

Coccidiostat and Anthelmintic Activities of *Allium sativum* Juice: *In Vitro* Study

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ABSTRACT

Eimeria species parasite resistance to drugs has been reported, which infects many animals and leads to great economic losses. Medicinal plants are a promising source of a cure for many diseases. This study aimed to investigate the effect of *Allium sativum* juice (ASJ) on the sporulation of oocysts and as an anthelmintic effector via *in vitro* study. Characterization of the plant was done by Fourier-transform infrared spectroscopy (FT-IR) and determination of phenolics, flavonoids, and antioxidant activity. The earthworm, *Eisenia fetida*, is used as a model worm to evaluate the anthelmintic activity of ASJ. The sporulation (%) of *Eimeria papillata* was assessed by ASJ compared to other detergents. FT-IR showed thirteen active compounds. Our results showed that paralysis and death of earthworms at ASJ (100 %) were 0.14 ± 0.007 and 0.18 ± 0.033 min, respectively, which highly significant than those with the reference drugs. In all treated worms, the histological study revealed obvious surface architecture abnormality. At 72 and 96 h, a high concentration of ASJ (100%) inhibits sporulation by 100%. At 96 h, other concentrations of ASJ (50%, 25%, 12.5%, 6.25%, and 3.12%), as well as amprolium, Dettol™, phenol inhibits oocyst sporulation by 42.29%, 38.02%, 22.44%, 11.63%, 8.50%, 26.66%, 75.45%, and 88.65%, respectively. Results suggest that ASJ possessed strong anthelmintic and coccidiostat properties.

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Authors' Contribution

RAG and EMA presented the concept of the study, planned methodology, performed formal analysis, and wrote, reviewed, and edited the manuscript. SNM, RAG and EMA arranged resources. RAG and SAQ investigated the trials. HMAA, RAG and SAQ curated data and did visualization. SAQ did funding acquisition. RAG administered the project.

Key words

Coccidiosis, Natural plants, Antiparasitic activity, Drugs

INTRODUCTION

Coccidiosis is a widespread, significant disease in ruminants (Khodakaram-Tafti and Hashemnia, 2017; Abdel-Gaber *et al.*, 2023), poultry (Quiroz-Castañeda and Dantán-González, 2015), rabbits (Li *et al.*, 2020), that results in production losses and disease prevention and treatment costs (Quiroz-Castañeda and Dantán-González, 2015). Additionally, coccidia can cause secondary infections to spread, such as clostridiosis, which causes severe necrotic enteritis (Moore, 2016; López-Osorio *et al.*, 2020). It is an intestinal illness brought by various *Eimeria* protozoan species which belong to the phylum Apicomplexa (Bakunzi *et al.*, 2010; Dakpogan *et al.*, 2019).

It has a life cycle involving stage separate exogenous (sporogony) and endogenous (schizogony and gametogony)

phases (Shen *et al.*, 2014). Unsporulated oocysts (non-infective) are shed in animal feces and undergo sporogony (sporulation) under appropriate conditions to become infective. After oral ingestion of sporulated oocysts, sporozoites are excysted and invade intestinal epithelia, after which enter an endogenous phase involving schizogony and gametogony. After fertilization, unsporulated oocysts are expelled into the environment with feces (Walker *et al.*, 2015; Su *et al.*, 2017; Abu Hawsah *et al.*, 2023). A double wall of proteins and lipids surrounds the oocyst, protecting against environmental mechanical and chemical harm (Quiroz-Castañeda and Dantán-González, 2015). It is also resistant to various detergents, disinfectants, and proteolysis (Ferguson *et al.*, 2003). As a result, sporulation process disruption is a crucial area where this parasite can be controlled (Mai *et al.*, 2009). Additionally, it has been reported that resistance against the drugs used in the treatment of *Eimeria* (Abbas *et al.*, 2012; Chapman, 2014). Therefore, research is now concentrating on finding new, efficient substitutes to control these pathogens (Al-Otaibi *et al.*, 2023). Medicinal plants are widely known for having antibacterial and antiparasitic properties, therefore they can be an effective tool against *Eimeria* (Cobaxin-Cardenas, 2018; Abdel-Tawab *et al.*, 2020). Plant extracts and their derivatives have demonstrated better coccidiostat effects as *Morus nigra* leaf extracts (Mulberry) (Thagfan *et al.*, 2020), *Saccharum officinarum* L. (Sugarcane)

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(Daneshmand *et al.*, 2021) and *Cinnamomum verum* (Qaid *et al.*, 2021).

The Liliaceae family includes the bulbous herbaceous plant known as garlic (*Allium sativum*). It is cultivated extensively worldwide and used as an additive, spice, and medicinal herb (Onyeagba *et al.*, 2004; Amagase, 2006). The therapeutic properties of garlic are a result of the increased concentration of sulfur compounds in garlic (allicin, diallyl disulfide, S-allyl cysteine, and diallyl trisulfide) (Tesfaye and Mengesha, 2015; Singh and Singh, 2019). Garlic was recommended as therapy for various diseases, such as cancers, infections, injuries, cardiovascular diseases, and gastrointestinal dysfunctions, and an immunostimulant (Bayan *et al.*, 2014; Arreola *et al.*, 2015).

Additionally, Garlic is recognized as an antibacterial (Chakraborty and Majumder, 2020), antiviral and fungicidal (Houshmand *et al.*, 2013), and antiparasitic such as *Trichomonas* and *Entamoeba* (Leitsch, 2017), *Trypanosoma* and *Leishmania* (Krstin *et al.*, 2018), schistosomiasis (Hamad and El-Moaty, 2021), and *Trichinella spiralis* (Abou Hussien *et al.*, 2022). Moreover, anthelmintic activity was reported for garlic against *Pheritima posthuma* (Dubey *et al.*, 2010), *Cotylophoron cotylophorum* (Radwan *et al.*, 2012), *Haemonchus contortus* (Iqbal *et al.*, 2001), *Cosmocerca ornata* (Aydin and Mammadov, 2022).

This study aimed to evaluate the *in vitro* coccidiostat activity of *Allium sativum* juice (ASJ) against coccidia (*Eimeria papillata*) infecting mice, and additionally, its anthelmintic activity.

MATERIALS AND METHODS

Plant collection, and preparation of its juice

The garlic cloves (*Allium sativum*) were obtained from the Alothaim markets in Riyadh, Saudi Arabia. The juice of garlic was prepared according to the method of Farhadi *et al.* (2015). Briefly, the garlic cloves were squeezed using a blender (Braun, Czech Republic), and then the juice was filtered using gauze, after that the juice was kept in the refrigerator (-20 °C) until it was used in the experiment. Also, a quantity of *A. sativum* juice (ASJ) was dried by placing it in the oven at a temperature of 37 °C for 24 h to obtain a powder which was used for Fourier-transform infrared spectroscopy (FT-IR) analysis.

An excess of potassium bromide powder (1:99 wt%) was added to a tiny portion of the sample, which was then processed to a homogeneous consistency before being finely ground and placed in a pellet formation. The instrument used for this analysis is Thermo Scientific's optical spectrometer NICOLET 6700 Fourier-transform

infrared spectroscopy (FT-IR). Maximum absorption was reported in the number of waves (cm⁻¹). Spectra were registered from 400 to 4000 cm⁻¹.

Total phenolic and flavonoid contents

The phenolic content was determined using the Folin–Ciocalteu technique as previously described by Abdel Moneim (2013). The measured value was compared to a calibration curve built with gallic acid solutions, and the results are given as mg gallic acid per gram of dry weight extract. Moreover, the total flavonoid content was determined using the aluminum chloride colorimetric method published by Abdel Moneim (2013). The total flavonoid content was calculated using a calibration curve and is reported as mg rutin per gram dry weight extract.

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The activity of ASJ was determined to scavenge DPPH radicals. Briefly, fresh DPPH radical solution (0.08 mM) in methanol was produced, and 950 ml of DPPH solution was combined with 50 ml of ASJ and incubated at 25°C for 5 min in dark. The absorbance was measured at 515 nm using a microplate reader. The antioxidant activity is expressed as the percent suppression of DPPH radicals according to Akillioglu and Karakaya (2010).

Anthelmintic activity of Allium sativum juice (ASJ)

The anthelmintic study was carried out using three doses (100, 50, and 25%) of ASJ against the earthworm, *Eisenia fetida*, according to Ajaiyeoba *et al.* (2001). Mebendazole (10 mg/ml) was used as the reference drug. Worms in distilled water were used as a control. Five worms of nearly the same body size were used per dose. The time to reach paralysis and death state was expressed in minutes (Dkhil, 2013).

Histological examinations

Small parts of the treated and control worms were fixed in 10% buffered formalin after that were embedded in paraffin wax and sectioned to 4 µm thicknesses. Hematoxylin and eosin (H & E) were used to stain the sections according to Drury and Wallington (1973), then examined and photography using an Olympus B×61 microscope (Tokyo, Japan). Measurements for the cuticular thickness of worms were taken using ImageJ 1.53e software and represented in micrometers (µm).

In vitro oocyst sporulation

Five laboratory male mice (*Mus musculus*) (aged 10–12 weeks) were inoculated with 1×10⁵ sporulated *Eimeria papillata* oocysts by oral gavage. Feces were collected at

5 days post-infection (p.i.), and oocysts were separated by floatation technique and then used for *in vitro* study.

The unsporulated oocysts (1×10^5) were incubated in 5 ml Dist. H₂O (negative control), 5 ml 2.5% K₂Cr₂O₇ (positive control), 5 ml of 100% ASJ, and finally 5 ml K₂Cr₂O₇ containing one of the following: ASJ (50, 25, 12.5, 6.25, and 3.125%), 8.3 mg amprolium (Veterinary Agriculture Products Company (VAPCO), Jordan), 109 µl Dettol™, 25 µl phenol, and 5% formalin. Sporulation of the oocysts was monitored by examining sporocysts using an Olympus compound microscope (Olympus Co., Tokyo, Japan). For each treatment, incubation was done for 72 and 96 h at 25 to 29 °C (Gadelhaq *et al.*, 2018). According to Thagfan *et al.* (2020), a total of 100 oocysts were counted for each treatment and control group to estimate the sporulation and inhibition (%) of oocysts.

Statistical analysis

The one-way analysis of variance (ANOVA) was used to evaluate the data with SigmaPlot® version 11.0 (Systat Software, Inc., Chicago, IL, USA), and the results were given as mean $\pm$ SD. At a *p*-value ≤ 0.05 , differences between groups were considered significant.

RESULTS

Chemical component of *Allium sativum* juice

Table I shows major bands after FT-IR analysis of *Allium sativum* juice indicating the presence of alcohol, alkane, carbon dioxide, carbodiimide, alkene, alkene, alkyl aryl ether, a tertiary alcohol, sulfoxide, and 1,4-disubstituted, compounds, respectively.

Table I. FT-IR for *Allium sativum* juice (ASJ).

Absorption (cm ⁻¹)	Transmittance (%)	Appearance	Group	Compound class
3411.63	1.239086	strong, broad	O-H stretching	alcohol
2930.09	4.376641	medium	C-H stretching	alkane
2338.31	14.99319	strong	O=C=C stretching	carbon dioxide
2119.54	14.8321	strong	N=C=N stretching	carbodiimide
1638.09	4.218665	strong	C=C bending	alkene
1454.32	6.405822	medium	C-H stretching	alkene
1412.83	6.039644	medium	C-H stretching	alkene
1274.79	8.676123	strong	C=O stretching	alkyl aryl ether
1132.47	4.47557	strong	C-O stretching	tertiary alcohol
1026.62	2.696528	strong	S=O stretching	sulfoxide
930.44	0.873064	strong	C=C stretching	alkene
817.69	13.52744	strong	C-H bending	1,4-disubstituted
598.75	9.788553	strong	C=C bending	alkene

The total concentration of phenolics and flavonoids in the ASJ was found to be 17.15 ± 0.04 mg gallic acid/g of the sample and 3.45 ± 0.03 mg rutin/g of the sample, respectively. In addition, the percentage of DPPH assay was 62.88 ± 2.72 .

Anthelmintic activity of ASJ

The ASJ produced a relatively comparable anthelmintic activity with mebendazole against *E. fetida* (Fig. 1). The most efficient dose, ASJ (100%) showed the time to paralysis and death were 0.14 ± 0.01 and 0.18 ± 0.03 min, respectively. However, mebendazole showed less effect (6.62 ± 0.42 and 9.39 ± 0.77 for paralysis and death time, respectively) (Table II). Moreover, histological sections showed no changes in the cuticular layers of the control group (97.43 ± 3.21 µm), while, there is a significant thickness reduction in the upmost layer of the treated groups as 40.22 ± 2.03 for ASJ and 53.72 ± 3.28 for the reference drug (Figs. 2, 3).



Fig. 1. *In vitro* anthelmintic effect of *Allium sativum* juice (ASJ) against *Eisenia fetida*.

Table II. Anthelmintic activity of *Allium sativum* juice (ASJ).

Test samples	Concentration	Time is taken for paralysis (min.)	Time is taken for death (min.)
Control (H ₂ O)	--	--	--
ASJ	25%	2.17 ± 0.12 ^{abde}	3.07 ± 0.34 ^{abde}
	50%	0.25 ± 0.01 ^{abce}	0.32 ± 0.01 ^{abce}
	100%	0.14 ± 0.01 ^{acd}	0.18 ± 0.03 ^{acd}
Mebendazole	10 mg/ml	6.62 ± 0.42 ^{acd}	9.39 ± 0.77 ^{acd}

Values are mean $\pm$ SD. All superscripts indicate significance at *p* ≤ 0.05 , ^a compared to untreated (H₂O), ^b compared to mebendazole, ^c compared to the lowest concentration of ASJ, ^d compared to the moderate concentration of ASJ, ^e compared to the highest concentration of ASJ.

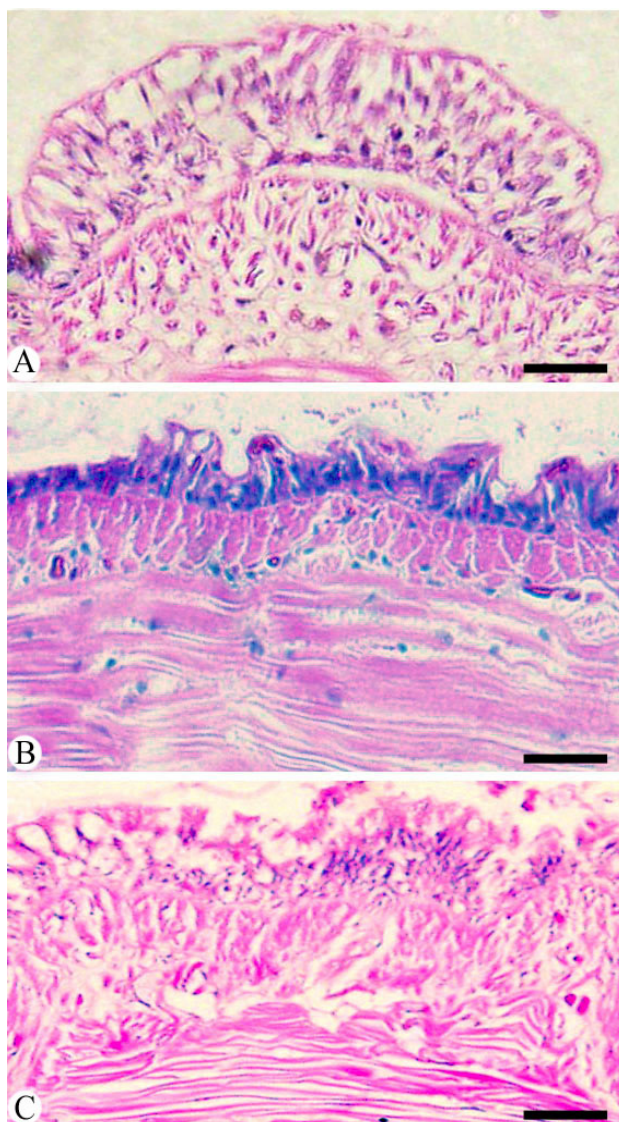


Fig. 2. Cuticle thickness of *E. fetida* with various treatments. (A) worms in dist. H₂O (control). (B) worms in ASJ (100%). (C) worms in mebendazole. Scale bar = 25 μ m.

In vitro study of ASJ on oocyst sporulation

At 72 and 96 h, a considerable level of *in vitro* oocysts sporulation in dist. H₂O was observed to be 83.44% and 88.78%, respectively. There was no change for oocysts incubated in ASJ (100%) at 72 and 96 h. Moreover, K₂Cr₂O₇, ASJ (50, 25, 12.5, 6.25, and 3.12%), amprolium, Dettol™, and phenol induced variable inhibition levels at 96 h of 3.95%, 42.29%, 38.02%, 22.44%, 11.63%, 8.50%, 26.66%, 75.45%, and 88.65%, respectively (Table III). After incubation with formalin, the unsporulated oocysts showed no rate of sporulation.

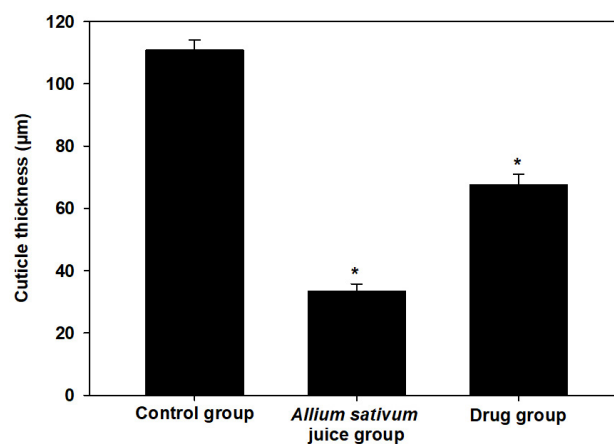


Fig. 3. The thickness of *E. fetida* cuticle (μ m) among the control group, as well as *Allium sativum* juice (ASJ) and drug-treated groups. * significance change concerning the control group.

Table III. Coccidiostat effects of *Allium sativum* juice (ASJ) on the sporulation and inhibition percentages (%) of *Eimeria papillata* oocysts.

Groups	Time	Sporulation of oocyst (%)	Inhibition of sporulation (%)	p value
Distilled H ₂ O	72 h	83.44 $\pm$ 2	6.42 $\pm$ 1	0.05
	96 h	88.78 $\pm$ 2	5.95 $\pm$ 1	0.05
Potassium dichromate (2.5%)	72 h	89.17 $\pm$ 2	5.82 $\pm$ 1	0.05
	96 h	94.04 $\pm$ 2	3.95 $\pm$ 0.2	0.05
ASJ (100%)	72 h	0	100 $\pm$ 0.1	-
	96 h	0	100 $\pm$ 0.1	-
ASJ (50%)	72 h	52.63 $\pm$ 1	46.88 $\pm$ 2	0.05
	96 h	53.70 $\pm$ 1	42.29 $\pm$ 2	0.05
ASJ (25%)	72 h	57.81 $\pm$ 1	35.16 $\pm$ 1	0.05
	96 h	58.28 $\pm$ 1	38.02 $\pm$ 2	0.05
ASJ (12.5%)	72 h	61.03 $\pm$ 2	31.83 $\pm$ 2	0.05
	96 h	72.93 $\pm$ 2	22.44 $\pm$ 1	0.05
ASJ (6.25%)	72 h	58.78 $\pm$ 1	34.08 $\pm$ 2	0.05
	96 h	83.10 $\pm$ 2	11.63 $\pm$ 1	0.05
ASJ (3.12%)	72 h	71.87 $\pm$ 2	19.40 $\pm$ 1	0.05
	96 h	86.04 $\pm$ 2	8.50 $\pm$ 1	0.05
Amprolium	72 h	62.67 $\pm$ 1	33.35 $\pm$ 1	0.05
	96 h	65.39 $\pm$ 1	26.66 $\pm$ 1	0.05
Dettol™	72 h	18.67 $\pm$ 1	79.06 $\pm$ 1	0.05
	96 h	23.08 $\pm$ 1	75.45 $\pm$ 1	0.05
Phenol	72 h	7.7 $\pm$ 0.1	91.36 $\pm$ 1	0.05
	96 h	10.67 $\pm$ 1	88.65 $\pm$ 1	0.05
Formaline	72 h	0	100 $\pm$ 0.1	-
	96 h	0	100 $\pm$ 0.1	-

DISCUSSION

Several studies have reported garlic as an anthelmintic (Hodge *et al.*, 2002; Veerakumari and Chitra, 2015; Raza *et al.*, 2016; Abu Almaaty *et al.*, 2021; Toni *et al.*, 2023). In the present study, different concentrations of ASJ showed anthelmintic efficacy against earthworms in comparison to Mebendazole, which is attributable to the presence of numerous bioactive phytochemical constituents, this agrees with Iqbal *et al.* (2001), Kanojiya *et al.* (2015), and Palacio-Landín *et al.* (2015). Freshly garlic extracts' main physiologically active ingredient is (ACSO, SAC, S-Allyl-L-cysteine sulfoxide). It easily passes through artificial and biological phospholipid membranes (Oosthuizen *et al.*, 2018). Allicin is a reactive sulfur species (RSS) that can oxidize cellular thiols, such as cysteine residues in proteins (Borlinghaus *et al.*, 2014; Miron *et al.*, 2000). *A. sativum* is rich in selenium and organosulfides (S-allylcysteine and S-allyl mercaptocysteine) which are strong antioxidant compounds capable of preventing lipid peroxidation (Yin *et al.*, 2002; Santhosha *et al.*, 2013). One of the byproducts of allicin's breakdown, ajoene, was shown to be antiparasitic; which interfered with fat and protein absorption in the parasite, causing the intracellular membrane system to rupture and cell lysis (Anthony *et al.*, 2005). Moreover, garlic oil dramatically reduced glucose uptake, glycogen content, and oxygen use in both *Ascaridia* and *Heterakis gallinarum* which led to their death (Raza *et al.*, 2016). *In vitro*, extract of *A. sativum* causes inhibition of enzyme activities of pyruvate kinase and phosphoenolpyruvate carboxykinase for *H. contortus*, which severely affects the energy-generating process of the worm and leads to its death (Navaneetha and Veerakumari, 2009). Also, garlic's sulfurous components can aid in the removal of tapeworms (Hodge *et al.*, 2002). In addition, several studies reported that garlic affects the phenotypic features of worms, where the change was evident in the structure of the tegument (Simonsen *et al.*, 1990; Sotillo *et al.*, 2010; Cortés *et al.*, 2017), and this agrees with our result, where ASJ led to noticeable changes that occurred in the skin with widespread shrinkage.

Moreover, different concentrations of ASJ had a significant effect on oocyst sporulation for *E. papillata*, which is consistent with several studies (Gadelhaq *et al.*, 2018; Sidiropoulou *et al.*, 2020; Abd-Elrahman *et al.*, 2022), this is due to garlic's (allicin) bioactive components can interact with cytoplasmic membranes and change their cation permeability, which disrupts vital processes in the parasite cells and ultimately leads to their death (El-Saber *et al.*, 2020). Our findings demonstrated that the hazardous chemical formalin (5%) fully prevented *E. papillata* sporulation. Previous studies showed that a

concentration of formalin 10% (Gadelhaq *et al.*, 2018) and 2% (Chroustová and Pinka, 1987) significantly affected the sporulation of *E. tenella* oocyst. Samaha *et al.* (2013) noted that phenol (10%) inhibits sporulation. On the other hand, Dettol did not affect oocyst sporulation, and these results may be explained by the impermeability of the oocyte wall to water-soluble compounds and resistance to proteolysis (Kuticic and Wikerhauser, 1996; Mai *et al.*, 2009).

CONCLUSION

This study concluded that *A. sativum* juice are having rather good anthelmintic and coccidiostat potentials. Relying on the use of medicinal plants as anthelmintic and coccidiostat effectors will provide a cheap, effective, and readily available alternative compared to conventional drugs, which showed parasite resistance to them. Therefore, the results of the current study may contribute to, the development of sustainable, safe alternatives and effective and/or additions to conventional anthelmintic and coccidiostat.

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IRB approval

Not Applicable.

Ethics statement

Not Applicable.

Data availability statement

All the datasets generated or analyzed during this study are included in this published article.

Statement of conflict of interest

The authors have declared no conflict of interest.

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