Jurnal Biodjati 11(1): 78–89, May 2026

e-ISSN : 2541-4208
p-ISSN : 2548-1606

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

In Silico Evaluation of Drug-Likeness, ADMET Profile, and Toxicity of Antimalarial Compounds from the Bark of *Alstonia scholaris*

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Received: December 18, 2025
Revise from: December 26, 2025
Accepted: April 22, 2026

DOI: 10.15575/biodjati.v11i1.53359

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***Abstract**. Malaria remains a major global health problem, particularly in tropical countries, highlighting the need for new antimalarial agents with favorable pharmacokinetic and safety profiles. This study aimed to evaluate the drug-likeness, ADMET properties, and toxicity of six bioactive compounds derived from Alstonia scholaris bark using an in silico approach. Computational analyses were performed using SwissADME, pkCSM, AdmetSAR, and ProTox-3.0 to assess absorption, distribution, metabolism, excretion, and toxicity parameters, as well as Lipinski's Rule of Five. The results showed that four compounds met all Lipinski criteria, while two exhibited logP values greater than 5, suggesting potential limitations in lipophilicity. Predicted VDss values suggested moderate and variable systemic distribution, and none of the compounds were predicted to interact with CYP2D6. Toxicity analysis indicated generally low toxicity, with α-Amyrin classified as non-toxic (LD_{50} = 70,000 mg/kg; toxicity class VI). Among the compounds, Spirost-8-en-11-one demonstrated the most favorable pharmacokinetic profile, with high gastrointestinal absorption and the highest total clearance. In conclusion, the evaluated compounds exhibit promising pharmacokinetic and safety profiles as potential antimalarial candidates. These findings provide preliminary computational evidence supporting further in vitro and in vivo validation to confirm their efficacy and safety.*

***Keywords**: Alstonia scholaris, in silico, malaria, toxicity*

INTRODUCTION

Malaria is one of the most widespread infectious tropical diseases globally. The World Health Organization (WHO) estimated that 224-276 million malaria cases occurred worldwide in 2021. The disease is caused by Plasmodium parasites that infect red blood cells and are transmitted to humans through mosquito bites (Hebber et al., 2021). In tropical and subtropical countries, malaria continues to contribute significantly to mortality rates, particularly among infants, young children, and pregnant women (Irawan et al., 2017). Indonesia, with its tropical

Citation
Haq, M. Z. A., Fahriza, M. R., Insani, G. T., Rahmatullah, M. F. P., Waluyo, S., Pratama, A. P., Wahid, N. B. A., Primandiri, P. R., & Santoso, A. M. 2026. In Silico Evaluation of Drug-Likeness, ADMET Profile, and Toxicity of Antimalarial Compounds from the Bark of *Alstonia scholaris*. *Jurnal Biodjati, 11*(1), 78-89.

climate, remains one of the countries affected by malaria and has designated malaria as a national priority health issue under the Ministry of Health Regulation No. 293/SK/IV/2009 (Isnani et al., 2021). Resistance of Plasmodium parasites to first-line antimalarial drugs has further complicated control efforts (Kakisina & Ukratalo, 2011), prompting the exploration of natural product-based therapies from medicinal plants as alternative strategies (Shadrack et al., 2016). Among these, *Alstonia scholaris* (L.) R. Br. (pule) has been widely documented as a traditional antimalarial plant used by communities across Indonesia (Mufidah & Zuhrotun, 2020).

A. scholaris is a widely distributed tree species in Indonesia whose bark has long been used ethnopharmacologically to treat malaria (Pratap et al., 2013). In Papua and Central Maluku, communities have traditionally consumed boiled pule bark decoctions to treat fever and alleviate malaria symptoms (Abdul M Ukratalo, 2025; Gunawan & Simaremare, 2016; Mayor & Wattimena, 2022). The antimalarial activity of pule bark is attributed to its alkaloid content (Kartika et al., 2023). Hebber et al., (2021) identified six bioactive compounds from *A. scholaris* bark through GC–MS analysis, namely Urs-12-en-24-oic acid, 3-oxo-, methyl ester; α-Amyrin, 9,19-Cyclolanost-24-en-3-ol acetate (3β); Spirost-8-en-11-one; Olean-12-en-3-ol acetate (3β); and 7-[β-D-ribofuranosyl]imidazo[4,5-d][1,2,3]triazin-4-one (2-azainosine). Although these compounds demonstrated potential antimalarial activity through molecular docking, their pharmacokinetic (ADMET) profiles have not been systematically evaluated. Hebber et al. (2021) identified these six compounds through GC-MS analysis and recommended further *in vitro* and *in vivo* studies to evaluate their pharmacological potential. The present study responds to this recommendation by providing a preliminary computational pharmacokinetic assessment as a foundational step before experimental investigation. Importantly, these traditional practices are carried out without standardized dosage guidelines or evidence-based safety references, raising concerns about the risks of unregulated consumption.

Scientific validation of traditional herbal medicines is essential to support their safe application and regulatory compliance (Khan et al., 2025; Shan et al., 2025). *In silico* approaches have been previously employed to evaluate the pharmacological potential of bioactive compounds from natural sources, including studies published in this journal (Fakih et al., 2021; Taupiqurrohman et al., 2022). In the case of *A. scholaris*, no systematic evaluation has been conducted on the drug-likeness, metabolic stability via cytochrome P450 enzyme interactions, blood-brain barrier permeability, or acute oral toxicity class of its key bioactive compounds, information critical for determining their pharmacological feasibility as drug candidates. This gap is particularly significant given the widespread traditional use of pule bark across Indonesia without any pharmacokinetic or toxicological safety basis. Therefore, this study aims to evaluate the *in silico* pharmacokinetic (ADMET) properties and toxicity profiles of bioactive compounds from *A. scholaris* bark to assess their preliminary drug-likeness and safety characteristics as potential antimalarial drug candidates.

MATERIALS AND METHODS

Research Design

This study employed a descriptive–exploratory research design to evaluate the *in silico* pharmacokinetic (ADMET) properties and toxicity profiles of bioactive compounds from *A. scholaris* bark. This design was selected to systematically describe and explore the pharmacolog-

ical characteristics of the selected compounds based on computational prediction data (Malikhana et al., 2021).

Procedure

The research procedure began with a literature search conducted using Google Scholar and PubMed databases to identify bioactive compounds from *A. scholaris* bark with reported antimalarial activity. Compounds were included based on the following criteria: (1) identified from *A. scholaris* bark through GC–MS analysis as reported by Hebber et al. (2021), (2) reported to have potential antimalarial activity in prior *in silico* studies, and (3) have canonical SMILES data available in public chemical databases. Based on these criteria, all six bioactive compounds reported by Hebber et al. (2021) were selected for analysis, namely Urs-12-en-24-oic acid, 3-oxo-, methyl ester, α-Amyrin, 9,19-Cyclolanost-24-en-3-ol acetate (3β); Spirost-8-en-11-one; Olean-12-en-3-ol acetate (3β); and 7-[β-D-ribofuranosyl]imidazo[4,5-d][1,2,3]triazin-4-one (2-azainosine). The canonical SMILES data for all six compounds were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/, accessed March 2025) and used as input for all subsequent computational analyses.

The drug-likeness of all six compounds was evaluated using Lipinski's Rule of Five through the SwissADME web tool (http://www.swissadme.ch/, accessed March 2025) using default platform settings (Daina et al., 2017). A compound was considered drug-like if it satisfied the following criteria: molecular weight ≤ 500 Da, logP ≤ 5, hydrogen bond acceptors (HBA) ≤ 10, and hydrogen bond donors (HBD) ≤ 5 (Lipinski, 2000).

The ADMET pharmacokinetic profiles of all six compounds were subsequently predicted using SwissADME (accessed March 2025) and pkCSM (https://biosig.lab.uq.edu.au/pkcsm/, accessed March 2025), with all analyses performed using default settings. Absorption was assessed based on gastrointestinal absorption (HIA); distribution was evaluated through blood-brain barrier (BBB) permeability and steady-state volume of distribution (VDss); metabolism was assessed through predicted interactions with cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4); and excretion was evaluated based on total clearance (TCl) rate (Shofi, 2022).

Toxicity analysis was performed using AdmetSAR (http://lmmd.ecust.edu.cn/admetsar1/home/, accessed March 2025) and ProTox-3.0 (https://tox.charite.de/protox3/, accessed March 2025), both using default prediction settings. The median lethal dose (LD_{50}) and toxicity classification of each compound were determined based on ProTox-3.0 output, following the GHS oral toxicity classification system (Malmfors & Teiling, 1983). The BOILED-Egg plot was generated directly using the SwissADME web tool.

RESULTS AND DISCUSSION

The analysis was conducted on six antimalarial compounds from the bark of *A. scholaris* (Figure 1), namely Urs-12-en-24-oic acid, 3-oxo-, methyl ester, α-Amyrin, 9,19-Cyclolanost-24-en-3-ol acetate (3β); Spirost-8-en-11-one; Olean-12-en-3-ol acetate (3β); and 7-[β-D-ribofuranosyl]imidazo[4,5-d][1,2,3]triazin-4-one (2-azainosine). The evaluation began with an assessment of these six compounds as potential drug candidates using Lipinski's rule of five via the SwissADME web platform.

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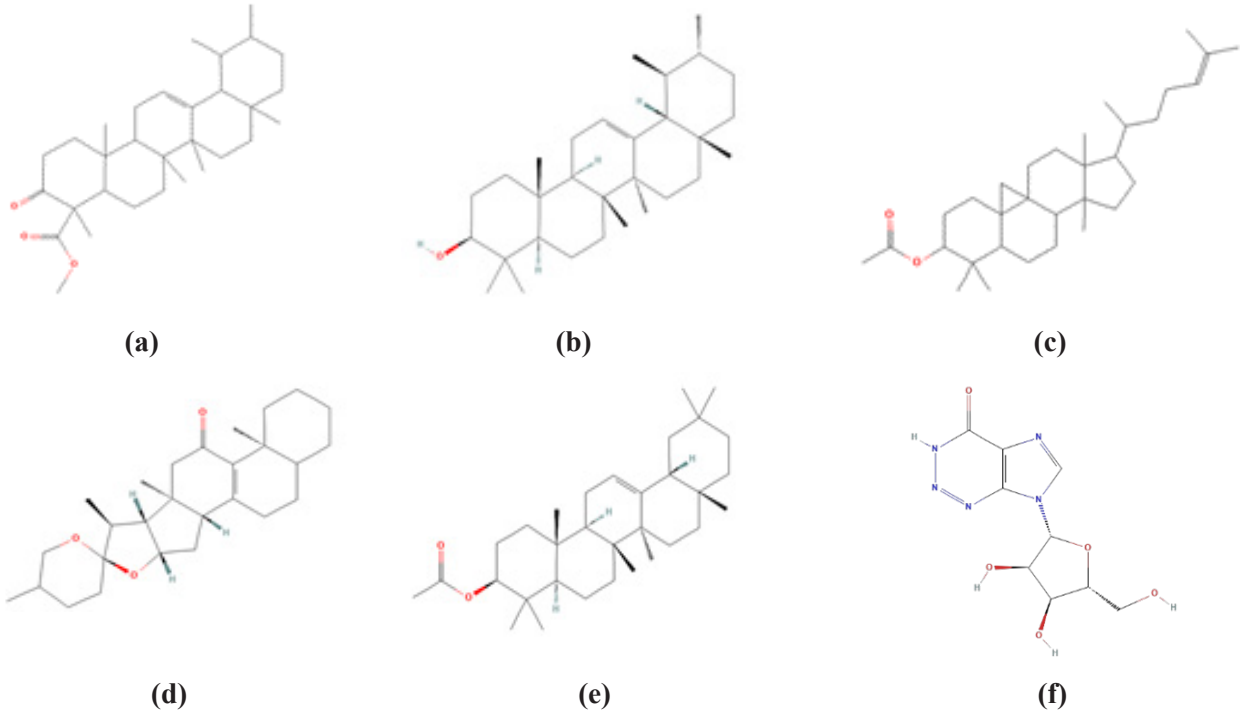

Figure 1. Two-dimensional structures of compounds: (a) Urs- 12-en-24- oic acid, 3-oxo-, methyl-ester; (b) α-Amyrin; (c) 9,19-Cyclolanost-24-en-3-ol, acetate, (3β); (d) Spirost-8-en-11-one; (e) Olean-12-en-3-ol, acetate, (3β); (f) 7-[β-D-ribofuranosyl]imidazo[4,5-d][1,2,3]triazin-4-one (2-azainosine).

Table 1. The lipinski's rule of five criteria to assess the bioactive compounds of *A. scholaris* L.

Compound	Lipinski's Rule				
	Molecular Mass ≤ 500 Da	**MlogP ≤ 4,15 or log P ≤ 5**	**Hydrogen Bond Acceptors (HBA) ≤ 10**	**Hydrogen Bond Donors (HBD) ≤ 5**	**Solubility in Water**
Urs-12-en-24-oic acid, 3-oxo-, methyl ester	468.71 g/mol	4.63	3	0	*Poorly soluble*
α-Amyrin	426.72 g/mol	4.77	1	1	*Poorly soluble*
9,19-Cyclolanost-24-en-3-ol, acetate, (3β)	468.75 g/mol	5.19	2	0	*Poorly soluble*
Spirost-8-en-11-one	412.60 g/mol	4.34	3	0	*Poorly soluble*
Olean-12-en-3-ol, acetate, (3β)-	468.75 g/mol	5.35	2	0	*Poorly soluble*
7-[β-D-ribofuranosyl] imidazo[4,5-d][1,2,3] triazin-4-one (2-azainosine)	269.21 g/mol	-1.45	8	4	*Very soluble*

The six bioactive compounds from *A. scholaris* bark were evaluated against Lipinski's Rule of Five criteria (Table 1) to assess their drug-likeness as potential oral drug candidates (Sinurat et al., 2021). Based on the SwissADME analysis, all six compounds had molecular weights below 500 g/mol, hydrogen bond acceptors ≤ 10, and hydrogen bond donors ≤ 5 (Coimbra et al., 2020). However, two compounds, 9,19-Cyclolanost-24-en-3-ol, acetate, (3β) and Olean-12-en-3-ol, acetate, (3β), exhibited logP values exceeding 5 (5.19 and 5.35, respectively), indicating a violation of the lipophilicity criterion that may reduce oral bioavailability due to poor aqueous solubility. The remaining four compounds fully complied with all Lipinski criteria.

In terms of water solubility, five compounds were categorized as poorly soluble, which may limit their absorption in the gastrointestinal tract (Bhalani et al., 2022; Halim et al., 2012). Notably, 2-azainosine was the only compound classified as very soluble (consensus logP = –1.45), a physicochemical advantage that may support higher gastrointestinal absorption compared to the other five compounds, as further reflected in the predicted ADMET results shown in Table 2. These findings indicate that although most compounds meet the general criteria for drug-likeness, variations in lipophilicity and solubility may influence oral bioavailability, which is an important consideration in the development of antimalarial drug candidates.

Table 2. Predicted ADMET results

Compound	Gi Absorption	BBB Permeat	VDss (logL/kg)	TCl	Pgp substrate	CYP Inhibitor					Bioavailability score
						1A2	2C19	2C9	2D6	3A4	
Urs-12-en-24-oic acid, 3-oxo-, methyl ester	Low	No	-0,054	0,069	No	No	No	Yes	No	No	0,55
α-Amyrin	Low	No	0,266	0,119	No	No	No	No	No	No	0,55
9,19-Cyclolanost-24-en-3-olacetate, (3β)	Low	No	0,1	-0,134	No	No	No	No	No	No	0,55
Spirost-8-en-11-one	High	No	0,278	0,205	No	No	No	No	No	No	0,55
Olean-12-en-3-ol, acetate, (3β)-	Low	No	-0,194	0,134	No	No	No	No	No	No	0,55
7-[β-D-ribofuranosyl]imidazo[4,5-d] [1,2,3] triazin-4-one (2-azainosine)	Low	No	0.044	0.51	No	No	No	No	No	No	0,55

Note : BBB permeant = blood–brain barrier; VDss = volume of distribution; TCl = total clearance; CYP inhibitor = cytochrome P450 enzyme inhibitor

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Three compounds, 9,19-Cyclolanost-24-en-3-ol acetate (3β); Olean-12-en-3-ol acetate (3β); and α-Amyrin are indicated as out of range in the BOILED-Egg plot due to their high WLOGP values exceeding the visualization boundary, consistent with their high lipophilicity profile (Daina et al., 2017). It is important to note that this study represents a secondary computational profiling analysis. The antimalarial potential of these compounds is inherited from prior experimental findings by Hebber et al. (2021) and is not directly demonstrated in this work. Rather, this study aims to characterize the pharmacokinetic and toxicological profiles of these compounds as a preliminary step toward evaluating their suitability as oral antimalarial drug candidates before target-based or docking studies.

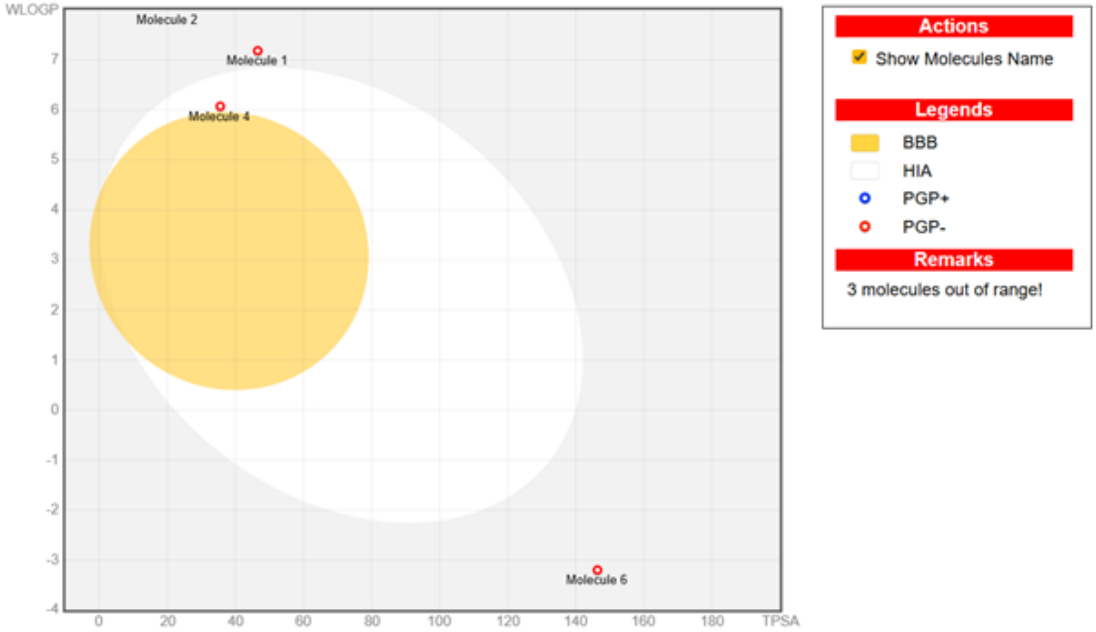

Figure 2. BOILED-Egg plot including BBB, HIA, and P-gp

Based on the SwissADME predictions for the six compounds analyzed, Spirost-8-en-11-one exhibited high GI absorption, indicating a strong ability for gastrointestinal absorption (HIA). This property facilitates the absorption process in the digestive tract, particularly in the intestines, followed by the compound's transfer into the circulatory system. Such a process plays a primary role in determining the bioavailability and pharmacokinetics of the bioactive compounds (Liu et al., 2022). Among the six predicted compounds, none were found to pass through the BBB (Figure 2). Figure 2 presents the BOILED-Egg plot generated directly from SwissADME. In this visualization, the white region (egg white) represents compounds predicted to have high gastrointestinal absorption (HIA+). In contrast, the yellow region (egg yolk) indicates compounds predicted to penetrate the blood-brain barrier (BBB+). Compounds located outside both regions are predicted to exhibit low absorption and no BBB penetration. As shown in Figure 2, Spirost-8-en-11-one is positioned within the white region, confirming its high HIA prediction, while the remaining compounds fall outside both zones. Three compounds were noted as out of range in the BOILED-Egg visualization due to their exceptionally high lipophilicity (WLOGP > 8), which places them beyond the graphical boundaries of the plot. This does not indicate a computational error but rather reflects their extreme lipophilic character, already captured in the Lipinski analysis (Lipinski, 2000), suggesting that these compounds are predicted

to have limited penetration into the central nervous system via passive diffusion. However, it should be noted that the lack of BBB penetration does not entirely rule out neurological or systemic toxicity through other mechanisms, and this prediction requires further experimental validation (Shofi, 2022).

As shown in Table 2, the predicted VDss values of the six compounds ranged from –0.194 to 0.278 log L/kg, suggesting varying degrees of systemic distribution rather than uniform plasma distribution. These values indicate that the compounds tend to distribute moderately into tissues relative to plasma, and further pharmacokinetic validation is required to confirm their in vivo distribution behavior (Pires et al., 2015). The VDss reflects the extent to which a compound distributes into tissues relative to its plasma concentration; a higher VDss value indicates greater tissue distribution than plasma concentration (Pires et al., 2015). The total clearance (TCl) analysis showed that Spirost-8-en-11-one possessed the highest clearance rate among the six compounds. This finding implies that Spirost-8-en-11-one exhibits the most efficient rate of drug elimination from the body among the other compounds (Blake et al., 2005).

Based on SwissADME and pkCSM predictions, none of the six compounds were identified as P-glycoprotein (P-gp) substrates, suggesting that their distribution and bioavailability are unlikely to be affected by P-gp-mediated efflux mechanisms, In general, drug metabolism primarily occurs in the liver and intestines, where the cytochrome P450 (CYP) enzyme plays a main role not only in the oxidation but also elimination of drugs from the cells. These two vital organs significantly influence the efficacy, safety, and absorption of therapeutic compounds (Song et al., 2021). Among the six compounds, only one was identified as a potential CYP enzyme inhibitor. According to Chen et al. (2024), cytochrome P450 (CYP) inhibitors have a substantial impact on drug metabolism by altering the enzymatic activity responsible for the transformation of various drugs and xenobiotics. The CYP groups (e.g., CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) are involved in about 75% of cellular metabolic processes. Inhibition of these enzymes may result in elevated plasma concentrations of drugs metabolized through these pathways. It increases the risk of adverse drug reactions and toxicity (Zhao et al., 2021).

CYP inhibition analysis revealed that among the six compounds, only Urs-12-en-24-oic acid, 3-oxo-, methyl ester was identified as a CYP2C9 inhibitor. In contrast, the remaining five compounds, α-Amyrin, 9,19-Cyclolanost-24-en-3-ol acetate (3β), Spirost-8-en-11-one, Olean-12-en-3-ol acetate (3β), and 2-azainosine, exhibited no inhibitory activity, including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. As a major hepatic enzyme, CYP2C9 metabolizes approximately 15% of clinically used drugs, such as warfarin and several nonsteroidal anti-inflammatory drugs (NSAIDs). Inhibition of CYP2C9 can elevate the plasma concentrations of co-administered drugs metabolized via the same pathway, thereby increasing the risk of drug-drug interactions (Chen et al., 2024). Consequently, the potential co-administration of Urs-12-en-24-oic acid, 3-oxo-, methyl ester with CYP2C9 substrates warrants careful consideration during further drug development. Conversely, the lack of inhibition by the other five compounds indicates a favorable metabolic safety profile with minimal risk of interfering with hepatic drug metabolism (Zhao et al., 2021). None of the compounds inhibited CYP2D6, suggesting no predicted interaction with CYP2D6 in this computational model; however, this finding alone does not establish hepatic safety, as hepatotoxicity is a multifactorial endpoint that cannot be concluded from a single CYP interaction prediction (Kim et al., 2015). Overall, the CYP inhibition profiles of these compounds are considered safe, although the specific CYP2C9 inhibitory activity of Urs-12-en-24-oic acid, 3-oxo-, methyl ester requires further in vitro and in vivo investigation.

Table 3. Toxicity test results

Compound	Predicted LD50	Toxicity Class	Probability of acute oral toxicity
Urs-12-en-24-oic acid, 3-oxo-, methyl ester	1190 mg/kg	IV	71,38%
α-Amyrin	70000 mg/kg	VI	82,99%
9,19-Cyclolanost-24-en-3-ol, acetate, (3β)	1190 mg/kg	IV	87,04%
Spirost-8-en-11-one	1190 mg/kg	IV	61,47%
Olean-12-en-3-ol, acetate, (3β)-	1190 mg/kg	IV	77,49%
7-[β-D-ribofuranosyl]imidazo[4,5-d][1,2,3] triazin-4-one (2-azainosine)	3390 mg/kg	V	68,07%

The toxicity prediction results presented in Table 3, obtained through ProTox-3.0, indicate that among the six analyzed compounds, four, Urs-12-en-24-oic acid, 9,19-Cyclolanost-24-en-3-ol acetate (3β), Spirost-8-en-11-one, and Olean-12-en-3-ol acetate (3β), were classified under toxicity class IV with an LD_{50} value of 1190 mg/kg. According to the ProTox classification system, class IV compounds are considered harmful if swallowed, characterized by an LD_{50} of more than 300 mg/kg but less than 2000 mg/kg. One compound, α-Amyrin, was classified as class VI with an LD_{50} of 70,000 mg/kg, indicating a non-toxic classification, as class VI compounds are defined by an LD_{50} exceeding 5000 mg/kg (Malmfors & Teiling, 1983). According to Deora et al. (2010), LD_{50}, also known as the median lethal dose, is a significant parameter in toxicology, indicating the amount of a substance required to cause death in 50% of the test organisms. This metric plays an essential role in assessing the acute toxicity of pharmacological compounds. Among the six analyzed compounds, 9,19-Cyclolanost-24-en-3-ol, acetate (3β), exhibited the highest acute oral toxicity. This finding is consistent with the results reported by Mvondo et al. (2021), who evaluated nine quinine derivatives as potential antimalarial candidates using the same SwissADME and pkCSM platforms and found that compounds classified under toxicity class IV with LD_{50} values between 1192–1618 mg/kg still demonstrated acceptable pharmacokinetic profiles as oral drug candidates. The present findings align with this precedent, suggesting that class IV classification alone does not preclude further development of these compounds as antimalarial candidates, provided that additional experimental validation is conducted. Among the compounds, 9,19-Cyclolanost-24-en-3-ol acetate (3β) showed the highest predicted toxicity. 2-azainosine was classified under toxicity class V with a predicted LD_{50} of 3390 mg/kg, indicating a relatively low acute oral toxicity compared to class IV compounds. It should be noted, however, that this prediction was associated with a lower accuracy (68.07%) and average similarity score (63.67%) in ProTox-3.0, suggesting that the structural analogs of this compound are underrepresented in the training dataset. Therefore, this result should be interpreted with greater caution compared to the other compounds.

Compared to similar *in silico* profiling studies on plant-derived antimalarial compounds, the ADMET profiles obtained in this study are generally consistent with reported findings. Shadrack et al. (2016) previously identified antimalarial compounds from Hoslundia opposita with promising in silico inhibitory activity against PfLDH enzyme through molecular docking, and concluded with a recommendation for pharmacokinetic evaluation a gap that the present study addresses through systematic ADMET profiling of compounds from *A. scholaris*. Furthermore, Mvondo et al. (2021), who evaluated quinine derivatives as antimalarial candidates using the same SwissADME and pkCSM platforms, reported that compounds classified under toxicity class IV with LD_{50} values between 1192–1618 mg/kg still demonstrated acceptable pharmacokinetic profiles as oral

drug candidates, a finding consistent with four of the six compounds analyzed in this study. The high GI absorption observed in Spirost-8-en-11-one is particularly noteworthy, as favorable intestinal absorption is considered a key prerequisite for oral bioavailability in antimalarial drug development (Liu et al., 2022).

The utilization of *A. scholaris* as an antimalarial remedy by local communities across various regions of Indonesia has been extensively reviewed. Mayor & Wattimena (2022) reported that in Central Maluku (Indonesia), the bark of the pule tree is traditionally used as an antimalarial medicine by boiling it before consumption. Similarly, Kiat et al. (2019) noted that people in some tribes in Raja Ampat (Indonesia) use boiled pule bark as a traditional remedy for ailments such as toothache and high cholesterol. Furthermore, Abdul M Ukratalo (2025) documented that residents of Saleman Village in Central Maluku also use pule bark decoctions to treat fever, stimulate appetite, and alleviate malaria symptoms.

However, based on ethnopharmacological observations, these traditional practices often lack standardized dosage guidelines for pule bark use as an antimalarial treatment. The toxicity predictions from this study provide a preliminary computational indication of relative acute oral toxicity, but no safe human dosage can be concluded from these data alone. Further *in vivo* toxicological studies and pharmacokinetic validation are essential before any therapeutic dosage recommendation can be established for human use.

CONCLUSION

This study evaluated six bioactive compounds from *Alstonia scholaris* bark as potential antimalarial candidates using in silico analyses of drug-likeness, ADMET properties, and toxicity. Four compounds fulfilled all Lipinski's Rule of Five criteria, while two compounds showed minor violations related to lipophilicity. Among the evaluated compounds, Spirost-8-en-11-one demonstrated the most favorable pharmacokinetic profile, particularly in gastrointestinal absorption and total clearance, whereas α-Amyrin exhibited the lowest toxicity (toxicity class VI). Overall, the analyzed compounds show promising pharmacokinetic feasibility and safety profiles as potential antimalarial agents. However, these findings are based on computational predictions, and further experimental validation is required to confirm their efficacy and safety.

AUTHOR CONTRIBUTION

M.Z.A.H, **M.R.F.**, **G.T.I.**, and **M.F.P.R.** designed the research project, collected the data, and wrote the manuscript. **S.W.** & **P.R.P.** validated the method; **A.T.P.** & **N.B.A.W.** reviewed the paper; and **A.M.S.** supervised the project-based learning, conceptualized the design research, and finalized the article.

ACKNOWLEDGMENTS

This article is one of the products of students who took part in a project-based biocomputational class implemented through the Ethno-ASICC learning model. The authors thank the Directorate of Research and Community Service (DPPM) of Kemdiktisaintek Republik Indonesia for funding this research through the Applied Research Scheme (Number: 109/ES/PG.02.00.PL/2024).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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