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Original Article

Antiplasmodial Activity of Ethanol Extract and Fractions of *Nauclea Latifolia* Smith *(Rubiacea)* Roots

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ABSTRACT

Malaria is a parasitic disease that is endemic in Nigeria with one of the greatest challenges being the development of resistance by *Plasmodium falciparum* against chloroquine and Artemisinin combination therapy (ACT). Since, ethnobotanical studies have given insight on the use *Nauclea latifolia* (Pin cushion tree) to treat malaria, this study was aimed at validating or rejecting this claims through the extraction and fractionation of the secondary metabolites in the roots of this plant as well as evaluation of the *in vivo* antiplasmodial activity by comparing their effects with amodiaquine and artesunate. The extract and fractions were administered orally to albino mice by adopting the standard procedures of prophylactic, suppressive and curative antiplasmodial assay models. The following phytochemicals were relatively abundant: alkaloids, saponins, flavonoids, glycosides, cardiac glycosides and carbohydrates. The analysis showed that all treatment groups had significant prophylactic activities but the best three were amodiaquine, dichloromethane and ethyl acetate fractions. However, all the treatment groups showed good chemosuppresive activity against *Plasmodium berghei* as follows: amodiaquine (99.2%), artesunate (98.8%), ethanol extract (96.2%) and aqueous fraction (91.3%) which showed similarity with the standard drugs. Moreover, the curative abilities of the plant roots in treating malaria infection showed that all the treatment groups significantly reduced ($P < 0.05$) parasite densities in mice when compared with the negative control group with % growth inhibitions as amodiaquine (98.2%) > butanol fraction (90.8%) > artesunate (90.3%) > aqueous fraction (33.8%) etc. Therefore, *Nauclea latifolia* roots may be used in prophylaxis, suppression of parasite growth as well as in the treatment of malaria.

Keywords: Malaria; *Plasmodium berghei*; *Nauclea latifolia*, parasite density, mean survival, % parasite growth inhibition.

INTRODUCTION

Malaria is a parasitic disease caused in humans by protozoa of the genus *Plasmodium* that invade and destroy red blood cells [39]. The disease continues to be a major global burden to both public health and economic development with an estimated 217 million malaria cases every year, resulting in about 0.7 million deaths [39]. However, the actual incidence of malaria is probably underestimated in some important endemic areas [13]. About 40 % of the world population is at risk of the malaria disease. An estimated 1.2 billion is at high risk of transmission (≥1 case per 1000 population), half of which live in the African regions; 80% of such cases are concentrated in 13 countries, and over half in Nigeria, Congo, Ethiopia, Tanzania and Kenya [39]. Nigeria accounts for a quarter of all malaria cases in Africa [38]. In the southern part of Nigeria, transmission occurs all year round while in the north it is more seasonal. Almost all malaria cases in the country are caused by *Plasmodium falciparum*, considered to be the leading cause of death worldwide from a single infectious agent. Antimalarial drugs remain the mainstay for malaria treatment and control [34, 28, 42]. Artemisinin-based combination therapy (ACT) is recommended as first-line treatment for uncomplicated *Plasmodium falciparum* malaria, [40] and its implementation has contributed significantly to reducing the malaria burden in many endemic countries and countering resistance to key antimalarial medicines [41, 40]. Yet, the recent reports of artemisinin resistance in the Cambodia–

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Thai border [14, 26, 11] highlights the need for the continued development of new medicines [40, 11]. *Plasmodium falciparum* has developed resistance against most conventional drugs like Chloroquine, ACT etc. So, the search for novel antimalarial drugs is therefore imperative. Balick *et al*. [5] observed that tropical rainforest plants have higher concentrations of natural chemical defences and a greater diversity than plants from any other biome. There is therefore an advocacy for researches on plants from these regions since, the major proportion of malaria-attributable deaths occur in sub-Saharan African regions in which Nigeria is a part. This advocacy is strengthened by the fact that popular antimalarial drugs such as quinine and Artemisinin were obtained from plants, *Cinchona ledgeria* and *Artemisia annua,* respectively.

Ethnopharmacological studies have shown that one of the plants used in Nigeria, Mali and Ghana to treat malaria is *Nauclea latifolia* Smith (Rubiacea) [6, 18]. Asanga *et al*. [4] have reported the antihyperglycemic potentials of the leaves of this plant. *Nauclea latifolia* is an evergreen multi-stemmed shrub or tree; it belongs to the family Rubiaceae and grows up to an altitude of 200 m. It is widespread in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa. Moreover, ethanol extract of the leaves of *Nauclea latifolia* and fractions contain diverse phytochemicals such as alkaloids, flavonoids, steroids and glycosides, saponins. Moreover, Zirihi *et al*. [44] reported that out of thirty three plants commonly used in west tropical Africa by traditional healers for the treatment of malaria, *Nauclea latifolia* showed a good antiplasmodial activity and a weak toxicity. Most of the antimalaria studies carried out on this plant have been on the aerial parts.

The primary objective of this study was to carryout extraction, fractionation and phytochemical screening of the *Nauclea latifolia* plant roots extracts and fractions as well as the evaluation of the *in vivo* antiplasmodial activity of the extracts and fractions of the plant roots through prophylactic, suppressive and curative test models.

MATERIALS AND METHODS

Preparation of the extracts/fractions: The roots of *Nauclea latifolia* were collected in August 2015 from a bush in Ikot Andem Ididep, Ibiono Ibom Local Government Area, Akwa Ibom State, Nigeria. They were identified and authenticated by Mr. Etefia (a technologist) in the Department of Pharmacognosy and Natural medicine, University of Uyo, Nigeria. Voucher specimen with herbarium number UUH67G was deposited at the Department of Pharmacognosy and Natural medicine, University of Uyo, Nigeria. They were carefully selected, washed, cut and dried under shade for days. The dried roots were pulverized using mortar and pestle into moderately coarse particles, stored in air-tight container.

The dried plant material (4.741kg) was macerated with 12.5 litres of 70% ethanol at room temperature for 72 hours with ocassional shaking. The extract was filtered and concentrated using a rotary evaporator at 40^{o}C and dried in a desiccator containing self-indicating silica gel. The ethanol extract (108.2g) was dissolved in distilled water (200ml) and successively partitioned with dichloromethane (19 x 250 ml), ethyl acetate (25x250 ml), and butanol (40 x 250 ml) using separating funnel to yield upon evaporation of the solvents 15.3g (14.14%) of dichloromethane fraction, 8.3g (7.7%) of ethyl acetate fraction, 33.7g (31.2%) of butanol fraction, and 6.5g (6%) aqueous fraction.

Phytochemical evaluation of the root of *Nauclea latifolia:* Qualitative tests were carried out on the ethanol whole extract and the various fractions of *Nauclea latifolia* using standard procedures to identify their constituents [35].

Acute toxicity determination: The acute toxicity test of the plant root extract and fractions in mice were evaluated by determining their effective and median lethal dose (LD_{50}) using the Lorke's method [24].

Animals: Albino mice of both sexes, weighing between 14 and 23 g obtained from Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria were used as in the study. The NIH guide for the care and use of laboratory animals (NIH Publication No. 80-23; revised 1978) was observed. They were given free access to food (standard pellet diet) and water and were kept under a 12:12 hour light/dark cycle at ambient temperature. The experimental protocol was approved by the Animal ethics committee of the Faculty of Pharmacy University of Uyo, Nigeria.

Parasites: Samples of Chloroquine sensitive *Plasmodium berghei* (NK-65) obtained from National Institute of Medical Research (NIMR), Lagos, Nigeria, maintained by sub-passage were used for the research to evaluate the anti-malaria activities of the plant extract and fractions used in the study.

Inoculum preparation: A stock of parasitized erythrocytes was obtained from infected mice, with

a minimum peripheral parasitemia of 20 % by cardiac puncture in heparin-coated tube. The percentage parasitaemia was determined by counting the number of parasitized red blood cells against the total number red blood cells. The cell concentration of the stock was determined and diluted with physiological saline such that 0.2 mL of the final inoculum contained 1 x10^7 parasitized red blood cells which are the standard inoculums for the infection of a single mouse [27].

Drugs: Tablets of artesunate (100 mg) (from Mekophar Chemical Pharmaceutical Joint–stock Company, Ho Chi Minh City- Vietnam.) were dissolved in distilled water and administered at a dose of 5 mg/kg body weight of mice. Amodiaquine (200 mg) (from Pfizer Afrique de l' Ouest Dakar R. P. Senegal) was dissolved in distilled water and administered at a dose of 30 mg/kg body weight of mice. Both artesunate and amodiaquine were used as positive control.

***In vivo* antiplasmodial determination:** The antiplasmodial property of the extract and fractions were evaluated through suppressive, prophylactic and curative tests for their *in vivo* antiplasmodial properties using a modified method earlier described by Okokon *et al.* [27] and Tekalign *et al*. [33] as follows:

Suppressive activity on early infection (4 - day test): The mice were each inoculated on the first day (day 0) intraperitoneally with 0.2 ml of infected blood containing about 1 × 10^7 *Plasmodium berghei* parasitized erythrocytes. The animals were then randomly divided into eight groups of six animals each. After twenty minutes, the mice in Group 1 were orally administered with distilled water at a dose of 10 ml/kg of mice and served as the negative control group. Groups 2 and 3 received amodiaquine (30 mg/kg/day body weight) and artesunate (5 mg/kg) body weight of mice, respectively. Both served as the positive control groups. Group 4 received ethanol whole extract at a dose of 150 mg/kg body weight of mice, while groups 5, 6, 7 and 8 were administered with dichloromethane, ethyl acetate, butanol and aqueous fractions (150 mg/kg) body weight of mice respectively. The oral administration of the extract, fractions and drugs were continued daily for 4 days (D_0 – D_3) between 10 am and 11.00 am. On the fifth day (D_4), thick and thin blood films were made from tail blood obtained from each mouse. Parasite density was estimated by counting the number of *Plasmodium* parasites against 200 or 500 white blood cells and expressing the resultant number of parasites/µl of blood assuming a white blood cell count of 8000 per µl of blood according to the relative value method [15]. Parasite density was deduced by using the formula: No. of parasites counted X 8000/ WBC Counts = Parasites /µL

Repository/Prophylactic Activity: Mice were divided randomly into eight groups of six animals each. Mice in group 1 (negative control group) were orally administered with distilled water at a dose of 10 ml/kg body weight of mice. Groups 2 and 3 received amodiaquine (30 mg/kg/day body weight) and artesunate (5 mg/kg/day) body weight of mice, respectively. Both served as the positive control groups. Moreover, group 4 received the whole extract at a dose of 150 mg/kg body weight of mice, while groups 5, 6, 7 and 8 were administered with dichloromethane, ethyl acetate, butanol and aqueous fractions (150 mg/kg) body weight of mice, respectively. All the groups were orally treated for three consecutive days (D_0 - D_2) and on day 4 (D_3), the mice were intraperitoneally injected with 0.2 ml of infected blood that contained 1 × 10^7 *Plasmodium berghei* parasitized red blood cells (RBCs). The level of parasitaemia was assessed using thick and thin films obtained from tail blood of each mouse 72 hours after the parasite inoculation. Parasite density was estimated by counting the number of *Plasmodium* parasites against 200 or 500 white blood cells and expressing the resultant number of parasites/µl by blood assuming a white blood cell count of 8000 per µl of blood according to the relative value method [15]. Parasite density was deduced by using the formula: No. of parasites counted X 8000/WBC Counts = Parasites/µL

Evaluation of antiplasmodial activity on established infection (Curative or Rane Test): Seventy two mice were inoculated intraperitoneally with standard inoculum of 1×10^7 *Plasmodium berghei* parasitized red blood cells on the first day (D_0). After 72 hours, the mice were randomized into 8 groups of eight animals each. Mice in group 1 received distilled water (10 ml/kg) body weight of mice and served as the negative control group. Groups 2 and 3 (positive control groups) received amodiaquine (30 mg/kg/day body weight) and artesunate (5 mg/kg) body weight of mice, respectively. Moreover, group 4 was administered with ethanol whole extract at a dose of 150 mg/kg body weight of mice, while groups 5, 6, 7, and 8 were administered with dichloromethane, ethyl acetate, butanol and aqueous fractions each at 150 mg/kg body weight of mice, respectively. The drugs, extract and fractions were orally administered to the animals once daily for 5 days. Tail blood samples from each mouse were collected daily for 4 days, stained with Giemsa's stain. Thick and thin films prepared were used to monitor the level of parasitaemia. Parasite density was estimated by counting the number of

Plasmodium parasites against 200 or 500 white blood cells and expressing the resultant number of parasites/µl by blood assuming a white blood cell count of 8000 per µl of blood according to the relative value method [15]. Parasite density was deduced by using the formula: No. of parasites counted X 8000/WBC Counts = Parasites /µL

Staining and examination of the thick and thin smears: Two films were prepared for each sample from mice. Each slide had a measured volume of 6µl of blood for thick film and 2µl for the thin film, respectively: 10% (1:9 ml) for 7 min and 3 % (3:97 ml) for 45-60. Working Giemsa stains were prepared from already prepared stock of Giemsa-staining solution. Thick and thin blood smears were stained with Giemsa after fixing the thin smear with absolute methanol. The entire film was first screened at a low magnification (10X×40X objective lens) to detect suitable fields with even distribution of WBC (10 – 20 WBC/field) [31]. Smears were then examined using X100 oil immersion. At least 100 high power fields were examined before a thick smear was declared negative. *Plasmodium berghei* parasites were counted per 200 or 500 leukocytes, which were used to estimate the parasite density per micro litre of blood.

Statistical analysis: The data were analysed by using GraphPad Prism version 5.0 (GraphPad Software, Inc. San Diego, CA, USA) and the results expressed as Mean + SEM. The significant differences between and within the groups were analysed statistically by One-way ANOVA followed by multiple comparisons of their means using Turkey's *post hoc* test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

The results of phytochemical screening showed that the ethanol extract and fractions contain alkaloids, saponins, flavonoids, glycosides, cardiac glycosides and carbohydrates (Table 1). The results of the prophylactic, suppressive and curative activities of the extract and fractions are shown in (Table 2, 3, 4 and Fig. 1, 2, 3), respectively. In the prophylactic model parasite densities/µL of the extract and fractions were significantly lower than that of the negative control. The prophylactic activity of dichloromethane fraction was the highest (902.0±169.8). It was higher than that of artesunate (6436.8±1169.5) and comparable to that of amodiaquine (780.0±17.7). In the suppressive model, % growth inhibitions of the parasite were 96.2, 88.9, 52.3, 70.4 and 91.3%, respectively, and 99.2 and 98.8% for amodiaquine and artesunate, respectively. The suppressive activity of both dichloromethane and aqueous fractions were statistically comparable to those of amodiaquine and artesunate. In the curative model, the % growth inhibition of the parasite on days 1, 3 and 5 was (-16.2%, -6.0%, 3.3%), (1.0%, 15.8%, 18.2%), (1.5%, 10.6%, 26.3%), (-4.6, 53.5, 90.8%), (-12.6, 13.5%, 33.8%), respectively.

Table 1. Results of the Phytochemical screening of the extract and fractions of the roots of *Nauclea latifolia*

Test	Observations	Ethanol extract	Dichloro methane fraction	Ethylacet ate fraction	Butanol fraction	Aqueous fraction
Alkaloids						
Dragendorff's reagent	Orange, brown ppt or turbidity	++	+	_	++	+
Hagger's reagent	Yellow ppt	++	+	_	++	+
Saponins						
Froth test	Steady froth formed	+++	++	+	++	+
Emulsion test	Emulsion formed	+++	++	+	++	+
Fehling's test	Reddish brown colour	+++	++	+	++	+
Tannins						
Ferric chloride	No blue black ppt	_	_	_	_	_
Flavonoids						
Magnesium metal	Orange colour formed	++	+	++	++	+
Ethylacetate	Yellow colour in NH_3 phase	++	+	++	++	+
Glycoside						

Hydrolysis	Brick red ppt observed	+	_	+	+	++
Cardiac glycoside						
Salkowski test	Reddish brown formed at interphase	+++	+++	++	++	+
Terpenes & steroids						
Liberman-Burchard	Brown colour and not greenish colour at interphase	_	_	_	_	_
Deoxysugar						
Keller-Killiani test	Brown ring at interphase	+	_	++	++	+
Anthraquinone	No red pink or violet colour formed in NH_3 interphase	_	_	_	_	_
Carbohydrate	Violet colour formed at interphase	++	++	++	++	++

+++ = high concentration, ++ = moderate concentration, + = low concentration, - = not detected

Table 2. Prophylactic activity of amodiaquine, artesunate, ethanol extract and fractions of *Nauclea latifolia* roots.

S/No.	Treatment groups	Average weights (g)	Parasite densities/µL	Mean survival time
1	Negative control (distilled water)	17.5±0.3	49960.0±6491.6	14.5±0.3
2	Amodiaquine (30 mg/kg)	19.8±0.8	780.0±17.7[a*]	19.5±1.0
3	Artesunate (5 mg/kg)	18.5±1.3	6436.8±1169.5[a*]	17.0±1.4
4	Ethanol extract (150 mg/kg)	19.0±1.5	24212.0±1973.4[a*, b**, c**]	19.5±1.3
5	Dichloromethane fraction (150 mg/kg)	19.8±1.3	902.0±169.8[a*]	19.3±1.8
6	Ethylacetate fraction (150 mg/kg)	19.3±1.1	1275.0±58.0[a*]	18.3±1.8
7	Butanol fraction (150 mg/kg)	19.0±1.3	21395.0±805.8[a*,b**, c**]	16.8±0.5
8	Aqueous fraction (150 mg/kg)	18.3±0.5	8784.0±405.3[a*]	14.5±1.5

a* = significant decrease at $P < 0.05$, n =6 when compared with the negative control group.
b** = significant increase at $P < 0.05$, n =6 when compared with the amodiaquine group.
c**= significant increase at $P < 0.05$, n =6 when compared with the artesunate group.

Table 3. Suppressive activity of amodiaquine, artesunate, ethanol extract and fractions of *Nauclea latifolia* roots.

S/No.	Treatment groups	Average weights (g)	Parasite densities/µL & % growth inhibition	Mean survival time
1	Negative control (distilled water)	15.5±0.7	47584.0±12360.0	12.8±1.6
2	Amodiaquine (30 mg/kg)	18.3±0.9	390.8±93.3^{a*} (99.2%)	18.8±1.7
3	Artesunate (5 mg/kg)	16.3±0.8	560.0±105.5^{a*} (98.8%)	19.3±0.8
4	Ethanol extract (150 mg/kg)	19.8±0.8	1794.0±354.3^{a*} (96.2%)	14.0±3.0
5	Dichloromethane fraction (150 mg/kg)	17.5±1.1	5272.0±1808.0^{a*} (88.9%)	10.5±0.9 $^{b*, c*}$
6	Ethylacetate fraction (150 mg/kg)	16.8±1.3	22681.0±3647.0$^{a*\ b**, c**}$ (52.3%)	9.3±0.5 $^{b*, c*}$
7	Butanol fraction (150 mg/kg)	18.3±0.8	14108.0±4198.0^{a*} (70.4%)	15.0±1.1
8	Aqueous fraction (150 mg/kg)	13.8±4.1	4119.0±383.1^{a*} (91.3%)	15.0±2.0

a* = significant decrease at P < 0.05, n =6 when compared with the negative control group.
b** = significant increase at P < 0.05, n =6 when compared with the amodiaquine group.
b* = significant decrease at P < 0.05, n =6 when compared with the amodiaquine group.
c* = significant decrease at P < 0.05, n =6 when compared with the artesunate group.
c**= significant increase at P < 0.05, n =6 when compared with the artesunate group.

Table 4. Curative activity of amodiaquine, artesunate, ethanol extract and fractions of *Nauclea latifolia* roots.

Treatment groups	Average weights (g)	Parasite densities/µL on D_0 & % growth inhibition	Parasite densities/µL on D_1 & % growth inhibition	Parasite densities/µL on D_3 & % growth inhibition	Parasite densities/µL on D_5 & % growth inhibition	Mean survival time (MST)
Negative control (distilled water)	18.4± 0.7	51680.0± 6211.0	57112.0± 7315.0 (-10.5%)	81272.0± 6269.0 (-57.3%)**	129640.0± 8686.0 (-150.9%)**	14.6± 0.4
Amodiaquine (30 mg/kg)	18.0± 0.4	40960.0± 3912.0	38280.0± 5195.0 (6.5%)	2747.0± 810.3^{a*} (93.3%)*	756.0± 250.6^{a*,} (98.2%)*	23.8± 0.3a**
Artesunate (5 mg/kg)	18.0± 0.9	39364.0± 3411.0	34710.0± 3028.0 (11.8%)	15090.0± 2576.0^{a*} (61.7%)*	3802.0± 1499.0^{a*} (90.3%)*	22.3± 1.1^{a**}
Ethanol extract (150 mg/kg)	18.3± 0.8	73470.0± 19248.0	85385.0± 17893 $^{b** c**}$ (-16.2%)	77878.0± 14510.0$^{a*\ b**,}$ ^{c**} (-6.0%)	71050± 13729.0$^{a*,\ b**,}$ ^{c**} (3.3%)	16.5± 1.2$^{b*, c*,}$
Dichloromethane fraction (150 mg/kg)	18.8± 1.1	40350.0± 3217.0	39965.0± 2488.0 (1.0%)	34000.0± 2087.0$^{a*\ b**,}$ (15.8%)	33020.0± 1532.0^{a*,} $^{b**, c**}$ (18.2%)	15.5± 0.3$^{b*, c*}$
Ethylacetate fraction (150 mg/kg)	18.3± 0.5	49190.0± 9927.0	48445.0± 8057.0 (1.5%)	43980.0± 6890.0^{a*,b**} (10.6%)	36240.0± 3834.0^{a*,b**,} ^{c**} (26.3%)	16.0± 0.6$^{b*, c*}$

Butanol fraction (150 mg/kg)	18.3± 1.2	32760.0± 4435.0	34280.0± 3510.0 (-4.6%)	15250.0± 2128.0^{a*} (53.5%)*	3000.0± 321.2^{a*} (90.8%)*	17.0± 0.4$^{b*, c*}$
Aqueous fraction (150 mg/kg)	17.5± 1.3	61239.0± 5081.0	68960.0± 6850.0 (-12.6%)	52990.0± 4757.0$^{a*, b**, c**}$ (13.5%)	40560.0± 3335.0$^{a*, b**, c**}$ (33.8%)	18.0± 0.4$^{a**,b*, c*}$

a* = significant decrease at P < 0.05, n =8 when compared with the negative control group.
a** = significant increase at P < 0.05, n =8 when compared with negative control group.
b* = significant decrease at P < 0.05, n =8 when compared with the amodiaquine group.
b** = significant increase at P < 0.05, n =8 when compared with the amodiaquine group.
c* = significant decrease at P < 0.05, n =8 when compared with the artesunate group.
c**= significant increase at P < 0.05, n =8 when compared with the artesunate group.
*= significant decrease at P < 0.05, n =8 when compared with the baseline parasite density on day 1 (D_0).
**= significant increase at P < 0.05, n =8 when compared with the baseline parasite density on day 1 (D_0).

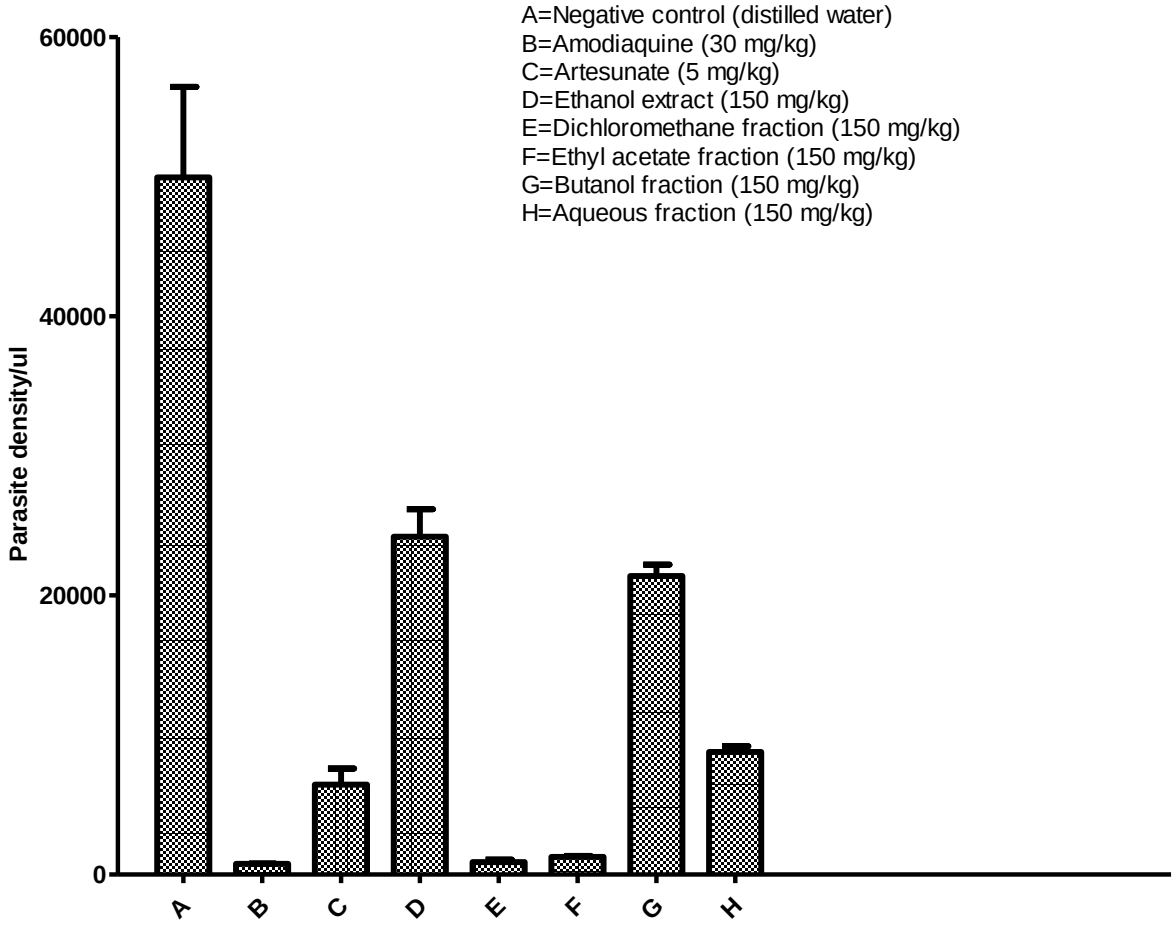

Fig. 1. Prophylactic activity of amodiaquine, artesunate, ethanol extract and fractions of the root of *Nauclea latifolia*.

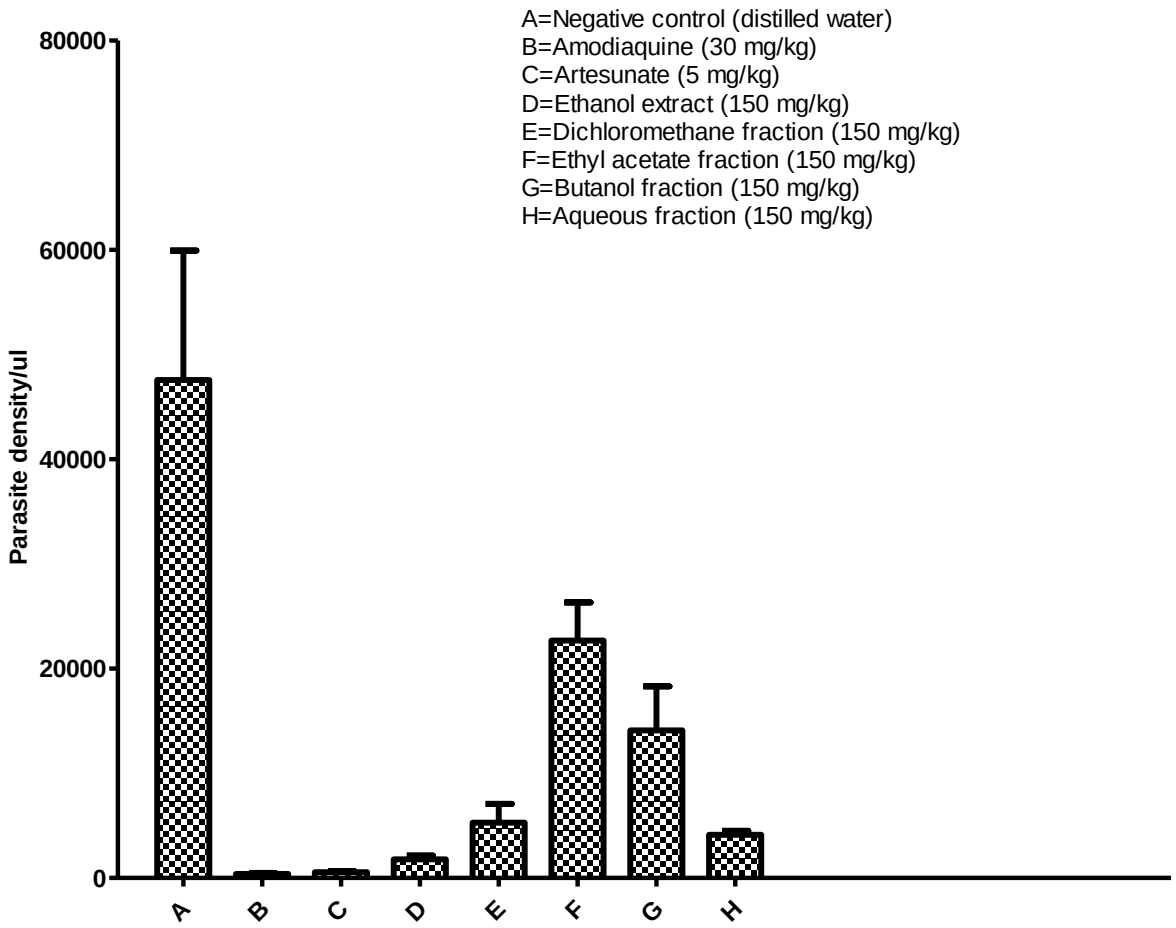

Fig. 2. Suppressive activity of amodiaquine, artesunate, ethanol extract and fractions of the root of *Nauclea latifolia*.

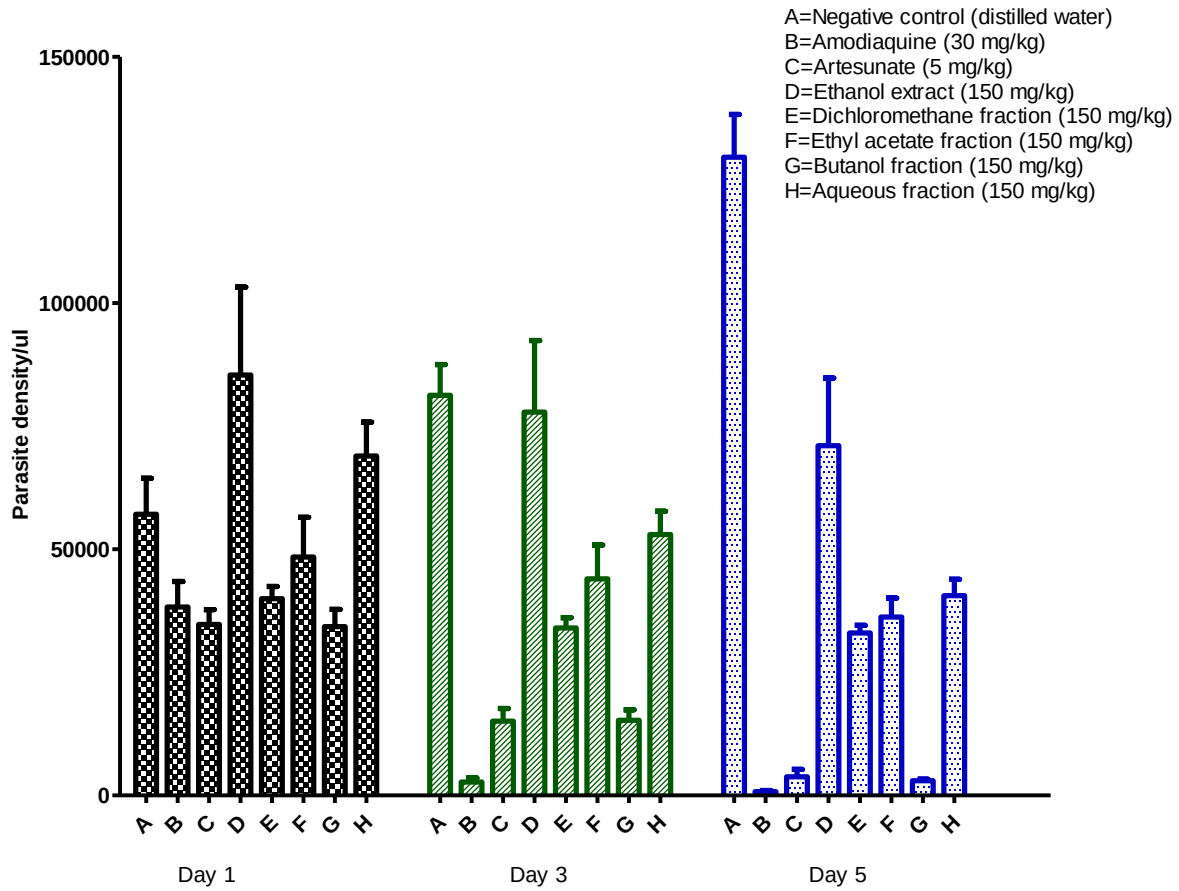

Fig. 3. Curative activity of amodiaquine, artesunate, ethanol extract and fractions of the root of *Nauclea latifolia*.

Amodiaquine and artesunate had (-10.5%, -57.3%, -150.9%), (6.5%, 93.3%, 98.2%), (11.8%, 61.7%, 90.3%), respectively. This showed that all the fractions had significant curative activity on days 3 and 5. Butanol fraction had the highest activity on day 5 comparable to that of artesunate.

Mean survival time by Negative control (distilled water), Amodiaquine (30 mg/kg), Artesunate (5 mg/kg), Ethanol extract, Dichloromethane fraction, Ethyl acetate fraction, Butanol fraction and Aqueous fraction for the prophylactic model are 14.5±0.3, 19.5±1.0, 17.0±1.4, 19.5±1.3, 19.3±1.8, 18.3±1.8, 16.8±0.5, 14.5±1.5, respectively. The values for suppressive activity are 12.8±1.6, 18.8±1.7, 19.3±0.8, 14.0±3.0, 10.5±0.9, 9.3±0.5 15.0±1.1, and 15.0±2.0, respectively. While 14.6±0.4, 23.8±0.3, 22.3±1.1, 16.5±1.2, 15.5±0.3, 16.0±0.6, 17.0±0.4, 18.0±0.4, respectively, are for curative activity.

DISCUSSION

Malaria is an inflammatory cytokine driven disease that sometimes results in death due to lyses of red blood cells. It is marked with oxidative changes in animals due to the parasites' numerous metabolic activities. *In vivo* screening provides an encompassing system in which drug efficacy can be evaluated in the biological milieu though, often times affected by the host factors like drug disposition and some other intrinsic drug antiparasitic activities. Nevertheless, it does not necessarily ascertain what a good antimalaria drug should be in terms of disposition. For instance, artemisinin is very efficacious *in vivo* despite its poor disposition in animals [37]. *Plasmodium berghei* is the species of choice for *in vivo* screening because of its higher accessibility and acceptability in drug discovery [17]. Some specific genes of the rodent adapted to *Plasmodium berghei* may have significantly diverged from the human pathogen *Plasmodium falciparum*.

The LD_{50} of the extract and fractions in mice was calculated to be 837.19 mg/kg body weight of mice. The result of the phytochemical analysis (Table 1) showed that almost all the extract and fractions had an abundance of saponins, flavonoids, alkaloids, and cardiac glycosides. Some of these secondary metabolites have been suggested to act as primary antioxidants or free radical scavengers that can mitigate the oxidative damages induced by

the malaria parasites. Free radicals also play roles in cellular signalling and as carriers for iron required by parasites for survival in the host system. Since iron is a necessity for the survival and replication of microorganisms such as *plasmodium* parasites, the potency of an antioxidant in chelating iron needed by these parasites will adversely negate the survival of the parasite in the host system.

A number of alkaloids such as oxyacanthine, alstonerine isolated from *Dehaasia incrassate, Alstonia angustifolia* respectively had been reported to possess antiplasmodial activity [23]. Others such as saponins, flavonoids, cardiac glycosides, steroids etc are reported to be responsible for the antiplasmodial activity of many medicinal plants [29]. Moreover, alkaloids are also known to show antimalaria properties by blocking protein synthesis in *Plasmodium falciparum* [25] whereas saponins, flavonoids and tannins have been reported to act as primary antioxidants or free radical scavengers that can counteract the oxidative damage induced by the malaria parasite [12]. However, Kirby [23] had reported that some plant extracts exerts their antiplasmodial actions by either causing elevation in red blood cell oxidation or by the inhibition of their protein synthesis. Therefore, the relative abundance of alkaloids, flavonoids, cardiac glycosides and saponins in the extract and fractions of the roots of *Nauclea latifolia* could be responsible for the antiplasmodial activity of the root.

The prophylactic activity of the treatment groups.
The result presented in Table 2 and Figure 1 showed that the parasite density in all the treatment groups were significantly decreased ($P < 0.05$) when compared with that of the negative control, pointing to the fact that the extract and all fractions had varying degrees of repository activity hence, suggesting that the consumption of *Nauclea latifolia* roots prior to malaria infection may help to reduce the upsurge of the signs and symptoms that result from of malaria attack. The dichloromethane fraction has the highest activity and its activity was better than that of artesunate. The result of this study was consistent with the report by Ettebong *et al*. [16] on 49.58% activity of ethanol extract of *Nauclea latifolia* stem bark at 300 mg/kg. Therefore, the prophylactic efficacy of these extract/fractions is indicative of their non-selectivity on the different stages of malaria parasite, since prophylactic drugs work either by the disruption of the initial development of malaria parasite in the liver or by suppressing the emergent asexual blood stages or even by the prevention of the relapses induced by hypnozoites [19]. Moreover, cardiac glycosides have been implicated in prophylactic activity of extracts as they attack the broadest age range of parasites from the tiniest rings that have invaded the erythrocytes to more mature stages of parasites such as developing trophozoites and schizonts [43]. Their relative broad stage specificity of action had been reported to extend the ability to impede developments of gametocytes. Therefore, the relative abundance of cardiac glycosides (Table 1) in the dichloromethane fraction may be the reason for its enhanced prophylactic activity.

The suppressive activity of the treatment groups.
The study which produced the result presented in Table 3 and Figure 2 was aimed at investigating the chemosuppressive abilities of the extract and the various fractions, in suppressing and preventing the rapid replication of *Plasmodium berghei* in mice model. The result (Table 3 and Figure 2) showed that the extract and all fractions had significantly reduced ($P < 0.05$) parasite density when compared with that of the negative control group. It had been reported that some plants extracts exerted their antiplasmodial action by the inhibition of protein synthesis in parasites [23]. Also, since alkaloids have been implicated in blocking protein synthesis in *Plasmodium falciparum* [25] it presupposes that the relative abundance of alkaloids in the extract and fractions with the exception of ethyl acetate fraction may have contributed to the chemosuppression of the *Plasmodium berghei* possibly by inhibiting the replication processes and blocking protein synthesis in the parasite thereby affecting growth and reproduction processes in the parasites.

The % growth inhibition of the parasite in the study is as follows: amodiaquine (99.2%), artesunate (98.8%), ethanol extract (96.2%), aqueous fraction (91.3%), dichloromethane fraction (88.9%), butanol fraction (70.4%) and ethyl acetate fraction (52.3%). When compared to similar studies on some other plants earlier reported: 55.6% at 600 mg/kg for the aqueous extract of *Parkia biglobosa* [10], 57.9% for Vernonia species [7], 61.86% and 82.17% for *Clerodendrum myricoides* root and leaves, respectively [20], 50% at 100 mg/kg and 68.4% at 200 mg/kg for *Cissampelos mucronata* [21]; the roots of *Nauclea latifolia* had a better activity. The % growth inhibition of *Plasmodium berghei* for amodiaquine in this study (99.2) was very close to that of Chloroquine (100%) earlier reported by Ettebong *et al*. [16]. This is most likely due to the fact that both drugs belong to the class of quinolines with similar mechanism of action. The fact that ethanol extract as well as aqueous, butanol and dichloromethane fractions showed almost similar % growth inhibition with

artesunate and amodiaquine is an indication that they have very good chemosuppressive activity.
The curative activity of the treatment groups.

The result (Table 4 and Figure 3) showed that the extract and all fractions used in the study had significantly reduced ($P < 0.05$) parasite densities when compared with that of the negative control group. The result of this study was consistent with that earlier reported for the aqueous extract of *Nauclea latifolia* stem bark (75.4 % at 500 µg /ml) [6], for *Lophira lanceolata* (79.7% at 400 mg/kg) [30], for *Clerendendrum polycephalus* (86.4% at 600 mg/kg) [1], for *Zizyphus spinschristi* root (100 mg/kg) [2]. Malaria is known as a complicated syndrome involving many inflammatory responses which may enhance cell-to-cell interaction, cell stimulation involving malaria derived antigens/toxins and the host derived factors such as cytokines [8]. The inflammatory conditions of malaria are marked with free radical generation, activation of phospholipase activity resulting in the generation of prostaglandins or even tumour necrotic factors. Therefore, the established curative properties of these extract/fractions may either be due to the inhibition of the production and/or the release of these inflammatory mediators associated with malaria or even by direct cytotoxic effect on the parasites. It is worthy of note that butanol fraction almost completely cleared the parasites from the blood of mice after the 5^{th} day of administration and the result compared favourably with those of the conventional drugs (amodiaquine and artesunate).

In addition, there were insignificant increases in the mean survival time (MST) of all the treatment groups when compared with that of the negative control group. The result showed consistency with that earlier reported on the MST of mice treated with fractions of *Nauclea latifolia* stem bark [16]. Therefore, the root of *Nauclea latifolia* is efficacious, as MST is used as an indicator for drug efficacy in antimalaria drug researches.
The result of mean survival time (MST) in the curative model (Table 4) showed that the groups of mice treated with amodiaquine, artesunate, and aqueous fraction significantly increased in their MST when compared with the negative control group, the result was in tandem with that earlier reported by Udobre *et al*. [36] on the significant increase ($P < 0.05$) in the MST of mice treated with methanol leaf extract of *Nauclea latifolia* leaves. The prolonged survival times of mice is evidenced by the high parasitemia clearance by amodiaquine, artesunate, and aqueous fraction of *Nauclea latifolia* roots. Mean survival time (MST) has often been used as one of the indicators for evaluating drug efficacy mostly, as it provides an insight to the potency of a drug in clearing the host system of parasite infection. The longer the survival time, the higher the chance of the animal surviving and recovering from the oxidative changes induced by the parasite and hence, the better the activity of the extract or drug.

Conclusion

The results of this study provides scientific basis for the folkloric use of *Nauclea latifolia* roots in the treatment of malaria. The dichloromethane, aqueous and butanol fractions showed the best prophylactic, suppressive and curative activity, respectively. The plant may be used for prophylaxis, chemosuppression of malaria parasites as well as in the treatment of established malaria cases in patients. Hence, this plant root may contribute to new drug discovery so; the mechanisms of action of the various fractions as well as efforts to isolates and characterize the antiplasmodial constituents responsible for these activities should be considered.

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Conflict of interests

There was no declaration of interest and no conflict among the authors in presenting this article for publication.

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