



# Scolicidal Effects of Barberry (*Berberis vulgaris*), Wild Rue Seed (*Peganom harmala*) and Shirazian Thyme (*Zataria multiflora*) Extracts on Protoscolices of Hydatid Cysts

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## Abstract

**Background:** Surgery is one of the most common strategies to treat hydatidosis reserved as a complementary treatment with chemical agents. Due to low efficacy and the adverse effects of these chemical agents, it is necessary to develop a new and effective scolicidal agent to prevent the recurrence of hydatidosis after surgery.

**Objectives:** The current study aimed at investigating the scolicidal effects of *Berberis vulgaris*, *Peganom harmala*, and *Zataria multiflora* methanolic extracts on protoscolices of hydatid cyst.

**Methods:** Protoscolices were aseptically obtained from sheep livers infected with hydatid cyst in Hamadan slaughterhouse (Hamadan, Iran). After methanol extraction of herbs, different concentrations of extracts were used in various exposure time for mortality rate assay of protoscolices in laboratory.

**Results:** The obtained results showed that various concentrations of *B. vulgaris* and *Z. multiflora* extracts could kill 100% of protoscolices after five minutes of exposure and the mean of mortality rate of protoscolices was remarkably higher in the experiment group comparison with that of the control group ( $P < 0.05$ ). However, the current study findings showed that *P. harmala* extract at a higher concentration (100 mg/mL) killed 14%, 12%, 12.67%, 15.67%, and 17% of the protoscolices after 5, 10, 15, 20 and 40 minutes of exposure, respectively. There was no significant difference between the experiment and control groups in terms of the mean of mortality rate ( $P > 0.05$ ).

**Conclusions:** The current study findings showed that *B. vulgaris* and *Z. multiflora* extracts can be natural sources to develop new scolicidal agents used in hydatid cyst surgery to prevent recurrence of the disease. However, the in vitro examination revealed that the *P. harmala* extract had no strong scolicidal activities.

**Keywords:** Hydatid Cyst, Scolicidal, *Berberis vulgaris*, *Peganom harmala*, *Zataria multiflora*

## 1. Background

Hydatidosis caused by a cosmopolitan parasitic tapeworm *Echinococcus granulosus*, is one of the serious parasitic infections in human. Infection with *E. granulosus* indicates a major public health problem with worldwide distribution. Humans are infected with this tapeworm as an intermediate host by ingesting the eggs existing in the feces of dog through eating the contaminated vegetables and other foods (1). The ingested eggs contain an oncosphere released in the intestine and after penetrating the intestinal mucosa are disseminated in the liver, lungs, spleen, heart, or other organs via the portal system (2).

Currently, surgery is the preferred treatment world-

wide, including Iran (3). However, one of the most important concerns in the hydatid cyst surgery is the recurrence of the disease caused by the incomplete removal of the germinal layer from the cyst cavity and spillage of the cyst contents that happens in 10% of the surgery cases. In such patients, it seems obligatory to use the most effective scolicidal agents (4, 5).

Inactivation of the cyst contents was performed using the common scolicidal agents such as silver nitrate, cetrimide, hypertonic saline, and ethanol, but these agents present dangerous side effects including necrosis in liver and methemoglobinaemia (6). Therefore, it is necessary to develop new scolicidal agents with low side effects and

more efficacies to apply in surgery cases.

Herbal drugs can be useful therapeutic agents in medicine due to their astonishing structural diversity, low side effects, and the presence of unlimited sources (7). Previous studies revealed that *Berberis vulgaris*, *P. harmala*, and *Z. multiflora* when used as a medicine for parasitic diseases have a remarkable therapeutic effect on healing of the disease (8, 9).

*P. harmala* is a perennial plant that widely grows in most parts of the world and commonly grows in Eastern Mediterranean. Also, it has dietary and medicinal properties and is used as analgesic and antiseptic in folk medicine (10). Previous studies reported that the extract of *P. harmala* seeds had antiseptic properties that can kill fungi, bacteria, *Plasmodium*, and *Leishmania* (11).

*B. vulgaris*, known with different names and called “Z-ereshk” in Persian, has biochemical and pharmacological activities related to its leaf, root, and fruit. Recently, scientific research results demonstrated that *B. vulgaris* and its related components possess antifungal, antiprotozoal, and antibacterial properties; also according to recent studies in Iran, anti-candida and anti-leishmania activities of the *B. vulgaris* extract are proved (5, 12).

*Z. multiflora* commonly grows in Iran and is used as a flavor agent in many foods (13). It is greatly used for medicinal and condimental purposes in some countries. Similar to the two herbal drugs mentioned above, *Z. multiflora* also has antifungal, antimicrobial, antibacterial, and anti-leishmanial properties (14-17). Aqueous and alcoholic extracts of *Z. multiflora* are medicinally used for alleviating nociceptive pain (18).

## 2. Objectives

Due to the presented activities of these herbal medicines and regarding the above-mentioned points about hydatidosis, the current study aimed at investigating the scolicidal activity of the methanol extracts of *B. vulgaris*, *P. harmala*, and *Z. multiflora*.

## 3. Methods

Liver sheep samples naturally infected with hydatid cyst were collected from Hamadan slaughterhouse in Iran. The separation of the protoscolices was performed by settling down the livers for 30 minutes at the bottom of the cylinders after aseptically conveying the hydatid fluid into glass cylinders. After removing the supernatant, the yielded protoscolices were washed three times with normal saline. The staining test with eosin 0.1% was performed to evaluate the primary viability of protoscolices. After

exposure to the stain, the mortality rate of protoscolices was measured; the dead protoscolices were stained and appeared to be red, while the live ones appeared clear. The samples with more than 95% live protoscolices were transferred into a dark container and stored at 4°C in normal saline solution for further use.

The aerial parts of *Z. multiflora*, as well as the seeds of *P. harmala* and *B. vulgaris* were provided from the garden of Bu-Ali-Sina University of Hamadan, Iran. The plants species were identified and authenticated by a botanist at the Botany Department of Bu-Ali-Sina University, Hamadan, Iran.

Plants were dried in an air conditioned dark place and then 200 g of each dried plant was separately extracted by percolation method with 80% methanol for 72 hours in room temperature. Plants debris was removed by passing the extracts through a filter paper (Whatman No. 3, Sigma, Germany), then solvent removing of filtered solutions were performed using distillation unit in vacuum. Finally, the concentrated extracts were stored at -20°C until testing (19).

In the current study, protoscolices exposure was performed using three concentrations of each herbal extract (25, 50, and 100 mg/mL) at five different times (5, 10, 15, 20 and 40 minutes). After preparing the above concentrations, to investigate the scolicidal effects of each extract; one drop of the sediment containing at least 1000 protoscolices was inoculated into 2 mL of the herb extract in a small test tube and then was mixed gently. After mixing, the tubes were incubated at 37°C for 5, 10, 15, 20 and 40 minutes. At the end of each incubation time, the supernatant was carefully removed not to disturb the protoscolices and the remains were washed three times using normal saline and then stained with eosin 0.1% (1 L of distilled water + 1 g of eosin). After adding eosin, the mixture was incubated for 15 minutes and then the supernatant was carefully discarded; and the stained protoscolices were smeared on a manually scaled glass slide. The glass slide was covered with a cover slide and examined with light microscope to determine the mortality rate. A minimum of 100 protoscolices were counted to calculate the mortality rate of parasite in each concentration and each exposure time as well. The control group only received normal saline and all experiments in the experiment group were performed in triplicate to increase the accuracy.

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### 3.1. Statistical Analysis

All the tests were performed in triplicate for each concentration and time of exposure to increase the accuracy. The mean  $\pm$  standard deviation (SD) of mortality rate of

protoscolices for triplicate tests was calculated and then, the mean scolical activity for different concentrations and different exposure time were compared. The statistical analysis was performed with SPSS version 16. Differences between the experiment and control groups were determined with One-way ANOVA, and independent-samples *t* test was used for further analysis (20). In the current study,  $P < 0.05$  was considered statistically significant.

## 4. Results

### 4.1. The Scolical Effects of *B. vulgaris* Methanol Extract

Table 1 illustrates the scolical activity of *B. vulgaris* methanol extract at different exposure time following several concentrations. As shown in Table 1, the results indicated that *B. vulgaris* methanol extract had high scolical activity and could be effective to kill the hydatid cyst protoscolices at all three concentrations. The exposures of protoscolices to *B. vulgaris* extract at three concentrations (25, 50, and 100 mg/mL) caused 100% mortality in protoscolices after 5, 10, 15, 20, and 40 minutes. The study findings also revealed that *B. vulgaris* extract at all concentrations had remarkable ( $P < 0.05$ ) scolical effects compared with the control group.

### 4.2. The Scolical Effect of *Z. multiflora* Methanol Extract

The results of the scolical activity at three different concentrations (25, 50, and 100 mg/mL) of *Z. multiflora* methanol extract are shown in Table 2. All concentrations of the extracts were highly effective. The results of the current study revealed that the *Z. multiflora* extract had 100% mortality at all three concentrations after five minutes. In various exposure time and three concentrations of the extract, the mortality rate of protoscolices significantly elevated compared with that of the control group ( $P < 0.05$ ).

### 4.3. The Scolical Effect of *P. harmala* Methanolic Extract

The mortality rate of protoscolices of hydatid cysts after exposure to different concentrations (25, 50, and 100 mg/mL) of *P. harmala* extract in different exposure times (5, 10, 15, 20, and 40 minutes) are presented in Table 3. These findings revealed that the exposure of protoscolices to the *P. harmala* extract at the concentration of 25 mg/mL caused the mortality rate of protoscolices to 6%, 6%, 7%, 11.3%, and 14.33% after 5, 10, 15, 20, and 40 minutes, respectively. Exposure of the protoscolices of hydatid cysts to 50 mg/mL of *P. harmala* extract killed 9%, 9%, 10%, 14%, and 16.67% of the protoscolices; and at the concentration of 100 mg/mL killed 14%, 12%, 12.67%, 15.67%, and 17% of the protoscolices after 5, 10, 15, 20, and 40 minutes of incubation, respectively.

Further analysis and comparison of the results of the mortality rate in all concentrations at various time with negative control revealed no significant differences between the two studied groups ( $P > 0.05$ ).

## 5. Discussion

Hydatidosis caused by the tapeworm *E. granulosus* is categorized as a helminthic infection with worldwide distribution. Currently, treatment with surgery is still the most effective method to treat hydatidosis. Despite many surgical advances to treat hydatidosis; leakage of protoscolices may occur during surgical operation that leads to relapse of the disease in patients (4). Previous studies reported that the recurrence rate of hydatidosis is about 10% - 30% after surgical operation of hepatic hydatid disease (8). Therefore, the employment of effective protoscolical agents to prevent secondary hydatid disease is crucial. In various studies, scolical activity of some substances such as silver nitrate (21) and mannitol (6), 95% ethyl alcohol (22), selenium nanoparticles (23), chlorhexidine gluconate (24), and some plant extracts are confirmed (19, 25, 26). However, these protoscolical agents associated with high toxicity and their efficacy is controversial; therefore, the development of new protoscolical agents with high efficacy and low adverse effects especially from natural sources with high accessibility and low cost is of great interest.

In the past decades, herbal extracts and plant-derived compounds were used as herbal remedies to treat a variety of complaints (27). The studies focused on herbal medicine and components of plants due to possessing the above-mentioned properties; thus, the current study aimed at investigating the scolical effects of methanol extract of three plants including *B. vulgaris*, *Z. multiflora*, and *P. harmala* at the concentrations of 25, 50, and 100 mg/mL after 5, 10, 15, 20, and 40 minutes on an in vitro model.

The study findings revealed that *B. vulgaris* methanol extract had significant scolical activity at 25, 50, and 100 mg/mL concentrations after different exposure time, it was observed that methanol extract of *B. vulgaris* at 25, 50 and 100 mg/mL concentrations had scolical activity of 100% after five minutes. The mortality rate of 100% after exposure to methanol extract of *B. vulgaris* showed that this plant had high scolical activity compared with those of the existing scolical agents mentioned above. In accordance with the current study results, Mahmoudvand et al. (25) reported that *B. vulgaris* methanol extract killed 100% of the protoscolices in a dose dependent manner. Also, Rouhani et al. (8) demonstrated that the scolical activity of *B. vulgaris* aqueous extracts was very high in a low concentration (4 mg/mL) after five minutes of exposure. On

**Table 1.** Scolicidal Effects of *B. vulgaris* Extract at Various Concentrations and Exposure Times<sup>a</sup>

Concentration, mg/mL	Exposure Time <sup>b</sup> , Min				
	5	10	15	20	40
25 <sup>c</sup>	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
50 <sup>c</sup>	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
100 <sup>c</sup>	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
Normal saline	3.67 ± 0.57	5.67 ± 1.15	7.00 ± 1.00	8.00 ± 1.00	9.67 ± 0.57

<sup>a</sup> Results are expressed as mean ± SD (n = 3).<sup>b</sup> Mean of mortality rate (%).<sup>c</sup> The mortality rate is significant (P < 0.05) compared with that of the control group.**Table 2.** Scolicidal Effects of *Z. multiflora* Methanol Extract on Protoscolices of Cyst at Various Concentrations and Exposure Times<sup>a</sup>

Concentration, mg/mL	Exposure Time <sup>b</sup> , Min				
	5	10	15	20	40
25 <sup>c</sup>	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
50 <sup>c</sup>	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
100 <sup>c</sup>	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
Normal saline	4.33 ± 0.57	6.00 ± 1.00	7.33 ± 0.57	9.33 ± 0.57	10.67 ± 0.57

<sup>a</sup> Results are expressed as mean ± SD (n = 3).<sup>b</sup> Mean of mortality rate (%).<sup>c</sup> The mortality rate is significant (P < 0.05) compared with that of the control group.**Table 3.** Scolicidal Effects of *P. harmala* Extract on Protoscolices of Cyst at Various Concentrations and Exposure Times<sup>a</sup>

Concentration, mg/mL	Exposure Time <sup>b</sup> , Min				
	5	10	15	20	40
25 <sup>c</sup>	6.00 ± 1.00	5.67 ± 0.57	7.00 ± 1.00	11.33 ± 1.15	14.33 ± 1.15
50 <sup>c</sup>	9.00 ± 1.00	9.00 ± 1.00	10.00 ± 1.00	14.00 ± 1.15	16.67 ± 0.57
100 <sup>c</sup>	10.00 ± 3.46	12.00 ± 1.00	12.67 ± 0.57	15.67 ± 1.15	17.00 ± 4.58
Normal saline	4.33 ± 0.57	5.67 ± 0.57	6.67 ± 0.58	8.67 ± 0.58	10.00 ± 1.00

<sup>a</sup> Results are expressed as mean ± SD (n = 3).<sup>b</sup> Mean of mortality rate (%).<sup>c</sup> The mortality rate is significant (P < 0.05) compared with that of the control group.

the other hand, previous study results revealed that this herbal medicine was used in clinics and had no toxicity or adverse effects (28). Therefore, with regards to the results of the current study, the extract of *B. vulgaris* could be a natural source to develop a new scolicidal agent for application to hydatid cyst surgery. However, further studies are needed to explain the exact mechanism of scolicidal activity of *B. vulgaris*, since it is unclear (5). In addition, more studies are needed to effectively report the optimal dose of the extract.

The result of the current study also revealed that the *Z. multiflora* methanol extract had strong scolicidal effect in vitro examination when using three concentrations of 25, 50, and 100 mg/mL after various exposure times. The current study findings showed that *Z. multiflora* extract killed

100% of protoscolices existing within the first five minutes of the examination and the mortality rate was significantly high compared with that of the control group (treated with normal saline). Nowadays, an ideal scolicidal agent was introduced with high efficacy in a shorter exposure time, potency at lower doses, higher availability, lower adverse effects, stability in the presence of cystic fluid, and scolicidal ability inside a cyst (19). Recent studies introduced the *Z. multiflora* methanol extract containing thymol, rosmarinic acid, and carvacrol as the potential agent with high scolicidal activity due to both thymol and rosmarinic acid extracted only into methanol (18). *Z. multiflora* as an herbal medicine is used in traditional medicine to treat various diseases (29). Jahanbakhsh et al. (19) showed that *Z. multiflora* extract, at various concentrations, killed

100% of protoscolices after 10 minutes of exposure. Results of another study by Moazeni et al. (26) revealed that the mortality rate of protoscolices was higher compared with that of the control group.

Nowadays, due to the recurrence rate of hydatidosis after surgery and the requirement for new, safe, and more effective scolicidal agents, and regarding to the current study results, it is suggested that *Z. multiflora* could be an effective scolicidal agent during surgery.

The current in vitro study examined the scolicidal activity of *P. harmala* methanol extract. For this purpose, three concentrations (25, 50, and 100 mg/mL) of *P. harmala* extract were used to expose to protoscolices in different intervals. Unlike the two methanolic extracts mentioned above, *P. harmala* extract was not an effective scolicidal agent and after various exposure times no significant effect compared with the control group was observed ( $P > 0.05$ ). However, increasing the exposure time led to higher mortality rate, but the increase was not remarkable (compared with the control group). Recent studies revealed that *P. harmala* contains bioactive natural components such as catechin, rutin, and p-coumaric acid (11). Biological activity of *P. harmala* may be due to such components. Antioxidant, antidiabetic, antiviral, and antimicrobial activities of *P. harmala* are determined in different recent studies (11). Nematollahi and Ghazi (30) reported that *P. harmala* extract had no significant scolicidal activity that was in accordance with the current study finding. Yones et al. also reported that the concentration of 2500  $\mu\text{g/mL}$  of *Thymus vulgaris* and *Salvia officinalis* alcoholic extracts had a significant protoscolicidal activity against *E. granulosus* protoscolices in vitro (20).

### 5.1. Conclusion

The current study findings suggested that methanol extracts of *Z. multiflora* and *B. vulgaris* due to their rapid and strong scolicidal activities were the likely sources of new compounds that could be used as the effective scolicidal agents. Further studies are needed to determine the main active compounds of these herbal medicines. In contrast to *Z. multiflora* and *B. vulgaris*, the *P. harmala* extract had no significant scolicidal activity at the examined concentrations.

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### Footnotes

**Authors' Contribution:** Roya Sohrabi: performing the experiments in research laboratory, Monir Taheri Moghadam: collecting the materials from slaughterhouse and preparing the parasite, Amirhossein Maghsood: performing laboratory works, Mohammad Matini: performing laboratory works to prepare the extracts, Shirin Moradkhani: supervising the herbal extract preparation, Mohammad Fallah: taking grant and supervising all steps of the research.

**Conflict of Interests:** The authors declared no conflict of interests.

**Ethical Considerations:** This research was done in the laboratory on the non-human materials and no need for Ethics Committee approval.

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