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Antimalaria Potential and Toxicological Evaluation of *Synclisia scabrida* (Miers) Ex Oliv Methanol Root Extract in *Plasmodium berghei*-infected Mice Model

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Abstract

Despite the artemisinin-based combination therapy for malaria treatment, it remains a public health concern globally. Therefore, there is an urgent need for novel antimalarial drugs to arrest this perceived threat to global malaria control. This study aims to investigate the antimalarial potential of *S. scabrida* (SS) and evaluate its possible effect on haematological and biochemical parameters. Malaria was inoculated in mice using *Plasmodium berghei*. After 72 h, they were orally treated for four days as follows: Group 1: 10 mL/kg % Dimethyl sulphoxide (DMSO); Group 2: 140 mg/kg Artemether/lumefantrine; Groups 3, 4 and 5: 500, 250, 125 mg/kg SS methanolic extract, respectively. SS methanolic extract significantly reduced parasite density and percentage parasitemia ($p < 0.05$) at all doses used in the study, non-significantly increased haematological, biochemical parameters and distorted liver architecture; when compared to the negative control. SS methanolic extract exhibited good antimalarial activity against *Plasmodium berghei*. Lower SS dose did not cause significant changes to the lipid profiles. However, the high dose may not be safe since there were some adverse effects shown on the liver and kidney status. Further studies are warranted to determine the effect of the middle and the lowest doses on the liver and kidney.

Keywords: *Synclisia scabrida*; antimalaria; *Plasmodium berghei*; lipids; haematology.

Abbreviations: WHO: World Health Organisation, FRIN: Forestry Research Institute of Nigeria, PRBCs: Packed Red Blood Cells, DMSO: Dimethyl sulphoxide, MST: Mean survival time, SPSS: Statistical Package for Social Sciences, ANOVA: Analysis of variance, CV: Central vein, HPS: hepatic sinusoids, HPC: Hepatocytes, AST: Aspartate aminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, A/L: artemether/lumefantrine.

INTRODUCTION

Malaria remains a significant global health concern, with Plasmodium species causing substantial morbidity and mortality. Resistance against the artemisinin-based combination therapy, the gold standard for malaria treatment has recently been reported in Africa (Balikagala et al., 2021), the region with 94% of the world's malaria cases (WHO, 2023). Therefore, there is an immediate need for novel antimalarial drugs to counter this trend, since if allowed to spread, would be a serious threat to malaria control globally.

Globally in 2022, approximately 249 million incidents of malaria were reported in 85 malaria endemic countries and estimated deaths of 608 000. With an estimated 233 an approximated 233 million cases, the World Health Organisation (WHO) African Region accounted for approximately 94% of cases worldwide and was projected to cause 580,000 deaths by 2022. Being the most seriously impacted in the same year with 27% and 31% of all malaria cases and fatalities respectively, worldwide, Nigeria was placed on the list of eleven African nations designated as high-burdened nations (WHO, 2023). Malaria is a major cause of poverty and it reduces the rate of growth in the economy by up to 1.3% per year in endemic countries (WHO, 2021).

Studies have shown hematological and biochemical alterations in malaria parasite-infected blood with common complications associated with this disease (Al-Salahy et al., 2016). In the discovery of novel antimalarials, therapeutic plants have had vital role, for instance the antimalarial drugs artemisinin and quinine were isolated from *Artemisia annua* and Cinchona bark, respectively (Ceravolo et al., 2021). *Synclisia scabrida*

belongs to the family Menispermaceae. Its root, leaf, stem and liana were found to have been used in the management of gastric ulcer, mental disorders, venereal diseases, prostate problems, asthma, hernia, upper abdominal pains, dyspepsia, menstrual pains, prevention of spontaneous abortion and as an aphrodisiac (Onwudiwe *et al.,* 2012; Anowi *et al.,* 2013).

Traditional healers in Nigeria have claimed they use *Synclisa scabrida* for the treatment of malaria. However, no scientific investigations have been done to confirm this claim. Hence, in the search for more potent malaria drugs, this study aims to investigate the antimalarial potential of *S. scabrida* and also evaluate its possible effect on the haematological and biochemical parameters, along with the kidney and liver.

MATERIALS AND METHODS

Plant Sample Collection

A plant specimen was collected by Mr Samuel Oyebanji from a farm in the Ugbine community, Edo State, Nigeria. The plant was dried and sent to the Forestry Research Institute of Nigeria (FRIN) for identification and authentication. It was identified and authenticated as S*ynclisia scabrida* Miers ex Oliv. by Mr S.A. Odewo of FRIN, where the herbarium specimen with voucher number FHI – 111106 was deposited.

Experimental Animals

Albino mice (50) of both sexes with no signs of ill-health were obtained from Federal Polytechnic Owerri, Imo State, and then brought to the animal house of the Faculty of Pharmacy, Madonna University, Nigeria where the research was carried out and then acclimatised for 14 days. The animals were housed in ventilated cages at room temperature of 24 ± 2°C and kept under standard conditions of a 12 h light/dark cycle. The animals were fed with standard laboratory feed and water.

They were also handled in accordance with the international animal care and welfare guidelines (OECD, 2008; National Institute for Laboratory Animal Research, 2011).

Extract Preparation

The roots of *S. scabrida* were supplied from Benin state. It was air-dried for 1 week and then oven dried for three days at 30^0C. After which it was extracted using methanol as solvent using the maceration method, at room temperature (30 ± 2°C) for 72 h. Then filtered using a Buchner funnel and Whatman no.1 filter funnel and filter paper. The obtained extract was concentrated by evaporating the solvent by exposing to air and also oven drying at controlled temperature not exceeding 40ºC.The resulting dried extract was stored in the refrigerator at 4°C prior to use. The percent yield (%w/w) of the extract was calculated using the following formula

$$Percentage\ Yield = \frac{\text{Weight of the extract}}{\text{Weight of the root powder}} \times 100\%$$

Parasites inoculation

The chloroquine-resistant strain of *Plasmodium berghei* was acquired from the Animal house of the Department of Pharmacology and Toxicology, University of Uyo, Nigeria. Two cycles of PRBC passage in rats and mice were used to prepare the parasites. Donors with parasitaemia levels of 50-70% were sacrificed, and a heart puncture was used to obtain their blood into heparinised tubes. After that, the blood was diluted with normal saline based on parasitaemia level of each donor and the red blood cell (RBC) count of normal mice, such that 1 mL blood contained 5×10^7 parasites. The experimental animals were each treated with 1×10^7 Packed Red Blood Cells (PRBCs) by intraperitoneal (*ip*) injection (Basir *et al*., 2012).

Pharmacological Screening

Rane's Test (Curative Model)

A total of 50 albino mice were selected and randomly grouped into 5 groups of 10 animals per group. The % parasitemia levels of every group were gotten before all the animals were infected with *Plasmodium berghei* (a zoonotic Plasmodium sp) as described above. The animals were then left for 72 h for the infection to establish. Percentage parasitemia was also determined after induction before treatment. After 72 h, the animals were given treatment by oral administration of the following, for four days:

Group 1 (Negative control): 10 mL/kg body weight of 1% Dimethyl sulphoxide (DMSO)

Group 2 (Positive control): 140 mg/kg Artemether/lumefantrine combination

Group 3 (High dose): 500 mg/kg of methanolic extract of *S. scabrida*

Group 4 (Middle dose): 250 mg/kg of methanolic extract *S. scabrida*

Group 5 (Low dose): 125 mg/kg of methanolic extract *S. scabrida*

Determination of Body Weight, Water and Food Intake While conducting the treatment, the water and food consumption of every group was documented to determine whether the experimental animals' intake increased or decreased. Additionally, the weight of all experimental animals in each group were taken before and after the treatment to assess any changes in their weight.

$$\%\ \text{Weight change} = \frac{\text{weight after treatment} - \text{weight before treatment}}{weight\ before\ treatment} \times 100$$

Determination of the Level of Parasite Density and Percentage Parasitemia

The blood from the tail of the infected mice was collected and placed on a clean glass slide placed horizontally on the working bench. The slide and the spreader were held at a suitable angle, pulled back to touch the dropped blood on the slide and spread along it. The thin layer film was fixed with methanol and lowered into the already prepared Giemsa stain (10 mL of Giemsa + 90 mL of distilled water) and allowed to stain for 5 min. The slide was lifted off the stain solution with the aid of forceps, excess stain was washed off, allowed to drain and air dried at room temperature. Then parasitaemia was observe with an oil immersion lens under a microscope, to examine the blood sample. The focus is on counting the number of red blood cells that are infected with parasites, and this count is then used to determine the parasitized level in the blood sample. The counting is standardized by considering a specific number of red blood cells (200) in a random field view. The % parasitemia, parasite density were calculated using the following formulae:

$$\%\ \text{Parasitemia} = \frac{parasitised\ RBC}{Total\ RBC} \times 100$$

$$\text{Parasite Density} = \frac{parasitised\ RBC}{WBC} \times 5000$$

Then the % inhibition was calculated using the following formula:

$$\%\ \text{inhibition} = \frac{\%\ parasitemia\ of\ negative\ control - \%\ parasitemia\ of\ treated\ group}{\%\ parasitemia\ of\ negative\ control} \times 100$$

Determination of mean survival time (MST)

From the time of infection until death, the mortality of each mouse was monitored and recorded regardless of the group in which the mouse was allocated throughout the follow-up period (30 days). Mean survival time of mice of each group was determined using the following formula:

$$\text{MST} = \frac{\text{sum of survival time of all mice in a group (days)}}{\text{sum of mice in the group}}$$

Statistical analysis

Data were analysed using Statistical Package for Social Sciences (SPSS Version 23). Data for parasite density, % parasitemia and hematological parameters were analysed using One-way ANOVA, followed by Turkey post hoc test. The results were presented as mean ± SD in tables. Mean values with $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of the methanol root extract of *Synclisia scabrida* on weight, daily water and food intake of *P. berghei*-infected mice

To determine the effect of the methanol extract of *S. scabrida* roots on weight changes in *P. berghei* infected vehicle-treated and extract-treated mice, animal weights were recorded and comparisons made between the day of infection (day 0) and day 8 post infection (24 h after administering the final dose of treatment). This is shown in Table 1. The weight loss observed in positive control and extract-treated groups was not significantly different from that seen in vehicle-treated group.

The treatment of the mice with crude methanolic root extract of *S. scabrida* resulted to negligible change or increased water and food intake throughout the period of the treatment (Figure 1). Treatment with crude extract of *S. scabrida* root and artemether/lumefantrine on daily water and food intake is not significantly different from each other. This is shown on Table 1.

Table 1. Effect of methanol root extract of *Synclisia scabrida* on weight, daily water and food intake of *P. berghei*-infected mice.

Treatment	Weight (g) on day 0 (infection day)	Weight (g) on day 8 (post infection)	Weight change (%)
Negative control (Vehicle)	21.2 ± 2.2	19.0 ± 2.5	10.4
Artemether/lumefantrine (140 mg/kg)	21.8 ± 2.9	19.4 ± 2.8	11
500 mg/kg SS	29.1 ± 2.6	24.1 ± 3.9	17.2
250 mg/kg SS	25.5 ± 3.3	23.7 ± 4.8	7.1
125 mg/kg SS	24.6 ± 3.6	17.4 ± 1.1	29.3

Data expressed as mean ± SD, n=10, SS = *Synclisia scabrida.*

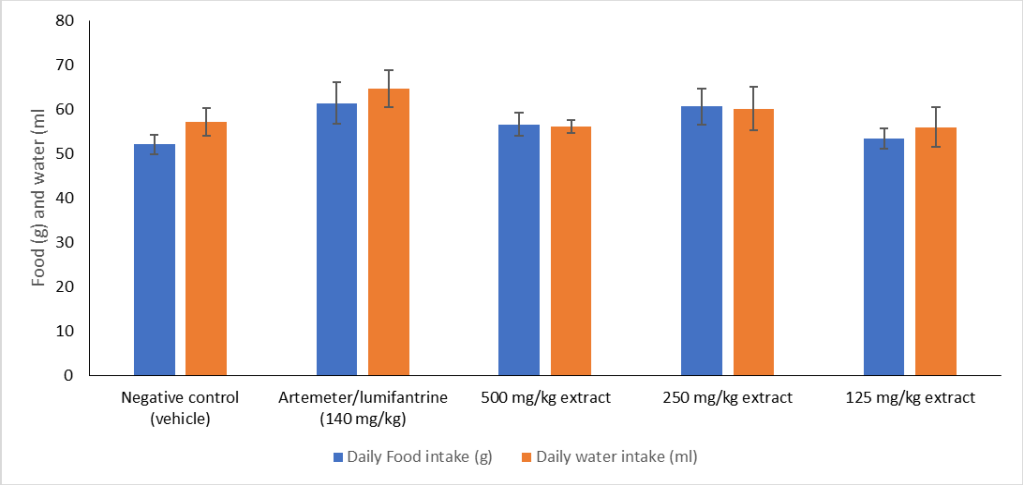

Figure 1. Effect of methanol root extract of *Synclisia scabrida* on daily water and food intake of *P. berghei*-infected mice.

The purpose of this study was to examine the impact of *Synclisia scabrida* on malaria parasites, hematology, biochemical parameters, lipids and histological parameters of liver and kidney of *P. berghei-infected* albino mice. Since the curative test allows for established infection, it was used in this investigation. *P. berghei* is frequently employed for preclinical antimalarial research of drug candidates with measurable treatment outcomes (Fidock et al., 2004). *Plasmodium berghei* serves as a crucial model organism for the study of malaria, offering crucial insights into the disease's pathogenesis, host immune responses, and potential therapeutic targets. (Otun et al.,2024.) because it makes it possible to investigate illness development, which appears challenging in humans. This is why *P. berghei* was utilised to induce malaria in mice. Additionally, it permits evaluations of organs like the liver, where parasites can hibernate (Albohiri & Alzanbagi, 2021). The most often used technique to study the antimalarial effect of a possible antimalarial drug moiety is in-vivo antimalarial research.

Prevention of loss in body weight is one of the parameters to confirm the antimalarial potential of new natural or synthetic antimalarial drugs as body weight loss is a characteristic of *Plasmodium berghei* infected mice as a result of increased parasitamia (Kifle et al., 2020) resulting from appetite loss, metabolic disturbance, and hypoglycemic effect of the parasite (Deharo *et al*., 2001). There is no significant change in weight of the infected mice treated with methanolic extract of *S. scabrida* when compared to vehicle-treated mice. This is an indication of little or no improvement in weight loss due to infection. Therefore, regarding weight as a measure, the extract might not have had a significant impact on the overall health or well-being of the infected mice compared to the control group. This is an indication that the extract preserved the weight of the animals; which could be due to its ability to prevent appetite loss. This is confirmed by the result of this study which showed that the extract increased food consumption at all doses.

Curative Potential of Extract *Synclisia scabrida* Root on *Plasmodium berghei*-infected Mice

Treatment with crude extract of *S. scabrida* root significantly reduced parasite density and percentage parasitemia ($p < 0.05$) at all doses used in the study; when compared to the negative control. The highest dose (500 mg/kg) showed the strongest reduction which is comparable to that of the standard antimalarial drug (artemether/lumefanthrine) used. Similarly, 500, 250, and 125 mg/kg *S. scabrida* crude methanol extract caused 78.0%, 58.0%, and 61.20% parasitemia inhibition, respectively. The percentage parasitemia produced by 500 mg/kg (16.7%) is very similar to that of the standard antimalarial drug (9.8 ± 4.4) used, but significantly different from that of 250 (32.0 ± 5.4; $p < 0.05$) and 125 mg/kg (29.5 ± 2.1; $p < 0.001$), when compared to the standard drug. Similarly, the extent of parasite density reduction by 250 and 125 mg/kg *S. scabrida* extract is significantly different ($p < 0.05$; ($p < 0.004$, respectively) when compared to the standard. Again, the parasite density reduction produced by 500 mg/kg (816.7 ± 275.4) is very similar to that of the standard antimalarial drug (768.7 ± 104.0) used (Table 2). Mean survival time of the rats treated with crude extract of *S. scabrida* root was prolonged at all doses when compared to the control group, although not significantly different.

Table 2. Parasite density, % parasitemia, % inhibition and mean survival time (MST) of *Plasmodium berghei*-infected mice treated with extract of *Synclisia scabrida* root.

Treatment	Parasite density (parasites/µL)	% Parasitemia	% Inhibition	MST (days)
Negative control (Vehicle)	3349.8 ± 591.1	76.2 ± 7.9	0	15.7±3.2
Artemether/lumefantrine (140 mg/kg)	768.7 ± 104.0	9.8 ± 4.4	87.1	30±0.0
500 mg/kg SS	816.7 ± 275.4[a]	16.7 ± 8.5[a]	78.0	16.7±3.2
250 mg/kg SS	1417.2 ±2 54.8[ab]	32.0 ± 5.4[ab]	58.0	17.0±2.0
125 mg/kg SS	1358.0 ± 59.4[ad]	29.5 ± 2.1[ac]	61.2	24.0±5.2

Data expressed as mean ± SD, SS = *Synclisia scabrida*, [a] $p < 0.05$ when compared to negative control, [b] $p < 0.05$, [c]$p < 0.001$, [d] $p < 0.004$, when compared to positive control, n=10.

Parasitemia is the number of parasites in the blood. The number of asexual forms of parasite about a blood volume is the traditional definition of parasite density, which is a measure of infection level (e.g., microliter) or a percentage of white blood cells (WBCs). It serves as a gauge of the organism's parasite burden and a sign of how severe an active parasitic infection is (Oyibo et al., 2023). Parasite density and percentage parasitemia were significantly reduced at all doses of the extract. *Synclisia scabrida* has thus been shown to possess good activity towards *P. berghei* infection and therefore confirms the anecdotal report of its use in the treatment of malaria. There is no significant difference between reductions in parasite density and percentage parasitemia produced by 500 mg/kg *S. scabrida* extract, when compared to the standard drug. This implies that the activity of the two is comparable, 500 mg/kg being as efficacious as the artemether/lumefantrine with percentage inhibition of 78.0%. According to Rasoanaivo *et al*. (2004), the extract at this dose possesses a good activity against *P. berghei.* This anti-malarial activity was classified according to Rasoanaivo *et al*. (2004); classification which states that extracts with very good or good activity should possess 90–100% parasitaemia inhibition, extracts with good to moderate activity should have 50–90% inhibition, extract with moderate to weak activity should have 10–50% inhibition and extracts that are inactive have 0% inhibition.

In accordance with (Maiga et al., 2021), artemether/lumefantrine (A/L) is one of the first line artemisinin-based combination therapies recommended for the treatment of malaria. Its antiplasmodial activity is attributed to the release of free radicals by its artemether component, which subsequently binds covalently to parasite proteins and heme. Additionally, it has been proposed that A/L inhibits the calcium ATPase (sarcoplasmic endoplasmic reticulum calcium ATPase) of malarial parasites (Moore et al., 2022). But its lumefantrine component builds up in the feeding vacuole of the parasite, where it disrupts heme polymerization by forming complexes that result in toxic heme, which ultimately leads to the death of the parasite (Kumar et al., 2007).

Cycleanine, a major compound present in *S. scabrida* has been shown to suppress the levels of parasitemia and increase mean survival times significantly compared to those of the control groups (Uche *et al*., 2021). The ethanol extract of *S. scabrida* was reported to possess high amounts of flavonoids, tannins, saponins, terpenes (Orumwense et al., 2022). Akande et al. (2021) reported that presence of saponin, flavonoid, alkaloids, tannin, triterpene, carbohydrates, steroids, glycosides and cardiac glycosides in methanol extract *Jatropha curcas* leaf resulted to significant suppression of parasitaemia. Alkaloids have over overtime been identified as significant phytoconstituents with notable biological properties, including antimalarial activity and excellent free radical scavenging activity. The initial effective antimalarial medication was quinine, an alkaloid which was obtained from the extraction of the Cinchona tree (Uzor, 2020).

MST is a crucial metric for the antiplasmodial evaluation of possible drug moieties. The results of the current investigation show MST was prolonged at all doses; 250 mg/kg of *Synclisia scabrida* extract prolonged it to the same extent as artemether/lumefantrine. It may reveal how well novel medications or therapy approaches work. A medication may be useful in controlling or lessening the severity of an illness if it lengthens the infected mice's survival period. The histology of the liver of the vehicle-treated mice shows a hepatic lobule having a central vein (CV). From the central vein, hepatocytes (HPC) (liver cells) radiate outwards like the spokes of a wheel. The hepatocytes form sheets and in between the sheets run hepatic sinusoids (HPS). The result on the histology of the liver suggests that the treatment of malaria-infected mice with the *Synclisia scabrida* root extract at a dose of 500 mg/kg induced some histological changes in the liver. These changes include alterations in the appearance and arrangement of hepatocytes, dilatation of the central vein, and changes in the staining characteristics of cell nuclei and cytoplasm, indicating potential effects on liver tissue integrity and cellular health due to the treatment.

Effect of methanol root extract of *Synclisia scabrida* on haematological parameters of *P. berghei*-infected mice

Synclisia scabrida extract increased WBC and RBC, although not significantly. Artemether/lumefantrine significantly increased the level of RBC ($p < 0.00$), when compared to the negative control (Table 3).

Table 3. Effect of *Synclisia scabrida* extract on haematological parameters.

Treatment	RBC (per µL)	WBC (per µL)	PCV (%)
Negative control (Vehicle)	69.0±13.5	78.8±11.4	15.0±7.0
Artemether/lumefantrine (140 mg/kg)	200.3±74.2[a]	115.3±29.1	47.7±1.5
500 mg/kg SS	130.0±26.5	133.3±57.7	13.3±1.5
250 mg/kg SS	97.2±32.2	111.7±43.6	15.0±8.9
125 mg/kg SS	90.0±14.1	97.5±3.5	7.0±2.8

Data expressed as mean ± SD, [a]$p < 0.05$, when compared to control, n=10.

It has been demonstrated that malaria infections modify the plasma biochemical indicators (Saftawy et al., 2022). Studies have shown alterations in the biochemistry and hematology of malaria parasite-infected blood with common complications associated with this disease. Haematological changes that are associated with malaria infection include anaemia, thrombocytopenia and disseminated intravascular coagulation (Nlinwe & Nange, 2020). PCV is measured to assess the effectiveness of a crude drug in preventing hemolysis due to the rising parasitemia level by measuring the proportion of red blood cells (Chidozie et al., 2020). Malaria infection is characterised by possible hemolysis and anaemia (Atanu *et al*., 2022). The main causes of anaemia in mice and humans include the clearance or destruction of infected RBCs, the clearance of uninfected RBCs, erythropoietic suppression, and dyserythropoiesis. The malaria parasite is known to cause cells to produce free radicals which compromise the red blood cell membrane. Therefore, the capacity of the extract to stabilise PCV in mice with infection could be due to the antioxidant compounds present in the plant extracts. In this study, the extract could not improve the PCV, nevertheless, it prevented further reduction in PCV of the extract-treated mice. RBC was increased in extract-treated and artemether/lumefantrine-treated infected mice. This suggests that the extract and the standard drug may affect the production of RBCs. Further, WBC in the infected extract-treated and artemether/lumefantrine-treated mice was higher than in the infected vehicle-treated mice. This is a suggestion that the extract and the standard drug were able to boost the immune system of the mice, hence improving the ability of the mice to fight infection as a result of the treatment with the extract and artemether/lumefantrine increasing the WBC levels. It implies that the increase in WBC was due to the administration of the extract which was able to reverse the damaging effects of the parasites.

Effect of methanol root extract of *Synclisia scabrida* on liver and kidney functions of *P. berghei*-infected mice

The effect of *Synclisia scabrida* extract on kidney and liver function parameters is shown in Table 4. *Synclisia scabrida* extract and the artemether/lumefantrine increased the levels of Aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), and creatinine, although only significant at 500 mg/kg and 125 mg/kg for AST.

Table 4. Effect of methanol root extract of *Synclisia scabrida* on liver and kidney functions of *P. berghei*-infected mice.

Treatment (mg/kg)	AST (µ/L)	ALT (µ/L)	ALP (µ/L)	Creatinine (mMol/L)
Negative Control	131.4±46.5	50±11.8	199±38.0	38.9±7.5
Artemether/lumefantrine (140 mg/kg)	225.3±87.0	56±15.3	250±71.3	50.4±12.5
SS whole root extract (500 mg/kg)	608.5±100.0[a]	376.25±37.0[a]	289.75±41.0	82±0.0[b]
250 mg/kg	696.3±92.4[a]	166.3±58.0[b]	266.7±81.5	54.7±23.7
125 mg/kg	714±119.6[a]	172±12[b]	399.8±38.7[b]	51.3±20.5

Data expressed as mean ± SD, [a]$p < 0.0001$, [b]$p < 0.02$; when compared to control, n=4.

S. scabrida extract increased the levels of AST, ALT, ALP, and creatinine as also seen with artemether/lumefantrine. This indicates toxicity to liver tissues, this corresponds with the histology of the liver as shown in Figure 1A. AST and ALT test is commonly used to check for liver disease, to monitor liver disorder,

to ascertain treatment efficacy and to make sure that medications are not causing liver damage.

Effect of methanol root extract of *Synclisia scabrida* on lipid profile of *P. berghei*-infected mice

The effect of *Synclisia scabrida* extract on lipid profile in treated albino mice is shown in Table 5. The crude extract (500 mg/kg and 250 mg/kg) increased the high-density lipoprotein (HDL) and low-density lipoprotein (LDL) when compared with the negative control. Also, all doses of the extract increased total cholesterol (TC) and triglyceride (TG) in comparison to the negative control. At 500 mg/kg, TC (7.5±1.4 mMol/L) and LDL (4.2±0.7 mMol/L) were significantly increased ($p < 0.004$). Similarly, there was a significant increase in TC (8.4±0.7 mMol/L; $p < 0.001$) and LDL at 125 mg/kg (4.5±1.6 mMol/L; $p < 0.002$), but not at 250 mg/kg. It is important to note that the extract at 500 mg/kg increased HDL far more than artemether/lumefantrine.

Table 5. Effect of methanol root extract of *Synclisia scabrida* on lipid profile of *P. berghei*-infected mice.

Treatment	TC (mMol/L)	TG (mMol/L)	HDL (mMol/L)	LDL (mMol/L)
Negative Control	3.1±0.8	1.9±0.6	1.3±1.2	0.9±1.3
Artemether/lumefantrine (140 mg/kg)	4.1±0.7	1.8±0.5	0.5±0.2	2.9±0.9
SS whole root extract (500 mg/kg)	7.5±1.4[a]	2.7±1.5	2.1±1.0	4.2±0.7[a]
SS whole root extract 250 mg/kg	5.3±1.9	2.8±1.2	1.3±1.2	2.5±2.0
SS whole root extract 125 mg/kg	8.4±0.7[c]	4.3±1.9	1.9±0.7	4.5±1.6[b]

Data expressed as mean ± SD, [a]$p < 0.004$, [b]$p < 0.002$, [c]$p < 0.001$; when compared to control, n=4.

The crude extract (500 mg/kg and 250 mg/kg) increased the high-density lipoprotein (HDL), which could probably be a result of flavonoids as increased flavonoids reduce the occurrence of low high-density lipoprotein (Ahn et al., 2020; Anaka et al., 2013); flavonoid have been found to be one of the major constituents of *S. scabrida* (Orumwense et al., 2022). The crude extract (500 mg/kg and 250 mg/kg) increased the high-density lipoprotein (HDL), which could probably be a result of flavonoids as increased flavonoids reduce the occurrence of low high-density lipoprotein (Ahn et al., 2020; Anaka et al., 2013) present in the plant (Orumwense et al., 2022). Flavonoids can also reduce cholesterol synthesis by inhibiting the activity of the enzyme acyl-CoA cholesterol acyl transferase (ACAT) in HepG2 cells. This enzyme reduces cholesterol esterification in the intestine and liver (Simorangkir et al., 2022). All the lipid parameters were not significantly affected at 250 mg/kg, the result of this study, indicating safety at a lower dose.

Effect of methanol root extract of *Synclisia scabrida* on liver and kidney functions of *P. berghei*-infected mice

The histology of liver of the vehicle treated mice is shown in Figure 1Ai. The photomicrograph shows a hepatic lobule having a central vein (CV). From the central vein, hepatocytes (HPC) (liver cells) radiate outwards like the spokes of a wheel. The hepatocytes form sheets and in between the sheets run hepatic sinusoids (HPS). Hepatocytes have one or two darkly stained nuclei and deeply eosinophilic cytoplasm. The photomicrograph of the liver of 500 mg/kg *Synclisia scabrida* crude extract treated mice shows dilated central vein. Liver cell architecture is a distorted compared to negative control, hepatocytes are sequestered, cell outline are indistinct. Cell nuclei are darkly stained and the cytoplasm is poorly eosinophilia (Figure 1Aii).

The histology of kidney of the vehicle treated mice is shown in Figure 1Bi. The photomicrograph shows renal corpuscles and renal tubules. Renal corpuscles consist of glomeruli and Bowman's capsule. Renal tubules (RT) have complete epithelial lining. The photomicrograph of the kidney of 500 mg/kg *Synclisia scabrida* crude extract treated mice shows renal corpuscle (RC) and renal tubules. Renal corpuscle is normal. There are areas of fibrosis of renal tubules (Figure 1Bii).

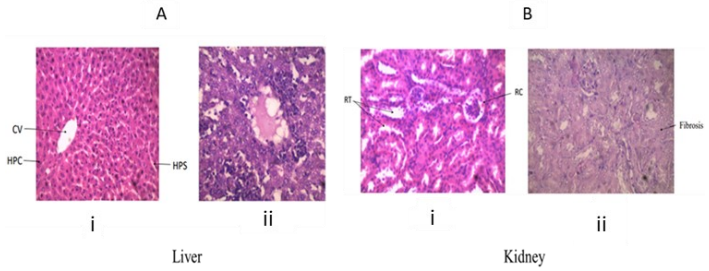

Figure 1. A; Liver of *P. berghei*-infected mice treated with vehicle (i) and 500 mg/kg crude methanolic root extract of *Synclisia scabrida* (ii). B; Kidney of *P. berghei*-infected mice treated with vehicle (i) and 500 mg/kg crude methanolic root extract of *Synclisia scabrida* (ii). CV= central vein, HPC= hepatocytes, HPS= hepatic sinusoids, RT=renal tubule, RC=renal corpuscle. [Mag: X500]

The result on the histology of the kidney suggests that treatment with the Synclisia scabrida root extract at a dose of 500 mg/kg in malaria-infected mice has induced some histological changes in the kidney. While the renal corpuscles, which include the glomeruli and Bowman's capsule, appear normal, there are areas of fibrosis in the renal tubules, indicating damage or scarring in these tubular structures. These changes in the kidney tissue suggest potential effects on kidney health due to the treatment. Given the effect of 500 mg/kg S. scabrida on the liver and kidney, the lowest dose (125 mg/kg) could be used in treatment since it significantly reduced the parasite density and percentage of parasitemia.

The results of this study revealed that the methanolic root extract of *Synclisia scabrida* exhibited good antimalarial activity against *Plasmodium berghei* as it significantly reduced parasite density and the level of parasitemia. Lower dose of the extract did not cause significant changes to the lipid profiles. However, the high dose may not be safe since there were some adverse effects shown on the liver and kidney status but not on the haematological parameters. Further studies are warranted to determine the effect of the middle and the lowest doses on the liver and kidney.

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REFERENCES

Ahn S, Jun S, Joung H (2020) Association of total flavonoids intake with hypo-HDL-Cholesterol among Korean adults: Effects modification by polyunsaturated fatty acid intake. Nutrients 12(1): 195.

Akande OA, Ndams IS, Natala AJ, Babamale OA (2021) In-vivo anti-plasmodial activity of methanolic leaf extracts of jatropha curcas on plasmodium berghei (NK 65) infected mice. J. Phytomed. Ther 20(2): 702– 713.

Albohiri HH, Alzanbagi NA (2021) Systematic review: The pathogenicity of plasmodium berghei in the liver and spleen of the experimental mice Journal of Pharmaceutical Research International 33(47B): 515-529.

Al-Salahy M, Shnawa B, Abed G, Mandour A, Al-Ezzi A (2016) Parasitaemia and its relation to hematological parameters and liver function among patients malaria in Abs, Hajjah, Northwest Yemen. Interdiscip Perspect Infect Dis 2016: 5954394.

Amaratunga C, Sreng S, Suon S, Phelps ES, Stepniewska K, Pharath Lim, Zhou C, Mao S, Anderson JM, Lindegardh N, Jiang H, Song J, Su Z, White NJ, Dondorp AM, Anderson TJC, Fay MP, Mu J, Duong S, Fairhurst RM (2012) Artemisinin-resistant Plasmodium falciparum in Pursat province, western Cambodia: a parasite clearance rate study. Lancet Infect Dis 12(11): 851–858.

Anaka ON, Owolabi OJ, Ikpefan E, Okhiokpamwonyi O (2013) Hypolipidemic activity of ethyl acetate fraction of methanolic seed extract of Persea americana mill (Lauraceae) in rats. J. Pharm. Allied. Sci 10(3): 1905-1917.

Anowi FC, Onyekaba TU, Eze CC, Ike C, Ezenachi VC (2013) Preliminary Phytochemical investigations and evaluation of Antimicrobial activity of n-hexane extract of the leaves of Synclisia scabrida family menispermaceae. Res. J. Pharmaceutical Sci 2(3): 1-5

Atanu FO, Rotimi D, Ilesanmi OB, Al Malki JS, Batiha GE, Idakwoji PA (2022) Hydroethanolic extracts of Senna alata leaves possess antimalarial effects and reverses haematological and biochemical pertubation in plasmodium berghei-infected mice. J Evid Based Integr Med 27: 2515690X221116407

Basir R, Rahiman SF, Hasballah K, Chong WC, Talib H, Yam MF, Ahmad Z (2012) Plasmodium berghei ANKA infection in ICR mice as a model of cerebral malaria**.** Iran. J. Parasitol *7*(4): 62.

Bays HE, Kirkpatrick CF, Maki KC, Toth PP, Morgan RT, Tondt J, Christensen SM, Dixon DL, Jacobson TA (2024) Obesity, dyslipidemia and cardiovascular disease: A joint expert review from the obesity medicine association and the national lipid association 2024**.** J. Clin. Lipidol 18(3): e320-e350.

Balikagala B, Fukuda N, Ikeda M, Katuro OT, Tachibana S-I, Yamauchi M, Opio W, Emoto S, Anywar DA, Kimura E, Palacpac NMQ, Odongo-Aginya EI, Ogwang M, Horii T, Mita T (2021) Evidence of Artemisinin-Resistant Malaria in Africa. N Engl J Med 385(13): 1163-1171.

Birnbaum J, Scharf S, Schmidt S, Jonscher E, Hoeijmakers WAM, Flemming S, Toenhake CG, Schmitt M, Sabitzki R, Bergmann B, Fröhlke U, Mesén-Ramírez P, Blancke Soares A, Herrmann H, Bártfai R, Spielmann T (2020) A Kelch13-defined endocytosis pathway mediates artemisinin resistance in malaria parasites. Science 367(6473): 51-59.

Catherine MM, Wang J, Li, Q, Ferreira P, Avery MA, Elokely K, Staines H, Kris S (2022) Selective inhibition of *plasmodium falciparum* ATPase 6 by Artemisinins and identification of new classes of inhibitors after expression in yeast. Antimicrob. Agents Chemothe 66(5): e02079-21.

Ceravolo IP, Aguiar AC, Adebayo JO, Krettli AU (2021) Studies on Activities and Chemical Characterization of Medicinal Plants in Search for New Antimalarials: A TenYear Review on Ethnopharmacology. Front Pharmacol 12: 734263.

Chidozie,VN, Okwori AEJ, Oluwatayo BO, Adekeye AM, Kinjir H, Okeke C, Abah LM, Salako Y (2020) Assessment of packed cell volume among students of federal college of veterinary and medical laboratory technology, Vom, Plateau state. Int J. Adv. Res 8(5): 457-460.

Choudhari AS, Mandave PC, Deshpande M, Ranjekar P, Prakash O (2020) Phytochemicals in cancer treatment: From preclinical studies to clinical practice. Front Pharmacol 10: 1614.

Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, Ringwald P, Silamut K, Imwong M, Chotivanich K, Lim P, Herdman T, An SS,

Yeung S, Singhasivanon P, Day NP, … (2009) Artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med 361: 455–467.

Deharo E, Bourdy G, Quenevo C, Muñoz V, Ruiz G, Sauvain M (2001) A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. J Ethnopharmacol 77(1): 91-98.

Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S (2004) Antimalarial drug discovery: efficacy models for compound screening. Nat Rev Drug Discov 3(6): 509-520.

Kifle ZD, Adinew GM, Mengistie MG, Gurmu AE, Enyew EF, Goshu BT, Amare GG (2020) Evaluation of antimalarial activity of methanolic root extract of *Myrica salicifolia*: A rich (Myricaceae) against plasmodium berghei-infected mice. J Evid Based Integr Med 25: 2515690X20920539.

Kumar S, Guha M, Choubey V, Maity P, Bandyopadhyay U (2007) Antimalarial drugs inhibiting hemozoin (beta-hematin) formation: a mechanistic update. Life Sci 80: 813-828.

Kyaw MP, Nyunt MH, Chit K, Aye MM, Aye KH, Aye MM, Lindegardh N, Tarning J, Imwong M, Jacob CG, Rasmussen C, Perin J, Ringwald P, Nyunt MM (2013) Reduced susceptibility of *Plasmodium falciparum* to artesunate in southern Myanmar. PLoS ONE. 8(3): e57689.

Maiga FO, Wele M, Toure SM, Keita M, Tangara CO, Refeld RR, Thiero O, Kayentao K, Diakite M, Dara A, Li J, Toure M, Sagara I, Djimde A, Mather FJ, Doumbia SO, Shaffer JG (2021) Artemisinin-based combination therapy for uncomplicated plasmodium falciparum malaria in Mali: a systematic review and meta-analysis. Malar J 20: 356.

Moore CM, Wang J, Lin Q, Ferreira P, Avery MA, Elokely K, Staines HM, Krishna S (2022) Selective Inhibition of Plasmodium falciparum ATPase 6 by Artemisinins and Identification of New Classes of Inhibitors after Expression in Yeast. Antimicrob Agents Chemother 66(5): e0207921.

Simorangkir M, Silaban S, Roza D (2022). Anticholesterol activity of ethanol extract of Ranti Hitam (Solanum blumei Nees ex Blume) Leaves: In vivo and In silico study. Pharmacia. 69(2):485-492.

National Institute of Health USA: Public Health Service Policy on Humane Care and Use of Laboratory; 2002

Nlinwe NO, Nange TB (2020) Assessment of hematological parameters in malaria, among adult patients attending the Bamenda regional hospital. Anemia 2020:3814513.

Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM (2008) Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med 359: 2619–2620.

Onwudiwe TC, Ughachukwu PO, Unekwe PC, Ogamba JO (2012) Evaluation of antiulcer properties of ethanolic and hot aqueous stem extracts of Synclisia scabrida on experimentally induced ulcer models in albino mice. Ann Med Health Sci Res 2(2): 134-139.

Orumwense GE, Osagie AM, Omage SO, Omage K, Azeke MA (2022) *Synclisia scabrida* protects against oxidative stress, hepatotoxicity and hyperglycermia in alloxan induced diabetic rats. J. Diabetes Metab. Disord, 21(1): 669-680.

Otun SO, Graca R, Onisuru O, Achilonu I (2024) Evaluation of plasmodium berghei models in malaria research. J. Cell Signal 5(3): 96-113.

Oyibo W, Latham V, Oladipo O, Ntadom G, Uhomoibhi P, Ogbulafor N, Okoronkwo C, Okoh F, Mahmoud A, Shekarau E, Oresanya O, Cherima YJ, Jalingo I, Abba B, Audu M, David JC (2023) Malaria parasite density and detailed qualitative microscopy enhances large-scale profiling of infection endemicity in Nigeria. Sci Rep 13: 1599.

Rasoanaivo P, Deharo E, Ratsimamanga-Urverg S, Frappier F (2004) Guidelines for the non clinical evaluation of the efficacy of traditional antimalarials. In: Willcox M, Bodeker G, Raoanaivo P, editors. Traditional Medicinal Plants and Malaria. Boca-Raton: CRC pp. 225–270.

Saftawy EE, Farag FM, Gebreil HH, Abdelfatah M, Aboulhoda EB, Alghamdi M, Albadawi EA, Elkhalek MAA (Malaria: biochemical, physiological, diogonostic and therapeutic updates. Peer J 12: e17084.

Scheinmann F, Scriven EFV, Ogbeide ON (1980) Cycleanine from Synclisia scabrida: conformational information from the 1H NMR spectrum at 300 MHz. Phytochemistry 19:937–1940.

Uche FI, Guo X, Okokon J, Ullah I, Horrocks P, Boateng J, Huang C, Li W-W (2021) In vivo efficacy and metabolism of the antimalarial cycleanine and improved in vitro antiplasmodial activity of semisynthetic analogues. Antimicrob Agents Chemother, 19(8): 1837-1840.

Uzor PF (2020) Alkaloids from plants with antimalarial activity: a review of recent studies. Evid Based Complement Alternat Med, 2020:8749083.

Venkatesan P (2024). The 2023 WHO World malaria report. The Lancet Microbe. 5(3): e214.

WHO guidelines for the treatment of malaria. Geneva: World Health Organization, 2006.

World Health Organization. (2021). WHO initiative to stop the spread of Anopheles stephensi in Africa (No. WHO/UCN/GMP/2022.06). World Health Organization.

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