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Research Article

Activity Prediction of Bioactive Compounds Contained in *Etlingera elatior* Against the SARS-CoV-2 Main Protease: An *In Silico* Approach

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Abstract

The COVID-19 pandemic has become a serious problem today, with its prevalence increasing every day. The SARS-CoV-2 main protease (M^{Pro}) is a promising therapeutic target to inhibit replicating and spreading the virus that causes COVID-19. The compounds contained in the *Etlingera elatior* plant has the potential. This study aimed to examine the compounds' activity in *E. elatior* against SARS-CoV-2 M^{Pro} using *in silico* methods. A total of seven compounds contained in *E. elatior* were obtained from the Knapsack database. The compounds were then docked into the SARS-CoV-2 M^{Pro} receptor's active site with the PDB ID 6LU7. Afterward, the biological activities were predicted by the PASS prediction webserver. The molecular docking results showed that ergosterol peroxide and sitostenone had the best binding energy with -10.40 kcal/mol and -9.17 kcal/mol, respectively. The *in silico* PASS prediction showed it has potential as antiviral therapy. It concluded ergosterol peroxide and sitostenone has the potential as SARS-CoV-2 M^{Pro} inhibitor candidate.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a disease caused by infection with the SARS-CoV-2 virus, which in January 2020 began to spread from Wuhan around the world (Hui *et al.*, 2020). Globally, on August 30th 2020, there have been 24,854,140 confirmed cases of COVID-19, including 838,924 deaths (World Health Organization, 2020). One of the targets that have become a focus of research in the world for COVID-19 therapy was SARS-CoV-2 main protease (M^{Pro}) (Dai *et al.*, 2020; Jin *et al.*, 2020; Wu *et al.*, 2020; Zhu *et al.*, 2011). The main protease is a key enzyme in the viral replication cycle, which proteolytically cleaves overlapping polyproteins pp1a and pp1ab into functional proteins, an essential step during viral replication (Du *et al.*, 2004). Inhibition of these target proteins can result in disruption of the SARS-CoV-2 replication cycle (Mahmud *et al.*, 2020; Pratama *et al.*, 2020). The SARS-CoV-2 M^{Pro} in COVID-19 is not the same as the main protease in humans, so it becomes a promising therapeutic target (Ullrich & Nitsche, 2020).

Many compounds derived from medicinal plants have not been discovered and have great potential as therapeutic candidates (Mushtaq *et al.*, 2018; Pan *et al.*, 2013). In the discovery of COVID-19 therapy candidates, many natural products were used; some of them examined compounds from herbs against the SARS-CoV-2 using in silico method (Aanouz *et al.*, 2020; Enmozhi *et al.*, 2020; Joshi *et al.*, 2020; Prasanth *et al.*, 2020; Sukardiman *et al.*, 2020). However, the discovery of

compounds from medicinal plants with potential activity as SARS-CoV-2 M^{Pro} inhibitors is still being carried out today.

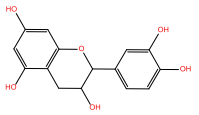
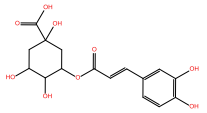
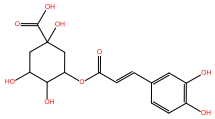
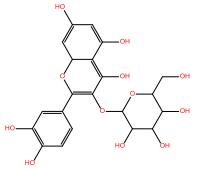
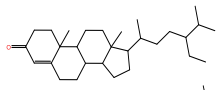
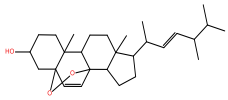
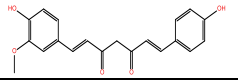
Etlingera elatior (known as Wualae in Tolakinese) is a medicinal plant from Indonesia which are found mainly on the island of Sulawesi, particularly Southeast Sulawesi (Fristiohady *et al*., 2020; Fristiohady *et al*., 2019). *Etlingera elatior* contains various types of compounds, including flavonoids and steroids with various pharmacological activities, one of them as antimicrobial (Sahidin *et al*., 2019; Wahyuni *et al*., 2018). However, there has been no specific development of its antiviral activity until now, especially to the SARS virus family. Flavonoid and steroid group compounds as found in *E. elatior* have been widely researched on their activity to the SARS-CoV-2 and have shown promising results as a drug candidate for COVID-19 (Islam *et al*., 2020; Suwannarach *et al*., 2020). Therefore, this study aims to identify compounds from the flavonoid and steroid group contained in *E. elatior*, which have potential as SARS-CoV-2 M^{Pro} inhibitors.

MATERIALS AND METHODS

Ligand preparation

The ligands used in this study were compounds contained in *E. elatior*. A total of seven compounds contained in *E. elatior* were selected in this study based on the KNApSAcK database (http://www.knapsackfamily.com/KNApSAcK/) (Afendi *et al*., 2012). The compound identity, as well as their two-dimensional structure, can be seen in **Table I**. The three-dimensional ligand conformation was obtained from the KNApSAcK 3D database (http://knapsack3d.sakura.ne.jp/) (Nakamura *et al*., 2013). The compounds saved in .mol format, then converted into .pdb format using the OpenBabel 2.4.1 GUI software.

Table I. Identity of the compounds contained in *E. elatior* based on the KNApSAcK database

No	C_ID KNApSAcK	Compounds	2D structure
1	C00000947	Catechin	
2	C00002724	Chlorogenic acid	
3	C00005141	Kaempferol 3-glucuronide	
4	C00005373	Isoquercetin	
5	C00029821	Sitostenone	
6	C00030200	Ergosterol peroxide	
7	C00037023	Demethoxy curcumin	

Receptor preparation

The three-dimensional structure of the SARS-CoV-2 M^{Pro} was obtained from the Protein Data Bank (PDB) website http://www.rcsb.org/pdb/. The receptor with the PDB ID 6LU7 and resolution 2.16 Å was chosen. Furthermore, the unique ligands and water molecules were removed from the receptor. Then, the receptor was added with polar hydrogen and given a charge (Kollman charge). All preparation procedures were performed using AutoDock 4 software (Morris *et al*., 2009).

Validation method

The native ligand from the 6LU7 receptor (N-[(5-methylisoxazol-3-yl)carbonyl]alanyl-l-valyl-N~1~-((1R,2Z)-4-(benzyloxy)-4-oxo-1-{[(3R)-2-oxopyrrolidin-3-yl]methyl}but-2-enyl)-l-leucinamide; an inhibitor N3) (Jin *et al*., 2020) was separated from the protein, then re-docked using AutoDock 4 software into the previous active site. The native ligand conformation from the

docking procedure was taken and overlayed with the native ligand conformation before docking. Furthermore, validation is done by looking at root-mean-square deviation (RMSD) parameters, calculated using PyMOL software. An RMSD value of fewer than 2.0 Å indicates that the method is valid and can be used for the docking process (Bell & Zhang, 2019).

Molecular docking simulation

The simulation was carried out using previously valid parameters. The three-dimensional structure of the compounds in *E. elatior* was docked to the SARS-CoV-2 M^{Pro} receptor's active site, which is then analyzed for its binding energy and amino acid interactions. The active site coordinates were x=-9.732, y=11.403, and z=68.925, with the dimensions of the grid box, were 64, 60, and 60 Å, respectively. The docking process in AutoDock 4 has been performed with 100 number of GA runs for each ligand.

Prediction of Activity Spectra for Substances

The *in silico* Prediction of Activity Spectra for Substances (PASS) were carried out to get biological activity spectra of compounds accessed through PASS web server (http://www.way2drug.com/PASSOnline/predict.php) (Marwaha *et al*., 2007). This server estimates the predicted activity spectrum of a compound as probable activity (Pa) and probable inactivity (Pi). Prediction of this spectrum by PASS is based on structure-activity relationship analysis of the training set containing more than 205,000 compounds exhibiting more than 3,750 kinds of biological activities. For probabilities range, the Pa and Pi values vary from 0.000 to 1.000. The PASS prediction results were interpreted and used flexibly:

1. Only activities with Pa > Pi are considered as possible for a particular compound.
2. If Pa >0.7, the chance to find activity is experimentally high.
3. If Pa is >0.5 but less than <0.7, the chance to find activity is experimentally low, but the compound is probably different from known pharmaceutical agents.
4. If Pa <0.5, the chance to find activity is experimentally is low, but the chance to find structurally NCE's is high.

RESULTS AND DISCUSSION

Validation method

Validation was carried out to see the strength of affinity prediction through re-docking the native ligand into its binding site. The validation results can be seen in **Figure 1**. It showed that the RMSD value of the native ligand was 1.3 Å, with the binding energy obtained was -7.30 kcal/mol. This shows that the molecular docking method used is valid because of the RMSD value below 2.0 Å.

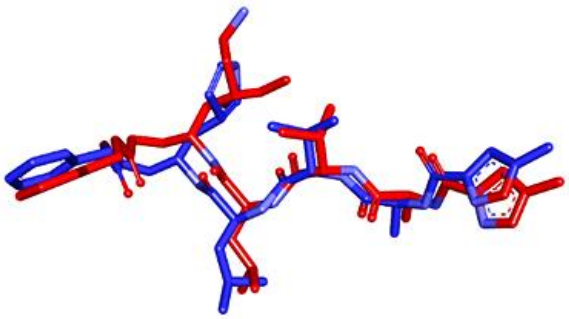


Figure 1. Validation of the SARS-CoV-2 M^{Pro} receptor PDB ID 6LU7, the native ligand position before docked (red) and after docked (blue) overlayed with RMSD 1.3 Å

Molecular docking simulation

The bioactive compounds in *E. elatior* after docked showed varied binding energies as shown in **Table II**. In general, *E. elatior* compounds from the database were dominated by polyphenols such as flavonoids and phenylpropanoid, then the steroid compounds. The flavonoid compounds such as quercetin, curcumin, etc., have been previously studied *in silico* against the SARS-CoV-2 M^{Pro} receptor, as Cherrak *et al*. (2020) reported.

Meanwhile, the type of steroid has been previously studied by Ghosh *et al*. (2020). As shown by the docking results in **Table II**, the steroid group showed better inhibitory activity than the flavonoid groups to SARS-CoV-2 M^{Pro} receptor. The docking results show that the steroids' binding energy, namely ergosterol peroxide and sitostenone, was -10.4 and -9.17 kcal/mol, respectively. Compared to the flavonoid group, the lowest binding energy was shown by catechins (-8.64 kcal/mol), while the binding energy of the other compounds was in the range of -6.53 to -7.9 kcal/mol.

Table II. The binding energy of the compounds in *E. elatior* to the SARS-CoV-2 M^{Pro} receptor

Compounds	Compound group	Binding energy (kcal/mol)	Ki (nM)
Catechins	Flavonoid	−8.64	467,520
Chlorogenic acid	Phenyl-propanoid	−6.53	16,380
Kaempferol 3-glucuronide	Flavonoid	−7.08	6,450
Isoquercetin	Flavonoid	−7.09	6,310
Sitostenone	Steroid	−9.17	188.59
Ergosterol peroxide	Steroid	−10.4	23.62
Demethoxycurcumin	Polifenol	−7.9	1,630

Molecular interactions

The top two compounds that ranked by their binding energy, ergosterol peroxide and sitostenone, were analyzed for their amino acid interactions with the SARS-CoV-2 M^{Pro} receptor and compared with the interactions of the native co-crystal ligand (N3 inhibitor). The interactions' tabulation data can be seen in **Table III**, while the two-dimensional interaction can be seen in **Figure 2**. The native ligand forms hydrogen bonds with amino acids Gly-143; Cys-145; Glu-166; Gln-189; and Thr-190. The native ligand also forms several types of hydrophobic interactions with residues His-41; Met-49; Met-165; Leu-167; Pro-168; and Ala-191, while the rest were Van der Waals interactions with ten residues. The steroid sitostenone forms a hydrogen bond with the amino acids Gln-192. Ergosterol peroxide forms hydrogen bonds with Thr-26 and Gly-143. Sitostenone forms hydrophobic interactions with six amino acid residues Leu-27; His-41; Met-49; Cys-145; His-163; and Met-165, as well as Van der Waals interactions with 13 other amino acid residues. Ergosterol peroxide compounds form hydrophobic interactions with five amino acid residues, specifically His-41; Met-49; Cys-145; Met-165; and Arg-188, and form Van der Waals interactions with ten other amino acid residues. Overall, the interaction on sitostenone is more similar to the interaction of native ligands with a similarity level of 82.6% compared to ergosterol peroxide with 56.52%.

The interaction pattern shows that the hydrophobic group on the ligands influences the SARS-CoV-2 M^{Pro} receptor's affinity. This is what causes the steroid class compounds to give good affinity for this molecular docking simulation. This is also related to the native ligand structure, an inhibitor of SARS-CoV-2 M^{Pro}, which is composed of several non-polar peptide groups. In terms of three-dimensional interactions (**Figure 3**), ergosterol peroxide does not occupy the protein's surface as large as the native ligand. However, the functional groups on its compounds bind to a larger hydrophobic region in the protein surface (brown area), and one oxygen group can interact in the polar region (blue area). Simultaneously, sitostenone compounds form a similar orientation to the native ligand in occupying the surface of the receptor's binding pocket.

Table III. Amino acid interactions of native and test ligands

Interaction residues		
N3 inhibitor (native ligand)	Sitostenone	Ergosterol peroxide
Thr-24	-	-
Thr-25	-	Thr-25
Thr-26	Thr-26	Thr-26
-	Leu-27	Leu-27
His-41	His-41	His-41
Met-49	Met-49	Met-49
-	-	Pro-52
-	-	Tyr-54
Phe-140	-	-
Leu-141	Leu-141	-
Asn-142	Asn-142	Asn-142
Gly-143	Gly-143	Gly-143
Ser-144	Ser-144	-
Cys-145	Cys-145	Cys-145
His-163	His-163	-

His-164	His-164	His-164
Met-165	Met-165	Met-165
Glu-166	Glu-166	Glu-166
Leu-167	Leu-167	-
Pro-168	Pro-168	-
Asp-187	-	Asp-187
Arg-188	Arg-188	Arg-188
Gln-189	Gln-189	Gln-189
Thr-190	Thr-190	-
Ala-191	Ala-191	-
Gln-192	Gln-192	-
The similarity of interaction with native ligand (%)	82.6	56.52

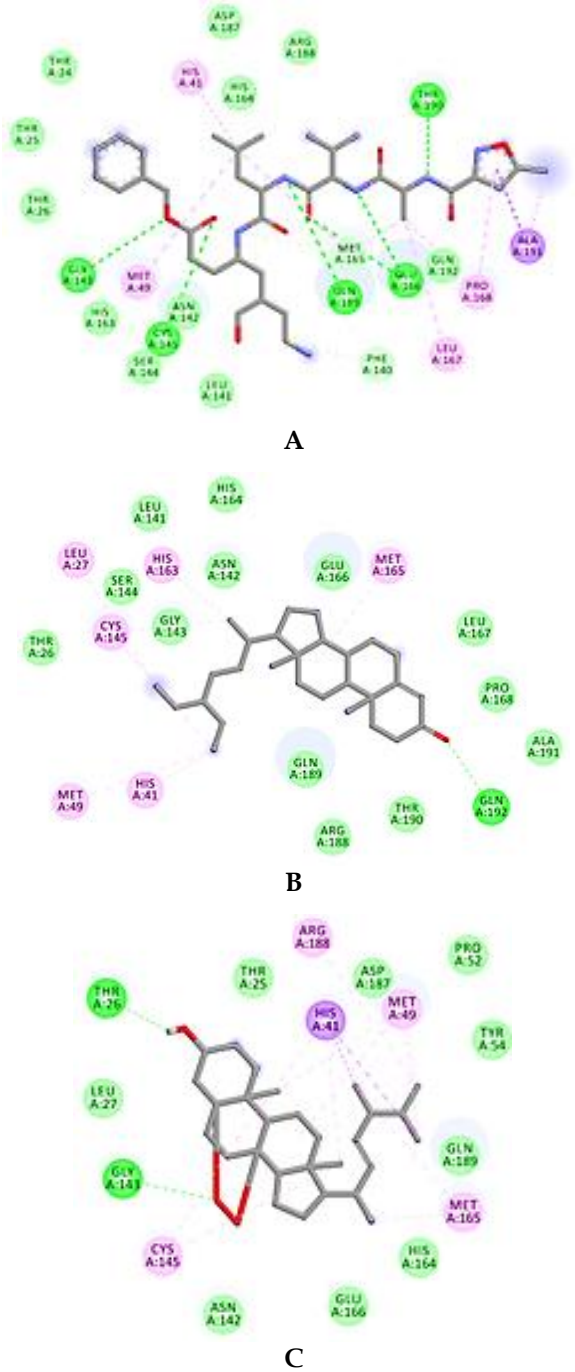


Figure 2. Two-dimensional interactions of native ligand (**A**), sitostenone (**B**), and ergosterol peroxide (**C**) to SARS-CoV-2 M^{Pro} receptor

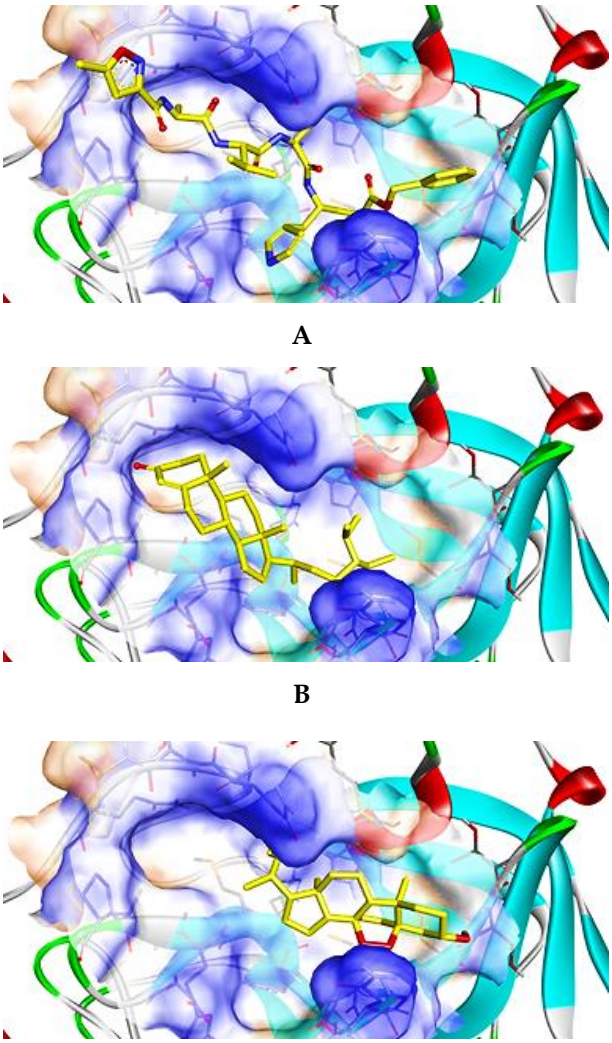


Figure 3. Three-dimensional interactions of native ligand (**A**), sitostenone (**B**), and ergosterol peroxide (**C**) to SARS-CoV-2 M^{Pro} receptor

Prediction of Activity Spectra for Substances

The PASS prediction was carried out on native ligand, ergosterol peroxide, and sitostenone compounds to see and compare their probability level as COVID-19 therapy (**Table IV**). The native ligand was predicted to have activity in severe acute respiratory syndrome treatment, 3C-like protease (Human coronavirus) inhibitor, protease inhibitor, and antiviral, with Pa value of 0.357; 0.358; 0.321; and 0.312, respectively. However, ergosterol peroxide and sitostenone were only predicted to have antiviral activity with a Pa value of 0.418 and 0.495, respectively.

Table IV. *In Silico* PASS Prediction results

Ligands	Activities prediction	Pa	Pi
Native	Severe acute respiratory syndrome treatment	0.357	0.005
	3C-like protease (Human coronavirus) inhibitor	0.358	0.006
	Protease inhibitor	0.321	0.008
	Antiviral	0.312	0.033
Sitostenone	Antiviral	0.495	0.026
Ergosterol peroxide	Antiviral	0.418	0.073

CONCLUSION

Compounds in *E. elatior* (ergosterol peroxide and sitostenone) showed potential as inhibitor candidates for the SARS-CoV-2 M^{Pro} receptor. Although the PASS server's *in silico* prediction does not show specific activity against the receptor like native ligand, the bioactive compounds were predicted to have potential as antiviral. Moreover, from the molecular docking simulation, ergosterol peroxide and sitostenone showed better affinity against SARS-CoV-2 M^{Pro} compared to flavonoid groups and the native ligand.

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