CONTROL OF GASTRO-INTESTINAL NEMATODES IN RUMINANTS USING PLANT EXTRACTS

D. Kimani[1], P. G. Kareru[2], F. K. Njonge[3], H. L. Kutima[4], G. C. Nyagah[5], S. O. Rechab[6], R. W. Wamburu[7] and J. M. Karanja[8]

[1,2,5,6,7,8] Chemistry Department, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

[3]Land Resource and Animal Sciences Department, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

[4]Zoology Department, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

E-mail: davykym@yahoo.com

Abstract

Helminthes infestation has been recognised as a major constraint to livestock production. Increasing anthelmintic resistance and the impact of conventional anthelmintics on the environment has led to increased interest on ethnobotanical approach to come up with new novel compounds. The synthetic drugs are toxic, expensive and sometimes beyond reach of most rural small holder farmers and pastoralists. The economic losses due to helminthes infestation are enormous and unnecessary since they are preventable. The aim of this study was to test for *in vitro* anthelmintic activities of the test plants with the aim of formulating a novel herbal anthelmintic drug for ruminants. Egg hatch inhibition (EHI) tests were done to test for *in vitro* anthelminitic activities of ethanolic extracts of *Entada leptostachya*, *Albizia anthelmintica* and *Prosopis juliflora*. Graduated doses of between 0.5 and 6 mg/ml were prepared using distilled water as solvent. Fresh nematode eggs were harvested using simple salt floatation method and eggs of mixed nematode species of *Haemonchus spp.*,*Trichostrongyle spp.* and *Oesophagostomum spp.* were obtained. The *in vitro* anthelmintic activities of the plant extracts were compared to albendazole. *Entada leptostachya* inhibition was 91% and 100% and was comparable to albendazole which was 98% and 100% at 2 and 6 mg/ml respectively. *Albizia anthelmintica* showed 100% inhibition and was comparable to albendazole whose inhibition was 100% at 6mg/ml. *Prosopis juliflora* inhibition was 100% and 97% and was comparable to albendazole whose inhibition was 98% and 100% at 2 and 6mg/ml respectively. All the three test plants showed lower inhibitory anthelmintic activities at the lower concentrations compared to albendazole. The ethanolic extracts of the three plants have potential as novel anthelmintic drugs for gastro-intestinal nematodes.

Key words: Ethnobotanical, gastrointestinal nematode, anthelmintic, ruminants

1.0 Introduction

Helminthiasis is one of the most common setbacks in production and reproductive performance of livestock (Agaie and Onyeyili, 2007; Dawo and Tibo, 2005). In the developed world, with exception of countries in the southern hemisphere, the greatest impact is found in the costs of control, mostly in the case of helminth parasitoses (Githiori, 2004). Most of these effects go unnoticed because of sub-clinical or chronic nature of the diseases they cause unless the parasites cause death of the animal (Dawo and Tibo, 2005). Control of gastro-intestinal nematode (GIN) parasitism is usually based on the use of chemical anthelmintics, whose effectiveness and consistent use has been limited by high levels of anthelmintic resistance and high cost. The earliest documentation of anthelmintic resistance (AR) was to phenothiazine in 1957 followed by thiobendazole in 1964 (Fleming *et al.,* 2006). A recent survey carried out by FAO and the Office Internationale des Epizooties (OIE) in 77 out of 151 OIE member countries, revealed that over 50 per cent of countries are affected by parasite resistance (FAO, 2006). This has led to increasing popularity of herbal de-wormers for GIN control (Burke *et al.,* 2009). As a result, pastoralists and small holder farmers (SHFs) continue to use indigenous plants as livestock de-wormers drawn from centuries of traditional beliefs. These herbal preparations are much cheaper and readily available than synthetic drugs and have been used for a long time for treatment of livestock against helminth parasites (Githiori, 2004).

Entada leptostachya is found in several parts of Kenya (Machakos, Embu and Mbeere districts) and other parts of Africa such as Somalia, Ethiopia and Tanzania. The communities in Embu and Mbeere districts of Eastern Province, Kenya use the root bark decoction to treat worms in humans and animals (Kareru, 2008). The root bark of *Albizia anthelmintica* is used for intestinal worms. It is a common shrub in dry bushland and in Maasai-land. Saponins from its bark have been reported in South West Africa to be effective against intestinal worms (Githiori, 2004). *Prosopis juliflora* (locally known as 'Mathenge') was introduced in Kenya in the early 1970s (Ebenshade and Grainger 1980, Maghembe *et al.,* 1983) and is generally considered a noxious weed locally. This study, therefore, sorts to find out possible pharmacological application of the plant as a way of mitigating its negative attributes.

2.0 Materials and Methods

2.1 Collection and Preparation of Medicinal Plant Material

Entada leptostachya and *Albizia anthelmintica* root barks were collected from Embu and Mbeere areas of Eastern province, Kenya. *Prosopis juliflora* leaves were collected from Marigat, Baringo County. The plant specimen were identified in the field and authenticated by a plant taxonomist from the Botany department at Jomo Kenyatta University of Technology (J.K.U.A.T.) where voucher specimens were also deposited. The samples were sorted, cleaned and air dried on the laboratory benches away from direct sunlight before being ground into fine powder that were then separately stored in air-tight plastic bags for further use to avoid contact with moisture.

2.2 Preparation of Ethanolic Plant Extracts

Ethanol extracts were prepared by soaking 50g of each of the various plant powders in 500ml of distilled ethanol for 72 hours. The ethanol extracts were then filtered under vacuum using a Buchner funnel and concentrated under vacuum using a rotary evaporator at 40°C. The extracts were then stored at -4°C until needed for bioassay.

2.3 Screening of the Ethanolic Plant Extracts for *In Vitro* Anthelmintic Activities

Nematode egg recovery was done using simple floatation method (Hansen and Perry, 1994) with a few modifications. Goodwin's solution was prepared by dissolving 4.25g of NaCl and 0.5g of glucose in 50ml of distilled water and this was used as egg diluent and physiological solution. The egg solution was adjusted to 16 eggs per 5µL of egg solution using Goodwin's solution. The eggs were quantified by counting and averaging the number of eggs present in 5µL of egg solution. Five microliters (5µL) of egg solution each was placed on three slides and coverslips placed on them and counting done under a microscope (X10). The egg hatch inhibition assay was done following a method by Thoithi *et al.* (2002) with slight modifications. Five microliters (5µL) of the egg solution was measured into each well of a 96-well microtitre plate. Hundred microliters (100µL) of the plant extracts at the various graduated concentrations (0.5 to 6mg/ml) were then added. Distilled water and 0.5% DMSO was used as negative control. The activities of the plant extracts were compared to albendazole at the same concentration range. The experiment was done in triplicate. The hatched eggs were then counted in each of the wells under a microscope (X10) after 48 hours.

3.0 Results and Discussion

3.1 Extraction Yield

The ethanolic extract yields for the three plants are shown in Figure 1. *Entada leptostachya* gave the highest crude extract yield (11.39%) while *Albizia anthelmintica* gave the lowest yield (5.24%). This could possibly be as a result of *Entada leptostachya* having more phytochemical principles whose polarity corresponds to that of ethanol.

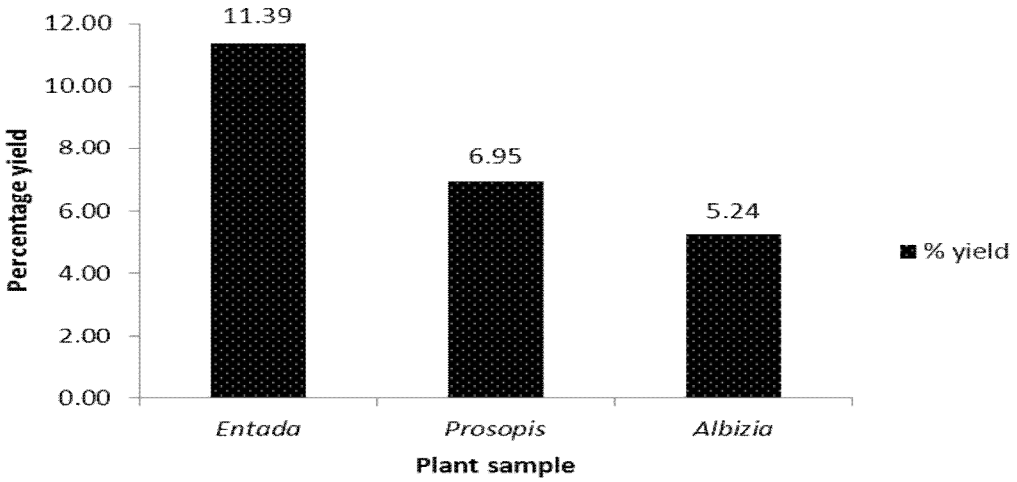

Figure 1: Percentage yields of ethanol extracts of Entada leptostachya, Prosopis juliflora and Albizia anthelmintica

3.2 Results for Egg-Hatch Inhibition (EHI) Assay

Mixed nematode eggs containing *Haemonchus spp., Trichostrongyle spp.* and *Oesophagostomum spp.* were used for EHI assay. The plant extracts showed concentration-dependent anthelmintic activities in inhibiting egg hatching as shown in Figure 2. From Table 1, of the three plants, *Prosopis juliflora* showed the highest anthelmintic activity (LC_{50}=0.251 mg/ml) while *Albizia anthelmintica* showed the least activity (LC_{50}=0.330 mg/ml). There were no statistically significant differences (P>0.05) in the activities of the plant extracts compared to albendazole (LC_{50}=0.245 mg/ml).

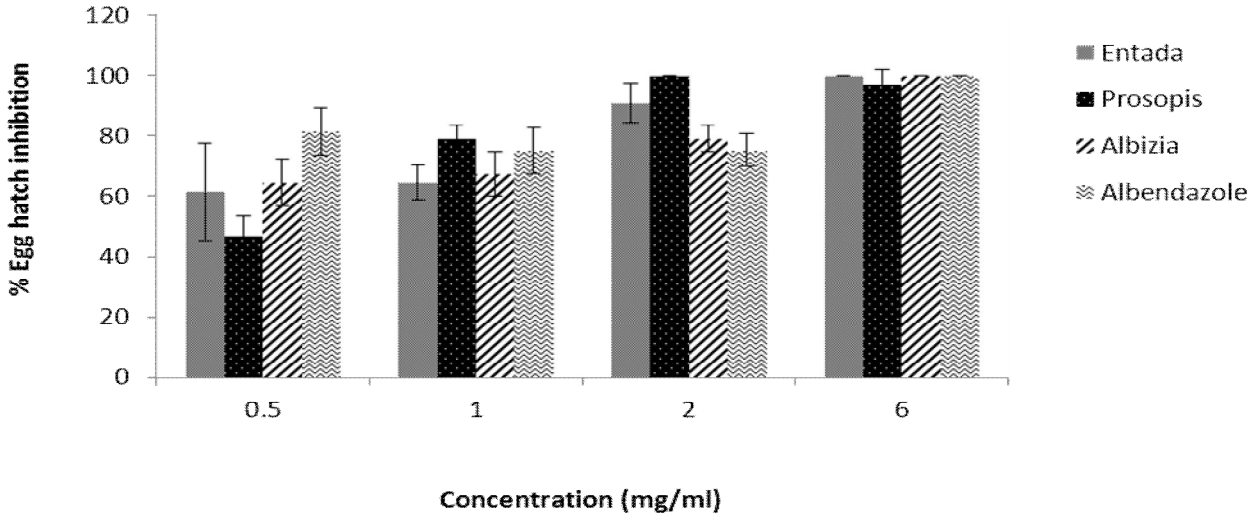

Figure 2: In vitro egg-hatch inhibition assay of Entada leptostachya, Prosopis juliflora and Albizia anthelmintica at the concentrations of 0.5, 1, 2 and 6mg/ml

Table 1: LC_{50} values of Entada leptostachya, Prosopis juliflora and Albizia anthelmintica at 95% confidence limit with the upper and lower boundaries

Plants and control	95% confidence limits for concentration (mg/ml)		
	LC_{50}	Lower boundary	Upper boundary
Entada leptostachya	0.317	0.168	0.498
Prosopis juliflora	0.251	0.127	0.401
Albizia anthelmintica	0.330	0.178	0.509
Albendazole	0.245	0.119	0.400

There was also high positive correlation of the activities of the extracts when compared to albendazole as shown in Table 2. This shows that the anthelmintic activities of the test plants compared very well with albendazole.

Table 2: Paired samples correlations (Plant extracts vs Albendazole) of Entada leptostachya, Prosopis juliflora and Albizia anthelmintica

Paired samples	Correlation, R^2
Entada leptostachya and Albendazole	0.967
Prosopis juliflora and Albendazole	0.979
Albizia anthelmintica and Albendazole	0.963

Other similar work on numerous herbal plants has been reported. *In vitro* anthelmintic activity study on *Terminalia arjuna* bark showed a dose dependent anthelmintic activity against *Haemonchus contortus* ova with an LC_{50} value of 0.646 mg/ml (Zafar *et al.,* 2009). A similar trend was observed with the *in vitro* anthelmintic activity of ethanolic plant extracts from Northern Cameroon on inhibition of *Haemonchus contortus* eggs (Monglo *et al.,* 2006).

The anthelmintic activities of the plants under investigation can be attributed to the presence of phytochemicals antagonistic toward gastro-intestinal nematodes. Some of the phytochemicals known to be active against gastro-intestinal nematodes include polythienyls, isothiocyanates, glucosinolates, cyanogenic glycosides, polyacetylenes, alkaloids, lipids, terpenoids, sesquiterpenoids, diterpenoids, quassinoids, triterpenoids, simple and complex phenolics (Chitwood, 2002), thymol (Lateef *et al.,* 2006), steroids (Badmanaban and Patel, 2010), saponins (Kareru, 2008), tannins (FAO, 2006; Githiori, 2004; Ramalingam *et al.,* 2010) amongst other classes of phytochemicals. Saponins were first reported to kill worms as early as 1962 by Watt and Brayer, using an extract from *Albizia anthelmintica* (Kareru, 2008). Sesquiterpene and kosotoxins have also been isolated from the bark and root bark extracts of *Albizia anthelmintica* (Hussain, 2008). Kareru (2008) reported the presence of triterpenes, tannins, saponins and glycosides in *Entada leptostachya* while triterpenes, saponins, tannins and anthraquinones (bound) were found in *Albizia anthelmintica*. Phytochemical studies have revealed presence of saponins, tannins, flavonoids and alkaloids in *Prosopis juliflora*. These phytochemicals could have been responsible for their anthelmitic activities.

4.0 Conclusion

Egg hatch inhibition assay has shown that the ethanol extracts of *Entada leptostachya*, *Albizia anthelmintica* and *Prosopis juliflora* exhibit *in vitro* anthelmintic activity that is sufficiently comparable to albendazole and therefore have potential as novel anthelmintic drugs for control of gastro-intestinal nematodes in ruminants.

Acknowledgement

The authors thank Research Production and Extension Division of Jomo Kenyatta University of Agriculture and Technology for funding this project. The laboratory technicians at Zoology department (Evelyne, Eunice, Mary, Kierugara, Samuel, Christine and Wasike) are also acknowledged for helminth egg identification and dedicated facilitation during the egg-hatch inhibition assay.

References

Agaie, B. M. and Onyeyili, P. A. (2007). Anthelmintic activity of the crude aqueous leaf extracts of *Anogeissus leiocarpus* in sheep, *African Journal of Biotechnology,* **6**: pp 1511-1515.

Badmanaban, R. and Patel, C. N. (2010). Studies on anthelmintic and antimicrobial activity of the leaf extracts of *Lagenaria siceraria Mol, Journal of Global Pharma Technology,* **2**: pp 66-70.

Burke, J. M., Wells, A., Casey, P. and Kaplan, R. M. (2009). Herbal dewormer fails to control gastrointestinal nematodes in goats, *Veterinary Parasitology,* **160**: pp 168-70.

Chitwood, D. J. (2002). Phytochemical based strategies for nematode control, *Annual Review of Phytopathology,* **40**: pp 221-249.

Dawo, F. and Tibbo, M. (2005). Anthelmintic effect of *Halothamus somalensis* in Arsi-Bale goats, *Livestock Research for Rural Development,* **17**.

Ebenshade, H.W. and Grainger, A. (1980). The Bamburi reclamation project, *International Tree Crops Journal:* **1**: pp 199-202.

Food and Agriculture Organization of the United Nations (FAO). Naturally occurring plant tannins as a means of controlling intestinal nematode infections in ruminants, http//:www.fao.org/teca/content/naturally-occurring-plant-tannins-means-controlling-intestinal-nematode-inections-ruminants. Accessed on March 11, 2011.

Fleming, S. A., Craig, T., Kaplan, R. M., Miller, J. E., Navarre, C. and Rings, M. (2006). Anthelmintic resistance of gastrointestinal parasites in small ruminants, *Journal of Veterinary Internal Medicine,* **20:** pp 435-444.

Githiori, J. B. (2004). *Evaluation of anthelmintic properties of ethnoveterinary plant preparations used as livestock dewormers by pastrolists and smallholder farmers in Kenya,* Ph.D. Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Hansen, J. and Perry, B. (1994). The epidemiology, diagnosis and control of helminth parasites of ruminants, pp. 62-63. International Laboratory for research on Animal Diseases (ILRAD), Nairobi, Kenya.

Hussain, A. (2008). *Evaluation of antelmintic acitivity of some ethnobotanicals,* PhD thesis in parasitology, University of Agriculture, Faisalabad, Pakistan.

Kareru, P. G. (2008). *Ethnomedicine practices, analysis and standardization of a herbal anthelmintic drug used by the Embu and Mbeere peoples of Kenya,* PhD thesis, Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya.

Lateef, M., Iqbal, M., Rauf, U. and Jabbar, A. (2006). Anthelmintic activity of *Carum capticum* seeds against gastro-intestinal nematodes of sheep, *J. Anim. Pl. Sci.,* **16**: pp 35-36.

Maghembe, J. A., Kariuki, E. M. and Haller, R. D. (1983). Biomass and nutrient accumulation in young *Prosopis juliflora* at Mombasa, Kenya, *Agroforestry Systems*: **1**: pp 313-321.

Monglo, D., Njongmeta, L. M., Musongong, G., Ngassoum, M. and Nukenine, E. N. (2006). Evaluation of anthelmintic potential of ethanolic plant extracts from Northern Cameroon against eggs and infective larvae of *Haemonchus contortus*, *J. Boil. Sci.*, **6**: pp 426-433.

Ramalingam, R., Bindu, K. H., Madhavi, B. B., Nath, A. R. and Banji, D. (2010). Phytochemical and anthelmintic evaluation of corm of *Amorphophallus campanulatus*, *International Journal of Pharma and Bio Sciences:* **1**: pp 3-8.

Thoithi, G. N., Maingi, N., Karume, D., Gathumbi, P. K., Mwangi, J. W. and Kibwage, I. O. (2002). Anthelmintic and other pharmacological activities of the root bark extracts of *Albizia anthelmintica* Brongn, *East and Central African Journal of Pharmaceutical Sciences,* **5**: pp 60-66.

Zafar, I., Allah, H. B., Nisar, M. K., Jabbar, A., Hassan, A. G., Ud, I. D. (2009). *In vitro* and *in vivo* activity of Terminalia *arjuna* bark, *International Journal of Agriculture and Biology,* **11**: pp 273-278.