

IN VIVO ANTIMALARIAL ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *ACACIA AURICULIFORMIS*

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ABSTRACT

The *in vivo* antiplasmodial activity of the ethanol leaf extract of *Acacia auriculiformis* use in the treatment of malaria in Niger Delta region of Nigeria was evaluated in *Plasmodium berghei* infected mice. The antimalarial activity of the ethanol leaf extract of *Acacia auriculiformis* (350 – 1050 mg/kg/day) was evaluated for suppressive activity during early infection in 4-day test and curative activity in established infection (Rane's test). *Acacia auriculiformis* (350 – 1050mg/kg/day) exhibited significant ($P < 0.05$) blood schizonticidal activity both in 4- day early infection test and in established infection with a considerable mean survival time though not comparable to that of the standard drug, chloroquine, 5mg/kg/day. The leaf extract possesses significant ($P < 0.05$) antiplasmodial activity which confirms its use in folkloric medicine in the treatment of malaria.

Keywords: *Acacia auriculiformis*, Malaria, antiplasmodial

INTRODUCTION

In Most part of Nigeria, human malaria is endemic and constitutes one of the major causes of death for all ages, with an estimated 100,000 malaria attributable deaths yearly [1]. The resistance of malaria parasites to the available antimalarials particularly, chloroquine, the cheapest among them and the relatively high cost of the newer more effective treatment options necessitates many Nigerians to opt for herbal remedies against the disease. Many plants preparations currently used in Nigeria are without scientific evidence of efficacy.

Acacia auriculiformis A. Cunn. ex Benth (Fabaceae) also known as earpod wattle, is a resilient, vigorous growing evergreen tree up to 30 ft in height. The leaves are alternate, simple, blade-like and slightly curve. The plant is well distributed all over the world [2] and is use for shade and ornamental

purposes. The Ibibios of Niger Delta region of Nigeria claims that the plant is useful as malarial remedy. Acaciaside A and B, two acylated Bisglycoside saponnins isolated from the funicles of *A. auriculiformis* are reported to be antihelminthic [3]. The antioxidant [4] antifungal and antibacterial activities [5], antifilarial activity [3,6], cestocidal activity [7] as well as antimutagenic and chemopreventive activity [8] of *A. auriculiformis* have been reported. We investigated the antiplasmodial activity of the leaves of this plant to confirm its folkloric use.

Materials and Methods

Plant Material

Fresh leaves of *Acacia auriculiformis* were collected on a street in Uyo – Akwa Ibom State of Nigeria in June, 2006 and authenticated by Dr Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo, Nigeria. A voucher specimen has been deposited in the Faculty of Pharmacy herbarium, University of Uyo, Uyo. The

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plant materials were air dried at room temperature and then powdered.

Preparation of extract

The dried and powdered leaf of *Acacia auriculiformis* (1kg) was exhaustively macerated in 70% ethanol for 72 hours. The liquid extract obtained was concentrated in vacuum at 40°C. The yield was 0.48%. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study. The dry ethanolic extract was dissolved in distilled water to make the stock solution from which the various doses administered were prepared for use by serial dilution.

Phytochemical screening

Phytochemical screening of the extract was carried out employing standard procedures [9,10].

Animals

Albino swiss mice (21-28g) 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

Parasite inoculation

The chloroquine – sensitive *Plasmodium berghei berghei* was obtained from National Institute of Medical Research, Lagos, Nigeria and maintained in mice. The inoculum consisted of 5×10^7 *P. berghei berghei* parasitized 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. Each mouse was inoculated on day 0, intraperitoneally,

with 0.2 mL of infected blood containing about 1×10^7 *P. berghei berghei* parasitized red blood cells.

Determination of LD₅₀

The LD₅₀ of the extract was determined using albino mice by intraperitoneal (i.p.) route using the method of Lorke [11].

Evaluation of Schizontocidal Activity on early infection (4 – day test)

Schizontocidal activity of the extract was evaluated using the method described by Knight and Peters [12]. Each mouse was inoculated on the first day (day 0), intraperitoneally, with 0.2ml of infected blood containing about 1×10^7 *P. berghei berghei* parasitized erythrocytes. The animals were divided into five groups of five mice each and orally administered, shortly after inoculation with 350, 700 and 1050mg/kg/day doses of the *Acacia auriculiformis* leaf extract, chloroquine 5mg/kg/day and an equivalent volume of distilled water (negative control) for four consecutive days, (day 0 to day 3). On the fifth day (day 4), thin films were made from the tail blood of each mouse and the parasitaemia level was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Average percentage chemosuppression was calculated as

$$100 \times \frac{[A - B]}{A} \dots\dots\dots[1]$$

A

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

Evaluation of schizontocidal activity established infection (Curative or Rane test)

Evaluation of curative potential of the extract was done using a method similar to that described by Ryley and Peters [13]. The mice were injected intraperitoneally with standard inoculum of 1×10^7 *P. berghei berghei* infected erythrocytes on the first day (day 0). Seventy-two hours later, the mice were

divided into five groups of five mice each. The groups were orally administered with *Acacia auriculiformis* leaf extract (350,700, 1050mg/kg/day), chloroquine (5mg/kg) was given to the positive control group and an equal volume of distilled water to the negative control group. The drug/extract was given once daily for 5 days. Thin films stained with Giemsa stain were prepared from tail blood of each mouse daily for 5 days to monitor the parasitaemia level. The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post inoculation) in each group over a period of 28 days (day 0 to day 27).

Statistical Analysis

Data obtained from the study were analyzed statistically using Student's test and values of $P < 0.05$ were considered significant.

Results

Acute toxicity

The extract (500-5000mg/kg) produced physical signs of toxicity such as writhing, gasping,

palpitation, decreased respiratory rate, body and limb bone and death depending on the dose. All the mice treated with 4000mg/kg dose of the extract and above died. The I.p LD₅₀ of the extract in mice was calculated to be 3741.7mg/kg.

Phytochemical Screening

Phytochemical screening of the ethanolic leaf extract of *Acacia auriculiformis* revealed the presence of compounds like terpenes, flavonoids, anthraquinones, saponins, cardiac glycosides and tannins.

4-day test

Ethanolic leaf extract of *Acacia auriculiformis* produced a dose dependent chemosuppressive effect at various doses employed in this study. The chemosuppression were 69.26, 72.47 and 76.37% for 350,700 and 1050 mg/kg/day doses. The chemosuppression produced by the extract were significant ($P < 0.05$) compared to control and uncomparable to that of the standard drug (chloroquine 5mg/kg/day) with a chemosuppression of 87.8% (table 1).

Table 1: Antiplasmodial activity of *Acacia auriculiformis* during 4-day test.

Drug/Extract	Dose (mg/kg/day)	Average (%) parasitaemia	Average (%) suppression
<i>Acacia auriculiformis</i> extract	350	13.4± 3.29*	69.26
	700	12.0± 4.08*	72.47
	1050	10.3 ± 6.02*	76.37
Chloroquine (standard)	5	5.13 ± 0.38*	88.20
Distilled water (control)	0.2ml	43.6 ± 3.16	-

Data are expressed as mean ± S.D for five animals per group. $P < 0.05$ when compared to control.

Curative test

On established infection, it was observed that there was a daily increase in parasitaemia of the control group. However, there was a daily reduction in the parasitaemia levels of the extract treated group as well as that of positive control (chloroquine).

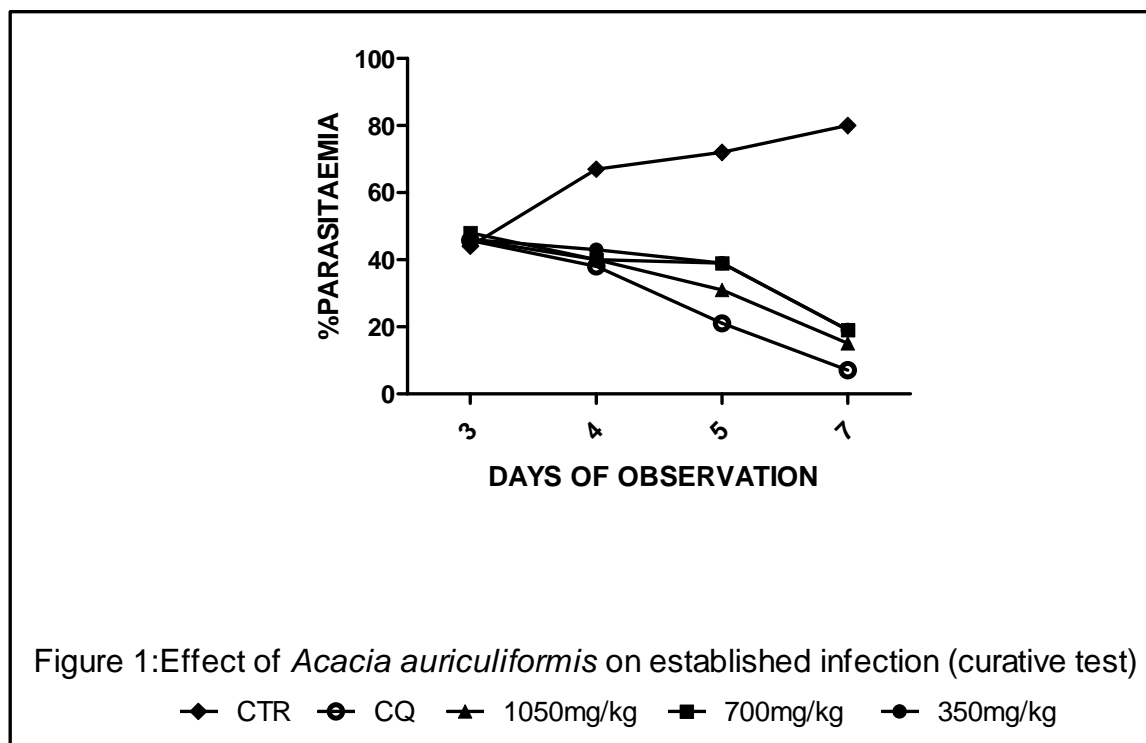
On day 7, the average percentage parasitaemia for the groups were 25, 19, 15, 7.0 and 80.0% for 350,

700, 1050mg/kg/day of the extract, chloroquine and control groups respectively (figure 1). The mean survival time (m.s.t) of the extract treated groups were significantly ($P < 0.05$) longer than that of control and was comparable to that of the standard drug, chloroquine. The values are given in table 2.

Table 2: Mean survival time of mice receiving various doses of ethanolic leaf extract of *Acacia auriculiformis*.

Drug / Extract	Dose (mg/kg/day)	Mean survival time (day)
<i>Acacia auriculiformis</i> extract	350	15.0± 1.41 *
	700	18.0± 8.64*
	1050	20.6 ± 6.64*
Chloroquine (standard)	5	28.0 ± 0.00*
Distilled water (control)	0.2ml	9.81 ± 1.32

Data are expressed as mean ± S.D for five animals per group. P<0.05 when compared to control.



Discussion

In this study phytochemical screening, acute toxicity and evaluation of in vivo antiplasmodial activity of ethanolic leaf extract of *Acacia auriculiformis* were carried out. Saponins, flavonoids, tannins, terpenes and cardiac glycosides were found to be present in the extract. However, reports of isolation of triterpenoid saponins from *Acacia auriculiformis* have been published [14, 15]. The LD₅₀ value of 3741.7 mg/kg shows that the extract is slightly toxic [16]. In vivo evaluation of the antiplasmodial activity of the leaf extract of *Acacia auriculiformis* revealed that the leaf extract possesses a significant (P<0.05) blood schizonticidal activity as evident from the chemosuppression obtained during early

and established infection as well as by the significant mean survival time of the mice treated with the extract compared to control. This is due to the ability of the extract to suppress the development and maturity of the parasite. Antimalarial compounds like flavonoids, terpenes and triterpenoids implicated in antimalarial activity of plants [17, 18, 19] were found present in the leaf extract. These compounds maybe responsible for the observed antiplasmodial activity of this extract. Although, the mechanism of action of this extract has not been elucidated, some plants are known to exert antiplasmodial activity by causing elevation of red cell oxidation [20] or by inhibiting protein synthesis [21]. The extract could have elicited its

action through either of the two mechanism mentioned above or by some other unknown mechanism.

Conclusion

The result of this study shows that ethanolic leaf extract of *Acacia auriculiformis* possesses antiplasmodial activity and also support the use of this plant in the treatment of malaria traditionally. Therefore, it would be interesting if the active principle could be identified and the mechanism of action elucidated from this promising medicinal plant.

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References

- 1) Ogunbona FA, Akata KJ, Fadiran EO, Femi-Oyewo MN. (Eds). *Malaria Chemotherapy In Nigeria: Problems And Prospects*. A. U.P. Ibadan, Nigeria. 1990; p131.
- 2) Langeland KA, Burks KC. *Identification and Biology of Non-native plants of Florida's natural areas*. University press of Florida, Gainesville, FL.1998.
- 3) Ghosh M, Babu S P, Sukul N C, Mahato S B, Antifilarial effect of two triterpenoid saponins isolated from *Acacia auriculiformis*. *Indian journal of experimental biology*, 1993; 31 (7):604-606.
- 4) Singh R, Singh S, Kumar S, Arora S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. *Food and Chemical Toxicology*. 2007;45 (7):1216-1223..
- 5) Mandal P, Sinha Babu SP, Mand NC. Antimicrobial activity of saponins from *Acacia auriculiformis*. *Fitoterapia* 2005; 76(5):462 – 465.
- 6) Mahato SB. Saponins with antifilarial activity from *Acacia auriculiformis*. *Journal of Natural Products*. 1996; 55(4):500 – 502.
- 7) Gosh NK, Babu SP, Sukul NC, Ito A. Cestocidal activity of *Acacia auriculiformis*. *Journal of Helminthology*.1996; 70(2):171 –172.
- 8) Kaur K, Arora S, Hawthorne ME, Kaur S, Kumar S, Mehta RG. A correlative study on antimutagenic and chemopreventive activity of *Acacia auriculiformis* A.Cunn and *Acacia nilotica* (L)Willd ex Del.Chem.Toxicol. 2002; 25(1):39 – 64.
- 9) Harbone J.B. *Phytochemical Methods. A guide to Modern Techniques of plant Analysis*. London, Chapman and Hall.1983.
- 10) Trease A, Evans W. C., *Trease and Evans Pharmacology*. 13th edn. London. Bailliere Tindal.1989.
- 11) Lorke D., *A new approach to practical acute toxicity test*. *Arch. Toxicology*. 1983; 54:275-286.
- 12) Knight DJ, Peters W. The antimalarial action of N-benzyloxy dihydrotriazines. The action of cycloquanil (BRL50216) against rodent malaria and studies on its mode of action. *Ann. Trop. Med. Parasitol*. 1980; 74:393-404.
- 13) Ryley, J F, Peters W. The antimalarial activity of some quinone esters. *Ann. Trop. Med. Parasitol*. 1970; 84:209-222.
- 14) Uniyal SK, Badoni V, Sati O.P. A new triterpenoidal saponin from *Acacia auriculiformis*. *Journal of Natural Products*.1992; 55(4):500 – 502.
- 15) Garai S, Mahato S.B. Isolation and structural elucidation of three triterpenoid saponins from *Acacia auriculiformis*.*Phytochemistry*.1997; 1:137 – 140.
- 16) Homburger, F. In vivo testing in the study of toxicity and safety evaluation. In: *A Guide to General Toxicology*. Marquis J. K. (Ed). 2nd Edn Karger, New York.1989.
- 17) Christensen SB, Kharazmi A. Antimalarial natural products. Isolation, characterization and biological properties. In. *Bioactive compounds from natural sources: Isolation, characterization*

and biological properties. Ed. Tringali, C. London, Taylor & Francis. 2001; Pp 379-432.

- 18) Philipson JD, Wright CW. Antiprotozoal compounds from plants sources. *Planta Medica*. 1991; 57:553-559.
- 19) Saxena S, Pant N, Jain DC, Bhakun RS. Antimalarial agents from plant sources. *Current Science*. 2003; 85(9):1314 – 1329
- 20) Etkin NL. Antimalarial plants used by Hausa in Northren Nigeria. *Tropical Doctor*. 1997; 27(suppl.1):12 – 16.
- 21) Kirby GC, O'Neil MJ, Philipson JD, Warhurst DC. In vivo studies on the mode of action of quassinoids with activity against chloroquine resistant *Plasmodium falciparum*. *Biochem.Pharmacol*.1989; 38:4367 – 4374.

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