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Anthelmintic activity of preparations derived from *Myrsine africana* and *Rapanea melanophloeos* against the nematode parasite, *Haemonchus contortus*, of sheep

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Abstract

Myrsine africana L. and *Rapanea melanophloeos* L. belong to the plant family Myrsinaceae. Various rural communities in Kenya, such as smallholder farmers and pastoralists, use them to treat their livestock. The anthelmintic effects/activities of leaves and fruits of *M. africana* and fruits of *R. melanophloeos* were tested in sheep experimentally infected with the nematode parasite *Haemonchus contortus*. Male lambs were infected with 3000–5000 third stage larvae of *H. contortus* and treated 28 days after inoculation with concoctions made from leaves or fruits of the plants. No significant reduction in faecal nematode egg counts was observed with any of the concoctions at any of the doses tested. Packed red cell volume decreased and live weight increased at similar rates in treated and control groups, thus there was no significant effect of treatment. The results showed that the tested extracts of the *M. africana* and *R. melanophloeos* were not efficacious against *H. contortus* in sheep.

Keywords: *Myrsine africana*; *Rapanea melanophloeos*; *Haemonchus contortus*; Sheep; Anthelmintic activity; Traditional medicine

1. Introduction

A major animal health constraint to livestock production, especially small ruminants, is gastrointestinal nematode infection. The greatest losses associated with nematode parasite infections are sub-clinical, and economic assessments show that financial costs of internal parasitism are enormous due to increase in mortality, and a reduction in growth rate and wool production (McLeod, 1995; Preston and Allonby, 1979). Control of internal parasites often relies on the use of anthelmintic drugs. In Kenya, for instance, there is a widespread dependence, and generally frequent use, of anthelmintics by livestock owners. This is because grazing management strategies, which can extend the effectiveness of drug treatment, cannot be readily practiced due to limited size of land (smallholders) and communal grazing where land is communally owned (pastoralists). Widespread intensive use of sometimes poor quality drugs (Monteiro et al., 1998) has led to development of a high level of multiple anthelmintic resistance in many parts of the developing world (Waller, 1997a). The combination of these factors has stimulated the search for alternative control strategies (Hammond et al., 1997; Newton and Munn, 1999; Niezen et al., 1996; Waller, 1997b). Amongst these strategies is the use of traditional plant remedies. In Kenya, the use of plants as anthelmintics by smallholder farmers and pastoralists is practiced widely (Anonymous, 1996; Wanyama, 1997a,b). This is particularly so in regions of the country where modern anthelmintics may not be available, or, when available, are too expensive for these farmers. The use of plants as anthelmintics could, therefore, be a potential alternative for livestock owners, provided that the reputed efficacy is proven.

Myrsine africana is a small shrub or tree (1–5 m high) that is widespread in Kenya, particularly in upland dry forests and rocky hillsides (Beentje, 1994). *Rapanea*

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melanophloeos is an evergreen tree (4–20 m high) that is widespread in upland forests near the edges of moorlands. They both belong to the plant family, Myrsinaceae. There are reports that concoctions made from the bark, roots and fruits of these trees have been widely used as anthelmintics in humans and livestock (Kokwaro, 1993; Beentje, 1994). Particular emphasis of their role for livestock was made by Gachathi (1993), who reported the use of fruits of both plants as anthelmintics in sheep and goats. The use of leaves of *M. africana* and the fruits of *R. melanophloeos* as anthelmintics for small stock has also been reported (Anonymous, 1996). The aim of the present study was to validate the anthelmintic/nematodocidal efficacy of *M. africana* and *R. melanophloeos* by administration of plant extracts to penned sheep harboring monospecific infections with the nematode parasite *Haemonchus contortus.*

2. Material and methods

2.1. Animals and monitoring

Male Dorper lambs, 3–4 months old at the time of purchase, were used. After purchase the lambs were moved indoors and given a period of 3 weeks to get used to feeding on pellets and hay. Over this period the animals were dosed with injectable ivermectin (Ivomec®, MSD) at 200 μg/kg live weight (LWT), treated with a long-acting tetracycline at 20 mg/kg LWT (Tenaline LA®, Sanofi) and sprayed with flumethrin (Bayticol®, Bayer), according to manufacturers' instructions.

Monitoring of nematode infections by faecal egg count (FEC) showed that all animals were not shedding nematode eggs 7 days post anthelmintic treatment (AT). After an additional two weeks, when the lambs were acclimatised to hay and pellet feeding, they were inoculated with 3000 *H. contortus* infective third stage larvae (L3), with the exception of animals that were subsequently treated with *M. africana* leaves and their control, which were orally dosed with 5000 L3 (Table 1). The parasite was isolated from sheep grazing on the Kapiti plains, Machakos District, 60 km east of Nairobi, in an area where none of the plant materials used in the trials grow. Three weeks post-infection the animals were blocked based on their FEC and LWT and randomized into control and treatment groups. A week later, the animals were then treated with the herbal preparation according to the methods described by Anonymous (1996) or those used by traditional healers (personal communication). Each treatment and control group had at least 15 animals. In all instances, dose-titration was conducted for all plant preparations. This was achieved by determining the 'traditional' dose rate of the plant compound. Five sheep were administered this dose rate and five were administered the compound at both half and double this dose rate. Faeces and blood were taken from each animal for estimating nematode FEC using the McMaster method (Urquhart et al., 1996) and red cell packed cell volume (PCV) using the microhaematocrit method. Animals were sampled four times in the first week post-treatment and twice during the second week. LWT changes were recorded weekly from infection to the end of the experiments. Following treatment with the plant preparations/extracts, close monitoring of all the animals' feeding, behavior and general activities were observed daily until the termination of the trial two weeks post treatment.

Table 1
Experimental design showing the inoculum, the plants and traditional dose used in each of the four experiments to evaluate anthelmintic efficacy of *M. africana* and *R. melanophloeos* plants in lambs

Experiment	Inoculum	Group	Traditional dose (g)
1	5000 L3	Control	
		M. africana leaves	125
2	3000 L3	Control	
		M. africana fruits	50
3	3000 L3	Control	
		R. melanophloeos fruits	125
4	3000 L3	Control	
		R. melanophloeos fruits	50

2.2. Plants preparation

A total of four experiments were conducted, two on each plant (Table 1). Two different methods of preparation of *M. africana* were used. The method of preparation was based on that given by Anonymous (1996), supplemented with information gathered from interviews with traditional healers in Baragoi Division, Samburu District, Kenya. In the first experiment *M. africana* leaves were collected from the foothills of the Aberdare ranges, 100 km to the West of Nairobi, in August, September and early October 1999. This period coincided with the start and middle of the fruiting season for this shrub. All samples were collected between midday and 17:00 h. The leaves of *M. africana* were collected and shade dried after which they were ground using a coffee mill. The traditional dose used was 125-g dry weight (DW) of leaves mixed with 500 ml of water per animal. Shade dried *M. africana* fruits collected from Mount Nyiro in Samburu District were used in the second experiment. The traditional dose was 50 g of the dried pounded fruits ground using a coffee mill and mixed in 250 ml of water after which the mixture was heated up to 70 °C for 5 min. The mixture was stirred to cool for 10 min and then used to drench each animal.

R. melanophloeos fruits used in the third experiment were collected from the Aberdare in December 1999 when the trees were fruiting. The fruits were dried under the shade and then ground using a coffee mill. The traditional dose described by Anonymous (1996) was composed of 125 g of fruits, which were ground and mixed in half a liter of water. The mixture was boiled for 15 min and allowed to cool for 20 min thereafter. Fruits for the fourth experiment were collected from Mt Nyiro in the Samburu district. The fruits were dried under the shade, ground with a hammer mill, mixed in 250 ml of water, heated at 70 °C and then allowed to cool before drenching. A traditional dose of 50 g DW was used for each animal.

Specimens of all the plants collected were given to the East African herbarium, Nairobi for authentication.

2.3. Statistical analysis

Faecal egg counts (FEC) were $\log_{10}$ transformed prior to statistical analyses. Weekly FEC, PCV and LWT values were then analyzed by least squares analysis of variance (ANOVA) to compare control and treatment means. Linear regression analysis was carried out to see whether there were dose responses for treatment. Faecal egg count reduction was determined by the method described by Coles et al. (1992) using the formula $\mathrm{FECR}\% = 100 \times (1 - T/C)$, where T is the treatment geometric mean and C is the control geometric mean post treatment. The sample size of 15 animals per group was chosen to be able to detect a FEC reduction of 70% between a treatment group and the control as significant ($P < 0.05$).

3. Results

All sheep were healthy and showed no clinical signs of haemonchosis in the first four weeks post-inoculation (PI) with L3. The FECs rose in animals treated with *M. africana* leaves to levels as high as in the control at the termination of Experiment 1 and there was no significant difference ($P > 0.05$) between the treatment and control groups (Table 2). In lambs treated with *M. africana* fruits (Experiment 2), FECs decreased two weeks post treatment, but so did FECs in the control lambs. Lower FECs ($P < 0.05$) were observed in *R. melanophloeos* treated lambs in Experiment 3 than Experiment 4 (Table 2). However, there were no significant differences between treated and control groups in either experiment. No linear relationship could be determined across the different doses used for any of the plant extracts tested.

A progressive decline in PCV in all infected lambs was observed from the second week PI but without significant ($P > 0.05$) differences between treated and control groups either on the day of treatment or two weeks later in all four experiments (Table 3).

A general increase in the LWT was observed in both treated and control groups from the time of infection up to the fourth week PI. However, no significant LWT differences were observed between any of the *M. africana* or *R. melanophloeos* treated and control groups two weeks post treatment in all experiments (Table 3). In addition, no behavioral changes were observed in any of the four experiments after application of the herbal treatments.

4. Discussion

These experiments were carried out to assess the effectiveness of *M. africana* leaves and dried fruits and *R. melanophloeos* fruits against *H. contortus* infection in sheep. The two plants did not exhibit significant anthelmintic activity as measured by faecal egg count reduction at even the highest dose used of 250 g per animal for either of the plants. Although several authors (Anonymous, 1996; Beentje, 1994; Gachathi, 1993; Kokwaro, 1993) have described that plants belonging to the family Myrsinaceae have anthelmintic activity, this was not shown to be the case in the current study. Phytochemical analysis of crude extracts of these plants has shown embelin to be one chemical constituent of these trees with the greater concentration of the compound found in the fruits than the leaves (Ghebremeskel, 1991; Manguro, 1994). Gupta et al. (1976) indicated that this compound had an anthelmintic activity in vitro. Embelin isolated from *Embelia schimperi*, another member of the family Myrsinaceae, had anthelmintic activity against the cestode parasites *Hymenolepis diminuta*, both in vivo and in vitro (Bogh et al., 1996). However, it showed little activity against the trematode *Echinostoma caproni* and the nematode *Heligmosomoides polygyrus* in rats and mice in the same study (Bogh et al., 1996).

In vitro studies by Kakrani and Kalyani (1983) demonstrated that aqueous and alcoholic extracts of *M. africana* fruits had very little or no effect against the nematode *Oesophagostomum columbianum,* while the alcoholic extracts were highly efficacious against the nematode *Bunostomum trigonocephalum* and the cestode *Taenia solium.* Furthermore, Desta (1995) demonstrated that *Taenia saginata* was expelled from humans after dosing with aqueous or ethanolic extracts of *M. africana* fruits. Thus, there is evidence that the anthelmintic activity of embelin is somewhat species specific and generally more efficient against cestode parasites. Therefore, these two plants may have similar effects on ruminants' tapeworms, for example, *Monezia spp*. It is likely that these plants are considered efficacious by pastoralists against all worms when they see tapeworm

Table 2
Geometrical mean faecal egg counts per gram of faeces for *M. africana and R. melanophloeos* treated and control lambs at the time of treatment and 2 weeks post treatment

Experiment	Group	Number of lambs	Weeks post treatment	
			0	2
1	Control	15	20 300 (14 500–28 400)[a]	34 400 (29 100–40 700)
	M. africana leaves	16	26 500 (19 100–36 600)	37 840 (32 400–44 300)
2	Control	17	26 100 (20 800–32 800)	20 400 (15 700–26700)
	M. africana fruits	16	25 400 (20 100–32 100)	22 400 (21 800–23 100)
3	Control	16	13 000 (10 800–15 700)	18 100 (16 000–21 900)
	R. melanophloeos fruits	17	10 800 (9100–13 000)	14 700 (12 600–17 100)
4	Control	17	26 100 (20 800–32 800)	20 400 (15 700–26 700)
	R. melanophloeos fruits	16	26 300 (20 500–33 000)	25 100 (19 100–33 100)

[a] 95% Confidence interval for geometric mean.

segments in the faeces after treatment. Thus, destrobilation or total expulsion of the tapeworms alone could easily be mistaken as evidence of a good de-worming effect. There is some evidence to suggest that this misconception that presence of tapeworms or their segments in faeces are a good indication of anthelmintic activity maybe widespread (personal observation in Samburu).

A problem associated with the present study is the lack of consistency of the dose used by different traditional healers. This was overcome by evaluating each plant extract at different doses in an effort to cover possible ranges of doses used by pastoralists.

These experiments used methods described by Anonymous (1996) and Samburu and Turkana traditional healers. There were differences in the method of preparations as well as quantities used. In the second and fourth Experiments, Samburu and Turkana healers described the methods of plant preparation. These methods utilized far less plant materials than in Experiments one and three, using methods described by Anonymous (1996). Again this did not result in significant differences between the control and treated groups.

Unknown factors may have influenced the negative outcome of these trials. For instance, it is well known that the chemical constituents of plants vary, not only within the parts of the plant, but also between plants in relation to location, age, and stage of growth, condition and whether the plant material is fresh or preserved (McCorkle et al., 1996). Thus, it could be possible that material from other regions, prepared in different ways, may yield positive responses. However, if this proves to be the case, then the utility of using such plant material as an alternative to present anthelmintics must be questioned. Owners of livestock need to be assured that any plant preparations used to treat their animals for internal parasites will be efficacious.

In conclusion, the two plants *M. africana* and *R. melanophloeos* did not exhibit any anthelmintic activity

Table 3
Mean Packed cell volume (PCV) and live weight (LWT) in *M. africana* and *R. melanophloeos* treated and control groups of male lambs at the time of treatment and two weeks post treatment

Experiment	Group[a]	PCV (%)		LWT (kg)	
		Weeks post treatment			
		0	2	0	2
1	Control	19.8	15.4	16.1	16.3
	M. africana leaves	19.7	16.2	16.9	16.8
	Average SED	2.11	2.12	1.41	1.36
2	Control	16.8	17.6	19.5	19.7
	M. africana fruits	18.5	17.5	19.7	19.9
	Average SED	2.00	1.75	2.18	2.27
3	Control	22.5	19.7	19.7	19.7
	R. melanophloeos fruits	21.4	19.0	19.6	20.4
	Average SED	1.45	1.23	1.72	1.70
4	Control	16.8	17.6	19.5	19.7
	R. melanophloeos fruits	18.6	16.8	20.5	20.4
	Average SED	1.57	1.32	1.77	2.09

[a] Number of lambs per experiment shown in Table 2.

at the doses and preparation method used. Neither fruits nor leaves produced any significant reduction in FEC of the nematode *H. contortus* in sheep. *H. contortus* is the most economically important nematode parasite of sheep and goats in the tropics. For any plant concoction to be of any practical value for farmers, it must have activity against this parasite.

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