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Antimalarial potentials of *Stemonocoleus micranthus* Harms (leguminoseae) stem bark in *Plasmodium berghei* infected mice

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ABSTRACT

Background: Malaria is a leading cause of death in Nigeria.
Aim: Antimalarial activity of *Stemonocoleus micranthus* stem bark was evaluated in mice with an objective to finding scientific evidence for its use as antimalarial remedy in South-east Nigeria.
Methods: Antiplasmodial activities of hydro-methanolic extract and solvent fractions (hexane, chloroform, ethyl acetate and aqueous) of *S. micranthus* stem bark against chloroquine-sensitive *Plasmodium berghei* infected mice were determined using suppressive and curative procedures. Chloroquine was used as positive control. *In vitro* models, DPPH (1, 1-diphenyl-2- picrylhydrazyl) radical scavenging, FRAP (ferric reducing antioxidant power) and TPC (total phenolic content) were used to assay antioxidant activity of the test samples. Phytoconstituents of the active fractions were analysed by GC-MS.
Results: Chemosuppressive effect exerted by extract (50, 100, 200, 400 mg kg^{-1}) and fractions (20, 40, 80 mg kg^{-1}) ranged between 54.14 − 67.73% and 59.41−94.51% respectively. Curative effects was also dose dependent. In both models, ethyl acetate was the active fraction. At low doses the animals lived longer but not protected ($D_0 - D_{29}$). At high doses, extract (400 mg kg^{-1}), active fractions (80 mg kg^{-1}) and chloroquine (5 mg kg^{-1}) the animals were fully protected.
The extract and fractions exhibited antioxidant potentials which could have contributed individually or synergistically to antimalarial activities reported in this study. Oral LD_{50} was estimated to be greater than 4000 mg kg^{-1}, in mice.
Conclusion: The results of this study may have provided support on traditional therapeutic use of the plant in treatment of malaria.

1. Introduction

Malaria is a disease of global concern. World Health Organization (WHO) estimated that about 41% of total world population lives in areas with malaria risk particularly within the less economic empowered regions.[1,2] Malaria is caused by blood parasite of *Plasmodium* species such as *Plasmodium falciparum, P. ovale, P. malariae, P. knowlesi* and *P. vivax*.[3] It is an infection associated with high morbidity and mortality rate in spite of various efforts made over the past 50 years to eliminate or/and eradicate it. This can be attributed to the ease at which the malaria parasites develop resistance to available antimalarial drugs, compromised vector control agents and high human movement.[4] It is estimated that malaria kills about 437 000 African children before their fifth birthday and is responsible for one out of every ten pregnancy oriented deaths in Africa.[5,6] Immunoprophylaxis offers a long term control option, but effective vaccines against malaria have not been developed.

Reactive oxygen species (ROS) are essential for many physiological processes and could cause oxidative stress when excessive in the body.[7,8] Atamna et al., (2014)[9] reported increase in production of $OH^{\bullet}$ radicals and H_2O_2 in erythrocytes infected with *P. falciparum* compared to normal erythrocytes. This suggested that malarial pathogenesis may be associated with free radicals formation and decrease of antioxidant level in the biological

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system.[8,10,11] Plants and compounds with antioxidant activity may ameliorate the progress or have beneficial effect in the management of malaria infection and probably prevent its sequelae.[12,13]

Stemonocoleus micranthus Harms (Leguminosae- Caesalpinioideae) is a large forest tree (up to 45 m high) found along West – Central Africa coast. It is known as "nre" in South-east of Nigeria.[14] The stem bark of *S. micranthus* (SM) is used in ethnomedicine to manage various fertility problems in women, as remedy for contaminated wounds, pains, rheumatoid arthritis and joint pains.[15] The analgesic, antibacterial, anti-ulcer and local anesthetic properties of the stem bark extract have been reported.[16,17] However documented information on the pharmacological and traditional use of the plant is limited.

This study was aimed at evaluating the antiplasmodial activity of 80% methanol stem bark crude extract and solvent fractions of *S. micranthus* on *Plasmodium berghei berghei* infected mice. Also included in the study was antioxidant capacity of the plant using various quantitative models. Phytochemical profiles of the active solvent fractions were determined using GC-MC (Gas chromatography - Mass spectrometry) analysis. Extractable secondary metabolites of the plant in different extraction solvent systems were investigated using the conventional laboratory phytochemical procedures.

2. Materials and methods

2.1. Reagents and chemicals

DPPH (1, 1-diphenyl-2- picrylhydrazyl), ferric chloride, potassium ferricyanide, trichloroacetic acid, chloroquine phosphate were obtained from Sigma Aldrich (Germany). Solvents used were of analytical grade. DMSO (Dimethyl sulphoxide) was obtained from Qualikems laboratory reagents, India.

2.2. Plant collection and identification

Stem bark of SM was collected at Eha-Alumona village, Nsukka, Enugu state, Nigeria in February 2016. The plant was identified and authenticated by a taxonomist, Mr. Ozioko, of International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu state. The herbarium specimen was prepared and deposited with voucher number InterCEDD/877.

2.3. Plant extraction

The stem bark was air dried, reduced into coarse powder (1 kg) using a grinding mill (Hamburg 76 West Germany) and extracted exhaustively with 80% methanol by Soxhlet apparatus (2000 mL) for 70 h. The filtrate was concentrated under vacuum using a Buchi rotary evaporator (Buchi Vacuum module V-801 Easy Vac) at 45 rpm and 40 °C to obtain the crude extract. The dried extract (156.36 g, crude extract) was stored in a refrigerator at 4 °C in air tight plastic container until used.

Extraction of smaller quantities (3 g), using cold maceration process was done using 10 mL of different solvents (hexane, chloroform and ethyl acetate) with the sole aim to compare the secondary metabolites extractable by different solvents.

2.4. Solvent fractionation

Crude 80% methanol extract (40 g) was subjected to solvent-solvent fractionation using different solvents (3 × 300 mL) according to polarity gradient resulting in n-hexane (FH), chloroform (FC), ethyl acetate (FE) and aqueous (FA) fractions, respectively.

2.5. Parasites

Chloroquine sensitive, rodent parasite *Plasmodium berghei berghei* (NK - 65), was obtained from National Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria. The *Plasmodium* was maintained in University Animal House Laboratory by serial passage of the parasite from donor mice to uninfected mice. A standard inoculum of 1×10^7 of parasitized erythrocytes from a donor mice (0.2 mL) was used to infect the experimental animals intraperitoneally.

2.6. Animal handling

The animals used in this study were Swiss albino mice of both sexes, weighing between 20 and 24 g. They were procured from the Laboratory Animals Center, College of Medicine, University of Lagos. The animals were accommodated in clean and well plastic ventilated cages. The animals were allowed to acclimatize for 7 days preceding the experiments. The ethical guidelines for handling of laboratory animals were followed in the study. Ethical protocol was approved and issued by the Health Research Ethics committee of College of Medicine of the University of Lagos in line with the international accepted principles for laboratory animal use and care.

2.7. Phytochemical screening

Phytochemical screenings were carried out on the four extracts to detect the presence of secondary metabolites using standard phytochemical methods as described by Sofowora 1993.[18]

2.8. Acute toxicity

A modified method as described by Lorke, (1983)[19] was used to test for toxicity of the extract. The mice were starved for 24 h prior to drug administration. They were divided into five groups of five mice each. The first four groups received a single doses of 500, 1000, 2000 and 4000 mg kg^{-1} of crude extract while the untreated group (negative group) was given the vehicle only (5% DMSO). The animals were given food and water 4 h post drug administration and observed closely for signs of distress or death. The observation was continued for up to 7 days.[20]

2.9. Antimalarial assays

2.9.1. Suppressive test

The four day suppressive test described by Peters (1965)[21] was employed for antiplasmodial activity evaluation of crude extract of SM stem bark against *P. berghei*. Animals were infected intraperitoneally with blood containing 1×10^7 parasitized red blood cells contained in 0.2 mL inoculum on the first day (D_0). They were randomly divided into 6 groups of 5 mice each. Two hours post infection, 4 test groups were administered orally with SM crude extract at four dose levels (50, 100, 200 and 400 mg kg^{-1}), 1 test group received the reference drug (chloroquine 5 mg kg^{-1}), while another group received 0.2 mL of the vehicle, 5% DMSO (negative control). Drug administration was repeated 24, 48, and 72 h post infection (D_1 to D_3). A uniform drug volume of 0.2 mL was used for all the animals. On D_4 (day 5 post treatment), tail blood smears were prepared, stained with 10% Giemsa in phosphate buffer, pH 7.2 for 15 min and examined under microscope at 100 x. The percentage (%) suppression of parasitaemia was calculated by comparing the parasitaemia present in infected controls with those of test mice.

The above 4-day suppressive test was repeated for each of the

fractions (FH, FC, FE and FA) at 3 dose levels of 20, 40 and 80 mg kg^{-1}. A total of 100 mice were used for this 4-day suppressive antimalarial test for both SM crude extract and the four fractions.

2.9.2. Curative test

Evaluation of the curative potential of SM stem bark extract against established infection was carried out as described by Ryley and Peters, (1970)[22] and modified by Iyiola et al., 2011.[23] The animals were infected intraperitoneally with standard inoculum of 1×10^7 *Plasmodium berghei* NK - 65 infected erythrocytes on the first day (D_0) and left for 72 h before commencement of treatment (D_3). The mice were divided into 6 groups of 5 mice each. Four test groups were administered with the crude extract orally at four dose levels of 50, 100, 200 and 400 mg kg^{-1} daily for 5 days ($D_3 - D_7$). Positive control group received chloroquine 5 mg kg^{-1} daily while the negative control group received 0.2 mL of 5% DMSO. Blood smears were collected and examined microscopically on D_3 to establish parasitaemia level. Then parasitaemia level in each mouse was monitored daily for the remaining 4 dosing days ($D_4 - D_7$) and D_8 (24 h after last drug administration) using Geimsa stained thin smear film.

The above procedure was repeated for the antimalarial test for FH, FC, FE and FA fractions. Each fraction was tested at 3 dose levels of 20, 40 and 80 mg kg^{-1}. A total of 100 mice were used for this test.

Thereafter, the animals were fed ad libitum and observed till day 30 ($D_0 - D_{29}$) post inoculation of parasite for death. Any death that occurred during this period was noted and used to determine the survival rate and mean survival time during this period.

Survival rate is the number of death that occurred in each group between D_0 and D_{29}. Mean survival time (MST) for each group was determined by calculating the average survival time (days) of mice from date of infection over a period of 30 days ($D_0 - D_{29}$).

$$\text{MST} = \frac{\text{Sum of survival time of all mice in each group (days)}}{\text{Total number of mice in that group}}$$

2.10. Antioxidant assay

2.10.1. Determination of total phenolic content

Total phenolic content was measured by using the Folin-Ciocalteu reagent according to the method described by McDonald et al., 2001, with some modifications.[24] Gallic acid was used as standard. Various diluted concentrations of ethanolic gallic acid solutions (1 mL) were mixed with freshly diluted 10-fold Folin-Ciocalteu reagent (5.0 mL) and 4 mL (75 g L^{-1}) sodium carbonate. The reaction mixture was kept in the dark for 1 h with intermittent shacking, absorbance was read at 610 nm and gallic calibration curve drawn. Ethanolic test extract (100 mg/mL, 1 mL) and blank solution (negative control) were done following the same method. All determinations were performed in triplicates ($n = 3$). The total phenolic content was calculated and expressed as milligrams of gallic acid equivalent (GAE) per gram of the plant extract.

2.10.2. Scavenging activity on DPPH radicals

DPPH (1, 1-diphenyl-2- picrylhydrazyl) radical scavenging activity was measured according to Mensor et al. 2001.[25] Various concentrations of SM crude extract (0.05–1.00 mg/mL) in methanol were prepared. To 1 mL of each solution, 3 mL of methanol and DPPH solution (1 mL, 1 mM) was added to make up to 5 mL. The resulting solution was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The experiment was repeated for all the various concentrations of the extract, ascorbic acid (positive control) and a blank solution (without the extract) as the negative control. All determinations were carried out in triplicates.

The above *in vitro* assay was repeated for the various fractions of crude extract of SM.

The decrease in absorbance was converted to percentage scavenging activity (%SA) using the formula:

$$\text{DPPH radical scavenging activities percentage (\%SA)} = [(A_b\text{-}A_s)/A_b] / x100$$

Where:

A_b = Absorbance of the blank solution
A_s = Absorbance of the test (extract) solution or the standard (ascorbic acid)

2.10.3. Ferric reducing antioxidant power ability (FRAP)

The reducing power was assessed referring to the method described in literature by Yen and Chen (1995) with slight modifications.[26] Various concentrations of SM extract, (0.1–2.0 mg/mL) in methanol were prepared. Extract (1 mL) was mixed with sodium phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%, w/v) and the mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (2.5 mL, 10%, w/v) was added, and the mixture was centrifuged at 3000 rpm for 10 min. Upper layer fraction (2.5 mL) was mixed with deionized water (2.5 mL) and freshly prepared ferric chloride (0.5 mL, 0.1%, w/v) and thoroughly mixed. Absorbance was measured at 700 nm. A blank was prepared without adding extract while ascorbic acid was used as positive control. A higher absorbance indicates a higher reducing power. Increase in absorbance of the reaction mixture (test extract and standard) compared with that of the blank indicates increased reducing power.

2.11. Gas chromatography–mass spectrometry (GC–MS) profile of S. micranthus fractions

The qualitative and quantitative analysis of the constituents of solvent – solvent fractions (FH, FE and FA) from *S. micranthus* stem bark extract (SM) were analysed on Agilent Technologies 7890 USA, equipped with MS detector 5975 Agilent Technologies and HP5 MS capillary column (30.00 m × 0.320 mm inner diameter × 0.25 μm film thickness). The injection, mass transfer line and ion source were set at 250 °C. The oven temperature was programmed from 80 °C, held for 2 min at a rate of 10 °C/min to 240 °C and held for 6 min. Helium was used as carrier gas with a constant flow rate of 2 mL/min. The injected volume of test sample was 1 μL with a splitless mode. The volatile components of the test fractions were identified on the basis of GC retention time on fused silica capillary column, by comparison of their mass fragmentation pattern with literature reports and by computer matching with standard spectra (NIST 2014 version 2.1.0).

2.12. Statistical analysis

All experiments results were evaluated by analysis of variance (ANOVA), followed by Dunnett's (post-hoc test) which was used to determine statistical significance of means and $p < 0.05$ was regarded as statistically significant.

Table 1
Phytochemical constituents of various solvent crude extracts of *S. micranthus* (SM) stem bark.

Test	Hexane	Chloroform	Ethyl acetate	80% MeOH
Saponin	−	−	−	+
Alkaloid	−	−	−	+
Tannin	+	+	+	+
Phenol	−	+	+	+
Flavonoid	−	−	+	+
Terpenoid	−	−	+	+
Steroid glycoside	+	+	+	+
Cardiac glycosides	+	+	+	+
Anthraquinone	+	+	+	+
Reducing sugar	+	+	+	+

KEY: Absence + Presence.

3. Results

3.1. Qualitative phytochemical analysis of the extracts

Results of the phytochemical analysis (Table 1) showed the variation in the constituents extracted by various solvent extractions. Hydro-alcoholic solvent (80% MeOH) extracted more phyto-constituents, followed by ethyl acetate, then chloroform and hexane.

3.2. Fractionation yield

Solvent − solvent fractionation of 40 g of crude extract of SM yielded 3.03, 21.75, 46.65, 28.56% of hexane fraction (FH), chloroform fraction (FC), ethyl acetate fractions (FE) and aqueous fraction (FA) respectively.

3.3. Acute toxicity study

There was no mortality at all dose levels tested within the first 24 h and thereafter 7 days. No adverse clinical symptoms was observed. The median lethal dose LD_{50} was estimated to be $\geq 4.00\ g\ kg^{-1}$ body weight.

3.4. The suppressive activity of extract/fractions of S. micranthus

The results of the study showed that crude extract of SM displayed significant ($p < 0.05$) chemosuppressive activity against *P. berghei* compared to vehicle treated group (Table 2). The extract caused chemosuppression of 55.14, 69.50, 66.40 and 67.73% for dose levels of 50, 100, 200 and 400 mg kg^{-1} respectively, when compared to the control. Doses above 100 mg kg^{-1} failed to elicit significant higher response. The standard drug, chloroquine caused chemosuppression of 93.09%, which was higher than those of the extract treated groups (Table 2).

All the fractions (20, 40 and 80 mg kg^{-1}) exhibited dose-dependent chemosuppression of the parasite compared to the negative control (Table 3). However, 40 and 80 mg kg^{-1} of FC and FE were significantly ($p < 0.05$) better chemo-suppressants compared to others and comparable to the standard antimalarial agent used.

Table 2
Suppressive activity of stem bark extract of *S. micranthus* against *P. berghei* infection in mice.

Drug	Conc. mg kg-1	% Parasitaemia	% Chemosuppression
5% DMSO	0.2 mL	11.26 ± 0.39	–
Chloroquine	5	0.78 ± 0.02*	93.09
SM	50	5.06 ± 0.3*	55.14
SM	100	3.44 ± 0.26*	69.50
SM	200	3.79 ± 0.18*	66.40
SM	400	3.64 ± 1.02*	67.73

Values are expressed as mean ± SEM; n = 5.
Significant at $^*p < 0.05$ with reference to vehicle treated mice (negative control).

Table 3
Suppressive activity of fractions of *S. micranthus* against *P. berghei* infection in mice.

Drug	Dose Mg kg^{-1}	% Parasitaemia	% Chemosuppression
5% DMSO	0.2 mL	10.2 ± 0.39	0
Chloroquine	5	0.74 ± 0.02*	94.75
FH	20	4.14 ± 0.13*	59.41
	40	3.48 ± 0.11*	65.88
	80	3.26 ± 0.12*	68.04
FC	20	2.94 ± 0.14*	71.18
	40	2.05 ± 0.15*	79.9
	80	1.78 ± 0.09*	82.54
FE	20	2.17 ± 0.07*	78.73
	40	1.37 ± 0.06*	86.57
	80	0.56 ± 0.05*	94.51
FA	20	3.14 ± 0.18*	69.22
	40	2.84 ± 0.12*	72.16
	80	2.14 ± 0.05*	79.10

Values are expressed as mean ± SEM; n = 5.
Significant at $^*p < 0.05$ with reference to vehicle treated mice (negative control).

3.5. Curative effect of extract/fractions of S. micranthus

The result of the effect of crude extract of SM stem bark on established parasite infection showed reduction in parasite load on a daily progress format and dose-dependently, comparable to the vehicle treated group. Parasitaemia clearance was noted on D_4 (24 h post first dose of drug) at all dose levels of SM crude extract and chloroquine. Thus on D_8 (24 h after the last dose was administered) there was 74.43, 90.52, 91.04 and 92.02% parasitaemia clearance in 50, 100, 200 and 400 mg kg^{-1} SM extract treated groups respectively (Table 4). Similar parasitaemia reduction was observed for chloroquine, where reduction was also observed on D_4, (18.89%) and on D_8 total clearance was recorded (100%). The animals treated with extract 200 and 400 mg kg^{-1} were fully protected (no death occurred) 30 days (D_{29}) post drug treatment (Table 6). Untreated negative control group that showed daily increase in parasitaemia, death occurred in this group between day 5 and 9 post treatment. Chloroquine (CQ) treated group displayed a mean survival time of 27.01 ± 2.31 days (Table 6).

There was dose-dependent decrease in parasite load on D_8 in the 4 different fractions compared to the negative control group, indicating curative therapeutic potentials of the fractions (Table 5). Chloroform (FC) and FE percentage of cure at 40 and 80 mg kg^{-1} on D_8 were comparable to that of CQ, the reference drug. Malaria mortality was not reduced in FH and FA fractions and mean time of survival increased in relation to non-treated group (Table 6). Both survival parameters (reduced mortality and mean time of survival) improved significantly ($P < 0.05$) in the two active fractions (FC and FE). The most active fraction, FE at 40 and 80 mg kg^{-1} exhibited 94.08 and 95.95% parasitaemia clearance; improved mean time of survival of 27.99 and 30 days respectively compared to the negative control (Tables 5 and 6). Only 1 animal death was observed on day 23 (D_{22}) for ethyl acetate fraction 40 mg kg^{-1}. Chloroquine (CQ) treated group displayed a mean survival time of 28 ± 0.02 days (Table 6).

3.6. Scavenging ability on DPPH radicals

The percent inhibition of DPPH radicals (scavenging effect) of SM stem bark test extract and standard antioxidant compound (Vit C) at different concentrations. Both indicated good antioxidant activities with strong DPPH radical scavenging activity. The IC_{50}

Table 4
Curative effect of stem bark extract of *S. micranthus* against *P. berghei* infection in mice.

Drug	Dose mg kg $^{-1}$	Parasitaemia	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
5% DMSO	0.2 mL	% Parasitaemia	13.18 ± 0.07	15.35 ± 0.24	18.30 ± 0.04	20.47 ± 0.51	21.31 ± 0.11
		% Cure	0	0	0	0	0
SM (Crude extract)	50	% Parasitaemia	12.11 ± 0.25	9.37 ± 0.20	8.58 ± 0.36	7.67 ± 0.41	5.45 ± 0.27
		% Cure	8.12	38.96	53.11	62.53*	74.43*
	100	% Parasitaemia	12.19 ± 1.03	8.55 ± 2.01	5.86 ± 0.62	3.71 ± 1.07	2.02 ± 0.38
		% Cure	7.51	44.30	67.98	81.88*	90.52*
	200	% Parasitaemia	12.41 ± 1.24	9.05 ± 0.10	6.09 ± 0.41	3.73 ± 0.16	1.91 ± 0.02
		% Cure	5.84	41.04	66.72	81.78*	91.04*
	400	% Parasitaemia	12.36 ± 0.03	7.89 ± 0.14	5.31 ± 1.02	3.54 ± 0.05	1.70 ± 0.23
		% Cure	6.22	48.60	70.98	82.71*	92.02*
CQ	5	% Parasitaemia	10.69 ± 0.06	4.38 ± 1.30	1.39 ± 1.24	0.00 ± 0.00	0.00 ± 0.00
		% Cure	18.89	71.47	92.40	100.00*	100.00*

Values are expressed as mean ± SEM; n = 5.
Results are expressed as the percent clearance of parasitaemia with reference to non-treated mice, 5% DMSO (negative control).
Significant at $P < 0.05$ with reference to vehicle treated mice (negative control).

Table 5
Curative activity of fractions from hydro-methanolic stem bark extract of *S. micranthus* against *P. berghei* infection in mice.

Drug	Dose mg kg $^{-1}$	Parasitaemia	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
5% DMSO	0.2 mL	% Parasitaemia	10.44 ± 0.18	14.71 ± 0.44	18.05 ± 0.83	20.47 ± 0.21	22.21 ± 0.46
		% Cure	0	0	0	0	0
FH (Hexane fraction)	20	% Parasitaemia	10.58 ± 0.36	8.75 ± 0.10	7.94 ± 0.10	6.93 ± 0.22	6.28 ± 0.21
		% Cure	−1.34	40.51	56.01	66.15*	72.06*
	40	% Parasitaemia	9.93 ± 0.38	8.57 ± 0.12	7.43 ± 0.05	6.64 ± 0.12	5.68 ± 0.20
		% Cure	4.89	41.74	58.84	67.56*	74.73*
	80	% Parasitaemia	11.28 ± 0.57	9.72 ± 0.02	7.6 ± 0.16	6.3 ± 0.15	4.67 ± 0.09
		% Cure	−8.05	33.92	57.89	69.22*	79.23*
FC (Chloroform fraction)	20	% Parasitaemia	11.28 ± 0.00	8.16 ± 0.40	6.60 ± 0.24	4.8 ± 0.27	3.004 ± 0.22
		% Cure	−8.05	44.53	63.45	76.55*	86.48*
	40	% Parasitaemia	10.3 ± 0.13	8.07 ± 0.12	6.36 ± 0.08	4.53 ± 0.16	2.86 ± 0.14
		% Cure	1.34	45.14	64.76	77.87*	87.28*
	80	% Parasitaemia	9.99 ± 0.49	8.15 ± 0.17	5.84 ± 0.11	3.83 ± 0.03	1.89 ± 0.15
		% Cure	4.31	44.6	67.65	81.28*	91.59*
FE (Ethyl acetate fraction)	20	% Parasitaemia	10.9 ± 0.11	9.11 ± 0.24	7.59 ± 0.18	5.46 ± 0.10	3.28 ± 0.23
		% Cure	−4.41	38.07	57.89	81.39*	85.41*
	40	% Parasitaemia	9.76 ± 0.32	7.72 ± 0.18	5.7 ± 0.25	3.48 ± 0.20	1.33 ± 0.15
		% Cure	6.51	47.52	68.42	83.00*	94.08*
	80	% Parasitaemia	9.83 ± 0.18	7.55 ± 0.18	5.49 ± 0.23	3.14 ± 0.14	0.91 ± 0.05
		% Cure	5.75	48.67	69.58	84.66*	95.95*
FA (Aqueous fraction)	20	% Parasitaemia	9.69 ± 0.29	8.41 ± 0.45	8.24 ± 0.27	7.25 ± 0.36	6.32 ± 0.46
		% Cure	7.18	42.83	54.35	64.58*	71.89*
	40	% Parasitaemia	10.04 ± 0.25	9.7 ± 0.17	9.06 ± 0.08	7.01 ± 0.23	5.34 ± 0.21
		% Cure	3.83	34.06	49.81	65.75*	76.25*
	80	% Parasitaemia	9.75 ± 0.07	8.42 ± 0.34	7.26 ± 0.27	5.09 ± 0.26	3.28 ± 0.18
		% Cure	6.61	42.76	59.78	75.13*	85.41*
CQ	5	% Parasitaemia	9.03 ± 0.13	3.92± 1.04	1.32 ± 0.6	0.00 ± 0.00	0.00 ± 0.00
		% Cure	13.51	73.35	92.67	100.00*	100*

Values are expressed as mean ± SEM; n = 5.
Significant at $P < 0.05$ with reference to vehicle treated mice (negative control).

values of crude extract of SM and Vit. C were calculated to be 4.49 μg mL^{-1} and 3.08 μg mL^{-1}, respectively (Fig. 1 A). The FC, FE and FA were checked for scavenging activity, while the FH was devoid of enough yield for evaluation of its activity (the limited yield was used for antimalarial potential evaluation).

3.7. Ferric reducing antioxidant power ability (FRAP)

The reducing power of crude extract of SM is dose dependent and indicated that SM is an effective antioxidant (Fig. 1C). The reducing power effect of SM extract and Vit. C increased with concentration in a strong linear manner ($R^2 = 0.9889$ and 0.9809 respectively). The IC_{50} values for reducing power of SM extract and Vit. C were calculated to be 419.66 and 396.66 μg/mL respectively.

3.8. GC-MS identification of chemical fingerprint of S. micranthus fractions: FC, FE, FA

The GC-MS spectra of FC, FE and FA of *S. micranthus* showed 8, 21 and 12 peaks respectively indicating different phytochemical compounds. The results revealed the presence of compounds such as hexadecanoic acid and methyl stearate that are common among the three fractions.

This study is only a preliminary study on the phytoconstituents composition of SM stem bark extract, an in-depth study will provide a more revealing result.

Table 6
Survival rate and Mean Survival Time (MST) of each group on Day 30 (D_0-D_{29}) during curative test.

Drug	Dose mg Kg $^{-}$	Survival rate	Survival (%) on day 30	Mean Survival Time (MST) of each group in days
5% DMSO	0.2 mL	0/5	0	5.67 ± 1.74
SM crude extract	50	0/5	0	17.20 ± 0.80*
	100	3/5	60	24.67 ± 1.80*
	200	5/5	100	30.00 ± 0.00*
	400	5/5	100	30.00 ± 0.00*
CQ[a]	5	4/5	80	27.01 ± 2.31*
FH	20	0/5	0	15.67 ± 0.87*
	40	0/5	0	16.67 ± 0.80*
	80	0/5	0	17.60 ± 0.24*
FC	20	0/5	0	20.01 ± 0.20*
	40	3/5	60	23.00 ± 0.94*
	80	5/5	100	30.00 ± 0.00*
FE	20	0/5	0	21.67 ± 0.53*
	40	4/5	80	27.99 ± 2.00*
	80	5/5	100	30.00 ± 0.00*
FA	20	0/5	0	14.67 ± 0.33*
	40	0/5	0	15.53 ± 0.88*
	80	0/5	0	17.80 ± 0.20*
CQ[b]	5	4/5	80	28.00 ± 1.02*

Values are expressed as mean ± SEM; n = 5.
Significant at $P < 0.05$ with reference to vehicle treated mice (negative control).
CQ[a]: Chloroquine, reference used for SM crude extract.
CQ[b]: Chloroquine, reference used for the fractions.

4. Discussion

Phytoconstituents are chemicals produced by plants that protect them from the environment and diseases. They have been recognized as having healing and protective values against humans diseases.[27] Phytochemical analysis of stem bark of SM from various solvent extractions demonstrated that the choice of solvent of extraction plays a determinant role in the active compounds extracted. Result from this study showed an increase in the number of secondary metabolites extracted as the polarity of the solvent of extraction increased. Anti-malarial herbal decoction of the plant under study (SM) is prepared locally mainly by boiling in water or soaking in dilute local alcohol which are polar solvents. Phytochemical analysis showed that phytoconstituents extracted with hydro-alcoholic solvent in this study is comparable to the report of Mbaoji et al., 2016 research team that extracted with dichloromethane–methanol (1:1).[15]

The GC-MS spectra (Fig. 2) showed high concentration of fatty acids. Unsaturated fatty acids are not produced in humans, but have been shown to possess diverse pharmacological properties and aid in general functioning of the human body. Kumaratilake and collaborators reported on antimalarial properties of polyunsaturated fatty acids, especially the essential fatty acids and their bio-activity increases with their degree of unsaturation.[28,29] Polyunsaturated fatty acids such as methyl ester, 9,12-octadecadienoic acid methyl ester (linoleic acid) and 9-Octadecenoic acid (Z)-, methyl ester (Oleic acid) were among the identified compounds in the active antiplasmodial fractions of SM stem bark extract.[29–31]

Two *in vivo* models commonly used in antimalarial screening are, 4 day suppressive test which determines the effect of a drug on early infection and Rane's test, which evaluates the curative capability of the drug on established infection.[2,13] The two animal models were used for this study because they take into consideration the possible prodrug effect and possible involvement of various bio-defense systems in eradication of infection and elimination of drugs.[2] The results obtained from the study showed significant ($P < 0.05$) percentage inhibition of parasitaemia in *P. berghei* infected mice treated with 80% methanol crude extract and fractions of *S. micranthus* (SM) stem bark in both models. Hiben et al. 2016, reported antiplasmodial activity of hydroalcoholic (70% ethanol) extract of *Senna singueana* which is in line with observed antimalarial activity of hydro-methanolic extract of SM in this study.[13] The significant ($P < 0.05$) chemosuppression observed between 50 and 100 mg kg^{-1} of the crude extract increased with dose while the effect between 100 and 400 mg kg^{-1} was not significant which may be indicating that at 100 mg kg^{-1} the peak therapeutic effective concentration may have been achieved in the body system. The four fractions all exhibited chemosuppression effect at dose dependent format as showed in Table 3. However, FC and FE were found to possess higher blood schizontocidal effect than the extreme non-polar and polar fractions (FH and FA). This suggests the possible localization of the active phytochemicals in these fractions. Semi purification of SM stem bark crude extract improved its early stage antiplasmodial activity as shown in the active fractions (Table 3).

The curative test of SM stem bark crude extract, showed an early onset of parasite clearance (D_4) for all the tested dose levels, which progressed daily till D_8 (Table 4). The early onset of parasite reduction suggest possible use of extract as drug of emergency and loading dose may not be needed. This characteristic is not exhibited by all antimalarial plant extract as demonstrated with *Cronton macrostachyus* in which parasite clearance started after 2 doses.[2] The percentage parasitaemia clearance obtained 24 h post last drug administration (D_8) in the various dose levels of crude extract/fractions indicated possible prolonged (long acting) bio-active antimalarial activities. Extending duration of dosing/drug administration to 6–7 days may give the desired 100% parasite clearance in curative model. Long acting effect may be indicating that SM could be a good candidate in combination therapy with another antimalarial agent with fast but short duration of activity. The observed higher curative efficacy of the stem bark crude extract of SM treated groups (74.43–92.04%) than its suppressive activity (Table 2) may in part be due to its selectivity of activity (established infection). The animals were fully protected at 200 and 400 mg kg^{-1} of the crude extract but at lower doses, the days of survival were only prolonged compared to the untreated group.[29] Death at lower doses instead of higher doses likely indicated that death occurred due to malaria infection and not toxicity. Occurrence of mortality after a prolonged survival could mean recrudescence of *P. berghei* after apparent cure. Similar result on

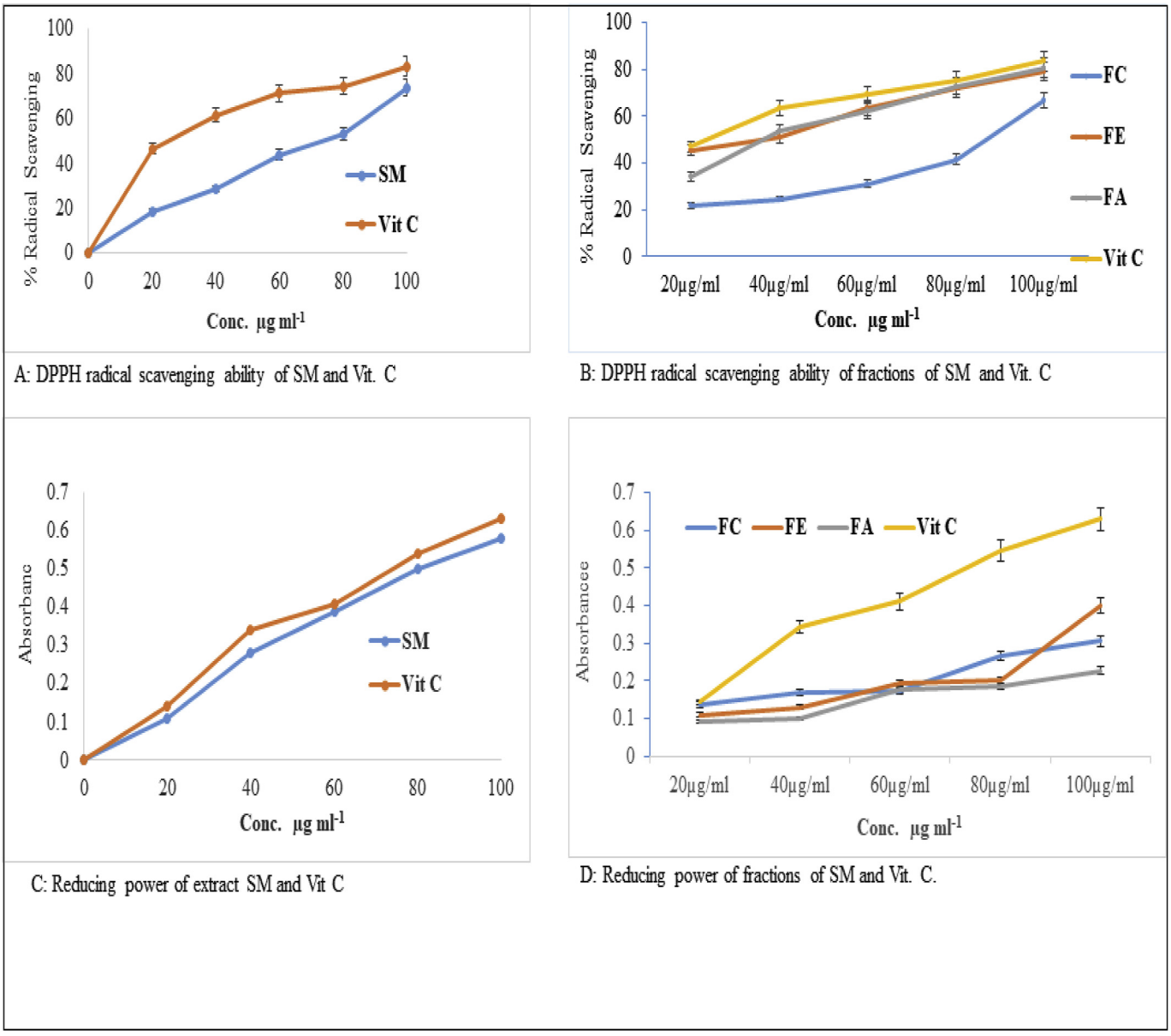

Fig. 1. Antioxidant profile of crude extract and fractions of crude hydro-methanolic extract of. *S. micranthus* compared with a standard drug Vit C. Data are means ± standard deviation of triplicate experiments. FC: Chloroform fraction of crude hydro-methanolic extract FE: Ethyl acetate fraction of crude hydro-methanolic extract FA: Aqueous fraction of crude hydro-methanolic extract.

prolonged MST but not fully protected was also reported of *Strychnos mitis* leaf extract.[32]

Onset of curative activity was delayed in 20 mg kg^{-1} dose of most of the fractions indicating the need of higher dose for therapeutic purpose. Comparing the curative results of these active fractions with their suppression activity indicated that both fractions have better effect on established infection as observed in the crude extract activity profile. This indicates that the herbal treatment has a stronger effect as curative than plasmodial growth suppressant and may be less effective as prophylaxis.

Active fractions, FC and FE in curative assay offered partial to full protection to the animals till D_{29} (Table 6) depending on dose. The most active fraction, FE is a good candidate for future therapeutic evaluation and possibly isolation procedure considering its high fractionation percentage yield (46.65%), relatively good antimalarial property (early and established phases), increased MST and increased survival rate.

The absence of death at 4000 mg kg^{-1} in toxicity test probably indicated that LD_{50} of SM is greater than 4000 mg kg^{-1} when given orally. However toxicological studies on the crude extract will give a stronger evidence of this observation.

Plants are reservoir of novel antioxidant molecules especially those that have a high concentration of phenolic compounds.[33,34] The results of this study indicated that the stem bark of SM possesses antioxidant activity that is comparable to that of ascorbic acid (Vit. C). Researchers studying the possible effects of antioxidant in malarial management have suggested antioxidants may exhibit mopping of free radicals (Fig. 1 A and B), defend against singlet and triplet oxygen damage (Fig. 1 D), or decomposition of peroxides that are initiators of various disease states.[35,36] Thus the antioxidant effect of crude extract/ethyl acetate fraction of SM during malarial chemo-therapy could play protective role,

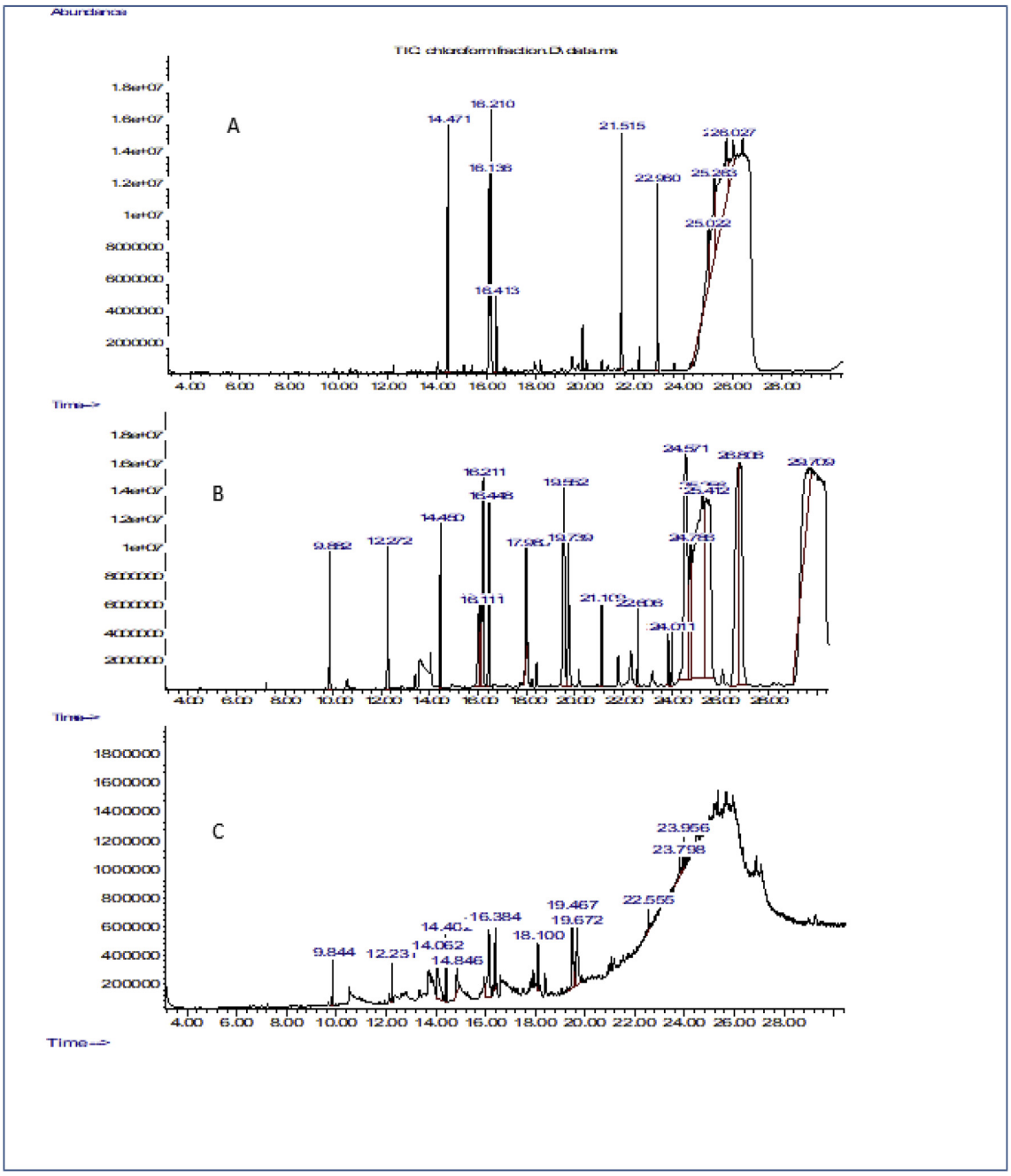

Fig. 2. GC-MS chromatograms of fraction of *S. micranthus* showing the retention time (min) of the compounds in X-axis and percentage (%) of peak area in the Y-axis. A: Chloroform fraction (FC); B: Ethyl acetate fraction (FE); C: Aqueous fraction (FA).

minimize malaria associated systemic complications by minimizing the damaging effects of free radicals associated with malarial fever and possibly potentiate drug efficacy and fast healing of the body.[37,38]

5. Conclusion

Evidence based on these findings suggest that *S. micranthus* possesses potent anti-plasmodial effect against *P. berghei* infection in mice, thereby supporting its folkloric usage in the management of malaria. The results also indicated that SM possesses antioxidant potential which has the ability to decrease the free radical formation and possibly prevent the development of malaria related complications. To the best of our knowledge, this is the first time antimalarial activity is being reported on *S. micranthus.*

Conflict of interests

The authors declare no conflict of interests.

Ethical approval

All authors hereby declare that “principles of laboratory animal care” (NIH publication no. 8523, revised 1985) were followed, as well as specific national laws where applicable. All experiments were examined and approved by the Health Research Ethics

committee of College of Medicine of the University of Lagos, protocol ID number CMUL/HREC/05/17/129.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2019.03.001.

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