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In Silico Study: the Potential of Apigenin Compounds from Moringa Plants as Inhibitors of Dihydroorotate Dehydrogenase Enzyme of Plasmodium Falciparum in Hepatic Malaria Therapy

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ABSTRACT

Malaria is a global health problem caused by Plasmodium infection, with hepatic malaria representing a serious complication due to liver dysfunction, particularly from Plasmodium falciparum. One promising therapeutic target is the enzyme dihydroorotate dehydrogenase (PfDHODH), which plays a crucial role in the parasite's de novo pyrimidine synthesis. Moringa oleifera is known to contain various flavonoids with antimalarial and hepatoprotective activities, including apigenin. This study aimed to evaluate the potential of apigenin as an inhibitor of PfDHODH for hepatic malaria treatment using an in silico approach. The research employed a one-shot experimental design involving molecular docking simulations with AutoDock Vina, followed by pharmacokinetic, drug-likeness, and toxicity predictions using SwissADME, pkCSM, and ProTox3. Results showed that apigenin exhibited strong binding affinity to PfDHODH (-8.9 kcal/mol), comparable to the primary control ligand (-9.3 kcal/mol) and higher than artemisinin (-8.7 kcal/mol). Apigenin demonstrated favorable ADME properties, complied with Lipinski's rule, and was classified as low toxicity (class 5) with no predicted carcinogenic effects. These findings indicate that apigenin from Moringa oleifera has promising potential as a PfDHODH inhibitor and may serve as a candidate for the treatment of hepatic malaria, warranting further experimental validation.

KEYWORDS *Apigenin; PfDHODH; hepatic malaria; in silico.*

INTRODUCTION

Malaria is a tropical and subtropical disease caused by *Plasmodium* infection that poses a global health problem. According to WHO data in 2023, there were 263 million cases, and the death toll reached 597,000 in malaria-endemic countries (World Health Organization, 2024). According to data from the Ministry of Health, there were 443,530 positive malaria cases in Indonesia in 2022 and 71 deaths due to malaria, with high-endemic areas in the provinces of Papua and East Nusa Tenggara (NTT) (Directorate General of Disease Prevention and Control, 2023). Malaria, caused by the *Plasmodium* parasite, can be transmitted through the bite of the *Anopheles* mosquito, with *P. falciparum* being the main species causing severe, life-threatening malaria.

Plasmodium has a complex life cycle that depends on two hosts: the *Anopheles* mosquito and humans. In humans, there are two phases of the cycle: the exo-erythrocytic cycle and the erythrocytic cycle (CDC, 2024). Based on this cycle, malaria can cause serious complications from severe malaria due to *P. falciparum* infection, which can affect various organ functions, including the liver—also known as *hepatic malaria* or *hepatopathy malaria*—with typical symptoms such as metabolic disorders, elevated liver enzymes, and hepatomegaly that can lead to liver failure. *Hepatic malaria* occurs due to parasite invasion of hepatocytes and immune dysfunction, causing inflammation and impaired liver function. Liver damage is often associated with cases of severe malaria, but it has now been confirmed that uncomplicated

malaria can also cause liver injury. Liver dysfunction is characterized by elevated liver enzymes (ALT/AST) in 69% of patients upon hospitalization (Reuling et al., 2018).

Hepatic malaria, particularly in *P. falciparum* infections, is frequently associated with severe complications such as cerebral malaria, shock, hyponatremia, acute respiratory distress syndrome (ARDS), and disseminated intravascular coagulation (DIC); moreover, acute kidney injury can occur across malaria types, while *hepatic malaria* caused by *P. falciparum* is significantly associated with prolonged hospitalization, delayed parasite clearance, slower resolution of fever, and persistent jaundice, unlike vivax malaria (Jain et al., 2016). The survival and metabolism of *P. falciparum* rely on key enzymes, including *dihydroorotate dehydrogenase* (DHODH), a mitochondrial flavoprotein essential for de novo pyrimidine biosynthesis required for RNA and DNA replication, such that inhibition of DHODH disrupts parasite replication and viability (Owoloye, 2020).

According to the WHO, the primary treatment for malaria, particularly *P. falciparum*, generally uses antimalarial drugs, namely Artemisinin-based Combination Therapies (ACTs) (World Health Organization, 2023). However, resistance to several antimalarial drugs, such as ACTs and chloroquine—which are used clinically against malaria parasites—has been reported. This occurs in *P. falciparum*, which is resistant to artemisinin-based ACTs due to a polymorphism in the Kelch-13-propeller protein that inhibits parasite control (Owoloye, 2020).

Drug resistance in malaria therapy reduces the sensitivity and effectiveness of antimalarial drugs due to the adaptive ability of *Plasmodium* species, while liver injury in malaria patients may arise not only as a complication of severe disease but also from hepatotoxic antimalarial agents that further impair liver function (Reuling et al., 2018). In response to the increasing burden of malaria, alternative therapeutic strategies have been explored using natural resources such as *Moringa oleifera*, which contains flavonoids—including apigenin—that can inhibit *P. falciparum DHODH*, a key enzyme in de novo pyrimidine synthesis required for parasite DNA replication, by binding to its active site and disrupting the oxidation of dihydroorotate to orotate (Owoloye, 2020; Sergey et al., 2016). Previous studies have reported that apigenin, kaempferol, rutin, and quercetin exhibit promising antimalarial activity in both in vitro and in vivo models (Bezerra et al., 2023).

Moringa oleifera, often referred to as the “tree of life” or “miracle tree,” is rich in diverse bioactive compounds, including alkaloids, flavonoids, anthraquinones, vitamins, glycosides, and terpenes, which underpin its antioxidant, anticancer, antihypertensive, hepatoprotective, and nutritional properties (Pareek et al., 2023). Flavonoids present in *Moringa* flowers and leaves—such as apigenin, quercetin, kaempferol, isoquercetin, and rhamnetin—are particularly associated with hepatoprotective and antioxidant effects (Pareek et al., 2023; Van Quan et al., 2023). Apigenin (4,5,7-trihydroxyflavone), one of the most abundant flavonoids in plants, has demonstrated antimalarial potential by inhibiting β-hematin formation and interfering with parasite development through inhibition of de novo pyrimidine biosynthesis, disruption of membrane formation during the intraerythrocytic phase, and suppression of hemoglobin degradation, thereby preventing parasite growth and malaria-associated anemia (Adeoye et al., 2019; Veronica et al., 2020; Owoloye, 2020).

In this study, the authors used an *in silico* method, hoping to demonstrate that the flavonoid-derived compound apigenin can interact with the active site of the *DHODH* enzyme in *Plasmodium* and has the potential to inhibit its function. Apigenin's effects, *as the title*

suggests, are interrelated: not only as an antiparasitic but also as a hepatoprotective agent, thus potentially preventing or treating liver damage caused by malaria.

Based on the background described, the *in silico study* method is crucial because it is expected to provide baseline data for further in vitro and in vivo research, thus supporting the potential development of phytopharmaceutical-based drugs in Indonesia as more selective, safe, and readily available malaria therapies due to the readily available ingredients, particularly in Indonesia. Furthermore, this research serves as an effort to prevent and treat malaria, thereby reducing malaria cases nationally and internationally.

This research is expected to provide significant benefits academically, appliedly, and socially. Scientifically, this study provides basic computational data on the potential of apigenin as an inhibitor of the *PfDHODH* enzyme, as well as enriching knowledge about the use of flavonoids from local Indonesian plants for the treatment of malaria accompanied by hepatic complications. These findings could be a strong foundation for further in vitro and in vivo research. From the applied side, the identification of new compound candidates that work through essential enzyme inhibition mechanisms has the potential to address the problem of existing antimalarial drug resistance. Furthermore, apigenin offers a dual advantage because it not only acts as an antiparasite but also has a hepatoprotective effect that can prevent or restore liver damage due to infection. This supports the development of phytopharmaceuticals based on Indonesian natural resources that are more selective, safe, and easy to obtain. From a social and public health perspective, this research contributes to efforts to reduce malaria illness and mortality in endemic areas, encourage the utilization and cultivation of economically valuable *Moringa* plants, and support national and global malaria control programs that align with sustainable development goals.

METHOD

This research used a One-Shot Experimental Study to test the potential of apigenin as an inhibitor of the enzyme dihydroorotate dehydrogenase (PfDHODH) in the de novo biosynthesis process of pyrimidine in *Plasmodium falciparum* as a therapy of hepatic malaria, with each variable observed once. The method used is an in silico study which includes the preparation of active compounds, prediction of compound potential, molecular docking, prediction of ADME (Absorption, Distribution, Metabolism, and Excretion), and prediction of toxicity. The 3D structure of the PfDHODH protein (PDB ID: 6GJG) was obtained from RCSB PDB and prepared using the Discovery Studio Client 2024 by removing water molecules and ligands, while the apigenin ligand structure (CID 5280443) was downloaded from PubChem, minimized in energy, and converted to .pdb format using Open Babel at PyRx 0.8. Molecular docking was carried out using AutoDock Vina at PyRx 0.8 with coordinate parameters center_x = 14.2554; center_y = 10.3713; center_z = 25.2089 and exhaustiveness 50, using control ligands in the form of native ligands and artemisinin, with the strength of the interaction assessed based on the increasingly negative bond energy and visualized in 2D and 3D using the Discovery Studio Client 2024.

ADME prediction was carried out using the PKCSM webserver to evaluate the pharmacokinetic properties of the compound, then analyzed based on Lipinski's rule which includes molecular weight, lipophilicity, hydrogen bond donors and acceptors, and molecular

refractivity (Long et al., 2019), with the aim of assessing the ability of compounds to penetrate the blood brain barrier, water solubility, skin permeability, intestinal absorption through Human Intestinal Absorption, and the excretory process. Furthermore, toxicity prediction was performed using the Pro-Tox and PKCSM webservers to determine potential long-term toxicity in cells and organs, including an estimated lethal dose value of 50 (LD50) as an indicator of the dose that caused death in 50% of test subjects after administration of the compound.

There are six categories of toxicity based on data on the Globally Harmonized System (GHS), as follows:

Table 1. Category LD50

Classes	Properties	Toxic Dose /LD50
1	Fatal if swallowed	LD50 ≤ 5 mg/kgBB
2	Fatal if swallowed	5 mg/kgBB ≤ LD50 ≤ 50 mg/kgBB
3	Toxic when ingested	50 mg/kgBB < LD50 ≤ 300 mg/kgBB
4	Dangerous when ingested	300 mg/kgBB < LD50 ≤ 2,000 mg/kgBB
5	May be dangerous if ingested	2,000 mg/kgBB < LD50 ≤ 5,000 mg/kgBB
6	Harmless and non-toxic	LD50 > 5,000 mg/kgBB

Source: adapted from the Globally Harmonized System/GHS

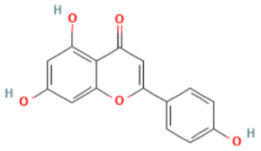
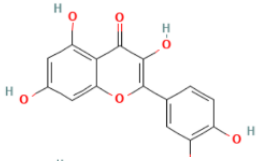
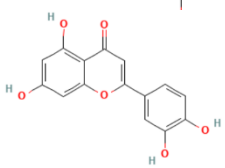
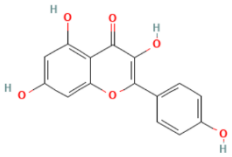
RESULT AND DISCUSSION

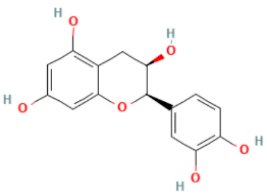
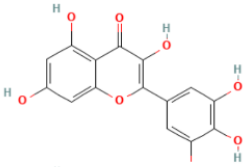
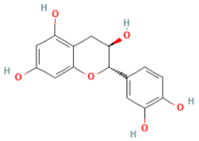
Identification of the Chemical Structure of Active Compounds in Moringa oleifera

The Moringa plant contains 46 active compounds, primarily found in the leaves and seeds. Previous research has identified several flavonoids with potential antimalarial properties, including quercitrin, naringin, apigenin, quercetin, myricetin, epicatechin, luteolin, catechin, and kaempferol. Flavonoids have a hydroxyl group (-OH) bound to an aromatic ring, and their structural patterns influence interactions with enzymes or proteins of the Plasmodium parasite.

Moringa contains various bioactive compounds with antimalarial properties, classified as flavonoids. The antimalarial effects of these bioactive compounds are also influenced by their chemical structure. The following are the chemical structures of the active compounds in the Moringa plant, obtained from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/).

Tabel 2. Struktur kimia senyawa aktif dalam kelor sebagai inhibitor PfDHODH

No	Substance	2D Structure	CID Pubchem	Molecule Mass
1.	Apigenin		5280443	270.24 g/mol
2.	Quercetin		5280343	302.23 g/mol
3.	Luteolin		5280445	286.24 g/mol
4.	Kaempferol		5280863	286.24 g/mol

No	Substance	2D Structure	CID Pubchem	Molecule Mass
		C15H10O6		
5.	Epicatechin		72276	290.27 g/mol
6.	Myricetin		5281672	318.23 g/mol
7.	Catechin		9064	290.27 g/mol

Source: PubChem

Stages of Analysis of the Interaction of Apigenin Compounds with Dihydroorotate Dehydrogenase Protein

Analyzing the predicted effects of apigenin compounds from Moringa oleifera and control compounds (3,6-Dimethyl-N-(4-(trifluoromethyl)phenyl)oxazolo[5,4-d]pyrimidin-4-amine and artemisinin) on inhibiting the growth and development of Plasmodium falciparum by performing several prediction stages, namely: 1) Predicting the binding energy value of the active compound with the target protein; 2) Predicting the binding of the active compound with the PfDHODH protein by molecular docking using AutoDock Vina software integrated with PyRx 0.8; 3) 2D and 3D visualization of the binding of the active compound with the target protein Dihydroorotate Dehydrogenase of Plasmodium falciparum using Discovery Studio Client 2024 software.

1. **Prediction Results of the Binding Energy Value of Apigenin Compounds with Target Proteins**

The binding affinity value obtained from molecular docking aims to predict the strength of a binding energy interaction between a ligand and a protein. The active site of the protein structure was prepared using Discovery Studio Client 2024 software by removing water molecules and ligands. The interaction between the ligand and the receptor is said to be stronger if the value obtained is more negative. Docking was performed with Protein 3D. The target protein, PfDHODH, had its sequence downloaded from https://www.rcsb.org/ (Owoloye, 2020), with artemisinin as the control (PubChem accession code CID68827), and 3,6-Dimethyl-N-(4-(trifluoromethyl)phenyl)oxazolo[5,4-d]pyrimidin-4-amine from the webserver https://www.rcsb.org/ (code 6GJG) used as a comparison.

The molecular docking results of apigenin have a lower binding affinity than 3,6-Dimethyl-N-(4-(trifluoromethyl)phenyl)oxazolo[5,4-d]pyrimidin-4-amine, but apigenin has a greater value than artemisinin as the main drug for malaria.

2. Prediction Results of Apigenin Compound Binding Type with PfDHODH

Docking values generated from AutoDock Vina integrated on PyRx 0.8 between proteins and ligands. The PfDHODH protein interacted with apigenin and control compounds at coordinates center_x = 14.2554; center_y = 10.3713; and center_z = 25.2089 with exhaustiveness 50. The control compound 3,6-Dimethyl-N-(4-(trifluoromethyl)phenyl)oxazolo[5,4-d]pyrimidin-4-amine used ligands obtained from synthesis results based on isoxazolopyrimidine fragments in previous research by Kokkonda et al. (2018) and artemisinin was obtained from the PubChem website. The interaction between proteins and ligands was visualized in 2D and 3D visualizations using Discovery Studio Client 2024 software.

Table 1. Predicted Results of Interaction of Apigenin, 3,6-Dimethyl-N-(4-(trifluoromethyl)phenyl)oxazolo[5,4-d]pyrimidin-4-amine, and Artemisinin with PfDHODH Protein

Ligand	Energy Bonding	Interaction	Distance (A)	Types of Bonds	Bond Type
Apigenin	-8,9 kcal/mol	:LIG1:H - A:GLY181:O	2.69348	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:H - A:PHE188:O	2.7013	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:H - A:CYS233:SG	2.93208	Hydrogen Bond	Conventional Hydrogen Bond
		A:VAL532:CG1 - :LIG1	3.9415	Hydrophobic	Pi-Sigma
		A:VAL532:CG2 - :LIG1	3.91557	Hydrophobic	Pi-Sigma
		:LIG1 - A:PHE188	4.51107	Hydrophobic	Pi-Pi T-shaped
		:LIG1 - A:PHE227	5.11231	Hydrophobic	Pi-Pi T-shaped
		:LIG1 - A:PHE227	4.9908	Hydrophobic	Pi-Pi T-shaped
		:LIG1 - A:LEU531	5.39517	Hydrophobic	Pi-Alkyl
		:LIG1 - A:CYS184	5.01236	Hydrophobic	Pi-Alkyl
3,6-dimethyl-~{N}-[4-(trifluoromethyl)phenyl]-[1,2]oxazolo[5,4-d]pyrimidin-4-amine	-9,5 kcal/mol	A:F1T603:H - A:LEU531:O	2.96484	Hydrogen Bond	Conventional Hydrogen Bond
		A:GLY181:CA - A:F1T603:O	3.40767	Hydrogen Bond	Carbon Hydrogen Bond
		A:ARG265:CD - A:F1T603:O	3.5261	Hydrogen Bond	Carbon Hydrogen Bond
		A:PHE188:O - A:F1T603:F	3.21296	Halogen	Halogen (Fluorine)
		A:PHE188:O - A:F1T603:F1	3.22865	Halogen	Halogen (Fluorine)
		A:F1T603:C4 - A:HIS185	3.95308	Hydrophobic	Pi-Sigma
		A:VAL532:CG1 - A:F1T603	3.70734	Hydrophobic	Pi-Sigma
		A:VAL532:CG1 - A:F1T603	3.65208	Hydrophobic	Pi-Sigma

Ligand	Energy Bonding	Interaction	Distance (A)	Types of Bonds	Bond Type
		A:VAL532:CG2 - A:F1T603	3.55453	Hydrophobic	Pi-Sigma
		A:F1T603 - A:PHE188	4.79129	Hydrophobic	Pi-Pi T-shaped
		A:F1T603 - A:PHE227	4.5919	Hydrophobic	Pi-Pi T-shaped
		A:F1T603:C12 - A:LEU197	5.45424	Hydrophobic	Alkyl
		A:F1T603 - A:CYS184	3.96501	Hydrophobic	Pi-Alkyl
		A:F1T603 - A:CYS184	5.39721	Hydrophobic	Pi-Alkyl
		A:PHE227 - A:F1T603:C12	5.18255	Hydrophobic	Pi-Alkyl
Artemisinin	-8,7 kcal/mol	A:ASN342:ND2 - :LIG1:O	3.04819	Hydrogen Bond	Conventional Hydrogen Bond
		A:LYS429:NZ - :LIG1:O	3.12162	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:C - A:CYS276	4.75322	Hydrophobic	Alkyl
		:LIG1:C - A:ALA224	4.28655	Hydrophobic	Alkyl
		A:CYS276 - :LIG1	5.31065	Hydrophobic	Alkyl
		A:PHE278 - :LIG1:C	4.97883	Hydrophobic	Pi-Alkyl
		A:TYR528 - :LIG1	4.05753	Hydrophobic	Pi-Alkyl
		A:TYR528 - :LIG1:C	4.52841	Hydrophobic	Pi-Alkyl
		A:TYR528 - :LIG1:C	5.39124	Hydrophobic	Pi-Alkyl

Source: docking simulation results using AutoDock Vina/PyRx

3. Molecular Docking Visualization Results

The docking results of proteins and ligands using AutoDock Vina integrated with PyRx 0.8, docking visualizations with 2D, 3D views, and interactions were carried out using Discovery Studio Client 2024 software.

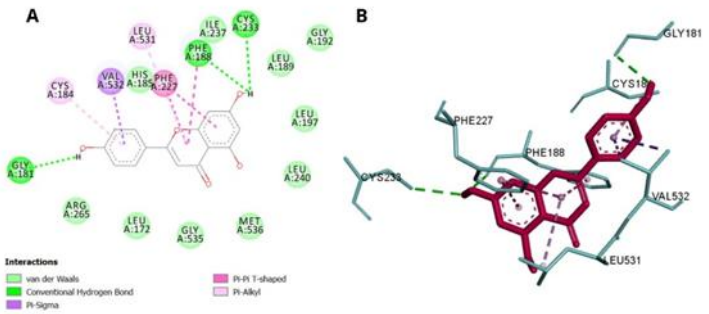


Figure 1. Interaction between apigenin compounds and PfDHODH proteins

Description: A) 2D Structure and B) 3D Structure

Source: visualization using Discovery Studio Client 2024

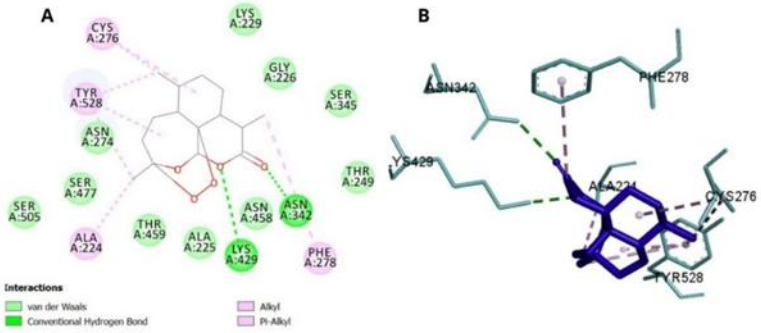
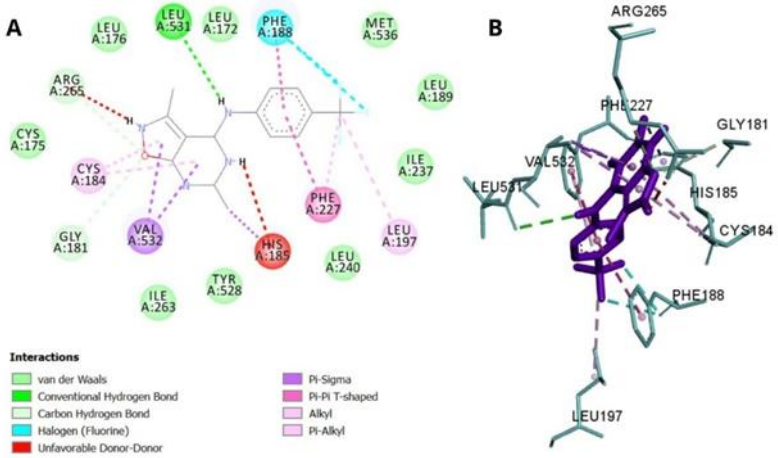


Figure 2. Interaction between artemisinin compounds and PfDHODH proteins
Description: A) 2D Structure and B) 3D Structure
Source: visualization using Discovery Studio Client 2024



Gambar 3. Interaksi antara senyawa *3,6-dimethyl-~{N}-[4-(trifluoromethyl)phenyl]-[1,2]oxazolo[5,4-d]pyrimidin-4-amine* dengan protein PfDHODH
A) 2D Structure and B) 3D Structure
Source: visualization using Discovery Studio Client 2024

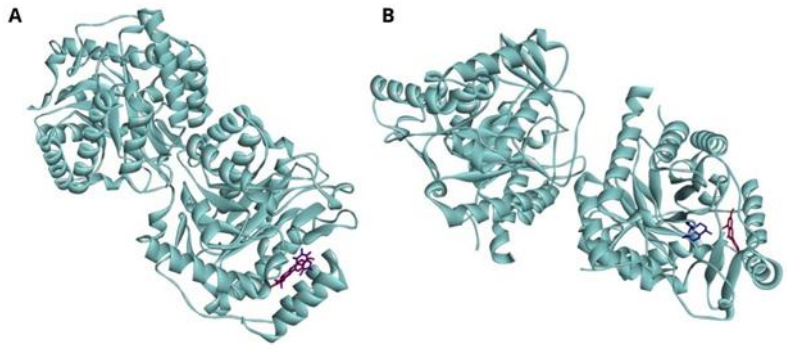


Figure 4. Interaction of inhibitory compounds with PfDHODH
Source: visualization using Discovery Studio Client 2024

Predicted Results of Bioactivity of Target Compounds

The bioactivity value of the target compound is obtained by inputting data using the WAY2DRUG PASS prediction tool (http://www.pharmaexpert.ru/passonline/predict.php). The Pa (Probability to be Active) value describes the potential of a tested compound. This value reflects the accuracy of the prediction function obtained. The higher the Pa value, the better the accuracy. This bioactivity value is determined by comparing the structure of the input

compound with compounds that have been proven to be effective as a specific treatment (Filimonov et al., 2014).

Table 2. Prediction Results of Bioactivity of Apigenin and Artemsinin Compounds

Bioactivity	Apigenin	Artemisinin
Antiprotozoal	0,342 Pa	0,992 Pa
Antiprotozoal (*Plasmodium*)	0,263 Pa	0,954 Pa

Source: analysis using PASS Online

The PASS prediction results are presented in the form of Pa (probability of active compounds) and Pi (probability of inactive compounds) values in the range of 0.000–1.000, with the provision that Pa > Pi indicates potential biological activity and in general Pa + Pi ≠ 1 (Arabi & Kawsar, 2023). The interpretation of PASS results refers to Filimonov et al. (2014), where a Pa value > 0.7 indicates a high chance of experimental confirmation and generally has structural similarities to known drugs, a value of 0.5 < Pa < 0.7 indicates a lower chance of experimental activity but with a smaller degree of similarity to existing pharmaceutical agents, while Pa < 0.5 indicates a low chance of experimental activity, shows the potential of the compound as a parent candidate for the development of new chemical classes with certain biological activities.

Predictive Results of Absorption, Distribution, Metabolism, Excretion, (ADME) & Druglikeness of Target Compounds

The ADME (Absorption, Distribution, Metabolism, and Excretion) properties of drug candidates play a crucial role in the drug discovery process. The PKCSM online tools database (https://biosig.lab.uq.edu.au/pkcsm/) is an in silico approach used to predict the pharmacokinetic properties of a drug compound (Pires et al., 2015). Another parameter used in druglikeness prediction is Lipinski's rules of five using Protox 3 (Banerjee et al., 2024).

Table 5. Results of Adsorption, Distribution, Metabolism, and Excretion of Apigenin and Artemisinin

Property	Model Name	Unit	Apigenin	Artemisinin
Absorption	Water solubility	Numeric (log mol/L)	-3.329	-3.678
	Caco2 permeability	Numeric (log Papp in 10- 6 cm/s)	1.007	1.295
	Intestinal absorption (human)	Numeric (% Absorbed)	93.25	97.543
	Skin Permeability	Numeric (log Kp)	-2.735	-3.158
	P-glycoprotein substrate	Categorical (Yes/No)	Yes	No.
	P-glycoprotein I inhibitor	Categorical (Yes/No)	No.	No.
	P-glycoprotein II inhibitor	Categorical (Yes/No)	No.	No.
Distribution	VDss (human)	Numeric (log L/kg)	0.822	0.457

	Fraction unbound (human)	Numeric (Fu)	0.147	0.4
	BBB permeability	Numeric (log BB)	-0.734	0.235 d
	CNS permeability	Numeric (log PS)	-2.061	-2.909
Metabolism	CYP2D6 substrate	Categorical (Yes/No)	No.	No.
	CYP3A4 substrate	Categorical (Yes/No)	No.	Yes
	CYP1A2 inhibitior	Categorical (Yes/No)	Yes	Yes
	CYP2C19 inhibitior	Categorical (Yes/No)	No.	No.
	CYP2C9 inhibitior	Categorical (Yes/No)	No.	No.
	CYP2D6 inhibitior	Categorical (Yes/No)	No.	No.
	CYP3A4 inhibitior	Categorical (Yes/No)	No.	No.
Excretion	Total Clearance	(log ml/min/kg)	0.566	0.98
	Renal OCT2 substrate	Categorical (Yes/No)	No.	No.

Description:
VDss = *Volume of Distribution at Steady State*
BBB = *Blood Brain Barrier*

Source: analysis using pkCSM

Table 6. Pharmacokinetic and Physicochemical Results of Apigenin and Artemisinin according to Lipinski's Rule

Remarks	Apigenin	Artemisinin
Molweight (g/mol)	270.24	282.33
Hydrogen Bond Acceptors	4	5
Hydrogen Bond Donors	3	0
Lipophilicity	<5	<5
Molecular Refractivity (Generally: 40-130)	73.99	70.38
Rotable Bonds (<10)	1	0
GI Absorption	High	High
BBB Permeant	No.	Yes
Number of Atoms	20	20

Source: analysis using pkCSM and ProTox3

Results of Apigenin Compound Toxicity Prediction

1. Results of Prediction of Toxicity of Compounds Based on GHS Category

Compound toxicity prediction uses the Protox 3 webserver (https://tox.charite.de/protox3/). This webserver helps determine the lethal dose (Lethal Dose 50), meaning 50% of test subjects die when exposed to the compound. The safety level of a drug is determined by its LD50 value (Banerjee et al., 2024).

Table 3. Results of Prediction of Toxicity of Compounds Based on GHS Category

Parameter	Apigenin	Artemisinin
LD50 (mg/kg)	2500	4228
Toxicity Class	5	5

Source: analysis using ProTox-3

2. Results of Prediction of Toxicity of Compounds to Organs and Environment

Prediction of toxicity to organs and the surrounding environment of target compounds was carried out using the Canonical SMILES structure of apigenin and artemisinin compounds inputted into the PKCSM tool (https://biosig.lab.uq.edu.au/pkcsm/) (Pires et al., 2015).

Table 8. Results of Predicting the Toxicity of Apigenin and Artemisinin Compounds to Organs and the Surrounding Environment

	Substance	Apigenin	Artemisinin
Toxicity	AMES toxicity	No.	Yes
	Max. tolerated dose (human) (log mg/kg/day)	0.328	0.065
	hERG I inhibitor	No.	No.
	hERG II inhibitor	No.	No.
	Oral Rat Acute Toxicity (LD50) (mol/kg)	2.45	2.459
	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	2.298	1
	Hepatotoxicity	No.	No.
	Skin Sensitisation	No.	No.
	T.Pyriformis toxicity (log ug/L)	0.38	0.322
	Minnow toxicity (log mM)	2.432	1.406

Source: analysis using pkCSM

Discussion of the Chemical Structure of Active Compounds in Moringa with Potential Antimalarial Properties

The Moringa oleifera plant has long been used in traditional medicine for its phytochemical content and pharmacological activities, including antiplasmodial activity. This study identified several active flavonoids—catechin, quercetin, apigenin, kaempferol, luteolin, myricetin, and epicatechin—that exhibit biological activities such as antioxidant, anti-inflammatory, and antiplasmodial activity through inhibition of the dihydroorotate dehydrogenase (PfDHODH) enzyme in Plasmodium falciparum. Flavonoids have a C6–C3–C6 basic skeleton, with two aromatic rings and one heterocyclic ring; this structure supports hydrogen bond formation, hydrophobic interactions, and π–π stacking at the enzyme's active site. Apigenin ($C_{15}H_{10}O_5$) is in focus because its hydroxyl group is able to interact with the catalytic residue of PfDHODH, while the planar aromatic ring system increases stability and binding affinity.

Apigenin has a relatively smaller number of OH groups, making it more structurally stable than other flavonoids; this is relevant because highly polar hydroxyl groups can decrease the membrane permeability of drugs (Hu et al., 2025; Bhanot & Sundriyal, 2021). Although

molecular weight is often associated with protein-ligand binding strength, this relationship is not always linear; the larger-mass myricetin has been reported to have a lower binding energy than apigenin (Owoloye, 2020), confirming that the stability and chemical nature of the functional groups are more important determinants of affinity (Karnan et al., 2023; Kenny, 2019). Inhibition of PfDHODH disrupts de novo pyrimidine synthesis—specifically the formation of dUMP and dTMP—thus inhibiting parasite DNA replication. Thus, apigenin exhibits structural stability and interaction efficiency that support its potential as an oral antimalarial, strengthening the prospects of Moringa leaves as an antiplasmodial source.

Discussion of the Results of the Molecular Docking Stages of Apigenin Compounds in *Moringa oleifera*

The PfDHODH (Plasmodium falciparum dihydroorotate dehydrogenase) enzyme is an important target in the development of antimalarial drugs because it plays a crucial role in the de novo biosynthesis of pyrimidines required by the parasite for DNA and RNA synthesis, considering that Plasmodium does not have an alternative pathway (salvage pathway) to form pyrimidines. The 3D structure of PfDHODH was obtained from RCSB PDB with the code 6GJG, while the structures of the apigenin ligand and reference compounds were taken from PubChem. Ligand-protein interactions were analyzed in silico through molecular docking using AutoDock Vina integrated in PyRx 0.8, with a focus on apigenin as the active compound of moringa. As a comparison, the PfDHODH inhibitor 3,6-dimethyl-N-[4-(trifluoromethyl)phenyl]-[1,2]oxazolo[5,4-d]pyrimidin-4-amine and artemisinin as an antimalarial drug that has been widely used were used. Validation of the docking results showed an RMSD value = 0 Å (≤ 2 Å), indicating that the docking configuration was identical to the cocrystal ligand and the analysis results were declared valid.

Discussion of the results of predicting the energy value of apigenin compound bonds with target proteins

The molecular docking prediction results in Table 5.2 show that apigenin from the Moringa plant has a binding energy of -8.9 kcal/mol for PfDHODH. This value demonstrates the potential for significant affinity between apigenin and the active site of the enzyme.

For comparison, the control compound 3,6-dimethyl-~{N}-[4(trifluoromethyl)phenyl]-[1,2]oxazolo[5,4-d]pyrimidin-4-amine, a synthetic compound shown to inhibit PfDHODH in previous studies, exhibited a binding energy of -9.5 kcal/mol. Artemisinin also served as a control compound, with a binding energy of -8.7 kcal/mol. Artemisinin is a commonly used antimalarial drug, and resistance to artemisinin has been reported in some cases of malaria. Taking this comparison into account, apigenin shows a superior affinity value compared to artemisinin, although slightly below the synthetic compound, meaning that apigenin is a potential antimalarial candidate as a competitive inhibitor for PfDHODH.

The more negative the binding energy value, the stronger the attraction of the compound (ligand) to its target receptor (Casarrubias et al., 2025). The value of -8.9 kcal/mol in apigenin indicates that this compound can interact stably and has a strong enough attraction to inhibit the active site of PfDHODH. The DHODH compound plays an important role in the de novo pyrimidine biosynthesis process, which is an important metabolic pathway for the survival of Plasmodium falciparum. Therefore, if DHODH is inhibited, the life cycle of the malaria parasite

can be disrupted in the exoerythrocytic (in the liver) and erythrocytic (in red blood cells) phases. This is in accordance with the way the PfDHODH inhibitor works, which is selective for the parasite's DHODH enzyme, which has a different structure from DHODH in humans.

Thus, it is concluded that the apigenin compound has the potential to be a PfDHODH inhibitor because its affinity value is close to the value of the control compound 3,6-dimethyl-~{N}-[4 (trifluoromethyl)phenyl]-[1,2] oxazolo[5,4-d]pyrimidin-4-amine. This can be seen from the binding energy value, although lower than the control compound 3,6-dimethyl-~{N}-[4 (trifluoromethyl)phenyl]-[1,2]oxazolo[5,4-d]pyrimidin-4-amine, but the apigenin value is higher than the control compound artemisinin. This potential of apigenin supports its role as an antimalarial by inhibiting the replication of Plasmodium parasites in the body. The reduced number of parasites can prevent complications of hepatic malaria, where the liver is one of the locations of malaria replication in the exoerythocyte phase.

Discussion of the prediction of the interaction of apigenin compounds with PfDHODH proteins

The interaction between ligand compounds and PfDHODH was identified through molecular docking using AutoDock Vina integrated in PyRx 0.8. The PfDHODH protein interacted with apigenin and control compounds at coordinates center _x = 14.2554; center _y = 10.3713; and center_z = 25.2089 with exhaustiveness 50. The X, Y, and Z axes in molecular docking are a three-dimensional coordinate system to determine the center position and motion of the ligand in finding the best position to bind to the protein. According to the analysis of the interaction between the active compound and the target protein (PfDHODH), these compounds have the ability to form various types of bonds with important residues in the active site of the enzyme. These types of bonds include hydrogen bonds (types: conventional hydrogen and carbon hydrogen), hydrophobic bonds (types: pi-sigma, pi-Alkyl pi-T-shaped), and halogen bonds, all of which play a role in the stability of the ligand-receptor complex.

The interaction of apigenin with PfDHODH shows that apigenin forms three hydrogen bonds with residues GLY181 (2.69 Å), PHE188 (2.70 Å), and CYS233 (2.93 Å). These three conventional hydrogen bonds stabilize apigenin within the active site of the PfDHODH enzyme. The interatomic distance is approximately 2.6–2.9 Å, indicating the ideal range for strong and specific hydrogen bonds.

Furthermore, apigenin forms several hydrophobic bonds with residues VAL532 (1st methyl group: 3.94 Å and 2nd methyl group: 3.91 Å), PHE188 (7 Å), PHE227 (5.11 Å), LEU531 (5.39 Å), and CYS184 (5 Å). These hydrophobic bonds can enhance non-covalent interactions within the active site of the target protein. The type of interaction shows the ability of apigenin to interact with different chemical properties, namely polar (GLY181, CYS233, and PHE188) and nonpolar (VAL532, PHE188, PHE227, LEU531, and CYS184) on the target protein.

Compared with the control compound, the synthetic inhibitor 3,6-dimethyl-~{N}-[4(trifluoromethyl)phenyl]-[1,2]oxazolo[5,4-d]pyrimidin-4-amine has a greater number of interactions compared to apigenin, this compound forms three hydrogen bonds, two halogen bonds, and ten hydrophobic bonds (types: alkyl, sigma, and T-shaped). The second control compound, artemisinin, forms two hydrogen bonds and seven hydrophobic bonds (dominated

by the alkyl type), with more scattered positions and longer distances. This indicates that the affinity and interaction values of artemisinin with PfDHODH are less specific than apigenin.

Based on the results of the research that has been done, some apigenin interactions occur binding to residues in the active site of the PfDHODH enzyme (GLY181, PHE188, VAL532, PHE227, and CYS184) have bonds that are also found in the interaction of the compound 3,6-dimethyl-~{N}-[4 (trifluoromethyl)phenyl]-[1,2]oxazolo[5,4-d]pyrimidin-4-amine as the first control compound as a ligand of the native protein itself, meaning that if many of the same bonds indicate that the apigenin compound has potential as an antiplasmodium. Meanwhile, when compared with the second control compound, namely artemisinin, it shows that there is no similar residue bond between artemisinin and apigenin, but the affinity and type of apigenin interaction are stronger against PfDHODH. This supports that apigenin not only attaches to the protein surface, but can also stop the enzyme from working at its important location. The hydrogen and hydrophobic interactions together show that apigenin has a stable binding strength, so it has the potential to be a candidate for antimalarial drugs that can be developed.

Discussion of Visual Prediction of Molecular Docking

Molecular docking visualization between apigenin and the target protein PfDHODH provides a clear picture of the ligand orientation and the types of interactions in the ligand-receptor complex displayed in 2D and 3D forms. 2D structural analysis shows that apigenin forms three conventional hydrogen bonds with key residues in the active site of the enzyme, namely GLY181, PHE188, and CYS233, which play an important role in the catalytic activity of PfDHODH. These hydrogen bonds are at an optimal distance ($R \leq 2.7$ Å), which theoretically lowers the potential energy barrier and increases the stability of the bond through proton distribution and quantum effects (Zhou & Wang, 2019). In addition, apigenin also interacts hydrophobically with nonpolar residues such as VAL532, PHE188, PHE227, LEU531, and CYS184 through van der Waals forces and water exclusion from the active site, which also increases the stability of the complex. 3D visualization shows apigenin is tightly bound to the active site of the enzyme with a combination of polar and nonpolar interactions, indicating the ability of apigenin to disrupt the stability of the active structure of PfDHODH and potentially inhibit the biological activity of the enzyme effectively.

Figure 5.2 shows that the compound 3,6-dimethyl-~{N}-[4 (trifluoromethyl)phenyl]-[1,2]oxazolo[5,4-d]pyrimidin-4-amine has the most complex interactions due to the formation of hydrogen, halogen, π–π, and π–alkyl bonds. The polar and branched chemical structure makes this molecule more flexible in forming various types of interactions. 3D visualization shows that this compound has a very suitable and precise location on the active site of the target protein, this is what causes this compound to have the highest affinity value (-9.5 kcal/mol) among the other two compounds.

Figure 5.3 shows that artemisinin forms fewer hydrogen bonds, only interactions occur at the ASN342 and LYS429 residues. In addition to hydrogen bonds, it is also composed of bonds dominated by van der Waals and alkyl interactions, without π–π bonds because it does not form a planar aromatic structure (a C ring structure with conjugated double bonds in one plane, usually arranged 6 sides). 3D visualization shows that artemisinin is not located in a suitable active site location or according to the active pocket of the protein, but the position of

this ligand is more sideways from the target protein, this is the reason for its lower affinity (-8.7 kcal/mol) compared to the other two compounds.

Discussion of the Results of Bioactivity Prediction of Apigenin Compounds in Moringa oleifera.

The results of the prediction of the bioactivity of the apigenin compound were obtained using the PASS program by providing potential biological activity results based on its chemical structure, the term type of compound activity is read in the form of "active" (Pa) or "inactive" (Pi) to describe biological activity. The Pa value is not directly linear with the Pi value, meaning the sum of Pa and Pi is not equal to one (Arabi & Kawsar, 2023). Filimonov et al. (2014) created a PASS program accompanied by three categories of biological activity results, namely Pa > 0.7 (high probability); 0.5 < Pa < 0.7 (moderate probability); Pa < 0.5 (low probability).

Apigenin showed an antiprotozoal Pa value of 0.342 and an antiplasmodial Pa value of 0.263, both of which are categorized as Pa < 0.5 according to the PASS program criteria, thus indicating that the opportunity to prove antimalarial activity through experimental and in vivo tests is still relatively low (Filimonov et al., 2020). In contrast, artemisinin as a comparison produced very high Pa values, namely 0.992 for antiprotozoal activity and 0.954 for antiplasmodial activity, confirming its role as a clinically proven first-line antimalarial agent. Although the Pa value of apigenin is relatively low, this result does not preclude its further development, because apigenin can still be positioned as an early candidate for flavonoid-based antimalarial compounds that require further validation through experimental studies.

Discussion of Predicted Results of Absorption, Distribution, Metabolism, and, Excretion (ADME) and Drug-Likeness of Apigenin Compounds in *Moringa oleifera*

ADME prediction using PKCSM tools is carried out in silico to assess the pharmacokinetic properties of compounds and reduce the risk of failure in drug development. In general, apigenin and artemisinin show good and relatively comparable absorption profiles, with apigenin having a higher water solubility (−3.329 log mol/L) than artemisinin (−3.678 log mol/L), which favors oral absorption. The Caco-2 apigenin permeability of 1.0007 Papp logs (>0.9) as well as high human intestinal absorption (93.25% in apigenin and 97.543% in artemisinin) confirms optimal gastrointestinal absorption capability. Both also show good skin permeability. However, apigenin has been identified as a P-glycoprotein (P-gp) substrate that has the potential to lower oral bioavailability through the efflux mechanism, although this can be overcome with a combination of natural P-gp inhibitors such as quercetin which are also found in moringa leaves and are synergistic (Singh et al., 2021). In terms of distribution, apigenin had a higher Vdss value (0.822 log L/kg) than artemisinin (0.457 log L/kg), indicating a wider tissue distribution, although the plasma-free fraction (Fu) of apigenin was lower indicating stronger plasma protein bonds. The low BBB permeability of apigenin (−0.734 log BB) compared to artemisinin (0.235 log BB) and the equally low CNS permeability values indicate the small potential of both compounds for penetration into the central nervous system, which supports the safety of apigenin for peripheral targets such as hepatic malaria.

Metabolism is assessed based on the metabolism of cytochrome P450 enzymes. Cytochrome P450 enzymes are enzymes that play a role in detoxification in the body, generally

found in the liver. The main function of this enzyme is to oxidize xenobiotics to facilitate excretion. Based on the data, apigenin is not metabolized by CYP2D6 and CYP3A4 enzymes, which are two of the main enzymes in cytochrome P450. Apigenin functions as an inhibitor of CYP1A2 and CYP2C19, indicating the possibility of interactions with other drugs processed by these enzymes. The absence of metabolism via the CYP3A4 enzyme indicates that apigenin's elimination is slower than that of artemisinin, so that the concentration and pharmacological effects in the body can be more stable and persist for a longer period.

Excretion is assessed based on two aspects: total clearance and organic cation transport substrate (OCT2) in the kidneys (renal OCT2). Total clearance is the excretion of drugs through hepatic clearance (metabolism in the liver and bile), and renal clearance (excretion through the kidneys). This property is related to the bioavailability of the compound. Based on the data obtained, apigenin has a total clearance value of 0.566 log mL/min/kg, lower than artemisinin (0.98 log mL/min/kg). These data indicate that the body has a slower rate of elimination of apigenin compared to artemisinin. This causes the pharmacological effects of the drug in the body to last longer. Renal OCT2 substrate is a protein in the kidney that is responsible for transporting substances from the blood to the renal tubules for excretion through urine. The results of renal OCT2 substrate in apigenin and artemisinin show that both compounds do not have this substrate, meaning that apigenin and artemisinin are not excreted through renal proteins.

Based on Table 5.6, it can be seen that apigenin and artemisinin both meet all of Lipinski's criteria, indicating the presence of potential as oral drug candidates. Apigenin has a molecular weight of 270.24 g/mol and artemisinin is slightly higher, at 282.33 g/mol, both of which are still far from the maximum limit of 500 g/mol. Apigenin has 4 hydrogen bond acceptors and 3 hydrogen bond donors, while artemisinin has 5 hydrogen bond acceptors but no donor. This figure is still within the permissible limit (HBA $\leq$ 10 and HBD $\leq$ 5), so it does not violate Lipinski's terms (Long et al., 2019). This difference suggests that apigenin is more capable of forming hydrogen bonds than artemisinin, which may affect the affinity of its interaction with the target protein. In terms of lipophilicity, both compounds showed a logP value of $\leq$ 5, which indicates the ability of the compound to pass through the lipid membrane of the cell without causing solubility problems (Ivanović et al., 2020). Apigenin has one rotatable bond, while artemisinin does not, which suggests that apigenin has a slightly more flexible spatial structure. The molecular refractivity values of apigenin (73.99) and artemisinin (70.38), which are in the general range (40–130), indicate the presence of electron stability and potential molecular interactions with biological targets (Kaviyarasu et al., 2025).

Apigenin and artemisinin both exhibit high gastrointestinal absorption (GI absorption), making them potentially good for oral formulations, but they differ significantly in blood–brain barrier (BBB) permeability. Artemisinin is able to penetrate the BBB, whereas apigenin is not, which suggests that artemisinin is more suitable for central nervous system targets, while apigenin is more relevant for peripheral targets such as the liver, in line with the therapeutic focus of hepatic malaria. The low permeability of BBB in apigenin is beneficial because it can lower the risk of CNS-related side effects such as drowsiness, dizziness, or cognitive impairment. Although the two compounds have the same number of atoms (20) and a relatively comparable level of chemical complexity, differences in functional groups lead to different pharmacokinetic characteristics. Overall, apigenin meets Lipinski's criteria and has good drug-

likeness , strengthening its potential as a PfDHODH inhibitor candidate for the development of oral antimalarial therapies.

Discussion of Toxicity Test Prediction Results of Apigenin Compounds in *Moringa oleifera*

Prediction of apigenin toxicity using the ProTox-3 tool to determine the toxic effects of compounds with important parameters such as LD50 (Lethal Dose 50%) on their toxicity level using the GHS (Globally Harmonized System) category. In addition, tools in the form of online PKCSM are also used to see the effects of toxicity on other parameters such as, AMES toxicity, maximum tolerated dose, hERG I-inhibitor, hERG II-inhibitor, oral rat acute toxicity, oral rat chronic toxicity, hepatotoxicity, skin sensitisation, T. pyriformis toxicity, and minnow toxicity.

Based on ProTox-3 data, it shows an LD50 value of 2500 mg/kg and artemisinin has an LD50 of 4228 mg/kg, both of which are classified as the fifth toxicity class according to the GHS system. Toxicity class 5 means that the compound has low toxicity but has potentially harmful effects if ingested, especially at high doses. This value suggests that apigenin has a safe toxicity profile like artemisinin, thus supporting the potential of apigenin as an alternative to malaria therapy.

Based on PKCSM toxicity predictions, apigenin is classified as non-toxic in the AMES test, indicating low mutagenic and carcinogenic potential, whereas artemisinin shows a positive AMES result; apigenin also has a higher tolerated dose (0.328 log mg/kg/day) than artemisinin (0.065 log mg/kg/day). Acute oral toxicity in rats (LD_{50}) is similarly low for both compounds, with high and comparable values for apigenin (2.45 mol/kg) and artemisinin (2.459 mol/kg), indicating very low acute toxicity. Chronic toxicity assessment using LOAEL demonstrates that apigenin can be administered at relatively high daily doses over long periods (2.298 log mg/kg_bw/day) before adverse effects appear, suggesting lower chronic toxicity and a wider safety margin compared with artemisinin (Kumar, Ojha, & Roy, 2024). In terms of environmental toxicity, both compounds show Tetrahymena pyriformis toxicity values below the harmful threshold (0.38 log µg/L for apigenin and 0.322 log µg/L for artemisinin) and are also non-toxic to flathead minnows based on LC_{50} values (2.432 log mM for apigenin and 1.406 log mM), indicating minimal acute aquatic toxicity.

CONCLUSION

In silico studies on apigenin and other flavonoids (e.g., quercetin, luteolin, kaempferol, epicatechin, myricetin, catechin) from *Moringa oleifera* demonstrate their potential as inhibitors of *PfDHODH* in *P. falciparum* for hepatic malaria therapy, owing to their C6–C3–C6 framework, hydroxyl groups (-OH), and planar aromatic structure, with apigenin showing superior stability due to fewer hydroxyl groups. Molecular docking revealed the strongest binding affinity for the control compound 3,6-dimethyl-N-(4-(trifluoromethyl)phenyl)oxazolo[5,4-d]pyrimidin-4-amine, followed by apigenin and then artemisinin. Although apigenin exhibited relatively low predicted antiprotozoal and antiplasmodial activity, its favorable ADME profile adheres to Lipinski's rule of five, and toxicity predictions indicate low risk (LD50 of 2500 mg/kg, class 5, non-carcinogenic with minimal organ toxicity), positioning it as a promising, safe drug candidate. For future research, validation through in vitro and in vivo assays, including liver-specific models of *P. falciparum*

infection, is recommended to confirm efficacy, hepatoprotective synergy, and pharmacokinetics in malaria-endemic contexts like Indonesia.

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