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In vitro anthelmintic potential of *Vernonia amygdalina* and *Secamone africana* on gastrointestinal nematodes

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ABSTRACT

In-vitro studies were conducted to determine the anthelmintic activity of ethanolic and water extracts of *Vernonia amygdalina and Secamone africana* used by agro-pastoralists in semi-arid land Uganda. The plant materials were collected from Nakasongola district and extracted using 70% ethanol and water. Efficacy and potency of crude extracts was determined using extracts' serial dilutions ranging 4mg/ml to 64mg/ml parallel to serial dilutions of albendazole ranging: 6.25-100mg/ml in three replicates. *Ascaris suum* model was used for the assays. Standard phytochemical methods were used for qualitative phytochemical analysis. The plants' extracts caused a dose-dependent motility inhibition with highest concentration of both ethanolic and water extracts of *V.amygdalina* causing 90% inhibition compared with 100% inhibition caused by albendazole. The corresponding median effective doses of ethanolic, water and albendazole were 5.94mg/ml, 13.70mg/ml and 15.12mg/ml respectively and significantly differed ($F_{(2, 53)}$ =257.43, p=0.001). There was a significant difference in motility inhibition in all dose levels ($F_{(5, 53)}$ = 14.01, P = 0.001; R^2 = 0.93). Similarly, the water and ethanolic extracts of *S.africana* caused a 93.3% and 80% motility inhibition with median effective doses of 40.08mg/ml and 25.41mg/ml respectively which also significantly differed ($F_{(2, 53)}$ =183.26, p= 0.001). There was also a highly significant difference in motility inhibition in all dose levels ($F_{(5, 53)}$ = 8.00, P = 0.001; R^2 = 0.92). The phytochemical screening revealed presence of tannins, alkaloid, saponins, flavonoids, steroids glycosides, triterpenes, coumarin derivatives, anthocyanocides, anthracenocides, and reducing sugars. In conclusion, *V.amygdalina* and *S.africana* are potential sources for novel antihelmintics and the secondary metabolites present justify their ethno-veterinary use.

Keywords: Helminths, motility inhibition*, Ascaris suum,* Nakasongola

INTRODUCTION

Traditional use of medicinal plants in treatment of animal and human diseases is gaining global interest very fast. In Uganda, rural and urban communities have continued to use or consult medicine-men for remedies to a variety of diseases. Helminths infections are one of the major health conditions affecting humans and livestock although are some of the diseases that herbalist have confident in treating of which they have continued to claim effective. *Secamone africana* (Oliv.) Bullock (Ascleradaceae) locally called "Akatakura in Luganda" and *Vernonia amygdalina Del* (*Asteraceae*) locally called "Omululuza in Luganda" are some of the most used plants in treating gastro-intestinal parasites of both humans and livestock (Nalule *et al*., 2011a).

Vernonia amygdalina commonly called bitter leaf is a shrub of 2-5 m tall sometimes a tree to 10 m that grows in secondary scrub, forest edges, thickets and invades cultivated areas (Katende *et al*., 1995). In Uganda, the plant is traditionally for medicine, nutriceutical and construction purposes (Tabuti, 2009) as well as for increasing uterine contraction during child birth in human (Kamatenesi and Oryema-Oringa, 2007). The cold leaves water extract is characteristically very bitter, though the bitterness reduces on boiling (Burkill, 1985). In West and East Africa, *V. amygdalina* is used in treatment of constipation, fever, gastro-intestinal parasites (Nalule *et al*., 2011a), urinary tract inflammations and as a purgative (Akinpelu, 1999) and as soup vegetable (Aregheore *et al*., 1998; Yeap *et al*., 2010). Both water and alcoholic extracts of the stem, bark, roots and leaves are used as a purgative, antimalarial and

for treatment of eczema (Kupcham, 1971). In western Kenya, the leaf concoction is used to treat local chicken of *Ascaridia galli* (Siamba *et al*., 2007). In Tanzania, the local people use the plant to malaria fever, stomache, schistosomiasis, amoebic dysentery, and other intestinal parasites (Huffman and Seifu, 1989). It has also been reported to significantly reduce glucose levels in diabetic patients (Akah and Okafor, 1992; Asuquo *et al*., 2010). Huffman, 2003 reported the wild chimpanzees use the bitter leaf to relieve stomach pain probably involving internal parasites. Anti-malarial and antihelminthic properties (Abosi and Raseroka, 2003); anti-tumour properties (Izevbige *et al*., 2004) and hepato-protective activity Arhoghro *et al*., (2009); Ojiako and Nwanjo 2006) and Anti-bacterial (Hamil *et al*., 2003) have been demonstrated. Pig farmers use the plant to treat pigs' intestinal parasites (Engel, 2007). The roots of *V. amygdalina* have been used for treatment of gingivitis and toothache (Elujoba *et al*., 2005).

Secamone africana is a lianar that climbs on trees and bushes; has smooth leaves and produce milky latex on damage. *S. africana* is common around Lake Victoria area and in Central Africa it can easily be confused vegetatively with *Secamone afzelii,* the most common *Secamone* species in West Africa (Kémeuzé, 2010). The plant has been widely used in traditional folk medicine in many parts of Uganda (Tabuti, 2009). The leaves and stems are used as anthelmintic and purgative (Nalule *et al*., 2011a) and for treatment of hypertension in humans (Hamill *et al*., 2003). In Ivory Coast, the leaves are used to maintain pregnant women till childbirth, and for treating swellings in children (Zabri *et al*., 2008). The leaves or leafy twigs of a related species *S. afzelii* are used in West Africa to treat or act as a quick-acting but gentle laxative, treat colic and oedema (Kémeuzé, 2010). In Côte d'Ivoire a leaf infusion is also taken as an antispasmodic and treatment of diarrhoea and excessive purging caused by *Anchomanes difformis* (Blume) Engl. (*Araceae*) (Kémeuzé, 2010). It is therefore likely that *S.africana* could provide similar roles although there are limited experimental studies and documentation on the claims.

Despite several reports on the traditional medicinal use of *V.amygdalina* and *S.africana,* experimental reports on anthelmintic efficacy are limited or varied, yet communities continue to claim efficacy. Different studies employing different parasites and methods have revealed different efficacies. Thus one study cannot be used to generalise recommendation on the anthelmintic activity of a single plant species. This study was undertaken to determine *in vitro* anthelmintic activity of ethanolic and water crude extract on nematode helminths using *Ascaris suum* model and to determine its qualitative phytochemical composition. The study contributes to the knowledge base of *materia medica* and strategies for sustainable animal health management and the well-being of people whose livelihoods are livestock based industries.

MATERIALS AND METHODS

Collection and preparation of plant materials: The whole aerial plant parts of *S. africana* and the leaves of *V. amygdalina* were collected from Nakasongola District of Uganda basing on the study conducted between January and March 2010 (Nalule *et al*., 2011a). The plants were ranked by the agro-pastoralists in the district as the most preferred and efficacious. The plants were identified by a botanist in Department of Botany Herbarium of Makerere University. They were sorted out of any extraneous material before they were air dried at room temperature for ten days. On drying all samples were milled into powder, kept in tight lidded containers.

Dosage adopted by community: The amounts of the plant parts used by the community were collected from five individuals. The flesh plant part amounts of each individual were weighed and weights recorded. The individual materials were oven dried at 60^0C and thereafter re-weighed and the mean weights recorded. The amount of the water solvent used by the community for extracting active ingredient was considered and this was considered in dosage determination.

Extraction of crude plant active ingredients and extraction efficiency determination: Two hundred fifty grams (250g) of dry plants material were macerated in 2000ml of 70% ethanol for 72hours with intermittent shaking in duplicates. Filtration through cotton wool was done to remove coarse particles and finely through filter paper (Whatman®, England). The filtrate was concentrated on Rota-vapor type Buchi-R, Switzerland under reduced pressure at 40^0C. The extracts were transferred to previously weighed kidney and petri dishes and put into an oven to dry completely at 50^0C to produce solid materials. The mean yield of the duplicate samples were determined and recorded. Thereafter dried extracts were packed into universal bottles and kept at 4^0C till needed for bioassay tests.

Similarly, 250g of fresh dried materials of the study plants were soaked in 2liters of distilled water with intermittent shaking for 72 hours. Thereafter, filtering was done to remove coarse material first with cotton wool and finely with Whatman filter paper (12.5mm). The filtrate was concentrated under reduced pressure in a rotar evaporator as above. The concentrated filtrates were then evaporated to dryness in an oven at 50^0C and yield recorded. The water extracts were used shortly after drying to avoid spoilage since it was not freeze dried.

Collection and maintenance of *Ascaris suum:* Adult Ascaris suum worms were collected from small intestines of pigs obtained from Wambizi slaughter house in Kampala. Immediately after slaughter, adult worms were collected and transported in flask containing Goodwin's solution Lamson and Brown, 1936; Donahue *et al*. 1981) at about 37^0C to the pharmacology laboratory, School of Veterinary Medicine, Makerere University. The active worms were selected and washed in warm water at 37^0C and maintained in Goodwin's solution at 37^0C before setting the experiment.

Effect of Plant Extracts on Adult Worms: Motility inhibition test was selected due to its suitability for use in field or laboratory settings and ease of parasite identification as well as previous reports of its application to detect resistance to both the benzimidazole and macrocylic lactone drug groups (Gill *et al*., 1991). In preliminary experiments, a Criteria used for assessing the effects of crude plant extracts on the motility of adult *Ascaris suum* was developed and combined the procedures described by Kotze *et al*. (2004); Paolini *et al*. (2004) and Marie-Magdeleine *et al*. (2009).

Helminth motility inhibition assay experimental design: Eighteen (18) sets of 250ML conical flasks were grouped into six groups with three replicates each for each plant species. The dosage was adjusted following the preliminary study results and the plant yields. To each of the three flask of group one, 100ml of Goodwin's solution was added to act as negative control. To groups 2-6 serial dilutions of *S. africana* ethanolic crude plant extract ranging 4 to 64mg/ml were added. A similar set was prepared for *V. amaygdalina* ethanolic extract. In parallel, 18 flasks were also divided into six groups to cater for the negative control and the five level serial dilutions (concentrations from 6.25 to 100mg/ml) of positive control where *Albendazole* (Valbazen®) 10% was used. At the end of the experiment, the procedure was repeated with serial dilutions of water crude extract also ranging 4 to 64mg/ml of *S. africana* and *V. amaygdalina* respectively. The lowest dose level represents half of the community adopted dosage and on extraction efficiency. Stock solutions were prepared by dissolving a weighed amount of extract in 10ml of dimethyl sulfoxide (DMSO) then diluted by Goodwin's physiological solution to 600ml mark to make the highest concentration (mg/ml) with the same solution. The volumes in each of the flask were made to 100ml mark of calculated dose to fully submerge the heavy parasites. Ten average size motile adult worms were randomly placed in each of the flask. The flasks and their contents were incubated at 37^0C and checked for motility at 24 and 48hours during which all the parasites in each flask were assessed for paralysis, death or motility (active) and recorded. A motility index was calculated as the ratio between the numbers of immotile worms/total number worms in each flask of the 3 flask replicates per concentration.

A worm was considered to be motile if it moved in a sinusoidal motion when stimulated by water at 50°C. Similarly it was considered paralyzed if on stimulating it by water at 50^0C only part of the body responded either by raising the head and whether some parts showed autolysis and change of colour to pale white. Motility was also assessed using water at 50 - 60^0C. Death of worms was ascertained by the absence of motility for an observation period of 5-6 seconds. To differentiate dead from paralysed the worm were dipped in water at 60^0C for 30 seconds that would be followed by sudden regaining of motility for those paralyzed while the dead ones do not respond. The number of motile (alive) and immotile (dead/or paralysed) worms were counted and recorded for each concentration. A mortality index was calculated as the number of dead worms divided by the total number of worms per flask.

Preliminary phytochemical screening: The water and ethanol extracts were qualitatively phytochemically analyzed using the standard methods described by Cieule (1964); Harbone (1973); Mojab *et al*. (2003) and Tchamadeu *et al*. (2010) for presence of alkaloids salts (Meyer's and drangedorffs test), tannins (Styassny's reagent), saponins (foaming test), flavonoids (Shibata's reaction), reducing sugars (Fehling's tests), anthracenocides (Borntagen's reaction), coumarins (colour fluorescence under UV light), glycosides and triterpenoids (Liebermann-Burchard's test) and anthocynosides .

Data analysis and determination of median effective dose (ED_{50}): The bioassay data was analyzed by the Generalised Linear Model procedures for regression, Nonlinear regression curves of treatments are defined as; Percentage motility inhibition (Y) = A + C/(1 + EXP(-B*(X – M))).

Where; Y is proportion of worm motility inhibited by ethanol, water extracts and albendazole. A is Y intercept; C is the top – bottom of the curve i.e X=0 and X= maximum; B is a rate constant expressed as reciprocal of X; M is random error and X is the dose of treatment (ethanolic, water and albendazole). ED_{50} determination and percent mean comparisons was carried out using Bonferroni test in the Graph Pad Prism version 5.01 software (Inc San Diego, CA USA) and Genstat 13th edition, VSN (www.genstat.co.uk). The soft wares were used to determine the means of percent motility inhibition, regression equations and 95% confidence intervals (CI) and to generate the dose-response curves. Two-way analysis of variance was carried out and P value < 0.05 was used for significance level.

RESULTS

Community adopted dosage and extraction efficiency of the plants: Community adopted dosage, amount of solvent used and extraction efficiency of *Vernonia amygdalina* and *Secamone africana* in 70% ethanol and water solvents are given in Table 1.

Table 1 Community adopted dosage and extraction efficiency (yield) of the plant species in water and 70% ethanol solvents (g/250g of dry plant material)

Plant name	Community dry weights used (g) Mean ± SEM	Volume of solvent used by community	Volume of crude extract administered*	Water extract yield (g) Mean ± SEM	Ethanol extract yield (g) Mean ± SEM
S. africana	66.30 ± 6.08	1L	0.3 - 0.5L	25.00 ± 1.67 [a]	52.05± 6.95 [a]
V.amygdalina	65.52 ± 8.39	3L	0.75 - 1.5L	39.17 ± 2.50 [b]	37.45± 3.95 [b]

*Lower amount for calves and small ruminants. All values represent mean ± standard error of means (SEM); Comparison for significance between the solvents was done using paired sample t-test. [a-b]Means with same superscript in a row are not significant (p> 0.05)

In vitro anthelmintic activity of ethanolic and water extracts of the plants

Vernonia amygdalina: After 48 hours of exposure of adult *Ascaris suum* to different concentrations of plant extracts, both *ethanolic and* water extracts of plants produced high worm motility inhibition proportions that were dose-dependent compared to the negative control group. The 48 hour percent motility inhibition by different concentrations of both water and ethanolic extracts of *V.amygdalina* compared with negative control is given in Table 2. The dosage adopted by the community also inhibited worm motility by 23.33 ± 3.33 of the parasites by 48 hours post treatment.

There was a significant difference in all dose levels of both plant extracts on motility inhibition that was dose-dependent irrespective of solvent used when compared with negative control observed using generalized linear model ($F_{(5, 53)} = 14.01$, $P = 0.001$; $R^2 = 0.93$). There was a significant difference in potency of *V.amygdalina* extracts from the different solvents used in extraction of active ingredients and albendazole ($F_{(2, 53)} = 257.43$, $p=0.001$). However, comparing the crude extracts on worm motility with 10% albendazole a conventional anthelmintic drugs commonly used in the study area positive control, there was no significant difference ($p > 0.05$).

The dose response-curve of the ethanolic and water of *V. amygdalina* crude extracts and albendazole revealed that the ethanolic extract was more potent than the water extracts compared with albendazole as demonstrated by the shift of the ethanolic extract curve to the left (Figure1).

Table 2: Effect of concentration of crude extracts of *Vernonia amygdalina* on motility of *Ascaris suum* 48 hours post treatment

Treatment	Dose mg/ml*	% Motility inhibition Mean ± SEM	95%confidence interval	
			Lower bound	Upper bound
Ethanolic extract	0.00 4.00 8.00 16.00 32.00 64.00	0.00 ± 0.00 16.67 ± 6.67 23.33 ± 3.33 63.33 ± 3.33 86.67 ± 8.82 90.00 ± 5.77	-12.24 1.09 11.09 71.09 74.43 77.76	12.24 25.57 35.57 95.57 98.91 102.24
Aqueous extract	0.00 4.00 8.00[cd] 16.00 32.00 64.00	0.00 ± 0.00 0.00 ± 0.00 23.33 ± 3.33 80.00 ± 8.82 83.33 ± 5.77 90.00 ± 5.77	-12.24 -12.24 11.09 67.76 71.09 77.76	12.24 12.24 35.57 92.24 95.57 102.24
Albendazole	0.00 6.25 12.50 25.00 50.00 100.00	0.0 ± 0.00 30.00 ± 10.0 46.67 ± 12.20 76.67 ± 8.82 90.00 ± 5.77 100.00 ± 0.00	-8.91 17.76 34.43 64.43 77.76 87.76	8.91 42.24 58.91 88.91 102.24 112.24

*Three replicates per treatment dose; Number of worms used, N = 10; [cd] dose adopted by community; Goodwin's solution used in negative control

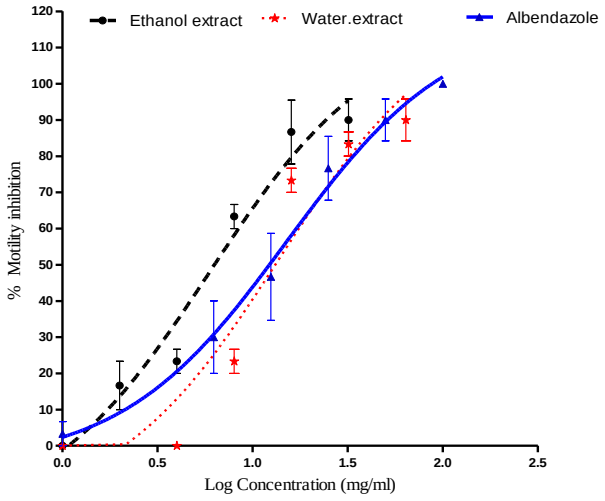

Fig 1: **Dose-response curves of adult *Ascaris suum* motility inhibition by ethanol and water crude extracts of *Vernonia amygdalina* 48 hours post treatment.**

Secamone Africana: The extracts of *S.africana* produced high worm motility inhibition proportions that were dose-dependent compared to the negative control group. The percent motility inhibition by different concentration of water and ethanolic extracts of *Safricana* are given in table 3. The highest concentration of the water and ethanol extracts inhibited 93.3% and 80% respectively compared with negative control. The dosage adopted by the community inhibited worm motility by 13.33 ± 8.82 percent of the parasites.

The dose response-curve of the ethanolic and water of *S.africana* crude extracts and albendazole revealed that the water extract was more potent than the ethanolic extracts compared with Albendazole as demonstrated by the shift of the water extract curve to the left of ethanolic extract (Figure 2).

Table 3: Effect of concentration of crude extracts of *Secamone africana* on motility of *Ascaris suum*48 hours post treatment

Treatment	Dose mg/ml*	% Motility inhibition Mean ± SEM	95% Confidence interval	
			Lower bound	Upper bound
70% Ethanol	0.0	0.00 ± 0.00	-11.60	11.60
	4.0	13.33 ± 3.33	1.73	24.93
	8.0	23.33 ± 3.33	11.73	34.93
	16.0	36.67 ± 3.33	25.07	48.27
	32.0	73.33 ± 6.67	61.73	84.93
	64.0	90.00 ± 6.67	78.40	101.60
Aqueous	0.0	0.00 ± 0.00	-11.60	11.60
	4.0	6.67 ± 6.67	-4.93	18.27
	8.0[cd]	13.33 ± 8.82	-2.24	22.24
	16.0	56.67 ± 6.67	45.07	68.27
	32.0	73.33 ± 3.33	61.73	84.93
	64.0	93.33 ± 6.67	81.733	104.93
Albendazole	0.00	0.0 ± 0.00	-8.91	8.91
	6.25	30.00 ± 10.0	17.76	42.24
	12.50	46.67 ± 12.20	34.43	58.91
	25.00	76.67 ± 8.82	64.43	88.91
	50.00	90.00 ± 5.77	77.76	102.24
	100.00	100.00 ± 0.00	87.76	112.24

*Three replicates per treatment dose; Number of worms used, N = 10; [cd] dose adopted by community; Goodwin's solution used in negative control

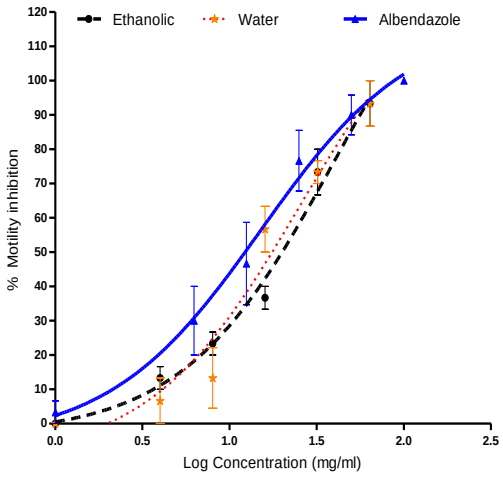

Fig 2: Dose-response curves of adult *Ascaris suum* motility inhibition by ethanol and water crude extracts of *Secamone africana* 48 hours post treatment

There was also a significant difference motility inhibition in all dose levels of both plant extracts that was dose-dependent irrespective of solvent used when compared with negative control observed using generalized linear model ($F_{(5, 53)} = 8.00$, $P = 0.001$; $R^2 = 0.92$). There was also a significant difference in potency of ethanol and water extracts of *S.africana* and albendazole ($F_{(2, 53)} = 183.26$, $p = 0.001$). However, comparing the crude extracts on worm motility with 10% albendazole there was no significant difference ($p > 0.05$). A weak interaction between dose effect and the solvent used in extraction of active ingredients on motility inhibition was also observed ($F_{(10, 53)} = 2.657$, $p = 0.015$; $R^2 = 0.93$).

The median effective doses of the extracts compared with albendazole: The study revealed variation in median effective doses of the plant extracts. The median effective doses (ED_{50}) of *V. amygdalina* and *S.africana* ethanol and water extracts are given in Table 4. *V.amygdalina* ethanol extract was more potent than water extract while the water extract of *S. africana* was more potent than ethanol extract. The two plant species ED_{50} were also different.

Table 4: The median effective doses (ED_{50s}) of ethanolic and aqueous extracts of the crude plants' extracts 48 hours post treatment

	Ethanolic extract		Water extract	
Plant species	**ED_{50} mg/ml**	**95% CI of ED_{50}**	**ED_{50} mg/ml**	**95% CI of ED_{50}**
Secamone africana	40.08	19.18 - 83.72	25.41	10.28 - 62.80
Vernonia amygdalina	5.94	2.56 to 13.80	13.70	5.87 - 32.00

Phytochemical screening of chemical ingredients: Chemical analysis of ethanolic and water extracts of *S. africana* and *V. amygdalina* revealed presence of a number of secondary plants metabolites whose intensity varied with solvent used (Table 5). However, *S. africana* ethanol extract lacked anthocyanosides while the water extract lacked saponins. Anthracenocides were weakly detected in ethanol extract of *S. africana* while flavonoids were weakly detected in *V. amygdalina* extract. On the other hand, reducing sugars test was weakly reactive in water extract of *V. amygdalina.*

Table 5. Phytochemical constituents of water and ethanol extracts of the five anthelmintic plants

Compound	*S. africana*		*V.amygdalina*	
	Water	Ethanol	Water	Ethanol
Tannins	++	++	++	++
Reducing sugars	++	++	+	++
Saponins	-	x	+++	x
Alkalloid salts	++	++	++	++
Anthracenosides	++	+	++	++
Coumarin derivatives	++	++	++	++
Flavonosides	++	++	++	+
Steroid glycosides	++	++	++	++
Triterpenes	++	++	++	++
Anthocyanosides	++	-	++	++

Legend: (+) weakly present, (++) moderate, (+++) strongly present, (-) absent or undetected, (x) not tested in ethanol extract

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

DISCUSSION

The results of this study showed that *V.amygdalina* and *S.africana* have higher anthelmintic potential. The results of this study support the claims by the Nakasongola agro-pastoral farmers and previous reports that these plants treat helminths infections in livestock (Wasswa and Olila, 2006; Nalule *et al.,* 2011). However, the community dosage effect was low by both plants probably indicating under dosing. This probably explains the persistent worm burden despite use of these herbs. This may lead to worm resistance.

Both the ethanolic and water extracts of *V. amygdalina* and *S. africana* inhibited *Ascaris suum* motility in a dose dependent response by paralyzing them or causing their death by 48 hours (table 2 and table 3) compared with albendazole. It was also evident that *S.africana* was less potent than *V.amygdalina* extracts, an activity that could be

attributed to the lack of saponins and the anthocyanosides (Table 5) despite high yield in ethanol and water solvents.

The median effective dose (ED_{50}) of ethanolic and water crude extracts of *V. amygdalina* and *S. africana* varied with solvent used in extraction of active ingredients with ethanolic extract of *V. amygdalina* and water extract of *S.africana* being the most potent despite comparable efficacies (Table 5). This could probably be related to the different chemical ingredients extracted in the different solvents and their biological effects on parasites. The variation in potency may also be attributed to source of parasites and previous exposure to the plants. Similar variation in potency and efficacy were observed by Gakuya (2001) and Costa *et al*. (2008) when they used different solvents for extraction of active ingredient and observed varying bioactivity results. Similarly, Tuwangye and Olila (2006) used methanol to extract *V. amygdalina* for anthelmintic bioassay and achieved 50% kill at 6mg/ml and ED_{50} of 3.533 mg/ml, all different from the findings of this study. The study showed that efficacy of extracts increased with increasing concentration of extract. Increasing motility inhibition with increasing concentration could be due to the saturation of target receptors. Similar observation were made by Lullman *et al*. (1993) who said that the receptors get saturated with increasing dose of active ingredient that increases with incubation period. It is likely that at higher concentration all binding receptors on the worms were occupied thus leading to hyperpolarisation of membranes limiting excitation and impulse transmission causing flaccid paralysis of worm muscles, a similar observation made by Wasswa and Olila, (2006). This study revealed that despite the community having knowledge on use of the plants for helminthes control, they under dose the parasites. The low dose adopted by the agro-pastoral community could be responsible for the persistent helminths infections and low livestock productivity. Continued administration of low doses of plant crude extract to parasites may lead to risks of development of helminths resistance from repeated exposure to low doses.

The antihelmintic property of *V. amygdalina* was also observed when the juice from the leaves was found to reduce mixed infection worm egg production in goats by 64% using fecal egg reduction count test (Nalule *et al*., 2011b). Nfi *et al.* 1999 reported anthelmintic efficacy of *V. amygdalina* in ethno-veterinary to be 52.4%. Alawa *et al*. (2010) when used *V.amygdalina* water extract at a dose concentration of 1.1g/kg body weight on calves naturally infected, achieved 59.5% reduction in eggs per gram (EPG) of faeces. However, Alawa *et al.* (2003) previously reported that the hot water extract did not possess anthelmintics effect *in vitro* fecal culture of eggs at concentrations up to 11.2mg/ml against *Haemonchus contortus* and *Trichostrongylos colubriformis* and the author attributed it the high temperature subjected to the extract that could have degraded the active compounds in the leaves. A study by Huffman and Seifu, (1989) reported that the feacal egg count reduced from 130 to 15 within 24 hour post Chimpanzee chewing of the *V. amygdalina* pith. A study by Molgaard *et al*. (2001), using water extract of leaves, stem, root and root bark of *V. amygdalina* were able to kill cestodes of *Hymenolepis diminuta* after 24 hours of treatment. A recent study by Ademola and Eloff (2011), revealed a significant effects on different development stages of *Haemonchus contortus* that varied with solvent used for extraction of active ingredient.

The anthelmintic properties of *V.amygdalina* and *S.africana* crude extracts could be attributed to the variety of secondary metabolites present. Preliminary phytochemical screening of the plants extracts revealed presence of tannins, alkaloid salts, glycosides, triterpenoids, flavonoids, anthracenoides, anthracyanins, coumarin derivatives and saponins whose intensity varied among the ethanolic and water extracts (Table 5). Notwithstanding, Waterman (1992) reported that plant metabolites are unstable molecules and their biological activity are dependent on their structure, physical and chemical properties.

It is therefore possible that the parasite paralysis and/or death observed may have been attributed to secondary metabolites (Makut *et al.,* 2008) like tannins, alkaloids salts and saponins among others. These plant metabolites may have worked singly or in combination to cause the motility inhibition, paralysis or death of the worms that was achieved in all the studied crude extracts. Kaufman *et al*. (1999) accredited the synergistic interactions to underlie the effectiveness of phyto-medicines that lead to better activity of some individual constituents. Briskin (2000) and Wynn and Fougere, (2007) acknowledged that the plant metabolites action may be additive, synergistic or antagonistic in manner acting at single or at multiple target sites. It is therefore likely that a number of compounds could have contributed to the anthelmintic activity observed in both plants'extracts. However, Engel (2007) reported that *V. amygdalina*

contain seven steroid glycoside as well as four sesqueterpene lactones that could be attributed to the killing of the parasites that cause schistosomiasis, malaria and Leshmaniasis. In addition, the plant was reported to have high levels of vernoniaside B1 that may be toxic to animals (Engel, 2007). It is therefore likely that the worms in this study could have been killed by these compounds.

Nevertheless, it is well documented that some anthelmintic drugs like the benzimidazoles (BZD) kill the parasites by binding to a specific building block, the beta tubulin and prevent its incorporation into micro-tubules which are essential for energy metabolism (Schoenian, (2008). Barrowman *et al*. (1984) also reported that Benzimidazole anthelmintics act by interfering with the microtubule system in *Ascaris suum*. Thus, these compounds could have caused their effect through the same mechanism. Paralysis of worm tissues makes them unable to feed leading to death as result of lack of energy. It is also likely that alkaloids present in the plants could also have contributed to the paralysis and consequent death of the worms. The nematocidal activity of alkaloids had also been demonstrated by Satou *et al.* (2002) when they used two rat nematodes; *Strongyloides ratti* and *S. venezuelensis* models for human nematodes. Alkaloids salts on the other hand are competitive antagonists at muscarinic acetylcholine receptor preventing the binding of acetylcholine and are reportedly physiologically active with sedative and analgesic properties in addition to leading to excitation of cells and neurological dysfunction (Tarnopolsky and Beal, 2001). On the other hand the saponins present in the crude extract of *V.amygdalina* could have caused feed refusal and starvation of the parasites leading to their death from lack of energy thus the variation in potency compared with *S.africana* that lacked saponins. There is however, no literature on *S.africana* anthelmontic study for comparison with this study finding. Similar views on saponins' effects on feeding were held by Dalsgaard *et al*. (1990) and Francis *et al.* (2002) who also reported that saponins kill protozoans and mollusks. It is also probable that *in vivo* paralysis lead to loss of grip of parasites on the gut wall leading to the spontaneous expulsion of parasites together with feaces.

The roles of tannins in helminths control have been documented (Athnasiadou *et al*., 2001a; Molan *et al*., 2003a; Hoste *et al*., 2006; Cenci *et al*., 2007; Kotze *et al*., 2009; Forbey *et al*., 2009). The nematocidal activity of tannin extracts has also been reported with evidence of anthelmintic properties of condensed tannins by series of in vitro studies (Dawson *et al*., 1999; Athanasiadou *et al*., 2001a; Molan *et al*., 2003b; Ademola and Idowu, 2006) and in vivo studies (Athanasiadou *et al*., 2000; Butter *et al*., 2001; Paolini *et al*., 2003a and 2003b; Kotze *et al*., 2009). Chemically tannins are polyphenolic compounds (Bate-Smith, 1962) and synthetic phenolic anthelmintics like niclosamide and oxyclozanide are said to interfere with energy generation in helminths parasites by uncoupling oxidative phosphorylation (Martin, 1997). It is possible that tannins contained in ethanol and water extracts of plants produced similar effects. It was also suggested that tannins bind to free proteins in the gastrointestinal tract of host animal (Athnasiadou *et al*. 2001b; Hoste *et al*., 2006) or glycoprotein on the cuticle of the parasite disturbing the physiological functions like motility, feed absorption and reproduction (Thompson and Geary, 1995; Aerts *et al*., 1999; Githiori *et al*. 2006) or interference with morphology and proteolytic activity of microbes (Min *et al*., 2003; Waghorn and McNabb, 2003) and cause death.

The study further showed variation in potencies based on the ED_{50s} obtained despite the many compounds that were extracted in the different extracts of the two plants. It is likely, only a few could be active as anthelmintics. The compounds could be specific to each plant structurally as an isomer or derivatives of the bigger molecules and their concentration in different plant species and plants parts could be different. Similar observations were made by Waterman *et al*. (2010). It is likely that the solvent used in extraction of active ingredients could be responsible for the crude extract potency. This is in agreement with observations made by Malu *et al*. (2009).

CONCLUSION AND RECOMMENDATION

The overall findings of the study showed that ethanolic and water extracts of *S. africana and V. amygdalina,* exhibit evidence of *in vitro* anthelmintic activity against *Ascaris suum* revealing the anthelmintic potential justifying their traditional ethno-veterinary use by the pastoral communities in the cattle corridor of Uganda. However, potency of plant extract was dependent on the solvent used to extract the active ingredients. Nevertheless, the community adopted dosage explicitly fell far below the required quantity to produce beneficial helminths control. Further studies are needed to determine, bioactivity

in vivo and against other developmental stages of parasites in addition to determining the toxic effect in animals, bioavailability and bio-acceptability. Feasibility studies would give more insight into the recommendation for broader use or integrated into the current clinical treatments. There is also need to standardize and improve on the community adopted dose through intensive sensitization of stakeholders to harmonise the traditional knowledge and the scientific inference.

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