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In Silico Study of Bioactive Compounds from Tangerines (*Citrus reticulata*) as Protein Inhibitor for 3CLpro SARS-CoV-2 : Omicron Variant

Victor Alvianoes Guterez Hose[1], Shella Zahra Kumala Azmi[1], Muhammad Faiz Maulana[1], Daniel Tumbur Hamonangan Sirait[1], Ailsa Calista Salsabila[1], Rahmat Grahadi[1] and Fatchiyah Fatchiyah[1 2]*

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University,
Jalan Veteran, Malang, East Java, Indonesia, 65145
[2]Research Center of Smart Molecules of Natural Genetic Resources (SMONAGENES), Brawijaya University,
Jalan Veteran, Malang, East Java, Indonesia, 65145

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***Corresponding author**: Fatchiyah Fatchiyah; **e-mail**: fatchiya@ub.ac.id

ABSTRACT. *COVID-19 is a disease caused by coronavirus that was identified as a new strain of coronavirus last December 2019. Coronavirus disease can be handled by blocking its protein using bioactive compounds, it is known that there are compounds from Citrus reticulata that have potential as antiviral. An in silico evaluation of the active compounds available in Citrus reticulata was carried out to compare their anti-inflammatory effects based on the molecular characteristics of docking with nirmatrelvir as a control compound. In addition, analysis of binding affinity, binding site, ADME prediction, and molecular dynamics simulation was also carried out. The results obtained indicate that neohesperidin compounds can bind to receptors that bind to catalytic sites on Cys145 and His41 as well as nirmatrelvir. In addition, there is also phlorin which can bind to the catalytic site of the His41 receptor. Active site analysis of the interaction suggests neohesperidin and phlorin compounds may have a therapeutic effect against COVID-19. However, from the results of ADME predictions, not all of these compounds qualify Lipinski's rule criteria such as neohesperidin and diosmin.*

Keywords: *Citrus reticulata, COVID-19, in silico study*

INTRODUCTION

COVID-19 is a disease caused by a coronavirus that was identified as a new strain of coronavirus in last December 2019, namely Sars-CoV-2 [1]. Sars-CoV-2 is part of the *Coronavirinae* subfamily and belongs to the beta-coronavirus member with one of the largest positive sense single-stranded RNA genomes, ranging from 30 kilobase pairs and having more than 10 open reading frames (ORFs) [2]. Sars-CoV-2 continues to mutate and produce various variants. One of the groupings of these variants is called the Variant of Concern (VOC), which is classification for variants with evidence of an increase in transmissibility or caused more severe disease. There are 2 variants classified as VOC, including Delta and Omicron. Omicron is a variant that was first identified in South Africa and is estimated to have high transmission ability and can reduce the effectiveness of the vaccine [3]. Beta-coronavirus generates ~800 kDa polypeptide after genome transcription. These polypeptides are proteolytically cleaved to produce various proteins [4]. Papain-like protease (PLpro) and 3-chymotrypsin (3CLpro), also known as main protease, are two important components for post-translational processing of Sars-CoV-2 polypeptides and production of 16 non-structural proteins. These non-structural proteins play a fundamental role in viral replication, transcription, and recombination during infection. This makes 3CLpro the main target most often used as the main target in the development of COVID-19 drugs [2].

Citrus reticulata or commonly known as mandarin orange or "*jeruk keprok*" in Indonesian is one of the imported oranges from China which has been widely cultivated in various regions in Indonesia. Tangerine contains various compounds that can be used as flavoring, medicine, antibacterial, and antiviral [5]. Several active compounds such as quercetagetin, diosmin, and neohesperidin are known to have antiviral abilities. The use of quercetagetin has been shown to inhibit RNA replication and functional proteins of the Enterovirus A-71 virus [6]. Other studies have shown that the tangeretin compound found in tangerines affected the intracellular replication of RSV through inhibiting the expression of RSV P protein [7].

Based on the background described previously and due to the Sars-CoV-2 virus which is still mutating, another effort and treatment is needed to break the chain of Sars-CoV-2 virus replication. Therefore, this study aims to determine the potential of the active compounds in *Citrus reticulata* as inhibitors for the 3CLpro protein, especially in the omicron variant.

RESEARCH METHODS

Compound Screening and Antivirus Ability Prediction

The active compounds in *Citrus reticulata* were collected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Canonical SMILES data of the selected compounds were recorded and tested on the Way2Drug website with the AntiVir-Pred service (http://www.way2drug.com/antivir/) which functions to predict antivirus abilities (Table 1).

Table 1. Compound Screening

Compound	CID
Nirmatrelvir (Control)	155903259
Neohesperidin	442439
Diosmin	5281613
Myricetin	5281672
Quercetagetin	5281680
Phlorin	476785
Haplogenin	15907673
Diosmetin	5281612
Linderoflavone B	97151
Corniculatusin	5280695
Sudachitin	12443122
4',5-Dihydroxy-7,8-dimethoxyflavone	14585506
8-Hydroxy-4',5,7-trimethoxyflavone	5318368
Cirsilineol	162464
Gardenin D	3080750
Limocitrin	5489485
Vicenin 2	442664
5-Hydroxy-7,8,4'-trimethoxyflavone	14353376
5-dimethyl sinensetin	152430
Cirsimaritin	188323
7-Demethylnobiletin	10452846
3-[2-(Dimethylamino) ethyl]-1H-indole-5-yl beta-D-glucopyranoside	23266018
Bufotenine-O-glucoside	74039123
Salvigenin	161271
6-Demethoxytangeretin	629964
6-Hydroxy-3, 3', 4', 5,7,8-hexamethoxyflavone	131753119

Molecular Docking

Docking is done using active compounds that have been filtered and are predicted to have the highest confidence value. Furthermore, the ligand data is retrieved and downloaded in SDF format from its 3D structure section on the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Then, the receptor data used, namely 3CLpro, was taken from RCSB PDB (https://www.rcsb.org/) with ID : 7TLL and then uploaded its structure in the form of PDB. The receptor was prepared with the Discovery Studio Software by removing water molecules and pre-adhered ligands. Meanwhile, the ligand preparation itself was carried out using the PyRx Software to minimize the energy in the structure, this was useful in order to make the ligand able to bind to new receptors. Docking can be done with Autodock Vina which is already integrated with the PyRx application. The success of docking is seen from the bonds interaction formed and their binding affinity bonds.

Data Visualization and ADME Prediction

Docking results can be visualized using Discovery Studio to determine the interaction of the ligands. Visualization can be in the form of 2D diagrams and 3D diagrams. 3D structures can also be colored and labeled for simplified analysis. The results that have the affinity between the receptor and the ligand were analyzed, then compared with the control compound. After that, the docking results were continued with ADMET analysis using pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) to further predict the pharmacokinetics of each selected compound.

Molecular Dynamics Simulation

Molecular dynamics simulations were carried out to ensure that the docking results had maximum potential in its function as an inhibitor, one of which was an analysis of the Root Mean Square Fluctuations (RMSF) value. RMSF value use to explain the fluctuations of each amino acid residue. RMSF value carried out using CABS-flex 2.0 website (http://biocomp.chem.uw.edu.pl/CABSflex2). Each of the five compound complex with 3CLpro receptors submitted to CABS-flex with the selection chain A and B. Wait until the simulation process is complete and the result form of residual fluctuation plot data is stored in CSV format and graphed.

RESULTS AND DISCUSSIONS

Bioactive and Antivirus Ability from Compound of *Citrus reticulata*

Compounds that have been screened are being tested for the bioactive and antivirus ability using Way2Drug. The potential compounds are Neohesperidin, Diosmin, Myricetin, Quercetagetin and Phlorin that are shown on (Table 2). These compounds are the ones that have the highest confidence value of antivirus among the compounds that collected on *Citrus reticulata*.

Table 2. Compounds Screening Value

Compound	Confidence Value
Neohesperidin	0,97
Diosmin	0,97
Myricetin	0,96
Quercetagetin	0,95
Phlorin	0,94

Binding Affinity of *Citrus reticulata* Compounds

The binding affinity results obtained from the receptor-ligand docking can be seen in Figure 1. Compounds from each ligand consisting of quercetagetin, neohesperidin, myricetin, diosmin, and phlorin to the 3CLpro receptor variant of omicron showed binding affinities -9.2, -8, respectively. 7, -9.3, -10.2, and -7.1 (kcal/mol). Negative binding affinity values indicate the stability of the result from complexes with a receptor, and this shows the important characteristics of drugs that have efficacious [9].

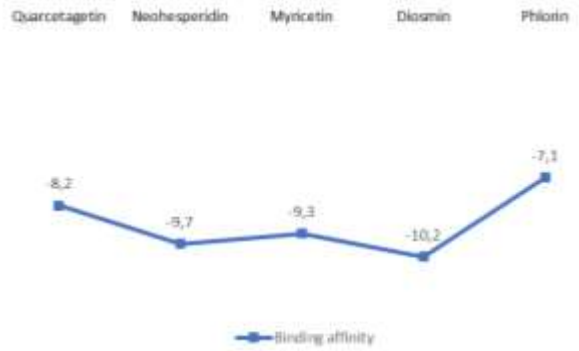


Figure 1. Binding affinity between 3CLpro and compounds

3CLpro and Compounds Interaction

In this study, nirmatrelvir was chosen as the control compound because nirmatrelvir is a drug compound that has so far been used to treat COVID-19 patients, especially for the Omicron variant. The visualization results of the interaction (Figure 2) between the control compound nirmatrelvir and 3CLpro showed the binding of His41 and Cys145 which are amino acid residues in the LBD 3CLpro enzyme and act as catalytic sites that inhibit virus replication [10].

The 2D interactions are shown in Figure 3. The bonds shaped in neohesperidin compounds include hydrogen bonds in Glu166, Phe140, Ser144, Cys145, Thr26, and Thr190. Unfavorable donor giver bonds in Gln192, Pi-Alkyl bonds in Met49 and Pro168, whereas hydrogen bonds to carbon in His41, Ala191, and Asn142. Diosmin compound forms hydrogen bonds at the amino acid residue Lys5 then, there are also several other bonds such as a carbon hydrogen bond in Pro122, and three hydrogen bonds in Lys12, Glu14, Phe3, and Gln49. Myricetin compound has hydrogen bonds attached to Gln127 and Val125. In addition, it also binds to Amide-Pi Stacked with Ala7, favorable donor-donor bonds with Arg4, van der Waals bonds with Met6, and Pi-Cation bonds with Lys5. Quercetagetin compounds binds van der Wals bonds in Met6, hydrogen bonds in Val125 and Gin127, Unfavorable donor bonds in Arg4 and Ala7, Pi-Cation bonds in Lys5, Amide-Pi bonds in Lys5 and Pi-Alkyl bonds in Ala7. 3CLpro with phlorin compounds forms hydrogen bonds at the amino acid residues Ser144, His41, and Arg188. In addition, there are also several other bonds such as a Pi-Pi T-shaped bond in Met165, a carbon-hydrogen (C-H) bond in His164 and a non-unfavorable acceptor bond in Leu141.

ADME Prediction

Based on the data obtained from the analysis of the five Lipinski's rules, that each compound analyzed according to the rules consisting of the Lipinski Rule consists of a molecular weight (MW) $\leq$ 500 daltons, hydrogen acceptor $\leq$ 10, hydrogen donor $\leq$ 5, lipophilicity (LogP) $\leq$ 5, molar refraction (MR) between 40-130. From these rules it is explained that compounds that have a high probability of being drug-like molecules must follow at least two of Lipinski's rules [11]. From the results of the prediction of ADME with Lipinski's the rules are pressed by the observed parameters, where the five compounds. Diosmin and Neohesperidin are not in accordance with Lipinski's rules.

Molecular Dynamics Simulation

Table 4. Average RMSF Value

Compounds	Average RMSF	
	A	B
Diosmin	1,526	0,394
Myricetin	1,869	0,871
Neohesperidin	0,108	0,694
Nirmatrelvir	0,651	0,332
Phlorin	0,375	1,101

Generally, RMSF analysis results showed that the ligand target is sufficiently stable and the conformation has low flexibility. It is also stated that if the RMSF result has a value above 2,5 Å, it indicates that the bond and conformation formed is unstable [13]. Based on Fig.3, it is shown that phlorin is a compound that has a lower RMSF value and showed least fluctuations (below 2,5 Å). This is also supported by the data in Table 4 which shows that phlorin has the lowest average RMSF value in A chain, which shows that phlorin is one of the most potentially compounds that can bind very stable to 3CLpro.

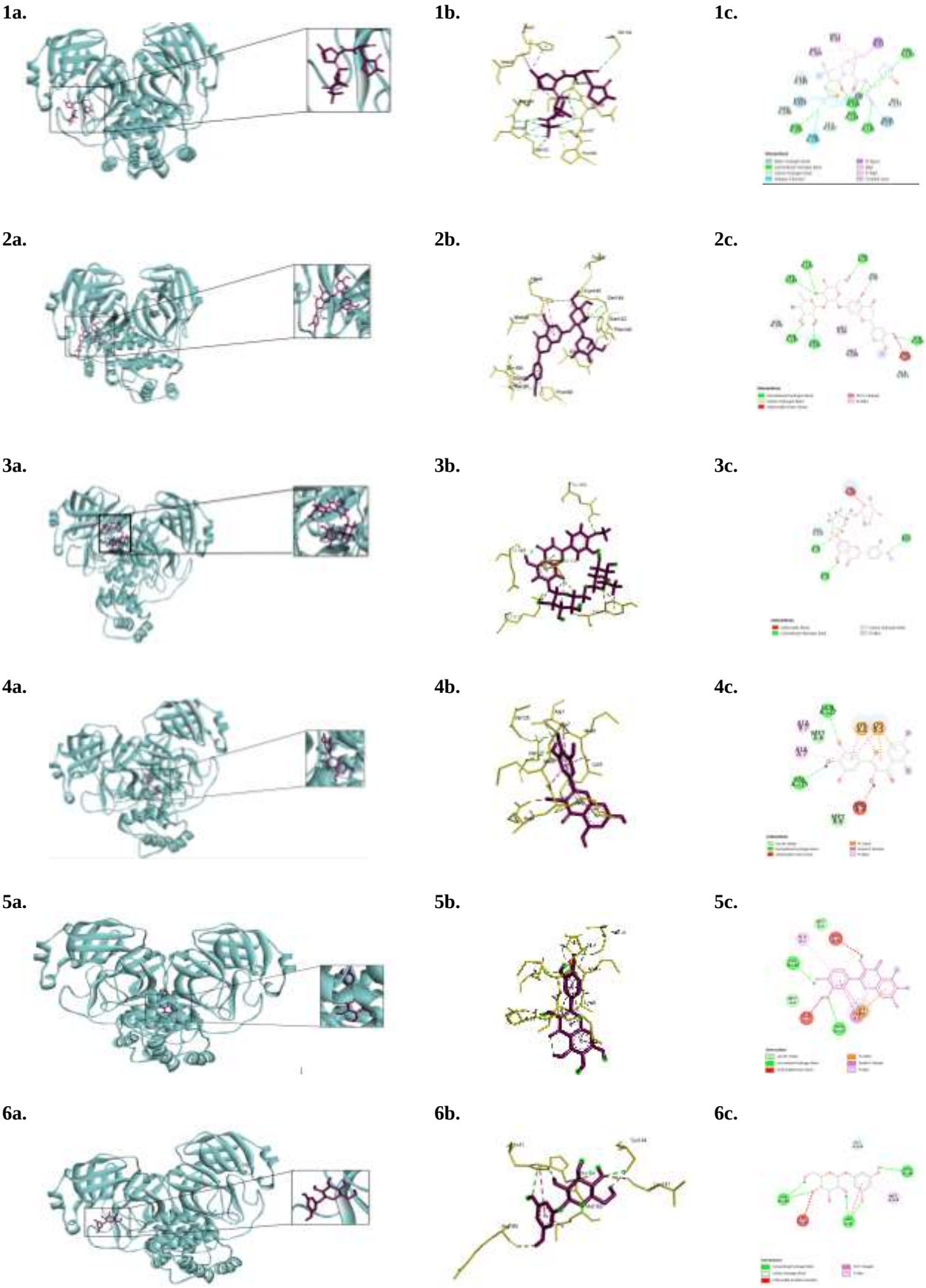


Figure 2. 3D and 2D interaction visualization between 3CLpro and compounds: 1a-c) nirmatrelvir (control); 2a-c) neohesperidin; 3a-c) diosmin; 4a-c) myricetin; 5a-c) quercetagetin; and 6a-c) phlorin.

Table 3. Information about ADME prediction from compound

Compound	MW	LogP	Rotatable Bonds	Acceptors	Donors
Neohesperidin	620.565	-1.1566	7	15	8
Diosmin	608.549	-1.0897	7	15	8
Myricetin	318.237	1.6936	1	8	6
Quercetagetin	318.237	1.6936	1	8	6
Phlorin	288.252	-1.7235	3	8	6

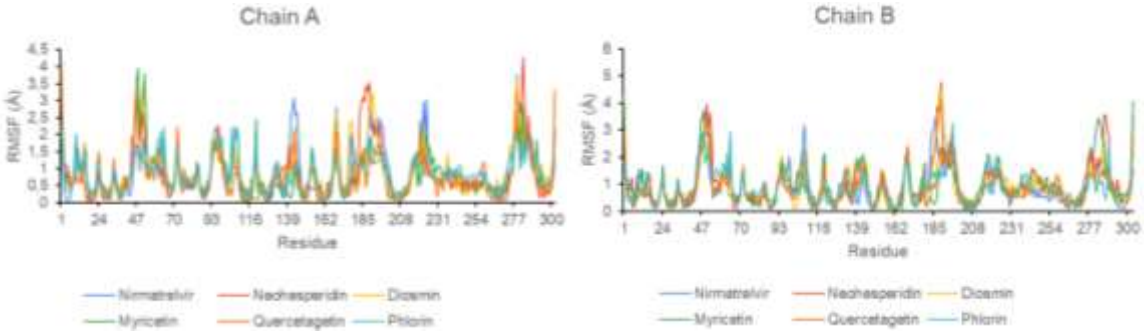


Figure 3. RMSF analysis for 3CLpro with compound.

CONCLUSION

We found that Neohesperidin and Phlorin interacted with 3CLpro active site and could potentially inhibit the activity of 3Clpro. However, the ADME prediction found that only Phlorin was suitable for drug development.

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