Iranian Journal of Veterinary Science and Technology

Received: 2024- Sep-23
Accepted after revision: 2024- Nov-30
Published online: 2024-Nov-31

RESEARCH ARTICLE

DOI: 10.22067/ijvst.2024.86720.1347

# A Green Way to Combat *Echinococcus granulosus*: Exploring the Scolicidal Effects of *Lycopus europaeus* and *Lythrum salicaria* Extracts

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## ABSTRACT

This study assessed the scolicidal activity of Lythrum *salicaria* and *Lycopus europaeus* methanolic extracts on the protoscoleces of *Echinococcus granulosus* using ultrasound-assisted extraction. Protoscoleces were obtained from sheep livers and lungs and were exposed to extracts at concentrations of 125, 250, and 500 mg/mL for 1, 10, 20, and 30 minutes. Over the exposure period, both plant extracts demonstrated progressively stronger scolicidal activity at all tested doses. At the lower concentrations of 125 and 250 mg/mL, *L. salicaria* generally exhibited a higher protoscolicidal effect compared to *L. europaeus*. However, the difference in efficacy was more pronounced at 125 mg/mL. On the other hand, at the 500 mg/mL concentration, the *L. europaeus* extract showed considerably greater scolicidal activity than the *L. salicaria* extract. Statistical analysis revealed that concentration had the biggest impact on mortality, followed by plant species and exposure time. The interaction between concentration and plant type impacted mortality the most, indicating that both factors influenced the overall effectiveness. In conclusion, both *L. salicaria* and *L. europaeus* methanolic extracts showed promise as potential candidates for future studies aimed at developing natural agents to control *E. granulosus*.

## Keywords

*Echinococcus granulosus, Hydatidosis, Methanolic extract, Medicinal plants, Protoscolex*

Number of Figures: 2
Number of Tables: 2
Number of References:: 43
Number of Pages: 10

## Abbreviations

E. granulosus: Echinococcus granulosus
L. salicaria: Lythrum salicaria
L. europaeus: Lycopus europaeus
PAIR: Puncture, Aspiration, Injection, Reaspiration



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## Introduction

Hydatidosis, or echinococcosis, is a zoonotic parasitic infection caused by the metacestode stage of the tapeworm *E. granulosus*. In the life cycle of the parasite, adult cestodes reside in the intestines of carnivores from the Canidae family, releasing eggs through the feces of these definitive hosts. Intermediate hosts, including humans and herbivorous mammals, may inadvertently ingest these eggs through contact with contaminated food, water, or soil, leading to the development of the larval stage (metacestode) within their tissues. The larval stage results in hydatid cyst formation, which can have severe health consequences and may be fatal if left untreated [1-5].

Despite the range of treatment options available, surgery remains the preferred approach for managing human hydatidosis, especially in cases involving large, infected cysts or those located in critical organs. Surgical intervention provides a direct method for cyst removal, reducing the risk of complications and recurrence associated with untreated cysts. However, alternative therapeutic methods, such as PAIR and chemotherapy, are recommended for patients with multiple cysts affecting several organs or in cases where cysts are deemed inoperable. The PAIR technique, a minimally invasive approach, is effective in reducing cyst size and rupture risk, while chemotherapy with agents, such as albendazole and mebendazole, targets parasite viability, complementing other interventions for non-surgical cases or to prevent recurrence [6]. The use of effective scolicidal agents during surgical intervention is crucial to mitigate the risk of secondary infection caused by the accidental release of protoscoleces from hydatid cysts. An ideal scolicidal compound should possess high parasiticidal efficacy while minimizing potential adverse effects on the host tissue. A variety of compounds have been explored for their scolicidal properties, including formalin, 10% polyvinylpyrrolidone-iodine, 3% hydrogen peroxide, mannitol, 20% silver nitrate, 95% ethyl alcohol, hypertonic glucose, a combination of 1.5% cetrimide with 0.15% chlorhexidine, and 20% saline solution. Each of these agents offers varying degrees of effectiveness and safety, providing options tailored to specific surgical contexts in hydatidosis treatment [7-14]. While many scolicidal agents have demonstrated effectiveness, several compounds, including ethanol (70%–90%), cetrimide (0.5%), silver nitrate, and hypertonic saline (15%–20%), have been discontinued in clinical practice due to toxicity concerns and undesirable side effects [7-9, 12, 14].

The limitations and safety issues associated with these conventional agents highlight the urgent need for alternative scolicidal solutions. Herbal extracts have emerged as promising candidates, offering potential advantages, such as cost-effectiveness, accessibility, and a lower incidence of adverse effects. Therefore, expanding research into plant-based scolicidal agents represents a crucial step toward developing safer and more effective treatments, ultimately reducing the disease burden on both human and animal health.

*L. salicaria*, commonly referred to as purple loosestrife, is a perennial herbaceous plant of the Lythraceae family, native to regions across Europe, North Africa, and Asia. In Iran, multiple Lythrum species grow abundantly, particularly in wetland areas adjacent to streams. L. salicaria has a long history of use in traditional medicine, primarily for managing gastrointestinal disorders, such as diarrhea and dysentery. Its extract is also utilized topically to treat inflammatory conditions, such as eye inflammation, sinusitis, varicose veins, hemorrhoids, menorrhagia, hemorrhages, leucorrhoea, and ulcers. Beyond these applications, L. salicaria has been recognized for its effectiveness in urogenital inflammation, rheumatism, rabies, fever, benign prostatic hyperplasia, pruritus, dermatitis, and eczema. The phytochemical profile of *L. salicaria* reveals a rich array of bioactive compounds, predominantly polyphenolic constituents, such as tannins, flavonoids, anthocyanins, catechins, phenolic acids, and coumarins. In addition, various secondary metabolites have been identified, including steroids, triterpenes, phthalates, and alkaloids. This diverse phytochemical composition supports the plant's wide-ranging therapeutic applications, highlighting its potential as a valuable source of natural compounds for medicinal use [15-17].

*L. europaeus*, commonly known as bugleweed, gypsywort, bitter bugle, or water horehound, is an herbaceous perennial plant in the Lamiaceae family, native to Europe and Western Asia, including Iran. Traditionally, *L. europaeus* has been employed in herbal medicine for mild hypothyroidism and alleviating minor nervous disorders. Recent studies have further substantiated its medicinal potential, highlighting analgesic, antitussive, and anti-inflammatory properties, alongside its anti-thyrotropic and anti-gonadotropic effects. Additional pharmacological benefits include cardiotonic, antioxidant, and antimicrobial activities, which underscore its therapeutic versatility. Although the health benefits of *L. europaeus* are attributed to its complex phytochemical profile, not all bioactive constituents have been fully elucidated. Phytochemical investigations have identified a range of active compounds, including terpenoids, various phenolic compounds (such as phenolic acids, flavonoids, coumarins, and tannins), as well as alkaloids, glycosides, saponins, and sterols. These constituents collectively contribute to the plant's pharmacological effects, supporting its traditional and contemporary medicinal

applications [18-25].

To date, the protoscolicidal potential of extracts from *L. salicaria* and *L. europaeus* has not been investigated. This study aimed to evaluate whether extracts from these plants exhibit protoscolicidal effects against *E. granulosus* protoscoleces in an in vitro setting, with the objective of identifying novel natural protoscolicidal agents. If effective protoscolicidal activity be demonstrated, subsequent studies would focus on isolating and characterizing active constituents, elucidating the mechanisms of action and exploring structure-activity relationships to inform further development. Promising in vitro findings would also support the need for in vivo efficacy and safety assessments, essential for future consideration in clinical applications.

## Result

The protoscolicidal activity of *L. salicaria* and *L. europaeus* extracts was evaluated against *E. granulosus* protoscoleces at the concentrations of 125, 250, and 500 mg/mL, with exposure times ranging from 1 to 30 minutes (Table 1). Statistical analysis demonstrated significant scolicidal activity for both plant extracts ($p < 0.01$), with mortality rates exhibiting dose- and time-dependent characteristics.

At 125 mg/mL, both extracts exhibited protoscolicidal activity, with *L. salicaria* consistently outperforming *L. europaeus* at all the time points. After 1 min, *L. salicaria* demonstrated 83.87% activity compared to 55.82% for *L. europaeus*. At 5 min, the activity of *L. salicaria* increased slightly to 84.49%, while *L. europaeus* improved to 59.84%. By 10 min, the activity of *L. salicaria* rose to 85.5%, significantly higher than the 63.47% observed for *L. europaeus.* This trend persisted at 20 min, with *L. salicaria* reaching 88.73% activity versus 64.43% for *L. europaeus.* After 30 min, *L. salicaria* retained superior activity at 89.68%, compared to 69.14% for *L. europaeus.*

Both *L. salicaria* and *L. europaeus* displayed protoscolicidal activity at the concentration of 250 mg/mL, but *L. salicaria* was more effective in killing protoscolices compared to *L. europaeus*. Following a 1-min exposure, *L. salicaria* exhibited an 85.87% protoscolicidal activity, while *L. europaeus* showed a lower efficacy of 69.1%. The difference in efficacy between the two plants became less prominent with increasing the exposure time. After 5 min of exposure, *L. salicaria had* a protoscolicidal activity of 87.24%, whereas *L. europaeus* had a lower efficacy of 83.27%. After 10 min of exposure, *L. salicaria* showed a protoscolicidal activity of 90.44%, which was significantly higher than *L. europaeus* with 87.41% protoscolicidal activity. Similarly, after 20 min of exposure, *L. salicaria* had a protoscolicidal activity of 93.68%, while *L. europaeus* showed 88.95% protoscolicidal activity. After a 30-min exposure period, *L. europaeus* demonstrated a protoscolicidal activity of 95.98%, while *L. salicaria* showed 95.16% activity. Despite the absence of statistical significance in the protoscolicidal activity difference between *L. salicaria* and *L. europaeus* at this concentration and exposure time, the result contradicts previous findings at lower concentrations and exposure times where *L. salicaria* exhibited greater effectiveness.

At 250 mg/mL, the efficacy of both extracts improved, though *L. salicaria* continued to show higher protoscolicidal activity at shorter exposure times. After 1 min, *L. salicaria* demonstrated 85.87% activity,

**Table 1.**
Protoscolicidal effect of the methanolic extract of *Lythrum salicaria* and *Lycopus europaeus* at the different concentrations following various exposure times

| | | Protoscolicidal activity (%) | | | | |
|---|---|---|---|---|---|---|
| Plant | Concentration (mg/ml) | 1 min | 5 min | 10 min | 20 min | 30 min |
| *L. salicaria* | 125 | 83.87jk | 84.49j | 85.5i | 88.73g | 89.68fg |
| | 250 | 85.87i | 87.24h | 90.44f | 93.68e | 95.16d |
| | 500 | 90.35f | 93.5e | 96.86bc | 100a | 100a |
| *L. europaeus* | 125 | 55.82o | 59.84n | 63.47m | 64.43m | 69.14l |
| | 250 | 69.1l | 83.27k | 87.41h | 88.95g | 95.98cd |
| | 500 | 95.35d | 97.45b | 100a | 100a | 100a |

Values with the same letter have no significant difference.

while *L. europaeus* exhibited 69.1%. The efficacy gap narrowed with longer exposure, with the activities of 87.24% for *L. salicaria* and 83.27% for *L. europaeus* at 5 min. By 10 min, *L. salicaria* reached 90.44% activity, slightly exceeding the 87.41% observed for *L. europaeus*. After 20 min, the activities increased to 93.68% for *L. salicaria* and 88.95% for *L. europaeus*. At 30 min, *L. europaeus* surpassed *L. salicaria,* with the activities of 95.98% and 95.16%, respectively. This reversal suggests that relative efficacies may shift at higher concentrations and longer exposure times.

At 500 mg/mL, *L. europaeus* displayed greater protoscolicidal activity, reversing the trend seen at lower concentrations. After 1 min, *L. europaeus* achieved 95.35% activity compared to 90.35% for *L. salicaria*. By 5 min, *L. europaeus* improved to 97.45%, while *L. salicaria* reached 93.5%. At 10 min, *L. europaeus* achieved 100% activity, whereas *L. salicaria* lagged at 96.86%, only reaching 100% activity after 20 min of exposure.

Table 2 highlights that extract concentration, plant type, and exposure time significantly influenced protoscoleces mortality, both individually and through interactions. Among these, concentration had the largest mean square value, making it the most critical factor, followed by plant type and exposure time. The two-way interaction between concentration and plant type produced the highest mean square value among the interactions, indicating that the efficacy of the extracts was strongly influenced by their concentration and botanical source. Interactions between concentration and time, and plant type and time, were also significant, albeit with smaller mean square values. The three-way interaction had the smallest mean square value but was still highly significant, reflecting a combined effect of all three factors.

**Table 2.**
Analysis of variance for the factors influencing protoscoleces mortality of Echinococcus granulosus, including the concentration of the extract, plant type, and exposure time individually and through interactions

| Effect | DF | MS | Sign. F |
|---|---|---|---|
| *Plant* | 1 | 1826.82256 | ** |
| *Concentration* | 2 | 3949.18783 | ** |
| *Time* | 4 | 366.226815 | ** |
| *Plant × Concentration* | 2 | 1367.98903 | ** |
| *Plant × Time* | 4 | 31.587735 | ** |
| *Concentration × Time* | 8 | 27.90843 | ** |
| *Plant × Concentration × Time* | 8 | 27.320355 | ** |
| *Residual* | 60 | 0.208333333 | |
| *Total* | 89 | 162.9973569 | |

**: $P < 0.01$

## Discussion

Over the past few decades, there has been significant interest in researching natural scolicidal compounds with favorable safety profiles and without adverse effects. This interest stems from the necessity for effective and safer alternatives to conventional scolicidal agents in treating parasitic infections. For instance, Moazeni and Nazer (2010) evaluated the protoscolicidal efficacy of methanolic extract derived from *Allium sativum*. Their results showed that at the concentrations of 25 and 50 mg/mL, the extract completely killed protoscoleces after 60 and 10 min of application, respectively. Similarly, Moazeni *et al.* (2012) investigated the scolicidal effects of *Rhus coriaria* and *Zataria multiflora* methanolic extracts at varying concentrations and exposure times. Another study by Zibaei *et al.* (2012) reported that the hydroalcoholic extract of *Satureja khuzestanica* leaves demonstrated greater scolicidal activity than the aqueous extract of *Olea europaea* leaves. Furthermore, Taran *et al.* (2013) examined the scolicidal activity of *Hymenocarter longiflorus* methanolic extract against the metacestode of *E. granulosus* and found it to be a potent scolicidal agent. Baqer *et al.* (2014) investigated the scolicidal effect of *Zingiber officinale* ethanolic extract and observed that the concentrations of 50, 100, and 150 mg/mL resulted in the complete killing of protoscoleces after 120, 90, and 60 min, respectively. Moreover, Mahmoudvand *et al.* (2014) demonstrated that the methanolic root extract of *Berberis vulgaris* at the concentrations of 2 and 5 mg/mL killed all protoscoleces after 10 min of exposure. However, the scolicidal effect of the methanolic extracts of *Ocimum bacilicum* and *Allium cepa* was found to be insufficient in a study

by Haghani (2014). Abdel-Baki et al. (2016) investigated the scolicidal activity of *Salvadora persica* root extract and reported the highest scolicidal effect at the concentrations of 30 mg/mL after 30 min, and 50 mg/mL after 20 and 30 min of exposure, respectively [26-34].

Screening the medicinal plants with established antimicrobial properties represents a logical approach to discovering novel natural scolicidal agents. Previous research has validated the antimicrobial efficacy of *L. salicaria* and *L. europaeus* extracts [16, 21, 22, 25], though their potential scolicidal activity had yet to be investigated. Therefore, this study aimed to assess the in vitro protoscolicidal potential of methanolic extracts from *L. salicaria* and *L. europaeus* against *E. granulosus* protoscoleces.

It has been shown that the scolicidal effect of herbal extracts is both dose- and time-dependent. Increasing the concentration of the extracts while maintaining a constant incubation time, has been reported to increase the mortality rate of protoscoleces. Similarly, prolonged exposure to each concentration has resulted in a significant increase in the scolicidal activity of the extracts. As a result, it can be deduced that raising the extract concentration along with prolonging the application time will result in more potent scolicidal effects.

The difference in efficacy between the two plant extracts was particularly pronounced during shorter exposure durations, specifically at 1 and 5 min. However, this distinction became less significant as the exposure time increased to 20 and 30 min. Furthermore, when tested at a concentration of 125 mg/mL, both plant extracts demonstrated reduced scolicidal activity. Conversely, at higher concentrations of 250 and 500 mg/mL, both extracts exhibited more potent scolicidal activity.

According to the findings, compounds in the *L. salicaria* extract likely exhibit more rapid onset of action against the parasite as well as a more potent overall effect, achieving markedly higher protoscolicidal activity than *L. europaeus* at a concentration of 125 mg/mL and all exposure times tested. In contrast, *L. europaeus* showed a faster initial rate of action but consistently lower activity at all time points, indicating weaker protoscolicidal effects at this concentration. At the concentration of 250 mg/mL, the differences in protoscolicidal activity between the two extracts were less pronounced but *L. salicaria* still demonstrated consistently stronger effects at all time points tested except after 30 min, where *L. europaeus* exhibited 95.98% activity compared to 95.16% for *L. salicaria*. These findings indicated that at a concentration of 250 mg/mL and longer exposure times, *L. europaeus* can achieve comparable or slightly higher protoscolicidal activity compared to *L. salicaria*, as the differences in activity between the two extracts were diminished at 250 mg/mL after 30 min. Interestingly, the relative efficacies of the two extracts reversed at 500 mg/mL, and *L. europaeus* demonstrated significantly higher protoscolicidal activity than L. salicaria, achieving near-complete inhibition more rapidly. This result suggests that compounds in *L. europaeus* may act faster against the parasite and/or exhibit greater overall potency at a concentration of 500 mg/mL.

In this experiment, the concentration of the extract emerged as the most influential factor in determining mortality rates, with plant type and exposure time following in importance. The dose-response profiles of the two plant extracts were significantly different, with *L. salicaria* demonstrating more potent effects at lower concentrations and *L. europaeus* displaying greater activity at higher concentrations. Notably, *L. salicaria* exhibited time-independent kinetics, reaching maximum activity within similar timeframes across all tested concentrations, in contrast to *L. europaeus*. These findings highlight the importance of optimizing the dosage and duration of exposure to the herbal extracts when considering their potential use as scolicidal agents. The underlying reasons for these varying concentration-effect relationships remain incompletely understood and necessitate further investigation into the specific active components present in each extract.

In traditional medicine, it is a common practice to use the mixtures or extracts of plants as herbal medicines instead of isolated compounds. This approach is based on the understanding that plant

extracts contain a complex mixture of bioactive molecules that may act synergistically to produce more potent therapeutic effects than those obtained using isolated compounds alone. Furthermore, using plant extracts rather than isolates can mitigate the risk of drug resistance because multiple bioactive agents act simultaneously and target different pathogenic mechanisms. Therefore, it is not surprising that the methanolic extracts of *L. salicaria* and *L. europaeus* demonstrated strong scolicidal activity as the extract contains a diverse mixture of active components that may collectively disrupt the viability and integrity of the parasite (Lila, 2014; Rasoanaivo et al., 2011).

The active components found in *L. salicaria* and *L. europaeus* extracts, particularly phenolics, and terpenes, have been extensively studied and shown to possess antibacterial, antifungal, and antiparasitic properties. For example, luteolin-7-O-glucuronide and rosmarinic acid have all demonstrated antibacterial activity in several investigations [25, 35-37]. On the other hand, certain terpenes, such as eugenol, α-terpinene, terpinolene, caryophyllene, and α-pinene have all exhibited potent protoscolicidal activity [33, 38, 39]. These bioactive molecules can interfere with the growth, replication, or metabolic activity of microorganisms or parasites, leading to their destruction or inhibition. Therefore, it is likely that the presence of these compounds in *L. salicaria* and *L. europaeus* extracts contributes to their observed protoscolicidal effects [40, 41].

While further research is necessary to identify and isolate the bioactive components responsible for the observed scolicidal activity, the results of this study suggest that these two plants possess potent scolicidal properties. Consequently, they have the potential to be utilized in the development of new scolicidal agents for application in hydatid cyst surgery or the PAIR technique. Further research is required to elucidate the mechanisms underlying this activity and identify the specific active components responsible.

Conclusion

This study demonstrated that the methanolic extracts of *L. salicaria* and *L. europaeus* exhibited significant protoscolicidal activity against *E. granulosus* protoscoleces in a dose- and time-dependent manner. At lower concentrations, *L. salicaria* consistently showed higher efficacy, while at higher concentrations, *L. europaeus* surpassed *L. salicaria* in activity. The interaction between concentration and plant type significantly influenced the mortality rate, with concentration emerging as the most critical determinant.

These findings highlight the potential of both plant extracts as natural scolicidal agents, offering promising alternatives to conventional chemical treatments for hydatid disease. Further investigation into their active components and in vivo applications is essential for their potential clinical use.

## Materials and Methods

### *Collection of protoscoleces*

Sheep livers and lungs containing hydatid cysts were collected from a slaughterhouse (Amol abattoir) and were brought to the Parasitology Lab at the Veterinary School of Amol University of Special Modern Technologies. The surface of the hydatid cysts was washed with sterile 0.9% NaCl solution (normal saline), and the protoscoleces-containing hydatid fluid was aspirated using a 50 mL syringe and was placed into a sterile conical urine glass. After settling for 30 min, the protoscoleces sank to the bottom. The supernatant was carefully discarded, and the protoscoleces were twice washed with normal saline. The vitality of the harvested protoscoleces was checked under an ordinary light microscope, and the number of protoscoleces was adjusted to $3\times10^3$ in 1 mL of normal saline with a minimum viability rate of 95%. The protoscoleces were subsequently transferred to a 50 mL Falcon centrifuge tube containing sterile 0.9% NaCl solution and stored at 4°C for future use [32].

### *Viability test*

To measure the percentage of viable protoscoleces, the eosin exclusion test was employed. To measure the percentage of viable protoscoleces, the eosin exclusion test was employed. Briefly, the eosin exclusion test was conducted by mixing equal parts of 0.1% (W/V) eosin stain solution and protoscolex. Dead protoscoleces absorbed the stain and turned red after approximately 10 min, while live protoscoleces remained colorless (Figures 1 and 2). Viability of the protoscoleces was additionally confirmed through observing the flame cell activity and body movement [42, 43].

### *Preparation of plant extracts*

In 2022, the aerial parts of *L. salicaria* and *L. europaeus* were collected from Amol and transported to the Faculty of Medicinal Plants at Amol University of Special Modern Technologies. The plant materials were washed, air-dried, and homogenized into a uniform particle size at room temperature before extraction. The samples were then suspended in 80% methanol (v/v) and vortexed for 5 min. Next, the mixtures were placed in an ultrasonic bath (Elmasonic S40H, 340 W, 37 kHz) at 30°C and sonicated for 1 h. Finally, the extracts were dried using a vacuum rotary evaporator in a water bath set at 40°C. The resulting dried samples

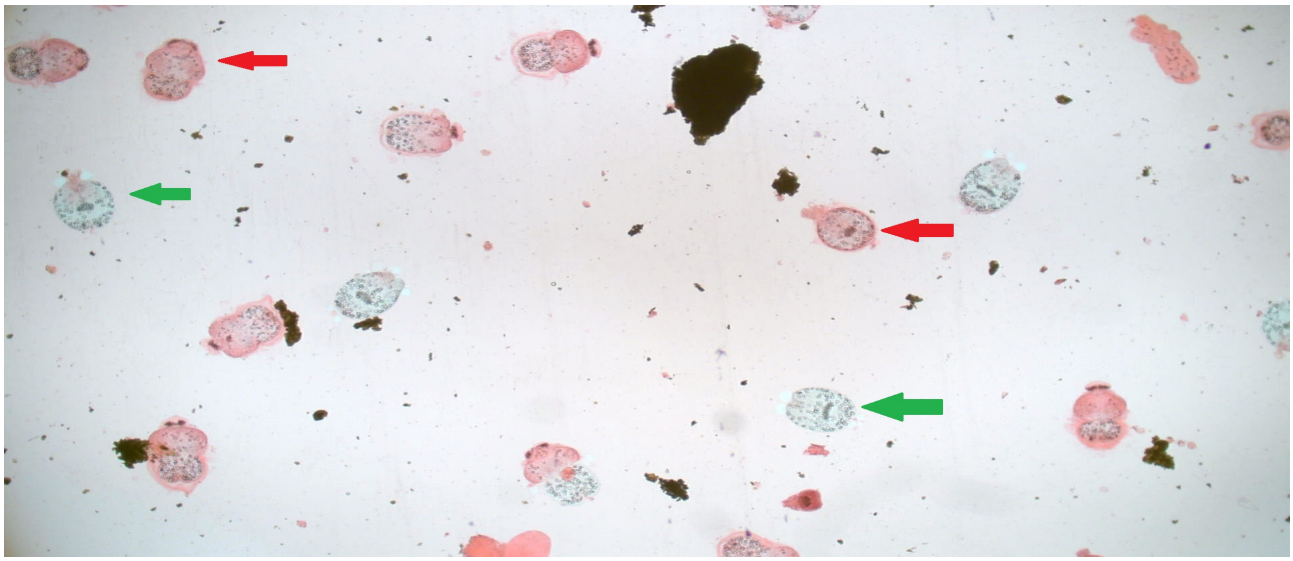

**Figure 1.**
A comparison of live and dead protoscolex stained with 0.1% eosin. The green arrow points to a live protoscolex, while the red arrow indicates a dead one.

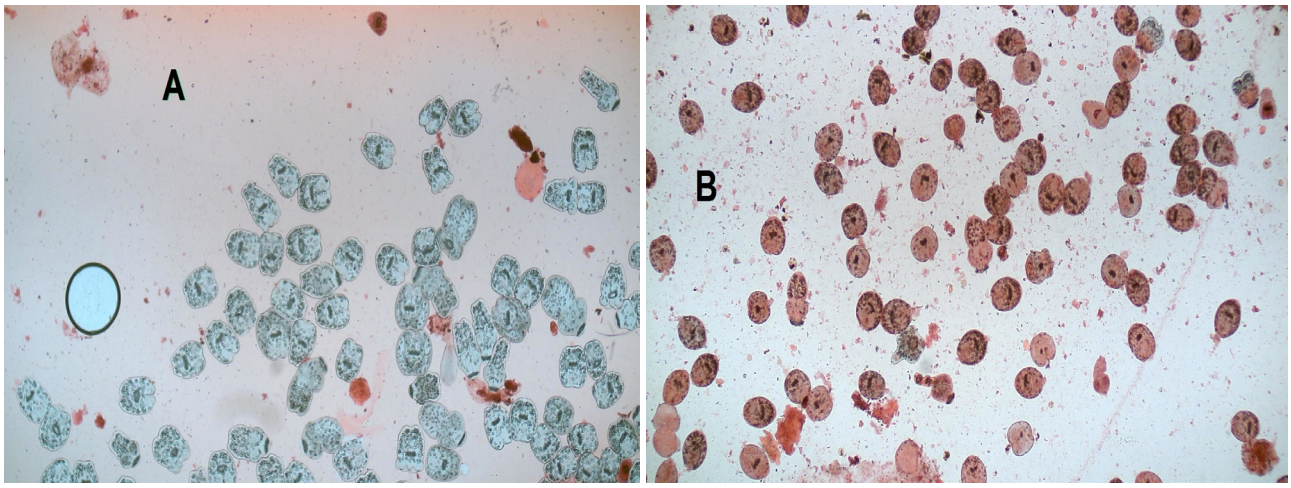


**Figure 2.**
In the negative control group, untreated living protoscoleces were stained with 0.1% eosin (A). In the positive control group, dye penetration was observed in dead protoscoleces after staining with 0.1% eosin (B).

were weighed, transferred into microtubes, and kept at 4°C until use [42, 43].

### *Scolicidal activity*

To determine the protoscolicidal activity of the methanolic extracts of *L. salicaria* and *L. europaeus*, three concentrations (125, 250, and 500 mg/mL) were tested. In each experiment, 1 mL of each concentration was placed in test tubes, and 1 ml of the protoscolex mixture was added to the tubes and gently mixed. The tubes were then incubated at 37°C for 1, 5, 10, 20, and 30 min. Following incubation, the upper part of the solution was removed with a pipette, and the sediment was washed twice with 2 mL of normal saline. Next, the supernatant was discarded, and the sediment was treated with 2 mL of 0.1% eosin stain solution. After 10 min, one drop of the sedimented protoscoleces was smeared on a glass slide, covered with a cover glass, and examined under a light microscope. The protoscolicidal effect of each treatment was determined by counting 500 protoscoleces. In addition, hypertonic and normal saline solutions were used as positive and negative controls, respectively (Figure 2).

### *Statistical analysis*

All experiments were performed in triplicates, and the data were analyzed using the analysis of variance (ANOVA). Significant differences between the means at $p < 0.01$ were determined by the Duncan's test using SPSS software version 16 (SPSS Inc., USA).

## Authors' Contributions

A.N. and M.R. conceived and planned the experiments. A.N. carried out the experiments. M.K. contributed to sample preparation. M.R. contributed to the interpretation of the results. M.K. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

## Acknowledgements

This research was supported by a research grant from the Amol University of Special Modern Technologies, Amol, Iran.

## Competing Interests

The authors declare that there is no conflict of interest.

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**How to cite this article**
Nikpay A, Kiani M, Ranjbar M. A Green Way to Combat *Echinococcus granulosus:* Exploring the Scolicidal Effects of *Lycopus europaeus* and *Lythrum salicaria* Extracts. Iran J Vet Sci Technol. 2024; 16(4): 49-58.
DOI: https://doi.org/**10**10.22067/ijvst.2024.86720.1347
URL:https://ijvst.um.ac.ir/article_46106.html