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THE ANTHELMINTIC EFFECTS OF *Buchholzia coriacea* SEED

NWEZE, N.E. AND ASUZU, I.U.

Faculty of Veterinary Medicine
University of Nigeria, Nsukka

SUMMARY

The dry pulverized seeds of *Buchholzia coriacea* were exhaustively extracted in 70% ethanol over night at 40°C using a soxhlet extractor. The extract was concentrated in a vacuum using a rotary evaporator. The extract was a thick, brown, honey–like paste. Acute toxicity test was performed using two-week-old White Harco cockerel chicks, which weighed between 53.6 and 86.8 g. The extract showed a wide safety margin as no death was recorded up to the maximum dose of 2000 mg /kg. Brine shrimp lethality assay gave an LC_{50} of 117.46 ppm. *In vitro* anthelmintic assay using the L_3 larvae of *Haemonchus contortus* and *Heligmosomoides polygyrus* gave 94% death and 100% death at the concentration of 100 mg of extract/ml, respectively. Their LC_{50} values using probit analysis were 16.82 mg/ml and 11.20 mg/ml, respectively. In the *in vivo* test the extract did not reduce the post-mortem worm count and the faecal egg count in the treatment groups at doses of 200, 400, 800 and 1000 mg/kg when compared with Fenbendazole-treated birds. The extract exhibited larvicidal activity.

KEY WORDS: Anthelmintic, *Buchhlozia coriacea*, extract, *Haemonchus contortus*, *Heligmosomoides polygyrus*.

INTRODUCTION

Infection with gastro-intestinal helminths, especially nematodes results in reduced productivity and considerable economic losses. *Ascaridia galli* (the large roundworm of poultry) is commonly found in birds reared on deep litter and those under the extensive poultry management. Young, growing birds are more severely affected than older ones.

Poultry production is a major source of income in SouthEastern Nigeria. Most households have backyard poultry farms. Infection with *A. galli* causes poor growth, unthriftiness, diarrhoea, loss of weight (which is important in broiler production) and reduced egg production in layers.

Traditionally, some plants are reputed to have anthelmintic effects and are used both in the treatment of man and animals. The plants are readily available, cost very little and are claimed to be effective. The seeds of *Buchholzia coriacea*, popularly known as wonderful cola, are claimed to have potent anthelmintic effect. They are soaked over night in the local gin and the infusion is used in the treatment of human helminth infections. They are sometimes given as part of a multi-component therapy. *Buchholzia coriacea* leaves have been shown to have anthelmintic activity on *Fasciola hepatica, Pheritima posthuma and Taenia solium* (Ajaiyeoba *et al;* 2001). This study aims at

assessing the anthelmintic effects of *Buchholzia coriacea* seeds on *Haemonchus* contortus and *Heligmosomoides polygyrus*.

MATERIALS AND METHODS

Preparation of the plant extract

Sun dried seeds of *B. coriacea* were pulverized into fine powder and extracted with 70% ethanol overnight at 40°C using a soxhlet extraction apparatus. The percolate was concentrated using a rotary evaporator and the brown oily liquid obtained was stored in a glass bottle at 4°C.

Brine shrimp lethality assay

The eggs of brine shrimp, *Artemia salina* LEACH, were placed in sea water and they hatched within 48 hours, providing large numbers of larvae (nauplii) according to the method of Meyer et al (1982). The extract was tested at 3 concentrations of 1000, 100 and 10 g/ml. Three bijou bottles were prepared for each concentration for a total of 9 bottles. Twenty milligrams of the extract was dissolved in 2ml of seawater. From this solution 500, 50 and 5 l were transferred to bijou bottles and the volume adjusted to 5 ml/vial corresponding to 1000, 100 and 10 g/ml, respectively. Then ten nauplii were counted and placed in each bottle, and 24 h later the survivors were recorded. This test was repeated once more to give a total of six replicates for each concentration. The data obtained were analyzed using Finney computer programme to determine LC_{50} values at 95% confidence intervals.

Acute toxicity test

Thirty White Harco cockerels were used according to the method of Asuzu and Onu (1994). At 14 days of age, the chicks were randomly divided into five groups with six birds in each group. The chicks weighed between 53.6 g and 86.8 g. Graded doses (250, 500, 1000 and 2000 mg/kg) of the extract were administered by gastric intubation. The control group received equal volumes of distilled water. The birds were fed *ad libitum* and allowed access to clean water. They were observed for acute toxicity signs like behavioural changes or death over 24 hours.

In vitro anthelmintic assay of B. coriacea using H. polygyrus (L3) larvae

This was done according to the method of Njoku and Asuzu (1998). *Buchholzia coriacea* extract was tested at doses of 100, 50, 25 and 12.5 mg/ml. Mebendazole, a positive control, was tested at a concentration of 10 mg/ml. An average of 14 larvae/0.1ml was put in each well of a flat-bottomed microtitre plate. 0.1ml of each concentration of the extract and drug were added to larvae containing wells. Each concentration was replicated six times. After 24 h incubation at 4°C, the larvae were examined at 40 times magnification and classified as normal if moving or dead if no observable motion occurs during a five second interval.

The percentage of larvae dead or paralyzed was calculated using the following formula:

$$\frac{\text{Number dead}}{\text{Total number of parasite}} \times \frac{100}{1}$$

Probit analysis was used to determine the LC_{50}.

In vitro anthelmintic assay of B. coriacea using Haemonchus contortus (L3) larvae

This was done according to the method of Njoku and Asuzu (1998). Briefly, an average of 11 larvae /0.1ml were put in each well of a flat-bottomed microtitre plate, containing 0.1ml of the various concentrations of the extract (100, 50, 25 and 12.5 mg/ml) or mebendazole (10 mg/ml). Probit analysis was used to determine the LC_{50}. Percentage death was calculated as above.

In vivo anthelmintic evaluation of B. coriacea in experimentally infected chicken.

This was done according to the method of Asuzu and Onu (1994). Sixty White harco cockerels were used. At 16 days of age with mean body weight of 147.3 g, they were inoculated orally by gastric intubation with approximately 1,000 *Ascaridia galli* infective

eggs in two divided doses within an interval of one week. The birds were randomly allotted into six groups of ten cockerels each. Forty-six days post-infection, groups 1, 2, 4 and 5 were treated with graded doses (200, 400, 800 and 100 mg/kg, respectively) of the *B. coriacea* extract for five consecutive days. Group 3 served as positive control and was treated with a standard anthelmintic Fenbendazole (Panacur ®) suspension at a dose of 20 mg/kg body weight. Group 6 remained as infected, non-treated negative control. The test extract and drug were administered orally to each bird. Faecal evaluation was done by the salt floatation technique while egg counts were carried out by the Mcmaster method (Anon, 1977).

The results obtained were analyzed using the critical test (Asuzu and Onu, 1994)

$$\text{Percent efficacy} = \frac{\text{Number of worms passed}}{\text{Total number of worm}} \times \frac{100}{1}$$

$$\text{Percent egg reduction} = \frac{\text{Average pre-treatment egg/g} - \text{post-treatment egg/g}}{\text{Average pre-treatment egg/g}} \times \frac{100}{1}$$

Faecal egg counts in extract-treated, panacur-treated and untreated groups were compared and plotted as a graph.

Data obtained were subjected to statistical analysis using the analysis of variance (ANOVA).

RESULTS

Acute Toxicity Test

No signs of toxicity were observed. Birds dosed with the extract continued to feed normally. In the *in vitro* test using *Haemonchus contortus* L_3 larvae, a dose-dependent lethal action of *Buchhlozia coriacea* extract was observed (Fig. 1).

At 100 mg/ml, the highest concentration of the extract tested, the percentage mortality of the larvae was 94%. Then at 50, 25 and 12.5 mg/ml of extract, there were 87.80% 71.90% and 61.70% mortality recorded. The positive control drug (Mebendazole) showed 100% larval mortality at the concentration of 10 mg/ml. There were significant differences between groups A and C; B and C; C and E; D and E; C and F; D and F at $P < 0.05$.

There was also a dosedependent lethal action of *B. coraicea* extract on the L_3 larvae of *Heligmosomoides polygyrus*. At 100mg/ml of the extract, there was 100% mortality recorded (Fig 1). Then at 50, 25 and 12.5 mg/ml, there were 97.66%, 82.81% and 69.44% mortality, respectively. In the Mebendazole-treated positive control wells,100% mortality was recorded at the concentration of 10 mg/ml. Deaths in the untreated negative control wells were 10.62%. When the percentage deaths were compared statistically using ANOVA, significant difference was obtained between groups A and C; A and D and A and F at $P < 0.05$.

In the *in vivo* anthelmintic evaluation using *Ascaridia galli* infected cockerels, the ethanolic extract of *B. coriacea* was not able to reduce the faecal egg counts of the treated birds at the doses tested (Fig 2). The Fenbendazole (Panacur®)-treated birds had the lowest egg count and percentage egg per gram reduction of -15.

Also the ethanolic extract of *B. coriacea* had no effect on the host worm burden and, therefore, had zero percentage efficacy. Fenbendazole (Panacur®) had 100% efficacy (Table1).

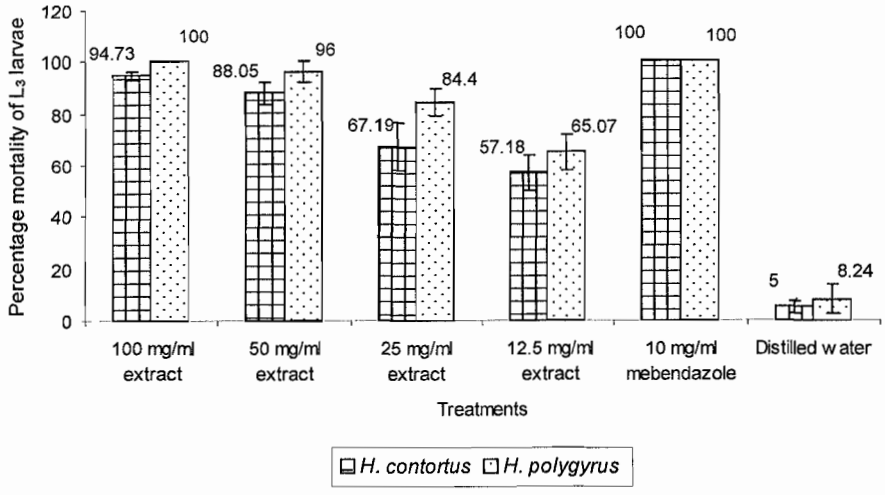
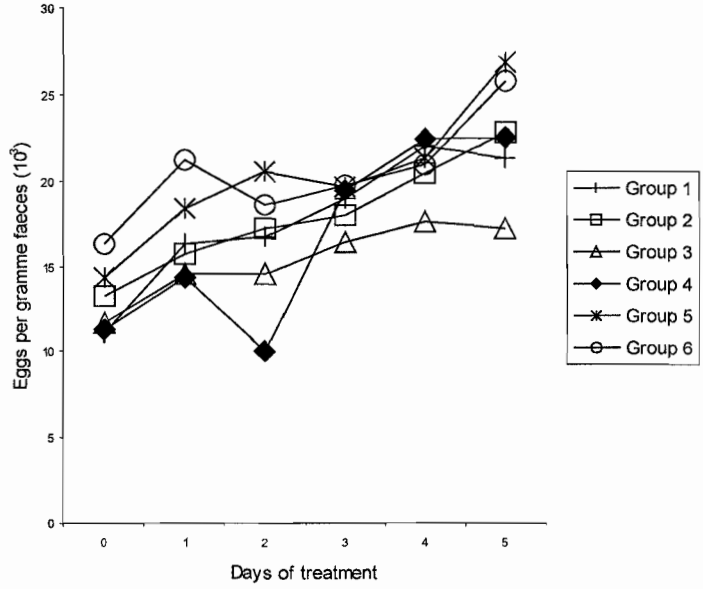


Figure1. The *in-vitro* anthelmintic (larvicidal) activities of *B. coriacea* extract using L_3 larvae of *Haemonchus contortus* and *Heligmosomoides polygyrus*



Treatment Groups:
Group 1 = 200mg/kg bw. of extract; Group 2 = 400mg/kg bw. of extract;
Group 3 = Panacur® (20mg/kg bw.); Group 4 = 800mg/kg bw. of extract;
Group 5 = 1000mg/kg bw. of extract; Group 6 =

Figure 2: Effect of *Buchholzia coriacea* extract and Panacur® on faecal egg count (eggs per gram faeces).

Table I: The effect of graded doses of *B. coriacea* and Fenbendazole (Panacur ®) on host worm burden and percentage efficacy values.

A	B	C	D	E(C+D)	F
Group no	Dose (mg/kg of extract/panacur	Total no of worms expelled	No of worms at necropsy	Total worm burden	% Efficacy
1	200	-	42	42	0
2	400	-	76	76	0
3	20(panacur)	1	0	1	100
4	800	-	42	42	0
5	1000	-	61	61	0
6	No treatment - ve control)	-	57	57	0

DISCUSSION

In the acute toxicity study of the test extract, no death or toxic sign was observed at the highest dose of 2000mg/kg P/O for 24h. This suggests that the extract is safe for use in this species (*Gallus gallus).* This extract had lethal actions on the nauplii of brine shrimp (*Artemia salina).* The LC_{50} was 117.4ppm(335.66 - 40.65ppm). This is an indication of the presence of biological activity in extract, since according to Lewis (1995) a wide variety of biologically active compounds are lethal to brine shrimps.

The ethanolic extract of *Buchholzia coriacea* exhibited larvicidal activities on the L_3 (infective) stage larvae of *Heligmosomoides polygyrus* and L_3 stage larvae of *Haemonchus contortus*. Their LC_{50} values were 11.20 mg/ml (8.98 13.41 mg/ml) and 16.82 mg/ml (12.04 21.60 mg/ml), respectively. The larvae of *H. polygyrus* showed greater susceptibility to the extract than the larvae of *H. contortus*.

The test extract had no effect on the adult worms and on their eggs. This was evident in the inability of the extract to either kill or expel the *Ascaridia galli* worms. Also, there was no reduction in the faecal egg count of the treated birds. This means that the extract has a narrow spectrum of anthelmintic activity.

Egg counts were lowest in the Fenbendazole treated birds compared to the other treatment groups. The egg counts increased from day zero to day 2 when there was a decrease in all the treatment groups. From day 3, egg counts increased up till day 5 in all the groups, except the Fenbendazole (Panacur®) treated group where there was a decrease from the 4th to the 5th day. This decrease in the egg count coincided with the expulsion of one live worm. Treatment was terminated on the same day and four days later, the birds were slaughtered and necropsied. On necropsy, no worms were found in the lumen of the intestine of the Panacur® treated birds.This suggests that the intestinal enzymes

probably digested the other dead worms in the lumen of the intestine, since no worm was seen on necropsy.

The continued presence of the eggs of *A. galli* in the faecal droppings despite the fact that there was no worm found in the intestine was rather unexpected. However, it could not be ascertained in the present study the exact time the worms died or were digested to release the eggs in the faecal droppings.

The anthelmintic effect ascribed to *B. coriacea* seed in folklore may be actually due to other components included in the therapy. *Buchholzia coriacea* seed may act synergistically with those other components to give the acclaimed result. The parasite, *Ascaridia galli* was used because of its close resemblance to *Ascaris lumbricoides,* (the human roundworm) but finer specie differences exist which may be responsible for the differences in susceptibility observed.

Also in Ibo folklore, there is no differentiation between the different types of worms-nematodes, cestodes and flukes. It could be that the extract might have greater effects on cestodes or flukes than on nematodes.

The methanolic extract of the roots of *Ritchiae capparoides* var. *Longipecellata*, (family, Capparaceae) which are closely related to *B. coriacea,* was found to have *in vitro* anthelmintic activity against tapeworms, earthworms and round worms. The extract was more active on tapeworm and had the least effect on round worms (Ajaiyeoba and Okogun, 1996).

CONCLUSION

The ethanolic extract of *Buchholzia coriacea* showed larvicidal action on the infective larval stages of *Heligmosomoides polygyrus* and *Haemonchus contortus*. Further work needs to be done to evaluate the activity of *B. coriacae* seed extract on tapeworms and flukes. The work should be done using the purified extract, which may have more anthelmintic activity. This would give the overall picture of its anthelmintic activity.

REFERENCES

AJAIYEOBA, E.O. and OKOGUN, J.I. (1996): Anthelmintic activity of a root extract of *Ritchiea cappariodes var longipedicellata. Phytother. Res.*, **10**(5): 436 437.

AJAIYEOBA, E.O. ONOCHA, P.A AND OLARENWAJU, O.T. (2001): *In vitro* anthelmintic properties of *Buchholzia coriacae* and *Gynandropsis gynandra* extracts. *Pharmaceutical Biology.*, **39**(3): 217-220

ANON, (1977): Manual of Veterinary Parasitological Laboratory Techniques.Technical Bulletin No 18, Her Majesty Stationery Office.

ASUZU I.U. and ONU, I.O (1994): Anthelmitic activity of the ethanolic extract of *Piliostigma thonningii* bark in *Ascardia galli* infected chickens. *Filtoterapia.*, **LXIV** (4): 291-297.

NJOKU, C.J. and ASUZU, I.U (1998). The Anthelmintic effect of the leaf extract of *Ocimum gratisimum* (L). *Phytomedicine.*, **5** (6): 485-488.

MEYER, B.N;FERRIGNI, N.R; PUTNAM, J.E; JACOBSON, L.B; NICHOLAS D.E AND Mclaughlin, J.L (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica.*, **45**: 31-34.

LEWIS, G. E. (1995). Testing the toxicity of extracts of Southern African plants using brine shrimp (*Artemia salina*). *South African Journal of Science.*, **91**: 382-384.