Full Length Research Paper

In vitro anthelmintic potential and phytochemical composition of ethanolic and water crude extracts of *Euphorbia heterophylla* Linn.

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In vitro studies were conducted to determine the anthelmintic activity of ethanolic and water extracts aerial whole plant parts of *Euphorbia heterophylla.* Efficacy and potency of crude extracts was determined using 70% ethanol and water extracts in serial dilutions: 3 mg/ml to 64 mg/ml parallel to serial dilutions of albendazole 6.25 to 100 mg/ml in three replicates. *Ascaris suum* was used for the assays. The phytochemical screening of the extracts were carried out using standard laboratory methods. Both crude extracts of *E. heterophylla* and albendazole reduced worm motility by 100% in 48 h post treatment in a dose-dependent response when compared with negative control with median effective dose being 26.85 mg/ml, 4.60 mg/ml and 15.12 mg/ml respectively. All dose levels of *E. heterophylla* extracts caused a significant adult worm motility inhibition ($F_{(5, 53)}$ = 4.41, P= 0.003; R^2 = 0.92). A significant difference in motility inhibition by the ethanolic, water extracts and albendazole treatments as measured by median effective doses of *E. heterophylla* ($F_{(2, 53)}$ = 140.43, p= 0.001) was observed although water extract effect did not differ from albendazole effect (p= 0.878). Phytochemical screening revealed presence of tannins, alkaloid, saponins, flavonoids, steroids glycosides, triterpenes, coumarin derivatives, anthocyanocides, anthracenocides and reducing sugars whose intensity varied with solvent used for extraction. The study revealed the anthelmintic potential of *E. heterophylla* and that water extract was more potent than ethanolic extract. The phytochemical compounds present justify the plant's ethno-veterinary use although *in vivo* efficacy evaluation and toxicity studies need to be carried to ascertain their bioavailability and safety to the animals.

Key words: *Ascaris suum*, medicinal plants, motility inhibition, Nakasongola.

INTRODUCTION

Helminthes infections remain a big challenge both in developed and developing countries because of their chronic debilitating nature and their epidemiological characteristic of continuous contamination of environment.

Helminthes infections are the most neglected among the healthcare systems. In developing countries, the disease may be attributed to lack of resources to regularly deworm affected individuals in addition to development of

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parasite resistance to conventional drugs resulting from poor use of drugs. Moreover, parasites infections are likely to increase in the face of climate change (Weaver et al., 2010; Tinsley et al., 2011). It is well documented that parasites undergo evolution to adapt to opportunities presented by climate change or anthelmintic use or undoubtedly as a manifestation of 'survival of the fittest' (Sargison et al., 2007; Davey et al., 2009). The different control strategies including the use of anthelmintics, grazing management and improvements in sanitation, are available for gastrointestinal nematode infections but these control methods are associated with many problems, such as development of resistance to the currently available chemotherapeutic anthelmintic drugs (Kaplan, 2004; Wolstenholme et al., 2004; Gasbarre et al., 2009). Ian et al. (2007) concluded that "whether poor or rich livestock farmers, depending on their production systems and market conditions, the value of the animals in question may not warrant the cost of the professional veterinary care and inputs".

Consequently rural communities resort to using medicinal plants to treat symptomatic clinical signs of which they have continued to claim effectiveness. Studies and field trials have been conducted in the region on plants used as anthelmintics and their reports give interesting reading (Mbaria et al., 1998; Bizimenyera et al., 2008; Gradé et al., 2008).

Previous studies indicated several plants in Uganda are used in treatment of livestock and humans helminths although some were considered more potent (Nalule et al., 2011). However, efficacies of the claimed potent plants have not been investigated to validate their traditional use as anthelmintics. One of such plant is *Euphorbia heterophylla* Linn (Euphorbiaceae or spurge family), commonly called milkweed (Parsons and Cuthbertson, 1992). The plant is native to Central and South America, but now widely distributed throughout the tropics and subtropics and is a common crop weed across the world (Parsons and Cuthbertson, 1992; Mosango, 2008). It occurs throughout most of tropical Africa and the Indian Ocean islands, as well as in the Mediterranean region and South Africa (Mosango, 2008).

The Ugandan agro pastoralists also use the plant to treat livestock and human constipation while the pigs feed on it (Tabuti, 2009). In West Africa and India, *E. heterophylla* and a related species, *Euphorbia hirta* are traditionally used to treat constipation, bacterial and inflammatory disease conditions such as arthritis and rheumatism (Ogueke et al., 2007; Falodun et al., 2008; Anilkumar, 2010; Karimi et al., 2010). In Africa a decoction or infusion of the stems and fresh or dried leaves is taken as a purgative and laxative to treat stomach-ache and constipation and to expel intestinal worms (Mosango, 2008). In Nigeria the latex and preparations of the leaves and root are applied to treat skin tumours, while in East Africa the roots are used in the treatment of gonorrhoea or to increase milk production in breast-feeding women (Mosango, 2008). In 2010, Oluduro and Olumide demon-

strated anti-typhoid activity of aqueous and methanolic leaf extracts. Treatment of diarrhea and dysentery especially amoeba have been reported in Africa. In Tanzania, the plant is used for fungal infection (Moshi et al., 2007). The plant is also reported to have diuretic and purgative action in addition to having anti inflammatory actions caused by asthma by causing bronchial relaxation (Johnson et al., 1999; Edeoga et al., 2005). Edeoga et al. (2005) further reported, the plant vegetable and latex are used in insect bites, treatment of erysipelas, treatment of cough, bronchial paroxymal asthma, hay fever and catarrh. *E. heterophylla* was reported useful in rheumatism, dropsy, gout, neuropathy, deafness and cough (Kirtikar and Basu, 1975). The plant leaves are used medically as laxative, anti gonorrhea, migraine and wart cures (Falodun et al., 2008; Omale and Friday, 2010) as well, the plant lattices are used as fish poison, insect repulsive and ordeal poisons (Rodriguez et al., 1976). Despite several reports on the traditional medicinal use of *E.heterophylla*, experimental reports on anthelmintic efficacy are limited. This study was therefore undertaken to determine *in vitro* anthelmintic activity of ethanolic and aqueous crude extract of whole aerial plant parts on gastrointestinal nematodes using *Ascaris suum* model and to determine its qualitative phytochemical composition.

MATERIALS AND METHODS

Collection of Plant Materials

The plant parts were collected from the Ugandan cattle corridor in Nakasongola District based on the study conducted between January and March 2010. Sample of the plant species used were collected and identified by a plant taxonomist and specimens were deposited under accession number-39698 at the Department of Botany Herbarium, Faculty of Science, Makerere University.

Dosage adopted by community

The amount of the plant parts used by the community was collected from five individuals. The fresh plant parts of each individual were weighed and weights recorded. The individual materials were oven dried at 60°C and thereafter re-weighed and the mean recorded. The amount of the water as 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 and fifty grams (250 g) of dry plants material were macerated in 2000 ml of 70% ethanol for 72 h with intermittent shaking in duplicates. Filtration through cotton wool was done to remove coarse particles (residues) and finely through filter paper (Whatman®, England). The filtrate was concentrated on Rota-vapor type Buchi-R, Switzerland under reduced pressure at 40°C. The extracts were transferred to previously weighed kidney and petri dishes and put in an oven to dry completely at 50°C 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°C till needed for bioassay tests.

Similarly, 250 g of fresh dried materials of the study plants were soaked in 2 L of distilled water with intermittent shaking for 72 h. Thereafter, filtering was done to remove coarse material first with cotton wool and finely with Whatman filter paper (12.5 mm). 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°C and yield recorded. The water extracts were used shortly after drying to avoid spoilage since it was not freeze dried.

Collection and maintenance of worms

Adult worms were collected from small intestines of pigs obtained from the Wambizi slaughter house in Kampala, Uganda. The adult worms were collected and transported in flask containing Goodwins solution to the Pharmacology Laboratory, School of Veterinary Medicine, Makerere University. In the laboratory, the active worms were washed in warm water at 37°C and placed in a clean flask where they were maintained in Goodwin's solution according to Lamson and Brown (1936); Donahue et al. (1981); Wasswa and Olila (2006) till setting of experiment.

Preparation of Goodwin's physiological solution

Goodwin's physiological solution was prepared from a number of chemical compounds including Calcium chloride (0.20 g), Glucose (5.0 g), Magnesium chloride (0.10 g), Potassium chloride (0.20 g), Sodium bicarbonate (0.15 g), Sodium chloride (8.0 g) and Sodium hydrogen phosphate (0.5 g). All were dissolved in 1000 ml of distilled water. Calcium chloride was added after dissolving other salts since it would precipitate other salts before they are dissolved. Glucose was added shortly before use to avoid fermentation and the solution was pre warmed to 37°C before putting in the warms.

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).

Bioassay experimental design

Eighteen (18) sets of 250 ml conical flasks were distributed into six groups in three replications each. To group one flask, 100 ml of Goodwin's solution was added to act as negative control. To groups 2 to 6 flasks, serial dilutions of ethanolic crude plant extract concentrations (ranging from 3 to 48 mg/ml) based on yield in 70% ethanol were added. In a parallel set up, 18 flasks were divided into six groups to cater for the serial dilutions (6.25 to 100 mg/ml) of positive control where 10% *Albendazole* was used. At the end of the experiment, the procedure was repeated with water crude extract instead of ethanolic extract with serial dilution concentrations (ranging from 4 to 64 mg/ml) dosage based on yield in water and dosage adopted by community. The lowest dose level being half of the community dose based on extraction efficiency. Stock solutions were prepared by dissolving appropriate weighed amount of extract in 5 to 10 ml of dimethyl sulfoxide (DMSO) and then topped to 200 ml mark to make the highest concentration (mg/ml) with Goodwins' physiological solution. The volumes were made to 100 ml mark with Goodwin's solution to fully submerge the heavy parasites. Ten average size motile worms were randomly placed in each of the flask. The flasks and their contents were incubated at 37°C and checked for motility at 24 and 48 h 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 immobile worms/total number worms in the 3 flask per concentration.

Phytochemical screening

The aqueous and ethanol extracts were qualitatively phytochemically analyzed using the standard methods described by Ciulei (1964); Harborne (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 ED_{50} of the extracts

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) = $A_{(1,2,3)} + C_{(1,2,3)}/(1 + EXP(-B_{(1,2,3)} \times (X - M(1,2,3))))$.

Where:
1,2,3 represent ethanol, water and albendazole graphs
Y is proportion of worm motility inhibited by ethanol, water extracts and albendazole.
A is Y intercept
C is the top to bottom of the curve,
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 softwares 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

Extraction efficiency of *E. heterophylla Linn* in water and 70% ethanol solvents

The mean dry weight of the whole aerial plant parts used by the community was established to be 56.26 ± 9.18 (g) which is boiled in 2 liters of water. The mean yields of 250 g of the plant dry material in ethanol and water solvents and their percent equivalent are shown in Table 1. There was a significant variation (p= 0.004) in the yields by the two solvents.

Table 1. Extraction efficiency (yield) of dry *E. heterophylla* in water and 70% ethanol solvents (g/250 g of dry whole aerial plant material).

Extract	Community adopted dry weights used Mean ± SEM(g)	Extract yield Mean ± SEM(g)	Yield efficiency (%)
Water	56.26 ± 9.18	56.67 ± 1.67[a]	22.7%
70% Ethanol	NA	38.80 ± 4.30[b]	15.6%

All values represent mean ± standard error of means (SEM); Comparison was done between the solvents used and values with different superscript are statistically significant (p< 0.05).

Table 2. Effects of crude extracts of *Euphorbia heterophylla* Linn on motility of *Ascaris suum* 48 h post treatment.

Method of extraction	Dose mg/ml*	% Motility inhibition Mean ± SEM	95% Confidence interval of the mean	
			Lower bound	Upper bound
70% Ethanol	0.00	0.00 ± 0.00	-8.14	8.14
	3.00	6.67 ± 3.33	-4.84	18.17
	6.00	20.00 ± 5.77	8.49	31.51
	12.00	33.33 ± 3.33	21.83	44.84
	24.00	96.67 ± 3.33	85.16	108.17
	48.00	100.00 ± 0.00	88.49	111.51
Aqueous	0.0	0.00 ± 0.00	-8.14	8.14
	4.0	40.0 ± 5.77	28.49	51.51
	8.0[cd]	56.67 ± 6.67	45.16	68.17
	16.0	70.00 ± 5.77	58.49	81.51
	32.0	76.67 ± 3.33	68.49	91.51
	64.0	100.00 ± 0.00	88.49	111.51
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.

Anthelmintic activity of ethanolic and water crude plant extracts

The 48 h exposure of adult *Ascaris suum* to different concentrations of both *ethanolic* and aqueous crude extracts of *E. heterophylla* produced high worm motility inhibition proportions compared to the negative control group. The 48 h percent motility inhibition by the different concentrations of plants extracts and albendazole are given in Table 2. The highest concentration of 48 and 64 mg/ml for ethanol and water extracts and albendazole gave maximum mean percent anthelmintic activity by 48 h as 100.00 ± 00.00% compared with negative controls. However, the community concentration equivalent (8.0 mg/ml) of water extracts inhibited motility of the worms by 56.67 ± 6.67%, 48 h post treatment.

A significant difference in all dose levels of all tested plants species on motility inhibition that was dose-dependent when compared with negative control was observed using generalized linear model; *E. heterophylla* ($F_{(5, 53)}$ = 4.41, p= 0.003; R^2 = 0.92). A significant difference in motility inhibition by the ethanolic and aqueous extracts of *E. heterophylla* and albendazole treatments as measured by median effective doses was observed ($F_{(2, 53)}$ = 140.43, p= 0.001) although water extract effect did not differ from albendazole effect (p= 0.878). There was however, no significant interaction between solvent of extraction and the dose effect on motility inhibition in *E. heterophylla* ($F_{(10, 53)}$ = 0.65, p = 0.762; R^2 = 0.92).

Table 3. The median effective doses (ED$_{50}$) of the ethanol and water extracts and albendazole.

Extract/treatment	Worm motility inhibition (%)	Median effective dose (ED$_{50}$) mg/ml	95% CI of ED$_{50}$
Ethanol	100	26.85[a]	10.09 - 71.45
Water	100	4.60[b]	2.37 - 8.92
Albendazole	100	15.12[b]	6.95 - 32.90

Comparison was done between the ED$_{50}$ of solvents used and values with different superscript are statistically significant (p< 0.05).

Table 4. Phytochemical constituents of water and ethanol extracts of the whole aerial parts of *Euphorbia heterophylla*.

Phytochemical constituents tested	Solvent used for extraction	
	Water extract	70% ethanol extract
Tannins	++	+
Reducing sugars	++	++
Saponins	++	x
Alkalloid salts	++	++
Anthracenosides	+	+
Coumarin derivatives	++	++
Flavonosides	++	+
Steroid glycosides	++	+
Triterpenes	++	+
Anthocyanosides	+	++

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

Median effective dose (ED$_{50}$) and Anthelmintic potency of plant crude extracts

The median effective dose (ED$_{50}$) (defined as the dose required to kill or inhibit motility of fifty percent of the test subjects), of the plant extracts and positive control are given in Table 3. The results showed that *E. heterophylla* crude water extract was more potent than the ethanolic extract and albendazole demonstrated by the shift of the graph to the left of both curves as illustrated in Figure 1 despite both achieving 100% kill of the parasites by the highest dose.

A$_1$, A$_2$, A$_3$ are parameter estimates for ethanol extracts, aqueous extracts and albendazole respectively given as 8.15, -9.9, 3.66 with S.E of 3.64, 26.5, 6.02 respectively. C$_1$, C$_2$, C$_3$ are also parameter estimates for the same treatments given as: 93.72, 206, 102.7 with S.E of 7.70, 3.34, 13.3 respectively. B$_1$, B$_2$, B$_3$ are given as, 4.94, 0.434 and 1.401with S.E of 1.86, 0.526, 0.404 respectively and M$_1$ M$_2$, M$_3$ = 2.6772, 3.91, 2.671 with S.E of 0.0958, 6.36 and 0.233 respectively. X = treatment doses (level 1 to level 6) for ethanol, aqueous and albendazole. Percentage variance accounted for is 92.4 with a standard error of observations estimated at 10.4. The error bars show the standard error of the percent worm motility inhibition.

Phytochemical analysis of *E. heterophylla*

The result of the qualitative phytochemical screening of *E. heterophylla* ethanolic and water crude extracts is presented in Table 4. However, the study showed that the concentration of some compounds varied with the solvent used.

DISCUSSION

This *in vitro* adult worm motility inhibition assay showed that *E. heterophylla* used in ethno-veterinary medicine could be of value in the treatment of livestock helminthosis irrespective of solvent used to extract the active ingredients. Extraction efficiency of ethanol and water was relatively high for both solvents (Table 1) demonstrating both solvents could equally be good for bioactive ingredient extraction. Although the yields were different, their biological activity did not display the same trend probably indicating the bulk of the extracts may not be biologically active. It is likely that the compounds determining yield are more soluble in ethanol than water solvents compared to compounds responsible for the bioactivity. This could be attributed to high solubility of reducing sugars that may not participate in the pharma-cological activities of chemical compounds. The
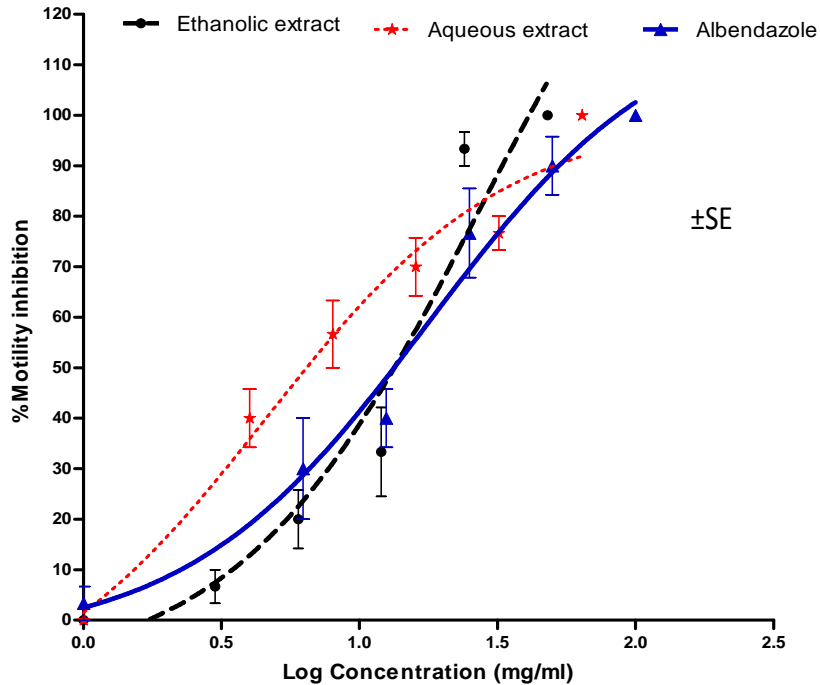
Figure 1. Dose-response curves of adult *Ascaris suum* motility inhibition treated with serial dilutions (3 mg/ml to 64 mg/ml) of water and ethanol crude extract of *Euphorbia heterophylla* compared with serial dilutions of *albendazole* (6.25 mg/ml to 100ml) in three replicates. N=10 *Ascaris suum.*

variations in yield probably indicate that different individual using the different solvents may achieve different control benefits. However, it also appears that different solvent determine the potency of different extract despite lack of significant difference in extraction efficiency.

The study indicated that *E.heterophylla* has anthelmintic potential against adult *Ascaris suum* as both ethanolic and water crude extracts were 100% effective in inhibiting worm motility. The fact that the crude extracts' effect were not different from the conventional drug on market shows the noble value of this plant. According to Vercruysse et al. (2001), development of new drug is only if the current drug kills less than 90% or 80% by WAAVP of the parasites as indication possibility of resistance. However, this study has shown that by using this plant crude extract, there would be no call for seeking for alternative drug.

The lack of significant difference in motility inhibition of the water extract compared with the Albendazole indicates the plant's unexploited potential as a noble anthelmintic. It is therefore, probable that a compound with similar mode of action to Albendazole (benzimidazole) may be present and may be more soluble in water than ethanol. This finding calls for more studies to identify the underlying active principles, mode of action and probably development of anthelmintic drug based on this plant for the benefit of poor societies. The study also revealed that the dose level given by the community was able to kill more than 50% of the parasites although still fell below the required dosage that would produce an effect close to the conventional drug dosage. However, the community probably uses that amount due to the fear that the plant may be toxic in higher doses. This also points to the fact that herbalist's recommend that the patient on the "drug" should drink a lot of water at least when used in human beings and should be taken mixed with food (Nalule et al., 2011). This calls for standardize and improvement of the dosage being guided by the scientific studies, to a dose that provide beneficial results while at the same time protects the animals.

The anthelmintic activity observed could be attributed to the secondary plant metabolites that may act in additive, synergistic or antagonistic manner at single or multiple target sites unlike the synthetic drugs as was recognized by Briskin (2000)**.** Makut et al. (2008) also acknowledged that the medicinal values rely on certain chemical substances that produce the physiological effect on the animal body.

Since the parasites were similarly affected by the both extracts, it is likely that some compounds in the extracts probably bound to the tubulin and inhibited the polymerization of tubulin into micro-tubulins as what happens in the benzimidazoles drugs. This is in agreement with findings of Barrowman et al. (1984) who reported that Benzimidazole anthelmintics act by interfering with the microtubule system in *Ascaris suum*. It has been recognized that animals take advantage

of secondary plant metabolites such as condensed tannins on biological activity to mitigate parasites infection and enhance reproduction (Forbey et al., 2009). Kaufman et al. (1999) accredited the synergistic interactions to underlie the effectiveness of phyto-medicines that lead to better activity as well as decrease potential toxicity of some individual constituents. However, it should be noted that susceptibility of parasites may vary according to species, development stage of parasites, strain or previous exposure to anthelmintics in addition to bioactive extraction methods. Ebrahimzadeh and Bahramian (2009) also noted differences in biological activity based on solvent used for extraction and the concentration of the extracts. Sonali and Shekhawat (2010) also observed that ethanol extract was more potent than the aqueous crude extract of the plant when they conducted an *in vivo* and *in vitro* antibacterial screening of *Anethum graveolens* that was found rich in tannins, terpenoids, cardiac glycosides and flavonoids.

The worm motility decreased with increasing extract concentration although with the low rate of change of worm response at bottom and peak concentrations. Similar trends were observed by Abukakar et al. (2008) when they studied the antibacterial activity of *Tamarindus indica* pulp extract using hot water extraction method. A study on chloroform, ethanol and water extracts of, *Cyphostemma glaucophilla* on total protein and membrane stabilisation also revealed dose dependent increases of concentrations of plasma and liver proteins with water extract being more potent than ethanol and chloroform respectively (Ojogbane and Chiletugo, 2010). This behavior probably relates to deficient and saturation of receptors with active ingredients respectively. Paralysis of worms was very evident in treated groups that progressed till death. The paralysis could probably be linked to the action of sitosterol, sitosterolln and proanthocyanidins reported to be present in this plants (Brookes and Katsoulis, 2006).

The revealed phytochemical compounds probably explain the biological activity observed in this study since the plant was was rich in flavonoids, tannins and alkaloids. Al-Mustafa and Al-Thunibat, (2008) acknow-ledged differences in biological activity based on solvent used for extraction when they studied antioxidant activity of methanol and aqueous extracts of some Jordanian medicinal plants used for treatment of diabetes. All the phytochemical contituents detected in this plant have been known to have medicinal activity and to show physiological effects (Edeoga et al., 2005; Mariita et al., 2011).

The alkaloids, tannins, saponins and triperpens could be responsible for the high potency of water extract that was observed in the study although synergy by other compounds could have enhanced the activity. The role of tannins in helminthes and bacterial control has been widely documented (Athnasiadou et al., 2001; Hoste et al., 2006; Cenci et al., 2007; Sibi et al., 2012). Chemically tannins are polyphenolic compounds (Bate-Smith. 1962) and synthetic phenolic anthelmintics like niclosamide and oxyclozanide are said to interfere with energy generation in helminthes parasites by uncoupling oxidative phosphorylation (Martin, 1997). It is possible that tannins contained in both extracts of *E. heterophylla* produced similar effects. It was also suggested that tannins bind to free proteins in the gastrointestinal tract of host animal (Athnasiadou et al., 2001; 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. Similar effects were reported by Aiyegoro et al. (2008) who suggested that the concentrations of extracts resulted in protein leakages in the test organisms and they proposed disruption of cell membrane as a mechanism of action of the plant extract.

Alternatively, the presence of alkaloids salts which are physiologically active with sedative and analgesic properties could have contributed to the paralysis and consequent death of the worms. Alkaloids have been reported to be toxic as result of their stimulatory effects that lead to excitation of cells and neurological dysfunction (Izaddoost et al., 1975; Rujjanawate et al., 2003).

A study conducted by *Paolini* et al. (2004) reported that the anthelmintic plants' effects differ depending on the parasitic species and/or stages of maturity. For instance, a study by Sritong et al. (2005) using adult *Ascaridia galli* in layers revealed efficacy of *E. heterophylla* to be 63%. However, previous exposure of parasites to the test material, solvent used for bioactive ingredients, source of the test material and its maturity, seasoning and maintenance of parasites to the experimental conditions may influence the efficacy. Ikhiri et al. (1992) also noted that bioactive compounds responsible for efficacy can vary depending on the plant part used.

Differences in methods of bioactive ingredients extraction may affect the ingredients extracted thus affecting their bioactivity. For instance, Gakuya (2001) used water, methanol and chloroform solvents to extract Albizia *anthelmintica* for lethality test on shrimp. He found that toxicity decreased in order of chloroform, water and methanol. In their study of *A. anthelmintica* from different parts of Kenya by Githiori et al. (2003) and study of *Hedera helix* in Ethiopia by Eguale et al. (2007) found differences in efficacy between solvent preparations. On the other hand the active ingredients responsible for parasite destruction may not be affected by the different solvents since different compounds have different solubilities and perform different medicinal roles. In this study both extracts achieved the same maximum effect despite the difference in potency. The community

dosage (8 mg/ml) was able to inhibit motility in 56.67 % of worms still proving their claimed use in traditional human and veterinary helminthes control. However, the study has disclosed that the community under doses the parasites and this probably explains the continued helminths related problems and persistent low livestock productivity despite the revealed anthelmintic potential of this plant.

CONCLUSION AND RECOMMENDATIONS

This study indicated that administration of E. heterophylla holds great potential as an effective treatment for adult helminths parasites. The community dose (8 mg/ml) administered was found to be much less than what would provide more beneficial results. The phytochemical constituents present in the plant provide the basis for their use as anthelmintic "drugs" and direct to plant's role in medicinal uses. If the community adopted dosage could be standardized, then it could be integrated in the veterinary extension as part of routine helminths parasite control in developing countries. The study has confirmed the community claims of this plant as anthelmintic. However, the plant needs to be studied in vivo to establish the effect of digestive enzymes on efficacy in addition to determining toxicity and safety of this noble plant species.

ACKNOWLEDGEMENT

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