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Antihelmentic Effects of the Essential oil Extracts of Selected Medicinal Plants against *Haemonchus contortus*

[1]Jemal Hussien, [2]Kaba Urgessa, [3]Fayissa Regassa, [4]Awol Jemal, [5]Sultan Abajebel and [4]Nezif Hussien

[1]Department of Pharmacy, College of Public Health and Medical Sciences, Jimma University, Ethiopia
[2]Department of Natural Resources Management, College of Agriculture and Veterinary Medicine, Jimma University, Ethiopia
[3]Department of Animal Science and Veterinary Medicine, College of Agriculture and Veterinary Medicine, Jimma University, Ethiopia
[4]Department of Pharmacy, College of Public Health and Medical Sciences, Jimma University, Ethiopia
[5]Jimma Town Health Bureau, Jimma, Ethiopia

Corresponding Author: Jemal Hussien, Department of Pharmacy, College of Public Health and Medical Sciences, Jimma University, P.O. Box 378, Jimma, Ethiopia Tel: +251-917-800964 Fax: +251-47-1114484

ABSTRACT

The present study was aimed at investigating the antihelmentic effects of the essential oil extracts of *Coriandrum sativum* Linn., *Ocimum gratissimum* Linn., *Ocimum lamifolium* Hochst. ex Benth, *Ruta chalpensis* Linn., *Thymus schimperi* Ronniger and *Echinops kebericho* Mesfin against *H. contortus*. *In vitro* bioassay employing the egg hatching test was conducted to determine the antihelmentic potential of the essential oils at 1, 0.5, 0.25, 0.125 and 0.0625% (v/v) concentration. The concentration of the extracts that inhibited 50% of test organisms (IC_{50}) was determined using probit analysis of the mean percent egg hatch inhibition followed by linear regression analysis. Accordingly, the plants demonstrated inhibitory effects on hatching of eggs at all concentration levels. The mean percent inhibition at 1% conctration ranged from 81.8±0.6 (*E. kebericho*) to 100±0 (*O. gratissimum* and *R. chalpensis*). At each test concentration of the essential oils, the observed biological effect was statistically significant ($p<0.05$). Among the extracts, the essential oils of *O. gratissimum* (IC_{50} 0.0784% v/v), *R. chalpensis* leaf (IC_{50} 0.0876% v/v) and fruit (IC_{50} 0.0944% v/v) were found to be the most active against *H. contortus* egg hatching. The intensity of egg hatching inhibitory effect of the essential oils was observed to vary in a dose dependent fashion ($p<0.05$) and 1% concentration of the essential oils of most of the plants investigated was generally best efficacious with comparable efficacy to the positive antihelmentic compound, thiabendazole (0.5 μg mL^{-1}). The result indicated the potential utility of the plants in the control of egg shedding into the environment to prevent infection of new hosts during grazing. *In vivo* antihelmentic efficacy and toxicity studies of the plants should be established.

Key words: *In vitro* bioassay, antihelmentic activity, egg hatch test, essential oils, *Haemonchus contortus*

INTRODUCTION

Ethiopia is generally considered to has the largest population of livestock of any country in Africa. It contributes 12-16% of the total GDP and 30-35% of agricultural GDP. Livestock are also estimated to contribute to the livelihoods of 60-70% of the Ethiopian population (Solomon *et al.*, 2003). Gastrointestinal parasitic infestations especially with *Haemonchus contortus* are worldwide problems for both small and large-scale farmers (Akhtar *et al.*, 2000). It has been established that animal diseases are a major constraint to livestock production in Ethiopia (Perry *et al.*, 2002). Consequently, the country has benefited little from this enormous resource owing to a multitude of problems, disease being the most important. Diseases alone accounts for mortalities of 30% in lambs and 20% in adults. A loss of US$ 81.8 million is reported annually due to helminth parasites (Biffa *et al.*, 2006).

The control of gastrointestinal nematodiasis is usually made with synthetic anthelmintics. However, widespread resistant ruminant nematodes to many anthelmintic drugs and have been reported in many parts of the globe and thus became the threat to sustainable helmenth control (Axford *et al.*, 2000; Schnyder *et al.*, 2005). The current synthetic drugs are also expensive and moreover, these agents also have the negative impact in terms of leaving residues in animal products and causing environmental pollution (Biswas *et al.*, 2010; Maphosa and Masika, 2010).

This triggers the search of alternatives, such as medicinal plants. Many plants synthesize substances that are useful to the maintenance of health in humans and animals (Falodun, 2010). Plants constitute major part of traditional veterinary practices and have been found to be a rich source of botanical antihelmentics for centuries (Assefa *et al.*, 2010). Among the major plant secondary metabolites, essential oils received much attention due to their diverse biological activities. They have been reported to possess various bioefficacies such as insect repellent and biocidal activities including ovicidal and larvicidal against various parasites, insects and helmenths (Asha *et al.*, 2001; Pessoa *et al.*, 2002; Hierroa *et al.*, 2006; Costa *et al.*, 2008). Other studies also indicated that optimal essential oil supplementation improved nutrient digestibility, daily gain, milk-to-feed ratios and productive performances in ruminants (Soltan *et al.*, 2009; Patra, 2011).

The flora of Ethiopia is rich and variable due to the vast area of the country's diverse ecological and climatic conditions (Hedberg and Hedberg, 2003). *Coriandrum sativum* L. (Apiaceae) (*Dimbilal* in Amharic) is an erect aromatic annual herb which is cultivated in most parts of Ethiopia for its aromatic seeds. The seed has a wide range of daily use in food stuff. It is traditionally used against stomachache (Hedberg and Hedberg, 2003) and for treatment of ascariasis and hepatitis (Dessisa, 2001; Giday *et al.*, 2007).

Members of the genus *Ocimum* (Lamiaceae) are also widely used in Ethiopian herbal medicine for the treatment of various diseases. *Ocimum gratissimum* Linn. (*Anchabi* in Afaan Oromoo) is an aromatic plant traditionally used in treating different digestive disorders, as anthelmintic and physical drench/balls (Abera, 2003). Similarly, *Ocimum lamifolium* Hochst. ex Benth (*Damakesee* in Amharic) finds traditional indication in the treatment of intestinal disorders (Abera, 2003).

Thymus schimperi (Lameaceae, called *Tossign* in Amharic) is a small herb that is widely used in Ethiopian cooking. In Ethiopia, it is used medicinally and as culinary herbs (Asfaw *et al.*, 2000). A tea made by the herb in water has been recommended as medicinal remedy for respiratory problems (cough, bronchitis, sore throat), gastrointestinal disorders, (colic, dyspepsia gastritis, flatulence, diarrhea and as anthihelmentic) and liver disease (Abebe and Ayehu, 1993).

Echinops kebericho Mesfin (Asteraceae) is claimed to be useful in the treatment of migraine, diarrhea and intestinal worm infestation (Hymete and Kidane, 1991; Abebe and Ayehu, 1993; Assefa *et al.*, 2010). The plant *Ruta chalepensis* Linn. (Rutaceae) (*Tena Adam* in Amharic) is a leafy, branched shrublet with an aromatic or pungent odour. It is traditionally claimed to be useful in treating stomach ache (Teklehaymanot *et al.*, 2007).

Previous scientific reports indicating the antihelmentic effects of the volatile extracts of the selected Ethiopian medicinal plants against *H. contortus* have not been obtained. Thus, the present study was conducted to investigate the *in vitro* antihelmentic activity using egg hatch test as a bioassay protocol, of the essential oil extracts of six medicinal plant species growing in Ethiopia against *H. contortus* ova.

MATERIALS AND METHODS

Study area and design: The study was conducted in Jimma town, South Western Ethiopia from August 2009 to June 2010. *In vitro* experimental study design was conducted to investigate the antihelmentic activity of selected medicinal plants against *H. contortus*. The outcome parameters were percent egg hatch inhibition and the median inhibitory concentration in 50% test organisms (IC_{50}).

Plant materials: The plant parts (leaf, fruit, inflorescence and/or root) of the selected plants were collected from *Jiren area,* Jimma town (between September to November, 2009) or purchased from local markets and authenticated by taxonomist and specimen were deposited with the following voucher number at Jimma University Herbarium: *Corriandrum sativum* L. (BT01/2009), *Thymus schimperi* R. (BT02/2009), *Ocimum gratissimum* L. (AJ01/2009), *Ocimum lamifolium* (JH02/2009), *Ruta chalpensis* L. (JH05/2009) and *Echinops kebericho* M. (AB/2009).

Chemical and reagents: Sodium sulphate (anhydrous), Tween-80 (BDH® Laboratory Supplies, Lot ZA2088516 647, Poole, England), freshly prepared Phosphate Buffer Saline (PBS, pH 7.4, in 0.5% Tween-80), Lugol's iodine solution (USP 30/NF25, 2007), saturated solution of sodium chloride (USP 30/NF25, 2007), 0.5% Tween-80 and distilled water were used.

Apparatus and equipments: Microscope (Olympus, binocular model CX21, Japan), McMaster slide (AVE SE ISSAQUAH, WA 98029 USA), essential oil determination apparatus (Clevenger type), centrifuge (Heraeus type B 1620, USA), incubator (Clifton targa NE 0106, England), micro pipette (Socorex, Swizerland), Enzyme-linked immunosorbent assay (ELISA) titre plates (Neo/SCI corporation, New York) were used.

Test specimen: Ova of the strongyline parasite *H. contortus* recovered abomasums of naturally infected sheep were collected from Jimma town abattoir and employed for the *in vitro* antihelmentic activity test.

Control agents: Control agents involving thiabendazole (0.5 μg mL^{-1}, positive control), 0.5% Tween-80 in PBS (negative control) and distilled water (control) were used.

Hydro distillation of essential oils: Freshly collected parts of *Ocimum gratissimum* Linn., (leaf) *Ocimum lamifolium* Hochst. ex Benth. (leaf and infruitescence), *Ruta chalpensis* Linn. (leaf and fruit) and shade dried morphological parts of *Coriandrum sativum* Linn., (seeds), *Thymus schimperi* Ronniger (leaf) and *Echinops kebericho* Mesfin (root) were subjected to hydro distillation in a Clevenger type of apparatus for three hours after the mixture started boiling. The distillation apparatus consists of a heating mantle, a 3 L round-bottom extraction flask, a 5 mL graduated receiver (Dean-Stark type apparatus) and a condenser (jacketed coil). At the end of the distillation process, the volume of essential oil collected in the receiver was measured and the percentage yield was calculated. The essential oils obtained were treated with anhydrous sodium sulphate to remove any dissolved water from the oils and then stored in refrigerator at 4°C preserved from exposure to direct light until use for bioassay.

Egg recovery: Adult *H. contortus* worms were collected after giving longitudinal incision along the greater curvature of abomasum of naturally infected sheep and placed in a bottle containing cool Phosphate Buffered Saline (PBS) (pH 7.4) to be transported to the veterinary parasitology laboratory, Jimma University College of Agriculture and Veterinary Medicine (JUCAVM). Female worms were separated from males by grossly witnessing the blood filled intestine and triturated using mortar and pestle to liberate eggs. The suspension was filtered and the filtrate was centrifuged for 2 min at about 300x g (gravitational force) and the supernatant was discarded. The tubes were agitated in 0.5% Tween-80 solution to loosen the sedimented eggs and the concentration of the eggs were adjusted using the McMaster technique (Hansen and Perry, 1994) to 500 eggs per millilitre for use in egg hatch assay.

Egg hatch test: The *in vitro* egg-hatching test for the extracts was employed according to the standard guidelines of World Association for the Advancement of Veterinary Parasitology (WAAVP) to evaluate the efficacy of anthelminthics in ruminants (Coles *et al*., 1992).

Statistical analysis: All the tests were performed three times for each treatment and control. The data obtained were analyzed using Statistical Package for Social Science (SPSS) version 16.0 for windows and results presented as Mean±SD. Probit transformation of the mean percent hatch inhibition was done using Finney's table (Vincent, 2010) and linear regression was undertaken to determine the extract concentration required to prevent 50% (IC_{50}) of eggs from hatching.

RESULTS

The yields of the essential oils expressed in relation to the fresh weight of the plant materials are shown in Table 1. The highest (1% v/w) and lowest (0.04% v/w) yields were obtained from *T. schimperi* leaves and *O. lamifolium* infruitscence, respectively.

Table 1: The yield and colour of the essential oils of the plant species studied

Plant species	Parts used	Colour of oil	Yield (%v/w)♣
Echinops kebericho	Root	Yellow	0.60
Corriander sativum	Seeds	Pale yellow	0.37
Ocimum gratissimum	Leaves	Pale yellow	0.30
Ocimum lamifolium	Leaves	Pale yellow	0.12
Ocimum lamifolium	Infruitscence	Pale yellow	0.04
Ruta chalpensis	Leaves	Pale yellow	0.14
Ruta chalpensis	Fruits	Colourless	0.17
Thymus schimperi	Leaves	Pale yellow	1.00

♣ % v/w = No. of parts by volume of the essential oil per 100 parts by weight of the plant material

Table 2: The effectiveness of plant essential oils against *Haemonchus contortus* egg hatching (n = 3)[a]

The plant species and control agents	The concentration (% v/v)[b] of essential oil	Mean percent egg hatch inhibition[c]
Ocimum gratissimum leaf	1	100.00±0.00
	0.5	93.47±1.72
	0.25	80.40±3.08
	0.125	68.93±1.69
	0.0625	51.20±0.95
Ocimum lamifolium leaf	1	92.43±3.77
	0.5	77.87±0.46
	0.25	64.13±1.62
	0.125	55.10±1.67
	0.0625	42.03±1.72
Ocimum lamifolium infruitscence	1	90.50±1.65
	0.5	75.63±4.38
	0.25	59.80±1.91
	0.125	43.97±3.27
	0.0625	28.60±2.25
Ruta chalpensis leaves	1	100.00±0.00
	0.5	91.90±1.57
	0.25	77.83±1.94
	0.125	62.07±1.39
	0.0625	48.70±1.32
Ruta chalpensis fruit	1	99.30±1.21
	0.5	87.60±1.74
	0.25	72.67±1.14
	0.125	58.17±3.20
	0.0625	43.73±1.55
Corrianderum sativum seeds	1	83.63±2.05
	0.5	68.60±2.11
	0.25	54.33±0.70
	0.125	41.27±1.50
	0.0625	26.37±0.57
Thymus shimperi leaves	1	88.67±3.29
	0.5	76.77±2.25
	0.25	59.86±0.83
	0.125	47.37±2.49
	0.0625	31.10±1.28
Echinops kebericho root	1	81.80±0.60
	0.5	69.53±0.61
	0.25	53.67±1.90
	0.125	41.23±1.27
	0.0625	24.63±1.76
Thiabendazole (μg mL^{-1})	0.5	100.00±0.00
Tween-80 solution	0.5	6.67±1.89
Distilled water	-	2.77±0.40

[a]No. of experiments. [b]v/v: No. of parts by value of the essential oil per 100 parts of resulting solution [c]Values are presented as Mean±SD ($p<0.05$)

Antihelmentic activity of the essential oils: All of the essential oils of the investigated plants demonstrated inhibitory effects on hatching of eggs at the test concentrations, 1, 0.5, 0.25,

Table 3: Regression equation and IC_{50} values of the Essential oils

Plant species	Regression equation *Y = Intercept + BX	R^2	**IC_{50} (% v/v)
O. gratissimum leaf	Y = 7.61 + 2.359x	0.894	0.078
O. Lamifolium leaf	Y = 6.27 + 1.282x	0. 951	0.103
O. lamifolium infruitscence	Y = 6.25 + 1.541x	0. 986	0.155
R. chalpensis leaves	Y = 7.58 + 2.438x	0.8510	0.088
R. chalpensis fruit	Y = 7.02 + 1. 973x	0. 912	0.094
C. sativum seeds	Y = 5.94 + 1.325x	0. 994	0.195
T. schimperi leaves	Y = 6.18 + 1.422x	0. 993	0.147
E. kebericho root	Y = 5.91 + 1.306x	0. 998	0.199

*Y = Probit; x = The log concentration of the oil extracts; B = Slope of the graph, **IC_{50} = Inhibitory concentration in 50% of test organisms; % v/v = The number of parts by volume of the essential oil per 100 parts of the resulting solution

0.125 and 0.0625% v/v (Table 2). The intensity of egg hatching inhibitory effect of the essential oils was observed to vary in a dose dependent fashion ($p<0.05$). At 1% concentration, the mean percent inhibition ranged from 81.8±0.6 (*E. kebericho*) to 100±0 (*O. gratissimum* and *R. chalpensis*). At the minimum concentration (0.0625%) of the oils used, the mean percent egg hatch inhibition ranged from 24.6±1.75 (*E. kebericho*) to 51.2±0.95 (*O. gratissimum*). At each test concentration of the essential oils, the observed biological effect was statistically significant ($p<0.05$).

More than 50% egg hatch inhibition was observed at a concentration of 0.0625% for *O. gratissimum* leaf, 0.125% for *O. lamifolium* leaf, *R. chalpensis* (leaf and fruit) and 0.25% for *O. lamifolium* infruitscence, *C. sativum, T. schimperi and E. kebericho*. 0.5% Tween-80 (negative control) and distilled water (control) exhibited 6.67±1.89 and 2.77±0.40 egg hatch inhibition (Table 2). Thus, in comparison to the negative control, significant egg hatch inhibitory effects were observed by the essential oil extracts ($p<0.05$).

At a concentration 0.125% or higher, the tested essential oils generally produced 40 to 100% egg hatch inhibition (Table 2). One percent essential oil concentration was best efficacious in all plant species investigated. The result obtained for these plants at 1.0% concentrations were comparable to 0.5 μg mL^{-1} thiabendazole, the positive control.

The concentration of the essential oils required to inhibit 50% egg counts from hatching (IC_{50}) were found to range between 0.078 (*O. gratissimum*) to 0.199 (*E. kebericho*) (Table 3). The IC_{50} values indicated that the two species, *O. gratissimum* leaf (IC_{50} 0.078% v/v) and *R. chalpensis* (IC_{50} 0.088 and 0.094%v/v, respectively for leaf and fruit) were more potent in their inhibitory effect against egg hatching.

DISCUSSION

As observed in this study, all of the essential oils of the studied plants were found to be active against egg hatching process. As shown in Table 2, *O. gratissimum* exerted 93.47 and 100% inhibition of ecludibility at 0.5 and 1% concentrations, respectively. Potent anthelmintic activity of the essential oil of the closely related plant species, *O. sanctum* has also been reported by Asha *et al.* (2001) and Pessoa *et al.* (2002). Moreover, Njoku and Asuzu (1998) reported 15% paralytic effects of the ethanol extract of *O. gratissimum* leaves on the third-stage larvae of *H. contortus*, underscoring the antihelmentic activity of the plant.

Though the biological effect observed in this study is similar with previous findings, the intensity of effect is slightly lower compared to the result reported by Pessoa *et al.* (2002) who reported a maximum (100%) inhibition of eclodibility at and above 0.50% concentration of the essential oil of *O. gratissimum*. However, 100% inhibition by *O. gratissimum* was recorded at 1% concentration level of the essential oils in this study. The relatively lower activity in present result could be due to the lower concentration of the biologically active constituents. Recent studies on the extracts of *O. gratissimum* also indicated the effect on biochemical and hematological parameters of animal cells (Obianime *et al.*, 2011). The volatile oil of the plants of the genus *Ocimum* contain mostly phenolic compounds, particularly eugenol and thymol (Sainsbury and Sofowora, 1971; Pessoa *et al.*, 2002; Javanmardi *et al.*, 2003) and could be responsible for the observed antihelmentic effects of *O. gratissimum* and *O. lamifolium*.

Similarly, the essential oil of the plant *C. sativum* exhibited egg hatch inhibition ranging from 26-83%. According to Eguale *et al.* (2007), the crude aqueous and hydro-alcoholic extracts of *C. sativum* inhibited hatching of eggs completely at a concentration less than 0.5 mg mL^{-1} which marks the antihelmentic potential of the *C. sativum*. Linalool, the major volatile component (Ramadan and Moersel, 2002) reported to occur in the plant alone or in synergy with other minor components could be responsible for the inhibition of egg hatching.

The observed antihelmentic effect of the volatile oils of *E. kebericho* against *H. contortus* ova is also in agreement, at least in part with previous reports (Hymete and Kidane, 1991). Alcoholic extract of the roots of *E. kebericho* has been shown to have a very strong lethal activity against earthworms, helmenths that share similar anatomic and physiological similarity with many intestinal worms. The same worms kept in a cabinet together with the powdered roots of *E. kebericho* were found dead after a few hours (Hymete and Kidane, 1991) which suggest that the volatile constituents could be responsible for the lethal activity. In the hydro distilled essential oil of *E. kebericho*, eudesm-7 (11)-en-4-ol (14.3%), followed by caryophyllene oxide (9.7%) and τ-cadinol (8.3%) were reported to occur as the main constituents (Hymete *et al.*, 2007). These volatile components could be implicated to the activity of the plant.

Though published reports are unavailable, the traditional claim regarding the antihelmentic properties of *R. chalpensis* and *T. shimperi* can also be substantiated in this study. The principal compounds identified from *R. chalpensis* were 2-nonanone, 2-undecanone, 2-decanone and 2-dodecanone (African Laboratory for Natural Products, ALNAP, 2001). On the other hand, the main constituents of the essential oils of T. schimperi were identified as p-cymene (9-23%), γ-terpinene (8-17%), thymol (6-38%) and carvacrol (5-63%) (Asfaw *et al.*, 2000). These chemical entities might be responsible for the biological effect observed in this study.

CONCLUSION

The results of the present bioassay on *H. contortus* ova indicated that the investigated plants in general, *O. gratissimum* and *R. chalpensis* in particular were shown to be efficacious against egg hatching. This observation is an indicator in that the plants could interrupt the parasite's life cycle and could play a role in the control of egg shedding into the environment and thus prevent infection of new hosts during grazing. Further work is recommended to elucidate the *in vivo* antihelmentic activity and toxicity of the extracts.

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