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Ascaricidal Activity of Some Medicinal Plants Used by The Karimojong: A Nomadic Pastoralist Community in Uganda

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Abstract: Many rural people world-wide depend on traditional methods of treatment of livestock diseases. In the Karamoja region of Uganda, the pastoralists have accumulated a vast amount of knowledge on natural products and traditional livestock management systems. But little work has been done to evaluate and establish a pharmacological basis for their use. In this study, some of the plants that are used by the Karimojong pastoralists were evaluated for ascaricidal activity *in vitro*. Among the plant extracts evaluated in the study *Athroisma* sp., *Sarcocephalus latifolius*, *Pseudocedrela kotschyi* had significant ascaricidal activity. But there was variation in the efficacy of the plant extracts. *Athroisma sp., Pseudocedrela kotschyi and Sarcocephalus latifolius* all achieved 100% activity against *Ascaris suum. Athroisma* sp. achieved 100% activity against the *Ascaris* at concentrations of 2 , 4 and 8 mg mL^{-1}. 100% activity was achieved after 36 h of incubation for the 8 mg mL^{-1} concentration and after 48 h of incubation for the 2 and 4 mg mL^{-1} concentrations. *Pseudocedrela kotschyi* achieved 100% mortality at concentrations of 4 and 8 mg mL^{-1}. 100% mortality was achieved after 36 h of incubation and after 48 h of incubation of the Ascaris for the 4 and 8 mg mL^{-1} concentrations, respectively. *Athroisma* sp. Sarcocephalus latifolius. Two of the plants (*Pseudocedrela kotschyi* and *Terminalia brownie*) with the highest activity may therefore have some potential for the treatment of nematode infections in ruminants.

Key words: *Pseudocedrela kotschyi*, *sarcocephalus latifolius*, athroisma sp. terminalia brownie, ascaricidal, medicinal plant

INTRODUCTION

Many rural people world-wide depend on traditional methods of treatment of livestock diseases. In the past ethno veterinary medicine has received little attention. But of recent, the western world researchers and development partners have taken a serious and sustained scientific interest in traditional livestock health care systems and related management practices[1].

In the Karamoja region of Uganda, the pastoralists have accumulated a vast amount of knowledge on natural products and traditional livestock management systems. The constraints that face the Karimojong livestock keepers include lack of veterinary personnel, poor infrastructure and insecurity created by inter-clan conflicts and rampant raids. This has made delivery of veterinary services by the inadequate veterinary personnel difficult. Migration of the pastoralists in search of pasture and water has also hampered delivery of veterinary services in the area. Fortunately the Karimojong livestock keepers posses vast knowledge on medicinal plants which they have used to treat their livestock in the absence of conventional veterinary drugs and this has helped bridge the gap created by the lack of adequate veterinary services.

Ethno veterinary management practices, mostly utilizing medicinal plants, would therefore appear to be more cost-effective, efficient and available than conventional medicine[2]. But not much work has yet been put into pharmacological validation of the plants that are used by these indigenous societies. Here we report findings of a study designed to evaluate the ascaricidal activity some of the plants commonly used by the pastoralists of Karamoja for the treatment of worms, using an in-vitro Ascaris model.

MATERIALS AND METHODS

The area of study was in the four sub counties of Chekwi County that is in Namalu, Nakapiripirit, Moruita and

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Kakomongole sub-county of the Karamoja region of Uganda. The target group included 22 farmers/livestock owners and 8 herbalists.

Plant collection and extract preparation: Information on the plants used by the farmers and pastoralists in Chekwi County was obtained using a questionnaire administered to them. The plants mentioned in the questionnaire were then ranked according to the number of times they were mentioned in the questionnaires using Epi Info 6 (version 6.04d-January 2001). The plants with the highest frequencies were selected for the study. The plant parts that were commonly used for treatment of helminth infection by the farmers and livestock owners were collected for analysis in the laboratory. The laboratory procedures adopted were as described before[3-5]. Briefly, following collection of the required plant parts, preparation of the collected plant parts was done by crushing them using a pestle and motor to reduce their particle size. After the crushing the plant parts were dried in the oven to drive out any moisture this also prevented fungal growth on the plant parts during storage. The weights of the plants parts for each plant were determined. The prepared roots of *Athroisma* sp. (Ethiaput) and barks of Pseudocedrela kotschyi (Ekooti), Terminalia brownie (Epie) and Sarcocephalus latifolius (Ewonokori) were each soaked in a conical flask containing one liter of methanol. The conical flasks containing the different mixtures were soaked for a period of 24 h, with duration of 4 h allowed for the mixtures in the electrical shaker (Mitamara model-s 102). Filtration of the mixtures to remove the plant residues was done using Whatman filter paper No. 1. The recovered filtra tes were concentrated using a rotary evaporator (Heidolph-wb-200) to obtain semi solid extracts.

Ascaricidal assays

Collection and preparation of Ascaris suum: Glucose was added to the Goodwins solution on the morning of collection of the worms to prevent its fermentation. The worms were collected from Natete pig slaughter house. Recovery of the worms from the pigs was done by squeezing the worms from small intestines of the pigs which had been slaughtered and immediately put in a vacuum flask containing Goodwins solution at 37° C. The collected Ascaris Suum were transported to the pharmacology laboratory and washed in Goodwins solution to remove the fecal matter from them. Ascaris suum was chosen as the worm of choice because its easy to maintain in Goodwins solution[5].

Preparation of the stock solution: Preparation of the stock solutions was done as described before[5]. Briefly, 5gms of each extract was dissolved in 200 mL of Goodwins solution to give a concentration of 25 mg mL^{-1}. Three sets of 250 mL flasks were labeled according to the desired concentration for each flask. That is 2.0, 4.0 and 8.0 mg mL^{-1}, respectively. 20 mL of the solution was withdrawn from the stock solution, put in 250 mL flask and topped to the 250 mL mark using Goodwins solution to make a 2 mg mL^{-1} concentration of the extract. The same procedure was repeated to obtain 4.0 and 8.0 mg mL^{-1} of the extract but withdrawing 40 and 80 mL, respectively from the stock solution in each case. Eight Ascaris of approximately the same size were gently introduced in each of the conical flasks containing the plant extracts. The worms were then incubated for a period of 48 h with observation for motility being done after every 12 h.

Testing for viability of the worms: Here following incubation for a particular period of time, the worms in the conical flasks were slowly introduced into a basin and observed for motility. Observation for motility was done by gently stroking the heads of the worms, worms that showed motility following stroking of their heads were regarded to be motile and those that did not show any motility were presumed to be dead.

RESULTS AND DISCUSSION

After 12 h of incubation no ascaricidal activity was observed for the 2 mg mL^{-1} concentration of the extract, however the 4 and 8 mg mL^{-1} concentrations showed activity after 12 h of incubation (Table 1). A 100% activity was observed for the 2 and 4 mg mL^{-1} extract concentrations after 48 h of incubation and after 36 h of incubation for the 8 mg mL^{-1} Concentration. All the extract concentrations had achieved 50% death of the total number of worms after 24 h of incubation. 25% of the worms at 0 concentration (negative control) of the extract died after 48 h of incubation.

Ascaricidal activity of for 8 mg mL^{-1} concentration of the extract was observed after 12 h of incubation Table 2. No activity was observed for the 2 and 4 mg mL^{-1} concentrations after 12 h of incubation. Ascaricidal activity for the 2 and 4 mg mL^{-1} extract concentrations started after 24 h of incubation. 100% activity was observed for the 4 mg mL^{-1} extract concentration after 48 h of incubation and after 36 h of incubation of the 8 mg mL^{-1} extract concentration. 12.5% of the Ascaris died after 48 h of incubation at 0 concentration (negative control) of the extract

No activity was observed after 12 h of incubation for the 2 and 4 mg mL^{-1} concentrations of the extract. Activity for the 8 mg mL^{-1} concentration of the extract

Table 1: Ascaricidal activity of *Athroisma* sp.(Ethiaput) extract.

Concentration of extract mg mL^{-1}	Number of Ascaris used	Number of Ascaris dead with time (h)				% of Ascaris dead with time (h)			
		12	24	36	48	12	24	36	48
0	8	0	0	0	2	0	0	0	25
2	8	0	4	7	8	0	50	87.5	100
4	8	2	6	7	8	25	75	87.5	100
8	8	3	7	8	8	37.5	87.5	100	100

Table 2: Ascaricidal activity of *Pseudocedrela kotschyi* (Ekooti) extract

Concentration of extract mg mL^{-1}	Number of Ascaris used	Number of Ascaris dead with time (h)				% of Ascaris dead with time (h)			
		12	24	36	48	12	24	36	48
0	8	0	0	0	1	0	0	0	12.5
2	8	0	1	2	6	0	12.5	25.0	75.0
4	8	0	1	4	8	0	12.5	50.0	100
8	8	1	6	8	8	12.5	75.0	100	100

Table 3: Ascaricidal activity of *Sarcocephalus latifolius* (Ewonokori) extract.

Concentration of extract mg mL^{-1}	Number of Ascaris used	Number of Ascaris dead with time (h)				% of Ascaris dead with time (h)			
		12	24	36	48	12	24	36	48
0	8	0	0	1	1	0	0	12.5	12.5
2	8	0	1	2	4	0	12.5	25.0	50.0
4	8	0	3	5	7	0	37.5	62.5	87.5
8	8	1	4	7	8	12.5	50.0	87.5	100

Table 4: Ascaricidal activity of *Terminalia brownii* (Epie) extract

Concentration of extract mg mL^{-1}	Number of Ascaris used	Number of Ascaris dead with time (h)				% of Ascaris dead with time (h)			
		12	24	36	48	12	24	36	48
0	8	0	0	0	0	0	0	0	0
2	8	0	1	1	3	0	12.5	12.5	37.5
4	8	0	2	2	5	0	25.0	50.0	62.5
8	8	1	3	5	7	12.5	37.5	62.5	87.5

was observed after 12 h of incubation Table 3. Hundred percent activity was not achieved for the 2 and 4 mg mL^{-1} extract concentrations even after 48 h of incubation. 100% activity was observed after 48 h of incubation for the 8 mg mL^{-1} concentration of the extract. 12.5% of the Ascaris died after 36 h of incubation at 0 concentration of the extract (negative control).

After 12 h of incubation Table 4 of the extract, no activity was observed for the 2 and 4 mg mL^{-1} concentrations of the extract. Activity for the above concentrations of the extract was first observed after 24 h of incubation of the Ascaris. At 8 mg mL^{-1} concentration of the extract activity against the Ascaris was observed after 12 h of incubation. A 100% activity was not observed for any of the extract concentrations. At 2 and 4 mg mL^{-1} concentrations of the extract both achieved a maximum activity of 100% after 48 h of incubation. The highest concentration of the extract (8 mg mL^{-1}) achieved maximum activity after only 36 h of incubation. The median effective dose for *Athroisma* sp. (EC_{50}) was 1.276 mg mL^{-1}. 100% percent mortality of the Ascaris was achieved after 48 h of incubation for the 2 and 4 mg mL^{-1} concentrations and after 36 h of incubation for the 8 mg mL^{-1} concentration of the extract. The median effective dose (EC_{50}) of Sarcocephalus latifolius was determined to be 4.978.

The median effective dose of Teminalia brownii was 7.709. Terminalia brownii (MED = 7.709) was the least potent among the plant extract analyzed for ascaricidal activity and *Athroisma* sp. (Ethiaput) (MED = 1.276) was the most efficacious (least dose killed the highest number of worms). Therefore, while all the plants evaluated showed some ascaricidal activity, there were variations in the efficacy of the different plant extracts. These observations agree with other findings[6] which indicated that time is a crucial factor that increases the amount of active compounds and their distribution within the ascarid body. Other studies[7] have reported that the amount of substrate that binds to the receptor sites within the Ascaris cuticle increases with time hence the increase in total deaths of the Ascaris with time. Analysis of variance for trend (Kruskal Willis test) showed that for *Athroisma* sp. Pseudocedrela kotschyi, *Sarcocephalus latifolius* extracts, there were significant ($p<0.05$) variation of ascaricidal activity of the different extracts with time. The median effective dose of Terminalia brownii was 7.709 mg mL^{-1}, which was the lowest among the plant extracts analyzed for ascaricidal activity. The efficacy of this plant

was also the lowest among the plants evaluated for ascaricidal activity in this study. Terminalia spp have been reported to contain tannins[8], which have been implicated in yellow wood toxicity following consumption of leaves of Terminalia brownii[9]. Like many medicinal plants Terminalia brownii can be considered to have both beneficial and detrimental effects. However, the plant has been used by several pastoral communities for treatment of livestock. The Masai use a decoction of crushed leaves dissolved in water administered orally to treat worms and the barks are dissolved in water to treat babesioisis in both goats and cattle[10]. The Shukria of northern Sudan use a bark decoction dissolved in water to treat helminth infestation in cattle[11]. Tannins isolated from *Terminalia* sp. have been shown to protect ruminants against bloat and also to have anthelmintic activity[12]. The results of this study agree with reports from[13] who carried out in-vivo evaluation of tanniferous plants against Trichostrongylus colubriformis and Teladorsagia circumcincta 12 days after tanniferous plant administration there was a significant reduction of Trichostronglus infestations and close to significant reduction in Teladorsagia infestations. Others[8] also stated that tannins isolated from plants like Terminalia chebula and Terminalia alata have activity against bacteria, filamentous fungi and yeasts their activity has been attributed their to ability to inactivate microbial adhesions, enzymes and cell envelope transport proteins.

This study evaluated the effect of *Terminalia brownii* on adult *Ascaris* suum *in vitro*. However, previous studies[14] showed that various tanniferous plants were able to inhibit feeding, migration and development of immature nematodes but also reduce the viability of adult nematodes. Further studies on this plant therefore are needed to assess its effect on immature nematodes. Excessive consumption or prolonged administration of *Terminalia* and other tanniferous plants can lead to decreased nutrient utilization, in particular protein utilization, they also lead to decreased in growth fortunately, ruminants have a lower intake of tannins rich feeds owing to their astringent taste[15]. The plant should therefore be administered for shorter periods of time if it is to be of benefit.

Sarcocephalus latifolius with a median effective dose of 4.978 mg mL^{-1} had a higher efficacy and Log dose response curve placed above that of Terminalia brownii. The activity of *Sarcocephalus latifolius* is said to be due to indole alkaloids and sugars. The fractionated the root extract[16] of the plant and obtained angustidine, quinovic, angustidine, ethylangustoline, strictosamide and nauclefide among other indole alkaloids, as earlier documented[17]; characterizing the sugar fraction of Sarcocephalus latifolius leading to isolation of D-fructose derivatives, Xylose, B-D pyranose forms of glucose, arabinose perbenzoate glycerol and D-erythriol perbenzoate. Anthelmintic properties of the plants have been attributed to isoquinolone alkaloids (Hollowel, 2004) hence the use of the plant as a vermifuge and antidysenteric in Guyana. Sarcocephalus latifolius has much usage has an antimalarial herb due to its alkaloid content[16]. These reports signify that the plant may have additional uses in addition to its reported anthelmintic activity; necessitating further detailed studies.

There are very few reports of ascaricidal activity of Pseudocedrela kotschyi in literature. The plant is however, commonly used as a tropical chewing stick for oral hygiene by indigenous people. Owing to its common use for oral hygiene in the tropics, other researchers[18] have evaluated the plant and found it had potency against *Staphylococus* areus and *Staphylococcus* auriculus. The antibacterial activity of this plant against other common bacteria veterinary importance therefore needs to be evaluated. In other reports the methanolic and evaporated crude water extracts of the plant have been screened for molluscidal activity on laboratory reared Lymnae natalensis and the plant has showed molluscidal activity. *Athroisma* sp. (with a median effective dose of 1.276 mg mL^{-1}) was the most efficacious of the plants evaluated for ascaricidal activity.

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