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Biological Activity of Kencur (Kaempferia Galanga L.) against SARS-CoV-2 Main Protease: In Silico Study

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***Abstract*---**COVID-19 is a syndrome affecting pulmonary function but rather in serious conditions leads to death. Kencur (*Kaempferia galanga* L.) is a type of rhizome plant in Indonesia that is used as an herbal medicine called Jamu because it is believed to be able to cure various types of diseases. One of which is for anti-virus. The goal of this study was to see how effective the compounds in kencur are against COVID-19 with a molecular docking strategy. Kencur biological activities were obtained from the library and the design of the Acute Respiratory Syndrome Main protease (M^{pro}) has been gained from the protein data bank website. In addition, the biological activities in kencur were examined utilizing Lipinski's five-point concept was used to evaluate their substance molecular characteristics. Molecular docking analysis was performed with the PyRx Virtual Screening Tool software. The PyRx program was used for molecular docking simulation. While, the Discovery Studio Visualizer program was used to visualize the interaction between SARS-CoV-2 (M^{pro}) and the pharmacologically active metabolites in kencur. The docking evaluation on three antiviral substances revealed that Quercetin had the lowest binding energy when bound with Mpro and thus had the greatest potential as a viral inhibitor.

***Keywords*---**Bioinformatics, COVID-19, Kaempferia galanga L., Kencur, SARS-CoV-2.

Introduction

Nowadays, the disease that is a threat to the world in various fields of life is COVID-19. This disease by experts is called the disease of a thousand faces

because of its ability to make many variations of its type. This disease was induced by a new type of Acute Respiratory Infection in Humans (SARS-CoV-2) that is an infectious pathogen belonging to the Betacoronaviruses (Beta-CoVs) group. This virus can be spread through a variety of interactions including those between living organisms (humans) and others (Ansori et al., 2020). The signs and symptoms of a person contaminated with COVID-19 are a five-day time of incubation chest infection (38.1-39 °C), chronic cough, and difficulty breathing. However, in severe cases, it has the potential to cause bronchitis, severe respiratory disease, renal failure, and even destruction (Kocaadam & Şanlier, 2017).

Coronavirus polyproteins are encoded by two types of protease protein, namely Plpro and main protease (M^{pro}) protein (Kandeel & Al-Nazawi, 2020). M^{pro} protein is a type of enzyme that has 11 cleavage sites and it's important in the replication and transcription of proteins in viruses. Then, when the activity of this enzyme is inhibited, it will block RNA replication which will cause the death of the virus. In addition this type of protease enzyme is not found in humans. Then, M^{pro} inhibitors will not have a negative impact in the form of being toxic to humans. This is the reason why this enzyme is used as among the most important drug candidates of COVID-19 antivirals (Zhang et al., 2020).

Many different ways to handle and even reduce the number of COVID-19 patients widely have been carried out one of which is by utilizing plants that have active medicinal compounds that are used as anti-fungal, anti-bacterial, and anti-viral. One of these plants is kencur (*Kaempferia galanga* L.) which contains many active compounds which were Myricetin, Quercetin, and Catechin (Mustafa et al., 2010). Because it includes kaempferol (flavonol group) which suppresses the reproduction of the rabies virus (Chen et al., 2021). Molecular docking is one of method of bioinformatics that is often used to determine the potential of bioactive substances from organism which possess the ability of suppress virus replication (Umesh et al., 2020; Shaghagi 2020; ul Qamar et al., 2020). This method is used to predict how proteins (receptors) will interact with active compounds (ligands) (Earlia et al., 2019). Furthermore, molecular dynamics is a modeling system used to explore the physical and chemical aspects of molecular docking outcomes in a complicated way (Liguori et al., 2020). The purpose of this study is to find out how effective the compounds in the *K. Galanga* L. plant play a role in inhibiting COVID-19 using Molecular Docking which can later be used as reference material for further research.

Materials and Methods

Ligand Preparation

The compounds that are used in this research are Myricetin. Quercetin. and Catechin that the major compound in *K. galanga L.* (Mustafa et al., 2010). The three-dimensional PDB format of Myricetin (CID: 5281672), Quercetin (CID: 5280343), and Catechin (CID: 9064) structure could be accessed in PubChem database website (http://pubchem.ncbi.nlm.nih.gov). Myricetin, Quercetin, Catechin, Remdesivir, and N3 three-dimensional format is opened in the Discovery Studio application and saved in PDB format.

Protein preparation

The SARS-CoV-2 main protease (M^{pro}) or 3C-like-protease-3CLpro which used as a drug target is achieved and downloaded in the protein data bank website (http://www.rcsb.org//pdb). The three-dimensional SARS-CoV-2 crystal structure could be downloaded from protein data bank website in SDF format with the protein code PDB: 6LU7. The protein is prepared in Discovery Studio to remove water and other ligands. Subsequently. the M^{pro} protein energy is minimized with PyRx Virtual Screening Tool software and continued by the OpenBabel menu with default parameters. PyRx and Discovery Studio are open access software to do docking between ligands and M^{pro} protein.

QSAR analysis and Toxicity level of the compounds

The ligand that conducted to be a drug were tested for QSAR analysis with the PASS server website (http://way2drug.com/passonline/predict.php). In this website. the drug candidates were analyzed with Quantitative Structure-Activity Relationship (QSAR) to examine the antiviral activity and 3C-like-protease-3CLpro inhibitor (Parikesit & Nurdiansyah, 2020). The toxicity level of the compounds can be determined by searching the name of the compound on the PubChem website (http://pubchem.ncbi.nlm.nih.gov).

Molecule screening with insilico method

The insilico method was used to determine compounds in *K. galanga L.* which have a high energy binding with the main protease (M^{pro}) protein. The procedure was carried out using Autodock Vina 1.1.2 menu on PyRx application. This method was used to determine the energy minimum of molecules with the SARS-CoV-2 protein. In contrast to myricetin and quercetin, Remdesivir and N3 inhibitors or Michael acceptor inhibitors were used as benchmarks in the docking method (Ghersi & Sanchez, 2009; Jin et al., 2020; Wijaya et al., 2021). The Lipinski's rule in the docking method is used to describe the properties of molecules that have the best interaction of SARS-CoV-2 (Kumar et al., 2020).

Molecular visualization

The Discovery Studio Visualizer software is used to investigate the types of interactions which exist between the active molecules of *K. galanga* L. and the main protease protein (Sharma et al., 2021). After the docking process. the complex with PDB extension will be obtained. Subsequently, the complex PDB opened in Discovery Studio software to see the interaction between the molecules and the main protease protein. The molecular visualization of the complex will be showing the 2D and 3D structures of protein and active molecules.

Molecular dynamics simulations

Molecular dynamic simulation is an usual procedure for modeling protein dynamics; CABS-flex is a common tool for protein modeling. In CABS-flex, protein structure with PDB format extension is required. CABS-flex only accepts single and continuous protein chains. The documentation in the CABS-flex website is

accessible online and connected to a user email, the RMSF data and protein simulation will get from CABS-flex simulation (Jamroz et al., 2013).

Results and Discussions

Ligand and protein preparation

Myricetin, quercetin, and catechin are the major composition of *K. galanga* L. and remdesivir downloaded from the PubChem website. Remdesivir, which also is frequently used for docking analysis against the main protease M^{pro} protein. The N3, which is a potent inhibitor of the main protease protein attached to M^{pro} protein is detached from Mpro protein and docked with PyRx. The 3D structure of myricetin and quercetin is acquired from PubChem database in SDF format. After that, the compounds opened in Discovery Studio software to analyze the 3D structure and save with PDB extension. The compound list was docked with the main protease protein described in Table 1.

Table 1
Compounds List which was Docked with Main Protease Protein

Compound Name	Class	Protein	Citation
Remdesivir	Standard	6LU7	(Naik et al., 2021)
N3	Inhibitor	6LU7	(Jin et al., 2020)
Myricetin	Flavonoid	6LU7	(Cherrak et al., 2020)
Quercetin	Flavonoid	6LU7	(Cherrak et al., 2020)
Catechin	Flavonoid	6LU7	(Mustafa et al., 2010)

The main component of *K. galanga* L. performed drug-like prediction with Lipinski's Rules of Five on (scfbio-iitd.res.in/ software/drug-design/Lipinski.JSP) website. The Lipinski Rule of Five is often used to predict the chemical bond properties of the compound which are molecular weight (MW). Log P-value, the number of hydrogen bond donors, and the number of hydrogen acceptors. Lipinski Rule of Five in docking analysis has become standard to access drug-like properties in the compound (Petit et al., 2012). The rule of five of the compounds must consist of several rules which was the atomic mass of the compounds should be less than 500 Da, Log P should be less than 5, hydrogen donor and acceptor must be less than 5 and the molar refractivity has to be in the range 40-130 (Wijaya et al., 2021). The Lipinski's Rules result of myricetin and quercetin is explained in Table 2. Since the hydrogen bond donors and acceptors of myricetin, quercetin, and catechin disobey Lipinski's Rule of Five, these compounds could not be taken orally but could be inhaled (Choy & Prausnitz, 2011).

Table 2
Lipinski's Rule of *K. galanga* L. compounds

Compound Name	Molecular Mass (Da)	Log P	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Molar Refractivity
Myricetin	318.00	1.71	6	8	75.71
Quercetin	302.00	2.01	5	7	74.05
Catechin	290.00	1.54	5	6	72.62

The main protease (M^{pro}) protein was obtained from the protein data bank database (http://www.rcsb.org//pdb) with PDB ID: 6LU7. The protein was downloaded with the .pdb extension and opened with Discovery Studio software to remove the ligand that naturally attaches to the protein and removes the water. In previous research, the main protease protein (M^{pro}) has highly similar to the SARS-CoV-2 virus until 96 % (Zhang et al., 2020). And also main protease protein attracts to essential drugs with the coronaviruses family (Naik et al., 2021).

QSAR analysis and toxicity level results

Myricetin, Quercetin, and Catechin were observed with the PASS server that resulted as shown in Table 3. Myricetin and Quercetin knew to have activity with 3C-like-protease-3CLpro inhibitors. But, Catechin did not have an activity with 3C-like-protease-3CLpro inhibitor. Then, the toxicity level (LD_{50}) of Myricetin, Quercetin, and Catechin was obtained from https://pubchem.ncbi.nlm.nih.gov/. Furthermore, the LD_{50} value of the compounds could determine how should the drug candidate compound administered. Myricetin has an interperitoneal LD_{50} value of 1410 mg/kg, Quercetin has an oral LD_{50} value of 159 mg/kg and intervena LD_{50} value of 18 mg/kg. Then, the Catechin has an oral LD_{50} value of > 10 gm/kg and intravenous LD_{50} of > 100 mg/kg. The lower LD_{50} value has the most toxical compound properties, but, the higher LD_{50} value has the lowest toxical compound properties. Furthermore, the lowest dose of LD_{50} value of the compound will make the animals death but, the highest doses of LD_{50} value of the compound will make the animals keep lived (Kharisma et al, 2022).

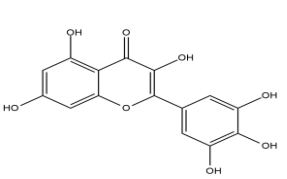
Table 3
QSAR Analysis and Toxicity Level Results

No	Ligand	3C-like-protease-3CLpro inhibitor	Toxicity level (LD_{50}) in mouse	
			Oral	Intravenous/ Intraperitoneal
1	Myricetin	Yes	NA	1410 mg/kg
2	Quercetin	Yes	159 mg/kg	18 mg/kg
3	Catechin	NA	>10 gm/kg	>100 mg/kg

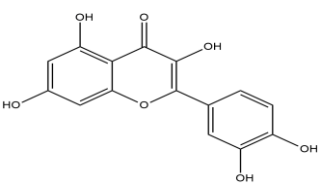
Docking analysis

In this research, the main compounds in *K. galanga L.* were used as a ligand, remdesivir as a drug standard, N3 as a useful inhibitor, and the main protease

(M^{pro}) protein for docking analysis. Docking analysis was completed by PyRx software, the protein target has to be determined as macromolecules and ligand energy was minimized with the OpenBabel menu. After that, ligand and molecules were added to then Vina Wizard menu and the grid box center was set intospecific docking mode X= -26.284, Y= 12.5976, Z= 58.9679. and dimensions was set in X= 25.000 Å, Y= 25.000 Å, Z= 25.000 Å. Quercetin has the highest energy binding than myricetin against the main protease (M^{pro}) protein. The docking result between myricetin and quercetin against the M^{pro} explained in Table 3. Molecular docking was conducted to identify the binding energy and RMSD value. RMSD value is used to predict the good interaction between ligand and the protein, the best RMSD value of docking analysis was less than 2 Å (Rizvi et al., 2013). From Table 3 below, the binding energy of quercetin is lower than myricetin, chatechin, and also remdesivir as a potent drug of COVID-19 nowadays against the main protease protein (M^{pro}). The lower binding energy theoritically can give the complex between protein and ligand is more steady and it make the ligand is fit with the protein target (Dewi & Sanjaya, 2018). N3 is a potent inhibitor of main protease protein (M^{pro}) of SARS-CoV-2, N3 or Michael acceptor inhibitor specifically prevent main protease protein from coronaviruses. In previous research, the N3 inhibitor is suitable with the SARS-CoV-2 main protease protein substrate and has a stable covalent bond with M^{pro} protein (Jin et al., 2020).



A



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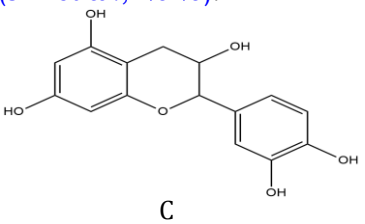
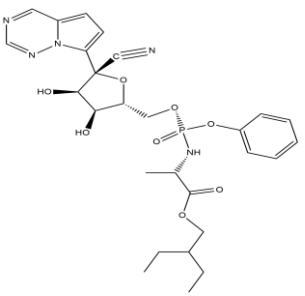


C



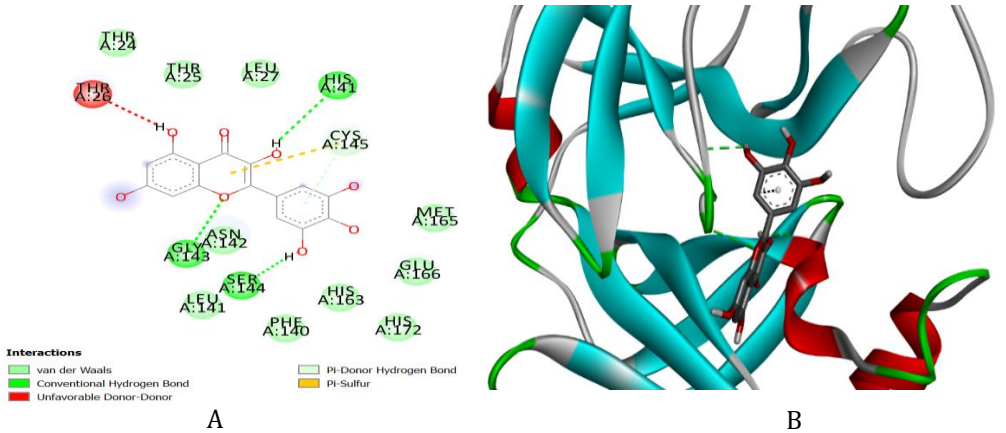
D

Figure 1. Compound Structure A. Myricetin. B. Quercetin. C. Catechin. D. Remdesivir (Standard)

Table 4
Molecular docking result between ligand and main protease protein (M^{pro})

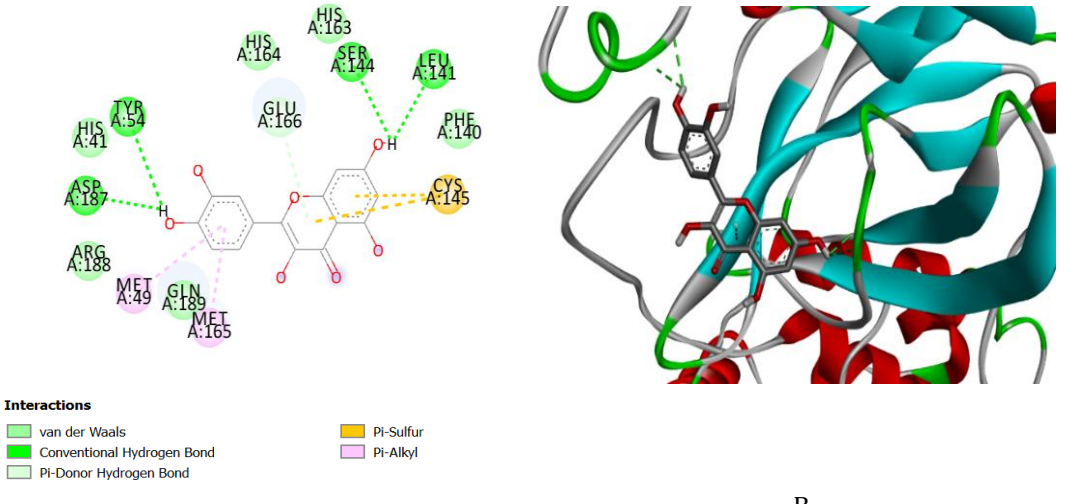
Ligand	Target	Binding energy (kcal/mol)	RMSD (Å)
Remdesivir	M^{pro}	-6.3	0
N3 Inhibitor	M^{pro}	-0.9	0
Myricetin	M^{pro}	-7.0	0
Quercetin	M^{pro}	-7.4	0
Catechin	M^{pro}	-7.1	0

The docking result has been visualized by Discovery Studio software, the complex from docking results was read by Discovery Studio software to analyze the amino acid residue that is attached to the ligand. Figure 2A was the 2D image visualization of myricetin against the main protease protein, myricetin has the hydrogen bonding interaction with His:41, Gly A:143, and Ser A:144. Quercetin (Figure 3A) has the hydrogen bonding interaction with Tyr A:54, Asp A:187, Ser A:144, and Leu A:141. Catechin (Figure 4A) has the hydrogen bonding interaction with Arg A:188, Ser A:144, Leu A:141 amino acid residue. Subsequently, Remdesivir is a coronavirus common drug and is used as a control. Remdesivir has a hydrogen bonding interaction with amino acids including Thr A:199, Tyr A:239, Lys A:137, Asp A:289. The ligand and receptor hydrogen bonding is assumed to be the most significant component which is used to determine receptor and ligand specificity. In drug research, strong hydrogen bonding is achieved through polar interaction because a drug must be able through polar interaction together with lipophilicity to penetrate the lipid bilayer (Davis & Teague, 1999). Of the three compounds tested, it was seen that quercetin had lower binding energy, including when compared to remdecivir. This makes quercetin a potential compound as a COVID-19 drug. This potential ability is due to this compound forming a stable bond on the active site of M^{pro} so that the strength of this bond can affect mutations in amino acids that will inhibit the activation and function of the M^{pro} virus (Gu et al., 2021; Saakre et al., 2021). In addition, another reason that makes quercetin more potent is that this compound binds to the active site of the M^{pro} amino acid residue in the smallest order there by accelerating viral inhibitors.



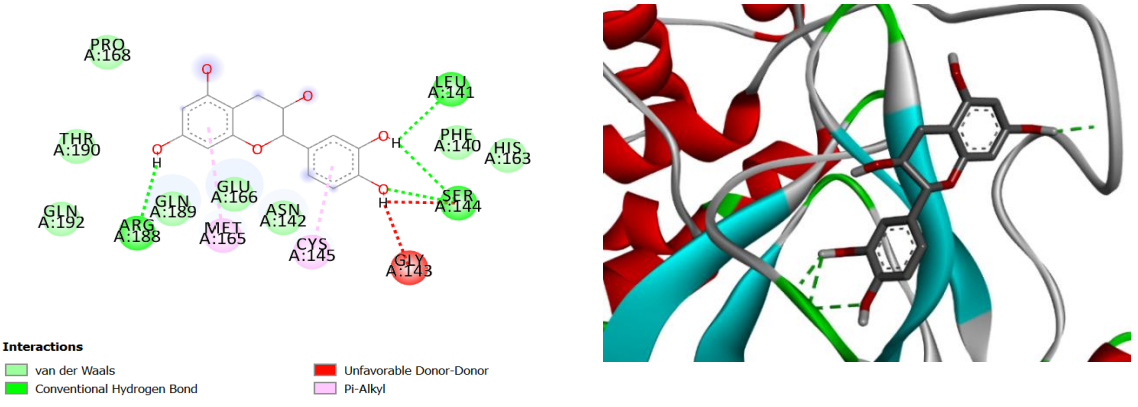
A B

Figure 2. A. 2D image visualization of Myricetin and B. 3D image of Myricetin against M^{pro} protein (6LU7)



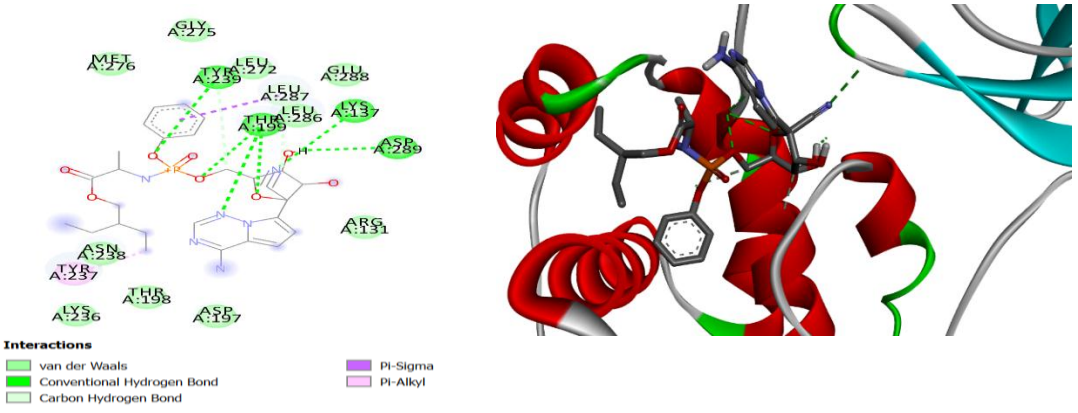
A B

Figure 3. A. 2D image visualization of Quercetin and B. 3D image of Quercetin against M^{pro} protein (6LU7)



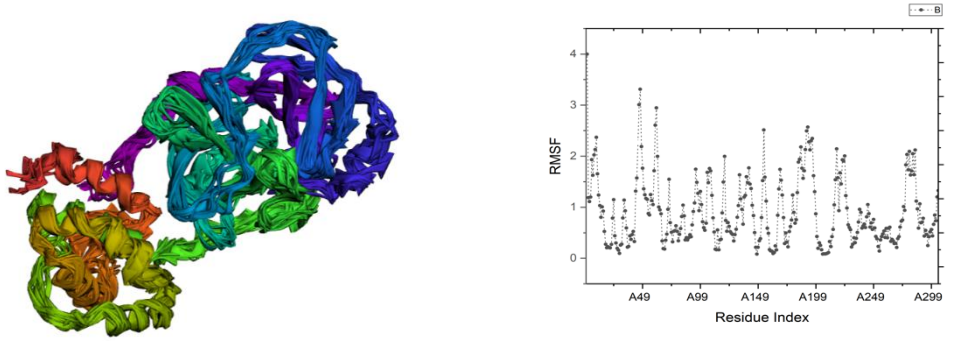
A B

Figure 4. A. 2D image visualization of Catechin and B. 3D image of Catechin against M^{pro} protein (6LU7)



A B

Figure 5. 2D image visualization of Remdesivir and B. 3D image of Remdesivir against M^{pro} protein (6LU7)



A B

Figure 6. *A. structure 3D and B. RSMF value of main protease (M^{pro}) protein*

Molecular Dynamic Simulation

Figure 6A shows the 3D in main protease protein structure with molecular dynamics simulation. Ligands such as catechin. myricetin and quercetin are attached to chain A. The RMSF result is got from the CABS-flex simulation with the x-axis showing the residue index of chain A and the y-axis (Figure 6B) is the RMSF values. The main protease protein shows the RMSF result below 4 Å and it is suitable with the RMSF criterion is has valued 1-3 Å (Parikesit & Nurdiansyah, 2021).

Conclusion

COVID-19 is a disease that spreads very fast throughout the country. This makes the researchers immediately get the right drug to prevent infections from replicating. Several investigations have been conducted using various methods. In this study, the biologically active in kencur (*Kaempferia galanga* L.) had been used to examine Lipinski's five-point concept molecular analysis. Molecular Design molecular dynamic simulation for determine the interaction between bioactive compounds and M^{pro} on Sars-CoV-2 as a target. From the 3 existing bioactive compounds, it was found that Quercetin has a very potential anti-viral activity as a M^{pro} inhibitor as shown from the results of molecular docking analysis and molecular dynamic simulation. So that the compound quercetin is recommended to analize further to find out its effectiveness in more detail.

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