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ANTHELMINTHIC EFFICACY OF AQUEOUS EXTRACT OF *ACANTHUS MONTANUS* LEAF AGAINST STRONGYLID NEMATODES OF SMALL RUMINANTS.

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Abstract

The anthelminthic efficacy of the crude aqueous extract of *Acanthus montanus* (Nees) T. Anders (*Acanthaceae)* against strongylid nematodes of small ruminants was investigated using the *in-vitro* egg hatch and larval growth inhibition assays. Faecal samples collected per rectum from sheep and goats were subjected to parasitological examination using the McMaster counting technique with a yield of 700 eggs per gram (E.P.G.) of faeces. Crude aqueous leaf extract of *Acanthus montanus* was extracted using cold water extraction with a yield of 13.01% w/w. Egg hatch assay revealed a 91.75% reduction in egg hatch at concentration of 25mg/ml of the extract. The extract had a 100% inhibition, at 200mg/ml concentration which was equivalent to the activity of 3.125mg/ml albendazole. The distilled water control however, showed a 0% inhibition. Larval growth inhibition assay results showed the extract on Day 1 at 25mg/ml and 200mg/ml concentrations yielded 67.02% and 85.26% larval inhibition. On Day 2, 100% inhibition was obtained on all concentrations of the extract except for 25mg/ml that yielded 88.30%. Albendazole however, had a 100% larval inhibition for all concentrations on Day 2. A 100% larval inhibition was recorded on Day 3 from the 25mg/ml concentrations. The mean percentage larval inhibition of the extract at 200mg/ml (92.63%) was comparable to the standard anthelminthic (albendazole) at 12.50mg/ml (92.28%). These findings showed that there is a pharmacological basis for the folkloric claim of the anthelminthic effect of *Acanthus montanus*.

Key words: Anthelminthic, Extract, Strongylid Nematodes, *Acanthus montanus*

Introduction

Helminths are a major cause of reduced production in livestock in many countries, particularly the tropics (Hammond et al., 1997). Basically, three classes of helminths exist: Cestoda, Trematoda and Nematoda (Soulsby, 1982). The class Nematoda contains the most pathogenic helminths to livestock and companion animals, hence a threat to successful and sustainable livestock production worldwide (Perry and Raudolph, 1999).These helminth infestation cause direct and in direct losses. Direct losses are due to drop in production and death of animals. Indirect losses are due to increased cost of control strategies such as cost of drugs, labour and drenching equipment. Other helminth related set backs include delay in achieving target weights, reduced quality of carcass and predisposition to other diseases (Soulsby, 1982; Kassai, 1999; Mc Gaw and Eloff, 2008).

Annually, a huge amount of money is spent worldwide to combat helminth parasites in livestock (Coles, 2005). Scillhorn van Veen (1975) reported an annual loss of 14 million US dollars in Nigeria. Four years later Akerejola et al., (1979) reported a loss of over 60 million US dollars. In other African countries an estimated 700 million Ethiopian Birr was lost due to helminth infestation in Ethiopia (Habte-Silasie et al., 1991).

Systemic anthelminthics have long been considered the most effective way of controlling helminth infestation, to minimize losses. However, the threats of anthelmintic resistance, risk of residue in meat and milk is of concern. The availability and affordability of systemic anthelminthics to small holder farmers and pastoralist is a major problem in many developing countries. These set backs justify the need for alternative control methods (Baker et al.,1992 ;Wayangu et al.,1996 ;Waller 1997).Options such as, biological control,(Chandrawathani et al.,2003), vaccination (Bain, 1999) and the use of traditional medicinal plants (Githiori, 2004) are being examined in different parts of the world. The screening and proper evaluation of medicinal plants could offer possible alternative that may both be sustainable and environmentally acceptable (Eguale et al., 2007).

The plant *Acanthus montanus* (Nees) T. Anders(Acanthaceae) is a vigorously thinly branched perennial with basal clusters of oblong to lance-shaped, glossy, dark green leaves reaching up to 12 inches long (Huxley, 1992). The leaves have silver marks with wavy margins. The plant grows up to 6 feet tall and about 24 inches wide, with spikes of pale pink flowers (Huxley, 1992). This plant is common in Nigeria. It has been used in folk medicine to relieve aches, pains and to treat furuncles (Igoli et al, 2004; Igoli et al, 2005; Ibe and Nwufo, 2005). In Benue State, Nigeria, the plant is utilized by the Etulo natives to treat worms in children and adults (Agishi, 2004).

Pharmacological studies shows that the plant has spasmolytic (Adeyemi et al., 1999), analgesic (Adeyemi et al., 2004), anti-inflammatory and antipyretic (Asongalem et al., 2004) activities. The anthelminthic activity however has not being scientifically tested. The aim of this study therefore was to investigate the *in vitro* anthelminthic activity of the leaves of *Acanthus montanus* on the eggs and larvae of strongylid nematodes of small ruminants.

Materials and Methods

Collection of plant materials

The leaves of *Acanthus montanus* with stalk were harvested in Katsina-Ala, Benue State, Nigeria in the month of November, 2008. The plant was identified and authenticated by a plant taxonomist in the Department of Forestry of the University of Agriculture, Makurdi where a voucher specimen UAM V 200 was deposited.

Preparation of extract

The leaves of *A. montanus* were air-dried in the laboratory for 10 days and then pounded in mortar with pestle to yield 984g of the pulverized product. The extract was prepared according to standard methods (Mittal, et al., 1981) and stored at 4^{o}C until used. The w/w yield of *A.montanus* was 13.01%.

Preparation of stock solutions and serial dilutions of aqueous extracts

10g of crude aqueous extract of *A. montanus* leaf was dissolved in 50ml of distilled water to obtain a stock solution with a concentration of 200mg/ml. Serial dilutions of stock solution was performed to yield 10ml each of 100mg/ml, 50mg/ml and 25mg/ml concentrations of the extract. Similarly, 3 boluses of albendazole (Albidol®) weighing 750mg were crushed with mortar and pestle and dissolved in 30ml of distilled water to obtain a concentration of 25mg/ml stock solution. By serial dilution, 10ml of 12.50mg/ml, 6.25mg/ml and 3.125mg/ml concentrations of albendazole were placed in separate test tubes and labelled. 10ml of distilled water was also placed in a test tube as control.

Collection of faecal materials

Faecal materials (pellets) were collected per rectum from sheep and goats with natural acute/ sub-acute parasitic gastro-enteritis due to mixed nematode species. Samples were placed in labelled polythene bags and transported to the laboratory for examination.

Determination of faecal egg count

Faecal Samples were examined for helminth eggs using the modified McMaster technique with saturated sodium chloride solution as the floating medium (Hansen and Perry, 1990; Soulsby, 1982).

Egg hatch assay

The pooled faecal sample with EPG of 700 was used for this assay. Egg hatch assay was conducted following Sloss and Kemp, 1978) and Kelly et al. (1981). The eggs in the faecal materials were incubated at room temperature for five days. A drop of Lugol's iodine solution was added to stop the eggs from hatching. All the larvae in each test tube were counted. There were duplicates for each treatment and control. The number of unhatched eggs in each treatment and control were evaluated by relating the number of hatched larvae in the water control to the number of hatched larvae in the extract and albendazole.

Faecal culture and larval recovery

Faecal materials were cultured in the Petri dishes and were subjected to the modified Baermann technique with larvae harvested (Hansen and Perry, 1990). Active larval movement was observed when viewed under the microscope with ×10 objective. Six centrifuge tubes were filled to two thirds with medium and larvae was spinned at 1500rpm for 5 minutes in an electrically powered centrifuge (Centurion Scientific Ltd., K_3 System) to concentrate the larvae to the bottom. The larvae were recovered and pooled together after decantation of the supernatant fluid. A drop of the recovered larvae was stained with Lugol's iodine, viewed and counted, under light microscope at × 40 objectives.

Larval inhibition test

Various concentrations of the water extract of *A. montanus* and albendazole were prepared as described previously. 1ml of the water containing larvae was placed in each test tube already containing different concentrations of *A. montanus*,

albendazole and distilled water. Equal amount (1ml) of the test samples was placed in the corresponding test tubes and observations recorded after one hour. Subsequently for 4 days at room temperature. A drop of each test sample was observed microscopically and active and dead larvae counted for each time. Percentage inhibition of larvae in the test samples were calculated as shown below

$$\%\ Inhibition\ of\ \ larvae\ in\ Extract/Drug = \frac{No.\ of\ larvae\ dead}{Total\ \ No.\ of\ \ larvae\ counted} \times \frac{100}{1}$$

The mean percentage (%) inhibition of larvae in extract/drug for 4 days was calculated to determine the anthelminthic activity of the extract/drug. The results were compared with water control.

Results

Faecal egg hatch assay

Faecal samples with a minimum of 500 eggs per gram (EPG) of faeces were pooled. The egg count of pooled faecal sample was 700EPG.The results of the egg hatch assay using aqueous extract of the leaf of *A. montanus*, albendazole and their water controls are shown in Table I. The results show a 91.75% reduction in egg hatch at a concentration of 25mg/ml of the extract and at 100mg/ml the extract had a 100% inhibition, equivalent to 3.125mg/ml of albendazole.

Table 1: Percentage Inhibition of Strongylid nematode egg hatch in various concentrations of leaf extract of *A. montanus*, albendazole and distilled water.

Extract/ Drug (mg/ml)	**No. of Larvae Hatched**	**% Reduction in egg Hatch**
Distilled Water Control	5,150	**0***
Extract		
25.00	425	**91.75**
50.00	300	**94.18**
100.00	0	**100.0**
200.00	0	**100.0**
Albendazole (Albidol ®)		
3.125	0	**100.0**
6.25	0	**100.0**
12.50	0	**100.0**
25.00	0	**100.0**

***** Larval recovery from Distilled water control cultures was used as standard (0% reduction in egg hatch).

Larval growth inhibition assay

The larvae recovered by the modified Baermann technique after centrifugation at 1500rpm for 5 minutes averaged 285 per 0.5ml. The results of the larval growth inhibition tests are shown in Tables 2 to 5. The larvae were classified into active and dead. There was a 35.09% inhibition of larvae in the distilled water control as against 67.02 % and 85.26% for the 25mg/ml and 200mg/ml concentrations of the extract respectively. Albendazole had an inhibition of 74.74% and 93.68% at 3.125mg/ml and 25mg/ml concentration after 24 hrs of culture. There was a steady progress in the inhibition of larvae with attainment of 100% in the extract by day 3 of the study as against day 2 for albendazole. Table 6 shows the mean % inhibition.

Discussion

The results of this study revealed that the aqueous leaf extract of *Acanthus montanus* inhibit egg hatch and larvae survival at various concentrations when compared with control. The inhibition of egg hatch in vitro is an indication of the possible usefulness of this plant as a potential anthelminthic. This activity was dose dependent suggesting it has a pharmacological basis. The extract with higher concentrations shows more activity when compared to extract with lower concentrations. Thus the activity

Table 2: Percentage inhibition of Strongylid larvae in aqueous extract of *Acanthus montanus*, Albendazole and Distilled Water on Day 1.

Extract/ Drug (mg/ml)	No.of larvae showing activity	No.of larvae dead	% Inhibition of larvae
Distilled Water	285	100	**35.09**
Extract			
25.00	285	191	**67.02**
50.00	285	211	**74.04**
100.00	285	229	**80.35**
200.00	285	243	**85.26**
Albendazole (Albidol®)			
3.125	285	213	**74.74**
6.250	285	237	**83.16**
12.50	285	241	**84.56**
25.00	285	267	**93.68**

Table 3: Percentage inhibition of strongylid larvae in aqueous extract of *A. montanus*, albendazole and distilled water on Day 2.

Extract/ Drug (mg/ml)	No. of larvae showing activity	No.of larvae dead	% Inhibition of larvae
Distilled Water	185	78	**42.16**
Extract			
25.00	94	83	**88.30**
50.00	74	74	**100.0**
100.00	56	56	**100.0**
200.00	42	42	**100.0**
Albendazole (Albidol®)			
3.125	72	72	**100.0**
6.250	48	48	**100.0**
12.50	44	44	**100.0**
25.00	18	18	**100.0**

may be due to active substances found in the extract. The benzimidazole anthelminthics prevent embryonation and hatching of nematode eggs (Taylor et al., 2002). This suggest therefore that extract such as *A. Montanus* with ability to inhibit egg hatch may be useful for further evaluation as a possible anthelminthic. Egg inhibition will reduce pasture contamination by nematode egg during grazing by livestock. This is of practical importance in overall helminth control.

Further evaluation of the extract of the leaf of *Acanthus montanus* also reveals the inhibition of nematode larvae at various concentrations. The inhibition shows a graded dose dependent activity suggesting it has a pharmacological basis. The water control had some mortality of the larvae due to lack of nutrients or exhaustion and not associated with any activity (Hubert and Kerboeuf, 1992). This graded dose response activity was reported in plant extracts by Onyeyili et al. (2001) and Nwosu et al. (2005) on strongylid nematodes of small ruminants.

Table 4: Percentage Inhibition of Strongylid larvae in aqueous extract of *Acanthus montanus,* Albendazole and Distilled water on Day 3.

Extract/ Drug (mg/ml)	No. of larvae showing activity	No.of larvae dead	% Inhibition of larvae
Distilled Water	107	51	**47.66**
Extract			
25.00	11	11	**100.0**
50.00	-	-	-
100.00	-	-	-
200.00	-	-	-
Albendazole (Albidol®)			
3.125	-	-	-
6.250	-	-	-
12.50	-	-	-
25.00	-	-	-

Table 5: Percentage Inhibition of Strongylid larvae in aqueous extract of *Acanthus montanus,* Albendazole and Distilled water on Day 4.

Extract/ Drug (mg/ml)	No. of larvae showing activity	No. of larvae dead	% Inhibition of larvae
Distilled Water	51	25	**49.02**
Extract			
25.00	-	-	-
50.00	-	-	-
100.00	-	-	-
200.00	-	-	-
Albendazole (Albidol®)			
3.125	-	-	-
6.250	-	-	-
12.50	-	-	-
25.00	-	-	-

The activity of *Acanthus montanus* may be related to pharmacologically active substances such as alkaloids and saponins previously reported from the leaf of the plant (Anam, 1997a, b). This activity can also be due to toxicity from the plant. Further study, therefore need to evaluate the toxicity of this extract using the brine shrimp lethality assay. Also there will be need to evaluate the activity of this extract *in vivo* using small ruminant animal model following the FECRT (Coles et al., 1992). In conclusion, the leaf of *Acanthus montanus* produced a dose dependent inhibition of egg hatch and larvae of strongylid nematodes of small ruminants. This provides a pharmacological basis, for the folkloric medicinal application of this plant.

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Table 6: Mean Percentage Inhibition of Strongylid larvae in aqueous extract of *Acanthus montanus*, Albendazole and Distilled water on Day 1, 2, 3, and 4.

Extract/ Drug (mg/ml)	**Percentage Inhibition**				**Mean % Larval Inhibition**
	Day 1	**Day 2**	**Day 3**	**Day 4**	
Distilled Water	35.09	42.16	47.66	49.02	**43.48**
Extract					
25.00	67.02	88.30	100.0	-	**85.11**
50.00	74.04	100.0	-	-	**87.02**
100.00	80.35	100.0	-	-	**90.16**
200.00	85.26	100.0	-	-	**92.63**
Albendazole (Albidol®)					
3.125	74.74	100.0	-	-	**87.37**
6.250	83.16	100.0	-	-	**91.58**
12.50	84.56	100.0	-	-	**92.28**
25.00	93.68	100.0	-	-	**96.84**

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