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Studies of *Achyranthes aspera* Shoot Phytochemicals on *Plasmodium falciparum* Dihydrofolate Reductase (*PfDHFR*) Enzymes Targeted in Antimalarial Drug Design

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ABSTRACT

Resistance of *Plasmodium falciparum* to the first-line antimalarial drug (artemisinin) has been reported in several endemic regions, highlighting the need for new therapeutic alternatives. Medicinal plants are potential sources of bioactive compounds with antimalarial properties. *Achyranthes aspera* (Amaranthaceae) which is widely distributed in Africa and Nigeria, has shown significant antimalarial activity. This study investigated the *in silico* inhibitory effects of phytochemicals from the chloroform fraction of *A. aspera* against the *Plasmodium falciparum* dihydrofolate reductase (*PfDHFR*) enzyme. Bioactive compounds were identified and screened using molecular docking via AutoDock Vina 4.2 to evaluate their binding affinities to *PfDHFR*. Twelve phytochemicals demonstrated promising interactions with the target enzyme. Notably, Chikutsesusaponin iva (-9.4 kcal/mol) and Gypsogenin-3-O-glucuronide (-9.0 kcal/mol) exhibited stronger binding energies than standard inhibitors pyrimethamine (-5.6 kcal/mol) and cycloguanil (-5.8 kcal/mol). These lead compounds formed multiple traditional hydrogen bonds and other interactions within the *PfDHFR* binding pocket, suggesting a higher spontaneity and stability of binding. Some of the additional active compounds included are Betulin (-7.2 kcal/mol), Causapogenin (-7.8 kcal/mol), Gypsogenic acid (-7.9 kcal/mol), Kaempferol-3-O-galactoside (-6.9 kcal/mol), Lupeol (-7.8 kcal/mol), Lutein (-7.1 kcal/mol), Oleanolic acid (-7.6 kcal/mol), Squalene (-6.6 kcal/mol), and α-Spinasterol (-7.8 kcal/mol). These findings suggest that Chikutsesusaponin iva and Gypsogenin-3-O-glucuronide are promising candidates for antimalarial drug development targeting *PfDHFR*.

INTRODUCTION

Malaria is a parasitic disease caused by *Plasmodium parasites* and transmitted through the bite of infected female Anopheles mosquitoes (Paul *et al.,* 2023; Tripathi *et al.,* 2023; WHO, 2024). The World Malaria Report 2024 estimated 263 million cases of malaria in 2023, with 597,000 resulting deaths (WHO, 2025). Nigeria bears a disproportionate share of this burden, which accounts for over 30% of global cases, and with 97% of its population at risk (Bayode & Siegmund, 2022).

The treatment of malaria has primarily relied on artemisinin-based combination therapies (ACTs), along with other antimalarial drugs like lumefantrine, mefloquine and sulfadoxine-pyrimethamine (SP) for preventive treatment (Nosten & White, 2007). SP targets dihydrofolate synthase (DHFS) and dihydrofolate reductase (DHFR) enzymes in the folate synthesis pathway by inhibiting the parasite's DNA synthesis (Heinberg & Kirkman, 2015). However, growing resistance to these drugs and their associated side effects in the liver and kidney necessitate the search for safer and more effective alternatives (CDC, 2024; Yeung *et al.,* 2004).

Plants have long served as a source of bioactive compounds for drug discovery (Aware *et al.,* 2022; Chaachouay & Zidane, 2024). In Nigeria, *Achyranthes aspera* has traditionally been used for malaria treatment with a promising ant plasmodial activity (Mankilik *et al.,* 2021, 2025). Preliminary in vivo studies on aqueous, methanol, and chloroform fractions of *A. aspera* confirmed its potential against P. berghei infection in mice (Mankilik *et al.,* 2021, 2025). Therefore, this study investigates the in-silico binding potential of phytochemicals from the chloroform fraction of *A. aspera* with *Plasmodium falciparum* DHFR, with the aim of identifying promising compounds for future antimalarial drug development.

LITERATURE REVIEW

Drug resistance in malaria parasites continues to challenge global malaria control and eradication efforts (CDC, 2024). Resistance arises when parasites survive and multiply despite appropriate drug administration (Vestergaard & Ringwald, 2007; White, 2013). In *P. falciparum*, mutations in the *PfDHFR* gene significantly reduce the effectiveness of antifolate drugs such as pyrimethamine and cycloguanil. Specific point mutations at positions 108, 59, 51, 164 and 540 have been linked to reduced binding affinity of these drugs, making them less effective (Choowongkomon *et al.,* 2010; Fernandes *et al.,* 2007; Nwankwo *et al.,* 2024; Rastelli *et al.,* 2000). In addition to genetic mutations, factors such as immunity,

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parasite density, transmission intensity, and the use of substandard drugs contribute to the emergence and spread of resistance (Witch *et al.,* 2020).

Antifolates like pyrimethamine function by inhibiting DHFR and preventing the conversion of dihydrofolate to tetrahydrofolate which is essential for DNA synthesis in the parasite (Hadni & Elhallaoni, 2021). Although these drugs were effective in the past, the growing incidence of resistance has limited their clinical usefulness. Moreover, their use has been associated with adverse effects on bone marrow and other vital organs (Blessborn *et al.,* 2010; Marrelli & Brotto, 2016).

The search for alternative therapies for malaria treatment has led to increased interest in natural products, particularly plant-derived compounds with known therapeutic properties (Habibi *et al.,* 2022) as several antimalarial drugs like quinine and artemisinin were originally derived from plants (Uzor *et al.,* 2020). This is because, natural compounds are generally more accessible especially in rural areas and are associated with fewer side effects compared to synthetic drugs (Sarkar *et al.,* 2024; Shrestha *et al.,* 2025). In this context, research has shifted toward identifying and characterizing phytochemicals with potential antimalarial properties.

Achyranthes aspera, commonly known as "Chaff flower", belongs to the Amaranthaceae family and has been widely used in traditional medicine for various ailments including malaria, asthma, and hypertension (Adeleye *et al.,* 2021). Phytochemical screening has revealed the presence of secondary metabolites such as alkaloids, tannins, phenols, flavonoids, and saponins, which are believed to contribute to its pharmacological effects (Mankilik *et al.,* 2021). The presence of high concentrations of saponins in particular, has been linked to the inhibition of malarial parasite proteins such as plasmepsins I, II, and IV (Mankilik *et al.,* 2025).

Further investigation into the chloroform fraction of *A. aspera* using LC-MS analysis revealed 37 distinct phytocompounds. These compounds were subjected to molecular docking studies to assess their binding interactions with *PfDHFR*. Findings from earlier in vivo experiments highlighted that the chloroform fraction at a dose of 200 mg/kg body weight showed the highest antimalarial efficacy (Mankilik *et al.,* 2021). The identification and analysis of these compounds could lead to the development of effective antimalarial agents that act on the parasite's folate pathway while minimizing adverse effects in patients.

MATERIALS AND METHODS

Ligand Identification

A total of 37 compounds previously identified from the chloroform fraction of *Achyranthes aspera* shoot using LC-MS analysis (Mankilik *et al.,* 2025) were subjected to computational evaluation. The compounds were searched and retrieved from the PubChem database, where their chemical details such as canonical SMILES and PubChem CIDs were obtained and downloaded in SDF file format. Two-dimensional structures were retrieved and later confirmed in three-dimensional format. The SDF files were converted to PDBQT format using Open Babel's online format converter available at https://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html.

Drug-Likeness Screening

Drug-likeness of the compounds was evaluated using Lipinski's Rule of Five through an online server (Lipinski *et al.,* 2001). The screening considered physicochemical parameters such as cLogP, hydrogen bond donors and acceptors, and molar refractivity. Further prediction of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties was conducted using the ADMETlab server at https://admetmesh.scbdd.com, following the approach outlined by (Guan *et al.,* 2018; Jung *et al.,* 2024; Xiong *et al.,* 2021)

Target Identification

The *Plasmodium falciparum* dihydrofolate reductase (*PfDHFR*; PDB ID: 7CTW) was selected as the target enzyme. The protein structure was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/). The PDB file was prepared for docking by removing water molecules, adding hydrogen atoms, and assigning charges using AutoDock Tools. The protein was then saved in PDBQT format. The docking grid was defined around the active site with the following coordinates: x = -1.569 Å, y = -2.223 Å, z = 24.213 Å. Energy minimization of the ligands was conducted using the Merck Molecular Force Field and steepest descent optimization. Ligands were transformed into PDBQT format using a universal force field. Protonation states of the targets were adjusted using PROPKA, which estimates the pKa values of ionizable groups based on protein and ligand complex structures (Bas *et al.,* 2008). Protonation was set to physiological pH (~7.0), accounting for different tautomeric and stereoisomeric states of the compounds.

In Silico Docking Study

Docking simulations were performed using AutoDock Vina to predict the inhibitory potential of the phytochemicals. Each ligand (in PDBQT format) was docked with the prepared enzyme target. Binding affinity scores and interaction conformations were used to determine the best binding ligands, following the method described by Trott and Olson (2020). Docked complexes were visualized and analyzed using Discovery Studio Visualizer and Protein Plus software, as described by Adasme *et al.* (2021).

RESULTS AND DISCUSSION

Table 1: Chemical Constituents of the Chloroform Fraction of *Achyranthes aspera* (Shoot) from PubChem

S/N	Name of compound	Mol. Formula	Mol. Weight	CID	Canonical SMILES
1.	17-Pentatriacontane (Alkane)	$C_{35}H75$	490.9	5365022	CCCCCCCCCCCCCCCCC/C=C/CCCCCCCCCCCCCCCC
2.	1-pentacontanol phosphate ester	NA	NA	NA	NA
3.	27-cyclohexy-hepacosy-7-0L	NA	NA	NA	NA
4.	4-(3-hydroxy-1-propenyl-2-methoxy (phenol)	$C_{20}H_{40}O$	180.2	9983	COC1=C(C=CC(=C1)C=CCO)O
5.	5,7, dimethoxyflavone -4 – 0 0 alpha-6-rhamnopyranosyl glycosyl (flavonoids)	NA	NA	NA	NA
6.	9,12-octadecanoic acid (linoliec acid)	$C_{18}H_{36}O_2$	284.5	5362793	CCCCCCCCCCCCCCCCCC(=O)O
7.	Acetoxy-6-benzoxyloxy apangamide	NA	NA	NA	NA
8.	Betaine	$C_5H_{11}NO_2$	117.15	247	C[N+](C)(C)CC(=O)[O-]
9.	Betain	$C_5H_{11}NO_2$	117.15	247	C[N+](C)(C)CC(=O)[O-]
10.	Betulin (triternoid flavonoid)	$C_{30}H_{50}O_2$	442.7	72326	CC(=C)C1CCC2(C1C3CCC4C5(CCC(C(C5CCC4(C3(CC2)C)C)(C)C)O)C)CO
11.	Cauasapogenin	$C_{30}H_{48}O_4$	472.7	73299	CC1(CCC2(CCC3(C(=CCC 4C3(CCC5C4(CCC(C5(C)CO)O)C)C)C2C1)C)C(=O)O)C
12.	Chikutsesusaponin iva	$C_{42}H_{66}NO_4$	794	13909684	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)OC6C(C(C(C(O6)C(=O)O)O)O)O)C)C)C2C1)C)C(=O)OC7C(C(C(O7)CO)O)O)O)C
13.	Dexapanthenol	$C_9H_{19}NO_4$	205.25	131204	CC(C)(CO)C(C(=O)NCCCO)O
14.	Dibuthylphathalate (pthalic acid)	NA	NA	NA	NA
15.	Eugenol acetate (phenol)	$C_{12}H_{14}O_3$	206.24	7136	CC(=O)OC1=C(C=C(C=C1)CC=C)OC
16.	Gypsigenin-3-0-D glucoronide (saponin)	NA	NA	NA	NA
17.	Gypsogenic acid (Pentacyclic triterpenoid saponins	$C_{30}H_{46}O_5$	486.7	15560324	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C(=O)O)O)C)C)C2C1)C)C(=O)O)C
18.	Gypsogenin - 3 - 0 glucoronides	$C_{37}H_{56}O_{10}$	660.8	3086515	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C=O)OC6C(C(C(C(O6)C(=O)O)O)O)O)O)C)C)C2C1)C)C(=O)O)C
19.	Hepta-cosan-2-one (ketone)	$C_{29}H_{54}O$	394.7	547857	CCCCCCCCCCCCCCCCCCCCCCCCC(=O)C
20.	Hexatriacontane II methyl	NA	NA	NA	NA

21.	Hydrooxyphyto-laccagenin (saponin)	NA	NA	NA	NA
22.	Kemferol 3-0-galactoside (flavonoids)	$C_{21}H_{20}O_{11}$	448.4	5282149	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O[C@H]4[C@@H]([C@H]([C@H]([C@H](O4)CO)O)O)O)O
23.	Lupeol (triterpene)	$C_{30}H_{50}O$	426.7	259846	CC(=C)C1CCC2(C1C3CCC4C5(CCC(C(C5CCC4(C3(CC2)C)C)(C)C)O)C)C
24.	Lutein	$C_{40}H_{56}O2$	568.9	5281243	CC1=C(C(CC(C1)O)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2C(=CC(CC2(C)C)O)C)C)C
25.	Oleonolic acid (triterpene aglycone saponin)	$C_{30}H_{48}O_3$	456.7	10494	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1)C)C(=O)O)C
26.	P. benzoquinone (Alkaloid)	$C_6H_4O_2$	108.09	4650	C1=CC(=O)C=CC1=O
27.	Pentatriacontane (Alkane)	$C_{35}H_{72}$	492.9	12413	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
28.	Phytol (deterpene alcohol)	$C_{20}H_{40}O$	296.5	5280435	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C
29.	Quercetin monoisotope (flavonoids)	NA	NA	NA	NA
30.	Rotundioside B (triterpenoid Saponin)	$C_{54}H_{87}O_{26}S$	1184.3	441943	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)OS(=O)(=O)[O])C)C)C2C1)C)C(=O)OC6C(C(C(C(O6)COC7C(C(C(C(O7)CO)O)O)OC8C(C(C(C(O8)COC9C(C(C(C(O9)CO)O)O)O)O)O)O)O)O)O)C
31.	Spathulenol (Alkaloid)	$C_{15}H_{24}O$	220.35	92231	CC1(C2C1C3C(CCC3(C)O)C(=C)CC2)C
32.	Squalene	$C_{30}H_{50}$	410.7	638072	CC(=CCC/C(=C/CC/C(=C/CC/C=C(/CCC=C(C)C)\C)\C)/C)/C)C
33.	Tetradecane (alkane)	$C_{14}H_{30}$	198.39	12389	CCCCCCCCCCCCCC
34.	Tetraflourenthylene	C_2F_4	100.01	8301	C(=C(F)F)(F)F
35.	Triacontanol (Aliphatic alcohol)	$C_{30}H_{62}O$	438.8	68972	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCO
36.	α-ionone (compound of volatile oil)	$C_{13}H_{20}$	192.3	5282108	CC1=CCCC(C1C=CC(=O)C)(C)C
37.	α-spinasterol-3-glycoside	$C_{35}H_{58}O_6$	574.8	12960505	CCC(C=CC(C)C1CCC2C1(CCC3C2=CCC4C3(CCC(C4)OC5C(C(C(C(O5)CO)O)O)O)O)C)C)C)C
38.	Pyrimethamine	$C_{12}H_{13}ClN_4$	248.71	4993	CCC1=C(C(=NC(=N1)N)N)C2=CC=C(C=C2)C
39.	Cycloguanil	$C_{11}H_{14}ClN_5$	251.71	9049	CC1(N=C(N=C(N1C2=CC=C(C=C2)Cl)N)N)C

NA signifies no detail available from the PubChem data based

Phytochemical Diversity in the Chloroform Fraction

Table 1 presents 27 out of the 39 compounds identified from the chloroform fraction of *Achyranthes aspera* (shoot) which were selected for further analysis based on the availability of complete chemical data from the PubChem database. These compounds are listed along with their chemical properties, such as PubChem CID, molecular formula, molecular weight, and canonical SMILES. These compounds also belong to various chemical classes like the alkanes, triterpenoids, flavonoids, saponins, alkaloids, phenols, ketones, and alcohols, with varying molecular weights and structural features. The diversity observed in the compounds showed the chemical richness of the extract and its potential as a source of bioactive molecules. The compounds such as Betulin, Gypsogenic acid, and α-spinasterol-3-glycoside have been documented to have pharmacological relevance among the constituents (Jonnalagadda *et al.,* 2017; Yang *et al.,* 2017). This broad phytochemical profile highlights the potential of *A. aspera* in drug discovery, especially for developing novel antimalarial agents with alternative mechanisms of action.

Drug-likeness evaluation of compounds based on Lipinski's Rule of Five and molar refractivity range (40–130).

Table 2: Drug-Likeness of *Achyranthes aspera* (Shoot) Compounds Based on Lipinski's Rule of Five

S/N	Name of compound	Molecular Mass ≤500 Dalton	Hydrogen Bond Donor ≤5	Hydrogen Bond Acceptor ≤10	High lipophilicity expressed as Log P ≤ 5	Molar Refractivity less should be between 40-130	Status
1.	17-Pentatria contane (Alkane)	490.9	0	0	15.15	0.00	Accepted
2.	4-(3-hydroxy-1-propenyl-2-methoxy	180.2	2	3	1.38	46.45	Accepted
3.	9,12-octade canoic acid (linoliec acid)	284.5	1	2	7.57	37.30	Accepted
4.	Betaine	117.15	0	2	0.94	31.84	Accepted
5.	Betulin (triternoid flavonoid)	442.7	3	3	6.70	152.99	Not accepted
6.	Cauasapogenin	472.7	4	6	-0.05	77.15	Accepted
7.	Chikutsesu saponin iva	794	4	4	1.51	55.40	Accepted
8.	Dexapanthenol	205.25	0	3	2.40	54.10	Accepted
9.	Eugenol acetate (phenol)	206.24	3	5	6.36	151.45	Not accepted
10.	Gypsogenic acid	486.7	5	6	0.05	77.14	Accepted
11.	Gypsogenin - 3 - 0 glucoronides	660.8	3	5	6.36	151.45	Not accepted
12.	Hepta-cosan-2-one (ketone)	394.7	0	1	11.09	17.07	Accepted
13.	Kemferol 3-0-galactoside (flavonoids)	448.4	7	11	0.83	190.29	Not Accepted
14.	Lupeol (triterpene)	426.7	1	1	7.83	153.44	Not accepted
15.	Lutein	568.9	2	2	9.34	189.85	Not accepted
16.	Oleonolic acid (triterpene aglycone)	456.7	2	3	7.01	152.05	Not accepted
17.	P. benzoquinone (Alkaloid)	108.09	0	2	0.38	23.73	Accepted

18.	Pentatriacontane (Alkane)	492.9	0	0	15.92	0.00	Accepted
19.	Phytol (deterpene alcohol)	296.5	1	1	5.72	109.17	Accepted
20.	Rotundioside B (triterpenoid Saponin)	1184.3	5	6	-0.05	77.15	Accepted
21.	Spathulenol (Alkaloid)	220.35	1	1	3.67	75.94	Accepted
22.	Squalene (vitamin E)	410.7	0	0	10.07	0.00	Accepted
23.	Tetradecane (alkane)	198.39	0	0	7.59	0.00	Accepted
24.	Tetraflouren thylene	100.01	0	0	1.55	0.00	Accepted
25.	Triacontanol (Aliphatic alcohol)	438.8	1	1	12,68	20.23	Accepted
26.	α-ionone (compound of volatile oil)	192.3	0	1	3.24	64.81	Accepted
27.	α-spinasterol-3-glycoside	574.8	2	2	7.52	154.31	Not accepted

Compounds exceeding limits in molecular mass (>500 Da), H-bond donors (>5), H-bond acceptors (>10), log P (>5), or molar refractivity between 40 or 130 are marked Not accepted.

Evaluation of Drug-Likeness Properties

Drug-likeness analysis using Lipinski's Rule of Five in Table 2 revealed that 19 out of the 27 compounds met the acceptable parameters. Most of these compounds had molecular weights below 500 Da and exhibited acceptable ranges for hydrogen bond donors, acceptors, and molar refractivity. Compounds like 4-(3-hydroxy-1-propenyl-2-methoxy) phenol, Betain, and Dexapanthenol fully met the selection criteria, indicating favourable oral bioavailability. Others, such as Betulin and Kemferol 3-O-galactoside, violated one or more parameters due to their high lipophilicity or excessive molecular flexibility. This filtering step demonstrates a practical approach to narrowing down phytochemical candidates for further analysis and emphasizes that despite the complexity of some natural products, many still hold promise as drug-like molecules. Such findings are important in the context of current challenges in antimalarial therapy, where physicochemical balance is key to effective bioavailability and therapeutic action (Ikegbunam & Okoye, 2022).

This table summarizes key physicochemical properties, oral bioavailability predictions (EGAN and VEBER models), and the acceptance status for various compounds

Table 3: ADMET Analysis of *Achyranthes aspera* (Shoot) Compounds

S/N	Name of compound	Rotatory Atom	Hetero Atom	Solubility (mg/L)	Oral (Bioavailability (EGAN)	Oral (Bioavailability (VEBER)	Ratio (H/C)	3-75	Status
1.	17-Pentatriacontane (Alkane)	31	0	$3.69x10^{-11}$	Bad	Bad	0.94	Bad	Rejected
2.	4-(3-hydroxy-1-propenyl-2-methoxy (phenol)	13	6	22863.0	Good	Good	0.30	Warming	Accepted
3.	9,12-octadecanoic acid	20	0		Bad	Bad	0.94	Bad	Rejected

4.	Betaine	8	3	82544.3	Good	Good	0.60	Warming	Accepted
5.	Betulin (triternoid flavonoid)	32	0		Bad	Good	0.93	Bad	Accepted
6.	Cauasapogenin	34	4	459.8	Bad	Good	0.13	Warming	Accepted
7.	Chikutsesusaponin iva	56	0		Bad	Bad	0.90	Bad	Rejected
8.	Dexapanthenol	14	5	176739.5	Good	Good	0.56	Good	Accepted
9.	Eugenol acetate (phenol)	15	6		Good	Good	0.23	Good	Accepted
10.	Gypsogenic acid	35	5	942.2	Good	Good	0.28	Warming	Accepted
11.	Gypsogenin - 3 - 0 glucoronides	46	0		Bad	Bad	0.86	Bad	Rejected
12.	Hepta-cosan-2-one (ketone)	28	0		Bad	Bad	0.96	Bad	Rejected
13.	Kemferol 3-0-galactoside (flavonoids)	32	16		Bad	Bad	0.29	Bad	Rejected
14.	Lupeol (triterpene)	31	0		Bad	Good	0.93	Bad	Accepted
15.	Lutein	42	0		Bad	Good	0.45	Bad	Accepted
16.	Oleonolic acid	33	0		Bad	Good	0.90	Bad	Accepted
17.	P. benzoquinone (Alkaloid)	8	2	57215.3	Good	Good	0.33	Warming	Accepted
18.	Pentatriacontane (Alkane)	35	0		Bad	Bad	1.00	Bad	Rejected
19.	Phytol (deterpene alcohol)	21	01	749.7	Good	Good	0.05	Warming	Accepted
20.	Rotundioside B (triterpenoid Saponin)	81	27	3491.9	Low	Low	0.50	Good	Accepted
21.	Spathulenol (Alkaloid)	16	01	9297.3	Good	Good	0.07	Bad	Accepted
22.	Squalene (vitamin E)	30	0		--	--	0.60	Bad	Rejected
23.	Tetradecane (alkane)	14	0		Bad	Good	1.00	Bad	Accepted
24.	Tetraflourenthylene								
25.	Triacontanol (Aliphatic alcohol)	31	0		Bad	Bad	1.00	Bad	Rejected
26.	α-ionone (volatile oil)	14	1	6712.3	Good	Good	0.08	Bad	Accepted
27.	α-spinasterol-3-glycoside	41	0		Good	Good		Good	Accepted

Pharmacokinetics and ADMET Profile Assessment

The 19 drug-like compounds were further evaluated for pharmacokinetics and toxicity profiles using ADMET screening as shown in Table 3. The results showed that 17 of the 19 compounds have favourable ADMET properties. Compounds such as Betain and Dexapanthenol exhibited high aqueous solubility, good intestinal absorption, and acceptable bioavailability based on Egan and Veber filters (Alagga *et al.,* 2024; Daina *et al.,* 2017; Pereira *et al.,* 2018). Even structurally larger compounds like Gypsogenic acid and Phytol passed most parameters, suggesting potential for systemic activity. However, highly lipophilic molecules like Pentatriacontane and Hepta-cosan-2-one were excluded due to poor solubility and low predicted oral absorption. These results reinforce the importance of integrating both drug-likeness and ADMET profiling in early-phase screening to identify viable drug candidates (Sun & Shahrajabian, 2023).

Table 4: Docking results of *A. aspera* compounds and standard inhibitors with *PfDHFR*

S/N	Name of Compound	Binding Energies (kcal/mol)	Ligand-Amino acid Interactions	Type of Interaction	Distance of Interaction (Å)
1.	17-Pentatriacontane (Alkane)	-3.2	-LysB321, TyrB322, ProB324, LysB359	-Alkyl	3.55 to 3.74
2.	4-(3-hydroxy-1-propenyl-2-methoxy (phenol)	-4.8	-HisA323 -AspA212, LysA321 -ProA324 -LysA359, GlnA542, GlnA364, PheA360, AspA361, TyrA326, TyrA322, GlnA327	-Conventional hydrogen bonding -Carbon hydrogen bonding -Pi-alkyl -Val der waals	2.76 to 4.04
3.	9,12-octadecanoic acid (linoliec acid)	-4.1	-Tyr A356, ProB326, AsnB330, IleB335, AspA336, AspA212, PheB360, IlyB358, TyrB322, HisB323, GlnB327, TyrB326, LysB323, MetB358, IleB357, TyrB356	-Val der waals	3.80 to 3.82
4.	Betaine	-3	-GlnA542, AspA361 -AspA212 -PheA360, GlnA327, TyrA365, LysA359, ProA324	-Carbon hydrogen bonding -Attractive charge -Val der waals	2.30 to 4.82
5.	Betulin (triternoid flavonoid)	-7.2	-IleA357, LysA359, TyrA322, ProA324 -AspA212 -LysB297, LysA321, hisA323, TyrA365, AspA361, PheA360, GlnA542, TyrB356, GlyB355, Ile B 357, Gln A 327, MetA354.	-Alkyl -Carbon hydrogen bonding -Van der waals	3.76 to 3.90
6.	Cauasapogenin	-7.8	-IleB357 -IlsB359 -lysB321 -IleB321, TyrB322, TyrB326, HisB323, ProB324, GlnB327, AsnB330, MetB358, IleA357, tyrA356, TyrB356	-Conventional hydrogen bonding -Alkyl -carbon hydrogen bonding -Van der waals	2.12 to 2.25

7.	Chikutsesusaponin iva	-9.4	-LysB321, AsnB330, IleA357 -LysA359, IleB331 -GlnA542, TyrA368, IleA331, AsnA330, MetA358, GlnA452, TyrB356, GlyB355, TyrA326, AspA334, IleB357, metB358, GlnB327, TyrB326, HisB323, ProB324, TyrB323	-Conventional hydrogen bonding -Alkyl -Val der waals	2.13 to 3.89
8.	Dexapanthenol	-4.2	-AspA212, AspA361 -TyrA322, LysA321, HisA323, GlnA327, ProA324, TyrA326, GlnA542, TyrA365, PheA358	-Conventional hydrogen bonding -Val der waals	2.16 to 3.78
9.	Eugenol acetate (phenol)	-4.6	-AspA361 -AspA232 -LysB297, LysA321, Gln327, HisA323, PheA324, LysA359, GlnA542 -TyrA322, ProA324	-Conventional hydrogen bonding -Carbon hydrogen bonding -Val der waals -Pi-alkyl	-3.68 to 3.99
10.	Gypsogenic acid (Pentacyclic triterpenoid saponins	-7.9	-IleA357 -IleA331, MetA358, TyrA356 -IleB357, GlnA327, TyrA326, AsnA330, AspA334, TyrA333, AsnA338, TyrA315	-Conventional hydrogen bonding -Alkyl -Val der waals	2.08 to 3.82
11.	Gypsogenin - 3 - 0 glucoronides	-9	-HisA323, TyrA322, GlnA542, IleB357, - LysA339, IleA357 -GlnA327 -ProA324, PheA360, AspA212, AspA361, GlyB355, tyrB356, IleB331, MetB358, GlnB327, AsnB330, AspB334, TyrA326, IlyA321.	-Conventional hydrogen bonding -Alkyl -Carbon hydrogen bonding -Van der waals	2.31 to 3.28
12.	Hepta-cosan-2-one (ketone)	-3.7	-TyrA358, IleB331 -GlyA355, LysB326, GlnA327, IleA357, AsnA330, TyrA326, MetB358, TyrB356, IleB331, AsnB330	-Alkyl -Van der waals	2.56 to 3.79
13.	Kemferol 3-0-galactoside (flavonoids)	-6.9	-LysB359 -GlnB327 -Lysa297, AspB212 -AsnA294, PheA290, GlnB542, AspB361, PheA360, ProB324, TyrB365, glnB327, IlyB321, HisB323, TyrB326, AsnB330	-Conventional hydrogen bonding -Carbon hydrogen bonding Pi-carbon Val der waals	2.01 to 3.48

14.	Lupeol (triterpene)	-7.8	-GlnB327 -LysB359, IleB357, TyrA356, IleA332 -AspA334, AsnA330, GlnA327, MetA358, IleA357, GlyA355, MetB358	-Carbon hydrogen bonding -Alkyl -Van der waals	3.58 to 3.70
15.	Lutein	-7.1	-AspB212 -IleB357, LysA329, IleA327, LysB357, ProB324 LysB291, AspA361, GlnA342, AspA212, TyrA365, ProA324, GlnA327, GlyB355, GlnB327, PheB360, TyrB365, tyrB214, IleA297, TyrA356, GlnA355, AspB361	-Conventional hydrogen bonding -Alkyl -Van der waals	2.35 to 3.64
16.	Oleonolic acid (triterpene aglycone saponin)	-7.6	-TyrA322 -AspA334, TyrA356, AsnA330, LysA321, IleA357, MetA358, GlnA327, ProA324, TyrA326, LysB297	-Pi-donor hydrogen bonding -Val der waals	3.04 to 3.97
17.	P. benzoquinone (Alkaloid)	-3.5	-AsnA330 -IleA331 -TyrA356, IleA357, MetA358, GlnA327, TyrA326	-Conventional hydrogen bonding -Pi-alkyl -van der waals	2.10 to 3.77
18.	Pentatriacontane (Alkane)	-3.6	-GlnB327, AsnB330, IleB331, AsnB334, TyrA356, IleA357, IleB357, LysB359	-Alkyl	3.48 to 3.95
19.	Phytol (deterpene alcohol)	-4.6	-AspA330, GlnA327 -IleA357, IleB357, TyrB356, Ileb331, -MetB358, LysB358, TyrA356, Tyra326, LysA358, GlyB355, AsnB330, AspB334	-Conventional hydrogen bonding -Alkyl -van dar waals	2.32 to 3.74
20.	Rotundioside B	Not docked	No interaction	No interaction	No result
21.	Spathulenol (Alkaloid)	-5.6	-AspA212 -ProA324, lysA359, TyrA322 -HisA323, TyrA365, PheA360, GlnA327, GlnA542, AspA361, LysB297	-Conventional hydrogen bonding -Alkyl -Van der waals	2.85 to 3.87
22.	Squalene	-6.5	-IleA391, TyrA356, TyrA322, LysA321 -AspA334, TyrA333, GlnA327, AsnA330, MetA326, TyrA326, IleA327, HisA323, ProA324, TyrA320, LysA319	-Alkyl -Val der waaals	3.23 to 3.98

23.	Tetradecane (alkane)	-3.8	-TyrA356 -HisA323, GlnA327, PheA360 -ProA324 -TyrA322, AspA334, LysA359	-Conventional hydrogen bonding -Van der waals -Carbon hydrogen bond-ing -hydrogen-fluorine interaction	3.54 to 3.89
24.	Tetraflourenthylene	-2.9	GlnA327	-Alkyl	3.55
25.	Triacontanol (Aliphatic alcohol)	-3.2	-AsnA330 -ProA324, TyrA322 -LysB297, AspA212, HisA323, GlnA327, TyrA326, LysA321	-Conventional hydrogen bonding -Alkyl -Van der waals	3.51 to 3.96
26.	α-ionone (compound of volatile oil)	-4.9	-LysA357 -IleB357, IleA357, TyrA356, IleA335, -LysA358, GlyA355, AspA334, LysB297, TyrA365, GlnA342, AspA361, ProA324, PheA369, LysA358, TyrA322, GlnA327, TyrA326, LysA321, MetA358, AsnA330	-Conventional hydrogen bonding -Alkyl -Van der waals	2.55 to 3.88
27.	α-spinasterol-3-glycoside	-7.8	-IleB357, TyrA356, IleA331 -GlyA355, IleA357, GlnA327, AspA334, AsnA330, LysB359	-Alkyl -Val der waals	2.32 to 3.97
28.	Pyrimethamine	-5.6	-AsnA330 -ProA324, TyrA322 -AspA212, HisA323, LysA359, PheA360, TyrA365, GlnA327, TyrA326, LysA321	-Conventional hydrogen bonding -Alkyl -Val der waals	2.54 to 5.58
29.	Cycloguanil	-5.8	-AspA212 -TyrA322, LysA359 -GlnA327, GlnA542, AspA361, PheA360, TyrA365, ProA324, LysA321	-Conventional hydrogen bonding -Alkyl -Val der waals	2.77 to 4.08

Binding affinities, interaction types, and contact residues of 29 *Achyranthes aspera* compounds and standard inhibitors docked with *P. falciparum* DHFR (PDB ID: 7CTW).

Docking Evaluation of 29 Phytocompounds Against *Plasmodium falciparum* DHFR

Table 4 presents the docking results of 29 phytochemicals from the chloroform fraction of *Achyranthes aspera* shoots against the *Plasmodium falciparum* DHFR enzyme (PDB ID: 7CTW), revealing several compounds with strong binding affinities and favourable interactions. Among these, Chikutsesusaponin iva (−9.4 kcal/mol) and Gypsogenin-3-O-glucuronides (−9.0 kcal/mol) exhibited the highest binding affinities, significantly stronger than standard DHFR inhibitors Pyrimethamine (−5.6 kcal/mol) and Cycloguanil (−5.8 kcal/mol). Other notable compounds, including Cauasapogenin (−7.8), Lupeol (−7.8), α-spinasterol-3-glycoside (−7.8), Gypsogenic acid (−7.9), and Betulin (−7.2), also demonstrated strong interactions. These top binders formed conventional hydrogen bonds, alkyl, and van der Waals interactions with key catalytic residues such as Asp212, Lys321, Tyr322, Gln327, and Ile357, suggesting stable ligand-enzyme

binding. In contrast, compounds like Tetrafluoroethylene (−2.9) and Betaine (−3.0) had weak binding affinities with minimal residue interactions. Overall, the docking analysis pointed out multiple *A. aspera*-derived phytochemicals as promising *PfDHFR* inhibitors, supporting their potential as leads in antimalarial drug development.

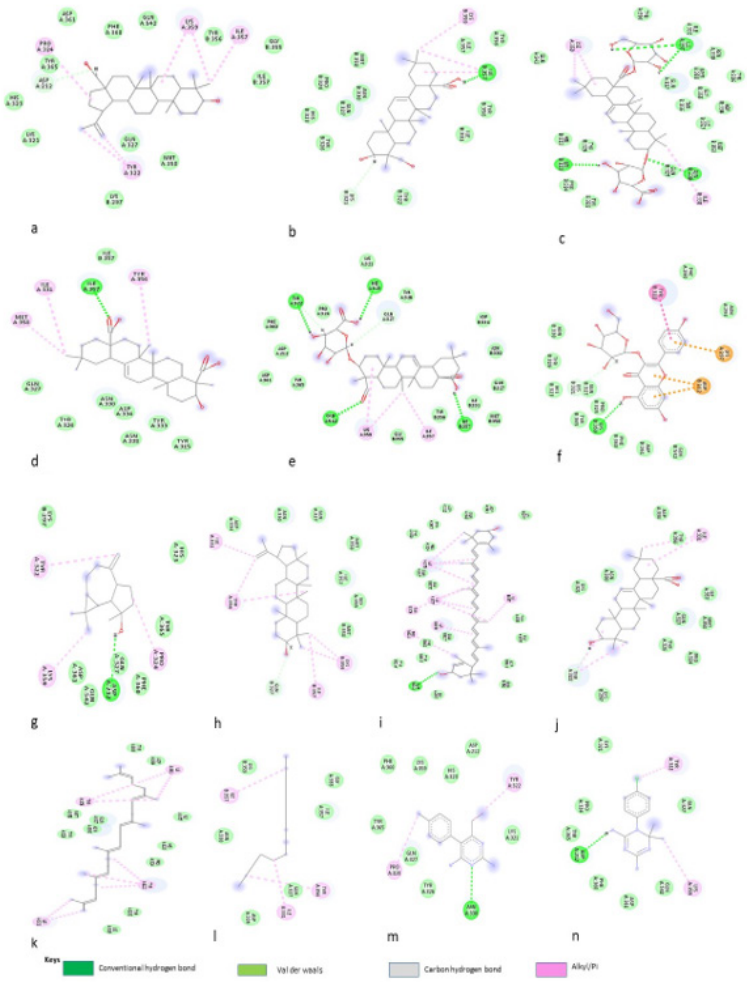

Figure 1: Binding interactions of *A. aspera* shoot phytochemicals and standard inhibitors with *PfDHFR* active site residues a–f represents docking complexes of *PfDHFR* with Betulin (a), Cauasapogenin (b), Chikutsesusaponin iva (c), *Gypsogenic acid (d), Gypsogenin-3-O-glucuronides (e), and Kaempferol 3-O-galactoside (f), Lupeol (g), Lutein (h), Oleonolic acid (i), Spathulenol (j), Squalene (k), a-Spinasterol-3-glycoside (l), Pyrimethamine (m), and Cycloguanil (n).*

Ligand–*PfDHFR* Interaction Profiles of Selected Phytochemicals and Inhibitors

Figure 1 illustrates the molecular docking interactions between *Plasmodium falciparum* dihydrofolate reductase (*PfDHFR*) and selected phytochemicals from the chloroform fraction of *Achyranthes aspera*, alongside standard inhibitors. The visualizations (a to n) revealed diverse interaction patterns like the conventional hydrogen bonds, van der Waals forces, carbon–hydrogen bonds, and alkyl/π interactions. Phytochemicals such as Betulin (a), Gypsogenic acid (d), and Lupeol (g) formed stable complexes with key active site residues, particularly Lys321, Gln327, and Tyr326, through favourable docking poses and multiple non-covalent bonds. Compounds containing glycosidic and triterpenoid moieties demonstrated enhanced binding through multiple donor and acceptor sites, indicating strong molecular recognition. Comparisons with standard inhibitors Pyrimethamine (m) and Cycloguanil (n) showed that certain *A. aspera* compounds such as Chikutsesusaponin iva (c) and Gypsogenin-3-O-glucuronides (e) engaged more residues and exhibited greater interaction diversity, suggesting superior stabilization of the enzyme-inhibitor complex. These findings reinforce the potential of plant-derived compounds as effective *PfDHFR* inhibitors and align with earlier reports supporting the antimalarial relevance of structurally novel phytochemicals (Adam *et al.,* 2023; Akinnusi *et al.,* 2023; Ikegbunam *et al.,* 2022).

CONCLUSION

This study demonstrates that phytochemicals from *Achyranthes aspera* shoots particularly Chikutsesusaponin iva and Gypsogenin-3-0 glucuronides adhere to drug-likeness criteria and exhibit strong binding affinities to

the *PfDHFR* enzyme primarily through hydrogen bonds. These interactions suggest their potential as potent enzyme inhibitors offering promising new avenues for malaria treatment and addressing drug resistance, consistent with findings on other plant-derived antimalarials.

REFERENCES

Adams, L., Afiadenyo, M., Kwofie, S. K., Wilson, M. D., Kusi, K. A., Obiri-Yeboah, D., Moane, S., & McKeon-Bennett, M. (2023). *In silico* screening of phytochemicals from Dissotis rotundifolia against *Plasmodium falciparum* Dihydrofolate Reductase. *Phytomedicine Plus, 3*(2), 100447. https://doi.org/10.1016/j.phyplu.2023.100447

Adasme, M. F., Linnemann, K. L., Bolz, S. N., Kaiser, F. S., & Haupt, V. J. (2021). Expanding the scope of the protein–ligand interaction profiler to DNA and RNA. *Nucleic Acids Research, 49*(W1), W530–W534. https://doi.org/10.1093/nar/gkab294

Adeleye, O. A., Femi-Oyewo, M. N., Bamiro, O. A., Bakre, L. G., Alabi, A., Ashidi, J. S., Balogun-Agbaje, O. A., Hassan, O. M., & Fakoya, G. (2021). Ethnomedicinal herbs in African traditional medicine with potential activity for the prevention, treatment, and management of coronavirus disease 2019. *Future Journal of Pharmaceutical Sciences, 7*(1). https://doi.org/10.1186/s43094-021-00223-5

Akinnusi, P. A., Olubode, S. O., Adebesin, A. O., Osadipe, T. J., Nwankwo, D. O., Adebisi, A. D., Titilayo, I. B., Alo, Y., Owoloye, A. J., & Oyebola, K. M. (2023). Structure-based scoring of anthocyanins and molecular modeling of PfLDH, PfDHODH, and *PfDHFR* reveal novel potential *P. falciparum* inhibitors. Informatics in Medicine Unlocked. https://doi.org/10.1016/j.imu.2023.101206

Alagga, A. A., Pellegrini, M. V., & Gupta, V. (2024, February 27). *Drug absorption. StatPearls - NCBI Bookshelf*. https://www.ncbi.nlm.nih.gov/books/NBK557405/

Aware, C. B., Patil, D. N., Suryawanshi, S. S., Mali, P. R., Rane, M. R., Gurav, R. G., & Jadhav, J. P. (2022). Natural bioactive products as promising therapeutics: A review of natural product-based drug development. *South African Journal of Botany, 151*, 512–528. https://doi.org/10.1016/j.sajb.2022.05.028

Bas, D. C., Rogers, D. M., & Jensen, J. H. (2008). Very fast prediction and rationalization of pKa values for protein–ligand complexes. *Proteins: Structure, Function, and Bioinformatics, 73*(3), 765–783. https://doi.org/10.1002/prot.22102

Bayode, T., & Siegmund, A. (2022). Social determinants of malaria prevalence among children under five years: A cross-sectional analysis of Akure, Nigeria. *Scientific African, 16*, e01196. https://doi.org/10.1016/j.sciaf.2022.e01196

Blessborn, D., Romsing, S., Bergqvist, Y., & Lindegardh, N. (2010). Assay for screening for six antimalarial drugs and one metabolite using dried blood spot sampling, sequential extraction and Ion-Trap detection. *Bioanalysis, 2*(11), 1839–1847. https://doi.org/10.4155/bio.10.147

Centers for Disease Control and Prevention (CDC). (2024, April 8). *Drug resistance in the malaria-endemic world*. Malaria. https://www.cdc.gov/malaria/php/public-health-strategy/drug-resistance.html

Chaachouay, N., & Zidane, L. (2024). Plant-derived natural products: A source for drug discovery and development. *Drugs and Drug Candidates, 3*(1), 184–207. https://doi.org/10.3390/ddc3010011

Choowongkomon, K., Theppabutr, S., Songtawee, N., Day, N. P., White, N. J., Woodrow, C. J., & Imwong, M. (2010). Computational analysis of binding between malarial dihydrofolate reductases and anti-folates. *Malaria Journal, 9*(1). https://doi.org/10.1186/1475-2875-9-65

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports, 7*(1). https://doi.org/10.1038/srep42717

Fernandes, N., Figueiredo, P., Rosário, V. E. D., & Cravo, P. (2007). Analysis of sulphadoxine/pyrimethamine resistance-conferring mutations of *Plasmodium falciparum* from Mozambique reveals the absence of the dihydrofolate reductase 164L mutant. *Malaria Journal, 6*(1). https://doi.org/10.1186/1475-2875-6-35

Guan, L., Yang, H., Cai, Y., Sun, L., Di, P., Li, W., Liu, G., & Tang, Y. (2018). ADMET-score – A comprehensive scoring function for evaluation of chemical drug-likeness. *MedChemComm, 10*(1), 148–157. https://doi.org/10.1039/c8md00472b

Habibi, P., Shi, Y., Grossi-De-Sa, M. F., & Khan, I. (2022). Plants as sources of natural and recombinant antimalaria agents. *Molecular Biotechnology, 64*(11), 1177–1197. https://doi.org/10.1007/s12033-022-00499-9

Hadni, H., Bakhouch, M., & Elhallaoui, M. (2021). 3D-OSR molecular docking, DFT and ADMET study on quinazoline derivatives to explore novel DHFR inhibition. *Journal of Biomolecular Structure and Dynamics,* 1–15. https://doi.org/10.1080/0739102.2021.2004233

Heinberg, A., & Kirkman, L. (2015). The molecular basis of antifolate resistance in *Plasmodium falciparum*: Looking beyond point mutations. *Annals of the New York Academy of Sciences, 1342*(1), 10–18. https://doi.org/10.1111/nyas.12662

Ikegbunam, M. N., Ejiofor, I. M., Onwuakpa, V., Duru, V. C., Amarachi, A., Oranu, E., Okoyeh, J., & Egieyeh, S. (2022). *Virtual screening of selected phytochemicals from four antimalarial plants predicted potential inhibitors of wild type and mutant forms of dihydrofolate reductase-thymidylate synthase in Plasmodium sp*. Preprints. https://doi.org/10.20944/preprints202202.0107.v1

Jonnalagadda, S., Suman, P., Morgan, D., & Seay, J. (2017). Recent developments on the synthesis and applications of betulin and betulinic acid derivatives

as therapeutic agents. *In Studies in Natural Products Chemistry* (pp. 45–84). https://doi.org/10.1016/B978-0-444-63930-1.00002-8

Jung, W., Goo, S., Hwang, T., Lee, H., Kim, Y., Chae, J., Yun, H., & Jung, S. (2024). Absorption distribution metabolism excretion and toxicity property prediction utilizing a pre-trained natural language processing model and its applications in early-stage drug development. *Pharmaceuticals, 17*(3), 382. https://doi.org/10.3390/ph17030382

Lipinski, C. A. (2004). Lead- and drug-like compounds: The rule-of-five revolution. *Drug Discovery Today: Technologies, 1*(4), 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007

Mankilik, M. M., Longdet, I. Y., & Luka, C. D. (2021). Evaluation of *Achyranthes aspera* shoot extract as an alternative therapy for malaria. *The Journal of Basic and Applied Zoology, 82*(1). https://doi.org/10.1186/s41936-021-00211-4

Mankilik, M. M., Paul, J., Mhya, D. H., Luka, C. D., & Longdet, I. Y. (2025). In vivo antiplasmodial studies of *Achyranthes aspera* fractions and molecular docking studies of its phytochemicals against Plasmodium aspartic proteases targeted in antimalarial drug design. *American Journal of Bioscience and Bioinformatics, 4*(1), 11–25. https://doi.org/10.54536/ajbb.v4i1.4516

Marrelli, M. T., & Brotto, M. (2016). The effect of malaria and anti-malarial drugs on skeletal and cardiac muscles. *Malaria Journal, 15*(1). https://doi.org/10.1186/s12936-016-1577-y

Nosten, F., & White, N. J. (2007, December 1). Artemisinin-based combination treatment of falciparum malaria. Defining and Defeating the Intolerable Burden of Malaria III: Progress and Perspectives - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK1713/

Nwankwo, N. J., Paul, J., Gaknung, S. K., Fakdul, T., Simon, M. O., Davou, D. T., Mankilik, M. M., Kutshik, R. J., Yakubu, B., & Longdet, I. Y. (2024, May 31). Genetic insights into *Plasmodium falciparum* resistance to sulfadoxine-pyrimethamine through a study of the 540-dihydropteroate synthetase gene mutations in Jos, Nigeria. *Asian Journal of Biotechnology and Genetic Engineering*. https://journalajbge.com/index.php/AJBGE/article/view/131

Paul, J., Nwankwo, N. J., Simon, M. O., Gaknung, S. K., Fakdul, T., Davou, D. T., Mankilik, M. M., Kutshik, R. J., Yakubu, B., & Longdet, I. Y. (2023). Non-synonymous mutations associated with *Plasmodium falciparum* artemisinin resistant gene (Pfkelch13) in malaria cases, Jos Nigeria. *Journal of Advances in Biology & Biotechnology, 26*(10), 28–38. https://doi.org/10.9734/jabb/2023/v26i10661

Pereira, V. S. de S., Emery, F. S., Lobo, L., Nogueira, F., Oliveira, J. I. N., Fulco, U. L., Albuquerque, E. L., Katzin, A. M., & de Andrade-Neto, V. F. (2018). In vitro antiplasmodial activity, pharmacokinetic profiles and interference in isoprenoid pathway of 2-aniline-3-hydroxy-1,4-naphthoquinone derivatives. *Malaria Journal, 17*(1). https://doi.org/10.1186/s12936-018-2615-8

Rastelli, G., Pellecchia, M., Sirawaraporn, W., & Parenti, M. D. (2000). Interaction of pyrimethamine, cycloguanil, WR99210 and their analogues with *Plasmodium falciparum* dihydrofolate reductase: Structural basis of antifolate resistance. *Bioorganic & Medicinal Chemistry, 8*(5), 1117–1128.

Sarkar, R., Nayak, S. L., Suthar, M. K., & Das, M. (2024). Nutraceutical formulations from medicinal plants: A potential therapeutic agent. In *Nutraceuticals and Natural Product Derivatives* (pp. 391–417). https://doi.org/10.1007/978-981-97-2367-6_19

Shrestha, K. K., Tiwari, N. N., & Ghimire, S. K. (2025). *MAPDON–Medicinal and aromatic plant database of Nepal*. ResearchGate. https://www.researchgate.net/publication/281288981_MAPDON-Medicinal_and_aromatic_plant_database_of_Nepal

Sun, W., & Shahrajabian, M. H. (2023). Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. *Molecules, 28*(4), 1845.

Tripathi, H., Bhalera, B., Arya, H., Alotaibi, B. S., Rashidi, S., Higa, M. R., & Bhalt, T. K. (2023). Malaria therapeutics: Are we close enough? *Parasites & Vectors, 16*(130), 289–294.

Trott, O., & Olson, A. J. (2020). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry, 31*, 455–461. https://doi.org/10.1002/jcc.21334

Uzor, P. F., Prasastry, V. D., & Agubats, C. O. (2020). Natural products as sources of antimalarial drugs. *Evidence-Based Complementary and Alternative Medicine*. https://doi.org/10.1155/2020/9286362

Vestergaard, L. S., & Ringwald, P. (2007, December 1). Responding to the challenge of antimalarial drug resistance by routine monitoring to update national malaria treatment policies. Defining and Defeating the Intolerable Burden of Malaria III: Progress and Perspectives – NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK1708/

White, N. J. (2013). Malaria. In *Elsevier eBooks* (pp. 532–600.e1). https://doi.org/10.1016/B978-0-7020-5101-2.00044-3

Witcht, K. J., Mok, S., & Fidock, D. A. (2020). Molecular mechanism of drug resistance in *Plasmodium falciparum* malaria. *Annual Review of Microbiology, 74*, 431–454. https://doi.org/10.1146/annurev-micro-020518-115546

World Health Organization. (2024). *World malaria report 2024*. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2024

World Health Organization. (2025). *World Malaria Day 2025*. https://www.who.int/campaigns/world-malaria-day/2025

Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., Yin, M., Zeng, X., Wu, C., Lu, A., Chen, X., Hou, T., & Cao, D. (2021). ADMETlab 2.0: An integrated online platform for accurate and comprehensive predictions

of ADMET properties. *Nucleic Acids Research, 49*(W1), W5–W14. https://doi.org/10.1093/nar/gkab255

Yang, X., Zhou, J., Wang, T., Zhao, L., Ye, G., Shi, F., Li, Y., Tang, H., Dong, Q., Zhou, X., Xu, M., Rong, Q., Chen, H., Yang, X., & Cai, Y. (2017). A novel method for synthesis of α-spinasterol and its antibacterial activities in combination with ceftiofur. *Fitoterapia, 119*, 12–19. https://doi.org/10.1016/j.fitote.2017.03.011

Yeung, S., Pongtavornpinyo, W., Hastings, I. M., Mills, A. J., & White, N. J. (2004, August 1). *Antimalarial drug resistance, artemisinin-based combination therapy, and the contribution of modeling to elucidating policy choices*. The Intolerable Burden of Malaria II: What's New, What's Needed - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK3762/