



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203




Human Journals

Research Article


October 2020 Vol.:19, Issue:3

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In Vitro Assessment of the Anthelmintic Activity of the Crude Extracts of *Coriandrum sativum* (Linn.): Seeds



IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals



ISSN 2349-7203
HUMAN

Kamal Jaiswal, Suman Mishra, Jyoti Pandey*

*Department of Zoology, Babasaheb Bhimrao Ambedkar
University
Lucknow-226025, India.*

Submission: 22 September 2020
Accepted: 28 September 2020
Published: 30 October 2020



HUMAN JOURNALS

www.ijppr.humanjournals.com

Keywords: *Coriandrum sativum* (L.), Helminths parasite, Anthelmintic potential, Helminthiasis

ABSTRACT

The study aims to assess *in-vitro* anthelmintic potential of the aqueous and methanolic extract of *Coriandrum sativum* (L.) using helminth parasite (*Haemonchus* sp.). Various concentrations of extracts ((1 mg/ ml, 2.5 mg/ ml, 5 mg/ml, and 10 mg/ml)) were tested based on time of paralysis and time of death of the parasite. Albendazole (1 mg/ ml) was set as a standard and PBS as a control group. The result of this study disclosed that both extracts of the *Coriandrum sativum* (L.) potentiate to paralyze the parasite and after sometime caused its death. The minimum time of paralysis noticed at the higher concentration (10 mg/ml) of both aqueous and methanolic extracts was found to 388.3 ± 3.8 and 194.6 ± 1.7 respectively. The result evaluates that the methanolic seed extract of the *Coriandrum sativum* (L.) showed higher anthelmintic potential than its aqueous extract. Thus, this study revealed that the seed extract of *Coriandrum sativum* (L.) could be used as an alternative in the place of the synthetic anthelmintic drug to control helminthiasis.

INTRODUCTION

Anthelmintic medicines work on the parasitic worms either to expel from the body or killing them. The WHO stated that approximately two billion people suffered from parasitic worm infections^[1]. Livestock is the mainstay of poor farmers, consumers, and marginal community's people throughout the developing world for their livelihoods. The improvement of livestock production systems will have an imperative effect to favour sustainable growth and conditions of the marginal population^[2]. Gastrointestinal helminths infections are of significant economic importance, eliciting clinical disease with mortalities as well as severe losses in production yield as a result of weight loss, and reduced milk production^[3].

Among the gastrointestinal helminths encountered the livestock, the nematode parasites signify as the dominant parasitic species in large and small ruminants^[4, 5, 6, 7] causes massive economic losses like damages in gastric function, loss of appetite, weight loss, anemia, and alterations in internal body metabolism^[8, 9]. The main prophylactic method used to treat these parasites is modern anthelmintic drugs. Conversely, the repeated use of these medicines has inevitably led to the commencement of drug resistance, and currently, it is becoming a global phenomenon^[10]. The emergence of drug resistance in the parasite, as well as the accumulation of chemical residue and toxicity in the host body, leads the researchers to pay attention to plant medicine as an alternative to cure helminthiasis. Medicinal plants have been used to treat parasitism without any side effects from time immemorial. Many researchers used different herbal plants to treat helminthiasis throughout of the World, such as; *Vernonia amygdalina* and *Annona senegalensis*^[11], *Butea frondosa*^[12], *Chenopodium album*^[13].

Coriandrum sativum (L.) comes under the family Apiaceae and locally known as dhania in Hindi. It is an annual herb and cultivated worldwide but in India widely grown in, Rajasthan, Andhra Pradesh, Gujarat, and Madhya Pradesh^[14]. This plant has immense medicinal values and works as antioxidants^[15, 16], antifertility^[17], antidiabetic^[18], agents from ancient times. It is used to cure hepatitis and ascariasis in human beings in many countries of the world^[19]. Moreover, many researchers from different parts of the world identified anthelmintic potency of this plant against, *Hymenolepis nana*^[20], *Haemonchus contortus*^[21], *Pheretima posthuma*^[1]. Keeping the ethnoveterinary uses as well as literature reports in mind, the present study was designed to evaluate the anthelmintic efficacy of *Coriandrum sativum* (L.) against *Haemonchus* spp.: A helminth parasite of the small ruminant.

MATERIALS AND METHODS

In vitro study was done to evaluate the anthelmintic efficiency of both Aqueous Extract (AE) and Methanolic Extract (ME) of the seeds of *Coriandrum sativum* (L.) against the gastrointestinal (GI) nematode parasite (*Haemonchus* spp.) of the goat. Albendazole was considered as a positive control (standard drug) and PBS alone as a negative control for the assay. The experiment was carried out at the Parasitology and Silkworm Pathology Laboratory of the Department of Zoology (formerly Dept. of Applied Animal Sciences) Babasaheb Bhimrao Ambedkar University (B. B. A. U.), Lucknow, Uttar Pradesh.

Collection of plant material:

Seeds of *Coriandrum sativum* (L.) were purchased from the local markets of Lucknow and validation was done in the Department of Applied Plant Sciences of the same University.

Preparation of extract:

The plant seeds were washed properly in tap water after that dried in the oven at 45°C for 2 to 3 days until the seeds are completely dry. The dried seeds were processed to a fine powder by using the electric grinder. 100 g of processed powder of the plant was subjected to extraction using 500 ml each of water and methanol separately, as solvents, for 48 h at room temperature. The particular extracts were concentrated by evaporation in the water bath, then dried at 20°C- 25°C and finally, stored in airtight bottles at 4°C^[22, 23].

Primary phytochemical analysis:

Seed extracts of *Coriandrum sativum* (L.) subjected for phytochemical analysis by following the standard procedures^[24-29].

Collection of test parasites:

Gastrointestinal tracts (GI) were collected from freshly slaughtered goats and rapidly transported to the Parasitology Laboratory of the Department of Zoology, B.B.A.U., Lucknow, Uttar Pradesh. The GI tracts were dissected carefully to look at the presence of parasite (*Haemonchus* sp.) by following the standard method. Adult and actively motile worms were collected, washed with normal saline (0.9%), and kept in phosphate-buffered saline (PBS, pH- 7.5-8.0) until further use within the anthelmintic analyses.

***In - vitro* anthelmintic assay:**

The anthelmintic analyses of both aqueous extract (AE) and methanolic extract (ME) of *Coriandrum sativum* (L.) were directed by following the standard procedure^{19,30-32} with few modifications. Drug solution and extract concentrations are freshly prepared before the starting of the assay. Ten same-sized actively motile, worms were preferred and placed in Petri dishes containing 1mg/ml, 2.5 mg/ml, 5 mg/ml, and 10 mg/ml concentration of both aqueous and methanolic extract of the *Coriandrum sativum*(L.). PBS alone was set as the negative control and standard drug Albendazole was considered as a positive control. Three replicates of each concentration were taken. Worms were examined by noting the time (minutes) taken for paralysis and death of the individual parasite at 1, 2, 3, 4, 5, 6, 7, and 12 hours of the time interval. The paralyzed parasites were kept in PBS for 30 minutes after each interval of time, for the achievement of the possible rescue of the worm motility. After accomplishment of the assay (after 12 hours), alive and dead worms were counted under a dissecting microscope and recorded. The paralysis time was evaluated based on the activities of the parasites i.e. no recovery in motility even after placing in PBS whereas death was confirmed based on the complete loss of motility with the alteration in body color^[30,33,34].

Statistical Analysis

Data are presented as Mean \pm S.E.M of all worms for each concentration. One way ANOVA followed by Tukey- post hoc test was performed with the help of statistics software SPSS version 20.00. The difference in the value at $P \leq 0.05$ was considered statistically significant.

RESULTS

The results of the primary phytochemical investigation of both aqueous and methanolic extract of the seeds of *Coriandrum sativum* (L.), revealed the presence of different groups of the phytochemical compound. The aqueous extract showed the presence of flavonoids, terpenoids, and phenol whereas methanolic extract has shown the presence of flavonoids, terpenoids, tannin, phenol (Table 1).

Anthelmintic assay results revealed that both extracts of the plant showed significant ($P \leq 0.05$) anthelmintic potential as compared to the negative control. The plant exhibited dose as well as time - dependent anthelmintic efficiency in both extracts, causing paralysis along with the death of the parasite under all concentration tested as compared to the positive control (Albendazole). The most proficient anthelmintic activity of both extracts was exhibited at the

concentration of 10 mg/ml at which aqueous extract took 211.3±2.0 min for paralysis and 388.3±3.8 min for the death of the worms, conversely, the methanolic extract showed 111.3±1.8 for paralysis whereas 194.6±1.7 min for the death., In the control (negative control) group, the parasites were found to be efficiently motile and indicated no paralysis or death within the 12 hour study period. The standard drug (positive control) at the concentration of 1 mg/mL was very effective, showing paralysis at 32.0±2.3 min and death at 57.3±5.5 min. (Table 2, Graph.1 & 2).

Table No. 1: Primary phytochemical analysis of both Aqueous Extract (AE) and Methanolic Extract (ME) of *Coriandrum sativum* (L.)seeds

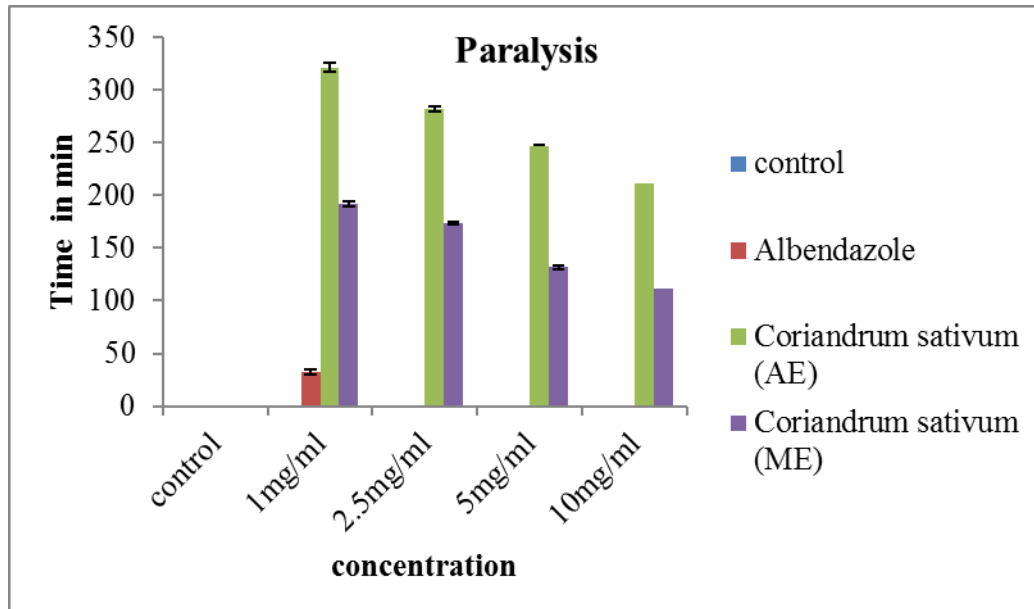
Phytochemical constituents	Aqueous Extract (AE)	Methanolic Extract (ME)
Alkaloids	–	–
Flavonoids	+	+
Terpenoids	+	+
Tannins	–	+
Phenols	+	+
Saponin	–	–
Glycosides	–	+

Where; + :Positive, - : Negative

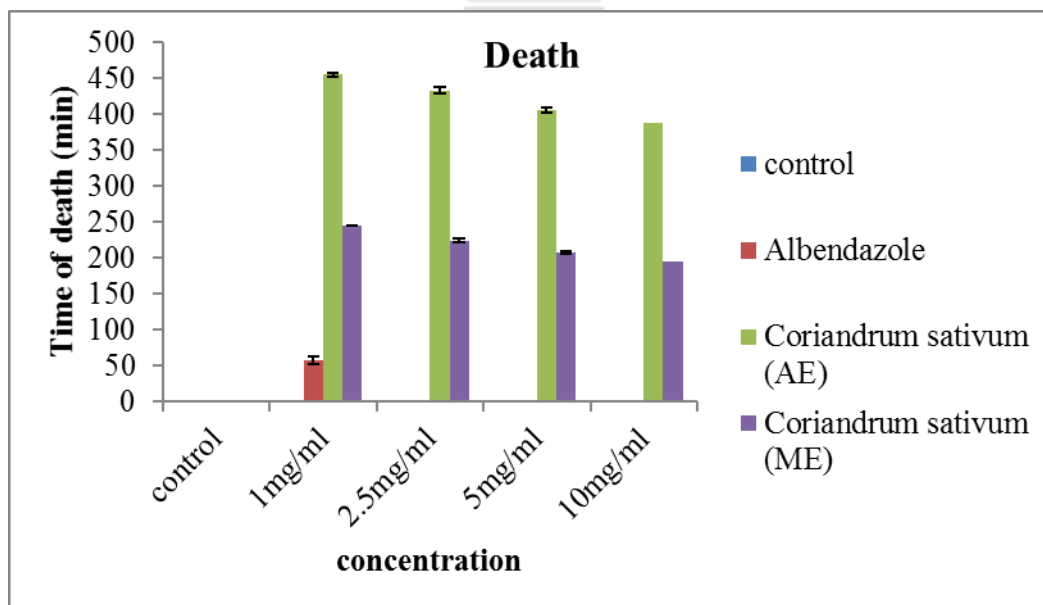
Table No. 2: *In – vitro* anthelmintic assay of Aqueous Extract (AE) and Methanolic Extract (ME) of *Coriandrum sativum* (L.) seeds against test parasite

Treatment	Concentration (mg/ml)	Paralysis time (min) (mean±SEM)	Death time (min) (mean±SEM)
Control		0	0
Albendazole		32±2.3*	57.3±5.5*
<i>Coriandrum sativum</i> (AE)	1	321.6±6.0* ^a	455.0±1.1* ^a
	2.5	282.3±4.3* ^a	433.0±2.0* ^a
	5	246.3±2.3* ^a	406.3±4.4* ^a
	10	211.3±2.0* ^a	388.3±3.8* ^a
<i>Coriandrum sativum</i> (ME)	1	192.3±1.8* ^a	245.0±2.3* ^a
	2.5	173.6±2.4* ^a	224.3±0.6* ^a
	5	132.0±1.5* ^a	207.3±3.2* ^a
	10	111.3±1.8* ^a	194.6±1.7* ^a

Values are mean±SEM, (n=10), *p≤0.05 as compared to control group, ^ap≤0.05 as compared to reference drug (one-way ANOVA followed by Tukey *post hoc* test),. AE: Aqueous extract, ME: Methanolic extract.



Graph. 1: Anthelmintic assay of aqueous extract and methanolic extract of *Coriandrum sativum* (L.) seeds as compared to standard drug (albendazole): Worm paralysis



Graph. 2: Anthelmintic assay of aqueous extract and methanolic extract of *Coriandrum sativum* (L.) seeds as compared to standard drug (albendazole): Worm death

DISCUSSION:

Pharmaceutical industries as well as scientific societies have recently paid attention to the medicinal plants being used as herbal remedies. The main advantages of using them are generally safe with fewer side effects if used in the proper therapeutic way^[35]. These plants are important resources for various types of bioactive compounds with abundant biological characteristics as medicine or nourishment^[36].

The present *in-vitro* study demonstrated that both aqueous and methanolic seed extracts of *C. sativum* showed significant anthelmintic activity against the nematode parasite (*Haemonchus* spp). They might be work on the worm by initiating drooping paralysis that result in the ejection of the worm by peristalsis. The standard drug albendazole works on the parasites by binding itself to free protein in the intestinal tract or on the body surface which leads to reduced glycogen levels in the parasite probably by blocking the glucose uptake which finally leads to the death of the worms^[37].

Earlier reported study revealed that the various phytochemical constituents namely tannins, saponins, alkaloids, and phenols^[27, 38] have been isolated from the medicinal plants which either in isolation or in synchronization may be responsible for the anthelmintic activity^[39,40]. Tannins can bind to the cuticular surface of the parasite, initiating paralysis, and leading to death^[41]. Alkaloids have antioxidating properties that create hindrance in nitrate generation which is used for protein metabolism, hamper the sucrose translocation, and hindered the glucose uptake process of helminths that affect the central nervous system causing paralysis^[42]. Saponin initiates the vocalization and destruction of helminths cuticular surface^[43]. Many allopathic anthelmintics which have phenolic bases such as niclosamide and bithionol are shown to hinder the path of energy generation system in helminth parasites as a result of uncoupling oxidative phosphorylation, and this reason probably supported the anthelmintic power of tannins which is responsible for obstructive ATP synthesis in these parasites^[44, 45].

The study disclosed that the qualitative phytochemical analysis *Coriandrum sativum* revealed the presence of flavonoids, terpenoids, tannins, phenols, and glycosides in methanolic extract whereas aqueous extract showed the presence of flavonoids, terpenoids, phenols, and glycosides^[46]. The results also reported time and dose-dependent mode of anthelmintic efficiency of both extracts of the plant as compared to the reference drug Albendazole^[40, 47]. The study revealed the methanolic extract of *Coriandrum sativum* showed significantly ($p \leq 0.05$) higher anthelmintic potential as compared to the aqueous extract. The reason for this

disparity might be due to the polarity differences of the solvents [48] that cause a huge variation in the extraction of bioactive compounds in the extract^[49]. Methanol as a solvent extracts both the polar as well as few non-polar bioactive compounds in a higher yield which may be reasons for the anthelmintic activity of the methanolic extract of *Coriandrum sativum*^[50, 51]. Finally, this study manifested the anthelmintic property of the seed of the *Coriandrum sativum* plant.

CONCLUSION:

Based on the above findings, the present study concluded that both aqueous and methanolic seeds extracts of *C. sativum* have promising anthelmintic potential. Besides, this study also concludes that methanolic extract of *C. sativum* has significant anthelmintic activity than aqueous extract. Therefore, this study must be validated by *in - vivo* study to assess the anthelmintic potency of the plant on a large - scale. Further, it is necessary to explore the plant in the order that can lead to knowing the mechanism of action of the bioactive compound of against all the endo-parasitic helminth and developed a low cost, eco-friendly herbal medicine.

ACKNOWLEDGMENT:

The author (Jyoti Pandey) is thankful to UGC for the non-NET research fellowship.

CONFLICT OF INTEREST

The authors proclaim that they have no conflict of interest.

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