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Antimalarial Studies Of The Ethanol Extract Of The Leaves Of *Nauclea Latifolia (Rubiaceae)*

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ABSTRACT

A large percentage of Nigeria population especially those in rural areas depends on traditional medicine as a source of primary health care including malaria. *Nauclea* latifolia is one of those plants used in the treatment of malaria especially by the people of Ogidi in Anambra State of Nigeria. They claim that the plant is an effective anti-malaria drug. To verify this claim, there is the need for the investigations into the anti-malarial potential of the leaves extracts of *Nauclea latifolia* as it is claimed by the natives to have antimalarial activity

The leaves of *Nauclea latifolia* were collected from the wild, and dried for two weeks. 500g was pulverized and marcerated in 1000mls of ethanol for 48hrs with constant shaking. it was then filtered and the procedure was 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.

It was observed from the work that the leaves contain the following secondary metabolites - flavonoids, saponins, alkaloids, Carbohydrates, steroids and terpenoids.

The ethanol extract of the leaves of *Nauclea latifolia* was found to be effective against malarial parasites as claimed by the natives from the work done.

Key words – Nauclea latifolia, Plasmodium berghei, albino mice, marc.

INTRODUCTION

Malaria deaths may be falling globally, but Nigeria remains one of the ten countries where the anopheles mosquito- borne disease is a major killer (Burkhill HM 1985), according to a new report released by the World Health Organization (WHO, 2012). Although the Report did not give the specific figures of the fatal cases of mosquito infections, about 165 million Nigerians are all susceptible to the malaria parasite, with less than 20 percent of the population, covered by preventive measures such as the use of treated nets (Cohen et al 1972; Garnham PCC 1980). In the same league with Nigeria is the Democratic Republic of Congo, now ravaged by crisis (Abeku 2007). WHO said the fight against malaria has saved 3.3 million lives worldwide since 2000, but the mosquito- borne disease still killed 627,000 people last year, mainly children in Africa (Batoloni and Zammarchi 2012; Caraballo Hector 2014). A shortage of funding and basic remedies like bed nets mean that malaria is still a major threat, particularly in Africa and Southeast Asia, according to the World Health Organization's Malaria Report 2013.

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). This has led to increase in research for investigation of a new alternative source of treatment for malaria including medicinal plants (Maje, 2007; Abdulela and Zaina 2007). 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; Ali et al 2011). Anti-plasmodial activity observed in many plants was assumed to result from single or combined actions of these metabolites (Okokon et al, 2005), which could be the same for the present study. *Cinchona succiruba* 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 presence 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 and antioxidant effect, which might play a role in disease resistance.

MATERIALS AND METHODS

REAGENTS

Dragendoff reagent, Wagner reagents, Hager's reagents, ammonium chloride solution, Fehling's solution 1&2, sulphuric acid, ferric chloride, lead sub-acetate, olive oil, ethanol (BDH LABS England), million's reagent, picric acid, molisch reagent, iodine and ethanol

EQUIPMENT

Analytical weighing balance, Water bath (Serological, UK), Test tubes, Beakers, Measuring cylinder, Funnels, Conical flask, Syringes(1ml), Cannula, Crucible, Rotary evaporator (Buchi, Germany), Evaporating dish, No 1 Whattman 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 November 2014 at Agulu, Anambra State, Nigeria. The plant was identified by Mr. Ozioko, a Taxonomist at University of Nigeria, Nsukka, Enugu State, Nigeria.

ETHANOL EXTRACT

The leaves were collected from Agulu near Awka in Anambra State, Nigeria in November 2014, air dried in room temperature for two weeks and then powdered. 500g of the powder was macerated in 1liter of ethanol for 48hrs and filtered. The procedure was repeated with the marc and the combined filtrate was concentrated using rotary evaporator at reduced pressure.

EXPERIMENTAL ANIMALS AND HOUSING

Albino mice(20) of both sexes with weight ranging from 24-35g were used for the experiment. They were obtained from the Zoology Department, University of Nigeria, Nsukka, Enugu State, Nigeria. 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 experiments were 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 Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

TEST FOR ALKALOIDS

To test for the presence of alkaloid in a plant material, 5g 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 0.2g 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

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 dry 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.

- **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,1g 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 (0.1g) 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 SAPONINS

To test for the presence of saponins,1g 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

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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

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

ANTI-MALARIAL ACTIVITY OF NAUCLEA LATIFOLIA

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

CURATIVE TEST

In this test, 20 albino mice were selected and grouped into 8 groups of 5 animals per each group. All the animals were infected with *Plasmodium berghei (*approximately $1x10^7$ infected red cells) by intra-peritoneal route. Then the animal were left for 72 hours before given treatment as follows:
Group 1 received 250mg/kg ethanol
Group 2 received 500mg/kg ethanol
Group 3 received 100mg/kg quinine
Group 4 received 10ml/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 ± 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	++

Table KEY + = presence_= absence

GROUP	BASAL PARASITAEMIA	DAY 4	DAY 7
1	11.000 ± 0.524	4.833 ± 0.667	1.750 ±0.327
2	10.200 ± 0.464	3.166 ± 0.441	0.833 ± 0.167
3	10.300 ± 0.339	2.000 ± 0.204	0.750 ± 0.144
4	8.600 ± 0.534	10.166 ± 0.441	11.833 ± 0.441

PERCENTAGE PARASITAEMIA INHIBITION FOR THE CURATIVE TEST WITH STANDARD ERROR IN MEAN

DISCUSSION AND CONCLUSION

Medicinal plants have provided significant clinical antimalarial effects such as artemisinin derived from *Artemisia annua* and quinine from *Cinchona succiruba*.
This showed that nature can serve as a potential lead to the development of new and safe antimalarial drug. The study showed that the extract has curative effects in the mice infected with *Plasmodium berghei*. Its curative effect is almost equivalent to that of quinine, a standard drug used as a positive control. This research 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.

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