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In vitro Evaluation of Anthelmintic Activities of Crude Extracts of Selected Medicinal Plants Against Haemonchus Contortus in Alemgena Wereda, Ethiopia

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Abstract: The present study was carried out to evaluate the anthelmintic activities of crude aqueous and methanol extracts of leaves of *Myrsine Africana, Rhus glabrous, Jasminum abysinicum, Rhus vulgaris*, *Acokanthera schimperi* and aerial parts of *Foenicum vulgare* on nematode parasite, *Heamonchus contortus.* For this purpose, Egg Hatch Test (EHT) and Larval Development Test (LDT) were conducted on nematodes ova to investigate the *in vitro* ovicidal and larvicidal effects of crude extracts. Significant variation in yield among different plant species with different methods of extraction was observed. The highest yield was recorded for methanol extract of *A. schimperi* leaves (36,93%) and lowest yield for methanol extract was observed with *R. glabrous* leaves (4.8%). The highest yield for aqueous extraction was that of leaves of *F. vulgare* leaves which was (22.17%) and lowest yield for aqueous extraction was that of *A. schimperi* leaves (1.72%). Of all tested plants, both aqueous and alcoholic extracts of aerial part of *F. vulgare* showed best performance, producing nearly 100% egg hatch inhibition at concentration of 1mg/ml while none of the other plants were able to induce complete inhibition even at concentration of 2mg/ml. The performance of aqueous and methanol extracts of most plants were significantly different ($p<0.05$) except that of *F. vulgare* were both extracts induced nearly similar effect. In general, all used plants in the current study induced over 50% inhibition of egg hatch of *H. contortus* at highest used concentration (2mg/ml). Albendazole required a maximum concentration (0.25µg/ml) to induce 100% egg hatch inhibition. The effective doses required to induce 50% and 90% (ED_{50} and ED_{90}) inhibition of egg hatching were calculated by probit analysis. Of all investigated plants, both extracts of *F. vulgare* induced 50% inhibition of egg hatching at lower concentrations (0.24 and 0.27 mg/ml) for aqueous and alcoholic extracts, respectively. Aqueous extract of *R. vulgaris* and alcoholic extract of *A. schimperi* have also performed the next remarkable inhibition of egg hatching at concentration of 0.64 and 0.85 mg/ml, respectively. Of all plants, alcoholic extract of *R. glabrous* induced 50% inhibition at higher concentration. ED_{50} value for the aqueous and methanol extracts of most plants in the current study did not show statistically significant variation. ED_{50} for egg hatch inhibition of Albendazole was 0.09µg/ml. Both aqueous and alcoholic extracts of most plants had shown variable effect on development of larvae of *H. contortus.* The highest larval development inhibition observed was for alcoholic extract of *R. glabrous* (97.7%) while the least effective plant was aqueous extract of *R. vulgaris* (10%) at the maximum tested concentration. Despite high dose required to inhibit larval development, except for both extracts of *R. vulgaris*, almost all plant extracts induced dose dependent inhibition of larval development. Unlike the effect of plants on egg hatching, there was statistically significant variation in activity of aqueous and methanol extracts of most plants ($p<0.05$) at most tested concentrations. In most cases, alcoholic extracts are more effective than their aqueous counterparts.

Key words: Helminthosis · *Haemonchus contortus* · Medicinal Plants · Anthelmintic Activities · Aqueous Extract · Methanol Extract

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INTRODUCTION

Helminthosis is one of the major problems of livestock production throughout the world, particularly in tropical and subtropical areas. The disease is especially prevalent in developing countries in association with poor management practices and inadequate control measures [1]. Most of the parasite control programs are based upon a combination of chemotherapeutic control, grazing management, dietary management, biological control, vaccination and ethnoveterinary treatment [2].

Chemotherapeutic control practices have evolved a number of problems including resistance of helminthis to various groups of anthelmintic [3]. Studies have shown that anthelmintic resistance has developed to most of these drugs in different parts of the world, especially in major sheep producing countries, like Australia, South Africa and Kenya. For instance worms in 80% of sheep farms in Australia have developed resistance to benzimidazole, imidazothiazole and tetrahydropyrimidine [4]. In South Africa 90% of the sheep farms have parasite strains resistant to at least one anthelmintic group and 40% have parasite strains resistant to all the three major groups of anthelmintics [5, 6].

The major anthelmintic drugs commonly used in Ethiopia for the control of helminth parasites in small ruminants are benzimidazolees (albendazole, triclabendazole, fenbendazole) and levamisole (tetramisole) and Oxyclozanide. In spite of long use of anthelmintics, there are a few reports of suspect for development of anthelmintic resistance against these drugs. Hussein *et al.* [7] and Bayu [8] reported resistant nematodes to tetramisole in goats at Adami Tulu and around Addis Ababa, respectively whereas Ademe [9] reported the occurrence of albendazole resistant population of *Haemonchus contortus* in goats at Awassa. Biffa *et al.* [10] also reported low activity of albendazole in sheep with mixed nematode infection, speculating the development of resistance. Since country wide surveys for anthelmintic resistance have not yet been carried out, the current prevalence of anthelmintic resistance in Ethiopia might be underestimated. In addition to anthelmintic resistance, inadequate availability and high cost of commercial anthelmintics are the other important constraints of helminth control in developing countries like Ethiopia. Such problem diverted the researchers' attentions towards the development of alternate methods for the treatment of helminthosis.

Several ethnoveterinary surveys conducted in Ethiopia indicate that several traditional healers use medicinal plants for treatment of various animal health problems including treatment of helminth infections [11-13]. However, very few efforts have been made to scientifically screen and evaluate the effect of these medicinal plants.

Due to the good efficacy and cost effectiveness, herbal medicine has gained much importance in recent years. Plants provide a huge part of traditional veterinary practices and are a rich source of herbal anthelmintics of veterinary importance for centuries [14, 15]. Moreover, many currently available therapeutic compounds are plant derived and/or synthetic analogues derived from plants [16].

In the present study, crude aqueous and hydro-alcoholic extracts of leaves *Myrsine Africana, Rhus glabrous, Jasminum abysinicum, Rhus vulgaris*, *Acokanthera schimperi* and aerial parts of *Foenicum vulgare* were screened for possible anthelmintic activity against nematode *Heamonchus contortus.*

M. africana, (*Myrsinaceae*), kurjan seed (Eng) locally called "*Kechemo*" (Amharic). It is an errect, densely branched, ever green shrub, usually 1-3m high. leaves are alternate, elliptic or abrogated, usually leathery As hair dye; charred powder of fruits, seed, leaf, fruit and leaf and wing (crown) applied daily far 3 days [17]. The fruit is edible and has anthelmintic property and is particularly effective for expulsion of the tape worms. Decopction of the leaves are used as blood purifier [18].

J. abysinicum (Oleaceae) locally called "Tembelel' is climbing shrub, with compounded leaves with 5 leaflets and white flower. This plant is claimed for its anthelmintic activity in Africa. A study in Kenya indicated that ground leaves of *Jasminum abyssinicum* induced 69% reduction of *H. contortus* in naturally infected sheep [19].

F. vulgare (Apiaceae) locally called "insilal" is a weed of cultivated or disturbed grounds, sometimes common in grassland areas too. Plant is green and blooms when it is dry for anything else. The boiled or roasted roots are used in the treatment of gonorrhea in human [20].

A. schimperi (Apocynacaea) locally called "Mirenz" belongs to a family of Apocynaceae and is a small ever green tree or shrub growing up to 6m high, with elleptic leaves. It is native to East Africa and Yemen. *A. schimperi* contains ouabaïne, a cardiotonic glycoside. It is used by the local people to poison the tips of arrows. In traditional African medicinal practice, it is used for treatment of snake bite and tape worm infection [21].

R. glabrous is trifoliate hairy shrub locally called "Kimo" belongs to a family of anacardiacae with alternate, pinnately compound leaf and dioucious small with pale yellow petals borne in a dense up right cluster up to 8 inches long flower that appearing in mid to late summer [20].

R. vulgaris (Anacardiaceae) is dioecious shrub up to 5 m tall.

The wood, bark of roots and branches and leaves of *R. vulgaris* are used in tanning and the bark also produces fiber for rough rope. *Rhus* species are widely used as anthelmintic in Africa [21].

MATERIALS AND METHODS

Plant Material Collection and Extraction: Leaves of *M. Africana, R. glabrous and R. vulgaris* were collected around Portugise bridge about 100 Km North of Addis Ababa along Addis Ababa Fiche road and aerial parts of *Foenicum vulgare* from 25 Km south west of Addis Ababa along Addis Ababa-Butajira road. Leaves of *J.abysinicum* were collected from Entoto mountain which lies immediately north of Addis Ababa and leaves of *A. schimperi* from Gibe-Tollay in south west Ethiopia. Parts of all the plant species were collected and transported to National Animal Health Diagnostic and Investigation Center (NAHDIC). All plants were identified by plant taxonomist and voucher specimens of each species deposited at the Herbarium of the Addis Ababa University, Biology Department. The garbled plants were air dried at room temperature, powdered using pestle and mortar and kept in amber colored bottle until use. Extraction was conducted at the drug research department of Ethiopian Health and Nutrition Reaserch Institute (EHNRI). Aqueous extraction was performed by soaking a weighed amount of the dry powder (100-150g) in distilled water and shaken for three hours by electric shaker. The suspension was filtered through muslin gauze and the filtrate kept in deep freezer for 24 hours, which was then lyophilized using lyophilizer. The lyophilized dry powder was then collected in a stoppered sample vial, weighed and kept in a desiccator to avoid absorption of water until use in the assay. Hydro-alcoholic extraction was performed by percolate a weighed amount of the dried and powdered plant material using 80% methanol for 5 days, which was then be filtered through Whatman filter paper No.1. The solvent was then evaporated using a Rota vapor to remove the solvent. The extracts were then kept in a stoppered sample vial at 4°C until use.

Experimental Infection of Sheep: Adult females parasites of *Haemonchus contortus* were collected from abomasum of infected slaughtered sheep obtained from Addis Ababa Abattoir. The worms were washed and crushed to liberate eggs. The eggs were cultured in a glass jar filled with autoclaved sheep faeces for eight days at room temperature. About 3000 larvae were inoculated to two worm free sheep that were kept indoor in separate house in the animal house of the NAHDIC throughout the study period. These sheep were serving as *H.contortus* egg donors for subsequent *in vitro* trials.

In vitro Experiments

Collection of Eggs: Briefly, faecal pellets were collected from the rectum of donor sheep and placed in small bucket. Warm water was slowly added to the faeces and the pellets stirred until a relatively liquid suspension was obtained. The suspension was mashed through sieve with 3mm aperture. The suspension that passed through the sieve was collected and washed through 100-mesh (150µm pore size) sieve. The suspension was then poured into 15ml test tubes and centrifuged for 2 minutes at 1000 RPM and the supernatant was decanted. The tube was agitated by vortex mixer to loosen the sediment. Saturated sodium chloride solution was then added to the test tube until the meniscus forms above the tube on which the cover slip was placed. After 5 minutes the cover slip was carefully taken off the tube and the eggs washed into glass centrifuge tubes, filled with water and centrifuged for 2minutes at 1000 RPM. Most of the water was then decanted and the number of eggs per ml was determined and diluted to the required concentration for use in Egg Hatch Assay (EHA) and Larval Development Test (LDT).

Egg Hatch Assay (EHA): The Egg Hatch Assay was conducted according to the World Association for The Advancement of Veterinary Parasitology (WAAVP) guidelines [22]. Aqueous and alcoholic extracts of the plant materials were used as the active treatment. Albendazole (pure standard reference) was used as positive control while untreated eggs in water used as negative control. The test was conducted in 5ml test tubes. In the assay, approximately 150 - 250 eggs in 1.5ml of water were placed in each test tube. Various serial concentrations of each plant extract in total volume of 0.5ml in distilled water were added. Albendazole originally dissolved in Dimethyl sulfoxide (DMSO) and diluted in distilled water at different concentrations was used.

The test tubes were covered and kept in incubator at 27°C for 48 hrs. The experiment was replicated four times for each concentration. Hatched larvae and unhatched eggs were then counted under dissecting microscope at 40X magnification.

Larval Development Test (LDT): The procedure used was a modification of the technique described by Costa *et al.* [23]. The experiment was conducted in plastic cups of 20 ml. Aqueous and alcoholic extracts of the plant material was used as the active treatment. Ivermectin 1% (10mg/ml) was used as positive control while untreated eggs in water were used as negative control. The collected eggs were incubated at 27°C for 24 hours. An aliquate of 1ml, containing 100-120 first stage larvae (L1) of *H. contortus*, was mixed with 5gm of feaces collected from a sheep free of gastrointestinal nematodes and various serial concentrations of each plant extract. Serial concentrations of, 50,25,12.5,6.25,3.125 and 1.562mg/ml of each plant were made in distilled water to make total volume of 7ml together with water containing L1 and volume of egg free feaces. Ivermectin dissolved in distilled water at the concentrations of 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.0156µg/ml was used. It was then incubate for 6 days at room temperature. At the end of 6th day the wall of each cup containing the sample was thoroughly rinsed with 10ml of water to collect the larvae. Then one drop of Lugol's iodine solution was added and all L3 stage larvae were counted under dissecting microscope at 40X magnification.

Data Management and Statistical Analysis: Data from EHA was transformed by probit transformation against the logarithm of extract concentration. The extract concentrations required to inhibit 50% (ED_{50}) and 90% (ED_{90}) egg hatching were calculated by probit analysis. Comparison of mean percentages of egg hatch inhibition and larval development at different concentrations with the control was performed by one-way ANOVA. All statistical analysis was performed by SPSS version 11.0. The post hoc statistical significance test employed was Least Square Difference (LSD), the difference between the means was considered significant at $p<0.05$.

RESULTS

Yield of Extracted Plant Materials: The percentage yield of different parts of plants with different extraction methods is shown in Table 1. Significant variation in yield among different plant species with different types of extraction was observed. The highest yield was recorded for alcoholic extract of *A. schemperi* (36.93%) and lowest yield observed for alcoholic extraction was extract of leave of *R. glabrous* (4.8%). The highest yield for aqueous extraction was that of *F.vulgare* leaves which was (22.17%) and lowest yield for aqueous extraction was that of *A. schemperi* leaves (1.72%) (Table 1).

Anthelmintic Activities

Ovicidal Effect: Mean percentage inhibition of egg hatching post exposure to eggs of *H. contortus* to various concentration of plant extracts are shown in Table 2. Of all plants tested, both aqueous and alcoholic extract of aerial part of *F. vulgare* showed best performance, producing nearly 100% egg hatch inhibition at concentration of 1mg/ml plant extract, while non of the other plants were able to induce complete inhibition even at concentration of 2mg/ml. The performance of aqueous and methanol extracts of most of the plants were significantly different ($p<0.05$) except that of *F. vulgare* were both extract types

Table 1: Percentage yield of different plants using aqueous and alcoholic extraction methods

Plant species	Used parts	Extract type	% yield (W/W)
Foenicum vulgare	Leaves	Aqueous	22.170
		Alcoholic	14.720
Acokanthera schimperi	Leaves	Aqueous	1.720
		Alcoholic	36.930
Rhus vulgaris	Leaves	Aqueous	7.380
		Alcoholic	23.700
Rhus glabrous	Leaves	Aqueous	10.620
		Alcoholic	4.795
Jasminium abysinicum	Aerial part	Aqueous	14.280
		Alcoholic	10.940
Myrsine africana	Leaves	Aqueous	8.830
		Alcoholic	26.570

Table 2: Mean percentage inhibition of egg hatch of *Haemonchus contortus* after 48 hours exposure to different concentration of plant extracts (mg/ml) and Albendazole (µg/ml)

		Mean ± SE at different concentration								
Plant type	Extr.	0.0	0.0156	0.0313	0.0625	0.125	0.25	0.5	1	2
Albendazole		1.8±0.006	3.8±0.123	7.1±0.007	16±0.060	84.30±.041	100±0.00	100±0.00	-	-
F. vulgare	Aq	3.3±0.010	-	-	15.6±0.042	36.6±0.031	59.2±0.070	87.2±0.006	99.4±0.006	99.4±0.006
	HA	2.3±0.012	-	-	3.5±0.007	13.4±0.015	26.5±0.080	93±0.010	100±0.00	100±0.00
A. schimperi	Aq	2±0.012	-	-	11.4±0.050	15.4±0.021	17±0.03	25.8±0.008	37.7±0.026	53.6±0.048
	HA	2.1±0.012	-	-	6±0.028	12±0.014	17±0.029	27.2±0.016	65.2±0.030	86.9±0.010
R. hairy	Aq	3±0.018	-	-	5.7±0.031	17.6±0.043	24.9±0.016	69.4±0.035	86.3±0.020	90.2±0.013
	HA	1.1±0.006	-	-	14±0.004	22.9±0.027	28.8±0.017	48.9±0.048	48.3±0.030	67.7±0.043
R. glabrous	Aq	3±0.007	-	-	6.7±0.018	13±0.020	17±0.036	25±0.021	41.3±0.066	66±0.036
	HA	1.1±0.012	-	-	3.7±0.008	9.4±0.023	15±0.023	22±0.046	29.7±0.070	51.9±0.056
J. abysinicum	Aq	3±0.005	-	-	13.2±0.011	15.4±0.021	25.5±0.031	37.8±0.001	44±0.044	51.8±0.056
	HA	1.4±0.012	-	-	7.6±0.027	15.5±0.010	18.4±0.017	30.6±0.044	47±0.067	70±0.026
M. africana	Aq	3.3±0.011	-	-	6.2±0.040	12.4±0.007	16.8±0.040	21.7±0.023	31.1±0.038	58.3±0.056
	HA	4±0.010	-	-	5±0.010	14.5±0.010	20±0.017	28±0.040	38.6±0.024	52.6±0.020

Table 3: *In vitro* anthelmintic activity of plant extracts and Albendazole expressed in ED50 and ED90 on the egg of *Haemonchus contortus* exposed for 48 hours

			95% confidence interval for mean			95% confidence interval for mean	
	Extr.	ED_{50}	LCL	UCL	ED_{90}	LCL	UCL
Albendazole (µg/mL)		0.90	0.86	1.0	0.2	0.17	0.25
F. vulgare (mg/mL)	Aqueous	0.24	0.16	0.38	0.50	0.37	0.87
	Alcoholic	0.27	0.13	0.53	0.65	0.44	1.62
A. schimperi (mg/mL)	AQ	1.69	1.35	2.25	3.46	2.74	4.86
	HA	0.85	0.48	1.57	2.22	1.52	4.81
R. vulgaris (mg/mL)	AQ	0.64	0.40	1.02	1.41	1.04	2.46
	HA	1.03	0.40	5.37	4.09	2.38	35.81
R. glabrous (mg/mL)	AQ	1.41	1.21	1.68	2.77	2.37	3.40
	HA	2.20	-	-	5.73	-	-
J. abysinicum (mg/mL)	AQ	1.58	1.16	2.60	3.60	2.60	6.63
	HA	1.22	0.70	3.52	3.49	2.16	13.02
M. africana (mg/mL)	AQ	1.66	1.42	2.00	3.23	2.73	4.02
	HA	1.95	-	-	5.54	-	

Table 4: Mean percentage inhibition of larval development of *Haemonchus contortus* exposed to different concentrations of plant extracts (mg/mL) and Ivermectin (µg/mL)

Plants and drug		Mean ± SE percent inhibition at different concentration						
Plants (mg/mL)	Extr	0.00	1.562	3.125	6.25	12.5	25	50
F. vulgare	Aq	11±0.01	12.3±0.04	14±0.014	19.6±0.032	22.7±0.027	25.9±0.005	31.8±0.08
	HA	3±0.01	11.8±0.027	16.8±0.014	25.9±0.014	46.8±0.014	73.6±0.009	90±0.01
A. schimperi	Aq	9±0.02	16.4±0.009	25.9±0.006	28.6±0.014	36.4±0.027	63.6±0.027	83.5±0.02
	HA	9±0.01	11.8±0.027	16.8±0.014	25.9±0.014	46.8±0.014	73.6±0.009	90±0.01
R. vulgaris	Aq	1±0.01	10.5±0.005	11. ±80.018	11.4±0.014	11.4±0.005	9.6±0.005	10±0.00
	HA	3±0.01	25±0.014	25.9±0.014	25.9±0.014	26.8±0.014	37.3±0.018	63.6±0.03
R. glabrous	Aq	7±0.01	14.4±0.023	45.5±0.009	50.5±014	57.7±0.032	75±0.032	86.4±0.01
	HA	9±0.01	53.6±0.018	63.6±0.009	72.7±0.009	77.7±0.022	86.8±0.050	97.7±0.01
J. abysinicum	Aq	13±0.02	36.4±0.027	36.4±0.009	38.2±0.018	40±0.027	42.7±0.036	67.3±0.03
	HA	12±0.01	43.2±0.032	56.4±0.027	68.6±0.014	79±0.009	78±0.014	84±0.01
M. africana	Aq	6±0.02	9±0.0036	9.6±0.005	11.8±0.009	12.7±0.036	24.6±0.027	51.4±0.03
	HA	15±0.02	25±0.014	25.9±0.014	26.8±0.014	37.3±0.018	63.6±0.027	89±0.09
Ivemectin*	-	0.15±0.01	14±0.023	19±0.036	52.3±0.032	85.5±0.018	89±0.009	95±0.013

induced nearly similar effect. For instance, the alcoholic extract of leave of *A. schimperi* required a maximum concentration of 2mg/ml to induce 87% egg hatch inhibition while aqueous extract of same plant induced 53.6% egg hatch inhibition at concentration 2mg/ml. In general, all plants used in the current study induced over 50% of inhibition hatch of egg of *H. contortus* at highest concentration used (2mg/ml). Albendazole required a maximum concentration 0.25µg/ml to induce 100% egg hatch inhibition (Table 2).

The effective doses required to induce 50% and 90% (ED_{50} and ED_{90}) inhibition of egg hatching calculated by probit analysis are presented in Table 3. Of all plants investigated, both extracts of *F. vulgare* induced 50% inhibition of egg hatching at lower concentration, i.e, 0.24 and 0.27 mg/ml for aqueous and alcoholic extracts, respectively. Aquaeos extract of *R. vulgaris and alcoholic extract of A. schimperi* have also performed the next remarkable inhibition of egg hatching at concentration of 0.64 and 0.85 mg/ml, respectively. Of all plants, alcoholic extract of *R. glabrous* induced 50% inhibition at higher concentration. ED_{50} value for the aqueous and alcoholic extracts of most of plants in the current study did not show statistically significant variation. ED_{50} for egg hatch inhibition of Albendazole was 0.09µg/ml (Table 3).

Larvicidal Effect: Mean percentage inhibition of larval development of *H. contortus* at different concentrations of plant extracts (mg/ml) and Ivermectin (µg/ml) is indicated in Table 4. Most of both aqueous and methanol extracts of the plants had shown variable effect on development of larvae of *Haemonchus contortus.* The highest larval development inhibition observed was for methanol extract of *R. glabrous* (97.7%) while the least effective plant was aquoeos extract of *R. vulgaris* (10%) at the maximum tested concentration. Despite high dose required to inhibit larval development, except for both extracts of *R. vulgaris*, almost all plant extracts induced dose dependent inhibition of larval development.

Unlike the effect of plants on egg hatching, there was statistically significant variation in activity of aqueous and methanol extract of most of the plants ($p<0.05$) at most of the tested concentrations. In most of the cases, methanol extracts are more effective than their aqueous counterparts.

DISCUSSION

Plant materials in the current study had been identified from various sources to serve as anthelmintic agents by traditional healers. All plants in the present study had shown significant variation in the yield of extracts. The observed variation was between different plants and with in the plant with different types of extraction. Variation in the yield of aqueous and methanol extracts of plants could be due to difference in chemical composition or solubility of the components in different solvents, so that some plants may contain active components that are more soluble in organic solvents while others may contain active components that are more soluble in water [24].

In vitro technique had advantage to evaluate anthelmintic activities of claimed medicinal plants over *in vivo* techniques due to simplicity and cost effectiveness of this technique. However *in vivo* test are not the best model to screen plants extracts with anthelmintic activities, since this tests are time consuming, expensive and present low precision and reproducibility due to inter animal variation and pharmacodynamics of the drugs in the host [25]. on the other hand, the *in vitro* test using free living stage of parasitic nematodes offer means of evaluating the antihelmintic activities of new plants components, as already reported by various authors [26].

The variation between anthelmintic activity of aqueous and methanol extracts of used plants, in current study, might be due to difference in the proportion of the active components responsible for anthelmintic activity resulting from the difference in solubility either in water or methanol. The result of the present study strengthens the report of [24]. Some plants in the current experiment induced inhibition of egg hatching of *H. contortus* at lower concentration compared to other plants evaluated previously. For example, 2.5 mg/ml of essential oil of *Ocimum gratissimum* induced 96.94% inhibition of egg hatching [27]. 7.1mg/ml aqueous extract of *Annona senegslensis* inhibited only 11.5% egg hatch [28], methanol extract of *Spigelia anthelmia* induced 97.4% egg hatch inhibition a t concentration of 50mg/ml [26]. On the other hand, most of plants investigated in the current experiment required m aximum concentration of 2 mg/ml to induce ED_{50} of egg hatching.

The concentration required to produce 50% egg hatch inhibition (ED_{50}) for the four most potent extracts in descending order were, methanol extract of *F. vulgare*, aqueous extract of *F. vulgare*, aqueous extract of *R. vulgaris* and methanol extract of *A. schimperi,* with ED_{50} of 0.24, 0.27, 0.64 and 0.85mg/ml respectively. Significant dose dependent effects observed for most of the plants in current experiments indicate the fact that increasing the dose of the plant extracts increases the

proportion of the chemical ingredient(s) with pharmaceutical value in the crude plant extract. Methanol extract of *M. africana* and *R. glabrous* have not shown significant dose dependent activity at various tested concentrations.

ED_{50}-value for the aqueous and alcoholic extracts of most plants in the current study did not show statistically significant variation. This might be due to the presence of similar or related chemicals having ovicidal property in both extract types in nearly equivalent proportion.

The minimum concentration of albendazole required to induce 100% egg hatch inhibition (0.25µg/ml) and the ED_{50} value of 0.09 µg/ml indicates that the susceptibility of the strain of *H.contortus* employed in the current study to benzimidazole anthelmintics. According to WAAVP, [22] eggs with ED_{50} value less than 0.1µg/ml are indicative of benzimidazole susceptibility.

The possible reason for the better performance of methanol extracts compared to aqueous extracts, on the larval development could be due to easy transcuticular absorption of the methanol extracts into the body of the parasite more than the aqueous extracts. Although distinct chemical profiles of the plant extracts are not known, in general, alcoholic extracts of plants may contain some non-polar organic chemicals with wide range of polarity than the aqueous extracts [29].

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