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In vitro anthelmintic efficacy of aqueous extracts from *Coriandrum sativum* seeds and *Swertia chirata* plant against *Haemonchus contortus* in Barbari goats

RAMAKANT[1], ARBIND SINGH[2], PREM SAGAR MAURYA[3], TARUN KUMAR SARKAR[4], VIKAS JAISWAL[5], DEVESH KUMAR YADAV[6] and AMIT KUMAR VERMA[7]✉

Sardar Vallabhbhai Patel University of Agriculture and Technology Meerut 250 110 Uttar Pradesh India

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ABSTRACT

The present study explored the laboratory-based anti-parasitic potential of water extracts of coriander (*Coriandrum sativum*) seeds and entire *Swertia chirata* plant against *Haemonchus contortus* parasite, collected directly from Barbari goats. The faecal samples were cultured to harvest infectious third-stage (L3) larvae. Subsequently, PCR testing on the ITS-2 ribosomal DNA region was utilized to verify the parasite as *H. contortus*. To evaluate the deworming potential of the plant extracts, both egg hatch assays (EHA) and larval paralysis assays (LPA) were performed at four different dosage levels: 12.5, 25, 50, and 100 mg/ml. Phosphate-buffered saline (PBS) was used as a negative baseline, while Closantel (0.5 mg/ml) served as the positive control. Findings revealed that the coriander seed extract showed dose dependent effectiveness, successfully preventing 27.49% to 86.94% of the eggs from hatching and paralyzing 35.07% to 89.93% of the larvae. However, aqueous extract of *S. chirata* proved to be considerably more effective. It restricted 43.64 and 93.12%, egg hatching and stopped larval movement by 47.40% to 94.10%. As expected, the closantel control achieved total elimination in both tests. Ultimately, these experiments demonstrated that both botanical extracts successfully cleared *H. contortus* in a lab setting, with *S. chirata* standing out as the highly superior candidate.

Keywords: Anthelmintic activity, Barbari goat, *Coriandrum sativum*, Haemonchus contortus, *In vitro* assay, *Swertia chirata*

For many smallholder and marginalized farmers across rural India, raising goats is an essential means of survival. This is largely because goats require very little upfront capital, reproduce quickly, and can thrive in a wide variety of environmental conditions (Brahma *et al.* 2015). However, these economic benefits are heavily threatened by gastrointestinal parasites, which can severely stunt the animals' growth, lower their milk production, hinder reproduction, and even lead to death. One of the most destructive of these parasites globally is the gastrointestinal nematode *Haemonchus contortus* (Shamim *et al.* 2018, Ramakant *et al.* 2024a). Commonly known as the "barber's pole worm," this blood-sucking parasite lives within the abomasum of goats and sheep. When infections are severe, the animals can suffer from drastic blood loss (anemia), fluid retention (edema), a drop in blood protein levels, and poor nutritional absorption. These symptoms often culminate in death, causing massive financial blows to the livestock sector (Edwards *et al.* 2016; Hou *et al.* 2022).

Control of *Haemonchus contortus* has traditionally relied on extensive use of broad-spectrum anthelmintics such as benzimidazoles, imidazothiazoles, macrocyclic lactones and salicylanilides. However, frequent and indiscriminate use of these drugs has resulted in widespread development of anthelmintic resistance throughout the world. Studies have documented resistance levels exceeding 70–90% in many small ruminant farms, making *H. contortus* one of the most drug-resistant nematodes of veterinary importance (Kaplan and Vidyashankar, 2012; Kotze and Prichard, 2016). This alarming increase in resistance necessitates the search for alternative, environmentally safe and sustainable anthelmintic agents. The botanical alternatives are appealing because they are relatively inexpensive, easy to source, safe for the environment, and less prone to triggering parasite drug resistance (Mahima *et al.* 2012, Dhama *et al*. 2013). A variety of plants used in traditional animal medicine are packed with powerful natural chemicals; like flavonoids, tannins, alkaloids, and glycosides; that naturally fight off parasites (Santos

Present address: [1]Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology Kumarganj-224 229 Ayodhya, Uttar Pradesh, India. [2]Department of Livestock Farm Complex, [3]Department of Veterinary Parasitology, [4]Department of Veterinary Medicine, [5]Department of Veterinary Pathology, [6]Department of Animal Genetics and Breeding, [7]Department of Veterinary Medicine, College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture and Technology Meerut-250 110, India. ✉Corresponding author email: drakverma79@gmail.com

et al. 2017). Researchers use both laboratory (*in vitro*) and live-animal (*in vivo*) tests. They are affordable, fast, and ethically responsible, allowing scientists to see how a plant affects a parasite at various stages of its life cycle before moving on to live animal trials (Dehuri *et al.* 2021; Demeler *et al.* 2013).

C. sativum (coriander) and *S. chirata* (chirayata) already have a strong reputation in traditional medicine for their healing, antioxidant, and anti-parasitic properties (Ramakant *et al*. 2024b, 2026). *S. chirata*, in particular, is rich in active compounds like swertiamarin and amarogentin, which are believed to drive its parasite-killing abilities. Although several such medicinal plants have demonstrated anthelmintic activity, limited information is available regarding the comparative in vitro efficacy of aqueous extracts of *C. sativum* (seeds) and *S. chirata* (whole plant) against molecularly confirmed *H. contortus* isolates. Therefore, the present study was undertaken to evaluate the *in vitro* anthelmintic activity of aqueous extracts of *C. sativum* (seeds) and *S. chirata* (whole plant) against *H. contortus* isolated from Barbari goats using egg hatch assay and larval paralysis assay. The efficacy of both extracts was also compared with the standard anthelmintic drug Closantel under identical experimental conditions.

MATERIALS AND METHODS

Ethical approval: Before initiating any animal-related procedures, full ethical clearance for this study was obtained from the Institutional Animal Ethics Committee (IAEC) at the Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India. The official approval (IAEC/SVPUAT/2022/125) was granted on December 3, 2022.

Microscopic examination of collected faecal samples: To gather the primary parasite samples, fresh faecal matter was safely collected directly from the rectums of Barbari goats, using sterile, disposable gloves. The feces were screened for strongyle-type eggs employing standard direct smear and floatation techniques (Soulsby, 1982).

Samples testing positive for strongyle eggs were then pooled together. To rear infectious third-stage (L3) larvae, these pooled samples were incubated using the established coproculture techniques detailed by Ramakant *et al.* (2025).

Molecular Identification of H. contortus: To genetically confirm the specific parasite species, roughly five L3 larvae were isolated from the sediment of the centrifuged cultures. To remove the larvae's protective outer sheaths, they were soaked in a 3.5% sodium hypochlorite solution for twenty minutes. Afterward, they were carefully rinsed three to five times with distilled water to ensure no chemical residue remained prior to DNA extraction. The genomic DNA extraction was performed using a QIAamp Blood Mini Kit (Qiagen, Germany). The manufacturer's base protocol was followed but incorporated an extra freeze-thaw cycle to improve DNA yield. The finalized DNA was stored securely at -20°C. To verify the isolates as *H. contortus*, the ITS-2 region of their ribosomal DNA was amplified. A forward primer specific to *Haemonchus* (5′-CAAATGGCATTTGTCTTTTAG-3′) was paired with a universal reverse primer (5′-TTAGTTTCTTTTCCTCCGCT-3′) (Bott *et al.* 2009). The PCR thermal cycling began with a 5-minute initial denaturation phase at 94°C. This was followed by 35 cycles consisting of denaturation at 94°C (30 seconds), annealing at 55°C (30 seconds), and extension at 72°C (30 seconds). A final extension was run at 72°C for 7 minutes (Bott *et al.* 2009; Tan *et al.* 2014). Finally, the PCR products were run on an agarose gel to visually confirm the successful amplification of the target genes.

Preparation of herbal extracts: Fresh seeds of coriander (*C. sativum*) were thoroughly washed under tap water and left to air-dry. The dry seeds were then pulverized in an electric grinder and the powder sifted to discard coarse fragments. One hundred grams of this fine powder were immersed into one liter of distilled water, heated at 100°C in a water bath for thirty minutes. After allowing it to cool, the liquid was filtered twice: first through a basic muslin cloth, and then through Whatman No. 1 filter paper. The extracts were concentrated under reduced pressure using a rotary evaporator maintained below 55°C to minimize degradation of heat-sensitive phytoconstituents. Similar procedure was applied to create *S. chirata* extract. The whole plants were cleaned to remove debris and dried at 50°C. Following grinding and sieving, 100 grams of the *S. chirata* powder were boiled in one liter of distilled water for thirty minutes. The same two-step filtration method was used, but the extract was concentrated at a slightly higher temperature of 55°C on the rotary evaporator. It was subsequently dried at 40°C and refrigerated at 4°C until the assays.

Egg Hatch Assay (EHA): The egg-hatching experiments were modelled on the standard protocols established by Coles *et al.* (2006). First, strongyle eggs were extracted from the feces by passing the matter through a nested series of sieves (250, 125, and 25 µm) (Thienpont *et al.* 1986). The eggs caught on the finest 25 µm sieve were washed, and the suspension was adjusted to contain exactly 100 to 200 eggs per milliliter, verifying the count with the McMaster technique (Soulsby, 1982). The prepared plant extracts were then introduced at four different strengths: 12.5, 25, 50, and 100 mg/ml. A positive control was established using closantel (0.5 mg/ml) and a negative control using standard phosphate-buffered saline (PBS). Each concentration, alongwith positive and negative controls, was tested in six replicates (n = 6). Approximately 100 eggs were added to each well of a 24-well plate, and the entire experiment was repeated three times independently. After incubating the plates at 27°C for 48 hours, a drop of Lugol's iodine was added to each well to completely halt the hatching process. Under 40X magnification of microscope, the unhatched eggs were counted against the newly emerged L1 larvae to calculate the precise percentage of hatching inhibition.

Larval Paralysis Assay (LPA): To assess how well the herbal treatments paralyzed mobile parasites, methodology

of Varady and Corba (1999) was followed. A 200 µl suspension containing approximately 50 infective L3 larvae (harvested from the coprocultures) was prepared. In the test wells, these larvae were combined with 800 µl of distilled water and 1000 µl of the plant extracts (testing the 12.5, 25, 50, and 100 mg/ml concentrations). Each treatment was performed in six replicates and repeated independently three times. Closantel (0.5 mg/ml) and PBS were again utilized as positive and negative controls. After letting these mixtures incubate at 27°C for 24 hours, the larvae were evaluated microscopically. Any larva that failed to exhibit movement for five consecutive seconds was officially classified as paralyzed. This allowed for the calculation of the overall percentage of larval motility inhibition.

Data analysis: All raw experimental data was organized Microsoft Excel. The statistical analyses were then performed using IBM SPSS Statistics software (version 20). Percentage egg hatch inhibition and larval paralysis were expressed as mean.

RESULTS AND DISCUSSION

Parasitological and molecular confirmation: Initial verification of the collected fecal samples under a microscope clearly displayed the typical morphology of strongyle-type eggs (Fig. 1). After successfully incubating the positive samples to cultivate infective third-stage larvae, PCR testing targeting the ITS-2 ribosomal DNA region was performed to ensure accurate molecular identification. The testing yielded a distinct 265 base-pair band, definitively confirming the specimens as *H. contortus* (Fig. 2).

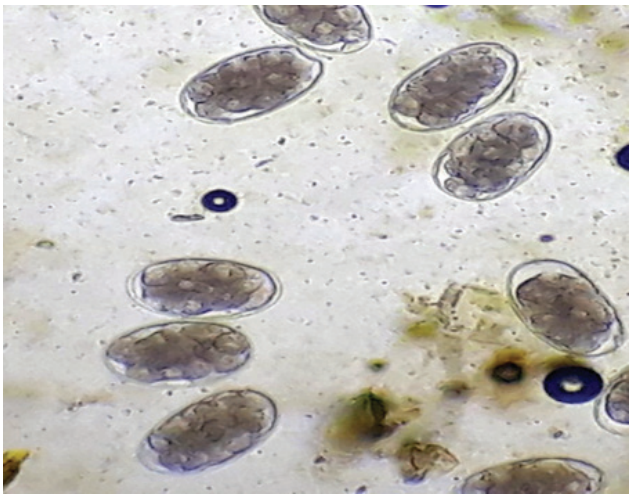


Fig. 1. Picture depicting the morphology of typical strongyle-type eggs

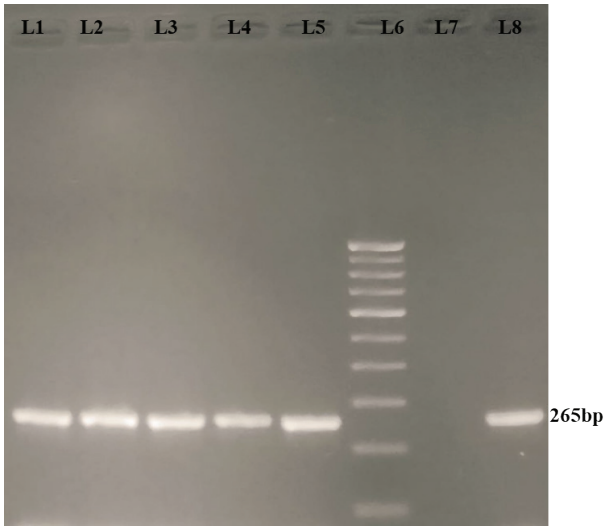


Fig. 2. PCR amplification of the ITS-2 gene of *H. contortus*
- L6: 100bp DNA marker
- L1-L5: *H. contortus* positive samples
- L7: Negative control
- L8: Control positive

Egg Hatch Assay: The results of the egg-hatching tests demonstrated that both botanical extracts successfully interrupted the parasite's development, with their effectiveness scaling directly alongside the dosage strength (Table 1). As anticipated, the negative control (PBS) barely impacted hatching (showing a mere 3% inhibition), while the positive control (Closantel at 0.5 mg/ml) halted it entirely. Closantel (0.5 mg/ml) produced complete (100%) inhibition of egg hatching, confirming its expected anthelmintic efficacy. Although neither plant extract achieved efficacy equivalent to closantel, both demonstrated dose-dependent inhibition. At 100 mg/ml, *S. chirata* produced 93.12% inhibition whereas *Coriandrum sativum* produced 86.94% inhibition, suggesting considerable anthelmintic potential but remaining inferior to the standard drug.

When treated with the *C. sativum* seed extract at increasing concentrations (12.5, 25, 50, and 100 mg/ml), hatching was suppressed by 27.49%, 32.65%, 66.32%, and 86.94%, respectively. This strong, dose-linked performance echoed previous findings by researchers examining

Table 1. Percentage strongyle egg hatching inhibition at different concentrations of aqueous extracts of *C. sativum* and *S. chirata* measured 48 hours after treatment

Groups	*Coriandrum sativum* seeds	*Swertia chirata* whole plant extract	Negative control	Positive control
T1(12.5 mg/ml)	27.49	43.64	3.00	100.00
T2(25 mg/ml)	32.65	63.23		
T3(50 mg/ml)	66.32	83.16		
T4 (100 mg/ml)	86.94	93.12		

Table 2. Percentage inhibition of larval motility at various concentrations of aqueous extracts, at 24 hours following treatment

Groups	*Coriandrum sativum* seeds	*Swertia chirata* whole plant extract	Negative control	Positive control
T1 (12.5 mg/ml)	35.07	47.40	4	100
T2 (25 mg/ml)	62.85	68.06		
T3 (50 mg/ml)	78.48	84.71		
T4 (100 mg/ml)	89.93	94.10		

coriander's anthelmintic traits (Ahmed *et al.* 2020; Eguale *et al.* 2007). It is highly probable that the rich blend of essential oils, phenolics, and flavonoids naturally found in coriander seeds interferes fundamentally with the nematode embryos' ability to develop (Laribi *et al.* 2015). However, the whole-plant extract of *S. chirata* proved even more formidable. Across the identical concentration gradient, it prevented 43.64%, 63.23%, 83.16%, and 93.12% of the eggs from hatching. This exceptionally high potency is likely to stem from the plant's bitter secoiridoid glycosides; namely sweroside, swertiamarin, and amarogentin; which are renowned for their aggressive anti-parasitic properties (Aleem and Kabir, 2018; Sinha *et al.* 2023). Ultimately, the data clearly illustrated that *S. chirata* is much more capable of stopping *H. contortus* eggs from hatching than the coriander seed extract.

Larval paralysis assay: To cross-verify the extracts' anti-parasitic strength, their ability to immobilize active larvae was also evaluated (Table 2). Following a 24-hour exposure window, the PBS baseline showed negligible effect (4% paralysis), whereas closantel achieved complete, 100% larval immobilization. Closantel produced complete larval paralysis (100%), whereas the highest concentrations of *Swertia chirata* and *Coriandrum sativum* induced 94.10% and 89.93% paralysis, respectively. *C. sativum* extract progressively diminished larval movement, recording paralysis rates spanning from 35.07% at the lowest dose up to 89.93% at the highest. This aligned seamlessly with existing data indicating that coriander treatments can significantly incapacitate *H. contortus* larvae, presumably by blocking their metabolic energy pathways or disrupting their neuromuscular signaling (Jaiswal *et al.* 2020). Echoing the egg hatch results, the *S. chirata* extract exhibited a vastly comparable paralyzing effect although slightly lower than closantel, halting movement in 47.40% to 94.10% of the larvae as concentrations increased. It is theorized that this remarkable larvicidal impact is the result of a powerful synergy among the plant's bitter glycosides and alkaloids, which are known to severely compromise parasite survivability (Khanal *et al.* 2014). Furthermore, other secondary plant metabolites like flavonoids and tannins tend to latch onto the protective outer cuticles of the parasites, scrambling their normal metabolic functions until paralysis and death occur (Niezen *et al.* 1995, Veerakumari and Munuswamy, 2000).

Taking both assays into account, there was a distinct, dose-responsive pattern indicating that water-based extracts from both *C. sativum* seeds and the complete *S. chirata* plant were highly effective *in vitro* agents against *H. contortus* isolated from Barbari goats. Both botanical extracts demonstrated significant dose-dependent *in vitro* anthelmintic activity against *H. contortus*. Although their efficacy remained lower than the standard anthelmintic closantel, particularly at lower concentrations, *S. chirata* exhibited greater activity than *Coriandrum sativum*. These findings suggested that both plants possess promising phytotherapeutic potential and warrant further in vivo investigations for development as complementary alternatives to synthetic anthelmintics.

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