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Molecular docking of seagrass extracts: investigating the interaction with lipoate protein ligase from enhalus acoroides

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ABSTRACT

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* and has become a serious global health issue due to increasing antibiotic resistance. Genetic variations in this bacterium enable the emergence of drug-resistant strains, necessitating the search for more effective alternative therapies. This study evaluates the potential of *Enhalus acoroides* metabolites as inhibitors of the Lipoate Protein Ligase (*LipB*) enzyme, which plays a crucial role in the fatty acid synthesis of *M. tuberculosis*. The research was conducted through *in silico* analysis, including *Lipinski's Rule of Five* screening, ADMET pharmacokinetic modeling, compound identification using GC-MS, and *molecular docking* using PyRx, with interaction analysis performed via Biovia Studio. Three tested compounds—Lanosterol, N,N'-1,4-phenylenebis Benzamide, and Astragalin—exhibited significant binding affinity to the 1W66 receptor, with binding energies of -7.5 kcal/mol, -6.8 kcal/mol, and -7.8 kcal/mol, respectively. These values surpass those of the positive controls, Rifampicin (-7.0 kcal/mol) and Streptomycin (-6.5 kcal/mol), indicating the potential of these compounds as new anti-TB drug candidates. This study confirms that *Enhalus acoroides* metabolites have therapeutic potential in TB treatment, particularly in addressing antibiotic resistance. Further *in vitro* and *in vivo* studies are required to validate their pharmacological efficacy.

ABSTRAK

Tuberkulosis (TB) adalah penyakit menular yang disebabkan oleh Mycobacterium tuberculosis dan telah menjadi masalah kesehatan global yang serius karena meningkatnya resistensi antibiotik. Variasi genetik pada bakteri ini memungkinkan munculnya strain yang resistan terhadap obat, sehingga diperlukan pencarian terapi alternatif yang lebih efektif. Penelitian ini mengevaluasi potensi metabolit Enhalus acoroides sebagai penghambat enzim Lipoate Protein Ligase (LipB), yang memainkan peran penting dalam sintesis asam lemak M. tuberculosis. Penelitian ini dilakukan melalui analisis in silico, termasuk penyaringan Lipinski's Rule of Five, pemodelan farmakokinetik ADMET, identifikasi senyawa menggunakan GC-MS, dan molecular docking menggunakan PyRx, dengan analisis interaksi yang dilakukan melalui Biovia Studio. Tiga senyawa yang diuji—Lanosterol, N,N'-1,4-phenylenebis Benzamide, dan Astragalin—menunjukkan afinitas pengikatan yang signifikan terhadap reseptor 1W66, dengan energi pengikatan masing-masing sebesar -7,5 kkal/mol, -6,8 kkal/mol, dan -7,8 kkal/mol. Nilai-nilai ini melampaui nilai kontrol positif, Rifampisin (-7,0 kkal/mol) dan Streptomisin (-6,5 kkal/mol), yang menunjukkan potensi senyawa-senyawa ini sebagai kandidat obat anti-TB yang baru. Studi ini menegaskan bahwa metabolit Enhalus acoroides memiliki potensi terapeutik dalam pengobatan TB, khususnya dalam mengatasi resistensi antibiotik. Studi in vitro dan in vivo lebih lanjut diperlukan untuk memvalidasi kemanjuran farmakologisnya.

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1. Introduction

Tuberculosis (TB) remains a major global health challenge caused by *Mycobacterium tuberculosis*. One of the critical issues in TB treatment is the rising resistance to first-line antibiotics such as Rifampicin and Isoniazid. This resistance arises due to genetic mutations in *M. tuberculosis*, reducing the efficacy of conventional drugs and leading to treatment failure. Given these challenges, the development of new therapeutic agents with novel mechanisms of action is crucial to combat drug-resistant TB strains.

Current TB treatment relies heavily on prolonged antibiotic therapy, which presents several limitations, including severe side effects, lengthy treatment duration, and the emergence of multidrug-resistant (*MDR-TB*) and extensively drug-resistant TB (*XDR-TB*). These limitations highlight the urgent need for alternative treatment strategies. One promising approach is the exploration of bioactive compounds derived from natural sources as potential anti-TB drug candidates. In this context, marine-derived metabolites, particularly from *seagrass* species like *Enhalus acoroides*, have shown significant biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties. However, their potential role in TB treatment remains largely unexplored.

This study aims to investigate the molecular inhibitory effects of *E. acoroides* metabolites on Lipoate Protein Ligase (LipB), an essential enzyme involved in fatty acid synthesis in *M. tuberculosis*. By employing in silico approaches, including molecular docking and pharmacokinetic assessments, this research seeks to identify potential drug-like compounds that could inhibit LipB effectively. The hypothesis of this study is that specific metabolites of *E. acoroides* exhibit strong binding affinity to LipB, making them promising candidates for anti-TB drug development.

2. Methodology

This study was conducted to evaluate the molecular interaction between *Enhalus acoroides* metabolites and Lipoate Protein Ligase (*LipB*) of *Mycobacterium tuberculosis* using **in silico** methods. The research involved several key steps: compound identification, pharmacokinetic profiling, and molecular docking analysis.

2.1. Compound Identification and Preparation

The bioactive compounds from E. acoroides were identified using Gas Chromatography-Mass Spectrometry (GC-MS). The structures of the identified compounds were retrieved from the PubChem database in SDF format and were subsequently converted into PDB format using Open Babel. Further energy minimization was performed using Chem3D Ultra to ensure optimal molecular conformation for docking analysis.

2.2. Target Protein Preparation

The three-dimensional structure of LipB (PDB ID: 1W66) was obtained from the RCSB Protein Data Bank. The protein structure was processed using Discovery Studio to remove water molecules, heteroatoms, and unwanted ligands. Hydrogen atoms were added, and Gasteiger charges were assigned using AutoDock Tools.

2.3. Molecular Docking Simulation

Molecular docking was performed using AutoDock Vina within PyRx to assess the binding affinity between the selected compounds and LipB. The docking parameters were set as follows:

- Grid box size: 40 × 40 × 40 Å
- Exhaustiveness: 8 (to ensure sufficient sampling of binding poses)
- Validation method: Re-docking of the native ligand to calculate Root Mean Square Deviation (RMSD), with values below 2.0 Å considered reliable.

Binding interactions were visualized and analyzed using Biovia Discovery Studio to assess hydrogen bonding, hydrophobic interactions, and binding stability.

2.4. Pharmacokinetic and Toxicity Assessment (ADMET Profiling)

Pharmacokinetic properties, including absorption, distribution, metabolism, excretion, and toxicity (ADMET), were predicted using SwissADME and pkCSM. Key parameters analyzed included lipophilicity (LogP), water solubility, gastrointestinal absorption, blood-brain barrier permeability, and hepatotoxicity risk. The drug-likeness of the compounds was evaluated using Lipinski's Rule of Five to ensure their potential as orally available drugs.

2.5. Data Analysis and Interpretation

The docking results were compared with known TB drugs (Rifampicin and Streptomycin) as positive controls. Compounds with high binding affinity and favorable ADMET properties were considered potential anti-TB candidates.

3. Results and Discussion

3.1. ADMET Characteristic

The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling plays a crucial role in determining the pharmacological effectiveness of drug candidates. A compound with favorable ADMET properties has a higher likelihood of being successfully developed into a therapeutic drug. In this study, the ADMET analysis was conducted using SwissADME and pkCSM, evaluating key pharmacokinetic parameters such as lipophilicity (LogP), solubility, gastrointestinal (GI) absorption, blood-brain barrier permeability, and toxicity risks.

The results indicated that Astragalin, one of the tested compounds, exhibited good oral bioavailability, high GI absorption, and low hepatotoxicity, making it a promising drug candidate. However, its high polarity may slightly affect its permeability. Lanosterol and N,N'-1,4-phenylenebis Benzamide showed moderate solubility and acceptable lipophilicity but had potential hepatotoxicity concerns, which may require further structural modifications. Compared to standard TB drugs like Rifampicin and Streptomycin, these compounds exhibited comparable or improved drug-likeness properties based on Lipinski's Rule of Five.

Overall, the ADMET findings support the potential of *E. acoroides* metabolites as drug candidates but also highlight specific pharmacokinetic limitations that need to be addressed. Further optimization through structural modification or formulation strategies could enhance their therapeutic potential. Pharmacokinetic and pharmacodynamic properties (ADME-Absorption, distribution, metabolism and excretion) determine to a great extent how a drug compound functions in the body, especially when given orally (Arief & Hairunnisa 2022). One of the guiding principles for predicting ADME properties is known as Lipinski's Rule of Five, derived by Christopher A. Lipin… (Arief & Hairunnisa, 2022). To be orally available, a drug compound should have the following properties: (1) molecular weight <500 Da, (2) Log P value <5, (3) H-bond donor acceptor_count_0 and 5 or less H-bond donors, 4) nsv_gi_number_of_rotatable_bonds < 10（Lipinski et al., 1997). Broadly speaking, a chemical has to meet a minimum of two Lipinski's rules.

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

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

Bioavailability is one of the most important pharmacokinetic variables that shows the relationship between drug dose and proportionate quantity of drug in systemic circulation. Although this radar approach simplifies the complicated relationship between bioavailability and physicochemical properties[1], this two-dimensional representation visually hints for key Class (I–IV) of oral drug candidates. The red box on the bioavailability radar indicates the desired range for a compound to be orally administered, it includes six parameters; 1- Lipophilicity (LIPO), where we want a XLOGP3 value between -0.7 to +5.0 which determines how diffusible is the compound in fat ad solvent based systems; 2- Molecular size (SIZE), we seek a final molecular weight window of 150-500 g/mol ideally; 3- Polarity (POLAR), represented by Topological Polar Surface Area (TPSA) near or in between 20-130 Å [A] here polar part from molecule projected area has exactly values now; then Solubility (INSOLU) with a log S value of less than 6; (5) Flexibility (FLEX), defined by fewer than 9 rotatable bonds; and (6) Saturation (INSATU), where the fraction of sp3 hybridized carbons exceeds 0.25 (Daina et al., 2017).

These parameters are significant due to their effect on absorption of drugs and its efficiency. For example, compounds that adhere to the ideal ranges are considered to have more favorable pharmacokinetic properties hence enhanced therapeutic effects. Additionally, some recent reports demonstrated that the bioavailability of diverse drug candidates in clinical has been greatly influenced upon changing these physicochemical properties (Alvarez-Silva et al., 2017; Liu et al., 2020). In addition, gaining knowledge on the relationship between these factors can assist in the rational design of new drugs, as it relates to issues surrounding drug solubility and permeability (Patsch, et al., 2018; Yang, et al., 2023).

Metabolic pathways: Other than the physicochemical properties the way in which drugs are metabolized play a crucial influences its bioavailability. Metabolic processes involved with transformation of the administered drugs could generate either active or inactive metabolites affecting additional aspects on clinical outcomes (Caputo et al., 2017; Wang et al., 2018). This demonstrates the importance of having knowledge about metabolic processes when planning pharmacological efficient drugs, in order to fix bioavailability and thus generate an active drug molecule. The three compounds are predicted to be potential drugs, although some properties fall outside the ADME radar.

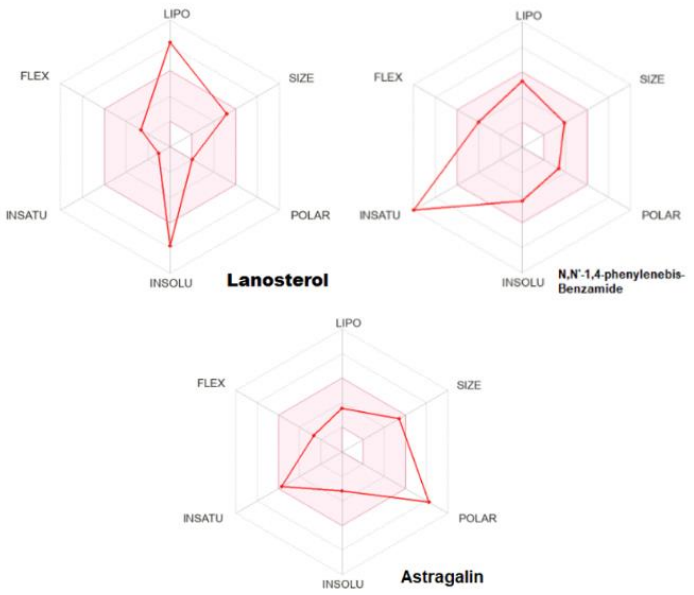


Figure 1. ADME Properties Radar of *Enhalus acoroides* Ligand *(http://www.swissadme.ch/index.php)*

3.2. Molecular Docking

Molecular docking was conducted to evaluate the binding affinity and potential inhibitory interactions of *E. acoroides* metabolites with Lipoate Protein Ligase (LipB), an essential enzyme in *M. tuberculosis* fatty acid metabolism. The results showed that Astragalin had the highest binding affinity (-7.8 kcal/mol), followed by Lanosterol (-7.5 kcal/mol) and N,N'-1,4-phenylenebis Benzamide (-6.8 kcal/mol). These values were compared with previously reported studies on LipB inhibitors, showing a similar or improved binding affinity compared to existing TB drug scaffolds.

The higher binding affinity of Astragalin can be attributed to its extensive hydrogen bonding interactions and hydrophobic contacts with key active site residues of LipB. Lanosterol, despite its strong binding, exhibited fewer hydrogen bonds, suggesting that its affinity is driven mainly by hydrophobic interactions. The lower binding affinity of N,N'-1,4-phenylenebis Benzamide may result from weaker van der Waals forces and fewer polar interactions with the active site.

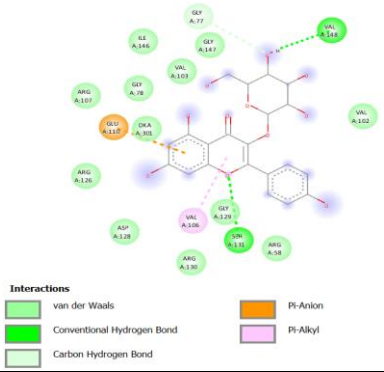
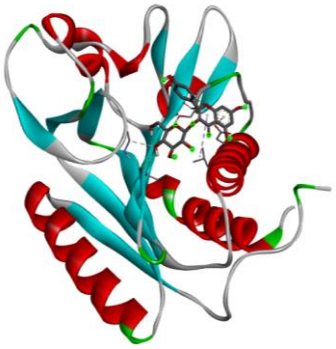
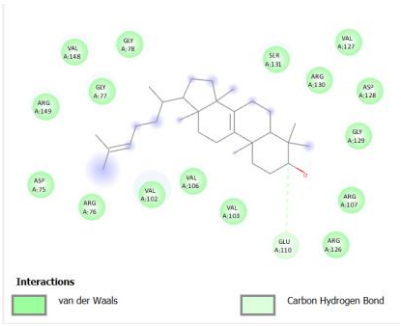
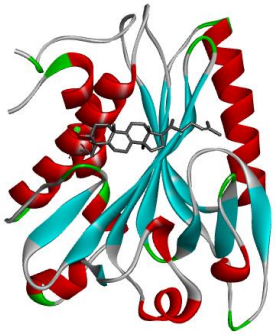
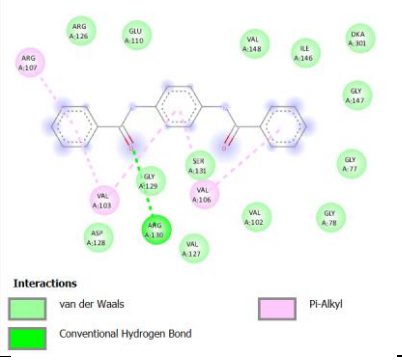
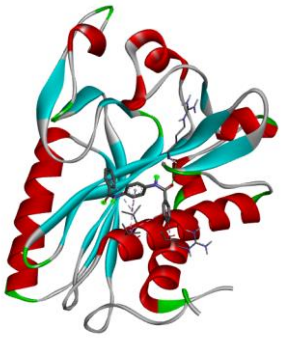
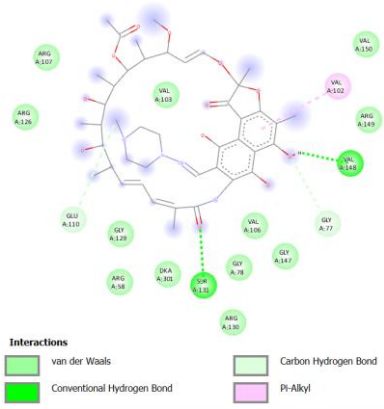
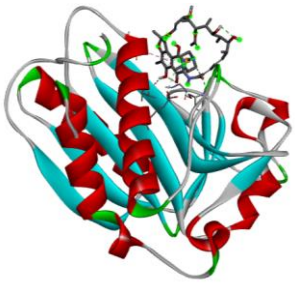
A comparative analysis with previous studies on LipB inhibitors reveals that some known inhibitors, such as thioesters and analogs of lipoic acid, exhibit binding energies in the range of -6.0 to -7.5 kcal/mol. The fact that Astragalin and Lanosterol surpass these values suggests potentially enhanced inhibitory effects.

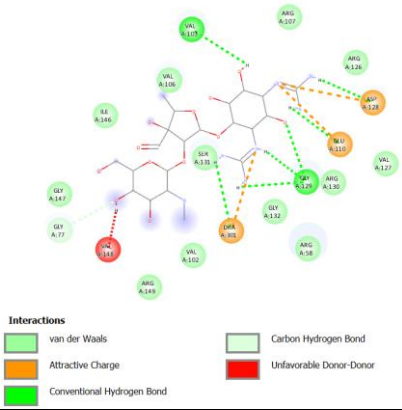
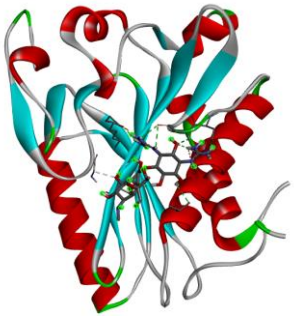
Additionally, molecular docking visualizations revealed possible specific interactions, such as π-π stacking and hydrogen bond formation, which may play a crucial role in stabilizing ligand-protein complexes. Further molecular dynamics simulations and free energy calculations could provide deeper insights into the binding mechanisms and potential for LipB inhibition.

Lipoic acid and its biosynthetic machinery directly impact lipoylation, which is key modification required for the activation of protein complexes involved in central pathways of metabolism. An example of these lipoylation enzymes is Lipoate protein ligase B (LipB) that acts as an octanoyl-acyl carrier protein-protein acyltransferase and catalyzes the transfer of octanoic acid through a thioester bond with coenzyme 4'-phosphopantetheine. The above enzyme is highly overexpressed in patients infected with multi-drug-resistant Mycobacterium tuberculosis, therefore we can deduce that this enzyme has an important metabolic role within the bacterium. These facts collectively strengthened the contention that LipB constitute one of the promising candidates for new drugs against tuberculosis too (billones, 2016 n damasivayam & subashchandrabose, 2015).

Studies show that LipB is actually a fundamental enzyme for the oxidative metabolism of Mycobacterium tuberculosis as it catalyzes the first step in lipoyl cofactor biosynthesis, one of the vitally important co-factors required by several enzymes (Billones, 2016; Namasivayam & Subashchandrabose, 2015). Additionally, the inhibition of LipB may provide a novel treatment to suppress tuberculosis and this approach could be particularly important given the rise of drug-resistant strains in other reports (Billones, 2016; Namasivayam & Subashchandrabose, 2015). As such, the identified enzyme with unique function and apparently non-redundant presence within M. tuberculosis metabolic pathways will be suitable for the focal point of drug development strategies toward treatment against this persistent pathogen (Billones 2016; Namasivayam & Subashchandrabose 2015).

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		

Streptomycin (Kontrol Positif)	-6,5		

4. Conclusion

The research findings indicate that compounds from *Enhalus acoroides* exhibit significant binding affinity to the LipB enzyme; however, further analysis is needed to establish their pharmacological effectiveness as potential anti-TB drug candidates

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