IOSR Journal of Polymer and Textile Engineering (IOSR-JPTE)
e-ISSN: 2348-019X, p-ISSN: 2348-0181, Volume 2, Issue 1 (Jan - Feb. 2015), PP 06-10
www.iosrjournals.org

In-vivo anthelmintic evaluation of a processed herbal drug from Entada leptostachya (Harms) and Prosopis juliflora (Sw.) (DC) against gastrointestinal nematodes in sheep

Jackson Mutembei[1], Patrick Kareru[1], Francis Njonge[2], Githira Peter[1], Hellen Kutima[3], Joseph Karanja[1], David Kimani[1]
[1]*Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology, Kenya*
[2]*Department of Land Resource, Planning and Management, Jomo Kenyatta University of Agriculture and Technology, Kenya*
[3]*Department of Zoology, Jomo Kenyatta University of Agriculture and Technology, Kenya*

Abstract: *In vivo anthelmintic activity of a herbal drug processed through spray-drying from Prosopis juliflora (Sw.) DC) and Entada leptostachya (Harms) against gastrointestinal nematodes of sheep is described and reported for the first time in this work. Maximum percent egg count reduction (%ECR) of 80.95% in egg per gram of faeces (EPG) on day 11 post-treatment (PT) was recorded in sheep treated with a dose of 250 mg/kg b.w. of the herbal drug mixture. This was followed by a reduction ($P \leq 0.05$) of the same dose treatment (59.80%) on day 8 PT. The positive control exhibited the highest egg count reduction ($P < 0.01$) with an EPG of 88.31% on day 14 PT ($P \leq 0.05$). It was found that the herbal drug had a comparable anthelmintic activity with the conventional anthelmintic drug, Nilzan (1.5% Levamisole HCl). Although the herbal drug had a far much higher concentration than 1.5% Levamisole HCl (5.0 mg/Kg b.w.), the difference in activity could be attributed to high purity of Levamisole. Phytochemical analysis of the herbal drug revealed the presence of alkaloids, steroids, phenolic compounds and tannins, flavonoids and saponins. It was concluded that the processed herbal drug mixture had appreciable anthelmintic activity and could therefore be used as an alternative dewormer in livestock.*

Keywords: *Anthelmintic resistance, gastrointestinal nematodes, herbal drug processing, phytochemicals*

I. Introduction

Helminth control in domestic animals is widely based on the use of synthetics anthelmintics [1, 2]. However, the current efficacy of these drugs has been reduced because of development of resistant nematode strains [3, 4]. Furthermore, the high cost of these drugs, chemical residual concern in animal products and environmental pollution have awaken interest in medicinal plants as an alternative source of anthelmintic drugs [5].

Animal diseases are a major constraint to livestock production in Kenya [6, 7]. Prevention and control of animal diseases therefore have been of critical concern in Kenya like in other African countries. Natural plant-derived products have been known for many decades to possess anthelmintic properties and yet generally these have been inadequately researched and none have been taken to the market stage. Cysteine proteinases found in fruits such as papaya (*Carica papaya*), figs (*Ficus* spp.) and pineapple (*Ananas comosus*) for example have been demonstrated to damage intestinal nematodes of rodents targeting the cuticle which at first blisters, and then is disrupted and weakened sufficiently to enable the internal hydrostatic pressure to rupture the body wall and result in the disintegration of the worms [8].

Use of plant extracts has advantages that make them attractive for use in developing countries such as low cost, access to large amounts of raw material and easy integration into traditional cultural practice. However, scientific validation of these traditional treatments lacks [9]. Most studies that have investigated the anthelmintic potential of traditional medicinal plants focuses on crude extracts from a limited selection of plants, with only a summary analysis of the chemical constituents, and no further investigation of the active compounds [10]. Thus, there is a need for more systematic studies to identify and validate the use of plants as anthelmintics.

Entada leptostachya is a climbing shrub or tree. It is widely distributed in arid and semi-arid areas of Mbeere, Embu, Kamba and Tharaka communities of Kenya. Its roots used together with the roots of *Hamsonia abyssinica* result into a mixture which is used as a treatment against tuberculosis and for relief from chest pains [11, 12]. Powder made from its roots extracts exhibit high activities of anthelmintic properties. *In-vitro* anthelmintic evaluation of methanolic extract of *E. lepostachya* was found to be effective against *Haemonchus contortus* adult worms [13].

Prosopis juliflora (Sw.) DC on the other hand is an evergreen tree with a large crown and an open canopy, growing to a height of 5-10 m. The plant is native to South America, Central America and the

Caribbean [14 - 16]. It is fast growing, nitrogen-fixing and tolerant to arid conditions and saline soils [17]. *P. juliflora* was introduced in Kenya in the early 1970s in order to remedy environmental problems, improve biomass cover and rehabilitate degraded soils [18, 19], but in later years, it spread rapidly and colonized agricultural lands and pastures [20].

The purpose of this study was to evaluate the anthelmintic activity of a herbal drug processed through spray-drying from *Entada leptostachya* (Harms) and *Prosopis juliflora* (Sw.) DC). The spray-dried powders are stable compared to water decoctions that the local communities use to administer herbal drugs. On the other hand, the anthelmintic drug of plant origin are safer and affordable to resource limited small scale livestock keepers, and thus there is the need to scientifically validate their use through research.

II. Materials And Methods

2.1 Sample collection and preparation

The leaves of *Prosopis juliflora* were collected from Marigat District in Baringo County, while the roots of *Entada leptostachya* were collected from Mbeere District in Embu County. The plant materials from both plants were then dried at a room temperature (20–25°C) in the laboratory benches after which they were pulverised into fine powders.

2.2 Extraction and processing of the herbal drug

A weighed amount of the ground powders of *P. juliflora* and *E. leptostachya* was subjected into aqueous extraction process. The resultant aqueous extracts were concentrated by evaporation of excess solvent (water) and then spray-dried in a spray-drier into a powder. The resultant powders of *P. juliflora* and *E. leptostachya* were then blended in various ratios into a herbal drug and kept at 4°C until further use. The latter powders will hereafter in the text be referred to as "processed" herbal drug

2.3 *In-vivo* bioassay

A total of 20 sheep of both sexes (female and male) aged less than a year and weighing 18–24 kg were used for *in-vivo* trials. Before the start of the experiment, the animals were confirmed to be infected with mixed species of gastrointestinal nematodes (*Strongyle, Nematodirus battus, Haemonchus contortus, Nematodirus filicollis* and *Strongyloides papillosus*). The animals were kept in paddocks for three weeks prior to the experiment for acclimatization while being fed with fresh grass, mineral salt and water *ad libitum*. The sheep (n = 20) were randomly divided into 4 groups of 5 animals each and treated with various concentrations of the processed herbal drug. Group 1 acted as the positive control and was treated with 5.0mg/Kg body weight (b.w.) of Nilzan (1.5% Levamisole) while group 2 was the negative control and received no treatment. Group 3 and 4 were treated with 250mg/Kg and 500mg/Kg b.w. respectively of the herbal drug. Faecal egg count reduction (FECR) was monitored using McMaster technique for fourteen days by counting the nematodes eggs in faeces collected from the animals directly from the rectum. Fecal samples of each group were collected in the morning, starting from day 0 pre-treatment and at day 2, 4, 6, 8, 11 and 14 post-treatment (PT) and were evaluated for the presence of nematode eggs by salt floatation technique. Faecal egg counts were presented as mean ± standard deviation. The eggs were counted by the McMaster technique and percent egg count reduction (%ECR) was calculated. The data were statistically analyzed using SPSS.

2.4 Phytochemical screening of the herbal drug

Qualitative phytochemical analysis of the processed herbal drug from *P. juliflora* and *E. leptostachya* was carried out according to established standard methods [21 - 24].

These tests are usually based on visual observation of color or precipitate formation after addition of specific reagents. One gramme of the herbal drug was re-dissolved in 10mL of distilled water and subjected into qualitative phytochemical tests for saponins, alkaloids, phenolic compounds and tannins, flavonoids, phytosterols and glycosides.

III. Results And Discussion

Anthelmintic activities of various botanical anthelmintics have been reported for example *Vernonia anthelmintica* seeds [25], *Artemisia brevifolia* [26], *Calotropis procera* [27], *Nicotiana tabacum* [28] and *Cocos nucifera* L. [29]. The anthelmintic property of these medicinal plants is associated with bioactive secondary metabolites [30]. Phytochemical analysis of the herbal drug (Table 1) revealed the presence of alkaloids, steroids, phenolic compounds and tannins, flavonoids and saponins. Tannins are polyphenolic compounds derived from plant secondary metabolism [29]. Several experiments have demonstrated their anthelmintic activity [31, 32]. Thus, the presence of tannins in the processed herbal drug in this study could be attributable to its anthelmintic activity. 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 death [13, 31].

Triterpenoid saponins, alkaloids and phenolic acids have been purported to be responsible for the anthelmintic activity of *Chenopodium album* [2]. Even though glycosides were found to be present in both *P. juliflora* and *E. leptostachya* from which the herbal drug was processed, they were absent in the drug. This is probably due to their presence in the individual plants in low concentration. *In-vivo* anthelmintic activity of *Coriandrum sativum* is considered to be as a result of classes of secondary metabolites of alkaloids and flavonoids [33]. Anthelmintic activity of *Vernonia anthelmintica* has been attributed to its sesquiterpene lactones vernodalin, vernolide, and hydroxyvernolide, and steroid-related constituents [25]. Bioactive plants may contain large numbers of plant secondary metabolites that may act singly or in combination to produce direct [31] and /or indirect effects on parasites in the alimentary tract, leading to reduced nematode survival, growth and fecundity. Plant secondary metabolites are increasingly recognized as important in the health, welfare and nutrition of animals as well as in people [34].

Table 1: Phytochemical profile of the processed herbal drug, Prosopis juliflora and Entada leptostachya

Phytochemical	Test	Herbal drug	P. juliflora	E. leptostachya
Alkaloids	(a.) Mayer's	+	+++	-
	(b.) Wagner's	+	+++	-
	(c.) Dragendorff's	+	+++	-
Glycosides	(a.) Borntrager's	-	-	-
	(b.) Keller-Kilian	-	+	+
Steroidal nucleus	(a.) Lieberman's	+	+	+
	(b.) Salkowski's	+	+	+
Phenolics & tannins	(a.) Ferric chloride	++	++	+
	(b.) Lead acetate	++	++	-
	(c.) Alkaline reagent	++	++	+
	(d.) Mg & HCl reduction	+	++	+
Flavonoids	(a.) Alkaline reagent	++	++	+
Phytosterols	(a.) Salkowski's	+	+	+
	(b.) Liebermann-Burchard	+	+	+
Saponins	(a.) Foam	+++	++	+++

Key: '**-**' Not present, '**+**' Present, '**++**' Present in high concentration, '**+++**' Present in the highest concentration

The *in-vivo* test results (Table 2) revealed time dependent activity with a maximum reduction (80.95%) in egg per gram of faeces (EPG) on day 11 post-treatment (PT) in sheep treated with a dose of 250 mg/kg b.w. of the herbal drug. This was followed by a reduction ($P \leq 0.05$) of the same treatment (59.80%) on day 8 PT. The positive control exhibited the highest egg count reduction (P□ 0.01) with an EPG of 88.31% on day 14 PT. A significant anthelmintic activity was observed on day four, and this can be attributed to the serum concentration of the drug being at the peak on this day. There were no physical clinical signs of toxicity (for example salivation, diarrhoea, skin reaction, and so on) throughout the study period in all the groups of sheep treated with the herbal drug.

Table 2: Effects of a processed herbal drug administration on eggs per gram (EPG) of faeces in sheep infected with mixed species of gastrointestinal nematodes.

Day PT*	Control group		Experimental group	
	Untreated	**Levam. 5mg/kg b.w.**	**250mg/kg b.w.**	**500mg/kg b.w.**
Day 0	2710±642.53 (0%)	3080±1597.47 (0%)	1020±411.58 (0%)	2760±987.72 (0%)
Day 2	3290±636.47 (-21.40%)	2210±962.34 (9.52%)	2040±988.61 (-28.43%)	3720±1036.41 (5.23%)
Day 4	1720±540.97 (-11.44%)	660±224.94 (78.57%)	1270±414.00 (0.14%)	3460±1299.65 (41.00%)
Day 6	1860±410 (-16.61%)	460±199.62 (85.00%)	610±168.37 (40.20%)	2070±823.50 (25.00%)
Day 8	1820±231.08 (-26.20%)	423.4±155.33 (86.25%)	410±111.13 (59.80%)	1450±606.42 (47.46%)
Day 11	3330±1101.09 (-22.88%)	440±84.26 (87.71%)	1710±2179.99 (80.95%)	1820±743.40 (34.06%)
Day 14	3320±1159.81 (-22.51%)	360±62.05 (88.31%)	1950±740.27 (-34.20%)	4370±1830.55 (9.41%)

PT* = Post treatment; Levam.= Levamisole; b.w. = body weight.
Figures in parenthesis against ± show % reduction with respect to day 0.

The inconsistency in activity manifested by increase in egg load in some days (days 2 and 14 PT at a dose of 250mg/Kg b.w. experimental group) contrary to the expected decrease after treatment can be attributed to the mode of action of the various phytoconstituents present in the herbal preparations against the mixed species of gastrointestinal nematodes in the sheep. Some classes of nematodes might be prone to particular group of phytochemicals and thus inhibit egg production, while those that are not affected continue to lay eggs thereby causing an increase in egg load. Unlike synthetic anthelmintic drugs that are pure, the synergistic effect of plant compounds present in various plant extracts may be active against the parasitic nematodes thus decreasing egg production. Other plants have been reported to have anthelmintic activity. Crude methanol extract (1g/kg) of *Nicotiana tabacum* L. leaves for example has been reported to exhibit a maximum anthelmintic activity of 73.6% reduction in EPG on day 5 of post-treatment at a dose of 3g/kg b.w. This was associated with nicotine, an alkaloid present in the leaves of *Nicotiana tabacum* L. Nematode muscles contain excitatory neuromuscular junctions containing ganglion-type nicotinic receptors with acetylcholine as their neurotransmitter. Any ganglion stimulant would tend to activate these neuromuscular junctions causing a spastic paralysis in the worms leading to their death and expulsion from the host [28]. Crude aqueous extract of *Vernonia anthelmintica* seed at 3g/kg b.w. on the other hand was found to exhibit a maximum percent egg count reduction of 73.9% on day 5 PT [25].

IV. Conclusion

The processed herbal drug had an anthelmintic activity that was comparable with the conventional anthelmintic drug levamisole, and can thus be used by the resource limited farmers to deworm their livestock since the plants are readily available locally and easy to process. In addition, spray-dried powders are advantageous than water decoctions that farmers use in that they are stable and can last longer. The anthelmintic activity of the herbal drug may be associated with the various phytochemicals present in the hebal drug such as tannins and phenolics compounds, alkaloids and saponins. However, further studies on pharmacokinetics and pharmacodynamics of the herbal drug to establish its metabolism need to be caried out. Repeated dose investigation also need to be carried out to establish the time the drug is most effective. The main limitations experienced in the course of the experiment was breakdown of the spray-drier mortar which caused delay in the processing of the drug. Moreso, during the acclimatisation period of the animals, mating occurred and this led to the pregnancy of the female sheep and this delayed the experiment since they could not be used while expectant.

Acknowledgments

This research was funded by the research, production and extension (RPE) division of Jomo Kenyatta University of Agriculture and Technology (JKUAT) and the The National Commision of Science, Technology and Innovation (NACOSTI) of Kenya.

References

[1]. C. T. C. Costa, C. M. L. Bevilaqua, M. V. Maciel, A. L. F. Camurca-Vasconcelos, S. M. Morais, M.V. B. Monteiro, V. M.Farias, M. V. da Silva, and M. M. C. Souza, Anthelmintic activity of Azadirachta indica A. Juss against sheep gastrointestinal nematodes, Veterinary Parasitology, 137, 2006, 306–310.

[2]. A. Jabbar, A. M. Zamana, Z.,Iqbal, M. Yaseen, and A. Shamima, Anthelmintic activity of Chenopodium album (L.) and Caesalpinia crista (L.) against trichostrongylid nematodes of sheep, Journal of Ethno pharmacology 114, 2007, 86–91.

[3]. R. Artho, M. Schnyder, L. Kohler, P. R. Torgerson, and H. Hertzberg, Avermectin resistance in gastrointestinal nematodes of Boer goats and Dorper sheep in Switzerland, Veterinary Parasitology 144, 2007, 64–73.

[4]. M. J. Behnke, J. D. Buttle, G. Stepek, A.Lowe, and R. I. Duce, Developing novel anthelmintics from plant cysteine proteinases, Parasites and Vectors, 1, 2008, 29.

[5]. E. S. Bizimenyera, J. B. Githiori, J. N. Eloff, and G. E. Swan, In vitro activity of *Peltophorum africanum* Sond (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode Trichostrongylus colubriformis, Veterinary Parasitolology, 142, 2006, 336–343.

[6]. J. B. Githiori, Evaluation of anthelmintic properties of ethno-veterinary plants preparations used as livestock dewormers by pastoralists and small holder farmers in Kenya, doctoral diss.,Thesis, Swedish University of Agriculture Science, Uppsala, Sweden, 2004.

[7]. N. G. Njoroge, and W. R. Bussmann, Herbal usage and informant consensus in ethnoveterinary management of cattle diseases among the Kikuyus (Central Kenya), Journal of Ethnopharmacology, 2006, 4271.

[8]. D. J. Buttle, J. M. Behnke, Y. Bartley, H. M. Elsheikha, D. J. Bartley, M. C. Garnett, A. A. Donnan, F. Jackson, A. Lowe, I. R. Duce, Oral dosing with papaya latex is an effective anthelmintic treatment for sheep infected with Haemonchus contortus, Parasites & Vectors, 4, 2011**,** 36.

[9]. R. Williams, A. C. Fryganas, A. Ramsay, I. Mueller-Harvey, S. M. Thamsborg, Direct anthelmintic effects of condensed tannins from diverse plant sources against Ascaris suum, PLoS ONE, 9 (5), 2014. Pp.1-16.

[10]. J. B. Githiori, J. Hoglund, and P. J. Waller, Ethnoveterinary plant preparations as livestock dewormers: practices, popular beliefs, pitfalls and prospects for the future, Animal Health Research Reviews, 6, 2005, 91–103.

[11]. J. O. Kokwaro, Medicinal Plants of East Africa (3rd Edition, Nairobi; University of Nairobi Press, 2009).

[12]. P. G. Kareru, Ethnomedicine practices, analysis and standardization of herbal anthelmintic drugs used by Embu and Mbeere peoples of Kenya, doctoral Theses, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya, 2008.

[13]. O. Ombasa, P. G. Kareru, G. Rukunga, J. Mbaria, J. M. Keriko, F. K. Njonge, and B. O. Owuor, In vitro antihelmintic effects of two Kenyan plant extracts against Heamonchus contortus adult worms, International Journal of Pharmacological Research 2 (3), 2012.

[14]. D. Admasu, Invasive Plants and Food Security: the case of Prosopis juliflora in the Afar region of Ethiopia, prepared by (FARM-Africa) for the International Union for Conservation of Nature, IUCN, 2008.

[15]. E. Mwangi and B. Swallow, Invasion of prosopis juliflora and local livelihoods: Case study from the Lake Baringo area of Kenya, Working Paper – no. 3. Nairobi: World Agroforestry Centre, 2005.

[16]. N. M. Pasiecznik, P. Felker, P.J.C. Harris, L.N. Harsh, G. Cruz, J.C. Tewari, K. Cadoret, and L.J. Maldonado, The Prosopis juliflora-Prosopis pallid Complex: A Monograph. HDRA Coventry, UK, 2001.
[17]. Andersson, Spread of the introduced tree species Prosopis juliflora (Sw.) DC in the Lake Baringo area, Kenya, World Agroforestry Center (ICRAF), 2005.
[18]. N. M. Oduor, and J. K. Githiomi, Fuel-wood energy properties of Prosopis juliflora and Prosopis pallid grown in Baringo District, Kenya, African Journal of Agricultural Research 8(21), 2013, 2476-2481.
[19]. J. A. Maghembe, E. M. Kariuki, and R. D. Haller, Biomass and nutrient accumulation in young Prosopis juliflora at Mombasa, Kenya. Agro forestry Systems 1, 1983, 313-321.
[20]. S. K. Choge, N. M. Pasiecnik, M. Harvey, J. Wright, S. Z. Awan, and P. J. C. Harris, Prosopis pods as human food, with special reference to Kenya. Waters SA 33 (3), 2007, 419-424.
[21]. J. B. Harborne, Phytochemical methods, a guide to modern techniques of plant analysis (2^{nd} Edition, Chapman and Hall, London New York, 1984).
[22]. M. Kavit, B. N. Patel, and B. K. Jain, Phytochemical analysis of leaf extract of Phyllanthus fraternus, Research Journal of Recent Sciences, 2**,** 2013, 12-15.
[23]. A. Wadood, M. Ghufran, S. B. Jamal, M. Naeem, A. Khan, R. Ghaffar, and Asnad, Phytochemical analysis of medicinal plants occurring in local area of Mardan, Biochemistry and Analytical Biochemistry, 2 , 2013, 4.
[24]. R. N. S. Yadav, and M. Agarwala, Phytochemical analysis of some medicinal plants, Journal of Phytology, 3, 12, 2011, 10-14.
[25]. Z. Iqbal, M. Lateef, A. Jabbar, M. S. Akhtar, and M. N. Khan, Anthelmintic Activity of Vernonia anthelmintica Seeds against Trichostrongylid Nematodes of Sheep, Pharmaceutical Biology, 44, (8), 2006, 563-567.
[26]. Z. Iqbal, M. Lateef, M. Ashraf, and A. Jabbar, Anthelmintic activity of Artemisia brevifolia in sheep, Journal of Ethnopharmacology 93, 2004, 265-268.
[27]. Z. Iqbal, M. Lateef, A. Jabbar, G. Muhammad, and M. N. Khana, Anthelmintic activity of Calotropis procera (Ait.) Ait. F. flowers in sheep, Journal of Ethnopharmacology 102, 2005, 256-261.
[28]. Z. Iqbal, M. Lateef, A. Jabbar, M. N. Ghayur, and A. H. Gilani, In vitro and In vivo Anthelmintic Activity of Nicotiana tabacum L. Leaves against Gastrointestinal Nematodes of Sheep, Phytotherapy Research 20, 2006, 46–48.
[29]. L.M.B. Oliveira, C. M. L. Bevilaqua, C. T. C. Costa, I. T. F. Macedo, R. S. Barros, A. C. M Rodrigues, A. L. F. Camurca-Vasconcelos, S. M. Morais, Y. C. Lima, L. S. Vieira, and A. M. C. Navarro, Anthelmintic activity of Cocos nucifera L. against sheep gastrointestinal Nematodes, Veterinary Parasitology 159, 2009, 55–59.
[30]. H. Hoste, F. Jackson, S. Athanasiadou, M. S. Thamsborg, and S. O. Hoskin, The effects of tannin-rich plants on parasitic nematodes in ruminants, Trends in Parasitology 22 (6), 2006.
[31]. S. Athanasiadou, I. Kyrazakis, F. Jackson, and R. L. Coop, Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: In-vitro and in-vivo studies, Veterinary Parasitolology, 99, 2001, 205-219.
[32]. V. Paolin, J.P. Bergeaud, C. Grisez, F. Prevot, P. H. Dorches and H. Hoste, Effects of condensed tannins on goats experimentally infected with Haemonchus contortus, Veterinary Parasitology, 113(3-4), 2003a, 253-261.
[33]. T. Eguale, G. Tilahun, A. Debella, A. Feleke, and E. Makonnen, In vitro and in vivo anthelmintic activity of crude extracts of Coriandrum sativum against Haemonchus contortus, Journal of Ethnopharmacology 110, 2007, 428–433.
[34]. J. J. Villalba, F. D. Provenza, A. K. Clemensen, R. Larsen, and J. Juhnke, Preference for diverse pastures by sheep in response to intraruminal administrations of tannins, Saponins and alkaloids, Grass and Forage Science 66, 2010, 224-236.