**BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY** ISSN 2089-6514 (paper)
Volume 14, Number 2, October 2025 | Pages: 1579-1597 | DOI: 10.14421/biomedich.2025.142.1579-1597 ISSN 2540-9328 (online)

# Identification of Potential Antimalarial Compound(s) from Alkaloid, Flavonoids and Phenolics Rich Extracts of *Piliostigma reticulatum* Leaf

**Abdulhafiz Lamiya[1]*, Adamu Jibril Alhassan[2], Maryam Abdulkadir Dangambo[2], Hauwa Ahmed Zailani[3]**
[1]Department of Science Laboratory Technology, Modibbo Adama University Yola, Nigeria
[2]Department of Biochemistry, Bayero University Kano, Nigeria
[3]Departemnt of Biochemistry, Modibbo Adama University Yola, Adamawa state, NIgeria.

**Corresponding author***
alamiya@mau.edu.ng

Manuscript received: 18 June, 2025. Revision accepted: 26 January, 2026. Published: 30 January, 2026.

**Abstract**

Malaria is a deadly vector born disease caused by *Plasmodium spp* with increasing morbidity and mortality in sub-Saharan Africa. Ethnobotanical studies and phytochemical screening remains the mainstay in the hunt of bioactive secondary metabolites from natural sources needed to confront the growing challenges faced by available drugs used in the treatment of many infectious diseases malaria inclusive. This study aimed at identifying potential antimalarial compound(s) in some *Piliostigma reticulatum* extracts. *In vivo* technique was used to assay for antimalarial activities of *P. reticulatum* extracts and spectroscopic technique was used to characterize compounds present in the extract. Top hit compound against some *Plasmodium* spp targets were identified using *in silico* technique. *In vivo* study revealed phenolic rich extract to exhibit significant antimalarial activity ($P<0.05$) compared to other extracts and twenty five compounds were identified in the extract spectroscopically. Molecular docking of the compounds against some *Plasmodium falciparum* targets (PMII, HAP, FP-2 and PfENR) revealed ethylene brassylate as the top hit compound that inhibited PMII, FP-2 and PfENR with binding affinity of -7.3, -7 and -6.5Kcal/mol respectively, whereas 2-hexadecanol was the top hit for HAP with binding affinity of -6.4Kcal/mol. *In silico* ADMET analysis of the top hits revealed that they both possess drug-like property, metabolizable and relatively safe. This finding gives credence to the use of the plant in malaria treatment and identified two compounds Ethylene brassylate and 2-hexadecanol as promising antimalarial drug candidates which can be harnessed for discovery of novel antimalarial drug.

**Keywords:** Antimalarial compounds; *Piliostigma reticulatum*; Molecular docking; *in silico* analysis; Antimalarial activity.

## INTRODUCTION

Malaria though an ancient disease, remains a major public health challenge in the developing nations with sub-Saharan Africa bearing the largest burden(Tajbakhsh et al., 2021). It is the most common infectious disease caused by *Plasmodium* spp namely *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax, and Plasmodium knowlesi* viz which are vectored through the bite of infected female anopheles mosquito(Oladeji et al., 2020). *P. falciparum* is the most virulent of these species and the most predominant in sub-Saharan Africa. It has also been reported to be responsible for the morbidity and mortality sub-Saharan Africa has been bearing on malaria global outlook(Tajbakhsh et al., 2021). This single-cell eukaryote has a complicated life cycle and is an obligatory intracellular parasite of hepatocytes and erythrocytes with tendencies of causing variety of complications such as severe anemia and cerebral malaria(Chaurasia & Pandey, 2022), consequently children and pregnant women become the most at risk of malaria related morbidities and mortalities. According to 2023 world health organization (WHO) malaria report, an estimated 249 million cases of malaria occurred in 85 malaria-endemic countries in 2022, a case incidence of 58 per 1000 population risk. For comparison, in 2019 there were an estimated 233 million global cases, a case incidence of 57 per 1000 population at risk. The numbers for 2022 were 55% higher than they should have been if the 2025 Global technical strategy for malaria targets are to be met. Of the 249 million cases noted in 2022, 233 million (around 94%) were in the WHO African Region, with Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), and Mozambique (4%), accounting for nearly 50% of all cases (Venkatesan, 2024). Chemotherapy remains a major strategy in the fight against malaria. However, the emergence of multi-antimalarial drug resistant *Plasmodium falciparum* and the emergence of insecticide resistant Anopheles mosquitoes is not only threatening the treatment of malaria in endemic areas, but cause the spread of malaria to new areas and also its re-emergence in areas where it

had previously been eradicated(Madara et al., 2010), thereby posing a great set back in the malaria eradication agenda. Plants have been and is still being an important source of medication across the globe for the treatment and management of many infectious diseases, malaria inclusive. This has made plants pivotal in drug discovery process and have provided value to the pharmaceutical industry over the years. Many orthodox drugs used in the treatment/management of infectious diseases, cancer, and other debilitation diseases caused by metabolic disorders were initially developed based on active compounds from plant sources. Quinine, isolated from *Cinchona succiruba* and artemisinin from *Artemisia annua*, and their synthetic derivatives which are the mainstay of anti-malarial chemotherapy, are perfect examples of clinically important orthodox drugs derived from plant sources (Shibi et al., 2016). Therefore, the diverse plant species across the different geographical regions of the world still holds the promise of providing novel antimalarial compounds that will help mankind sustain the fight against this deadly infectious disease.

Ethnobotanical survey of plants used in the treatment of malaria have reported several plants to have been used in the treatment or management of malaria in the traditional settings out of which many have been validated by scientific studies(Tajbakhsh et al., 2021). It is noteworthy that *Piliostigma thonningii* Schum. (Caesalpiniacea) is one of the plants used in the traditional setting of Nigeria for the treatment of common ailments such as cough, dysentery, sores, snake bites, malaria, stomach upsets, pain reliever(Abdulsalami et al., 2022) that have been validated for it's antimalarial activity in preliminary studies. Crude leaf extracts of *Piliostigma thonningii* were reported to exhibit *in vivo* antimalarial activity in mice infected with chloroquine sensitive *Plasmodium berghei* NK65(Madara et al., 2010) and when tested *in vitro* by (Kwaji et al., 2016) against chloroquine resistant *Plasmodium falciparum clone (W2 – Indochina isolates)*, a significant inhibition was observed. Thus, it can be theorized that *Piliostigma thonningii* Schum. (Caesalpiniacea) contains active substance that holds the potential of surmounting the challenges faced by the current antimalarial drugs specifically antimalarial resistance. *Piliostigma thonningii* Schum. (Caesalpiniacea) belongs to the family caesalpiniaceous. It is a shrubby tree with alternate compound leave found growing abundantly as a wild uncultivated tree in many parts of Nigeria and other tropical regions of the world. Common names of *Piliostigma thonningii* include camel's foot (English), Abefe (Yoruba), Kalgo (Hausa) and Okpoatu (Igbo)(Abdulsalami et al., 2022). In spite the numerous traditional benefits of this plant, it remains an underexploited plant and research is relatively scanty on the active components associated with it's pharmacological activities.

Malaria has been declared a public health emergency with high morbidity and mortality particularly among children and women with sub-Saharan Africa bearing the highest burden(Zailani, et al., 2020a). According to World Health Organization (2024), there were an estimated 249 million cases of malaria and 608, 000 deaths in 2022 with 94% of cases coming from WHO African region and Nigeria bears the largest malaria burden in Africa and globally, with 66.7 million cases in 2022(World Health Organization, 2024). Failure of malaria eradication strategies such as use of insecticide and insecticide treated nets due to insecticide resistance developed by the vector of the parasite as well as resistance to front line antimalarial drugs by the parasite have contributed to the morbidity and mortality associated with malaria (Shibi et al., 2016). In addition to these, side effects of some of these conventional antimalarial drugs such as primaquine, atovaquone, doxycycline among others have made them contraindicated in pregnant women and children (Tajbakhsh et al., 2021). Also, the high cost of most of the conventional frontline antimalarial drugs have contributed to low compliance which increases morbidity and mortality associated with malaria particularly in low-middle income countries where the disease is endemic (Erhirhie et al., 2021). It is interesting to also mention that most of the anti-malarial plants reported in literature remains underexploited despite the potentials they hold. This instigates the unrelenting search for effective antimalarial compounds from local plants that will be cost-effective, handy, acceptable, and with possibly new targets.

Many drugs including antimalarial drugs such as quinine and artemisinin, isolated from *Cinchona succiruba* and *Artemisia annua*, are classical examples of natural product derived drugs(Shibi et al., 2016). Thus, plant materials still hold the promise of providing many more potent drugs. *In vivo* studies carried out by Abdulkadir et al. (2022) on antimalarial activity of methanol leaf extract of *Piliostigma reticulatum* reported a significant activity and Pharmacologically important classes of phytochemicals including alkaloids, flavonoids, phenolics, tannins among others were reported to be present in the extract. However, paucity of information on the specific compounds present in the plant's leaf responsible for it's antimalarial efficacy continues to prevail in literature nor the possible mechanism of action elucidated. Thus, this study seeks to evaluate the antimalarial activity of *Piliostigma reticulatum* (alkaloid, flavonoids and phenolics) rich extracts and identify the most potent compound(s) responsible for the antimalarial activity that will serve as substitute or complement to the frontline antimalarial drugs confronted by growing number of challenges.

## MATERIALS AND METHODS

### Materials

### Collection and preparation of plant material

The leaves of *Piliostigma reticulatum* were collected from Hong local government area Adamawa state in the month of November. It was then taken to the Herbarium Unit of the Department of Plant Biology, Bayero University Kano for identification and was assigned a voucher specimen number; BUKHAN 0072. The leaves were then washed with distilled water to remove debris, air dried and ground into powder in pestle and mortar. The powdered leaf was then placed in a dry plastic container and stored in moist free environment.

### Chemicals and reagents

All chemicals and reagents used in this research were of analytical grade and were obtained from Northern Scientific. Chemicals include methanol (98%), ethanol (98%), chloroform (98%), ethyl acetate (98%), formic acid (98%), chloroquine tablets, distilled water (100%) and Hydrochloric acid (98%).

### Equipment

Equipment used in the study include analytical weighing balance, water bath, automated biochemical analyzer (URIT 880), automated hematology analyzer (Beckman Coulter DxH 800), UV/Visible spectrophotometer (Perkin Elmer, USA Model: Lambda 950), gas chromatography mass spectrophotometer (QP 2010 Plus SHIMADZU), Fourier transform infrared spectrophotometer (Nicolet iS10, Thermo Fisher Scientific, Madison, USA) and HP laptop computer (HP 260).

### Parasite Strain

Chloroquine sensitive strain of *Plasmodium berghei* (NK-65) was obtained from the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Science, Ahmadu Bello University Zaria, Kaduna State Nigeria. The parasites were maintained weekly by serial passage of blood from the donor-infected mice to healthy uninfected mice via intraperitoneal (IP) injection.

### Ethical clearance

Ethical clearance was obtained from the department of veterinary medicine, ministry of Agriculture Adamawa state (see appendix).

### Experimental animals

Ninty (90) Swiss albino mice (about 6 to 8 weeks old) of both sexes were obtained from the animal breeding unit of the University of Jos Plateau State for antimalarial suppressive study, and thirty six (36) Swiss albino rats (about 6 to 8 weeks old) of both sexes were obtained from the Department of Biochemistry Modibbo Adama University Yola for acute toxicity study. The animals were housed in plastic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water *ad libitum* for acclimatization for a period of one-week prior commencement of the experiment.

### Methods

### Phytochemical extraction

#### *Extraction of alkaloids*

One hundred grams (100 g) of powdered leaf of *Piliostigma reticulatum* was macerated in 500 mL of 1% hydrochloric acid for 24 hours with occasional shaking. It was then filtered using what man filter paper and concentrated in water bath. Ammonia solution was then added to liberate free alkaloids. Alkaloids was then extracted using chloroform (1:1 v/v). The organic layer containing the alkaloid was then collected and evaporated to dryness to obtain an alkaloid-rich extract (Harborne, 1997).

#### *Extraction of flavonoids*

Fifty grams (50 g) of the powder *Piliostigma reticulatum* leaf was macerated in 500 mL of 70% methanol for 48 hours at room temperature with occasional stirring. It was then filtered using whatman filter paper and the filtrate was concentrated in water bath at 40°C. The filtrate was then partially purified through partitioning with ethyl acetate (1:1 v/v), which selectively extracts flavonoid, aglycones and glycosides. The ethyl acetate phase was then dried through evaporating the solvent to obtain a flavonoid-rich fraction(Harborne, 1997).

#### *Extraction of phenolic compounds*

Fifty grams (50 g) of powdered *Piliostigma reticulatum* leaf was cold macerated in 500 mL of 80% methanol acidified with 1% formic acid for 48 hours with gentle agitation. The extract was filtered using whatman filter paper and the filtrate was concentrated in water bath at temperatures not exceeding 40°C to preserve phenolics integrity until dryness (Harborne, 1997).

### Experimental design for acute toxicity study

In phase I, twenty seven Swiss albino rats were grouped into three groups of three rats per group for each of the alkaloid, flavonoid and phenolics rich extracts and administered three graded doses of the respective extracts. In phase II, nine Swiss albino rats were grouped into three groups of one rat per group for each of the phytochemical extracts and administered three different doses of the respective extracts base on the outcome of the phase I.

### Acute toxicity test

The Median lethal dose ($LD_{50}$) of the three phytochemical extracts were determined according to the method described by Lorke, (1983). The protocol consists of two phases. In the first phase, three groups of three rats per group for every phytochemical extract were

treated orally with graded doses of the three respective extracts at 100, 500 and 1000mg/kg. The rats were observed for signs of toxicity and/or death for the first 4h and then over 24h. In the second phase, three rats were divided into three groups of one rat each for every phytochemical extract and treated with 2000, 3000 and 5000mg/kg of the extract, based on the outcome of the phase I. The animals were also observed for signs of toxicity and/or death over 24 h. The $LD_{50}$ was calculated as the geometric mean of the lowest lethal dose and that of the highest non-lethal dose.

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

$D_0$ = highest dose that gave no mortality
$D_{100}$ = lowest dose that produced mortality Experimental

**Experimental design for antimalarial suppressive study**

For each phytochemical class, the mice were divided into six groups (A-F) of five mice per group. Group 1 was not infected with the parasite and was not administered any treatment. This group served as the normal control group. Group 2 was infected with the parasite and treated with chloroquine (5mg/Kg) and served as standard drug control group. Group 3 was infected with the parasite and was not treated and served as negative control group; Groups 4, 5 and 6 of the respective phytochemical classes were infected with the parasite and administered 50, 100 and 200 mg/kg body weight of the respective phytochemicals orally once daily base on the outcome of the acute toxicity study.

| Groups | Alkaloid rich extract | Flavonoid rich extract | Phenolic rich extract |
|---|---|---|---|
| **Group I (NC)** | NN | NN | NN |
| **Group II (DC)** | 5mg/Kg.b.w CQ | 5mg/Kg.b.w CQ | 5mg/Kg.b.w CQ |
| **Group III (NG)** | IN | IN | IN |
| **Group IV (D1)** | 50 (mg/Kg b.w) | 50 (mg/Kg b.w) | 50 (mg/Kg b.w) |
| **Group V (D2)** | 100 (mg/Kg b.w) | 100 (mg/Kg b.w) | 100 (mg/Kg b.w) |
| **Group VI (D3)** | 200 (mg/Kg b.w) | 200 (mg/Kg b.w) | 200 (mg/Kg b.w) |

Key: NN = not infected and not treated, IN = infected and not treated, CQ = chloroquine, NC = normal control, DC = drug control, NG = negative control, D1 = first dose, D2 = second dose, D3 dose three.

**Animal infection and treatment**

The Peters 4-day antimalarial suppressive test was adopted (Peters, 1965) in this study. A total of ninety Swiss albino mice were used for the study. Seventy five (75) Swiss albino mice of both sexes were infected by intraperitoneal (I.p) injection with standard inoculum of *P. berghei* (1 x 10 $^{7}$). Four hours after infection, the infected mice were allocated to each of the three extract with each extract group having 25 mice. The 25 mice were then divided into five groups of five mice per group. The standard drug control group received 5mg/Kg bw chloroquine orally, the negative control group received no treatment, the treatment groups then received three graded doses of 50, 100 and 200mg/Kg bw of the respective phytochemical extracts orally base on the $LD_{50}$ of each extract. Five healthy uninfected mice were allocated to each of the phytochemical extracts and served as the normal control group. The treatment continued once daily for four consecutive days. On day 5 of the experiment, blood sample was collected from the tail of each mouse and smeared on to a microscope slide to make a film. The blood films were then fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 minutes. The slides were then examined microscopically to determine the parasitemia level (by counting the number of parasitized RBCs in three random microscopic felds) and percentage parasitemia for each mouse was calculated using the formular in equation 1. The percentage suppression of parasitaemia was calculated for each dose level using equation 2 by comparing the mean parasitaemia in negative controls with those of treated mice.

$$\% \text{ Parasitaemia} = \frac{\text{Total number of PRBC X 100}}{\text{Total number of RBC}} \ldots [1]$$

Where RBC= Red Blood Cells; PRBC= Parasitized Red Blood Cells (parasite count)

$$\% \text{ Chemosuppression} = \frac{A - B}{A} 100 \ldots\ldots\ldots [2]$$

Where;
A = Mean parasitemia of negative control,
B = mean parasitemia of treated group

**Hematological analysis**

Mice in the phytochemical extract group that exhibits the highest parasite suppression in the suppression test were subjected to hematological assay. A day after the completion of treatment, three (3) animals from each group (1-6) of the most active extract group were sacrificed by suffocation in chloroform vapor. Blood was obtained by cardiac puncture using sterile syringe and needle and transferred into clean EDTA containers for hematological analysis using automated electronic hematology analyzer (Beckman Coulter DxH 800). The analysis was carried out according to the manufactures

guide. Briefly, blood samples are collected into EDTA tubes, mixed and labeled. The samples are then loaded into the analyzer and the desired tests are selected. The analyzer then aspirates the blood sample and perform the analysis. Then it generated the result as a print out.

**Liver function analysis**

Mice in the phytochemical extract group that exhibits the highest parasite suppression in the suppression test were subjected to liver function assay. A day after treatment, three (3) animals from each group (1-6) of the most active extract group were sacrificed by suffocation in chloroform vapor. Blood was obtained by cardiac puncture using sterile syringe and needle and transferred into clean plain containers for the assessment of liver function biomarkers that include Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP) activities, bilirubin, albumin and total protein using automated electronic Biochemical analyzer (URIT 880 Biochemistry analyzer). Briefly, blood samples were collected in plain tubes, labeled and loaded into the analyzer's tray. The analyzer aspirates precise amount of sample and adds some reagents to the sample which are then incubated at a controlled temperature over a period of time. The analyzer then measures the absorbance or optical properties of the sample and then calculates the concentration of analyst base on the values.

**UV-Visible analysis of the most active phytochemical extract**

UV–Visible analysis of the most active phytochemical extract was carried out using a UV-visible spectrophotometer (Perkin Elmer, USA Model: Lambda 950) with a slit width of 2nm, using a 10-mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 200-750nm to observe possible electronic transitions (σ- σ*, σ- π*, π- π*, π- σ*, n- σ *, n- π *) of compounds base on $\lambda_{max.}$ Briefly, the sample was dissolved at a ratio of 1:10 in ethanol, centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper. It was then placed in a cuvette to two-third of the total volume of the cuvette. The cuvette was then placed in the spectrophotometer and scanned at a wavelength range of 200-750nm. The absorbance at different wavelengths were recorded and a graph of absorbance versus wavelength was plotted to obtain the UV-Vis spectra of the extract.

**FTIR analysis of the most active phytochemical extract**

FTIR (Nicolet iS10, Thermo Fisher Scientific, Madison, USA) equipped with temperature stabilized detector was used for the analysis of functional groups present in the phytochemical extract base on their different vibrational transitions (stretching, bending) in the wave number range of 400–4000 cm−1 which can provide information about the structure of molecules present. Briefly, the phytochemical extract was mixed in dry potassium bromide (KBr) and thoroughly mixed in a mortar and pressed at a pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory and scanned from 4000 to 400 cm-1, and the spectra were recorded. The FTIR spectra was obtained and peak values (wavenumber ($cm^{-1}$) and % transmittance) were used for the identification of functional groups present with the help of FTIR table.

**Gas chromatography-mass spectrometry (GC-MS) analysis of the most active phytochemical extract**

The GC–MS analysis of the phytochemical extract was carried out in a (QP 2010 Plus SHIMADZU) instrument at 70 eV to identify the active constituents of the phytochemical extract. Briefly, the dried extract was dissolved in methanol and filtered using syringe filter and diluted. It was then transferred in GCMS sample vial for the analysis. One microliter (1 uL) of the extract was injected into the GC–MS using a micro syringe and scanning was performed for 20 min. The column used was Rtx-5 of 30m X 0.25mm X 0.25µm size. The initial column temperature was $100^0$C rising 28°C at a rate of $5^0$C/min and the temperature was maintained for 3 minutes. The temperature was further increased to [28]$0^0$C at a rate of $15^0$C/min with a hold time of 35 minutes. The ion source of mass spectrometer was held at $230^0$C with an interface temperature of $270^0$C. Detection was performed in full scan mode from m/z 40 to 650.The identification of the peaks was computer generated by comparing their mass spectra obtained with those of the bibliography data of known compounds from the national institute of standards and technology (NIST) library mass spectra database.

**Molecular docking analysis of the major phytocompounds identified against falcipain-2**

Molecular docking was carried out to identify top hit antimalarial phytocompound from the library of compounds identified in the most active phytochemical extract (phenolics rich extract). The docking was carried out against some novel *Plasmodium falciparum* drug targets (plasmepsin II (PMII), histo-aspartic protease (HAP), falcipain-2 (FP2), and P. *falciparum* enoyl acyl carrier protein reductase (PfENR)).

**Ligand preparation**

The identified phytocompounds served as the ligand and they were downloaded from Pubchem database in SDF format. Structures of compounds not available in Pubchem were sketched using chemdraw software version 21 and converted to 3D structurce. The structures were then converted to PDB format and saved.

**Receptor preparation**

The 3D crystal structures of *Plasmodium falciparum* receptors that include plasmepsin II (PMII), histo-

aspartic protease (HAP), falcipain-2 (FP2), and P. falciparum enoyl acyl carrier protein reductase (PfENR) with PDB IDs 1LF2, 3FNS, 3BPF, and 1VRW respectively were downloaded from protein data bank (PDB database, https://www.rcsb.org) and imported into Biovia discovery studio for binding site prediction and protein preparations. Water molecules and Hetatm were removed and polar hydrogen added. The binding site of the respective receptor molecules were predicted and XYZ coordinates of the ligand binding site were obtained for grid box construction during docking in 'PyRx'. Prepared protein was saved in PDB format for the docking.

**Molecular docking and ligand-receptor visualization**

The prepared proteins were imported into PyRx and converted to pdbqt format ready for docking. All the prepared compounds were also imported into OpenBabel within the Python Prescription Virtual Screening Tool (PyRx) and subjected to energy minimization and then converted to pdbqt format ready for docking. The grid centers for PMII was X= 32.244893 Y= 33.373646 Z = 12.478201and grid box dimension of X = 22.3852, Y = 37.8529, Z = 18.6164; grid center for HAP was X=1.208455, Y=45.590238 and Z=26.931662 and grid box dimension of X=21.7836, Y=27.5750, and Z=25.7145; grid center for FP2 center x = -55.5956, center y = 3.8443, center z = -30.6489 and grid dimension was x = 34.2056, size y = 46.7523, size z = 19.4207; and grid center of PfENR were center X = 30.530889, Y = 95.630850, Z = 34.389203 with grid dimension of x = 24.3723, y = 25.0000, z = 21.9926 as obtained from Biovia discovery studio after prediction of the active site of the sites of the respective receptor molecules. This information were used to center the grid box on the binding site of the proteins and the docking was then executed using PyRx with an exhaustiveness of eight (8). Binding scores in the form of binding energy of the best binding pose of each compound was recorded and the best poses were visualized in Biovia discovery studio to observe the ligand-receptor interactions. Types of interactions and interacting atoms of ligands and amino acids of receptor were noted and 2D as well as 3D profile of the interactions saved.

**In-Silico Drug-Likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) Analysis**

The top hit phytocompounds were subjected to *in-silico* ADME analysis using swiss-ADME tools http://www.swissadme.ch, which is an online web tool used for ADME analysis. 'SMILES' formats of the most active phytocompound were used for the toxicity assessments using PROTOX-II, which is an *in silico* online toxicity assessment tool. ADMET profile of the compounds were then compiled.

**Data analysis**

Data analysis was carried out using statistical package for social sciences (SPSS) version 25 and results of replicates were expressed as mean ± SD. Two-way ANOVA with a post-hoc Tukey's HSD test was used to analyze significant difference between means and values were considered significantly different at $p \leq 0.05$.

## RESULT AND DISCUSSION

Table 1. Shows the results of acute toxicity of alkaloid, flavonoid and phenolic extracts of *Piliostigma reticulatum* leaf. In the phase I part of the test, no behavioral change was observed in all the groups that received 10, 100 and 1000mg/kg of the respective extracts and no death was recorded. In phase II part of the test, the same observation was made. No behavioral change nor death was recorded in all the animal groups that received 2000, 3000 and 5000mg/kg of the respective phytochemical extracts. Fayanju et al. (2020) mentioned that acute toxicity study accesses the adverse effects of substances on living organisms after a single or short-term exposure. This study is performed principally in rodents and provides information on safety and potential risks of substances pharmaceuticals inclusive which guide the handling, classification and administration of substances. It is an initial step in the evaluation of the toxic characteristics of a substance for both health and environmental effects. Acute toxicity studies carried out on the alkaloid, flavonoid and phenolic extract of *Piliostigma reticulatum* reveals that the three phytochemical extracts caused no behavioral change in all the doses administered and no mortality was recorded as well (Table 1). Thus, the $LD_{50}$ of the extracts were all concluded to be >5000mg/kg and the extracts are said to be non-toxic in line with Organization for Economic Co-operation and Development (OECD) guide for the classification of substances. The finding of this study is in agreement with previous findings by Abdulkadir et al. (2022) and Fayanju et al. (2020) who reported that the crude methanolic extract of the plant had $LD_{50}$ > 5000mg/kg and reported no behavioral change in the animals. The relative safety of this plant leaf may justify its wide usage and acceptability as medicine, feed and food by inhabitants of the sub-Saharan region Boualam et al. (2021). This finding was instrumental in calculating the dose to be administered to the experimental animals for antimalarial studies using the ≤ one tenth of $LD_{50}$ rule.

**Table 1.** Acute toxicity of alkaloid, flavonoid and phenolic extracted from *Piliostigma reticulatum* leaf.

| | PHASE 1 | | | |
|---|---|---|---|---|
| | Dose | Number of mice | Number of deaths | Behavioral change |
| Alkaloids | 10 | 3 | 0 | Non |
| | 100 | 3 | 0 | Non |
| | 1000 | 3 | 0 | Non |
| Flavonoids | 10 | 3 | 0 | Non |
| | 100 | 3 | 0 | Non |
| | 1000 | 3 | 0 | Non |
| Phenolics | 10 | 3 | 0 | Non |
| | 100 | 3 | 0 | Non |
| | 1000 | 3 | 0 | Non |
| | **PHASE 2** | | | |
| | Dose | Number of mice | Number of deaths | Behavioral change |
| Alkaloids | 2000 | 1 | 0 | Non |
| | 3000 | 1 | 0 | Non |
| | 5000 | 1 | 0 | Non |
| Flavonoids | 2000 | 1 | 0 | Non |
| | 3000 | 1 | 0 | Non |
| | 5000 | 1 | 0 | Non |
| Phenolics | 2000 | 1 | 0 | Non |
| | 3000 | 1 | 0 | Non |
| | 5000 | 1 | 0 | Non |

Table 2. shows the result of parasite suppression test of alkaloid, flavonoid and phenolic extracted from *Piliostigma reticulatum* in mice infected with Plasmodium berghei (NK-65). Mean %parasitemia and %chemosupression were calculated from the formulars mentioned in equation (1) and (2) respectively. All the phytochemical extracts were able to suppress the %parasitemia in dose dependent manner with phenolics rich extract exhibiting the highest %chemosuppression value of 80% at 200mg/kg next to the standard drug used (chloroquine) with a %chemosuppression of 82.89% at 5mg/kg.

**Table 2.** Mean %parasitemia and %chemosupression of alkaloid, flavonoid and phenolic extracted from *Piliostigma reticulatum.*

| GROUPS | MEAN % PARASITEMIA | % CHEMOSUPRESSION |
|---|---|---|
| **NC** | 0.00±00 | 100 |
| **DC** | 6.37±0.75 | 82.89 |
| **NG** | 37.23±5.70 | - |
| **Alkaloid 50mg/kg** | 19.03±1.22 | 48.89 |
| **Alkaloid 100mg/kg** | 14.40±1.01 | 61.32 |
| **Alkaloid 200mg/kg** | 10.03±1.30 | 72.33 |
| **Flavonoid 50mg/kg** | 17.67±0.75 | 52.53 |
| **Flavonoid 100mg/kg** | 14.47±0.65 | 61.13 |
| **Flavonoid 200mg/kg** | 10.37±1.25 | 72.15 |
| **Phenolics 50mg/kg** | 13.03±0.31 | 65.00 |
| **Phenolics 100mg/kg** | 9.23±1.17 | 75.21 |
| **Phenolics 200mg/kg** | 7.57±0.51 | 80 |

NC = normal control, DC = drug control, NG = negative control, D1 = first dose, D2 = second dose, D3 dose three.

Table 3. Shows the mean difference of the effects of the treatments on %parasitemia base on results of two-way ANOVA. Statistically significant difference between the treatments were observed ($P<0.05$). All the treatments had statistically significant ($P<0.05$) effect on %parasitemia compared to the negative group. There was no statistically significant difference ($P>0.05$) between the effect of standard drug and Phenolics rich extract on %parasitemia. Also, no significant difference ($P<0.05$) was observed between the effect of alkaloid and flavonoids on %parasitemia.

**Table 3.** Effect of treatments on % parasitemia.

| Phytochemical extracts | Mean parasitemia |
|---|---|
| Standard drug control | 6.37[a] |
| Negative control | 37.27[c] |
| Phenolics | 9.94[a] |
| Flavonoids | 14.16[b] |
| Alkaloids | 14.49[b] |

*Means with different superscript are statistically significantly different at P<0.05. F = 186.798, P = 0.00, $\eta^2 = 0.961$

Table 4 shows the effects of the various doses of the phytochemical extracts administered to the mice infected with *Plasmodium berghei* (NK-65) on % parasitemia. All the extract administered had statistically significantly different (P<0.05) effect on %parasitemia. However, there was a statistically significant difference (P<0.05) between the effect of the 10mg/kg of the extracts administered compared to 200 and 100mg/kg.

**Table4.** Effect of treatment doses on % parasitemia.

| Treatments | Mean % parasitemia |
|---|---|
| **50mg/kg** | **18.67[a]** |
| **100mg/kg** | **16.35[ab]** |
| **200mg/kg** | **14.32[b]** |

*Means with different superscripts are statistically significantly different at P<0.05. F = 10.078, P = 0.00, $\eta^2 = 0.402$

The antimalarial activities exhibited by the alkaloids, flavonoids and phenolics rich extracted from *Piliostigma reticulatum* leaf validates the report of Abdulkadir et al. (2022) who mentioned these phytochemicals as some of the constituents of the methanol extract of *Piliostigma reticulatum* leaf which exhibited dose dependent antimalarial activity *in vivo*. Although all the phytochemical extracts exhibited significant (P<0.05) antimalarial activity compared to the negative control group, the effects of phenolics rich extract on %parasitemia was more pronounced as it had no significant difference (P>0.05) with the standard drug control group. This finding highlights the

Potentials of this extract and the need for prioritizing it in the quest for novel antimalarial drug. Similarly, Zailani et al. (2020) reported that some phytochemicals including alkaloids, flavonoids and phenolic rich extracted from *Cola nitida* exhibited antimalarial activities *in vivo* and restores derangements in hematological parameters. In a review titled "Antiplasmodial , antimalarial activities and toxicity of African medicinal plants: a systematic review of literature" done by Tajbakhsh et al. (2021), it was reported that these phytochemicals constitute the major component of various plant extracts that exhibits antimalarial activity both *in vitro* and *in vivo.* These underscore the potentials of these phytochemicals in providing novel antimalarial drugs that can surmount the challenges faced by the current frontline antimalarial since even the earlier antimalarial drugs such as quinine which is an alkaloid isolated from the bark of *Cinchona officinalis* earlier used by the indigenous people of South America to treat malaria, artemisinin which is an a terpenoid isolated from the leaves of *Artemisia annua* which has been used for centuries in the treatment of malaria in traditional Chinese medicine were both of plant origin (Nureye et al., 2020).

Table 5. shows the effects of phenolics rich extract on hematological indices of mice infected with *Plasmodium berghei* (NK-65). The extract was able to restore the distorted hematological indices towards normal in a dose dependent manner. The effects of all the doses of phenolics rich extract on the parameters were statistically significantly different (P<0.05) from the negative control group except for PCV at 50mg/kg. When compared with the effect of standard drug on the hematological parameters, phenolic extract had no significant difference (P>0.05) at 200mg/kg.

**Table 5.** Effects of the most active phytochemical extract (phenolic extract) of *Piliostigma reticulatum* leaf on hematological parameters of mice infected with *Plasmodium berghei* (NK-65).

| Groups | PCV | Hb | RBC | WBC | Lymp. |
|---|---|---|---|---|---|
| **NC** | 44.06±0.98[a] | 13.36±0.65[a] | 8.40±0.46[a] | 13.03±0.11[ab] | 12.50±0.60[a] |
| **DC** | 42.26±0.53[a] | 12.74±0.90[a] | 7.34±0.49[ab] | 14.03±0.18[b] | [28].43±2.15[b] |
| **NG** | 26.96±1.19[c] | 9.60±0.63[c] | 5.06±0.15[d] | 18.66±0.89d | 68.42±0.90[d] |
| **D1** | 30.29±1.44[c] | 10.66±0.41[b] | 5.75±0.36[c] | 16.14±0.34[c] | 55.47±4.02[c] |
| **D2** | 34.48±2.34[b] | 12.12±0.51[ab] | 6.39±0.44[bc] | 12.53±0.32[ab] | 52.67±1.50[c] |
| **D3** | 41.23±1.73[a] | 12.90±0.86[a] | 7.10±0.22[b] | 12.39±0.40[a] | 31.30±1.05[b] |

*Means with different superscripts are statistically significantly different at P<0.05, n=3
NC = normal control, DC = drug control, NG = negative control, D1 = first dose, D2 = second dose, D3 dose three.

Phenolics rich extract treated groups exhibited the best antimalarial activity and was therefore was prioritized for further studies including hematological and liver function assays. Dysregulation of hematological parameters particularly red blood cell count, pack cell volume and hemoglobin concentration is a major clinical sign and cause of mortality in malaria (Enechi et al., 2016), and the intraerythrocytic stage of the malaria parasite's life cycle is responsible for this derangement due to invasion and subsequent destruction of the red blood cells by the parasite (Zailani, et al., 2020). The hematological parameters of animals in the phenolic extract treated group (group with the best antimalarial activity) were assayed to observe possible changes that

can be corroborative to the finding of the malaria suppressive effects. The findings of this study reported that all the animal groups treated with the phenolic extract had their red blood cell parameters including pack cell volume, hemoglobin concentration and red blood cell count restored towards normal in dose dependent manner and statistically significantly different (P>0.05) from negative control group (Table 5.). A statistically significant difference (P<0.05) was observed between the extract, standard drug treated groups and the negative control group (Table 5.). However, no statistically significant difference (P>0.05) was observed in the red blood cell parameters between the extract treated groups and group receiving the standard drug. The restoration of the hematological parameters towards normal in dose dependent manner can be attributed to the parasite clearance effect of the extract which allows for restoration of hematological parameters through improved hematopoiesis and decreased red blood cell destruction during the intraerythrocytic stage of the parasite life cycle (Mawson & Mawson, 2016). This finding corroborates the malaria suppressive effect of the phenolics rich extract of the studied plant. White blood cells and its differentials constitutes the body's immune system and responsible for fighting off invaders. Previous antimalarial studies on crude methanol leaf extract of this plant also reported association between antimalarial activity and restoration of hematological parameter(Abdulkadir et al., 2022). During infection by pathogens and parasites, white blood cells parameters normally rise to higher levels to increase the robustness of the system in fighting off the infection and ultimately protecting the body. This justifies the statistically significant increase (P<0.05) in the levels of white blood cells observed in the negative control group compared to the normal group (Table 5.). However, the groups that received the phenolic extract had their white blood cells level restored towards normal in a dose dependent manner. The statistically significant difference between the white blood cells level of the extract treated group and negative control group gives credence to the malaria suppressive effect of the phenolic extract as shown in table 3. This finding is in agreement with previous findings reported by (Enechi et al., 2016) and (Zailani, ., et al., 2020). Thus, it can be asserted that the corroborativeness of the hematological parameters to the malaria suppressive effect justifies the antimalarial activity of the phenolic extract of *Piliostigma reticulatum* leaves.

Table 6. shows the effect of administering the phenolic extract on liver function parameters of mice infected with *Plasmodium berghei* (NK-65). The different doses of the extracts were able to restore the distorted liver function parameters in dose dependent manner. At the highest dose (200mg/kg), all the liver biomarkers were not statistically significantly different (P>0.05) from the normal except alkaline phosphatase. However, they were all statistically different from the negative control group.

**Table 6.** Effects of the most active phytochemical extract (phenolic extract) of *Piliostigma reticulatum* leaf on liver function biomarkers of mice infected with *Plasmodium berghei* (NK-65).

| Groups | AST | ALT | ALP | T. B | D. B | Alb. | T. Prot. |
|---|---|---|---|---|---|---|---|
| **NC** | $31.67\pm3.05^{ab}$ | $32.73\pm3.00^{a}$ | $68.27\pm2.37^{a}$ | $5.53\pm0.46^{b}$ | $0.44\pm0.05^{a}$ | $30.67\pm2.08^{a}$ | $54.67\pm2.08^{b}$ |
| **DC** | $35.67\pm11.93^{ab}$ | $32.93\pm2.25^{a}$ | $160.67\pm6.66^{b}$ | $3.67\pm0.58^{a}$ | $0.46\pm0.04^{ab}$ | $25.00\pm2.00^{bc}$ | $50.00\pm1.00^{b}$ |
| **NC** | $44.93\pm1.88^{b}$ | $53.30\pm3.82^{b}$ | $155.67\pm6.11^{b}$ | $11.07\pm1.10^{c}$ | $0.59\pm0.02^{cd}$ | $21.00\pm2.65^{c}$ | $34.67\pm3.21^{c}$ |
| **D1** | $39.59\pm3.92^{ab}$ | $35.57\pm3.00^{a}$ | $72.00\pm3.00^{a}$ | $6.80\pm0.70^{b}$ | $0.62\pm0.04^{d}$ | $20.33\pm2.08^{c}$ | $35.67\pm4.93^{c}$ |
| **D2** | $30.00\pm3.61^{ab}$ | $32.30\pm2.56^{a}$ | $77.67\pm11.66^{a}$ | $6.34\pm0.41^{b}$ | $0.54\pm0.03^{bcd}$ | $[28].33\pm2.08^{ab}$ | $71.00\pm2.00^{a}$ |
| **D3** | $26.00\pm4.36^{a}$ | $33.73\pm1.70^{a}$ | $146.33\pm9.07^{b}$ | $6.11\pm0.[28]^{b}$ | $0.51\pm0.02^{abc}$ | $30.33\pm1.15^{ab}$ | $51.00\pm2.65^{b}$ |

*Means with different superscripts are statistically significantly different at P<0.05
NC = normal control, DC = drug control, NG = negative control, D1 = first dose, D2 = second dose, D3 dose three.

Liver is the first niche of *plasmodium spp* after being injected into the living system. The colonization of the liver involves invasion and subsequent destruction of hepatocytes. These leads to the leakage of hepatic enzymes into circulation observed as increased liver function biomarkers such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, total bilirubin among others (Odutuga et al., 2026). Therefore, it was not surprising that a statistically significant difference (P<0.05) was observed between the ALT and AST levels of the negative and those of extract, standard drug treated groups (Tbale 6.). However, both the drug and the extract were able to restore ALT and AST towards normal in a dose dependent manner. The extract was also able to restore alkaline phosphatase (ALP) towards normal in a dose dependent manner. However, the drug was not able to restore alkaline phosphatase (ALP) levels towards normal as done by the extract, and there was no statistically significant difference between the standard drug control and negative control groups. The unique metabolic role of the liver as detoxification organ exposes it to possible damage by foreign substances drugs inclusive. This indicates the possible hepatoxicity of the standard drug as previously reported in literature (Odutuga et al., 2026). Thus, Characterizing liver enzymes in uncomplicated malaria is becoming increasingly important, as clinical trials of novel

antimalarial NCEs have reported abnormalities in liver enzymes, specifically alanine aminotransferase (ALT) and/or total bilirubin (TB) (Cheaveau et al., 2019). Perturbation of bilirubin levels in malaria is associated with increased hemolysis, hepatocyte damage and sometimes bile duct obstruction due to parasite activities (Odutuga et al., 2026). This is observed as increased levels of total and direct bilirubin in the blood. This justifies the increased levels of total and direct bilirubin observed in the negative control group. However, both standard drug and extract treated groups were able to restore the bilirubin levels in a dose dependent manner as there was statistically significant difference ($P<0.05$) between both groups and the negative control group. The effect of the extract on albumin and total protein was not statistically different ($P>0.05$) compared to the normal group. The ability of the extract to restore the albumin and total protein levels towards normal is an indication of the efficacy of the extract in clearing the parasites and its relative safety to the liver which allows for proper protein metabolism by the liver. The ability of the extract to restore these liver function parameters towards normal consolidates the outstanding effect of the phenolic extract in the malaria suppressive test (table 2.). Similar findings have also been reported by Zailani et al., (2020) who reported that the antimalarial activity of some phytochemicals extracted from *Cola nitida* succeeded in restoring liver function parameters in mice infected with *Plasmodium berghie*. Also, Enechi et al., (2016) mentioned that liver function parameters were restored in mice infected with *Plasmodium berghei* and treated with crude methanol seed extract of *Buchholzia coriacea*.

**UV/Visible analysis of phenolics rich extract of *Piliostigma reticulatum***

Figure 1. shows the absorption of UV-Visible radiation by phenolics rich extract of *Piliostigma reticulatum.* Absorption bands were between 200-500nm.

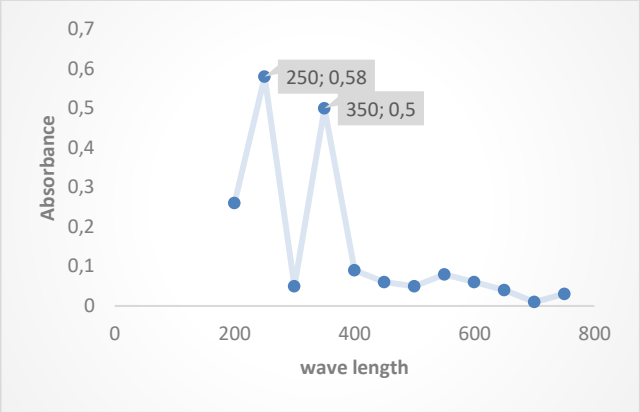


**Figure 1.** UV-Visible spectra of phenolics rich extract of *Pillostigma reticulatum.*

Figure 2. Shows the FTIR spectrum of phenolic extract. Various functional groups of the active compounds were identified base on the peak values in the infrared region. Information about peaks observed in the analytical (functional group) region of the spectrum useful in providing insight on the possible compounds present in the extract are compiled in table 8. Absorption was observed at 3277, 2849, 2917, 1731 and 1614cm$^{-1}$ indicating the presence of –OH, C-H, C=O and C=C stretching respectively.

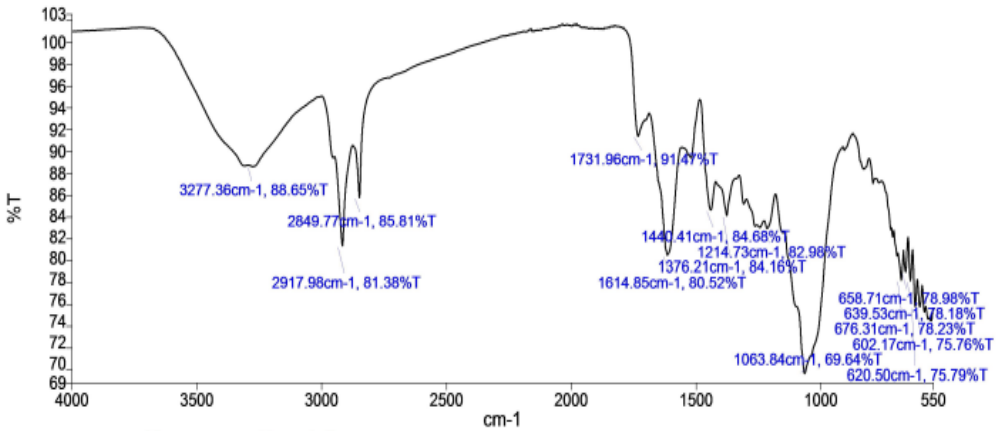


**Figure 2.** FTIR spectrum of phenolics (most active extract) extracted from *Pillostigma reticulatum.*

**Table 8.** Peak information of *Piliostigma reticulatum* leaf phenolic extract FTIR spectrum.

| Peak number | Peak value (cm$^{-1}$) | Reference range | Functional group/bond | Class of compound | Transmittance (%) | Peak intensity/ signal strength |
|---|---|---|---|---|---|---|
| **1** | 3277.36 | 3200-3450 | O-H stretching | Alcohol, acid | 88.65 | Weak |
| **2** | 2917.98 | [28]00-2980 | $SP^3$ C-H stretching | Alkyl | 81.38 | Weak |
| **3** | 2849.77 | [28]00-2980 | $SP^3$ C-H stretching | Alkyl | 85.81 | Weak |
| **4** | 1731.96 | 1700-1750 | C=O stretching | Carbonyl | 91.47 | Weak |
| **5** | 1614.85 | 1600-1680 | C=C stretching | Alkene, aromatic ring | 80.52 | weak |

Spectroscopic techniques such as FTIR, UV/Visible, mass spectroscopy, NMR are instrumental in the characterization of unknown substances such as isolated compounds as well as profiling of crude substances such as extracts of plant materials (Kalaichelvi, 2017). Figure 1 shows the UV-Visible analysis spectrum of the phenolics rich extract. Higher absorptions were observed at wavelengths of 250 and 350nm with absorbance of 0.58 and 0.5 respectively. Other wavelengths where lower absorptions were observed are 200, 400 and 550nm. Absorption at 200 and 250nm are indicative of σ → σ*, π → π* and n → σ* transitions which can be attributed to the presence of single C-H bonds, double bonds in aromatic rings and carbon-carbon double bonds as well as atoms containing lone pair of electrons that may include O, N, S; and absorption between 300-400nm can be attributed to n → π* transition indicative of the presence of compounds containing double bond involving hetero atoms C=O, C≡N, N=O inclusive. All these findings are indicative of important features of important class of plant secondary metabolites phenolics, flavonoids, terpenoids, carotenoids, tannins among many as mentioned in the work of Kumar et al. (2020) which are of pharmacological relevance.

The infrared spectrum of phenolics rich extract in the frequency range of 4000–400 $cm^{-1}$ was obtained to identify the characteristic absorption peaks corresponding to stretching vibrations of different functional groups (figure 2). Peak values in the functional group region (4000–1500 $cm^{-1}$) were useful in the identification of functional groups present in compounds in the extract. Peak values observed at various frequencies in the functional group region indicates the presence of various functional groups (table 8). Prominent peaks at 3277.36, 2917.98, 2849.77, 1731.96 and 1614.85 $cm^{-1}$ are indicative of O-H, C-H, C=O and C=C stretching vibrations (figure 2). This vibrational transitions are indicative of characteristic features of pharmacologically important classes of phytocompounds that include phenolics, terpenoids, carotenoids, flavonoids, tannins among others (Kalaichelvi, 2017). Previous studies have reported that many pharmacologically active phytochemicals belong to these groups of phytochemicals and they have been reported to be present in plant extracts with antimalarial activities *Piliostigma reticulatum* inclusive (Artasa et al., 2025).

Figure 3 shows the GCMS chromatogram of phenolics rich extract of *Piliostigma reticulatum* with different peaks that indicates different compounds base on their respective retention time. Major compounds were observed between the retention times (RT) of 1-25minutes. The various peaks in the chromatogram have their respective spectrum in the mass spectrophotometry that corresponds to specific compounds. The compounds were identified base on similarities of the individual spectrum with spectrum in the NIST data base. Table 9 shows the major compounds identified in the extract with their respective characteristic.

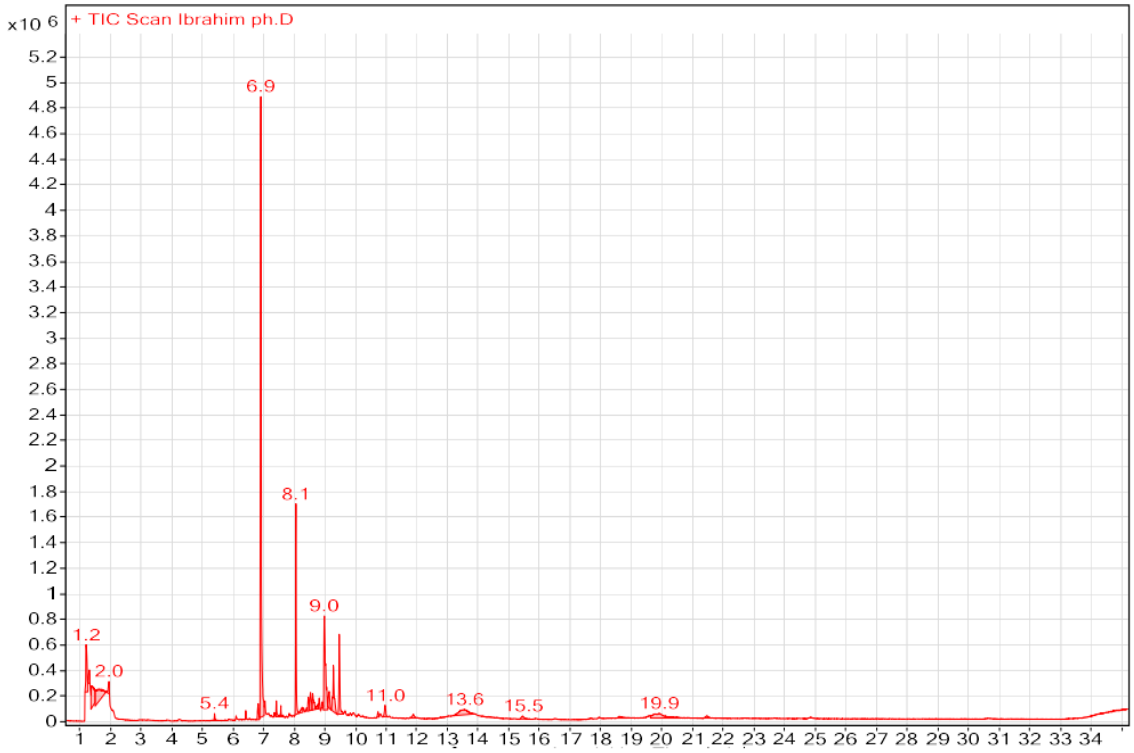


**Figure 3.** GCMS chromatogram of phenolics rich extract of *Piliostigma reticulatum.*

**Table 9.** GCMS identified compounds in phenolics rich extract of *Piliostigma reticulatum.*

| *Peak* | *Compounds* | *RT (minutes)* | *Area* | *Area %* | *Area Sum %* | *Height* |
|---|---|---|---|---|---|---|
| 1 | Propionic acid | 1.2 | 1113555.24 | 11.68 | 4.16 | 368375.67 |
| 2 | β-Hydroxyisovaleric acid | 1.3 | 244335.88 | 2.56 | 0.91 | 104975.39 |
| 3 | 4-Pentene-2-ol, 2-methyl | 1.4 | 1130145.63 | 11.85 | 4.22 | 174753.68 |
| 4 | Hordenine | 1.6 | 1472745.8 | 15.45 | 5.5 | 97079.38 |
| 5 | DL-Ephedrine | 2 | 217100.77 | 2.28 | 0.81 | 95177.24 |
| 6 | Phytol | 5.4 | 98208.14 | 1.03 | 0.37 | 53996.34 |
| 7 | 6-Hydroxynicotinic acid | 6.8 | 313705.76 | 3.29 | 1.17 | 124476.5 |
| 8 | Butylphthalide | 6.9 | 9535064.93 | 100 | 35.6 | 4856450.25 |
| 9 | 1-Decanol, 2-hexyl | 7.4 | 216989.12 | 2.28 | 0.81 | 123477.44 |
| 10 | (Z)-4-Decen-1-ol | 7.6 | 105207.77 | 1.1 | 0.39 | 84922.96 |
| 11 | Hexadecanoic acid, methyl ester | 8.1 | 2322763.21 | 24.36 | 8.67 | 1649348.32 |
| 12 | D-(-)-Ribose | 8.3 | 150185.33 | 1.58 | 0.56 | 39509.43 |
| 13 | 1-Decanol, 2-hexyl | 8.5 | 2701[28].45 | 2.83 | 1.01 | 107253.93 |
| 14 | Tetradecanoic acid, 12-methyl-, methyl ester | 8.5 | 459404.49 | 4.82 | 1.72 | 139399.73 |
| 15 | β-Citronellol | 8.6 | 437157.78 | 4.58 | 1.63 | 1[28]129.26 |
| 16 | Octadecanoic acid, 11-methyl-, methyl ester | 8.8 | 113700.5 | 1.19 | 0.42 | 35303.48 |
| 17 | Tetradecanoic acid, 10,13-dimethyl-, methyl ester | 8.8 | 166528.11 | 1.75 | 0.62 | 81933.13 |
| 18 | Ethylene brassylate | 8.9 | 101990.85 | 1.07 | 0.38 | 47551.07 |
| 19 | Methyl tetradecanoate | 9 | 2666370.99 | 27.96 | 9.96 | 7350[28].13 |
| 20 | 1,2-Octadecanediol | 9.1 | 392610.66 | 4.12 | 1.47 | 131008.77 |
| 21 | 9-Octadecenoic acid (Z)-, methyl ester | 9.3 | 1143196.67 | 11.99 | 4.27 | 359521.28 |
| 22 | Methyl stearate | 9.5 | 1375595.14 | 14.43 | 5.14 | 624897.08 |
| 23 | D-Gluconic acid | 10.7 | 106710.82 | 1.12 | 0.4 | 41754.39 |
| 24 | Tributyl acetylcitrate | 11 | 272397.25 | 2.86 | 1.02 | 89623.99 |
| 25 | Glycerol 1-stearate | 11.9 | 141905.45 | 1.49 | 0.53 | 29301.35 |

A total of twenty five major compounds were identified in the phenolics rich extract of *Pilistigma reticulatum* base on match between the mass spectrum of each of the peaks of the chromatogram with those in the national institute of standard and technology data base (Table 9). Other smaller peaks in the chromatogram have been attributed to the compounds present in small quantities as well as disintegrated major compounds[28] . Most of these compounds have been reported in literature to be present across diverse species of plants in the plant kingdom as secondary metabolites with varying functions essential to the adaptation of the plants(Kabera et al., 2014). Some of the compounds have been reported to be present in the work of (Boualam et al., 2021). Pharmacological activities of these compounds that include antibacterial, antifungal, antioxidant, antidepressant, anti-inflammatory, antihypertensive among many others. Some of the compounds like phytol have been documented to be present in *Andrographis paniculata* and was active against various *Plasmodium falciparum* receptors *in silico* (Afolayan & Oladokun, 2024).

Table 10 shows the result of molecular docking of the twenty five prominent compounds identified in the most active extract of *Piliostigma reticulatum* by GCMS. All the compounds were active against their respective receptors. Compounds identified with the highest binding affinity against plasmepsin II (PMII), falcipain-2 (FP2), and P. *falciparum* enoyl acyl carrier protein reductase (PfENR) was ethylene brassylate with binding affinities of -7.3, -7, and -6.5Kcal/mol. respectively. 2-hexadecanol was the compound with highest activity against histo-aspartic protease (HAP) with binding affinity of -6.4Kcal/mol. Figure 4a-9a shows the 3D complexes of the respective top hit compounds and their respective targets, and the 3D complexes of the respective known inhibitors bound to their respective receptors (figure 4b-9b). The 3D complexes shows the various types of interactions between the ligands and their respective protein targets.

**Table 10.** Docking scores of GCMS identified compounds against *P. falciparum* receptors.

| S/N | Ligands | Binding Affinity to PMII (Kcal/mol) | Binding Affinity to HAP (Kcal/mol) | Binding Affinity to FP-2(Kcal/mol) | Binding Affinity to PfENR (Kcal/mol) |
|---|---|---|---|---|---|
| **1** | Known inhibitors/binding affinity | 3-amino-n-{4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide -9.9 | Pepstatin A -8.2 | N-[N-[1-hydroxycarboxyethyl-carbonyl]leucylamino-butyl]-guanidine -7 | Triclosan -6.0 |
| **2** | Ethylene brassylate | **-7.3** | -5.6 | **-7** | **-6.5** |
| **3** | DL-Ephedrine | -6.1 | -5.7 | -5.8 | -5.1 |
| **4** | phytol | -6 | -5.3 | -5.1 | -4.7 |
| **5** | Glycerol 1-stearate | -5.9 | -6.1 | -5.2 | -4.7 |
| **6** | 2-Hexadecanol | -5.8 | **-6.4** | -6.5 | -5.9 |
| **7** | 1,2-Octadecanediol | -5.6 | -5.8 | -4.8 | -4.4 |
| **8** | 1-Decanol, 2-hexyl | -5.6 | -4.5 | -4.5 | -4.1 |
| **9** | 9-Octadecenoic acid (Z)-, methyl ester | -5.6 | -4.8 | -5.1 | -4.1 |
| **10** | Butylphthalide | -5.6 | -6.1 | -5.9 | -5.1 |
| **11** | Tributyl acetylcitrate | -5.5 | -5.9 | -6.1 | -4.7 |
| **12** | Octadecanoic acid, 11-methyl-, methyl ester | -5.4 | -5.7 | -4.7 | -4.2 |
| **13** | Hexadecanoic acid, methyl | -5.2 | -5.3 | 5.1 | -4.0 |
| **14** | Methyl stearate | -5.2 | -4.5 | -4.5 | -4.3 |
| **15** | Tetradecanoic acid, 10,13-dimethyl-, methyl ester | -5.2 | -5.0 | -4.6 | -4.0 |
| **16** | Tetradecanoic acid, 12-methyl-, methyl ester | -5.1 | -5.7 | -4.6 | -4.3 |
| **17** | D-Gluconic acid | -5 | -6.0 | -5.3 | -5.1 |
| **18** | Hordenine | -5 | -5.8 | -5.5 | -4.3 |
| **19** | 6-Hydroxynicotinic acid | -4.9 | -5.6 | -5.8 | -4.5 |
| **20** | beta-Citronellol | -4.9 | -5.0 | -4.7 | -4.0 |
| **21** | (Z)-4-Decen-1-ol | -4.7 | -4.5 | -4.4 | -3.7 |
| **22** | Methyl tetradecanoate | -4.7 | -5.3 | -4.1 | -4.1 |
| **23** | D-(-)-Ribose | -4.5 | -5.5 | -5.3 | -4.4 |
| **24** | Beta-Hydroxyisovaleric acid | -4.1 | -4.8 | 4.6 | -4.3 |
| **25** | 4-Pentene-2-ol, 2-methyl | -3.8 | -4.3 | -3.9 | -3.4 |
| **26** | propionic acid | -3.4 | -3.6 | -4.3 | -3.5 |

Molecular docking of these compounds against four novel drug targets of *Plasmodium falciparum* (PMII, HAP, FP-2 and PfENR) (Artasasta et al., 2025) revealed that all the compounds had positive interaction with PMII target molecule with a binding affinity ranging between -3.4 and -7.3Kcal/mol, ethylene brassylate exhibited the highest binding affinity with PMII with a binding energy of -7.3Kcal/mol making it the top hit inhibitor of PMII. However, the binding affinity of Ethylene brassylate was not as high as that of PMII known ligand (3-amino-n-{4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide) which has a binding affinity of -9.9Kcal/mol to the PMII receptor (table 10). Ethylene brassylate was found to interact with SER A218 and GLY A216 in the binding pocket of the receptor protein through hydrogen bonds with bond lengths of 2.46 and 3.52 Å respectively; and through Vandaawal's interactions with many amino acids in the binding pocket of the receptor (figure 4A). Hydrogen bonds contribute to stability, strength and specificity of ligand-receptors complexes(Majewski et al., 2019). These depends on the length of the bonds as they increase with decrease in bond length and decreases with increase in bond length (Medimagh et al., 2023). The ideal range of hydrogen bond length is 2.5–3.5 Å, a range in which the bond lengths of the ligand receptor complex in Figure 4A falls. These highlights the possible strength and the stability of the Ethylene brassylate-PMII complex. The higher binding affinity exhibited by the known inhibitor can be attributed to the higher number of interactions it had with the receptor molecule(Majewski et al., 2019). The known inhibitor made eight hydrogen bonds with THR A217, GLY A216, ASP A214, GLY A36, ASN A39, LEU A131 in the binding pocket of the receptor molecule with a bond length range of 1.80-3.25 Å. It also had eight hydrophobic interactions with ILE A32, ILE A123, TYR A77, LEU A131, TYR A192, ILE A300, ILE A212 and MET A75 with the amino acid

residues in the binding pocket of the receptor. The number of hydrogen bonds formed in ligand-receptor complex depends on several factors that include the number of polar functional groups (e.g., hydroxyl, amide, carboxyl, and amine) in the ligand and receptor, availability of hydrogen bonds donor (–OH, –NH) and acceptor (O, N) atoms in ligands and receptors as well as the presence of charged residues (e.g., Asp, Glu, Lys, Arg) in the receptor (Shanshan et al., 2013). The number of hydrophobic interactions made between ligands and receptors are attributed to larger the hydrophobic regions (e.g., aromatic rings, aliphatic chains) in ligand structures and nonpolar amino acids (e.g., Phe, Leu, Ile, Val) in the receptor binding sites. These factors can be responsible for the higher number of interactions made between the known inhibitor and the receptor (PMII) compared to the interaction between the top hit compound and the receptor which translates into the higher binding affinity the known inhibitor has with the receptor compared to the *Piliostigma reticulatum* GCMS detected compounds (table 10). Both the known inhibitor and top hit compound made interactions with the receptor molecule through Van der waals forces. Although Van der waals interaction is weak, the overall effects of the forces contribute significantly to binding stability and affinity, enhances shape complementarity and molecular recognition which leads to enhanced specificity(Ahsin et al., 2025). According to Harish et al. (2025) molecular docking simulations calculate Van der waals interactions to score and rank docking poses which highlights its significant contribution binding energy values.

Docking of the detected compounds against HAP revealed 2-hexadecanol as the top hit compound with binding affinity of -6.4Kcal/mol which is lower than the binding affinity of the known ligand pepstatin A which has binding affinity of -8.2 Kcal/mol with HAP (table 10). The higher binding affinity of the known inhibitor to HAP compared to that of the top hit compound is attributed to the presence of abundant hydrogen bonds and hydrophobic interactions in the former (Urbina & Slipchenko, 2025). Interaction studies revealed that the known inhibitor (pepstatin A) had seven hydrogen bonds with ALA B10, GLU A276, LEU A275, GLN A273 and ASN A159 in the binding pocket of the receptor, and had three hydrophobic interactions with ALA A10, VAL B220 and LEU A278 (figure 5B). Whereas the top hit compound only made one hydrogen bond with ASN B285, and two hydrophobic interactions with ALA B10 and PRO A277 (figure 5A). The higher numbers of interactions (Artasa et al., 2025) made, particularly hydrogen bonds by pepstatin A with the receptor compared to four interactions made between the top hit compound and the receptor accounts for the differences in the binding affinity. Both the known inhibitor and top hit compound made interactions with the receptor molecule through the weak Van der waals forces which additively contribute to binding specificity, stability and ultimately binding affinity (Shi et al., 2025).

Ethylene brassylate was identified as the top hit compound that inhibited falcipain-2 with a binding affinity score of -7Kcal/mol equivalent to the binding energy of the known inhibitor (N-[N-[1-hydroxycarboxyethyl-carbonyl] leucylamino-butyl]-guanidine) (table 10). Study of binding interaction between the known inhibitor and the receptor (FP-2) revealed that the known inhibitor made six hydrogen bond with GLN A36, LYS A37, ASN A16, ASP A35, TRP A206 in the binding site of the receptor, and several Van der waals interactions with many other amino acids in the binding pocket of the receptor protein (figure 6B). The top hit compound (Ethylene brassylate) made only one hydrogen bond with GLN A209 and several Van der waals interactions with many amino acids in the binding pocket of the receptor molecule (figure 6A). This phenomenon can be attributed to the presence of stronger van der waals forces and better steric complementarity between the top hit compound and the receptor as well as a consequence of weakness of the hydrogen bonds that exist between the known inhibitor and the receptor (Raja et al., 2025), hence the similarity in the binding energy (-7Kcal/mol) for both compounds.

Ethylene brassylate also came up to be the top hit compound that inhibited PfENR with a binding energy of -6.5Kcal/mol (figure 7A) slightly better than that of the known inhibitor (triclosan) with binding energy of -6.0Kcal/mol (figure 7B). The slightly higher binding affinity between the top hit and receptor can be attributed to two stronger hydrogen bonds the ligand formed with THR B410, one hydrophobic interaction formed with TYR A412 in the binding pocket of the receptor, as well as multiple Van der waals interactions formed between it and several amino acids in the binding pocket of the receptor (figure 7A) compared to two hydrogen bonds by the known inhibitor with THR B410 and two hydrophobic interactions between it with ILE A419 and TYR B412 all in the binding pocket of the receptor. It also formed several Van der waals interactions with many amino acids of the receptor binding site. Differences in binding affinity have not only been attributed to type of interactions between ligands and receptors in molecular docking, but also to the bond lengths(Alshahrani et al., 2025). This accounts for the differences in binding energy/affinity observed in Figure 7A and 9B.

Although most of the known inhibitors of the Plasmodium falciparum proteins targeted in this study have better binding affinity compared to the binding affinity of the top hit compound (table 10), the compounds identified in the extract remains potential candidates for antimalarial drug discovery due to positive interactions they exhibited with the parsaite's targets at varying binding energies. These may accounts for the best antimalarial activity the phenolics rich extract

exhibited during *in vivo* studies compared to other extracts. All the proteins targeted in this study (PMII, HAP, FP-2 and PfENR) are novel antimalarial targets (Iyabode et al., 2023) on which many researchers are actively working on in a bid to discover novel antimalarial drugs that will surmount the challenges the current antimalarial drugs are confronted with. The multitarget potentials of some of the most active ligands such as ethylene brassylate highlights the potential of novel antimalarial drugs discovered from *Piliostiga reticulatum*. This finding gives credence to the use of *Piliostiga reticulatum* in malaria treatment in traditional medicine as reported by Oderinde et al. (2022) and justifies the positive antimalarial as well as antiplasmodial activities reported in literature (Abdulkadir et al., 2022). The lower activities of the top hits observed in this study can be attributed to lesser number of pharmacophores needed for maximal affinity compared to those of the known inhibitors. However, presence of strong van der waals forces in all the interactions between the top hits and the respective receptors entails the steric complementarity and possible stability between them. Thus, pharmacophore modification which is an essential component of hit modification in the drug discovery pipeline(Klebe, 2013) can be carried out to improve the overall binding affinity, specificity, and ultimately the efficacy of the hit compounds.

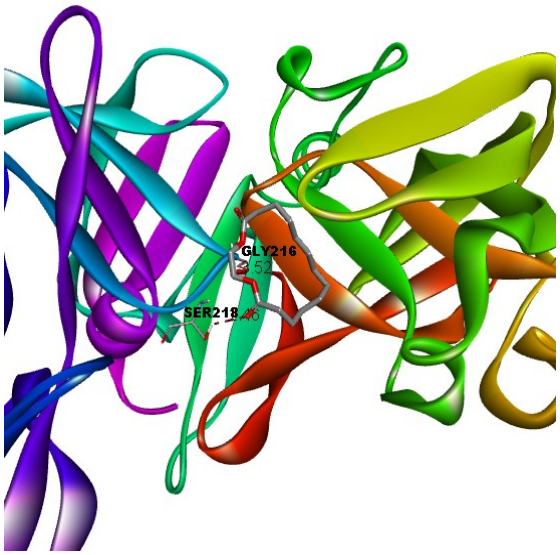


**Figure 4A.** 3D structure of Ethylene brassylate (top hit)-PMII complex.

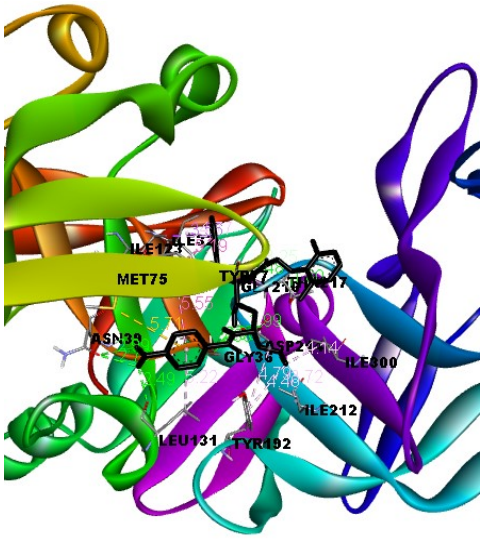


**Figure 4B.** 3D structure of 3-amino-n-{4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide (known inhibitor)-PMII complex.

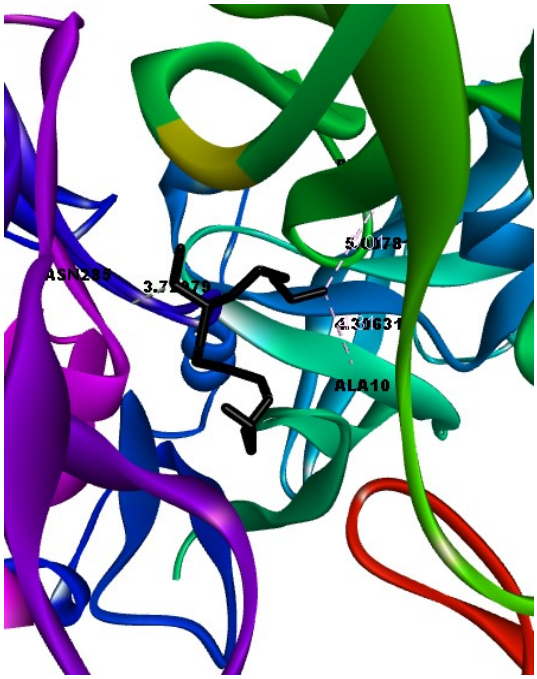


**Figure 5A.** 3D structure of 2-hexadecanol (top hit) -HAP complex.


**Figure 5B.** 3D structure of pepstatin A (known inhibitor)-HAP complex

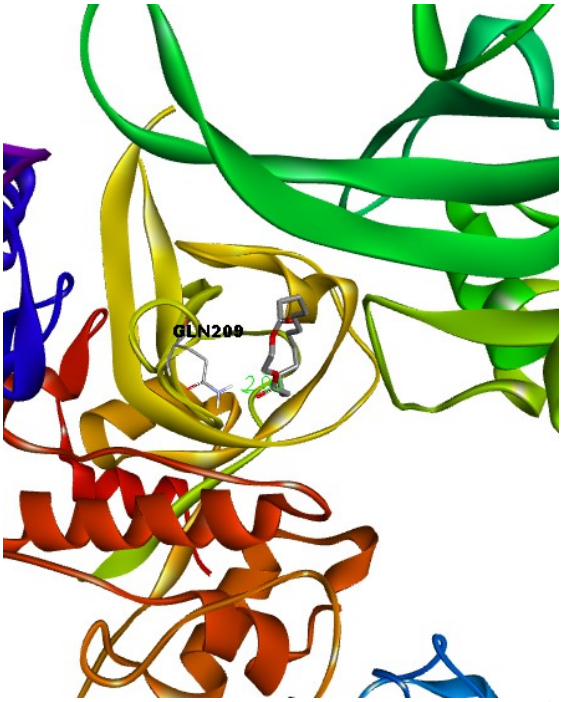


**Figure 6A.** 3D structure of ethylene brassylate (top hit) – FP-2 complex.

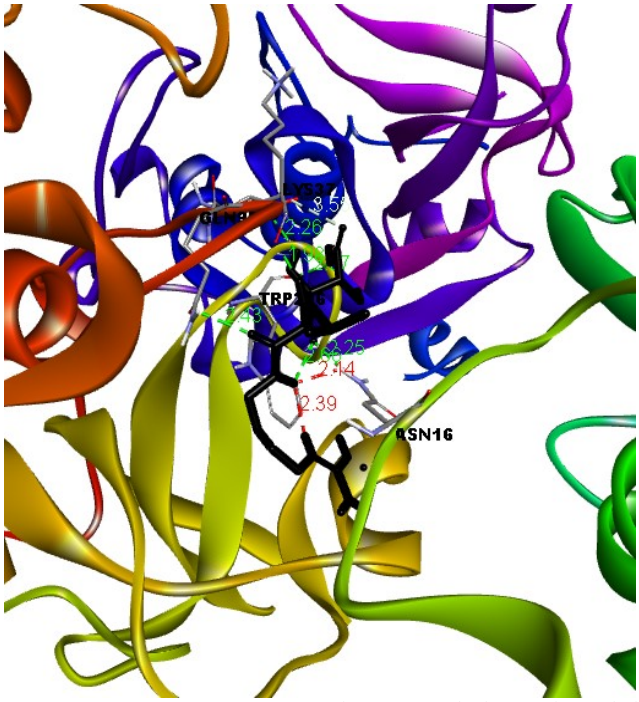


**Figure 6B.** 3D structure of N-[N-[1-hydroxycarboxyethyl-carbonyl]leucylamino-butyl]-guanidine (known inhibitor) – FP-2 complex.

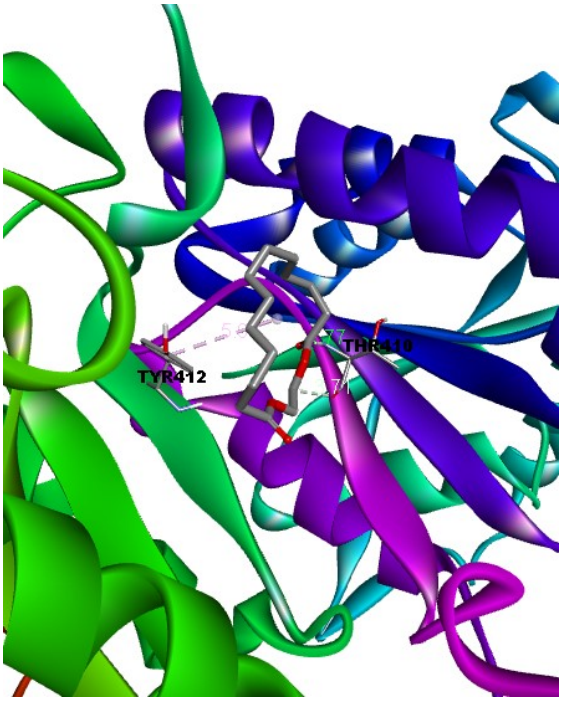


**Figure 7A.** 3D complex of ethylene brassylate (top hit) compound-PfENR complex.

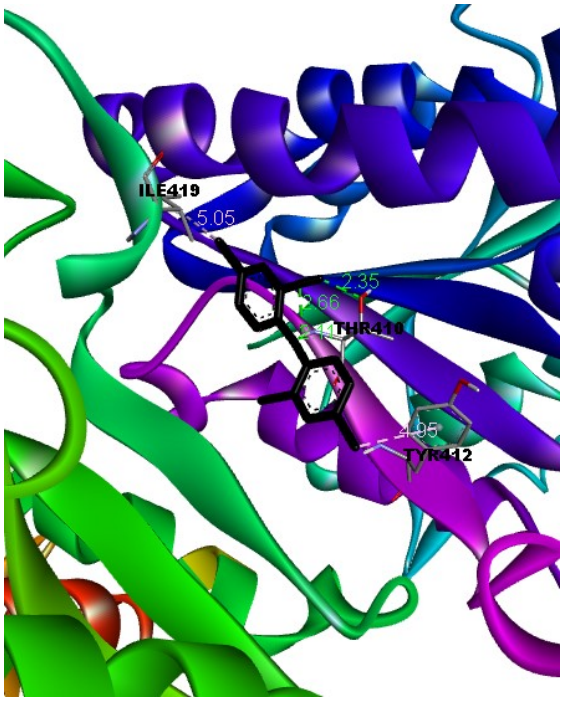


**Figure 7B.** 3D of Triclosan (known inhibitor)-PfENR complex.

Table 11 shows the result of *in silico* ADMET analysis of the top hit compounds. Drug-likeness, absorptions, metabolism and toxicity analysis revealed both compounds exhibit favorable pharmacokinetics and toxicity. They both passed Lipinski's rule of five for drug-likeness, had high GI absorption, non substarte to permeability glycoprotein (p-gp), ability to penetrate blood brainbarrier (BBB), metabolizable by the most important cytochrome isozyme (CYP3A4) and inactive against vital organs.

**Table 11.** ADMET result of the top hit compounds.

| ADMET properties | Ethylene brassylate | 2-hexadecanol |
|---|---|---|
| **Druglikeness (base on Lipinski's rule of five)** | Yes; 0 violation | Yes; 1 violation: MLOGP>4.15 |
| **Gastrointestinal absorption** | high | High |
| **BBB permeation** | yes | Yes |
| **P-gp substrate** | no | No |
| **CYP1A2 inhibitor** | No | No |
| **CYP2C19 inhibitor** | No | No |
| **CYP2C9 inhibitor** | Yes | Yes |
| **CYP2D6 inhibitor** | No | No |
| **CYP3A4 inhibitor** | No | No |
| **Toxicity class** | Class V | Class IV |
| **Predicted $LD_{50}$** | 4750mg/kg | 1000mg/Kg |
| **Hepatoxicity** | inactive | inactive |
| **Neurotoxicity** | Inactive | Inactive |
| **Nephrotoxicity** | Slightly active | inactive |
| **Respiratory toxicity** | Inactive | Inactive |
| **cardiotoxicity** | Inactive | Inactive |
| **Carcinogenicity** | Inactive | Inactive |
| **Immunotoxicity** | Inactive | Inactive |
| **Mutagenicity** | Inactive | Inactive |
| **cytotoxicity** | Inactive | Inactive |

Efficacy and toxicity have been the two major reasons drug candidates fail during drug discovery. This underscores the significance of ADMET study in all stages of drug discovery (Mourya & Pandey, 2024). The top hits of all the Plasmodium falciparum receptors targeted in this study were subjected to *in silico* ADMET analysis Vis-à-vis the low cost and short time frame of the method (Wang et al., 2025). ADMET analysis results of the top hit compounds ethylene brassylates which was the top hit inhibitor of PMII, FP-2 and PfENR, and 2-hexacadenol which was the top hit of HAP are presented in table 11. Ethylene brassylate passed Lipinski's rule of five for druglikeness of compounds with no violation. This implies that this compound is orally bioactive and bioavailable and oral administration of drug that will be formed from this compound can exert the desired effects (Nhlapho & Munien, 2024). It was also revealed that Ethylene brassylate has high GI absorption and was not a substrate of p-gp, which implies that the compound has high tendency of assimilation by the intestine and maintaining desirable concentration in it's targets. It was also observed that the compound has the potential of permeating BBB which highlights its potential of CNS activity which calls for caution in the use of the substance to avoid unfavorable consequencies such as neurotoxicity (Upadhyay, 2014). However, this compound can be advantageous in the treatment of cerebral malaria (CM). In *P. falciparum* CM, infected red blood cells (iRBCs) sequester in the brain's microvasculature. BBB-penetrant antimalarials can reach these sites directly, potentially reducing parasite burden faster(Shekarau et al., 2024). Nevertheless, the use of this compound has to be with caution to avoid tendencies of neurotoxicity. Cytochrome P450 (CYP) enzymes are a superfamily of heme-containing enzymes that play a crucial role in drug metabolism and pharmacokinetics. These enzymes, primarily found in the liver and intestines, influence the absorption, distribution, metabolism, and excretion (ADME) of drugs(Pandey et al., 2024). Ethylene brassylate was found not be inhibitor the cytochrome isoforms except for CYP2C9 which is involed in the metabolism of some drugs such as the NSAIDs. However, most critical isoform of the enzyme (CYP3A4/5) which metabolizes 50% of drugs was not inhibited by the compound which underscores the potentials of the system metabolizing the compound. The compound was also found to belong to class V toxicity base on globally harmonized system (GHS) which makes categorizes the compound into compound with low oral acute toxicity potentials. It was not also found to exhibit organ toxicity potentials for vital organs and end point toxicities (table 11) except for slight nephrotoxicity potential. This entails the degree of safety the compound exhibit and may only need some pharmacophore modifications to minimize the potentials of nephrotoxicity. Pharmacophore modification is a key component of hit and lead optimization. This process leads to the improvement of efficacy of substances and reduction of toxicity which ultimately optimizes drug potentials (Klebe, 2013). ADMET result for the top hit compound (2-hexadecanol) for HAP (table 11) revealed that the compound exhibited drug-likeness by passing Lipiski's rule of five with only one violation (MLOGP>4.15). This highlights the oral bioavailability and bioactivity potential of the compound, favorable pharmacokinetics and lower risk of metabolic issues. It was also revealed that the compound has the potential of high GI absorption and cellular retention (not p-gp substrate) which increases efficacy potentials through ensuring effective concentrations are attained in the target organ. The compound also has the potential of application in the treatment of cerebral malaria due to its ability to cross the BBB, though its application here has to be with caution to reduce the chances of central nervous system toxicity. The compound was also reported not to be an inhibitor of the most important cytochrome isoform (CYP3A4) which metabolizes 50% of drugs. This entails the potentials of human system in metabolizing the compound to enhance its efficacy and reduce its accumulation in the body that can lead toxicity(Pandey et al., 2024). The compound was also found to belong to IV toxicity base on GHS which implies that the drug has moderate toxicity when administered orally and can be harmful when consumed in large quantity. This calls for caution in does determination to ensure that the lowest effective dose and dosage is administered. Organ toxicity analysis revealed that the compound has no toxicity potential against all the vital organs under consideration (table 11). This entails the potentials of safety of the compound when used as drug. Although, the *in silico* results of this study

turns out to be impressive, it is imperative to note that all the *in silico* outcomes are based on computational models and calls for the need of *in vitro* and *in vivo* validation.

## CONCLUSION

The findings of these study reported the presence of several phytocompounds with antimalarial activities which gives credence to the use of *Piliostigma reticulatum* as antimalarial herb in traditional medicine and validates the previous findings that reported the antimalarial activity of the plant. Ethylene brassylate and 2-hexadecanol identified as the top hit compounds that binds the various *Plasmodium falciparum* targets are potential antimalarial compounds that can be channeled into drug discovery pipeline for the development of novel antimalarial drug.

***Funding:*** The funding was sponsored by the researchers. No funding was received from any sponsor.

***Author's Contribution Statement:*** Abdulhafiz Lamiya: conceptualization, molecular docking analysis, data analysis and writing of manuscript. Adamu Jibril Alhassan: Phytochemical studies and editing of manuscript. Maryam Abdulkadir Dangambo: collection of plant material and biological analysis. Hauwa Ahmed Zailani: Biochemical assay and editing of manuscript.

***Conflict of Interest:*** The authors wishes to declare that there is conflict of interest

## REFERENCE

Abdulkadir, S. S., Jatau, A. I., Abdussalam, U. S., & Bichi, L. A. (2022). In vivo antiplasmodial activity of the methanol leaf extract of Piliostigma reticulatum ( Dc .) Hochst ( Fabaceae ). *Bulletin of the National Research Centre*. https://doi.org/10.1186/s42269-022-00910-0

Abdulsalami, H., Daudu, Y. O. A., Adabara, N. U., & Hamzah, R. U. (2022). *Antisalmonellal Activity and GC-MS Analysis of Piliostigma thonningii leaf extract*. *2*(2), 1–9.

Afolayan, F. I. D., & Oladokun, S. E. (2024). *In silico antiplasmodial effects of phytocompounds derived from Andrographis paniculata on validated drug targets of different stages of Plasmodium falciparum*. *5*(2), 1–11. https://doi.org/10.53388/IDR2024006.Executive

Ahsin, A., Kurbanova, M., Ahmad, S., Qamar, A., Ashfaq, M., Tahir, M. N., Dege, N., Şahin, O., Abuelizz, H. A., Al-Salahi, R., & El Bakri, Y. (2025). Synthesis, structure, supramolecular assembly inspection by Hirshfeld surface analysis, DFT study and molecular docking inspection of 4,5-bis(2-chlorophenyl)-8a-phenylhexahydropyrimido[4,5-d]pyrimidine-2,7(1H,3H)-dithione. *Journal of Molecular Structure*, *1319*. https://doi.org/10.1016/j.molstruc.2024.139580

Alshahrani, M. Y., Zaid, A., Suliman, M., Bibi, S., Muhammad, S., & urRehman, S. (2025). In-silico discovery of efficient second-generation drug derivatives with enhanced antihistamine potency and selectivity. *Computational Biology and Chemistry*, *115*. https://doi.org/10.1016/j.compbiolchem.2024.108340

Artasasta, M. A., Djamaludin, H., Listyorini, D., Putra, W. E., Eviana, D., & Putri, K. (2025). *Molecular Docking and ADMET Analysis of Bioactive Compounds from Aspergillus nomius NC06 Against Plasmepsin Protein : Antimalarial Activity*. 511–517. https://doi.org/10.17957/IJAB/15.2230

Boualam, K., Ndiaye, B., Harhar, H., Tabyaoui, M., Ayessou, N., & Taghzouti, K. (2021). *Study of the Phytochemical Composition , the Antioxidant and the Anti-Inflammatory Effects of Two Sub-Saharan Plants : Piliostigma reticulatum and Piliostigma thonningii*. *2021*.

Chaurasia, S., & Pandey, A. (2022). *A Systematic In Silico Investigation of Phytochemicals from Artocarpus Species against Plasmodium falciparum Inhibitors †*.

Cheaveau, J., Marasinghe, D., Akakpo, S., Deardon, R., Naugler, C., Chin, A., & Pillai, D. R. (2019). *The Impact of Malaria on Liver Enzymes : A Retrospective Cohort Study ( 2010 – 2017 )*. 1–8. https://doi.org/10.1093/ofid/ofz234

Enechi, O. C., Okpe, C. C., Ibe, G. N., Omeje, K. O., & Okechukwu, P. C. U. (2016). *Effect of Buchholzia coriacea Methanol Extract on Haematological Indices and Liver Function Parameters in Plasmodium berghei -Infected Mice*. *16*(1), 57–66. https://doi.org/10.5829/idosi.gv.2016.16.01.10244

Erhirhie, E. O., Ikegbune, C., Okeke, A. I., Onwuzuligbo, C. C., Madubuogwu, N. U., & Chukwudulue, U. M. (2021). *Antimalarial herbal drugs : a review of their interactions with conventional antimalarial drugs*.

Fayanju, b., akinmoladun, a. C., & olaleye, m. T. (2022). *Toxicological evaluation of the methanolic leaf extract of piliostigma reticulatum ( dc .) Hochst in male wistar rats*. *03*(01), 3380–3384.

Harborne, J. B. (1997). *Phytochemical methodsitle. Chapman and Hall ltd., London*.

Harish, K. K., Khamees, H. A., Venkatesha, K., Nagaraja, O., & Madegowda, M. (2025). The pivotal role of the carbonyl group in methoxy chalcones: comprehensive analyses of the structure and computational insights into binding affinity towards monoamine oxidase enzymes. *Molecular Systems Design and Engineering*. https://doi.org/10.1039/d4me00135d

Iyabode, S., Ahmed, A., Ishola, A., & Bewaji, C. O. (2023). *Antimalarial Activities of a Therapeutic Combination of Azadirachta indica , Mangifera indica and Morinda lucida Leaves : A Molecular View of its Activity on Plasmodium falciparum Proteins*. *0123456789*.

Jain, P. K., Soni, A., Jain, P., & Bhawsar, J. (2016). *Phytochemical analysis of Mentha spicata plant extract using UV-VIS , FTIR and GC / MS technique Phytochemical analysis of Mentha spicata plant extract using UV-VIS , FTIR and GC / MS technique*. *March*.

Kabera, J. N., Semana, E., Mussa, A. R., & He, X. (2014). *Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Properties*. *2*, 377–392.

Kalaichelvi, K. (2017). *Screening of phytoconstituents , UV-VIS Spectrum and FTIR analysis of Micrococca mercurialis ( L .) Benth*. *5*(6), 40–44.

Klebe, G. (2013). Optimization of Lead Structures. *Drug Design*, 153–172. https://doi.org/10.1007/978-3-642-17907-5_8

Kumar, T., Shrivas, K., Kurrey, R., Upadhyay, S., Jangde, R., & Chauhan, R. (2020). Spectrochimica Acta Part A : Molecular and Biomolecular Spectroscopy Phytochemical screening and determination of phenolics and fl avonoids in Dillenia pentagyna using UV – vis and FTIR spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular*

*Spectroscopy*, *242*, 118717. https://doi.org/10.1016/j.saa.2020.118717

Kwaji, A., Bassi, P. U., Aoill, M., Nneji, C. M., & Ademowo, G. (2016). *Preliminary studies on Piliostigma thonningii Schum leaf extract : Phytochemical screening and in vitro antimalarial activity*. *2016*.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, *54*(4), 275–287. https://doi.org/10.1007/BF01234480

Madara, A. A., Ajayi, J. A., Salawu, O. A., & Tijani, A. Y. (2010). *Anti-malarial activity of ethanolic leaf extract of Piliostigma thonningii Schum . ( Caesalpiniacea ) in mice infected with Plasmodium berghei berghei*. *9*(23), 3475–3480.

Majewski, M., Ruiz-Carmona, S., & Barril, X. (2019). An investigation of structural stability in protein-ligand complexes reveals the balance between order and disorder. *Communications Chemistry*, *2*(1). https://doi.org/10.1038/s42004-019-0205-5

Mawson, A. R., & Mawson, A. R. (2016). *The pathogenesis of malaria : a new perspective The pathogenesis of malaria : a new perspective*. *7724*(December). https://doi.org/10.1179/2047773213Y.0000000084

Medimagh, M., Issaoui, N., Gatfaoui, S., Kazachenko, A. S., Al-Dossary, O. M., Kumar, N., Marouani, H., & Bousiakoug, L. G. (2023). Investigations on the non-covalent interactions, drug-likeness, molecular docking and chemical properties of 1,1,4,7,7- pentamethyldiethylenetriammonium trinitrate by density-functional theory. *Journal of King Saud University - Science*, *35*(4). https://doi.org/10.1016/j.jksus.2023.102645

Mourya, A. S., & Pandey, R. K. (2024). *A Study on Factors Attributed to Failure of Pharmaceutical Products*. *2*(2), 12–21.

Nhlapho, S., & Munien, I. (2024). *Sciences of Pharmacy Druggability of Pharmaceutical Compounds Using Lipinski Rules with Machine Learning*. *5*(5).

Nureye, D., Salahaddin, M., & Zewudie, A. (2020). *Current Medicines for Malaria Including Resistance Issues*. 3–10. https://doi.org/10.4103/jpp.JPP

Oderinde, O., Onu, A., Shagari, A., & Olaitan, O. (2022). Effect of Cymbopogon citratus and Piliostigma reticulatum Aqueous Leaf Extracts on Rat Liver Function and In vitro Schizont Maturation Inhibition. *Nigerian Journal of Basic and Applied Sciences*, *29*(2), 1–8. https://doi.org/10.4314/njbas.v29i2.1

Odutuga, A.A., Ukpanukpong, R.U. and Uyabeme, R. . (2016). Biochemical and Histological Studies on the effects of Lonart Ds on Plasmodium berghei Infected Mice. *JABU Journal of Science and Technology*, *2*(4), 69–77.

Oladeji, O. S., Oluyori, A. P., Bankole, D. T., & Afolabi, T. Y. (2020). *Natural Products as Sources of Antimalarial Drugs : Ethnobotanical and Ethnopharmacological Studies*. *2020*. https://doi.org/10.1155/2020/7076139

Pandey, S. K., Verma, S., Upreti, S., Mishra, A., Yadav, N., & Dwivedi-Agnihotri, H. (2024). Role of Cytochrome P450 3A4 in Cancer Drug Resistance: Challenges and Opportunities. *Current Drug Metabolism*, *25*(4), 235–247. https://doi.org/10.2174/0113892002312369240703102215

Peters, W. (1965). Drug resistance in Plasmodium berghei Vincke and Lips, 1948. III. Multiple drug resistance. *Experimental Parasitology*, *17*(1), 97–102.

Raja, B., Sivakumar, A., Mekata, T., Radhakrishnan, V., Asely, A. M. El, Abdelsalam, M., & Raja, S. (2025). *Research Article Bis ( Oxy )] - , ( 5 . Beta ) - as an Antiviral Candidate Against White Spot Syndrome Virus ( WSSV ): Molecular Docking and Simulation Approach*. *2025*. https://doi.org/10.1155/are/8855914

Shanshan, L., Yuan, X., Qiancheng, S., Xian, L., Jing, L., Yadong, C., Tao, L., Cheng, L., Xiaomin, L., Mingyue, Z., & Hualiang, J. (2013). Non-Covalent Interactions with Aromatic Rings: Current Understanding and Implications for Rational Drug Design. *Current Pharmaceutical Design*, *19*(36), 6522–6533. https://doi.org/10.2174/13816128113199990440

Shekarau, E., Uzoanya, M., Ogbulafor, N., Ntadom, G., Ijezie, S. N., Uzoanya, M. I., Seye, B., Fashanu, C., Eze, N., Nwidae, L., Mokuolu, O., Nwokenna, U., Nglass, I., Ishola-Gbenla, O., Okouzi, M., Fagbola, M., Oresanya, O., Getachew, D., Chukwumerije, J., … Rietveld, H. (2024). Severe malaria intervention status in Nigeria: workshop meeting report. *Malaria Journal*, *23*(1). https://doi.org/10.1186/s12936-024-05001-1

Shi, Y. H., Shen, J. X., Tao, Y., Xia, Y. L., Zhang, Z. B., Fu, Y. X., Zhang, K. Q., & Liu, S. Q. (2025). Dissecting the Binding Affinity of Anti-SARS-CoV-2 Compounds to Human Transmembrane Protease, Serine 2: A Computational Study. *International Journal of Molecular Sciences*, *26*(2). https://doi.org/10.3390/ijms26020587

Shibi, I. G., Aswathy, L., Jishaa, R. S., Masand, V. H., & Gajbhiyec, J. M. (2016). *Virtual Screening Techniques to Probe the Antimalarial Activity of some Traditionally Used Phytochemicals*. *December*. https://doi.org/10.2174/1386207319666160420

Tajbakhsh, E., Kwenti, T. E., Kheyri, P., Nezaratizade, S., & Lindsay, D. S. (2021). Antiplasmodial , antimalarial activities and toxicity of African medicinal plants : a systematic review of literature. *Malaria Journal*. https://doi.org/10.1186/s12936-021-03866-0

Upadhyay, R.K. (2014). Drug delivery systems, CNS protection, and the blood brain barrier. *BioMed Research International*, *2014*.

Urbina, A. S., & Slipchenko, L. V. (2025). Rationalizing protein-ligand interactions via the effective fragment potential method and structural data from classical molecular dynamics. *Journal of Chemical Physics*, *162*(4). https://doi.org/10.1063/5.0247878

Venkatesan, P. (2024). News The 2023 WHO World malaria report. *The Lancet Microbe*, *5*(3), e214. https://doi.org/10.1016/S2666-5247(24)00016-8

Wang, D., Jin, J., Shi, G., Bao, J., Wang, Z., Li, S., Pan, P., Li, D., Kang, Y., & Hou, T. (2025). ADMET evaluation in drug discovery: 21. Application and industrial validation of machine learning algorithms for Caco-2 permeability prediction. *Journal of Cheminformatics*, *17*(1). https://doi.org/10.1186/s13321-025-00947-z

World Health Organization. (2024). *Global Malaria Programme operational strategy 2024-2030*. 01–84.

Zailani, A. H., ., Hammanadama, I. I., & ., Adamu, M. G1., Lamiya, A. (2020). Ameliorative effects of some phytochemicals extracted from Cola acuminate leaf on altered liver function indices of mice infected with Plasmodium berghei (NK_65). *International Journal of Scientific and Research Publications*, *10*(7), 1–5. https://doi.org/10.29322/IJSRP.10.07.2020.p10315

Zailani, A. H., Adamu, M. G., Hammanadama, I. I., Dauda, E. M., & Lamiya, A. (2020). *Some phytochemicals extracted from Cola acuminata leaf have antimalarial activity and improve derangements in haematological indices of Plasmodium berghei -infected mice*. *10*(07), 1–8. https://doi.org/10.7439/ijpr.v10i7.5458

THIS PAGE INTENTIONALLY LEFT BLANK