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Antimalarial potency of the leaf extract of *Aspilia africana* (Pers.) C.D. Adams

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ABSTRACT

Objective: To investigate the antimalarial activity of ethanol extract of *Aspilia africana (A. africana)* leaf. **Methods:** The ethanol extract of *A. africana* leaf (100-400 mg/kg) was screened for blood schizonticidal effect against chloroquine-sensitive *Plasmodium berghei (P. berghei)* in mice both in early and established models of antimalarial studies. **Results:** The leaf extract exhibited significant ($P<0.05$) antiplasmodial activity in 4-day early infection and in established infection tests with a considerable mean survival time comparable to that of standard drug, chloroquine (10 mg/kg). **Conclusions:** The findings show that ethanol extract of *A. africana* leaf possesses potent antiplasmodial activity which justify the use in ethnomedicine and can be developed in malaria therapy.

1. Introduction

Malaria continues to cause morbidity and mortality on a large scale in tropical countries. The alarming rate at which the parasite, particularly *Plasmodium falciparium* (*P. falciparium*), has developed resistance to currently used antimalarial drugs makes it imperative to search for newer, more effective therapeutic agents. The selection of plants to be screened for antimalarial activity is done on the basis of traditional reputation of particular plants for efficacy in the treatment of malaria. Malaria is Africa's leading causes of under five mortality and constitutes 10% of the continents overall disease burden, malaria accounts for 40% of public health expenditure, 30% to 50% inpatient and up to 50% out-patient in areas with high malaria transmission. The global strategy for malaria mainly focuses on case management through provision of drugs capable of reducing the morbidity and mortality or eliminating parasites[1,2]. Despite the increase threat of malaria to lives especially in Africa, success in controlling the disease is possible[3]. Different approaches are currently being advocated to achieve this which includes: exploring evidence of immunity, revisiting the abandoned vector control methods and investigation into traditionally used herbal medicines[4].

Aspilia africana (*A. africana*) (Pers.) C.D. Adams (Asteraceae) is commonly found in tropical West Africa. This plant is commonly referred to as "hemorrhage plant" due to its ability to stop blood flow from fresh wounds, and has several uses in traditional medical practice. The crushed leaves have been used for patients suffering from rheumatic pains and to clean surfaces of sores by absorption of exudates[5]. Wound healing occurs when treated with the crushed leaves[6]. An infusion of the leaves of this plant is usually taken by pregnant women at child birth and used as cough remedy in children. *A. africana* is also used for traditional treatment of malaria symptoms in East and Central Africa[7]. The antibacterial activity of the leaf extract has been reported[8].

This study aimed at evaluating the antimalarial activity of ethanol leaf extract of *A. africana* on *P. berghei* infection in mice.

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2. Materials and methods

2.1. Collection and preparation of plant materials

Fresh leaves of *A. africana* were collected from plants growing within the campus of University of Calabar, Cross River State, Nigeria. The plant material was identified and authenticated by Mr. F.I. Apejoye of Botany Department, University of Calabar, where a voucher specimen (No. 252) is maintained. The international plant number index is Asteraceae *A. africana* (pers.) C.D. Adams-Webbia X11.236 (1956) (Ik).

The leaves were cleaned, cut into smaller pieces, air-dried at room temperature for 7 days and pulverized to dry powder using a mortar and pestle.

2.2. Extraction of plant material

Three hundred and fifty grams of the dry leaf powder was extracted with ethanol by maceration for 48 h with constant shaking. The resultant filtrate was dried on water bath to obtain 32 g (9.1% w/w) of ethanol extract. The leaf extract was subsequently reconstituted in water at appropriate concentrations for the experiment.

2.3. Phytochemical screening

The ethanol leaf extract of *A. africana* was subjected to phytochemical analysis for identification of constituents using conventional protocols[9,10].

2.4. Animals

Swiss albino mice (18-22 g) of both sexes obtained from the Animal Facility Centre, NIPRD, Abuja, were used for the study. The animals were housed in aluminum cages at room temperature and moisture, under naturally illuminated environment of 12:12 h dark/light cycle. They were fed on standard diet and had water *ad libitum*. The mice were used in accordance with NIH Guide for the care and use of Laboratory Animals[11], NIPRD-Standard Operation Procedures.

2.5. Acute toxicity test

The LD_{50} of the plant extract was tested to determine the safety of the agent using Lorke's method. The study was carried out in two phases. In the first phase, nine mice were randomized into three groups of three per cage and were administered 10, 100 and 1 000 mg/kg of the leaf orally. The mice were observed for signs of toxicity which include paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first four hours and subsequently for 4 days. In the second phase, another set of nine mice were also randomized into three groups of three mice per cage and were administered 1 600, 2 900 and 5 000 mg/kg of the leaf extract orally, based on the result of the first phase. The animals were also observed for signs of toxicity and mortality for the first four hours and thereafter for 4 days. The oral LD_{50} was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

2.6. Inocula

Parasitized erythrocytes were obtained from a donor infected mouse maintained at Animal Facility Centre, NIPRD, Abuja, Nigeria. Parasites are maintained by continuous reinfestation in mice. The inoculums consisted of *P. berghei* parasitized erythrocytes. This was prepared by determining both the percentage parasitemia and the erythrocytes count of the donor mouse and diluting them with normal saline in proportions indicated by both determinations. Each mouse was inoculated intraperitoneally with infected blood suspension (0.2 mL) containing 1×10^7 *P. berghei* parasitized red blood cells.

2.7. Suppressive test

A 4-day suppressive test was performed using the methods of David *et al*[12-14]. Thirty Swiss albino mice of both sexes weighing (18-22 g) were inoculated by intraperitoneal injection with standard inoculums of *P. berghei* containing 1×10^7 infected erythrocytes. The mice were randomly divided into 5 groups of 6 per cage and treated for 4 consecutive days with 100, 200 and 400 mg/kg of the extract, chloroquine 10 mg/kg and 0.2 mL normal saline, all administered orally. On the fifth day, blood was collected from the tail of each mouse and thin films made on a slide. The films were fixed with methanol, stained with Giemsa and parasitemia density examined by microscopically (Nikon YS2-H, Japan) counting the parasitized red blood cells on at least 1 000 red blood cells in 10 different fields.

2.8. Curative test

Evaluation of curative potential of *A. africana* leaf extract was done adopting the method described by Ryley *et al* with slight modification. Thirty mice were selected and intraperitoneally injected with 1×10^7 *P. berghei* infected erythrocytes on the first day. Seventy two hours after, the mice were grouped into 5 groups of 6 per cage and treated with 100, 200 and 400 mg/kg of the extract, chloroquine 10 mg/kg and 0.2 mL normal saline, all administered orally. Treatment continued daily until the seventh day when thin films were prepared with blood collected from the tail of each mouse. The films were fixed with methanol, stained with Giemsa and parasitemia density examined by microscopically counting the parasitized red blood cells on at least 1 000 red blood cells in 10 different fields.

2.9. Statistical analysis

Results obtained were expressed as mean±SEM. The data was analyzed using one-way ANOVA and differences between mean were considered significant when $P< 0.05$[15].

3. Results

3.1. Phytochemical test

Phytochemical screening of the ethanol extract of *A. africana* leaf revealed the presence of terpenes, steroids, flavonoids, alkaloids, volatile oils and carbohydrates. These classes' compounds are reported to show important biological activities[16,17].

3.2. Acute toxicity test

There was no mortality observed in mice after oral administration of the ethanol extract even at doses as high as 5 000 mg/kg signifying that the oral LD_{50} was 5 000 mg/kg. Thus the experimental doses used (100, 200 and 400 mg/kg *p.o.*) were within safe margin.

3.3. Suppressive effect

The ethanol extract of *A. africana* leaf exerted dose-dependent chemosuppressive effect at the various doses employed in this study (100, 200 and 400 mg/kg), with a chemosuppression of 79.42%, 84.28% and 92.23% respectively. The effect of this extract was significantly ($P<0.05$) when compared with the control. The standard drug, chloroquine (10 mg/kg), caused 95.30% suppression (Table 1).

3.4. Effect of the extract on established infection

Treatment of the *P. berghei* infected mice with the leaf extract of *A. africana* exhibited a dose-dependent daily decrease in parasitemia in the extract treated group similar to chloroquine-treated group unlike in the saline group in which there was a consistent increase in the blood parasite density. The mean survival time also increased dose-dependently. Death was observed in the control group on day 8, and by day 12, all mice in the group died. On the other hand, mice in the groups treated with 100, 200 and 400 mg/kg survived beyond 21 days. Chloroquine treated group survived the 30 days duration of observation (Table 2).

Table 1

Suppressive activity of *A. africna* in *P. berghei*-infected mice.

Treatment	Dose (mg/kg)	Mean parasitemia density (D_5)
Control	0.2 mL/kg	32.56±0.38
A. africana	100	6.70±0.20*
	200	5.12±0.50*
	400	2.53±0.27*
CQ	10	1.53±0.26*

*Significantly different from control at $P<0.05$. D_5=Day five, CQ=Chloroquine.

Table 2

Curative effect of *A. africana* in *P. berghei*-infected mice.

Treatment	Dose (mg/kg)	Mean parasitemia density		Survival time (Days)
		Pre (D_3)	Post (D_7)-treatment	
Control	0.2 mL/kg	32.25±3.28	40.32±2.40	12.00±2.30
A. africana	100	29.30±2.33	8.47±0.82	22.10±1.20*
	200	30.42±3.10	6.80±0.27	25.00±1.00*
	400	31.38±4.20	4.93±0.47	28.20±0.50*
CQ	10	29.28±2.00	2.56±0.32	30.00±0.00*

*Significantly different from control at $P<0.05$. D_3=Day three, D_7=Day seven, CQ=Chloroqiune.

4. Discussion

The results show that *A. africana* leaf extract has negligible toxicity, as shown in the LD_{50} value of 5 000 mg/kg. The results also show that the leaf extract possess significant antimalarial activity which was evident from the chemosuppression it produced during the 4-day early-infection test. The rodent parasite, *P. berghei* has been used in studying the activity of potential antimalarials in mice[18] and in rats[19]. It produces diseases similar to those of human plasmodial infection[20,21]. Rodent models of antimalarial study have been validated through the identification of several conventional antimalarials especially with the success of quinine and more recently artemisinin derivatives[22]. Our investigation of the scientific reasons behind the folkloric use of *A. africana* in the treatment of malaria attack in traditional African setting can be partially satisfied with this result[23]. In addition, the result of chemosuppressive study can be interpreted to be that the leaf extract of this plant can suppress parasite growth to non-detectable levels in erythrocytes. It is important that scientific evaluation of traditional medicine preparations for claimed antimalarial efficacy be carried out even up to the level of finding out the degree of suppression of growth in erythrocytes[24].

The leaf extract of this plant also exerted significant curative effect during established infection. Curative activity of potential antimalarial agents of ethnobotanical materials should be discernable during testing for antimalarial properties. The observed antimalarial activity of the plant extract is consistent with the traditional use of the plant as herbal medication against the disease and indicative of its potential as a chemotherapeutic antimalarial agent. This was confirmed by the mean survival time values which at employed doses were twice or more than that of control group. In the untreated mice, the parasitemia count increased daily until the death of the animals, which was also observed in our previous studies[25].

The antiplasmodial activity of this plant extract might be attributed to the presence of alkaloids, flavonoids and terpenes which have been variously implicated in antiplasmodial activities of many plants[26-28]. However, the active compounds known to exert the observed activity need to be identified. Some plants are known to exhibit antiplasmodial activity by either causing red blood cell oxidation or by inhibiting protein synthesis depending on their phytochemical constituents. These compounds may be acting singly or in synergy with one another to exert antiplasmodial activity observed in the study.

This study has however, established the rationale for the traditional use of this plant in Nigeria and like many others, showed that medicinal plants which have folkloric reputations for antimalarial properties can be investigated in order to establish their efficacy and to determine their potentials as sources of new antimalarial drug.

Conflict of interest statement

We declare that we have no conflict of interest.

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