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THE *IN-VITRO* ASCARICIDAL ACTIVITY OF SELECTED INDIGENOUS MEDICINAL PLANTS USED IN ETHNO VETERINARY PRACTICES IN UGANDA

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Abstract

Twenty-one plants were identified through preliminary field surveys and seven were selected for in vitro anti-helminthic activity against *Ascaris suum*. Of the seven plants that were initially screened, five gave appreciable positive results while two did not. The ED_{50s} obtained were: 1.62mg/ml (*Tetradenia riparia*), 4.13mg/ml (*Cassia occidentalis*), 12.50 mg/ml (*Carica papaya*) and 16.75mg/ml (*Momordica foetida).* The research findings showed that *Tetradenia riparia*, *Cassia occidentalis*, *Carica papaya*, *Momordica foetida* and *Erythrina abysinnica* may be of value in the treatment of helminthiasis; whereas *Moringa oleifera* and *Cannabis sativa* are probably ineffective or of limited value for the same purpose.

Key words: Ascaricidal, Uganda, Medicinal Plants

Introduction

Helminths are of major public health and economic importance to both man and livestock throughout the tropics. It is estimated that 60-80% of the world's population is affected by helminths with a vast majority of these in developing countries (Fansworth, 1988). In man, diseases such as schistosomiasis, ascariasis and ancylostomiasis cause untold suffering to millions of people worldwide, especially in Sub-Saharan Africa. The effects of such infestations include gastroenteritis, anemia, stunted growth, blindness and lameness among others. In the livestock industry, similar conditions are seen that greatly affect productivity and hence economic output of the livestock sector. Here, helminths cause a multitude of problems such as poor weight gain, diarrhea, respiratory problems and even mortalities in severe cases (Schmidt and Roberts, 1985). Ascariasis, for instance, affects about one quarter of the world's population, especially the growth and nutritional status of children (Latham, 1977).

Control of helminthiasis has therefore been the centre of focus in biomedical research since time immemorial. Both the medical and veterinary professions have tried to

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control helminthiasis by administration of synthetic drugs (Ssebuguzi, 2000). However, these drugs are becoming increasingly expensive with some having serious side effects (Siddiqui and Hussein, 1992).

Despite the large number of drugs and medicines available for treatment of all forms of diseases, the demand for herbal medicines has steadily increased over the past decade worldwide. However, a great majority of them are not assessed for their quality, safety or licensed as medicines (Alte, 1993). Little is known or documented about the usefulness, effectiveness or potential of such medicines. With the onset of modernization of agriculture and other western influences, such knowledge is greatly threatened and could totally be lost with the passing generations. It is prudent therefore to urgently research more on this field and generate vital data that could be necessary to revitalize and preserve such knowledge. Previous studies in our laboratories have demonstrated antibacterial and antifungal (Olila et al. 2001a) as well as antiviral and trypanocidal (Olila et al., 2001b; Odyek et al., 1993; Olila et al., 2002) activity in Ugandan medicinal plants.

Here we report findings from studies designed to screen local plants that are used in the treatment of helminthiasis in livestock in some parts of Uganda, where human and veterinary services are still very poor; being compounded by many people living in rural areas several kilometers from health centers. This has resulted in a large proportion of the population relying on traditional methods of treatment, using herbal extracts, which have been claimed to produce beneficial responses. These remedies are not only more readily available and acceptable but could also be cheaper, if their efficacy could be scientifically validated.

Materials and Methods

Selection of the plants

The plants were selected mainly on the basis of frequency of their being mentioned by the farmers. A total of twenty-seven respondents (including farmers and herbalists) were interviewed. Twenty-one plants were mentioned. Of these, seven were selected: *Tetradenia ripara* (kyewamala), *Cannabis sativa* (njaga), *Moringa oleifera* (moringa), C*arica papaya* (papaali), *Cassia occidentalis* (mutanjoka), *Momordica foetida (bombo) and Erythrina abyssinica (ejirikiti).* All the seven selected plants were collected from the field and transported to Makerere University Botany Department for botanical identification and voucher specimens (Waswa/04/ASCA) have been kept at the department of Veterinary Physiological sciences, Makerere University, Kampala, Uganda.

Extraction procedures

Fresh leaves and stems of the plants were ground separately in a mortar. Each of the plant tissues was soaked in approximately 400ml of 95% ethanol on an electrical shaker for three hours at room temperature and then left to stand overnight. The mixtures were filtered into conical flasks using Whatman filter paper No. 1. The filtrate was then concentrated on a

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rotary evaporator at 50^{o}C to yield semi-solid masses whose weights were determined. The extracts were then stored in a refrigerator at 4^{o}C.

Collection and maintenance of the worms (*Ascaris suum*)

The worms were collected from a pig abattoir (Wambizi -, Rubaga division, Kampala). They were got from intestines of freshly slaughtered pigs, whose contents were manually strained until all the worms were ejected. The worms were immediately placed in two thermos flasks containing Goodwin's physiological solution at 37^{o}C. In the laboratory, the worms were gently rinsed in distilled water at 37^{o}C and then placed in two large glass jars containing Goodwin's physiological solution. This setup was left in a water bath at 37^{o}C until 6 pm when the experiment commenced.

Screening for ascaricidal activity and determination of ED_{50} of the extracts

Seven conical flasks of 500ml capacity were labeled with different ethanol extracts. 15ml of the respective 1% crude ethanol extracts (prepared by diluting stock solution with distilled water in a ratio of 1:4) and 300ml of Goodwin's physiological solution were added to each of the flasks with one control containing only Goodwin's physiological solution. Ten worms were placed in each of the eight flasks and these were incubated at 37^{o}C in two water baths. A similar setup using 5% solutions was also done after the end of the first test. The worms were monitored every 12 hours for 48 hours. The ED_{50} was the dose that killed immobilized 50% of the worms within 24 h.

Five separate parts of 10ml solution were removed from each of the selected four stock solutions of the plant extracts and placed in different beakers. Each 10 ml sample was diluted with an appropriate volume of distilled water to make concentrations of 2%, 1%, 0.5%, 0.25% and 0.125%.

Five flasks were labeled and filled with 300ml of Goodwin's physiological solution and 15ml of the corresponding diluted extracts. Ten worms were then placed in each of the flasks, with a sixth flask as a control containing only Goodwin's physiological solution. The setup was then incubated at 37^{o}C for 48 h. The worms were monitored at 12 hourly intervals, which was at 7p.m. and 7 a.m. daily. This procedure was repeated for the other three plant extracts and the results obtained were recorded.

Results

Plants used for the treatment of helminthiasis

Tetradenia riparia was the most mentioned plant (over 50%) by the respondents, followed by *Erythrina abyssinica* (Table 1). The least mentioned plants included *Azadirachta indica* and *Ricinus communis*. There was a very small relationship between plants most frequently mentioned and their efficacy/ potency. Although *Tetradenia riparia* and *Cassia occidentalis* showed significant ascaricidal activity, *Erythrina abyssinica* was only moderately ascaricidal. *Carica papaya*, another less commonly mentioned plant (14.9%) showed relatively high ascaricidal activity.

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Table 1. Plants mentioned for the treatment of helminthiasis in the study area

	Plant (Luganda name in brackets)	**Frequency (f)**	**Percentage (%)**
1.	*Tetradenia riparia* (kyewamala)	14	51.9
2.	*Erythrina abyssinica* (Ejirikiti)	11	40.7
3.	*Cassia occidentalis* (Mutanjoka)	8	29.6
4.	*Momordica foetida* (Bombo)	8	29.6
5.	*Euphorbia hirta* (Kisandasanda)	8	29.6
6.	*Vernonia amgydalina* (Mululuza)	7	25.9
7.	*Phytolacca dodecandra* (Luwoko)	6	22.2
8.	*Moringa oleifera* (Moringa)	5	18.5
9	*Senna didyomobotrya* (Mukyula)	5	18.5
10.	*Steganotaenia araliacea* (Kinulangombe)	4	14.8
11.	*Cannabis sativa* (Njaga)	4	14.8
12	*Carica papaya* (Papaali)	4	14.8
13	*Combretum collinum* (Mukoola)	4	14.8
14.	*Nicotiana tabacum* (Tabba)	4	14.8
15.	*Bridelia micrantha* (Katazamiti)	3	11.1
16.	*Vangueria apiculata* (Amatuguda)	3	11.1
17	*Teclea nobilis* (Nzo)	3	11.1
18.	*Ricinus communis* (Nsogasoga)	2	7.4
19.	*Justica exigua* (kazunzanjuki)	2	7.4
20.	*Bidens pilosa* (Sere)	2	7.4
21.	*Azadarichta indica* (Niimu)	2	7.4

Preliminary screening of the extracts for anthelmintic activity

From the initial results obtained from screening of the seven plants, it was observed that only two plants, *Tetradenia riparia* and *Cassia occidentalis* showed activity at 1% concentration, with *Tetradenia riparia* killing three and *Cassia occidentalis* killing two worms respectively (Tables 2 and 3). The rest of the plants did not show any activity at this concentration after 48 h. At 5% concentration, *T. riparia* and *C. occidentalis* killed the worms within the first 12 h, *C. papaya* after 24 , *M. foetida* after 36 h and *E. abyssinica* after 48 h. *C. sativa* and *M. oleifera* achieved only a limited effect after 48 h. Hence *Tetradenia riparia* and *Cassia occidentalis* were potentially the most efficacious of the selected plants, with *Cannabis sativa* and *Moringa oleifera* on the opposite side of the scale.

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Table 2. Effect of 1% concentration of the seven selected plant extract on *Ascaris suum*

Plant extract	Total Worms used	Number of worms dead Time (h)			
		12	24	36	48
T.riparia	10	0	1	2	3
C.occidentalis	10	0	0	1	2
C.papya	10	0	0	0	0
M.foetida	10	0	0	0	0
E.abyssinica	10	0	0	0	0
C.sativa	10	0	0	0	0
M.oleifera	10	0	0	0	0

Table 3. Effect of 5% concentration of each plant extract on *Ascaris*

Plant extract	Total Worms used	Number of worms dead Time (hr)			
		12	24	36	48
T.riparia	10	10	10	10	10
C.occidentalis	10	10	10	10	10
C.papya	10	8	10	10	10
M.foetida	10	7	9	10	10
E.abyssinica	10	5	6	9	10
C.sativa	10	0	1	3	5
M.oleifera	10	0	0	1	2

Effect of various concentrations and incubation time of *Tetradenia riparia* extract on *Ascaris suum*

The ascaricidal effect of *T. riparia* extract increased with increasing concentration of the extract and the incubation time as shown in Table 4. The lowest concentration of 1.25 mg/ml showed some ascaricidal activity by 12 h. and maximum effect in 48 h. Doubling concentration achieved a similar effect 12 h earlier while a concentration of 20mg/ml killed all worms within 12 h. Analysis of variance revealed a significant difference in ascaricidal activity for the different concentrations of *T. riparia* extract ($P = 0.019$) but for different incubation periods ($P = 0.067$).

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Table 4. Effect of various concentrations and incubation time of *Tetradenia riparia* extract on *Ascaris*

Conc. (mg/ml)	Total worms used	Number of worms dead Time (hrs)			
		12	24	36	48
1.25	10	2	4	7	10
2.50	10	3	7	10	10
5.00	10	6	9	10	10
10.00	10	8	10	10	10
20.00	10	10	10	10	10

Table 5. Effect of various concentrations and incubation time of *Cassia occidentalis* extract on *Ascaris*

Conc. (mg/ml)	Total worms used	Number of worms dead Time (hrs)			
		12	24	36	48
1.25	10	0	1	3	6
2.50	10	2	3	5	9
5.00	10	4	6	8	10
10.00	10	7	10	10	10
20.00	10	10	10	10	10

Table 6. Effect of various concentrations and incubation time of *Carica papaya* extract on *Ascaris*

Conc. (mg/ml)	Total worms used	Number of worms dead Time (hrs)			
		12	24	36	48
1.25	10	0	0	1	2
2.50	10	0	0	3	4
5.00	10	1	2	4	5
10.00	10	3	4	6	7
20.00	10	5	8	10	10

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Table 7. Effect of various concentrations and incubation time of *Momordica foetida* extract on *Ascaris*

Conc. (mg/ml)	Total worms used	Number of worms dead Time (hrs)			
		12	24	36	48
1.25	10	0	0	0	1
2.50	10	0	0	1	3
5.00	10	0	2	3	4
10.00	10	2	3	5	6
20.00	10	4	6	8	10

Effect of various concentrations and incubation time of *Carica papaya* extract on *Ascaris suum*

There was a distinct relationship between the incubation time and concentration of *Carica papaya* extract with the ascaricidal activity of the extract as shown in Table 6. An increase in incubation time and concentration of the extract resulted in higher mortality of the worms. Both the 1.25 mg/ml and 2.5 mg/ml concentrations were effective in the first 24 h and neither achieved ED_{50}s of the end experiment. ED_{50}s were, however, achieved by all higher concentrations, 5 mg/ml in 48 h, 10 mg/ml in 36 h and 20 mg/ml in 12 h. Analysis of variance also revealed a significant difference in ascaricidal activity for both the different incubation periods of *C. papaya* extract ($P < 0.001$).

Effect of various concentrations and incubation time of *Cassia occidentalis* extract on the mortality of *Ascaris*

The ascaricidal activity of *C. occidentalis* increased with incubation time and concentration of the extract as shown in Table 5. While the minimum concentration of 1.25mg/ml killed 60% of the worms after 48 h., a double concentration of 2.5mg/ml killed 90% of the worms by the end of the experiment. The median dose of 5.0 mg/ml took effect by 12 hours, killing 40% of the worms and over 50% of the worms 24 hours. All worms were dead by 48 hours. A concentration of 10.00 mg/ml killed 70% of the worms by 12 hours and all by 24 hours. The highest concentration had killed all the worms by the first observation. Analysis of variance revealed a significant difference ($P = 0.000037$) in ascaricidal activity for different concentrations of *Cassia occidentalis* extract. Analysis of variance also revealed a significant difference ($P = 0.002$) in ascaricidal activity for different incubation periods of *Cassia occidentalis* extract.

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Effect of various concentrations and incubation time on *Momordica foetida extract* on the mortality of *Ascaris*

The ascaricidal activity of *M. foetida* is shown in Table 7. 1.25mg/ml killed 10% of the worms by 48 h; 2.5mg/ml killed 10% by 36 hours and 30% by 48 h. A dose of 5.0 mg/ml killed 20% by 24 h. and 40% by the end of the experiment; while 10.00 mg/ml killed 50% of the worms after 36 hours and only 10% more after 48 h. A dose of 20.00 mg/ml killed over 50% of the worms by 24 h. and 100% by 48 h. Analysis of variance revealed a significant difference (P=0.000037) in ascaricidal activity for different concentrations of *Momordica foetida* extract.
Analysis of variance also revealed a significant difference (P=0.002) in ascaricidal activity for different incubation periods of *Momordica foetida* extract.

The median effective doses ($ED_{50's}$) of the plant extracts

The median effective doses of the plant extracts were obtained (using the computer program Microsoft Graph 2000 Chart). The ED_{50} of *Tetradenia riparia* was found to be 1.62mg/ml, that of *Cassia occidentalis* was 4.13mg/ml, *Carica papaya* had a median effective dose of 12.50mg/ml and *Momordica foetida* had an ED_{50} of 16.75mg/ml.

Discussion

This study showed that some plants used in ethno veterinary medicine could be of value in the treatment of helminthiasis. Out of the seven plants studied, five yielded appreciably positive results, four of which had $ED_{50's}$ less than 20mg/ml within 12-36 h. These were *T. riparia*, *Cassia occidentalis*, *Carica papaya*, *Momordica foetida*, and *Erythrina abyssinica*. These findings agree with previous reports that indigenous plants are useful in the treatment of helminthiasis (Akhtar and Riffat, 1984). Two of the studied plants *(Cannabis sativa* and *Moringa oleifera)*, however, did not give satisfactory results, with ED_{50}s more than 50mg/ml in the initial screening.

The anthelmintic property of plants is dependent on numerous substances that are found in them. These could be alkaloids, sugars, saponins, aromatic oils, resins and other medicinally useful chemicals (Lejoly *et al.*, 1996). Oryema (1997) reported that substances like steroids, coumarins, tannins, and triterpoids and other chemical constituents of plants like alkaloids, glycosides, enzymes, anthraquinones, tannins, gums, fixed oils, fats, waxes, volatile oils, proteins and carbohydrates all have medicinal or pharmaceutical value. Many species of *Cassia* especially *Cassia occidentalis*, *Cassia senna*, and *Cassia tora* are commonly used in traditional medicine in tropical Africa for the treatment of worm infections, constipation, pleurisy, edema, ringworm and eruptive skin lesions (Weiser, 1994). *Cassia senna* contains anthracene glycosides (anthraquinone derivatives), one of the important plant drug constituents (Wagner et al., 1984). The milky juice of *Carica papaya* contains proteolytic ferments, which together with papain have successfully been used as an anthelmintic agent for the treatment of Ascariasis, Trichuriasis, and ancylostomiasis (Watt and Breyer-Brandrijk, 1962).

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The anthelmintic effects of *Cannabis sativa* was relatively low compared with other extracts in this study. This was probably due to the mode of action of the active principle. Balick and Cox (1996) stated that *Cannabis sativa* effects are almost completely confined to the cerebral hemisphere resulting in characteristic fibrillary tremors and ataxia due to motor in-coordination. Its most active compound is a resin containing delta-1-tetrahydrocannabinol (THC). *Erythrina abysinnica*, another of the tested plants yielded fairly low anthelmintic activity. Its seeds contain alkaloids like erythrovine, erythroline and erysothiopine (Watt and Breyer, 1962). *Momordica foetida*, a multipurpose plant, also gave moderate results. It is used as an abortifacient and ecbolic, among others. The plant has been shown to exhibit some antimalarial activity and the root extract contains foetidin as a major chemical constituent ((Waako *et al.*, 2005 and Marquis *et al.*, 1977).

In conclusion, therefore, this study has been able to demonstrate significant ascaricidal activity in some plants which could be used in ethno-veterinary medicine. It is recommended that the extracts of *T. riparia, E. abyssinica, M. foetida, C. papaya* and *C. occidentalis* should be further analyzed to isolate the probable anthelmintic principles in them. Toxicity studies of the effective plants should also be done to determine the safety indices of the extracts. Studies to determine the mechanisms of the action, compatibility with other drugs, side effects and other important parameters should be done.

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