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Computation Approach of Extract *Enhalus acoroides* Against Lipoate Protein Ligase: A Study of Molecular Docking and Pharmacophore

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Abstract

Tuberculosis (TB) is an infectious ailment instigated by the bacterium Mycobacterium tuberculosis. The escalating prevalence of antibiotic resistance poses a formidable challenge in the management of TB. Genetic variances within Mycobacterium tuberculosis lead to swift evolution and the formulation of strains that prove arduous to treat. The study is focused on discerning the molecular inhibitory potential of the secondary metabolite compound from *Enhalus acoroides* against the enzyme Lipoate Protein Ligase, pivotal in the lipoil binding process to the carrier protein during the synthesis of fatty acids in Mycobacterium tuberculosis. The determination of extract toxicity through BSLT testing, identification of the extract compound via GC-MS Analysis, followed by molecular inhibition in Silico using the PyRx application, and binding interpretation using the Biovia Studio application. The research findings reveal that the methanol extract of *Enhalus acoroides* manifests toxic properties with an LC50 value of 128.22 ppm, containing compounds such as Lanosterol, Benzamide, N, N'-1,4- phenylenebis, and Astargalin. These three compounds are anticipated to exhibit a substantial binding potential on the 1W66 receptor, with a binding affinity ranging from -7.5 to -6.8, -7.8 kcal/mol, ultimately resulting in the impediment of fatty acid synthesis in Mycobacterium tuberculosis cells.

Keywords: *Enhalus acoroides*, Lipoat Protein Ligase, Molecular Docking, Pharmacophere

INTRODUCTION

Tuberculosis (TB) is an infectious disease that affects the lungs and is caused by the bacterium Mycobacterium tuberculosis. According to the World Health Organization (WHO), tuberculosis remains one of the leading causes of death worldwide, ranking higher than HIV/AIDS. In 2021, Indonesia was ranked third globally with the highest number of tuberculosis cases, following India and China (WHO, 2022). In Indonesia, there are 969,000 TB cases with 144,000 deaths and 28,000 cases of drug-resistant TB (Republic of Indonesia Ministry of Health, 2022). In 2022, the province of Banten ranked fifth with a total of 23,343 reported TB cases (DataBoks, 2022).

Meanwhile, the commonly employed treatment for tuberculosis involves administering anti-tuberculosis drugs in the form of antibiotics. Tuberculosis treatment consists of two phases: the intensive phase and the continuation phase. In the intensive phase, drugs such as Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol are utilized, while the continuation phase involves Isoniazid and Rifampicin (Rizwani, 2017). This treatment typically needs to

be administered regularly for a period of 6 months. However, it may lead to various side effects, including nausea, vomiting, abdominal pain, joint pain, fever, decreased appetite, orange-colored urine, skin disturbances such as itching and rashes, with the most severe being hepatotoxicity (Verencia, 2022). Consequently, there is currently a significant effort to explore new compounds that can serve as alternative drugs in tuberculosis management.

Indonesia boasts a marine environment rich in biodiversity, much of which remains untapped, particularly for pharmaceutical development. One example is the seagrass plant (*Enhalus acoroides*). Seagrass (*Enhalus acoroides*) is a marine plant containing active compounds such as tannins, saponins, triterpenoids, flavonoids, and steroids (Permana *et al*., 2020). These compounds exhibit antimicrobial, antioxidant, anti-inflammatory, and antibacterial activities (Purnama & Brahmana, 2018; Yusuf *et al*., 2021).

The utilization of phytochemical compounds from seagrass plants has been explored for antibacterial effects against Escherichia coli, Staphylococcus aureus, and Bacillus subtilis (Purnama & Brahmana, 2018) and anticancer properties (Kristanti *et al*., 2024). Given the antibacterial activity of these compounds, preliminary computational research using molecular docking approaches aims to identify the most effective seagrass (*Enhalus acoroides*) compound as an antibacterial agent against Mycobacterium TB.

METHOD

This research was conducted in the Basic Chemistry Laboratory of the Untirta Faculty of Engineering, the Chemical Computing Laboratory of the Untirta Engineering Faculty. GC-MS analysis was carried out at the DKI Jakarta Regional Health Laboratory. The research took place from May 2023 to January 2024. This research includes GCMS testing, and bioinformatics studies through docking in silico.

Preparation the Proteins

The receptor protein was acquired from a website link (https;//www.rcsb.org/) with a code 1w66, for study and examination in a downloadable format known as *.pdb file extension format through Discovery Studio software application to refine and enhance its structure by eliminating any unnecessary ligands like water molecules attached to it. Subsequently during protein preparation process within AD4 framework tools were used to rectify any missing atoms and incorporate hydrogen atoms along with Gasteiger charge, into it ensuring completeness and accuracy of its molecular composition which was then finally saved in pdbqt file format for future reference.

Preparation the Ligands

The compounds arrangement was determined through GC MS analysis. Verified on PubChem at http;//pubChem.ncbi.nml.nih.gov before being saved in the SDF format (*.sdf). Following that Chem3D Ultra was utilized to reduce energy and save the structure in *.pdb format.

Docking Molecular

The receptors were set up in AutoDock Tools software. Converted to PDBQT format before converting the ligand into a torque formation and saving it in *.pdbqt format. Once the ligands and receptors were prepared accordingly the next step involved creating a working area (grid box) align, with the control grid box followed by initiating the docking process. Upon completion of the docking process the binding affinity was. The docking ligand was examined for its binding site using the Discovery Studio Visualizer tool. A comparison structure was set up in Pymol to ensure that the RMS deviation is kept below 2 A.

RESULTS AND DISCUSSION

The pharmacokinetic and pharmacodynamic aspects, represented by the properties of absorption, distribution, metabolism, and excretion (ADME), greatly influence how a drug compound works in the body, especially when administered orally (Arief & Hairunnisa, 2022). A primary reference for predicting the suitability of ADME properties is Lipinski's Rule of Five, formulated by Christopher A. Lipinski (Arief & Hairunnisa, 2022). A drug compound can be orally administered if it meets the following criteria: (1) a molecular weight of less than 500 Da, (2) a Log P value of less than 5, (3) no more than 5 hydrogen bond donors, (4) no more than 10 hydrogen bond acceptors, and (5) no more than 10 rotatable bonds (Lipinski *et al*., 1997). In general, at least two of Lipinski's rules must be fulfilled for a compound to be considered.

Table 1. Lipinski's Rule of Five Results for the Ligand Compounds of *Enhalus acoroides*

No	*Compound*	MW ≤500 da	*Hydrogen Bond Donor* < 5	*Hydrogen Bond Acceptors* <10	Log P ≤ 5	*Rotatable Bond* ≤ 10
1.	*Lanosterol*	426	1	1	8,479104	4
2.	*Benzamide, N, N'-1,4-phenylenebis*	316	2	4	4,191198	6
3.	*Astragalin*	448,38	7	11	-0,24	4

Source: http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp

Bioavailability reflects the relationship between the drug dose and the relative amount of the drug delivered into the bloodstream. The bioavailability radar parameters are visualized in 2D (Figure 1). The red area on the bioavailability radar indicates the optimal range for a compound to be considered as an oral drug, taking into account six physicochemical

properties, namely: (1) Lipophilicity (LIPO), which refers to the compound's ability to dissolve in fats (lipophilicity) with an XLOGP3 value ranging from -0.7 to +5.0, (2) Molecular size (SIZE) of approximately 150-500 g/mol, (3) Polarity (POLAR), indicated by a Topological Polar Surface Area (TPSA) value of around 20-130 Å, (4) Solubility (INSOLU), with a solubility parameter of log S < 6, (5) Flexibility (FLEX), with less than 9 rotatable bonds as the flexibility parameter, and (6) Saturation (INSATU), with a fraction of sp3 hybridized carbons greater than 0.25 as the saturation parameter (Daina *et al*., 2017)

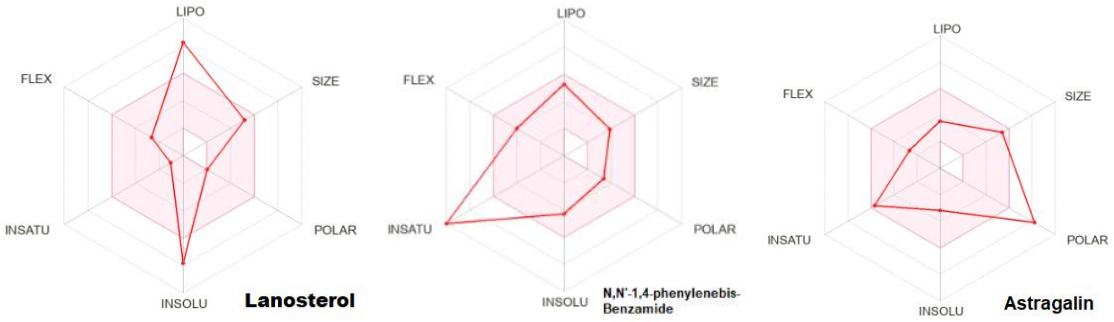


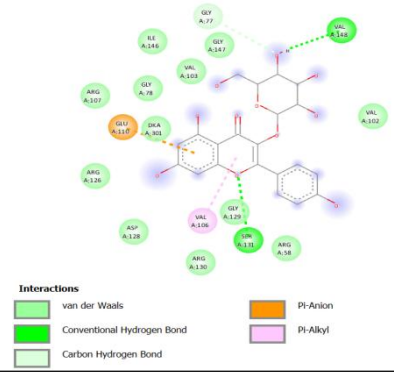
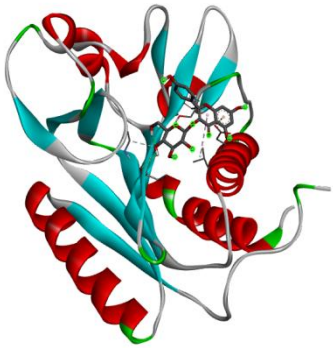
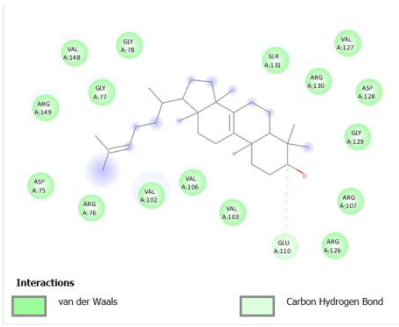
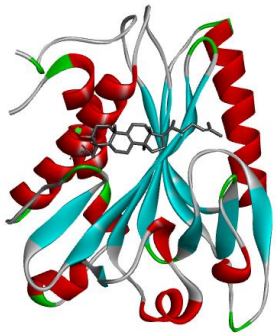
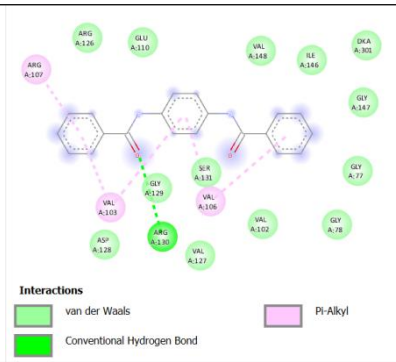
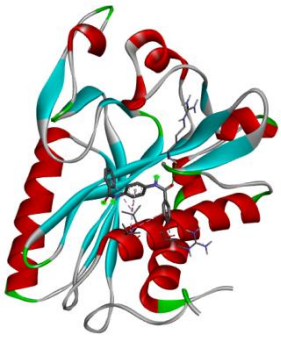
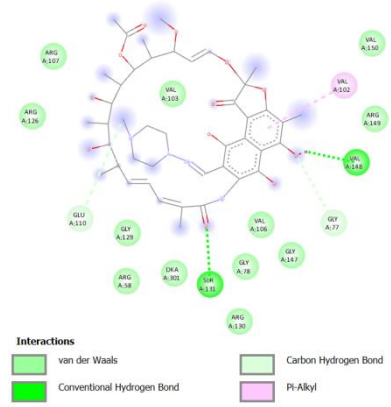
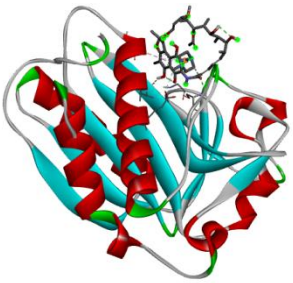
Figure 1. ADME Properties Radar of *Enhalus acoroides* Ligand (http://www.swissadme.ch/index.php)

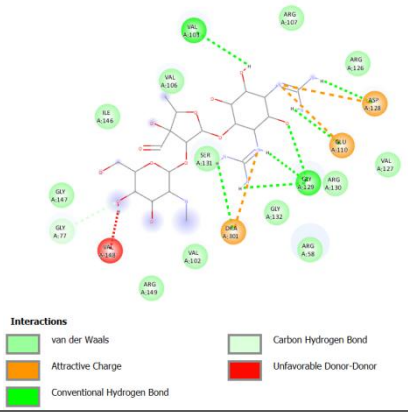
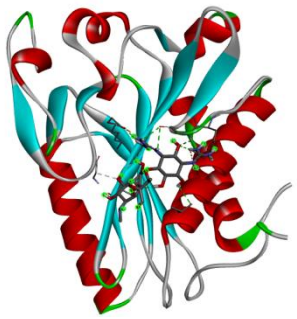
The three compounds are predicted to be potential drugs, although some properties fall outside the ADME radar.

Molecular Docking

The enzymes involved in the lipoylation process and lipoic acid, which are essential for activating several protein complexes that participate in key metabolic processes, have been linked to the growth and pathogenicity of various bacteria, including *Mycobacterium tuberculosis*. The enzyme model Lipoate protein ligase B (LipB) with PDB ID: 1W66 is known as octanoyl-[acyl carrier protein]-protein acyltransferase. This enzyme is responsible for transferring octanoic acid from one molecule to another (lipoyl domain) through a specific bond (thioester bond) that involves coenzyme 4'-phosphopantetheine as part of the acyl carrier protein (ACP). LipB expression shows a significant increase in patients affected by multi-drug-resistant *M. tuberculosis*. No alternative mechanism has been identified that can replace LipB's role in tuberculosis metabolism. This suggests that LipB plays a crucial role in the growth of *Mycobacterium tuberculosis* and can be a target for research into new anti-TB drugs (Billones *et al*., 2013).

Table 2. Molecular Docking Results of *Enhalus acoroides* Compounds with Receptor 1W66

No.	Ligan	*Binding Affinity (kkal/mol)*	*Binding Visualization*	
			2D *Image*	3D *Image*
1.	*Astragalin*	**-7,8**		
2	*Lanosterol*	**-7,5**		
3	*Benzamide, N, N'-1,4-phenylenebis*	**-6,8**		
	Rifampicin (Kontrol Posistif)	-7		

No.	Ligan	*Binding Affinity (kkal/mol)*	*Binding Visualization*	
			2D *Image*	3D *Image*
	Streptomycin (Kontrol Positif)	-6,5		

The Van der Waals interactions between amino acid residues of Lanosterol and Rifampicin share similarities at GLY78, ARG149, VAL106, VAL103, ARG126, ARG107, GLY129, and ARG130. These interactions play a key role in determining the stability of the ligand-receptor binding (Arwansyah *et al*., 2014; Ratu *et al*., 2021). Additionally, the Van der Waals interactions formed by Lanosterol also have similarities with Streptomycin (positive control) at amino acid residues ARG149, VAL102, VAL106, ARG126, ARG107, ARG130, SER131, and VAL127.

Astragalin shares similar binding characteristics with the positive control Rifampicin. Both compounds form hydrogen bonds, including a conventional hydrogen bond at amino acid residues SER131 and VAL148, and a carbon hydrogen bond at GLY77. This suggests that Astragalin may have a similar mechanism of action to Rifampicin. In addition to hydrogen bonds, Van der Waals interactions are also formed between Astragalin, Rifampicin, and Streptomycin at the amino acid residues GLY147, VAL103, ILE146, GLY78, ARG107, DKA301, ARG126, ARG130, GLY129, ARG58, and VAL102.

Moreover, Lanosterol and Benzamide, N, N'-1,4-phenylenebis also share Van der Waals interactions with Streptomycin at residues ARG126, GLY147, VAL102, VAL127, and SER131. The same result is observed with Benzamide, N, N'-1,4-phenylenebis and the positive control Rifampicin at amino acid residues ARG126, DKA301, GLY147, GLY78, and GLY129.

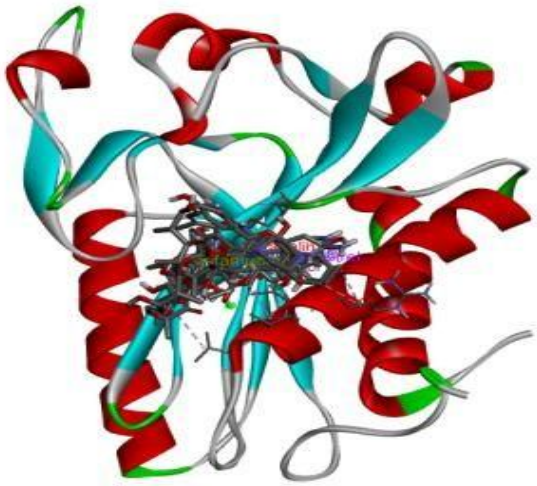


Figure 2. 3D Representation of the Position of 5 Ligands in Relation to Receptor

The similarities in amino acid residues between the active compounds, Lanosterol and Benzamide, N, N'-1,4-phenylenebis, and the comparators (Rifampicin and Streptomycin) are evident in Figure 2. These compounds occupy the same binding positions as the positive controls, indicating a similarity in the type of interactions and bonds with the target protein. A higher similarity in amino acid residues suggests a higher probability that the active compound ligands will exhibit similar interactions to the comparator ligands (Pratama *et al*., 2018).

CONCLUSION

The alignment of the drug characteristics of *Enhalus acoroides* compounds with Lipinski's Rule of Five and the bioavailability radar, along with the molecular docking results with the target protein receptor 1W66, indicates that Lanosterol, Benzamide, N, N'-1,4-phenylenebis, and Astragalin exhibit binding affinities to receptor 1W66 of -7.5, -6.8, and -7.8 kcal/mol, respectively. These values are lower than those of Rifampicin and Streptomycin, suggesting that these three compounds form stronger bonds with receptor 1W66 than the positive controls. Furthermore, the compounds share similarities with the positive controls in terms of surface position, indicating similar activity and binding to the same amino acids. This suggests that Lanosterol, Benzamide, N, N'-1,4-phenylenebis, and Astragalin have superior potential to inhibit the activity of receptor 1W66 and could be considered candidates for anti-tuberculosis drugs.

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