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IN SILICO IDENTIFICATION OF BREADFRUIT PLAN COMPOUNDS (*Artocarpus atilis*) AGAINST ANGIOTENSIN-CONVERTING ENZYME 2 AS A CANDIDATE MODEL OF COVID-19 INHIBITORS

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ABSTRACT

The spread of COVID-19 has continued to grow rapidly worldwide. This disease is characterized by the presence of severe respiratory syndrome disorders in humans. The ACE2 receptor is the gateway for the COVID-19 virus to target cells, where it is expressed in airway epithelial and vascular endothelial cells. Breadfruit (*Artocarpus altilis*) contains active flavonoids. Flavonoid derivatives found in breadfruit include *artonin-E, cycloaltilisin-7, cycloaltilisin, isocyclomorusin, cyclomorusin, cyclomulberrin, isocyclomulberrin, cyclocommunal, morusin, quercetin* and *artocarpin*. This study aimed to determine the flavonoid derivative of breadfruit (*Artocarpus altilis*), which has the most effective potential against the ACE2 receptor, as a candidate inhibitor of the COVID-19 virus in silico. The results showed that of the eleven flavonoid derivatives of breadfruit (*Artocarpus altilis*) tested, 3 compounds gave the lowest binding affinity value, namely *cycloaltilisin* -8.79 kcal/mol, *cyclomulberrin* -8.95 kcal/mol, and *artocarpin* -9.30. kcal/mol compared to Chloroquine -7.67 kcal/mol and Hydroxychloroquine -7.22 kcal/mol. The predicted results of the Lipinski Rule of Five showed that the 3 compounds and the comparison drug met the Ro5 rule. The compounds *cycloaltilisin, cyclomulberin*, and *artocarpine* have antiviral activity, as indicated by the Pa value in the range of 0.5<Pa<0.7. *Cycloaltilisin, cyclomulberin* and *artocarpine* are the most effective compounds to be developed as candidates for COVID-19 inhibitors.

Keywords: Breadfruit, ACE-2, Covid-19, In Silico

INTRODUCTION

The discovery of ligand-based and target-based drugs is the most widely applied view in modern drug discovery (Amin et al., 2021). In drug discovery for disease targets, bioactive compounds must have physicochemical and pharmacokinetic properties that have an important role in providing the effectiveness of a compound against disease targets related to solubility, permeability, and metabolic stabilization (Marulasiddaswamy et al., 2021).

The spread of COVID-19 continues to grow rapidly worldwide (Khayrani et al., 2020). SARS-CoV-2 is the virus that causes COVID-19, which infects millions of people worldwide (Rutwick et al., 2021). Protein modeling experiments on viral spike proteins soon demonstrated that SARS-CoV-2 has sufficient affinity for the angiotensin converting enzyme 2 (ACE2) receptor on human cells to use it as a cell entry mechanism. The ACE2 receptor is the gateway for the COVID-19 virus to target cells, where it is expressed in airway epithelial cells and vascular endothelial cells. ACE2 exists in two forms: a soluble form that represents circulating ACE2, and a structural transmembrane protein with an extracellular domain that is a receptor for the SARS -CoV-2 spike protein.

The potentially effective inhibition or regulation of ACE2 receptors has been used in the treatment of COVID-19 (Fauzi et al., 2021).

Breadfruit (*Artocarpus altilis*) contains active flavonoids. Flavonoid derivatives found in breadfruit include *artonin-E*, *cycloaltilisin-7*, *cycloaltilisin*, *isocyclomorusin*, *cyclomorusi*n, *cyclomulberrin*, *isocyclomulberrin*, *cyclocommunal*, *morusin*, *quercetin*, and *artocarpin* (Jagtap et al., 2010). Several comprehensive studies have been conducted on plants of the genus Artocarpus , such as anticancer (Fitriah et al., 2018), antioxidant (Soifoini et al., 2021), antibacterial (Prastiyanto et al., 2020), and antivirus (Wijayanti et al., 2013). This study aimed to determine the breadfruit flavonoid derivative (*Artocarpus altilis*) which has the most effective potential against the ACE2 receptor, as an in silico candidate inhibitor of the COVID-19 virus.

RESEARCH METHODS

Chemical structure preparation for In Silico Studies

The structure files for Breadfruit plant compounds *(Artocarpus atilis*) is *artonin E* (PubChem ID: 5481962), *cycloaltilisin-7* (PubChem ID: 11811595), *cycloaltilisin* (PubChem ID: 44258301), *isocyclomorusin* (PubChem ID: 5316261), *cyclomorusin* (PubChem ID: 5481969), *cyclomulberrin* (PubChem ID: 11742872) *isocyclomulberrin* (PubChem ID: 5316260), *cyclocommunal (PubChem ID:* 10315987), *morusin* (PubChem ID: 5281671), *quercetin* (PubChem ID: 5280343) and *artocarpin* (PubChem ID: 5458461) along with standart anti-COVID19 drugs chloroquine (PubChem ID: 2719) and hydroxychloroquine (PubChem ID: 3652).

Receptors preparation

The 1R4L receptor was used for the receptor preparation. The ACE2 receptor was downloaded from the Protein Data Bank website (http://www.rscb.org/pdb) (Towler et al., 2004). The receptors were visualized using the Discovery Studio 2016 Client® program. In this program, the downloaded receptor was prepared by removing the water molecules and their natural ligands. The result was a pure receptor, which was then saved in the Protein Data Bank (.pdb) format.

Physicochecimal properties and Pharmacocinetics parameters

Physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug similarity, and drug chemical parameters are well-established parameters of the drug discovery process for characterizing effective drug candidates. The bioavailability parameters were predicted using the Chemdraw application. The chemical structure of the compound was determined and so its structure is predicted using the parameters of relative molecular mass, partition coefficient, hydrogen donor, and acceptor. These results will determine the route of administration of the drug to the patient. Pharmacokinetic parameters were calculated using the preADMET® program which is accessed via website (https:/preadmet.bmdrc.kr/adme). The chemical structure of the compound was drawn or uploaded in a Mol file (.mol) format. The program automatically calculated the predictive value of the chosen parameters, namely, the permeability of human colon adenocarcinoma (Caco-2), Human Intestinal Absorption (HIA), protein binding, and carcinogenic properties (Lamie et al., 2021).

Biological activity prediction of Breadfruit Plant Compounds

PASS online, an in silico server for the prediction of biological properties and possible targets, was used to i investigate the biological activity spectrum of Fragransol B. The PASS algorithm with a training set of over 260,000 drug-like biologically active compounds (drugs, drug candidates, lead compounds, and toxic compounds) simultaneously predicts 3,678 kinds of activity (95% mean accuracy) based on multilevel neighbors of atom descriptors of the molecular structures of active compounds in comparison with the training set. The ratios of “probability to be active (Pa)” and “probability to be inactive (Pi)” were

used to predict and rank biological properties. A higher "Pa" indicates higher probability of a compound to be bioactive (Lagunin et al., 2000).

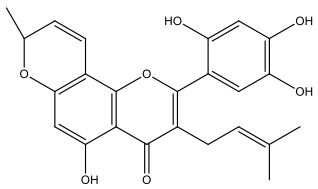
Anti-COVID19 molecular docking studies to predict the best

Docking was performed using the AutoDock 4 software. (run-autodock) by docking the ligand and receptor, and then edit cmd by removing the address of the working directory so that the cmd column contains (D:/Autodock/autodock4 –p dock.dpf –l dock.dlg &) then click launch. The docking calculation results can be observed in the notepad application. The conformation of the docking compound was determined by selecting its optimal configuration. The position and orientation of these ligands on macromolecules, as well as the amino acids bound to the ligands, were visualized using the Discovery Studio2016 Client® program to match their shape and anchorage sites in the form of 3D-pharmacophore modeling.

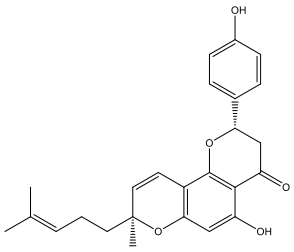
RESULTS AND DISCUSSION

In this study, an interaction study was conducted by docking the natural compounds contained in the breadfruit plant (*Artocarpus atilis*) with the ACE2 target receptor. The ACE2 receptor is a potential target for COVID-19 therapy, which is the main goal of the COVID-19 virus to transmit the virus to alveolar cells.

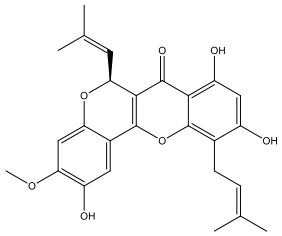
This process is a preliminary study where, in previous research studies, it has never been carried out on the target of ACE2. In this preliminary study, we investigated the content of compounds found in breadfruit plants, including *artonin-E, cycloaltilisin-7, cycloaltilisin, cyclocomunal, cyclomorusin, cyclomulberrin, isocyclomorusin, isocyclomulberrin, morusin, quercetin*, and *artocarpin.* The 11 compounds contained in breadfruit are flavonoids, as shown in **Figure 1**.



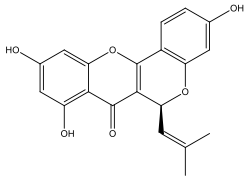
Artonin E



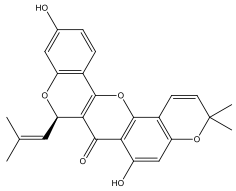
Cycloaltilisin-7



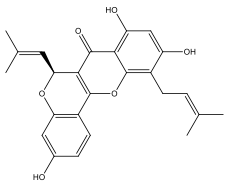
Cycloaltilisin



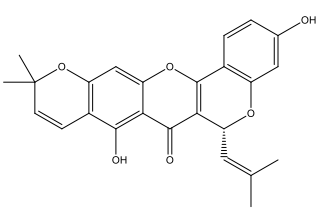
Cyclocomunal.



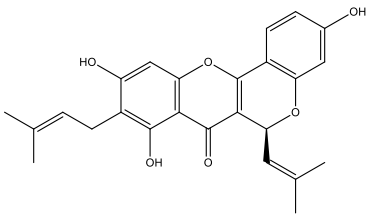
Cyclomorusin



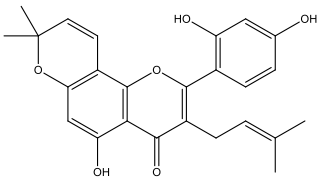
Cyclomulberrin



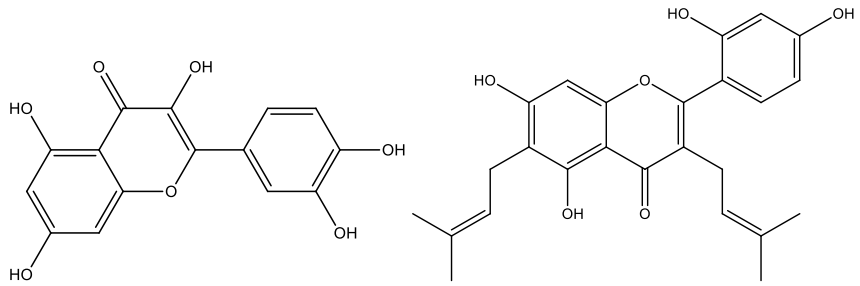
Isocyclomorusin



Isocyclomulberrin



Morusin



Quercetin Artocarpin

Figure 1. Compounds of Breadfruit (*Artocarpus atilis*)

The results showed that of the 11 docked breadfruit (*Artocarpus altilis*) flavonoid derivatives, 3 compounds gave the lowest binding affinity values, namely *cycloaltilisin* -8.79 kcal/mol, *cyclomulberrin* -8.95 kcal/mol, and *artocarpin* -9.30. kcal/mol compared with chloroquine -7.67 kcal/mol and hydroxychloroquine -7.22 kcal/mol, the results can be seen in **Figure 2** and **Figure 3**.

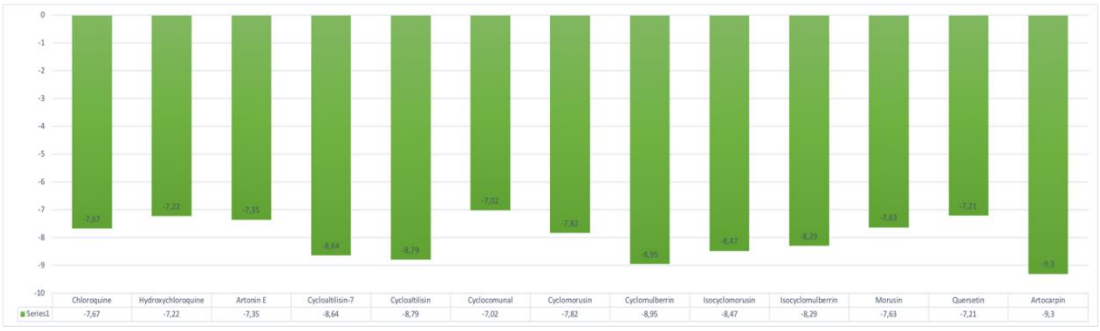


Figure 2. Value of binding affinity (kcal/mol) of docking compounds derived from breadfruit flavonoids (*A. altilis*) with ACE2 receptors

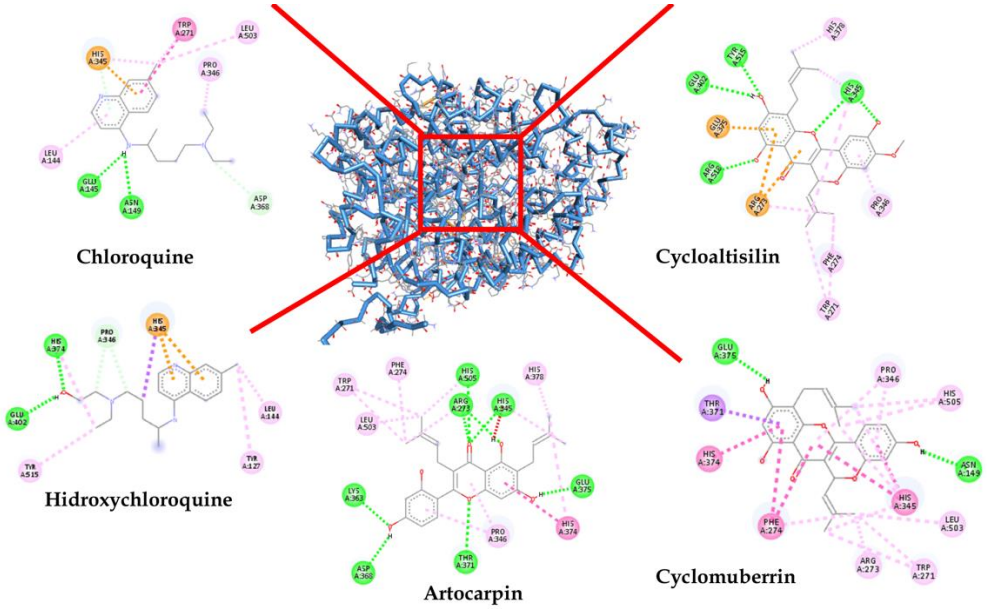


Figure 3. Comparative results of the interaction of comparison compounds and test compounds on the ACE2 receptor

Artocarpin provides the best activity based on binding affinity, and is able to inhibit ACE2 receptor targets. The interaction of amino acids formed from hydrogen bonds is stronger than that of the others and drug compounds, and the results are shown in Figure 3. The preliminary study will strengthen this research to continue to the next stage in the process of developing new drugs because the compound artocarpine provides very promising results. for inhibition of molecular targets.

Lipinski's Rule of Five is a rule of thumb for evaluating the physicochemical properties of compounds to be administered orally (Lagunin et al., 2021). This rule explains the physicochemical properties of the pharmacokinetic phase in the human body, which are predicted qualitatively to guide the design of medicinal compounds that are conveyed in several predictions, namely logP, molecular weight, and the number of hydrogen-bond donors and acceptors. Therefore, when designing drugs to be administered orally, it is hoped that they will comply with Lipinski's Rule of Five. Based on these rules, the results of 3 compounds, namely cycloaltilisin, cyclomulberin and artocarpine based on molecular docking predictions fulfill Lipinski's Rule of Five, the results are shown in Table I. The results of these 3 best compounds, based on the molecular docking results, can be given orally.

Table I. Prediction results of Lipinski Rule of Five (Ro5) ligand compounds

Compounds	Molecular Weight (Da)	Log P	H-Donor	H-Acceptor
Artonin E	422,14	3,38	4	7
Cycloaltilisin-7	406,47	4,24	2	5
Cycloaltilisin	450,48	4,02	3	7
Cyclocomunal	352,34	2,55	3	6
Cyclomorusin	418,44	3,57	2	6
Cyclomulberrin	420,16	4,15	3	6
Isocyclomorusin	418,44	3,57	2	6
Isocyclomulberrin	420,16	4,15	3	6
Morusin	420,45	3,99	3	6
Quersetin	302,04	0,35	5	7
Artocarpin	422,17	4,57	4	6

Molecular Weight ≤ 500 Da, Log P ≤ 5, H-donor ≤ 5, H-akseptor ≤ 10.[13]

Each compound contained in the breadfruit plant was predicted using the Prediction of Activity Spectra for Substances (PAAS) Prediction Server to observe its biological activity (Fakih et al., 2022). Server predictions are performed to identify biological activities that are relevant to the one being studied along with their probability spectrum. **Figure 4** shows the results of the biological activity analysis that has been carried out on *artonin-E*, *cycloaltilisin-7*, *cycloaltilisin*, *cyclocomunal*, *cyclomorusin*, *cyclomulberrin*, *isocyclomorusin*, *isocyclomulberrin*, *morusin*, *quercetin*, and *artocarpine*. Antiviral activity is the biological activity that is relevant to the molecular analysis of these compounds. All the compounds in this study showed active probability as antivirals, as indicated by the active probability value (Pa). The cutoff parameter Pa becomes a reference for determining the activity experimentally. The value of Pa varies from 0.000 to 1.000, indicating the possibility of the test compound being active or inactive (Ramadhan et al., 2020). A computer algorithm called the Prediction of Activity Spectra for Substances (PASS) was used to check for antiviral potential. Based on the structure–activity link between known chemical entities and chemical structures, including phytochemicals, this software predicts the biological activities of these structures. The biological activities of the compounds were verified and correlated using PASS software calculations. Using this technique, we conducted a structural study of the components of breadfruit plants to validate our findings. The Pa value of readfruit plant compounds for antiviral activity was based on the 3 best

compounds, namely, cycloaltilisin (PA 0.314), cyclomulberin (PA 0.334), and artocarpine (PA 0.352). Thus, these three compounds may act as antiviral agents.

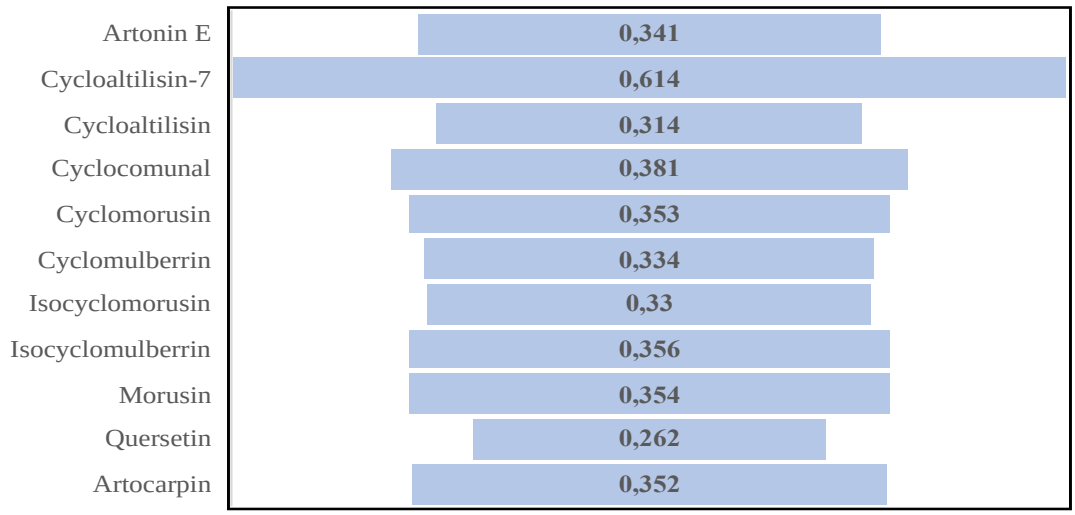


Figure 4. Pa value (Probable activity) antiviral activity of breadfruit (Artocarpus altilis) flavonoid derivative compounds

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that of the 11 predicted flavonoid derivatives of breadfruit (*Artocarpus altilis*), 3 compounds, namely *cycloaltilisin*, *cyclomulberin*, and *artocarpine*, are the most effective candidates for further development as COVID-19 inhibitors.

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