Trop J Nat Prod Res, September 2022; 6(9):1504-1510 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original ResearchArticle

Antimalarial Activities of Leaf Extract and Fractions of *Setaria megaphylla*(Willd.) Loes. in *Plasmodium berghei* Infected Mice

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ARTICLE INFO

Article history:
Received 07 July 2022
Revised 15 September 2022
Accepted 19 September 2022
Published online 01 October 2022

ABSTRACT

Setaria megaphylla (Steud) Dur&Schinz (Poaceae), a perennial grass used traditionally in the treatment of various diseases such as malaria was screened for antiplasmodial activity.The leaves extract (200–600 mg/kg) and fractions (hexane, dichloromethane, ethyl acetate and methanol; 400 mg/kg) were investigated for suppressive, prophylactic, and curative antimalarial activities against chloroquine-sensitive *Plasmodium berghei* infections in Swiss albino mice.Chloroquine (5 mg/kg) and pyrimethamine (1.2 mg/kg) were used as positive controls.Thin films made from tail blood of each of the mice were used to assess the level of parasitaemia of the mice. The extract/fractions progressively reduced parasitaemia induced by chloroquine-sensitive *P. berghei* infection in suppressive (11.12–46.94%), prophylactic (19.20 –47.96%) and curative (20.65–75.06%) models in mice. These reductions were statistically significant (p<0.01–0.001). They also improved significantly (p<0.01–0.001) the mean survival time (MST) from 13.33 to 18.60 d in suppressive, 10.33 to 26.00 in prophylactic and 12.50 to 22.66 days in curative models relative to respective controls. The activities of extract/fractions were not comparable to that of the standard drugs used (chloroquine and pyrimethamine) in all the models.The leaf of *S. megaphylla* may possess antimalarial effect which may in part be mediated through the chemical constituents of the plant.

Keywords*:Setaria megaphylla*, Antimalarial, Malaria, *Plasmodium berghei*.

Introduction

About 229 million malaria cases have been estimated to have occurred in 2019 in 87 malaria endemic countries globally.[1]Despite a significant decline in mortality due to malaria, the highest mortality rate of all malaria deaths globally in 2019 was recorded in Nigeria. This shows that malaria still threatens most countries of Africa particularly Nigeria despite the successes achieved in the global fight against malaria over the last two decades. Medicinal plants, therefore serve as an excellent reservoir for antimalarial remedies with the advantage of being safer and providing many therapeutic effects. *Setaria megaphylla* (Steud) Dur&Schinz (Poaceae), a perennial grass found in tropical and subtropical areas of the World,[2] is traditionally used for the treatment of diverse ailments including malaria and diabetes among others.[3] Preliminary reports of antiplasmodial activity on the leaf have been published[3,4] The leaf extract also possesses antidiabetic and hypoglycaemic,[5] anti-inflammatory, analgesic,[6] cytotoxic, immunomodulatory and antileishmanial,[7] antidepressant,[8] inhibitory effect on α-amylase and α-glucosidase[9] activities. Phytochemical analysis of the leaves extract revealed that the leaves extract contains specialized secondary metabolites such as flavonoids, carbohydrate, terpenes, saponins, tannins, anthraquinones, cardiac glycosides (Z,Z,Z)-8,11,14-eicosatrienoic acid, phthalic acid, diisooctyl ester, vitamin E, ϒ-elemene, urs-12-ene, bicyclogermacrene, α-muurolene, germacrene-A, and guaiol[3,7].

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Citation: William NB, Bassey AL, Daniel AO, Ekong O, Okokon JE. Antimalarial Activities of Leaf Extract and Fractions of *Setaria megaphylla* (Willd.) Loes. in *Plasmodium berghei* Infected Mice. Trop J Nat Prod Res. 2022; 6(9):1504-1510. http://www.doi.org/10.26538/tjnpr/v6i9.28

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

1-triacontanal, 1-triacontanol, 1-dotriacontanol, 1-triacontyl cerotate, and stigmasterol have also been isolated from the plant leaves.[5] We report the antimalarial potentials of the leaves extract and fractions of *Setaria megaphylla* in *Plasmodium berghei*-infected mice.

Materials and Methods

Collection and identification of plant material

The leaves of *Setaria megaphylla* were collected from bushes in the Uruan area of Akwa Ibom State, Nigeria in July, 2020. The plant was identified by Prof. Margaret Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. A voucher specimen (UUPH. 221 d) of the plant was deposited in the Department of Pharmacognosy and Natural Medicine herbarium at the University of Uyo, Nigeria.

Extraction

The leaves were washed and shade dried for two weeks. The dried leaves were cut into smaller pieces and pulverized to powder using electric grinder. The leaves powder was divided into two parts. One part (1.5 kg) was macerated in ethanol (7.5 L) for 72 hours, while the remaining part (1.5 kg) was successively and gradiently macerated for 72 hours in 7.5 L each of, n-hexane, dichloromethane, ethyl acetate and methanol respectively, which is along their polarities to give the corresponding gradient extract for each solvent. The liquid filtrates of the extract and fractions were concentrated and evaporated to dryness in *vacuo* 40^0C using a rotary evaporator. The different yields were calculated and the extract and fractions refrigerated at -4^0C, until used for the proposed experiments.

Microorganism (parasite)

The chloroquine-sensitive strain of *Plasmodium berghei*ANKA strain was obtained from the National Institute of Medical Research (NIMER), Yaba Lagos, Nigeria and maintained by subpassage of blood from infected to healthy mouse once every 7-8 days.

Parasite inoculation
The inoculum consisted of 5 x 10^7 *P. berghei* infected erythrocytes per milliliter prepared by diluting serially with normal saline, parasitized blood erythrocytes collected from an infected mouse with 20-30% parasitaemia to make a suspension of *P. berghei* parasitized erythrocytes.[10] Each mouse used in the experiment was inoculated intraperitoneally with 0.2 mL of infected blood containing about 1 x 10^7*P. berghei* parasitized erythrocytes. Parasitemia was monitored by standard methods; thin blood smears were made on glass slides, fixed using methanol, and stained with Giemsa stain. Parasitemia was counted using a microscope and was calculated as a percentage of infected red blood cells (RBCs) relative to the total number of cells in a microscopic field at ×100 magnification according to the formula given below[11]:

$$Parasitaemia\ (\%) = \frac{No\ of\ parasitised\ RBCs}{Total\ number\ of\ RBCs} \times 100$$

Experimental animals
Male and female Swiss albino mice, each weighing 21-32 g, were obtained from the University of Uyo's animal house. They were kept in standard cages and acclimatized for 10 days before use in the experiments. The mice were fed on a standard pelleted diet and water *ad libitum*. All animals were kept at room temperature in cross ventilated rooms. The care and use of animals were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication, 1996). Approval for the study was obtained from the University of Uyo's Animal Ethics Committee (UU/FP/AE/21/035).

Drug administration
The extract, fractions, chloroquine and pyrimethamine that were used in the antimalarial study, were administered orally with the aid of a stainless metallic feeding cannula.

Evaluation of the in vivo antimalarial activities of leaf extract and fractions of Setaria megaphylla:
Evaluation of suppressive activities of the leaf extract and fractions of Setaria megaphylla (4-day test)
This test was used to evaluate the schizontocidal activity of the crude extract and fractions as well as chloroquine against early *P. berghei* infection in mice. This was done as described by Ali et al.[11]Forty-five mice were randomly divided into nine groups of five (5) mice each. On the first day (D_0), the mice were infected with the parasite and randomly divided into various groups. These were administered the crude extract, fractions, chloroquine and distilled water. Based on previously determined LD_{50} by Okokon et al..[3] the mice in groups 1-3 were given 200 mg/kg, 400 mg/kg and 600 mg/kg of crude extract respectively, while groups 4, 5, 6, 7 were administered 400 mg/kg of n-hexane, dichloromethane, ethyl acetate, and methanol fractions respectively, group 8 was given 5 mg/kg of chloroquine (positive control) and group 9 was given 10 mL/kg of distilled water (negative control) for four consecutive days (D_0-D_3) between 8am to 9am. On the fifth day (D_4), thin film smears were made from the tail blood of each mouse. The films were stained with Giemsa stain to reveal parasitized erythrocytes out of 500 in a random field of the microscope. The average suppression of parasitemia was calculated as follows:

$$Average\ suppression\ of\ parasitaemia = \frac{\%\ parasitaemia\ positive\ control - \%\ parasitaemia\ negative\ control}{\%\ parasitaemia\ negative\ control}$$

The mean survival time of the mice in each treatment group was determined over 29 days (D_0-D_{28}), as follows:

$$MST = \frac{No\ of\ days\ survived}{total\ no.\ of\ days\ (29)} \times 100$$

Evaluation of prophylactic activities of the leaf extract and fractions of Setaria megaphylla
This was evaluated using the method described by Okokon et al.,[12]. The mice were randomly divided into nine groups of 5 mice per group. Groups 1-3 were given 200, 400, and 600 mg/kg of crude extract respectively, groups 4, 5, 6, and 7 were given 400 mg/kg of n-hexane, ethyl acetate, dichloromethane, and methanol fractions respectively, group 8 was given 1.2 mg/kg of pyrimethamine (positive control) and group 9 was given 10 mL/kg of distilled water (negative control). Administration of the extract and fractions continued for three consecutive days (D_0-D_2). On the fourth day (D_3), the mice were inoculated with 0.2 mL of infected blood containing about 1 x 10^7 *P. berghei* parasitized erythrocytes. The parasitemia level was assessed by blood smears 72 hours later. The mean survival time of the animals was calculated over 29 days.

Evaluation of the curative activities of the leaf extract and fractions of Setaria megaphylla
This test was used to evaluate the schizontocidal activity of the extract, fractions and chloroquine in established plasmodial infection. This was conducted according to the methods described by Evinemi et al.,[13]. Ninety (90) mice were injected intraperitoneally with 0.2 mL of infected blood containing about 1 x 10^7*P. berghei* parasitized erythrocytes on the first day *(D_0)*. Seventy-two hours later (D_3), the mice were divided into nine groups of ten mice per group. Groups 1-3 were given different doses of extract, 200, 400, and 600 mg/kg respectively, groups 4-7 were given 400 mg/kg of n-hexane, ethyl acetate, dichloromethane, and methanol fractions respectively. Group 8 was given 5 mg/kg chloroquine (positive control) and group 9 was given 10 mL/kg distilled water (negative control). The crude extract, fractions and chloroquine were administered once daily for 5 days. Giemsa stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor the parasitemia level. The rectal temperature of the mice was taken on days 0, 3, 5, and 7 to monitor changes in the body temperature of the mice. On the sixth day, five mice from each group were sacrificed under light diethyl ether vapour. Blood samples were collected by cardiac puncture into EDTA bottles and used for haematological analysis. The mean survival time (MST) of the mice in each group was determined over 29 days (D_0-D_{28}).

Hematological study
Blood samples collected from each mouse into ethylene diamine tetra-acetic acid (EDTA) – coated sample bottles were used to determine the effect of the extract/fractions on hematological parameters such as Red blood cell count (RBC), hemoglobin, (Hb), packed cell volume (PCV), platelet concentration (PLC) and total and differential white blood cell count (WBC). These parameters were analyzed using automatic hematological system (Sysmex Hematology – Coagulation system, Model MO-1000 I, Trans Asia, Japan).

Gas chromatography-Mass spectrometry analysis
GC-MS was carried out on ethyl acetate fraction on an Agilent 7890A gas chromatograph, coupled with an Agilent MS model 5975C MSD with triple axis detector (Agilent Technologies, USA). The system was equipped with a HP5-MS column 5 % phenyl-methyl polysiloxane, 30 m × 0.25 mm × 0.25 µm (Agilent Technologies, USA). The carrier gas was helium with a gas flow under a constant pressure of 10 psi. The injector temperature was set at 280 °C. The initial oven temperature was 160 °C and increased to 320 °C at 10 °C/min, and the final temperature was held for 6min at 320 °C. The mass spectrometer was operated in the electron ionization mode at 70 eV. The 0.2-0.4 mg of each compounds were dissolved in $CHCl_3$ (5 mg/mL) to attain a final concentration of 5 mg/ml for 20min at room temperature before injection of 1 uL to GC-MS system.[5] The compounds were identified by comparison of spectral data and fragmentation pattern with reference compounds in the NIST 2011 database.

Statistical analysis
Data obtained from this work were analysed statistically using ANOVA (one –way) followed by a post test (Tukey-Kramer multiple

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

comparison test). Differences between means were considered significant at a 5% level of significance ie $p \leq 0.05$

Results and Discussion

The percentage yields of the extract and fractions were; crude-7.38%, *n*-hexane- 0.15%, DCM -0.31%, ethyl acetate -0.24%,methanol -1.25%
The results of the suppressive activity of the leaf extract are shown in Table 1. The extract showed a dose-dependent and significant ($p<0.001$) reduction in parasitaemia levels of the treated mice at the different doses employed when compared to the control group in the study. The chemosuppression percentages were; 24.29, 30.76 and 46.94% for 200, 400 and 600 mg/kg of extract respectively. The standard drug, chloroquine showed relatively higher chemosuppression (92.78%).
The leaves fractions (n-hexane, DCM, ethyl acetate and methanol; 400 mg/kg); exerted prominent reductions in parasitaemia levels of the treated mice. The chemosuppressions were 11.12, 23.96, 20.81 and 41.00% for n-hexane, dichloromethane, ethyl acetate and methanol respectively. There was a significant ($p<0.05$-0.001) decrease in parasitaemia levels of groups treated with DCM, ethyl acetate and methanol fractions when compared to the control with the methanol fraction showing the highest activity. This was however lower than that of chloroquine, the standard drug as shown in Table 1. The extract and fractions exerted prominent protection to the animals as demonstrated in the significantly ($p<0.05$-0.001) prolonged MST of the treated mice with the highest dose (600 mg/kg) and methanol fraction having higher MST values of 18.6±0.66d and 17.6±0.88d respectively (Table 1).
The leaf extract showed a dose-dependent chemotherapeutic effect in the different doses used in this test with chemosuppressions of 19.20, 24.37 and 26.06% respectively for 200, 400 and 600 mg/kg doses of the extract. The reduction in parasitaemia was statistically significant ($p<0.01$) in all doses when compared to the control, as shown in Table 2, but less than that of the standard drug, pyrimethamine.The leaf fractions (400 mg/kg) showed significant ($p<0.001$) reductions in parasitaemia levels with 11.56±0.89, 10.66±0.85, 15.02±0.65, and 10.46±1.12% for n-hexane, dichloromethane, ethyl acetate and methanol respectively, and111 2.15±0.95 % for pyrimethamine.
There were significant ($p<0.001$) decreases in the parasitaemia level of all the fractions-treated groups compared to the control with the methanol fraction showing the highest activity though less than that of the standard drug, pyrimethamine, as shown in Table 2. The extract and fractions further demonstrated prominent protection on the treated infected mice with dichloromethane and methanol fractions treated group having elongated MST of 26.00±1.04 and26.00±2.00 respectively (Table 2). Progressive reductions in parasitaemia levels of the extract/fractions-treated groups of infected mice were observed in the study from day 0 to day 7 (Figure 1). The extract showed dose-dependent and statistically significant ($p<0.001$) chemotherapeutic effects when compared to control at 400 and 600 mg/kg doses of the extract on day 7. These reductions were, however, less than that of the standard drug (chloroquine) as shown in Figure 1.
Significant decreases ($p<0.05$-0.001) in parasitaemia levels of the infected mice were also observed on day 7 following treatment with the fractions (400 mg/kg) with ethyl acetate fraction showing the highest activity, compared to the control, though lower than that of the standard drug (chloroquine), as shown in Figure 1. There was a dose-dependent increases in the mean survival time of the leaf extract-treated groups. The extract increased the MST from 12 to 16 days when compared with the control. However, it was shorter when compared to that of the standard drug, chloroquine (28.50 days) as shown in Table 3.The leaf fractions (400 mg/kg) increased the MST from 12-22 days with the ethyl acetate fraction showing the highest activity compared to the control but lower than that of chloroquine the standard drug (28.50 days) as shown in Table 3.Administration of the leaf extract and fractions as well as chloroquine did not cause any significant difference ($p>0.05$) in the rectal temperatures of the treated mice when compared with that of control on days 5 and 7 (Table 4). Administration of the leaf extract and fractions of *S. megaphylla* to *P. berghei*-infected mice caused significant ($p<0.05$-0.001) increases in RBC, lymphocytes and platelets counts, Hb concentration and PCV percentages and MCH levels when compared to untreated infected mice though non-dose dependently. While the elevated WBC counts, neutrophils percentages, MCV and MCHC percentages in the untreated infected animals were reduced significantly ($p<0.05$-0.001) in the treated infected animals when compared statistically (Table 5).The results of GCMS analysis of the ethyl acetate fraction revealed that it contains some bioactive compounds such as (E)-β-ocimene, P-cymene, D:A-friedooleannan-3-ol,(3a)-, Bicyclo[2.2.1] heptan-2-ol,4,7,7 trimethyl, Stigmastone-3,6-dione,(5a)- and Phenol 2,6-dimethoxy among others Table 6).The leaves of *S. megaphylla* are used in Ibibio traditional medicine as malaria remedy and this work was designed to confirm and authenticate its antimalarial potential to provide the scientific basis for its usage as an antimalarial plant.The leaf extract and fractions of *S. megaphylla* were investigated for antimalarial activity against rodent malaria parasite, *P. berghei* infection in mice using standard *in vivo* models. It was found that the extract and fractions significantly reduced the parasitaemia in prophylactic, suppressive and curative models in a dose-dependent fashion with methanol fraction exhibiting the highest suppressive and prophylactic activities, while ethyl acetate fraction followed by DCM fraction exerted the highest curative effect confirming the antimalarial potential of this extract. These varying potencies of the fractions could have resulted from the activities of the various phytochemical constituents of each fraction. However, based on the classification of Tchatat *et al*,[14] their activities cannot be considered to be very good. The extract and fractions also prolonged the MST of the mice suggesting that they were able to offer a certain degree of protection to the mice. This activity could have resulted from plasmodicidal or plasmodistatic activity of the extract and fractions as reported earlier by Okokon *et al*.[4] The results of this study confirm and corroborate earlier report by Okokon *et al*,[3] in which significant antimalarial activity was preliminarily reported on the leaves extract, and also provide information on the most active fraction(s) where the active principles may likely be localised.

Table 1: Suppressive activities of leaf extract and fractions of *S. megaphylla* during early *Plasmodium berghei* infection in mice

Treatment	Dose (mg/kg)	Parasitaemia	Chemosuppression (%)	MST
Control	-	29.12 ±0.56	-	13.3 ±0.98
Extract	200	22.04±1.20^{c}	24.29	16.0±0.57^{a}
	400	20.16±0.98^{c}	30.76	16.66±0.33^{c}
	600	15.45±1.15^{c}	46.94	18.6±0.66^{c}
n-hexane	400	25.88 ±1.54	11.12	14.02±0.33^{b}
Dichloromethane	400	22.14±0.33^{a}	23.96	15.0±0.57^{b}
Ethyl acetate	400	23.06±0.96^{c}	20.81	14.6±0.60^{c}
Methanol	400	17.18 ± 0.78^{c}	41.00	17.6±0.88^{b}
Chloroquine	5	2.10 ± 1.38^{c}	92.78	30.00 ±0.00^{c}

Values are expressed as mean ± SEM. Significant relative to control. $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$. n = 6

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Prophylactic activities of leaf extract and fractions of *S. megaphylla*

Treatment	Dose (mg/kg)	Parasitaemia	Chemosuppression (%)	MST
Control	-	20.10 ± 0.86	-	10.33 ± 0.33
Extract	200	17.24 ± 1.26[b]	19.20	11.33 ± 0.33
	400	15.20 ± 1.42[b]	24.37	12.0 ± 0.57
	600	14.86 ± 1.50[b]	26.06	13.33 ± 0.66
n-hexane	400	11.56 ± 0.89[c]	42.48	19.30 ± 1.09
Dichloromethane	400	10.66 ± 0.85[c]	46.96	26.00 ± 1.04[a]
Ethyl acetate	400	15.02 ± 0.65[c]	25.27	12.66 ± 0.33
Methanol	400	10.46 ± 1.12[c]	47.96	26.00 ± 2.00[a]
Pyrimethamine	1.2	2.15 ± 0.95[c]	89.30	25.04 ± 0.29[c]

Values are expressed as mean ± SEM. Significant relative to control. [a]p<0.05; [b]p<0.01; [c]p<0.001. n = 6.

Table 3: Mean survival time of mice treated with leaves extract and fractions of *S. megaphylla* during established *Plasmodium berghei* infection in mice

Treatment	Dose (mg/kg)	Mean Survival Time (Days)
Control	-	12.50 ±0.28
Extract	200	12.75±0.75[b]
	400	14.50±0.28[c]
	600	16.75±1.43[c]
n-hexane	400	16.75±2.17[c]
Dichloromethane	400	14.05±0.75[c]
Ethyl acetate	400	22.66±4.34[c]
Methanol	400	17.75±1.03[c]
Chloroquine	5	28.50±1.19[c]

Values are expressed as mean ± SEM. Significant relative to control. [a]p<0.05; [b]p<0.01; [c]p<0.001. n = 6.

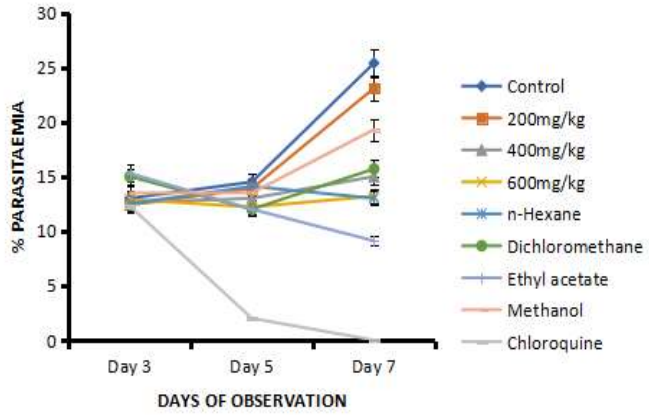


Figure 1: Effect of leaf extract and fractions of *Setaria megaphylla* on established *Plasmodium berghei* infection in mice

Thus, validating the use of the leaves extract decoction as malarial remedy. However, it was observed that lower chemosuppression percentages were recorded especially in the suppressive model in this study compared to previous reports; this could have been due to different seasons of collection of the plant materials which might have affected the composition of the phytochemical constituents. However, *in vitro* studies by Okokon *et al.*[4] carried out to evaluate the activities of the leaf extract and fractions against human malaria parasite, chloroquine-resistant strain of *P. falciparum* (*Pf*3D7) and chloroquine-resistant strain (*Pf* INDO), had reported the highest activity against both strains of *P. falciparum* from ethyl acetate fraction with IC_{50} of 8.15±1.10 µg/mL (Pf3D7) and 8.94±1.26 µg/mL (PfINDO). Chloroform fraction was found to be more active against Pf INDO with IC_{50} of 8.05±0.12 µg/mL (PfINDO), probably suggesting the localization of the active compounds in these fractions. However, ethyl acetate fraction was outstandingly more active during the *in vitro* test corroborating the *in vivo* studies in which ethyl acetate was most active in curative test, while methanol fraction was the most active in suppressive and prophylactic tests. The findings of the *in vivo* study corroborate the previous report of *in vitro* activity.[4] The slight variation in the activities of the fractions suggests the involvement of immunostimulating activity which may be due to the phytochemical constituents in these fractions especially those that were active during *in vivo* study. The reported phytochemical screening and GC-MS analysis of *n*-hexane, DCM, and ethyl acetate fractions revealed the presence of some pharmacologically active compounds such as tannins, flavonoids, alkaloids, terpenes, triterpenes like squalene, phenolics, β-sitosterol and polyunsaturated fatty acids (PUFAs), (E)-β-ocimene, P-cymene, D:A-friedooleannan-3-ol,(3a)-, Bicyclo[2.2.1] heptan-2-ol,4,7,7trimethyl, Stigmastone-3,6-dione, (5a)- and Phenol,2,6-dimethoxy among others.[4,7] These compounds are likely to be responsible for the observed activities of the extract and fractions. Some secondary metabolites of plants such as alkaloids, flavonoids and triterpenoids have been reported previously to have antiplasmodial properties.[15] Polyunsaturated fatty acids such as hexadecanoic acid, methyl ester, 9,12-octadecadienoic acid methyl ester (linoleic acid), 9,12,15-octadecatrienoic acid, methyl ester (linoleic acid), and 9-octadecenoic acid have been found in the active antiplasmodial fraction especially n-hexane fraction and other fractions. These PUFAs mentioned above have been implicated in antiplasmodial activity and this activity has been reported to increase with the degree of unsaturation.[16] Also, an earlier study by Okokon et al.[7] had shown the presence of ϒ-Elemene, Urs-12-ene, Bicyclogermacrene, α-muurolene, Germacrene- A, and Guaiol in the leaf fractions, which are mono and sesquiterpene compounds implicated in antiplasmodial activity of many plants.[17] These compounds mentioned above to be present in the extract and the active fraction maybe responsible for the observed antiplasmodial activities.

The findings of this study further suggest that leaf extract and fractions of *S. megaphylla* possess antimalarial activity which is due to the activities of its phytochemical constituents. This confirms and authenticates its use as malarial remedy in folkloric medicine. Fever is one of the cardinal symptoms of malaria especially in humans.

However, *P. berghei* infection in mice is reported to be associated with hypothermia rather than pyrexia[18].

Results of rectal temperatures of the infected mice in this study (curative test), showed that there was no significant difference between the mean temperature values of both the treated and untreated infected mice before and after treatment, suggesting that the mice were hypothermic.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 4: Effect of leaf extract and fractions of *Setaria megaphylla* on rectal temperatures of mice infected with *Plasmodium berghei* during established infection

Treatment	Dose (mg/kg)	Rectal Temperature (˚C)			
		D0	D3	D5	D7
Control	-	34.86 ± 0.46	35.22 ± 0.02	35.22 ± 0.02	35.75 ± 0.06
Extract	200	35.47 ± 0.07	35.52 ± 0.04	35.53 ± 0.02	35.42 ± 0.07
	400	35.47 ± 0.07	35.43 ± 0.02	35.33 ± 0.02	35.30 ± 0.04
	600	35.50 ± 0.04	35.32 ± 0.02	35.32 ± 0.02	35.23 ± 0.02
n-hexane	400	35.47 ± 0.04	35.40 ± 0.02	35.30 ± 0.02	35.50 ± 0.04
Dichloromethane	400	35.52 ± 0.04	35.30 ± 0.06	35.40 ±0.04	35.33 ± 0.06
Ethyl acetate	400	35.52 ± 0.02	35.45 ± 0.04	35.33 ±0.06	35.30 ± 0.06
Methanol	400	35.00 ± 0.20	35.30 ± 0.10	35.30 ± 0.10	35.31 ± 0.04
Chloroquine	5	35.18 ± 0.27	35.37 ± 0.07	35.37 ± 0.07	35.22 ± 0.08

Values are expressed as mean ± SEM.

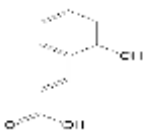
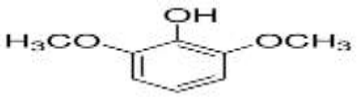
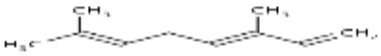
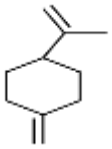
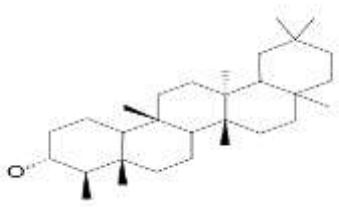
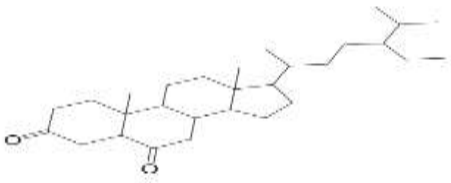
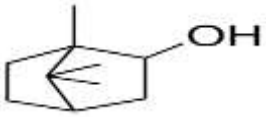
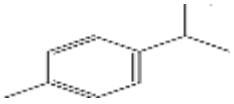
TABLE 5: Effect of *Setaria megaphylla* leaf extract/fractions on haematological parameters of *P. berghei*-infected mice

Treatment	WBC ($x10^9$/L)	LYM ($x10^9$/L)	NEUT ($x10^9$/L)	RBC ($x10^{12}$/L)	HGB (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT ($x10^9$/L)
Control	15.70 ± 4.30	32.53 ± 2.45	65.57 ± 1.52	5.34 ± 0.35	8.43 ± 0.96	38.07 ± 2.43	75.07 ± 0.62	11.83 ± 0.09	30.13 ± 0.33	454.33 ± 64.32
Extract(200mg/kg)	6.46 ± 0.90^c	54.85 ± 10.5^c	39.95 ± 11.5^c	7.62 ± 0.38	10.75 ± 1.25	41.85 ± 2.85	54.85 ± 0.95^b	14.05 ± 0.95	25.60 ± 1.30	674.50 ± 86.5^a
Extract (400mg/kg)	7.95 ± 1.78^b	40.0 ± 2.60	54.80 ± 1.78	8.24 ± 0.3^a	12.70 ± 0.10	49.0 ± 0.60^a	49.0 ± 0.60^b	19.50 ± 1.70^a	25.95 ± 0.55	696.01 ± 83.0^a
Extract (600mg/kg)	5.66 ± 0.06^c	51.15 ± 14.5^a	45.0 ± 14.60^b	7.66 ± 0.31	12.95 ± 0.15^a	54.55 ± 6.35^b	71.65 ± 11.0	17.0 ± 0.90	24.10 ± 2.50	968.56 ± 86.5^b
Hexane fraction	10.96 ± 2.88^a	45.90 ± 3.90^a	49.70 ± 3.80^b	8.41 ± 1.2^a	13.50 ± 0.20^a	50.50 ± 2.45^a	61.20 ± 11.0^a	16.40 ± 2.60	27.0 ± 1.00	673.0 ± 53.00^a
Dichloromethane fraction	6.89 ± 0.90^b	51.2 ± 6.30^a	44.20 ± 6.40^c	8.29 ± 0.0^a	12.65 ± 0.15^a	48.55 ± 1.85^a	58.50 ± 2.90^b	15.30 ± 0.40	26.05 ± 0.75	702.5 ± 32.50^b
Ethyl acetate fraction	12.26 ± 2.83	41.30 ± 2.70	53.0 ± 1.60^a	8.60 ± 0.7^b	14.55 ± 2.05^a	59.90 ± 9.10^b	69.35 ± 4.55	16.90 ± 0.90	24.40 ± 0.30	539.0 ± 11.00
Methanol Fraction	9.06 ± 4.30^a	44.85 ± 1.15	60.15 ± 3.05	7.47 ± 0.17	10.30 ± 0.20	40.90 ± 3.50	54.95 ± 5.95^b	13.80 ± 0.60	25.30 ± 1.60	1065.0 ± 25.0^c
Chloroquine	9.58 ± 1.39	40.66 ± 0.90	46.34 ± 0.39	8.53 ± 0.2^a	12.94 ± 0.70^a	48.72 ± 1.22^a	59.40 ± 0.84^b	18.88 ± 0.27^a	25.68 ± 0.71	631.40 ± 44.7^a

All values are presented as mean±S.E.M. for six rats in each group.compared with control group ^{a} $p<0.05$, ^{b} $p<0.01$, ^{c} $p<0.001$.WBC-white bllod cell, RBC-Red blood cell, HGB-Hemoglobin, PLT-Platelets, PCV-Packed cell volume, LYM-Lymphocytes, NEUT-Neutrophils, MCH-Mean corpuscular hemoglobin, MCV-Mean copuscular volume, MCHC-Mean corpuscular hemoglobin concentration.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

TABLE 6: GC–MS analysis of ethyl acetate fraction of *Setaria megaphylla*

S/No.	Name of compound	Retention Index	Mol.wt g/mol	Chemical formula/Chemical Structure
1.	2-propenoic acid, 3-(2 hydroxy-phenyl,(E)-	101	164	 $C_9H_8O_3$
2.	Phenol,2,6-dimethoxy	280	154	 $C_8H_{10}O_3$
3.	(E)-β-ocimene	810	136	 $C_{10}H_{16}$
4.	P-metha-1(7),8-diene	999	136	 $C_{10}H_{16}$
5.	D:A-friedooleannan-3-ol,(3a)-	1011	428	 $C_{10}H_{16}$
6.	Stigmastone-3,6-dione,(5a)-	1012	428	 $C_{29}H_{48}O_2$
7.	Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethyl	282	154	 $C_{10}H_{18}O$
8.	P-cymene	1012	134	 C_10H_{14}

This hypothermia in mice may have resulted from the serious physiological and metabolic effects of the malaria parasite on the host, leading to body heat loss and ultimately death of mice[19]. The extract/fractions however, were unable to attenuate these processes and hence the resultant hypothermia. Alterations in hematological indices of the infected mice such as decreases of RBC count, Hb level, PCV, and mean haemoglobin concentration levels were observed in infected animals which are common signs of anaemia.[20] The invasion of host's RBC by *Plasmodium* during malaria infection, shortens the lifespan of RBC through the digestion of Hb using glucose, oxygen and hemozoin formation and finally the bursting of the erythrocytes during the development of their asexual blood stage. [21] The treatment of animals with the leaf extract and fractions significantly improved the haematological parameters, portraying the leaf extract and fractions potentials to inhibit the parasites growth as confirmed by the decreased parasitaemia and thus, protecting animals from death. WBC was observed to increase significantly in the infected mice. This can be attributed to the immunogenic effects induced by the parasite and their pigment (hemozoin).[22] Decreased parasitaemia due to extract/fractions treatment caused corresponding reduction in WBC. The platelets counts of the treated infected mice were significantly improved compared to untreated *P. berghei*-infected mice. The treatment of infected mice with the *S. megaphylla* extract and fractions must have offered some degree of protection to the infected mice from the immune cells and platelets dysregulation, through the activities of its phytochemical constituents.

Conclusion

The results of this study show that the leaf extract and fractions of *Setaria megaphylla* possess antimalarial potentials which maybe attributed to the activities of its phytochemical constituents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors are grateful to Mr. Nsikan Malachy of Pharmacology and Toxicology Department for providing technical assistance.

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