

ORIGINAL ARTICLE

Scolicidal Efficacy of Combining Two Garlic Extracts Against *Echinococcus granulosus* Isolated from Sheep Livers

Shaimaa A. Al-Samir*, Ahmed. J. Mohammed, Azhar A. Ameen, Zainab J. Madhi

Department of Biology, College of Science, Basrah University, Basrah, Iraq

ABSTRACT

Key words:
Hydatidosis, Medicinal
plants, garlic,
Echinococcus granulosus

***Corresponding Author:**
Shaimaa A. Al-Samir
Department of Biology,
College of Science, Basrah
University, Basrah, Iraq
shaimaass1977@gmail.com

Background: Garlic is one of the most well-known extract for its remarkable therapeutic benefits. **Objective:** Evaluate the efficacy of a mixture containing ethyl acetate and aqueous extracts of garlic against *Echinococcus granulosus* protoscolices in vitro. **Methodology:** Sixty-three hydatidosis-infected sheep livers were collected from the slaughterhouse in Basrah City, located in southern Iraq, over three months. Protoscolices were isolated from the livers of hydatidosis-infected sheep and exposed to a combination of ethyl acetate and aqueous extracts (GEA) using four dilutions (25, 50, 100, and 150 mg/mL) for 5, 10, and 15 minutes, respectively. **Results:** The 150 mg/mL concentration presented the highest viability rates (20.3%, 10.00%, and 0.00%) at the 5, 10, and 15-minute marks, respectively, while the survival rate after 5, 10, and 15 minutes of extract concentration of 25mg/mL was the lowest (98.4%, 79.5%, and 70.0%) correspondingly, exposure to other concentrations at time intervals of 5, 10, and 15 minutes revealed viability rates of (68.6%, 60.2%, and 50.4%) for the concentration of 50 mg/mL and (40.7%, 31.0%, and 25.3%) for the concentration of 100 mg/mL. GAE extract improves scolicidal effects, yielding higher results than those of Albendazole sulfoxide, which showed lower fatality (88.70%, 56.8%, and 45.00%) at a dose of 100 mg/mL at the same time intervals. These results demonstrate that the new mixture of Garlic extracts has lethal in vitro effects on protoscolices. **Conclusion:** The in vitro lethal effect of garlic extract against protoscolices of *E. granulosus* is highly encouraging and may contribute to the development of innovative, side-effect-free treatments for humans.

INTRODUCTION

Echinococcosis is a parasitic platyhelminth larval infection of the tapeworm, *Echinococcus granulosus*. Humans become infected by ingesting eggs. This serious medical condition is a significant zoonotic parasitic disease with a high frequency in several regions, including Iraq¹. To prevent the spread of *E. granulosus* in endemic areas, dogs should be kept away from raw offal or carcasses. Dogs exposed to cadavers or fresh viscera from goats, camels, cattle, and sheep should be treated with an anthelmintic such as praziquantel every 6 weeks². Cystic echinococcosis primarily affects the organs of the intermediate host, including the lungs, liver, brain, kidneys, and spleen³. It infects both people and animals and spreads through wild carnivores/herbivores in sheep-raising regions, triggering global economic and public health issues. This infection is prevalent in India, the Middle East, South America, the Baltic region, northern China, Iraq, and other areas where sheep are raised³. Symptoms include increased pressure in the infected part. Liver Hydatid cysts can cause persistent abdominal pain, hepatomegaly, a palpable abdominal mass, portal hypertension, biliary cirrhosis, and ascites. Bacterial

infection of a cyst can lead to the formation of an abscess⁴. The infection is often detected through imaging and serologic testing. Surgery remains the primary treatment option, with chemotherapy as a supplementary approach which remains the most common treatment choice, especially for large cysts in the brain or heart⁵. Surgery is the preferred method for treating unilocular cysts in accessible areas of the body⁵. However, leakage of protoscolices after surgery can lead to subsequent hydatidosis. Protoscolicidal chemicals are injected into hydatid cysts during surgery or during the PAIR procedure to prevent live protoscolices from leaking into surrounding tissues⁶. They are used to eliminate the germinal layer and viable protoscolices of hydatid cysts. Several protoscolicidal drugs, including cetrime, silver nitrate, and hypertonic saline, have been used to sterilize cysts⁷.

Common protoscolicidal drugs, including formalin, chloride, 0.5% silver nitrate, cetrime, and 20% hypertonic sodium, can lead to serious consequences, such as cholangitis and liver necrosis⁷. The World Health Organization's expert group on hydatidosis emphasized the crucial need for novel protoscolicidal agents that are highly effective and have fewer side effects⁸. To limit recurrence rates, it is critical to prevent

cyst leakage and use effective medications accessible for non-urgent Hydatid cysts, such as Mebendazole, Albendazole, Nitazoxanide, and Furazolidone. These drugs can cause adverse side effects and treatment failures⁹. Although documented resistant patients who did not respond to therapy¹⁰. Therapeutic plants have been extensively utilised in developing countries due to their affordability and safety, so herbal medicines with scolical properties are gaining interest for pre-surgical therapy and the prevention of hydatid cyst recurrence¹¹.

Garlic has been shown to possess antiparasitic properties against various parasites, including *Giardia intestinalis*, *Ascaris lumbricoides*, and *Hymenolepis nana*¹². Garlic refers to the species *Allium sativum* has been used for its biological properties in the treatment of various illnesses because it possesses antibacterial, antiviral, antifungal, anticancer, and antithrombotic properties¹³. Garlic's antibacterial effect stems from allicin, which is produced by the enzyme alliinase (a cysteine sulfoxide lyase) when the clove is crushed or cut¹⁴. Allicin and other thiosulfates are thought to be responsible for garlic's medicinal properties. Garlic has been shown to inhibit the growth of both Gram-negative and Gram-positive bacteria¹⁴. Allicin also inhibited *Entamoeba histolytica*¹⁵. Also demonstrated its effectiveness as an anti-giardial agent¹⁶. In vitro, diallyl trisulfide, an organic sulfur compound found in garlic, suppresses the development of *E. histolytica*, *Giardia lamblia*, and *trypanosome*¹⁷. A study has found that feeding garlic powder to *Biomphalaria alexandrina* snails caused biological and biochemical alterations¹⁸. Garlic extract is a safe and effective protoscolicidal agent that may be used to treat hydatid cysts and avoid secondary cyst return before surgery¹⁹.

Garlic Nitric oxide (NO), a vascular and neuronal messenger, plays a crucial role in several biological activities²⁰. NO synthase produces a soluble free radical from arginine through three isoforms²¹. According to Liew *et al.*²², NO plays a key role in antimicrobial defence. Immunological stimuli, such as TNF or IFN- γ , can stimulate macrophages to produce large quantities of iNOS, which in turn kills parasites²³. Using Garlic as a treatment has an impact on immunity, as it may improve phagocytosis and natural killer cell activity, thereby strengthening the immune cells and defining the mechanism²⁴. Due to the limited treatment options for hydatid cysts with commercially available medications. The current study examined a novel herbal medicine of combining two garlic (*Allium sativum*) aqueous and ethyl acetate extracts against protoscolex in vitro.

METHODOLOGY

Preparation of Garlic ethyl acetate, and aqueous extracts (GEA)

Two extracts of *Allium sativum* were prepared and mixed. The first was the aqueous extract, prepared

according to Beni *et al.*²⁵, by crushing and weighing 15g of garlic, then combining it with 30 ml of distilled water and passing it through Whatman filter paper. The *Allium sativum* ethyl acetate extract was prepared by placing 20 g of plant powder into a paper container (Thimble), which was then inserted into a Soxhlet extractor filled with 200 mL of 95% ethyl alcohol. This process took 24 hours and was carried out at 450 °C. Following extraction, the mixture was concentrated using a rotary evaporator at 400 °C and stored in sterile bottles at 4 °C for later use²⁶. Finally, the two extracts were mixed in a 1:1 ratio and stored in a stock solution for future use.

Gas chromatography-mass spectrometry (GC-MS)

The analyses were performed by using a GC system connected to Mass Hunter workstation software (Agilent Technologies, USA; 7890B GC with a 5977A mass selective detector). The capillary column was used in a 5% phenyl-methyl-siloxane column and was held at a column pressure of 6.0799 psi. The programming of the temperature started with 40 °C and then it increased in a linear manner at a rate of 10 °C/min until 280 °C. The injector temperature had been maintained at 290 °C and solvent delay had been maintained at 4 min. Helium was the carrier gas and delivered at the rate of 1 ml/min. A pulsed splitless injection protocol was used to inject the samples; the mass spectral data were collected in a scan range of 35-650 m/z and the resolving power was 1562 m/z (N₂). Before injection, syringe filters were used to filter the extracts, and 1 μ L aqueous extract of *Allium sativum* was injected onto the resultant chromatograms and spectra were compared to the spectral library of National Institute of Standards and Technology (NIST)²⁷.

Protoscolices Collection

Livers of infected sheep were obtained from the Basrah slaughterhouse, and hydatid cysts were isolated from them after sterilising with 70% alcohol and rinsing with sterile phosphate-buffered saline three times (pH 7.2). The protoscolices were then separated by centrifugation at 3000 rpm for 15 min. An inspection was later performed using wet-mount drop microscopy to determine the presence of living protoscolices in the hydatid cyst fluid, stained with an aqueous eosin solution (0.1%)²⁸.

The effect of the extract on protoscolices. Different extract dilutions (25, 50, 100, and 150 mg/mL) were tested by adding 1.0 mL of each dilution to test tubes containing 1.0 mL of live protoscolices and incubating at 37°C for 5, 10, and 15 minutes. Finally, protoscolices were treated with varied extract concentrations for varying amounts of time. Their vitality was verified with 0.1% Eosin staining and inspected under a microscope. The proportion of killed protoscolices was measured using a haemocytometer. Unprocessed protoscolices served as the control group. The trials were repeated three times. The proportion of viable cells

was intended to be the percentage of alive protoscolices among the entire number.

Albendazole sulfoxide treatment

In the current study, commercially available Albendazole sulfoxide, at a concentration of 100 mg/mL, was used as a comparison drug for the prepared extract. The experiment was conducted as described by Soleymani *et al.*²⁹, with this medication added to the protoscolices at 5, 10, and 15 minutes. Finally, the survival rates were determined using a haemocytometer. The trials were repeated three times.

Statistical Analysis

The statistical analysis was performed using SPSS software version 24. A two-way ANOVA was used to compare the control and test groups; a p-value of less than 0.05 indicated statistical significance.

RESULTS

Bioactive Compounds Identified by GC-MS

GAE analysis by GC-MS revealed the presence of bioactive components with different retention times, areas, percentages, and various composites, as shown in (Table 1 & Fig. 1).

Table 1. GC-MS showing bioactive composites of a crude GAE extract of Garlic

Name	RT	%	Formula
Hexanoic acid	11.856	0.36	C ₆ H ₁₂ O ₂
4-Vinylphenol	15.211	0.43	C ₈ H ₈ O
Phenol, 5-ethenyl-2-methoxy-	16.472	0.7 ^y	C ₉ H ₁₀ O ₂
14-Pentadecenoic acid	19.747	0.44	C ₁₅ H ₂₈ O ₂
Geranyl isovalerate	19.946	0.5 ^o	C ₁₅ H ₂₆ O ₂
Cyclopentane carboxylic acid, 3-acetoxy-2-acetoxymethyl-4,4-dimethyl-, methyl ester	20.418	0.40	C ₁₄ H ₂₂ O ₆
9-Methoxy-3,4-dihydrophenanthren-1(2H)-one	21.148	4.7 ^y	C ₁₅ H ₁₄ O ₂
Oxacyclopentadecan-2-one, 15-methyl-	21.694	0.5 ^o	C ₁₅ H ₂₈ O ₂
7-Methyl-Z-tetradecen-1-ol acetate	21.997	1.14	C ₁₇ H ₃₂ O ₂
Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-	23.494	0.4 ^y	C ₁₉ H ₃₆ O ₃
l-(+)-Ascorbic acid 2,6-dihexadecanoate	24.224	12.7 ^y	C ₃₈ H ₆₈ O ₈
n-Hexadecenoic acid	24.534	17.0 ^y	C ₁₆ H ₃₂ O ₂
Oleic Acid	25.242	1.94	C ₁₈ H ₃₄ O ₂
6-Octadecenoic acid	26.326	32.7 ^y	C ₁₈ H ₃₄ O ₂
Pentosane	28.597	0.9 ^o	C ₂₅ H ₅₂
Hentriacontane	29.357	1.17	C ₃₁ H ₆₄
Heptacene	30.087	0.8 ^y	C ₂₇ H ₅₆
Nonacetate	31.489	0.77	C ₂₉ H ₆₀
Ethyl iso-alcoholate	36.268	0.93	C ₂₆ H ₄₄ O ₅

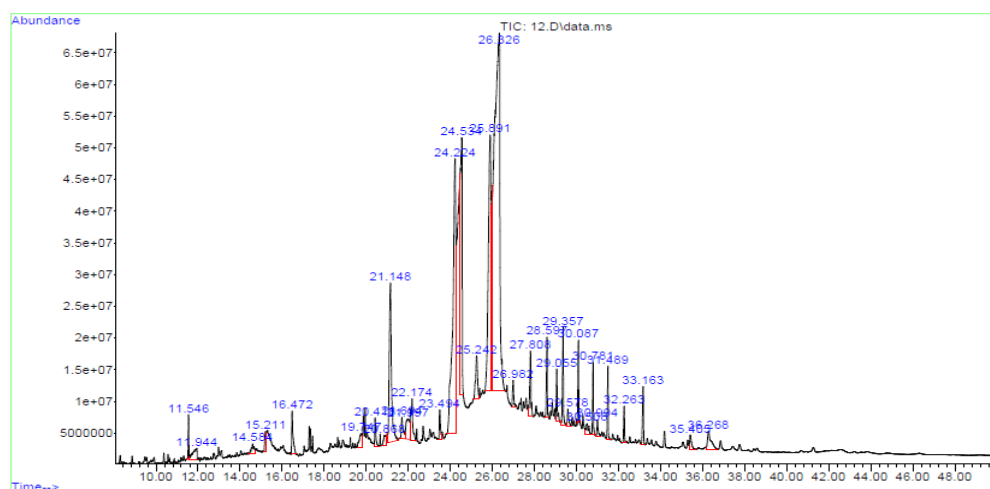


Fig. 1. GC-MS showing bioactive composites of a GAE extract

Morphologically, the liver severely affected by this parasite infection, comprising live hydatid cysts, showed uneven, liquid-bursting white sacs ranging from 1 to 10 cm in diameter (**Fig. 2**). These sacs were accompanied by yellow-white pellets known as 'hydatid sand'.

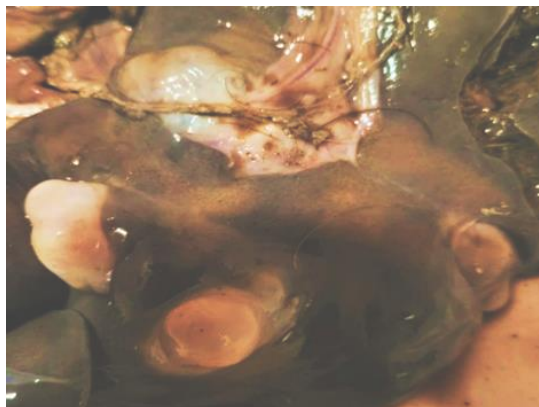


Fig. 2. Liver infected with hydatid cysts: numerous overlapping, pale multifocal lesions are apparent on the surface of the organ

As shown in (Table 2) the GAE scollicidal effect in the concentration of 150 mg/ml demonstrated the highest viability rates (20.3%, 10.00%, and 0.00%) at the 5, 10, and 15-minute marks, respectively, while the viability rates after 5, 10, and 15 minutes of exposure to an extract concentration of 25mg/ml were the lowest (98.4%, 79.5%, 70.0%, and 77.9%), respectively. Furthermore, exposure to other concentrations at time intervals of 5, 10, and 10 minutes revealed viability rates of (68.6%, 60.2%, and 50.4%) for the concentration of 50 mg/ml and (40.7%, 31.0%, and 25.3%) for the concentration of 100 mg/ml. At a dosage of 150 mg/ml, the extract effectively eliminated all protoscolices after 15 minutes of exposure. The investigation found that the extract had excellent efficacy in vitro. The number of live protoscolices decreased as the concentration increased. Additionally, prolonged exposure to a consistent dosage leads to fewer viable cysts. Finally, the control group exhibited a 100% viability rate. Statistical analysis (LSD) shows significant differences ($p < 0.05$). Furthermore, the study indicated that GAE extract has scollicidal properties.

Table 2. Survival rates of *E. granulosus* protoscolices treated with different quantities of Garlic GAE extract for varied durations of time in vitro compared to the standard Albendazole sulfoxide

Concentration (mg /ml)	Survival rate/ time			Mean survival rate in concentration	Comparison group
	5 min	10 min	15 min		
0	100%	100%	100%	100	$p < 0.05$
25	84.4%	79.5%	70.0%	77.9	$p < 0.05$
50	68.6%	60.2%	50.4%	59.7	$p < 0.05$
100	40.7%	31.0%	25.3%	32.3	$p < 0.05$
150	20.3%	10.00%	0.00%	10.1	$p < 0.05$
Mean survival rate in exposure time	62.8	58.14	49.14		
Albendazole sulfoxide (100 mg/ml)	88.70%	56.8%	45.00%		

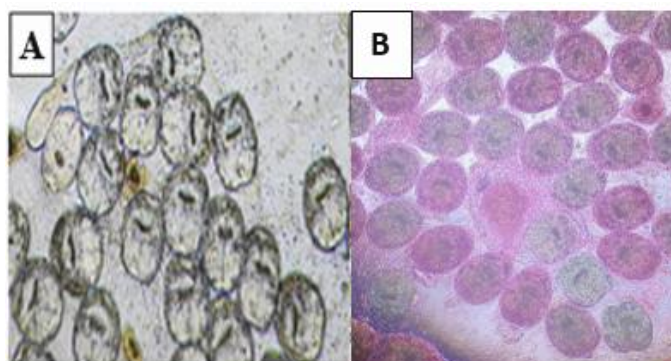


Fig. 3. *E. granulosus* protoscolices microscopic images (40X) showing, A- Alive, non-treated with GAE extract (control) appears white, B-treated with GAE extract, Alive (non-stained with eosin stain) appear green, and dead stained (pink coloured) after exposure time

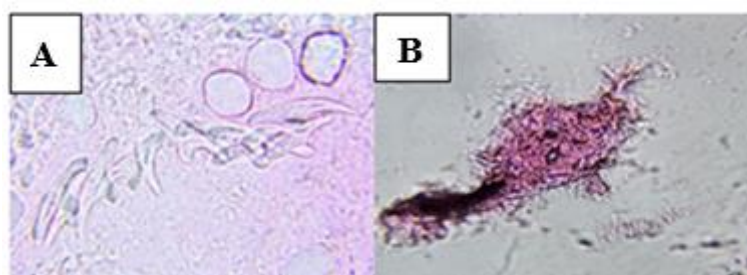


Fig. 4. *E. granulosus* hook A-intact hook before exposure to GAE extract (10X). B- Damaged hook after exposure to garlic AGE (40X)

DISCUSSION

During hydatidosis, alternative therapies have been attempted with variable degrees of success using certain drugs, such as benzimidazole, albendazole, and mebendazole, that can be particularly toxic²⁹. Furthermore, commercially available albendazole is poorly absorbed in the gastrointestinal tract, resulting in low hepatic concentrations and reduced efficacy²⁹. Also, it can cause significant side effects, including allergic purpura, flu-like symptoms, encephalitis syndrome, and drug rash²⁹. Unfortunately, this treatment is less effective against hydatid cyst protoscolices than the garlic GAE extract prepared in the current study, due to its bioactive molecules.

Garlic is a highly valued medicinal product that contains anthelmintic compounds and is a popular herb with numerous nutritional and therapeutic benefits. It has antioxidant, anticancer, antiviral, antifungal, antibacterial, antiprotozoal, anthelmintic, and antiprotoscolex properties³⁰. Garlic GAE extract has demonstrated effectiveness against parasites such as *Giardia intestinalis*, *Hymenolepis nana*, and *Leishmania*¹². Numerous studies have investigated the role of Hexanoic acid in the immune response, which can be either protective or harmful, depending on nematode infection³¹.

Additionally, oleic acid is effective against parasites, as demonstrated by Nunes *et al.*³², who reported in vitro killing of the schistosome parasite *S. mansoni*, suggesting a new treatment option. Destroying the protoscolex walls could also be achieved with Ascorbic acid, which has been shown to exhibit in vitro activity against many parasites³³. Shi *et al.*³³, used it to inhibit *Plasmodium* growth. The compound n-hexadecenoic acid may also play an important role in killing protoscolex, as indicated by Sajayan *et al.*³⁴, who demonstrated a potent bactericidal effect against *P. aeruginosa*. This bacterium infects marine fish.

In vitro, Garlic GAE extract has demonstrated vigorous protoscolicidal activity. Protoscolicidal effects were confirmed after at least 15 minutes. An in vitro study showed that garlic extract at 150 mg/mL exhibited effective protoscolicidal activity. The protoscolices of

hydatid cysts were affected after exposure for 15 minutes. However, such prolonged exposure periods are not feasible during hydatid cyst surgery. In this in vitro study, the protoscolicidal effects of GAE extract at 4 concentrations (25, 50, 100, and 150 mg/mL) were evaluated over 3 exposure times (5, 10, and 15 minutes). The 150 mg/mL concentration showed a 0.00% survival rate after 15 minutes of exposure. This suggests that this extract is effective and represents a promising novel protoscolicidal agent.

In the current study, the results of the extract were very efficacious at all doses tested despite the presence of bioactive composites in the garlic, such as oleic acid, which is found in high concentrations in this plant; this efficacy was similar to that indicated by Eskandarian when they conducted an in vitro study of garlic extract against *E. granulosus* protoscolices¹⁰. The current investigation used garlic extract exposure times that are considerably shorter than those in previous research. The mechanism of anti-parasitic activity of these active substances remains unclear; however, they are highly effective at inhibiting protoscolices.

This activity may be due to their ability to degrade lipids, which aligns with the findings of Lun *et al.*¹⁷ on garlic's antiparasitic activity. Additionally, Dibfiora *et al.*³⁰ discovered that garlic is effective in killing *Ascaris* in vitro. The extract demonstrated higher scolical activity than albendazole sulfoxide.

CONCLUSION

The Garlic GAE extract in vitro lethal effect against protoscolices of *E. granulosus* is highly encouraging and may contribute to the development of innovative, side-effect-free treatments for people. Furthermore, these protoscolices have developed resistance to albendazole, which is an ideal scolical therapy that should be effective immediately and completely, with no local or systemic adverse effects, and at a reasonable price. As a result, the study concluded that garlic is a useful therapeutic agent because its natural bioactive compounds effectively eliminate protoscolices. It suggests that it may be used as a natural treatment for hydatid cysts and to prevent the development of

secondary cysts. The study demonstrated that garlic GAE extract has a potent protoscolicidal effect, potentially replacing current treatments for parasitic infections.

Ethics Statement

The study was conducted at the University of Basra in accordance with the decision of the Scientific Research Ethics Committee, according to the protocol (EC-35) issued in February 2025.

Conflict of interest

The authors stated no conflicts of interest.

Funding

Self-funding

REFERENCES

1. Aldhafer AHS, Alsamir SA. Protoscolicidal Activity of The Crude Extract of Stem Bark of OAK (*Quercus* sp.). *Life Sci Arch* 2020;6(5):1969-1974 .
2. Lahmar S, Ben Chéhida F, Pétavy AF, Hammou A, Lahmar J, Ghannay A, et al. Ultrasonographic screening for cystic echinococcosis in sheep in Tunisia. *Vet Parasitol* 2006;143(1):42-49.
3. Tinsley B, Abbara A, Kadaba R, Sheth H, Sandhu G. Spontaneous intraperitoneal rupture of a hepatic hydatid cyst with subsequent anaphylaxis: a case report. *Case Rep hepatol* 2013;2013(1): 320418.
4. Cattaneo F, Graffeo M, Brunetti E. Extrahepatic textiloma long misdiagnosed as calcified echinococcal cyst. *Case Rep Gastrointest Med* 2013;2013(1):261685.
5. WHO Informal Working Group. International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. *Acta Trop* 2003;85:253–261.
6. Sokouti M, Sadeghi R, Pashazadeh S, Abadi SHE, Sokouti M, Ghojzadeh M, et al. A systematic review and meta-analysis on the treatment of liver hydatid cyst using meta-MUMS tool: Comparing PAIR and laparoscopic procedures. *Arch Med Sci* 2019;15(2):284-308.
7. Sadjjadi SM, Zoharizadeh MR, Panjeshahin MR. In Vitro Screening of Different *Allium Sativum* Extracts on Hydatid Cysts Protoscoleces. *J Invest Surg* 2008;21(6):318-322.
8. Kyo E, Uda N, Suzuki A, Kakimoto M, Ushijima M, Kasuga S, et al. Immunomodulation and antitumor activities of Aged Garlic Extract. *Phytomedicine* 1998;5(4):259-267.
9. Ulger S, Barut H, Tunc M, Aydin E, Aydinkarahaliloglu E, Gokcek A, et al. Radiation therapy for resistant sternal hydatid disease. *Strahlenther Onkol* 2013;189(6):508-509.
10. Eskandarian AA. Scolicidal effects of squash (*Corylus* spp) seeds, hazel (*Curcubia* spp) nut and garlic (*Allium sativum*) extracts on hydatid cyst protoscolices. *J Res Med Sci* 2012;17(11):1011-1014.
11. Sutton GA, Haik R. Efficacy of Garlic as an Anthelmintic in Donkeys. *J Vet Med* 1999;54:66-78.
12. Soffar SA, Mokhtar GM. Evaluation of the antiparasitic effect of aqueous garlic (*Allium sativum*) extract in hymenolepiasis nana and giardiasis. *J Egypt Soc Parasitol* 1991;21:497-502.
13. Samir EA, Alsamir SA, Rubaaie EM, Mahdi SWAMA. The effect of *Allium sativum* aqueous extract on *Echinococcus granulosus* protoscolices. *Int J Bio Res* 2024;9(1): 17-21.
14. Fani M, Kohanteb J, Dayaghi M. Inhibitory activity of garlic (*Allium sativum*) extract on multidrug-resistant *Streptococcus mutans*. *J Indian Soc Pedod Prev Dent* 2007;25(4):164-168.
15. Mirelman D, Monheit D, Varon S. Inhibition of Growth of *Entamoeba histolytica* by Allicin, the Active Principle of Garlic Extract (*Allium sativum*). *J Infect Dis* 1987;156 (1):243-244.
16. Eissa FM, Abdel-Shafi IR, El-Sayed SH, Negm MS, Ahmed JA. Assessment of therapeutic potential of *Allium sativum* and *Zingiber officinale* commercial supplements in experimental giardiasis models. *J Parasit* 2022;46(3):704–713.
17. Lun ZR, Burri C, Menzinger M, Kaminsky R. Antiparasitic activity of diallyl trisulfide (Dasuansu) on human and animal pathogenic protozoa (*Trypanosoma* sp., *Entamoeba histolytica* and *Giardia lamblia*) in vitro. *Ann Soc Belg Med Trop* 1994;74(1):51-59.
18. Mantawy MM, Mahmoud AH. Effect of *Allium cepa* and *Allium sativum* feeding on glucose, glycogen, protein bands profile, and phenol oxidase activity in *Biomphalaria alexandrina*. *J Egypt Soc Parasitol* 2002;32(1):271-283.
19. Rhadi HAJ. In Vitro Effects of Alcohol, Garlic Extraction, Formalin, and Glucose on Protoscolices of *Echinococcus Granulosus*. *Bas J Vet Res* 2012;11(1):137-147.
20. Moncada S, Higgs A. The L-Arginine-Nitric Oxide Pathway. *N Engl J Med* 1993;329(27):2002-2012.
21. Bogdan C, Thüning H, Dlaska M, Röllinghoff M, Weiss G. Mechanism of suppression of macrophage nitric oxide release by IL-13: influence of the macrophage population. *J Immunol* 1997;159(9):4506-4513.

22. Liew FY, Wei X, Proudfoot L. Cytokines and nitric oxide as effector molecules against parasitic infections. *Philos Trans R Soc Lond B Biol Sci* 1997;352(1359):1311-1315.
23. ElShenawy NS, Soliman FM, Reyad SI. The effect of antioxidant properties of aqueous garlic extract and *Nigella sativa* as anti-schistosomiasis agents in mice. *Rev Inst Med Trop São Paulo* 2008;50(1):29-36.
24. Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Intake of Garlic and Its Bioactive Components. *J Nutr* 2001;131(3):955-962.
25. Beni C, Casorri L, Masciarelli E, Ficociello B, Masetti O, Rinaldi S, et al. Characterization of garlic (*Allium sativum* L.) aqueous extract and its hypothetical role as biostimulant in crop protection. *J Food Agric Environ* 2018;16(3,4):38-44.
26. Alsamir SA, Albadran RM, Muthanna MM, Al-laeiby A. In vitro lethal activity of *Allium sativum* ethyl acetate extract on *Echinococcus granulosus* protoscolices isolated from sheep-infected liver. *J Appl Biol Chem* 2025;68:317-323.
27. Hameed MF, Mkashaf I, Al-Shawi AA, Hussein K. Antioxidant and Anticancer Activities of Heart Components Extracted from Iraqi Phoneix *Dactylifera* Chick. *Asian Pac J Cancer Prev* 2021;22(11):3533-3541.
28. Alsamir SA, Mahdi MA, Al-Ghanyem HS, Mohammed AJ. Investigates Bacterial Species Isolated from Hydatid Cyst Fluid in Sheep Livers in Basrah, Iraq. *Egypt J Med Microbiol* 2025;34(4):467-473.
29. Soleymani N, Sadr S, Rahdar A, Santucci C. Evaluation of the In-Vitro Effects of Albendazole, Mebendazole, and Praziquantel Nanocapsules against Protoscolices of Hydatid Cyst. *J Pathogens* 2024;13(9):790.
30. Dibfiora R, Situmorang EUM, Firmansyah RD. Efficacy of Shallot (*Allium cepa* L. Var. *aggregatum*) and Garlic (*Allium sativum*) as Herbal Anthelmintic against *Ascaris suum*. *J Med Health* 2021;3(1):34-45.
31. Ntalli N, Menkissoglu-Spirodi U, Doitsinis K, Kalomoiris M, Papadakis EN, Boutsis G, et al. Mode of action and ecotoxicity of hexanoic and acetic acids on *Meloidogyne javanica*. *J Pest Sci* 2020;93(2):867-877.
32. Nunes R, Campos PM, Moreira R, Mioduski J, Santos RD, Justus B, et al. The promising antischistosomal activity of oleic acid-loaded polymeric nanocapsules for oral administration. *J Drug Deliv Sci Technol* 2021;63:102429.
33. Shi X, Wei M, Xu Z, Liu Y, Zhang M, Lv L, et al. Vitamin C Inhibits Blood-Stage Plasmodium Parasites via Oxidative Stress. *Front Cell Deve Biol* 2021;9:1-13.
34. Sajayan A, Ravindran A, Selvin J, Ragothaman P, Kiran GS. An antimicrobial metabolite n-hexadecenoic acid from marine sponge-associated bacteria *Bacillus subtilis* effectively inhibited biofilm-forming multidrug-resistant *P. aeruginosa*. *Biofouling* 2023;39(5):502-515.