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Anthelmintic efficacy of selected medicinal plants against gastrointestinal nematodes in naturally infected sheep in Kenya

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ABSTRACT

The use of plants, or their extracts, for the treatment of human and animal ailments, including helminthosis is steeped in antiquity. Anthelmintic resistance in pathogenic helminths has been spreading in prevalence and severity to a point where there is multi-drug resistance against the three major classes of anthelmintics. This globe-wide phenomenon calls for urgent search for different approaches to the control of helminthosis including novel anthelmintic products.
The objective of this study was to evaluate the efficacy of plants, which are commonly used in the treatment and control of helminthosis, under the natural grazing conditions in Loitoktok Sub-County of Kenya. The plant species (*Albizia anthelmintica, Myrsine africana,* and *Embelia schimperi* were selected based on their availability and ethnopharmacological uses, as anthelmintics, by the traditional health practitioners in the area. Fifty sheep of mixed breeds, belonging to local herders, and naturally infected with mixed gastrointestinal nematodes were recruited for the study. The herbal remedies were prepared and administered by methods prescribed by the traditional practitioners. Their efficacy was determined using faecal egg count reduction test (FECRT). The percentage faecal egg count reduction against the mixed gastrointestinal nematodes was 59, -11, -31 and 87 for *Myrsine africana, A. anthelmintica, E. schimperi* and albendazole respectively. It was concluded that some of the remedies have some efficacy and further studies are needed to evaluate their potential as anthelmintics. There was also suspicion of development of anthemintic resistance to albendazole and herders needed to be advised accordingly on the proper use of conventional anthelmintic products.

Keywords: Anthelmintic plants, gastrointestinal nematodes, natural infection.

INTRODUCTION

Medicinal plants have been used to combat parasitism and other human and veterinary ailments for centuries and in many parts of the world (including Kenya), they are still used for this purpose. The World Health Organization estimates that 80% of the populations of developing countries rely on traditional medicine, mostly plant drugs, for their primary health care needs [1]. There has been a resurgence of interest in traditional health practices throughout the world, which mainly encompasses ethnobotany and the use of herbal remedies. The forces responsible for this momentum include the perception that "natural is nice", concerns of synthetic drug residues in the environment and the food chain, and particularly the spectre of rapid emergence of multiple resistant pest organisms through misuse and overuse of conventional drugs.

Renewed interest in traditional pharmacopoeias has meant that researchers are more concerned, not only with determining the scientific rationale for the plant's usage but also, with the discovery of novel compounds of pharmaceutical value. Instead of relying on trial and error as in random screening procedures, traditional knowledge help scientists to target plants that may be of medicinal value [2, 3].

Reports from around the world include exhaustive lists of medicinal plants that have been reported to have anthelmintic properties [4-13]. Although most of the evidence on the antiparasitic activity of these plants is based on anecdotal observations, there is growing number of controlled studies that aim to scientifically verify, validate and even quantify such bioactivity.

Two main approaches have traditionally been used in efficacy studies against helminths. The first one is through feeding plants or their parts to naturally or artificially infected animals [14, 15]. The second one is by testing extracts and concoctions from medicinal plants via *in vivo* and *in vitro* systems [16, 6].

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Kenya is endowed with a variety of indigenous medicinal plants which are used by the local herbalists for the treatment of various diseases among them helminthosis [17-19]. However, most of these herbal remedies have not yet been scientifically validated or developed into viable products for the market despite the looming threat of disappearance of traditional knowledge.

The purpose of the current study was to determine the anthelmintic efficacy of three medicinal plants most frequently used to treat and control helminthosis in Loitoktok Sub County of Kajiado County in Kenya. The three plants studied were *Albizia anthelmintica* Brongn, *Embelia schimperi* L. and *Myrsine africana* L. The first plant belongs to the Fabaceae family while the last two are Myrsinaceae. These plants were selected from an earlier ethnopharmacological study conducted in the area involving renowned traditional healers identified through key informants [20].

MATERIALS AND METHODS

Study site

Loitoktok Sub County comprises an area of 6,300km^2 and is home to the pastoralist Ilkisonko subgroup of the Maasai people. However, several non-Maasai groups, of which the Kikuyu and Kamba are the most numerous, now live in Loitoktok. Figure1 shows Loitoktok Sub County in relation to the map of Kenya. It is located in the southwestern part of the Rift valley province of Kenya and borders Kajiado central Sub County to the north, Namanga Sub County to the northwest, Tanzania to the southwest, Taita - Taveta and Makueni Sub Counties to the southeast and northeast respectively. Its highest point is the slopes of the snow-capped Mount Kilimanjaro (the highest mountain in Africa) and the Chyulu hills while its lowest point is the Amboseli basin.

Loitoktok has a bimodal rainfall pattern with the long rains falling between March and May and the short rains between October and December. High rainfall occurs around the slopes of Mt. Kilimanjaro and the Chyulu hills. Other areas, especially the rangelands are characterized by lower rainfall. The October-December rainfall accounts for 45% and the March-May for 30% of the total rainfall. The temperatures in Loitoktok, like rainfall, also vary with altitude and season. The hottest temperatures of 30^{o}C have been recorded around Lake Amboseli and the lowest mean minimums of 10^{o}C are experienced on the eastern slopes of Mt. Kilimanjaro.

The coolest period is June-August and the hottest is September-February. The vegetation of the Amboseli plains is dominated by bushland and open grasslands (*Acacia* – commiphora mosaic). Swamps are found at the base of Mt. Kilimanjaro. The vegetation composition has changed significantly over the last decade [21]. Most of the woodland has been converted into marginal crop farming areas, swamps into irrigated land and grassland to bush land due to overgrazing and overstocking.

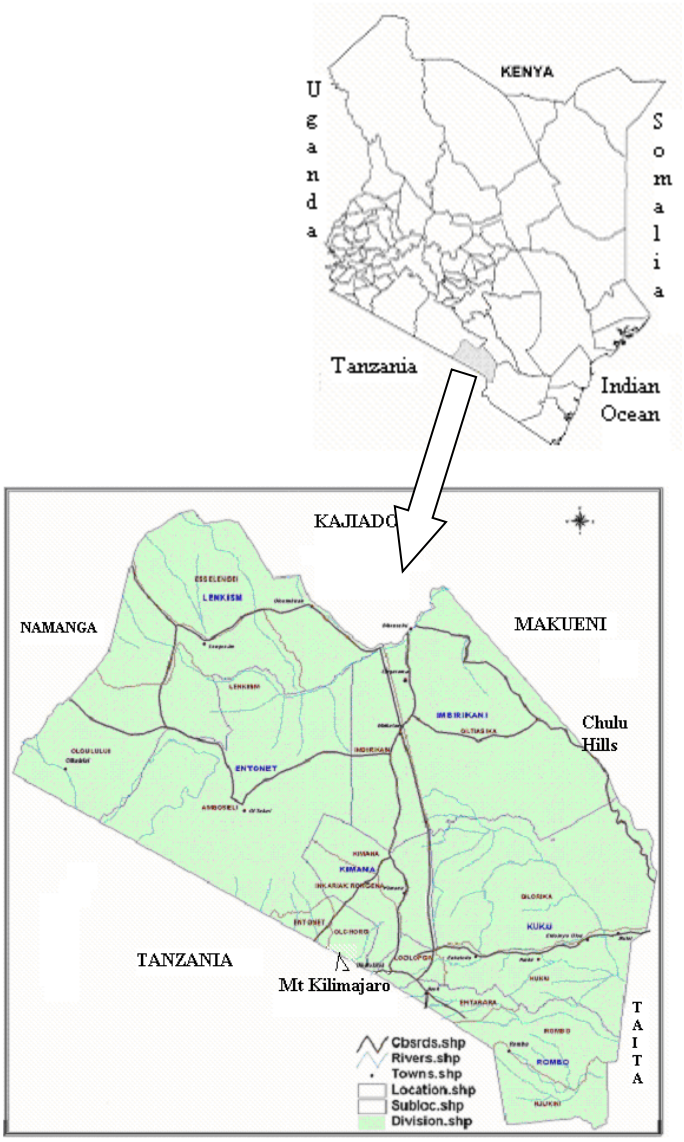

Figure 1: Map of Kenya showing the location of Loitokitok Sub County
Adopted from the Surveys of Kenya

Plant collection and Preparation of the anthelmintic remedies

The plant materials were collected with the help of the traditional healers (THs) from different parts of Loitoktok Sub County and dried under shade. Representative samples for each plant were collected and placed into a field press for transportation and identification at the University of Nairobi herbarium where voucher specimens were deposited.

The dry plant materials were crushed into powder using the traditional wooden mortar and pestle and anthelmintic remedies prepared and dosed according to methods traditionally used by the healers as follows:

1) *Albizia anthelmintica* remedy was prepared by boiling 130 grams

of powder in 5 litres of water for about 30 minutes and letting it cool and then sieving it with tea strainer. The dose for adult sheep was 300 ml of the filtrate

2) *Embelia schimperi* remedy was prepared by boiling 130 grams of powder in 5 litres of water for about 30 minutes and letting it cool and then sieving it with tea strainer. The dose for adult sheep was 300 ml of the filtrate
3) *Myrsine africana* remedy was prepared by boiling 800 grams of powder in 5 litres of water for about 30 minutes and letting it cool and then sieving it with tea strainer. The dose for adult sheep was 300 ml of the filtrate
4) One cup (approximately 300 ml) of cow milk was added to each of the five litres of the remedies before administering to the animals. The reason for this, according to the healers, was to forestall abortion in case some the animals involved in the trial were pregnant.

Animals used in the study

The participating sheep flocks, used in the study, were identified through the local Veterinary office in Loitoktok Sub County. The main breeds of the sheep flocks kept in the area were the Red Maasai and the Persian blackhead. Most of the sheep flocks were mixed and grazed together with goats. The selected flocks had not been dewormed for at least 90 days prior to the study. They were screened for the presence of gastrointestinal nematodes by examining faecal samples obtained directly from the rectum.

A pooled faecal sample was cultured in the laboratory and 100 larvae identified to estimate the prevalence of the nematodes that were present [22]. The study animals were selected, based on the faecal egg counts of samples obtained on the day of the treatment, and marked on the easily visible areas of the body using oil based paints of different colours. The selected animals (male and female of different breeds and ages) were randomly divided into five groups of 10 animals each. The test groups were for three anthelmintic herbal remedies (*Albizia anthelmintica, Embelia schimperi* and *Myrsine africana*), positive and negative controls. The Faecal egg count (FEC) of the sheep ranged from 100 to 1500 eggs per gram of faeces (epg) while the group means varied from 238 to 438 epg before treatment (Table 1).

The positive control group was given a synthetic commercial anthelmintic product valbazen® (2.5% albendazole formulation by Pfizer/Ultravetis) at the recommended dosage rate of 10 mg/kg body weight orally. The negative control group was left untreated. The treatment for each group was administered with help of the traditional healers. The study animals remained with the rest of the flock, under normal grazing conditions, as before for 10 days. On day 11 faecal samples were obtained directly from the rectum for FEC determination. Pooled group faecal samples were cultured to determine the species of the nematodes still shedding the eggs post treatment.

Table 1: Groups of sheep used in the anthelmintic efficacy against natural infection of mixed gastrointestinal nematodes

Group	Treatment	Parts used (where specified)	Dose/adult sheep (or as specified)	Mean eggs/gram (Range)
A	*Albizia anthelmintica*	Stem bark	300 ml	238 (100 -500)
E	*Embelia schimperi*	Fruits	300 ml	238 (100 – 400)
M	*Myrsine africana*	Fruits	300 ml	438 (100 – 1100)
V	Valbazen® (Albendazole)		10 mg/Kg body weight	357 (100 – 1400)
C	Untreated control		-	400 (100 – 1500)

Estimation of anthelmintic efficacy

The anthelmintic efficacy was estimated through percentage faecal egg count reduction (FECR %). The FECR% was calculated using the following equation:

$$FECR\% = (1-(T_2/T_1 \times C_1/C_2)) \times 100.$$

Where, T and C are the arithmetic means of the eggs per gram of faeces for the treated and control groups and subscripts 1 and 2 designate the counts before and after treatment, respectively [23, 24]. The confidence interval for the albendazole reduction was calculated according to the formula by Coles [25] to find out whether there was resistance as follows:

$$95\%\ CI\ limits;\ upper\ limit = 100[1-\bar{Y}_t/\bar{Y}_c \exp(-2.048\sqrt{Y^2})]$$

and $$lower\ limit = 100[1-\bar{Y}_t/\bar{Y}_c \exp(+2.048\sqrt{Y^2})].$$

Where, $\bar{Y}_t$ and $\bar{Y}_c$ are the arithmetic means of the treated and control groups respectively, and Y^2 is the variance of the reduction (log scale).

RESULTS

The results of the faecal egg count reduction test (FECRT) and their 95% confidence intervals (CI) are shown on Table 2. The FECR% varied from -11.3 to 58.9 for the herbal remedies and 87.2% for albendazole with a 95% confidence interval (CI) of 67.9 – 94.9. The genera of the gastrointestinal nematodes, cultured from pooled faecal samples, remained the same in all the groups before and after the treatments. They were Haemonchus (80%), Trichostrongylus (17%) and Oesophagostomum (3%).

Table 2: Anthelmintic efficacy against natural infection of mixed gastrointestinal nematodes of sheep in Loitoktok Sub County.

Treatment Group	Arithmetic mean eggs per gram (Range)				
	Before treatment (day 0)	After treatment (day 11)	FECR%	95% CI (Albendazole)	Remarks
A	238 (100-500)	1029 (200-1700)	-11.3	-	No efficacy
E	238 (100-400)	1213 (100-2800)	-31.1	-	No efficacy
M	438 (100-1100)	700 (0-2000)	58.9	-	Some efficacy
V	357 (100-1400)	200 (0-300)	87.2	67.9 – 94.9	Suspects resistance
C	400 (100-1500)	1543 100-4800)	0	-	Untreated Control

Key: A = *Albizia anthelmintica*; E = *Embelia schimperi*; M = *Myrsine africana*; V = Valbazen (albendazole); C = Untreated control; FECR % = Percentage faecal egg count reduction and CI = Confidence interval for the reduction

DISCUSSION

Only *Myrsine africana* remedy and albendazole had some anthelmintic efficacy at 58.9% and 87.2% respectively. However, the FECR by albendazole was less than 95% with a 95% confidence level of 67.9 – 94.9 making it suspect for resistance [25]. The results for the *Myrsine africana* anthelmintic remedy looked promising and together with others, became the subject of a further study under controlled laboratory conditions that followed this field study. In that study *Myrsine africana* remedy had a FECR of 83% while *Embelia schimperi* and *Albizia anthelmintica* had no significant reduction against mixed gastrointestinal nematodes in artificially infected sheep [26].

Other studies have reported varied results in the past. A similar study under the pastoral field conditions of Samburu County reported a FECR of 77 and 90% for *Myrsine africana* and *Albizia anthelmintica* respectively [6]. The Samburu study also reported 100% efficacy against Monezia tapeworms by both plants [6]. A similar study with naturally occurring mixed gastrointestinal parasites in northern Uganda had a FECR of 78% [27]. Further, extracts from the fruits of *Myrsine africana* have been reported to have good efficacy against the cestode *Taenia solium* and the nematodes *Bunostomum trigonocephalum* and *Oesophagostomum columbianum* [28]. However, a study done in Kenya using Dorper lambs artificially infected with a monoculture of *H. contortus* reported insignificant efficacy for for *Myrsine africana* and *Albizia anthelmintica* [16]

Another study with hydro alcoholic extracts of the fruits of *Embelia schimperi* exhibited significant anthelmintic activities against the dwarf tapeworm, *Hymenolepsis nana, in vivo* by clearing 100% of the parasites when administered at the dosage of 1000 mg/kg body weight [29]. Further, extracts of dried fruits of *Embelia schimperi* had significant effect on *Hymenolepsis diminuta* in rats when given at the dosage of 100 mg/kg but no in vivo effect against the nematode *Heligmosomoides polygyrus* in mice [30].

The differences in the reported efficacies could be as a result of the variation in the dosages given or the phytochemical composition of the plants obtained from different areas and ecosystems and also the methodologies, and possibly the composition and species of GI nematodes in the study animals [9, 31]. Variable conditions of collection and storage of the plants have also been shown to affect the physical and chemical properties of the plant secondary metabolites (PSM) and probably their bioactivity as well. In addition, seasonal and environmental variability will have an impact on the synthetic pathways of the PSM, which can potentially affect their physical and chemical properties [32].

The FECR for albendazole of 87.2% with a 95% CI 0f 67.9 - 94.9 led to the suspicion of anthelmintic resistance according to the criterion set by World Association for the Advancement of Veterinary Parasitology (WAAVP) [25]. Anthelmintic resistance has widely been reported globally in gastrointestinal nematodes of farm animals [33-35]. In Kenya, anthelmintic resistance has mainly been reported in institutional farms which also happen to be the main sources of breeding stock for other smaller farms with potential danger of spreading this problem [36-38].

CONCLUSION

The results of this study have indicated that some of the herbal remedies used in Loitoktok Sub County like *Myrsine africana* have potential for use as anthelmintics. Further studies are necessary to evaluate this potential. The GI nematodes in this study are suspected to be developing resistance to albendazole and that the herders in the area needed to be advised accordingly, on the proper use of the conventional anthelmintic products.

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