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Evaluation of antiplasmodial activity of ethanol extract and fractions of *Maesobotrya barteri* root

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Abstract

Maesobotrya barteri is a medicinal plant that is used by the Ibibios of Southern Nigeria, to treat various ailments including malaria. The objective of this study was to evaluate the *in vivo* antiplasmodial activity of ethanol extract and fractions of *M. barteri* root in *Plasmodium berghei berghei* infected Swiss albino mice. Phytochemical screening of the root revealed the presence of saponins, flavonoids, terpenes and cardiac glycosides. Ethanol extract (100 – 300mg/kg/day) was screened for antiplasmodial activity in mice. Schizonticidal effects in early and established infections were investigated. Antiplasmodial effect of butanol and n-hexane fractions of the root extract (200mg/kg/day) were also evaluated. Intraperitioneal administration of 100 – 300mg/kg of ethanol extract resulted in gradual, dose-dependent decrease in parasitaemia, in both the suppressive and curative tests. Reduction in parasitaemia was also observed when butanol and n-hexane fractions were administered. The antiplasmodial effects of 200 and 300mg/kg of the extract and the butanol fraction (200mg/kg) were statistically significant ($p<0.05$) when compared with their controls. Chloroquine (5mg/kg), performed better than the extracts and fractions in terms of antiplasmodial activity in all the tests carried out. Mice treated with the negative control (distilled water) survived fewer days (14.67 ± 0.90) than those treated with the extracts (18.00 ± 0.36 – 26.30 ± 0.56) and the fractions (25.00 ± 0.36 and 27.00 ± 0.36, for n-hexane and butanol respectively). Mice treated with chloroquine survived beyond the observation period of 28 days. The root of *M. barteri* holds antiplasmodial potential which should be further explored.

Keywords: Antimalarial, extract, fractions, *Maesobotrya barteri*

INTRODUCTION

Malaria is a complex disease that varies widely in epidemiology and clinical manifestations in different parts of the world. Despite recent advances in the development of a wide range of antimalarials, the disease remains a great threat to the people of tropical developing countries [1]. The problem of resistance has been a major concern in the chemotherapy of malaria. Herbal antimalarial drug development may hold more promise for the treatment of malaria because plant products have proved to be less toxic, more affordable and with minimal side effects.

Going by the popular maxim "prevention is better than cure" it is needful to also explore appropriate preventive measures, because treatment coverage of populations in

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most endemic countries is well below the level needed to interrupt transmission. Development of vaccines has been a long term objective of scientists but progress has been slow. The attitude of people to the use of insecticide treated nets has also contributed to the poor control of the disease. Besides, confirming status and monitoring treatment success using diagnostic aids is not routine and regular.

Vector control is one of the ways to reduce malaria transmission and it is an intervention that can reduce malaria transmission from very high levels to close to zero [2]. Some indigenous plant based products are very promising against mosquitoes including the malaria vector. Some of these have been used to effectively get rid of the larval stage of mosquitoes [3-5].

The rainforest plant, *Maesobotrya barteri* (Euphorbiaceae) commonly called "squirrel cherry", is known by the Ibibios of Akwa Ibom State, Nigeria, as "Nyanyated". It is the most widely distributed *Maesobotrya* species in Nigeria. The fruits are delicious and enjoyed by both children and adults. Its twigs and small branches are used as chewing sticks. The extract of the pulverized bark is drunk to treat dysentery and diarrhea while the root decoction is drunk to treat mumps, rheumatism and malaria [6].

This study has been carried out to evaluate the *in vivo* antiplasmodial efficacy of ethanol extract and fractions of the root of *M. barteri.*

EXPERIMENTAL

Plant collection and identification. *M. barteri* root was collected from a farmland at Itak Ikot Akap village, Ikono, Akwa Ibom State, Nigeria. Taxonomic keys for the identification of this experimental plant was obtained from the Department of Pharmacognosy and Natural Medicine, University of Uyo. Herbarium specimen with voucher number UUH 31h was prepared and deposited in the herbarium of the same department.

Extraction and fractionation. The experimental root sample was thoroughly washed and air dried, after which it was pulverized and cold-macerated in 50% ethanol, for 72 hours with periodic stirring. This was followed by filtration and each filtrate was separately transferred to a rotary evaporator to get rid of the organic solvent and then concentrated to dryness. A quantity of 30g of ethanol extract of *M. barteri* root was successively partitioned using n-hexane and butanol to obtain their respective fractions. Both fractions were concentrated to dryness and stored in a refrigerator until used for the experiments reported in this research.

Phytochemical screening. The roots of *M. barteri* were screened for bioactive constituents such as alkaloids, cardiac glycosides, saponins, tannins, terpenes and flavonoids using the methods of Evans [7] and Sofowora [8].

Animal stock. Adult Swiss albino mice weighing 17 – 25g were obtained from the Animal House of the Department of Pharmacology and Toxicology, University of Uyo, Nigeria. They were fed with growers' pellet feed with water given *ad libitum*.

Ethical clearance. Approval for the use of animals in this study was obtained from the Animal Ethics Committee, Faculty of Pharmacy, University of Uyo, Nigeria.

Malaria parasite. Chloroquine-sensitive strain of *Plasmodium berghei berghei* was obtained from Nigeria Institute for Medical Research (NIMR), Lagos. These parasites were maintained by sub passage in mice.

Preparation of inoculum. Blood was obtained from the parasitized donor mouse. This was done by first anaesthetizing the mouse with chloroform and through cardiac puncture blood was collected into sterile heparinised bottles, using sterile syringe.

Blood obtained was suitably diluted with sterile normal swine so that the final inoculum (0.2ml) for each mouse contained the required number of parasitized erythrocytes (1 x 10^7). This is the standard inoculum for the infection of a single mouse [9]. A volume of 1ml of the standard inoculum is expected to contain 5 x 10^7 parasitized erythrocytes [10].

Drug and extract administration. The standard drug, chloroquine, ethanol extract and fractions of *M. barteri* root used in this research were administered through the oral route using sterile feeding cannula.

Acute toxicity test. Acute toxicity test was carried out to determine the median lethal dose (LD_{50}) of the root of *M. barteri* using the method of Lorke [11]. The test was also carried out to determine the safety of the extracts using Swiss albino mice. Twenty-one (21) animals were divided into 7 groups of 3 animals per group. The groups received 50, 100, 500, 1000, 2000, 3000 and 5000mg/kg of ethanol extract of *M. barteri* root, respectively. Extract doses were administered intraperitoneally. Signs of acute toxicity were observed for 24 hours. The LD_{50} was calculated using the formula:

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

A = The maximum dosage that produced 0% mortality
B = The minimum dosage that produced 100% mortality

Determination of antiplasmodial efficacy. *Determination of suppressive activity of extract on early infection (4-day Test).* The method of Okokon *et al*. [12] was adopted for the determination of suppressive activity of the extract. A total of 30 mice were used for this study. Each mouse was inoculated on the first day (D_0), intraperitoneally with 0.2ml of infected blood containing 1 x 10^7, *P. berghei berghei* parasitized erythrocytes. The animals were thereafter randomly divided into five groups of six (6) mice each. Animals in groups 1 to 3 received 100, 200 and 300mg/kg/day respectively of the ethanol extract of *M. barteri* root. Group 4 animals received 5mg/kg/day of chloroquine orally. This served as the standard drug; Group 5 animals received 10ml/kg/day of distilled water. This served as the control. Extract, drug and distilled water were administered daily to the different groups for 4 days (D_0 – D_3), between 9.00am and 10.00am. On the fifth day (D_4), thin blood films were made from tail blood obtained from each mouse. The films were stained with Giemsa stain to reveal parasitized erythrocytes, under 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. The following formulae were used:

% parasitaemia = (No of parasitized erythrocytes ÷ Total No. of erythrocytes counted) X 100

Average percentage chemosuppression

$$= 100 \{(A - B) / A\}$$

Where A is the average percentage parasitaemia in the control group and B, average percentage parasitaemia in the test group.

Evaluation of antiplasmodial efficacy of the fractions using 4-day test. This test was carried out using the method of Ettebong *et al.,* [9]. Twenty-four (24) mice were used for the study. These mice were each infected intraperitoneally with 0.2ml of infected blood containing about 1 x 10^7 *P. berghei berghei*. Thirty (30) minutes after inoculation, they were randomly divided into four groups of 6 mice per group: Mice in groups 1 and 2 were given oral administration of 200mg/kg/day of n-hexane and butanol fractions of the root of *M. barteri* respectively. Group 3 mice received 5mg/kg/day of the standard drug (chloroquine). Mice in group 4 were given 10ml/kg/day of distilled water. These treatments were continued for 4 days (D_0 – D_3). Thin films were prepared from tail blood of each mouse on the fifth day (D_4) and the level of parasitaemia was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. The average

percentage chemosuppression was determined using the formula shown in the suppressive test, involving the extract.

Determination of the effect of extract on established infection (Curative or Rane Test). An evaluation of the schizonticidal efficacy of the ethanol extract of *M. barteri* root, during established infection was carried out using the method of Tekalign *et al.* [13]. Thirty (30) mouse were inoculated intraperitoneally with standard inoculum of 1 x 10^7 *P. berghei berghei* parasitized erythrocytes on the first day (D_0). Seventy-two (72) hours later, the mice were randomly divided into five groups of 6 mice per group. Mice in groups 1, 2 and 3 received 100, 200 and 300mg/kg/day respectively of the extract orally. Group 4 mice received 5mg/kg/day of chloroquine. Group 5 mice were administered with 10ml/kg/day of distilled water. Extract doses, drug, and distilled water were administered to all the animals once daily for 5 days. Tail blood samples were collected from each mouse on alternate days, after commencement of treatment i.e. D_2, D_4, D_6. Blood samples collected were used to prepare thin films; they were stained with Giemsa stain and observed under the oil immersion objective as described earlier for the suppressive test. The Mean Survival Time (MST) was determined for each group, over a period of 28 days (D_0 – D_{27}).

MST = (Number of days survived ÷ Total No. of days of observation) X 100

Data analysis. Data obtained from this research were analyzed using Graph pad instat and SPSS version 17. Results were expressed as mean ± SEM. Significance was determined using one-way analysis of variance (ANOVA). A probability level of $p < 0.05$ was considered significant.

RESULTS

Results of phytochemical screening. The phytochemical screening of the root of *M. barteri* revealed the presence of saponins, flavonoids, terpenes and cardiac glycosides as shown in Table 1.

LD_{50} value of the root of *M. barteri.* The median lethal dose (LD_{50}) of ethanol extract of *M. barteri* root which was determined using the method of Lorke (1983), was 1095. 4mg/kg.

Results of antiplasmodial studies. Administration of the different doses of ethanol extract of *M. barteri* root resulted in a dose dependent decrease in the levels of parasitaemia in the suppressive test. Administration of extract doses of 100, 200 and 300mg/kg/day resulted in percentage suppression of 13.68, 36.10 and 37.79% respectively; while the standard drug, chloroquine gave 92.79% suppression. Thus, the suppressive effect of the different doses of the extract was less than the effect of the standard drug. However, the suppressive effects demonstrated using 200 and 300mg/kg of the extract were statistically significant ($p< 0.05$) when compared with the results obtained from the control experiment (0% suppression). These results are presented in Table 2.

Results of 4-day test. Results of the 4-day test using the fractions revealed that 200mg/kg/day of the butanol fraction exhibited a higher (23.51%) chemosuppressive effect than the same dose of n-hexane fraction which exhibited a chemosuppression of 11.73%. Administration of chloroquine resulted in a chemosuppressive effect of 93.09%. No suppressive effect was observed in the control (distilled water). These results are presented in Table 3.

Results of curative test. Results obtained from the curative test (on established infection) indicated a progressive dose and time dependent reduction in parasitaemia on administration of the extract (Table 2).

Mean Survival Time (MST). The mean survival time (MST) of extract-treated groups

was also dose-dependent and significantly longer when compared with the control (18.00 ± 0.36 – 26.30 ± 0.56 days), but was shorter when compared with the standard drug, chloroquine, (Table 5). Mice treated with chloroquine survived beyond the observation period of 28 days.

DISCUSSION

The median lethal dose (LD_{50}) of 1095.4mg/kg obtained from acute toxicity test of the root of *M. barteri* indicates that the plant is only slightly toxic and hence, relatively safe, according to Loomis and Hayes [14]. Phytochemical screening of the root of *M. barteri* revealed the presence of saponins, flavonoids, cardiac glycosides and terpenes. Saponins and flavonoids have been reported to be responsible for the antimalarial activities of plants.

Table 1: Results of phytochemical screening of the root extract of *M. barteri*

Phytochemicals	Test	Inference
Alkaloids	Dragendoff	-
Saponins	- Frothing	+
	- Sodium bicarbonate	+
Tannins	0.1% Ferric chloride (Fe Cl_3)	-
Flavonoids	Shinoda test	+
Terpenes	Glacial acetic acid	+
Cardiac glycosides	- Steroidal ring test	+
	- Keller Killiani test	+
	- Lieberman's test	+

+ = Presence of secondary metabolite - = Absence of secondary metabolite

Table 2: Suppressive activity of ethanol extract of *M. barteri* root in *P. berghei berghei* infected mice

Treatment	Dose (mg/kg/day)	Mean Parasitaemia Density	% Suppression
Extract	100	76.96 ± 1.59	13.68
Extract	200	56.97 ± 0.96	36.10*
Extract	300	55.47 ± 0.38	37.79*
Chloroquine (Standard drug)	5	6.43 ± 1.72	92.79*
Distilled water	10ml/kg	89.16 ± 0.23	-

n = 6 * = Significant compared with control ($p < 0.05$)

Table 3: Suppressive activity of n-hexane and butanol fractions of *M. barteri* root in mice infected with *P. berghei berghei*

Treatment	Dose (mg/kg/day)	Mean parasitaemia density	% Suppression
n-Hexane	200	78.70 ± 1.80	11.73
Butanol	200	68.20 ± 0.42	23.51*
Chloroquine	5	6.40 ± 0.23	93.09*
Distilled water	10ml/kg	87.70 ± 0. 38	-

n = 6 * = Significant compared with control ($p < 0.05$)

Table 4: Antiplasmodial effect of different doses of ethanol extract of *M. barteri* root (during established infection)

Treatment	Dose (mg/kg)	Mean ± SEM		
		D_2	D_4	D_6
Extract	100	86.50 ± 0.81	84.93 ±0.66	82.30 ± 0.17
Extract	200	86.13 ± 0.59	81.16 ± 1.43	76.20 ± 1.81*
Extract	300	70.40 ± 0.22*	67.60 ± 0.15*	65.80 ± 0.18*
Chloroquine	5mg/kg	54.40 ± 0.25*	36.46 ± 0.53*	2.30 ± 0.87*
Control (Distilled Water)	10ml/kg	87.00 ± 0.63	88.70 ± 0.03	91.13 ± 0.12

n = 6 * = Significant compared with control ($p < 0.05$)

Table 5: Mean survival time of mice treated with ethanol extract of the root of *M. barteri (Mean* ± SEM, n = 6)

Treatment	Dose (mg/kg/day)	Survival Time (days)
Extract	100	18.00 ± 0.36*
Extract	200	22.67 ± 0.76*
Extract	300	26.30 ± 0.56*
n-Hexane	200	25.00 ± 0.36*
Butanol	200	27.00 ± 0.36*
Distilled water	10ml/kg/day	14.67 ± 0.90

* = significant compared with control ($p < 0.05$).

Flavonoids have been reported to chelate with nucleic acid base pairing of the parasite [15]. Flavonoids have also been reported to exhibit significant antiplasmodial activity against different strains of malaria parasite [16]. Azebaze *et al.,* [17] reported the antimalarial efficacy of *Allanblakia monticola*, of which stigmasterol-3-*O*-beta D-glucopyranoside saponin was detected. These secondary metabolites could have elicited the observed antiplasmodial activity either singly or in synergy with each other [18]. The crude ethanol extract and fractions of *M. barteri* root demonstrated some degree of antiplasmodial activity against *P. berghei berghei.* Although the extract and fractions did not completely clear the parasite, there was a gradual reduction in the level of parasitaemia on administration of the extract and fractions. The antiplasmodial activities of ethanol extract and fractions of *M. barteri* root did not compare favourably with the activity of the standard drug (chloroquine). However, the effects of 200 and 300mg/kg of the extract and 200mg/kg of butanol fraction were statistically significant ($p<0.05$) when compared with the control in the 4-day suppressive test. These doses of the extract also elicited antiplasmodial effect in the curative test, which also was significant compared with the control. The extract and fractions also sustained the mice for longer duration than the controls, thus demonstrating antiplasmodial activity. According to Krettli *et al.,* [19] and Adugna *et al.,* [20] a compound is considered active when suppression is 30% or above. In this study, 200 and 300mg/kg of the root extract of *M. barteri* produced suppression of 36.10 and 37.79% respectively in the 4-day test.

The antiplasmodial activity of this plant, from these results, appear to be relatively weak when compared with the standard drug chloroquine. Hence, its use as antiplasmodial could be enhanced if used in polypharmacy with other plants with antiplasmodial activity like *Nauclea latifolia* and *Clausena anisata* [21] or artesunate. Some seemingly weak antimalarial plant products have had their performance improved through combination with other antimalarial agents, which could be either herbal formulations or synthetic drugs. These combinations have resulted in positive synergistic or additive effects [22-24].

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