

IN-VITRO ANTIHELMINTIC EFFECTS OF TWO KENYAN PLANT EXTRACTS AGAINST HEAMONCHUS CONTORTUS ADULT WORMS

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Abstracts

This study was on evidence based information that *Entada leptostachya* Harms and *Rapanea rhododendroides* (Gil) Mez were used by the herbalists in Mbeere County, Kenya, for the treatment of gastrointestinal worms. The plants' aqueous and solvent extracts were tested for their *in-vitro* antihelmintic activity against *Haemonchus contortus* adult worms. Of the eight plant extracts investigated, four extracts exhibited adult worm mortality greater than 50% while the other four afforded mortality ranging between 60-77%. *E. leptostachya* methanol extract was the most active (77%). Albendazole was used as a positive control drug while Goodwin's physiological solution was used as negative control. Methanol extracts for both plants exhibited the highest anthelmintic activity at the test concentrations of 25mg/ml. Although *R. rhododendroides* was ranked third in general usage by the herbalists, *E. leptostachya* was solely used for the treatment of intestinal worms. The present results demonstrated that *E. leptostachya* and *R. rhododendroides* plant extracts had antihelmintic agents, and justified their traditional use as alternative drugs for the treatment of heamonchosis in ruminants

Keywords: Heamonchosis, Herbalists, Mbeere, Tannins, Saponins, *Entada leptostachya*

1. Introduction

Gastrointestinal (GI) nematodes are a major threat to sheep productivity and endanger animal welfare Worldwide particularly in developing countries¹. Haemonchosis has been identified as one of the top ten constraints to sheep and goat rearing in East Africa² and is the leading pathogenic and resistant helminth parasite in small ruminants that causes acute and high mortality³. Control of gastrointestinal helminths by use of synthetic anthelmintics has inherent challenges to the poor farmers of developing countries⁴. Furthermore, continuous usage of conventional anthelmintics leads to development of resistance, presence of residues in meat and milk with associated high environmental impact⁵. Resistance of *H. Contortus* to ivermectin and benzimidazoles has been reported, the parasite the occurrence being significantly higher in sheep than in goats^{6,7}. Anthelmintic resistance and other associated shortcomings of conventional drugs has necessitated search for alternative herbal remedies⁸. *In-vitro* activity of plant extracts against *H. contortus* adult worms, larvae development and hatching has been demonstrated^{9,10,11}. Numerous studies from various parts of the World have shown that

certain plant species effectively reduce the degree of parasite infestation in ruminants (sheep) and are promising alternatives to conventional anthelmintics^{12,13,14}. Many of these plant species owe their anthelmintic effects to plant secondary metabolites such as saponins, tannins and essential oils^{15,16,17,18,19}. These effects are time and dose dependent.

Entada leptostachya and *R. rhododendroides* are among the plants used by the traditional healers in Mbeere and Embu Counties of Kenya as dewormers and are found in East Africa. Hence the aim of the present study was to determine the *in-vitro* potency of *Entada leptostachya* Harms and *Rapanea rhododendroides* (Gil) Mez. plant extracts against *H. Contortus* adult worms in infected sheep.

2. Materials and methods

2.1 Collection of indigenous knowledge: Over 30 herbalists from Mbeere County, Kenya were interviewed for their indigenous knowledge on common plants used to treat worm infestation in humans and ruminant animals. The plant ranking was based on the frequency of usage and preference. From this information two plants with

the highest ranking were selected for the present study.

The root bark of *E. leptostachya* and stem bark of *R. rhododendroides* were collected after identification and authentication by a taxonomist of the Botany Department, Jomo Kenyatta University of Agriculture and Technology (JKUAT). Voucher specimens OOM/1 and OOM/2 were deposited in the herbarium of that department. The collected plant parts were dried under the shade and ground to a powder.

2.2 Plant extraction: The plant powders were subjected to hot water maceration to obtain aqueous extracts. solvent extracts were carried out using methanol, acetone and hexane in a Soxhlet extractor. Extracts were dried under vacuum, placed in separate dry marked vials and stored at 4°C for further use.

2.3 Collection of *H. contortus* worms: Mature *H. contortus* worms were collected from the abomasums of freshly slaughtered sheep at a local abattoir, identified by a meat inspector and later confirmed by a parasitologist at Zoology department, JKUAT. The worms were washed in distilled water then suspended in phosphate buffered saline (PBS) made by dissolving 0.85 g of sodium chloride and 1 g glucose in 1 litre distilled water. They were then transported to the laboratory in an air tight can and then left for 2hrs to acclimatize.

2.4 Dissolution of plant extracts: Plant extracts were dissolved in DMSO and made to the mark using distilled water to make 25mg/ml solutions. Filter paper discs (6 mm diameter) were dipped into each solution. The discs impregnated with the above extracts were dried at room temperature to evaporate the DMSO, leaving only the test compounds. The discs for water extracts were not dried.

2.5 Screening of saponins and tannins in plant extracts: Saponins and tannins were screened using the methods described by Edeoga *et al*²⁰.

2.6 Determination of *In-vitro* anthelmintic activity: Ten (10) adult *H. contortus* worms were placed into a sterile Petri dish containing 10ml of PBS. The filter paper disc containing the aqueous extract was added and agitated. After 24 hours, the worms were removed from the Petri dish and the parasites suspended in PBS for 30min for possible recovery of the parasite motility. The number of motile (alive) and immotile (dead) worms were counted using a hand lens and recorded. Death or paralysis of worms was ascertained by absence of motility for an observation period of 5–6 seconds²¹.

The above procedure was repeated for all the other plants' extracts. Three replicates were performed for each treatment. Albendazole (purchased from Kobian Ltd) at a concentration of 0.55 mg/ml was used as the reference drug (positive control) while PBS was used as a negative control. Worm motility and mortality were the rationale for anthelmintic activity.

3. Results and discussion

Table 1 shows the list of plants purported to treat worm infestation in ruminants as well as in humans. The ranking of 1-9 was on the basis of potency. There were claims, however, that plants with the highest ranking were used to treat stomach troubles in humans due to amoeba infections. There was unanimous agreement that *Entada leptostachya*, *Rapanea rhododendroides* and *Albizia anthelmintica* were used for deworming ruminant animals. The latter plants were reported to have anthelmintic activity²².

Table 1: Plants purported to treat worms in humans and animals

Local names	Scientific names	Ranking	Parts used
Mubarwa	<i>Albizia anthelmintica</i> *	3	Bark/roots
Mwinu	<i>Senna didymobotrya</i>	8	Leaves
Muvovo	<i>Leonotis mollissima</i>	5	Leaves
Mucaritha	<i>Entada leptostachya</i> *	1	Roots
Mugeeta	<i>Rapanea rhododendroides</i> *	2	Bark
Mururuku	<i>Terminalia brownii</i>	6	Bark
Terere	<i>Amaranthus hybridus</i>	9	Leaves
Mubera	<i>Psidium guajava</i>	4	Leaves
Mubiru	<i>Vangueria madagascariensis</i>	7	Leaves

*Plants commonly used to treat worms in ruminant animals

Anthelmintic activity of the *E. leptostachya* and *R. Rhododendroides* extracts against *H. contortus* adult worms were presented in Table 2 and in Figure 1. The samples were tested at a concentration of 25 mg/ml and compared to

Albendazole positive control, whose concentration was maintained at 0.55mg/ml as directed on the product label. All the plants' extracts tested positive for saponins and tannins, except the hexane extracts.

Table 2. *In-vitro* anthelmintic activity of plants extracts^a

<i>E. leptostachya</i>	Mean	^b Mean std. error	<i>R. rhododendroides</i>	Mean	^b Mean std. error
Acetone extract	27.78	3.74	Acetone extract	25.83	3.17
Aqueous extract	64.44	4.82	Aqueous extract	46.94	3.92
Hexane extract	20.56	3.03	Hexane extract	13.06	2.32
Methanol extract	76.67	3.78	Methanol extract	54.44	3.60
PBS	0.00	0.00	PBS	0.00	0.00
Albendazole	100.00	0.00	Albendazole	100.00	0.00

^aThree replicate determinations at a concentration of 0.25mg/l for each extract and 0.55mg/ml for Albendazole

^bStandard mean error using SAS software

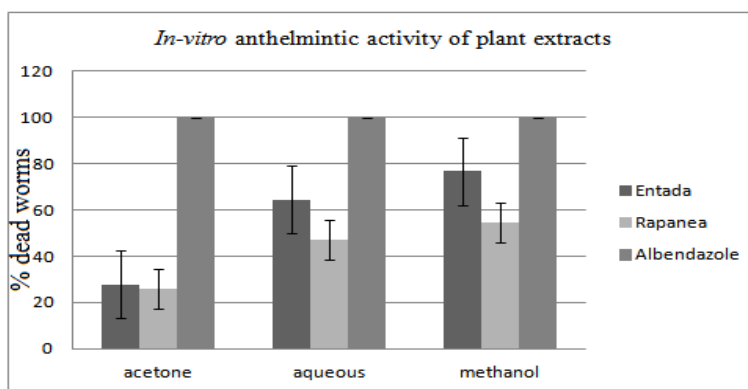


Figure 1. *In-vitro* anthelmintic activity of *E. leptostachya* and *R. Rhododendroides*

E. leptostachya and *R. rhododendroides* methanol extracts were more potent against *H. contortus* adult worms with mortalities of 77 and 54% respectively, when compared to other extracts. Hexane extracts were the least potent exhibiting mortalities of 21 and 13% for *E. leptostachya* and *R. rhododendroides* respectively. Aqueous extracts of both *E. leptostachya* and *R. rhododendroides* showed moderate potency against *H. contortus* adult worms. Acetone extracts were the second least potent, compared to other extracts. There were no significant differences ($p = 0.17$) in anthelmintic activity between acetone extracts of *E. leptostachya* and *R. rhododendroides*. However, aqueous, hexane and methanol extracts had significant differences ($p < 0.05$). The reference drug albendazole showed 100% mortality at a concentration of 0.55mg/ml while the negative control PBS showed no mortality. The high mortality values exhibited by the methanol extracts of *E. leptostachya* root bark and *R. rhododendroides* stem bark support their medicinal uses in the treatment of GI nematodes. Secondary metabolites of plant origin have been found to have both *in-vivo* and *in-vitro* anthelmintic activity^{12,13,14,15,16,17,18,19} against GI nematodes in a dose and time dependent manner. The exact mechanism of saponins action against GI nematodes is not very well known. But

Albendazole works by interference with the polymerization of microtubule²³. The drug binds to the protein tubulin of the *H. contortus* hence causing death by starvation²⁴. Tannins are reported to cause anthelmintic activity by binding to the proteins found in the gastro intestinal of the host or to glycoprotein on the cuticle of the parasite and may cause death²⁵.

4. Conclusions

The traditional use of *E. leptostachya* and *R. Rhododendroides* by herbalists of Mbeere County, Kenya, has been established in the present study. These plants have potential in drug development for GI nematodes affecting ruminants.

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References

- Dhar, D. N., Sharma, R. L. and Bansal, G. C. Gastrointestinal nematodes in sheep in Kashmir. *Veterinary Parasitology* 1982, 11: 271 - 277.
- Perry, B. D., Randolph, T. F., McDermott, J. J., Sones, K. R. and Thornton, P. K., Investing in animal health research to alleviate poverty. ILRI (International Livestock Research Institute), Nairobi, Kenya, 2002 p148.
- Allonby, E.W. and Urquhart, M. The epidemiology and the pathogenic significance of Haemonchosis in Merino Folk in Eastern Africa. *Veterinary Parasitology* 1975, 1: 1001 - 1007.
- Marley, C. L., Cook, R., Barrett, J., Kea tinge, R. and Lampkin, N. M. The effect of bird foot trefoil (*Lotus corniculatus*) and chicory (*Cichorium intybus*) on parasite intensities and performance of lambs naturally infected with Helminth parasites. *Vet. Parasitol.* 2003, 112, 147-155.
- Echevaria F A, Amour J, Borba M F, Duncal L. Response to ivermectin treatment of parasitic stages of *Haemonchus contortus* resistance or susceptible to ivermectin, *J. Parasitol.*, 2007, 78(5): 894-8
- Peter J Walker and Chandrawathani P. *Haemonchus contortus*: Parasite problem No.1 from Tropics-Polar Circle.. Problems and prospects for control based on epidemiology, tropical Biomedicine 2005, 22(2):131-137
- William J Blackhall. Genetic variation and multiple mechanisms of anthelmintic resistance to *H. Contortus*. PhD Thesis, 1998, McGill University, Quebec, Canada
- Oliveira L.M.B., C.M.L.Bevilaqua, C.T.C.Costa, I.T.F.Macedo, R.S. Barros, A.C.M.Rodrigues, A.L.F.Camurçã-Vasconcelos, S.M.Morais, Y.C.Lima, L.S.Vieira, A.M.C.Navarro. Anthelmintic activity of *Cocos nucifera* L. against sheep gastrointestinal nematodes, *Veterinary Parasitology* 2009, 159:55-59
- Zacharias F, J E Guimaraes, RR Araujo, M A O Almeida, M C C Ayres, M E Bavia and F W Mendonca-Lima. Use of homeopathic medicine, low cost, absence of residues in meat and milk and low environmental impact, *Homeopathy*, 2008, 97: 145-151
- Egualé, T. and Giday, M., *In vitro* anthelmintic activity of three medicinal plants against *Haemonchus contortus*. *International Journal of Green Pharmacy* 2009, 3(1):29 - 34
- Wahab A, R, R, Lee and S. F Sulaiman., *In-vivo* anthelmintic activity of Neem plant (*Azadirachta indica*) extract against third-stage *Haemonchus contortus* larvae from goats. *Global Veterinarian*, 2011, 7(1): 22-26.
- Githori, J. B. Athanasiadou, S. and Thamsborg, S. M., Use of plants in novel approaches in the control of gastrointestinal helminthes in livestock with emphasis on small ruminants. *Veterinary Parasitology*, 139: 308 - 320.
- Max, R. A., Kimambo, A. E., Kassaku. A. A., Mutenga, L. A and Buttery, P. J. Effects of tanniferous browse meals on nematode faecal egg counts and internal parasites burdens in sheep and goats. *S. Afr. J. Anim. Sci.* 2007, 37: 97 - 106.
- Jan, U., Lanislav, K., Iva, L and Jana, 2008. *In vitro* anthelmintic effect of medicinal plants used in Czech Republic, *Pharmaceut. Biol.*, 46: 808 - 813.
- Squires M Jill. The effects of naturally occurring plant products on experimental Heamonchus contortus infection in Gerbils and Sheep. MSc. Thesis, 2009, Blacksburg, VA
- Yadav P, A Kumar, V S Vihan and K Mahour. *In-vitro* adulticidal activity of various plant extracts against *H. contortus*, *Asian J. Exp. Biol. Sci.*, 2010, 1(4): 975-978
- Mehmood F, M. Qasim, Z Khan, N Iqbal, S Mehmood, M Lateef and P Shahzadi. *In-vitro* evaluation of anthelmintic activity of essential oils from different parts of *Skimmia laureola* (DC.) Zucc.Ex Walp., ver Nair, *Pak. J. Bot.*, 2011, 43(6): 2915-2918.
- Viola Maphosa, P J Masika. Anthelmintic screening of *Elephantorrhiza elephantina* root extract against *H. contortus*. *Tropical Animal Health and Production*, 2012, 44(1): 159-163
- Adamn Kahore, T Amadou, N Man, T H Hamidou, Belem AM. Gaston. Anthelmintic activity of *Daniellia oliveri* (Rolf) Hutch. and Daltz (Caesalpiniaceae) in sheep in Burkina Faso. *Journal of Research in Antimicrobials* 2012, 1: 049-055
- Edeoga, H. O., Okwu, D. E., Baebie, B. O. Phytochemical constituents of some Nigerian medicinal plants: *African Journal of Biotechnology*, 2005, 4(7):685-688.
- Rabel, B., McGregor, R., Douch, P. G. C., Improved bioassay for estimate of inhibitory effects of ovine gastrointestinal mucus and anthelmintic on Nematodes larval Migration. *Int. J. Parasitol.* 1994, 24:671- 676.
- Kareru P G, A N Gachanja, J M Keriko and G M Kenji. Screening of indigenous medicinal plants for anthelmintic activity. *Journal of Pharmacy and Pharmacology, Science Proceedings, Supplement 1, British Pharmaceutical Conference*, 2006.
- Harder, A. 2002. Chemotherapeutic approaches to nematodes: current knowledge and outlook. *Parasitology Research* 2006, 88: 272 - 277.
- Roos, M. H. The role of drugs in the control of parasitic nematode infections: must we do without? *Parasitology* 1997, 114: 137-1
- Kane S. R., Mohite, S. K., Rajarambapu, J. S. S. Anthelmintic activity of aqueous and methanol extracts of *Euphorbia tymifolia*. *International Journal of PharmTech Research*. 2009, 1 (3):666-669