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In Vitro Anthelmintic Potentials of Six Selected Medicinal Plants against Sheep Helminthiases in Koibatek and Mogotio Sub-Counties of Baringo County, Kenya

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Abstract: Helminthiasis is one of the most important diseases worldwide that cause high production losses in livestock. The disease is prevalent all over the world especially in developing countries, associated with poor management practices, lack of access to conventional anthelmintic drugs as a control or curative strategy, and greatly hampered by drug resistance exhibited by parasites. Farmers therefore resort to traditional medicinal plants for helminthiases treatment, which lack information on their effectiveness, toxicity levels, dosages, and safety. The current study aimed to determine anthelmintic potentials of methanolic and crude water extracts of six selected medicinal plants: *Leucas calostachys, Olinia rochetiana, Vepris simplicifolia, Olea capensis, Jasminum floribundum,* and *Asplenium aethiopicum,* used in the traditional management and treatment of sheep helminthiases in Koibatek and Mogotio sub counties, Baringo County, Kenya. The findings indicated positive results from both methanolic and water extracts from the six selected medicinal plants.
Keywords: Helminthiases, medicinal plants, sheep, *in-vitro* experiments.

INTRODUCTION

Background information

Parasitic nematodes are among the most common and economically important infectious disease organisms of grazing livestock, especially in ruminants around the world causing helminthiases [1, 2]. In developing countries like Kenya, the disease may be attributed to lack of resources to regularly deworm affected livestock in addition to development of parasite resistance to conventional drugs resulting to poor use of drugs.

Moreover, parasites infections are likely to increase in the face of climate change [3, 4]. 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" [5, 6].

Ovine gastrointestinal parasites are important pathogens affecting the health of animals and the income of their farmers [7, 8]. Clinical signs of diseases such as diarrhea, and even mortalities affect mainly young animals [9]. Sub clinical effects such as long term weight loss and reduced growth, are probably more important considerations to modern livestock production aiming for improvement of production through healthier animals [10-11]. To achieve such a lasting effect the farmers and veterinarians require knowledge of parasites affecting the sheep, risks affecting the presence of parasites and methods to detect and treat the infections in a suitable way [12].

Different control strategies including the use of anthelmintics, grazing management in livestock and improvements in sanitation are available for gastrointestinal nematode infections but these control methods are associated with development of resistance to available chemotherapeutic anthelmintic drugs [13-15]. Consequently, rural communities resort to using medicinal plants to treat symptomatic clinical signs of which they have continued to claim effectiveness. In most developing African countries like Kenya, livestock production remains crucial and represents a major asset among resource-poor small-scale farmers by providing milk, meat, skin, manure, and traction. However, the economic benefits of livestock populations remains marginal due to prevailing livestock diseases which are among the bottle neck of livestock performance and cause of high economic losses to the resource - poor farmers [16].

Ethno-veterinary medicine is a system that is based on folk beliefs, traditional knowledge skills, methods, and practices used for curing diseases and maintaining health of animals [17,18]. Traditional medicine knowledge like all other traditional knowledge systems is handed down orally from

generation to generation and it may disappear because of rapid social-economic, environmental, technological changes and as a result of the loss of cultural heritage under the guise of civilization [17,19]. The only solution is that it must be documented and conserved through systematic studies before it is lost forever. In third world countries, there is poor extensive network to provide modern veterinary facilities. Dwindling financial resources and poorly developed necessary infrastructure like roads, laboratories and cold chains to keep heat-sensitive vaccines refrigerated at all times/makes government-run veterinary services unable to provide good quality animal health services [20-22].

The bulk of material used for medicinal purposes are collected from the natural vegetation stock that continues to shrink, with majority of claimed ethno-veterinary medicinal plants collected from natural habitat (wild) without cultivation. The fact that the remedies are only found in the wild possesses a big threat to their existence as long as the mass destruction of their habitat continues [23] and the mode of transfer of indigenous knowledge is verbal from generation to generation. There is also an increase in threat to these medicinal plants due to frequent drought, agricultural expansion, and cultivation of marginal land.

The stock of vegetation of medicinal plants is shrinking rapidly and hence poses a big threat of their extinction due to combined effects of these factors [24]. Medicinal plants are cost-saving replacement of commercial drugs [25]. Not only the resource poor farmers, but intensive production units use the medicinal plants. Market and public demand of medicinal plants has been increased and there is great risk that many medicinal plants today, face either extinction or loss of genetic diversity [26]. In European Union and other countries where the use of antibiotics and other drugs is increasingly restricted in food animals, plant medicines are gaining importance. In developing countries, an interest in botanicals is reviving and different developmental organizations are supporting commercial or backyard cultivation. This is an established fact not only in developing countries, but even in the industrialized countries where medicinal plants will remain an integral part of veterinary therapeutics [27].

Control of helminthiases has been the centre of focus in biomedical research for a long time. Both the medical and veterinary professionals have tried to control helminthiases by administration of conventional drugs [28]. However, these drugs are becoming increasingly expensive while some have serious side effects [29]. The demand for herbal medicine has steadily increased over the past decade worldwide but, majority of them have not been assessed for their quality, safety or licensed as medicines [30]. Little is known or documented about usefulness, effectiveness, or potential of such medicines. With onset of modernization of agriculture and other western influence, such knowledge is greatly threatened and could totally be lost with the passing generations. It is prudent therefore to research more on this field to generate vital data that could be necessary to revitalize and preserve such knowledge.

METHODOLOGY

Identification of medicinal plants

Medicinal plants were identified in the Month of March to April 2013 to obtain an impression on vegetation characteristic (plants being leafy and flowery) of the study area using semi-structured questionnaires and interviews with individuals, local village elders and traditional medicine practitioners. The medicinal plants were selected mainly based on frequency of their mention by the respondents (farmers, herders, and herbalist), where six plants with the highest frequency percentage was selected for extraction namely: - *Leucas calostachys*, *Olinia rochetiana*, *Vepris simplicifolia*, *Olea capensis*, *Jasminum floribundum* and *Asplenium aethiopicum* (Figure 1).

a) **Ngechebchat (*Leucas calostachys)***

b)Kiptere (Jasminum floribundum)

Leaves Rhizomes

c) Sugumerie (*Asplenium aethiopicum)*

d) Kurionte (*Vepris simplicifolia*)

e) Masaita (*Olea capensis)*

f) Nerkwe (*Olinia rochetiana)*

Fig-1: Photographs of medicinal plant selected for extraction

Field collection of prioritized medicinal plants

Fresh plant parts used by the farmers in the treatment of helminthiases were collected from the field, dried in the shade, and kept in polythene bags after being chopped into small pieces. The polythene bags were labeled and transported to Kenyatta University- Department of Pharmacy for processing and extraction.

Processing and extraction of six-selected medicinal plant material

Each dry plant material (parts used) was grounded using an electric grinder into powder and stored in airtight bottles. Exposure to sunlight was avoided to prevent the loss of active components.

Crude extraction (water extracts)

Plants material grounded into fine powder was weighed and extracted in distilled water using the methods described by Anonymous [31]. Ten grams of the powder from each selected plant was immersed in 100 ml of water in labeled beakers and soaked overnight. The extracts were then filtered using a muslin cloth and filter papers to provide the traditional dose and stored in labeled bottles at 4^0C until used. To *Leucas calostachys*, ten grams of grounded plant material was mixed with 200 ml of water because it absorbed a lot of water. Treatment was similar to the other plant material.

Solvent extraction (methanol extracts)

For each of the dry sample of the plant material (powder), 50 grams from each plant material was soaked in 200 ml of methanol extraction fluid. The mixtures were kept for 2 days (48 hours) in tight sealed conical flasks (labeled) at room temperature protected from sunlight and mixed daily with a sterile glass rod. The mixtures were then filtered into conical flasks using whatman filter paper No. 10. The filtrate was then concentrated on a rotary evaporator at 50^0C to yield semi solid masses. The extracts were then stored in labeled bottles in a refrigerator at 4^0C until used.

Evaluation of anthelmintic activity of the selected medicinal plants

In vitro experiments were carried out at Kenya Agricultural and Livestock Research Organization (KALRO) - Muguga laboratories to test the biological activities of water and methanolic extracts of the six priority plants used by farmers to treat sheep helminthiases.

Collection of fresh eggs of *Haemonchus contortus*

At KALRO-Muguga North Veterinary Laboratories, at least 10 sheep were randomly selected from a flock of 36 and 100 grams of faecal samples were collected from rectum of each animal using gloved finger three times at an interval of three days. The faecal matter was then placed in plastic polythene bags and transported to the laboratory for extraction (Figure 2).

Fig-2: The author with assistance of KALRO staff removes feaces from two of the sampled sheep

Extraction of eggs from feaces

Forty milliliters (40 ml) of tap water was added to 50 grams of faecal material in a polythene bag and kneaded thoroughly by compression using a stomacher. The faecal suspension was passed through a series of sieves-strainer, 150 µm and 38 µm as minimum and retentate containing fine faecal debris was poured into polyallomer centrifuge tubes and centrifuged at 1000 revolution per minute (rpm) for 5 minutes. The supernatant was removed/discarded using a vacuum line, leaving approximately 1 ml of faecal debris and eggs. The sediment was re-suspended using 10-12 ml saturated sodium chloride solution and mixed thoroughly but gently (violent mixing causes more faecal debris to float); it was again centrifuged at 500 rpm for 5 minutes. Top third of supernatant (2 ml) was removed and retained by pipetting into another centrifuge tube. Water was added and centrifuged at 1000 rpm for five minutes, the supernatant was discarded. It was then resuspended in water and centrifuged again at 1000 rpm for 5 minutes and a repeat of resuspending in water was done to obtain final volume of approximately 2 ml. Twenty five microlitre (µl) was withdrawn and number of eggs counted. The extracted eggs were pooled to make up a total of 10 ml in a volumetric flask and the number of eggs counted in 100 µl (contains approximately 100 eggs).

Preparation of stock and working solutions

One gram (1g) each of methanolic extracts stored at 4^0C were removed, transferred into a 100 ml volumetric flask, and dissolved in 10 ml of DiMethylsulphoxide (DMSO) on the day of the experiments. It was mixed thoroughly and distilled water added to make 100 ml of stock solutions which was used to prepare different suitable range of dilutions for the purposes of evaluating anthelmintic activity. The stock solutions made were used to make the following concentration of working solutions for each methanolic plant extract in 96 micro-titre plates. The concentrations of 6.25 mg/µl, 12.5 mg/µl, 25 mg/µl, 50 mg/µl and 100 mg/µl were prepared and using clean pipettes 100 µl distilled water was added to each well. With the water extract, 10 ml each was removed and placed in beakers, then 200 µl was obtained from each beaker for egg hatch assay and larval development assay respectively [38].

Egg hatch test

Suspensions (0.2ml) containing approximately 100 eggs were distributed in 96 micro-titre plates in three replicates and mixed with same volume of different concentration (6.25 mg/µl, 12.5 mg/µl, 25 mg/µl, 50 mg/µl and 100 mg/µl) of plant extracts with control plates having 200 µl of distilled water and Levamisole10 mg/ml. Eggs were incubated in these

mixtures for 48 hours at 25^0C. One drop of lugols iodine solution was added to stop the eggs from hatching. Eggs (dead or embryonated) and first stage larvae (L1) in each plant extracts were counted as described before [39].

Larval development test

The isolated eggs that had been extracted were incubated in a micro-titre plate for seven days at 25^0C. To prevent dehydration, a wet sponge was introduced under the plate and the system covered with a pouch. The water in the wells was checked every day during incubation. After hatching, wells were supplemented with 20 µl of growth nutritive medium and 10 µl of distilled water was added to the control well. Plant extracts were prepared by diluting them with 1% DiMethylsulphoxide (DMSO) to make different dilutions of 6.25 mg/µl, 12.5 mg/µl, 25 mg/µl, 50 mg/µl and 100 mg/µl and 3 replicates were made for each set up. Ten living *H. contortus* larvae were introduced to each well and the plates were then returned into the incubator for 6 days. On the seventh day, all the larvae were counted as either living third stage larvae (L3) or dead larvae a modification as described by Hubert and Kerboeuf.

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) was used to determine whether there was a significance difference in plants extracts of varying/different concentration in egg hatchability and larval development test.

RESULTS

***In vitro* anthelminthic activity of the medicinal plant extracts**

a) Effect of concentrations of plant extracts in egg hatchability

The result showed that, *Olea capensis* had the least mean of eggs hatched (1.0 ± 1.00) at concentration of 50 mg/µl which was lower than the positive control (Levamisole) with a mean of 3.0 ± 5.02, while plant extract from *Jasminum floribundum* (mean 25.33 ± 3.51) had the highest mean of hatched eggs than the other plant extracts. *Olinia rochetiana* had the highest mean of eggs hatched (64.0 ± 2.65) at a concentration of 6.25 mg/µl and the least mean of eggs hatched at concentration of 100 mg/µl, indicating that effectiveness of plant extract increases with an increase in concentration which was showing the same trend for the six plants tested. In the experiment, two controls were used in three replicates, which included distilled water (negative control) and Levamisole (positive control). In distilled water, a mean of 94.0 ± 5.0 eggs hatched while a mean of 3.0 ± 5.02 eggs hatched in Levamisole (Table 1).

Table-1: The mean of eggs hatched after treatment with various methanolic concentrations of the plant extracts, water extract, and controls

Plant extract	Concentrations of methanolic extracts							
	6.25 mg/µl	12.5 mg/µl	25 mg/µl	50 mg/µl	100 mg/µl	Water extract	Distilled water	Levamisole
Jasminum floribundum	52.0± 4.58b	30.67± 1.53b	28.67± 2.52b	25.33± 3.51c	19.00± 2.65b	29.33± 3.06c	94.0± 5.0	3.0± 5.02
Vepris simplicifolia	51.63± 4.16b	35.33± 3.79b	31.67± 4.16b	24.33± 2.52c	29.33±5 .86c	28.67± 6.81c	94.0± 5.0	3.0± 5.02
Asplenium aethiopicum	17.67± 3.51a	16.00± 2.00a	13.67± 2.31a	14.00± 1.00b	18.67± 7.51b	16.67± 5.13b	94.0± 5.0	3.0± 5.02
Leucas calostachys	44.33±4 .73b	25.00±6 .56b	14.33± 1.53a	5.67± 2.31a	15.33± 1.16b	22.00± 2.65bc	94.0± 5.0	3.0± 5.02
Olea capensis	18.67± 2.52a	13.33± 2.08a	10.00± 2.00a	1.00 ± 1.00a	6.00 ± 1.00a	6.33± 1.53a	94.0± 5.0	3.0± 5.02
Olinia rochetiana	64.0± 2.65c	52.67±4 .16c	47.67±2 .89c	22.00± 1.73c	4.67 ± 2.08a	43.00± 6.08d	94.0± 5.0	3.0± 5.02

Mean values in same column denoted by similar letters are not significantly different at $P \leq 0.05$.

Using One-way ANOVA to establish the variations in the effect of the plant extracts on the mean of eggs hatched at concentration of 50 mg/µl, the results indicated that there was a significant difference ($F = 65.31$; $P = 0.0001$) in the mean eggs hatched following treatment in 50 mg/µl concentration of plant extracts. A Scheffe post hoc test revealed that *J. floribundum* had statistically higher mean of eggs hatched (25.3 ± 3.51) compared to *A. aethiopicum* (mean 14.0 ± 1.0); P=0.02, *L. calostachys* (mean 5.67 ± 2.31); P=0.000 and *O. capensis* (mean 1.0 ± 1.0); P=0.000. *Vepris simplicifolia* (24.3 ± 2.51) had a statistically higher

mean of eggs hatched as compared to *A. aethiopicum* (*P*=0.004), *L. calostachys* (*P*=0.000) and *O. capensis* (*P*=0.000). *Asplenium aethiopicum* had a statistically lower mean of eggs hatched as compared to *J. floribundum, V. simplicifolia and O. rochetiana* (22.0 ± 1.73). *Asplenium aethiopicum* also had statistically a higher mean of eggs hatched as compared to *L. calostachys* (*P*=0.018) and *O. capensis* (*P*=0.000). *Asplenium aethiopicum* had a statistically different mean of eggs hatched as compared to all the other plants tested (Table 2).

Table 2: Multiple comparison of egg hatched in various methanolic plant extract at concentration of 50 mg/µl

Row mean – column mean		*J. floribundum*	*V. simplicifolia*	*A. aethiopicum*	*L. calostachys*	*O. capensis*
Vepris simplicifolia	i-j	-1				
	P	0.997				
Asplenium aethiopicum	i-j	-11.33	-10.3333			
	P	0.002	0.004			
Leucas calostachys	i-j	-19.6667	-18.6667	-8.33333		
	P	0.000	0.000	0.018		
Olea capensis	i-j	-24.3333	-23.3333	-13	-4.66667	
	P	0.000	0.000	0.000	0.308	
Olinia rochetiana	i-j	-3.33333	-2.33333	8	16.3333	21
	P	0.641	0.88	0.023	0.000	0.000

There was no statistical difference between the mean of eggs hatched by *J. floribundum* at the concentration of 50 mg/µl and the mean eggs hatched by *V. simplicifolia* or *O. rochetiana* (*P* = 0.641). Further, there was also no significant difference between the mean of egg hatched by *L. calostachys* and *O. capensis* (*P* = 0.308). A scheffe post hoc test created three groups: (C) the greatest number of egg hatching occurred in plant *J. floribundum*, *V. simplicifolia* and *O. rochetiana*; (B) the second moderate number of egg hatching in plants occurred in *A. Aethiopicum;* (A) the lowest number of egg hatching occurred in *L. calostachys* & *O. capensis* (Table 3).

Table-3: Mean for groups of methanolic plant extracts in homogeneous subsets at the concentration of 50 mg/µl

Plant		Subset for alpha = 0.05		
		1	2	3
Scheffe[a]	*O. capensis*	1.00		
	L. calostachys	5.67		
	A. aethiopicum		14.00	
	O. rochetiana			22.00
	V. simplicifolia			24.33
	J. floribundum			25.33
	Sig.	.308	1.000	.641

Number of larvae recovered after treatment with various concentrations of plant extracts

Olea capensis had statistically the lowest mean of larvae recovered (1.0 ± 1.0) compared to *V. simplicifolia* (17.0 ± 1.0), *O. rochetiana* (16.0 ± 1.0) and *A. aethiopicum* (11.0 ± 4.58). *Vepris simplicifolia* had statistically the highest mean of larvae formed at concentration 50 mg/µl (17.0 ± 1.0) as compared to *A. aethiopicum* (11.0 ± 4.58) and *O. rochetiana* (16.0 ± 1.0). *Jasminum floribundum* (9.0 ± 2.0) had the highest mean of larvae recovered as compared to *L. calostachys* (4.0 ± 1.0) (Table 4).

Table-4: Mean number of larvae recovered in methanolic plant extracts at concentration of 50 mg/µl

Plant	Mean ± Standard Deviation (SD)
J. floribundum	9.0 ± 2.0
V. simplicifolia	17.0 ± 1.0
A. aethiopicum	11.0 ± 4.582576
L. calostachys	4.0 ± 1.0
O. capensis	1.0 ± 1.0
O. rochetiana	16.0 ± 1.0

Number of larvae formed in the treatments with different methanolic plant extracts at concentration of 50 mg/µl were significantly different (F = 25.24; *P* = 0.0001) according to ANOVA test. A Scheffe post hoc test showed that *J. floribundum* had a significantly lower mean of larvae formed as compared to *V. simplicifolia* (*P* = 0.023) and significantly higher compared to *O. capensis* (*P* = 0.023). *Vepris simplicifolia* had a significantly higher mean of larvae formed at a concentration of 50 mg/µl as compared to *J. floribundum* (*P* = 0.023), *L. calostachys* (*P* = 0.000) and *O. capensis* (*P* = 0.000). *Olea capensis* had statistically the lowest mean of larvae recovered as compared to *L. calostachys* but the two were not significantly different (*P* = 0.73) (Table 5).

Table-5: Multiple comparison of larvae recovered in various methanolic plant extract at concentration of 50 mg/µl

Row mean - column mean		*J. floribundum*	*V. simplicifolia*	*A. aethiopicum*	*L. calostachys*	*O. capensis*
V. simplicifolia	i-j	8				
	P	0.023				
A. aethiopicum	i-j	2	-6			
	P	0.933	0.118			
L. calostachys	i-j	-5	-13	-7		
	P	0.246	0.000	0.053		
O. capensis	i-j	-8	-16	-10	-3	
	P	0.023	0.000	0.005	0.73	
O. rochetiana	i-j	7	-1	5	12	15
	P	0.053	0.997	0.246	0.001	0.000

There was no significant mean difference in larvae recovered between *O. capensis* and *L. calostachys* (*P* = 0.730). There was no significant mean difference between *L. calostachys, J. Floribundum* and *A. aethiopicum* (*P* = 0.053). There was no significant mean difference between *J. Floribundum, A. aethiopicum* and *O. rochetiana* (*P* = 0.053). Finally, there was no statistical difference between *A. aethiopicum, O. rochetiana* and *V. simplicifolia* (*P* = 0.118). The most potent plant among the six plants was *O. capensis* followed by *L. calostachys* though not significantly different, *J. floribundum, A. aethiopicum, O. rochetiana* respectively, and least potent was *V. simplicifolia* (Table 6).

Table-6: Mean for groups of methanolic plant extracts in homogeneous subsets for larvae recovered at the concentration of 50 mg/µl

Plant		Subset for alpha = 0.05			
		1	2	3	4
Scheffe[a]	*O. capensis*	1.00			
	L. calostachys	4.00	4.00		
	J. Floribundum		9.00	9.00	
	A. aethiopicum		11.00	11.00	11.00
	O. rochetiana			16.00	16.00
	V. simplicifolia				17.00
	Sig.	0.730	0.053	0.053	0.118

Comparing various concentrations of methanol extract with the water extracts

Using independent t-test for the comparison of the various means of methanol extract in egg hatching at 50 mg/µl concentration from the six plants tested and the water extracts showed that, there was a significant difference in methanolic extract versus water extract in egg hatchability in plant extract *Leucas calostachys* (t = 7.00; *P* = 0.020), *Olea capensis* (t = 16; *P* = 0.004) and *Olinia rochetiana* (t = 6.53; *P* = 0.023), while there was no significance difference in methanolic versus water extract in *Jasminum floribundum* (t = 1.33; *P* = 0.314), *Vepris simplicifolia* (t = 1.00; *P* = 0.423) and *Asplenium aethiopicum* (t=0.79; *P*=0.513) (Table 7).

Table-7: Comparison of mean eggs hatched in the different concentration of methanol extract with water extract

	Mean of Methanolic plant extracts at 50mg/µl ± SD	Water extract	t- value	P values
Jasminum floribundum	25.33 ± 3.51	29.33 ± 3.06	1.33	0.314
Vepris simplicifolia	24.33 ± 2.52	28.67 ± 6.81	1.00	0.423
Asplenium aethiopicum	14.00 ± 1.00	16.67 ± 5.13	0.79	0.513
Leucas calostachys	5.67 ± 2.31	22.00 ± 2.65	7.00	0.020*
Olea capensis	1.00 ± 1.00	6.33 ± 1.53	16.00	0.004*
Olinia rochetiana	22.00 ± 1.73	43.00 ± 6.08	6.53	0.023*

*Indicate a significant independent t value at 95% CI between the rows (the plant extracts and water extracts)

Effects of plants extract concentrations on larval development

The results showed that *Olea capensis* extracts killed the highest number of living *H. contortus* larvae at 50 mg/µl and 100 mg/µl concentrations, while *Leucas calostachys* extracts killed all living larvae, when water extract was used. Rate of larvae killed increased as the plant extract concentration was increased. For the controls, distilled water killed none of the larvae while Levamisole killed all the living larvae (Figure 3).

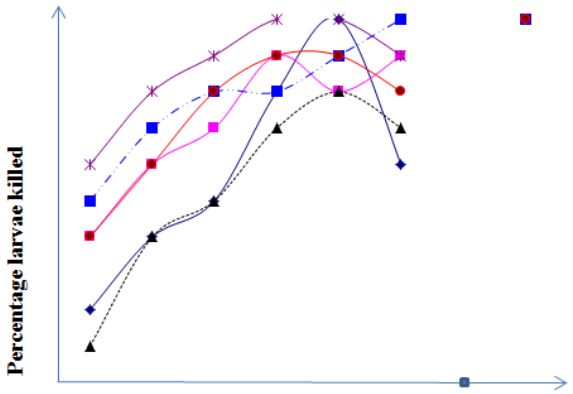

Fig-3: Percentage larvae killed by different concentrations of methanol plant extracts, water extracts, and controls (distilled water and Levamisole)

When the number of larvae killed was determined, there was no significant difference ($F = 2.613$; $P = 0.080$) in mean of larvae killed by the various methanolic plant extracts tested at 100 mg/µl concentration. *Olea capensis* plant extract killed the highest number of larvae with a mean of 9.33 ± 0.577 followed closely by *V. simplicifolia* and *O. rochetiana* with mean of 9.0 ± 1.0 (Table 8).

Table-8: Mean of *H. contortus* larvae killed by methanolic plant extracts at 100 mg/µl

Plant extract	Mean larvae killed ± SD in methanolic extract
Jasminum floribundum	8.0 ± 1.0
Vepris simplicifolia	9.0 ± 1.0
Asplenium aethiopicum	7.0 ± 1.0
Leucas calostachys	8.0 ± 1.03
Olea capensis	9.33 ± 0.577
Olinia rochetiana	9.0 ± 1.0

DISCUSSION

The present study shows that all the six plants extracts used in the tests (*in-vitro*) as ethno-veterinary medicine could be of value in treatment of livestock helminthiases irrespective of solvent used to extract the active ingredients because it showed levels of effectiveness in reducing egg hatching and larval development of *Haemonchus contortus* eggs and larvae respectively. The present study indicated that medicinal plants; *Jasminum floribundum*, *Vepris simplicifolia*, *Asplenium aethiopicum*, *Leucas calostachys*, *Olea capensis* and *Olinia rochetiana* could be of value in the treatment of helminthiases because they yielded appreciably positive results. Most potent among the six plants were *Olea capensis* and *Leucas calostachys*, which showed high level of activity in both tests, indicating presence of active components. These findings agree with previous reports that indigenous plants are useful in treatment of helminthiases [32].

Two of the studied plants (*Cannabis sativa* and *Moringa oleifera*) however, did not give satisfactory results in the initial screening. The present findings also agree with previous studies in Samburu District of Kenya on efficacy of *Myrsine africana*, *Albizia anthelmintica*, and *Hilderbrantia sepalosa* herbal remedies against mixed natural sheep helminthiases [33]. According to [34], all the herbal remedies had some efficacy against both nematodes and monezia species of helminths. The effects of plant extracts concentrations against nematodes were significantly different as compared to untreated control group, the efficacy against nematodes was 77%, 89.8% and 90% for *M. africana*, *A. anthelmintica* and *H. sepalosa*, respectively while albendazole had 100% efficacy. The current findings also concur with the previous study to assess herbal anthelmintics used by farmers in Kirinyaga County, Kenya, for treatment of helminthiases in cattle where *Entada leptostachya* exhibited highest *in-vitro* activity while *Erythrina abyssinica* had lowest activity. Active ingredients that caused anthelminthic activity were present [34].

Extraction efficiency of methanol and water was relatively high for both solvents. This demonstrated that both solvents could equally be good for bioactive ingredients for 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 active molecules are more soluble in methanol than water solvent compared to compounds responsible for bioactivity. This could be due to high solubility of reducing sugars that may not participate in pharmacological activities of chemical compounds. According to Gakuya [35], water, methanol and chloroform solvents were used to extract *Albizia anthelmintica* for lethality test on shrimps. The present study findings were similar with these findings, in that difference in method of bioactive ingredients extraction may have affected ingredients extracted thus affecting their bioactivity. Further, in conformity to the current study, *Albizia anthelmintica* from different parts of Kenya [36] and *Hedera helix* from Ethiopia [37] showed difference in efficacy between solvents preparations. However, different solvents may not affect active ingredients responsible for parasite destruction since different compounds have different solubility and perform different roles. In the present study, both extracts achieved relatively the same maximum effects despite the difference in potency.

CONCLUSIONS

Methanolic and water extracts from the six medicinal plants under investigation, showed biological activities in egg hatching and larval development in varying concentrations as compared to positive and negative controls. The findings indicated a significant difference in mean of eggs hatched ($F = 65.31$; $P = 0.0001$) in varying methanolic concentrations with the lowest concentration being significantly different from negative controls. *Olea capensis* displayed the least mean of eggs hatched (mean 1.00 ± 1.00 larvae); followed by *Leucas calostachys* (mean 5.67 ± 2.31 larvae). *Jasminum floribundum* had the highest mean of eggs hatched (mean 25.33 ± 3.51) followed by *Vepris simplicifolia* (mean 24.33 ± 2.52) and *Olinia rochetiana* (mean 22.00 ± 1.73) at concentration of 50 mg/ul. In larval development, there was no significant difference ($F=2.613$; $P=0.080$) in the mean number of larvae killed by the various methanolic plant extracts at 100 mg/ul. Plant extract from *O. capensis* had the highest number of dead larvae (mean of 9.33 ± 0.577 larvae) followed by extracts from *V. simplicifolia* (mean of 9.0 ± 1.0 larvae) and *O. rochetiana* (9.0 ± 1.0). *A. aethiopicum* had the least mean larvae killed (7.0 ± 1.0). The preliminary results on the six selected medicinal plant species (*Jasminum floribundum*, *Vepris simplicifolia*, *Asplenium aethiopicum*, *Leucas calostachys*, *Olea capensis* and *Olinia rochetiana*) used in traditional treatment of sheep helminthiases in *in-vitro* system displayed degrees of anthelmintic activity in reducing egg hatching and killing of larval stages (L3) of *Haemonchus contortus* worms, when both methanolic and water extract were used. This is a scientific proof of the presence of active ingredients in the plants studied.

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