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Original Article

IDENTIFYING PROPOLIS COMPOUNDS POTENTIAL TO BE COVID-19 THERAPIES BY TARGETING SARS-COV-2 MAIN PROTEASE

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ABSTRACT

Objective: The study aims to perform molecular docking to examine the interaction between propolis compound and SARS-CoV-2 main protease.

Methods: The protein target of this research was the crystal structure of SARS-CoV-2 main protease in complex with an inhibitor N3 (PDB ID: 6LU7). The ligand of this research was the bioactive compounds from Propolis of *Tetragonula* aff. *biroi.*

Results: The results showed that propolis compound which has the potential to inhibit SARS-CoV-2 protease activity was Sulabiroins A (binding affinity-8.1 kcal/mol), following by (2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone acid and broussoflavonol F (binding affinity-7.9 kcal/mol) with binding similarity more than 50% compared to N3-main protease interaction.

Conclusion: Molecular docking showed propolis compounds of *Tetragonula* aff. *biroi* potential to inhibit SARS-CoV-2 main protease activity. The highest binding affinity presented by Sulabiroins A, following by (2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone acid and broussoflavonol F, with values of-8.1 kcal/mol,-7.9 kcal/mol, and-7.9 kcal/mol, respectively, with binding similarity more than 50% compared to N3 and SARS-CoV-2 main protease interaction.

Keywords: COVID-19, SARS-CoV-2 main protease, Propolis compounds, Molecular Docking, Binding affinity

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INTRODUCTION

In late December 2019, there was a severe respiratory disease outbreak in Wuhan, China. This disease then spread to other country, became a global issue and declared as a pandemic by World Health Organization (WHO). Based on WHO's data, almost 15.3 million cases had been reported as of 24th July 2020 around the world and the amount increases every day. This disease then namely coronavirus disease 2019 (COVID-19).

Coronavirus belong to *Coronaviridae* family and *Orthocoronavirinae* subfamily [1]. Coronavirus is an enveloped virus with positive single stranded RNA. In 2003, there was associated case, namely severe acute respiratory syndrome (SARS-2003), caused by another beta-coronavirus. Another case related coronavirus was Middle East Respiratory Syndrome (MERS) in 2012. Both SARS-2003 and MERS were caused by coronavirus, the difference was the objects which transmitted the virus. SARS was transmitted from exotic animals in wet markets to humans, whereas MERS is transmitted to humans from camels [2].

Epidemiological investigations then revealed that COVID-19 was caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2) [3]. The exact origin, location, and natural host of SARS-CoV-2 is not clear. COVID-19 causes nonspecific symptoms, including fever, dry cough, and fatigue. Many systems may be involved, such as respiratory system (sore throat, short of breath, cough, rhinorrhea, hemoptysis, and chest pain), gastrointestinal system (nausea, diarrhea, and vomiting), musculoskeletal system (muscle ache), and neurologic system (headache or confusion) [4].

Currently, there is no validated therapy and no vaccine available for COVID-19. Some treatments are underway, including antiviral drug development for Ebola and SARS as COVID-19 treatment, using COVID-19 patient's plasma as convalescent therapies. But further trial needed to evaluate the effectiveness of those therapies [5]. Experimental trial needed to find safe and effective therapy for COVID-19 patients.

According to several reports, propolis have several biological activities, including antioxidant, anti-inflammatory, antifungal, and antiviral [6–9]. Propolis is a natural resinous substance collected by honey bees from buds and exudates of plant species, mixed with bee enzymes, pollen and wax. The chemical composition of the propolis biologically active compounds depends on the geographical and botanical origin, the type of bees, and the seasons in which propolis is collected [10]. Several compounds within propolis have been identified, including flavonoids, terpenes, phenols and their esters, sugars, hydrocarbons and mineral elements [11].

Sabanovic *et al.*, (2019) revealed that propolis have antiviral activity [9]. It acts at different levels and blocks the replication of certain viruses such as herpes simplex type 1 and 2, adenovirus type 2, influenza virus, human immunodeficiency virus (HIV), and others. Furthermore, Yildirim *et al.*, (2016) reported that Hatay propolis may be a potential drug for the Herpes Simplex Virus infections treatment [11]. The combination between Hatay propolis and acyclovir (standard drug) produce a stronger antiviral effect against HSV-1 and HSV-2 than acyclovir alone. Research by Banskota *et al.* (2001) showed that propolis can exhibit antiviral activity by causing partial blocking of viral penetration into the cell, affecting the steps in the viral replication cycle, and leading to degradation of the RNA virus before penetration in a cell or after its release into the supernatant [12].

Since the target protein is known, computational study can be operated to determine SARS-CoV-2 potential drug [11, 14]. The study of Yu *et al.*, (2020) found that molecular docking can be used to identify SARS-CoV-2 potential drug that has antiviral activity [1, 3]. It revealed that the main flavonoid of honeysuckle, luteolin can bind to the active sites of the SARS-CoV-2 main protease tightly with lower binding energy than the ligand of the protein crystal structure. Furthermore, molecular docking can be conducted to analyze the propolis compound that has antiviral activity and potential to be SARS-CoV-2 drug. Molecular docking is a computational procedure that tries to predict a macromolecule

(receptor) and a small molecule (ligand) noncovalent binding, so bound conformations and the binding affinity can be determined [13].

This research aims to examine the potential propolis compounds of *Tetragonula* aff. *biroi* as COVID-19 therapy by molecular docking. The protein target of this research is the crystal structure of SARS-CoV-2 main protease in complex with an inhibitor N3 (PDB ID: 6LU7). There is already an inhibitor in its active site. The ligand of this research is the bioactive compounds from propolis of *Tetragonula* aff. *biroi.* Bioactive compounds of propolis believed can interact with the crystal structure of SARS-CoV-2 main protease. To determine it, molecular docking is used to identify which compounds are bound into the binding site. The potential propolis compounds show the lowest molecular docking score toward SARS-CoV-2 main protein.

MATERIALS AND METHODS

Hardware instrument

Specification of laptop in this research is Acer Aspire V5, AMD C-70 APU with Radeon™ HD Graphics 1.00 GHz processor, 2.00 GB of RAM. Windows 8 Pro 32-bit Operating System, x64-based processor.

Software instrument

The software used in this research are Visual Molecular Dynamic (VMD, University of Illinois, Urbana-Champaign), PyMOL, Autodock Tools 1.5.6 (The Scripps Research Institute, USA), Autodock Vina (The Scripps Research Institute), MarvinSketch (ChemAxon, Budapest, Hungary), and LigPlot+(EMBL-EBI, UK).

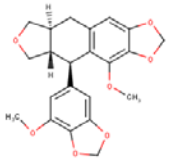
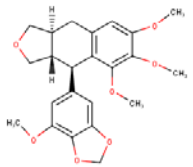
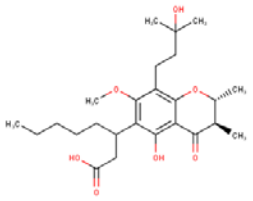
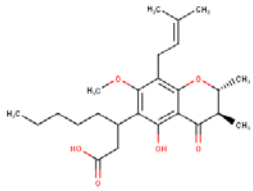
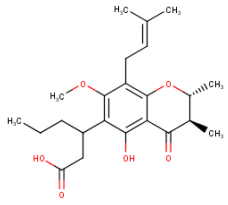
Propolis compounds selection

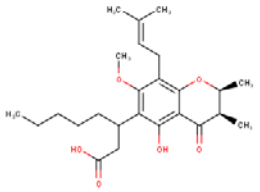
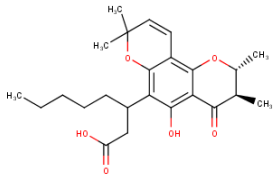
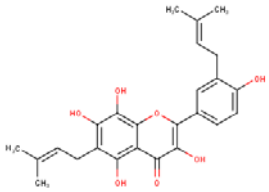
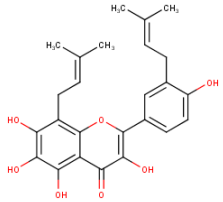
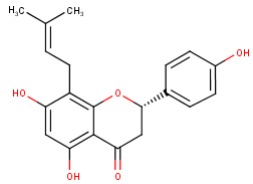
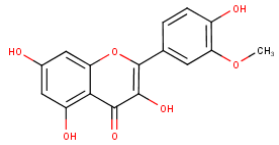
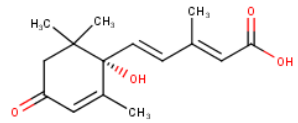
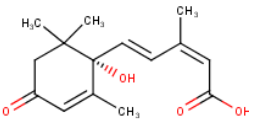
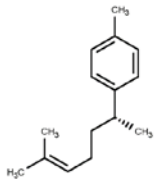
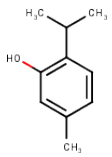
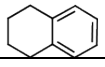
According to Miyata *et al.,* (2020), various compounds were identified from the propolis of stingless bees (*Tetragonula* aff. *biroi*) (table 1, Compound 1-13) [16, 17]. Meanwhile, Mahadewi *et al.* (2018) reported that Sulawesi propolis wax contained antifungal compounds (table 1, Compound 14-17) [18]. Furthermore, Sahlan *et al.* (2019) identified anti-inflammatory compounds from propolis of *Tetragonula sp.,* (table 1, Compound 18-20) [7].

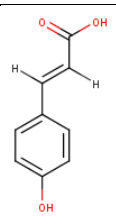
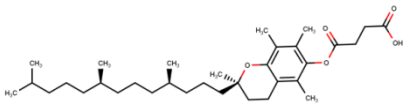
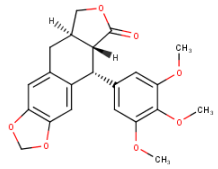
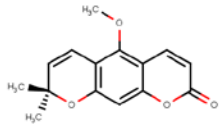
Protein structure preparation

The crystal structure of the examined protein was downloaded from RCSB Protein Data Bank (http://www.rcsb.org) in the PDB format. In this study, we obtain the crystal structure of SARS-CoV-2 main protease in complex with an inhibitor N3 (PDB ID: 6LU7). The protein and native ligand then separated using VMD (Hsin *et al.,* 2008), in this case, the native ligand is N3 [20]. The separated file was saved as*. pdb. These files are then loaded to Autodock Tools 1.5.6 to prepare the 3D structure of each protein and native ligand as*. pdbqt file format. The protein preparation, including the addition of polar hydrogens and charges.

Table 1: Propolis compounds

No	Compound	Molecular formula	Structure-2D
1	Sulabiroins A	$C_{22}H_{22}O_7$	
2	Sulabiroins B	$C_{23}H_{26}O_7$	
3	2',3'-Dihydro-3'-hydroxypapuanic acid	$C_{25}H_{38}O_7$	
4	(-)-Papuanic acid	$C_{25}H_{36}O_6$	
5	(–)-Isocalolongic Acid	$C_{24}H_{34}O_6$	

6	Isopapuanic acid	$C_{25}H_{36}O_6$	
7	Isocalopolyanic acid	$C_{24}H_{32}O_6$	
8	Glyasperin A	$C_{25}H_{26}O_7$	
9	Broussoflavonol F	$C_{25}H_{26}O_7$	
10	(2S)-5,7-Dihydroxy-4'-methoxy-8-prenylflavanone	$C_{20}H_{20}O_5$	
11	Isorhamnetin	$C_{16}H_{12}O_7$	
12	(1'S)-2-Trans,4-trans-abscisic acid	$C_{15}H_{20}O_4$	
13	(1'S)-2-Cis,4-trans-abscisic acid	$C_{15}H_{20}O_4$	
14	Curcumene	$C_{15}H_{22}$	
15	Thymol	$C_{10}H_{14}O$	
16	Tetralin	$C_{10}H_{12}$	

17	P-Coumaric acid	$C_9H_{10}O_3$	
18	α-Tocopherol succinate	$C_{32}H_{52}O_5$	
19	Deoksi podophyllotoxin	$C_{22}H_{22}O_7$	
20	Xanthoxyletin	$C_{15}H_{14}O_4$	

Ligand structure preparation

In total 21 ligands used in this research, one native ligand as a control (fig. 1) and 20 propolis compounds (table 1).

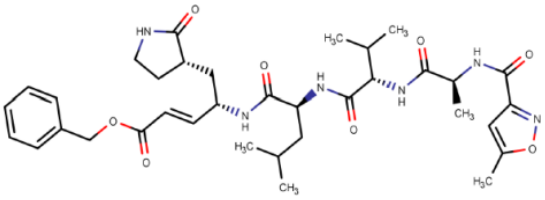


Fig. 1: Structure of native ligand N3 (http://www.rcsb.org)

The 2D structure of propolis compounds prepared by using Marvin Sketch (Csizmadia, 1999) and converted into 3D structure [21]. All files then saved as*. pdb format and loaded to Autodock Tools 1.5.6 to prepare*. pdbqt file by adding polar hydrogens and charges.

Re-docking

Docking propolis compounds, as a ligand, to the main protease required specific search space. This is the important step to determine the binding site of the ligand within the protein area, hence re-docking have to be done. This step involves separating native ligand and protein then dock it back to its place on protein by setting the size and the center of the search space box [22]. Re-docking and docking simulation performed by AutoDock Vina, automated docking tools with easy operational use, high processing speed and binding mode prediction accuration [15]. Autodock Vina was chosen because of its accuracy is higher than Autodock Tools in term of binding mode predictions [23].

Determining the specific search space in protein involves three criterions; number of points in x, y, z dimension (grid box size), grid box center and grid spacing. Re-docking simulation obtain some poses, the first generally poses the best pose with the lowest docking score. Then Root Mean Square Deviation (RSMD) between native ligand and re-docking result first pose was evaluated using PyMol, Python-based software that can be used for protein-ligand modelling [24]. The result is classified as the accurate one if the RSMD value is less than 2 Å [25]. The re-docking coordinate then used to dock propolis compounds into the protein target.

Docking and analysis

Propolis compounds were docked to each receptor using parameters and coordinates that had been obtained from re-docking simulation. The lowest docking score result was considered as the best pose which used to analyze the interaction between propolis compounds, as a ligand, and protein. The interaction profiles then represented in 2D visualization using LigPlot+. LigPlot+is an automatic program that generates multiple two-dimensional (2D) diagrams of ligand–protein interactions from docked complexes. It is utilized to identify the interacting residues, hydrogen bonds interaction, and hydrophobic contacts of ligands and the main-chain or side-chain of the protein [26].

RESULTS AND DISCUSSION

Selection of propolis compound

This study focused on identifying propolis compounds of *Tetragonula* aff. *biroi that* targeted the main protein (M^{pro}) of SARS-CoV-2. M^{pro} is a 33.8-kDa protease, it is a coronaviruses key enzyme and has a pivotal role in mediating viral replication and transcription functions through extensive proteolytic processing of two replicased polyproteins, pp1a (486 kDa) and pp1ab (790 kDa) (Yang *et al.,* (2003). Its important role makes it an interesting target for developing SARS-CoV-2 potential drugs. From several main protease structures which available at Protein Data Bank, PDB ID 6LU7 was chosen in this research because it has native ligand N3 that bind to the substrate-binding pocket of SARS-CoV-2 main protease. Jin *et al.,* (2020) found that N3 showed inhibition activity against SARS-CoV-2 with individual half-maximal effective concentration (EC_{50}) values of 16.77 μM.

The selection of 20 propolis compounds in table 1 based on previous researches that have been done.

Table 2: Parameter optimization by re-docking simulation

Coordinates	Grid box size (Å)	Grid box center	Grid spacing (Å)
x	22	-13.939	1
y	24	12.912	

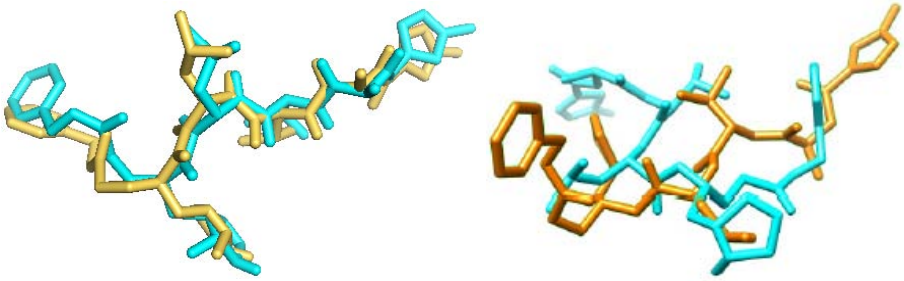
z	26	69.026

Binding site determination

Binding site then became an area where the propolis compounds bind to the protein. Native ligand re-docking obtained mode 1 as the best pose, which had a docking score-8.4 kcal/mol. The grid box was determined on the active site area, considering the presents of amino acids Thr190, Glu166, Gln189, Gln192, Gly143, His163, His172, His164, Cys145, Phe140, Phe185, accordance to previous studies with the crystallographic structure of the SARS-CoV-2 main protease [27, 28]. Re-docking evaluation performed by calculating Root-Mean Square Deviation (RMSD) value using Pymol and the result was 1.468 Angstroms. Meanwhile, Andrade *et al.* (2020) reported that the best confirmation of N3 presented RMSD of 1.94 Angstroms (fig. 2b) [29]. Fig. 2 shows the comparison position between crystallized N3 and re-docking confirmation result.

First, re-docking done by AutoDock Vina where the native ligand was docked back to protein in specific area so the binding site can be determined. Re-docking simulation found that the binding site apparently located at the following coordinate (table 2).

RMSD was used to validate the docking procedure. It represents the difference between the acquired docking pose and the crystallized ligand orientation of the same ligand molecule. The criterion of the correct orientation often uses RMSD 2Å [25]. The lower RMSD value, the docking pose getting more similar to crystallized ligand orientation. It means the docking procedure is validated and its accuracy getting higher.



(a) **(b)**

Fig. 2: Comparison position between crystallized N3 (yellow) and re-docked N3 (cyan), (a) research result position, (b) Andrade *et al.*, (2020) report

Docking procedure results of propolis compounds

Molecular docking performed by AutoDock Vina obtain 9 ligand poses with different docking score. AutoDock Vina determines the docking score, also known as binding affinity, automatically [15]. The lowest docking score represented the strongest binding affinity. Docking score results of propolis compounds towards SARS CoV-2 main protease in complex with an inhibitor N3 showed by table 3. The results present that all compounds bind to amino acid at binding site. The propolis compound which has the lowest docking score was Sulabiroins A, following by (2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone and broussoflavonol F, with values of-8.1 kcal/mol,-7.9 kcal/mol, and-7.9 kcal/mol, in succession.

Propolis and SARS-CoV-2 main protease interaction

The interaction between propolis compounds and main protease then analyzed using LigPlot+. The visualization results of three propolis compounds with the lowest docking score are presented in fig. 3. There are two kinds of molecular interactions in the visualization result; dashed lines and arcs. The green dashed lines illustrate hydrogen bonds, the length of the bond showed by the numbers above these lines. Meanwhile, the arcs with spokes radiating toward the ligand atoms mean hydrophobic interactions. Furthermore, ligand structure is represented by purple lines and amino acid residues showed by the brown line.

Table 3: Docking result of propolis compounds towards SARS CoV-2 main protease

No	Propolis compounds	Docking score
1	N3 (native ligand)	-8.4
2	Sulabiroins A	-8.1
3	Broussoflavonol F	-7.9
4	(2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone	-7.9
5	Sulabiroins B	-7.8
6	Glyasperin A	-7.8
7	Deoksi podophyllotoxin	-7.4
8	Papuanic acid	-7.4
9	Isorhamnetin	-7.3
10	Isocalolongic acid	-7.2
11	2',3'-dihydro-3'-hydroxypapuanic acid	-7.1
12	Isopapuanic acid	-7.0
13	Xanthoxyletin	-6.7
14	(1'S)-2-trans,4-trans-abscisic acid	-6.5
15	Isocalopolyanic acid	-6.4
16	α-Tocopherol succinate	-6.2
17	(1'S)-2-cis,4-trans-abscisic acid	-6.0
18	Curcumene	-5.5
19	P-coumaric acid	-5.1

20	Tetralin	-4.7
21	Thymol	-4.7

Molecular interaction between amino acid residues of main protease with various ligand represents in table 4. Native ligand N3 formed hydrogen bond with Thr190, Gln189, His41, Ser144, His163, Glu166 and Phe140. Yu *et al.,* (2020) reported that N3 formed a hydrogen bond with Cys145 and Gln189 [30]. N3 also formed hydrophobic interaction with Thr25, Leu27, Leu4, Val3, and Asn142.

The interaction between the three best propolis compounds with SARS-CoV-2 main protease indicated that each compound has a bound to binding site in the main protease. It is enhanced by the binding similarity result that represents each propolis compound has a similarity above 50% compared to N3 and SARS-CoV-2 main protease interaction. Compared with N3–main protease interaction, the interaction of Sulabiroins A, Broussoflavonol F, and (2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone with main protease represented the binding similarity of 64%, 59%, and 59%, respectively.

Sulabiroins A did not form hydrogen bond with any residues. It formed hydrophobic interaction with 14 amino acids in the active site region of SARS-CoV-2 main protease instead. Broussoflavonol F represented hydrogen bond with the residues Cys145, Gly143, Gln189, His163, Leu141 and Ser144. Furthermore, (2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone displayed five hydrogen interactions with Ser144, Leu141, Gly143, Glu166 and Asp187. It showed interaction with Leu27, which did not interact with other propolis compounds, including N3.

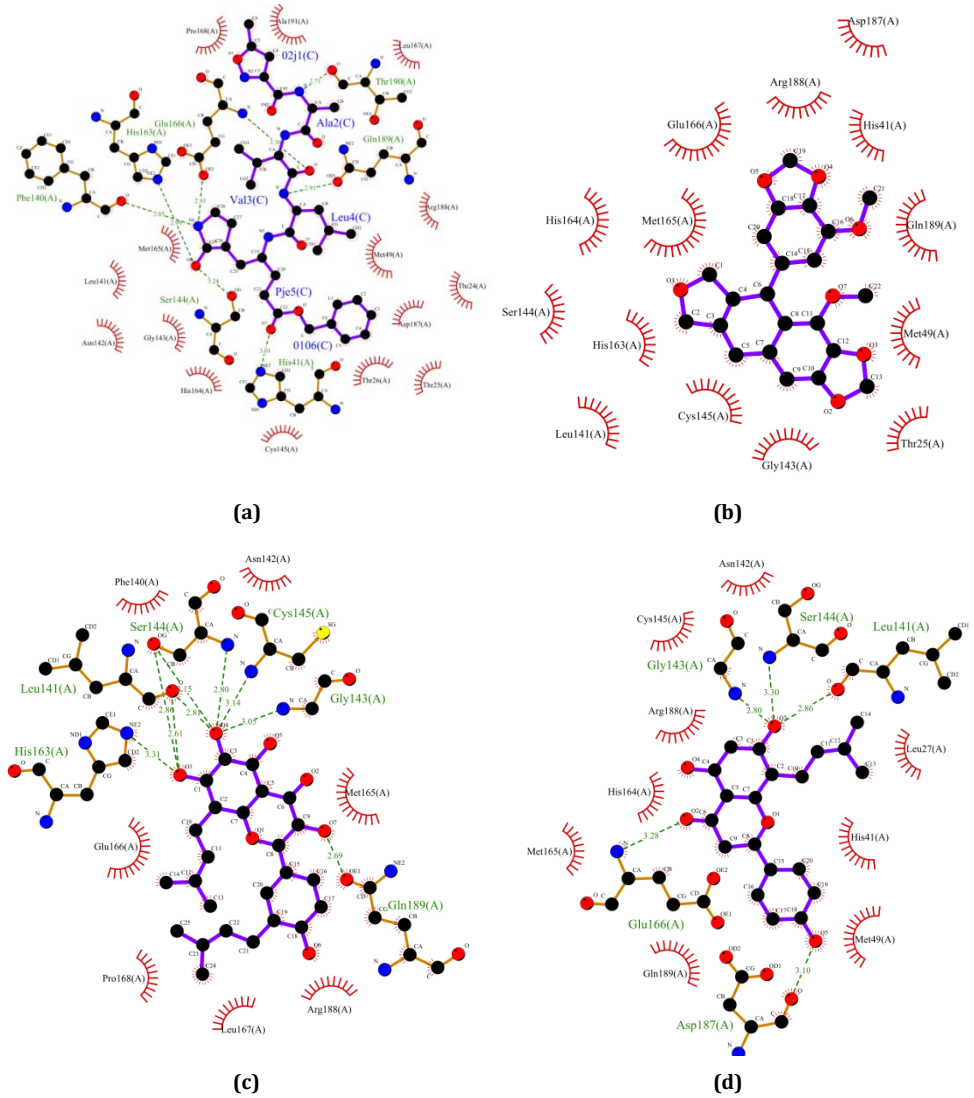


Fig. 3: Interaction visualization of main protease with various ligands. (a) N3; (b) Sulabiroins A; (c) Broussoflavonol F; (d) (2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone

Table 4: Molecular interaction of various ligands and main protease

Ligand	Binding analysis			Interactions with other residues
	Hydrogen bonding	Hydrophobic interactions	Binding similarity*	
N3 (native ligand)	Thr190, Gln189, His41,	Pro168, Ala191, Leu167, Arg188, Met49,	100%	-

	Ser144, His163, Glu166, Phe140	Thr24, Asp187, Thr25, Thr26, Cys145, His164, Gly143, Asn142, Leu141, Met165		
Sulabiroins A	-	Asp187, His41, Gln189, Met49, Thr25, Gly143, Cys145, Leu141, His163, Ser144, His164, Met165, Glu166, Arg188	64%	-
Broussoflavonol F	Cys145, Gly143, Gln189, His163, Leu141, Ser144	Asn142, Met165, Arg188, Leu167, Pro168, Glu166, Phe140	59%	-
(2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone	Ser144, Leu141, Gly143, Glu166, Asp187	Asn142, His41, Met49, Gln189, Met165, His164, Arg188, Cys145	59%	Leu27

*Binding similarity between propolis compounds–protease interaction and inhibitor N3–protease interaction

Jin *et al.*, (2020) reported that SARS-CoV-2 M^{pro} has a Cys-His catalytic dyad, and the substrate-binding site is located in a cleft between domain I (residues 8–101) and domain II (residues 102–184) [27]. This is supported by Hsu *et al.*, (2005) that the active site of SARS-CoV-2 main protease SARS-CoV consisting of His41 and Cys145 [31]. Sulabiroins A and (2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone have hydrophobic interaction His41 and Cys145. Meanwhile, Broussoflavonol F only has one hydrogen bonding with Cys145. Since His41 and Cys145 were known as catalytic site, so these three propolis compounds potential to inhibit main protease activity.

Further studies are needed to determine the efficacy and safety of these propolis compounds for COVID-19 therapy. There are numerous drugs and ongoing medication strategies under clinical trials related with COVID-19 with a hope for avoiding possible threatening of the lives of millions of human beings and providing directions for future research.

CONCLUSION

Molecular docking showed propolis compounds of *Tetragonula* aff. *biroi* potential to inhibit SARS-CoV-2 main protease activity. The highest binding affinity presented by Sulabiroins A, following by (2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone and broussoflavonol F, with values of-8.1 kcal/mol,-7.9 kcal/mol, and-7.9 kcal/mol, respectively, with binding similarity more than 50% compared to N3 and SARS-CoV-2 main protease interaction. These three compounds could be a promising drug candidate for COVID-19 as they interacted with His41 and Cys145 in the active site of SARS-CoV-2 main protease. Further studies are needed to determine the efficacy and safety of these propolis compounds for COVID-19 therapy [32].

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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