Research Article

In Vitro Anthelmintic Potential of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) Extracts against *Haemonchus contortus*: A Phytochemical Approach

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Abstract | The extensive use of synthetic anthelmintics, particularly benzimidazoles, macrocyclic lactones, and cholinergic agonists, has resulted in widespread resistance among gastrointestinal nematodes (GINs) of small ruminants. This study aimed to evaluate the phytochemical composition and in vitro anthelmintic potential of *Allium sativum* (Garlic) and *Zingiber officinale* (Ginger) against *Haemonchus contortus*. Phytochemical screening of aqueous, ethanolic, and methanolic extracts revealed the presence of alkaloids, saponins, glycosides, steroids, triterpenoids, carbohydrates, and amino acids, while flavonoids and tannins were absent in some extracts, notably in aqueous preparations. Anthelmintic efficacy was assessed using the Egg Hatch Assay (EHA), Larval Development Assay (LDA), and Adult Motility Test (AMT). All extracts exhibited significant ($p < 0.001$) concentration-dependent inhibition across all developmental stages. In the EHA, methanolic extracts of garlic and ginger demonstrated the highest ovicidal activity, reducing egg hatching to 19.16% and 14.66% at 100 mg/ml, respectively, approaching the efficacy of albendazole (5.50%). Similarly, in the LDA, methanolic extracts showed superior larvicidal activity, with minimal larval development (25% for garlic and 13% for ginger at 100 mg/ml). In the AMT, both plant extracts caused a time- and dose-dependent reduction in adult worm motility, with complete paralysis achieved at higher concentrations (60–100%) within 8 hours. Ethanolic and methanolic extracts consistently exhibited greater potency than aqueous extracts. Overall, *Zingiber officinale* demonstrated slightly higher anthelmintic efficacy compared to *Allium sativum*, particularly in larval and adult stages. The findings suggest their potential as effective botanical alternatives or adjuncts to conventional anthelmintics for controlling GIN infections and mitigating anthelmintic resistance in small ruminants.

Keywords | Nematode, Anthelmintic resistance, Ethnoveterinary, Phytochemical, *Haemonchus contortus*

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INTRODUCTION

Small ruminant production plays a vital role in the livelihoods of rural communities in developing countries; however, its productivity is significantly constrained by gastrointestinal nematodes (GINs). Among these, *Haemonchus contortus*, a highly pathogenic blood-feeding nematode of the family Trichostrongylidae, is a major cause of economic losses in sheep and goat farming worldwide (Fayaz *et al*., 2019). The parasite inhabits the

abomasum, where heavy infections can lead to severe anemia, weight loss, and mortality (Flay *et al*., 2022).

Haemonchosis is strongly influenced by environmental conditions and is widely distributed in warm and humid regions. However, recent climatic changes have facilitated the spread of *H. contortus* into previously low-risk areas, increasing its global significance (Arsenopoulos *et al*., 2021).

Control of gastrointestinal nematodes has traditionally relied on synthetic anthelmintics. However, their extensive and often indiscriminate use has resulted in the rapid development of anthelmintic resistance, posing a major challenge to sustainable livestock production (Githiori *et al*., 2006). This has led to growing interest in alternative strategies, particularly plant-based anthelmintics, which are considered environmentally friendly and potentially less prone to resistance development.

Medicinal plants have long been recognized as sources of bioactive compounds with antiparasitic properties. Several plant species, including neem (*Azadirachta indica*), coriander (*Coriandrum sativum*), and valerian (*Valeriana wallichii*), have demonstrated varying degrees of efficacy against gastrointestinal nematodes (Amin *et al*., 2012; Mukherjee *et al*., 2019; Singh *et al*., 2009; Upadhyay and Ahmad, 2012; Yamson *et al*., 2019). However, their efficacy often remains below the threshold required for replacing conventional anthelmintics, necessitating further investigation (Githiori *et al*., 2006).

Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) are widely used medicinal plants with well-documented pharmacological properties. Garlic contains bioactive compounds such as allicin and S-allyl cysteine, which exhibit antimicrobial and antiparasitic activities (Hyun Kim *et al*., 2019; Kayis *et al*., 2009; Kodera *et al*., 2002; Worku *et al*., 2017; Yavuzcan *et al*., 2019; Zhong *et al*., 2021). Similarly, ginger contains compounds such as gingerols, shogaols, and zingerone, which possess antioxidant, anti-inflammatory, and anthelmintic properties (Ali *et al*., 2008). Previous studies have reported promising antiparasitic effects of these plants against various helminths, including *H. contortus* (Abd El Wahab *et al*., 2021; Ahmed *et al*., 2021; Goswami *et al*., 2024; Iqbal *et al*., 2006; Sofowora *et al*., 2013).

Despite these findings, limited information is available regarding the comparative efficacy of different solvent-based extracts of garlic and ginger. Since solvent polarity significantly influences the extraction of phytochemicals, evaluating aqueous, methanolic, and ethanolic extracts may provide valuable insights into optimizing extraction methods and enhancing anthelmintic activity (Eguale *et al*., 2007; Githiori *et al*., 2005).

Therefore, the present study was designed to evaluate the *in vitro* anthelmintic potential of different extracts of *Allium sativum* and *Zingiber officinale* against *H. contortus*, with the aim of identifying effective plant-based alternatives for parasite control.

MATERIALS AND METHODS

Plant material collection and preparation

Fresh bulbs of garlic (*Allium sativum*) and rhizomes of ginger (*Zingiber officinale*) were procured from a local market. The plant materials were thoroughly washed with tap water to remove debris and subsequently surface sterilized using 70% ethanol. The cleaned materials were air-dried under shade at room temperature (25–28 °C) for 7–10 days. During drying, samples were regularly inspected to prevent fungal contamination. The dried materials were ground into a fine powder using an electric grinder and stored in airtight containers until further use.

Preparation of crude extracts (aqueous, methanolic, and ethanolic)

For crude extraction, 10 g of dried plant powder was mixed with 100 mL of respective solvents (distilled water, methanol, or ethanol) in separate flasks. The mixtures were stirred using a magnetic stirrer at 600 rpm for 1 hour and then kept at room temperature for 24 hours for maceration. The extracts were filtered using Whatman No. 1 filter paper (Azwanida, 2015).

The filtrates were concentrated by evaporating the solvent in a water bath at 50–60 °C until the final volume reached approximately 10 mL. These concentrated extracts were considered as stock solutions and stored at 4 °C in sterile bottles until further use. Working concentrations (2.5, 5, 10, 25, 50, and 100 mg/mL) were prepared by diluting the stock solutions with PBS containing 0.5% dimethyl sulfoxide (DMSO) where required (Cos *et al*., 2006).

Drying of plant material and extraction

The bulbs of garlic and rhizome of the ginger were washed with tap water three times and sterilized by using 70% alcohol as a spray. After sterilization, material was dried in shade at room temperature. During the dry process material was frequently checked for any fungal contamination. The dried material was subjected to prepare a fine powder with the help of grinder. The fine powder was used for the extraction of crude drug.

To prepare aqueous extract, firstly, 10 gm of powder was mixed with 100 ml of distilled water and then the mixture was stirred with a magnetic stirrer at 600 rpm for an hour

and was left for overnight. The mixture was then filtered, and condensed into 10 ml by the evaporation of solvent in a water bath at 50-60°C. This condensed extract was stored as a stock solution in refrigerator at 4°C until their use. Ethanol and methanol extract was prepared following the same procedure by mixing ethanol and methanol respectively instead of distilled water. Different concentrations in Phosphate Buffer Saline (PBS) such as 20,40,60,80 and 100%, solution of different extract was used for screening.

Soxhlet extraction of phytoconstituents

Soxhlet extraction was performed to obtain semi-purified extracts (Azwanida, 2015; Soxhlet, 1879). Approximately 20 g of dried plant powder was initially defatted using petroleum ether for 6–8 hours. The defatted material was then subjected to Soxhlet extraction using methanol and ethanol (100 mL each) for 24 hours.

The extracts were concentrated using a rotary evaporator under reduced pressure and dried in a desiccator to obtain a semi-solid residue. The final extracts were weighed to calculate yield and stored in sterile airtight containers at 2–4 °C until use.

Extraction of phytoconstituents by Soxhlet extraction

In this method, the dried material was crushed directly by grinder without adding any solvent. The powder was initially defatted with petroleum ether followed by 100 ml of methanol and ethanol by using a Soxhlet extractor for 24 hours and was dried in desiccators. The hydro-alcohol was evaporated by using a rotary evaporator leaving a small yield of extracted plant material in the glass bottom flask. The extracts were stored in sterile bottles and stored in refrigerator at 2-4 °C until further use.

Phytochemical screening

Qualitative phytochemical screening of the plant extracts was carried out using standard procedures with clearly defined reagent compositions and volumes (Sofowora *et al.*, 2013). This study was limited to qualitative phytochemical screening, and no quantitative estimation of individual bioactive compounds was performed. Therefore, the concentrations used in subsequent bioassays represent crude extract doses rather than standardized or purified active constituents.

Alkaloids (Mayer's test)

To 2–3 mL of each extract, a few drops of Mayer's reagent (prepared by dissolving 1.36 g mercuric chloride and 5 g potassium iodide in 100 mL distilled water) were added. The formation of a cream-colored precipitate indicated the presence of alkaloids.

Flavonoids (Alkaline reagent test)

Approximately 2–3 mL of the extract was treated with a few drops of sodium hydroxide solution. The appearance of an intense yellow color, which turned colorless upon addition of dilute acid, confirmed the presence of flavonoids.

Tannins (Gelatin test)

To 2–3 mL of extract, 1% gelatin solution containing 10% sodium chloride was added. The formation of a precipitate indicated the presence of tannins.

Saponins (Froth formation test)

About 2 mL of the extract was vigorously shaken with distilled water in a test tube. The formation of stable and persistent foam confirmed the presence of saponins.

Glycosides (Borntrager's test)

The extract (2–3 mL) was boiled with 1 mL of sulfuric acid for 5 minutes, then filtered and cooled. The filtrate was mixed with an equal volume of chloroform and shaken thoroughly to form two layers. The lower chloroform layer was separated and treated with dilute ammonia. The appearance of a rose-pink to red color indicated the presence of glycosides.

Steroids and Triterpenoids (Salkowski Test)

To 2–3 mL of extract, a few drops of concentrated sulfuric acid were carefully added along the side of the test tube. A red coloration in the lower layer indicated the presence of steroids, while a yellow coloration suggested triterpenoids.

Carbohydrates (Molisch's test)

A few drops of alcoholic α-naphthol were added to 2–3 mL of extract, followed by careful addition of concentrated sulfuric acid along the sides of the test tube. The formation of a violet or purple ring at the interface confirmed the presence of carbohydrates.

Amino acids (Millon's test)

To 2–3 mL of extract, 2 mL of Millon's reagent (mercuric nitrate solution) was added. The formation of a white precipitate indicated the presence of amino acids.

In vitro evaluation of anthelmintic potential of garlic and ginger

Egg hatch test

All adult *H. contortus* were recovered from the abomasum of goat slaughtered in the abattoir of Hyderabad (Gadahi *et al.*, 2016). For the preparation of egg suspension, all female *H. contortus* were morphologically and microscopically identified and separated. The isolated female worms were macerated to liberate the eggs. The eggs were successively sieved and finally diluted with phosphate-buffered saline (PBS)(Coles *et al.*, 1992). Every treatment was triplicated

in which a single replicate contained 10-15 random sample of eggs in 10 mL PBS. These eggs were exposed to various concentrations of plant extracts 2.5, 5, 10, 25, 50, 100 mg/ml and control groups (0.5% DMSO, 0.5% ethanol, PBS, and 0.5% albendazole). These were humidly incubated in a room at 27°C for 72 h (Coles *et al*., 1992; Geary *et al*., 2012). The development of eggs was stopped adding by adding a drop of Lugol's iodine. The culture material was checked and counted the number of eggs that remained morulated and turned into free L1. The ovicidal activity was expressed based on the percentage of eggs that failed to develop and hatch (Varady *et al*., 2009).

Larval motility test (L3)

The procedure for the culture of L3s will be based on the technique described by Rupa and Portugaliza (Rupa and Portugaliza, 2016), while the *in vitro* larvicidal assay will be adopted from Fernandez *et al*. (2009). Six replications per treatment and for each replicate an amount of 10 ml PBS with 10-15 alive L3s were randomly pipetted. The L3s will be exposed to various concentrations 2.5, 5, 10, 25, 50, 100 mg/ml of extracts in PBS supplemented with 0.5 % DMSO and control groups (0.5% DMSO, PBS, and various concentrations of % albendazole). These will be incubated at 27 ° C for 3 h. After 3 h, motility of L3s will be c. After than a drop of 1% Delafield's Hematoxylin stain will be added and re-incubated for 24 h. The L3s will be examined after 24 hours under the microscope for the uptake of stain. Immobile larvae will be considered on the basis of motility upon prodding and identified the larvae with cuticle damage based on the uptake of the red stain. The larvicidal activity will be expressed on the percentage of immobile L3s after exposure of plant extracts.

In vitro anthelmintic activity (Adult motility assay)

The *in vitro* anthelmintic evaluation will be conducted on adult *H. contortus* worms collected from the infected abomasum of sheep and goats (Tariq *et al*., 2009). The collected worms will be washed and suspended in Hank's balanced salt solution (HBSS). The 20-25 worms will be exposed in triplicate in petri dish containing 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% of extract prepared in 5 ml of HBSS and HBSS alone for the negative control group. The albendazole at the rate of 0.5 mg/ml will be used as reference drug of positive control. The petri dishes will be kept in an incubator at 37°C. The inhibition of motility, activeness and mortality of the worms will be observed at an interval of 0.5, 1, 2, 4, 6, 8 h. The number of motile (alive) and non-motile (dead) worms will be counted and recorded for each concentration.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical analyses were performed to determine significant differences among groups using analysis of variance (ANOVA) and Student's *t*-test for parametric data, employing GraphPad Prism software (GraphPad Software Inc., USA).

RESULTS

Phytochemical analysis

The extracts collected from the selected plants and herbs were checked for the presence of various phytoconstituents. Extracts collected from *Allium sativum L* show the presence of the tannins, saponins, glycoside, steroids and triterpenoids, carbohydrates and amino acids in all types of extracts such as aqueous extract, methanol and ethanol extracts. Whereas alkaloids were detected in aqueous extract and methanol extract. Alkaloids were not detected in ethanol extracts while Flavonoid was not traced in aqueous extracts. Phytochemical screening of *Zingiber officinale* extracts revealed the presence of alkaloids, saponins, glycosides, steroids and triterpenoids, carbohydrates, and amino acids across all types of extracts. Notably, flavonoids were absent in the aqueous extract, while tannins were not detected in the ethanol extract (Table 1).

Table 1: Phytochemical analysis of the extracts of Garlic (*Allium sativum L.*) and Ginger (*Zingiber officinale*).

Phytochemical	Aqueous extract		Methanol extract		Ethanol extract	
	Garlic	Ginger	Garlic	Ginger	Garlic	Ginger
Alkaloids	+	+	+	+	-	+
Flavonoids	-	-	+	+	+	+
Tannins	+	+	+	+	+	-
Saponins	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Steroids and triterpenoids	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Amino acids	+	+	+	+	+	+

Anthelmintic potential of various extracts of garlic (*Allium sativum L.*)

Inhibitory effect of different extracts of garlic (*Allium sativum* L.) on nematode egg hatching

The inhibitory effect of different extracts of *Allium sativum* L. on nematode egg hatching was evaluated (Table 2) and findings of current study revealed that negative controls, DMSO (0.5%) and PBS, exhibited high egg hatching percentages (90.83 % ± 1.44 to 93.33 % ± 1.44), with no significant differences among them ($p > 0.05$), indicating normal egg viability. Similarly, solvent controls for ethanolic (94.44 % ± 1.25) and methanolic extracts (88.83 % ± 1.25) did not differ significantly from the controls. In contrast,

the positive control, albendazole (0.5%), showed a marked and significant inhibition of egg hatching (5.50 % ± 1.80), confirming the validity of the assay. All garlic extracts demonstrated a significant, concentration-dependent reduction in egg hatching compared to controls.

Table 2: Inhibitory effect of different extracts of garlic (*Allium sativum* L.) on nematode egg hatching (%).

Treatment/ concentration	Aqueous extract (Mean % ± SEM)	Ethanolic extract (Mean % ± SEM)	Methanolic extract (Mean % ± SEM)
DMSO (0.5%)	90.83 ± 1.44[a]	90.83 ± 1.44[a]	90.83 ± 1.44[a]
PBS	93.33 ± 1.44[a]	93.33 ± 1.44[a]	93.33 ± 1.44[a]
Solvent control	—	94.44 ± 1.25[a]	88.83 ± 1.25[a]
Albendazole (0.5%)	5.50 ± 1.80[e]	5.50 ± 1.80[e]	5.50 ± 1.80[e]
2.5 mg/ml	75.00 ± 2.50[b]	68.33 ± 1.44[b]	65.00 ± 2.88[b]
5 mg/ml	75.66 ± 1.15[b]	55.00 ± 5.00[c]	56.66 ± 2.88[c]
10 mg/ml	66.66 ± 1.44[c]	48.33 ± 1.44[c]	38.33 ± 1.44[d]
25 mg/ml	46.66 ± 2.88[d]	40.00 ± 2.50[d]	30.00 ± 2.50[e]
50 mg/ml	43.33 ± 2.88[d]	27.50 ± 2.50[e]	27.50 ± 2.50[e]
100 mg/ml	32.50 ± 2.50[e]	22.50 ± 2.50[e]	2.88[e]

(Different superscript letters (a–e) within each column indicate significant differences ($p < 0.05$). Means sharing the same letter are not significantly different).

At the concentrations of 2.5 and 5 mg/ml, the aqueous extract showed relatively higher egg hatching percentages (75.00 % ± 2.50 and 75.66 ± 1.15), followed by ethanolic (68.33 % ± 1.44 and 55 % ± 5.00) and methanolic extracts (65.00 % ± 2.88 and 56.66 % ± 2.88). With increasing concentrations, a progressive decline in egg hatching was observed across all extracts. At 10 mg/ml, the methanolic extract (38.33 % ± 1.44) exhibited stronger inhibition compared to aqueous (66.66 % ± 1.44) and ethanolic extracts (48.33 % ± 1.44). At higher concentrations (25–100 mg/ml), all extracts significantly reduced egg hatching, with the methanolic extract showing the greatest inhibitory effect (30.00 % ± 2.50 to 19.16 % ± 2.88), followed by ethanolic (40.00 % ± 2.50 to 22.50 % ± 2.50) and aqueous extracts (46.66 % ± 2.88 to 32.50 % ± 2.50). The lowest egg hatching percentage was recorded at 100 mg/ml of methanolic extract (19.16 % ± 2.88), approaching the efficacy of albendazole.

Effect of different extracts of garlic (*Allium sativum* L.) on larval development

As shown in Table 3, the negative controls (DMSO and PBS) exhibited high larval development (98–99%), while solvent controls also showed no inhibitory effect. In contrast, albendazole (0.5%) caused a marked reduction in larval development (7.33 ± 2.44; $p < 0.001$). At lower concentrations (2.5 mg/ml), larval development remained relatively high across all extracts, with aqueous extract showing the highest value (95.11 ± 1.67), followed by methanolic (92.00 ± 1.67) and ethanolic extracts (91.11 ± 1.92). However, as the concentration increased, a progressive decline in larval development was observed. At 5 mg/ml, ethanolic (77.77 ± 1.92) and methanolic extracts (82.00 ± 1.76) exhibited greater inhibitory effects compared to the aqueous extract (90.22 ± 0.38).

Table 3: Effect of different extracts of garlic (*Allium sativum* L.) on larval development (%).

Treatment/ concentration	Aqueous extract (Mean % ± SEM)	Ethanolic extract (Mean % ± SEM)	Methanolic extract (Mean % ± SEM)
DMSO (0.5%)	99.55 ± 0.62[a]	99.55 ± 0.62[a]	99.55 ± 0.62[a]
PBS	98.00 ± 1.44[a]	98.00 ± 1.76[a]	98.00 ± 1.44[a]
Solvent control	—	98.00 ± 1.76[a]	97.77 ± 3.84[a]
Albendazole (0.5%)	7.33 ± 2.44[e]	7.33 ± 2.44[e]	7.33 ± 2.44[e]
2.5 mg/ml	95.11 ± 1.67[b]	91.11 ± 1.92[b]	92.00 ± 1.67[b]
5 mg/ml	90.22 ± 0.38[b]	77.77 ± 1.92[c]	82.00 ± 1.76[c]
10 mg/ml	88.88 ± 1.92[c]	68.88 ± 6.93[c]	62.00 ± 4.80[d]
25 mg/ml	61.11 ± 1.92[d]	53.33 ± 3.33[d]	46.88 ± 3.67[c]
50 mg/ml	57.77 ± 3.84[d]	36.66 ± 3.33[e]	36.66 ± 3.33[e]
100 mg/ml	43.33 ± 3.33[e]	30.00 ± 3.33[e]	25.55 ± 3.84[e]

(Different superscript letters (a–e) within each column indicate significant differences ($p < 0.05$). Means sharing the same letter are not significantly different).

At intermediate concentrations (10–25 mg/ml), the inhibitory effect became more pronounced, particularly for the methanolic extract (62.00 ± 4.80 at 10 mg/ml and 46.88 ± 3.67 at 25 mg/ml), followed by ethanolic and aqueous extracts. At higher concentrations (50–100 mg/ml), all extracts significantly suppressed larval development, with the methanolic extract showing the greatest inhibition (36.66 ± 3.33 to as low as ~25% at 100 mg/ml), followed by ethanolic (36.66 ± 3.33 to 30.00 ± 3.33) and aqueous extracts (57.77 ± 3.84 to 43.33 ± 3.33). The lowest larval development was observed at 100 mg/ml, particularly in the methanolic extract, approaching the inhibitory efficacy of albendazole.

Anthelmintic effect of garlic (*Allium sativum* L.) extracts on adult worm motility

The anthelmintic activity of *Allium sativum* extracts, evaluated through the percentage reduction in adult worm motility over an 0.5 to 8-hour period (Table 4). A time-dependent decline in motility was observed across all treatments. The control group (HBSS) exhibited only a moderate natural decline in motility, decreasing from 100% to 56% across all extract types, confirming minimal spontaneous paralysis. In contrast, the standard drug

Albendazole (0.5 mg/ml) showed a rapid and complete loss of motility (70.66% → 0 %), demonstrating its strong anthelmintic efficacy. Garlic extracts displayed a clear dose-dependent anthelmintic effect. At lower concentrations (20%), all extracts caused partial reduction in motility (30.66–37.33%), indicating moderate activity. At 40%, a stronger effect was observed, particularly in ethanolic and methanolic extracts, which achieved complete paralysis (0%), while the aqueous extract still retained some residual motility (22.66%). Higher concentrations (60–100%) resulted in complete inhibition of worm motility across all extract types within the experimental timeframe. Notably, the ethanolic and methanolic extracts generally exhibited slightly greater potency than the aqueous extract, achieving faster and more consistent paralysis at intermediate concentrations.

Table 4: Anthelmintic effect of garlic (*Allium sativum* L.) extracts on adult worm motility (%). Arrows (→) indicate progressive reduction in motility over time.

Treatment/ concentration	Aqueous extract (0.5 – 8 hrs.)	Ethanolic extract (0.5 – 8 hrs.)	Methanolic extract (0.5 – 8 hrs.)
HBSS (Control)	100 → 56[a]	100 → 56[a]	100→56 [a]
Solvent Control	70.66 → 0[e]	70.66 → 00 [e]	70.66→0[e]
Albendazole (0.5 mg/ml)	93.33 →30.66[b]	95.33→37.33[b]	95→33.66[b]
20%	69.33→22.66[c]	83 → 00[c]	83 → 00[c]
40%	60 → 00[d]	49.33→ 00[d]	53.33→00[d]
60%	34.66 → 00[d]	34.66 → 00[d]	34.66→00[e]
80%	30.66→ 00[d]	30.66 → 00[d]	22.33→00[d]

(Different superscript letters (a–e) within each column indicate significant differences ($p < 0.05$). Means sharing the same letter are not significantly different).

Anthelmintic potential of various extracts of ginger (*Zingiber officinale*)

Inhibitory effect of different extracts of *Zingiber officinale* on nematode egg hatching

The inhibitory effect of different extracts of *Zingiber officinale* on nematode egg hatching is presented in Table 5. The negative controls, DMSO (0.5%) and PBS, showed high egg hatching percentages (90.83 ± 1.44 to 93.33 ± 1.44), with no significant differences ($p > 0.05$), indicating normal egg viability. Similarly, solvent controls for ethanolic (93.33 ± 1.33) and methanolic extracts (88.83 ± 1.25) did not significantly affect egg hatching. As compared to the positive control, albendazole (0.5%), exhibited a marked reduction in egg hatching (5.50 ± 1.80), confirming strong ovicidal activity. All ginger extracts demonstrated a concentration-dependent decrease in egg hatching. At lower concentrations (2.5–5 mg/ml), relatively high egg hatching percentages were observed, particularly in the aqueous extract (87.00 ± 2.64 and 84.33 ± 1.73), followed by methanolic and ethanolic extracts. However, with increasing concentrations, a progressive decline in egg hatching was evident across all extracts. At 10 mg/ml, ethanolic (62.33 ± 2.08) and methanolic extracts (61.33 ± 2.64) showed greater inhibition than the aqueous extract (73.66 ± 1.44). At higher concentrations (25–100 mg/ml), all extracts significantly reduced egg hatching, with the methanolic extract exhibiting the strongest inhibitory effect, reaching the lowest value at 100 mg/ml (14.66 ± 1.46), followed by ethanolic (24.33 ± 2.43) and aqueous extracts (45.00 ± 3.33).

Table 5: Inhibitory effect of different extracts of *Zingiber officinale* on nematode egg hatching (%).

Treatment/ concentration	Aqueous extract (Mean % ± SEM)	Ethanolic extract (Mean % ± SEM)	Methanolic extract (Mean % ± SEM)
DMSO (0.5%)	90.83 ± 1.44[a]	90.83 ± 1.44[a]	90.83 ± 1.44[a]
PBS	93.33 ± 1.44[a]	93.33 ± 1.44[a]	93.33 ± 1.44[a]
Solvent control	—	93.33 ± 1.33[a]	88.83 ± 1.25[a]
Albendazole (0.5%)	5.50 ± 1.80[e]	5.50 ± 1.80[e]	5.50 ± 1.80[e]
2.5 mg/ml	87.00 ± 2.64[b]	82.66 ± 2.57[b]	85.33 ± 2.57[b]
5 mg/ml	84.33 ± 1.73[b]	77.00 ± 1.73[c]	73.66 ± 2.64[c]
10 mg/ml	73.66 ± 1.44[c]	62.33 ± 2.08[c]	61.33 ± 2.64[c]
25 mg/ml	60.00 ± 2.50[d]	44.00 ± 3.46[d]	54.33 ± 4.33[d]
50 mg/ml	53.33 ± 3.33[d]	34.33 ± 2.43[e]	32.33 ± 2.08[e]
100 mg/ml	45.00 ± 3.33[e]	24.33 ± 2.43[e]	14.66 ± 1.46[e]

(Different superscript letters (a–e) within each column indicate significant differences ($p < 0.05$). Means sharing the same letter are not significantly different).

Effect of different extracts of *Zingiber officinale* on larval development

The effect of different extracts of *Zingiber officinale* on larval development is presented in Table 6. The negative controls, DMSO (0.5%) and PBS, exhibited high larval development percentages (97.77–99.55%) with no significant differences ($p > 0.05$), indicating normal larval viability. Similarly, solvent controls for ethanolic (98.00 ± 1.44) and methanolic extracts (98.00 ± 1.76) showed no significant inhibitory effect. In contrast, the positive control, albendazole (0.5%), resulted in a pronounced reduction in larval development (7.33 ± 2.44), confirming strong larvicidal activity. All ginger extracts demonstrated a significant, concentration-dependent inhibition of larval development.

At the concentration of 2.5–5 mg/ml, larval development remained relatively high, particularly in aqueous (92.33 ± 2.08 and 88.00 ± 2.64) and ethanolic extracts (93.66 ± 3.21 and 70.00 ± 3.21), while the methanolic extract showed

comparatively greater inhibition (81.33 ± 3.00 and 64.00 ± 3.60). With increasing concentration, a progressive decline in larval development was observed across all extracts. At 10 mg/ml, the methanolic extract (42.66 ± 3.51) exhibited substantially stronger inhibition than aqueous (74.66 ± 4.26) and ethanolic extracts (62.00 ± 5.00). At higher concentrations (25–100 mg/ml), all extracts significantly suppressed larval development. The ethanolic and methanolic extracts demonstrated the greatest larvicidal activity, with minimal larval development observed at 100 mg/ml (13.00 ± 2.61 and 13.66 ± 1.52, respectively), followed by the aqueous extract (35.66 ± 3.05).

Table 6: Effect of different extracts of *Zingiber officinale* on larval development (%).

Treatment/ concentration	Aqueous extract (Mean % ± SEM)	Ethanolic extract (Mean % ± SEM)	Methanolic extract (Mean % ± SEM)
DMSO (0.5%)	99.55 ± 0.62^{a}	98.44 ± 0.62^{a}	99.55 ± 0.62^{a}
PBS	98.00 ± 1.44^{a}	97.77 ± 3.84^{a}	98.00 ± 1.76^{a}
Solvent control	—	98.00 ± 1.44^{a}	98.00 ± 1.76^{a}
Albendazole (0.5%)	7.33 ± 2.44^{e}	7.33 ± 2.44^{e}	7.33 ± 2.44^{e}
2.5 mg/ml	92.33 ± 2.08^{b}	93.66 ± 3.21^{b}	81.33 ± 3.00^{b}
5 mg/ml	88.00 ± 2.64^{b}	70.00 ± 3.21^{c}	64.00 ± 3.60^{c}
10 mg/ml	74.66 ± 4.26^{c}	62.00 ± 5.00^{c}	42.66 ± 3.51^{d}
25 mg/ml	63.66 ± 3.21^{d}	50.00 ± 2.88^{d}	37.00 ± 2.64^{d}
50 mg/ml	51.33 ± 3.21^{d}	23.33 ± 2.51^{e}	37.33 ± 2.08^{d}
100 mg/ml	35.66 ± 3.05^{e}	13.00 ± 2.61^{e}	13.66 ± 1.52^{e}

(Different superscript letters (a–e) within each column indicate significant differences ($p < 0.05$). Means sharing the same letter are not significantly different).

Anthelmintic effect of *Zingiber officinale* extracts on adult worm motility

The anthelmintic effect of different extracts of *Zingiber officinale* on adult worm motility is presented in Table 7. The negative control (HBSS) showed minimal reduction in motility over time (100 → 56%), with no significant differences ($p > 0.05$), indicating normal worm activity. Similarly, solvent controls for ethanolic (100 → 76.66%) and methanolic extracts (100 → 71%) exhibited no substantial inhibitory effects. In contrast, the positive control, albendazole (0.5 mg/ml), demonstrated a rapid and complete loss of motility (70.66 → 0), confirming strong anthelmintic activity. All ginger extracts exhibited a time- and concentration-dependent reduction in adult worm motility.

At lower concentrations (20%), moderate inhibition was observed, with motility decreasing from 52 → 6% (aqueous), 63.66 → 6.33% (ethanolic), and complete paralysis in the methanolic extract (51 → 0). With increasing concentrations, the rate and extent of motility reduction increased markedly. At 40% concentration, complete paralysis was achieved within the observation period for all extracts. At higher concentrations (60–100%), all extracts caused rapid and complete inhibition of motility (0%), with the ethanolic and methanolic extracts exhibiting faster effects compared to the aqueous extract. Overall, *Zingiber officinale* extracts showed strong anthelmintic activity against adult worms, with methanolic and ethanolic extracts being more potent, achieving complete paralysis at lower concentrations and shorter exposure times compared to the aqueous extract.

Table 7: Anthelmintic effect of *Zingiber officinale* extracts on adult worm motility (%). Arrows (→) indicate progressive reduction in motility over time.

Treatment/ concentration	Aqueous extract (0.5– 8h)	Ethanolic extract (0.5 – 8 h)	Methanolic extract (0.5 – 8 h)
HBSS (Control)	100 → 56^{a}	100 → 56^{a}	100 → 56^{a}
Solvent control	—	100 → 76.66^{a}	100 → 71^{a}
Albendazole (0.5 mg/ml)	70.66 → 00^{e}	70 .66→ 00^{e}	70.66 → 00^{e}
20%	52 → 6^{b}	63.66 → 6.33^{b}	51 → 00^{b}
40%	41.33 → 00^{c}	31.66 → 00^{c}	45 → 00^{c}
60%	24 → 00^{d}	13 → 00^{d}	34.33 → 00^{d}
80%	21.33 → 00^{d}	13 → 00^{d}	20.66 → 00^{e}
100%	12 → 00^{e}	6 → 00^{e}	6 → 00^{e}

(Different superscript letters (a–e) within each column indicate significant differences ($p < 0.05$). Means sharing the same letter are not significantly different).

DISCUSSION

The present study demonstrated that extracts of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) contain multiple bioactive phytochemicals, including alkaloids, saponins, glycosides, steroids, triterpenoids, carbohydrates, and amino acids. Variations observed among extracts, particularly the absence of certain compounds in specific solvents, highlight the influence of solvent polarity on phytochemical extraction. Similar solvent-dependent variations have been reported in previous studies (Ameh *et al.*, 2013; Ghasemzadeh *et al.*, 2016; Oboh *et al.*, 2007; Salawu *et al.*, 2021; Yusuf *et al.*, 2018).

The *in vitro* assays revealed significant, dose-dependent anthelmintic activity of both plant species against *Haemonchus contortus* across egg, larval, and adult stages. Among garlic extracts, methanolic and ethanolic preparations showed higher efficacy compared to aqueous extracts. This may be attributed to the enhanced extraction of bioactive organosulfur compounds such as allicin and

related metabolites, which are known to interfere with parasite metabolism and structural integrity (Ankri and Mirelman, 1999; Fredotović and Puizina, 2019; Lanzotti *et al*., 2014; Sharifi-Rad *et al*., 2016; Tsao and Yin, 2001). However, although garlic extracts exhibited strong activity, their efficacy remained lower than that of albendazole, which showed near-complete inhibition across all assays.

Similarly, ginger extracts demonstrated notable ovicidal, larvicidal, and adulticidal effects in a concentration-dependent manner. Methanol and ethanol extracts were more potent than aqueous extracts, likely due to their ability to extract lipophilic compounds such as gingerols, shogaols, and paradols. These compounds have been reported to disrupt neuromuscular function and metabolic pathways in helminths (Ali *et al*., 2008). At higher concentrations, the efficacy of organic extracts approached that of albendazole, particularly in larval and adult assays (Ahmed *et al*., 2021; Goswami *et al*., 2024; Sofowora *et al*., 2013).

The observed differences in efficacy among solvent extracts confirm that extraction method plays a critical role in determining the biological activity of plant-derived compounds. Organic solvents, particularly methanol and ethanol, appear more effective in extracting a broader spectrum of bioactive constituents, resulting in enhanced anthelmintic activity. These findings are consistent with previous studies reporting improved antiparasitic effects of organic extracts compared to aqueous preparations (Eguale *et al*., 2007; Iqbal *et al*., 2006; Upadhayay *et al*., 2012; Vilas, 2010; Zirintunda *et al*., 2022).

Although albendazole remained the most potent treatment, both garlic and ginger extracts exhibited significant activity against multiple developmental stages of *H. contortus*. This multi-stage activity is particularly important for parasite control, as it may help reduce transmission and infection intensity. However, the relatively lower efficacy compared to synthetic drugs suggests that these plant extracts are better suited as complementary rather than replacement therapies.

CONCLUSION

This study demonstrated that *Allium sativum* (garlic) and *Zingiber officinale* (ginger) extracts contain diverse bioactive phytochemicals, whose composition varies with extraction solvent. Methanolic and ethanolic extracts exhibited stronger ovicidal, larvicidal, and adulticidal activity against *Haemonchus contortus* compared to aqueous extracts, highlighting the influence of solvent polarity on bioactive compound efficacy. Although albendazole remained the most potent, garlic and ginger extracts showed significant, dose- and time-dependent anthelmintic effects, indicating their potential as complementary or alternative agents in integrated parasite management. Future studies should focus on isolating active compounds, optimizing extraction methods, and evaluating *in vivo* efficacy and safety to facilitate practical application in livestock parasite control.

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NOVELTY STATEMENT

This study demonstrates the potent in vitro anthelmintic activity of Allium sativum and Zingiber officinale against Haemonchus contortus, highlighting their potential as natural alternatives to conventional anthelmintics for controlling drug-resistant gastrointestinal nematodes in small ruminants.

AUTHOR'S CONTRIBUTION

HMS: Conducted the research and wrote the initial draft of the manuscript.
JAG: Conceived and conceptualized the research proposal and supervised the study.
BB: Performed data analysis.
MT: Provided technical input during the research and manuscript preparation.
SM: Assisted in laboratory experiments and data analysis.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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