

In vivo antiplasmodial activities of *Nauclea latifolia*

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Submitted: 15-05-2014

Revised: 29-10-2014

Published: 31-12-2014

ABSTRACT

Background: The plant *Nauclea latifolia* is widely used in the southern parts of Nigeria for the treatment of malaria. Malaria is the number one killer disease in Nigeria. WHO reports that malaria continues to cause approximately 207 million cases of infection around the world and kills yearly about 627,000 people. It still kills a child somewhere in the world every minute. The study was aimed at evaluating the *in vivo* antiplasmodial activities of the extract and fractions (n-hexane, chloroform, ethyl acetate, butanol, aqueous) of the stem bark in *Plasmodium berghei berghei* infected mice. **Materials and Methods:** Through oral administration of the extract (100, 200 and 300mg/kg) and fractions (200 mg/kg), antiplasmodial activities were screened using 4-day suppressive, 7-day curative and repository tests. Significance was determined using ANOVA followed by Tukey-Kramer multiple comparison post-test using Graphpad InStat 3.10. A probability level of less than 5% was considered significant. **Results:** The extract exhibited significant dose-dependent antiplasmodial activity in the suppressive and repository tests. A significant ($p < 0.05-0.001$) dose-dependent reduction in parasitaemia in extract-treated groups in curative test was also observed. The aqueous fraction had the highest percentage chemosuppressive effect (67.71%). The extract also dose-dependently increased the survival days of the infected mice. The preliminary phytochemical investigation showed the presence of alkaloids, saponins, tannins, flavonoids and cardiac glycosides. **Conclusion:** This result confirmed the ethnobotanical use of the plant as malarial remedy and calls for further investigation of its phytochemical components and its antimalarial potentials.

Key words: Antiplasmodial, Extract, Parasitaemia, *Plasmodium berghei berghei*

Access this article online

Website:

<http://nepjol.info/index.php/AJMS>

DOI: 10.3126/ajms.v6i3.11361

INTRODUCTION

Nauclea latifolia, known commonly as African peach, is a straggling shrub with rounded ovate leaves.¹ This plant is about 10 ft high with flowers and has fruits which are edible, but not appealing. It is a native of Africa but is widely distributed throughout the forest and tropical forests of Benin, Burkina Faso, Cameroon, Democratic Republic of Congo, Ghana and Nigeria.² Among the Ibibios of South-South Nigeria, the stem bark either as an infusion or decoction is used as antimalarial, antipyretic and aphrodisiac. The root bark is used as tonic, antipyretic, antidepressant and analgesic. The leaf has the potential to relieve dysentery and diarrhoea.¹ Different parts

of the plant including the inner bark, stem, sap, roots, fruits and bark of root have been used in the treatment of sleeping sickness, cough, febrile conditions, thrush, jaundice, piles, stomach and menstrual disorders as well as sores.³ In Guinea, West Africa, the root is used as tonic, stimulant and a restorative.² *Nauclea latifolia* is reported to contain monoterpene indole alkaloids and the major ones being naucleamides A-E such as tetrahydro- β -carboline monoterpene alkaloid glycosides, naucleorine and epimethoxy-naucleorine, isolated by chloroform from the dried stem of *N. latifolia*.⁴ Scientific reports show that it is antihypertensive and laxative,^{5,6} anti-inflammatory, analgesic, antipyretic;⁷ antidepressant, myorelaxant and anxiolytic,⁸ antimicrobial,^{9,10} antiplasmodial *in vitro*,¹¹

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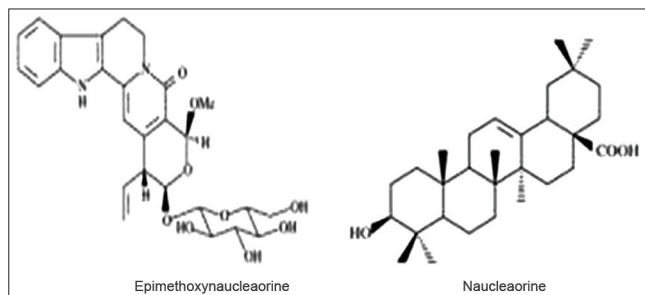
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anthelmintic,^{12,13} and antidiabetic.¹⁴ Literature from previous studies confirms the presence of tannins, alkaloids, glycosides and saponins in this plant.¹⁵ Much of the antiplasmodial work done on this plant is *in vitro* than *in vivo*. The aim of this study was to evaluate its *in vivo* antiplasmodial properties and to call the attention of the scientific world to a plant with such great but neglected potentials.



MATERIALS AND METHODS

Plant materials

The plant material of *Nauclea latifolia* was collected in September 2011 from Uyo, Akwa Ibom State, South-South Nigeria. The plant was identified and authenticated by Dr. (Mrs.) Margaret Bassey (a plant Taxonomist) in the Department of Botany and Ecological Studies, University of Uyo, where a voucher specimen with herbarium number UUH67G was deposited. The plant material was air-dried and then oven-dried at reduced temperature 35+2°C. It was thereafter ground into powder and cold-macerated in 70% ethanol for 72 h, and filtered. The filtrate was dried *in vacuo* using the rotary evaporator. 30 g of the dried extract was partitioned successively using various solvents such as n-hexane, chloroform, ethyl acetate, butanol and water to obtain their respective fractions. This partitioning yielded n-hexane (1.0 g), chloroform (2.1 g), ethyl acetate (1.6 g), butanol (3.2 g) and water (5.0 g) respectively. The crude extract and fractions were stored in a refrigerator at -4°C until required for use.

Phytochemical screening

The extract was screened for bioactive ingredients such as saponins, alkaloids, tannins, phlobotannins, flavonoids, anthraquinones, and cardiac glycosides as per the method described by Evans.¹⁶

Animal Stock

Adult albino mice (22-30 g) were obtained from the Animal House of the University of Jos, Jos, Plateau State and maintained in the University of Uyo Animal House and fed with growers pellet Feed (Bendel Feeds and Flour mills Ltd, Edo State) with water given *ad libitum*. Approval for the use of animals in the study was obtained from the

Animal Ethics Committee, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Micro-organisms

A chloroquine-sensitive strain of *P. berghei berghei* (NK-65) was obtained from National Institute of Medical Research (NIMR) in Lagos and maintained by subpassage in mice.

Inoculum preparation

The parasitized blood donor with high parasitaemia was obtained by first anaesthetizing the mouse with chloroform, and through cardiac puncture blood was collected using sterile syringe into sterile heparinised bottles. The percentage parasitaemia was determined by counting the number of parasitized red blood cells against the total number of red blood cells. The desired volume of blood then obtained from the donor mouse was suitably diluted with sterile normal saline so that the final inoculum (0.2 ml) for each mouse contained the required number of parasitized red blood cells (that is 1.0×10^7 parasitized red blood cells). Therefore, the 0.2 ml of the final inoculum contained 1×10^7 parasitized red blood cells which is the standard inoculum for the infection of a single mouse.^{17,18}

Drug administration

Drugs (chloroquine and pyrimethamine), extract and all fractions used in the current study were administered through the oral route using stainless metallic feeding cannula.

Acute toxicity studies

Acute toxicity study was carried out to determine the median lethal dose (LD_{50}) using the modified method of Miller and Tainter as described by Jigam *et al.*^{19,20}

Antiplasmodial activities of the extract

Suppressive activity on early infection (4 - day test)

To determine the suppressive activity of the extract, the method earlier described with modifications was adopted.²¹ The mice were each inoculated on the first day (day 0), intraperitoneally with 0.2 ml of infected blood containing about 1×10^7 *P. berghei berghei* parasitized erythrocytes. The animals were then randomly divided into six groups of six animals each. After ten minutes, the mice in the first group were orally administered with 10 ml/kg of distilled water and served as control. Groups 2, 3, and 4 received 100, 200 and 300 mg/kg of the extract orally. Group 5 received chloroquine 5 mg/kg/day as positive control while group 6 animals were administered with 200 mg/kg of extract conjointly with chloroquine 5 mg/kg/day. The administration of extract and drug was continued daily for 4 days (D0 - D3) between 8.00 am and 9.00 am. On the fifth day (D4) thin blood films were made from

tail blood obtained from each mouse. The films were thereafter stained with Leishman's stain to reveal parasitized erythrocytes. The percentage parasitaemia was obtained by counting the number of parasitized red blood cells out of 500 erythrocytes in random fields of the microscope.

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

Average percentage chemosuppression was calculated as

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

Where, A is the average percentage parasitaemia in negative control group and B, average percentage parasitaemia in the test group.

Repository/Prophylactic Activity

The methods earlier described with slight modifications were used to assess the prophylactic activity of the extract.^{21,22} Mice were divided randomly into 6 groups of six animals each. Group 1 animals received distilled water 10 ml/kg. Groups 2, 3 and 4 were administered with 100, 200 and 300 mg/kg of the extract orally. Group 5 animals served as positive control and were administered with 1.2 mg/mg/day of pyrimethamine. Group 6 animals received 200 mg/kg of the extract and Pyrimethamine (1.2 mg/kg/day). All the groups were treated for three consecutive days (D₀-D₂) and on day 4 (D₃), the mice were intraperitoneally injected with 1.2 ml of infected blood that contained 1 × 10⁷ *Plasmodium berghei berghei* parasitized red blood cells (RBCs). The level of parasitaemia was assessed using thin films obtained from tail blood of each mouse 72 h after parasite inoculation. Percentage parasitaemia and the average chemosuppression were calculated as stated above.

Evaluation of antiplasmodial activity on established infection (Curative or Rane Test)

To assess the schizonticidal activity of the extract during established infection, the methods earlier described and modified were used.^{21,22} Thirty-six mice were inoculated intraperitoneally with standard inoculum of 1 × 10⁷ *Plasmodium berghei* parasitized red blood cells on the first day (D₀). 72 h later, the mice were randomized into 6 groups of six animals each. Group 1 received 10 ml/kg of distilled water. Groups 2, 3 and 4 animals were administered with 100, 200 and 300 mg/kg/day of the extract orally. Group 5 animals were administered with 5.0 mg/kg/day of chloroquine. Group 6 animals received 200 mg/kg of extract along with 5 mg/kg/day of chloroquine. All the drugs were administered to the animals once daily for 5 days. Tail blood samples from each mouse was collected daily for 5 days, stained with Leishman's stain and thin films prepared were used to monitor the level of parasitaemia.

The Mean Survival Time (MST) of each group was determined over a period of 30 days (D₀-D₂₉).

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

Suppressive activity on fractions (4-day test)

Using the method earlier described²¹ various fractions of *Nauclea latifolia* (n-hexane, chloroform, ethylacetate, butanol, and aqueous) were administered orally at the dose of 200 mg/kg/day to different groups of six animals each 30 min after intraperitoneal injection of 0.2 ml of infected blood containing about 1 × 10⁷ *P. berghei berghei*. The negative control group received 10 ml/kg of distilled water. The administration of fraction/distilled water was continued for 4 days (D₀ - D₃). Thin films were prepared from tail blood of each mouse on the fifth day and the level of parasitaemia was determined by counting the number of parasitized red blood cells out of 500 RBCs in random field of the microscope. The average percentage chemosuppression was calculated as stated above.

Statistical analysis

Results were expressed as multiple comparisons of Mean + SEM. Significance was determined using One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison post-test using Graphpad InStat 3.10. A probability level of less than 5% was considered significant.

RESULTS

Phytochemical studies: Preliminary phytochemical studies showed the presence of alkaloids, saponins, tannins, flavonoids and cardiac glycosides.

Acute toxicity studies: The median lethal dose (LD₅₀) was determined to be 1,183 ± 0.01 mg/kg.

Antiplasmodial activities

Suppressive activity: The extract exhibited significant (p<0.001) dose-dependent antiplasmodial activity in the suppressive test when compared with control (Table 1).

Repository/prophylactic test: A significant (p<0.001) dose-dependent reduction in parasitaemia in extract-treated groups was observed relative to control (Table 2).

Curative or Rane test: The extract-treated groups showed a significant (p<0.05 - 0.001) dose-dependent reduction in parasitaemia compared to control (Figure 1).

Suppressive activity on fractions: The fractions exhibited various degrees of chemosuppressive effects. However, aqueous fraction had the highest percentage

Table 1: The suppressive activities of ethanol stembark extract of *Nauclea latifolia* (4-day test)

Drug extract	Dose (mg/kg)	Praasitaemia	% Chemosuppression
Control	10	71.00±1.83	-
Extract	100	41.67±2.43 ^a	41.31
	200	32.33±2.01 ^a	54.46
	300	25.67±2.69 ^a	63.84
	5.0	0.00±0.00	100.00
Chloroquine+extract	5.0+200	0.00±0.00	100.00

Values are expressed as mean±SEM. Significance relative to control ^ap<0.001, n=6

Table 2: Repository activity of ethanol stembark extract of *Nauclea latifolia* (prophylactic test)

Drug extract	Dose (mg/kg)	Parasitaemia	% Chemosuppression
Control	10 ml	78.00±3.29 ^a	-
Extract	100	59.67±2.74 ^a	23.50
	200	43.00±1.67 ^a	44.87
	300	39.33±2.57 ^a	49.58
	1.2	28.00±2.92 ^a	64.10
Pyrimethamine+ extract	1.2+200	23.67±2.20 ^a	56.83

Values are expressed as Mean±SEM. Significance Relative to control ^ap<0.001; n=6

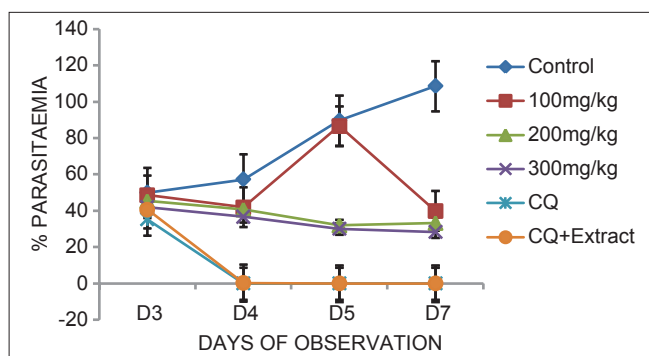


Figure 1: Effect of *N. latifolia* on established infection (Curative test)

chemosuppressive effect (67.71%) while butanol fraction had the least (15.68%) (Table 3).

Mean survival time: The mean survival times of extract-treated groups of mice increased dose-dependently and significantly (p<0.05-0.001) with the longest being 22.00 days observed with the highest dose (300 mg/kg) when compared to control but shorter when compared with standard drug chloroquine (27.33 days) (Table 4).

DISCUSSION

The preliminary phytochemical screening of *Nauclea latifolia*, stembark showed the presence of alkaloids, saponins, flavonoids, cardiac glycosides and tannins. Alkaloids, saponins and flavonoids are suggested as being responsible for the antimalarial activities of the plant. These

Table 3: Suppressive activity of fractions of *Nauclea latifolia*

Drug/fractions	Dose (mg/kg)	Parasitaemia	% Chemosuppression
Distilled water	100	74.33±2.37	-
Aqueous	200	24.00±1.46 ^b	67.71
n-hexane	200	51.33±1.92	30.94
Chloroform	200	34.00±1.42 ^a	54.25
Ethyl acetate	200	28.33±1.64 ^b	61.88
Butanol	200	62.67±2.85	15.68
Chloroquine	5.0	0.00±0.00 ^c	100

Values are expressed as mean±SEM. Significance relative to control ^ap<0.05; ^bp<0.01; ^cp<0.001, n=6

Table 4: The mean survival time (MST) of ethanolic extract of *Nauclea latifolia*

Drug/extract	Dose mg/kg	Mean survival time (MST): Days
Distilled water	100 ml	13.33±0.21
Extract	100	14.67±0.21
	200	16.67±0.56 ^b
	300	22.00±0.36 ^b
Chloroquine+extract	5.0+200	25.67±0.21 ^b
Chloroquine	5.0	27.33±0.56 ^a

Values are expressed as mean±SEM. Significance relative to control ^ap<0.05; ^bp<0.001, n=6

secondary metabolites could have elicited the observed antiplasmodial activity either singly or in synergy with each other.⁴ Alkaloids have been known to show antimalarial properties by blocking protein synthesis in *Plasmodium falciparum*.²³ Saponin, flavonoids and tannins have been suggested to act as primary antioxidant or free radicals scavengers that can counteract the oxidative damage induced by the malaria parasite.²⁴ It has been reported that extracts of *Nauclea latifolia* exhibited substantial antioxidant capacity. It is possible that the antioxidant activity might be due to the presence of vitamins.²⁵ Lenucci et al,²⁶ however, have demonstrated that the antioxidant activity is likely to be due to the presence of ascorbic acid, tocopherol and pigments. It is also possible that the antioxidant activity of extracts might be due to the presence of polyphenols.²⁷ The antioxidant property of this plant may represent yet another mechanism that contributes to its antiplasmodial activity.²⁸ Flavonoids are known to chelate with nucleic acid base pairing of malarial parasite.²⁹ Flavonoids have also been known to show significant antiparasitic activities against different strains of malaria, trypanosome and Leishmania.³⁰⁻³²

Aqueous extracts from stems and roots of *N. latifolia* have been tested in vitro in two *Plasmodium falciparum* strains, FcB1-Colombia (chloroquine resistant) and a Nigerian strain (chloroquine-resistant) and found to inhibit essentially the final developmental stages of the parasites. Two novel tetrahydro-β-carboline monoterpene alkaloid

glycosides, naucleaorine and epimethoxy-naucleaorine, isolated by chloroform from the dried stem of *N. latifolia*, strictosidine lactam, and oleanolic acid showed moderate *in vitro* activities against *Plasmodium falciparum*.⁴ The fact that aqueous fraction showed the highest chemosuppressive effect when compared with others suggests that the active ingredients of this plant responsible for its antimalarial activity may be localized here. The antiplasmodial activities of *Nauclea latifolia* extract and its fractions as observed in this study may, therefore, have resulted from one or more of these mechanisms.

Cloroquine has shown a greater chemosuppressive activity than the extract and fractions of *Nauclea latifolia* from this study, but it is no longer being used as an antimalarial agent in man because of resistance. The advantage that *Nauclea latifolia* has is that it is new, effective and with no known resistance.

In conclusion, the preliminary phytochemical screening of *N. latifolia* stem bark extract showed the presence of alkaloids, saponins, tannins, cardiac glycosides and flavonoids. The antiplasmodial activities of this plant may be ascribed to these constituents and further investigation is necessary to isolate, screen and determine the antiplasmodial compounds as well as their mechanism(s) of action. This study has established that *N. latifolia* stem bark extract obtained from South-South Nigeria, possesses antiplasmodial activities.

ACKNOWLEDGEMENTS

We acknowledge the technical assistance of Messrs Nsikan M. Udo, Susana Attah and Aniefiok Ukpong of the Department of Pharmacology and Toxicology, University of Uyo, Uyo.

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Authors Contribution:

Ettebong E.O – Designed the work, performed the laboratory tests, analysed the data, drafted the manuscript and revised the manuscript. **Ubulom P.E** – Performed the laboratory tests and assisted in designing the work. **Ekpenyong C.E** – Contributed to work design and assisted in review of manuscript. **Ekong U. S** – Contributed to work design. **Akpan O.E** – Assisted in laboratory tests. **Tambari V.D** – Assisted in laboratory tests.

Source of Support: Nil, **Conflict of Interest:** None declared.