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EVALUATION OF THE ANTIMALARIAL ACTIVITY OF *BRIDELIA FERRUGINEA* BENTH BARK

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ABSTRACT

The antimalarial activity of the methanolic extract of *Bridelia ferruginea* benth bark was evaluated at varying doses of 200mg/kg, 400mg/kg and 800mg/kg body weights in mice (*Mus musculus*) infected with chloroquine -sensitive *Plasmodium berghei*. The antimalarial activity of the extract during Prophylactic (pre-treatment before infection with the parasite), Suppressive (co-treatment with the infection of the parasite on day zero) and Rane test (established infection which involves post treatment after 72hours infection with the parasite) were investigated. Phytochemical screening revealed the presence of alkaloids, glycosides, phenolics, saponins, steroids, anthraquinones and tannins in the extract. The extract demonstrated a dose-dependent suppression of parasitaemia following administration to infected mice. An optimum dose of 400mg/kg body weight with established infection mode of treatment demonstrated 100% total clearance of parasitaemia comparing favourably with chloroquine, the reference drug over the 28 days period of observations. Conclusively, the bark extract has exhibited promising antimalarial activity which can be exploited in malaria therapy.

Keywords: *Bridelia ferruginea*, methanolic extract, phytochemicals, antimalarial.

INTRODUCTION

Malaria is an infection of the blood that is carried from person to person by mosquitoes. The disease has been recognized for thousands of years and once was found almost every where except in the most northern areas of the world (Wirth, 1998). Over the past hundred years, malaria has been one of the most serious and complex health problems facing humanity. Malaria remains the most important parasitic disease of humans, affecting approximately 40% of the human populations in tropical and subtropical areas of the world, as numbers of travelers of these areas increase (Sachs, 2002).

The disease is caused by *Protozoan* parasites of the subphylum *Apicomplexa,* belonging to the genus *Plasmodium* which is transmitted by female mosquitoes of the genus *Anopheles*. The major burden of approximately two million deaths annually occurs in sub-saharan Africa where 90% of all deaths are from *Plasmodium falciparum. Plasmodium falciparum* is the most lethal, accounting for over 90% of malaria associated deaths (Mesia *et al*., 2005).

The *Plasmodium* life cycle starts with a blood meal of an infected *Anopheles* mosquito and the coincident injection of sporozoites into human skin. After breaching blood vessels of the skin (Amino *et al.,* 2006) sporozoites are transported by the blood stream to the liver. There, the parasite infects liver parenchyma cells (hepatocytes) and differentiates into thousands of merozoites. To access the bloodstream, liver-derived merozoites must leave their host cell and cross the endothelium of the liver blood vessels. Within red blood cells (RBCs) each parasite replicates, depending on the *Plasmodium* species, into 16 or 32 merozoites, which then get released to directly infect other RBCs (Sturm and Heussler, 2007).

Although traditional medicine is widely used to treat malaria and is often more available and affordable than orthodox medicine, there are few clinical data on safety and efficacy. All cultures from ancient times to the present day have used plants as a source of medicine (Elujoba, 2005). Today, according to the World Health Organization (WHO), as many as 80% of the people of the world depend on traditional medicine for their primary health care needs. The greater part of traditional therapy involves the use of plant extracts or their active principles (Farnsworth and Soejarto, 1985). However, there is no consensus, even among traditional healers, on which plants, preparations, and dosages are the most effective. On the whole, medicinal plants are just taken based on local reputation; one such plant is *Bridelia ferruginea.*

Bridelia ferruginea of the family Euphorbiaceae is usually a gnarled shrub, which sometimes reaches the size of a tree in suitable condition. The down curved tip of the leaf is distinctive. In Nigeria, its common names are

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Iralodan (Yoruba), Ola (Igbo) and Kizni (Hausa). The plant has a geographical spread that runs from Guinea to Zaire and Angola. Its habitat is the savannah especially in the moister regions (Kolawole and Olayemi, 2003). Kolawole *et al.,* 2006, 2007 reported its efficacy in the reduction of total bacterial and coliform counts in river water and wastewater treatments. A decoction of the leaves is used to treat diabetes. It is also used as a purgative and a vermifuge (Cimanga *et al.,* 1999; De Bruyne *et al*., 1997). In Nigeria, the Yoruba people of Idofian, Ilorin South Local Government Area of Kwara State claimed that when the bark of *Bridelia ferruginea* is boiled in sizeable drinkable water and allowed to cool, it has been in use traditionally as antimalarial drug for more than a century ago. Recently, studies have reported the trypanocidal potentials of the methanolic extract (Ekanem *et al.,* 2008). Although this plant is used in the traditional treatment of malaria, there is no documented study of its antimalarial activity. This study was prompted in view of this to investigate the potency of the plant against blood stage of the plasmodium parasite using varying doses of the extract with different regime of treatments.

MATERIALS AND METHODS

Chemicals

Absolute methanol (Riedel-de Haën) was obtained from Sigma-Aldrich Laborchemikalien GmbH, Germany. Giemsa stain was obtained from Anosantec Laboratories, UK. Chloroquine diphosphate salt was obtained from Sigma Chemical Company, St. Louis, MO, USA. Other reagents used were of analytical grade and were prepared in all glass-distilled water.

Experimental Animals

Fifty five adult Swiss albino mice (*Mus musculus*) with an average weight of 20 ±2g were obtained from the animal breeding unit of the Department of Pharmacology, University of Ibadan, Oyo state. The mice were housed in plastic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water *ad-libitum*. The research adhered to the Principles of Laboratory Animal Care (NIH publication #85−23, revised in 1985).

Parasite strain

A chloroquine-sensitive strain of *Plasmodium berghei* (NK-65) was obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Oyo state. The parasites were maintained by weekly blood passage in mice.

Plant source and Identification

The barks of Bridelia ferruginea benth were obtained from Idofian town of Kwara state, Nigeria in October, 2008 and were authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo state.

Plant extract preparation and phytochemical screening

Fresh bark of *Bridelia ferruginea* were dried in the shade at room temperature and pulverized to powder using an electric blender. The extraction was done using the solvent methanol at ambient temperature (cold extraction) according to the method of Kolawole *et al., 2003.* Preliminary qualitative phytochemical screening of the plant extract was carried out employing standard procedures (Odebiyi and Sofowora, 1978).

Animal grouping and extract administration

The animals were divided into eleven groups A_a, A_b, A_c, B_a, B_b, B_c, C_a, C_b, C_c, D, and E of five mice each. The percentage parasitaemia of the donor mouse was first determined using a haemocytometer and an appropriate dilution of the infected blood with isotonic saline was done. Animals in all the infected groups were inoculated intraperitoneally with 0.2ml of infected blood containing about 1×10^7 *Plasmodium berghei* parasitized red blood cells depending on the regime of extract treatment. All the animals were infected from the same donor mouse. This study involved three regime of extract administration; the prophylactic (A_a, A_b and A_c), which involved pre-treatment stage (5 days before infection with parasite), the 4-day test or suppressive treatment (B_a, B_b and B_c) involving co-treatment starting on the day of infection with parasite and continued for 5-days; and Rane test or test of established infection (C_a, C_b and C_c) which involved post treatment after 72hours of infection with parasite. The test was conducted according to the method described by Ryley and Peters (1970).

Groups A_a, B_a, and C_a were given 800mg/kg body weight of the extract for 5 days.

Groups A_b, B_b, and C_b were given 400mg/kg body weight of the extract for 5 days;

Groups A_c, B_c, and C_c were given 200mg/kg body weight of the extract for 5 days;

Group D animals were infected with parasite but not treated.

Group E animals were given 4mg/kg body weight of the chloroquine diphosphate for 5 days, 72 hours after infection with parasite. All drugs administration was done orally with canula.

Sample collection and analyses

Daily blood films were screened for malaria parasites in tail blood of all the infected animals after fixing in methanol, stained with Giemsa and the percentage of parasites in the blood was determined through microscopic examination. The blood was screened from the fourth to the twenty eight days of post infection.

In calculating the percentage parasitaemia, the slide prepared in thin blood film was used. The parasitized red blood cells were then counted using the ×100 objective

lens (oil immersion). To calculate the percentage parasitaemia, the following formula was used

$$\% \text{ Parasitaemia} = \frac{\text{Total number of PRBC}}{\text{Total number of RBC}} \times 100$$

Where: PRBC = Parasitized Red Blood Cells
RBC = Total Red Blood Cells

Percentage chemosuppression
The percentage chemosuppression was calculated by subtracting the average percentage parasitemia in treated group from the average percentage parasitemia in control group and the value obtained was expressed as a percentage of the average percentage parasitemia in control group.

Mean survival time
The mean survival time for each group was determined arithmetically by finding the average of the survival time (days) of the mice post inoculation in each group over a period of 28 days (D0 to D28).

Statistical analysis
Values are expressed as mean ± SEM. Data from the test groups were compared with their respective controls and differences at $p<0.05$ were considered significant.

RESULTS

Some of the phytochemicals present in the methanolic extract of *Bridelia ferruginea* bark are shown in Table 1. The screening revealed the presence of alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, anthraquinones and tannins.

Table 1. Some phytochemicals in the methanolic extract of *Bridelia ferruginea* bark.

Phytochemicals	Status
Alkaloids	++
Tannins	++
Phenolics	++
Glycosides	++
Saponins	++
Flavonoids	+
Steroids	++
Anthraquinones	+

++ Strongly present, + Fairly present

Parasitaemia levels in infected untreated mice, infected mice treated with 800mg/kg, 400mg/kg, 200mg/kg body weight of methanolic extract of *Bridelia ferruginea* bark administered as prophylactic treatment, co-treatment (comparative with infected mice treated with 4mg/kg body weight of chloroquine) and post-treatment from the fourth to the twenty-eighth day of post infections are shown in Figures 1, 2, and 3. The results of the prophylactic treatment revealed that on day 4, groups treated with 800mg/kg, 400mg/kg and 200mg/kg had 1.45%, 2.02%, and 2.16% parasitaemia respectively. By day eight, there was percentage decrease in parasitaemia for 800mg/kg and 400mg/kg but with a progressive percentage parasitaemia increase for 200mg/kg along with the infected untreated mice continually till the end of the experiment. On day eight, all the remaining mice that received 800mg/kg of extract died, while those with 400mg/kg continued to experience a steady decrease in percentage parasitaemia compared to the infected untreated mice throughout the observation periods (Fig. 1).

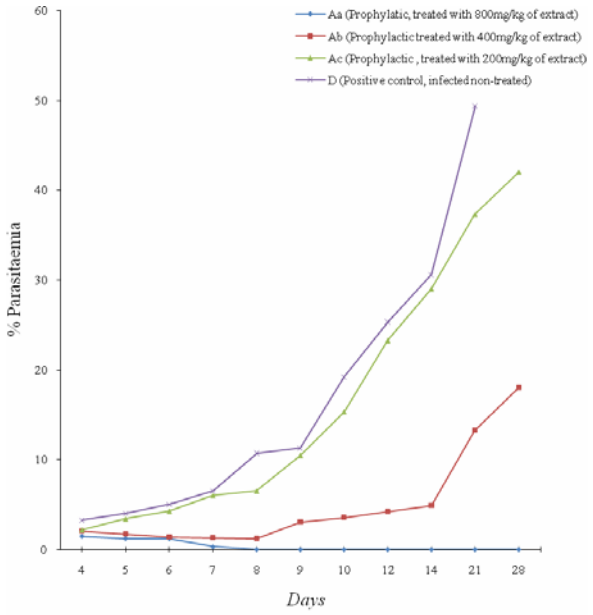

Fig. 1. Dose-dependent percentage parasitaemia of mice placed on prophylactic treatment. Each point is an average count from five infected mice (±SEM).

In the suppressive treatment, on day four, the percentage parasitaemia were 800mg/kg (1.50%), 400mg/kg (1.66%) and 200mg/kg (3.09%). By day eight, there was continual increase in percentage parasitaemia for all the treated groups but with considerable decrease compared to the infected untreated mice throughout the experimental period. It must be noted that at the fourteenth day, all the mice treated with 800mg/kg died (Fig. 2).

The results of established infection treatment shown in Figure 3 revealed that on day four the percentage parasitaemia were 800mg/kg (2.05%), 400mg/kg (2.98%) and 200mg/kg (3.02%). There was increase in parasitaemia in all groups for days 5, 6 and 7. By day 8, there was steady decline for 800mg/kg (2.03%) and 400mg/kg (2.01%) whereas 200mg/kg and infected

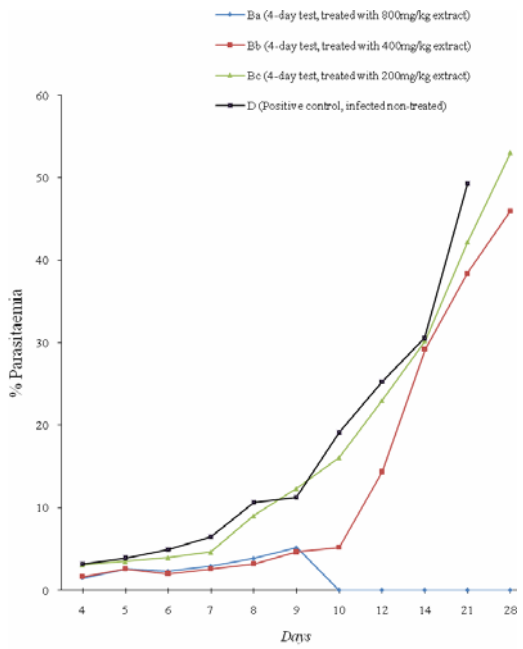

Fig. 2. Dose-dependent percentage parasitaemia of mice placed on 4-day/suppressive treatment. Each point is an average count from five infected mice (±SEM).

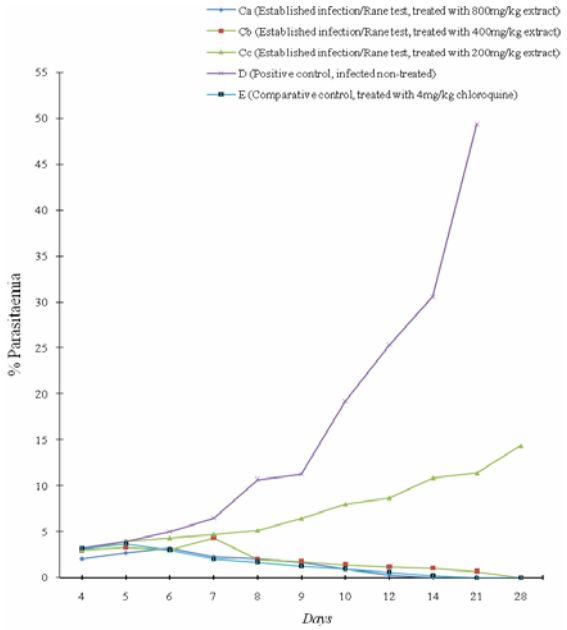

Fig. 3. Dose-dependent percentage parasitaemia of mice placed on established infection/ Rane treatment. Each point is an average count from five infected mice (±SEM).

Table 2. Percentage Chemosuppression at Optimum dose (400mg/kg) of methanolic extract of *Bridelia ferruginea* benth bark**.**

Group Treatment		Day 4 (%)	Day 5 (%)	Day 6 (%)	Day 7 (%)	Day 8 (%)	Day 10 (%)	Day 12 (%)	Day 14 (%)	Day 21 (%)	Day 28 (%)
Treated with Extract (Prophylaxis)	A_1	24.6	48.2	73.2	82.1	88.4	82.2	87.9	90.5	73.0	-
Treated with Chloroquine 4mg/kg (Prophylaxis)	A_2	44.4	46.6	77.1	88.5	95.5	100	-	-	-	-
Treated with Extract (4-Day test)	B	71.4	67.6	82.6	57.9	64.1	72.9	61.3	46.4	15.2	-
Treated with Extract (Established infection)	C	1.6	1.9	6.3	70.2	84.1	91.7	95.4	97.6	98.1	100
Treated with Chloroquine 4mg/kg (Established infection)	E	1.2	2.9	18.0	70.8	85.5	94.1	98.0	99.3	100	100

untreated experienced continual increased (5.13% and 11.19%) respectively. On day 14, all the mice for 800mg/kg died with 400mg/kg (1.00%) and 200mg/kg (10.86%) percentage parasitaemia. On day 21, 400mg/kg recorded 0.69% competing favourably with the positive control (4mg/kg chloroquine) recording 0.65%. However, the infected untreated mice experienced a continual increased (49.5%) percentage parasitaemia throughout the study.

The same trend was observed from the numerical values of percentage chemosuppression caused by treatment with the extract and chloroquine with the different regime of treatments. The established infection treated with extract and chloroquine respectively recorded 100% clearance of parasitaemia at the 28th day of the study while other mode of treatments recorded steady decline in the rates of parasite clearance (Table 2).

Table 3. Mean Survival Times (MST) of mice placed on different treatment at optimum dose (400mg/kg).

Group Treatments		MST (DAY 0 to 28)
Treated with Extract (Prophylaxis)	A_1	26.2 Days
Treated with Chloroquine 4mg/kg (Prophylaxis)	A_2	27.8 Days
Treated with Extract (4-Day test)	B	16.8 Days
Treated with Extract (Established infection)	C	27.4 Days
Infected Untreated (Positive Control)	D	10.6 Days
Treated with Chloroquine 4mg/kg (Established infection)	E	27.9 Days

The values for mean survival time of animals in the infected untreated group, infected extract-treated groups and infected chloroquine-treated groups for the three regimes of treatments over a period of twenty eight days is shown in Table 3. The animals in the extract-treated group for prophylactic and established infection test survived for a favourably comparable number of days as did the chloroquine-treated group.

DISCUSSION

The phytochemical screening of the methanolic extract of *Bridelia ferruginea* bark revealed the presence of alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, anthraquinones and tannins.

The observed antimalarial activity (Figs. 1 to 3) may be attributed to these compounds. Abo *et al.*, 1999 and Okokon *et al.,* 2005 have previously reported the antimalarial activities of these compounds in some medicinal plants. The antimalarial activity observed in this study could be attributed to a single or combined effect of these compounds although the isolation and structural identification of the active principle has not been carried out. The results revealed that the prophylactic treated mice recorded significant (p<0.05) decrease in percentage parasitaemia compared to the infected untreated over the period of observation. On the other hand, suppressive treated mice exhibited significant (p>0.05) increase in percentage parasitaemia slightly lowered than the infected untreated group over 28 days of observation. However, the established infection (Rane test) treated mice showed a gradual percentage parasitaemia increase at the onset with a later significant decrease (p<0.05) tending to total clearance of parasite at the 28th day comparing favourably with chloroquine treated mice, the reference drug.

The initial low percentage chemosuppression observed in the extract-treated group and the chloroquine-treated group (Table 2) may be due to the fact that the extract at the dose administered had not accumulated sufficiently to bring about considerable chemosuppression (Adebayo *et al.,* 2003). However, the prolonged administration of the extract led to the total clearance of the parasites. This result from accumulation of enough active compounds to effect total clearance of the parasites. Also, the calculated mean survival times suggests that the extract had an antimalarial activity which compared favourably to that of the reference drug (Table 3).

The finding from this study have been able to revealed that the optimum dose of extract to achieve total clearance of parasite with a mean survival time comparing favourably with the reference drug is 400mg/kg body weight (Tables 2 and 3). Although, 800mg/kg recorded significant (p<0.05) parasite clearance but was toxic to the animals and they could not live to the end of the observation period. This could possibly be due to the fact that the animals were overdosed at that concentration.

It is noteworthy that the extract exhibited antimalarial activity at all doses with all the regime of treatments but the established infection (Rane test) gave the best performance achieving 100% parasite clearance at the end of the experiment comparing favourably with chloroquine, the reference drug. This finding is contrary to the work of Okokon *et al.,* 2005 who reported that 5mg/kg of chloroquine and 200mg/kg of ethanolic leaf extract of *Croton zambesicus* respectively could not achieve 100% parasite clearance at 28th day of experimental observations.

The mechanism of action of methanolic bark extract of *Bridelia ferruginea* has not been elucidated. The extract could have exerted its action by causing elevation of red blood cell oxidation or by inhibiting protein synthesis (Etkin, 1997; Kirby *et al.,* 1989).

In conclusion, the plant has exhibited promising antimalarial activity confirming forklore report of its traditional use in the treatment of malaria. Therefore, it would be interesting to investigate its in vitro activity against *Plasmodium* parasite thereby establishing the isolation and structural identification of the active principle for possible exploitation in malaria therapy.

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