Antimalarial effects of ethanol leaf extract and fractions of *Peristrophe bicalyculata* in mice

Uwemedimo F. Umoh[a*] , Danladi N. Bala[a], Samuel J. Offor[a] and Chimezie Osuala[a]

[a] Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

ABSTRACT

The leaf infusion of *Peristrophe bicalyculata* (RETZ) NEES (Acanthaceae) is a commonly used remedy in the treatment of malaria by the Ibibio indigenes of Akwa Ibom State of Nigeria. The antimalarial effects of ethanol leaf extract and fractions of *Peristrophe bicalyculata* (RETZ) NEES (Acanthaceae) were investigated in mice infected with *Plasmodium berghei berghei* during early and established infections using suppresive, repository and curative animal experimental models. *Peristrophe bicalyculata* extract and fractions (374 – 1123 mg/kg/day) exhibited a significant ($P<0.05$) blood schizonticidal activity both in 4 day early infection and in established infection. The leaf extract and fractions possessed promising antiplasmodial activities which could be exploited in malaria therapy.

Key words: *Peristrophe bicalyculata* , antimalarial, established infection

INTRODUCTION

Malaria is one of the major killer diseases of the world. According to the World Health Organization (WHO), it is a significant public health problem in more than 90 countries inhabited by some 2400 million people which constitute about 40% of the world's population. There are an estimated 300-500 million clinical cases each year with more than 90% of these occurring in sub-Saharan Africa (Clark, 2002). Malaria causes up to 2.7 million deaths per year with the vast majority of these among children in Africa, especially in remote rural areas with limited or no access to medical care. In fact, in some parts of Africa, malaria kills 3000 children under 5 years of age each day and other high-risk groups include women during pregnancy, refuges and labourers entering endemic regions. Malaria also imposes a huge economic burden on countries where the disease is rife (Richard, 2006).

P. bicalyculata is a perennial herb which grows to a height between 0.6 m and 1.2 m. The leaf infusion of *P. bicalyculata has* been used in treating malaria by the Ibibio tribe of Akwa Ibom State of Nigeria. *Peristrophe* is a genus containing between 15 and 40 species of flowering plants in the family Acanthaceae. The species are shrubs or herbaceous plants native to warm temperate and tropical regions of Africa and Asia.

*Corresponding author: ufumoh@yahoo.com

The species found in Africa include *bicalyculata, cernua, angolensis, lanceolata, aculeate, cliffordii, hensii, decorticans, herereonsis, pilosa, mellerioides, namibiensis, serpenticola, teklei* etc. (Burkhill, 1996). This work is aimed at validating the folkloric use of *P. bicalyculata* in the treatment of malaria.

MATERIALS AND METHODS

Collection and preparation of plant material

Peristrophe bicalyculata was collected on July 2010 from Itak Ikot Ukap, Ikono Local Government Area in Akwa Ibom State, Nigeria. The plant was identified by Dr.(Mrs) M. E. Bassey, a plant taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria and a voucher specimen deposited in the herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Akwa Ibom State with voucher number UUH/028/14. The freshly collected leaves were air-dried, powdered and macerated in 70% ethanol for 72hours. The crude liquid extract was filtered and concentrated to dryness *in vacuo* at 40^0C. 40 g of plant extract (Pbe) was dissolved in 200ml distilled water and partitioned successively with n-hexane and butanol to obtained n-hexane, butanol and aqueous fractions denoted as N-hf, Butf and Aqf. The fractions were separately concentrated to dryness *in vacuo* at 40^0C and stored in a refrigerator from where they were being put to use.

Phytochemical analysis

Preliminary Phytochemical screening of *P. bicalyculata* leaf extract was carried out to detect the secondary metabolites present using standard procedures (Trease and Evans, 1999).

Animals

Adult albino mice of either sex weighing 20-30mg were used in this study. The animals were housed under standard environmental conditions with free access to Standard guinea feed mash and water, except for the day of experiments. The care and the handling of the animals were in accordance with the internationally accepted standard guide for the care and use of laboratory animals (1996) and as adopted and promulgated by the National Institute of Health and the related ethics regulation of Faculty of Pharmacy, University of Uyo, Nigeria. All animals were handled with humane care.

Acute Toxicity Study

Acute toxicity was estimated using the method of Lorke (1983) with slight modifications. Albino mice in groups of three weighing 20 – 30 g were treated with the extract (1000 mg/kg to 5000 mg/kg) intraperitoneally and animals were observed for physical signs of toxicity within 24hours.

Evaluation of suppressive activity (4 day test)

On the first day (D_0), forty eight (48) mice were infected with *Plasmodium berghei berghei* and randomly divided into eight (8) groups of six animals. Groups 1-3 mice were administered with extract (374mg/kg, 748 mg/kg and 1123 mg/kg) while groups 4, 5, and 6 were administered with 748.33 mg/kg of n-hexane, butanol and aqueous fractions respectively. Group 7, received 5mg/kg of Artesunate as standard and group 8 received 10 ml/kg of distilled water (negative control) for four consecutive days ($D_0 – D_3$). On the fifth day (D_4), thin blood film from the mice were stained with Leishman's stain to reveal parasitized erythrocytes out of 500 in

a random field of the microscope (Knight and Peters, 1980). The average percentage suppression of parasitaemia was calculated in comparison with the negative controls using the formula:

$$\frac{\text{Average \% parasitaemia in negative control - Average \% parasitaemia in positive groups}}{\text{Average \% parasitaemia in negative control.}}$$

Evaluation of repository or prophylactic activity

The protective activity of the extract and fractions was assessed by using mice which were randomly divided into eight groups of six mice each. Groups 1-3 were administered with extract (374 mg/kg, 748 mg/kg and 1123 mg/kg) while mice in groups 4-6 were treated with 748mg/kg of n-hexane, butanol and aqueous fractions respectively. Group 7 animals received 5 mg/kg of Artesunate as positive control and group 8, 10ml/kg of distilled water as negative control. The administration of the extract, fractions and drugs continued for three days (D_0-D_2). On the fourth day (D_3) the mice were inoculated with *Plasmodium berghei berghei*. The parasitaemia level was assessed by blood smears 72 hours later (Peters, 1965).

Evaluation of curative activity

This was used to evaluate the schizonticidal activity of the extract and fractions in established infection. *Plasmodium berghei berghei* was injected intraperitoneally into 48 mice on the first day (D_0). Seventy-two hours later (D_2), the mice were divided randomly into eight groups of six mice each. Different doses of the extract (374 mg/kg, 748 mg/kg and 1123 mg/kg) were orally administered to mice in groups 1-3. Groups 4-6 animals were pretreated with 748.33mg/kg of the n-hexane, butanol and aqueous fractions respectively, group 7 received 5mg/kg of Artesunate and group 8 was given 10ml/kg of distilled water. The extract, fractions and drug were administered 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 (Ryley and Peters, 1970). The mean survival time (MST) of the mice in each treatment group was determined over a period of 28 days ($D_0 - D_{27}$) using the formula below:

$$\text{MST} = \frac{\text{Number of days survived}}{\text{Total number of days (28)}} \times 100$$

Statistical analysis

Data were expressed as mean $\pm$ SEM and were statistically analyzed using one way ANOVA followed by Turkey Kramar multiple comparison test. Values of $P<0.05$ were considered significant.

RESULTS

Phytochemical screening

The result of phytochemical screening of ethanol leaf extract of *Peristrophe bicalyculata* is as shown in table 1.

Acute Toxicity Study

P. bicalyculata leaf extract showed physical signs of toxicity such as writhing, decreased motor activity, gasping, decreased limb/body tone, respiratory rate and death within 24hr after administration. The LD_{50} was calculated to be 3742 mg/kg according to Lorke's rule.

Table 4.2: Phytochemical constituents of ethanol leaf extract of *P. bicalyculata*

Metabolite	test	observation	inference
Tannins	ferric chloride	green ppt	+++
Phlobatanins	5% KOH	colourless solution	+++
Alkaloids	Dragendorff reagent	no brick red ppt	+++
Saponin	frothing	froth lasted 10min	++
Cardiac glycosides	salkowski	red colour at interphase	++
	Keller kiliani	brown ring at interphase	++
Terpenes	lieberman	dark green colour at interphase	+
Anthraquinones	Bontrager	no reddish colour	+
Flavonoid	Magnesium metal	crimson red colour	+

Key: + = present in trace
++ = present in abundance
- = absent

Suppressive Activity

Suppressive activity of crude extract and fractions of *P. bicalyculata* in mice during early infection revealed a dose-related chemosuppressive effect at the various administered doses (374 - 1123 mg/kg/day) with percentage chemosuppression of 12.77%, 28.72% and 37.77% respectively. For the partitioned fractions, butanol fraction showed a higher chemosuppressive effect of 57.44% (Table 2)

Table 2: Suppressive activities of ethanol extract and fractions of *P. bicalyculata* in early *P. berghei berghei* infection in mice

Drug/extract	Dose (mg/kg/day)	Average % parasitaemia	Average % suppression
Pbe	374	54.67 ± 0.41*	12.77
	748	44.67 ± 1.08*	28.72
	1123	39.00 ± 3.08*	37.77
N-hf	748	34.67 ± 0.82*	44.68
Butf	748	26.67 ± 3.36*	57.44
Aqf	748	50.00 ± 3.73	20.22
Artesunate	5	10.00 ± 0.71*	84.04
Water (control)	0.2ml	62.67 ± 1.47	-

Significance relative to negative control (*P< 0.05) n = 6

Repository Activity

The ethanol leaf extract and fractions showed a dose-dependent chemosuppressive effect on the parasitaemia. This effect was statistically significant relative to the control (P<0.05) and butanolic fraction exhibited the highest effect with percentage chemosuppression of 58.62% (Table 3)

Table 3: Repository activities of ethanol leaf extract and fractions of *P. bicalyculata* in *P. berghei berghei* infection in mice

Drug/extract	Dose (mg/kg/day)	Average % parasitaemia	Average % suppression
Drug/extract	**Dose**	**Average %**	**Average %**
Pbe	374	74.67 ± 2.48*	14.17
	748	55.67 ± 8.29*	36.01
	1123	45.67 ± 2.16*	47.51
N-hf	748	45.33 ± 1.08*	44.68
Butf	748	36.00 ± 3.54*	58.62
Aqf	748	75.33 ± 1.78	13.41
Artesunate	5	12.00 ± 0.61*	86.21
Water (control)	0.2ml	87.00± 1.41	-

Significance relative to negative control (*P< 0.05) n = 6

Figure 1: The curative effects of ethanolic leaf extract of *P. bicalyculata* on *Plasmodium berghei* established infection in mice

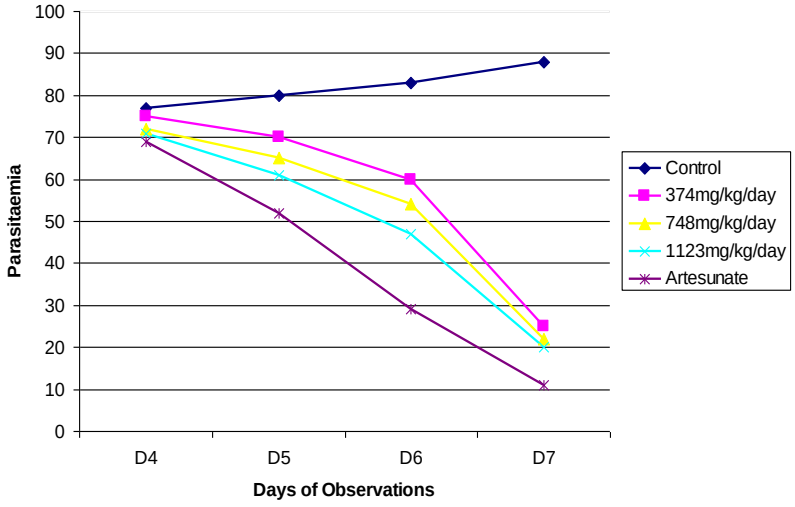


Figure 2: Curative effects of fractions of *P. bicalyculata* on *Plasmodium berghei* established infection in mice.

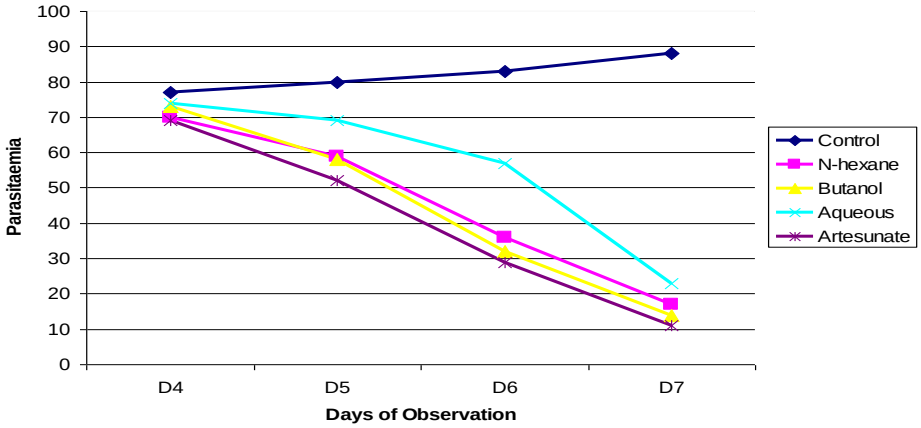
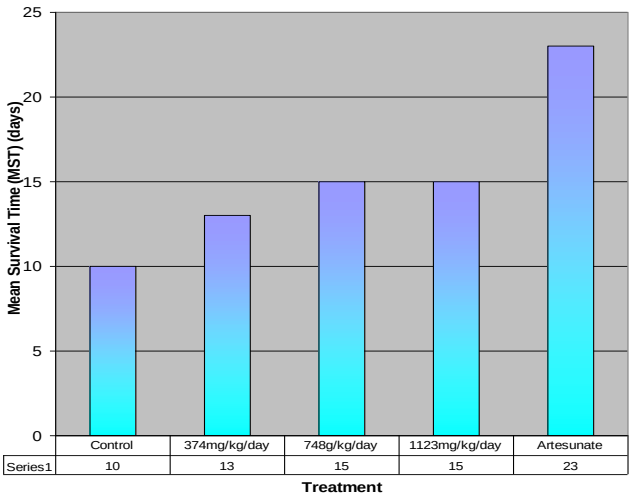


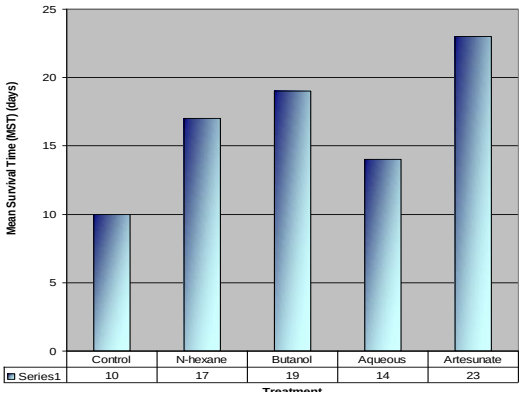
Figure 3: The mean survival time (mst) of ethanolic leaf extract of *P. bicalyculata* during established infection in mice



Curative Activity

There was a dose-dependent reduction in parasitaemia of extract treated groups of mice, while the control group showed a daily increase in parasitaemia. The mean survival time (MST) of the groups treated with extract, fractions and prototype drug was as shown in figures 1, 2, 3 and 4.

Figure 4: The mean survival time (mst) of fractions of *P. bicalyculata* during established infection in mice



DISCUSSION

Several studies have revealed the presence of bioactive agents in plants that function effectively as important drug base. From this study, Phytochemical screening of the leaves of *Peristrophe bicalyculata* showed the presence of alkaloids, tannins and phlobatannins, saponins, cardiac glycosides, flavonoids and anthraquinones. Alkaloids and flavonoids have been reported to be responsible for the antimalarial activities of plants. These secondary metabolites could have elicited the observed antiplasmodial activity either singly or in synergy with each other (Philipson and Wright, 1991).

The acute toxicity evaluation of the extract revealed that lethal dose (LD_{50}) of the extract was 3742 mg/kg.

The antiplasmodial properties of the extract and its fractions showed that both the extract and fractions significantly reduced parastaemia ($P<0.05$) in prophylactic, suppressive and curative models in a dose-dependent fashion. The results indicated that the leaf extract and its fractions possessed blood schizontocidal activity as evident from the chemosuppression obtained during the 4 day early infection test. The plant extract and its fractions also exhibited repository activity, though the dose used could not produce suppression comparable to that of the standard drug (Artesunate 5mg/kg/day). Butanol fraction demonstrated the highest chemosuppressive effect at the fraction level.

However, on established infection, the plant extract and its fractions exhibited significant ($P<0.05$) blood schizontocidal activity though not as potent as the prototype drug (artesunate), thus demonstrating a considerable antimalarial activity. Besides, this lower activity could have resulted from the crude nature of the extract which can be improved by further purification of the extract.

Although the mechanism of action of this extract and its fractions has not been elucidated, some plants are known to exert antiplasmodial action by causing elevation of red blood cell oxidation or by inhibiting protein synthesis (Kirby *et al*., 1989). The extract could have elicited its action through either of the two mechanisms mentioned above or by some other unknown mechanisms.

The results of this study support the use of this plant in the treatment of malaria traditionally but further studies is recommended to unravel the exact mechanism of action and identify specific constituents responsible for the observed activities.

Acknowledgement

The authors are grateful to Mr Nsikan Malachy for his technical assistance.

REFERENCES

Burkill, HM (1996). The Useful Plants of West Tropical Africa, Families A-D. Royal Botanic Gardens, Kew. Vol. 3, Pp. 320 - 323.

Clark, SB (2002). Antimalarial Natural Products. Isolation, Characterization and Biological Properties. Taylor and Francis, London. Pp. 379 -432.

Knight, D. J. and Peters, W. (1980). The antimalarial action of N-benzyloxydihydrotriazines and the studies on its mode of action. *Annals of tropical medicine and parasitology*. **74**:393-401.

Kirby, G. C., O'Neil, M. J., Philipson, J. D. and Warhurst, D. C. (1989). In vitro studies on the mode of action of quassinoids with activity against chloroquine resistant *Plasmodium falciparum*. *N Engl J. Med.* **38**: 4367 – 4374.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology* **54**: 275-286.

Peters, W. (1965). Drug resistance in *Plamodium berghei. Experimental parasitology*. **17**:80-89.

Philipson, J. D. and Wright, C. W. (1991). Antiprotozoal compounds from plants sources. *Planta Med.* **57**; 553 – 559.

Richard, D (2006). Malaria Mosquito *Anopheles gambiae*. *Science* **241**: 115-119.

Ryley, J. F. and Peters, W. (1970). The antimalarial activity of some quinolone esters. *Annals of Tropical Medicine and Parasitology*. **84:**209- 222.

Trease, G. E. and Evans, W. C. (1999). Pharmacognosy, 13th ed, Bailliere Tindal, London, Pp. 225-255.