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Original Research Article

Determination of Antimalarial Properties of the Extract and Fractions of *Brenania brieyi* (*Rubiaceae*) Root *In-Vivo* Using Curative Model

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ABSTRACT

Brenania brieyi (Rubiaceae) root is a medicinal plant, which has been widely used in ethno-medicine due to its antimalarial properties. The study aimed to evaluate the antimalarial potentials of *B. brieyi* root. Aqueous methanol extract of the roots of *Brenania brieyi* was prepared using cold maceration and dried *in vacuo* at 40 °C. The extract was fractionated using solvent-solvent partitioning with different solvents of varying polarity (*n*-hexane, ethyl acetate and methanol). Phytoconstituents of the extract and fractions were determined using standard procedures while the acute toxicity testing were carried out according to Lorkes method. Curative (8-day) test was employed for the antimalarial activity and the hematological parameters were also determined using standard procedures. Gas chromatography-mass spectroscopy (GC-MS) hyphenated technique was used to investigate volatile compounds present in the *n*-hexane fraction. Phytoconstituents present includes alkaloids, tannins, flavonoids, glycosides, carbohydrates, saponins while terpenoids is absent. Extracts at 200 and 400 mg/Kg dose levels caused a significant ($p<0.05$) comparable increase in parasitemia clearance of 86.36 % and 86.52 % respectively as opposed to 83.91 % clearance obtained from the standard (Arthemeter-lumefantrine). Ethyl acetate (87.89 %) and methanol (87.65 %) fractions gave higher clearance than Arthemeter-lumefantrine. The extract and fractions showed sufficient red blood cell restoration and reduction of white blood cell levels post treatment. GC-MS result shows the presence of twenty phyto-compounds, these scientific findings support the traditional use of *Brenania brieyi* root in management of malaria, for which the plant is known and used for.

***Keywords*:** Antiplasmodial activity, Artemisinin, *Brenania brieyi*, Hematological parameters, Parasitemia, *Plasmodium berghei*.

Introduction

Malaria is one of the deadliest disease affecting more than 200 million people yearly in the tropics.[1] Endemic to the tropics, especially Africa, it claims more than 400,000 lives yearly, especially among children and the elderly.[1] The genus, plasmodium is the protozoan responsible for malaria infection.[1] *P. falciparum, P. vivax, P. malariae, P. knowelesi* and *P. ovale* are the species implicated in human infection. In sub-Saharan Africa, *Plasmodium falciparum* is responsible for most deaths from malaria while *P. vivax* accounts for close to half of the malaria burden in South and East Asia.[2] Infection with malaria can lead to several clinical manifestations ranging from mild headaches to severe organ damage which may ultimately lead to death.[3] The major challenge facing the attempts to the eradication of malaria has been drug resistance.[4]

Plants are yet to be fully explored despite the fact that close to 80 % of the African populace employ plant remedies in different forms in the management of various illnesses.[5]

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With the evolving nature of the malarial plasmodium coupled with the increasing resistance to conventional therapies, there has been a renewed interest among researches to seek cheaper and less toxic alternatives from the rich plant biodiversity.

Brenania brieyi is an important plant in the African herbal practice especially in some parts of West Africa (mainly southern Nigeria) and central Africa up to Zaire. It is commonly called 'Mgbunsi' in Abagana, Njikoka Local Government Area of Anambra State, Nigeria and has been enlisted in the botanical inventory of Southern Nigeria[6] and Southeastern Cameroon.[7] It belongs to the family of *Rubiaceae* and grows up to 30 m height. Anthelmintic,[8] estrogenic,[9] and antibacterial activities[10] have been reported. Traditionally, its roots have been used as an effective remedy against malaria.[11]

Different approaches are used to determine new biologically active ingredients in medicinal plants for the preparation of safe drugs.[12] Evaluation of plants can be effectively analysed using these analytical tools such as High Performance Liquid Chromatography with UV (DAD), Gas Chromatography-Mass Spectroscopy (GC-MS), High Performance TLC-densitometry, Fourier Transform InfraRed (FTIR), Nuclear Magnetic Resonance (NMR) or a combination of these.[13,14] GC-MS technique is best used for quantitative and qualitative analysis of volatile compounds of plant origin.[15] The study aimed to evaluate the antimalarial potential of the roots of *B. brieyi in vivo* and to determine the phyto-compounds present.

Materials and Methods

Plant collection

Fresh roots of *B. brieyi* were collected in March, 2020 from Abagana in Njikoka Local Government Area of Anambra State Nigeria by Mr. Felix Nwafor a taxonomist with the Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka. The plant was

authenticated and voucher specimen: (PCG/UNN/0327) was deposited at the herbarium of the same department. The roots of *B. brieyi* were washed, cut into pieces, air dried and then pulverized into powder and kept in amber-coloured bottles before the extraction. Figure 1 shows *B. brieyi* in its natural habitat.

Reagents
All the chemicals, solvents and reagents used for the extraction were of analytical grade. *n*- Hexane (JHD UN1208, China), Ethyl Acetate (JHD UN1173, China) and Methanol (GHTECH, Guangdong Guanghua Sci-Tech CO. ltd, China), Artemeter/Lumefantrine (AC Drugs Ltd, Enugu Nigeria), microscope (XSZ – 107BN, India), counting chamber (MC Qiujing, China), micro – pipettes (Perfect, USA), rotary evaporator (Model 349/2, Corning Ltd, England), micro hematocrit centrifuge (Model SH120-1, England), slide, beaker, malaria parasites, mice, separating funnel, soxhlet apparatus, GC-MS analyzer (GC-MS-QP 2010 plus Shimadzu, Japan).

Extraction of plant material
The powdered roots of *B. brieyi* (2400 g) were thoroughly extracted with many portions (7.5 L) of 95 % v/v methanol using cold maceration for 72 h with repeated agitation and changing the solvent intermittently. The mixture was filtered using muslin and Whatman filter paper no. 1. The residue was reextracted for another 72 h twice and filtered. The extract was allowed to dry *in vacuo* at 40 ºC. [12,15]

Solvent fractionation of crude extract
N-hexane, ethyl acetate and methanol was used in preparing the fractions. The crude extract (150 g) was dispersed in 95 % v/v methanol. For the fractionation procedure, 300 ml of *n*-hexane, 3.8 L of ethyl acetate and 6.0 L of methanol were used successively to partition the dispersed crude extract using a separating funnel. The fractions were air dried and then weighed.[15]

Experimental Animals
Sixty (60) Swiss mice weighing 16-25 g and between 6-7 weeks old were used for this study. The animals were bred and kept in the animal house, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Nigeria, and Nsukka. Ethical clearance was obtained from the ethical committee board, Veterinary Medicine, University of Nigeria Nsukka: FPSRA/UNN/22/0081. The animals were acclimatized for seven (7) days in standard laboratory cages under natural illumination (12 h dark and light cycle) and given access to food and water freely. The animals were treated and taken care of in strict compliance with the guidelines on the care and use of laboratory animals under good laboratory practices.[16]

Parasites
The parasite used in this study was *Plasmodium berghei*, NK 65. It was obtained from National Institute for Medical Research (NIMR) Lagos, and kept at the Department of Veterinary Medicine, University of Nigeria Nsukka in an infected mouse. The parasites were maintained by continuous intra-peritoneal re-introduction into healthy mice every week to sustain the parasite infection.[17]

Acute Toxicity Testing
The acute lethal toxicity test of the crude plant extract of roots of *B. brieyi* was carried out, with a little modification; using the method as described by Lorkes.[18] Twelve animals were used for the study. The study was done in two phases instead of the standard three phases. In the first stage, nine (9) animals were divided into three groups of three animals each (n=3), with each group receiving 10, 100 or 1000 mg/Kg of the crude extract per oral. The animals were observed for any signs of toxicity and or lethality at 30 minutes interval for the first 4 h, for the next 24 h. In the second stage, three groups of one animals each were administered, 1600, 2900, 5000 mg/Kg of the crude extract per oral. Signs of toxicity and mortality were also observed in the second group. LD_{50} is the geometric mean of the highest non-lethal dose and the least toxic dose.

Figure 1: *Brenania brieyi* in its natural habitat.

Qualitative Phytochemical Tests
Tests to identify possible phytochemical constituents were done using standard procedure as described by Yadav and others.[19-21] The extracts and fractions of roots of *B. brieyi* were screened for tannins, saponins, glycosides, terpenoids, phenols, steroids, alkaloids, flavonoids and carbohydrates.

Parasite Inoculation
Parasitized erythrocytes were obtained from the tail of a donor mouse using heparinized syringes. The inoculum were diluted with normal saline to obtain a standard blood suspension containing 1 x 10^6 parasitized erythrocytes. Each mouse was administered a 0.2 ml of dilution through the intra-peritoneal route.[22]

Experimental Design and Grouping of Animals
The curative or Rane's test on the crude extracts and fractions of *Brenania brieyi* was carried out using the method described by Kolhatkar & Ochei (2008)[23] and also applied in Ryley J F& Peters (1970). [24] Fourty-five (45) animals divided into nine (9) groups of five animals (n=5) each were used in the study. The crude extract was tested at three dose levels (100, 200 or 400 mg/Kg) while the other three fractions used only one dose level (200 mg/Kg). Three control groups namely: normal (animals not infected and untreated), standard (animals infected and treated with artemether-lumefantrine 7mg/Kg) and negative (animals infected and treated with 0.2 ml normal saline).

Antimalarial screening
On Day 0, blood samples were collected into EDTA bottles from the medial cantus of the infected mice. They were subsequently inoculated, except for the normal group, with 0.2 ml of the standard dilutions of the *Plasmodium berghei* infected erythrocytes. Seventy-two (72) h later (Day 3), blood samples were again collected from the animals in all the groups and then treatment initiated except for the normal group. Treatment was continued for four days (Till Day 7). On Day 8, blood samples were finally collected from all the groups. Their rectal temperatures were taken on Day 0, then 72 hours after infection and then daily during the treatment course.

Study Parameters
The degree of infection was assessed using five different parameters: Parasitemia level, Mean Survival Time (MST), Packed Cell Volume (PCV), Red Blood Cell (RBC) count and White Blood Cell (WBC) count.

Parasitemia level and Parasitemia clearance
Parasitemia was monitored with a slight modification to the method already described in Ihekwereme *et al.* [22] Blood samples was collected on Day 3 and Day 8 from the medial cantus of the eyes of the animals by the aid of a capillary tube. Thin smears of the blood were made on the glass slides and allowed to air dry; then fixed with methanol for a minute and stained with Leishman stain for 30 minutes. Washing off the film with tap water, it was viewed in an oil immersion field at ×100 magnification. The number of parasitized erythrocytes out of at least

100 erythrocytes in 10 different random fields was counted. The percentage parasitemia was calculated as shown below:

$$Parasitemia(\%) = \frac{Number\ of\ parasitized\ RBC}{Total\ Number\ of\ RBC} \times 100$$

The percentage parasitemia clearance was then calculated using the formula:

$$Parasitemia\ Clearance(\%) = \frac{\%\ Parasitemia\ in\ Negative\ Control - \%\ Parasitemia\ in\ Test}{\%\ Parasitemia\ in\ Negative\ Control} \times 100$$

Determination of Packed Cell Volume (PCV), Red Blood Cells (RBC) and White Blood Cells (WBC)

All determinations were made by following the methods already described in Ochei & Kolhatkar.[24] Heparinized capillary tubes were used to take up blood samples and one end sealed with plasticine. The filled tubes were placed in a micro hematocrit centrifuge and spun at 10,000 rpm for 5 minutes. The tubes were read off on a specially designed scale and the PCV calculated as in below:

$$PCV(\%) = \frac{Packed\ RBC\ column\ height}{Total\ blood\ volume\ height} \times 100$$

The blood specimen was diluted 1:200 with RBC diluting fluid. Using a counting chamber and high-resolution power (40k) objective of a microscope, the cells were counted. The number of cells was calculated as shown below and reported as the number of cells per ml:

$$Total\ RBC = N \times \frac{200}{0.2 \times 0.1}$$

Where N = Number of cells counted

The blood specimen was diluted 1:20 in a WBC pipette with the diluting fluid. Using a counting chamber of 0.1mm in depth, the cells were counted under a low power microscope.

$$Total\ WBC\ (mm^3) = \frac{N \times 20}{0.1 \times A}$$

N = Number of cells counted; A = Area counted

Mean survival time

The animals were monitored for 28 days' post-treatment (Day 8-Day 36). The mean survival time for each group was calculated by:

$$Mean\ Survival\ Time = \frac{Total\ Survival\ Time\ in\ Days}{Number\ of\ animals\ in\ the\ group}$$

GC-MS Analysis

The *n*-Hexane fraction of *B. brieyi* was analyzed with the help of GC-MS analyzer (GC-MS-QP 2010 Shimadzu, Japan) at Shimadzu Training Centre for Analytical Instruments (STC) Lagos. The carrier gas helium (99.999 %) was used at a flow rate of 1 ml per min in split mode (10:1) v/v. n-Hexane fraction (8 µl) were injected into the column at 250 °C injector temperature. Temperature of oven started at 70 °C and held for 5 min. It was then raised at the rate of 10 °C per min to 280 °C without holding. Holding was allowed for 6 min at programmed rate of 5 °C per min. Temperature of ion sources was maintained at 200 °C. The injector temperature was set at 250 °C and detector temperature was set at 250 °C. The mass spectrum (MS) of compounds present in samples was obtained by electron ionization at 70 eV and detector operates in scan mode 50 to 600 Dalton atomic units. The MS table was generated through ACQ mode scan within 0.5 seconds of scan interval at the speed of 666 and a fragment from 30 to 350 Da was maintained. Total running time was 21 minutes.[25]

Statistical Analysis

All results were reported as the Mean ± SEM for all the groups and analyzed using SPSS version 22. The differences within and between groups were analyzed by the method of one-way ANOVA followed by Dunnetts Multiple Comparison post hoc test. P-values < 0.05 were considered statistically significant.

Results and Discussion

The phytochemical screening of *B. brieyi* root revealed the presence of glycosides, flavonoids, alkaloids, saponins, tannins, steroids, phenols, carbohydrates but terpenoids were absent (table 1). Results of PCV determination (table 2) revealed that at 400 mg/Kg of the extract, PCV of the mice increased from 25.75 (Day 3) to 41.75 (Day 7) after treatment. For the fractions the BBEF increased from 27. 00 (Day 3) to 39.20 (Day 7) while BBMF improved from 29.20 (Day 3) to 41.80 (Day 7). There was a dose-dependent increase in the RBC levels from day 3 to day 7. BBEF significantly ($p < 0.05$) increased from 6.60 (Day 3) to 10.55(Day 7) when compared with ACT 6.58 (Day 3) to 10.13 (Day 7). All the treated groups had a significant reduction in the WBC values from day 3 to day 7 while the RBC values rose from day 3 to day 7. The decrease in WBC and increase in RBC levels from day 3 to day 7 for the BBCE treated groups were dose dependent. The result in table 3, revealed that the extract produced a significant ($p < 0.05$) parasitemia clearance in the infected mice which are comparable for the 200 and 400 mg/kg doses. Ethyl acetate fraction produced the highest parasitemia clearance (87.89 %) followed by methanol fraction (87.65 %) and *n*-hexane fraction (36.55 %) respectively. The mean survival time result revealed that the crude extract at 200mg/kg gave the highest survival time (15.00 days) among all the group treated with the extract. Also, the BBEF (11.25 days) significantly ($p < 0.05$) prolonged the MST of the infected mice (table 3) when compared to other fractions, although the standard drug (Artemeter-lumefantrine at 7 mg/Kg) gave the highest MST (22 days) of all the treatment groups. Table 4, shows the GC-MS analysis result which revealed the presence of twenty (20) phytochemical compounds. The different classes of compound revealed include, alcohols (3), ketones (3), esters (7), fatty acids (5), and carboxylic acids (2).

Phytochemical studies of *Brenania brieyi* root showed the presence of tannins, flavonoids, saponins, carbohydrate, glycosides, alkaloids, steroids, and phenols (Table 1). Many plants and extracts with validated antimalarial activity owe these activities to the phytoconstituents they contain.[26,27] Other antimalarial study include (Odeja *et al.,* 2014),[28] (Ronan *et al*., 2009),[29] (Chukwujekwu *et al*., 2006),[30] (Sheeba *et al*., 2009)[31] and (Adaka *et al.*, 2021).[32] Uzor *et al.*, also reported that alkaloids are the most important phyto-constituents responsible for the antimalarial activity of various plants. [33] *B. brieyi* in this present study is rich in alkaloids across all the solvent extract and fractions (except the BBHF) and may have contributed to the observed antimalarial activity. There were no adverse signs and symptoms of toxicity observed in the extract treated mice. The extract could thus be considered as relatively safe according to the Lorke's classification of acute toxicity.

The PCV values for normal control remained same throughout the period of observation. All the treated groups had a significant rise in PCV value from day 3 to day 7. Anemia is the most common problem associated with malaria infection. Anemia was observed in all the groups on day 3 and this may be due to RBC destruction sequel to parasite multiplication associated hemolysis or the action of the spleens reticulo-endothelial system.[32] There was no significant difference between the PCV and RBC of the normal control on day 3 and day 7 (after treatment), this clearly shows that the plant has no intrinsic hematopoietic effect. Thus the rise in RBC and PCV levels back to normal observed in other groups (1-7) may be due to the receding parasitemia. The ability of the plant extract treated groups to restore the PCV and RBC levels to normal also suggest that the mechanism of action of this plant could be through inhibition of plasmodia proliferation and multiplication in the red cells during the erythrocytic cycle.[32]

The alteration in the WBC levels of mice including humans infected with plasmodia parasite is a well-documented across literature.[34, 35] The initial rise in WBC levels indicates a defense response to counter infection. On treatment, there was a significant reduction in the WBC levels which very well correlates with the receding parasitemia. There was a dose-dependent decrease in WBC levels in the BBCE treated groups (groups 1 to 3 (Table 2)). The significant ($p<0.05$) decrease in WBC displayed by the infected-standard-treated and infected-extract-treated mice indicates an improved ability of the drug extract and standard to combat the infection.

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Table 1: Phytochemical constituents present in the plant extract and fractions of *B .brieyi* roots

Test	BBCE	BBHF	BBEF	BBMF
Flavonoid	+	-	-	+
Alkaloid	+	-	+	+
Saponins	+	-	-	+
Tannins	+	-	+	+
Steroids	+	+	+	-
Terpenoid	-	-	-	-
Phenols	+	+	+	+
Carbohydrates	+	+	+	+
Glycosides	+	+	+	+

Brenania brieyi crude extract (BBCE); *Brenania brieyi n*-Hexane extract (BBHF); *Brenania brieyi* ethyl acetate fraction (BBEF); *Brenania brieyi* methanol fraction (BBMF).

Table 2: Effects of the different solvent extract and fractions on packed cell volume (PCV), Red Blood Cell (RBC) and White Blood Cell (WBC) of *Plasmodium berghei* infected mice

Group	Treatment (mg/Kg)	RBC (%)			WBC %			PCV (%)		
		Day 0	Day 3	Day 7	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
BBCE	100	10.48 ± 0.52	7.41 ± 0.29*	8.52 ± 0.16^{ab}	9400 ± 260.77	14520 ± 445.42*	13520 ± 376.03^{ab}	42.8 ± 0.86	29.80 ± 1.28*	27.60 ± 0.51^{*ab}
BBCE	200	10.59 ± 0.121	6.38 ± 0.14*	9.41 ± 0.23^{a}	9280 ± 416.41	15580 ± 414.00*****	11660 ± 389.36^{ab}	40.8 ± 0.73	26.80 ± 0.97*	34.80 ± 0.37^{*ab}
BBCE	400	10.62 ± 0.33	6.80 ± 0.28*	10.62 ± 0.14^{a}	9880 ± 351.28	15500 ± 355.90*****	9900 ± 353.55^{a}	43.0 ± 0.89	25.75 ± 1.18*	41.75 ± 1.18^{a}
BBHF	200	10.70 ± 0.27	7.71 ± 0.48*	9.26 ± 0.24^{ab}	9360 ± 413.04	16060 ± 323.42*****	11875± 228.67^{ab}	40.8 ± 1.24	29.80 ± 0.66*	36.25 ± 1.31^{*ab}
BBEF	200	10.98 ± 0.35	6.60 ± 0.26*	10.55 ± 0.12^{a}	9240 ± 229.35	15300 ± 339.12*****	10740± 166.13^{a}	41.80 ± 0.66	27.00 ± 0.89*	39.20 ± 1.24^{a}
BBMF	200	10.78 ± 0.76	7.55 ± 0.45*	10.37 ± 0.34^{a}	9060 ± 242.07	14580 ± 215.41*****	10380 ± 111.36^{a}	42.20 ± 1.11	29.20 ± 1.32*	41.80 ± 0.73^{a}
ACT7	7	10.52 ± 0.61	6.58 ± 0.22*	10.13 ± 0.16^{a}	9300 ± 397.49	15440 ± 358.61*****	10460 ± 227.16^{a}	40.00 ± 0.71	28.40 ± 1.21*	40.60 ± 0.60^{a}
Negative	Nil	10.49 ± 0.79	6.32 ± 0.27*	6.32 ± 0.34	10020 ± 341.17	16020 ± 280*****	16020 ± 181.66	41.80 ± 0.80	29.40 ± 0.51*	29.40 ± 0.51*
Normal	0.5 ml	10.64 ± 0.11	10.85 ± 0.23	10.80 ± 0.18	9960 ± 248.19	10140 ± 235.80	10140 ± 180.00	41.20 ± 0.86	43.40 ± 1.08	43.40 ± 0.73

Brenania brieyi crude extract (BBCE); *Brenania brieyi n*-Hexane extract (BBHF); *Brenania brieyi* ethyl acetate fraction (BBEF); *Brenania brieyi* methanol fraction (BBMF); ACT: Artemisinin Combination Therapy. Values are expressed as mean ± SEM (n=5). $^{*}P < 0.05$ treatment versus normal control, $^{a}P < 0.05$ extract versus negative control, $^{b}P < 0.05$ extract versus standard drug. SPSS version 22, one-way ANOVA was used followed by Dunnetts Multiple Comparison.

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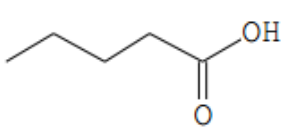
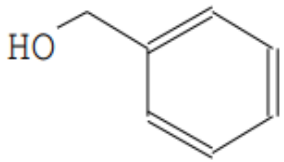
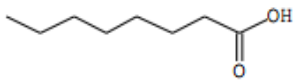
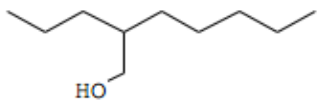
Table 3: Effects of different solvent extract on *B. brieyi* on parasitemia levels and mean survival times of *Plasmodium berghei* infected mice

Groups	Treatment (mg/Kg)	Parasitemia %			Parasitemia Clearance on Day 7 (%)	Mean Survival Time (Days)
		Day 0	Day 3	Day 7		
BBCE	100	0.00	36.00 ± 2.48	18.00 ± 2.18*	43.19b	11.60*b
BBCE	200	0.00	43.00 ± 3.92	5.00 ± 0.68*	86.37	15.00
BBCE	400	0.00	31.00 ± 2.04	4.50 ± 0.50*	86.53	10.00*b
BBHF	200	0.00	38.00 ± 4.51	22.00 ± 0.85*	36.55b	6.80*b
BBEF	200	0.00	38.00 ± 4.06	3.8.00 ± 0.80*	87.89	11.25*b
BBMF	200	0.00	47.00 ± 3.46	6.00 ± 1.34*	87.65	10.75*b
ACT 7	7	0.00	37.00 ± 2.31	6.00 ± 1.30*	83.91	22.00a
Negative	Nil	0.00	38.00 ± 3.30	41.00 ± 4.13	-10.19	8.80
Normal	0.5ml	-	-	-	-	24.00

Brenania brieyi crude extract (BBCE); *Brenania brieyi n*-hexane extract (BBHF); *Brenania brieyi* ethyl acetate fraction (BBEF); *Brenania brieyi* methanol fraction (BBMF); ACT: Artemisinin Combination Therapy. Values are expressed as mean ± SEM (n=5). *P < 0.05 treatment versus normal control, aP < 0.05 extract versus negative control, bP < 0.05 extract versus standard drug. SPSS version 22, one-way ANOVA was used followed by Dunnetts Multiple Comparison.

Table 4: Phyto-constituents identified in the GC-MS *n*-hexane fraction of root extract of *Brenania brieyi*

S/N	Retention Time	Name of Compound	Class of Compound	Molecular Weight	Compound Structure	Peak Area (%)	Molecular Formula	Biological Activity Reported
1	6.085	Pentanoic acid	Carboxylic acids	102		0.30	$C_5H_{10}O_2$	No Reported Activity
2	6.273	Benzyl alcohol	Alcohols	108		0.80	C_7H_8O	No Reported Activity
3	8.167	Octanoic acid	Carboxylic acids	144		0.21	$C_8H_{16}O_2$	No Reported Activity
4	8.483	2-propyl-1-Heptanol,	Alcohols	158		0.10	$C_{10}H_{22}O$	No Reported Activity

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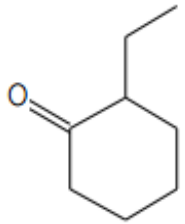
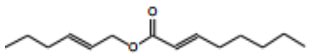
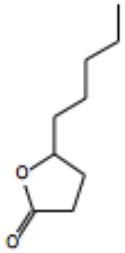
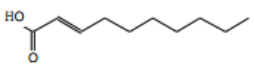
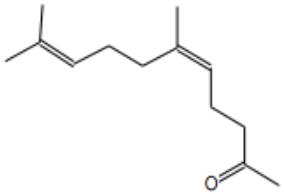
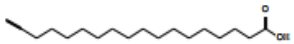
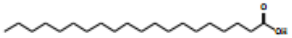
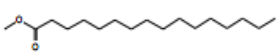
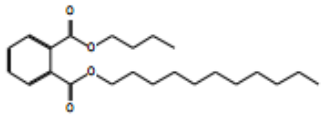
5	8.799	Cyclohexanone, 2-ethyl-	Ketones	126		0.33	$C_8H_{14}O$	No Reported Activity
6	9.525	E-2-Hexenyl E-2-octenoate	Unsaturated esters	224		0.30	$C_{14}H_{24}O_2$	No Reported Activity
7	9.724	2(3H)-Furanone, dihydro-5-pentyl-	Cyclic ketones	156		0.28	$C_9H_{16}O_2$	No Reported Activity
8	10.561	trans-2-Decenoic acid	Unsaturated fatty acids	170		0.45	$C_{10}H_{18}O_2$	Antibacterial, antifungal [37]
9	10.709	5,9-Undecadien-2-one, 6,10-dimethyl-, (Z)-	Ketones	194		0.15	$C_{13}H_{22}O$	No Reported Activity
10	11.055	17-Octadecynoic acid	Unsaturated fatty acids	280		0.25	$C_{18}H_{32}O_2$	Improves renal function [38]
11	11.870	Eicosanoic acid	Long chain saturated fatty acids	312		0.49	$C_{20}H_{40}O_2$	Repair neurons [39]
12	14.627	Hexadecanoic acid, methyl ester	Esters	270		1.59	$C_{17}H_{34}O_2$	Antimicrobial activity, [40] antifungal [41]
13	14.739	Phthalic acid, butyl undecyl ester	Esters	376		0.68	$C_{23}H_{36}O_4$	Antifungal [42]

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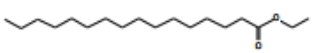
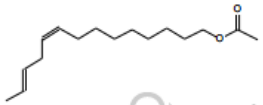
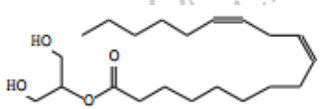
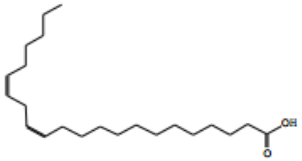
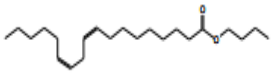
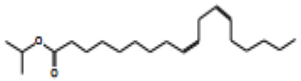
14	15.140	Hexadecanoic acid, ethyl ester	Esters	284		1.23	$C_{18}H_{36}O_2$	Antioxidant, antiandrogenic, hemolytic [43]
15	15.860	9,12-Tetradecadien-1-ol, acetate, (Z,E)-	Esters	252		8.80	$C_{16}H_{28}O_2$	No activity reported
16	16.404	9,12-Octadecadienoic acid (Z,Z)-	Fatty acids	280		14.84	$C_{18}H_{32}O_2$	Anti-inflammatory, antimicrobial, hypocholesterolemic [44]
17	17.773	cis-13,16-Docasadienoic acid	Fatty acids	336		63.96	$C_{22}H_{40}O_2$	No activity reported
18	19.448	Butyl 9,12-octadecadienoate	Esters	336		0.43	$C_{22}H_{40}O_2$	No activity reported
19	20.675	Isopropyl linoleate	Esters	322		2.85	$C_{21}H_{38}O_2$	No activity reported
20	21.236	Z,E-3,13-Octadecadien-1-ol	Long Chain alcohol	266		1.96	$C_{18}H_{34}O$	No activity reported

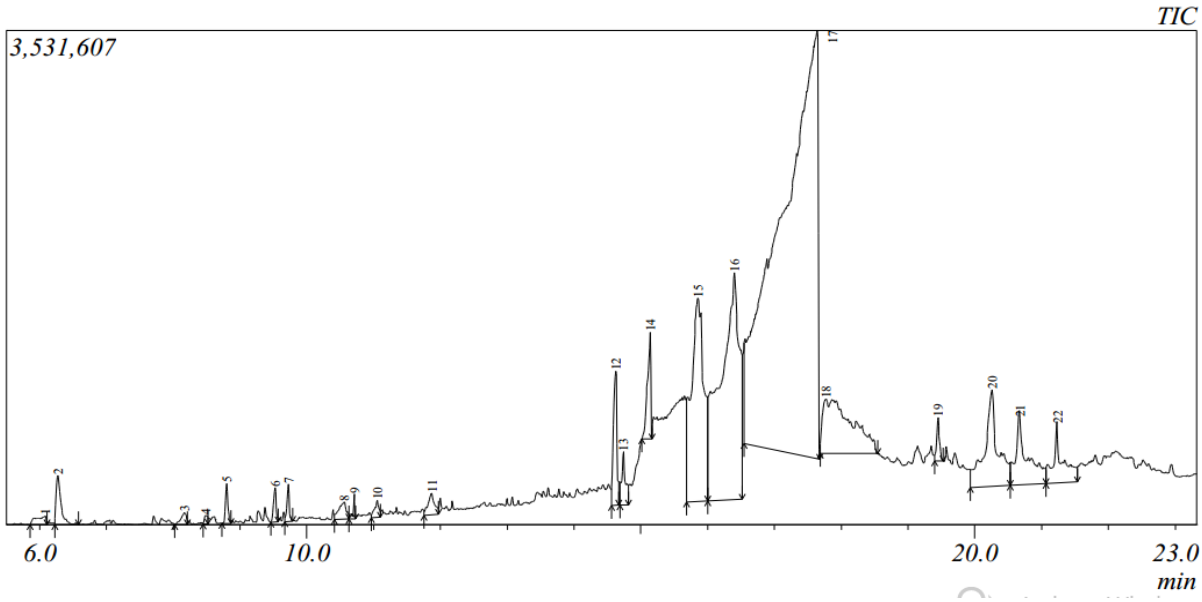


Figure 2: Chromatogram of *n*-hexane fraction of the roots of *B. brieyi*

The antiplasmodial activities of the plant extracts and fractions at all dose levels during the established infection were comparable to that of artemether-lumefantrine (the standard drug) used in the present study except that of the 100 mg/Kg BBCE and the 200 mg/Kg BBHF which gave a low percentage clearance of parasitemia of 43.2 % and 36.6 % respectively. In the infected and untreated mice (Negative control), the parasite counts increased daily which was consistent with the observations in literature.[17] In the BBCE groups, there is a dose dependent increase in the parasitemia clearance from BBME (100 mg/Kg) to BBCE (200 mg/Kg) while the clearance of the BBCE (400 mg/Kg) was not significantly different from that of the BBCE (200 mg/Kg) as shown in (Table 3). The clearance obtained for the BBMF (87.6 %) at 200 mg/Kg is also not very statistically different from that of the BBCE at 200 mg/Kg dose. This may suggest that 200 mg/Kg dose of the water miscible extract is the optimum dose for the antimalarial action of the plant. The parasitemia clearance obtained for the BBCE (200 mg/Kg and 400 mg/Kg), BBEF, and BBMF are higher, though still comparable with that of the ACT7 (standard). This clearly suggests that the plant has a higher antimalarial activity than that of the standard. Medicinal plants used in ethno-treatment of malarial infection shows that the plants have a better antimalarial activity[36]. Phenolic compounds also have anticancer, antiplasmodial and antioxidant potentials.[32] Alkaloids have been shown to have antiplasmodial potentials by inhibiting the actions of plasmodium from lysing the cell membrane.[32, 33]

Twenty (20) bioactive compounds were suggested in the *n*-hexane fraction of *B. brieyi* root (Table 4). Out of the twenty compounds present, Cis-13, 16-Docasadienoic acid (63.96 %), 9,12-Octadecadienoic acid (Z,Z)- (14.84 %), 9,12-Tetradecadien-1-ol, acetate, (Z,E) (8.80%), Z,E-3.13-Octadecadien-1-ol (1.96 %), Hexadecanoic acid, methyl ester (1.59 %) had the highest abundance as shown in their peak area. These constituents have been found to show promising biological activities against certain illness and pathogens e.g antibacterial and antifungal,[37]improves renal activity,[38] repairs neuron, [39] antimicrobial activity.[40]

Conclusion

The plant root extract has demonstrated antimalarial activity across all the extracts in its ability to clear parasitemia after full development of infection. This finding lends pharmacological support to the traditional use of the plant in the treatment of malaria. Further study are still required to elucidate the mechanisms of action and the actual phytochemical responsible for the antimalarial action seen in the plant.

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.

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