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Phytochemical Screening and *In vivo* Antiplasmodial Sensitivity Study of Locally Cultivated *Artemisia annua* Leaf Extract Against *Plasmodium berghei*

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ABSTRACT

Objective of the study
Medicinal plant research has become very important considering the herbal resources our rain forest is bestowed with which if harnessed could solve the challenges in malaria treatment in the area of accessibility and treatment cost. The present study on antimalarial activity of locally cultivated *Artemisia annua* in Langtang South area of Plateau State against *Plasmodium berghei* and the phytochemical screening of the leaf extract is to evaluate the efficacy, phytochemical content and possible indigenization of the herbal based medicine which for now is not native to Nigeria.
Methodology
In vivo anti-malarial sensitivity of plant extract was assessed using the 4-day test for anti-malarial activity against a chloroquine sensitive *Plasmodium berghei* NK65 strain in Swiss albino mice, Phytochemical analysis and the lethal dose (LD_{50}) in mice screening were also determined.
Results
There was a dose -dependent inhibition of parasitaemia in the *In vivo* antiplasmodial tests, with maximum effect at 300 mg/kg. LD_{50} in mice was estimated to be 2750 mg/kg and the phytochemical analysis revealed the presence of terpenes, steroids, cardiac glycosides, flavonoids and carbohydrate.
Conclusion
The leaf extract of *A. annua* contains biologically active principles that possess anti-malarial activities, therefore further studies of its active components will be carried out.
Keywords- Malaria, *Artemisia annua*, Parasitaemia, Phytochemicals, *Plasmodium falciparum, Plasmodium berghei.*

INTRODUCTION

Malaria is a parasitic disease caused by four main species of *plasmodium*: *Plasmodium ovale*, *Plasmodium* vivax, *Plasmodium malariae* and *Plasmodium falciparum*. It is spread by the female *anopheles* mosquito, *Anopheles gambiae*. Malaria infection is reported to have plagued man from prehistoric times and remains one disease with immense social costs, being responsible for unacceptably huge number of deaths[1]. The problem of malaria is particularly troublesome in tropical third world countries where together with HIV/AID and tuberculosis, they become the world worst communicable disease[2].

Globally malaria is responsible for 300-500 million clinical cases annually resulting in between 1-2.7 million deaths, majority of who are children. *Plasmodium falciparum* causes the most serious infections[3]. Malaria is severe in children if not treated adequately and promptly due to the absence of innate immunity in them[4]. Malaria has enormous economic costs. It accounts for 40% of public health costs in Africa, it costs Africa $12 Billion of its GDP annually[5].

A major problem associated with the chemotherapy of malaria is drug resistance. *Plasmodium* parasites soon develop resistance to new drugs, once released for widespread use. This leads to the introduction of artemisinine in the middle of the 1990s[6]. The introduction of artemisinine based therapy provided welcome alternatives to Chloroquine and Sulpha-doxine/Pyrimethamine, which had become totally ineffective in the face of widespread resistance. Artemisinine based therapy greatly reduced the morbidity and mortality due to acute severe malaria in endemic countries[7], thus prompting the World Health Organization to recommend the use of artemisinine based combination therapy for the treatment of uncomplicated malaria in endemic regions of the world[8].

Artemisinine is obtained from the Sweet wormwood, Artemisia annua (Qinqhao in Chinese) as shown in Figure 1.

Artemisinine is as effective as Chloroquine in multidrug resistant plasmodia and has good safety profile[7]. However, currently available artemisinine-based combination therapy is expensive beyond what the average African rural dweller can afford[10]. One reason for this is the cost of importing either finished drug products or raw materials from outside the continent. Studies have shown that increasing access to life saving artemisinine based combination therapy in endemic areas is critical in the control and reduction of malaria transmission[11].

The present study shows evaluation of antimalarial activity of locally grown Artemisia annua in Langtang South area of Plateau State against Plasmodium berghei and the phytochemical screening of the leaf extract in order to discover the efficacy, phytochemical content of the herbal based medicine.

MATERIALS AND METHODS

Collection of plants

The plant (*Artemisia annua*) used for this study was collected during the flowering season (June) from the University of Jos farm in gangnim in Langtang South area of Plateau state and were identified by a taxonomist in the department of Genetics and Biotechnology of the University of Jos. Sun dried plant specimens were deposited in the herbarium of the Pharma-cognosy Department, Pharmaceutical Sciences Faculty, University of Jos.

Plant extract preparation

The dry plant samples were pounded to powder in a wooden mortar with pestle and the extract was prepared by maceration

of the plant material using n-Hexane in a conical flask (50gm of powder in 150ml of n-Hexane) and kept at 4°C for 72 hours with intermittent shaking. The extract was finally evaporated to powder in a water bath set at 40°C, the residues was found to be 10.25% w/w and stored in a refrigerator at 4°C for further investigation.

Acute toxicity determination

The acute toxicity of the extract was evaluated using Lorkes method[12] with modifications. Briefly, the test was carried out in two parts. Part 1: Nine mice were divided into three groups of three mice per group. The three groups were administered orally with graded doses (10, 100 and 1000 mg/kg respectively) of the extract. Part 2: Another nine mice were divided into three groups of three mice per group, this received graded doses (1600, 2900 and 5000 mg/kg) of the extract respectively. The number of deaths in each group within 24 hours was recorded and the LD_{50} values were calculated.

Phytochemical tests

The phytochemical screening of hexanolic extract of *A. annua* leaves was carried out to determine the presence of the following phytochemicals, tannins, alkaloids, flavonoids saponins, glycosides, sterols, terpenes and carbohydrates using standard procedures[13]. *In vivo* anti-malarial sensitivity against *Plasmodium berghei.*

In vivo antimalarial study

A total of thirty mice were used for this study. Each mouse was administered intra -peritoneal inoculums of $1.0x10^7$ parasites. The animals were divided into five groups of six mice each and 75, 150 and 300 mg/kg/day of the extract were administered orally to these groups. Chloroquine 5 mg/kg/day was given as positive control and 0.2 ml of distilled water as negative control for four consecutive days (D0 to D3). On the fifth day (D4), thick blood smears were prepared, fixed by methanol and stained with Giemsa for the counting of the parasites[14].

Data analysis

Collected data from *In vivo* antimalarial activity were subjected to one – way analysis of variance (ANOVA) by scheffe's test Post - Hoc using SPSS version 16.

RESULTS

Acute toxicity test

The acute toxicity of *A. annua has* been investigated to determine any adverse effect that may arise as a result of a short time animal exposure to the water extract within a 24 h period. The LD_{50} obtained for the hexanolic extract of *A. annua* was found to be 2750mg/kg.

Phytochemical test

The phytochemical analysis results of the extracts showing the presence of some secondary metabolites are presented in Table 1. The result shows that the hexanolic extract of *A. annua* contains terpenes, steroids, cardiac glycosides, carbohydrates and flavonoids.

In vivo antiplasmodial study

The anti-malarial activity of the hexanolic extract of *Artemisia annua* against *Plasmodium berghei* infected Swiss albino mice is given in Table 2 and Table 3 for thick and thin film smear respectively. After 4 days of consecutive treatment with the dose of hexanolic extract of *Artemisia annua* (75-300mg/kg) mean parasitaemia (%) in the *Plasmodium berghei* infected mice ranged from 52.80 ± 2.17 to 36.83 ± 2.14 for thick film and 50.40 ± 0.89 to 38.5 ± 1.98 for thin film whereas the mean parasitaemia (%) in the control group was 72.00 ± 2.45 for thick film and 72.5 ± 3.02 for thin film.

The hexanolic extract showed significant inhibition of parasitaemia compared to that of control (overall analysis of variance significance, F = 370.703 and P = 0.000 for thick film and F = 764.130, P = 0.000 for thin film) by scheffe's test. Chloroquine (5mg/kg) was taken as positive control for the extract.

DISCUSSION

The acute toxicity of *A .annua* has been investigated to determine any adverse effect that mayarise as a result of a short time animal exposure to the hexane extract within 24 h period[15]. The results of this study indicated low toxicity in mice, which confirms its lowest side effects. The preliminary phytochemical screening of the hexane extract of *A. annua* revealed the presence of terpenes, flavonoids, steroids, cardiac glycosides and carbohydrates. Similar research had also been conducted by some researchers[16] though these results were similar but did not detect the presence of carbohydrate and cardiac glycosides. This difference may be attributed to certain clinical factors, which include time and place of plant collection and extraction technique. In the present studies the technique employed for extraction is maceration, the plant was appropriately obtained from Langtang South area of Plateau State where the plant is commonly used for the treatment of malaria and the plant was harvested in June which is their optimal time of collection.

The therapeutic benefits of traditional remedies are often attributed to the presence of bioactive constituents present in the crude material[15]. Many secondary metabolites of plants' origin have been shown to have antiplasmodial activity[16]. Triterpenoids such as meldenin, from *Azadirachta indica*, deacytlkhivorin from *Khaya grandifolia* and exiguaflavone from *Artemisia indica* have established antiplasmodial activities[17]. Similarly, a number of metabolites from plants such as flavonoids, cardiac glycosides and steroids are reported to be responsible for the antiplasmodial activity of many medicinal plants[18]. It is therefore, probable that some metabolites present in A. *annua* are responsible for its antiplasmodial activity.

This can be supported by the studies carried out by Badman *et al*. (1988) and Klayman (1985)[19,20] which show conclusively that artemisinin, the antipyretic principle/ingredient of the plant *A. annua*, possess anti-malarial activity. This artemisinin belongs to sesquiterpene lactone[21]. In a similar study carried out by Dikkason *et al*. (2006)[22] on the plant *Asparagus afrcicanus* Lam using the hydroalcoholic extracts *In vivo* in *P. berghei* infected mice, the extracts from the roots and aerial parts of the plant were observed to inhibit parasitemia in mice by 46.1 and 40.7% respectively. This is also in support of the anti-malarial activity observed with the plant extract in this study. It is likely that specific terpenoids are present in all these plants studied by other workers to show their anti-malarial activities.

Although the mechanism of action of the antiplasmodial activities of the extract has not been investigated, the presence of these bioactive secondary metabolites with antiplasmodial potential may mean that the antiplasmodial action of the extract may be by interplay of different mechanisms. Ayoola *et al*[23]. had reported high level of antioxidants in antiplasmodial activity, therefore, the presence of metabolites such as terpenes and flavonoids with antioxidant activities could be related to the antiplasmodial action of *A. annua.*

Plasmodium species that cause human disease are essentially unable to infect non primate animal models. Therefore, rodent malaria parasite is employed for the *In vivo* evaluation of antimalarial compounds, due to the

sensitivity of *P. berghei* parasite to chloroquine. A 4 day suppressive test was used to assess the efficacy of our extract by comparison of blood parasitaemia and mouse survival time in treated and untreated mice[15].

Chloroquine, which has been used for treatment of malaria, was employed as the standard in this study[24]. *A. annua extract* demon-strated similar anti-plasmodial activities to chloroquine. The extract displayed a significant dose dependent suppressive activity against *P. berghei* in mice and enhanced percentage survival of animals.

The decrease in parasitaemea with increasing concentration of the extract reflects an inhibitory activity on parasite replication. This may be an indicative of a significant potential for isolating a purer compound[25].

CONCLUSION

Preliminary phytochemical screening of the hexanolic extracts shows the presence of various phytoconstituents i.e. flavonoids, terpenes etc. These bioactive agents possess significant suppressive effects on *P. berghei* infection in Swiss albino mice. This study confirms the *In vivo* antiplasmodial activity of the extract of *A. annua*, further studies are in progress in our laboratory to isolate and characterize the relevant bioactive components in leaves of *A. annua*.

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Table 1. Analysis of phytochemicals of hexane extract of *Artemisia annua*

Constituents	Remarks
Saponin	-
Terpenes	+
Alkaloids	-
Steroids	+
Tannins	-
Cardiac glycosides	+
Carbohydrates	+
Flavonoids	+

(+) = Presence, (−) = Absence

Table 2. Effect of *A. annua Plasmodium berghei* infection in mice using thick film smear

Treatment	Dose mg/kg/day	Mean ±SD parasitaemia (%)	% suppression of parasitaemia	% survival of animal on day 10
Control	-	72.00 ± 2.45[a]	-	-
A. annua	75	52.80 ± 2.17[b]	26.02	66.66
A. annua	150	42.60 ± 3.72[c]	40.18	83.30
A. annua	300	36.83 ± 2.14[c]	43.73	100.00
Chloroquine	5	8.83 ± 3.76[d]	87.63	100.00

F = 370.703, P = 0.000
Values are represented as mean ± standard deviation. Values in the same column with the same letter as superscript do not differ significantly (P< 0.05).

Table 3. Effect of *A. annua Plasmodium berghei* infection in mice using thin film smear

Treatment	Dose mg/kg/day	Mean ±SD parasitaemia (%)	% suppression of parasitaemia	% survival of animal on day 10
Control	-	72.5 ± 3.02[a]	-	-
A. annua	75	50.40 ± 0.89[b]	29.82	66.6
A. annua	150	46.80 ± 1.64[b]	35.70	83.3
A. annua	300	38.50 ± 1.98[c]	44.48	100
Chloroquine	5	6.83. ± 2.14[d]	90.48	100

F = 764.130, P = 0.000
Values are represented as mean ± standard deviation. Values in the same column with the same letter as superscript do not differ significantly (P< 0.05).

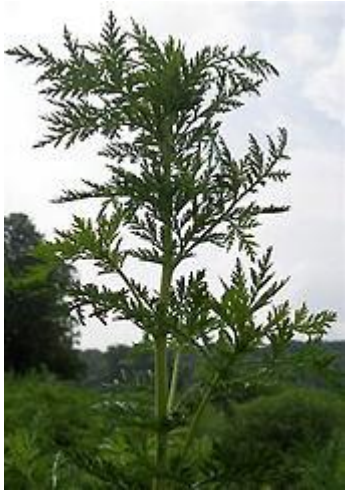

Figure 1. *Artemisia annua*[9].