

## TRYPANOCIDAL ACTIVITY OF THE ETHANOLIC EXTRACT OF *Buchholzia coriacea* SEED

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### SUMMARY

The ethanolic extract of *Buchholzia coriacea* seed was evaluated for trypanocidal activity in mice experimentally infected with *Trypanosoma brucei brucei*. Parasitaemia was monitored using the rapid matching technique and microscopic examination of the buffy coat. In the acute toxicity test no deaths were recorded. There were however signs of dizziness observed at 2000 mg/kg. The extract cleared trypanosomes in the blood between the 13<sup>th</sup> and 15<sup>th</sup> day post infection (PI), after 3 consecutive days of treatment at 1000 mg/kg. At day 15 PI, the mean level of parasitaemia in the latter was significantly ( $p < 0.05$ ) lower than those of the 250 mg/kg, 500 mg/kg and the infected untreated groups. There was no significant difference between the mean parasitaemia of the Diminazene aceturate (Berenil)(R) treated group and the 1000 mg/kg of extract treated group from the 11<sup>th</sup> - 17<sup>th</sup> day PI. From the 11<sup>th</sup> - 15<sup>th</sup> day PI the Berenil treated group had a significantly higher mean packed cell volume (PCV) than the extract treated groups. However, by the 15<sup>th</sup> day PI the 1000 mg/kg of extract treated group had a PCV significantly ( $p < 0.05$ ) higher than the other extract treated groups. In conclusion, the ethanolic extract of *B. coriacea* exhibited significant trypanocidal activity especially at 1000 mg/kg in mice infected with *T. b. brucei*. It also significantly increased the PCV of infected mice compared to the non-infected control.

**KEY WORDS:** Trypanosome, Extract, *Buchholzia coriacea*, Seed

### INTRODUCTION

Medicinal plants are known for their healing properties. In the rural areas especially, they are used in curing ailments of man and animals. They are a cheap source of medicine and many pharmaceutical companies derive their active principles from such plants. Approximately 119 pure chemical substances extracted from higher plants are used in medicine throughout the world (Arowolo, 1997).

According to the Food and Agriculture Organization of the United Nations, some 350 million people are affected by malaria (FAO, 1986). The seeds of *Buchholzia coriacea* Engler (Capparaceae) are folklorically used in Eastern Nigeria, to treat feverish conditions.

They are commonly called wonderful colas by the Ibos. They are chopped up and soaked overnight in the local gin. The infusion is drunk for the cure of such ailments as malaria. The already bottled preparations are sold in the village markets. Other healing properties ascribed to *Buchholzia coriacea* include anthelmintic properties and analgesic properties (Nweze and Asuzu, 2004). The oil contained in the seeds is claimed to be useful in the treatment of rheumatic pains.

*Buchholzia coriacea* leaves have been shown to have anthelmintic effects on *Fasciola hepatica* (Ajaiyeoba *et al.*, 2001). The ethanolic extract of *Buchholzia coriacea* seed caused the death of the infective stage larvae of *Haemonchus contortus* and *Heligmosomoides polygrus* at various

concentrations *in vitro* (Nweze and Asuzu, 2006).

Fractions prepared from the methanolic extract of *Buchholzia coriacea* stem bark exhibited high concentration-dependent antibacterial and antifungal activities comparable to Ampicillin and Tioconazole respectively (Ajaiyeoba *et al.*, 2001). Trypanosomiasis has continued to be a problem in Africa, south of the Sahara. This is more so with the development of resistance to the frequently used trypanocides like Diminazene aceturate (Berenil<sup>®</sup>) and Isometamidium chloride (Jamal *et al.*, 2005). Other drugs like Suramin and Homidium have also been affected (Codja *et al.*, 1993). This trypanosome resistance highlights the need for new, alternative trypanocides.

The present study was to find out if the seeds of *Buchholzia coriacea* used ethno medically to treat malaria have any effect on trypanosomiasis since both causative agents are blood protozoan parasites.

## MATERIALS AND METHODS

### Plants materials

The seeds of *Buchholzia coriacea* Engl. were purchased in February from Ogiye market in Nsukka, Enugu State. They were identified by Mr. P.O Ugwu of Botany Department, University of Nigeria, Nsukka, where voucher specimens are kept.

### Preparation of extracts

Sun-dried seeds of *B. coriacea* were reduced to coarse powder in mortar and were later pulverized into fine powder using a laboratory mill. Cold extraction was done with hexane for 72 h with intermittent shaking. The marc was left to dry under room temperature after filtration. It was re-extracted using 80% ethanol for another 72 h and thereafter re-filtered. The filtrate was poured into flat Petri dishes which were placed in a hot air oven at 40°C to evaporate. The extract was refrigerated until use.

### Animals

Sixty healthy albino mice of both sexes obtained from the Laboratory Animal Unit of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka were used. The animals were maintained in accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* (DHHS, NIH Publication No. 85-23, 1985). They were kept for a period of 7 days before the start of the experiments. They had access to clean drinking water and feed before and during the experiments.

### Acute toxicity test of the extract

Twenty-four mice weighing 28.6±1.5 g were housed in cages. They were divided into 4 groups of 6 mice each. Graded doses (250, 500, 1000, 2000 mg/kg) of the extract were administered to the different groups intraperitoneally. They were observed for acute toxicity signs like behavioural changes or death over 24 h.

### Evaluation of extract for trypanocidal activities

Thirty-six mice weighing 33±2.6 g were used for the experiment. They were divided into 6 groups of 6 mice each. Five groups were infected with *Trypanosoma brucei brucei* obtained from the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. They were inoculated with 0.2 ml of blood containing an absolute number of 7,943,000 trypanosomes/ml intraperitoneally. By six days post inoculation, infection had already established in all the mice. By eight days post-infection, treatment with graded doses of the extract (250, 500, 1000, mg/kg) was administered intraperitoneally for 3 consecutive days to mice in groups 1 to 3. Berenil<sup>®</sup> at 7 mg/kg was given intraperitoneally to the 4<sup>th</sup> group of mice. Mice in group 5 were infected but not treated. Those in group 6 were neither infected nor treated.

The level of parasitaemia was monitored in each mouse every other day by the rapid matching technique as described by Herbert and Lumsden (1976). A drop of mouse tail blood was examined at 40X magnification using a table microscope. The number of trypanosomes in each field was counted. Each counting per field was matched with log figures obtained from the reference table. The log figures were converted to antilog values which were subsequently converted to absolute numbers. This gave the number of trypanosomes per milliliter. Where no trypanosomes were seen in the blood, the buffy coat layers were then examined.

The tail blood of the mice was collected into heparinized haematocrit tubes. The tubes were spun in a haematocrit centrifuge and the packed cell volumes (PCV) were read off with a haematocrit reader.

#### **Analysis of data**

The data on the mean group parasitaemia and mean group PCV were analysed using one-way analysis of variance (ANOVA). Differences were considered significant at  $P < 0.05$ .

## **RESULTS**

The extract was thick, chocolate-brown syrup, with a yield of 5.26% w/w. No deaths were recorded in the acute toxicity test. Signs of dizziness were observed at the dose of 2,000 mg/kg body weight.

The results of the effect of the plant extract on the levels of parasitaemia of infected animals are presented in Table 1. In the 250 mg/kg and the 500 mg/kg extract treated groups, there was an increase in the level of parasitaemia from the 7<sup>th</sup> to the 9<sup>th</sup> day; thereafter, there was a decrease till the 11<sup>th</sup> and 13<sup>th</sup> days PI, respectively. In the 1000 mg/kg and the Berenil treated groups parasitaemia decreased from the onset of

treatment and by the 11<sup>th</sup> and 13<sup>th</sup> days PI respectively it was zero. In the former group there was a relapse by day 15, while in the latter group there was absence of parasites the end of the experiment. The level of parasitaemia in the 250 mg/kg treated group increased from the 13<sup>th</sup> day PI, up till the end of the experiment. In the 500 mg/kg extract treated group, there was an increase in parasitaemia from the 15<sup>th</sup> day PI to the end of the experiment. In the negative control group, this was the infected untreated group parasitaemia rose till the end of the experiment.

As from the 13<sup>th</sup> day PI, there was no significant difference between the 1000 mg/kg treated group and the Berenil treated group. By the 15<sup>th</sup> day PI, mean parasitaemia was significantly higher in the infected untreated group than in all the other treatment groups.

The results of the PCV are shown in Table 2. Mean PCV in both the 250 and 500 mg/kg extract treated groups decreased from infection till the 11<sup>th</sup> day PI. After this there was an increase till day 14 in the former and till the end of the experiment in the latter. In the 1000 mg/kg extract treated group, mean PCV decreased till day 11 PI, after which it increased till the end of the experiment. The mean PCV in the Berenil treated group decreased from the onset of infection till the 8<sup>th</sup> day after which it continued to increase till the termination of the work. The mean PCV value of the Berenil-treated group was higher than that of the 1000 mg/kg extract - treated group. The mean PCV of the uninfected untreated group was significantly higher than that of the infected untreated group whose mean PCV continued to decrease till the end of the experiment.

TABLE 1: Effect of varying doses of *B. coriacea* extract on the parasitaemia (mean±SEM) of mice infected with *Trypanosoma brucei brucei*

Post infection days	Group 1 (250 mg/kg)	Group 2 500mg/kg	Group 3 1000mg/kg	Group 4 Berenil 7mg/kg	Group 5 Infected-not-treated
5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
7	58.67 ± 39.55	53.33 ± 6.75	90.67 ± 17.36	117.33 ± 30.54	49.33 ± 17.36
9	91.33 ± 37.01 <sup>a</sup>	74.67 ± 17.85 <sup>a</sup>	74.67 ± 10.67 <sup>a</sup>	3.00 ± 2.62 <sup>b</sup>	60.00 ± 16.49 <sup>a,b</sup>
11	56.33 ± 40.28 <sup>a</sup>	21.67 ± 9.69 <sup>a</sup>	11.00 ± 4.78 <sup>a</sup>	0.00 ± 0.00	170.67 ± 39.34 <sup>b</sup>
13	72.00 ± 38.26 <sup>a</sup>	16.33 ± 10.84 <sup>a,b</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	204.80 ± 31.35 <sup>c</sup>
15	100.00 ± 28.00 <sup>a</sup>	58.71 ± 17.34 <sup>a</sup>	4.67 ± 1.61 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	192.00 ± 64.00 <sup>c</sup>
17	104.00 ± 24.00 <sup>a</sup>	117.33 ± 30.54 <sup>a</sup>	29.33 ± 8.68 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	256.00 ± 0.00 <sup>c</sup>

<sup>a,b,c</sup> Different superscripts in a row indicate significant differences between the means at P < 0.05.

TABLE II: Effects of varying doses of *B. coriacea* extract on the PCV(mean±SD) of mice infected with *T. b. Brucei*.

Post infection days	Group 1 (250mg/kg)	Group 2 (500mg/kg)	Group 3 (1000mg/kg)	Group 4 (Berenil®)	Group 5 (Infected untreated)	Group 6 (Uninfected untreated)
- 1	52.67 ± 2.58	53.17 ± 1.60	54.83 ± 2.32	54.17 ± 3.13	53.00 ± 2.76	55.50 ± 1.87
8	44.33 <sup>a</sup> ± 4.50	37.67 <sup>a,b</sup> ± 16.24	44.67 <sup>a</sup> ± 5.92	43.17 <sup>a,b</sup> ± 5.50	34.33 <sup>b</sup> ± 4.63	55.33 <sup>c</sup> ± 1.03
11	32.33 <sup>a</sup> ± 7.87	29.67 <sup>a</sup> ± 6.41	27.33 <sup>a</sup> ± 6.62	44.67 <sup>b</sup> ± 6.12	31.50 <sup>a</sup> ± 4.64	54.50 <sup>c</sup> ± 1.52
14	35.50 <sup>a</sup> ± 5.97	34.00 <sup>a</sup> ± 6.73	30.83 <sup>a</sup> ± 4.17	45.17 <sup>b</sup> ± 5.56	23.00 <sup>c</sup> ± 2.65	54.67 <sup>d</sup> ± 1.75
15	29.00 <sup>a,d</sup> ± 6.06	32.67 <sup>a</sup> ± 8.02	40.50 <sup>b</sup> ± 5.09	51.00 <sup>c</sup> ± 6.36	21.50 <sup>d</sup> ± 0.71	55.33 <sup>c</sup> ± 0.82

<sup>a,b,c,d</sup> Different superscripts in a row indicate significant difference between the treatment means at p < 0.05

## DISCUSSION

In the acute toxicity study of the extract, no deaths were recorded. Only signs of dizziness were observed at 2000 mg/kg. This suggests that the extract has a wide safety margin in mice.

The extract reduced the number of trypanosomes in the blood. The reduction was dose dependent. At 1000 mg/kg there was a complete clearance of the trypanosomes in the blood by the 13<sup>th</sup> day post-treatment. However, by the 15<sup>th</sup> day there was a

relapse of infection. The relapse may be as a result of the inability of the extract to affect those parasites in the tissues. This is known to be the case with *T. brucei brucei* where smears of body fluids, including cerebrospinal fluid (CSF) may contain many parasites even when they are undetectable in blood (Radostiis *et al.*, 1994).

One known clinical sign of trypanosomosis is anaemia (Holmes, 2005). This manifests as pallor of the mucous membrane. In this experiment, infected animals had reductions in

their PCV following infection. Also as treatment was administered, an increase in PCV was observed. The increase was dose-dependent and may have resulted from the effect of treatment as there was a concurrent reduction in parasitaemia. This finding suggests that the ethanolic extract of *Buchholzia coriacea* seed has antitrypanocidal activity in mice experimentally infected with *T. brucei brucei*.

It is not known which component of the extract exerts the observed trypanocidal activity. Work is presently in progress to identify the active component in the plant which is associated with trypanocidal activity, through bioassay-guided fractionation of the crude extract. The isolation of the active component will not only reveal its chemical constituent but will assist in determining its mechanism of trypanocidal action.

### CONCLUSION

In conclusion, the ethanolic extract of *Buchholzia coriacea* seeds has exhibited significant trypanocidal activity in mice infected with *T. b. brucei* organism. The effect seems to be dose dependent, with 1000 mg/kg showing the highest activity. The reduction in parasitaemia corresponded with the increase in the PCV of infected mice.

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