

The effect of *Allium sativum* aqueous extract on *Echinococcus granulosus* protoscolices

Esraa AHA Samir, Shaimaa A Alsamir*, Eman MAAL Rubaie, Sundus W Alabdullah Mohammed A Mahdi

Department of Biology, University of Basrah, Basrah, Iraq

Abstract

Allium sativum is one of the most famous medicinal plants. It has been commonly used throughout history for its medicinal importance. The aim of this paper is to evaluate the consequences of *Allium sativum* aqueous extract as an *in vitro* lethal agent used for *Echinococcus granulosus* protoscolices. Protoscolices were brought from infected sheep livers. The results have been obtained using three different concentrations of *Allium sativum* aqueous extract (50, 100, and 150 mg/mL) for 5, 10, and 15 minutes. The viability was 100% in the control group. At a concentration of 50 mg/ml, it was 62.85, 51.11, and 32.01 after 5, 10, and 15 minutes, respectively. Also, the viability rate was 45.95, 34.49, and 21.32% at a concentration of 100 mg/ml after the same time of extract exposure. Finally, the viability rate was found to be 01.86, 01.45, and 0.00% percent at 150 mg/mL after 15 minutes of contact. These results indicated that *Allium sativum* has lethal effects on protoscolices *in vitro*.

Keywords: Hydatid cyst disease, hydatidosis, medicinal, allium sativum

Introduction

Hydatidosis, is a parasitic infection of *Echinococcus granulosus* metacestode. This causes a disease of cystic infection in human or sheep lungs or liver through the intake of polluted water or food containing the egg stage that is released in stool from the adult tapeworm that lives in the dog intestine (Landa-Garacia *et al.*, 1997) [16].

Treatment of *Echinococcus granulosus* infection is usually done by surgical ways. However, in operations, the accidental spreading of the hydatid fluid viable protoscolices to the nearby tissues may cause reinfection (Arandes and Bertomeu, 2012) [6].

Allium sativum extract has been used for its medicinal properties all throughout history. Its primary ingredients are phenolic derivatives and volatile oil. Because of its several bioactive components, which include organic sulfides, saponins, phenolic compounds, and polysaccharides, it is grown as a prominent medicinal plant in many parts of the world (Stavěliková, 2008) [23]. Further, China has been used garlic as a traditional treatment (Jacob Song and Milner, 2001) [22]. Garlic's remarkable biological properties, such as its antibacterial, anti-inflammatory, anti-obesity, anti-inflammatory, anticancer, and anti-diabetic, have been the subject of numerous studies in recent decades (Tsai *et al.*, 2012) [25]. It is used as a treatment for a number of common illnesses, including the flue, the common cold, snake bites, and high blood pressure (Agarwal, 1996) [24]. It, furthermore, has a medicinal uses for treating lung conditions, whooping cough, stomach issues, colds, earaches, and helps against cardiovascular disease (Mallika *et al.*, 2014) [17]. Based on epidemiological data from human clinical studies, allium sativum and its active compounds play a major role in lowering the ability to get cardiovascular diseases and diabetes, and it also protects the immune system from microbial, fungal, cancer agents (Ambati, 2013) [5]. Various sulfur compounds have been reported to be present in raw garlic and its converted derivatives have been used in a variety of preparations (Dethier *et al.*, 2013) [12].

Materials and Methods

Protoscolices Collection

Sheep liver hydatid cysts were sterilised with 70% alcohol. Protoscolices have been collected from it by washing the cyst fluid with a sterile phosphate buffer saline pH 7.2 three times. protoscolices finally have been collected by centrifuging them at 3000 rpm for 15 minutes. wet mount drop was examined under a microscope to check for the occurrence of alive protoscolices in the cystic fluid (Smyth and Barrett., 1980) [21]. Staining them with 0.1% aqueous eosin stain which was considered a vital stain was done. After 5 minutes, the protoscolices were examined under a microscope, uncolored protoscolices were considered alive and stained protoscolices were considered dead. The protoscolices that were not treated (with plant extracts) were considered as the control group. The viability rate was calculated (as a ratio of number of viable protoscolices to total protoscolices).

Plant Material

A plant taxonomist from the University of Basrah, Department of Biological Science verified the garlic, which had been obtained commercially. *Allium sativum* aqueous extract has been prepared by crushing the garlic then weighing 15g, joining it with 30 ml of distilled water, and finally struggled it through filter paper type Watman. The mixture was kept at 20°C till used as a stock solution (Amagase *et al.*, 2001) [4].

In vitro study

Allium sativum aqueous extract stock solutions were employed in concentrations of (50, 100, and 150) mg/ml. Sets of twelve tubes were done for the *in vitro* enquiry. Each tube held 1.0 ml of live protoscolices (about 3000 viable protoscolices). Each tube also held 1.0 ml of plant extract for each concentration. The tubes were gently combined and allowed to incubate at 27 C°. The control group had a protoscolices solution without any extract plant. For every experiment, the percentages of viable

protoscolices or survival were calculated after five,10 and 15 minutes.the number of viable protoscolices was counted using haemocytometer in a three replications' mean value served as the basis for the data.

GC-MS.

Using GC-MS to identify the bioactive substances that were found in the garlic aqueous extracts. Five grams of Iraqi garlic were crushed in a plastic filling and left for the indicated times. 10 ml of petroleum ether solvent were then added to the extract, and they were allowed to stand at room temperature for thirty minutes. Next, one ml of the Iraqi garlic extract was injected into a GC-MS (split at ratio of one and a half) capillary column (cross band 5% diphenyl-95% dimethylpolysiloxane) with a 30 ml (L)×0.32 (i.d.) and a 0.25µm film thickness; the injection temperature was 250 °C. Finally, one ml of the extract was injected into a capillary column in the Rtx-5MS capillary column (cross band 5% diphenyl-95% dimethylpolysiloxane); the recorded intervals ranged from 30 to 170 m/z. The separated peaks were found to match the spectra database in the package library NIST08.LIB.

Statistical Analysis

The data of the study were assessed using the Least Significant Differences (L.S.D.) test at the level of (P<0.05) to indicate the significance of the results.

Results

Macroscopically, liver containing viable hydatid cysts displayed irregularly shaped, fluid-filled, oval to spherical, gray-white patches that ranged in size from 1 to 10 cm (Fig 1). These areas held yellowish-white granules (hydatid sand).

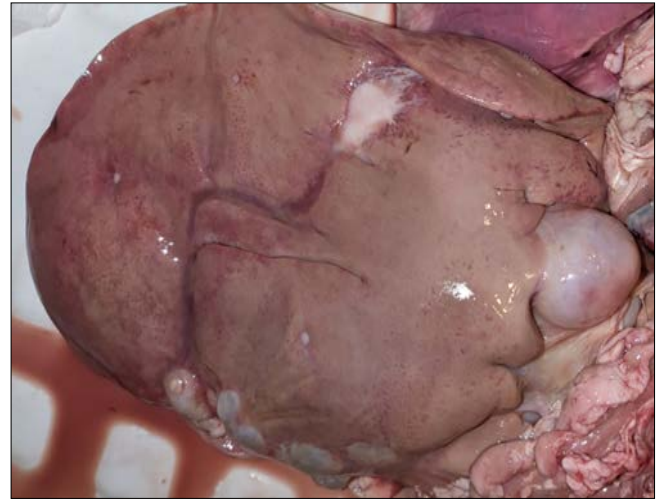


Fig 1: Fertile hydatid cyst-filled liver: a multitude of overlapping, pale, and projecting multifocal lesions are visible on the organ's exterior

The protoscolices treated with an extract of 50 mg/ml started to show a decline in viability 100% at zero concentration to 62.8,51.11, and 32.01% at time 5,10, and 15 minutes respectively and those have been treated with 100 mg \ ml showed a survival rate of 45.95,34.49, and 21.32% at 5, 10,15 minutes respectively. Finally It is clear from table (1) that the concentration with 150 mg/ml was the concentration that had the most impact on reducing the viability of the protoscolices showing a survival rate of 01.86,01.45 and 0.00% at 5,10, and 15 minutes respectively. The statistical analysis (L.S.D.) proved that there were a significant differences in P<0.05.

Table 1: Percentage of viability of *Echinococcus granulosus* protoscolices after being exposed to different concentrations of aqueous extract of *Allium sativum* for different time periods *in vitro*

| Concentration Mg/ml | Survival rate | | | Mean of survival rate in concentrations | Comparison groups |
|---|---------------|--------|--------|---|-------------------|
| | 5min | 10 min | 15 min | | |
| 0 | 100% | 100% | 100% | 100 | p>0.05 |
| 50 | 62.85% | 51.11% | 32.01% | 50.11 | p<0.05 |
| 100 | 45.95% | 34.49% | 21.32% | 33.90 | p<0.05 |
| 150 | 01.86% | 01.45% | 0.00% | 1.43 | p<0.05 |
| Mean of survival rate in exposure times | 61.47 | 52.20 | 52.20 | | |

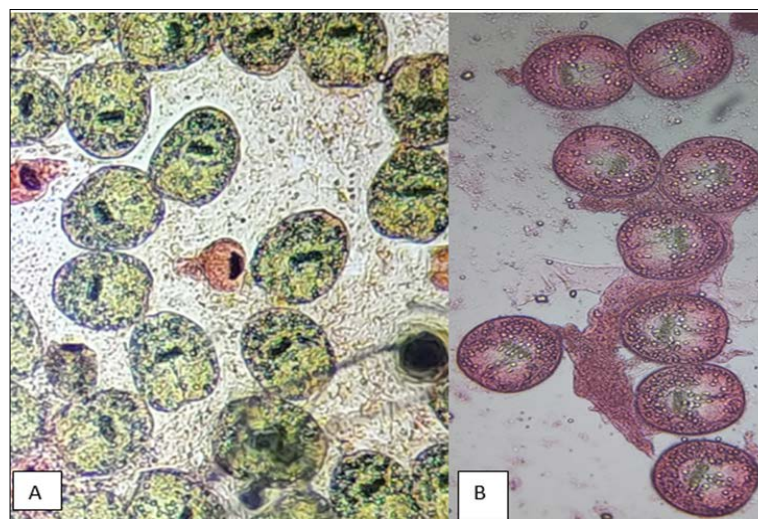


Fig 2: Microscopic images of *Echinococcus granulosus* Protoscolie, A-alive non-stained with 0.1% eosin stain (green colored), B- dead stained (red colored) after treatment with *Allium sativum* aqueous plant extract.

Diagnosing the bioactive Compounds of Allium sativum aqueous extract by GC-MS.

Results of the analysis of garlic by GC-MS have shown different bioactive compounds, as illustrated in fig 3.

| pk | Name | RT | Area | % | Formula |
|----|---|--------|----------|----------|------------|
| 1 | 3-Furanmethanol | 8.647 | 2243567 | 1.003507 | C5H6O2 |
| 2 | 1,1-Divinyl-1-silacyclobutane | 8.957 | 1027477 | 0.459572 | C7H12Si |
| 3 | endo-Borneol | 9.525 | 1164551 | 0.520883 | C10H18O |
| 4 | 2-Piperidinecarboxylic acid | 9.783 | 1791093 | 0.801123 | C6H11NO2 |
| 5 | 1-(3,3,3-Trifluoro-2-hydroxypropyl)piperidine | 10.063 | 1092211 | 0.488526 | C8H14F3NO |
| 6 | 2-Furancarboxaldehyde, 5-methyl- | 10.609 | 9684928 | 4.331892 | C6H6O2 |
| 7 | trans-3-Methyl-2-n-propylthiophane | 11 | 1036040 | 0.463402 | C8H16S |
| 8 | .epsilon.-N-Formyl-L-lysine | 11.398 | 983053 | 0.439702 | C7H14N2O3 |
| 9 | Benzaldehyde, 3-benzyloxy-2-fluoro-4-methoxy- | 12.151 | 1435521 | 0.642082 | C15H13FO3 |
| 10 | 2-Butene, 1,4-bis(ethylthio)-, (E)- | 12.49 | 740345 | 0.331143 | C8H16S2 |
| 11 | 1,2-Benzenediol, 3-fluoro- | 12.814 | 5616381 | 2.512105 | C6H5FO2 |
| 12 | 1,2-Benzenediol, 3-fluoro- | 12.94 | 3633258 | 1.62509 | C6H5FO2 |
| 13 | 4-Mercaptophenol | 13.441 | 525207 | 0.234916 | C6H6OS |
| 14 | 2-Azido-2,4,4,6,6-pentamethylheptane | 13.64 | 548866 | 0.245498 | C12H25N3 |
| 15 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 14.112 | 17351300 | 7.760921 | C6H8O4 |
| 16 | 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- | 14.732 | 2435483 | 1.089347 | C6H6O4 |
| 17 | .gamma.-Dodecalactone | 14.916 | 1321702 | 0.591173 | C12H22O2 |
| 18 | 4-Hexenal, 6-hydroxy-4-methyl-, dimethyl acetal, acetate, (Z)- | 15.197 | 1942930 | 0.869037 | C11H20O4 |
| 19 | 5-Hydroxymethylfurfural | 15.477 | 41084383 | 18.3763 | C6H6O3 |
| 20 | Melezitose | 16.318 | 564242 | 0.252375 | C18H32O16 |
| 21 | 2,2,6-Trimethyl-12-oxabicyclo[8.2.1]trideca-3,6,10(13)-triene-5,11-dione | 16.48 | 2408023 | 1.077065 | C15H18O3 |
| 22 | cis,trans-5,9-Cyclododecadiene-cis-1,2-diol | 17.018 | 1162848 | 0.520121 | C12H20O2 |
| 23 | Melezitose | 17.151 | 529250 | 0.236724 | C18H32O16 |
| 24 | Pyrrrolizin-1,7-dione-6-carboxylic acid, methyl(ester) | 17.468 | 506355 | 0.226483 | C9H11NO4 |
| 25 | trans-Traumatic acid | 18.213 | 1002221 | 0.448275 | C12H20O4 |
| 26 | (4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldecahydro-2H-benzo[d]oxecin-2-one | 19.12 | 542886 | 0.242823 | C14H24O4 |
| 27 | (4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldecahydro-2H-benzo[d]oxecin-2-one | 19.423 | 741850 | 0.331816 | C14H24O4 |
| 28 | (4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldecahydro-2H-benzo[d]oxecin-2-one | 19.865 | 627637 | 0.280731 | C14H24O4 |
| 29 | Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl- | 20.027 | 701897 | 0.313946 | C9H9F3O2 |
| 30 | (4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldecahydro-2H-benzo[d]oxecin-2-one | 20.182 | 605325 | 0.270751 | C14H24O4 |
| 31 | (4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldecahydro-2H-benzo[d]oxecin-2-one | 20.75 | 772139 | 0.345364 | C14H24O4 |
| 32 | Benzeneethanamine, 2-fluoro-4,5-dimethoxy- | 21.067 | 2661712 | 1.190535 | C10H14FNO2 |
| 33 | (4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldecahydro-2H-benzo[d]oxecin-2-one | 21.429 | 674895 | 0.301868 | C14H24O4 |
| 34 | 12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one | 21.65 | 682954 | 0.305473 | C14H24O3 |
| 35 | 12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one | 21.768 | 841811 | 0.376527 | C14H24O3 |
| 36 | Oleic Acid | 21.842 | 862976 | 0.385993 | C18H34O2 |
| 37 | (4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldecahydro-2H-benzo[d]oxecin-2-one | 22.1 | 1612036 | 0.721034 | C14H24O4 |
| 38 | Oleic Acid | 23.73 | 736236 | 0.329305 | C18H34O2 |
| 39 | n-Hexadecanoic acid | 23.929 | 9068211 | 4.056046 | C16H32O2 |
| 40 | Oleic Acid | 25.677 | 49971293 | 22.35125 | C18H34O2 |
| 41 | Oleic Acid | 25.824 | 4792548 | 2.14362 | C18H34O2 |
| 42 | Oleic Acid | 26.532 | 543767 | 0.243217 | C18H34O2 |
| 43 | Oleic Acid | 27.521 | 1203379 | 0.53825 | C18H34O2 |
| 44 | 9-Octadecenoic acid (Z)-, oxiranylmethyl ester | 28.421 | 674253 | 0.301581 | C21H38O3 |
| 45 | 2,3-Dihydroxypropyl elaidate | 29.918 | 1173122 | 0.524716 | C21H40O4 |
| 46 | 2,3-Dihydroxypropyl elaidate | 30.117 | 4173320 | 1.86665 | C21H40O4 |
| 47 | 9-Octadecenoic acid (Z)-, oxiranylmethyl ester | 33.576 | 878276 | 0.392837 | C21H38O3 |
| 48 | 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- | 36.275 | 1657846 | 0.741524 | C57H104O6 |
| 49 | 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- | 37.905 | 5246992 | 2.346884 | C57H104O6 |
| 50 | rac 1-Oleoyl-2-palmitoylglycerol | 43.282 | 26917402 | 12.03967 | C37H70O5 |
| 51 | 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester | 45.642 | 3376683 | 1.510329 | C21H40O4 |
| 52 | | | 2.24E+08 | 100 | |

Fig 3: GC-MS. of Allium sativum aqueous extract showing bioactive Compounds

Discussion

During hydatid cyst infections, surgery is the most used choice for handling a number of treatments that are being used in various degrees of success. However, the metabolites of certain medicines such as mebendazole, benzimidazole, albendazole sulfoxide, and albendazole are possibly lethal in some foci (Hayajneh *et al.*, 2014) [14]. However, the commercially used albendazole is poorly absorbed in the digestive tract, resulting in a low hepatic concentration, which lowers its efficiency because the liver

is most affected organ during infection by this parasite (Rokni, 2009). Uptake of albendazole may result in major side effects such as allergic purpura, influenza-like illness, encephalitis syndrome, drug rash. The treatment is, nonetheless, not successful. This results came in agreement with previous reports presented by Karakay (2007) [15]. In this study, we investigated the effect of garlic aqueous extract on the hydatid cyst protoscolices. Water was used as a solvent in the extraction process for two reasons: 1) it is a neutral liquid that does not negatively or positively affect

garlic bioactive compounds; and 2) it does not interfere with them, compared to organic solvents that may cause neither negative nor positive interference with these compounds (Al-Hilli, 2000^[2]. Alkaloids, phenols, and terpenes are among the substances found in plant extracts that are effective as bioactive materials because they block the respiratory process and interfere with the metabolism of carbohydrates (Delorenzi *et al.*, 2001)^[10]. Observing the results of the study, it became clear that the alkaloids have a strong and important effect on Tumbling the viability proportion of the protoscolices. This depends on the difference in concentration. These results are similar to that of Delorenzi *et al.*, (2001)^[10]. Furthermore, alkaloids could induce the damage of cell organelles and lead to the destruction of the cell nuclei and finally to death (Gidado *et al.*, 2007)^[13]. On the other hand phenols are active on the enzyme (acetylcholine esterase) that controls the cell membrane permeability and flexibility, they made the membrane lose its permeable feature's, yielding in loss of regulation and the entrance of various toxic component and finally the death of the parasite (Barzin *et al.*, (2019)^[7]. Phenols have the ability to denature proteins and altering enzymes actions that are responsible for a series of basic metabolic activities, thus causing the microorganism to lose its ability to continue living (Marizel *et al.*, 2012)^[18]. They have effect in reducing protoscolices vitality proportion. This is consistent with the result of (Zainab alaa *et al.*, 2024) which had a important consequence in dropping the viability of the acanthamoeba. This effect was depending on the period and concentration, as it led to a substantial reduction in the viability rate of the protoscolices. As for flavonoids, they work to reduce sugars, which leads to an imbalance in carbohydrate metabolism and thus a decrease of energy units (ATP) prepared for vital activities in the parasite (Sarkar *et al.*, 1996)^[20]. The effectiveness of flavonoids in the extract could be due to the presence of Quercetin, Kaempferol, and dihydroxyquercetin (Messaoudene *et al.*, 2011)^[19]. The process of inhibition done by alkaloid was clarified by the fact that they interfere in the series of protein metabolism reactions necessary for the continued vitality of the microorganism, and their ability to destroy the cell wall and the proteins and fats it contains, thus killing the parasite (Cowan, 1999)^[9]. The study concluded that high concentration of bioactive compounds play an important role in minimizing protoscolices viability rate compared to low concentrations. The results are also reliable with what Al-Azzawi, *et al.*, (1999)^[1] found, that when concentration increased, the reducing of parasite vitality will do so, The reason for this may be due to the plant extract bioactive compound content in a high level such as: terpenes, alkaloids, or it may be related to the high concentration providing more effect in killing the parasite comparing to a low concentration. Increasing the time period of exposure had an important role on the vitality of the protoscolices. This seems to be reliable with the results of Al-Omorani, (1995)^[3] who found out that the effect on the vitality of the protoscolices increases with an instant increase in the exposure period. This explanation might be related to the increase in the penetration of the active substances by penetrating the parasite's membranes and then destroying them, or lead to the weakening of the parasite (Carrique-Mas *et al.*, 2001)^[8]

Conclusions

The *in vitro* lethal effect of *Allium sativum* extract against *Echinococcus granulosus* protoscoleces was very valuable and could lead to the discovery of new medication that is safe for human, without any side effects. In addition, these protoscolices have also developed resistance to albendazole (Urrea-Paris *et al.*, 2000). For this reason, this study suggested that *Allium sativum* is a valuable medicinal therapy; its natural bioactive compounds work as an active agent in killing protoscolices.

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