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Analysis of Inhibitory Potential of Bioactive Compounds from Langusei (*Ficus minhassae* Tesym. & De Vr.) against SARS-CoV-2 using an in silico Approach

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Abstract
The langusei plant (Ficus minhassae (Teijsm. & De Vriese) Miq.) is widely believed to have numerous health benefits and is frequently processed into traditional medicine to treat a variety of diseases. This study aimed to investigate the potential inhibition of bioactive compounds from langusei leaves against SARS-CoV-2 using an in silico approach.The compounds docked to receptors were obtained from the PubChem site, while the receptors were obtained from the Protein Data Bank site. The docking process was carried out using Autodock Vina software. The best docking result was the lowest binding free energy value (kcal/mol). The ligands used in this study have a relatively high binding affinity, particularly the β-sitosterol with the receptor-binding domain, which has a binding affinity of -8.4 kcal/mol. Additionally, the phytyl fatty acid ester has a value of -7.3 kcal/mol when bound to the spike glycoprotein (closed state). According to the findings of this study, β-sitosterol and phytyl fatty acid esters had the highest binding affinity for several SARS-CoV-2 receptors. As a result, these two compounds found in langusei leaves have the potential to be developed as anti-SARS-CoV-2 drugs.
Keywords: *langusei; Ficus minahassae; β-sitosterol; phytyl fatty acid esters; SARS-CoV-2; COVID-19*

INTRODUCTION

SARS-CoV-2 is a new corona virus that causes COVID-19 (coronavirus disease 2019) which was first discovered in December 2019 in Wuhan, China (Tallei et al., 2020). This virus attacks the human respiratory system (Alberca et al., 2020). There is currently no drug that can be used to treat COVID-19 (Singhal, 2020). As a result, numerous studies have been conducted to develop drugs to treat COVID-19 using natural ingredients (Rakib et al., 2020; Sailah et al., 2021). Indonesia, with a high prevalence of COVID-19 cases, has the potential to become a new hotspot for this disease.

Plants have long been used by the public as a source of medicine because they have compounds with certain bioactive potentials. One of the medicinal plants that is endemic to North Sulawesi and the southern part of the Philippines is *Ficus minhassae*, which is called langusei by the local community (Tallei et al., 2020). Langusei leaf extract contains 2-hydroxyethyl benzoate, phytyl fatty acid ester, squalene, and β-sitosterol (Apostol et al., 2016).

The following measures can be taken to manage the infection of SARS-CoV-2: (i) inhibit viral entry into host cells, (ii) inhibit viral replication and survival in host cells, and (iii) attenuate an exaggerated host immune response (Shetty et al., 2020). Some of the receptors used in the search for drugs to inhibit SARS-CoV-2 include the main protease (M^{pro}), spike

glycoprotein (S) (closed state), spike ectodomain structure (open state), RNA-directed RNA polymerase (RdRp), and receptor-binding domain (RBD) (Dutta et al., 2021; Mahmud et al., 2021; Khairan et al., 2021). Therefore, this study aimed to investigate the inhibitory potential of the bioactive compounds found in langusei against these receptors.

MATERIALS AND METHODS

Ligands and Receptors Preparation

The ligand compounds used in this study were 2-hydroxyethyl benzoate, phytyl fatty acid ester, squalene, and β-sitosterol contained in langusei. The three-dimensional (3D) structures of these compounds were downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/) in sdf format. The receptors on SARS-CoV-2 used in this study were the main protease (M^{pro}) (PDB code: 6LU7), spike glycoprotein (S) (closed state) (PDB code: 6VXX), spike ectodomain structure (open state) (PDB code: 6VYB), RNA-directed RNA polymerase (RdRp) (code: 6M71) and receptor binding domain (PDB code: 6YLA). These receptors were downloaded from the Protein Data Bank website (http://www.rcsb.org/pdb/). The method of preparing ligands and receptors was based on previous research (Tumilaar et al., 2021; Tallei et al., 2021).

Molecular Docking

The ligands' binding to receptors was adjusted using Autodock Tools v1.5.6. The ligands were docked to the receptors at specific coordinates and grid-boxes, with a distance between the grids was 1 Å. These settings were written in notepad and then saved in txt format (conf.txt). Additionally, Autodock Vina v1.1.2 was used to perform molecular docking. The Vina program was launched from a command prompt and directed to the docking folder using the following formula: vina –config conf.txt –log log.txt. The results of the docking calculation were obtained from the output in notepad format (log.txt) and.pdbqt format (out.pdbqt).

Docking Analysis

From the docking results, the conformation with the lowest binding energy was chosen to determine the best interaction between the ligand and the receptor. A two-dimensional (2D) visualization was performed using the BIOVIA Discovery Studio 2020 to determine the position and orientation of the ligands on the receptor.

RESULTS AND DISCUSSION

Table 1 summarizes the results of the molecular docking of each ligand to each receptor. Figure 1-5 depicts a visualization of the interaction of ligands and amino acid residues on the receptor using the Biovia Discovery Studio Vizualizer. Table 1. The binding free energy (ΔG_{bind}) obtained from the interaction of langusei active compounds with several SARS-CoV-2 receptors.

Observations on the value of ΔG_{bind} (the binding free enery) and root mean square deviation (RMSD), as well as the interaction of ligands with protein residues, were included in this molecular docking investigation. The bond between the ligand and the receptor with the lowest binding energy is considered to have the highest binding affinity (Perumal et al.,

2014). This is demonstrated by the fact that a small value of ΔG_{bind} (more negative) indicates a stable conformation, whereas a large value of ΔG_{bind} (more positive) indicates a less stable complexity formed. β-sitosterol has the lowest ΔG_{bind} and RMSD values of the four ligands found in langusei leaves.

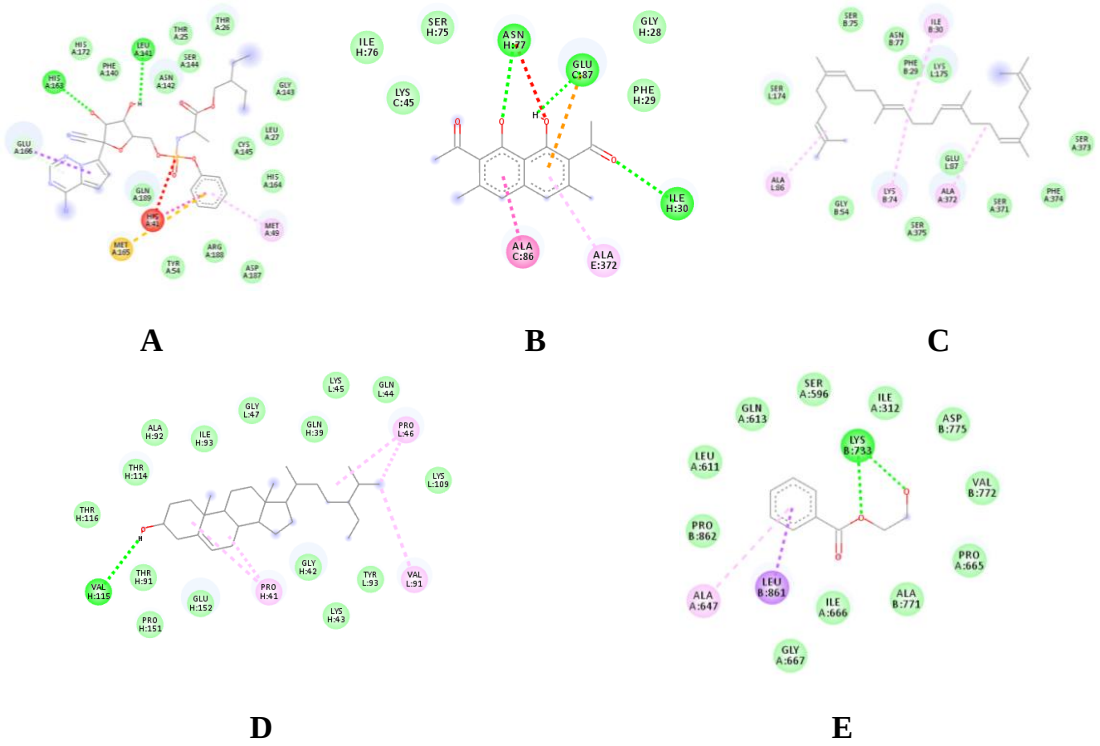
Table 1. The binding free energy ΔG_{bind} (kcal/mol) of each compound against their representative receptors

	Receptors				
Ligands	6VXX	6VYB	6YLA	6LU7	6M71
Remdesivir	-9,1	-7,5	-8,7	-8,1	-7,2
Phytyl fatty acid ester	-7,3	-7,1	-7,1	-6,8	-6,8
Squalene	-5,7	-6,1	-5,5	-5,2	-4,8
β-sitosterol	-6,9	-7,9	-8,4	-7,4	-6,9
2-hydroxyethyl benzoate	-5,5	-5,8	-5,4	-4,9	-5,0

As shown in Table 1, the ligands used in this study have a relatively high binding affinity, especially the β-sitosterol ligand with the receptor-binding domain which has a binding free energy value of -8.4 kcal/mol. On the other hand, the phytyl fatty acid ester has a binding free energy value of -7.3 kcal/mol when docked to spike glycoprotein (closed state). This value is close to the positive control ligand. Other receptors that are also attached to the β-sitosterol have good binding free energy values, especially the spike ectodomain structure (open State) with a value of -7.9 kcal/mol and the main protease receptor with a value of -7.4 kcal/mol.

When calculating molecular docking results, three parameters can be taken into account: binding affinity, hydrogen bond energy, and residual interactions of the amino acids involved (Ladokun et al., 2018). The ligand that binds to the receptor has a specific location, which is the active site (Rakib et al., 2020). This is demonstrated by the ligand's interaction with the amino acid residue on the receptor (Figures 1-5). The M^{pro} is a dimer in which each subunit contains a Cys145–His41 catalytic dyad, with His41 acting as a general base (Chang, 2009). Figure 1 shows that remdesivir binds to His41 and Cys145, β-sitosterol binds to His41, while other ligands do not bind to amino acids from the M^{pro} active site.

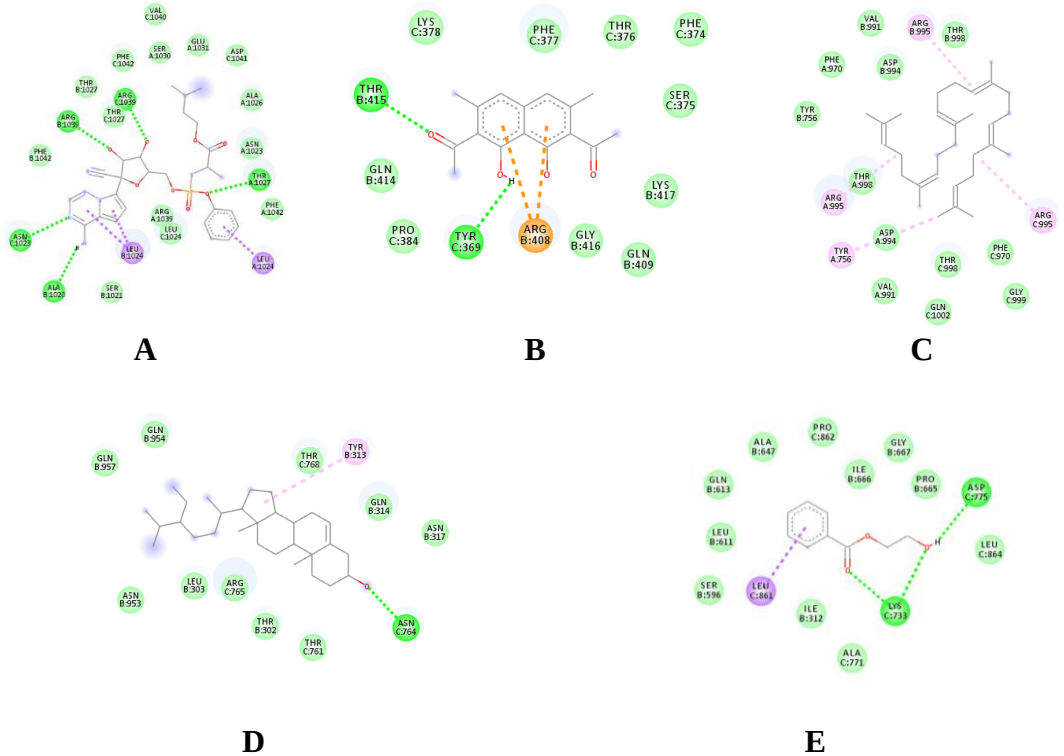
The spike (S) protein of SARS-CoV-2, which is involved in receptor recognition and cell membrane fusion, is made up of two subunits, S1 and S2. The RBD of the S1 subunit recognizes and binds to the host receptor angiotensin-converting enzyme 2, while the two-heptad repeat domain of the S2 subunit mediates viral cell membrane fusion by forming a six-helical bundle (Huang et al., 2020). Lys353 is a hotspot in SARS-CoV-2 (Jaiswal & Kumar, 2020). The enzyme RNA-dependent RNA polymerase (RdRp) is required for SARS-CoV-2 replication. During transcription and viral replication, RdRp can catalyze RNA synthesis. RdRp is thought to act as a cofactor (Subissi et al., 2014) .



A **B** **C**

D **E**

Figure 1. Visualization of the interaction between (A) remdesivir, (B) phytyl fatty acid ester, (C) squalene, (D) β-sitosterol, and (F) 2-hydroxyethyl benzoate with the main amino acids of the protease (M^{pro}).



A **B** **C**

D **E**

Figure 2. Visualization of the interaction between (A) remdesivir, (B) phytyl fatty acid ester, (C) squalene, (D) β-sitosterol, and (F) 2-hydroxyethyl benzoate with the main amino acids of the spike glycoprotein (closed state).

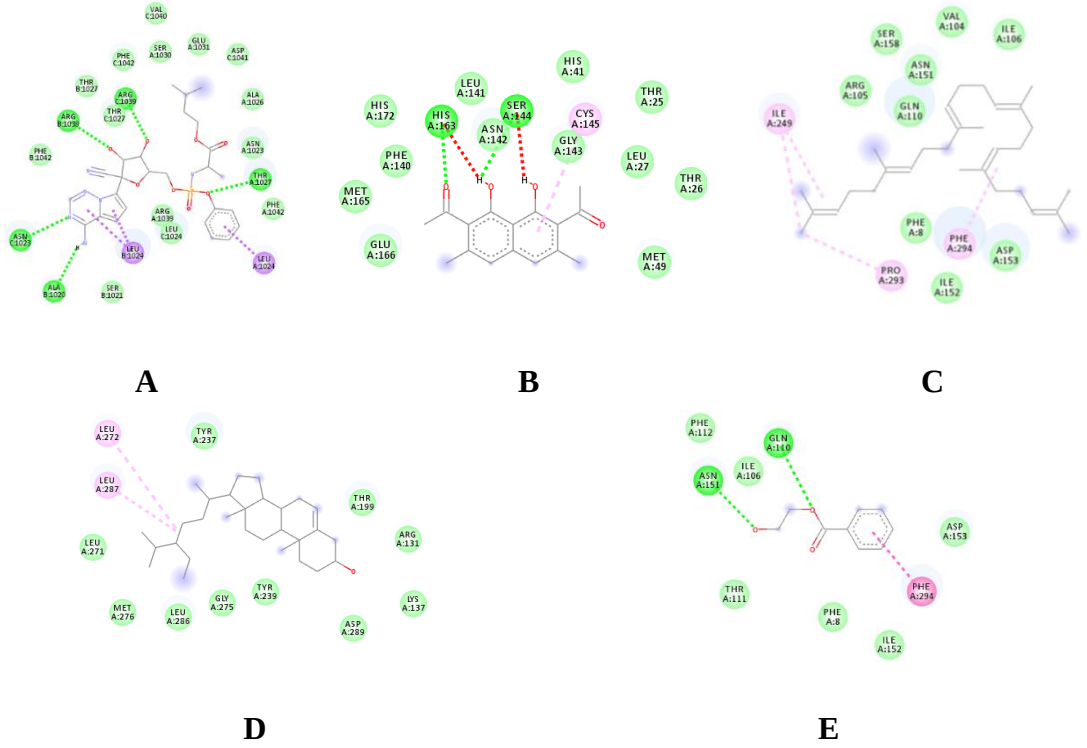


Figure 3. Visualization of the interaction between (A) remdesivir, (B) phytyl fatty acid ester, (C) squalene, (D) β-sitosterol, and (F) 2-hydroxyethyl benzoate with the main amino acids of the spike glycoprotein (open state).

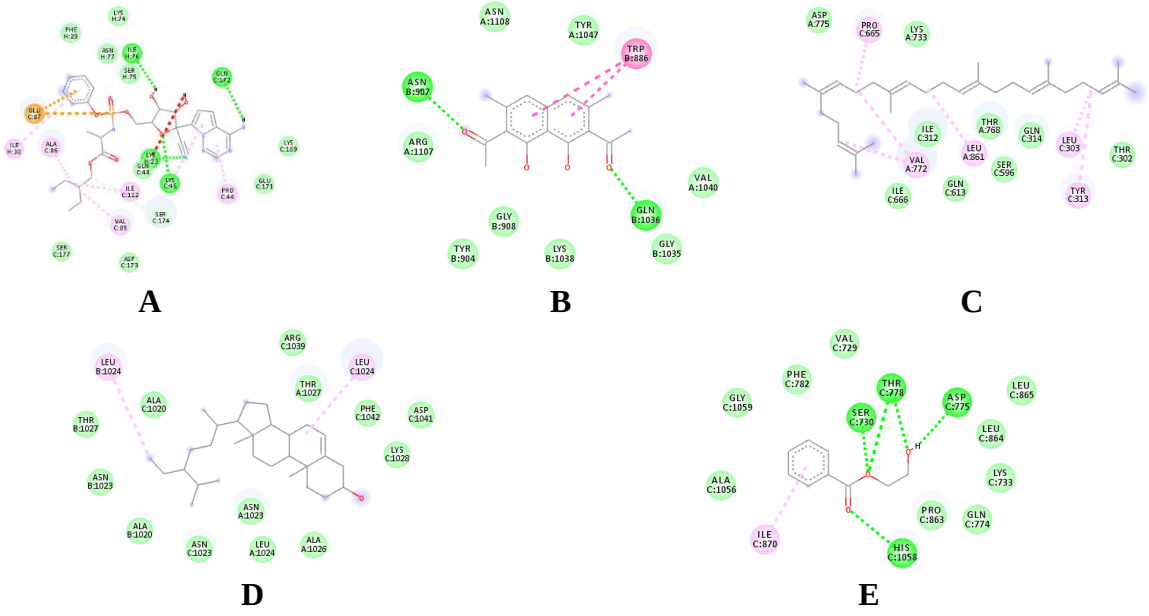


Figure 4. Visualization of the interaction between A) remdesivir, (B) phytyl fatty acid ester, (C) squalene, (D) β-sitosterol, and (F) 2-hydroxyethyl benzoate with the main amino acids of the RBD.

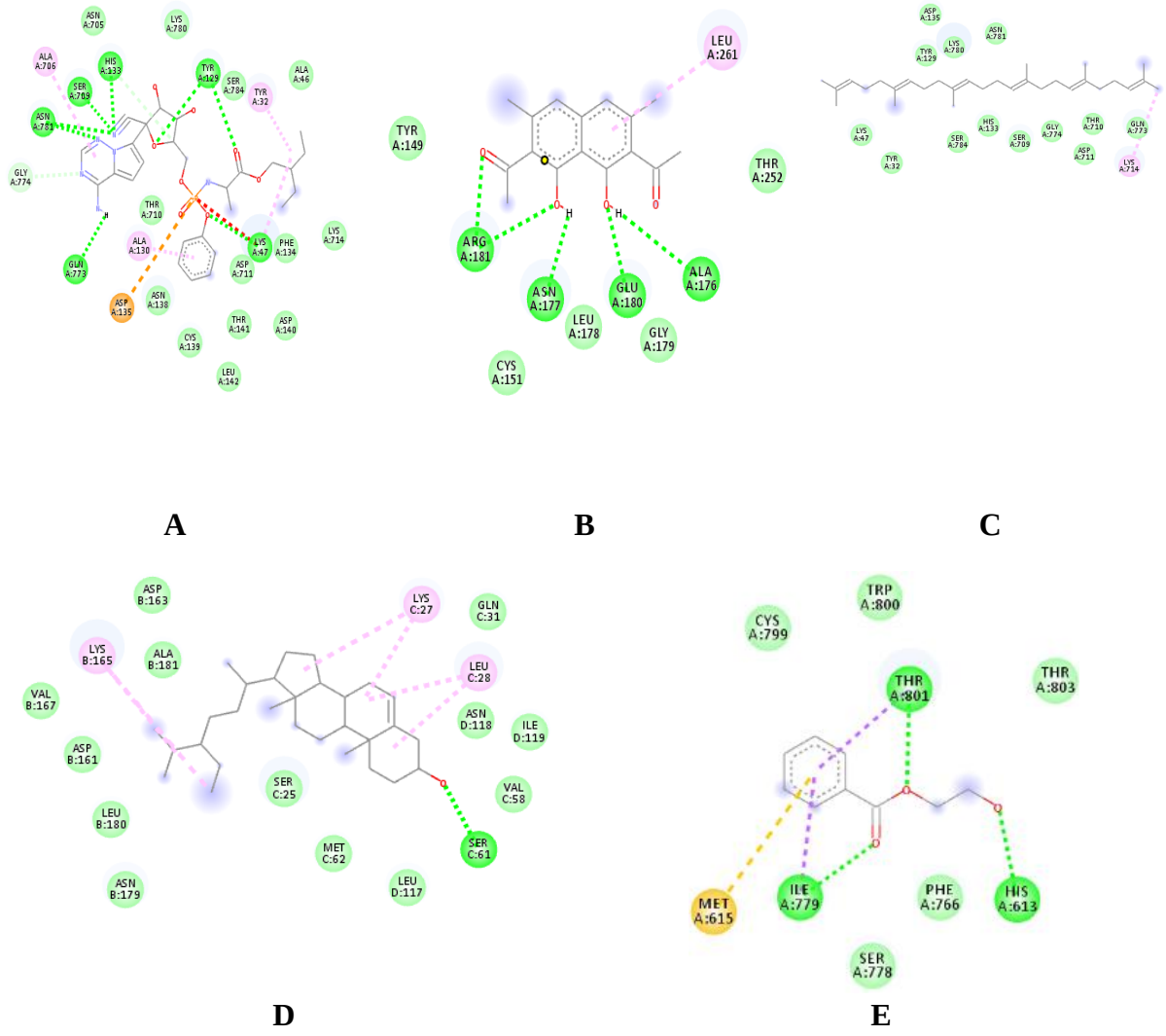


Figure 5. Visualization of the interaction between A) remdesivir, (B) phytyl fatty acid ester, (C) squalene, (D) β-sitosterol, and (F) 2-hydroxyethyl benzoate with the main amino acids of the RNA-directed RNA polymerase (RdRp).

Antiviral drugs can be designed to inhibit the activity of viruses' functional proteins or enzymes, to prevent viruses from binding to human cell receptors by targeting viral spike proteins, or to produce virulence factors in order to restore host innate immunity. On this basis, the M^{pro} is an excellent target. This is because inhibiting the M^{pro} activity can result in the loss of viral replication and transcriptional capability.

CONCLUSION

Langusei leaves contain compounds that exhibit favorable interactions with a variety of SARS-CoV-2 receptor targets, suggesting that they could be used as potential drugs. This is

due to the fact that β-sitosterol has a high affinity for the receptor-binding domain, whereas phytyl fatty acid esters have a high affinity for the spike glycoprotein (closed state).

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