol with 5 compounds have higher or similar affinity compared

to the standard, remdesivir, and N3. Amentoflavone showed the highest affinity with  $-9.6$  kcal/mol. Ligplum was utilized to visualize the protein's binding. The ligand interacts with the protein through hydrogen bond interactions. The strength of the bond affects the ligand-binding affinity.<sup>16</sup>

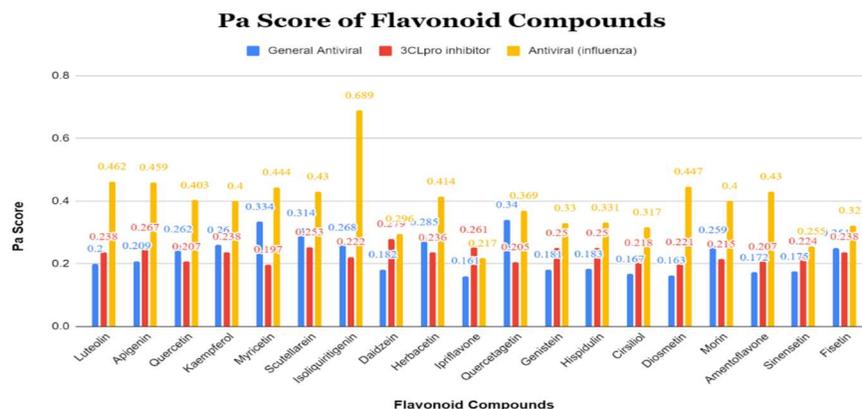


Fig.-1: Pa Score of Potential Flavonoid Compounds Based on PASS Online Screening

### Toxicity Prediction of Potential Ligand

The toxicity prediction is an important step in computer-aided drug discovery. In this research, toxicity analysis from SwissADME is conducted to analyze the drug-likeness and synthetic accessibility of the potential compound, toxicity analysis from Toxtree resulted in the Cramer's rule, carcinogenicity, in-vitro mutagenicity, and skin corrosion and irritation possibility. The result for SwissADME and Toxtree is archived in Zenodo.<sup>17,18</sup> Based on the results, 19 out of 20 potential compounds have the drug-likeness capability according to Lipinski's Rule of 5.<sup>19</sup> For synthetic accessibility, the potential ligands have a synthetic accessibility score between 2.5 - 4.0. The score ranged from 1 to 10 based on their easiness to be synthesized. Toxtree is another software that is used to conduct toxicity analysis of potential compounds which mainly focuses on the potential toxicity reaction that may arise based on the structure of the compound. On the first parameter, the Cramer's rule, all compounds are classified as class III. The second parameter that was used in the Toxtree analysis is the carcinogenicity analysis developed by Benigni in 2008.<sup>20</sup> From the prediction, 18 out of 20 compounds showed negative results for both genotoxic and non-genotoxic carcinogenicity potential. Amentoflavone showed significant structural alert for non-genotoxic carcinogenicity, whereas isoliquiritigenin showed significant structural alert for genotoxic carcinogenicity. The third parameter used is an assessment of *in vitro* mutagenicity (Ames test) which is also developed by Benigni in 2008.<sup>20</sup> Ames test is a commonly used biological assay that utilizes in vitro models to assess the potential of mutagenicity caused by a compound when given to bacteria. According to the result from the prediction, 16 out of 20 compounds showed negative mutagenicity potential against *Salmonella typhimurium*. The last parameter for Toxtree analysis is potential skin corrosion or irritation activity caused by the reaction from the compound.<sup>21,22</sup> All of the compounds tested negative for the potential activity of causing skin corrosion or irritation which showed that potential compounds are harmless to the skin.

### Molecular Dynamic Simulation

Root mean square deviation (RMSD) with a value under  $2\text{Å}$  or  $0.2\text{ nm}$  is considered to have good stability for the complex.<sup>23</sup> From the result which can be seen in Fig.-2, morin showed the highest RMSD score with a value of  $2.5\text{Å}$ , whereas apigenin and luteolin had peak RMSD values higher than  $2\text{Å}$ . The binding of 3CLpro with amentoflavone and kaempferol showed good stability based on their RMSD value lower than  $2\text{Å}$  during the simulation. Furthermore, kaempferol showed consistency in the RMSD value between  $1.2 - 1.4\text{ Å}$ . Root mean square fluctuation (RMSF) was used to analyze the residual protein mobility in the presence of selected ligands. Based on the result that can be seen in figure-3, morin, luteolin, and apigenin showed fluctuation on residue number 302 to 306. This indicates that there are structural changes when the ligand binds with the protein which affects the protein flexibility. On the other hand, kaempferol and amentoflavone remain stable and do not show any fluctuation in all residue numbers.

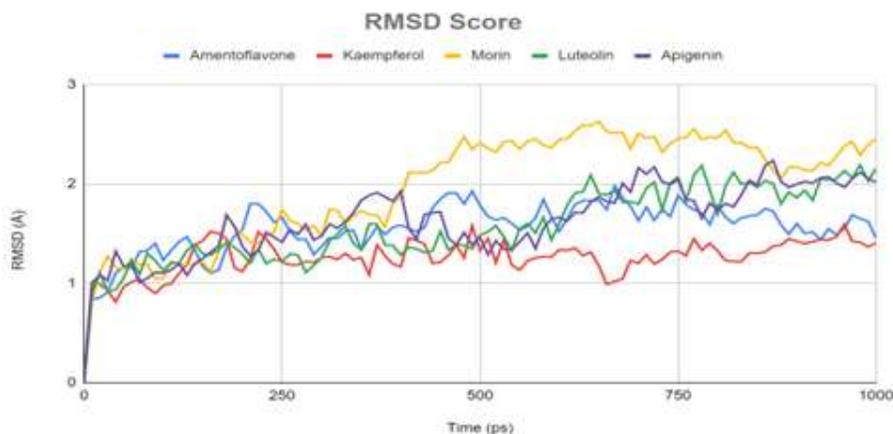


Fig.-2: RMSD Score of 3CLpro (PDB ID: 6LU7) Binding with Amentoflavone, Kaempferol, Morin, Luteolin, and Apigenin

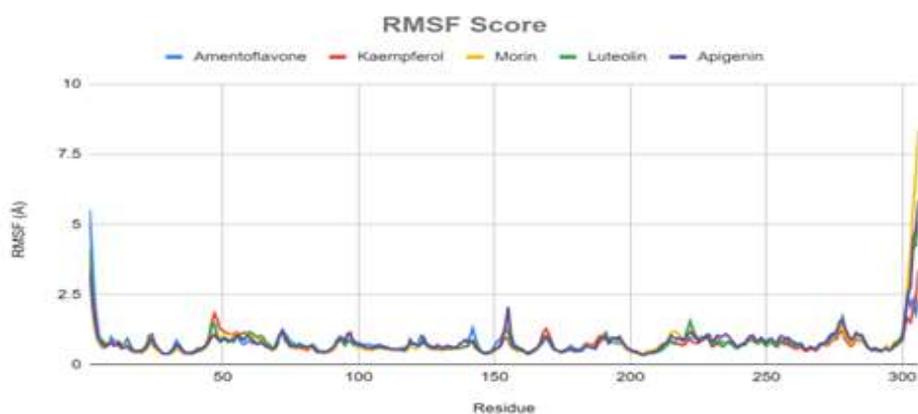


Fig.-3: RMSF Score of 3CLpro (PDB ID: 6LU7) Binding with Amentoflavone, Kaempferol, Morin, Luteolin, And Apigenin

## CONCLUSION

Through computational examination, 20 flavonoid compounds have the potential to inhibit the work of 3-chymotrypsin-like proteins of SARS-CoV-2. Most of the potential ligands are in line for the drug-likeness according to the SwissADME and tested negative carcinogenicity and mutagenicity from Toxtree. With the result from molecular dynamics, it is observed that kaempferol has the best RMSD and RMSF scores. Moreover, kaempferol does not violate any toxicity analysis from SwissADME and Toxtree. This showed that kaempferol is the best candidate among 20 potential compounds in this study. Further empirical research is needed to know the effect of flavonoid compounds binding with 3CLpro.

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