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Original article

Molecular interaction analysis of Sulawesi propolis compounds with SARS-CoV-2 main protease as preliminary study for COVID-19 drug discovery

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ABSTRACT

Coronavirus disease 2019 (COVID-19), a respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a global health concern, as the World Health Organization declared this outbreak to be a global pandemic in March 2020. The need for an effective treatment is urgent because the development of an effective vaccine may take years given the complexity of the virus and its rapid mutation. One promising treatment target for COVID-19 is SARS-CoV-2 main protease. Thus, this study was aimed to examine whether Sulawesi propolis compounds produced by *Tetragonula sapiens* inhibit the enzymatic activity of SARS-CoV-2 main protease. In this study, molecular docking was performed to analyze the interaction profiles of propolis compounds with SARS-CoV-2 main protease. The results illustrated that two compounds, namely glyasperin A and broussonol F, are potential drug candidates for COVID-19 based on their binding affinity of -7.8 kcal/mol and their ability to interact with His⁴¹ and Cys¹⁴⁵ as catalytic sites. Both compounds also displayed favorable interaction profiles with SARS-CoV-2 main protease with binding similarities compared to inhibitor 13b as positive control 63% and 75% respectively.

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1. Introduction

Coronavirus disease 2019 (COVID-19), is a respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is currently considered a global health emergency. Since first being identified in Wuhan, China in December 2019, SARS-CoV-2 has spread widely and infected millions of people globally. On March 11, 2020, the World Health Organization (WHO) declared this outbreak to be a global pandemic. To date, there have been around 1.24 million deaths reported according

to WHO as of November 7th, 2020 (World Health Organization, 2020) (Table 1).

SARS-CoV-2 is a single-stranded ribonucleic acid (RNA) enveloped virus from the genus *Betacoronavirus*, subfamily *Orthocoronavirinae*, and family *Coronaviridae* (Zheng, 2020). Two other viruses belong to this genus, namely Middle East respiratory syndrome virus and severe acute respiratory syndrome virus (SARS-CoV). SARS-CoV-2 was identified to have 82% RNA identity with SARS-CoV (Zhang et al., 2020). Both SARS-CoV and SARS-CoV-2 recognize the same receptor in the human body, namely angiotensin-converting enzyme 2. The SARS-CoV-2 genome encodes a trimeric structural spike protein, a homodimeric cysteine proteinase, an RNA polymerase, and several nonstructural proteins (Calligaris et al., 2020). The viral pathogenesis causes several symptoms, such as sore throat, running nose, cough, fever, and eventually respiratory failure.

Currently, there is no specific therapy with curative efficacy against the disease (Centers for Disease Control and Prevention,

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Table 1
Identified Sulawesi propolis compounds.

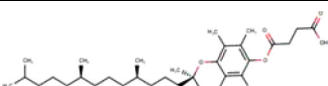
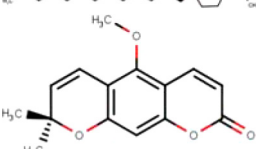
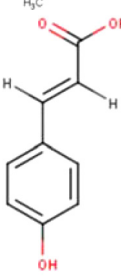
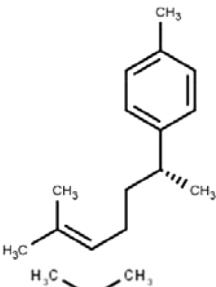
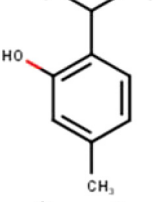
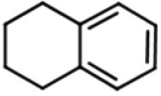
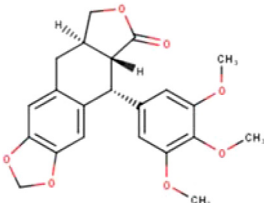
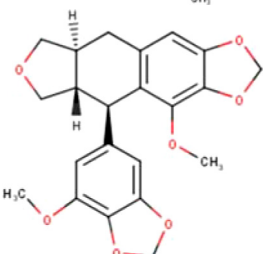
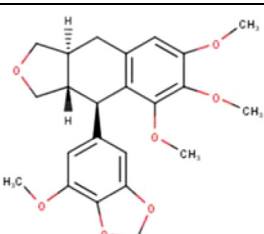
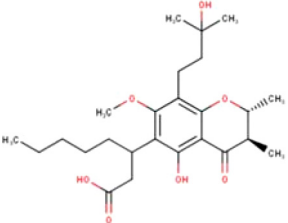
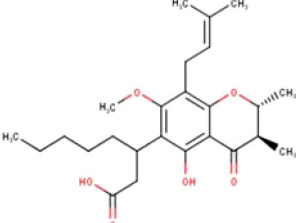
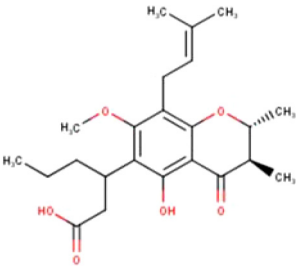
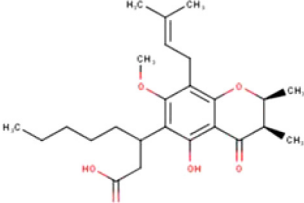
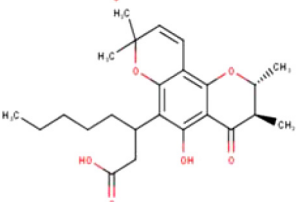
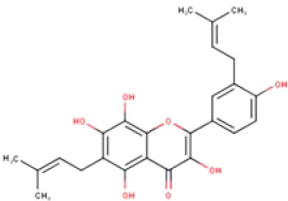
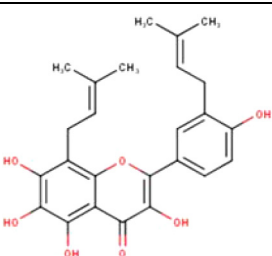
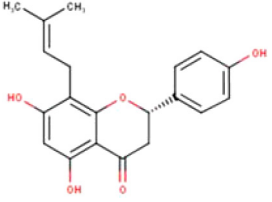
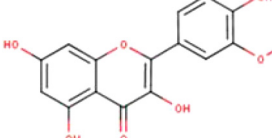
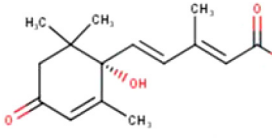
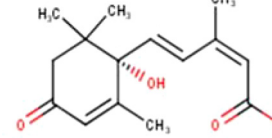
No	Compound	Molecular Formula	2-Dimensional Structure	References
1	α -Tocopherol succinate	C ₃₂ H ₅₂ O ₅		(Sahlan et al., 2019)
2	Xanthoxyletin	C ₁₅ H ₁₄ O ₄		(Sahlan et al., 2019)
3	P-Coumaric acid	C ₉ H ₁₀ O ₃		(Mahadewi et al., 2018)
4	Curcumene	C ₁₅ H ₂₂		(Mahadewi et al., 2018)
5	Thymol	C ₁₀ H ₁₄ O		(Mahadewi et al., 2018)
6	Tetralin	C ₁₀ H ₁₂		(Mahadewi et al., 2018)
7	Deoxypodophyllotoxin	C ₂₂ H ₂₂ O ₇		(Sahlan et al., 2019)
8	Sulabiroins A	C ₂₂ H ₂₂ O ₇		(Miyata et al., 2019, 2020a, 2020b)

Table 1 (continued)

No	Compound	Molecular Formula	2-Dimensional Structure	References
9	Sulabiroins B	C ₂₃ H ₂₆ O ₇		(Miyata et al., 2019, 2020a, 2020b)
10	2',3'-Dihydro-3'-hydroxypapuanic acid	C ₂₅ H ₃₈ O ₇		(Miyata et al., 2019, 2020a, 2020b)
11	(-)-Papuanic acid	C ₂₅ H ₃₆ O ₆		(Miyata et al., 2019, 2020a, 2020b)
12	(-)-Isocalolongic Acid	C ₂₄ H ₃₄ O ₆		(Miyata et al., 2019, 2020a, 2020b)
13	Isopapuanic acid	C ₂₅ H ₃₆ O ₆		(Miyata et al., 2019, 2020a, 2020b)
14	Isocalopolyanic acid	C ₂₄ H ₃₂ O ₆		(Miyata et al., 2019, 2020a, 2020b)
15	Glyasperin A	C ₂₅ H ₂₆ O ₇		(Miyata et al., 2019, 2020a, 2020b)

(continued on next page)

Table 1 (continued)

No	Compound	Molecular Formula	2-Dimensional Structure	References
16	Brousoflavonol F	C ₂₅ H ₂₆ O ₇		(Miyata et al., 2019, 2020a, 2020b)
17	(2S)-5,7-Dihydroxy-4'-methoxy-8-prenylflavanone	C ₂₀ H ₂₀ O ₅		(Miyata et al., 2019, 2020a, 2020b)
18	Isorhamnetin	C ₁₆ H ₁₂ O ₇		(Miyata et al., 2019, 2020a, 2020b)
19	(1'S)-2-Trans,4-trans-abcisic acid	C ₁₅ H ₂₀ O ₄		(Miyata et al., 2019, 2020a, 2020b)
20	(1'S)-2-Cis,4-trans-abcisic acid	C ₁₅ H ₂₀ O ₄		(Miyata et al., 2019, 2020a, 2020b)

2020). Several drugs, such as hydroxychloroquine and chloroquine, have been suggested as treatments. However, no studies were adequately powered to prove their efficacy (Pastick et al., 2020). In addition, several reports of serious arrhythmia have been described in patients with COVID-19 who received these compounds (United States Food and Drug Administration, 2020). In addition, efforts are underway to develop vaccines to control the outbreak. However, given the complexity of the virus and the rapid mutation of its single stranded RNA, it may take years to generate effective vaccines (Huanget al., 2020).

Thereby, other compounds should be examined to identify an effective treatment with few or no adverse effects. According to Marcucci, propolis, a resinous bee product, exhibits antiviral activity based on the presence of flavonoids, caffeic acid, and esters of aromatic acids (Marcucci, 1995). The antiviral activities of these compounds occur through the inhibition of viral transmission to other cells, inhibition of viral propagation, and destruction of the outer envelope of the virus (Marcucci, 1995). Various experiments have been conducted to analyze the antiviral activities of the compounds. Research by Yildirim et al. revealed that propolis significantly decreased the number of copies of herpes simplex virus 2 after 48 h of incubation (Yildirim et al., 2016). In addition, Gekker et al. demonstrated that propolis has a destructive effect on the outer envelope of human immunodeficiency virus (Gekker et al., 2005). In terms of antiviral activity in the respiratory tract, another study reported that some propolis compounds have lower IC₅₀ values against human rhinovirus than ribavirin (Kwon et al., 2020).

The potency of propolis as COVID-19 drug has also been observed through some research. Vardhan and Sahoo (2020) have shown that three propolis components, namely limonin, quercetin and kaempferol have inhibitory potential by binding to viral RNA-dependent RNA polymerase (RdRp) with binding energy −9 to −7.1 kcal/mol through molecular docking study (Vardhan and Sahoo, 2020). Molecular docking enables the virtual screening of millions of compounds in a time- and cost-efficient manner (Pinzi and Rastelli, 2019). Hence, this method is widely used as a preliminary study in drug discovery (Meng et al., 2011). Another molecular docking study by Güler et al. (2020) concluded that identified compounds from alcoholic extract of propolis are able to bind with angiotensin-converting enzyme (ACE)-2. Thus, the compounds may be potential to prevent SARS-CoV-2 to bind with ACE-2 (Güler et al., 2020).

Based on these findings, propolis compounds may have potency for treating COVID-19. Further research is needed to evaluate the ability of propolis compounds to inhibit SARS-CoV-2 replication. Other protein in SARS-CoV-2 that is also considered a potential therapeutic target is main protease (Anand et al., 2003). The enzyme cleaves polypeptides translated from viral RNA into 12 smaller proteins that participate in viral replication (Chen et al., 2020). Therefore, viral replication can be blocked by inhibiting this enzyme (Zhang et al., 2020).

Previously, Zhang et al. developed peptidomimetic α-ketoamides as potential broad-spectrum inhibitors of main protease in Betacoronaviruses and Alphacoronaviruses (Zhang et al.,

2020). One such compound, *tert*-butyl(1-((S)-1-(((S)-4-(benzylamino)-3,4-dioxo-1-((S)-2-oxopyrrolidin-3-yl)butan-2-yl)-amino)-3-cyclopropyl-1-oxopropan-2-yl)-2-oxo-1,2-dihydro-pyridin-3-yl) carbamate, also known as 13b, inhibited SARS-CoV-2 replication in human Calu3 lung cells. The crystal structure of SARS-CoV-2 main protease in complex with inhibitor 13b is available.

Propolis compounds are diverse according to the region (Alday et al., 2016). Beforehand, Sulawesi propolis compounds from North Luwu have been identified by several research (Mahadewi et al., 2018; Miyata et al., 2019, 2020a, 2020b; Sahlan et al., 2019). Sulawesi propolis previously was known to be produced by *Tetragonula aff. biroi*. However, recent study rectified that it is actually produced by *Tetragonula sapiens* (Sayusti et al., 2020). Some research has also proved the health benefits of Sulawesi propolis. Sulawesi propolis exhibits antifungal activity to *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. glabrata* and *Cryptococcus neoformans* (Sahlan et al., 2020). Other study showed that Sulawesi propolis is potential to be developed as a non-steroid anti-inflammatory drug and antioxidant agent (Christina et al., 2018; Sahlan et al., 2019). These facts encourage us to do more investigations regarding the health benefits of Sulawesi propolis. The components of antioxidant and flavonoid in Sulawesi propolis may perform antiviral activity. Up to date, there has been no research that aims to evaluate the potency of Sulawesi propolis compounds to treat COVID-19.

In this research, molecular docking was performed to analyze the molecular interaction between SARS-CoV-2 main protease (PDB ID: 6Y2F) and Sulawesi propolis compounds. Molecular docking aims to predict the conformation of the ligand within the receptor and assess the binding affinity (Guedes et al., 2014), which is represented by the docking score (kcal/mol). The docking score and binding characteristics of inhibitor 13b were used to judge whether the propolis compounds are potential inhibitors of SARS-CoV-2 main protease.

Furthermore, this research also performed molecular docking between SARS-CoV-2 main protease and 14b, a modified version of inhibitor 13b. 14b does not feature a Boc group, which predicted to protect the compound as it crosses the cellular membrane (Zhang et al., 2020). Although 14b was mostly inactive in human Calu3 lung cells, this compound may have lower affinity for plasma proteins than inhibitor 13b (Zhang et al., 2020).

2. Materials and methods

2.1. Hardware and software

Molecular docking was performed using an Asus laptop with an Intel® Core™ i7-8550U @1.80 GHz processor, 8 GB of RAM, the Windows 10 Home Single Language 64-bit operating system, and an Intel® UHD Graphics 620 graphics processing unit. The software used in the study included MarvinSketch (ChemAxon, Budapest, Hungary), Autodock Tools 1.5.6 (The Scripps Research Institute, USA), Autodock Vina (The Scripps Research Institute), LigPlot+

(EMBL-EBI, UK) and Visual Molecular Dynamics (University of Illinois, Urbana-Champaign).

2.2. Lipinski's rule of five (RO5) selection

First, 20 identified Sulawesi propolis compounds from North Luwu, South Sulawesi, Indonesia were selected based on Lipinski's RO5. The rules aim to assess the solubility and permeability of drug candidates. The RO5 stated that poor absorption or permeability are more likely to occur when a compound has molecular weight greater than 500 g/mol, Log P greater than 5, H-bond donors more than 5 and H-bond acceptors more than 10 (Lipinski et al., 1997). Sulawesi propolis compounds that violates more than 2 rules were thrown away. Lipinski's RO5 was assessed by using MarvinSketch (ChemAxon, 2018).

2.3. Protein preparation

The SARS-CoV-2 main protease in complex with the α -ketoamide 13b (PDB ID: 6Y2F) was obtained from the Protein Data Bank (<http://www.rcsb.org>) in the .pdb format (Zhang et al., 2020). The Protein Data Bank is an open access digital data resource that provides 3D structure data for large biological molecules (Berman et al., 2013). The protein and inhibitor 13b file was then separated into two different .pdb files using Visual Molecular Dynamics (Humphrey et al., 1996). Then, the separated protein files were loaded into Autodock Tools 1.5.6 for further preparation consisting of the addition of polar hydrogens, addition of kollman charges and conversion of the file to the .pdbqt format (Forli et al., 2016) for compatibility for docking using Autodock Vina.

2.4. Ligands preparation

Meanwhile, the test ligands were selected Sulawesi propolis compounds from North Luwu produced by *Tetragonula sapiens* and 14b using inhibitor 13b as control. The structure of inhibitor 13b and 14b are shown in Fig. 1. Excluding inhibitor 13b, the 2D structures of the ligands were constructed and converted into 3D structures in the .pdb format using MarvinSketch. Then, the ligands were loaded to Autodock Tools 1.5.6 to add polar hydrogens as well as gasteiger charges, and convert the file to the .pdbqt format.

2.5. Parameter optimization

Before performing molecular docking between propolis compounds and main protease, the optimal specific search space was determined. The search space is the area within the protein in which the docking simulation will be performed. It may represent the locations of the binding sites. The optimal search space can be predicted via docking between the native ligand of the protein structure, in this case 13b, using the protein that was separated in the protein preparation step. This method is also known as "redocking." Redocking and the subsequent docking simulation were

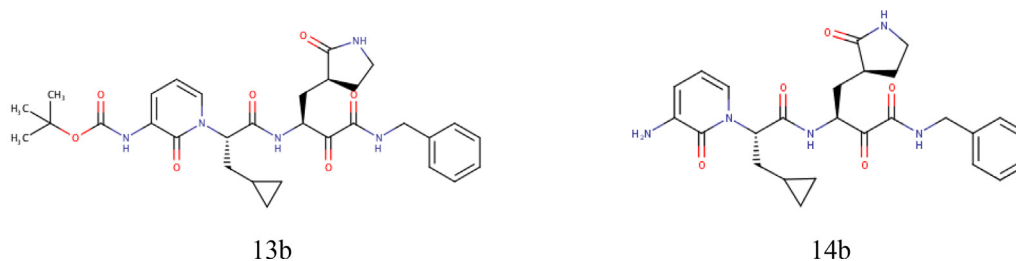


Fig. 1. Structure of inhibitor 13b and 14b (Zhang et al., 2020).

Table 2
Lipinski's RO5 selection.

No.	Compounds	Molecular weight (g/mol)	Log P	Number of H-bond acceptor	Number of H-bond donor	Number of violations
1	Sulabiroins A	398.411	2.74	7	0	0
2	Sulabiroins B	414.454	2.55	7	0	0
3	2',3'-Dihydro-3'-hydroxypapuanic acid	450.572	4.33	7	3	0
4	(-)-Papuanic acid	432.557	5.57	6	2	1
5	(-)-Isocalolongic acid	404.503	4.78	6	2	0
6	Isopapuanic acid	432.557	5.57	6	2	1
7	Isocalopolyanic acid	416.514	5.03	6	2	1
8	Glyasperin A	438.476	4.84	7	5	0
9	Brousoflavonol F	438.476	4.84	7	5	0
10	(2s)-5,7-Dihydroxy-4'-methoxy-8-prenylflavanone	340.375	4.19	5	3	0
11	Isorhamnetin	316.265	1.78	7	4	0
12	(1's)-2-Trans,4 trans-absicic acid	264.321	2.08	4	2	0
13	(1's)-2-Cis,4 trans-absicic acid	264.321	2.08	4	2	0
14	α -tocopherol succinate	530.790	9.18	4	1	2
15	Xanthoxyletin	258.273	2.01	3	0	0
16	P-coumaric acid	164.160	2.12	3	2	0
17	Curcumene	202.341	5.19	0	0	1
18	Thymol	150.221	3.42	1	1	0
19	Tetralin	132.206	3.27	0	0	0
20	Deoxypodophyllotoxin	398.411	2.63	6	0	0

performed using Autodock Vina. In terms of accuracy, Autodock Vina offers more accurate binding mode predictions than Autodock Tools (Vieira and Sousa, 2019).

Adjustment was performed for three parameters to obtain the optimal specific search space, namely the center of the grid box, number of points in the x, y, and z dimensions, and grid spacing. Based on the redocking simulation, it was found that the binding site had a higher probability to be located at the following coordinates: x = 11.476, y = -1.396, and z = 20.745. The number of points in the x, y, and z dimensions were all set to 25 Å, and the grid spacing was adjusted to 1.0 Å.

After the docking score was obtained, the root mean square deviation (RMSD) of the first rank ligand pose was evaluated. An RMSD of less than 2.0 Å indicates that the redocking procedure is accurate (Ramírez and Caballero, 2018). Thereby, the docking coor-

dinates could be used to dock the propolis compounds onto the protein.

2.6. Molecular docking analysis of propolis compounds onto main protease

To identify which propolis compounds are potential SARS-CoV-2 inhibitors, the compounds were docked into the protein individually using the coordinates and grid parameters obtained from the redocking simulation. The simulations were performed using Autodock Vina with exhaustiveness value set to 64. After the docking score for each compound was obtained, the interactions between the protein and the ligands with the lowest docking scores were analyzed using Ligplot+, which provides 2D visualization of molecular interactions.

3. Results and discussion

This research analyzed the molecular interactions between selected Sulawesi propolis compounds produced by *Tetragonula sapiens* and SARS-CoV-2 main protease in an effort to identify potential inhibitors. Main protease was selected because of its important role in viral replication. Several main protease structures were available in the Protein Data Bank. Of these, PDB ID 6Y2F was selected because it featured inhibitor 13b bound to the protein. Inhibitor 13b was previously proven to reduce SARS-CoV-2 RNA levels in infected human lung cells with an IC₅₀ of 0.67 ± 0.18 µm (Zhang et al., 2020).

3.1. Lipinski's RO5 selection result

First of all, 19 Sulawesi propolis compounds from North Luwu were selected based on Lipinski's RO5 in Table 2. According to the result, (-)-papuanic acid, isopapuanic acid, isocalopolyanic acid and curcumene have Log P of greater than 5.0. In the meantime, a compound, namely α -tocopherol succinate, violates two out of four rules, which are molecular weight and log P parameter. A molecular weight greater than 500 g/mol will lead to poor permeability when a drug molecule penetrates biological membrane through passive diffusion process (Qiu et al., 2016). Meanwhile, Log P represents octanol–water partition coefficient. A drug molecule with

Table 3
Docking score between main protease and test compounds.

No	Compounds	Docking score (kcal/mol)
1	* Inhibitor 13b	-8.2
2	14b	-7.2
3	Brousoflavonol F	-7.8
4	Glyasperin A	-7.8
5	Sulabiroins A	-7.6
6	Isorhamnetin	-7.5
7	Deoxypodophyllotoxin	-7.3
8	(2S)-5,7-Dihydroxy-4'-methoxy-8-prenylflavanone	-7.1
9	Sulabiroins B	-7.0
10	Isocalopolyanic acid	-6.8
11	Isopapuanic acid	-6.8
12	2',3'-Dihydro-3'-hydroxypapuanic acid	-6.7
13	(-)-Isocalolongic acid	-6.7
14	(-)-Papuanic acid	-6.6
15	Xanthoxyletin	-6.2
16	(1'S)-2-Trans-4-trans-absicic acid	-6.1
17	(1'S)-2-Cis-4-trans-absicic acid	-5.9
18	α -Tocopherol succinate	-5.1
19	P-Coumaric acid	-4.9
20	Curcumene	-4.7
21	Thymol	-4.7
22	Tetralin	-4.4

*) Native ligand as a control.

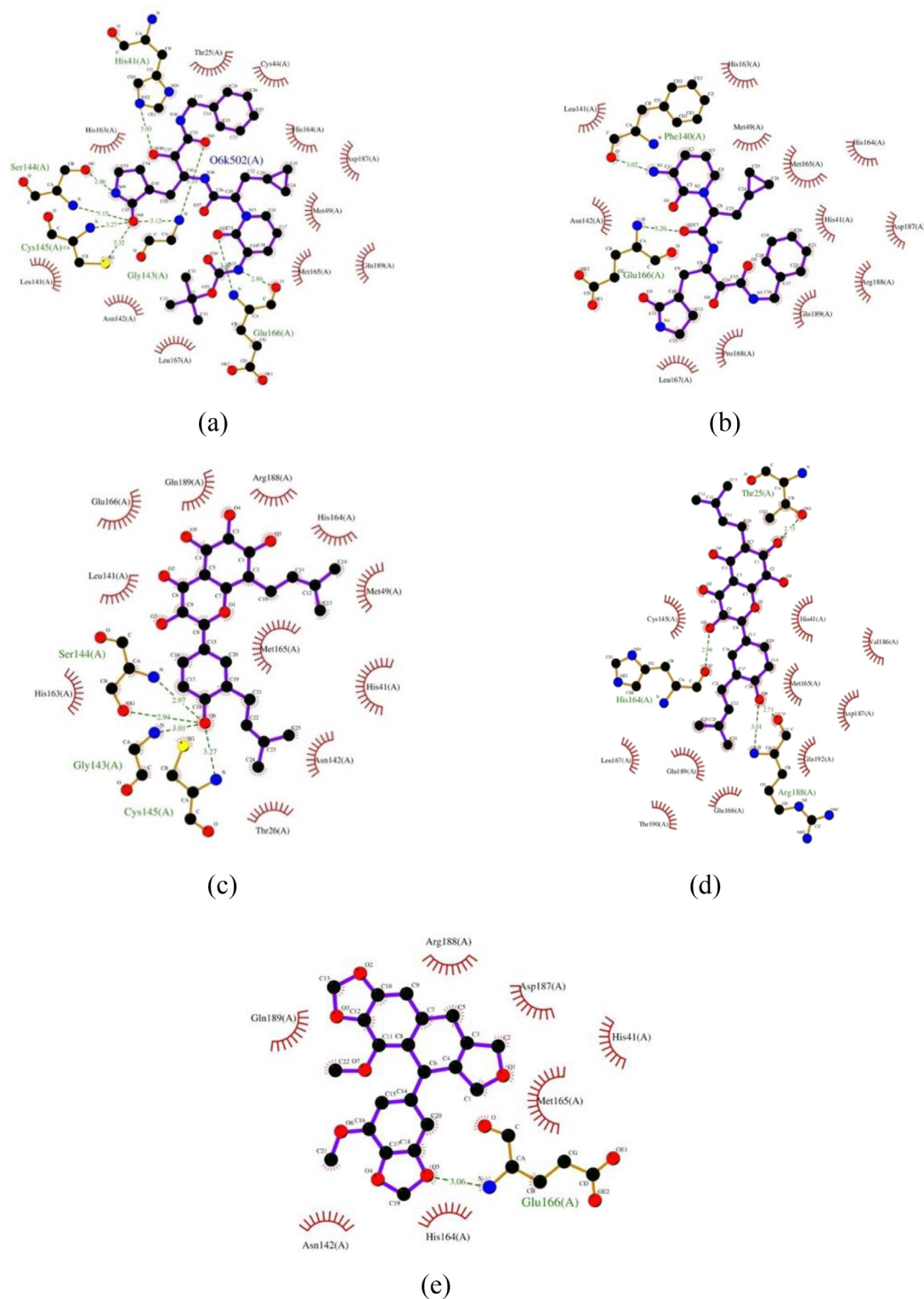


Fig. 2. Visualization of the molecular interactions of main protease with various ligands. (a) 13b, (b) 14b, (c) brousoflavonol F, (d) glyasperin A, (e) sulabiroids A. The purple lines denote the ligand structure, whereas the brown lines denote the structure of amino acid residues. The molecular interactions are reflected as dashed lines and arcs. The green dashed lines between atoms represent hydrogen bonds, and the numbers above these lines indicate the length of the bond. Meanwhile, the arcs with spokes radiating toward the ligand atoms represent hydrophobic interactions. The atoms involve in hydrophobic interactions are indicated by the presence of spokes radiating back (Wallace et al., 1995).

high Log P value tends to be more nonpolar and have poorer aqueous permeability (Templeton et al., 2015). Although there are several violations, the number of rules being obeyed is sufficient to

indicate that the compounds have good permeability. In addition, there are some tolerable Lipinski's RO5 parameter value for natural compounds.

3.2. Docking score

Selected Sulawesi propolis compounds, inhibitor 13b and 14b then were docked to main protease by using Autodock Vina. Docking simulation by using Autodock Vina produces two results, which are the most stable ligand poses and docking score. The docking score is generated from an empirical calculation that considers the number of hydrophobic interactions and hydrogen bonding (Trott and Olson, 2010). The greater the number of interactions, the greater the tendency for the docking score to be negative. The docking score may become a representative of binding affinity, and it is inversely proportional to the binding stability (Flamandita et al., 2020). The negative docking score indicates that the compound, which in this case acts as a ligand, has the ability to interact with the protein. Meanwhile, the most stable ligand pose will further be used to find out which amino acid residues in the protein interact with the ligand. Thereafter this is referred as interaction profile. By comparing interaction profile between SARS-CoV-2 main protease and propolis compounds with SARS-CoV-2 main protease and inhibitor 13b, the potency of propolis compounds to have the ability to inhibit the protein with the same pathway as inhibitor 13b may be evaluated.

The docking score between main protease and test compounds are available in Table 3. Based on the docking simulation, 13b and 14b bound to main protease with a binding affinity of -8.2 and -7.2 kcal/mol. Meanwhile, among the propolis compounds, the three lowest docking scores were obtained for broussonflavonol F, glyasperin A, and sulabiroids A, with values of -7.8 , -7.8 , and -7.6 kcal/mol, respectively. Broussonflavonol F and glyasperin A are identified as flavonoids, while sulabiroids A is a derivative of

podophyllotoxin compounds (Miyata et al., 2019, 2020a, 2020b). The interaction profiles of these three compounds, inhibitor 13b and 14b with main protease severally then were analyzed.

3.3. Interaction profile

The interaction profile analysis was focused on the existence of interaction with His⁴¹ and Cys¹⁴⁵. These amino acids have an important role as main protease catalytic sites. Thus, propolis compounds must interact with these sites in order to inhibit the activity of the enzyme. The two-dimensional (2D) visualization of the interaction of main protease with 13b, broussonflavonol F, glyasperin A, and sulabiroids A were presented in Fig. 2. The interaction profile between main protease and inhibitor 13b and 14b were firstly analyzed. According to the visualization, the docking simulation successfully captured the interaction of inhibitor 13b with both His⁴¹ and Cys¹⁴⁵, in line with the results reported by Zhang et al. (2020) as the crystallographer (Zhang et al., 2020). The shortest interatomic distance between main protease with His⁴¹ and Cys¹⁴⁵ are 3.03 and 3.27 Å respectively. In the meantime, 14b only interacts hydrophobically with His⁴¹. Based on this finding, although 14b may have a better *in vivo* potency due to lower affinity for plasma protein binding, it does not have favorable interaction towards the main protease.

The interaction profile between main protease and each propolis compounds were then analyzed. Based on the results, broussonflavonol F forms hydrogen bond with Cys¹⁴⁵ and interacts hydrophobically with His⁴¹. The interatomic distance between main protease and Cys¹⁴⁵ is 3.27 Å. The same value of interatomic

Table 4
Interaction profiles between main protease and test compounds

No.	Compounds	Hydrogen bonds	Hydrophobic interactions	Number of interactions	Binding similarity
1	* 13b	His ⁴¹ , Gly ¹⁴³ , Ser ¹⁴⁴ , Cys ¹⁴⁵ , Glu ¹⁶⁶	Thr ²⁵ , Cys ⁴⁴ , Met ⁴⁹ , Leu ¹⁴¹ , Asn ¹⁴² , His ¹⁶³ , His ¹⁶⁴ , Met ¹⁶⁵ , Leu ¹⁶⁷ , Asp ¹⁸⁷ , Gln ¹⁸⁹	16	100%
2	14b	Phe ¹⁴⁰ , Glu ¹⁶⁶	His ⁴¹ , Met ⁴⁹ , Leu ¹⁴¹ , Asn ¹⁴² , His ¹⁶³ , His ¹⁶⁴ , Met ¹⁶⁵ , Leu ¹⁶⁷ , Pro ¹⁶⁸ , Asp ¹⁸⁷ , Arg ¹⁸⁸ , Gln ¹⁸⁹	14	71%
3	Broussonflavonol F	Gly ¹⁴³ , Ser ¹⁴⁴ , Cys ¹⁴⁵	Thr ²⁵ , His ⁴¹ , Met ⁴⁹ , Leu ¹⁴¹ , Asn ¹⁴² , His ¹⁶³ , His ¹⁶⁴ , Met ¹⁶⁵ , Glu ¹⁶⁶ , Arg ¹⁸⁸ , Gln ¹⁸⁹	14	75%
4	Glyasperin A	Thr ²⁵ , His ¹⁶⁴ , Arg ¹⁸⁸	His ⁴¹ , Cys ¹⁴⁵ , Met ¹⁶⁵ , Glu ¹⁶⁶ , Val ¹⁸⁶ , Asp ¹⁸⁷ , Gln ¹⁸⁹ , Thr ¹⁹⁰ , Gln ¹⁹²	13	63%
5	Sulabiroids A	Glu ¹⁶⁶	His ⁴¹ , Asn ¹⁴² , His ¹⁶⁴ , Met ¹⁶⁵ , Asp ¹⁸⁷ , Arg ¹⁸⁸ , Gln ¹⁸⁹	8	44%

*) Native ligand as a control.

Table 5
Hydrogen bonds between main protease and test compounds

No.	Compounds	Hydrogen bond distance (Å)	Interacting amino acid	Binding ligand group	Binding amino acid group
1	* 13b	2.80	Glu ¹⁶⁶	-NH	-O
		2.86	Ser ¹⁴⁴	-NH	-OH
		3.03	His ⁴¹	-O	-NH
		3.12	Gly ¹⁴³	-O	-NH ₂
		3.15	Ser ¹⁴⁴	-O	-NH ₂
		3.20	Gly ¹⁴³	-O	-NH ₂
		3.27	Cys ¹⁴⁵	-O	-NH ₂
		3.30	Glu ¹⁶⁶	-O	-NH ₂
2	14b	3.32	Cys ¹⁴⁵	-O	-SH
		3.02	Phe ¹⁴⁰	-NH ₂	-O
		3.20	Glu ¹⁶⁶	-O	-NH ₂
		2.94	Ser ¹⁴⁴	-OH	-O
3	Broussonflavonol F	2.97	Ser ¹⁴⁴	-OH	-NH ₂
		3.01	Gly ¹⁴³	-OH	-NH ₂
		3.27	Cys ¹⁴⁵	-OH	-NH ₂
		2.73	Thr ²⁵	-OH	-OH
		2.73	Arg ¹⁸⁸	-OH	-OH
4	Glyasperin A	2.99	His ¹⁶⁴	-OH	-O
		3.01	Arg ¹⁸⁸	-OH	-NH ₂
		3.06	Glu ¹⁶⁶	-O	-NH ₂

*) Native ligand as a control.

distance compared to inhibitor 13b shows that brousoflavonol F may bind to Cys¹⁴⁵ as strong as inhibitor 13b. Meanwhile, glyasperin A does not form hydrogen bonds with any catalytic sites. It interacts hydrophobically with both catalytic sites instead. Hydrophobic interaction with the catalytic sites shows that although the binding affinity between the compound and the protein may not as strong as in hydrogen bonds, the compound may still have the potency to inhibit the activity of the enzyme. Lastly, for sulabiroids A, it is shown that this compound only interacts hydrophobically with one catalytic site, which is His⁴¹.

For further consideration, binding similarity between interaction profile of main protease with the three propolis compounds and 14b compared to interaction profile of main protease and inhibitor 13b were calculated. Based on the calculation, 14b, brousoflavonol F, glyasperin A and sulabiroids A bound to main protease with the similarity of 71%, 75%, 63% and 44% respectively. The summary of interaction profile between main protease and each test compounds are available in Table 4, while Table 5 summarizes the hydrogen bonds present between main protease and each test compounds.

4. Conclusion

Sulawesi propolis compounds from North Luwu that are produced by *Tetragonula sapiens* exhibit potential to inhibit SARS-CoV-2 main protease activity. Three compounds, namely brousoflavonol F, glyasperin A, and sulabiroids A, displayed the greatest docking score, with values of -7.8 , -7.8 , and -7.6 kcal/mol, respectively. The study results suggest that molecular dynamic simulation should be conducted for brousoflavonol F and glyasperin A given that both compounds interact with main protease catalytic sites and have the ability to bind with main protease with binding similarity of 75% and 63% respectively compared to potent inhibitor. Further research should be conducted to verify the potency and safety of brousoflavonol F and glyasperin A to the treatment of COVID-19.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Alday, E., Navarro-Navarro, M., Garibay-Escobar, A., Robles-Zepeda, R., Hernandez, J., Velazquez, C., 2016. Advances in pharmacological activities and chemical composition of propolis produced in Americas Beekeep. *Bee Conserv. Res.* Anand, K., Ziebuhr, J., Wadhwani, P., Mesters, J.R., Hilgenfeld, R., 2003. Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. *Science* (80-). 300, 1763–1767.

Berman, H., Kleywegt, G., Nakamura, H., Markley, J., 2013. How community has shaped the protein data bank. *Structure* 21 (9), 1485–1491. <https://doi.org/10.1016/j.str.2013.07.010>.

Calligari, P., Bobone, S., Ricci, G., Bocedi, A., 2020. Molecular investigation of SARS-CoV-2 proteins and their interactions with antiviral drugs. *Viruses* 12, 445.

Centers for Disease Control and Prevention, 2020. Information for clinicians on investigational therapeutics for patients with COVID-19. 2020-04-13. <<https://www.cdc.gov/coronavirus/2019-ncov/hcp/therapeutic-options.html>>.

ChemAxon. Marvin was used for drawing, displaying and characterizing chemical structures, substructures and reactions, Marvin 18.28, 2018, ChemAxon (<http://www.chemaxon.com>) (Version 18.28).

Chen, Y.W., Yiu, C.-P., Wong, K.-Y., 2020. Prediction of the SARS-CoV-2 (2019-nCoV) 3C-like protease (3CLpro) structure: virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates. *F1000Res* 9, 129. <https://doi.org/10.12688/f1000research.22457.1>.

Christina, D., Hermansyah, H., Wijanarko, A., Rohmatin, E., Sahlan, M., Pratami, D.K., Mun'im, A., 2018. Selection of propolis *Tetragonula* sp. extract solvent with flavonoids and polyphenols concentration and antioxidant activity parameters. In: AIP Conference Proceedings. <https://doi.org/10.1063/1.5023967>.

Flamandita, D., Lischer, K., Pratami, D.K., Aditama, R., Sahlan, M., 2020. Molecular docking analysis of podophyllotoxin derivatives in Sulawesi propolis as potent inhibitors of protein kinases. In: AIP Conference Proceedings. AIP Publishing LLC, p. 20010.

Forli, S., Huey, R., Pique, M.E., Sanner, M.F., Goodsell, D.S., Olson, A.J., 2016. Computational protein–ligand docking and virtual drug screening with the AutoDock suite. *Nat. Protoc.* 11 (5), 905–919. <https://doi.org/10.1038/nprot.2016.051>.

Gekker, G., Hu, S., Spivak, M., Lokensgard, J.R., Peterson, P.K., 2005. Anti-HIV-1 activity of propolis in CD4+ lymphocyte and microglial cell cultures. *J. Ethnopharmacol.* 102, 158–163.

Guedes, I.A., de Magalhães, C.S., Dardenne, L.E., 2014. Receptor–ligand molecular docking. *Biophys. Rev.* 6, 75–87.

Güler, H.I., Tatar, G., Yildiz, O., Belduz, A.O., Kolayli, S., 2020. An investigation of ethanolic propolis extracts: their potential inhibitor properties against ACE-II receptors for COVID-19 treatment by Molecular Docking Study. *Sci. Prepr.*

Huang, J., Song, W., Huang, H., Sun, Q., 2020. Pharmacological therapeutics targeting RNA-dependent RNA polymerase, proteinase and spike protein: from mechanistic studies to clinical trials for COVID-19. *J. Clin. Med.* 9, 1131.

Humphrey, W., Dalke, A., Schulten, K., 1996. VMD: visual molecular dynamics. *J. Mol. Graph.* 14, 33–38.

Kwon, M.J., Shin, H.M., Perumalsamy, H., Wang, X., Ahn, Y.-J., 2020. Antiviral effects and possible mechanisms of action of constituents from Brazilian propolis and related compounds. *J. Apic. Res.* 59, 413–425.

Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 23, 3–25.

Mahadewi, A.G., Christina, D., Hermansyah, H., Wijanarko, A., Farida, S., Adawiyah, R., Rohmatin, E., Sahlan, M., 2018. Selection of discrimination marker from various propolis for mapping and identify anti *Candida albicans* activity. In: AIP Conf. Proc. 1933. <https://doi.org/10.1063/1.5023939>.

Marcucci, M.C., 1995. Propolis: chemical composition, biological properties and therapeutic activity.

Meng, X.-Y., Zhang, H.-X., Mezei, M., Cui, M., 2011. Molecular docking: a powerful approach for structure-based drug discovery. *Curr. Comput. Aided Drug Des.* 7, 146–157.

Miyata, R., Sahlan, M., Ishikawa, Y., Hashimoto, H., Honda, S., Kumazawa, S., 2020a. Propolis components and biological activities from stingless bees collected on South Sulawesi, Indonesia. *HAYATI J. Biosci.* 27, 82.

Miyata, R., Sahlan, M., Ishikawa, Y., Hashimoto, H., Honda, S., Kumazawa, S., 2020b. Erratum: Propolis Components from Stingless Bees Collected on South Sulawesi, Indonesia, and Their Xanthine Oxidase Inhibitory Activity (Journal of Natural Products (2019) 82: 2 (205–210)). *J. Nat. Prod.*

Miyata, R., Sahlan, M., Ishikawa, Y., Hashimoto, H., Honda, S., Kumazawa, S., 2019. Propolis components from stingless bees collected on South Sulawesi, Indonesia, and their xanthine oxidase inhibitory activity. *J. Nat. Prod.* 82, 205–210. <https://doi.org/10.1021/acs.jnatprod.8b00541>.

Pastick, K.A., Okafor, E.C., Wang, F., Lofgren, S.M., Skipper, C.P., Nicol, M.R., Pullen, M. F., Rajasingham, R., McDonald, E.G., Lee, T.C., 2020. Hydroxychloroquine and chloroquine for treatment of SARS-CoV-2 (COVID-19). *Open Forum Infectious Diseases*. Oxford University Press, US, p. ofaa130.

Pinzi, L., Rastelli, G., 2019. Molecular docking: Shifting paradigms in drug discovery. *Int. J. Mol. Sci.* 20, 4331.

Qiu, Y., Chen, Y., Zhang, G.G.Z., Yu, L., Mantri, R.V., 2016. Developing Solid Oral Dosage Forms: Pharmaceutical Theory and Practice. Academic press.

Ramirez, D., Caballero, J., 2018. Is it reliable to take the molecular docking top scoring position as the best solution without considering available structural data? *Molecules* 23, 1038.

Sahlan, M., Devina, A., Pratami, D.K., Situmorang, H., Farida, S., Munim, A., Kusumoputro, B., Yohda, M., Faried, A., Gozan, M., 2019. Anti-inflammatory activity of *Tetragonula* species from Indonesia. *Saudi J. Biol. Sci.* 26, 1531–1538.

Sahlan, M., Mandala, D.K., Pratami, D.K., Adawiyah, R., Wijanarko, A., Lischer, K., Fauzi, A., 2020. Exploration of the antifungal potential of Indonesian Propolis from *Tetragonula* *biroi* bee on *Candida* sp. and *Cryptococcus neoformans*. *Evergr. J.* 7, 118–125.

Sayusti, T., Raffiudin, R., Kahono, S., Nagir, T., 2020. Stingless bees (Hymenoptera: Apidae) in South and West Sulawesi, Indonesia: morphology, nest structure, and molecular characteristics. *J. Apic. Res.* 1–14.

Templeton, A.C., Byrn, S.R., Haskell, R.J., Prinszano, T.E., 2015. Discovering and Developing Molecules with Optimal Drug-like Properties. Springer.

Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 31, 455–461.

- United States Food and Drug Administration. 2020. FDA cautions against use of hydroxychloroquine or chloroquine for COVID-19 outside of the hospital setting or a clinical trial due to risk of heart rhythm problems. 2020.
- Vardhan, S., Sahoo, S.K., 2020. Searching inhibitors for three important proteins of COVID-19 through molecular docking studies. *arXiv Prepr. arXiv2004.08095*.
- Vieira, T.F., Sousa, S.F., 2019. Comparing AutoDock and Vina in ligand/decoy discrimination for virtual screening. *Appl. Sci.* 9, 4538.
- Wallace, A.C., Laskowski, R.A., Thornton, J.M., 1995. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Eng. Des. Sel.* 8, 127–134.
- World Health Organization. 2020. Retrieved from <<https://www.covid19.who.int/>>.
- Yildirim, A., Duran, G.G., Duran, N., Jenedi, K., Bolgul, B.S., Miraloglu, M., Muz, M., 2016. Antiviral activity of hatay propolis against replication of herpes simplex virus type 1 and type 2. *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* 22, 422.
- Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauerhering, L., Becker, S., Rox, K., Hilgenfeld, R., 2020. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α -ketoamide inhibitors. *Science* (80-.). 368, 409–412.
- Zheng, J., 2020. SARS-CoV-2: an emerging coronavirus that causes a global threat. *Int. J. Biol. Sci.* 16 (10), 1678.