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In vivo antiplasmodial effect of ethanol leaf extract of *Hippocratea africana*

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Abstract

Medicinal plants are used by the Ibibios of Southern Nigeria, for the treatment of various diseases, including malaria. One of the plants used is *Hippocratea africana*. The aim of the study was to evaluate the *in vivo* antiplasmodial efficacy of ethanol leaf extract of this plant in experimental murine plasmodiasis. The suppressive (4-day test), prophylactic and curative activities of the leaf extract were determined. Doses of the extract used in this study were 70.71, 141.42 and 212.13mg/kg/day in mice. Artesunate (5mg/kg/day) and pyrimethamine (1.2mg/kg/day) were used as the standard drugs while distilled water (10ml/kg/day) served as the control. The suppressive test resulted in 37.50, 43.75 and 57.28% suppression for doses 70.71, 141.42 and 212.13mg/kg respectively. Artesunate gave percentage suppression of 64.59%. These results were significant ($p < 0.001$) when compared with the control. For the prophylactic test, the lowest dose (70.71mg/kg) resulted in percentage suppression of 22.91% while the highest dose (212.13mg/kg/day) gave 57.82% suppression. Results of the curative test also revealed a dose and time - dependent chemosuppression, which were also significant ($p < 0.001$) compared with the control. Parasitaemia increased in all the control experiments. Phytochemical screening of the leaf of *Hippocratea africana* revealed the presence of alkaloids, saponins, tannins and flavonoids. *Hippocratea africana* has antiplasmodial efficacy and should be further investigated.

Keywords: *In-vivo*, antiplasmodial, leaf extract, *Hippocratea Africana*

1. Introduction

Malaria which is caused by apicomplexan protozoans of the genus *Plasmodium* and transmitted by females of the dapple-winged mosquitoes (*Anopheles* spp), remains a nagging public health issue. Globally, about 3.2 billion people are at risk of malaria and the Sub-Saharan Africa carries a high share of the malaria burden [1]. Some degree of success has been achieved in the fight against this disease in the past years. This success has been attributed to the adoption of Artemisinin combination therapy (ACT) and intervention effects such as the use of long lasting insecticide treated nets, Intermittent Prevention Treatment (IPT), for pregnant women; vector control measures and more importantly increased funding [2]. However, malaria remains a major health challenge in Nigeria and some other tropical countries, with high morbidity and mortality.

A major constraint in the control of malaria is the acquisition of resistance to insecticides by mosquitoes and resistance to antimalarial drugs, by *Plasmodium*, spp, especially *P. falciparum*. Besides the problem of resistance problems associated with the use of synthetic agents are high cost of drugs, side effects such as headache, allergic reactions etc.

Malaria vaccines are considered amongst important modalities for potential prevention of malaria and reduction of its transmission. Research and development in this field have been an area of intense effort by many groups over the last few decades, but there is currently no licensed malaria vaccine due to the complexity of the malaria parasite [1]. This together with other constraints has necessitated the continued search for more effective antimalarial compounds especially from natural products.

Hippocratea africana (Celastrales: Hippocrateaceae), is commonly called African paddle pod, but the Ibibios of Southern Nigeria call it Ebaenangenang [3]. The Root *Hippocratea africana* has been evaluated for its antimalarial efficacy [4] as well as phytochemistry and *in vitro* antidiarrhoeal activity of both leaves and roots [5]. However, there is a paucity of scientific information on the antimalarial efficacy of the leaf of this plant.

The objective of this study was to evaluate the *in vivo* antiplasmodial potential of the ethanol leaf extract of *Hippocratea africana*, using Swiss albino mice.

2. Materials and Methods

2.1 Collection and Identification of Experimental Plant

Fresh leaves of *Hippocratea africana* were collected from Itak Ikot Akap Village, Ikono Local Government Area, Akwa Ibom State, Nigeria, in November, 2015.

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The Department of Pharmacognosy and Natural Medicine, University of Uyo provided taxonomic keys for the identification of this plant. A voucher specimen of this plant with herbarium number UUPH3a was prepared and kept in the same department for future referencing.

2.2 Plant Extraction

The fresh leaves of *Hippocratea africana* were washed and air-dried at room temperature for 21 days and then pulverized. The cold extraction method was employed in this study. A quantity of 300g of the pulverized leaves was soaked in 1.5 litres of 70% ethanol for 72 hours, using an extracting jar. The crude liquid extract was then separated from the marc by filtration. The filtrate was concentrated to dryness *in vacuo*.

2.3 Phytochemical Screening

Phytochemical screening of the leaf of *Hippocratea africana* was carried out using standard procedures [6, 7].

2.4 Animals

Swiss albino mice weighing 15 – 22g, of both sexes were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria. They were housed in standard cages and fed with growers pellet feed, with water given *ad libitum*.

2.5 Ethical clearance

The use of animals for the study was approved by the Animal Ethics Committee, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

2.6 Malaria parasites

Malaria parasites *Plasmodium berghei berghei* (NK 65 merozoites), used in this study were obtained from the Nigeria Institute of Medical Research (NIMR), Lagos, Nigeria. These parasites were maintained by sub passage in mice.

2.7 Preparation of inoculum

The donor mouse was first anaesthetized after which blood was collected through cardiac puncture, using sterile syringe. Blood obtained was stored in sterile heparinized bottles. This was followed by suitable dilution with sterile normal saline, such that the final inoculum (0.2ml) for each mouse contained the required number of parasitized erythrocytes (1×10^7). A volume of 1ml of the standard inoculum contains 5×10^7 parasitized erythrocytes [8].

2.8 Drugs and extract administration

The standard drugs artesunate and pyrimethamine and the ethanol leaf extract of *Hippocratea africana* were administered through the oral route, using sterile feeding cannula.

2.9 Acute toxicity study

This test was carried out to determine the median lethal dose (LD₅₀) of the leaf of *Hippocratea Africana* [9]. Swiss albino mice were divided into 7 groups of 3 animals per group. The groups received 50, 100, 500, 1000, 2000, 3000 and 5000mg/kg of ethanol leaf extract of *Hippocratea africana* respectively. The animals were observed for 24 hours to determine physical signs of toxicity.

The median lethal dose (LD₅₀) was calculated as shown:

$$LD_{50} = \sqrt{AB}$$

Where

A = Maximum dose that produced 0% mortality

B = Minimum dose that produced 100% mortality

2.10 Antiplasmodial studies

2.10.1 Evaluation of the suppressive effect of the extract on early infection (4-day test)

Prior to the test, the mice were inoculated with 0.2ml parasitized blood containing *P. berghei berghei*. They were then divided into 5 groups of 6 mice each. Groups 1, 2 and 3 were administered with 70.71, 141.42 and 212.13mg/kg/day of extract respectively. Mice in group 4 were treated with 5mg/kg/day of artesunate while those in group 5 were given 10ml/kg of distilled water (control), for four consecutive days (D₀ – D₃). Twenty four hours after the administration of the last dose, blood smear was obtained from the tail of each mouse to make thin films on microscope slides. The films were stained with Giemsa stain to reveal parasitized erythrocytes, with the aid of the oil immersion objective of the microscope. Percentage parasitaemia was obtained by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope [10].

These formulae were used:

$$\% \text{ Parasitaemia} = \frac{\text{Number of parasitized erythrocytes}}{\text{Total number of erythrocytes counted}} \times \frac{100}{1}$$

$$\text{Average percentage chemosuppression was calculated as: } 100 \left(\frac{A - B}{A} \right)$$

Where; A = Average percentage parasitaemia in the control group.

B = Average percentage parasitaemia in the test group.

2.10.2 Evaluation of the prophylactic effect of the leaf extract

Mice were divided into five groups of six mice each. Extract doses of 70.71, 141.42 and 212.13mg/kg/day were administered to mice in groups 1, 2, and 3 respectively mice in group 4 were given 1.2mg/kg/day of pyrimethamine while mice in group 5 were given 10ml/kg/day of distilled water to serve as the control. This test lasted for 72 hours (D₀ – D₂). On the fourth day (D₃), the mice were inoculated (intraperitoneally) with *P. berghei berghei* (0.2ml of infected blood). The parasitaemia level was assessed by obtaining blood smears 72 hours later. Percentage parasitaemia and the average percentage suppression were determined as earlier described in the four-day test [8].

2.10.3 Evaluation of the effect of the extract on established infection (Curative test)

In this method, mice were divided into five groups of 6 mice each. Prior to this, each mouse had been inoculated intraperitoneally with 0.2ml of parasitized blood. After 3 days, mice in groups 1, 2 and 3 were given 70.71, 141.42 and 212.13mg/kg/day of the extract, respectively. Mice in group 4 received oral administration of 5mg/kg/day of artesunate, while those in group 5 received 10ml/kg/day of distilled water (control). Treatment lasted for 5 days. Blood samples were obtained from the tip of the tail of each mouse on alternate days, after commencement of treatment. Blood smears were prepared as described above [10].

2.10.4 Determination of the mean survival time (MST)

The Mean Survival Time (MST) for each group was determined by finding the average survival time (in days) of the mice (post inoculation), over a period of 28 days ($D_0 - D_{27}$) as earlier described [8].

2.10.5 Statistical Analysis

Data obtained from this study were expressed as mean \pm SEM. Significance was determined using one-way analysis of

variance (ANOVA). Values of $p < 0.05$ were considered significant.

3. Results

3.1 Phytochemical constituents

The results obtained from the phytochemical screening of the leaf of *Hippocratea africana* revealed the presence of alkaloids, flavonoids, saponins and tannins. Anthraquinones, cardiac glycosides and terpenes were absent (Table 1).

Table 1: Phytochemical Constituents of *Hippocratea africana* leaf

Phytoconstituents	Test	Inference
Alkaloids	Dragendoff's test	+
	Hager's test	+
Combined Anthraquinones	Borntrager's test	-
Cardiac glycosides	Salkowski test	-
	Keller Killiani test	-
Flavonoids	- Shinoda Reduction test	+
	- Magnesium metal test	+
Saponins	- Froth test	+
	- Sodium bicarbonate test	+
Tannins	Ferric chloride test	+
	Lead acetate test	+
Terpenes	Liebermann's test	-

Key: + = positive, - = negative

3.2 The median lethal dose (LD₅₀)

The LD₅₀ value obtained was 707.11mg/kg

3.3.4 Day (Suppressive) test

The ethanol leaf extract of *Hippocratea africana* produced a significant ($p < 0.001$), dose dependent suppressive effect in

the 4-day test. Values of percentage chemosuppression obtained were 37.50, 43.75 and 57.28% for doses 70.71, 141.42 and 212.13mg/kg/day respectively. The standard drug, artesunate produced a chemosuppressive effect of 64.59% (Table 2).

Table 2: Suppressive Effect of Ethanol Leaf Extract of *Hippocratea africana* (4-day test results) in Infected Mice

Treatment	Dose (mg/kg/day)	Parasitaemia	Percentage (%) Suppression
Leaf extract	70.71	20.00 \pm 1.73	37.50*
	141.42	18.00 \pm 0.20	43.75*
	212.13	13.67 \pm 1.88	57.28*
Artesunate	5	11.33 \pm 0.67	64.59*
Distilled water (Control)	10ml/kg	32.00 \pm 2.31	-

Values are expressed as mean \pm SEM, n=6

* = significance relative to control ($p < 0.001$).

3.4 Prophylactic test

A dose dependent inhibition in the level of parasitaemia was also observed in this test. Extract doses 70.71, 141.42 and 212.13mg/kg/day produced chemosuppressive effects of

22.91, 38.56 and 50.59% respectively. The standard drug, pyrimethamine had the highest effect (57.82%). No reduction in parasitaemia was observed in the control group (Table 3).

Table 3: Prophylactic Effect of Ethanol Leaf Extract of *Hippocratea africana* in Infected Mice

Treatment	Dose (mg/kg/day)	Parasitaemia	Percentage (%) Suppression
Leaf extract	70.71	21.33 \pm 0.88	22.91*
	141.42	17.00 \pm 1.53	38.56**
	212.13	13.65 \pm 1.33	50.59**
Pyrimethamine	1.2	11.70 \pm 0.88	57.82**
Distilled water (Control)	10ml/kg	27.67 \pm 0.88	-

Values are expressed as mean \pm SEM, n = 6; significance relative to control (* $p < 0.01$; ** $p < 0.001$)

3.5 Curative test

Decrease in the levels of parasitaemia was observed in the extract treated groups as well as the group treated with

artesunate (Table 4). This decrease was dose and time-dependent and statistically significant ($p < 0.001$). There was a daily increase in parasitaemia in the control group.

Table 4: Curative Effect of Ethanol Leaf Extract of *Hippocratea Africana*

Treatment	Dose (mg/kg/day)	D2	D4	D6
Leaf extract	70.71	12.00 ± 0.53*	7.67 ± 0.66*	6.60 ± 0.33*
	141.42	11.68 ± 0.20*	6.00 ± 2.55*	4.00 ± 0.58*
	212.13	8.33 ± 0.27*	5.33 ± 0.80*	3.67 ± 2.33*
Artesunate	5	5.30 ± 0.33*	4.33 ± 0.88*	2.63 ± 0.30*
Distilled water (Control)	10ml/kg	15.60 ± 0.67	21.00 ± 1.15	24.33 ± 0.33

Values are expressed as mean ± SEM, n=6

* = significance relative to control ($p < 0.001$).

3.6 The mean survival time (MST)

Mice in the extract treated groups survived longer than those in the control group. However, the duration was shorter when

compared with mice treated with the standard drug. Results are presented in Table 5.

Table 5: Mean survival time of mice treated with ethanol leaf extract of *Hippocratea Africana*

Treatment	Dose (mg/kg/day)	Survival time (days)
Leaf extract	70.71	11.00 ± 0.50
	141.42	16.33 ± 0.33*
	212.13	18.00 ± 0.67*
Artesunate	5	26.00 ± 0.58*
Distilled water (Control)	10ml/kg	10.33 ± 0.20

Values are expressed as mean ± SEM, n=6

* = significance relative to control ($p < 0.001$)

4. Discussion

The leaf of *Hippocratea africana* tested positive to some phytochemical compounds. Alkaloids which were detected have been implicated in the antimalarial efficacy of many plants [11]. Often alkaloids act by blocking protein synthesis in the parasite (*Plasmodium*). Saponins have been reported to be detrimental to several infectious protozoans [12]. Nyemenya *et al.* [13] in their studies on the antiplasmodial activity of extracts from selected medicinal plants used in Cameroun, reported the significant antimalarial activity exhibited by flavonoids against different strains of the malaria parasite. Saponins, terpenes and flavonoids have been reported to have antiplasmodial properties [14]. The value obtained (707.11mg/kg) from acute toxicity study of the ethanol leaf extract revealed that it is moderately toxic [15].

The dose and time-dependent reduction in parasitaemia reported in the 4-day, prophylactic and curative tests corroborate results obtained from other antimalarial studies involving the use of crude extracts of medicinal plants [10, 16]. The chemosuppressive effects produced by the extract were significant compared with the control. The mean survival time (MST) of the extract treated groups which was longer compared with the control further demonstrated the efficacy of the extract.

5. Conclusion

This study shows that the leaf extract of *Hippocratea africana* has suppressive, prophylactic and curative potentials. These properties may in part be due to its phytochemical constituents. The extract is moderately toxic which implies that its long term use may not be safe.

Purification of the extract used in this study would undoubtedly improve its antimalarial efficacy.

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