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Computational Insights into the Dual Inhibition of PfATP6 and PfCRT by Bioactive Compounds from *Spatholobus littoralis* Hassk.

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Abstract

The current study examined the antimalarial activity of bioactive compounds extracted from *Spatholobus littoralis* Hassk. (bajakah wood) by an in-silico approach targeting the Plasmodium falciparum PfCRT and PfATP6 receptors. A total of forty-six phytochemicals were subjected to screening, resulting in the selection of six compounds based on bioactivity prediction, ADMET profiling, and molecular docking analyses. Ramachandran plots were used to check the accuracy of the model, which showed that the protein structures were reliable. Milbemycin A4-oxime exhibited the most significant binding affinity (−9.6 kcal/mol for PfCRT and −9.9 kcal/mol for PfATP6). These findings are comparable to or slightly better than those observed for artemisinin in this in silico model. These findings are preliminary and require further experiment. The molecule exhibited persistent interactions and favorable pharmacokinetic properties, indicating its potential use as a multitarget inhibitor. Quercetin and 8-O-methylretusin had significant efficacy. These results underscore the potential of S. littoralis metabolites, especially Milbemycin A4-oxime, as candidates for antimalarial drug development; however, additional in vitro and in vivo validation is necessary to establish efficacy and safety.

Keyword: *Spatholobus Littoralis* Hassk., Antimalarial Activity, In Silico Study, PfATP6 Receptor, PfCRT Receptor

1. Introduction

Malaria is an endemic parasitic disease caused by infection with the protozoan *Plasmodium*, transmitted through the bite of female Anopheles mosquitoes. It is prevalent in tropical and subtropical regions and remains a significant global health concern. Among more than 200 *Plasmodium* species, five infect humans, with *P. falciparum* being the primary cause of severe malaria.[1] In Indonesia, the Annual Parasite Incidence (API) of malaria in 2019 increased compared to 2018, namely from the initial 0.84 to 0.93 per 1,000 population.[2] The Indonesian Ministry of Health also said in 2023 that the military group (TNI) was one of the most impacted, with 6,718 positive cases (1.61%).

Malaria treatment in Indonesia has relied on artemisinin-based combination therapy (ACT) since 2004, following the widespread emergence of chloroquine resistance.[3] However, resistance to ACT is growing and has been become a global concern, with failure rates greater than 10% in several African regions.[4]

While the emergence of resistant *Plasmodium* strains induced by the continued use of drugs that share similar molecular targets. Taken together, these underscore the urgent need for novel antimalarial agents with distinct mechanisms of action. Finding these kinds of chemicals could lead to treatments that are not only more effective and targeted, but also long-lasting in the fight against long-term resistance.[5]

Indonesia, a megadiversity country, provides a reservoir for antimalarial development and discovery in numerous plant species with promising pharmacological potential, including *Spatholobus littoralis* Hassk. (locally known as bajakah). Indigenous communities in Central Kalimantan traditionally utilize it. Bajakah extracts contain flavonoids, tannins, and saponins with antiparasitic activities.[6] In line with this study, the ethanol fraction was reported to have notable antiplasmodial effects against *Plasmodium falciparum.*[7] Hence, the bioactive compounds of *Spatholobus littoralis* (bajakah) offer a promising new approach for developing antimalarial agents and could represent a meaningful step forward in addressing the growing challenge of drug resistance.

Building on these findings, *in silico* studies targeting key *Plasmodium falciparum* proteins—the chloroquine resistance transporter (PfCRT) and the calcium ATPase pump (PfATP6)—can provide critical insights into molecular interactions underlying antimalarial resistance.[8,9] Both protein has an important role in chloroquine and artemisinin work. So that, PfCRT and PfATP6 are targets for compounds from bajakah. Although initial findings are encouraging, the majority of studies on *Spatholobus littoralis* Hassk. are limited to whole-organism validation at the level. The different ways to obtain data and the absence of standard protocols make it even more challenging to reproduce results and compare studies. Further, information related to safety and pharmacokinetics is scarce, underscoring the need for systematic preclinical and clinical evaluations.

Nonetheless, the *in-silico* approach remains a powerful early-stage strategy for accelerating compound screening, reducing research costs, and predicting pharmacokinetic and toxicity profiles. Molecular docking, molecular dynamics, and quantitative structure–activity relationship (QSAR) modeling are some of the methods that quickly find potential inhibitors before expensive biological testing.[10,11] In this study, molecular docking was conducted to generate models of PfCRT and PfATP6 and to evaluate the binding affinities of bajakah-derived compounds.

2. Method

Data Collection

The data collection process began with a literature study, which identified 46 compounds contained in the plant *Spatholobus littoralis* Hassk.[12,13,14,15,16] The method employed was in silico analysis, involving the docking of compounds obtained from the PubChem database. Six main potential compounds from *Spatholobus littoralis* Hassk. were identified, selected based on their drug-likeness activity derived from in silico analysis results. The tested compounds included milbemycin A4 oxime (CID_145710042), quercetin (CID_5280343), 8-O-methylretusin (CID_5319771), 6-methoxyeriodictyol (CID_14034285), 3',4',7-trihydroxyflavon (CID_5322065), and (+)-medioresinol (CID_181681). Positive controls used for comparison were chloroquine and artemisinin. These tests were conducted on the PfCRT receptor (PDB ID 6UKJ) and PfATP6 receptor (PDB ID 6RDU), both obtained from the NCBI protein database.

Compound Bioactivity Analysis

The bioactivity of the compounds was predicted using the Way2Drug software, accessible at https://www.way2drug.com/passonline. The Simplified Molecular Input Line Entry System (SMILES) for each compound was obtained from the PubChem database, corresponding to each compound's PubChem ID. The data selected for analysis met specific criteria, with a requirement of Pa > 0.7.

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Analysis

In drug development, the use of in silico methods enables the ADMET profile to serve as a crucial tool for predicting the pharmacological and toxicological properties of potential drugs, particularly during the early stages of research. This computational model focuses on enhancing ADMET predictions, which are crucial for optimizing drug development and preventing late-stage failures. Such failures not only consume significant time and financial resources but can also be avoided by utilizing these predictive models. This experiment utilized the pharmacokinetic capabilities of the pkCSM-pharmacokinetics website (https://biosig.lab.uq.edu.au/pkcsm/), which enables the estimation and optimization of a compound's pharmacokinetic attributes. The method relies on combining graph-based screening and experimental data. The main parameters in ADMET analysis include Caco-2 permeability, human intestinal absorption, volume of distribution (Vdss), compound interaction with cytochrome enzymes, blood-brain barrier penetration, total clearance, and acute toxicity.

Molecular Modeling

The structures of PfCRT and PfATP6 were initially obtained from the Protein Data Bank (PDB), then further validated and optimized using AlphaFold. This approach was chosen because computational methods enable the prediction of three-dimensional structures based on experimental data, resulting in more accurate models. AlphaFold is an application that utilizes deep learning algorithms, revolutionizing structural biology by enabling the assembly of protein sequences using template structures as references. In this process, the target protein sequences were retrieved from the NCBI database to ensure suitability and accuracy before structural validation was performed.

Protein Analysis and Evaluation

To assess the quality of protein models, we apply geometric and stereochemical criteria. This evaluation is conducted using the PDBsum website, accessible at http://www.ebi.ac.uk/pdbsum. PDBsum plays a crucial role in validating PDB structures, focusing on stereochemical accuracy and the precision of predictive models. The assessment also relies on Ramachandran plot calculations, which examine the torsion angles Ø and ψ within protein amino acid residues. The results are expressed as percentages categorizing regions as core, allowed, generously allowed, or disallowed, thereby providing a comprehensive measure of protein structure quality.

Molecular Docking

All ligands were obtained from the PubChem database corresponding to each compound's PubChem ID. Docking procedures were performed using CB-DOCK2 (Cavity-detection guided Blind docking). CB-DOCK2 was chosen because it offers more advanced and diverse data submission options, as well as more convenient result visualization. To ensure docking reliability, validation was conceptually supported by the Root Mean Square Deviation (RMSD) criterion (≤ 2.0 Å), which indicates that the docked pose aligns with the native ligand conformation.[17] For milbewmycin A4-oxime, the Vina search box dimensions cover the X-axis (30,35), Y-axis (35,35), and Z-axis (35,35), with the box center located at coordinates X (148,142), Y (151,242), and Z (153,267). For quercetin, the Vina search box dimensions cover the X-axis (21,30), Y-axis (21,35), and Z-axis (21,21), while the box center is at coordinates X (152,184), Y (173,264), and Z (138,193). Next, for 8-O-methylretusin, the Vina search box dimensions include the X-axis (22,28), Y-axis (22,35), and Z-axis (22,35), with the box center at coordinates X (152,152), Y (173,278), and Z (138,264). The compound 6-

methoxyeriodictyol has Vina search box dimensions covering the X-axis (22,28), Y-axis (22,35), and Z-axis (22,35), with the box center at coordinates X (152,152), Y (173,278), and Z (138,264). Then, for 3',4',7-trihydroxyflavon, the Vina search box dimensions cover the X-axis (21,28), Y-axis (21,35), and Z-axis (21,35), with the box center at coordinates X (152,152), Y (173,278), and Z (138,264). Finally, for (+)-medioresinol, the Vina search box dimensions include the X-axis (24,24), Y-axis (24,35), and Z-axis (24,35), with the box center located at coordinates X (152,152), Y (173,278), and Z (138,264). Protein docking with compounds and control drugs was visualized using Discovery Studio Client 2025, while protein-protein interactions were analyzed using PDBsum.

3. Result

Bioactivity of Compounds in Spatholobus littoralis Hassk.

The Prediction of Activity Spectra for Substances (PASS) analysis (Figure 1) revealed that four compounds possess potential antiparasitic, antiprotozoal, and antimycoplasmal activities. These findings are supported by significant bioactivity scores that correspond to antiparasitic mechanisms. Notably, the compounds are predicted to interfere with key parasite metabolic enzymes, including dihydrofolate reductase (DHFR), enoyl-ACP reductase (FabI), and ATPase, which are essential for folate metabolism, fatty acid synthesis, and ionic homeostasis, respectively. This predicted bioactivity requires further validation through experimental assay.

ADMET Analysis of *Spatholobus littoralis* Hassk.

The pKCSM prediction of Caco-2 permeability indicated that five of the six compounds had values above the 0.90 threshold, suggesting good potential for intestinal absorption. In contrast, 8-O-methylretusin exhibited a permeability value of −0.229, reflecting limited intestinal membrane penetration. Nevertheless, all compounds demonstrated human intestinal absorption values exceeding 70%, classifying them as well-absorbed molecules. In terms of P-glycoprotein (P-gp) interaction, quercetin and (+)-medioresinol were predicted to act as inhibitors, whereas milbemycin A4-oxime and 3',4',7-trihydroxyflavone served as substrates.

The distribution profiles, as reflected by VDss values, showed that milbemycin A4-oxime (1.332) and 8-O-methylretusin (1.559) possess high tissue distribution, while 6-methoxyeriodictyol and (+)-medioresinol exhibited lower distribution (log VDss < 0.45). Among all compounds, only milbemycin A4-oxime displayed favorable blood–brain barrier (BBB) permeability (log BB = 0.349), whereas the others were below the threshold, indicating limited central nervous system (CNS) penetration.

Cytochrome P450 enzyme interaction analysis revealed that 8-O-methylretusin, 6-methoxyeriodictyol, and 3',4',7-trihydroxyflavone act as CYP1A2 inhibitors, suggesting possible modulation of drug metabolism mediated by this isoenzyme. Meanwhile, milbemycin A4-oxime, quercetin, and (+)-medioresinol were identified as CYP3A4 substrates, indicating potential involvement in hepatic metabolism and drug–drug interactions.

Total clearance predictions showed that (+)-medioresinol had the lowest value (0.05 mL/min/kg), indicating slow elimination, while 3',4',7-trihydroxyflavone exhibited moderate clearance (0.513 mL/min/kg). The remaining compounds demonstrated higher elimination rates. Predicted rat LD_{50} values ranged from 2.149 to 2.851 mol/kg, indicating moderate acute toxicity among the tested compounds. Collectively, these findings provide valuable insights into the pharmacokinetic characteristics and safety

profiles of the bioactive constituents of *Spatholobus littoralis* Hassk.

Evaluation and Molecular Modeling

Molecular modeling was performed using the PDBsum server (Figure 2) and successfully generated structural models for the target proteins PfCRT and PfATP6, along with the control ligands chloroquine and artemisinin. Quality assessment of the PfCRT model, which consists of 523 residues, showed that 100% of the residues were located within the favored and allowed regions of the Ramachandran plot, confirming the high structural reliability of the model for subsequent analysis. Similarly, the PfATP6 model exhibited 100% of residues within favored and allowed regions, indicating comparable structural integrity and suitability for molecular docking studies.

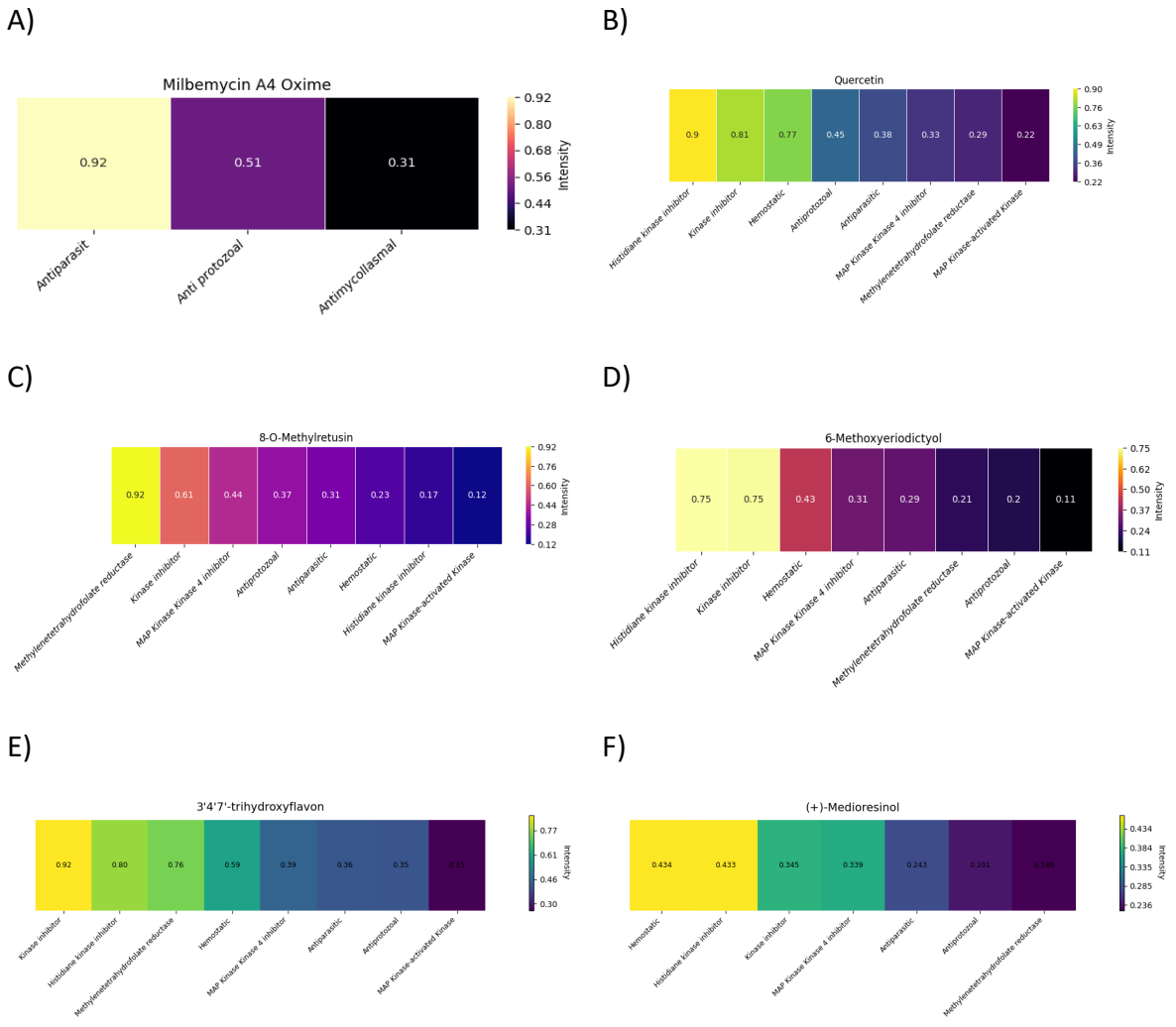


Figure 1. Predicted binding affinity heatmaps of selected Spatholobus littoralis compounds toward PfCRT and PfATP6 receptors obtained through Prediction of Activity Spectra for Substances (PASS). Compounds include (A) Milbemycin A4 oxime, (B) Quercetin, (C) 8-O-Methylretusin, (D) 6-Methoxyeriodictyol, (E) 3',4',7-Trihydroxyflavon, and (F) (+)-Medioresino.

A) B)

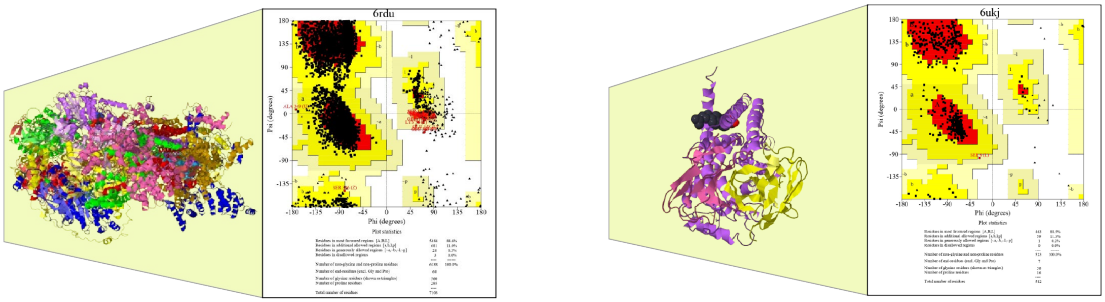
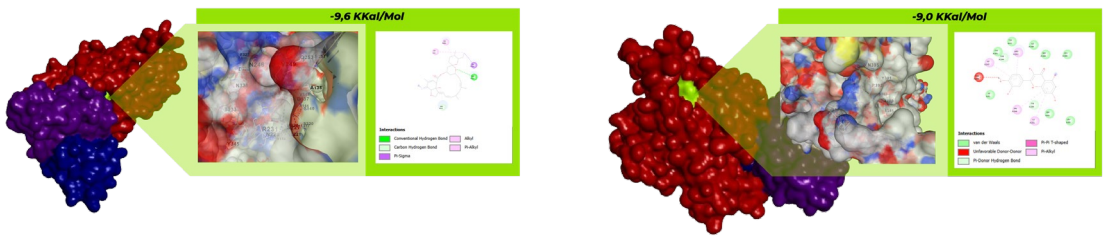


Figure 2. Three-dimensional structural models and Ramachandran plots of *Plasmodium falciparum* target proteins: (A) PfCRT and (B) PfATP6.

Protein Analysis and Evaluation

The molecular docking results (Figure 3) showed that the control compound, artemisinin (Figure 3(E)), exhibited a binding energy of −9.1 kcal/mol. Among the tested Spatholobus littoralis compounds, Milbemycin A4 oxime (Figure 2.4A) demonstrated the strongest affinity with a binding energy of −9.6 kcal/mol, suggesting its high potential as an inhibitor of key Plasmodium metabolic enzymes, including dihydrofolate reductase (DHFR), enoyl-ACP reductase (FabI), and ATPase, which are involved in folate metabolism, fatty acid synthesis, and ionic homeostasis. This compound formed carbon hydrogen bonds with several critical residues (Table 1), although an unfavorable donor–donor interaction was observed at residue GLN297, which may contribute to local protein instability and possible ligand dissociation. In addition, quercetin, 3’,4’,7-trihydroxyflavon, and (+)-medioresinol also exhibited inhibitory potential against essential parasite enzymes, albeit with weaker binding stability due to the presence of electrostatic repulsion between positively charged residues. These findings highlight Milbemycin A4 oxime as the most promising S. littoralis-derived candidate for further investigation to overcome artemisinin resistance in Plasmodium parasites.

A) B)



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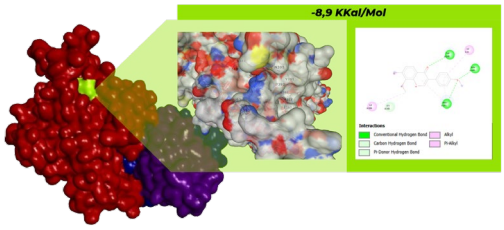

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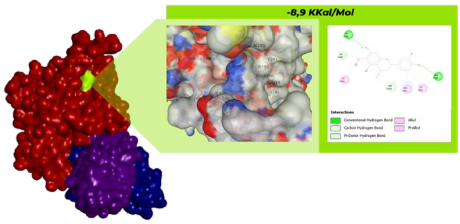

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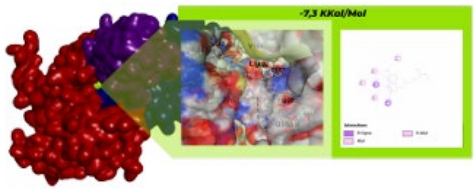


Figure 3. Binding conformations and interaction maps of *Spatholobus littoralis*-derived compounds within the PfCRT receptor binding pocket: (A) Milbemycin A4 oxime, (B) Quercetin, (C) 8-O-Methylretusin, (D) 6-Methoxyeriodictyol, and (E) Chloroquine (control). Hydrogen bonds, hydrophobic contacts, and electrostatic interactions are shown in the inset 2D interaction plots.

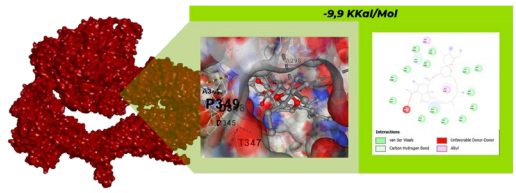
Table 1. Binding energies, key interacting residues, and interaction types of *Spatholobus littoralis* compounds docked with the PfCRT receptor.

Compound	Binding Energy	Key Residues	Type of Interactions
Chloroquine (positive control)	-7,3 kkal/mol	ARG 111, LEU 160, ALA 144, LEU 217, LEU 221, VAL 159	pi-sigma, dan alkyl
Milbemycin A4 oxime	-9,6 kkal/mol	GLN 253, ARG 111, TYR 110, VAL 141, HIS 97	Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Sigma, Alkyl, Pi-Alkyl
Quercetin	-9,0 kkal/mol	ILE 61, SER 65, LEU 69, LEU 385, SER 388, ILE 389, TYR 62, TYR 391, ASN 395, TYR 384, THR 344, VAL 348, ILE 351, ILE 347, ARG 392	van der Waals, Unfovorable Donor-Donor, Pi-Donor Hydrogen Bond, Pi-Pi T-shaped, Pi-Alkyl
8-O-Methylretusin	-8,9 kkal/mol	THR 344, ASN 395, ARG 392, LEU 385, ILE 389, ILE 61	Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Donor

			Hydrogen Bond, Alkyl, Pi-Alkyl
6-Methoxyeriodictyol	-8,9 kkal/mol	TYR 384, ASN 395, VAL 348, THR 344, ILE 351, ARG 392, ILE 61	Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Donor Hydrogen Bond, Alkyl, Pi-Alkyl

In addition, the molecular docking analysis for PfATP6 receptor (Figure 4) showed that the control compound, artemisinin (Figure 4(E)), exhibited a binding energy of −9.1 kcal/mol and formed alkyl interactions with residues TYR374, PHE453, and ALA450. This study highlights the potential of Spatholobus littoralis Hassk. bioactive compounds to interact with Plasmodium target proteins and therapeutic candidates against artemisinin-resistant strains. Milbemycin A4 oxime (Figure 4A) showed the most favorable binding energy (−9.9 kcal/mol), indicating strong affinity for the target site. It formed carbon-hydrogen bonds with several important residues (Table 2). This suggested Milbemycin A4 oxime potentially block important parasite metabolic enzymes like dihydrofolate reductase (DHFR), enoyl-ACP reductase (FabI), and ATPase, which is important for ionic homeostasis and membrane transport. An unfavorable donor–donor interaction was observed at residue GLN297, which may contribute to local instability and possible ligand dissociation. While, quercetin, 3’,4’,7-trihydroxyflavon, and (+)-medioresinol has comparable inhibitory potential against essential parasite enzymes but exhibited less stable binding due to electrostatic repulsion between positively charged residues.

A)



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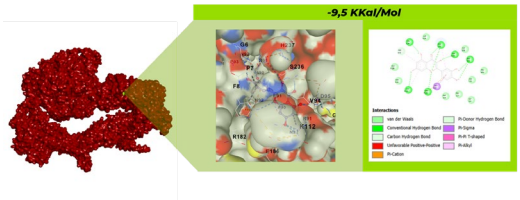

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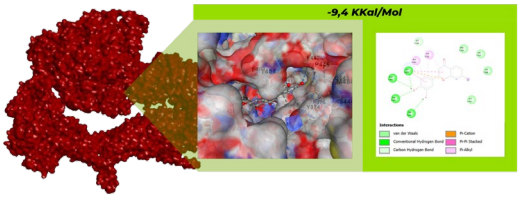

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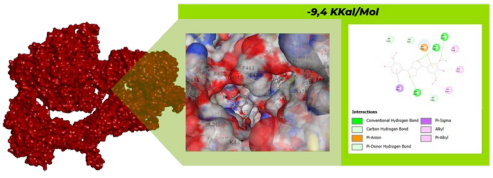

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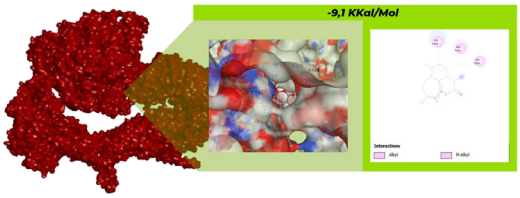

Figure 4. Molecular docking interactions of *Spatholobus littoralis* compounds with the PfATP6 receptor: (A) Milbemycin A4 oxime, (B) Quercetin, (C) 3',4',7-Trihydroxyflavon, (D) (+)-Medioresinol, and (E) Artemisinin (control).

Table 2. Binding energies, key interacting residues, and interaction types of *Spatholobus littoralis* compounds docked with the PfATP6 receptor.

Compound	Binding Energy	Key Residues	Type of Interactions
Artemisin (control)	-9,1 kkal/mol	TYR 374, PHE 453, ALA 450	Alkyl, Pi-Alkyl
Milbemycin A4 oxime	-9,9 kkal/mol	SER 391, ASP 345, ARG 294, ASP 389, ALA 392, GLN 386, THR 347, ALA 307, GLU 348, THR 301, THR 302, VAL 308, ARG 347, ASP 291, LYS 295, ALA 343, ALA 298, GLN 297	van der Waals, Carbon Hydrogen Bond, Unfavorable Donor-Donor, Alkyl
Quercetin	-9,5 kkal/mol	ARG 91 (C), SER 236, ARG 91 (B), ARG 182, HIS 237, ARG 91 (D), LYS 112, VAL 94, ASP 95, ALA 90, PRO 93, ASN 92 (C), GLU 186, ASN 92 (D), PRO 7, PHE 8, GLY 6	van der Waals, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Unfavorable Positive-Positive, Pi- Cation, Pi-Donor Hydrogen Bond, Pi-Sigma, Pi-Pi T-shaped, Pi-Alkyl
3',4',7-trihydroxyflavon	-9,4 kkal/mol	GLY 431, SER 428, VAL 427, ARG 429, TYR 374, GLN 445, PHE 447, THR 454, GLY 188, ALA 450, VAL 191	van der Waals, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi- Cation, Pi-Pi Stacked, Pi-Alkyl
(+)-medioresinol	-9,4 kkal/mol	ARG 335, GLN 297, THR 301, ASP 389, THR 347, GLU 348, SER 328, VAL 332, ALA 392, ALA 307, ALA 298	Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Anion, Pi-Donor Hydrogen Bond, Pi-Sigma, Alkyl, Pi-Alkyl

4. Discussion

In order to model PfCRT and PfATP6 and evaluate the binding affinities of chemicals from *Spatholobus littoralis*, this work used molecular docking. Their promise as new inhibitors is indicated by stable contacts at both active sites, which also serve as a foundation for the development of selective antimalarial drugs to counter drug resistance. Our study differs from previous ones, which mainly explored general biological potentials, such as antioxidants and anti-inflammatory, without focusing on specific molecular targets or computational analyses.[15,18] According to our research, two important compounds—quercetin and milbemycin A4-oxime—showed the strongest predicted affinities towards PfCRT and PfATP6. Quercetin was found to interact with the resistance variant site via van der Waals forces and hydrogen bonds, which supports this finding.[19] Additionally, milbemycin is also able to inhibit efflux pump ABC transporters, a resistance mechanism that is also present in PfCRT.[20] Then, both substances work in tandem to block Plasmodium sp. drug resistance pathways. However, these results require further study to confirm their antimalarial activity through in vitro and in vivo studies.

In addition to these two chemicals, 8-O-methylretusin, a flavonoid compound derived from retusin, is also present in *Spatholobus littoralis Hask*. The IC_{50} values range from 11.25 to 56.31 µM for the D6 strain and from 15.47 to 87.50 µM for the W2 strain of P. falciparum, which is a good sign for its antiplasmodial activity.[21] It works by inhibiting two important enzymes for parasite survival, enoyl-ACP reductase (PfENR) and dihydrofolate reductase (DHFR).[22] Moreover, 8-O-methylretusin may also target PfATP4, a pivotal membrane transporter for maintaining ion homeostasis. Their disturbance leads to ionic imbalance and even parasite mortality.[23]

This study also used ADMET profiling to evaluate the safety and pharmacokinetic appropriateness of candidate drugs, providing detailed computational information on their potential bioactivity. The best chemicals for oral bioavailability were found to be quercetin and milbemycin A4-oxime because of their high absorption efficiency and Caco-2 permeability. Their hepatotoxicity is also nonexistent, which makes them an exceptionally safe substance. Nonetheless, it was anticipated that they would have a favorable interaction with the well-known efflux transporter P-glycoprotein (P-gp). Multidrug resistance is thought to be exacerbated by lowering intracellular drug concentrations.[24] Although they demonstrated a promising foundation, more research is necessary to identify the ideal dose range and therapeutic index.

After verifying the ADMET profile, AlphaFold was used to thoroughly examine the interaction patterns. It is making remarkably accurate predictions about 3D protein structures. Significant changes in structural biology have been brought about by this approach, which has facilitated a deeper understanding of the relationships and functions of proteins.[25] Ramachandran plots were used to validate the PfCRT and PfATP6 models. This showed that there were allowed regions and favorable residue locations, indicating high-quality structures that were appropriate for docking. [5,26]

Docking study was conducted utilizing approved protein models based on validated protein models to assess the compound's interaction with PfCRT and PfATP6. Docking experiments for PfCRT revealed that milbemycin A4-oxime, quercetin, 8-O-methylretusin, and 6-methoxyeriodictyol have substantial inhibitory potential. Since Milbemycin A4-oxime had the highest binding affinity of all of them, indicating improved

complex stability, it stood out as a promising solution for overcoming chloroquine resistance. Quercetin exhibited advantageous inhibition, although a detrimental electron-donating interaction marginally diminished it. In the same way, 8-O-methylretusin and 6-methoxyeriodictyol showed strong binding energies, which could mean that they could stop PfCRT from working.

The following analysis of PfATP6 revealed that milbemycin A4-oxime and quercetin had binding affinities equal to or greater than those of artemisinin, indicating potential for multitarget inhibition. These interactions indicate the possibility that metabolites of Spatholobus littoralis can simultaneously modify multiple resistance pathways and enhance treatment efficacy against strains that are resistant to artemisinin. Although there was one negative donor–donor interaction at GLN297, milbemycin A4-oxime's overall binding stability was good. However, due to electrostatic repulsion between positively charged residues, quercetin exhibited somewhat reduced stability, while 3',4',7-trihydroxyflavon and (+)-medioresinol showed significant inhibitory potential.

Fortunately, the underlying mechanism of Milbemycin A4-oxime confirms its biological activity as a possible antimalarial medication. As a member of the macrolide lactone class, milbemycin A4-oxime works differently from artemisinin and chloroquine. By attaching to glutamate-gated chloride channels (GluCl) and GABA-related chloride receptors in invertebrate parasites, it stimulates chloride ion influx, membrane hyperpolarization, and parasite paralysis.[27,28] PfCRT and PfATP6, which are also involved in intracellular calcium regulation and ion transport, function similarly to this pathway. The chemical's strong interactions with these proteins raise the possibility that it could harm parasite ion homeostasis, intracellular signaling, and endoplasmic organelle stability, all of which could result in parasite mortality. Notably, Milbemycin A4-oxime may have similar antimalarial effects to its analog ivermectin, which interferes with Plasmodium's nucleocytoplasmic transport and liver-stage development.[29,30] By targeting disruption ion and calcium homeostasis, its unique mechanism works in tandem with current antimalarials to assist combat resistance and promote wider malaria control.[31] However, this analogy is proposed based on structural similarity within the macrolide lactone class. Further experiment is required to confirm whether comparable molecular targets and binding mechanisms are involved. Taken together, present study highlights Milbemycin A4-oxime as a promising lead for the development of new antimalarial agents. On the other hand, it only does in silico and predictive assessments. More in vitro and in vivo studies are mandatory to ensure its safety and pharmacological response.

This study conducted purely through using *in silico* approaches, including molecular docking, pharmacokinetic prediction, and bioactivity assessment. The present finding provides preliminary insights that require further confirmation through *in vitro* and *in vivo* experiments. It also should be note that, this study focused on PfCRT and PfATP6, without incorporating molecular dynamics simulations. These constraints may limit the understanding of ligand flexibility and binding stability.

5. Conclusion

This study demonstrated the antimalarial potential of bioactive compounds from *Spatholobus littoralis* Hassk. (bajakah wood) through an *in-silico* approach targeting PfATP6 and PfCRT proteins of *Plasmodium falciparum*. Among 46 compounds screened, Milbemycin A4-oxime emerged as the most

promising candidate based on bioactivity prediction, ADMET profiling, and molecular docking analyses. The compound exhibited strong binding affinity, stable target interactions, and favorable pharmacokinetic properties. Validation of the protein models through Ramachandran plots confirmed the reliability of the computational approach. These findings provide a solid foundation for developing novel antimalarial agents derived from Indonesian natural products.

References

1. Fikadu M, Ashenafi E. *Malaria: An overview*. Infect Drug Resist. 2023;16:3339–47.
2. Yayank Lewinsca M, Raharjo M, others. *Faktor Risiko yang Mempengaruhi Kejadian Malaria Di Indonesia: Review Literatur 2016-2020*. Jurnal Kesehatan Lingkungan. 2021;11(1):16–28.
3. Rosenthal PJ. *Are Artemisinin-Based combination therapies for malaria beginning to fail in Africa?*. Am J Trop Med Hyg. 2021;105(4):857–8.
4. Watson OJ, Muchiri S, Ward A, Meier-Sherling C, Asua V, Katairo T, et al. *Risk of selection and timelines for the continued spread of artemisinin and partner drug resistance in Africa*. bioRxiv. 2024;24312699
5. Coppée R, Sabbagh A, Clain J. *Structural and evolutionary analyses of the Plasmodium falciparum chloroquine resistance transporter*. Sci Rep. 2020;10(1):1–12.
6. Hadanu R, Irwansyah S, Sartika GP, Wahyuningrum R. *Compound characterization and evaluation of antioxidant potential of ethanol extract Spatholobus littoralis Hassk in Kolaka, Southeast Sulawesi, Indonesia*. J Pharm Res Int. 2023;35(28):59–70.
7. Anisa S, Wydiamala E, Hayatie L. *Efektivitas ekstrak etanol akar bajakah merah (Spatholobus littoralis Hassk) sebagai antimalaria secara in vitro terhadap Plasmodium falciparum*. Homeostasis. 2022;5(1):151–60.
8. Sanyaolu A, Marinkovic A, Prakash S, Balendra V, Shazley O, Gardellini T, et al. *Emerging molecular mechanisms in malaria pathogenesis and novel therapeutic approaches: A focus on P. falciparum malaria*. Biomolecules. 2025;15(7):1038.
9. Koukouikila-Koussounda F, Jeyaraj S, Nguetse CN, Nkonganyi CN, Kokou KC, Etoka-Beka MK, et al. *Molecular surveillance of Plasmodium falciparum drug resistance in the Republic of Congo: Four and nine years after the introduction of artemisinin-based combination therapy*. Malar J. 2017;16:155.
10. Ferreira LG, dos Santos RN, Oliva G, Andricopulo AD. *Molecular docking and structure-based drug design strategies*. Molecules. 2015;20(7):13384–421.
11. Kleandrova V V, Cordeiro MNDS, Speck-Planche A. *In Silico approach for early antimalarial drug discovery: de novo design of virtual Multi-Strain antiplasmodial inhibitors*. Microorganisms. 2025;13(7):1620.
12. Nursyafitri D, Ferdinan A, Rizki FS. *Skrining fitokimia dan parameter non-spesifik ekstrak etanol akar bajakah (Spatholobus littorilis Hassk.)*. Jurnal Farmasi IKIFA. 2021;1(1):64–70.
13. Iskandar A, Widodo N, Masruri M, Rollando R, Warsidah W. *Proposed functional activity of bioactive compounds from Spatholobus littoralis Hassk. in LC-MS-MS and in silico studies*. Materials Science Forum. 2022;1050:95–103.
14. Ariesanti Y, Wahyudina SP, Poedjiastoeti W, Angraini Y. *Antioxidant activity of roots, stems, and*

leaves of Spatholobus littoralis Hassk.: An experimental study. Padjadjaran Journal of Dentistry. 2023;35(3):206–10.

15. Az-Zahra SNR, Rahmania TA, Permana Y, Wardani AK. *Bajakah (Spatholobus littoralis Hassk.) as an anti-inflammatory herbal resource*. In: Proceedings of the International Conference on Pharmacy and Medical Applied Research (ICOPMAP 2025). 2025.
16. Sianipar RNR, Suryanegara L, Fatriasari W, Arung ET, Kusuma IW, Achmadi SS. *The role of selected flavonoids from bajakah tampala (Spatholobus littoralis Hassk.) stem on cosmetic properties: A review*. Saudi Pharm J. 2023;31(3):382-400.
17. Wang Z, Sun H, Yao X, Li D, Xu L, Li Y, et al. *Comprehensive evaluation of ten docking programs on a diverse set of protein – ligand complexes : the prediction accuracy of sampling power and scoring power*. Phys Chem Chem Phys. 2016;18(18):12964-75
18. Mahfudh N, Utami D, Nashihah S, Ahda M, Andika A, Sabilla GA. *Variability and pharmacological potential of bajakah (Spatholobus sp.) as an indigenous medicinal plant: A review*. Int J Public Health Sci. 2024;13(3):1470-1479
19. Sulyman AO, Aje OO, Ajani EO, Abdulsalam RA, Balogun FO, Sabiu S. *Bioprospection of Selected Plant Secondary Metabolites as Modulators of the Proteolytic Activity of Plasmodium falciparum Plasmepsin V*. Biomed Res Int. 2023:2023:6229503.
20. Aubry L, Brandalise D, Louvet M, Coste AT, Sanglard D, Lamoth F, et al. *Impact of milbemycin oxime on fluconazole resistance in Candida auris*. JAC Antimicrob Resist. 2025;7(2):dlaf060
21. Bekono BD, Ntie-Kang F, Onguéné PA, Lifongo LL, Sippl W, Fester K, et al. *The potential of anti-malarial compounds derived from African medicinal plants: A review of pharmacological evaluations from 2013 to 2019*. Malar J. 2020;19(1):183
22. Silva DAA, da Costa DM , Oliveira LM, Brandão HN , Alves CQ, Santos Jr. AF. *Identification of flavonoids as inhibitors of Plasmodium falciparum enoyl-ACP reductase by hierarchical virtual screening*. J Braz Chem Soc. 2020;31(9):1888–1899.
23. Rosling JEO, Ridgway MC, Summers RL, Kirk K, Lehane AM. *Biochemical characterization and chemical inhibition of PfATP4-associated Na+-ATPase activity in Plasmodium falciparum membranes*. J Biol Chem. 2018;293(34):13327–13337.
24. Si K, He X, Chen L, Zhang A, Guo C, Li M. *The structure of Plasmodium falciparum multidrug resistance protein 1 reveals an N-terminal regulatory domain*. PNAS. 2023;120(32):e2219905120.
25. Malhotra Y, John J, Yadav D, Sharma D, Vanshika V, Rawal K, Mishra V. *Advancements in protein structure prediction: A comparative overview of AlphaFold and its derivatives*. Comput Biol Med. 2025;188:109842.
26. N. N, C. GPD, Chakraborty C, V. K, D. TK, V. B, et al. *Mechanism of artemisinin resistance for malaria PfATP6 L263 mutations and discovering potential antimalarials: An integrated computational approach*. Sci Rep. 2016 Jul 29;6(1):30106.
27. DrugBank. *Milbemycin Oxime*. 2024.
28. Merola VM, Eubig PA. *Toxicology of Avermectins and Milbemycins (Macrocyclic Lactones) and the Role of P-Glycoprotein in Dogs and Cats*. Vet

Clin North Am Small Anim Pract. 2012;42(2):313-33

29. Mendes AM, Albuquerque IS, Machado M, Pissarra J, Meireles P, Prudêncio M. *Inhibition of Plasmodium liver infection by ivermectin*. Antimicrob Agents Chemother. 2017;61(2):e02005–16.
30. Singh L, Singh K. *Ivermectin: A promising therapeutic for fighting malaria. Status and perspective*. J Med Chem. 2021;64(14):9711–31.
31. de Oliveira LS, Alborghetti MR, Carneiro RG, Bastos IMD, Amino R, Grellier P, et al. Calcium in the Backstage of Malaria Parasite Biology. Front Cell Infect Microbiol. 2021;11(July):1–15.