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Antimalarial Activity of n-Hexane, n-Butanol Fractions of *Spilanthes filicaulis* in Swiss Mice Infected with *Plasmodium berghei*

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ABSTRACT

Malaria, a serious disease that can be fatal if left untreated, is caused by *Plasmodium* parasites. Malaria poses a significant life threat due to growing parasite resistance to drugs and the prohibitive cost of treatment, particularly in high-prevalence African countries. The prospect offered by the exploration of botanicals as alternatives necessitated this study to examine the antimalarial activities of the n-hexane fraction of *Spilanthes filicaulis* (HFSF) and its butanol fraction (BFSF) on *Plasmodium berghei*-infected mice. Swiss mice of both genders were infused with a chloroquine-sensitive *P. berghei* (NK-65) strain intraperitoneally. Antimalarial activity (*in vivo)* of *S. filicaulis* fractions was evaluated against early and established infection employing 4-day suppressive and curative antimalarial models at 250, 500 and 750 mg/kg dose levels respectively. Rectal temperature (RT), packed cell volume (PCV), body weight (BW), parasitemia level and mean survival time (MST) were variables determined. Findings herein demonstrated marked prevention of BW, RT, and PCV reduction at the treated doses relative to the untreated controls. Moreover, both fractions significantly suppressed parasitemia dose-dependently. The highest antimalarial chemosuppression was demonstrated by the HFSF producing 60.59%, 69.29% and 71.17% inhibition in the 4-day suppression and the BFSF yielded 45.44%, 43.96% and 47.97% chemosuppression in the curative, at the treated doses respectively. Similarly, the fractions delayed the mean survival duration of treated infected mice relative to the untreated group. Therefore, the results herein suggest that both fractions demonstrate dose-dependent and statistically significant suppression of parasitemia and improved clinical parameters in mice infected with *P.berghei* against murine malaria.

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INTRODUCTION

Malaria continues to pose a major public health challenge to people of sub-Saharan African countries despite the elephantine efforts towards its eradication (Ofeniforo *et al*., 2024a). It is an infection commonly associated with high morbidity and mortality rates in young ones lower than age five and expectant women (Wondafrash *et al*., 2019). In humans, this mosquito-borne disease is engendered by five species of *Plasmodium* namely, *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae,* and *P. knowlesi*. However, *P. falciparum* and *P. vivax* cause the greatest lethality and the majority of malaria infections worldwide (Misganaw *et al*., 2020). This disease could be preventable and curable if adequately controlled (WHO 2020). Common controlled measures include the use of treated mosquito nets, insecticide indoor spray, antimalarial medications, bush clearing, and removal of stagnant water (Alaribe *et al*., 2020).

Nature is as old as human existence providing us not just with food but also with medicines in abundance, such that humans could eat their food as medicine prevalent in plants (Adeleke and Ndah, 2022). Secondary metabolites are the most striking and promising elements in plants on which humans depend (Osuntokun and Faniyi, 2020). The cultural trust, readily availability and direct accessibility of medicinal herbs within the environment, and poor economy relative to the high cost of over-the-counter antimalarial drugs, may have encouraged the local populations of Southwestern States of Nigeria to continue to practice the use of traditional medicines.

The genus *Spilanthes* commonly distributed in the tropics has over 300 species (Jayaraj *et al.,* 2013). *Spilanthes filicaulis* belongs to the *Asteraceae* family. The aerial part of this small creeping plant (*Spilanthes filicaulis)* is orally administered and local traditional healers utilize the plant material in their practice in Igbara-Odo Ekiti Nigeria where it is envisaged that a high prevalence of this disease occurs. However, Akinwunmi, Ofeniforo, and Omisore (2018) reported limited scientific information regarding the antimalarial activity of the crude methanolic extract of *S. filicaulis*. Therefore, given the increasing resistance to conventional antimalarial drugs and the traditional use of *S. filicaulis* in malaria treatment among local populations in Ekiti State, this study aimed to evaluate the in vivo antimalarial potential of its n-hexane and n-butanol fractions in *Plasmodium berghei*-infected mice. This will provide a scientific basis for its traditional use and contribute to the search for new plant-based antimalarial agents.

MATERIALS AND METHODS

Chemicals/reagents for this study were sourced from reputable and certified vendors (Sigma-Aldrich, HPLC grade). Fresh aerial parts of S. filicaulis were collected from Igbara-Odo Ekiti, Ekiti State, Southwest Region of Nigeria (December 2022). It was identified and authenticated at IFE Herbarium, Obafemi Awolowo University Ile-Ife, Nigeria with voucher specimen number IFE/17571. The aerial parts were thoroughly washed, air-dried at room temperature and grounded into smooth powder using an electric miller. The smooth granular material (800 g) underwent extraction using maceration process with 2.5 L of 80% methanol for 72 hours at room temperature. Thereafter, the plant extract was lyophilized to a solid mass known as crude methanolic extract of *S. filicaulis* (MESF). 50g portion of MESF was later fractionated by solvent–solvent extraction with *n*-hexane followed by n-butanol successionally. Respective fractions at 40 °C were concentrated *in-vacuo* to get the HFSF and BFSF. The percentage yield was calculated using the equation below.

$$\text{Percentage yield } = \frac{\mathrm{W2} - \mathrm{W1}}{\mathrm{W0}} \times 100$$

Where W2 is the weight of the extract and the Petri dish, W1 is the weight of the petri dish alone and W0 is the total weight of the sample partitioned.

To pinpoint constituents in HFSF and BFSF preparatory qualitative screening of secondary metabolites was done using basic known methods (Senthilkumaran *et al.,* 2024). Methods that are well-known were also used to determine phytoconstituents quantitatively: alkaloids (Shubhra *et al*., 2019), terpenes (Das *et al*., 2022), Saponins (Chen *et al*., 2010), tannins (Devi *et al.,* 2019), steroids (Oyawaluja *et al*., 2024), flavonoids (Madaan *et al*., 2011) and glycosides (Ademoye, Lajide, and Owolabi, 2018).

Ninety-six adult Swiss mice (weighing 18-25g) were sourced from the Department of Pharmacology Animal Breeding Unit, University of Ibadan, Oyo State Nigeria. All the mice for this study were kept in well-ventilated cages (plastic) and acclimatized for seven days. The mice were housed in a temperature controlled room (temperature 25-30 ºC), with a relative humidity of 40-45%, and a 12-hour light-dark cycle, with unlimited access to pelletized growers mash and water at all times.

At the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria; a chloroquine-sensitive strain of *P. berghei* (NK-65) was obtained. Blood was taken from the heart of a donor mouse exhibiting increasing parasitemia levels of 25-30% through cardiac puncture using a sterile needle inserted into the left ventricle and the collected blood in the syringe was transferred into a capillary tube containing 0.1 ml of Acid Citrate Dextrose (ACD). The blood was subsequently diluted with 0.9 % physiological saline, taking into account the parasitemia level of the donor mouse. An intraperitoneal injection of 0.2 ml of diluted parasitized erythrocytes was administered to the experimental mice, which is equivalent to 1×10^7 red blood cells infected with *P. berghei*.

Infected mice were randomly grouped into five groups of six mice (n=3 female and n=3 male) each immediately after inoculation. Treatment administration in each group consisted of:

Group I (Negative control group): Infected untreated mice with 0.2 ml *P. berghei* thus serving as negative control (received pelletized feed and water).

Group II (Positive control group): Infected mice with 0.2 ml *P. berghei* but later treated with 10 mg/kg body weight chloroquine.

Group III-V (Test group): Mice infected with 0.2 ml *P. berghei* but later receive treatment with

250, 500, and 750 mg/kg body weight respectively of HFSF and BFSF extracts.

Early Infection (4-Day Suppressive Treatment)

The model was carried out using the method of Nureye *et al.* (2018). Adult Swiss mice (18-25 g) were randomly assigned to 5 groups (n=6) and were inoculated with 0.2 ml of parasitized blood containing (1 x 10^7), *P. berghei* NK-65 intraperitoneally (i.p.) on day zero D_0. Treatment with HFSF, BFSF extract and chloroquine started 3 hours after inoculation orally, once a day for 4 days. On the fifth day of study (D_4), thin blood smears were made from the tail blood of each mouse onto a clean slide, fixed with methanol and stained with 5% Giemsa, washed under a running tap, and allowed to dry. Microscopic examination was used to count the number of parasitized cells as described by Aarthi and Murugan (2011). On days 6 and 8 post-inoculation, additional smears were taken.

Established Infection (Curative Treatment)

The model was carried out using the method of Nureye *et al.,* (2018). Adult Swiss mice (18-25 g) were randomly assigned to 5 groups (n=6) and were inoculated with 0.2 ml of parasitized blood containing (1 x 10^7) parasites, *P. berghei* NK-65 intra-peritoneally (i.p.) on day zero D_0. Treatment with HFSF, BFSF extract and chloroquine commenced orally once daily 72 hours (D_3) after infection and it continued for 4 consecutive days. By the fourth day which is now (day D_7), thin smears of blood were prepared, methanol-fixed, and stained with 5% Giemsa stain. Thereafter, microscopic examination was used to count the number of parasitized cells as described by Aarthi and Murugan (2011). On days 8 and 10 post-inoculation, additional smears were taken.

Parasitemia Evaluation

The prepared blood films were mounted on the microscope stage, and a drop of immersion oil was gently applied. It was observed using an x100 objective lens, to locate a suitable field for enumeration of parasitized and unparasitized erythrocytes. The total number of red blood cells (TRBC) and total number of parasitized red blood cells (PRBC) were counted in a particular field. Parasitemia suppression was estimated to be a percentage for each tested dose by comparing the level of parasitemia in the untreated group with its treated counterpart.

Parasitemia percentage in each field was calculated as reported by Mengiste *et al*., 2012 thus.

$$\%\ \text{Parasitemia} = \frac{\text{Total number of PRBC}}{\text{Total number of TRBC}} \times 100\%$$

$$\text{PR}\ (\%) = \frac{\%\ \text{Poc} - \%\ \text{Pot}}{\%\ \text{Poc}} \times 100$$

Description:
Poc = Parasitemia of control
Pot = Parasitemia of test

Mean Survival Time (MST) Determination

Mortality rate in days was assessed daily at the onset of inoculation of the parasite until death was recorded for all mice across all groups. MST for all groups was estimated as described by Mengiste *et al*. (2012) thus;

$$\text{MST} = \frac{\text{Sum of survival time (Days) of all mice in a group}}{\text{Total number of mice in the group}}$$

Body Weight (BW) Determination

BW of each mouse was determined, to check whether HFSF and BFSF extracts prevented weight loss or not, as described by Belay, Gurmu, and Wubneh (2018). Briefly, the BW of the individual mouse across all groups was checked before to infection with the parasite (day 0) and on the fifth day after infection (day 4) in the suppressive treatment, while days 3 and 7 after infection will be used for curative treatment using a sensitive electronic balance.

$$\text{BW} = \frac{\text{Sum of BW of mouse in each group (g)}}{\text{Total number of mice in group (g)}}$$

Rectal Temperature (RT) Determination

The RT of mice was assessed with a digital handheld thermometer with a flat probe to ascertain the effect of HFSF and BFSF extract on averting body temperature reduction due to malaria. The colonic temperature attained was measured by inserting a probe >2 cm deep through the mouse anus which gives a measure of deep (core) body temperature. Briefly, the RT of mice across all groups was checked prior infection with the parasite (day 0) and on the fifth day after infection (day 4) in the suppressive treatment, while days 3 and 7 after infection used for curative treatment as reported by Kumar *et al*., 2017.

$$\text{RT} = \frac{\text{Sum of RT of mouse in each group (°C)}}{\text{Total mice per group (g)}}$$

Packed Cell Volume (PCV) Determination

The PCV was also assessed before and after treatments. In determining the PCV, samples of blood were taken from the terminal tail tip region of each mouse (from each group during suppressive and curative tests) using heparinized capillary tubes for microhematocrit analysis. The capillary tubes were filled to the desired volume (three-fourths) with blood and closed with plasticin at their outlet. It was later spun at 11,000 rpm for 10 minutes using a microhematocrit centrifuge. Finally, the sealed tubes were removed and PCV was estimated with the help of a hematocrit reader and the percentage PCV was calculated as reported by Mengiste *et al*., (2012) using the formula below.

$$\text{PCV}\ (\%) = \frac{\text{Erythrocytes volume fraction (ml)}}{\text{Total blood volume examined (ml)}} \times 100$$

Data Analysis

Results of our investigation were reported as mean ± standard error of mean (SEM) for six mice per group. The arithmetic mean value of parasitemia suppression, BW, RT, PCV and MST significance between groups was computed by one-way ANOVA. Duncan's *post hoc* multiple comparisons were used to analyze and compare the results at a 95% confidence level. Values of $p< 0.05$ were considered significant.

RESULTS AND DISCUSSION

Phytochemical screening of HFSF and BFSF extracts gave positive tests for the presence of steroids, alkaloids, flavonoids, tannins, saponins, terpenoids, and cardiac glycosides but their quantities vary (Table 1). The efficiency of extraction is a determinant of the method of extraction, extraction timing, the phytochemicals composition, temperature and the used solvent (Ngo *et al*., 2017), thus our investigation used n-hexane and butanol to partition the hydromethanolic extract of *S. filicaulis*. The percentage yield resulted in BFSF (22.56%) and HFSF (16.98%). The highest extraction yield was observed in BFSF suggesting itis a better solvent for the extraction of *S. filicaulis* phytoconstituents.

The therapeutic promise of plants is attributed to the presence of a diverse combination of varying secondary metabolites such as flavonoids, glycosides etc., that may act singly, collectively, or synergetic to establish an effect that may be harmful or beneficial to health (Merlin *et al*., 2019). Results from our study showed that HFSF and BFSF are rich in phytochemicals but in contrasting concentrations (Table 1). The abundance of some of the phytoconstituents in HFSF may have accorded better antimalarial activity than its counterpart, this supports the notion that the quality of bioactive content is more important than the quantity of fractions. However, these phytoconstituents identified have been reported to attribute antiplasmodial activities (Ibukunoluwa, 2017). Thus validating earlier reports of Agbafor who reported that *Spilanthes* is rich in phenolic compounds, tannins, alkaloids, flavonoids and glycosides (Agbafor *et al*., 2015). Albeit the fact that medicinal plants are perceived to be beneficial and safe, some are toxic potentially (Jothy *et al*., 2011). To substantiate this fact, a report on an earlier acute toxicity test on crude methanolic extract of *S. filicaulis* by Akinwunmi, Ofeniforo, and Omisore, (2018), showed that the evaluated median lethal dose (LD_{50}) was ≥ 5000 mg/kg bwt on laboratory mice.

Analysis of BW and RT of parasitized untreated control group in suppressive treatment showed a significant loss in BW and RT. All treated dose levels of HFSF and BFSF exhibited dose-dependently protection in opposition to BW loss and RT reduction which is significant at ($p<0.05$). The highest BW and reduction in temperature protection was demonstrated by the BFSF at the highest dose of 750 mg/kg bwt administered in comparison to its HFSF (Figure 1-4). HFSF produced peak PCV protection at 750 mg/kg bwt (Figure 5-6). In comparison to the parasitized untreated group, all treated dose levels of HFSF and BFSF in the curative treatment exhibited a dose-dependently protection in opposition to BW loss and RT reduction which is significant at ($p<0.05$). The highest BW and reduction in temperature protection was demonstrated by the BFSF at 750 mg/kg bwt (Figure 7-10). The HFSF and BFSF protected PCV infection-induced reduction with the BFSF at 750 mg/kg bwt producing the highest protection (Figure 11-12), and the least protection of the two fractions was demonstrated by HFSF at 250 mg/kg bwt (Figure 11).

Table 1. Phytochemicals Composition in HFSF and BFSF

Phytochemicals	Table Column Head	
	HFSF	**BFSF**
Steroid	10.38 ± 0.32	19.65 ± 0.80
Glycosides	0.08 ± 0.15	0.94 ± 0.23
Flavonoid	49.46 ± 1.02	31.07± 0.36
Terpenoids	16.92 ± 0.04	7.19 ± 0.85
Tannins	1.78 ± 0.15	0.82 ± 0.05
Saponins	2.63 ± 0.11	1.08 ± 0.02
Alkaloids	14.27 ± 0.42	11.21 ± 0.28

Values are expressed as the mean of three replicates ± S.E.M

Table 2. Effects of oral administration of HFSF on *Plasmodium berghei* (NK65)-infected mice (4-day Suppressive Test)

Treatment	% Parasitemia (% Inhibition)			MST
	Day 4^{o}	**Day 6^{o}**	**Day 8^{o}**	
Control	6.62 ± 0.41	7.85 ± 0.30	9.77 ±1.23	10.50 ± 0.87
10 mg/kg bwt CQ	1.72 ± 0.11 (74.07)	0.81 ± 0.23 (89.57)	0.12 ± 0.12 (98.74)	28.75 ± 0.63
250 mg/kg bwt extract	4.87 ± 0.32 (26.49)	4.62 ± 0.36 (40.81)	3.85 ± 0.15 (60.59)	14.00 ± 1.47
500 mg/kg bwt extract	3.65 ± 0.57 (44.86)	4.17 ± 0.66 (46.49)	3.00 ± 0.70 (69.29)	17.75 ± 2.87
750 mg/kg bwt extract	3.45 ± 0.10 (47.89)	3.27 ± 0.12 (58.03)	2.82 ± 0.15 (71.17)	18.25 ± 2.86

CQ = chloroquine; bwt = body weight; o = post-inoculation day. Values showed as the mean of six replicates ± S.E.M

Table 3. Effects of oral administration of BFSF on *Plasmodium berghei* (NK65)-infected mice (4-day Suppressive Test)

Treatment	% Parasitemia (% Inhibition)			MST
	Day 4°	Day 6°	Day 8°	
Control	6.62 ± 0.41	7.85 ± 0.30	9.77 ± 1.23	10.50 ± 0.87
10 mg/kg bwt CQ	1.72 ± 0.11(74.07)	0.81 ± 0.23 (89.57)	0.12 ± 0.12 (98.74)	28.75 ± 0.63
250 mg/kg bwt extract	5.63 ± 0.61 (14.90)	4.19± 0.33 (46.19)	3.81 ± 0.38 (61.00)	14.75 ± 1.25
500 mg/kg bwt extract	4.84 ± 0.34 (26.84)	4.31± 0.22 (44.78)	3.68 ± 0.13 (62.38)	15.50 ± 1.85
750 mg/kg bwt extract	4.15 ± 0.58 (37.36)	3.96 ± 0.54 (49.23)	3.92 ± 0.18 (59.88)	17.00 ± 2.16

CQ = chloroquine; bwt = body weight; o = post-inoculation day. Values showed as the mean of six replicates ± S.E.M

Table 4. Effects of oral administration of HFSF on *Plasmodium berghei* (NK65)-infected mice (Curative Test)

Treatment	% Parasitemia (% Inhibition)			MST
	Day 4°	Day 6°	Day 8°	
Control	5.05 ± 0.53	9.04 ± 0.24	10.16 ± 0.32	9.75 ± 0.48
10 mg/kg bwt t CQ	6.13 ± 0.81	1.67 ± 0.33 (81.56)	0.36 ± 0.17 (96.42)	26.75 ± 0.95
250 mg/kg bwt extract	6.76 ± 0.79	6.31 ± 0.15 (30.19)	5.95 ± 0.62 (41.44)	13.25 ± 0.48
500 mg/kg bwt extract	6.70 ± 0.28	6.03 ± 0.64 (33.93)	6.10 ± 0.55 (39.93)	13.75 ± 0.47
750 mg/kg bwt extract	5.95 ± 0.41	6.1 ± 0.39 (32.52)	5.63 ± 0.74 (44.59)	15.25 ± 1.89

CQ = chloroquine; bwt = body weight; o = post-inoculation day. Values showed as the mean of six replicates ± S.E.M

Table 5. Effects of oral administration of BFSF on *Plasmodium berghei* (NK65)-infected mice (Curative Test)

Treatment	% Parasitemia (% Inhibition)			MST
	Day 4°	Day 6°	Day 8°	
Control	5.05 ± 0.53	9.04 ± 0.24	10.16 ± 0.32	9.75 ± 0.48
10 mg/kg bwt CQ	6.13 ± 0.81	1.67 ± 0.33 (81.56)	0.36 ± 0.17 (96.42)	26.75 ± 0.95
250 mg/kg bwt extract	6.08 ± 0.35	6.03 ± 0.62 (33.26)	5.54 ± 0.84 (45.44)	13.50 ± 0.96
500 mg/kg bwt extract	6.62 ± 0.87	5.77 ± 0.18 (36.17)	5.69 ± 0.80 (43.96)	14.00 ± 2.09
750 mg/kg bwt extract	6.48 ± 0.86	5.16 ± 0.49 (42.92)	5.29 ± 0.51 (47.97)	15.75 ± 1.19

CQ = chloroquine; bwt = body weight; o = post-inoculation day. Values showed as the mean of six replicates ± S.E.M .

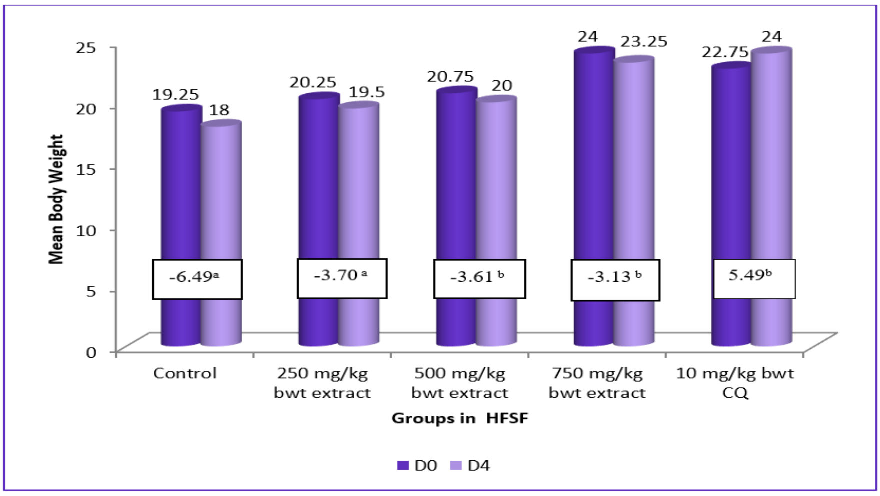

Figure 1. Effect of HFSF on body weight of *P. berghei*-infected mice (4-day Suppressive Test). Values showed as mean of six replicates ± S.E.M (n= 6). Column means with the same letter do not differ significantly ($p<0.05$). D0=Day 0, D4=Day 4, CQ= Chloroquine diphosphate. The numbers in rectangles between the graphs show the change in mean body weight between D0 and D4.

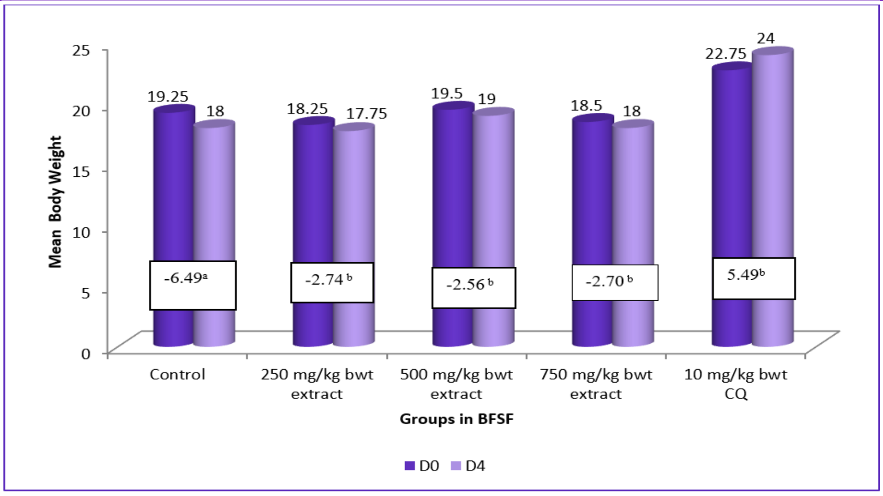

Figure 2. Effect of BFSF on body weight of *P. berghei*-infected mice (4-day Suppressive Test). Values showed as mean of six replicates ± S.E.M (n= 6). Column means with the same letter do not differ significantly (p<0.05). D0=Day 0, D4=Day 4, CQ= Chloroquine diphosphate. The numbers in rectangles between the graphs show the change in mean body weight between D0 and D4.

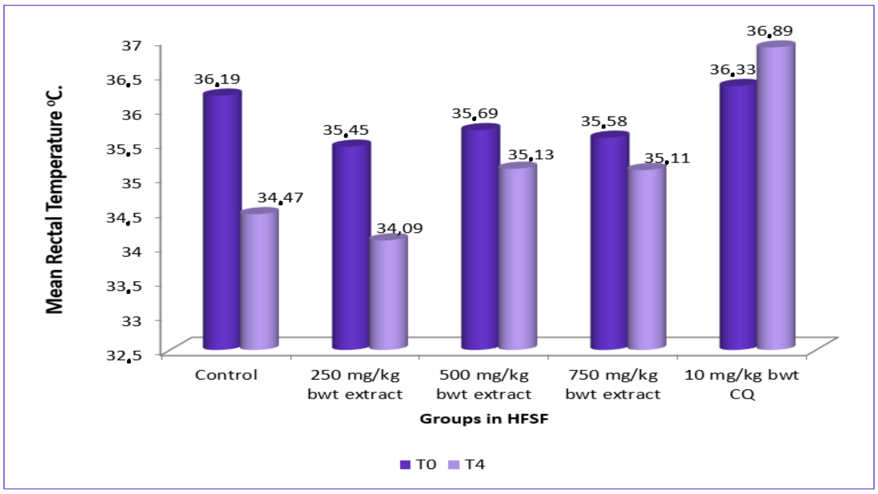

Figure 3. Effect of HFSF on Rectal temperature of *P. berghei*-infected mice (4-day Suppressive Test). Values showed as mean of six replicates ± S.E.M (n= 6). Column means with the same letter do not differ significantly (p<0.05). T0=Day 0, T4=Day 4, CQ= Chloroquine diphosphate. The numbers in rectangles between the graphs show the change in Rectal temperature between D0 and D4.

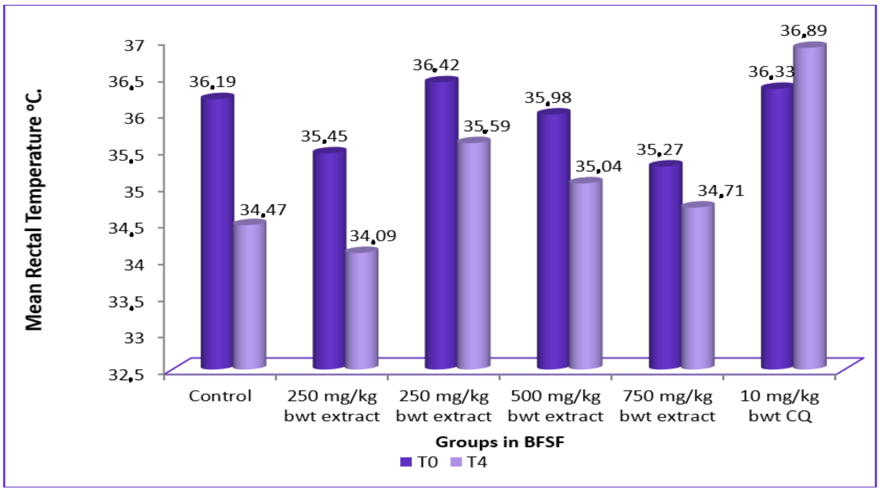

Figure 4. Effect of BFSF on Rectal temperature of *P. berghei*-infected mice (4-day Suppressive Test). Values showed as mean of six replicates ± S.E.M (n= 6). Column means with the same letter do not differ significantly (p<0.05). T0=Day 0, T4=Day 4, CQ= Chloroquine diphosphate. The numbers in rectangles between the graphs show the change in Rectal temperature between D0 and D4.

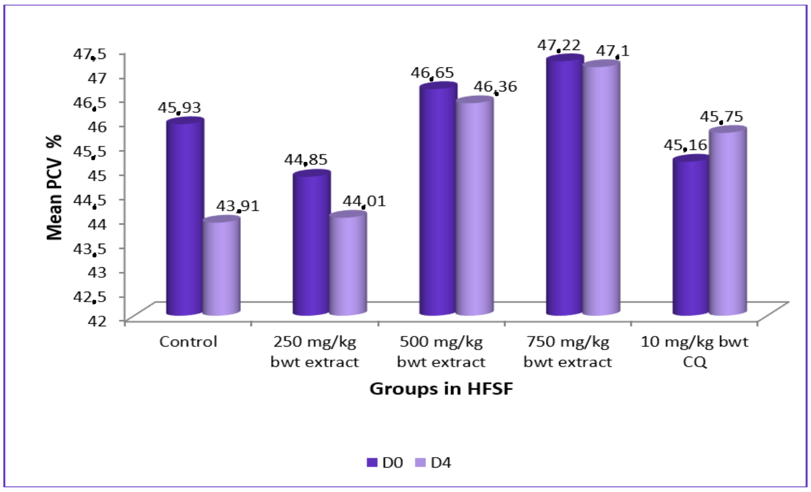

Figure 5. Effect of HFSF on packed cell volume of *P. berghei*-infected mice (4-day Suppressive Test). Values showed as mean of six replicates ± S.E.M (n= 6). Column means with the same letter do not differ significantly (p<0.05). D0=Day 0, D4=Day 4, CQ= Chloroquine diphosphate. The numbers in rectangles between the graphs show the change in packed cell volume between D0 and D4.

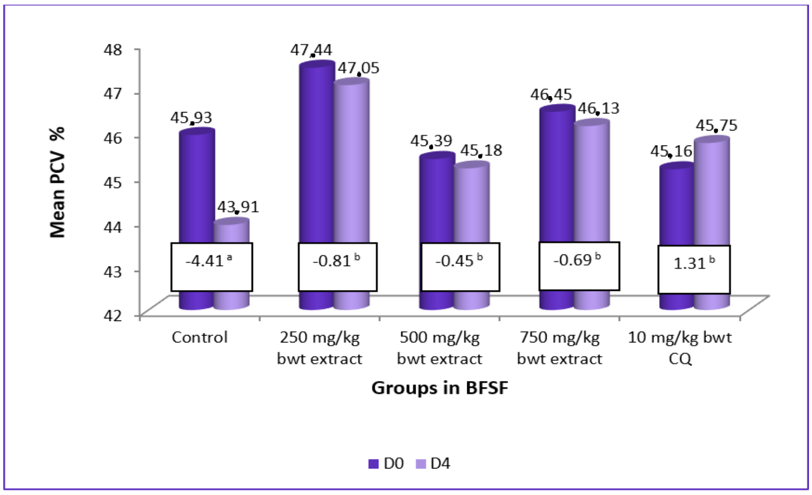

Figure 6. Effect of BFSF on packed cell volume of *P. berghei*-infected mice (4-day Suppressive Test). Values showed as mean of six replicates ± S.E.M (n= 6). Column means with the same letter do not differ significantly (p<0.05). D0=Day 0, D4=Day 4, CQ= Chloroquine diphosphate. The numbers in rectangles between the graphs show the change in packed cell volume between D0 and D4.

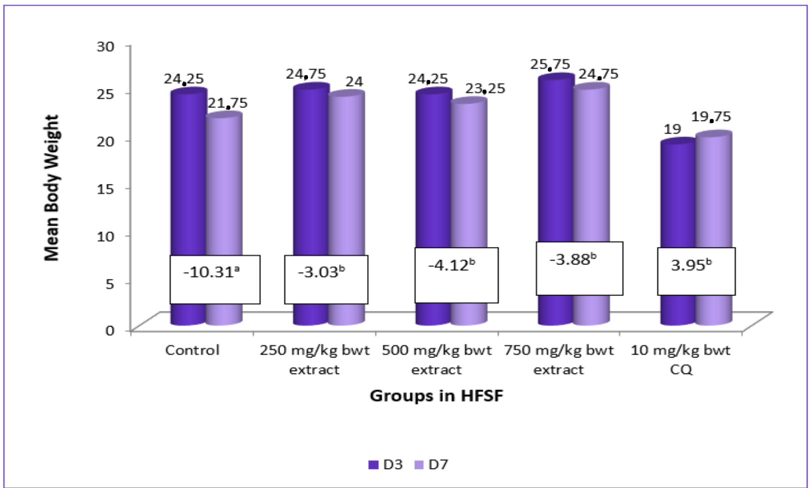

Figure 7. Effect of HFSF on mean body weight of *P. berghei*-infected mice (Curative Test). Values showed as mean of six replicates ± S.E.M (n= 6). Column means with the same letter do not differ significantly (p<0.05). D3=Day 3, D7=Day 7, CQ= Chloroquine diphosphate. The numbers in rectangles between the graphs show the change in mean body weight between D3 and D7.

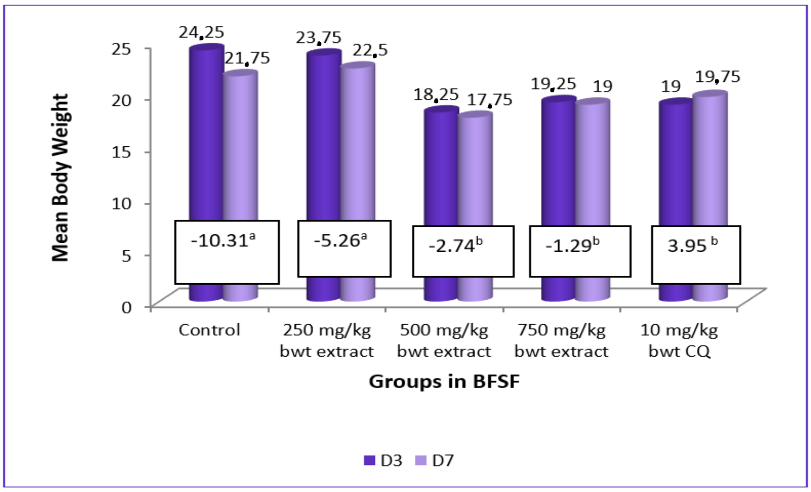

Figure 8. Effect of BFSF on mean body weight of *P. berghei*-infected mice (Curative Test). Values showed as mean of six replicates ± S.E.M (n= 6). Column means with the same letter do not differ significantly (p<0.05). D3=Day 3, D7=Day 7, CQ= Chloroquine diphosphate. The numbers in rectangles between the graphs show the change in mean body weight between D3 and D7.

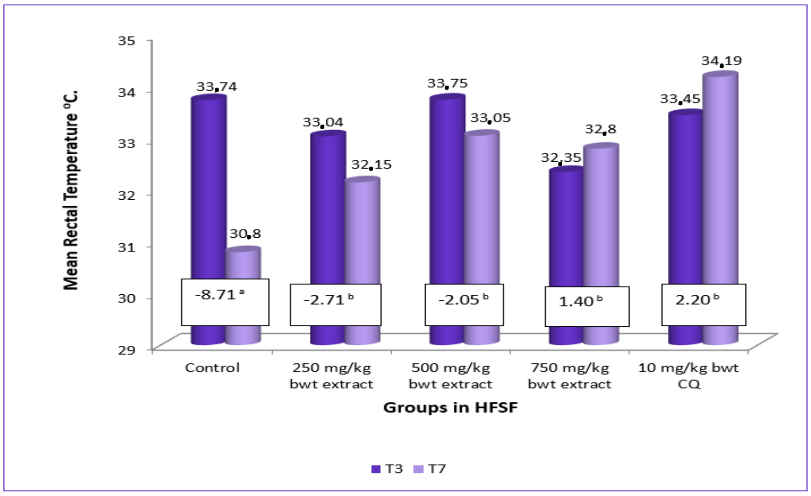

Figure 9. Effect of HFSF on Rectal temperature of *P. berghei*-infected mice (Curative Test). Values showed as mean of six replicates ± S.E.M (n= 6). Column means with the same letter do not differ significantly (p<0.05). T3=Day 3, T7=Day 7, CQ= Chloroquine diphosphate. The numbers in rectangles between the graphs show the change in Rectal temperature between T3 and T7.

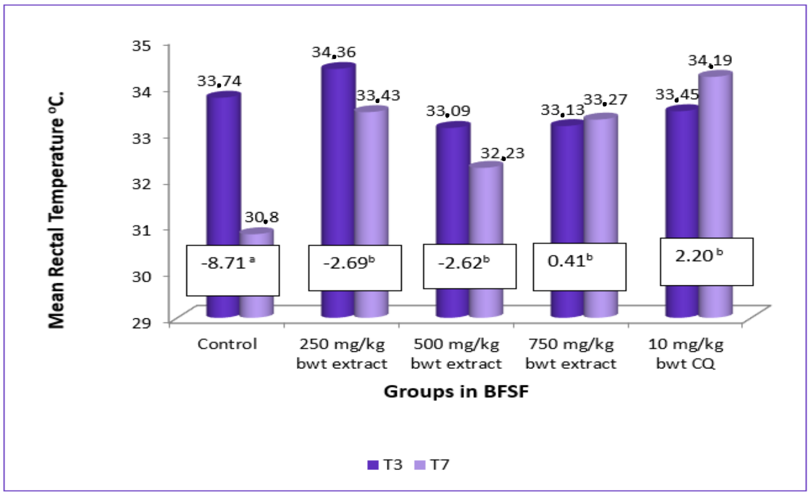

Figure 10. Effect of BFSF on Rectal temperature of *P. berghei*-infected mice (Curative Test). Values showed as mean of six replicates ± S.E.M (n= 6). Column means with the same letter do not differ significantly (p<0.05). T3=Day 3, T7=Day 7, CQ= Chloroquine diphosphate. The numbers in rectangles between the graphs show the change in Rectal temperature between T3 and T7.

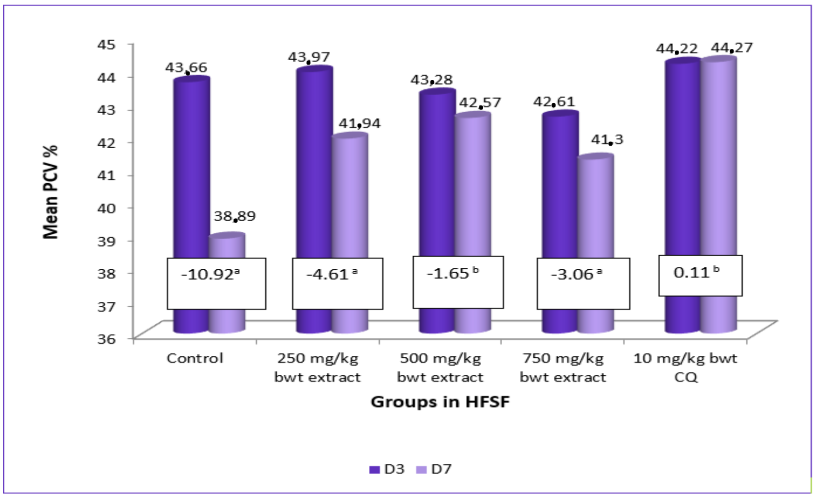

Figure 11. Effect of HFSF on packed cell volume of *P. berghei*-infected mice **(Curative Test).** Values showed as mean of six replicates ± S.E.M (n= 6). Column means with the same letter do not differ significantly ($p<0.05$). D3=Day 3, D7=Day 7, CQ= Chloroquine diphosphate. The numbers in rectangles between the graphs show the change in packed cell volume between D3 and D7.

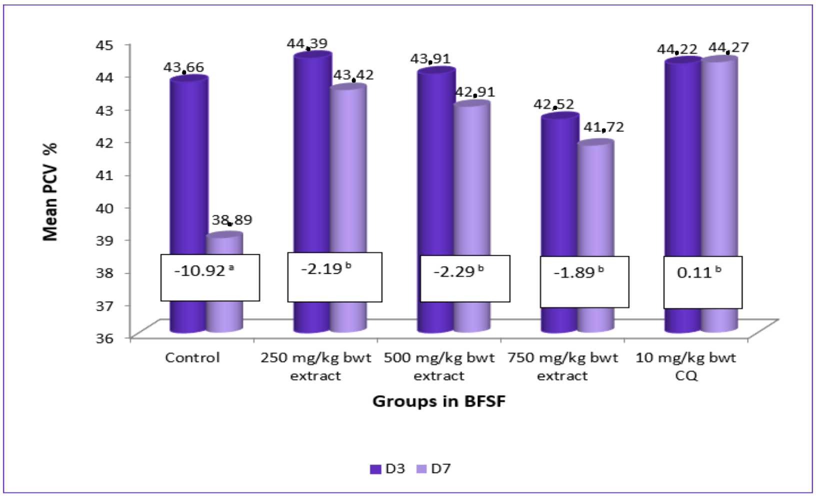

Figure 12. Effect of BFSF on packed cell volume of *P. berghei*-infected mice (Curative Test). Values showed as mean of six replicates ± S.E.M (n= 6). Column means with the same letter do not differ significantly ($p<0.05$). D3=Day 3, D7=Day 7, CQ= Chloroquine diphosphate. The numbers in rectangles between the graphs show the change in packed cell volume between D3 and D7

BW and RT are key health metrics that should be closely monitored in laboratory animals. In human malaria, fever is one key manifestation but in mouse models infected with *P. berghei* malaria is accompanied with reduction in body temperature (Mengiste *et al*., 2012). Reduction in BW and RT is one of the common pointer subsequent to the appearance of an adverse effect in mice infected with *P. berghei* as decrease in BW and RT arises from the rise in parasitemia and this reduction process could be averted by plants that have antimalarial properties (Bantie *et al*., 2014). The fractions of *S. filicaulis* significantly prevented BW and RT loss dose-dependent figure on D_4 as compared to D_0 for the 4-day suppressive treatment. Likewise, dose-dependent activity was reported in the curative treatment on day 7 in relative to day 3. Mengiste reported that infected mice displayed a decreasing metabolic rate before death consequential to a drop in core body temperature and weight (Mengiste *et al*., 2012). Active principle(s) in plants are expected to obviate the rapid falling of BW and RT. In 4-day suppressive and curative treatment, the highest administered doses in the HFSF and BFSF showed a noticeable increment in BW and RT in comparison to the untreated infected mice. The improved activity might have come from the enhancement in BW, RT, PCV and clearance of parasite in treated mice which agrees with the work reported by Jacob *et al*. (2016) and Ofeniforo *et al*., (2024b).

Haematological abnormalities ranging from destruction of the erythrocytes by reticuloendothelial cell action by the spleen or parasite multiplication resulting from many abnormal erythrocytes energizing the spleen to generate numerous phagocytes are considered a major hallmark of malaria (Qaiser *et al*., 2025). Opajobi *et al*., (2022) reported that *P. berghei* increases erythrocyte fragility thus resulting in a significant reduction of PCV in mice. This reduction occurred approximately two days post-infection

(Mace *et al*., 2015). These attributes altogether are the chief cause of malaria-induced anaemia in animals.

Antimalarial plants are anticipated to avert a reduction in PCV secondary to averting haemolysis. Interestingly, results from this study notes that HFSF and BFSF substantially prevented abatement in PCV in 4-day suppressive and in curative treatments dose dependently relative to the untreated group (Figure 5-6, & 11-12). This change in PCV abatement by the fractions may be associated with its ability to decrease parasitemia of infected mice which may be attributed to the abundance of flavonoids, alkaloids, steroids, and other metabolites which have antioxidant and membrane protecting effects. This study's results align with an earlier report by Balogun *et al*. (2012) who reported an improved PCV, hemoglobin (Hb) and RBC of *P. berghei*-induced alteration in infected mice. However, in untreated mice parasitemia level escalated, resulting in reduced PCV level on daily basis until their death, this agrees with observed previous studies reports (Asrade *et al* 2017; Olanlokun *et al*., 2021).

Based on the percentage parasitemia suppression the efficiency of an *in vivo* antimalarial test could be categorized into three distinct groups viz; moderate (≥ 50 % parasitemia suppression at 500-250 mg/kg bwt), good (≥ 50 % parasitemia suppression at 250-100 mg/kg bwt) and very good (≥ 50 % parasitemia suppression at < 100 mg/kg bwt) (Nureye *et al*., 2018). Moreover, the effectiveness of test materials in standard screening tests could also be determined if a test material could delay mortality in treated mice in comparison to the untreated group which agrees with our findings.

The HFSF exhibited partial suppressive antimalarial activity causing less than 50% chemosuppression reduction against infected mice on the 4th and 6th days following inoculation. Improved antimalarial activity beyond 50% chemosuppression was shown by the peak treated dose of 750 mg/kg bwt on the 6th day after inoculation and all other tested doses on the 8th day after inoculation. Optimal chemo suppressive response was 71.17% on day 8 post-inoculation at 750 mg/kg bwt (Table 2). The BFSF exhibited inactive antimalarial activity against *P.berghei* NK-65 causing below 30% chemosuppression on day 4 after inoculation. An enhancement in partial activity was noted on day 6 after inoculation at all treated doses, but on the 8th day after inoculation all doses showed good antimalarial activity with the highest chemosuppression of 62.38% at 500 mg/kg bwt (Table 3). The HFSF exhibited partial curative antimalarial on days 8 and 10 post-inoculation at all doses causing less than 50% chemosuppression on *P. berghei* NK-65 infected mice. The highest chemosuppression was 44.59% at the 750 mg/kg bwt treatment level (Table 4). The BFSF exhibited partial antimalarial activity causing less than 50% chemosuppression at all treatment level on the 8th and 10th day after inoculation. Optimal chemo suppressive response of 47.97% was reported at 750 mg/kg bwt (Table 5).

In the four-day suppressive tests, administered fractions suppressed parasitemia dose-dependently indicating that HFSF and BFSF have antimalarial activity. However, HFSF results in higher percentage chemosuppression probably as a result of higher abundance of phytoconstituents which agrees with study by Amelo, Nagpal, and Makonnen (2014).

Complementing the demonstrated parasitemia suppression of both fractions in the 4-day suppressive treatment, our investigation brings to light that categories of animals that were administered with the various fractions have a prolonged life than the untreated control group. Longest survival period of 18 days for the HFSF was associated with the maximum parasitemia inhibition of 71.17% (Table 2). Nevertheless, the extract-treated mice had reduced survival times relative to the chloroquine control. This possibly stems from the quick elimination phase of the fractioned extracts.

Results obtained in the curative treatment revealed that the fractions suppressed parasitemia dose-dependently confirming the antimalarial properties of the plant. The BFSF exhibited the best curative activity on day 10 post-inoculation with maximum parasitemia inhibition of 47.97% and a MST of 15 days (Table 5). Generally, overall higher suppressive activity in this study relative to curative activity of the fractions agrees with reports from other related findings where bioactive compounds showed higher pronounced activity on early antimalarial infection relative to their established counterpart (Olorunniyi, 2013; Taherkhani *et al*., 2013). In the curative treatment, the fractions exerted significant parasitemia suppression after the administration of the second dose. However, chloroquine onset its effects immediately after the first dose. A possible explanation for the delayed activity may be reflective for a certain amount of cumulative starting dosage or possibly the extract may be having a slower commencement effect in clearing the parasite compared to chloroquine.

Chloroquine, a standard antimalarial drug used in this study subdued parasitemia to a non-detectable level, which agrees with an earlier report by Nureye *et al*. (2018) and Madara *et al*. (2010). Accordingly, all tested doses of the extract fractions restrain parasitemia levels and extended survival period of infected mice demonstrating both suppressive and curative activities dose-dependently which agrees with the findings of Balogun *et al*. (2010) and Akinwunmi, Ofeniforo, and Omisore, (2018).

Previous phytochemical studies of *S. filicaulis* have reported the presence of alkaloids,flavonoids,terpenoids, saponin as the major bioactive component of the plant (Akinwunmi, Ofeniforo, and Omisore, 2018). Alkaloids inhibit protein and DNA synthesis in Plasmodium parasites, hindering their growth and replication (Zareen *et al*. 2021). Terpenoids disrupt isoprenoid biosynthesis and membrane function, compromising parasite survival. Similarly, flavonoids scavenge free radicals, reducing oxidative stress (Adesina *et al*., 2020). The observed antimalarial activity of the extracts could be attributed to its rich phytoconstituents profile, which may act singly or synergistically to target various stages of the Plasmodium life cycle (Vanaja *et al.,* 2025). Likewise, the significant reduction in parasitemia levels and improvement in PCV, survival time, and body weight suggest that *S. filicaulis* extracts may be interfering with the parasite's ability to invade and replicate within the erythrocyte. Therefore, the promising antimalarial activity exhibited by HFSF and BFSF suggests its potential as a natural candidate against early and established malarial infection at which the first line of malaria onset could be managed.

CONCLUSION

Conclusively, HFSF and BFSF demonstrated quality antimalarial activity, with the former demonstrating a higher level of parasitemia suppression relative to chloroquine. Specifically, the fractions exhibited highest parasitemia reduction rates of 71.17% and 62.38% and significantly prolonged survival times, reduced PCV loss, averted temperature, and weight loss. Given these promising results, further bio-guided isolation of lead compound/(s) is recommended to unlock the latent medicinal value of *S. filicaulis* for futuristic development of innovative antimalarial therapies.

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AUTHORS' CONTRIBUTIONS

This work was carried out by all authors. BEO: conceptualization, original draft writing, editing & review, investigation and methodology. OBO: data curation, investigation, methodology, review of manuscript. IOA: conceptualization, writing, drafting and editing of manuscript. EAB: Supervision and Review of Manuscript.

CONFLICT OF INTEREST

Authors declare no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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REFERENCES

Aarthi, N. and Murugan, K. (2011) 'Antimalarial activity and phytochemical screening of ethanolic leaf extract of Phyllanthus niruri and Mimosa pudica,' *International journal of pharmaceutical research and development,* 3(8), pp. 198-205.

Adeleke, M.T.V. and Ndah, S. (2022) 'Comparative phytochemical analysis of four medicinal plants traditionally used for malaria therapy in Nigeria,' *World Journal of Biology Pharmacy and Health Sciences,* 10(01), pp. 080–085, Available at: http://dx.doi.org/10.30574/wjbphs.2022.10.1.0069.

Ademoye, M.A., Lajide, L., and Owolabi B.J. (2018) 'Phytochemical and Antioxidants Screening of *Chrysophyllum albidum*, *Mezoneuron benthamianum*, *Phyllanthus muellerianus* and *Acalypha fimbriata,' International journal of science,* 7(11), pp. 8-18, Available at: http://dx.doi.org/10.18483/ijSci.1803.

Adesina, D.A. *et al.* (2020) 'Antiplasmodial effect and sub-acute toxicity of alkaloid, flavonoid and phenolic extracts of *Sida acuta* leaf on *Plasmodium berghei*-infected animals', *Journal of Taibah University for Science*, 14(1), pp. 943–953, Available at: https://doi.org/10.1080/16583655.2020.1790912

Agbafor, K. *et al*. (2015) 'Antioxidant Activities of Ethanoilc Extractsof *Spilanthes uliginosa*, *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus*against Swiss Mice Exposed to *Plasmodium berghei A*nka 65', *American Journal of Plant Sciences*, 6(1), pp. 64-72, Available at: http://dx.doi.org/10.4236/ajps.2015.61008

Akinwunmi, K.F., Ofeniforo, B.E., and Omisore, N.O. (2018) 'Evaluation of Antioxidant, Anti Inflammatory and Antimalarial Activities of the Methanolic Leaf Extract of *Spilanthes filicaulis* (Schumach & Thonn)', *Journal of pharmaceutical biology,* 8(1), pp. 1-11, Available at: https://doi.org/10.1089/vbz.2024.0039

Alaribe, C. *et al*. (2020) 'Suppressive and curative antiplasmodial properties of *Nauclea latifolia* root extract and fractions against erythrocytic stage of mice-infective chloroquine-sensitive *Plasmodium berghei* NK-65', *Journal of Medicinal Plants for Economic Development,* 4(1), pp. 1-6, Available at: http://dx.doi.org/10.4102/jomped.v4i1.72.

Amelo, W., Nagpal, P. and Makonnen, E. (2014) 'Antiplasmodial activity of solvent fractions of methanolic root extract of *Dodonaea angustifolia* in *Plasmodium berghei* infected mice', *BMC Complementary and Alternative Medicine*, 14(1), pp. 462, Available at: http://dx.doi.org/10.1186/1472-6882-14-462.

Asrade, S. (2017) 'In vivo antiplasmodial activity evaluation of the leaves of *Balanites rotundifolia* (Van Tiegh.) Blatter (Balanitaceae) against *Plasmodium berghei*', *Journal of Experimental Pharmacology*, 9, pp. 59–66, Available at: http://dx.doi.org/10.2147/JEP.S130491.

Balogun, E. *et al.* (2010) 'Activity of ethanolic extract of *Clerodendrum violaceum* leaves against *Plasmodium berghei* in mice', *Agriculture and Biology Journal of North America*, 1(3), pp. 307–312, Available at:

http://dx.doi.org/10.5251/abjna.2010.1.3.307.312.

Balogun, E. *et al*. (2012) 'Biochemical and histological changes associated with treatment of malaria and diabetes mellitus in mice with extracts of *Mormodiaca charantia*', *An International Journal of the Nigerian Society for Experimental Biology*, 24(I), pp. 38–47

Bantie, L. *et al*. (2014) 'In vivo antimalarial activity of the crude leaf extract and solvent fractions of *Croton macrostachyus* Hocsht (Euphorbiaceae) against *Plasmodium berghei* in mice', BMC Complement Altern Med, 14(79), Available at: http://dx.doi.org/10.1186/1472-6882-14-79.

Belay, W., Gurmu, A., and Wubneh, Z. (2018) 'Antimalarial activity of stem bark of *P. linearifolia* during early and established Plasmodium infection in mice', *Evidence-Based Complem & Alt Med*, 2018(1), pp. 1-7, Available at: http://dx.doi.org/10.1155/2018/4169397.

Chen, Y.F. *et al.* (2010) 'Foam properties and detergent abilities of the saponins from Camellia oleifera', *Inernational Journal of Molecular Science*, 11, pp. 4417–4425, Available at: https://doi.org/10.3390/ijms11114417

Das, S. *et al.* (2022) 'Quantitative estimation of Terpenoid content in some Tea Cultivars of North East India and Their in vitro Cell Cultures using an optimized Spectrophotometric method', *Journal of Advanced Scientific Research*, 13(03), pp. 112-117. Available at: https://doi.org/10.55218/JASR.202213318

Devi, C.B. *et al.* (2019) 'Effect of drying procedures on nutritional composition, bioactive compounds and antioxidant activity of wheatgrass (*Triticum aestivum* L)', *Journal of Food Science and Technology,* 56(1), pp. 491-496. Available at: https://doi.org/10.1007/s13197-018-3473-7

Ibukunoluwa, M.R. (2017) 'In vivo antiplasmodial activity and histopathological analysis of water and ethanol extracts of a polyherbal antimalarial recipe', *Journal of Pharmacognosy and Phytotherapy*, 9(6), pp. 87-100, Available at: http://dx.doi.org/10.5897/JPP2017.0449

Jacob, J. *et al*. (2016) 'Nutritional composition of selected wild fruits from Minna Area of Niger State, Nigeria', *International Journal of Food Science and Biotechnology*, 10(1), pp. 37–42

Jayaraj, P., Raghavan, G. and Pushpangadan P. (2013) 'The Genus *Spilanthes* Ethnopharmacology, Phytochemistry, and Pharmacological Properties', *A Review. Advances in Pharmacological Sciences*, pp. 1-23, Available at: http://dx.doi.org/10.1155/2013/510298.

Jothy, S. *et al*. (2011) 'Acute oral toxicity of methanolic seed extract of *Cassia fstula* in mice', *Molecules*, 16(6), pp. 5268–5282, Available at: http://dx.doi.org/10.3390/molecules16065268.

Kumar, S. *et al*. (2017) 'Antiplasmodial potential and quantification of aloin and aloe-emodin in Aloe vera collected from different climaticregions of India', *BMC Complementary and Alternative Medicine*, 17(1), pp. 369. Available at: https://doi.org/10.1186/s12906-017-1883-0.

Mace, K. *et al*. (2015) 'Evaluation of sulphadoxin–pyrimethamine for intermittent preventive treatment of malaria in pregnancy, a retripetive birth outcome study in Mansa', *Malaria Journal,* 14(69), pp. 1–11, Available at: http://dx.doi.org/10.1186/s12936-015-0576-8.

Madaan, R. *et al*. (2011) 'Estimation of total phenols and flavonoids in extracts of Actaea spicata roots and antioxidant activity studies', *Indian Journal of Pharmaceutical Sciences,*73(6), pp. 666. Available at: https://doi.org/10.4103/0250-474X.100242

Madara, A. *et al*. (2010) 'Anti-malarial activity of ethanolic leaf extract of *Piliostigma thonningii* Schum. (Caesalpiniaceae) in mice infected with *Plasmodium berghei*', *Afr J Biotechnology*, 9, pp. 3475–3480. Available at: http://www.academicjournals.org/AJB

Mengiste, B., Makonnen, E., and Urga, K. (2012) '*Invivo* antimalarial activity of *Dodonaea angustifolia* seed extracts against *Plasmodium berghei* in mice model', *Momona Ethiopian Journal of Science*, 4(1), pp. 47-63, Available at: https://doi.org/10.4314/mejs.v4i1.74056

Merlin, L.K.M. *et al*. (2019) 'Toxicity and Safety Implications of Herbal Medicines Used in Africa', *Intechopen Herbal Medicine*, Available at: http://dx.doi.org/10.5772/intechopen.72437.

Misganaw, D., Amare, G.G., and Mengistu, G. (2020) 'Chemo Suppressive and Curative Potential of Hypoestes forskalei against *Plasmodium berghei*: Evidence for in vivo Antimalarial Activity', *Journal of Experimental Pharmacology*, 2020(12), pp. 313–323, Available at: http://dx.doi.org/10.2147/JEP.S262026.

Ngo, T. *et al*. (2017) 'Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of *Salacia chinensis* L.', *Journal of Food Quality*, 2017(1), pp. 1-8, Available at http://dx.doi.org/10.1155/2017/9305047

Nureye, D. *et al*. (2018) '*In vivo* antimalarial activity of 80% methanol root bark extract and solvent fractions of *Gardenia ternifolia* Schumach & Thonn (Rubiaceae) against *Plasmodium berghei* infected mice', *Evidence-Based Complementary and Alternative Medicine*, 2018(1), pp. 1-10, Available at: http://dx.doi.org/10.1155/2018/9217835.

Ofeniforo, B. *et al*. (2024a) 'Assessing the Oxidative Stress Reducing Potential of Spilanthes flicaulis (Schumach & Thonn) Ethyl Acetate Sub fractions on Plasmodium berghei Infected Female Mice,' *Acta Parasitologica*, 2024(69), pp. 1990-1997, Available at: https://doi.org/10.1007/s11686-024-00925-9.

Ofeniforo, B. *et al*. (2024b) 'Phytochemical analysis and *in vivo* antimalarial activities of ethyl acetate fraction of *Spilanthes flicaulis* on mice subjected to *Plasmodium berghei*,' *Vector-Borne Zoonotic Disease*, 25(1), pp. 26-33, Available at: https://doi.org/10.1089/vbz.2024.0039.

Olanlokun, J.O., Balogun, A.A., and Olorunsogo, O.O. (2021) 'Influence of Artesunate Combinative Therapy CoAdministration with Rutin on Inflammatory Cytokines and Immunoglobulins in *Plasmodium berghei* Infected Mice', *Journal of Parasitology,* 107(4), pp. 639–647, Available at: http://dx.doi.org/10.1645/20-87.

Olorunniyi, O. (2013) 'In vivo antimalarial activity of crude aqueous bark extract of *Trichilia monadelpha* against *Plasmodium berghei berghei* (NK65) in mice,' *International Journal of Pharmaceutical and Bio Medical Science,* 2(4), pp. 2278–5221.

Opajobi, A. O. *et al*. (2022) 'Inhibition of Plasmodium berghei growth by alkaloid extract of *Phyllanthus amarus* in mice increased haem level and stabilized erythrocyte membrane', *International Journal of Health Sciences*, 6(S2), pp. 13028–13043. Available at: https://doi.org/10.53730/ijhs.v6nS2.8427

Osuntokun, O.T. and Faniyi, I.T. (2020) 'Curative, Suppressive and Prophylactic Phyto-Medicinal Therapy/Synergistic Efficacy of *Alstonia boonei*/*Capsicum frutescens* Ethanolic Extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice', *Asian Journal of Biotechnology and Genetic Engineering,* 3(2), pp. 19-34.

Oyawaluja, B.O. *et al.* (2024) 'Antioxidant Profiling, Phytochemical Investigation and Pharmacognostic Evaluation of N*ephrolepis biserrata* (SW.) Schott (Nephrolepidaceae)', *Tropical Journal of Phytochemistry and Pharmaceutical Sciences*, 3(2):208-215. Available at: http://www.doi.org/10.26538/tjpps/v3i2.8

Qaiser, A. *et al*. (2025) 'Evaluation of Hematological changes and types of anaemias in Malaria,' *JPTCP*, 32(4), pp. 466-471. Available at: https://doi.org/10.53555/hmjkew89

Senthilkumaran, P. *et al.* (2024) 'Qualitative phytochemical analysis of medicinal plants selected from Kolli hills Namakkal,' *Journal of Medicinal Plants Studies*, 12(3), pp. 171-173.

Shubhra, R. *et al*. (2019) 'Investigation of Phytoconstituents and Antioxidants activity of *Diospyros malabarica* fruit extracts', *Advances in Bioscience and Biotechnology*, 10(12), pp. 431-454, Available at: http://dx.doi.org/10.4236/abb.2019.1012031

Taherkhani, M. (2013) 'Comparison of antimalarial activity of Artemisia turanica extract with current drugs in vivo', *Journal of Vector Borne Diseases*, 50(1), pp. 51-56.

Vanaja, P. *et al.* (2025) 'Metabolite profiling, antimalarial potentials of *Schleichera oleosa* using LC-MS and GC-MS: *in vitro*, molecular docking and molecular dynamics', *Frontiers in Molecular Biosciences,* 12:1543939. Available at: https://doi.org/10.3389/fmolb.2025.1543939

Wondafrash, D.Z. (2019) 'Antimalarial Activity of *Cordia africana* (Lam.) (Boraginaceae) Leaf Extracts and Solvent Fractions in *Plasmodium berghei*-Infected Mice', *Evidence-Based Complementary and Alternative Medicine,* 2019(1), pp. 1-14, Available at: http://dx.doi.org/10.1155/2019/8324596

World Health Organization (2020) *Key facts World Malaria Report 2020*. Available at: https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2020.

Zareen, S. *et al.* (2020) 'Antiplasmodial potential of Eucalyptus oblique leaf methanolic extract against Plasmodium vivax:An in vitro study', *De Gruyter Open Chemistry*, 19, pp. 1023–1028. Available at: https://doi.org/10.1515/chem-2021-0091