



E-ISSN: 2278-4136  
P-ISSN: 2349-8234  
JPP 2015; 4(4): 68-71  
Received: 01-09-2015  
Accepted: 02-10-2015

**Anowi Chinedu Fredrick**  
Dept of Pharmacognosy and  
Traditional Medicine, Faculty of  
Pharmaceutical Sciences,  
Nnamdi Azikiwe University,  
Awka, Nigeria.

**Chibueze Ike**  
Dept of Pharmacology and  
Toxicology, Faculty of  
Pharmaceutical Sciences,  
Nnamdi Azikiwe University,  
Awka, Nigeria

**Emmanuel Ezeokafor**  
Dept of Physiology, Faculty of  
Medical Sciences, Nnamdi  
Azikiwe University, Awka,  
Nigeria.

**Anikpe Nwabunwanne Alloysius**  
Dept of Pharmacognosy and  
Traditional Medicine, Faculty of  
Pharmaceutical Sciences,  
Nnamdi Azikiwe University,  
Awka, Nigeria.

**Correspondence**  
**Anowi Chinedu Fredrick**  
Dept of Pharmacognosy and  
Traditional Medicine, Faculty of  
Pharmaceutical Sciences,  
Nnamdi Azikiwe University,  
Awka, Nigeria.

## Investigation into the anti-malarial activity of the aqueous leaf extract of *Nauclea latifolia* (Rubiaceae) using curative method

**Anowi Chinedu Fredrick, Chibueze Ike, Emmanuel Ezeokafor, Anikpe Nwabunwanne Alloysius**

### Abstract

Malaria is a disease caused by *Plasmodium* parasites that infect about 154 to 289 million people per year, resulting in approximately 660,000 – 2 million deaths worldwide. Infants, children, and pregnant females, along with immune-suppressed patients are at higher risk for worse outcomes when infected with malaria parasites. Malaria is a particular problem and a major one in areas of Asia, Africa, and Central and South America. To solve this problem, the natives of Ogidi in Anambra state of Nigeria use herbs to cure malaria and one of such herbs is *Nauclea latifolia*. With the rising problems of side effects, cost and limited efficacy of anti-malarial drugs, there is an urgent need for the development of alternative anti-malarial substances and researchers are nowadays turning to natural products from plants, as their main source of bioactive compounds with anti-malarial properties to complement the existing synthetic anti-malarials that are gradually becoming less potent against pathogenic parasites. Hence, there is the need for the phytochemical screening and investigations into the anti-malarial potential of the leaves extracts of *Nauclea latifolia* as it is claimed by Ogidi people to have antimalarial activity

The leaves of *Nauclea latifolia* were collected and dried at ambient temperature and pulverized. Exactly 500 g of the powdered drug was extracted with 1000 ml of water using the cold maceration technique for 48 hours with occasional shaking. This was filtered and the procedure repeated with the marc. The combined filtrates were concentrated under reduced pressure with rotary evaporator. The preliminary phytochemical tests were carried out using standard methods. The anti-malarial screening was conducted using the curative test (established infection) method to assess the efficacy of the extract as therapeutic agent.

The following secondary metabolites were present - flavonoids, saponins, alkaloids, Carbohydrates, steroids and terpenoids. The aqueous extract exhibited complete elimination of all the parasites present (100% cure)

From the research, it is found that the aqueous extract of the *Nauclea latifolia* is an effective anti-malarial agent as claimed by the natives of Ogidi people.

**Keywords:** *Nauclea latifolia*, *Plasmodium berghei*, albino mice

### Introduction

Malaria is caused by protozoa of genus *Plasmodium* and four species are responsible for disease in humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*. Malaria has been a major disease of mankind for thousands of years. Despite the availability of drugs for treatments, malaria is still one of the most important infectious diseases of humans with approximately 200-500 million new cases and 1-2.5 million death each year. Malaria is endemic in virtually all parts of the tropic and is responsible for a considerable proportion of morbidity and mortality in all developing countries (Adebayo, 2010) [1]. A large percentage of Nigeria population especially those in rural areas depends on traditional medicine as a source of primary health care including malaria (Sofowora, 1993) [13]. *Nauclea latifolia* is one of those plants used by the natives in the treatment of malaria especially in Nigeria and other parts of Africa (Sofowora, 1993) [13].

### Materials and Methods

#### Reagents Used For Phytochemical Test

Dragendorff reagent, Wagner reagents, Hager's reagents, ethyl acetate, ammonium chloride solution, Fehling's solution 1&2, sulphuric acid, ferric chloride, lead sub-acetate, olive oil, ethanol, water, million's reagent, picric acid, molisch reagent, iodine.

### Equipment

Analytical weighing balance, Water bath (Serological, UK), Test tubes, Beakers, Measuring cylinder, Funnels, Conical flask, Syringes (1 ml), Cannula, Crucible, Rotary evaporator (Buchi, Germany), Evaporating dish, No 1 Whatman filter paper, Porcelain cloth, Refrigerator, Filter paper and Electronic weighing balance (OHAUS, China) Microscope, glass slide, 5ml syringe, test tube, spatula.

### Collection of Plant and Identification

Fresh leaves of *Nauclea latifolia* were collected in February 2014 at Agulu, Anambra State. The plant was identified by Pharmacist Charity Ezea, a lecturer, Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka and further confirmed by Mr. Ozioko, a Taxonomist at University of Nigeria, Nsukka.

### Water Extract

The leaves of *Nauclea latifolia* were collected and dried at ambient temperature and pulverized. Exactly 500 g of the powdered drug was extracted with 1000 ml of water using the cold maceration technique for 48 hours with occasional shaking. This was filtered and the procedure repeated with the marc. The combined filtrates were concentrated under reduced pressure with rotary evaporator.

### Experimental Animals and Housing

Albino mice (20) of both sexes with weight ranging from 21-30 g were used for the experiment. They were obtained from the Zoology Department, University of Nigeria, Nsukka, Enugu State. They were housed in a wooden cage under room temperature. They were properly fed with growers mash, allowed free access to water. Good hygiene was maintained by constant removal of feces, spilled feed in the cage and cleaning of the environment. The mice were allowed to acclimatize for a period of 7 days before the work was conducted

### Preliminary Phytochemical Analysis

Chemical tests were carried out in the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993)<sup>[13]</sup>, Trease and Evans (1989)<sup>[15]</sup> and Harborne (1973)<sup>[5]</sup>.

### Test for Alkaloids

To test for the presence of alkaloid in a plant material, 5 g of the powdered leaves was placed in the test tube and 20 ml of methanol was poured into the test tube. The mixture was allowed to boil for 2 minutes in a water bath, cooled and filtered. The filtrate was then used for the following test:

- To the 2 ml of the filtrate, two drops of Dragendorff's reagent (solution of potassium bismuth iodide) was added and the color changed was noted
- To 2 ml portion of the filtrate, two drops of Mayer's reagent (potassium mercuric iodide solution) was added and color change was noted
- To 5 ml portion of the filtrate, two drops of Wagner's reagent (solution of iodide and potassium iodide) was added and color change was noted

- To 5 ml portion of the filtrate, two drops of Hager's reagent (saturated solution of picric acid) was added and the color change was noted

### Test for Flavonoid

To test for the presence of flavonoids, 10 ml of ethyl acetate was added to 0.2 g of the powdered plant material and heated on water bath for 3 minutes and then filtered. The filtrate was used for the following test:

#### • Ammonium Test

To 1 ml of dilute ammonia solution, 4 ml volume of filtrate was added, shaken and then the colors of the layers formed was noted

#### • Ammonium Chloride Solution

To 1 ml of 1% aluminium chloride solution, 4 ml portion of the filtrate was added, shaken and the layer of the color formed was noted

#### • Dilute Ammonia Solution

To 1 ml of dilute ammonia solution, 5 ml of aqueous filtrate of plant sample was added, then concentrated sulphuric acid was added and the color change was noted

### Test for Saponins

To test for the presence of saponins, 1 g of the powdered sample was boiled with 10 ml of water for 10 minutes, filtered and the following tests were performed:

#### • Frothing Test

To 2 ml of the filtrate, 10 ml of water was added and shaken vigorously for 2 minutes. Frothing was noted

#### • Emulsion Test

Few drops of olive oil was added to the filtrate solution and the content was shaken thoroughly, then the formation of emulsion was noted

### Test for Proteins

#### • Million's Test

To 2 drops of million's reagent, little drops of the filtrate of the powdered material was added, in a test tube and change in color of the precipitate was noted

### Test for Starch

#### • Molisch Test

The powdered material (0.1 g) was boiled with 2 ml of water and filtered. A few drops naphthol solution in ethanol (Molisch's reagent) was added, concentrated sulphuric acid was then gently poured down into the test tube to form a lower layer and the color formed was noted

#### • Iodine Test

A drop of iodine solution was added to 0.1g of the powdered material and the color change was noted

- **Fehling's Test**

To 1 ml portion of the filtrate, was added equal volume of Fehling's solution 1 and 2, and boiled on water bath for few minutes and the color change was noted

**Test for Resins**

- **Precipitation Test**

The powdered material (0.2g) was extracted with 1.5 ml of 96% ethanol. The alcoholic extract was then poured into 20 ml of distilled water in a beaker. A formation of precipitate was noted

**Test for Tannins**

- **Ammonium Chloride Solution**

To 1 ml of 1% aluminium chloride solution, 4 ml portion of the filtrate was added, shaken and the layer of the color formed was noted

- **Dilute Ammonia Solution**

To 1 ml of dilute ammonia solution, 5 ml of aqueous filtrate of plant sample was added, then concentrated sulphuric acid was added and the color change was noted

**Test for Tannins**

The filtrate (1 ml) was diluted to 5 ml with water, followed by addition of 2 drops of ferric chloride (0.1%) and the color change was noted

- **Ferric Chloride Test**

The filtrate (1 ml) was diluted to 5 ml with water, followed by addition of 2 drops of ferric chloride (0.1%) and the color change was noted

- **Lead Sub-Acetate Test**

To 1 ml of the original solution, 2 drops of lead sub-acetate was added and color change was noted

**Method of Staining**

The blood from the tail of the infected was collected and placed on a clean glass slide placed horizontally on the working bench. The slide and the spreader was held at a suitable angle, pulled back to touch the dropped blood on the slide and spread along it. The film was fixed with methanol and lowered into the already prepared Giemsa stains (1ml of Giemsa +19ml of buffer) and allowed to stain for 45 minutes. The slide was lifted off the stain solution with the aid of forceps, excess stain was washed off, allowed to drain and air dried at room temperature. Then parasitaemia was examined microscopically under oil immersion lens and the parasitized level was determined by counting red blood cells out of 200 red blood cells in a random field of microscope.

**Anti-Malarial Activity of *Nauclea Latifolia***

*In-vivo* evaluation of the anti-plasmodial activity of *Nauclea latifolia* was studied in this model - Curative test

**Curative Test**

In this test, 20 albino mice were selected and grouped into 4 groups of 5 animals per each group. All the animals were

infected with *Plasmodium berghei* (approximately  $1 \times 10^7$  infected red cells) by intra-peritoneal route. Then the animal were left for 72 hours before given treatment as follows:

Group 1 received 250 mg/kg aqueous extract

Group 2 received 500 mg/kg aqueous extract

Group 3 received 100 mg/kg quinine

Group 4 received 10 ml/kg 5% Tween 80

For four days. Then thin blood smears from the tail were made and staining processes was carried out, the parasitaemia was examined under the microscope. The extracts are said to have curative effect if the treated animals showed no parasitaemia or survived at least twice as long as the controls.

**Statistical Analysis**

The data was analyzed using one-way analysis of variance (ANOVA). Data were tabulated as Mean  $\pm$  SEM (Standard error of mean). P value  $< 0.05$  was considered significant if the q value is  $> 2.610$ .

**Result**
**Phytochemical Analysis**

Test	Results
Alkaloids	++
Flavonoids	+
Saponins	++
Tannins	-
Steroids	+
Terpenoids	++
Cardiac glycosides	++

Key

+ = presence

\_ = absence

**Percentage Parasitaemia Inhibition for the Curative Test with Standard Error in Mean**

Group	Basal Parasitaemia	Day 4	Day 7
1	12.750 $\pm$ 1.569	12.375 $\pm$ 1.087	8.750 $\pm$ 1.250
2	13.125 $\pm$ 0.315	10.167 $\pm$ 0.727	6.167 $\pm$ 0.441
3	10.300 $\pm$ 0.339	2.000 $\pm$ 0.204	0.750 $\pm$ 0.144
4	8.600 $\pm$ 0.534	10.166 $\pm$ 0.441	11.833 $\pm$ 0.441

**Discussion and Conclusion**

Malaria is one of the major diseases of the world (WHO, 2012) with wide spread anti-malarial drug resistance which pose great challenge against malaria control (Clarckson *et al*, 2004) [3]. This has led to increase in research for investigation of a new alternative source of treatment for malaria including medicinal plants (Maje, 2007) [8]. The preliminary phytochemical test done on the leaf of *Nauclea latifolia* extract showed that it contained different secondary metabolites such as alkaloids, flavonoids, cardiac glycosides, saponins and terpenoids. Some of these secondary metabolites have been found in other natural plant products to possess anti-plasmodial activity (Ayoola G.A, 2008) [2]. Anti-plasmodial activity observed in many plants was assumed to result from single or combined actions of these metabolites (Okokon *et al*, 2005) [11], which could be the same for the present study. *Cinchona succirubra* contains alkaloids which has anti-

plasmodial, bactericidal and analgesic effects. Therefore, the anti-plasmodial properties of the alkaloids may explain the relevance of *Nauclea latifolia* in the treatment of malaria. The result of the phytochemical analysis showed the present of flavonoids. Flavonoids detected in this plant could as well be responsible for the anti-plasmodial effect as these metabolites have been proved to possess potential immunomodulatory effect in other plant (Krotoski *et al* 1982) [6] and antioxidant effect (Enayati and Hemingway, 2010) [4], which might play a role in disease resistance (Mueller *et al*, 2007) [9]. The anti-plasmodial effect of *Nauclea latifolia* on the established malaria infection in mice, had shown significant malaria suppression when compared to the negative group. In this study, the quinine used as positive control showed significant decrease in parasitaemia level in the infected mice at the rate of approximately 93% when compared to the negative control group. The curative effects obtained with the leaf extracts of *Nauclea latifolia* agreed with previous reports of its anti-plasmodial activity and its traditional use. The mechanism of action of these extracts have not been elucidated, some plants are known to exert anti-plasmodial activity either by causing red blood cells oxidation (Murray *et al* 2012) [10] or by inhibiting protein synthesis (Sokomba *et al*, 1986) [14] depending on their active constituents or through other unknown mechanism

Medicinal plants have provided significant clinical antimalarial effects such as artemisinin derived from *Artemisia annua* and quinine from *Cinchona succirubra*. The results obtained from the study indicated that aqueous extract of *Nauclea latifolia* leaf extracts has a promising anti-plasmodial activity. This showed that nature can serve as a potential lead to the development of new and safe antimalarial drug by synthetic approaches. The study showed that the extract has both chemo curative effects in the mice infected with *Plasmodium berghei*. Its curative effect is effective the experiment about the effect of *Nauclea latifolia* leaf extract on the infected mice with *Plasmodium berghei* showed that it agreed with the traditional use of the plant. It also provided a scientific basis for its continuous use in the management of malaria in parts of Nigeria. This present study would encourage further research into *Nauclea latifolia* which has exhibited anti-plasmodial activity with the view to develop a new anti-plasmodial drug

## References

1. Adebayo lamikanra. Essential microbiology for students and practitioners of pharmacy, medicine and microbiology. 3rd edition, 2010.
2. Ayoola G, Coker H, Adesegun S, Bello A, Obaweya K, Ezennia C. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop. J Pharmaceut Res. 2008; 7(3):1019-1024.
3. Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG *et al*. Folb PI. *In vitro* antiplasmodial activity of medicinal plants native to or naturalized in South Africa Clarkson, 2004.
4. Enayati A, Hemingway J. Malaria management: Past, present, and future. Annual Review of Entomology 2010; 55:569-91.
5. Harborne JB. Phytochemical methods. (Third edition). Chapman and Hall Ltd, London, 1973, 135-203.
6. Krotoski WA, Granham PCC, Bray RS, Krotoski DM, Killick Kendrick R, Gwadz RW *et al*. Observations on early and late post-sporozoite tissue stages in primate malaria. I discovery of a new latent form of *Plasmodium cynomolgi* (the hipnozoite) and failure to detect hepatic forms within the first 24 hours after injection, Am. J Trop Med Hyg. 1982; 31:211-225.
7. Kyle R, Shampe M. Discoverers of quinine. Journal of the American Medical Association. 1974; 229(4):462.
8. Maje IM, Anuka JA, Hssaini IM. Evaluation of the anti-malarial activity of the ethanolic leaves extract of *Paullinia pinnata* linn (Sapindaceae). Nig. J Pharm Sci. 2007; 6(2):67-72.
9. Mueller I, Zimmerman PA, Reeder JC. *Plasmodium malariae* and *Plasmodium ovale*—the bashful malaria parasites Trends in Parasitology 2007; 23(6):278-83.
10. Murray CJ, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D *et al*. Global malaria mortality between 1980 and 2010: A systematic analysis. Lancet 2012; 379(9814):413-31.
11. Okokon J, Ofodum KC, Ajibesin KK, Danlandi B, Gamaneil KS. Pharmacological screening and evaluation of antiplasmodial activity of *Croton zambesicus* against *P. berghei* infection in mice. Indian J. Pharmacol, 2005.
12. Organization, World Health Guidelines for the treatment of malaria (2nd ed. ed.). Geneva: World Health Organization. p. ix. ISBN 9789241547925, 2010.
13. Sofowora H. Screening Plants for Bioactive Agents In: Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Ltd., Sunshine House, Ibadan. Nigeria, 2nd Ed., 1993, 134156.
14. Sokomba E, Wambebe C, Chowdhury BK, Iriah J, Ogbeide ON, Orkor D. Preliminary phytochemical, pharmacological and antibacterial studies of the alkaloidal extracts of leaves of *Synclisia scabrida* Miers. Journal of Ethnopharmacology. 1986; 18(2):173-185.
15. Trease GE, Evans WC. Textbook of Pharmacognosy. 14th Edition. W.B. Sanders, London, 1989.