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Antiplasmodial and antipyretic effects of ethanol root extract and fractions of *Setaria megaphylla* (Steud) T. Dur and Schinz (Poaceae)

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ABSTRACT

Objective: To evaluate various antiplasmodial and anti-inflammatory activities of *Setaria megaphylla* to authenticate its potential in traditional medicine.
Methods: The crude ethanol extract was divided into two portions. Partitioning of one portion into *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol fractions was carried out. The suppressive, prophylactic and curative effects of 150, 300, 450 mg/kg of the remaining extract portion were evaluated, while suppressive and curative activities were evaluated at 300 mg/kg of each of the above mentioned fractions. Artesunate and pyrimethamine at doses of 5 mg/kg and 1.2 mg/kg respectively were the standard drugs. Gas chromatography mass spectroscopy (GC-MS) was used to perform a qualitative analysis of dichloromethane and *n*-butanol fractions which had the highest antiplasmodial activity. Antipyretic effect of *Setaria megaphylla* extract was investigated using the D-amphetamine, 2,4-dinitrophenol and yeast-induced pyrexia models with ASA (100 mg/kg) as standard drug.
Results: The results on the antiplasmodial and antipyretic activities revealed significant ($P < 0.05$–0.001) and dose-dependent effects when compared to control. It was observed that the antipyretic activity was quite comparable to the standard drug while the antiplasmodial activity was not. It was also seen that a greater proportion of the components in the fractions subjected to GC-MS were monoterpenes and sesquiterpenes.
Conclusions: This research work on *S. megaphylla* extract and fractions revealed the presence of phytochemical constituents that also accounted for its observed antiplasmodial and antipyretic effects. This result justifies its usage by the Ibibio Tribe in traditional medicine to treat malaria, pains and diverse fevers.

1. Introduction

Interest towards scientific investigation of plants used in traditional medicine to treat diseases such as malaria, fevers and pain has been rekindled and renewed with tremendous vigour. The Ibibio people in Southern Nigeria have for ages been using different plants and their parts to treat malaria and other fevers even in the face of available conventional drugs. The use of *Setaria megaphylla* (*S. megaphylla*) has been done without much documentation[1]. *S. megaphylla* is an erect perennial broad-leafed bristle grass, cane-like with very coarse and robust roots mostly found along rivers, areas with plenty of moisture and in tropical and subtropical Africa with high rainfall[2], *e.g.* Nigeria. In Southern Nigeria, Ibibio Tribe in Akwa Ibom State used it for treatment of malaria, pain, fevers and diabetes[1]. The *in vitro*[3] and *in vivo*[1] researches have shown that *S. megaphylla* leaves have antiplasmodial effects. The results of phytochemical screening revealed the presence of flavonoids, deoxy-sugars, terpenes, saponins, tannins, anthraquinones and cardiac glycosides in the root extract[1]. Data from GC-MS studies of *n*-hexane fraction of the leaves showed that it contained components such as 8,11,14-eicosatrienoic acid (Z,Z,Z), phthalic acid, diisooctyl ester, vitamin E, γ-elemene, Urs-12-ene, bicyclogermacrene, α-muurolene, germacrene-A, and guaiol among others[4].

The cytotoxic, immunomodulatory and antileishmanial activities of the ethanol leaf extract of *S. megaphylla* have been reported[4]. There is little available information about the activities of *S. megaphylla* roots. Therefore, the present research aimed to evaluate and provide information on the medicinal potentials of *S.*

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megaphylla root extract and fractions. Evaluation of antiplasmodial and antipyretic activities, as well as the GC-MS profile were carried out to reveal its active components and medicinal potentials.

2. Materials and methods

2.1. Plant materials

S. megaphylla roots taken from Anwa Forest in Uruan, Akwa Ibom State, Nigeria were identified, authenticated and assigned with number of FPHUU 221 by Dr. Margaret Bassey, a plant taxonomist in University of Uyo. A sample was deposited in the Herbarium.

2.2. Extraction

The roots were washed, cut into small pieces and air-dried until a steady weight was obtained, then ground into powder with electric blender. The powder was soaked in 70% ethanol for 72 h, concentrated until dried at 40 ℃, and then divided into two portions and weighed. One part (1.5 mg) was stored in the refrigerator at –4 ℃, with the other portion of 1.5 kg being partitioned for 72 h in 2.5 L of each of *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol in sequence. The filtrates of the fractions were dried at 40 ℃, weighed, then stored at –4 ℃ until required.

2.3. Animals

Male and female adult Swiss albino rats and mice were acquired and fed with grower pellet feed and water was provided *ad libitum*. The experiments in the present study were approved by the Ethics Committee of the College of Health Sciences, University of Uyo and followed recommended guidelines.

2.4. Microorganisms

This work was done with chloroquine-sensitive *Plasmodium berghei berghei* from the National Institute of Medical Research, Lagos.

2.5. Determination of median lethal dose (LD_{50})

Miller and Tainter[5] model was used to evaluate the LD_{50}. The extract (500–5 000 mg/kg) was administered intraperitoneally (*i.p.*) to mice in 5 groups (six mice per group). Signs of toxicity and the number of deaths per group within 24 h were all recorded.

2.6. Parasite inoculation

Donor blood with about 5×10^7 *P. berghei berghei* parasitized erythrocytes/mL was diluted with isotonic saline[6] to produce inoculum that contained 1×10^7 *P. berghei berghei* parasitized erythrocytes/mL. Then 0.2 mL of the diluted one was given intraperitoneally to each mouse.

2.7. Antiplasmodial activities of the extract and fractions

2.7.1. Determination of suppressive activity of S. megaphylla extracton against early infection (4-day test)

The parasitaemia in this test was determined according to the following formula:

$$\% \text{ Parasitaemia} = \frac{\text{No. of parasitized RBC}}{\text{Total No. of RBC}} \times 100$$

Average percentage chemosuppression was calculated as:

$$100\left(\frac{\text{A-B}}{\text{A}}\right)$$

where, A = percentage parasitaemia in control group; B = percentage parasitaemia in the test group.

2.7.2. Determination of suppressive activity of S. megaphylla root fractions against early infection (4-day test)

The method of Knight and Peters[7] as reported by Bantie *et al*.[8] was used to assess the suppressive activity of *S. megaphylla* root fractions. Exactly 0.2 mL of infected blood was given intraperitoneally to mice in 6 groups (six mice per group). After 30 min, *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol fractions of *S. megaphylla* (300 mg/kg/day) were then given per oral. Distilled water (10 mL/kg) was given to the control group while artesunate (5 mg/kg/day) was used as the standard drug. Fractions, distilled water and artesunate were given continuously for 4 days (Day 0–Day 3). On the fifth day (Day 4), thin films were prepared from each mouse and the percentage parasitaemia estimated by using the Neubauer counting chamber to randomly count the number of parasitized RBCs out of 500 RBCs. Percentage chemosuppression was estimated as above.

2.7.3. Determination of prophylactic activity of S. megaphylla extract

The method earlier described by Peters[9] and reported by Udobre *et al*.[10] was employed in assessing the prophylactic effect of the extracts. Thirty mice were randomly divided into 5 groups (six mice per group). Group 1 (control) animals were given distilled water (10 mL/kg), while Groups 2–4 respectively received 150, 300 and 450 mg/kg of extract per oral. Group 5 mice were given the standard drug pyrimethamine (1.2 mg/kg). The mice all received the treatment daily for three days (Day 0–Day 2), and on the forth day (Day 3), 0.2 mL of infected blood was intraperitoneally administered. Parasitaemia was evaluated with thin films of blood from each mouse 72 h after parasite inoculation, and the percentage of parasitaemia and average chemosuppression were estimated.

2.7.4. Determination of effect of S. megaphylla root extract on established infection

The method of Liu *et al*.[11] as reported by Bantie *et al*.[8] was used for this experiment. Thirty adult Swiss albino mice were inoculated intraperitoneally on the first day (Day 0). After 72 h, the mice were randomly divided into 5 groups (six mice per group). Group 1 (control) animals were given distilled water (10 mL/kg), while Groups 2–4 respectively received 150, 300 and 450 mg/kg of extract per oral. Group 5 mice were given the standard drug artesunate (5 mg/kg). The mice all received the treatment daily for five days. Parasitaemia was evaluated with thin films of blood taken from each mouse daily for five days, and processed using Giemsa stain. Every group had its mean survival time (MST) calculated after thirty days (Day 0–Day 29) according to the following formula:

$$\text{MST} = \frac{\text{No. of days survived}}{\text{Total No. of days (30)}} \times 100$$

2.7.5. Determination of effect of S. megaphylla root fractions on established infection

The method of Liu *et al*.[11] as reported by Bantie *et al*.[8] was used for this experiment. Thirty six adult Swiss albino mice were inoculated intraperitoneally on the first day (Day 0). After 72 h, the mice were randomly divided into 6 groups (six mice per group). Group 1 (control) animals were given distilled water (10 mL/kg), while Groups 2–5 respectively received 300 mg/kg of various fractions per oral. Group 6 mice were given the standard drug artesunate (5 mg/kg). The mice all received the treatment daily for five days. Parasitaemia was evaluated with thin films of blood taken from each mouse daily for five days, and processed using Giemsa

stain. Every group had its MST calculated after thirty days (Day 0–Day 29) according to the formula mentioned above.

2.8. Evaluation of antipyretic activity of S. megaphylla extract

2.8.1. Amphetamine-induced pyrexia in rats

Thirty Swiss albino rats were fasted for 24 h but given access to water *ad libitum*. Their temperature was measured rectally and 5 mg/kg of D-amphetamine was administered. After 30 min of D-amphetamine administration, rats that had temperature raised by at least 1 ℃ were randomly divided into 5 groups (six mice per group). Group 1 (control) animals were given distilled water (10 mL/kg) per oral, while Groups 2–4 respectively received 150, 300 and 450 mg/kg of extract intraperitoneally. Group 5 mice were given the standard drug acetyl salycylic acid (ASA) (100 mg/kg *p.o.*). The rats all had their rectal temperatures measured at 30 min, then every hour for 5 h[12].

2.8.2. 2,4-Dinitrophenol (DNP)-induced pyrexia in rats

Thirty Swiss albino rats were fasted for 24 h but given access to water *ad libitum*. Their temperature was measured rectally and 10 mg/kg of DNP administered intraperitoneally. After 30 min of DNP administration, rats that had their temperature raised by at least 1 ℃ were randomly divided into 5 groups (six mice per group). Group 1 (control) animals were given distilled water (10 mL/kg, *p.o.*), while Groups 2–4 respectively received 150, 300 and 450 mg/kg of extract intraperitoneally. Group 5 mice were given the standard drug acetyl salycylic acid (ASA) (100 mg/kg *p.o.*). The rats all had their rectal temperatures measured at 30 min, then every hour for 5 h[13,14].

2.8.3. Yeast-induced pyrexia in rats

Thirty Swiss albino rats were fasted for 24 h but given access to water *ad libitum*, and had their rectal temperature measured using digital clinical thermometer. They were then given yeast suspension (10 mL/kg *s.c.*) in the back. Food was immediately withdrawn, and temperature increase was recorded 18 h after yeast administration. Rats that had their temperature raised by at least 1 ℃ were randomly divided into 5 groups (six mice per group). Group 1 (control) animals were given distilled water (10 mL/kg *p.o.*), while Groups 2–4 respectively received 150, 300 and 450 mg/kg of extract intraperitoneally. Group 5 animals were given the standard drug acetyl salycylic acid (ASA) (100 mg/kg *p.o.*). The rats all had their rectal temperature measured at 30 min, then every hour for 5 h. The differences between the actual and starting values were noted for each time interval[15].

2.9. Gas chromatography-mass spectrometry (GC-MS) analysis

Qualitative data were determined by GC-MS. The fraction was injected into a Shimadzu GC-17A system equipped with an AOC-20i autosampler and a split/splitless injector. The column used was DB-5 (Optima-5), 30 m, 0.25 mm i.d., 0.25 μm df, coated with 5% diphenyl-95% polydimethylsiloxane, operated with the following oven temperature programme: 50 ℃, held for 1 min, rising at 3 ℃/min to 250 ℃, held for 5 min, rising at 2 ℃/min to 280 ℃, held for 3 min; injection temperature and volume were 250 ℃ and 1 μL, respectively; injection mode, split; split ratio, 30:1; carrier gas, nitrogen at 30 cm/s linear velocity and inlet pressure 99.8 kPa; detector temperature, 280 ℃; hydrogen flow rate, 50 mL/min; air flow rate, 400 mL/min; make-up (H_2/air) flow rate, 50 mL/min; sampling rate, 40 MS/s. Data were acquired by means of GC solution software (Shimadzu). Agilent 6890N GC was interfaced with a VG Analytical 70-250s double-focusing mass spectrometer. Helium was the carrier gas. The MS operating conditions were: ionization voltage 70 eV, ion source 250 ℃. The GC was fitted with a 30 m × 0.32 mm fused capillary silica column coated with DB-5. The GC operating parameters were identical with those of GC analysis described above.

The constituents in the fractions were identified by directly comparing the retention times and mass spectral data with those of standard compounds, and by computer matching with the Wiley and Nist Library, as well as comparing the mass spectra fragmentation patterns with those earlier published[16,17].

2.10. Statistical analysis and data evaluation

Data obtained were analyzed using One-way ANOVA followed by Tukey-kramer multiple comparison test. Differences between means were considered significant at 5% level of significance ($P \leqslant 0.05$).

3. Results

3.1. Determination of median lethal dose (LD_{50})

The result showed an LD_{50} of (1 500 ± 35) mg/kg. Toxic effects noted included excitation, paw licking, tachypnea, reduced movement, gasping, coma and then death.

3.2. Phytochemical analysis

The GC-MS result of the dichloromethane and *n*-butanol fractions of *S. megaphylla* roots showed 14 components in each fraction (Tables 1 and 2). While borneol and α-terpineol were found in both fractions, the *n*-butanol fraction had hexadecanoic acid, carvacrol, linalool, camphor, menthofuran, menthone and α-eudesmol among others, and the dichloromethane fraction had constituents such as astaxanthin, terpinen-4-ol, β-*cis*-bergamotene, citronellol and germacrene D. These constituents are mostly monoterpenes and sesquiterpenes.

Table 1
GC-MS analysis of dichloromethane fraction of *S. megaphylla.*

No.	Name of compound	Molecular weight	Chemical formula	RI
1	Benzaldehyde, 3-methyl	164	C_8H_8O	200
2	2,4,6-trimethyl octane	156	$C_{11}H_{24}$	310
3	Tridecane, 6-methyl	198	$C_{14}H_{30}$	360
4	Decane, 5-propyl	184	$C_{13}H_{26}O_2$	473
5	Undecanoic acid, ethyl ester	214	$C_{13}H_{26}O_2$	646
6	1-octen-3-ol	128	$C_{17}H_{36}O$	967
7	*trans-p*-Menth-2-en-1-ol	154	$C_{18}H_{36}O_2$	1116
8	Borneol	154	$C_{10}H_{18}O$	1138
9	Astaxanthin	598	$C_{48}H_{52}O_4$	1179
10	α-Terpineol	154	$C_{10}H_{18}O$	1180
11	Terpinen-4-ol	154	$C_{10}H_{18}O$	1185
12	β-*cis*-bergamotene	204	$C_{15}H_{24}$	1404
13	Citronellol	156	$C_{10}H_{20}O$	1212
14	Germacrene D	204	$C_{15}H_{24}$	1484

RI: Refractive index.

Table 2

GC-MS analysis of *n*-butanol fraction of *S. megaphylla*.

No.	Name of compound	Molecular weight	Chemical formula	RI
1	Benzaldehyde, 3-methyl	120	C_8H_8O	200
2	Octane, 2,4,6-trimethyl	156	$C_{11}H_{24}$	320
3	9,10-secocholesta-5,7,10(19)-triene, -3,24,25-triol (3a',5Z,7E)	416	$C_{27}H_{44}O_3$	350
4	3-methyl-undecane	170	$C_{12}H_{26}$	490
5	Hexadecanoic acid	256	$C_{16}H_{32}O_2$	660
6	1,2-Benzenedicarboxylic acid, isodecyl octyl ester	418	$C_{26}H_{42}O_4$	906
7	Carvacrol	150	$C_{10}H_{14}O$	1076
8	Linanlool	154	$C_{10}H_{18}O$	1084
9	Camphor	152	$C_{10}H_{16}O$	1128
10	Borneol	154	$C_{10}H_{18}O$	1139
11	Menthofuran	150	$C_{10}H_{14}O$	1140
12	Menthone	154	$C_{10}H_{18}O$	1149
13	α-Terpineol	154	$C_{10}H_{18}O$	1180
14	α-Eudesmol	222	$C_{15}H_{26}O$	1650

3.3. Antiplasmodial activities of the extract and fractions

3.3.1. Suppressive activity of extract of S. megaphylla against early infection

The result revealed a dose-dependent effect. The chemosuppression was 42.66%, 53.67%, and 66.12% for 150, 300 and 450 mg/kg doses respectively. The extract produced a significant ($P < 0.05$) effect, which was weak when compared to the standard drug with chemosuppression of 86.70% (Table 3).

Table 3

Suppressive activity of ethanol root extract of *S. megaphylla* against *P. berghei berghei* infection in mice (4-day test).

Treatments	Parasitaemia	% Chemosuppression
Distilled water (10 mL/kg)	72.66 ± 5.54	-
Crude extract (150 mg/kg)	41.66 ± 3.38[a]	42.66
Crude extract (300 mg/kg)	33.66 ± 2.18[a]	53.67
Crude extract (450 mg/kg)	24.61 ± 1.85[a]	66.12
Artesunate (5 mg/kg)	9.66 ± 2.45[a]	86.70

Values are expressed as mean ± SEM. [a]: $P < 0.05$ compared with control. $n = 6$.

3.3.2. Prophylactic activity of extract of S. megaphylla against P. berghei berghei infection

The result revealed that the extract exerted a dose-dependent prophylactic effect with statistically significant ($P < 0.05$) reductions of parasitaemia in test groups. Chemosuppression of 50.00%, 64.49% and 78.50% for 150, 300 and 450 mg/kg doses was respectively recorded. The chemosuppressions by the extract were not comparable to pyrimethamine (1.2 mg/kg) with chemosuppression of 80.88% (Table 4).

Table 4

Prophylactic activity of ethanol root extract of *S. megaphylla* on *Plasmodium berghei berghei* infection in mice.

Treatments	Parasitaemia	% Chemosuppression
Distilled water (10 mL/kg)	61.00 ± 4.04	-
Crude extract (150 mg/kg)	30.00 ± 1.15[a]	50.00
Crude extract (300 mg/kg)	21.66 ± 3.18[a]	64.49
Crude extract (450 mg/kg)	14.33 ± 1.45[a]	76.50
Pyrimethamine (5 mg/kg)	11.66 ± 1.76[a]	80.88

Values are expressed as mean ± SEM. [a]: $P < 0.05$ compared to control. $n = 6$.

3.3.3. Antiplasmodial activity of extract on established infection

The results showed a dose-dependent significant ($P < 0.01$–0.001) reduction in parasitaemia in the test groups comparable to the artesunate group. The parasitaemia for Day 7 was 42.60%, 27.66%, 25.66%, 21.00% and 88.60% for 150, 300 and 450 mg/kg, artesunate and control groups respectively (Figure 1). The MST of the test groups (Table 5) was significantly ($P < 0.01$–0.001) longer than control. The mean MST for artesunate group was 21.66 days.

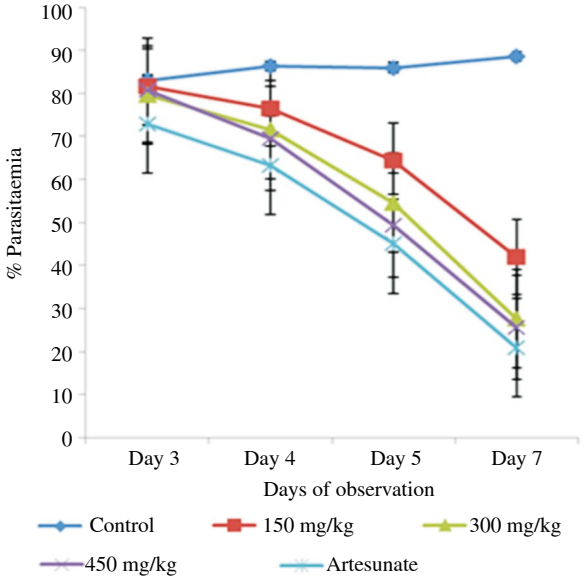

Figure 1. Antiplasmodial activity of ethanolic root extract of *S. megaphylla* on *P. berghei berghei* infection in mice.

Table 5

MST of mice treated with various doses of root extract of *S. megaphylla* during established infection.

Treatments	Mean survival time (days)
Distilled water (10 mL/kg)	11.33 ± 0.33
Crude extract (150 mg/kg)	13.33 ± 0.33[b]
Crude extract (300 mg/kg)	16.00 ± 0.00[c]
Crude extract (450 mg/kg)	18.00 ± 0.57[c]
Artesunate (5 mg/kg)	21.66 ± 0.25[c]

Values are expressed as mean ± SEM. [b]: $P < 0.01$, [c]: $P < 0.001$ compared to control. $n = 6$.

3.3.4. Suppressive activity of fractions of S. megaphylla on early infection

The result showed the potency of the fractions in the order of *n*-butanol > dichloromethane > ethyl acetate > *n*-hexane with chemosuppression of 60.57%, 42.82%, 15.66% and 14.66% respectively. The activity of fractions was weak compared to

artesunate (Table 6).

Table 6

Suppressive activities of root fractions of *S. megaphylla* (4-day test).

Drug/extract	Parasitaemia	% Chemosuppression
Distilled water (10 mL/kg)	12.96 ± 1.45	-
n-Hexane (300 mg/kg)	11.06 ± 1.22[a]	14.66
Dichloromethane (300 mg/kg)	7.41 ± 1.39[a]	42.82
Ethyl acetate (300 mg/kg)	10.93 ± 0.36[a]	15.66
n-Butanol (300 mg/kg)	5.11 ± 0.55[a]	60.57
Artesunate (5 mg/kg)	1.88 ± 0.44[a]	85.49

Values are expressed as mean ± SEM. [a]: $P < 0.05$ compared to control. $n = 6$.

3.3.5. Antiplasmodial activity of fractions of S. megaphylla against established infection

Figure 2 shows the results of the antiplasmodial activities of the root fractions against established infection.

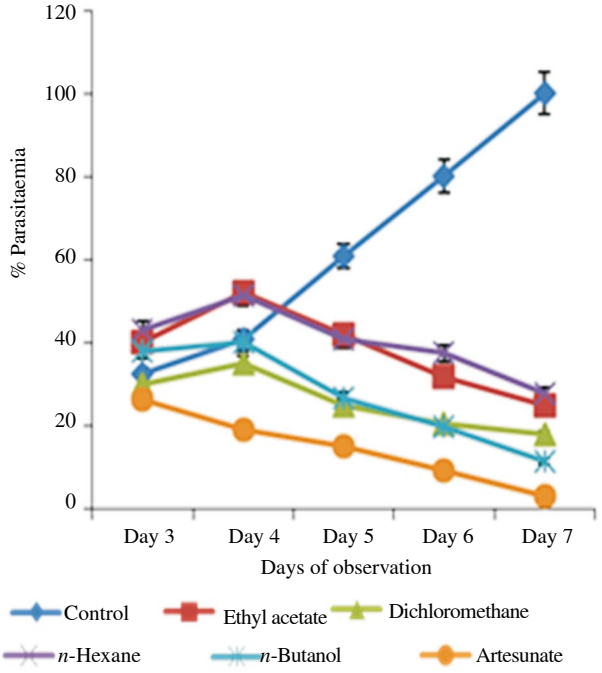

Figure 2. Antiplasmodial activity of root fractions of *S. megaphylla*.

The potency order was *n*-butanol > dichloromethane > ethyl acetate > *n*-hexane with percentage parasitaemia of 11.50%, 18.40%, 29.00% and 35.30% respectively on Day 7. The MST of *n*-butanol and dichloromethane fractions treated groups (16.50 and 14.25 days respectively) as well as artesunate treated group (25.00 days) were significantly ($P < 0.05$–0.001) longer than the control group (10.00 days) (Table 7).

Table 7

MST of mice receiving different fractions of *S. megaphylla.*

Drug/extract	MST (days)
Distilled water (10 mL/kg)	10.00 ± 0.40
n-Hexane (300 mg/kg)	11.00 ± 0.70
Dichloromethane (300 mg/kg)	14.25 ± 0.85[a]
Ethyl acetate (300 mg/kg)	12.00 ± 0.70
n-Butanol (300 mg/kg)	16.50 ± 1.32[b]
Artesunate (5 mg/kg)	25.00 ± 2.91[c]

Values are expressed as mean ± SEM. [a]: $P < 0.05$, [b]: $P < 0.01$, [c]: $P < 0.001$ compared to control. $n = 6$.

3.4. Antipyretic studies

3.4.1. Amphetamine-induced pyrexia in rats

The results (Table 8) showed a statistically significant ($P < 0.05$–0.001) reduction in the elevated temperatures of the extract treated rats when compared with the control. A dose-dependent antipyretic effect which was not comparable to ASA (100 mg/kg) was seen.

3.4.2. DNP-induced pyrexia in rats

The result (Table 9) shows that the extract caused a significant ($P < 0.001$) decrease in the elevated temperatures of the treated groups. A dose-dependent antipyretic effect by extract (450 mg/kg) comparable to ASA (100 mg/kg) was seen.

3.4.3. Yeast-induced pyrexia in rats

The extract demonstrated a significant ($P < 0.05$–0.001) decrease in the elevated temperatures of the test groups. A dose-dependent antipyretic effect not comparable to ASA (100 mg/kg) was seen (Table 10).

Table 8

Effect of extract on D-amphetamine-induced pyrexia in rats.

Treatment	Basal	Time intervals (h)						
		0	0.5	1	2	3	4	5
Control	36.90 ± 0.05	38.90 ± 0.82	39.46 ± 0.05	39.45 ± 0.08	39.36 ± 0.08	38.31 ± 0.14	38.13 ± 0.08	38.23 ± 0.10
Extract (150 mg/kg)	36.23 ± 0.12	37.56 ± 0.29	37.70 ± 0.20[c]	37.80 ± 0.17[c]	37.06 ± 0.14[c]	36.66 ± 0.03[c]	36.66 ± 0.29[a]	36.43 ± 0.03[c]
Extract (300 mg/kg)	35.75 ± 0.18	36.90 ± 0.10	36.70 ± 0.10[c]	36.80 ± 0.20[c]	37.00 ± 0.11[c]	36.36 ± 0.16[c]	35.90 ± 0.26[c]	35.80 ± 0.05[c]
Extract (450 mg/kg)	35.56 ± 0.29	36.83 ± 0.14	37.06 ± 0.13[c]	37.13 ± 0.08[c]	37.10 ± 0.05[c]	36.70 ± 0.05[c]	36.30 ± 0.15[b]	35.70 ± 0.05[c]
ASA (100 mg/kg)	35.63 ± 0.95	37.50 ± 0.41	37.53 ± 0.18[c]	37.36 ± 0.34[c]	36.33 ± 0.47[c]	35.96 ± 0.24[c]	35.76 ± 0.57[c]	35.41 ± 0.05[c]

Data are expressed as mean ± SEM. [a]: $P < 0.05$, [b]: $P < 0.01$, [c]: $P < 0.001$ compared to control. $n = 6$.

Table 9

Effect of extract on DNP-induced pyrexia in rats.

Treatment	Basal	Time intervals (h)						
		0	0.5	1	2	3	4	5
Control	36.03 ± 0.03	38.67 ± 0.27	38.95 ± 0.47	38.78 ± 0.08	38.50 ± 0.08	38.43 ± 0.06	38.35 ± 0.10	37.70 ± 0.10
Extract (150 mg/kg)	36.35 ± 0.14	38.77 ± 0.11	38.30 ± 0.20	37.67 ± 0.03[c]	37.40 ± 0.04[c]	37.15 ± 0.07[c]	36.65 ± 0.07[c]	36.60 ± 0.03[c]
Extract (300 mg/kg)	36.15 ± 0.09	38.72 ± 0.28	38.40 ± 0.80	37.30 ± 0.05[c]	36.95 ± 0.10[c]	36.70 ± 0.15[c]	36.60 ± 0.05[c]	36.48 ± 0.05[c]
Extract (450 mg/kg)	36.22 ± 0.10	39.15 ± 0.09	39.35 ± 0.11	37.75 ± 0.14[c]	36.80 ± 0.05[c]	36.50 ± 0.05[c]	36.35 ± 0.04[c]	36.28 ± 0.05[c]
ASA (100 mg/kg)	36.32 ± 0.10	39.15 ± 0.09	39.85 ± 0.11	37.70 ± 0.24[c]	36.85 ± 0.05[c]	36.60 ± 0.05[c]	36.40 ± 0.05[c]	36.20 ± 0.05[c]

Data are expressed as mean ± SEM. [c]: $P < 0.001$ compared to control. $n = 6$.

Table 10
Effect of extract on yeast-induced pyrexia in rats.

Treatment	Basal	Time intervals (h)					
		0.5	1	2	3	4	5
Control	35.80 ± 0.07	36.82 ± 0.20	37.82 ± 0.37	38.35 ± 0.22	38.55 ± 0.18	38.70 ± 0.18	38.77 ± 0.16
Extract (150 mg/kg)	35.92 ± 0.17	37.15 ± 0.23	36.85 ± 0.10[a]	37.03 ± 0.11[c]	36.87 ± 0.18[c]	36.82 ± 0.20[c]	36.75 ± 0.20[c]
Extract (300 mg/kg)	35.92 ± 0.16	37.12 ± 0.21	36.87 ± 0.21[a]	36.60 ± 0.22[c]	36.60 ± 0.22[c]	36.40 ± 0.22[c]	36.32 ± 0.18[c]
Extract (450 mg/kg)	35.97 ± 0.04	37.20 ± 0.09	36.77 ± 0.17[a]	36.40 ± 0.17[c]	36.40 ± 0.17[c]	36.12 ± 0.21[c]	36.07 ± 0.20[c]
ASA (100 mg/kg)	35.90 ± 0.04	37.05 ± 0.11	36.97 ± 0.14	36.57 ± 0.08[c]	36.57 ± 0.08[c]	36.35 ± 0.06[c]	35.95 ± 0.15[c]

Data are expressed as mean ± SEM. [a]: $P < 0.05$, [c]: $P < 0.001$ when compared to control, $n = 6$.

4. Discussion

Acute toxicity testing, phytochemical analysis, antiplasmodial and antipyretic evaluations of *S. megaphylla* roots were performed. The LD_{50} value of (1 500 ± 35) mg/kg indicated negligible toxicity[18]. Reported phytochemical constituents of medicinal importance in the root extract include alkaloids, terpenes, tannins, flavonoids, saponins and deoxy sugars[1].

The crude root extract of *S. megaphylla* showed significant ($P < 0.05$–0.001) antiplasmodial activities in all three models (suppressive, repository and curative) evaluated. These observed effects were weak when compared to the standard drugs (artesunate and pyrimethamine) used. The antiplasmodial effect could be attributed to the presence of alkaloids, flavonoids, terpenes and tannins[19,20]. Asnaashari *et al*.[21] have reported the antimalarial effects of flavonoids against *Plasmodium* strains resistant to chloroquine. Flavonoids are believed to interfere with the fatty acid biosynthesis (FAS II) of the *Plasmodium*[21], inhibit entry of L-glutamine and myoinositol into infected red blood cells[22], elevate erythrocytes oxidation and interfere with *Plasmodium* protein synthesis[23]. Alkaloids are known to block protein synthesis in *Plasmodium falciparum*[24,25], and the alkaloid conessine from *H. antidysentrica* has shown enormous antimalarial action with minimal cytotoxic activity[26]. Also several 1-aminopolycyclic β-carbolin alkaloids are known to exhibit substantial *in vitro* and *in vivo* antimalarial activity[27]. The phytochemical constituents present in this extract could have acted through one or more of the above mechanisms to exert the observed antiplasmodial activity.

The order of antiplasmodial potency of the four fractions was *n*-butanol > dichloromethane > ethyl acetate > *n*-hexane. Additionally, the fact that the most active fractions-treated group had longer MST also suggests that they contain the active ingredients. The monoterpenes identified in these fractions included borneol, α-terpineol, terpinen-4-ol, citronellol, carvacrol, linalool, menthofuran, menthone and *trans-p*-menth-2-en-1-ol, while germacrene D, β-*cis*-bergamotene and α-eudesmol were the sesquiterpenes identified. Other identified terpenes are camphor and astaxanthin. This result indicates that the antiplasmodial effects of *S. megaphylla* could have resulted from the presence of the discovered constituents.

Sesquiterpenes with monoterpenes have long been known to have antiplasmodial activity[28,29] and have been implicated in plasmocidal activity which results in death of plasmodia cells[30]. Linalool, a monoterpene found in *S. megaphylla* probably exerts its antimalarial effects by inhibition of isoprenyl diphosphate synthases[31].

Linalool is known to suppress dolichol biosynthesis in *P. falciparum* with strong effects on some developmental stages of the schizont[31]. Thymol in *Satureja thymbra* (savory, Lamiaceae) has shown larvicidal and adulticidal activities against *P. falciparum*[32].

The monoterpene limonene is known to inhibit protein isoprenylation in *P. falciparum*[33]. Artemisinin (ART), a sesquiterpene endoperoxide with described mechanism of action[34] induces fast decrease in parasitaemia, soon after being given[35]. ARTs destroy all parasites by acting upon haem to yield free radicals which alkylate proteins and destroy micro-organelles and parasite membranes. Iron then cleaves the peroxide bridge (C–O–O–C) and generates carbon-centred radicals which cause enzyme inactivation and parasite death[36,37]. The components found in this extract could be responsible for the observed antiplasmodial effect and therefore validate its ethnomedicinal use for treatment of malaria.

Antioxidation has been implicated as one of the mechanisms of antiplasmodial activity of plants[38]. The sesquiterpenes, α-eudesmol and monoterpenes such as borneol, astaxanthin, carvacrol and menthone found here do possess antioxidant properties[39], which may have contributed to their antiplasmodial action. Astaxanthin, a terpene, possesses the highest antioxidant effect compared with any known carotenoid[40], and borneol is known to possess radical scavenging properties[41], showing antioxidant effects by suppressing intracellular ROS production, depressing nitric oxide elevation, increasing inducible nitric oxide synthase enzymatic activity and upregulating inducible nitric oxide synthase expression[42]. The activities of these compounds found in this plant could account for its antiplasmodial activity through one or all of the mechanisms described here.

The extract (150–450 mg/kg) showed statistically significant ($P < 0.05$–0.001) and dose-dependent reduction in the elevated temperatures of the extract treated rats in the three models (amphetamine, DNP and yeast-induced pyrexia) evaluated. The antipyretic effect was sustained throughout the duration of the work with the effect being quite comparable to ASA (100 mg/kg). Pyrexia (fever) is the body's response to tissue damage, inflammation, malignancy or graft rejection that results in the formation of large amounts of cytokines, interleukin, interferon and TNF-α, and

increasing PGE_2 to trigger the hypothalamus and then cause fever.

In the brain, amphetamine causes the release of biogenic amines that are stored in nerve terminals leading to increases in the cAMP level, resulting in prostaglandins synthesis from arachidonic acids in neurons through hydrolysis of phospholipids. This results in hyperthermia[4]. DNP causes hyperthermia by uncoupling oxidative phosphorylation resulting in calcium release from mitochondrial stores and preventing calcium reuptake. This leads to increased intracellular calcium level, muscle contraction and hyperthermia[43]. The extract through its components may have caused stimulation of the sarcoplasmic reticulum Ca^{2+}-ATPase thus promoting calcium reuptake into the sarcoplasmic reticulum, muscle contraction and hypothermia[44]. Yeast induced pyrexia which is pathogenic and caused by PGE_2 production, which then resets the thermoregulatory center in the hypothalamus to a higher level[45].

Antipyretics lower elevated body temperature by suppressing cyclooxygenase actions and decreasing PGE_2 levels in the hypothalamus[46]. Temperature regulation involves a delicate balance between heat production and loss, and the hypothalamic thermostat[46]. Cyclooxygenase (COX_2) produces PGE_2 which is an imperative mediator of fever within the hypothalamus and most NSAIDs antipyretic activity results from suppression of prostaglandin synthetase in the hypothalamus. The resultant antipyretic effects could be due to reduced PGE_2 levels in the hypothalamus acting on COX_2, or through increased production of substances such as vasopressin and arginine that reduce temperature[47]. The extract may have caused hypothermia by acting through any of these mechanisms.

Another possible antipyretic mechanism of the extract is the mediation of the dilation of superficial blood vessels which causes improved heat loss from resetting of hypothalamic thermostat[47]. Flavonoids such as baicalin exhibit antipyretic activity by inhibiting tumour necrosis factor[45], and related compounds also suppress arachidonic acid peroxidation, resulting in decreased prostaglandin levels and fever reduction[48]. The phytochemical constituents of this extract may be responsible for its antipyretic effect through any or all mechanisms described here.

Finally, the findings of this work show that the root extract and fractions of *S. megaphylla* possess antimalarial and antipyretic activities which justify its ethnomedicinal use.

Conflict of interest statement

We declare that we have no conflict of interest.

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