



Antimalarial activities of *Breynia Nivosa*

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ABSTRACT

Background & Aim: Antiplasmodial activity of leaf extract of *Breynia nivosa* was evaluated to ascertain the folkloric claim of its antimalarial activity.

Experimental: The crude leaf extract (75 – 225 mg/kg), of *Breynia nivosa* was investigated for antiplasmodial activity against chloroquine sensitive *Plasmodium berghei* infections in mice. The antiplasmodial activity during early and established infections as well as prophylactic were investigated. Artesunate 5 mg/kg and pyrimethamine 1.2 mg/kg were used as positive controls. Thin films made from tail blood of each mouse were used to assess the level of parasitaemia of the mice.

Results & Discussion: The extract dose-dependently reduced parasitaemia induced by chloroquine sensitive *Plasmodium berghei* infection in prophylactic, suppressive and curative models in mice. These reductions were statistically significant ($p < 0.001$). They also improved the mean survival time (MST) from 11 to 27 days relative to control ($p < 0.01 - 0.001$). The activities of extract was comparable to that of the standard drugs used (pyrimethamine). The antiplasmodial and antipyretic effects may in part be mediated through the chemical constituents of the plant.

Industrial and practical recommendations: The plant, *Breynia nivosa*, possesses antimalarial property which can be exploited in the treatment of malaria.

1. Introduction

Breynia nivosa (W. Bull) Small syn. *Phyllanthus nivosus* W. G. Sm., *Breynia disticha* J.R.Forst. & G.Forst. (Euphorbiaceae), is a rounded shrub, about 2 m high, that is primarily used for its attractive foliage and found in villages and towns. It has small, mottled, multi-colored variegated leaves with white, green and red coloration leaves (Smith, 1981). In southern Nigeria, it is used as chewing stick (Amadi et al., 2007). Ethnomedicinally, *Breynia nivosa* is used in

treating headaches, toothaches and tooth infections (Onyebule et al., 2014) and in the treatment of fever and malaria by the Ibibios of Niger Delta region of Nigeria. The leaf extract of the plant has been reported to contain flavonoids, tannins, alkaloids, glycosides and starch, saponins and proteins (Onyebule et al., 2007). Reported biological activities of the leaf include; antimicrobial (Amadi et al., 2007; Onyebule et al., 2014), analgesic, anti-inflammatory, and antioxidant activities (Onyebule et al., 2014). In this study we report the antimalarial activity of the leaf

extract of *B. nivos*a to ascertain the folkloric uses of the plant as malarial remedy.

2. Materials and Methods

2.1. Plants collection

The plant material *Breynia nivos*a (leaves) was collected in a compound in Uyo metropolis, Akwa Ibom State, Nigeria in September, 2014. The plant was identified and authenticated by Dr. Margaret Bassey of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen (FPUU) was deposited at Department of Pharmacology and Natural Medicine Herbarium.

2.2. Extraction

The plant parts were washed and shade-dried for two weeks. The dried plants' materials were reduced to powder using mortar and pestle. The powdered material was soaked in 50% ethanol. The liquid filtrates were concentrated and evaporated to dryness in vacuo 40°C using rotary evaporator. The extract (2g) was partitioned with a 50:50 mixture of distilled water and chloroform. The aqueous fraction was evaporated to dryness in a water bath at 60°C and the chloroform fraction air-dried. The ethanol extract, the aqueous and chloroform fractions were stored at - 4°C. until used in a refrigerator.

2.3. Animals

Albino Swiss mice (15 – 20g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

2.4. Determination of median lethal dose (LD₅₀)

The median lethal dose (LD₅₀) of the extract was estimated using albino mice by intraperitoneal (i.p) route using the method of Lorke (1983). This involved intraperitoneal administration of different doses of the extract (1000-5000 mg/kg) to groups of three mice each. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 hours was recorded. The LD₅₀ was calculated as geometrical means of the maximum dose

producing 0% (a) and the minimum dose producing 100% mortality (b).

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

2.5. Parasite inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.2ml of infected blood containing about 1×10^7 *P. berghei berghei* parasitized erythrocytes. The inoculum consisted of 5×10^7 *P. berghei berghei* erythrocytes per ml. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations (Odetola and Basir, 1980).

2.6. Drug administration

The drugs (artesunate and pyrimethamine), extract and fractions used in the antiplasmodial study were orally administered with the aid of a stainless metallic feeding cannula.

2.7. Evaluation of antiplasmodial activity of the extract

2.7.1. Evaluation of suppressive activity of the extract (4-day test).

This test was used to evaluate the schizontocidal activity of the extract, fractions and artesunate against early *P. berghei berghei* infection in mice. This was done as described by Knight and Peters (1980). Forty-two mice were randomly divided into five groups of six mice each. On the first day (D₀), the thirty mice were infected with the parasite and randomly divided into various groups. These were administered with the extract, fractions and artesunate. The mice group 1 were administered with the 112 mg/kg, the group 2, 224 mg/kg and group 3, 448 mg/kg of crude extract, groups 5 and 6 were administered with the 224 mg/kg of the aqueous and chloroform fractions respectively, while group 6 was administered with 5 mg/kg of artesunate (positive control), and 10 ml/kg of distilled water to group 7 (negative control) for four consecutive days (D₀ – D₃) between 8am and 9am. On the fifth day (D₄), thin blood film was made from tail blood. The film was then stained with leishman stain to reveal parasitized erythrocytes out of 500 in a random field of the microscope. The average percentage suppression of parasitaemia was calculated in comparison with the controls as follows:

Average % parasitaemia - Average % parasitaemia
in negative control in Positive groups
 Average % parasitaemia in negative control

2.7.2 Evaluation of prophylactic or repository activities of extract

The repository activity of the extract and pyrimethamine (daraprim) was assessed by using the method described by Peters (1965). The mice were randomly divided into five groups of six mice each. Groups 1 - 3 were administered with 112, 224 and 448 mg/kg/day of the extract respectively, while group 4 - 7 were respectively given 224 mg/kg/day of the aqueous and chloroform fractions, 1.2 mg/kg/day of pyrimethamine (positive control) and 10 ml/kg of distilled water (negative control). Administration of the extract/drug continued for three consecutive days ($D_0 - D_2$). On the fourth day (D_3) the mice were inoculated with *P. berghei berghei*. The parasitaemia level was assessed by blood smears seventy-two hours later.

2.7.3 Evaluation of curative activities of extract (Rane's test)

This was used to evaluate the schizontocidal activity of the extract, fractions and artesunate in established infection. This was done as described by Ryley and Peters (1970). *P. berghei berghei* was injected intraperitoneally into another 30 mice on the first day (D_0). Seventy-two hours later (D_3), the mice was divided randomly into seven groups of six mice each. Various doses of the extract; 112 mg/kg, 224 mg/kg and 448 mg/kg were orally administered respectively to mice in groups 1-3, 224 mg/kg of the aqueous and chloroform fractions were administered to groups 4 and 5 respectively, 5 mg/kg/day of artesunate to the group 6 (positive control) and group 7 was given 10 ml/kg of distilled water (negative control). The extract and drugs were administered once daily for 5 days. Leishman's stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor parasitaemia level. The mean survival time (MST) of the mice in each treatment group was determined over a period of 29 days ($D_0 - D_{28}$).

$\frac{\text{No of days survived}}{\text{Total No. of days (29)}} \times 100 = \text{MST}$

2.8 Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using Students' t-test and ANOVA (One- or Two- way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means will be considered significant at 1% and 5% level of significance i.e $P \leq 0.01$ and 0.05 .

3. Results and discussion

The median lethal dose (LD_{50}) was calculated to be 2242.07 mg/kg. The physical signs of toxicity included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death.

On the suppressive test, the extract and its fractions showed a dose-dependent chemosuppressive effect on the parasitaemia. These effects were statistically significant relative to the control ($p < 0.001$). The chloroform fraction showed a high chemoinhibitory percentage of 69.86 % which was incomparable to that of the standard, artesunate, 5 mg/kg. (Table 1).

On the prophylactic/ repository activity test, the ethanol crude extract showed a dose-dependent reduction of parasitaemia in the extract-treated groups. These reductions were statistically significant relative to the control ($p < 0.01 - 0.001$). The chloroform fraction demonstrated a high antiplasmodial activity which was significant ($p < 0.001$) when compared to control but was incomparable to that exhibited by the standard drug, pyrimethamine, 1.2mg/kg. (Table 2).

On established infection, there was a progressive dose-dependent reduction of parasitaemia in all the extract/fraction-treated group relative to control. These reductions were statistically significant relative to the control ($p < 0.001$) (Figure 1). Though the aqueous fraction and the highest dose of the crude extract (448 mg/kg) exhibited a high chemosuppression these were uncomparable to that of the standard, artesunate.

The crude extract and fractions demonstrated a significant ($p < 0.001$) protective potentials on the animals as was seen in the mean survival time of the animals. The groups treated with chloroform and highest dose of the crude extract had a longer mean survival time, 24.92 ± 0.68 and 20.86 ± 0.78 days respectively, though less than that of the standard drug, artesunate (29.83 ± 0.12 days) (Table 3).

The major folkloric uses of *Breynia nivosa* have been in the treatment of headaches, toothaches and tooth infections (Onyegbule et al., 2014) and in the

treatment of fever and malaria. These prompted the need to evaluate the in vivo antiplasmodial potential of the crude leaf extract of *Breynia nivos*a to confirm its ethnobotanical uses.

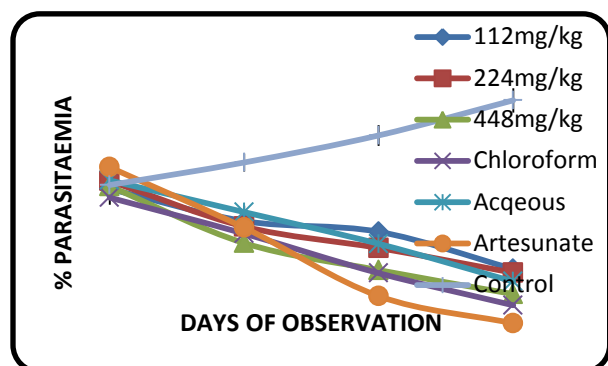


Figure 1: Curative effect of ethanolic leaves extract and fractions of *Breynia nivos*a on *Plasmodium berghei* established infection in mice.

Table 1. Suppressive activity of ethanolic leaves extract and fractions of *Breynia nivos*a on *Plasmodium berghei* infection in mice (4-day test)

Treatments	Dose (mg/kg)	Parasitaemia	% Chemosuppression
Normal saline	10ml/kg	23.00 ± 0.84	-
<i>B. nivos</i> a crude extract	112	12.3 ± 0.89 ^a	46.52
	224	11.0 ± 0.58 ^a	52.17
	448	9.3 ± 0.19 ^a	59.56
Aqueous fraction	224	10.25 ± 1.02 ^a	55.43
Chloroform fraction	224	6.93 ± 0.10 ^a	69.86
Artesunate	5.0	3.98 ± 1.19 ^a	82.69

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

In this work, median lethal dose (LD₅₀) was determined to be 2242.07 mg/kg, and the extract was relatively safe (Homburger, 1989).

The antiplasmodial properties of the extract and its fractions were investigated using standard models. It was found that both the extract and its fractions significantly reduced the parasitaemia in suppressive,

prophylactic and curative models in a dose - dependent fashion.

Table 2. Repository/Prophylactic activity of ethanolic leaves extract and fractions of *Breynia nivos*a on *Plasmodium berghei* infection in mice

Treatments	Dose (mg/kg)	Parasitaemia	% Chemosuppression
Control	10ml/kg	33.0 ± 1.57	-
<i>B. nivos</i> a leaf extract	112	25.37 ± 0.68 ^a	23.12
	224	15.66 ± 0.40 ^a	52.54
	448	13.33 ± 0.45 ^a	59.60
Aqueous fraction	224	13.72 ± 1.04 ^a	58.42
Chloroform fraction	224	9.34 ± 0.26 ^a	71.69
Pyrimethamine	1.2	3.16 ± 0.93 ^a	90.42

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

Table 3. Mean survival time of mice receiving the various doses of ethanol leaves extract and fractions of *Breynia nivos*a during established *P.berghei* infections in mice.

Treatments	Dose (mg/kg)	MEAN SURVIVAL TIME(days)
Normal saline	10ml/kg	9.0±0.65
<i>B. nivos</i> a crude extract	112	13.43 ± 0.53 ^{ns}
	224	15.44 ± 0.82 ^a
	448	20.86 ± 0.78 ^a
Chloroform fraction	224	24.92 ± 0.68 ^a
Aqueous fraction	224	16.78 ± 0.44 ^a
Artesunate	5.0	29.83 ± 0.12 ^a

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

These results further confirm the ethnobotanical use of this plant. Some secondary metabolites of plants are said to have antiplasmodial activity. The leaf extract of the plant has been reported to contained flavonoids, tannins, alkaloids and saponins (Onyegbule *et al.*, 2007). Phytochemical compounds such as alkaloids and terpenes and their derivatives such as monoterpenes have been implicated in antiplasmodial activity of many plants (Philipson and Wright, 1991; Christensen and Kharazmi, 2001). Among these metabolites are flavonoids and triterpenoids such as quassinoids (Philipson and Wright, 1991; Christensen and Kharazmi, 2001; Kirby *et al.*, 1989). Flavonoids are reported to chelate with nucleic acid base pairing of the parasite (Lui *et al.*, 1992) and triterpenes like quassinoids are potent protein inhibitors (Liao *et al.*, 1976). These compounds (flavonoids and alkaloids) present in this plant extract may in part have contributed to the plasmocidal activity of this extract and therefore explained the mechanism of antiplasmodial effect of the extract and its fractions.

4. Conclusion

The results of this study demonstrated that *Breynia nivos*a possesses considerable antiplasmodial activity. These confirm its use to treat malaria and fever in folkloric medicine. Therefore, it would be interesting if the the active principle is isolated, identified and characterised.

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