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Anthelmintic screening of fractions of *Spigelia anthelmia* Linn extracts against experimental *Nippostrongylus braziliensis* in rats

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ABSTRACT

This study was undertaken to screen fractions of extracts of *Spigelia anthelmia* to find out its anthelmintic activity against experimental *Nippostrongylus braziliensis* infection in rats. 1000 g of the ethanolic crude extract of *Spigelia anthelmia* yielded 199 g (19.9%) while fractionation with chloroform, water and ethylacetate yielded 120 g (60%), 64 (32%) and 9 g (4.5%) respectively. In the anthelmintic trial in rats, the aqueous and ethylacetate fractions gave a highly significant ($P<0.001$) deparasitization; while the chloroform fraction gave non-significant ($P>0.05$) deparasitization when compared with the control. However, comparing the fractions with each other, chloroform and ethylacetate fractions gave a non significant ($P>0.05$) deparasitization while the aqueous fraction gave a highly significant ($P<0.001$) deparasitization. The aqueous fraction was therefore found to possess the active anthelmintic ingredient.

Key words: *Spigelia anthelmia,* anthelmintic, *Nippostrongylus braziliensis,* rats

Introduction

The prevalence of helminth diseases in Nigeria is very high, especially during the wet season when infection is as high as 100% in cattle. Such high infection rates prevent them from attaining optimum productivity, especially under the traditional husbandry system (FAKAE, 1990).

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Parasitic infections of the gastrointestinal tract of ruminants with nematodes remain a main issue in developing countries since these worms are responsible for major production losses in livestock (BAKUNZI and SERUMAGA-ZAKE, 2000; HOUNZANGBE-ADOTE et al., 2005).

Nematode infections not only cause clinical disease and mortalities but also affect production. Some of these effects have been reported to include impairment of the normal physiological behaviour of the animals and reduced feed intake and nitrogen retention leading to decreased efficiency of utilization of feed, resulting in decreased performance in terms of reduced growth rates by up to 30% or more, low fertility of ewes and cows, low birth weight and reduced weight gain of lambs and calves, reduced milk and wool production, and a decrease in the percentage of ewes rearing lambs to weaning (PROVOST, 1989; MEYERS, 1991; AGEI, 1993; GITHIGIA et al., 1995). These insidious effects of chronic helminthosis have important implications for the attainment of maximum productivity in livestock (CHIEZEY, 1998).

To date, the principal mode of control of parasites in the digestive tract has been based on chemical treatments with anthelmintics. Due to the increasing development of anthelmintic resistance within worm populations (JACKSON and COOP, 2000) and also the cost of such treatments in developing countries, there is currently a growing interest in an ethnoveterinary approach to examine the anthelmintic properties of the plants which are traditionally used by local farmers (HAMMOND et al., 1997; WALLER, 1997; AKHTAR et al., 2000; JEGEDE et al., 2007).

This study was, therefore, carried out to screen three fractions of ethanolic extracts of *Spigelia anthelmia* Linn against experimental *Nippostrongylus braziliensis* infection in rats, in order to determine the fraction with the highest anthelmintic activity.

Materials and methods

Collection and preparation of plant material. All the plant material was collected from the premises of the Ahmadu Bello University, Zaria, Nigeria in July, 2005. Taxonomic identification was established by a botanist with the Soil Survey Unit of the Department of Soil Science, Faculty of Agriculture, Ahmadu Bello University, Zaria. It was authenticated by comparing it with the herbarium sample at the National Animal Production Research Institute herbarium, Shika, Zaria. A voucher specimen was deposited there and labeled Specimen number 1.

Extraction. Immediately after collection, the plants were sorted, cleaned and dried in the open air for one week. The dried materials were pulverized into powder using a Lab mill machine in clean containers and labeled for easy identification.

The extraction was carried out by the method as described by WAGNER et al. (1993), using ethanol as the solvent. Briefly, 1 kg of the dried powdered plant material was packed

in 2 L separatory funnels (250 g packed each time) and extracted with 95% ethanol for one week. The filtrate was evaporated to dryness *in vacuo* at 60 °C using a Buchi rotary evaporator coupled to a thermo regulator. The residue was then partitioned between chloroform and water. The aqueous phase was further partitioned using ethylacetate and later concentrated in a water bath. The solid extract obtained was removed and stored in labeled beakers at 4 °C until required.

Experimental animals. Albino rats (Wistar strain) weighing between 80-200 g were bred in the animal house, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria They were allowed to adapt to laboratory conditions for two weeks. They were maintained *ad libitum* on a ration containing commercial poultry feed growers mash (Silver feeds), groundnut cake ("kuli kuli") and maize bran ("dusa") at a ratio of 1:0.5:2 respectively. Water was also given *ad libitum* supplied by the Ahmadu Bello University water works. All the rats were dewormed using Albendazole® (Concept Pharmaceuticals, India) at 7.5 mg/kg to establish a worm- free colony. Sawdust was used as bedding and was changed every three days. The rats were identified by marks on their tails and cages.

Acute oral toxicity studies. Acute toxicity studies were conducted using the method described by LORKE (1983). Briefly, the rats were divided into 5 groups of three rats in each. The aqueous solution of the extract was administered through the oral route. The rats in groups 1, 2, 3 and 4 were given an aqueous solution of the extracts orally at a dose of 10, 100, 1000, and 10000 mg/kg respectively. The fifth group, which was used as the control, received distilled water at 5 mL/kg. The animals were observed for signs suggestive of toxicity within 72 hours. The animals that survived were further monitored for two weeks for toxic effects. The test was terminated after two weeks and all the animals were humanely sacrificed and postmortem examinations carried out on them.

Anthelmintic screening of the three fractions of extract of Spigelia anthelmia. Anthelminthic trials and screening were conducted for the three fractions (Ethylacetate, Chloroform and Aqueous) of the ethanolic extract of *Spigelia anthelmia*. Twenty nematode-free rats were infected subcutaneously in the cervical region each with 200 L_3 of *Nippostrongylus braziliensis*. Fresh faecal pellets were obtained on the seventh day post infection from each rat by manipulating them out of the rectum into labeled plastic tubes. The faecal samples were examined qualitatively by flotation to establish infection. Treatment commenced on the tenth, eleventh and twelfth days post experimental infection (IBRAHIM, 1984).

Five rats were used to test each of the three fractions i.e., Chloroform fraction (6.25 g/kg) Aqueous fraction (5.0 g/kg) Ethyl acetate fraction (2.5 g/kg). Animals were dosed orally on the 10^{th}, 11^{th} and 12^{th} days post experimental infection using an 18-gauge needle fitted with a plastic cannula. The rats in the fourth group were dosed with water at the

rate of 5 mL/kg and used as the control. On the 14th day post infection, the animals were starved overnight to empty the small intestine and to make worm counting easier. They were humanely euthanized on the 15th day. The rats were autopsied and the first 15 cm length of the small intestine removed, sectioned longitudinally, enclosed between two plates of thick glass and examined under a dissection microscope. All the worms present and visible were counted.

Results were analyzed according to the method recommended by CAVIER (1973). The percentage deparasitization was calculated using the formula:

$$\frac{N - n}{N} \times 100$$

Where:

N = average number of worms found in control animals and n = average number of worms found in groups of treated animals. A deparasitization of 70% was considered significant.

Statistical analysis. Data obtained from the experiment were subjected to statistical analysis (ANOVA) using GraphPad prism version 4 (2003), subsequently Bonferroni's Multiple Comparison Test was used to compare the groups. $P<0.05$ was considered statistically significant.

Results

Description of the extract. The ethanolic crude extract was green in colour and gave a yield of 199 g (199.9%). While the partitioning with chloroform also produced a greenish colour with a yield of 60%, the aqueous portion was brownish in colour giving a yield of 32% and the ethylacetate portion yellowish in colour yielding 4.5%.

Acute toxicity study of Spigelia anthelmia in rats. There were no deaths by the oral route even at a dose of 10,000 mg/kg (Table 1).

The clinical signs of intoxication observed included restlessness, anorexia, prostration, hyperpnoea, and reduced movement. At post mortem, the dead animals showed enlarged and congested livers, enteritis, and the spleen and kidneys were enlarged and congested. There was no visible gross lesion seen in the hearts.*Result of anthelmintic trial.* Results of the anthelmintic trials using three fractions of the ethanolic extract of *Spigelia anthelmia* and dosages administered are represented in Fig. 1.The average worm counts obtained from the control rats was 40.00 ± 3.54, while from rats given Ethylacetate fraction 26.00 ± 4.30, from rats given Aqueous fraction and Chloroform fraction were 4.00 ± 1.58 and 33.00 ± 6.71 respectively (Table 2).

Table 1. Evaluation of toxicity of *Spigelia anthelmia* L administered orally in rats

	Dose (mg kg^{-1})	N^{o} of animals	N^{o} of deaths	Survival	Mortality ratio
Control	0	3	0	3	0/3
	10	3	0	3	0/3
	100	3	0	3	0/3
	1000	3	0	3	0/3
	10000	3	0	3	0/3

Table 2. Table of average worm count and percentage deparasitization

	Dose (g kg^{-1})	Average wormcount*	Deparasitization %
Control	0.0	40 ± 3.54	0.0
Chloroform fraction	6.25	33 ± 6.71	17.5
Aqueous fraction	5.0	04 ± 1.58	90.0
Ethylacetate fraction	2.5	26 ± 4.30	35.0

*Worm count data expressed as mean ± SEM

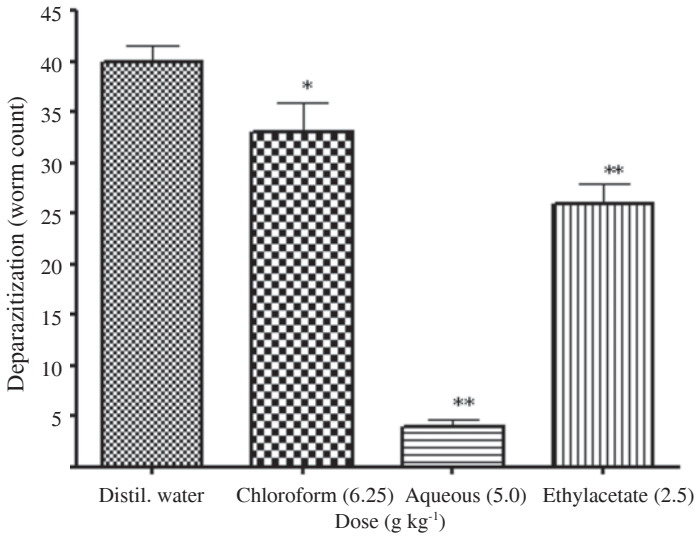

Fig. 1. Bar diagram showing anthelmintic activity of the three fractions of the extract of *Spigelia anthelmia* in rats experimentally infected with *Nippostrongylus braziliensis* 3 days post treatment. Activity of the fractions were compared with negative control (distilled water). Values shown are mean ± SEM, n = 5; *Not significant (P>0.05), **Significant at P<0.001.

Statistical analysis by the one-way analysis of variance (ANOVA) revealed that the means of deparasitization among the different doses used were significantly different ($P<0.05$), however, the variances did not differ significantly ($P>0.05$). Using the Bonferroni's multiple comparison test between the control and the three fractions used in this study, the analysis revealed a highly significant ($P<0.001$) difference in deparasitization of the aqueous and the ethylacetate fractions and a non significant ($P<0.05$) deparasitization between the control and the chloroform fraction. This result indicated that the anthelmintic activity of the aqueous and ethylacetate were almost similar ($P<0.001$) but different from the anthelmintic activity of the chloroform fraction when the fractions were compared with the control. However, comparing the fractions with each other, the statisticals result indicated the highly significant ($P<0.001$) deparasitization of the aqueous fraction when compared with the chloroform fraction. However, there was no statistically significant difference ($P>0.05$) between the ethylacetate fraction and the chloroform fraction. When compared, there was a highly statistically significant difference ($P<0.001$) in deparasitization between the aqueous fraction and the ethylacetate fraction. At a dose of 6.25 g/kg, the chloroform fraction of the ethanolic extract of *Spigelia anthelmia* caused deparasitization of 17.5 percent, the ethylacetate fraction caused 35.0 percent deparasitization at a dose of 2.5 g/kg and the aqueous fraction caused 90.0% deparasitization at a dose of 5.0 g/kg (Table 2). The percentage deparasitization obtained in this study compares very well with the 76% deparasitization reported by SULEIMAN et al. (2005) when they tested the crude methanol extract of *Xylopia aethiopica* at a dose level of 2.0 g/kg against experimentally infected *Nippostrongylus braziliensis* in rats. It can therefore be inferred from the results of the present study that the aqueous fraction of the ethanolic extract of *Spigelia anthelmia* has a good anthelmintic activity against adult *Nippostrongylus braziliensis* in rats.

Discussion

The observation that there was no death recorded even at 10,000 mg per kg body mass by the oral route suggests that *Spigelia anthelmia* is less toxic by this route.

The results of the acute toxicity studies carried out in this research are in sharp contrast to the reports of DALZIEL (1937) and OLIVER (1959) who reported that the plant is distinctly poisonous in its fresh state by the oral route and that it is capable of causing death in domestic animals in one to two hours. However, JOHNSON (1963) reported that some work done in Sierra Leone shows that *Spigelia anthelmia* L is not poisonous by the oral route. He went ahead to investigate the toxicity of *Spigelia anthelmia* L in small laboratory animals by feeding leaves, stem and seeds respectively to rabbits, and reported that only the seeds were found to be toxic. He therefore concluded that the toxic properties of *Spigelia anthelmia* L are contained in the seeds, which were not part of this study.

In the anthelmintic trial, the chloroform and ethylacetate fractions gave non-significant ($P>0.05$) deparasitization when compared with the aqueous fraction. The aqueous fraction gave highly significant ($P<0.001$) deparasitization. This result indicates that the probable active ingredient of the plant (against worms) is concentrated in the aqueous fraction. This therefore confirms the report of DALZIEL (1937), HUTCHINSON and DALZIEL (1963) that the local people boil the plant in water for treatment against nematodes.

From the results obtained in this study, it could be concluded that the probable active anthelmintic ingredient(s) of *Spigelia anthelmia* L is contained in the aqueous fraction. This may be an indication of the presence of water - soluble active anthelmintic principle(s) in the extract of *Spigelia anthelmia* L. It can be concluded from this study that *Spigelia anthelmia* L has a very good and promising potential as an anthelminthic in livestock. A full investigation into the anthelminthic activity of *Spigelia anthelmia* L in higher livestock species is therefore recommended.

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References

AGEI, A. D. (1993). Studies on the epidemiology of gastrointestinal parasites of lambs in Ghana. Proceedings of an IFS/SIPATH Workshop. Animal diseases of the gastrointestinaland liver. An African perspective. Addis Ababa, Ethiopia. 20-25th September, pp. 82-86.

AKHTAR, M. S., Z. IQBAL, M. N. KHAN, M. LATEEF (2000): Anthelmintic activity of medicinal plants with particular reference to their use in animals in the Indo Pakistan subcontinent. Small Rum. Res. 38, 99-107.

BAKUNZI, F. R., P. A. E SERUMAGA-ZAKE (2000): The effect of strategic anthelmintic treatment on internal parasites in communally grazed sheep in a Semi-Arid area as reflected in the faecal nematodal egg count. Trop. Anim. Health Prod. 32, 295-302.

CAVIER, R. (1973): Chemotherapy of Helminthiasis Vol. 1. Pergamon Press, Oxford, 215-436 .

CHIEZEY, P. N. (1998): The peripaturient increase in Trichostrongyle egg counts in Yankassa ewes in Zaria, Northern guinea savannah zone of Nigeria. Ph.D. Thesis, Ahmadu Bello University, Zaria. p. 2.

DALZIEL, J. M. (1937): The usefull plants of West tropical Africa. Crown agents, London. pp. 612.

FAKAE, B. B. (1990): Seasonal changes and hypobiosis in *Haemonchus contortus* in the West African Dwarf sheep and goats in the Nigerian derived savannah. Vet. Parasitol. 3, 123-130.

GITHIGIA, S. M, C. O. KIMORO, G. M. MWANGI, J. GICHANYA (1995): Prevalence and economic significance of oesophagostomum and other helminth parasites of ruminants surveyed in selected abattoirs around Nairobi, Kenya. Bull. Anim. Health Prod. Afr. 43, 29-33.

HAMMOND, J. A., D. FIELDING, S. C. BISHOP (1997): Prospects for plant anthelmintics in tropical veterinary medicine. Vet. Res. Comm. 21, 213-228.

HOUNZANGBE-ADOTE S., I. FOURASTE, K. MOUTAIROU, H. HOSTE (2005): *In vitro* effects of four tropical plants on the activity and development of the parasitic nematode, *Trichostrongylus colubriformis.* J. Helminthol. 79, 29-33.

HUTCHINSON, J., J. M. DALZIEL (1963): Flora of West Tropical Africa. 2nd ed. Vol. II. Crown Agents London. pp 45.

IBRAHIM, M. A. (1984): Evaluation of the activities of some African traditional anthelmintic herbs against *Nippostrongylus braziliensis* in rats M.Sc. Thesis, Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria. pp. 119.

JACKSON, F., R. L. COOP (2000): The development of anthelmintic resistance in sheep nematodes. Parasitol. 120, 95-107.

JEGEDE, O. C., I. E. IKANI, I. I. DAFWANG, P. I. BOLORUNDURO, A. I. ANNATTE (2007): Traditional animal healthcare practices in disease prevention and control by small ruminant farmers in Oyo State, Nigeria. J. Food Agricult. Environ. 5, 163-164.

JOHNSON, S. W. (1963): The toxicity of *Spigelia anthelmia* for small laboratory animals. Trop. Agric. 40, 165-167.

LORKE, D. (1983): A new approach to practical acute toxicity testing Archi. Toxicol. 54, 275-287

MEYERS, G. H. (1991): Parasite control importance in grazing beef cattle. Feedstuffs 63, 14.

OLIVER, B. (1959): Nigeria's usefull plants. The Nigerian Field. 24, 160-182.

PROVOST, A. (1989): Constraints to livestock production due to diseases. Proceedings of Integration of Livestock with Crops in Response to increasing Population Pressure on available Resources. Mauritus. (Preston, T. R., R. M. Mauricio, H. Osorio, Eds.) 11th - 14th July, 1989. pp. 64-87.

SULEIMAN, M. M., M. MAMMAN, Y. O. ALIU, J. O. AJANUSI (2005): Anthelmintic activity of the crude methanol extract of *Xylopia aethiopica* against *Nippostrongylus brasiliensis* in rats. Vet. arhiv 75, 487-495.

WAGNER, H., K. SEEGERT, K. P. ODENTHAL, M. ESPOSITO AVELLA, E. VILLARREAL, P. SOLIS, M. P. GUPTA (1993): Preliminary pharmacologic evaluation of *Spigelia anthelmia* aerial parts. Int. J. Pharmacognosy 31, 7-14.

WALLER, P. J. (1997): Nematode parasite control of livestock in the tropics/subtropics: the need for novel approaches. Int. J. Parasitol. 27, 1193-1201.

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SAŽETAK

Istraživana je protuhelmintička aktivnost pojedinih frakcija iscrpaka biljke *Spigelia anthelmia* na pokusnu invaziju oblićem *Nippostrongylus brasiliensis* u štakora. Iz 1000 g etanolnoga sirova iscrpka dobiveno je 199 g (19,9%) suhe tvari, dok je frakcioniranjem kloroformom dobiveno 120 g (60%), vodom 64 g (32%) i etilacetatom 9 g (4,5%). Vodene i etilacetatne frakcije imale su visoku aktivnost (P>0,001) dok kloroformna frakcija nije imala značajan učinak (P<0,05) u odnosu na kontrolu. Ipak, uspoređujući međusobnu aktivnost pojedinih frakcija, kloformna i etilacetatna nisu imale značajan (P>0,05), dok je vodena frakcija imala značajan protuhelmintički učinak (P<0,001) iz čega proizlazi da samo vodena frakcija sadrži aktivne protuhelmintičke sastojke.

Ključne riječi: *Spigelia anthelmia*, antihelmintik, *Nippostrongylus brasiliensis*, štakori