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Anti-plasmodial Activity of Aqueous Extract of *Bridelia ferruginea* (Benth) Stem Bark in *Plasmodium berghei*-infected Mice

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ABSTRACT: Aqueous extract of *Bridelia ferruginea* stem bark was investigated for its antimalarial activity using Rane test established infection; the phytochemical constituents were also determined. Thirty five albino mice were infected by intraperitoneal injection of standard inoculum of 1x 10^7 choloroquine-sensitive strain of *Plasmodium berghei* (NK-65). The animals were randomly divided into 7 of 5 mice each. Groups (B_1 to B_5) were administered orally with 0.5ml of 50, 100, 200, 400 and 800mg/kg body weight respectively of the extract. Group C were given 0.5ml of 5mg/kg body weight of chloroquine and Group D which served as the control group were infected with parasite but not treated. The phytochemical screening of the extract revealed the presence of alkaloids, glycosides, flavonoids, phenolics, steroids, saponins, anthraquinones and tannins. There was 100% parasite clearance in the group that received choloroquine, 98.60% parasite clearance in the group that received 800 mg/kg body weight of extract and 99.07% in the animals that received 400 mg/kg body weight of the extract. The 100% total clearance of parasitaemia demonstrated by the 400 mg/kg body weight of the extract over the 28 days of observation compared favourably with that of the reference drug, chloroquine. The study thus concludes that aqueous extract of *Bridelia ferruginea* stem bark has antiplasmodial activity and can be explored in the management of *Plasmodium berghei* infected animals.

KEYWORDS: Anti-plasmodial, *Plasmodium berghei*, *Bridelia ferruginea*, Malaria, Parasitaemia, Euphorbiaceae

1.0 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 was found almost everywhere 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. It is a public health problem in more than 90 countries inhabited by 2.4 billion people, and is responsible for more than 500 million clinical cases and 1.5–2.7 million deaths per year, most of whom are children under 5 years of age and pregnant women (Schwartlander, 1997; WHO, 1996). Every year, 10% of the global population is infected with malaria, and many (99.4%) of them survive after 10–20 days of illness.

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Plasmodium species are protozoan parasites responsible for malaria, an illness killing about millions of people per year (WHO, 2005). 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).

Chemotherapy and chemoprophylaxis remain the main methods for disease control, due to the absence of an operational vaccine for malaria or leishmaniasis in the immediate horizon. Current anti-protozoal drugs are inadequate due to parasite resistance, toxicity, lack of efficacy and inability to eliminate all stages of parasites from the host (Tasdemir *et al*., 2005). However, with the increase in cases of drug resistance and failure, there is an increase in the use of herbal medicine. Approximately 80% of the people in

the developing countries depend on traditional medicine for the management of disease conditions (Phillipson and Wright, 1991). The discovery of quinine and artemisinin from *Cinchona succiruba* (Rubiaceae) and *Artemisia annua*, followed by their development into powerful anti-malarial drugs represent milestones in the history of anti-parasitic drugs from plants (Kayser *et al*., 2003). Today's researchers are exploring the plant kingdom to lay hands on the bioactive phytochemicals, which can be used to cure malaria.

Bridelia ferruginea (family Euphorbiaceae) is a gnarled shrub. In Nigeria, its common names are *Iralodan* (Yoruba), *Ola* (Igbo) and *Kizni* (Hausa). 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 can cure malaria. The plant has been claimed to be used traditionally as antimalarial drug for more than a century ago. The trypanocidal potential of the methanolic extract has been reported (Ekanem *et al.*, 2008). Also the antimalarial potentials of the methanolic extract was reported by Kolawole and Adesoye (2010).

The traditional use in Nigeria of *Bridelia ferruginea* stem bark as an antimalarial plant prompted us to carry out this research. This study was therefore carried out to scientifically evaluate the *in vivo* antimalarial activity of aqueous extract of *Bridelia ferruginea* stem bark against *Plasmodium berghei* in mice.

2.0 Materials and Methods

2.1 Collection and Authentication of Plant Material

The study plant was obtained from a residential house in 'Ita Alamu' Ilorin, Nigeria where it was home grown, and was authenticated at the Department of Plant Biology, University of Ilorin. A voucher specimen (UIH/313) was deposited at the herbarium of the Department.

2.2 Malaria Parasite

The chloroquine sensitive, *Plasmodium berghei* (NK 65 strain) was obtained from the Iinstitute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria. The parasites were maintained by weekly blood passage in mice by infecting uninfected mice with the blood from plasmodium-infected mice.

2.3 Experimental Animals

Adult Swiss mice (*Mus musculus*), weighing 25-28 g of both sexes were obtained from the small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in wire mesh cages under standard conditions (ambient temperature, 23.0 ± 2.0^{o}C, and humidity 46%, with a 12 hour light/dark cycle). The study was conducted in accordance with the recommendations from the declaration of Helsinki on guiding principles in the care and use of animals (NIH, 1985).

2.4 Preparation of Extract

The stem barks were cleaned, air-dried at room temperature until constant weight was achieved. The air-dried samples were then pulverised into coarse powder using pestle and mortar. Two hundred and twenty grams of the powdered material was macerated with distilled water (1500 ml) for 72 h with constant shaking using the GFL shaker (No. 3017MbH, Germany) for 24 hours. The liquid extract obtained was concentrated to dryness in vacuum at 40^{o}C (Adesokan and Akanji, 2010). The yield was calculated to be 14.2%w/w. The residue was stored in a refrigerator at 4^{o}C until used for the experiment reported in this study. The resultant residue was dissolved in distilled water to make the stock solution from which the various doses were prepared for use by serial dilution. This aqueous extract was used in the present study with doses expressed in milligrams per kilogram body weight of the animal. Tween 80 (2.5 %) in distilled water was used as control (vehicle). The doses were so adjusted as to administer 0.25 ml in each mouse; chloroquine

diphosphate in vehicle was used as standard.

2.5. Phytochemical Screening

A portion of the stem bark powder of *Bridelia ferruginea* was subjected to phytochemical analysis, using standard procedure as described earlier by Odebiyi and Sofowora (1978) and Trease and Evans (1989).

2.6 Evaluation of Curative Activity (Rane Test)

Peter and Ryley's method (1970) with slight modification was used in the evaluation of curative activity on established infection. On the first day (day 0), standard inoculum of 1x 10^7 *Plasmodium berghei* infected erythrocytes was injected intraperitoneally into the mice after confirmation of parasitaemia, 72h post-inoculation. The animals were randomly divided into 7 groups of 5 mice each, Groups (B_1, B_2, B_3, B_4 and B_5) were administered orally with 0.5ml of 50, 100, 200, 400 and 800 mg/kg body weight respectively of *Bridelia ferruginea* stem bark aqueous extract for five days. Group C received 5mg/kg body weight of chloroquine base daily for 5 days. Group D, (control) was left untreated but administered appropriate volume of distilled water.

2.7 Collection of Blood Samples and Determination of Parasite Count

Daily blood films were screened for malaria parasites in tail blood of all the infected animals after fixing in methanol, stained with Geimsa and the percentage of parasites in the blood was determined through microscopic examination. 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).

2.7.1Estimation of Percentage Parasitaemia

Percentage parasitaemia was estimated on days 4, 5, 6, 7, 8, 10, 12, 14, 18, 21 and 28 post-infection using the expression described by Abosi and Raseroka (2003):

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

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

2.7.2 Percentage Chemosuppression

The percentage chemosuppression was calculated by subtracting the average percentage parasitaemia 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 (Peter, 1970).

2.7.3 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) (Peter, 1970)

2.8 Statistical Analysis

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

3.0 Results

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

There was no parasitaemia in the blood of mice in the chloroquine-treated group and the groups that received 400 and 800 mg/kg body body weight of extract of *Bridelia ferruginea* benth bark. Less than 2% parasitaemia was found in the blood of mice that received 100 and 200 mg/kg body weight of the extract. There

was 43% parasitaemia in the infected untreated group (Figure 1).

The percentage chemosuppression achieved by the administration of 50, 100, 200, 400 and 800 mg/kg body weight of *Bridelia ferruginea* stem bark extract respectively over a period of twenty-eight (28) days showed a significant increase in percentage chemosuppression was also achieved (Table 2). Administration of 5 mg/kg body weight of chloroquine for five days showed a 100% chemosuppression on day 28 post infection which was significantly different from all extract treated groups ($p < 0.05$) (Table 2).

Table 3 showed the mean survival time for the animals in each group. The least MST of 12.5 days was recorded for the control group that was left untreated. The mice in the chloroquine treated group recorded the highest MST of 27.9 days. MST of 27.0 and 27.6 days were recorded for the groups that received 400 and 800 mg/kg body weight of the extract respectively while the group that received 50mg/kg body weight of the extract survived for 16.2 days.

Table 1: Some phytochemicals in the aqueous crude extract of *Bridelia ferruginea* stem bark

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

++ Strongly present; + Fairly present

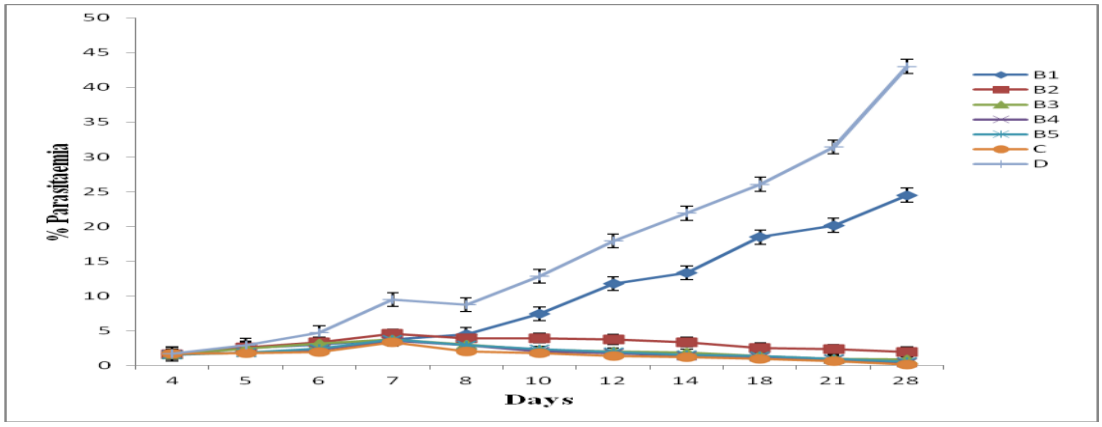

Fig. 1: Percentage parasitaemia of *Plasmodium berghei* infected mice administered different dose of *Bridelia ferruginea* stem bark extract and chloroquine

Each point is a percentage average count from five infected mice (±SEM); **Key:** B_1, B_2, B_3, B_4 and B_5 are the 50, 100, 200, 400 and 800 mg/kg body weight of the extract, C = chloroquine control, D = Infected, not treated

Table 2: Percentage chemosuppression of aqueous extract of *Bridelia ferruginea* stem bark

Group	Number of days (%)										
	4	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**18**	**21**	**28**
C	7.78	37.90	57.40	64.20	76.40	86.20	92.20	94.50	96.20	94.50	100
B_1	2.39	10.30	13.79	36.12	60.00	49.40	42.30	34.30	-	-	-
B_2	2.99	10.34	27.66	51.58	56.20	69.20	78.89	84.46	90.00	92.36	-
B_3	3.59	17.24	34.04	60.00	53.93	82.30	88.33	91.36	94.62	96.82	97.99
B_4	4.19	34.48	51.06	62.10	65.17	83.85	90.00	93.64	95.38	97.45	99.07
B_5	5.40	34.48	40.00	70.50	66.29	81.54	88.88	92.73	94.62	96.82	98.60

KEY: B_1 = 50 mg/kg b.wt. of *Bridelia ferruginea*; **B_2** = 100 mg/kg b.wt. of *Bridelia ferruginea*; **B_3** = 200 mg/kg b. wt. of *Bridelia ferruginea*; **B_4** = 400 mg/kg b. wt. of *Bridelia ferruginea*; **B_5** = 800 mg/kg b. wt. of *Bridelia ferruginea*; **C** = chloroquine control.

Table 3: Mean Survival Times (MST) of *P. berghei* infected mice treated with *B. ferruginea*

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GROUPS	MST (DAYS)
50mg/kg b.wt. *Bridelia ferruginea*	14.0
100mg/kg b.wt. *Bridelia ferruginea*	20.0
200mg/kg b. wt. *Bridelia ferruginea*	26.4
400 mg/kg b. wt. *Bridelia ferruginea*	27.0
800mg/kg b. wt. *Bridelia ferruginea*	27.6
Chloroquine control	27.9
Infected not treated	12.5

4.0 Discussion

The phytochemical screening of the aqueous extract of *Bridelia ferruginea* stem bark revealed the presence of alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, anthraquinones and tannins. The observed antimalarial activity (Figure 1) may be attributed to these compounds (Abo *et al.,* 1999 and Okokon *et al.,* 2005). The antimalarial activity observed in this study could be attributed to a single or combined effect of these compounds. Studies have attributed the antiplasmodial activity of plants to alkaloids, terpenes and flavonoids (Philipson and Wright, 1991, Christensen and Kharazmi, 2001).

Results from this study showed that aqueous extract of *Bridelia ferruginea* possess potent dose dependent antimalarial activities that were comparable to that of chloroquine. It was revealed that the extract-treated mice showed a gradual percentage parasitaemia increase at the onset with a later significant decrease ($p<0.05$) of 99.07% clearance of parasite at the 28th day post infection comparing favorably with chloroquine-treated mice, the reference drug (Figure 1). This result is in agreement with the findings of Kolawole and Adesoye, (2010) who reported that the methanolic extract of *Bridelia ferrugunea* stem bark reduced the parasitaemia levels of *Plasmodium* infected mice. The stem bark extract also exerted significant curative effect in established infection and this property is comparable to that of the standard drug (chloroquine) as shown in the percentage parasitaemia (Figure 1) of the mice in both the extract and chloroquine treated animals.

The initial low percentage chemosuppression in the extract-treated group and the chloroquine 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 the substantial accumulation of active compounds to effect total clearance of the parasites.

Furthermore, the Mean Survival Time of 27.6 days in the group that received 800 mg/kg body weight was similar to 27.9 days for the chloroquine group. MST of 27.0 days in the group that was administered 400 mg/kg body weight of the extract has proven that the extract possess potent antimalarial activity, due to the survival of experimental animals beyond the minimum of 12 days (Peters, 1980; Obih and Makinde, 1985; Abosi and Raseroka, 2003; Ajaiyeoba *et al.,* 2006).

The result of this study shows that aqueous stem bark extract of *Bridelia ferruginea* possesses antiplasmodial activity and also supported the use of this plant in the treatment of malaria traditionally. Therefore, it would be interesting to investigate the *in vitro* activity against both chloroquine sensitive and chloroquine resistant *Plasmodium* parasite. Also the isolation and structural identification of the active principle present in this plant could be identified and the mechanism of action elucidated from this promising medicinal plant for possible exploitation in malaria therapy.

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