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The Anthelmintic Activity of Selected Indigenous Medicinal Plants Used by The Banyankole of Western Uganda

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Abstract: Recently, there has been growing interest in the traditional cures of livestock diseases. This is because industrially produced drugs are too expensive for some sectors of the farming community especially in the developing world. Medicinal plants are often cheaper and more easily available than the commercially produced drugs. The self-help study in form of traditional medicines (especially from medicinal plants), offer a way out by making use of resources available within the communities themselves. Despite the steady increase in demand for herbal medicines over the past decade worldwide, a great majority of herbal products are not pharmacologically assessed for their quality, safety and efficacy, nor are they licensed as medicine. In this study some of the medicinal plants used by the Banyankole (an ethnic group with a long history of cattle-keeping and the use of medicinal plants) have been tested *in vitro* using the Ascaris model. Seven plants were studied (*Vernonia amygdalina*, *Cassia didymobotrya*, *Rhoicissus tridentata*, *Phytolacca dodecandra*, *Euphorbia hirta*, *Aspilla africana* and *Cymbopogon nardus*). *Aspillia africana* and *Cymbopogon nardus* did not show anthelmintic activity in this test system. The other five showed anthelmintic activity (*Vernonia amygdalina*, *Cassia didymobotrya*, *Rhoicissus tridentata, Phytolacca dodecandra* and *Euphorbia hirta*). Extracts of *Vernonia amygdalina*, *Rhoicissus tridentata* and *Cassia didymobotrya* showed higher activity than *Euphorbia hirta* and *Phytolacca dodecandra (Vernonia amygdalina* (ED_{50}) of 3.533 mg mL^{-1}, *Rhoicissus tridentata* (ED_{50}) of 4.355 mg mL^{-1} and *Cassia didymobotrya* (ED_{50}) of 4.880 mg mL^{-1}; Euphorbia *hirta* (ED_{50}) of 5.866 mg mL^{-1} and *Phytolacca dodecandra* (ED_{50}) of 7.151 mg mL^{-1}). Further studies on the five plants are needed. Phytochemical analysis to determine the active principles that are responsible for anthelmintic activity are urgently called for. This would help in identifying the spectrum of activity of the extracts as well as determining their mechanism of action. Studies should also be carried out on these plants to determine their toxicity and also their effect on other helminthes apart from Ascaris.

Key words: Ascaricidal, *Vernonia amygdalina*, *Cassia didymobotrya*, *Rhoicissus tridentata*, *Phytolacca dodecandra*, *Euphorbia hirta*, *Aspillia Africana*, *Cymbopogon nardus*

INTRODUCTION

Recently, there has been growing interest in the traditional cures of livestock diseases. This is because industrially produced drugs are too expensive for some sectors of the farming community. They are also not always available. The self-help approaches in form of traditional medicines, especially from medicinal plants, offer a way out by making use of resources available within the communities themselves[1]. Despite the steady increase in demand for herbal medicines over the past decade worldwide, a great majority of herbal products are not assessed for their quality, safety and efficacy, nor are they licensed as medicine[2]. There is now consensus about the need and significance of recording and evaluating different therapeutic and control procedures adapted in deserts and forests of Africa[3].

In 1997, the 30th World Health Assembly adopted a resolution urging all interested governments to give adequate importance to the utilization of their traditional systems of medicine with appropriate regulations as suited to their traditional health systems[4]. In Kenya, indigenous knowledge has greatly contributed to the recognition of the importance of medicinal clinics in various parts of the country[5].

In Uganda, traditional health care systems are mostly based on the use of medicinal plants that has been acquired and developed over a long time through experimentation and has been passed on from generation to generation mainly by word of the mouth. A number of studies are currently

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ongoing to pharmacologically validate the efficacy of the medicinal plants[6,7].

Here we now report the findings of a study on some medicinal plants used by the Banyankole herdsmen of Western Uganda for the treatment of helminth diseases in cattle. The *in vitro* anthelmintic activity of the extracts from these plants was established and the ED_{50} values of extracts determined using the Ascaris model. The findings should aid in making decisions on the rational use of medicinal plants in livestock production in these communities since these medicinal plants are easily accessible and available to them.

MATERIALS AND METHODS

The study area and target group: Rwampara County is located in Mbarara District in Western Uganda. It has five sub counties namely; Ndeija, Rugando, Bugamba, Nyakayojo and Mwizi. It is found along Mbarara-Kabale highway. The study targeted herdsmen, herbalists and traditional livestock farmers.

Plant collection and preparation: Plants were collected from Rwampara County using a self-administered questionnaire. Leaves of *Vernonia amygdalina* (Omubirizi), *Cassia didymobotrya* (Omugabagaba), *Phytolacca dodecandra* (Omuhoko), *Euphorbia hirta* (Omutanu), *Cymbopogon nardus* (Omutete), *Aspilla Africana* (Ekarwe) and the bark of the roots of *Rhoicissus tridentate* (Ekimara) were identified at the Botany herbarium, Makerere University. These plants were from a list of plants that were obtained from the target group (Table 1). These were chosen because during the interview, they were mentioned to be having excellent activity against helminths. These plant parts (leaves and the roots) were preserved in the refrigerator since they were required fresh. They were then pounded using a pestle and mortar in the pharmacology research laboratory, Faculty of Veterinary Medicine, Makerere University. The pounded leaves were placed into Erlenmeyer Flasks and extracted using methanol on an electrical shaker for three h and then left to stand overnight. The extract was then filtered using Whatman No 1 filter paper into conical flasks and concentrated in a Rotary evaporator at 40°C to give a semi-solid mass which was used for the bioassay.

Worm collection and maintenance: *Ascaris suum* was used as a worm of choice because it is easy to maintain in a laboratory environment for approximately 14 days in Goodwin's solution[8,9]. The worms were collected from a pig slaughterhouse in Kampala city (Wambizzi, Nateete). After collection the worms were immediately put in vacuum flasks containing Goodwin's solution prepared as described[9]. The worms were then transported to the pharmacology laboratory, washed with Goodwin's solution at 37°C, kept in large beakers containing Goodwin's solution and placed in a water bath at 37°C until when used. The solution was changed after 24 h.

Ascaricidal assay: Standard *in vitro* assays[9] were adopted and used. Briefly, Stock solutions were prepared by dissolving 10 gm of each concentrated extract in 200 ml of distilled water giving a concentration of 50 mg mL^{-1}.

Table 1: Indigenous knowledge about the plants studied

Plant name	Description	Part used	Preparation and administration route	Dose
Vernonia amygdalina	A shrub or small tree with petiolate leaves that are elliptic in shape	Leaves roots	Leaves are crushed and mixed with water to make a cold water extract that is given orally. Same for roots	3 to 4 liters in adults
Phytolacca dodecandra	Small tree with slender stems and shiny green leaves	Leaves Leaves	are pounded and a water mixture made and administered orally.	Less than ½ a liter
Cassia didymobotrya	Bushy shrub with heart shaped leaves that grows on rangelands and has a characteristic smell like butter with yellow flowers.	Leaves	Leaves are crushed and mixed with water and orally given.	2-3 cups
Rhoicissus tridentata	Grows in bushy areas and develops thick underground tubers (roots).	Leaves	Roots are pounded and mixed with water. Also a dry paste may be made and given orally.	2 ½ to 3 liters
Euphorbia hirta	Plant with a hairs on it's stem and opposite elliptic leaves	Leaves	Crushed and given orally after mixing with water.	1 to 2 cups
Aspilla africana	A small shrub with small light green leaves	Leaves	Squeezed with water to get extract and given orally.	½ a liter
Cymbopogon nardus	A grass that grows in rangelands and grows to about a meter high	Leaves	Leaves pounded and mixed with water	1 to 2 liters

Four sets of six 250 mL^{-1} flasks were labeled according to the doses. Doses of 2.0, 4.0, 6.0, 8.0 and 10.0 mg mL^{-1} of the extracts of different plants were added to five flasks and the sixth one was left as the control. The control contained Goodwin's solution (200 mL^{-1}). Ten *Ascaris* of about the same size were placed in each of the six flasks and doses of the extracts were topped to 200 mL^{-1} with Goodwin's physiological solution. The flasks were put in an incubator at 37°C. The worms were observed for motility after every 12 h for 48 h. This was done after pouring the flask content in a basin and allowing the worms to move freely. By tapping the ends of each worm with the index finger and applying a bit of pressure, the worms that were a live would be seen to move and those that were dead would not move. The motile worms were then returned to the flask and the incubation process carried out again. In the untreated controls the worms were viable for at least twelve days. The computer program, Graph Pad Prism, [Graph Pad Software, Inc., San Diego, CA, USA] was used to determine median effective doses of these plant extracts.

RESULTS AND DISCUSSION

Yields of the plant materials: The yields from the pounded plant materials were determined as follows: *Phytolacca dodecandra* (6.0%), *Cassia didymobotrya* (5.1%), *Rhoicissus tridentate* (10.7%), *Vernonia amygdalina* (4.5%), *Aspilla africana* (5.1%), *Cymbopogon nardus*(5.2%), *Euphorbia hirta* (5.8%).

Preliminary extract screening: During the preliminary screening, *Rhoicissus tridentata* extract had killed 100 % of the worms after 24 h (at 10 mg mL^{-1}); at 2 mg mL^{-1} it had killed only 40% worms. *Cassia didymobotrya* extract of 10 mg mL^{-1} had killed all the 10 *Ascaris* while 2 mg mL^{-1} killed only 3 worms after 24 h. Also the extract of *Phytollacca dodecandra* of 10 mg mL^{-1} had killed 8 worms and 2 mg mL^{-1} had killed only 2 worms after 24 h. *Vernonia amygdalina* extract of 2 mg / ml had not killed any of the worms and 10 mg mL^{-1} had killed only 7 worms. 10 mg mL^{-1} of *Euphorbia hirta* had killed 6 worms and 2 mg mL^{-1} had killed only 2 worms. During the preliminary screening, some of the plants (*Aspillia africana* and *Cymbopogon nardus*) did not show anthelmintic activity after 24 h so they were not studied any further.

The ascaricidal activity of *Rhoicissus tridentata* extracts is shown in Table 2. The highest dose of 10 mg mL^{-1} first showed activity at 12 h achieving a maximum response of 100% at 24 h. Doses of 4, 6 and 8 mg mL^{-1} first showed activity at 12 h. The maximum response for doses 4 and 6 mg mL^{-1} was achieved at 48 h and that of 8 mg mL^{-1} was achieved at 36 h. The lowest dose of 2 mg mL^{-1} started showing at 24 h and had only achieved a 90% response at 48 h.

The ascaricidal activity of *Vernonia amygdalina* extracts is shown in Table 3. The highest dose of 10 mg mL^{-1} first showed activity at 12 h achieving a maximum response of 100% at 24 h. Doses of 4, 6 and 8 mg mL^{-1} first showed activity at 12 h. The maximum response of 6 mg mL^{-1} was achieved at 48 h and that of 8 mg mL^{-1} was achieved at 36 h. The lowest dose of 2 mg mL^{-1} started showing activity at 12 h and had only achieved a 70% response at 48 h.

The ascaricidal activity of *Cassia didymobotrya* extracts increased with incubation time and the concentration used as shown in Table 4. The highest dose of 10 mg mL^{-1} first showed activity at 12 h achieving a maximum response of 100% at 36 h. Doses of 6 and 8 mg mL^{-1} first showed activity at 12 h. The maximum response for doses 6 and 8 mg mL^{-1} was achieved at 48 h and 4 mg mL^{-1} had achieved a 70% response at 48 h. The lowest dose of 2 mg mL^{-1} started showing activity at 36 h and had only achieved a 40% response at 48 h.

The ascaricidal activity of *Phytolacca dodecandra* extracts is shown in Table 5 above. The highest dose of 10 mg mL^{-1} first showed activity at 12 h achieving a maximum response of 100% at 48 h. Doses of 4 and6 mg mL^{-1} first showed activity at 24 h. 8 mg mL^{-1} started showing activity at12 h and achieved maximum response of 100% at 48 h. The maximum response for doses 4 and 6 mg mL^{-1} was 50% and 80% respectively at 48 h. The lowest dose of 2 mg mL^{-1} started showing at 36 h and had only achieved a 40% response at 48 h.

The ascaricidal activity of *Euphorbia hirta* extracts is shown in Table 6. The highest dose of 10 mg mL^{-1} first showed activity at 12 h achieving a maximum response of 100% at 36 h. Doses of6 and 8 mg mL^{-1} first showed activity at 24 h. The maximum response of 100% for doses 6 and 8 mg mL^{-1} was achieved at 48 h.4 mg mL^{-1} started activity at 36 h and achieved maximum response of 70% at 48 h The lowest dose of 2 mg mL^{-1} started showing activity at 36 h and had only achieved a 60% response at 48 h.

Determination of ED_{50}: The median effective dose (ED_{50}) of *Rhoicissus tridentata* extract was found to be 4.355 mg mL^{-1}, *Vernonia amygdalina* extract had 3.533 mg mL^{-1} as ED_{50}, *Cassia didymobotrya* had ED_{50}, of 4.880 mg mL^{-1}, *Phytolacca dodecandra* was found to be having ED_{50}, of 7.151 mg mL^{-1} and *Euphorbia hirta* extract had 5.866 mg mL^{-1} as ED_{50}.

Therefore, most of the plants which were screened showed anthelmintic activity. This could indicate that they could have medicinal value. The findings are in

Table 2: Anthelmintic activity of *Rhoicissus tridentata*

Concentration of extract (mg mL^{-1})	Number of *Ascaris* used	Number of Ascaris dead after				% of Ascaris dead			
		12 h	24 h	36 h	48 h	12 h	24 h	36 h	48 h
0	10	0	0	0	0	0	0	0	0
2	10	0	3	5	9	0	30	50	90
4	10	2	5	7	10	20	50	70	100
6	10	4	7	9	10	40	70	90	100
8	10	5	9	10	10	50	90	100	100
10	10	7	10	10	10	70	100	100	100

Table 3: Anthelmintic activity of *Vernonia amygdalina* extract

Concentration of extract (mg mL^{-1})	Number of *Ascaris* used	Number of Ascaris dead after				% of Ascaris dead			
		12 h	24 h	36 h	48 h	12 h	24 h	36 h	48 h
0	10	0	0	0	0	0	0	0	0
2	10	1	2	5	7	10	30	50	70
4	10	2	3	8	9	20	30	80	90
6	10	5	6	9	10	50	60	90	100
8	10	7	9	10	10	70	90	100	100
10	10	8	10	10	10	80	100	100	100

Table 4: Anthelmintic activity of *Cassia didymobotrya* extracts

Concentration of extract (mg mL^{-1})	Number of *Ascaris* used	Number of Ascaris dead after				% of Ascaris dead			
		12 h	24 h	36 h	48 h	12 h	24 h	36 h	48 h
0	10	0	0	0	0	0	0	0	0
2	10	0	0	3	4	0	0	30	40
4	10	0	1	4	7	0	0	40	70
6	10	1	2	7	10	10	20	70	100
8	10	3	5	9	10	30	50	90	100
10	10	4	6	10	10	40	60	100	100

agreement with several other reports that medicinal plants are sometimes effective[10,11]. However, some of the plants (*Aspillia africana* and *Cymbopogon nardus)* did not show any anthelmintic activity on preliminary screening. This can be explained by the fact that some anthelmintics do not exhibit wormicidal activity in their original forms but first require to be hydrolyzed in the host gut to form active compounds. The anthelmintic activity of such compounds cannot be easily demonstrated in an in vitro assay procedure of the kind used here.

There were significant differences in ascaricidal activity at different incubation times. These differences in ascaricidal activity probably arise because the amount of active compounds bound to receptor sites would be expected to increase with the period of incubation[12]. There were also significant differences in ascaricidal activity of the extracts with increase in concentration, probably for the same reason as above. The occupation of the receptor sites by active compounds in the extracts as the concentration of extracts increased up to 10 mg mL^{-1}, all binding sites were occupied, thereby causing hyperpolarisation of the membrane, rendering it unexcitable and abolishing impulse transmission inducing flaccid paralysis of the *Ascaris* muscle. A similar finding has been[13] that hyperpolarisation of membranes increases action potential of membranes thus rendering them unexcitable and causing paralysis. This agrees with observations[12] that time is a crucial factor that increases the amount and distribution of active compounds in the body. During the process of screening the worms for their motility, some worms were observed to regain motility after removal from the test extracts. This happened in some cases after relatively short times. This can be explained by the fact that some of the plant materials may act like like piperazine, paralyzing the Ascaris muscles leading to expulsion of the worms by peristalsis[13].

Although *Phytolacca dodecandra*, *Rhoicissus tridentata*, *Vernonia amygdalina*, *Cassia didymobotrya* and *Euphorbia hirta* have been intensively used for a long time in this country for the treatment of gastrointestinal ailments, there have been no previous laboratory investigations done on them in Uganda. *Vernonia amygdalina* is a common woody shrub in Uganda, growing in the sub-humid wooded savanna or wetter highlands. In Uganda it also grows in secondary shrub forest edges as thickets and invades cultivated areas. In Kenya, the plant is used as anti-trematode and also applied onto wounds to facilitate healing. The leaves are pounded, mixed with *Aspillia pluriseta* and a solution of this is taken orally as an anti-trematode or applied onto wound margins[5]. A cold infusion of the root bark of

Table 5: Anthelmintic activity of of *Phytolacca dodecandra* extract

Concentration of extract (mg mL^{-1})	Number of *Ascaris* used	Number of Ascaris dead after				% of Ascaris dead			
		12 h	24 h	36 h	48 h	12 h	24 h	36 h	48 h
0	10	0	0	0	0	0	0	0	0
2	10	0	0	2	4	0	0	20	40
4	10	0	1	3	5	0	10	30	50
6	10	0	2	4	8	0	20	40	80
8	10	2	3	8	10	20	30	80	100
10	10	4	6	9	10	40	60	90	100

Table 6: Anthelmintic activity of *Euphorbia hirta*

Concentration of extract (mg mL^{-1})	Number of *Ascaris* used	Number of Ascaris dead after				% of Ascaris dead			
		12 h	24 h	36 h	48 h	12 h	24 h	36 h	48 h
0	10	0	0	0	0	0	0	0	40
2	10	0	0	3	6	0	0	30	60
4	10	0	0	4	7	0	0	40	70
6	10	0	1	5	10	0	10	50	100
8	10	0	2	6	10	0	20	60	100
10	10	2	4	10	10	20	40	100	100

Vernonia amygdalina mixed with *Vigna sinensis* is drunk in daily regime as a remedy against schistosomiasis[14]. In Kenya, *Vernonia amygdalina* is used in the treatment of bloat, East coast fever and footrot[14].

Many plants mentioned in this study have also been mentioned by other researchers to be used medically in other parts of the world for example species of Cassia e.g. *Cassia occidentalis* is used in traditional topical medicine for the treatment of worm infestations, constipation; pleurisy, edema, ring worm and eruptive skin conditions[14]. *Vernonia amygdalina* is used to treat footrot, worm infestations, retained placenta, East coast fever, bloat, foot and mouth disease[15]. *Euphorbia hirta* is used to treat worm infestations, wounds, poor milk production[16]. This plant has also been reported to be active against *Staphylococcus aureus, Eschericia coli and Pseudomonas aeroginosa*[10].

Some of these plants are also used in traditional human medicine for example *Vernonia amygdalina* is used to treat fever. These livestock farmers also consult traditional birth attendants for conditions like agalactia, placenta retention. This has also been indicated[10] that where ethnoveterinary practices are found that in most cultures, disease concepts apply more or less equally to animals and people. Given this congruency, healers of people often treat livestock and vice versa.

Many of the plants were reported by the farmers to be toxic if used in excessive doses. Toxicological studies are therefore urgently required before concrete recommendations can be made on the use of the plants. The extracts of these plants (especially *Vernonia amygdalina*, *Euphorbia hirta*, *Cassia didymobotrya*, *Rhoicissus tridentata* and *Phytolacca dodecandra)* should be analyzed chemically in order to determine the active principles that are responsible for anthelmintic activity. Further studies should also be done on other helminthes apart from *Ascaris* to evaluate the effect of plant extracts on these. This would help in identifying the spectrum of activity of the extracts as well as determining their mechanism of action.

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