

Full Length Research Paper

Trypanocidal potential of methanolic extract of *Bridelia ferruginea* benth bark in *Rattus norvegicus*

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An *in vivo* study of the trypanocidal potential of *Bridelia ferruginea* benth bark was investigated to determine its possible use against African sleeping sickness. Phytochemical screening revealed the presence of alkaloids, tannins, saponins, steroids and phlobatanins. Four groups of male albino rats weighing 220-250 g were used. The groups included the control, infected-untreated, uninfected-treated, and infected-treated. A daily dose of 20 mg/Kg body weight methanolic extract of *B. ferruginea* benth bark was administered intraperitoneally at 72 h post-infection with *Trypanosoma brucei brucei* and parasitaemia was monitored daily. A continuous rise in parasite count was observed in the infected-untreated group. The infected-treated group showed a progressively lowered parasitaemia on Days 5 to 8 post-infection, with percentage parasite reduction between 11 and 76%. Treatment also extended the lifespan for 2 days. Results also showed that treatment significantly ameliorated hematological parameters that were studied. We suggest that methanolic extract of *B. ferruginea* benth bark can be useful in the management of African sleeping sickness.

Key words: *Bridelia ferruginea* benth bark, *Trypanosoma brucei*, sleeping sickness, management.

INTRODUCTION

African trypanosomes cause trypanosomiasis, also known as sleeping sickness in human for which about 300,000 new cases are reported annually in some 36 developing African countries south of the Sahara where about 60 million people in some 200 locations are exposed to the risk of infection (Chretien and Smoak, 2005). The parasites cause *nagana* in livestock. Human African trypanosomiasis (HAT) is caused by the haemoflagellate, *Trypanosoma brucei gambiense* in west and Central Africa and *Trypanosoma brucei rhodisiense* in Eastern Africa. In regions where HAT prevails, several other trypanosome species, including *Trypanosoma vivax* and *Trypanosoma congolense* are prevalent which affect health of cattle and other livestock (Picozzi et al., 2002).

Trypanosomiasis is fatal if left untreated and chemotherapy which forms the most important and major aspect of control and eradication of the disease in African countries is beset with problems of toxicity and increasing incidence of resistance among the trypanosomes to the

existing drugs (Kioy and Mattock, 2005; Moore, 2005). The search for new drugs and formulations which are safe, affordable and effective against both early and late stages of the disease is recommended (Jannin and Cattand, 2004; Chibale, 2005; Pink et al., 2005).

Bridelia ferruginea is the commonest savannah *Bridelia*. It is usually a gnarled shrub which sometimes reaches the size of a tree in suitable condition. Its common names are Kirni, Kizni (Hausa); Marehi (Fulani); Iralodan (Yoruba); Ola (Igbo); Kensange abia (Boki). Its habitat is the savannah, especially in the moister regions extending from Guinea to Zaire and Angola. The tree is 6-15 m high, up to 1.5 m in girth and bole crooked branching low down. The bark is dark grey, rough and often marked scaly (Rashid et al., 2000).

B. ferruginea has diverse uses. A decoction of the leaves has been used to treat diabetes. It is also used as a purgative and a vermifuge (Cimanga et al., 1999). The bark extract has been used for the coagulation of milk and also lime juice for the formulation of a traditional gargle "ogun efu" (Orafidiya et al., 1990). Kolawole and Olayemi (2003) reported its potential for water treatment. In Togo, the roots of the plant are used as chewing sticks and the root bark is used for intestinal and bladder

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disorders as well as skin diseases (De-Bruyne et al., 1997).

Other reported activities of the bark extract include molluscicidal (Iwu, 1984); antimicrobial (Adeoye et al., 1988) and anti-inflammatory (Olajide et al., 1999).

In a recent *in vitro* work that involved the testing for trypanocidal activities in petroleum ether, chloroform, methanolic and aqueous extracts of *B. ferruginea* stem bark, the methanolic extract was reported to have shown the highest trypanocidal activity (Atawodi, 2005). The work was built on an earlier survey by Atawodi et al. (2003) on trypanocidal activities of extracts of some Savanna plants.

This present study intends to determine *in vivo* the trypanocidal activity of methanolic extract of *B. ferruginea* stem bark and to ascertain its efficacy using some haematological and biochemical parameters.

MATERIALS AND METHODS

Plant material

B. ferruginea stem bark was collected from the tree of *B. ferruginea*, family Euphorbiaceae from the residential quarters of the University of Ilorin, Nigeria. A voucher sample was deposited at the Plant Biology herbarium of the university.

Preparation of bark extract

The bark was cut into small pieces and dried in an oven at 40°C for 48 h (Gallenkamp oven Bs size 2). The dried pieces were then pulverized using the laboratory mill (Christy and Norris Ltd. machine type 8) and the powder obtained was stored as stock. 100 g of the powder was dissolved in 500 ml absolute methanol for 48 h followed by further extraction by shaking for 2 h on Wrist Action Shaker. The suspension was then decanted and filtered using glass wool. The residue was evaporated to dryness at room temperature under electric fan. The extract was stored in the refrigerator at 4°C until required.

Phytochemical screening

The screening procedures were adapted from Sofowora (1977) and Trease and Evans (1983). They are briefly described below.

Alkaloids

0.5 g of the powdered leaves was stirred with 5 ml of 1% of aqueous hydrochloric acid in a steam bath. This was filtered and 1 ml of the filtrate was treated with a few drops of Mayer's reagent. Another 1 ml portion was treated similarly with Dragendorff's reagent. Turbidity or precipitation with either of these reagents was observed for as evidence of the presence of alkaloids (Sofowora, 1977).

Tannins

5 mg of the powdered leaves was stirred with 10 ml of distilled water, filtered and ferric chloride was added to the filtrate and observed for a blue-black, blue-green or green precipitate (Sofowora, 1977).

Saponins

0.1 g of the powdered leaves was boiled in 10 ml distilled water for 5 min and decanted while still hot. The filtrate was used for the following tests (Trease and Evans, 1983).

1. Frothing test: 1 ml of the filtrate was diluted with 4 ml of distilled water and the mixture shaken vigorously and observed on standing for stable froth.

2. Emulsion test: This was performed by adding 2 drops of olive oil to the frothing solution and shaking vigorously and observed for emulsion.

Steroids

The test for steroids was done by the Liberman acid test. A portion of the extract was treated with drops of acetic anhydride. Concentrated H₂SO₄ was carefully added to the side of the test tube. The presence of a brown ring at the boundary of the mixture was taken as positive result (Trease and Evans, 1983).

Phlobatannins

Deposition of a red precipitate when an aqueous extract of the plant was boiled with 1% aqueous hydrochloric acid was observed for the presence of phlobatannins (Sofowora, 1977).

Parasite strain

T. brucei brucei was obtained from the Veterinary and Livestock Studies Department of the Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria. The parasite was maintained by continuous passage in other rats. 0.5 ml of parasite suspension in 0.9% saline solution was introduced intraperitoneally into albino rats. The suspension contained 3 or 4 parasites per view at X 100 magnification.

Determination of parasitaemia

Parasitaemia was monitored daily in blood obtained from the tail of the rats. A thin smear of blood was made on glass slides and viewed at x 100 magnification under light microscope and the parasite count was taken.

Experimental animals

Male albino rats (*Rattus norvegicus*) weighing between 220-250 g were obtained from the Animal Holdings of the University of Ibadan, Nigeria. They were acclimatized for four weeks and fed with animal feed obtained from Bendel Feed and Flour Mills Ltd., Ewu, Edo State, Nigeria, and water was provided ad libitum.

Enzyme kits

Assay kits for glutamate; oxaloacetate transaminase (GOT) and glutamate: pyruvate transaminase (GPT) were obtained from Randox Laboratories Ltd., United Kingdom.

Enzyme assay

GOT activity was measured by monitoring the concentration

Table 1. Phytochemical screenings of methanolic extract of *Bridelia ferruginea* stem bark.

Active Constituents	Amount
Tannins	++
Saponins	+
Alkaloids	++
Phlobatannins	++
Steroids	+

Key: + = Positive; ++ = Strongly positive.

oxaloacetate hydrazone formed with 2, 4 – dinitrophenylhydrazine based on the procedure of Reitman and Frankel (1957) that was modified by Schmidt and Schmidt (1963). GPT activity was monitored using the same procedure. The activities of these enzymes were measured in the serum and liver.

Haematological studies

Parameters including packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hb), white blood cell (WBC), platelets, neutrophils and lymphocytes were determined using the Automated Haematologic Analyzer, Sysmex, KX-21 (Japan).

Design

The experiment consisted of four groups of five rats each. The groups included the control (uninfected-untreated); the infected-untreated; the uninfected-treated; and the infected-treated. The experiment was carried out in two phases: in the first phase, the infected-treated group was administered a daily dose of 20 mg/kg body weight of the bark extracts from Day 3 post-infection when the parasite was first sighted in the blood. Treatment was administered till the rats died on day 12. Also the infected-untreated group was monitored till they died on day 10. The second phase involved all four groups. Whole blood, serum and liver were collected from all the groups on day 9 post-infection.

Statistical analysis

Data are mean of 5 replicates \pm S.E.M. Statistical analysis was carried out using the Duncan Multiple Range Test (DMRT) (Mahajan, 1997). Results were compared and differences were considered at $p < 0.05$ level of significance.

Collection of blood and isolation of tissue

The rats were anaesthetized by dropping them in a jar containing cotton wool soaked with chloroform. Per rat, 2 samples of blood were collected immediately after jugular puncture, one to heparinized bottles and were used for haematological studies, and the other was allowed to coagulate followed by centrifugation at 1,000 rpm for 10 min to get clear serum which was used for enzyme studies. The rats were dissected and the liver was encapsulated into sample bottle containing ice-cold 0.25 M sucrose solution.

Preparation of liver homogenate

1 g of liver was sliced into small pieces and then homogenized

using pre-cooled pestle and mortar placed in a bowl of ice chips. The homogenized tissue was diluted with 0.25 M sucrose solution to give a final volume (1:5 w/v) and centrifuged at 1000 rpm for 10 min. The supernatant was collected and stored at 4°C for analysis.

RESULTS

In this study, phytochemical screening of methanolic extract of *B. ferruginea* stem bark revealed the presence of alkaloids, tannins, saponins, steroids and phlobatanins (Table 1). Daily monitoring of parasitaemia (Figure 1) revealed a parasite-combating potential under the test condition. Table 2 showed percentage parasite reduction in infected rats treated with methanolic extract of *B. ferruginea* stem bark. Percentage parasite reduction of 11% was recorded on day 5, 40%; on day 6, 54%; on day 7, 76%; on day 8 and 60% on day 9. The highest percentage parasite reduction of 76% was recorded on day 8 post-infection and treatment extended the lives of the animals by 2 days over the infected-untreated group.

Haematological results (Table 3) showed that administration of the plant extract to the infected group did not produce a significant change ($p > 0.05$) in RBC and factors relating to it (Hb, MCHC and PCV) when compared with the infected-untreated group (Table 3). On the other hand, platelet count significantly increased ($p < 0.05$) in the infected-treated group over the infected-untreated. WBC showed a marked increase ($p < 0.05$) in the infected-untreated group and a marked decrease ($p < 0.05$) in the infected-treated group. Differential WBC count (Table 4) revealed that there was a significant rise in lymphocytes only in the infected-untreated group, and that neutrophil levels significantly increased ($p < 0.05$) only in the treated groups.

Specific activities of GPT and GOT in the liver and serum of all the experimental groups were analyzed (Table 4). Results showed that over the control, the GPT levels in the liver and serum rose significantly ($p < 0.05$) in the infected-untreated group. Treatment with extract showed significantly lower ($p < 0.05$) levels of GPT in the liver and serum. However, compared with the infected-untreated group, treatment of the infected group lowered serum GPT to levels of no significant difference ($p > 0.05$) with the control.

GOT levels in the liver differed significantly ($p < 0.05$) only in the treated groups which showed a decrease compared with the infected-untreated. In the serum, treatment produced no significant change ($p > 0.05$) in GOT in the treated groups. GOT levels however increased significantly in the infected-untreated group.

DISCUSSION

This study has revealed a trypanocidal potential in methanolic extract of *B. ferruginea* stem bark. This finding confirms an earlier *in vitro* work by Atawodi (2005) involving the plant and the same solvent of extraction.

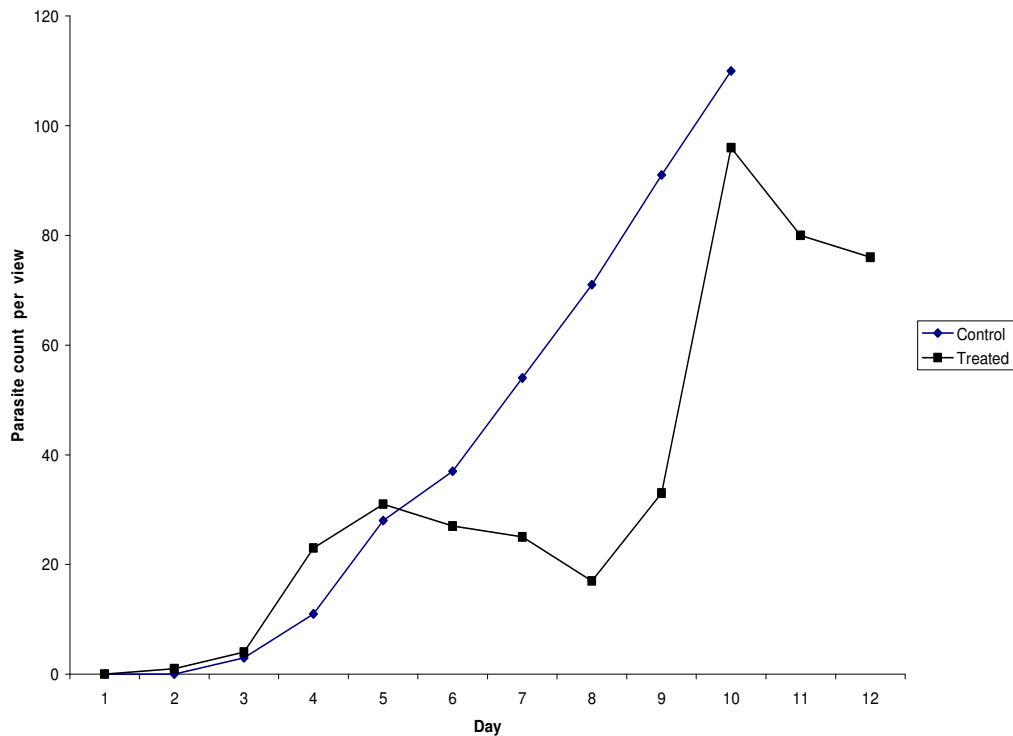


Figure 1. Parasitaemia of *T. brucei*-infected rats. Rats treated with methanolic extract of *Bridelia ferruginea* stem bark were inoculated at 20 mg/Kg body weight once daily until the animals died. Each point is an average of count from five infected rats.

Table 2. Percentage parasite reduction in infected rats treated with methanolic extract of *Bridelia ferruginea* stem bark.

Day	Parasite count per view		Parasite reduction (%)
	Untreated	Treated (20 mg/kg)	
1	0	0	0
2	0	0	0
3	3	3	0
4	11	23	-52
5	35	31	11
6	45	27	40
7	54	25	54
8	71	17	76
9	91	33	64
10	110	96	13
11	-	80	27*
12	-	76	40*
13	-	-	-

*Calculated using the parasite count of day 10 of the untreated group.

This indicates that the bark extract could contain potent trypanocidal constituents. Phytochemical screening of the

stem bark in this work revealed the presence of alkaloids, tannins saponins, steroids and phlobatanins. This con-

Table 3. Changes in haematological parameters of infected rats treated with methanolic extract of *Bridelia ferruginea* stem bark.

Animal grouping	Hb (g/dl)	MCHC (g/dl)	PCV (%)	RBC ($\times 10^{12}/L$)	WBC ($\times 10^9/L$)	Platelet ($\times 10^9$)
Control	13.87 \pm 0.88 ^a	31.1 \pm 0.00 ^a	45.67 \pm 0.20 ^a	7.79 \pm 0.20 ^a	15.33 \pm 3.67 ^b	1089.33 \pm 97.19 ^a
Infected-untreated	9.57 \pm 0.26 ^b	29.33 \pm 0.26 ^b	29.00 \pm 0.058 ^b	5.05 \pm 0.02b	16.00 \pm 0.06 ^a	121.33 \pm 1.76 ^c
Uninfected-treated	13.50 \pm 0.31 ^a	31.67 \pm 0.33 ^a	43.00 \pm 1.15 ^a	6.96 \pm 0.43 ^a	15.93 \pm 1.62 ^b	1043.33 \pm 1.56 ^a
Infected-treated	9.60 \pm 0.66 ^b	31.00 \pm 0.58 ^a	33.00 \pm 2.08 ^b	5.01 \pm 0.37 ^b	10.00 \pm 1.36 ^c	658.33 \pm 10.84 ^b

Means along the same column with different superscripts are significantly different at $p < 0.05$. Values are means of 5 determinations \pm S. E M.

Table 4. Differential count of white blood cells of infected rats treated with methanolic extract of *Bridelia ferruginea* stem bark.

Animal grouping	Lymphocytes (%)	Neutrophils (%)
Control	74.33 \pm 3.28 ^b	23.67 \pm 3.18 ^a
Infected-untreated	77.33 \pm 0.88 ^a	22.33 \pm 0.88 ^a
Uninfected-treated	61.67 \pm 3.72 ^b	37.00 \pm 3.00 ^b
Infected-treated	71.33 \pm 6.96 ^b	28.67 \pm 6.96 ^b

Means along the same column with different superscripts are significantly different at $p < 0.05$. Values are means of 5 determinations \pm S. E M.

Table 5. Specific activities of GPT and GOT in liver and serum of infected rats treated with methanolic extract of *Bridelia ferruginea* stem bark.

Animal grouping	GPT (IU/L)		GOT (IU/L)	
	Liver	Serum	Liver	Serum
Control	235.27 \pm 18.37 ^a	10.60 \pm 0.62 ^b	230.27 \pm 30.05 ^a	23.43 \pm 7.37 ^b
Infected-untreated	265.60 \pm 0.00 ^a	22.53 \pm 1.57 ^a	248.57 \pm 5.73 ^a	35.47 \pm 0.13 ^a
Uninfected-treated	177.70 \pm 7.31 ^c	8.83 \pm 2.24	170.43 \pm 22.37 ^b	25.50 \pm 0.13 ^a
Infected-treated	141.83 \pm 18.09 ^d	12.80 \pm 0.67 ^b	107.33 \pm 13.19 ^b	15.33 \pm 1.45 ^b

Means along the same column with different superscripts are significantly different at $p < 0.05$. Values are means of 5 determinations \pm S. E M.

conforms to an earlier work by Kolawole and Olayemi (2003). The reported trypanocidal activity of the plant extract in this study (Figure 1 and Table 2) may probably be due to the action of one of these constituents or two or more acting in concert. Anaemia is a known symptom of trypanosome infection. The plant extract under the test condition did not effectively improve on the anaemic condition of the infected rats (Table 3). The red blood cell and indices relating to it (packed cell volume, haemoglobin and mean cell haemoglobin concentration) in the infected rats were not significantly improved by treatment. However, treatment significantly increased the platelet count in infected rats (Table 3). Platelets function to activate the clotting mechanism and prevent loss of blood from the blood vessels through coagulation (Pasternek, 1979). Since anaemia is characteristic of trypanocidal infection and further possibility of blood loss either due to coagulation in the blood vessels or impairment of the blood clotting mechanism as a result of reduced platelet count may be highly detrimental to the animal. The

extract has shown significant potentials of boosting platelet production in infected animals and thereby increasing the survival chances of the animals. This is in consonance with the work done by Yakubu et al. (2007) which reported a significant increase in platelet levels in albino rats using aqueous extract of *Fadogia agrestis* stem.

Differential white blood cell count revealed that lymphocyte count was only significantly altered in the infected-untreated group which recorded an increase (Table 4). Lymphocytes are the main effector cells of the immune system (McKnight et al., 1999). This finding suggests that the plant extract must have possibly combated the parasite directly without demanding further production of the effector cells of the immune system. The significant increase in the percentage neutrophils may be adduced to a boost in the ability of the neutrophils to phagocytose (Dacie and Lewis, 1995).

Results from analysis of specific enzyme activity showed that the infected-untreated group had significant increase in serum GPT and GOT levels as opposed to

the control and the infected-treated group which had values that did not differ significantly (Table 5). This indicates that administration of the plant extract to the infected rats may have reduced the values of the enzymes in the serum in relation with the control. This finding is in consonance with the work done by Lin et al. (1995) which recorded that the plant *Curcuma xanthorrhiza* reduced significantly the acute elevation of serum transaminase levels induced by acetaminophen and carbon tetrachloride in mice.

In conclusion, the phytochemistry and *in vivo* trypanocidal activity of methanolic extract of *B. ferruginea* stem bark have been assessed with a view to establishing the possibility of developing the novel natural compound from the plant extract for effective and safe trypanocidal agents for combating trypanosomiasis, a disease that has continued to be of immense economic and health importance in many tropical countries of the world, especially in Africa.

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