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Original Article

In vivo Antiplasmodial and Cytotoxic Activity of *Achyranthes aspera* and *Ficus thoningii* in Mice Infected with *Plasmodium berghei*

Moses Zaruwa[1], Joshua Osuigwe Madu[2*], Abdulhakeem AdebayoYusuf[3], Abdurrasheed Ola Muhammed[4]

1. Department of Biochemistry, Adamawa State University, Mubi, Nigeria.

2. Department of Chemistry, American University of Nigeria, Yola, Nigeria.

3. Microbiology Unit, Medical Laboratory Services Department, Federal Medical Centre, Yola, Nigeria.

4. Department of Histopathology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

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Malaria remains one of the leading causes of morbidity and mortality in tropical countries, and the alarming spread of the drugs resistant malaria parasite underscores the need to develop new antimalarial compounds. In this study, the phytochemical constituents, *in vivo* antiplasmodial, and cytotoxic activity of methanolic extracts of *Achyranthes aspera* and *Ficus thoningii* were evaluated using chloroquine sensitive *Plasmodium berghei* in mice. The extract of *F. thoningii* exhibited significant ($P<0.05$) blood schizontocidal activity a dose dependent manner; in a four day treatment test. Likewise, *A. aspera* extract resulted in high suppressions of schizonts (74%, 100% and 87%) at 50, 100, and 200 mg/Kg body weight, respectively ($P<0.05$). Neither death nor toxic signs was recorded in the mice in the acute toxicity study. These findings provide scientific rationale for the traditional use of these plants against malaria symptoms, and also make the plant a candidate for bioactivity guided phytochemical analyzes to identify the active principles.

Keywords: Malaria, *Ficus thoningii*, *Achyranthes aspera*, antiplasmodial activity

Malaria is a major public health problem in tropical and sub-tropical regions of the world. Over half a million (627,000) people, mostly children younger than five years old die from malaria each year. There are estimated 207 million cases of malaria each year and the vast majority (90%) of malaria cases occur in sub-Saharan Africa" (1) In pregnant women, malaria may cause ill health, still birth and miscarriage (2). In Nigeria, malaria accounts for the major loss of work hours and absenteeism by employees; it also constitutes a major healthcare burden in families (3).

Although artemisinin based combination therapy remains the treatment of choice against malaria infection, the efficacy of artemisinins is threatened by the emergence of resistant *Plasmodium spp* (6) and resistance to artemisinins have been reported in four countries in South Eastern Asia (WHO 2015). Like the artemisinin based combination therapy, many other cheap and readily available antimalarial drugs (chloroquine, pyrimethamine, sulfadoxine, mefloquine, quinine) are used in the treatment of malaria, but parasites resistance have also been recorded for these drugs (7-8). The increasing spread of resistance has generated a need to search for new anti-malarial treatment in order

*Correspondence: Department of Chemistry, American University of Nigeria, Yola, Nigeria. E-mail: joshuamadu11@gmail.com

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to reduce the menace of infection by these parasites.

Medicinal plants are a promising source for the discovery, and development of new drugs. They constituted the basis of traditional medicine systems for many years, and have continued to be a good source of lead compounds for drug development (4). Nigeria is rich in terms of diversity of her botanical resources, many of these medicinal plants are utilized by the local population for therapeutic purposes; several of these medicinal plants are used alone or in combination by the populace to treat malaria, either for their effectiveness or their availability at very little cost (5). However, many of these plants are yet to be scientifically investigated for their effectiveness. In line with this, we investigated the plasmodicidal and cytotoxic effects of methanolic extracts of *Ficus thoningii*, and *Achyranthes aspera* in albino mice infected with *Plasmodium berghei*.

Materials and methods

Plant materials and extracts preparation

Fresh leaves of *Achyranthes aspera* and *Ficus thoningii* were collected from a botanical garden in Yola town where they are used traditionally for treatment of malaria. The plants were authenticated in the Department of Plant Science, Modibbo Adama University of Technology, Yola. All samples collected were air-dried at room temperature (28±3 ºC) for five days.

Methanolic extracts were prepared as described by Lusakibanza et al. (2). Briefly, 100 g of each powdered plant sample was macerated in 500 ml of methanol, for 72 h at room temperature. The extracts were then filtered with Muslin cloth. The filtrates were evaporated to dryness at 40 °C in a hot air oven, and the methanol extract obtained was preserved at 4ºC in a refrigerator until required.

Phytochemical screening

Tannins, saponins, flavonoids, terpenoids, steroids, phenolics, alkaloids, and glycosides were determined in the methanolic extract as described previously (5, 9).

Determination of acute toxicity

The mice were allowed to acclimatize before the study, and were divided into five groups of two mice for each dose level (0 mg/Kg, 500 mg/Kg, 1500 mg/Kg, 2900 mg/Kg, and 4000 mg/Kg) for each plant extract. The mice were fasted overnight before extracts administration (10). The mice were monitored for signs of toxicity, and mortality for 24 h. Animals were treated according to the institutional animal guidelines.

Malaria induction using *Plasmodium berghei*

Mice were infected with chloroquine sensitive *Plasmodium berghei* (NK65 strain) sourced from the National Institute of Medical Research (NIMR), Lagos. Fifty adult albino mice of both sexes weighing between 18-25 g were used in this study. Each mouse was inoculated intraperitoneally with 0.2 ml of infected blood containing $1x10^7$ *P. berghei* parasitized red blood cells as described by Ryley and Peters (11). The inoculated mice were allowed for 24 h before commencing treatment with the plant extract.

Experimental design

Adult mice weighing between 18-25 g were randomly divided into 8 groups of 5 animals each. Groups 1-3 were administered with 50, 10, and 200 mg/Kg body weight of methanol extract of *A. aspera*, respectively for four days. Similarly, groups 4-6 were administered 50, 10, and 200 mg/Kg body weight of methanol extract of *F. thoningii*, respectively for four days, while groups 7 and 8 serving as malaria control and drug control respectively, were administered distilled water, and 10 mg/Kg body weight chloroquine, respectively for four days.

Parasitaemia determination and histological examination

Parasitaemia was monitored in blood obtained from the tail of each mouse, pre-sterilized with methylated spirit after four days treatment with the plant extracts. Using the Giemsa staining technique, the number of parasitized

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erythrocytes in each of the 10-50 fields were counted three times, and the average was calculated to give the parasitaemia of each mouse. Percentage of suppression was calculated as described by Dawit et al. (10). Sections were also taken from liver and kidney of animals, and stained with haematoxylin and eosin.

Statistical analysis

Data on parasitaemia were analyzed using Statistical Package for Social Sciences Version 20 (SPSS Inc. Chicago, IL, USA). The one way ANOVA was used to compare results among and within groups for differences between initial and final results. All data were analyzed at 95% confidence interval ($\alpha = 0.05$). $P < 0.05$ was considered as statistically significant.

Results

Phytochemical composition of plants extracts

Results for the qualitative phytochemical screening of plants revealed that only alkaloids were detectable in both plants (Table 1).

Antiplasmodial activity of plant extracts

Evaluation of the *in vivo* antiplasmodial potency of the methanolic extract of *A. aspera* against *Plasmodium beirghei* infection showed that the 100 mg/Kg body weight dose has antiplasmodial activity comparable to chloroquine, and the parasitaemia percentages at other concentrations tested were statistically non-significant ($P > 0.05$) compared to chloroquine (Table 2). The methanolic extract of *F. thoningii* showed a dose dependent chemo-suppression with 91% inhibition at 200 mg/Kg body weight (Table 3).

Table 1. Phytochemical composition of *A. aspera* and *F. thonigii*

Phytochemical composition	Plants Screened	
	A. aspera	***F. thonigii***
Saponins	+	-
Phenols	_	+
Tannins	-	+
Flavonoids	+	-
Quinolones	-	-
Glycosides	-	+
Terpenoids	+	-
Steroids	-	+
Alkaloids	+	+

+ : presence of the component; - : absence of the component.

Table 2. Antiplasmodial potency of the methanol extract of *A. aspera* against *P. berghei* infection in mice

Test substance	Dose (mg/Kg)	% Parasitaemia	% Inhibition
A. aspera	50	3.0 ± 1.73^{c}	74
	100	ND^{a}	100
	200	1.5 ± 0.96^{b}	87
Chloroquine	10	ND^{a}	100
Control	H_2O	11.6 ± 0.40^{d}	-

Values are expressed as mean ± SEM. ND: not detected. Different superscripts down the column are significantly different ($P < 0.05$). Same superscripts are not significantly different.

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Table 3. Antiplasmodial potency of the methanol extract of *F. thoningii* against *P. berghei* infection in mice

Test substance	Dose (mg/Kg)	% Parasitaemia	% Inhibition
F.thoningii	50	5.8 ± 1.11^{d}	55
	100	1.6 ± 0.75^{c}	82
	200	1.0 ± 1.00^{b}	91
Chloroquine	10	0.00 ± 0.00^{a}	100
Control	H_2O	11.6 ± 0.40^{e}	-

Values are expressed as mean ± SEM. Different superscripts down the column are significantly different ($P < 0.05$). Same superscripts are not significantly different.

Results of acute toxicity of the plants extract administered to the mice at 4000 mg/Kg, 2900 mg/Kg, 1500 mg/Kg, and 500 mg/Kg did not show any sign of toxicity within and after 24 h of observation. In addition, sections taken from liver and kidney did not show any pathological changes.

Discussion

The increasing number of people at risk of malaria infection in Africa, and the increasing resistance of plasmodium parasite to available antimalarial therapies such as artemisinin, and its derivatives have given rise to an urgent need to re-evaluate traditional medicine by extensive researches on different plant species, and their active therapeutic principles (12-14). The abundance of plants can represent an opportunity to find newer compounds exerting significant therapeutic activities. The major advantage of herbal medicine is their efficacy, limited adverse effects, and low cost (15).

In this study, the phytochemical screening of the methanolic extract of *A. aspera* revealed the presence of bioactive compounds with ethnopharmacological significance. These results agree with the reports of Saurabh et al. (16) and Mali and Wadekar (17), but are in contradiction with the findings of Purushothaman (18) who found tannins, phenols, saponins, flavonoids, glycosides, carbohydrates, and alkaloids in *A. aspera*'s extract. The compounds obtained from phytochemical analysis of *F. thoningii* were different from the findings of Ndukwe (19), who demonstrated the presence of glycosides, carbohydrates, saponins, alkaloid, steroids, tannins, and flavonoids. The difference observed in phytochemical composition of the extracts may be attributed to their origin, and climatic condition of the area (20).

A. aspera is an important plant due to its large number of medicinal properties (16). The present study showed that *A. aspera* was active against *P. berghei*, indicating its antimalarial activity, similar to *Agerantum conyzoides* another antimalarial plant studied previously (21). The results obtained in the present study indicated that the treatment of infected mice with the methanolic extract of *A. aspera* reduced the erythrocytic stage of malaria parasites. At different concentrations tested, the results observed with *A. aspera* were comparable to that of the standard drug (chloroquine) at 10 mg/Kg body weight.

The methanolic extract of *F. thoningii* demonstrated a dose dependent suppression in the *P. berghei* infected mice. The improvement observed by the inhibition of methanolic extract of *F. thoningii* as compared to *A. aspera*, may be due to the bioactive compounds present in *F. thoningii* leaf extract. Our observation corroborated with the finding of Tiwari et al. (20) who showed that increased plant extract activity may be due to the bioavailability of some phytochemical constituents. The observed higher efficacy of the standard drug chloroquine (100%) in comparison with *F. thoningii* treated group, may in part be due to non-selectivity of the extract or slow absorption, and poor bioactivity of the crude extract. Similar

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observations had been made by other researchers (22-23).

Although the mechanisms of action of these extracts have not been elucidated, the antiplasmodial activity of natural plant extracts have been shown to depend on their active phytochemical compounds, especially saponins, flavonoids, and tannins that are antioxidants, and free radicals scavengers able to counteract the oxidative damage caused by malaria parasite (22). These phytochemicals may act alone or in synergy, to cause the observed antimalarial activity.

All the mice treated in this study with various doses of the plant extracts (10 to 4000 mg/Kg) were carefully observed for 24 h in order to investigate any sign of toxicity, behavioural change, and mortality. However, no visible symptoms of acute toxicity; change in behaviour, and mortality were observed when plant extracts were orally administered to mice. Aarthi and Murugan (24) reported that plants or plant products with LD50 values higher than 2000 – 3000 mg/Kg are considered free of any toxicity. This report supports the logical use of *A. aspera* and *F. thoningii* in folk medicine practices as the LD50 of the plants was found to be more than 4000 mg/Kg body weight of albino mice. Furthermore, sections taken from liver and kidney appeared normal when stained with haematoxylin and eosin. This finding is in line with an earlier observation showing that these plants do not cause cytotoxicity (25).

In conclusion, the present study justifies local claims on the efficacy of the plant leaves in malaria infection treatment. It thus makes the plant an ideal candidate for investigating the active substances, and the optimum dosage regimen, possibly in combination with other antimalarial plants.

Conflict of interest

The authors declare that they have no competing interest.

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