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IN VIVO ANTIMALARIAL ACTIVITY OF METHANOLIC EXTRACT OF YOUNG FRONDS OF PTERIDIUM AQUILINUM L. KUHN IN MICE INFECTED WITH PLASMODIUM BERGHEI.

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Abstract

The decoction of Young fronds of *Pteridium aquilinum* (bracken fern) is orally taken after boiling as remedy for malarial fever, dysentery, diabetes and stomach disturbances by the tribal people of Ogoni. It is also eaten as vegetable. *In vivo* antimalarial activity of the methanolic extract of young fronds of *pteridium aquilinum* was evaluated using chloroquine sensitive *plasmodium berghei* infection in mice, with a view to finding scientific evidence for the use of this plant as traditional remedy for malaria infection. Methanolic extract of young fronds of *P. aquilinum* (400 – 800 mg/kg) was administered orally to *P. berghei* infected mice in both the early and established phases of infection and the antimalarial effect on the parasites and survival time of the infected mice determined. The extract from the young fronds of *P. aquilinum* at difference doses caused 8.74 – 61.24% inhibition in the level of parasitaemia in the suppressive tests and 48.48 – 79.45% inhibition of parasitaemia in the curative tests, with a survival time of 26 – 30 days. These results show that the methanolic extract of the young fronds of *P. aquilinum* possesses significant ($P<0.05$) antimalarial activity thus rationalizing its traditional use in malaria therapy.

Key words: Antimalarial, Mice, *Plasmodium berghei, Pteridium aquilinum*.

Introduction

Malaria remains an important public health concern in the world. Each year, there are more than 250 million reported cases of malaria killing between 2 – 3 million people, the majority of whom are children below 5 years of age in Africa [1]. Malaria still remains an ever-continuing epidemic in Africa claiming thousands of lives each year, majority of which are due to severe falciparium malaria.

The widespread availability of cheap and effective antimalarial drugs, particularly chloroquine and primethamine-sulpadossina, has undoubtedly limited both morbidity and mortality, and also encouraged the development and spread of resistance. Drug resistance has also played an important role in the occurrence and severity of epidemics in most parts of the world. Due to the rising cases of resistance of antimalarial drugs, there is the need to develop more effective new antimalarial drugs that are not expensive and are readily available to people especially those in the developing countries [2]. Because of the problems of drug resistance, high cost and access to effective antimalarial drugs, traditional medicines particularly plant based antimarial products has become an important and sustainable source of treatment [3]. In Africa, the use of these indigenous plants still plays an important role in malarial treatment and which could be interesting sources for the development of novel antimalarial agents [4].

Pteridium aquilinum L. Kuhn (bracken fern) is a ubiquitous fern belonging to the family Dennstaedtiaceae and is distributed worldwide. In southern Nigeria, the Ogonis called it "Kebewii". The processed young fronds (fiddleheads) of *P. aquilinum* are used as human food in some areas of the world, though there are reported cases of its toxic effects on livestock [5, 6, 7]. Its analgesic, antibacterial and antiparasitic properties have been reported [8, 9, 10]. Young fronds of *P. aquilinum* are use as vegetable by the tribal people of Ogoni. Also its decoction is orally taken after boiling as remedy for malarial fever, dysentery, diabetes and stomach disturbances by the Ogonis. Our main objective in this present research work is to find out whether the methanolic extracts of *P. aquilinum* young fronds possess antimalarial activity in mice infected with *P. berghei*, since results of such investigations have not been reported.

Methods

Collection and Identification of Plant Materials.

Fresh young fronds of *Pteridium aquilinum* were collected from Wii-luere farm land in Nyogor-Beeri, Ogoni, Khana Local Government Area of Rivers State, South-South Nigeria, in the month of February, 2011 and were authenticated by Dr. N.L. Edwin-Wosu, of the department of Plant science and biotechnology, University of Port Harcourt, Nigeria, where voucher specimen (UPHV-1032) was deposited.

Preparation of Plant Extract.

Fresh young fronds of *P. aquilinum* were washed with distilled water, macerated and air-dried at room temperature for 28 days and then pulverized into powder form using electric blender (Bruders BL-133). Extraction was done on 300g of the powdered young fronds of *P. aquilinum* using soxhlet extractor [11] and 150ml of methanol as the solvent at about 110°c for 24 hours. The extract obtained was concentrated to dryness using water bath at 45°c for 72 hours and then stored in a refrigerator at 4°c until required for experiment. The percentage yield was calculated and the dry methanolic extract was dissolved in distilled water, and at different concentration to make stock solutions from which the various doses administered were prepared for use by serial dilution.

Experimental Animals

Eighty four 84 Swiss albino mice of either sex weighing between 17.4 – 22.5g, obtained from the animal house of the department of pharmacology, university of Nigeria, Enugu State, Nigeria, were used for the Experiments. The animals were maintained under standard conditions of humidity, temperature and 12 hours light and darkness cycle at the department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria. They were also fed with animal finisher feed and given free access to clean water *ad libitum* during the two weeks of acclimatization. The United States National Institute of Health "Principles of laboratory animal care" were strictly adhered to in the study [12].

Phytochemical Screening

The methanolic extract of young fronds of *P. aquilinum* was subjected to quantitative phytochemical tests for various plant constituents using standard procedures and identification was done by characteristic colour changes. The extract was tested for the presence of alkaloids, saponin, Tannins, flavonoids, glycosides and essential oil [13 - 16].

Parasite Inoculation

Chloroquine sensitive *plasmodium berghei* was obtained from the department of pharmacology, college of medicine, University of Nigeria, Enugu State. The parasites were maintained through weekly passage in mice. The inoculums consisted of $5x10^7$ *P.berghei* parasitized red blood cells per ml, which was prepared by determining both the percentage parasitaemia and the red blood cell count of the donor mouse, and diluting the blood with physiological saline solution. Each mouse was inoculated intraperitoneally on day 0 (the first day), with 0.2ml of infected diluted blood containing approximately $1x10^7$ *P. berghei* parasitized red blood cells. Also, the newly inoculated mice were monitored daily to determine their level of parasitaemia.

Determination LD_{50}

The LD_{50} of the extract of young fronds of *P. aquilinum* was determined using 24 albino mice by intraperitoneal route according to the methods of Lorke [17].

Evaluation of Schizontocidal Activity in early Infection (Chemosuppressive test).

Chemosuppressive activity of the methanolic extract of young fronds of *p. aquilinum* was evaluated using peter's 4-day suppressive test against *P. berghei* infection in mice [18, 19]. Each mouse was inoculated on the first day (day 0), intraperitoneally with 0.2ml of infected blood containing about $1x10^7$ *P. berghei* parasitized red blood cells. The mice were then divided into five groups (A-E) of six mice each shortly after inoculation with 1 x 10^7 *P. berghei* parasitized red blood cells. The mice were administered with 400, 600 and 800 mg/kg/day body weight doses of methanolic extract of young fronds of *P. aquilinum*, chloroquine (5mg/kg/day body weight) which serve as the positive control and an equivalent volume of physiological saline solution (negative control) for four consecutive days (D_0 - D_3). On the fifth day (D_4), thin blood films were prepared from the tail blood of each mouse and thin films fixed with methanol, stained with 4% Giemsa at P^H 7.2 for 30 minutes were prepared. The parasitaemia level was determined by counting the number of parasitized red blood cells out of 200 red blood cells in 8 random fields of microscope. The average percentage chemosuppression was calculated using the following formula:

$$A = (B - C)/B \times 100$$

Where;

A = % chemosuppression

B =Average % parasitaemia in negative control group.

C = Average % parasitaemia in test group.

Evaluation of schizontocidal Activity in established infection (Rane test).

The evaluation of curative potential of the methanolic extract was done using the method described by Ryley and Peters [20]. Thirty (30) Mice infected with 0.2ml of standard inoculums of 1 x 10^7 *P.berghei* infected red blood cells were grouped into five groups on the first day (D_0). Seventy two hours later, the infected mice were treated with 400, 600 and 800mg/kg/day body weight doses of methanolic extract of young fronds of *P. aquilinum* , chloroquine (5mg/kg/day), and an equivalent volume of normal physiological saline was given to the negative control group. Treatment with the methanolic extract/drug was given once daily for five days ($D_0 - D_4$). Thin films stained with Giemsa stain were prepared from the tail blood of each mouse daily for 5 days to monitor the parasitaemia level. The mice were further observed for more thirty days (30 days). Mortality was monitored daily during the period of the test and any death was recorded and used arithmetically to determined the means survival time (days) of the mice in each group over a period of 30 days ($D_0 - D_{29}$).

Statistical Analysis

Data were analyzed using student's t-test and the differences between means were considered statistically significant at values of $P<0.05$.

Results

Percentage yield

The percentage yield of methanolic extract of young fronds of *P. aquilinum* obtained was 83.3%. The extract was dark greenish jelly-like substance.

Phytochemical analysis

The phytochemical screening of the methanolic young fronds extract of *P. aquilinum* revealed the presence of alkaloids, saponin, tannins, glycosides, flavonoids and essential oil.

Acute Toxicity

The methanolic extract of young fronds exhibited an LD_{50} of 1200mg/kg. No major clinical signs of toxicity in the mice were recorded at all the doses tested. So the extract was assumed to be safe.

Chemosuppressive Activity

The result of this study indicated that *in vivo* methanolic extract of young fronds of *P. aquilinum* displayed some activity against *P. berghei* malaria parasite. The plant extract produced potent dose dependent chemosuppressive effect of 400mg/kg/day, 600mg/kg/day and 800mg/kg/day doses employed in this study. The chemosuppression produced were 8.74, 48.75 and 61.24% respectively at these doses.

The chemosuppressions produced by the extract were significant ($P<0.05$) at 600mg/kg, and 800mg/kg compared to the negative control. The standard drug (chloroquine 5mg/kg/day) caused 77.50% chemosuppression, which was significantly ($P<0.05$) higher than those obtained for the plant extracts treated groups (Figure 1.0).

Curative Effect (Rane Test)

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

On day 4 (D_3), the average percentage reduction in parasitaemia for the treated groups were; -41.41, -26. 92, -31.82 and –55.69% for 400, 600, 800mg/kg/day of extract, and 5mg/kg/day of standard drug (chloroquine). On 5 (D_4), 4.71, 11.53, 37.00, and 9.53% for 400, 600, 800mg/kg/day of extract, and 5mg/kg/day standard drug.

On day6 (D_5), 30.39, 35.49, 48.48, and 47.18% for 400, 600, 800mg/kg/day of extract, and 5mg/kg/day of standard drug. On day 7 (D_6), 52.05, 55.10, 68.04 and 68.04% for 400, 600, 800mg/kg/day of extract, 5mg/kg/day of standard drug. On day 8 (D_7), the average percentage reduction in parasitaemia for the treated groups recorded were 71.16, 65.90, 79.45% for 400, 600 and 800mg/kg/day of the methanolic extract of young fronds of *p. aquilinum* and 85.27% for the chloroquine (5mg/kg/day) respectively (fig. 2.0). The mean survival times of the extract treated groups were significantly ($P<0.05$) longer than that of the negative control (normal saline) and standard drug, chloroquine (positive control) (Table 1.0)

Discussion

The result of the phytochemical screening showed that the extract of *P. aquilinum* young fronds contained different classes of secondary metabolites that may be responsible for the medicinal use of this plant in African traditional settings. Among the phytochemcals were alkaloids, saponin, tannins, glycosides, flavonoids and essential oils which have been variously implicated in antimalarial and antibacterial activities [10, 21].

It was observed that the methanolic extract of *P. aquilinum* young fronds caused no lethality or gross behavior and physical changes to mice at LD_{50} value of 1200mg/kg, which is three times more than the minimum effective dose (MED) of 400mg/kg for the extract. This indicated that the test extract is safe for oral use [22].

Our results show that *P. aquilinum* young fronds exhibited promising antimalarial activity which can be exploited in malaria therapy. Methanolic extract of *p. aquilinum* young fronds possess a significant ($P<0.05$) antimalarial activity as evident from the chemosuppression obtained during the 4 – day early infection (Figure 1.0). The young fronds extract also produced significant ($P<0.05$) curative effect in established infection as compared to the standard drug, chloroquine (5mg/kg/day).

The extract prolonged the mean survival time of the mice in the treated groups, indicating that the extract suppressed *p. berghei* growth. The result on mean survival time also show that the extract of *P. aquilinum* young fronds prolonged the mean survival time better than the standard drug (chloroquine) used.

The antimalarial activity of *P. aquilinum* young fronds might be attributed to the presence of phytochemicals. Constituents such as alkaloids, saponin, flavoniods, and essential oils, are implicated in antimalarial activities of many plants [23 - 26]. A compound is considered active when reduction in parasitaemia is 30% [27], which supports the results of the parasite suppression in the study (see figures 1.0 and 2.0). The effect of the extract of *P. aquilinum* young fronds on parasitaemia level in this study is similar to the ones reported on *Nigella sativa* [28], *Anona senegalensis* [29], and *Azadirachta indica* [30].

Conclusion

The results of this study have shown that the methanolic extract of *P. aquilinum* young frond possesses antimalarial activity as seen in its ability to suppress *P. berghei* infection in mice. The extract exhibited minimal acute toxicity. This plant could represent potential source of lead molecules with antimalarial potential for the development of new novel drugs for treatment or prophylaxis against malaria. Therefore, this study justifies the acclaimed traditional use of this plant for the treatment of malaria.

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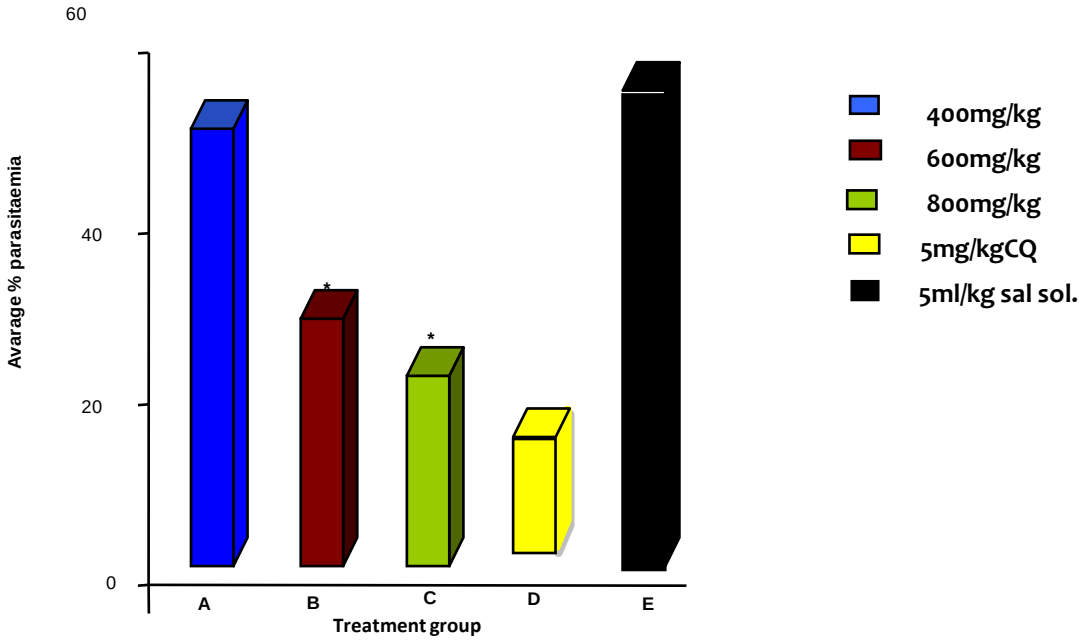

Fig.1: Effect of *P. aquilinum* extract On schizontocidal activity in early Infection of mice (4-day suppressive test)

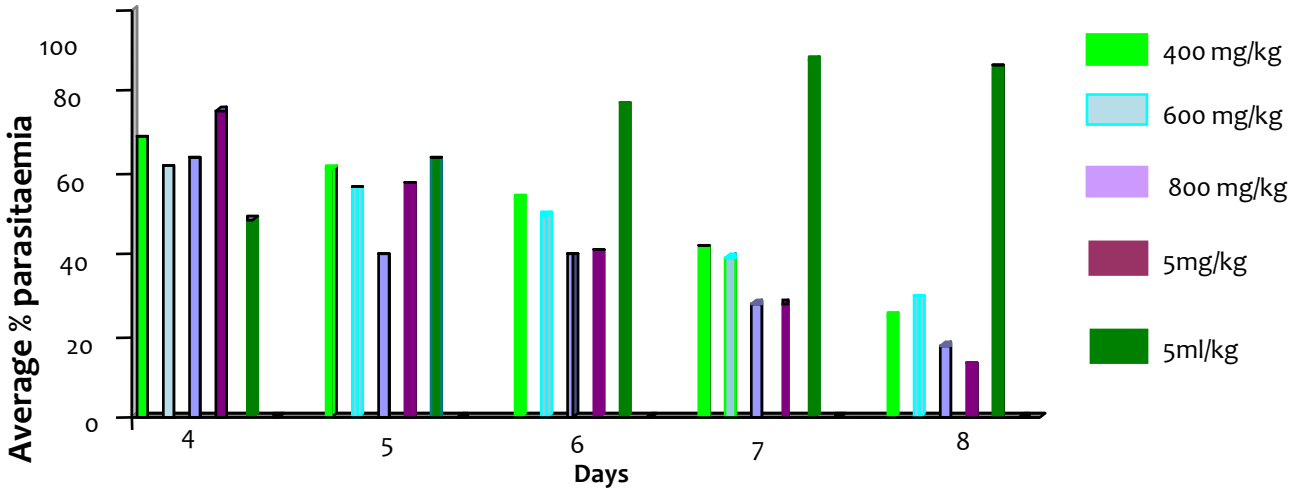

Fig. 2: Effect methanolic extract of tender frond of P.aquilinum on

Table 1. Mean survival time (days)

Group	Dose (mg/kg)	Total no. of Death	Mean survival time (Days)
A	400	1	26.00 ±23.73
B	600	0	30.00±27.38
C	800	3	28.83±26.31
D	5	5	14.67±13.39
E	5ml	6	14.83+13.54

Results are expressed as mean values ± SEM (n=6)