



# Evaluation of the Effects of *Bunium persicum* and Albendazole on Killing Hydatid Cyst Protoscolices *In Vitro*



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## Abstract

**Objectives:** Hydatidosis is a parasitic disease of zoonoses and the best treatment for this parasitic disease is cryosurgery. From time immemorial, many protoscolice-destroying chemicals have been used to inhibit the distribution of protoscolices within the cyst during surgery. However, the side effects of chemical compounds and medicinal plants should always be considered. Due to the antiparasitic properties of *Bunium persicum* (Boiss), this study was performed to evaluate and compare the effects of *B. persicum* (Boiss) essential oil and albendazole on protoscolices inside hydatid cyst *in vitro*.

**Materials and Methods:** In this study, the essential oil used from the desired plant seeds was prepared. Gas chromatography-mass spectrometry was performed to determine its components. Hydatid cyst protoscolices were extracted from hydatid cyst-infected livers and exposed to *B. persicum* (Boiss) (2.5, 5, and 10 µg/mL) for 10, 30, 60, and 120 minutes, respectively. The viability of protoscolices was measured by the effect of plant essential oil by eosin staining. Albendazole was also used as an effective antiparasitic drug in this study.

**Results:** The amount of essential oil protoscolices at 2.5 µg/mL in 10, 30, 60, and 120 minutes was 48.5%, 68.2%, 79.5%, and 96.1%, respectively. The antiparasitic drug albendazole showed less toxic effects at similar doses at the same intervals, but both treatment groups had a significant effect ( $P < 0.001$ ). The highest toxic effects of essential oil were observed at a concentration of 10 µg/mL 30 minutes after treatment. For albendazole, this effect was achieved at a dose of 10 µg/mL after 2 hours.

**Conclusions:** The results and statistical data of this study showed that the essential oil of *B. persicum* (Boiss) in comparison with the antiparasitic drug albendazole has an acceptable scolicide effects in hydatidosis and can be used as an alternative to scolicide compound.

**Keywords:** Hydatid cyst, Effects, Albendazole, *Bunium persicum*, *In Vitro*

## Introduction

Hydatidosis is one of the most important and common parasitic diseases of zoonoses and livestock in all countries of the world and is caused by the larval stage of *Echinococcus* cestodes. This parasitic disease is more common in rural areas and livestock with a closer relationship between dogs, livestock and humans (1,2). Hydatidosis is considered a major public and economic health challenge by reducing livestock products, causing serious damage to human health and incurring medical costs, and the risk of death. In hydatid cyst surgery technique, if the necessary preventive measures are not observed, there is a risk of spreading of protoscolices and penetration into viscera and tissues during surgery, which is one of the main reasons for the recurrence of this parasitic disease and the formation of secondary cysts (3,4). In order to prevent widespread dissemination of cyst contents, effective scolicides is essential to reduce the rate

of disease recurrence. So far, many chemical scolicides have been used, such as 2% formalin, 20%-30% hypertonic saline, silver nitrate, and betadine (5). In some cases, the use of these chemical compounds leads to serious side effects such as bile duct fibrinization, liver necrosis and healthy tissues adjacent to the cyst. The antiparasitic drugs benzimidazoles are used to treat human hydatid cysts with varying degrees of therapeutic success. Mebendazole was one of the first of these compounds, but due to the low absorption of the drug, its serum and intravesical levels were lower and not enough antiparasitic drug penetrated into the hydatid cysts (6-9). Albendazole is a newer, broader spectrum antiparasitic drug that is more effective than mebendazole in treating human hydatid cysts. An alternative treatment for human hydatid cyst could be the formulation of new compounds that kill protoscolices using compounds derived from medicinal plants, including essential oils extracted from them (10, 11). Very

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## Key Messages

- ▶ Hydatidosis is caused by the larval form of tapeworms of the genus *Echinococcus*.
- ▶ *Bunium persicum* (Boiss) essential oil has an acceptable scolicidal effect compared to albendazole.

few studies have been performed on the potential use of essential oils that kill hydatid cyst protoscolices (12,13). In this study, the seeds of *Bunium persicum* were collected in June 2019 from the wild plants, which found in the many regions of Iran. Numerous studies have also shown the antiparasitic activity of this aromatic plant against various species of parasites, for example; arthropods and protozoa (4,6). The present study investigated the scolicidal activity of essential oil of *B. persicum* oil on human hydatid cyst protoscolices in vitro conditions.

## Material and Methods

### Plant Material

To start the practical work of this study, first the *B. persicum* plant was collected from different regions of Iran and after determining and confirming the plant species, the plant essential oil was extracted by water distillation. To prepare different dilutions of *B. persicum*, essential oils were dissolved as a solvent in olive oil (14,15). Essential oil components were identified using mass spectrum and gas chromatography-mass spectrometry system (16).

### How to Prepare Protoscolices From Hydatid Cyst

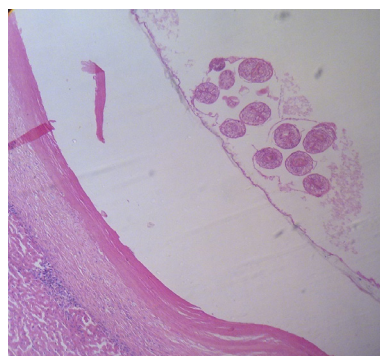
For this purpose, a number of sheep liver infected with hydatid cyst were prepared from the slaughterhouse and transferred to a parasitology laboratory. After disinfecting the surface of the liver, the contents of the cysts, including the protoscolices of cestode *Echinococcus granulosus* and hydatid fluid, were drained into a sterile container. Then, we waited a few minutes for the protoscolices to settle.

### Protoscolex Viability Test

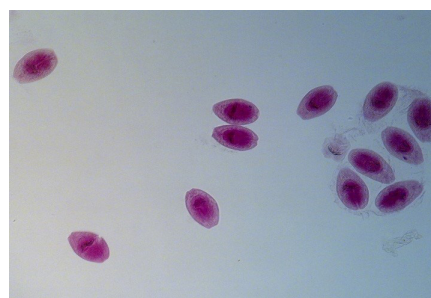
To estimate the viability of protoscolices, some of the liquid containing protoscolices was poured into a glass slide by pasteurization pipette and the same volume of 0.1% eosin dye solution was placed next to the sample. Then gently place the lamellae on the prepared solution containing the dye. After 10 minutes, they were evaluated by counting the number of live protoscolices (Figures 1 and 2).

### Investigation of Scolicidal Activity in Laboratory

In our study, the killing effects of 3 dilutions of 25 and 50 and 100 mg/mL of essential oil of *B. persicum* plant at 10, 30, 60, and 120 minutes on hydatid cyst proto-scolexes was evaluated. To prepare the dilutions of 25 and 50 and 100 mg/mL of essential oil of *B. persicum* in 9.7 mL of normal saline with 0.3 mL, Twin 80 was dissolved separately in each tube. After 10 minutes, the ratio of dead to live protoscolices was measured on the entire lamellar surface



**Figure 1.** Unilocular Hydatid Cyst With protoscolices in Liver (H&E Staining, ×400).



**Figure 2.** Microscopic Images of Eosin-Stained (Dead) Protoscolices After Exposing to Plants Products (×400).

using a light microscope (4,6). The scolicidal effects of essential oil were studied and compared with albendazole at similar doses.

## Results

The gas chromatography-mass spectrometry analysis results showed that in the first period of 10 minutes, the lowest dose of *B. persicum* essential oil (2.5 µg/mL) resulted in almost half (48.5%) of protoscolices. Albendazole with the same dose and in the same period showed a less lethal effect (22.3%) but compared to the control (0%), both treatments showed a significant effect ( $P=0.021$ ). With increasing dose, the lethal activity increased so that at a dose of 5 mg/mL of essential oil of *B. persicum* killed 96.1% and albendazole killed 51.1% of protoscolices ( $P<0.001$ ).

## Discussion

The present study results showed that *B. persicum* essential oil has an acceptable scolicidal effect compared to the standard drug albendazole. Some studies showed the toxicity of essential *Artemisia sieberi* oil against protozoa *Trichomonas gallinae* in vitro and in vitro conditions. They reported a concentration of 10 µg/mL of essential oil of *A. sieberi* in the culture medium as the minimum inhibitory concentration for 24 hours (17). Other studies showed that the *Artemisia* plant at a concentration of 0.001 at the beginning of cultivation has a complete effect on *Trichomonas vaginalis* (18). In the present study,

the toxicity of *B. persicum* essential oil on hydatid cyst protoscolices was shown. The results of other researchers were positive for evaluating the protoscolicidal effect of thymol on hydatid cysts, and thymol had a lethal effect on *Echinococcus granulosus* protoscolices. Still, this killing took longer than usual (19). In another study the effect of *B. persicum* extract on hydatid cyst protoscoleces during surgery showed that at a dose of 25 µg/mL in 100 minutes, 100% of protoscolices were killed (20).

Some other researches showed that the methanolic extract of ginger has a very good effect on hydatid cyst protoscoleces. Still, methanolic extract of *Artemisia aucheri* did not have a good scolical effect on protoscoleces of hydatid cysts. However, the present study showed an acceptable effect of *B. persicum* essential oil on the killing property of scolex of hydatid cyst protoscoleces. The difference in the findings of another studies is probably due to the differences in the plant species studied and the constituent chemicals (21). researchers in their study of the simultaneous effect of albendazole and thymol against hydatid cyst protoscoleces, showed a positive therapeutic effect and described the synergistic effect of this drug combination (22).

Our study showed that *B. persicum* essential oil could be considered as a suitable scolicide, and after passing hepatotoxicity and biliary system tests if it is safe to eliminate and inhibit protoscoleces be used. Further studies are needed to investigate the scolicide components of essential *B. persicum* oil, their mechanism of action, and the possible synergistic activity of essential *B. persicum* oil or its components with the standard drug albendazole (23). Due to the natural origin of this treatment and the extent of growth of this plant in Iran, further studies will provide the use of this substance during hydatid cyst surgery alone or with standard medicine.

## Conclusion

The results of our study and those of other researchers showed that medicinal plants and their derivatives have many antiparasitic effects, and that these chemical compounds have the power to inactivate the protoscolices of cestode *E. granulosus*. It should be noted that there is still disagreement among various scientists about the mechanism of action of these plant extracts in killing protoscolices and more research is needed in this regard. Another very important issue about the extracts of medicinal plants is the degree of toxicity and their destructive effects on the cells of the human body, which is still a lot of research.

## Authors' Contribution

YG presented the study proposal and wrote and edited the manuscript, AKH, FS and MH collaborated in gathering the required information, and finally PB statistically analyzed and edited the final version of the manuscript.

## Conflict of Interests

The authors declare that they have no conflict of interest.

## Ethical Issues

The ethics committee of the Islamic Azad University, Tabriz Branch reviewed and confirmed the results of this experimental parasitology study in all research stages. (No. 396127).

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