

In vitro and in vivo anthelmintic activity of crude extracts of *Coriandrum sativum* against *Haemonchus contortus*

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Abstract

In vitro anthelmintic activities of crude aqueous and hydro-alcoholic extracts of the seeds of *Coriandrum sativum* (Apiaceae) were investigated on the egg and adult nematode parasite *Haemonchus contortus*. The aqueous extract of *Coriandrum sativum* was also investigated for in vivo anthelmintic activity in sheep infected with *Haemonchus contortus*. Both extract types of *Coriandrum sativum* inhibited hatching of eggs completely at a concentration less than 0.5 mg/ml. ED₅₀ of aqueous extract of *Coriandrum sativum* was 0.12 mg/ml while that of hydro-alcoholic extract was 0.18 mg/ml. There was no statistically significant difference between aqueous and hydro-alcoholic extracts ($p > 0.05$). The hydro-alcoholic extract showed better in vitro activity against adult parasites than the aqueous one. For the in vivo study, 24 sheep artificially infected with *Haemonchus contortus* were randomly divided into four groups of six animals each. The first two groups were treated with crude aqueous extract of *Coriandrum sativum* at 0.45 and 0.9 g/kg dose levels, the third group with albendazole at 3.8 mg/kg and the last group was left untreated. Efficacy was tested by faecal egg count reduction (FECR) and total worm count reduction (TWCR). On day 2 post treatment, significant FECR was detected in groups treated with higher dose of *Coriandrum sativum* ($p < 0.05$) and albendazole ($p < 0.001$). On days 7 and 14 post treatment, significant FECR was not detected for both doses of *Coriandrum sativum* ($p > 0.05$). Significant ($p < 0.05$) TWCR was detected only for higher dose of *Coriandrum sativum* compared to the untreated group. Reduction in male worms was higher than female worms. Treatment with both doses of *Coriandrum sativum* did not help the animals improve or maintain their PCV while those treated with albendazole showed significant increase in PCV ($p < 0.05$).

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Keywords: Anthelmintic activity; *Coriandrum sativum*; *Haemonchus contortus*; Sheep

1. Introduction

Helminth infection is a major threat to small ruminant production leading to enormous economic losses particularly in areas where extensive grazing is practiced (Waller, 1997). Helminthosis is responsible for 28% of mortality and 3–8% of weight loss in

Ethiopian highland sheep (Bekele et al., 1992). The annual financial loss due to helminthosis in Ethiopia was also estimated to be about 700 million Ethiopian Birr (Habte-Silasie et al., 1991).

Compared to other nematodes, *Haemonchus contortus* is a highly pathogenic parasite of small ruminants, and is capable of causing acute disease and high mortality in all classes of stock (Allonby and Urquhart, 1975). Haemonchosis is characterized by hemorrhagic anemia attributable to blood loss via the blood-sucking activities of worms in the abomasum (Urquhart et al., 1996). *Haemonchus contortus* is highly prevalent in sheep and goats in most parts of East Africa (Tembely et al., 1997).

Commercial anthelmintics have been used for some decades throughout the world to minimize the losses caused by helminth infections. However, the threats of anthelmintic resistance, risk of residue, availability and high cost especially to farmers of

Abbreviations: AL-IPB, Akililu Lemma Institute of Pathobiology; ANOVA, analysis of variance; DMSO, dimethyl sulfoxide; ED₅₀, effective dose 50; EHA, egg hatch assay; EPG, egg per gram of faeces; FECR, faecal egg count reduction; LD₅₀, lethal dose 50; PBS, phosphate buffered saline; PCV, packed cell volume; SEM, standard error of the mean; TWCR, total worm count reduction; WAAVP, World Association for Advancement of Veterinary Parasitology

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low income in developing countries have led to the need of other alternative control methods (Baker et al., 1992; Waller, 1997). Options like, biological control, vaccine and traditional medicinal plants are being examined in different parts of the world (Bain, 1999; Chandrawathani et al., 2003; Githiori, 2004). Screening and proper evaluation of the claimed medicinal plants could offer the possible alternatives that may both be sustainable and environmentally acceptable.

Coriandrum sativum L. (Apiaceae) is an erect annual herb 20–70 cm tall with strong smell. It is widespread throughout the world as a result of cultivation for its aromatic seeds. It is cultivated at altitude of 1700–2500 m above sea level in most parts of Ethiopia. The seed has a wide range of daily use in foodstuff. It is also used against stomach ache (Hedberg and Hedberg, 2003). Extracts from seeds of *Coriandrum sativum* have several pharmacological effects such as anti-fertility, anti-diabetic, anti-hyperlipidemic, antioxidant, and hypotensive activities (Al-Said et al., 1987; Chithra and Leelamma, 1997; Gray and Flatt, 1999; Melo et al., 2003). In Ethiopia, it is traditionally used for treatment of ascariasis and hepatitis in human (Dessisa, 2001). Phytochemical screening indicated the presence of chemicals such as quercetin 3-glucuronide, linalool, camphor, geranyl acetate, geraniol and coumarins. The major fatty acid was petroselinic acid (65.7% of the total fatty acid methyl esters) followed by linoleic acid (Ramadan and Morsel, 2002). Taniguchi et al. (1996) isolated three isocoumarins, coriandrones C–E, from whole plants of *Coriandrum sativum*. Two types of 2-C-methyl-D-erythriol glycosides were also recently isolated from the seed of *Coriandrum sativum* (Kitajima et al., 2003). The objective of the current study was therefore to assess the in vitro and in vivo anthelmintic potential of the seeds of *Coriandrum sativum* on nematode parasite *Haemonchus contortus*.

2. Materials and methods

2.1. Plant material preparation

Seeds of *Coriandrum sativum* were purchased from Debre Birhan, 138 km North of Addis Ababa and transported to Aklilu Lemma-Institute of Pathobiology (AL-IPB), Addis Ababa University. It was then identified by a taxonomist and voucher sample representing Herbarium No. MG/004/05 was deposited at the Herbarium of the Addis Ababa University, Biology Department. The seeds were air dried at room temperature, ground and kept in amber colored bottle until processed. Preliminary qualitative phytochemical screenings for major secondary metabolites were conducted according to Debella (2002). The seed powder was screened for the presence of polyphenols, cyanogenic glycosides, saponins, phytosteroides and withanoids, phenolic glycosides, flavonoids, tannins, alkaloids and antraquinone glycosides. Aqueous extraction was performed by soaking a weighed amount of the dry powder 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 h, which was then lyophilized. The lyophilized dry powder was then collected in a stoppered sample vial, weighed and kept in a desiccator to avoid absorption of water until used. Hydro-

alcoholic extraction was conducted by percolating 200–300 g of the dried and powdered plant material using 80% methanol for 5 days, which was then filtered through whatman filter paper No.1. The solvent was evaporated using a Rotavapor and the extract was kept in a stoppered sample vial at 4 °C until used.

2.2. Parasites

Adult female parasites of *Haemonchus contortus* were collected from abomasums of infected sheep obtained from Addis Ababa Abattoir. The worms were washed and crushed to liberate eggs. The eggs were then cultured in a glass jar filled with autoclaved sheep faeces for 8 days at room temperature. At the end of the 8th day, infective larvae were harvested by rinsing the side of the culture jar with drops of water. About 3000 larvae were inoculated to two worm free sheep that were kept indoor in separate house in the animal facilities of the AL-IPB throughout the study period. These sheep served as *Haemonchus contortus* egg donors for subsequent in vitro and in vivo trials.

2.3. In vitro experiments

2.3.1. Egg hatch assay

Eggs used in the present assay were collected from previously mentioned donor sheep according to World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Coles et al., 1992). Fresh eggs were then washed repeatedly with distilled water. Aqueous and hydro-alcoholic extracts of the seeds of *Coriandrum sativum* were used as the test treatment. Albendazole (99.8 % pure standard reference) was used as positive control while untreated eggs in water were used as negative control. In the assay, approximately 200 eggs in 1.5 ml of water were placed in each test tube. Aqueous and hydro-alcoholic extract of *Coriandrum sativum* at concentrations of 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml in a total volume of 0.5 ml in distilled water was used. Albendazole was dissolved in Dimethyl sulfoxide (DMSO) and diluted at the concentrations of 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.0156 µg/ml. The test tubes were then covered and kept in incubator at 27 °C for 48 h. The experiment was replicated six times for each concentration. Hatched larvae (dead or alive) and unhatched eggs were then counted under dissecting microscope with 40× magnification.

2.3.2. Effect of plant extracts on adult worms

Adult *Haemonchus contortus* were collected from the abomasums of sheep slaughtered at the Addis Ababa Abattoir. Immediately after slaughter, the abomasums were collected and transported to the laboratory. The collected parasites were then washed and kept in phosphate buffered saline (PBS). Ten actively moving worms were placed in petridishes filled with 8.0, 4.0, 2.0, 1.0, 0.5, and 0.25 mg/ml of the aqueous and hydro-alcoholic extracts of *Coriandrum sativum* in PBS and PBS alone for the control group in a total volume of 4 ml. Albendazole dissolved in DMSO and diluted in PBS at the concentrations of 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml was used as a positive control. Three replications per each treatment concentration were employed. After 24 h, the extracts and albendazole were

washed away and the parasite resuspended in PBS for 30 min for possible recovery of the parasite motility. The number of motile (alive) and immotile (dead) worms were counted under dissecting microscope, and recorded for each concentration. Death of worms was ascertained by absence of motility for observation period of 5–6 s. A mortality index was calculated as the number of dead worms divided by the total number of worms per petridish.

2.4. In vivo anthelmintic efficacy test

2.4.1. Study animals, infection and treatment

Twenty-four 6–8 months old male Menz sheep were purchased from Debre Birhan. They were brought to animal house of AL-IPB and ear tagged. They were kept indoor on concrete floor and fed with hay and concentrate provided with water *ad lib* during the day time. The animals were provided with an adaptation period of 3 weeks before initiation of the experiment. During this period, all the animals were dosed with albendazole (Expitol[®], ERFARs.a., Greece) at 10 mg/kg and dipped with acaricide, Fenvalerate (VAPCOCIDIN 20 EC, Jordan) according to manufacturers instruction. Each animal was inoculated orally with 1750 *Haemonchus contortus* infective larvae (L₃). The infective larvae were obtained by culturing *Haemonchus contortus* eggs collected from previously mentioned two mono-species infected donor sheep.

Four weeks after infection, the sheep were divided in to four groups of six animals each by blocking based on live weight and faecal egg count taken 1 day ahead. The groups were randomly allocated to two dose levels of *Coriandrum sativum* 0.45 g/kg (group 1) and 0.9 g/kg (group 2), one positive control treated with albendazole (Expitol[®], ERFARs.a., Greece) at 3.8 mg/kg (group 3) and the last group served as untreated control (group 4). Plant extract was drenched by the use of stomach tube after dissolving in distilled water whereas albendazole was administered with balling gun.

2.4.2. Post treatment monitoring

Faecal sample was collected on day zero pretreatment and on days 2, 7, and 14 post treatment directly from the rectum. Faecal egg count per gram of faeces (EPG) was determined by modified McMaster Technique according to Coles et al. (1992). The animals were weighed on day 0 before treatment, on days 7 and 14 post treatment. They were bled from the marginal ear vein on days 0, 7 and 14 and Packed cell volume (PCV) was determined by microhaematocrit method. On day 15-post treatment all the animals were humanely killed and the male and female *Haemonchus contortus* collected and counted according to (Kassai, 1999).

2.5. Statistical analysis

ED₅₀ for egg hatch inhibition was calculated by probit analysis. Comparison of mean percentages of egg hatch inhibition and mortality of adult parasites with the control was performed by one-way ANOVA. Mean EPG, PCV and body weight of groups of sheep at different days, and mean worm burden at necropsy

were compared with the control group by one-way ANOVA. Variation in mean EPG, PCV and body weight for each group over time was analyzed using General Linear Model by repeated measures analysis of variance.

Efficacy test using faecal egg count reduction (FECR) and worm count reduction (WCR) was determined according to the method described by Coles et al. (1992). All statistical analysis was performed by SPSS Version 13.0. The post hoc statistical significance test employed was least square difference (LSD) and the difference between the means were considered significant at $p < 0.05$.

3. Results

3.1. Effect on egg hatching

Percentage yield of the aqueous seed extract of *Coriandrum sativum* was 7.75% while that of hydro-alcoholic extract was 5.54%. Phytochemical screening revealed the presence of polyphenols, phytosteroides and withanoids, flavonoids and alkaloids. The maximum concentration required to induce total (100%) egg hatch inhibition for both extracts was 0.5 mg/ml. ED₅₀ for inhibition of egg hatching are shown in Table 1. There was no statistically significant difference in the activity of the two extracts ($p > 0.05$) based on ED₅₀ for egg hatching inhibition.

3.2. In vitro effect on adult parasites

Both extracts did not produce in vitro dose dependent activity on adult parasites. The hydro-alcoholic extract, however, killed more worms than the aqueous one at all concentrations tested ($P < 0.05$). Hydro-alcoholic extract induced 85% mortality at the highest concentration tested while aqueous extract induced only 45% at the same concentration (Fig. 1).

Table 1

In vitro anthelmintic activity of *Coriandrum sativum* and albendazole expressed in ED₅₀ on the eggs of *Haemonchus contortus*

Treatment	Extract	ED ₅₀ (LCL–UCL)
Albendazole (μg/ml)		0.04 (0.02–0.05)
<i>Coriandrum sativum</i> (mg/ml)	Aqueous	0.12 (0.09–0.19)
	Hydro-alcoholic	0.18 (0.14–0.26)

LCL: lower confidence limit; UCL: upper confidence limit.

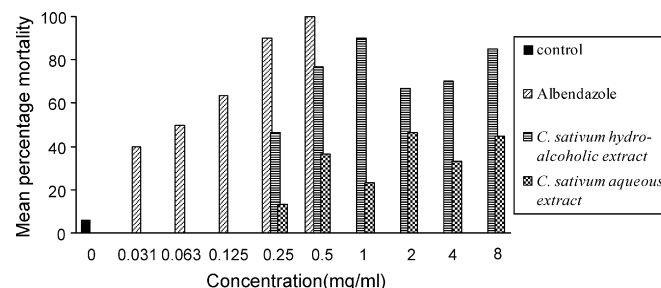


Fig. 1. In vitro anthelmintic activity of extracts of *Coriandrum sativum* and albendazole on adult *Haemonchus contortus*.

Table 2
Mean faecal EPG counts before and after treatment with aqueous extracts of *Coriandrum sativum* and albendazole

Group	Dose	Mean ^a EPG counts			
		Pretreatment		Post treatment	
		Day 0	Day 2	Day 7	Day 14
<i>Coriandrum sativum</i>	0.45 g/kg	10491.67 ± 1046.06	11483.33 ± 772.29	9258.33 ± 1538.40	9000 ± 33
<i>Coriandrum sativum</i>	0.90 g/kg	10625 ± 1017.25	9766.67 ± 772.30*	9450.00 ± 1252.20	8825.00 ± 1242.20
Albendazole	3.8 mg/kg	10900 ± 1464.87	266.67 ± 189.15**	16.67 ± 16.67**	0***
Untreated	–	10758.33 ± 1528.31	12933.33 ± 1137.40	10258.33 ± 443.70	8983.33 ± 347.67

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

^a Mean ± S.E.M.

3.3. In vivo experiment

3.3.1. Effect on faecal egg count

There was no physical clinical sign of toxicity in all groups of sheep treated with *Coriandrum sativum*. On day 2 post treatment higher dose of *Coriandrum sativum* ($p < 0.05$) and albendazole ($p < 0.001$) reduced EPG significantly. However, significant difference was not detected on days 7 and 14 post treatment for both doses of *Coriandrum sativum* ($p > 0.05$) (Table 2). The maximum FECR observed was 100% for albendazole on day 14 and 24.79% for *Coriandrum sativum* dose II, at day 2 post treatment (Table 3).

3.3.2. Effect on worm burden

Significant reduction in mean total worm count and male worm count was observed for higher dose of *Coriandrum sativum* ($p < 0.05$), while no significant reduction in female worm count was seen in both groups treated with plant extract. In the cases of sheep treated with albendazole, no worm was detected (Table 4).

Lower dose of *Coriandrum sativum* induced 8.96% reduction while higher dose induced 25.56% reduction of total worm bur-

Table 3
Percentage efficacy based on faecal egg count reduction test

Group	Dose	% reduction		
		Day 2	Day 7	Day 14
<i>Coriandrum sativum</i> (dose I)	0.45 g/kg	11.21	9.75	–0.19
<i>Coriandrum sativum</i> (dose II)	0.90 g/kg	24.49	7.88	1.76
Albendazole	3.8 mg/kg	97.79	99.84	100

den. Male worms were more susceptible to *Coriandrum sativum* at both doses compared to females (Table 5).

3.3.3. Effect on PCV and body weight

Mean PCV of group of sheep treated with lower dose of *Coriandrum sativum* and untreated group decreased significantly as of day 7 post treatment, while those treated with higher dose decreased significantly ($p < 0.05$) on day 14 post treatment. There was no significant difference between the groups on days 0 and 7. On day 14, group of sheep treated with albendazole had significantly higher PCV compared to other groups ($p < 0.05$) (Table 6).

Table 4
Geometric mean worm count of groups of sheep treated with aqueous extract of *Coriandrum sativum*, albendazole and untreated control

Treatment type	Dose	Geometric mean ^a worm count		
		Male	Female	Total
<i>Coriandrum sativum</i>	0.45 g/kg	426.56 ± 64.40	611.69 ± 96.43	1039.72 ± 158.1
<i>Coriandrum sativum</i>	0.90 g/kg	288.95 ± 32.36*	551.39 ± 33.74	850.18 ± 32.58*
Albendazole	3.8 mg/kg	0***	0***	0***
Untreated	–	507.74 ± 12.37	632.90 ± 32.70	1141.99 ± 41.23

* $p < 0.05$; *** $p < 0.001$.

^a Mean ± S.E.M.

Table 5
Efficacy of the treatment based on percentage reduction of worm burden

Group	Treatment type	Dose	% reduction		
			Male	Female	Total
1	<i>Coriandrum sativum</i> dose I	0.45 g/kg	15.99	3.35	8.96
2	<i>Coriandrum sativum</i> dose II	0.90 g/kg	43.09	12.88	25.56
3	Albendazole	3.8 mg/kg	100	100	100

Table 6
Mean PCV of sheep treated with aqueous extracts of *Coriandrum sativum* and albendazole

Group	Dose	Mean ^a PCV before and after treatment		
		Day 0 Bt	Day 7 Pt	Day 14 Pt
<i>Coriandrum sativum</i> (dose I)	0.45 g/kg	24.75 ± 0.36*	21.67 ± 1.36	19.83 ± 1.72c
<i>Coriandrum sativum</i> (dose II)	0.90 g/kg	22.67 ± 0.76	23.08 ± 1.39	19.33 ± 1.02 c*
Albendazole	3.8 mg/kg	21.83 ± 1.82	24.67 ± 0.72	26.00 ± 1.32 b*
Untreated control	–	25.33 ± 1.05*	21.33 ± 1.59	21.67 ± 0.88 c

Across rows, * means with significant difference at $p < 0.05$. Within columns, means with different letters have significant difference at $p < 0.05$.

^a Mean ± S.E.M., Bt: before treatment; Pt: post treatment.

Table 7
Mean live weight of sheep treated with aqueous extracts of *Coriandrum sativum* and albendazole compared to untreated control group

Group	Dose	Mean ^a body weight before and after treatment (kg)		
		Day 0 Bt	Day 7 Pt	Day 14 Pt
<i>Coriandrum sativum</i> (dose I)	0.45 g/kg	14.58 ± 0.50	14.63 ± 0.51	14.02 ± 0.62
<i>Coriandrum sativum</i> (dose II)	0.90 g/kg	14.68 ± 0.71	14.53 ± 0.79	14.01 ± 0.78
Albendazole	3.8 mg/kg	14.55 ± 1.02	14.83 ± 1.14	14.15 ± 1.12
Untreated control	–	14.52 ± 0.79	14.28 ± 0.78	14.25 ± 0.97

^a Mean ± S.E.M., Bt: before treatment; Pt: post treatment.

Statistically significant change in mean live weight was not detected among the groups ($p > 0.05$) (Table 7).

4. Discussion

Both the aqueous and hydro-alcoholic extracts of *Coriandrum sativum* showed good egg hatching inhibition. Both extracts inhibited egg hatching at low concentration compared to other plants studied previously. For example, 30% alcoholic extracts of leaves of *Zanthoxylum zanthoxiloides*, *Morinda lucida*, *Newbouldia levis* and seeds of *Carica papaya* inhibited hatching of *Haemonchus contortus* eggs only to the level of 40–60% at concentration of 2.4 mg/ml (Hounzangbe-Adote et al., 2005). Essential oil of *Ocimum gratissimum* at 0.5% concentration produced 100% of egg hatching (Pessoa et al., 2002). The difference in the ED₅₀ of the extracts of *Coriandrum sativum* was not statistically significant ($p > 0.05$), which could be due to the presence of similar or related chemicals having ovicidal property in both extracts in nearly equivalent proportion.

Transcuticular diffusion is a common means of entry into helminth parasites for non-nutrient and non-electrolyte substances in nematodes. It has also been shown that this route is predominant for the uptake of major broad-spectrum anthelmintics; benzimidazole, levamisole and ivermectin by different nematodes, cestode and trematode parasites as opposed to oral ingestion (Geary et al., 1999). The possible explanation for the better activity of the hydro-alcoholic extract compared to the aqueous extract on adult parasites in the current study could be due to easier transcuticular absorption of the hydro-alcoholic extracts into the body of the parasite than the aqueous extracts. Although distinct chemical profiles of the two extracts of seed of *Coriandrum sativum* are not known, in general, hydro-alcoholic extracts of plants contain some non-polar organic chemicals with lower polarity than the aqueous

extracts (Debella, 2002), rendering them more lipid soluble than the aqueous extracts and hence better anthelmintic activity. Lipophilic anthelmintics have a greater capability to cross the external surface of the helminths than the hydrophilic compounds (Geary et al., 1999). Methanol extract of *Artemisia brevifolia* at concentration of 25 mg/ml exhibited significant in vitro anthelmintic activity on adult *Haemonchus contortus*, while the aqueous extract did not produce significant effect which is in agreement with the current study (Iqbal et al., 2004). Dose dependent activity was not observed for both types of extracts. The absence of dose dependent in vitro activity was also reported for other plants investigated earlier (Hounzangbe-Adote et al., 2005).

In vivo efficiency of aqueous extract of higher dose of *Coriandrum sativum* on *Haemonchus contortus* infection in sheep based on FECRT on day 2 post treatment did not persist long. This might be due to the effect of the extract on fecundity of female parasites at early days when concentration of the extract was high in the animals (Athanasiadou et al., 2001). Efficacy test based on TWC confirmed significant reduction only in the group treated with higher dose of *Coriandrum sativum* ($p < 0.05$). The efficacy might be improved by increasing the dose or by repeated treatment for a few days (Prichard et al., 1978). Reduction in male worms was higher than the female worms at both dose levels tested. The increased susceptibility of male worms could be attributed to their smaller size. Males are 10–20 mm and females are 18–30 mm long (Soulsby, 1986). Males with smaller body size and larger surface area to volume ratio could more easily absorb plant extracts through their cuticle than females. The observation that there was significant reduction in EPG on day 2 post treatment irrespective of absence in significant reduction in female worm count in group of sheep treated with higher dose of *Coriandrum sativum*, justifies the effect of the plant extract on fecundity of the parasites.

Sheep treated with both doses of *Coriandrum sativum* neither maintained nor improved their PCV, probably because of low reduction in worm burden. Each worm is responsible for daily loss of about 0.05 ml of blood through ingestion and seepage from lesions (Urquhart et al., 1996). Treatment with *Coriandrum sativum* as well as albendazole did not show statistically significant effect on live weight of the animals. The reason could be due to confinement of animals to dry feed as well as leakage of blood and plasma protein to gastrointestinal tract by the parasites.

In conclusion, extracts from seeds of *Coriandrum sativum* showed some in vitro and in vivo anthelmintic activities against *Haemonchus contortus* at concentrations and dose levels tested. But the observed efficacy is not to the therapeutically required level. Classes of secondary metabolites 1, like alkaloids and flavonoids found in the current experiment, are considered the sources of chemical components responsible for wide therapeutic activities of several medicinal plants (Debella, 2002). The active principles that induced the observed anthelmintic activity might be found in one or both of these classes of chemicals. Further investigation on isolated fractions, at different dose levels and against different parasite species should be pursued.

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