



Original Article

# *In Vitro* Scolicidal Effects of *Artemisia Dracunculus* Hydroalcoholic Extract Against *Echinococcus Granulosus* Protoscoleces

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**Abstract**

**Introduction:** Cystic echinococcosis is a significant zoonotic infection that poses a substantial public health and economic burden worldwide. While surgical intervention is the primary treatment modality, intraoperative leakage of viable protoscoleces remains a leading cause of recurrence, occurring in approximately 10% of cases. Traditional chemical scolicides, though effective, are frequently associated with serious side effects, including sclerosing cholangitis and liver tissue damage. Consequently, this study aimed to evaluate the *in vitro* scolicidal efficacy of the purified organic extract of *Artemisia dracunculus* (Tarragon) against the protoscoleces of *Echinococcus granulosus*.

**Methods:** Protoscoleces were aseptically aspirated from naturally infected sheep livers obtained from a local slaughterhouse. Moreover, the scolicidal activity of the purified organic extract was tested at four concentrations (0.1%, 0.01%, 0.001%, and 0.0001%) over different exposure durations (5, 10, 15, 20, 25, and 30 minutes). Viability was also determined using the 0.1% eosin exclusion test, where dead protoscoleces absorbed the dye and appeared red under light microscopy. Silver nitrate (AgNO<sub>3</sub>) and normal saline served as positive and negative controls, respectively.

**Results:** The findings revealed a pronounced time- and dose-dependent scolicidal effect. The 0.1% concentration of the *A. dracunculus* extract was the most potent, achieving 100% mortality of protoscoleces at the 30-minute interval. Conversely, the 0.01% concentration reached only 40% mortality within the same duration. Although the positive control (AgNO<sub>3</sub>) showed a more rapid onset of action, reaching 100% lethality at nearly all tested points, the plant extract demonstrated significant inhibitory potential at its highest tested dose.

**Conclusion:** *A. dracunculus* extract exhibits potent *in vitro* scolicidal properties. While further research is required to standardize its bioactive components and assess *in vivo* safety, this extract represents a promising biological alternative for reducing surgical recurrence in hydatid disease.

**Keywords:** *Echinococcus granulosus*, *Artemisia dracunculus*, *In vitro*, Hydatid cyst



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## Introduction

Cystic echinococcosis (CE), commonly known as hydatid disease, is a neglected zoonotic infection caused by the larval stage of the tapeworm *Echinococcus granulosus*. This disease poses a significant public health challenge and an economic burden globally, particularly in endemic regions such as the Mediterranean, South America, and the Middle East, including Iran (1,2). Humans serve as accidental intermediate hosts for this infection, acquiring it through the ingestion of parasite eggs found in contaminated food or water or through direct contact with infected definitive hosts, primarily dogs (3). Moreover,

the clinical manifestation of CE is characterized by the slow development of cysts, predominantly in the liver (approximately 70%) and lungs (20%), although other organs, such as the spleen, kidneys, heart, and brain, may be affected as well (4). While many infections remain asymptomatic for years, the progressive enlargement of cysts can lead to compression of adjacent tissues and organ dysfunction. The rupture of the cysts, whether traumatic or spontaneous, is a critical complication that can result in anaphylactic shock or the dissemination of protoscoleces, resulting in secondary echinococcosis (5,6). Currently, surgery remains the gold standard for the treatment of



uncomplicated cysts, particularly when they are large or at risk of rupture. However, the intraoperative spillage of viable protoscoleces into the peritoneal cavity is a major cause of recurrence, observed in nearly 10% of surgical cases (2,7). To mitigate this risk, surgeons employ the puncture, aspiration, injection, and re-aspiration technique or open surgery alongside the instillation of scolical agents into the cyst cavity. Commonly used chemical scolical agents include hypertonic saline, silver nitrate ( $\text{AgNO}_3$ ), cetrimide, and formalin. Despite their efficacy, these agents are associated with severe and occasionally life-threatening side effects, such as sclerosing cholangitis and liver necrosis, particularly if there is a communication between the cyst and the biliary tract (8,9). Given the limitations and toxicity of synthetic scolical agents, there has been a paradigm shift toward exploring natural therapeutics. Specifically, medicinal plants have been utilized for millennia in traditional medicine, offering a promising reservoir for novel bioactive compounds with potentially fewer side effects, higher availability, and cost-effectiveness (10,11). In recent years, numerous studies have investigated the efficacy of various plant extracts against *E. granulosus* protoscoleces, aiming to identify safe and potent alternatives to conventional chemicals (12,13). *Artemisia dracunculus* L., commonly known as Tarragon, is a perennial herb belonging to the Asteraceae family. It is widely cultivated for culinary purposes and has a long history of use in traditional medicine for treating gastrointestinal disorders, inflammation, and infections (14). Likewise, phytochemical analyses have revealed that *A. dracunculus* is rich in essential oils, coumarins, flavonoids, and phenolic acids, with estragole and methyl eugenol being major constituents (15). Additionally, previous pharmacological studies have demonstrated its broad spectrum of biological activities, including antibacterial, antifungal, anti-inflammatory, and immunomodulatory effects (16,17). Furthermore, extracts from this plant have shown antiparasitic potential against other protozoa, such as *Leishmania major* (18,19). Despite the documented antimicrobial properties of *A. dracunculus*, its specific effect on the protoscoleces of *E. granulosus* has not been fully elucidated yet. Considering the urgent need for safer scolical agents and the promising bioactivity of the *Artemisia* genus, this study aims to evaluate the *in vitro* scolical effect of the purified organic extract of *A. dracunculus* on hydatid cyst protoscoleces. The findings of this research could contribute to the development of novel, plant-based adjuncts for the surgical management of CE.

## Materials and Methods

### Study Design and Plant Material Preparation

This experimental *in vitro* study was conducted to evaluate the scolical effects of *A. dracunculus*. To this end, fresh aerial parts of the plant (Tarragon) were collected from cultivated fields in South Khorasan Province, Iran. The plant material was taxonomically confirmed by a botanist at the Herbarium of Birjand University of Medical

Sciences. The leaves were separated, air-dried in the shade at room temperature, and then ground into a fine powder. In addition, an organic solvent fractionation and maceration workflow was employed to isolate distinct bioactive fractions. Further, 500 g of the powdered leaves was immersed in acetone, and the mixture was subjected to vigorous stirring for 10 hours. It should be noted that this initial maceration process was repeated twice to maximize the extraction of lipophilic and intermediate-polarity components. Next, the accumulated acetone fractions were filtered and subsequently concentrated under reduced pressure using a rotary evaporator at a controlled temperature of 40°C until a completely dry crude residue was obtained. Moreover, the resulting dry crude powder was sequentially washed with dichloromethane and methanol to further isolate and purify the targeted phytochemical fractions. This washing procedure selectively partitioned the target bioactive compounds from the dense matrix. Likewise, the final solvent-purified organic extract was subjected to evaporation under a vacuum in order to completely clear any residual volatile solvents, yielding a final dry extract mass of 42.5 g. This resulted in a total extraction yield of 8.5% w/w calculated relative to the primary dry plant biomass weight. The finalized, solvent-free purified extract was stored at 4°C under dark, airtight conditions until it was systematically reconstituted for the *in vitro* bioassays.

### Preparation of *Echinococcus Granulosus* Protoscoleces

Hydatid cysts were obtained from the livers of naturally infected sheep slaughtered at the industrial slaughterhouse in Birjand, Iran. The infected organs were immediately transported to the Parasitology Laboratory of the Faculty of Medicine, Birjand University of Medical Sciences. Next, cyst fluid containing protoscoleces was aseptically aspirated from a total of 5 independent cysts collected from 2 infected animals, using a sterile syringe after surface sterilization with 70% ethanol. The aspirated fluid was then transferred to sterile test tubes and allowed to settle by gravity. Subsequently, the supernatant was discarded, and the protoscolex-rich sediment was washed three times with sterile normal saline in order to remove host tissue debris. Afterward, the viability of the protoscoleces was assessed prior to each experiment using the standard 0.1% eosin exclusion test (20,21), in which dead protoscoleces with compromised membrane integrity absorb the dye and appear red under light microscopy. However, viable protoscoleces exclude the dye and remain translucent. Only protoscolex preparations with a viability rate exceeding 90% were used in the experiments.

### Preparation of Concentrations

All experimental treatment concentrations were prepared on a strict weight-to-volume (w/v) basis to achieve rigorous mathematical consistency and ensure absolute reproducibility. A master stock solution of 0.1% (w/v) was freshly prepared by precisely weighing 10 mg of the completely dried purified *A. dracunculus* extract powder

on a calibrated analytical balance and dissolving it in a total volume of 10 mL of sterile distilled water supplemented with Tween 20 to reach a final emulsifier concentration of 5% (v/v). This 0.1% (w/v) stock solution was directly used as the highest tested concentration, and serial ten-fold dilutions were subsequently prepared using distilled water containing the same 5% (v/v) Tween 20 matrix to obtain the lower experimental concentrations of 0.01%, 0.001%, and 0.0001%.

### ***In Vitro* Scolicidal Assay**

The scolicidal activity was evaluated using a standard *in vitro* incubation protocol adapted from previously published methods (20). In each experiment, 100  $\mu$ L of protoscoleces sediment (containing a minimum of 100 protoscoleces) was combined with 1,000  $\mu$ L of each prepared extract concentration (0.1%, 0.01%, 0.001%, and 0.0001%). Then, the mixtures were incubated at  $25 \pm 2^\circ\text{C}$  for predetermined intervals of 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, and 30 minutes. At the end of each incubation period, viability was assessed by the eosin exclusion method; a drop of 0.1% eosin solution (1 g eosin powder per 1,000 mL distilled water) was applied to the sample on a glass slide. After one minute of contact, the samples were examined under a light microscope. Subsequently, dead protoscoleces, identified by red eosin uptake, and viable protoscoleces, identified by dye exclusion and active flame cell motility, were counted. The percentage mortality was calculated as: Mortality (%) = (number of dead protoscoleces/total protoscoleces counted)  $\times$  100. All experiments were performed in triplicate.

### **Experimental Control Groups**

Three control groups were included to validate the experimental results:

- **Positive Control:** A 1% (w/v) aqueous stock solution of  $\text{AgNO}_3$  was prepared to serve as a baseline for maximum parasitic mortality. This master solution was sequentially diluted ten-fold in sterile distilled water to yield concentration-matched working solutions ranging from 0.1% to 0.0001%. These chemical controls were evaluated under identical temporal intervals as the test groups.
- **Negative Control:** Sterile normal saline (0.9% NaCl) was utilized parallel to the experimental groups to establish the natural baseline survival parameters and monitor any spontaneous viability loss of the protoscoleces over the maximum 30-minute processing window.
- **Vehicle Control:** A dedicated baseline control comprising solely distilled water and 5% (v/v) Tween 20 completely absent of any plant extract fractions was concurrently evaluated across all designated exposure intervals (5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, and 30 minutes). This group was explicitly integrated into the assay design to isolate and rule out independent surfactant-

mediated membrane permeabilization, thereby confirming that the emulsifier matrix exerts no confounding cytotoxic or scolicidal effects on the isolated protoscoleces.

### **Ethical Considerations and Statistical Analysis**

The study protocol was approved by the Ethics Committee of Birjand University of Medical Sciences (ethical code: IR.BUMS.REC.1401.446). All experiments were performed in triplicate to ensure reproducibility, and statistical processing was performed utilizing IBM SPSS statistics software (version 22). Quantitative data defining the mortality rates of *E. granulosus* protoscoleces were formally modeled and expressed as means  $\pm$  standard deviations (SD). A two-way analysis of variance (ANOVA) was executed to evaluate the independent and interactive contributions of the primary experimental variables (i.e., extract concentration and exposure duration). Following the determination of significant main effects, Tukey's honestly significant difference post-hoc multiple comparison test was applied to resolve individual pairwise statistical variations between specific treatment arms and control groups across each individual temporal interval. Finally, differences were defined as statistically significant at a threshold of  $P < 0.05$ .

### **Results**

#### ***Scolicidal Efficacy of Artemisia Dracunculus***

Two-way ANOVA revealed highly significant main effects for both treatment concentration ( $F_{9,120} = 4128.64$ ,  $P < 0.0001$ ) and exposure duration ( $F_{5,120} = 1542.18$ ,  $P < 0.0001$ ), as well as a statistically marked interaction between concentration and time ( $F_{45,120} = 186.42$ ,  $P < 0.0001$ ). At the maximum tested concentration of 0.1%, the mean mortality rate of *E. granulosus* protoscoleces progressively increased from  $10.0 \pm 1.2\%$  at 5 minutes to  $100.0 \pm 0.0\%$  at 30 minutes (Tukey's honestly significant difference pairwise comparison between 5 minutes and 30 minutes:  $P < 0.0001$ ).

Conversely, lower concentrations exhibited significantly weaker lethality within matching exposure windows. Specifically, the 0.01% concentration achieved a maximum mean mortality of  $40.0 \pm 2.8\%$  at 30 minutes, which was significantly lower than the  $100.0 \pm 0.0\%$  mortality achieved by the 0.1% extract at the identical time point ( $P < 0.0001$ ). The lowest concentrations (0.001% and 0.0001%) resulted in marginal 30-minute mortality rates of  $20.0 \pm 2.1\%$  and  $10.0 \pm 1.2\%$ , respectively, showing no statistically significant deviation from the negative control group across the initial 10 minutes of exposure ( $P = 0.942$  and  $P = 1.000$ , respectively).

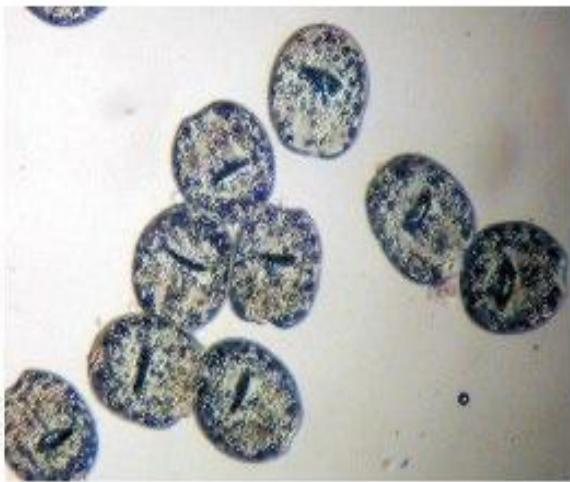
Throughout the incubation periods from 5 minutes to 30 minutes, the baseline mortality rate of protoscoleces exposed to the vehicle control remained extremely low, ranging from  $0.0 \pm 0.0\%$  at 5 minutes to  $1.8 \pm 0.4\%$  at 30 minutes. Similarly, the negative control showed negligible mortality, reaching  $1.5 \pm 0.4\%$  at 30 minutes. Moreover, statistical comparison using two-way ANOVA coupled

**Table 1.** Comparative in Vitro Mortality Rates (Mean±SD, %) of *Echinococcus granulosus* protoscoleces Treated With *Artemisia dracuncul* Extract, Silver Nitrate, and Controls

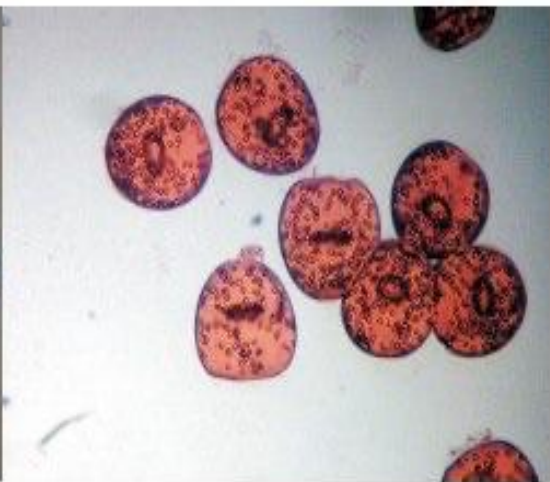
| Experimental Group                    | Concentration (%) | 5 Minutes (%)          | 10 Minutes (%)         | 15 Minutes (%)         | 20 Minutes (%)         | 25 Minutes (%)         | 30 Minutes (%)         |
|---------------------------------------|-------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| <i>Artemisia dracuncul</i>            | 0.1               | 10.0±1.2 <sup>a</sup>  | 35.0±2.5 <sup>b</sup>  | 68.0±3.1 <sup>c</sup>  | 88.0±2.0 <sup>d</sup>  | 96.0±1.5 <sup>e</sup>  | 100.0±0.0 <sup>f</sup> |
|                                       | 0.01              | 5.0±0.8 <sup>s</sup>   | 12.0±1.5 <sup>s</sup>  | 22.0±2.0 <sup>h</sup>  | 29.0±2.5 <sup>h</sup>  | 35.0±3.0 <sup>i</sup>  | 40.0±2.8 <sup>i</sup>  |
|                                       | 0.001             | 2.0±0.5 <sup>s</sup>   | 5.0±1.0 <sup>s</sup>   | 9.0±1.2 <sup>s</sup>   | 14.0±1.8 <sup>s</sup>  | 18.0±2.0 <sup>s</sup>  | 20.0±2.1 <sup>s</sup>  |
|                                       | 0.0001            | 0.0±0.0 <sup>s</sup>   | 2.0±0.5 <sup>s</sup>   | 4.0±0.8 <sup>s</sup>   | 6.0±1.0 <sup>s</sup>   | 8.0±1.2 <sup>s</sup>   | 10.0±1.2 <sup>s</sup>  |
| Positive control (AgNO <sub>3</sub> ) | 0.1               | 100.0±0.0 <sup>f</sup> | 100.0±0.0 <sup>f</sup> | 100.0±0.0 <sup>f</sup> | 100.0±0.0 <sup>f</sup> | 100.0±0.0 <sup>f</sup> | 100.0±0.0 <sup>f</sup> |
| Vehicle control                       | 5% Tween 20       | 0.0±0.0 <sup>s</sup>   | 0.5±0.2 <sup>s</sup>   | 1.0±0.3 <sup>s</sup>   | 1.2±0.4 <sup>s</sup>   | 1.5±0.4 <sup>s</sup>   | 1.5±0.4 <sup>s</sup>   |
| Negative control                      | 0.9% saline       | 0.0±0.0 <sup>s</sup>   | 0.0±0.0 <sup>s</sup>   | 0.5±0.2 <sup>s</sup>   | 1.0±0.3 <sup>s</sup>   | 1.2±0.4 <sup>s</sup>   | 1.5±0.4 <sup>s</sup>   |

Note. ANOVA: Analysis of variance; HSD: Honestly significant difference; SD: Standard deviation; AgNO<sub>3</sub>: Silver nitrate. Data are expressed as means±SD of three independent experimental replicates (n=3). Moreover, different superscript letters (\*\*) indicate statistically significant differences between treatments and exposure times evaluated via two-way ANOVA followed by Tukey's HSD post-hoc test ( $P<0.05$ ). No statistically significant difference was observed between the vehicle control (5% Tween 20) and negative control (0.9% normal saline) at any exposure time ( $P<0.05$ ).

Alive Protoscoleces



Dead Protoscoleces



**Figure 1.** Microscopic View of *E. granulosus* Protoscoleces  
Note. *E. granulosus*: *Echinococcus granulosus*. The red-stained organisms display death following exposure to the scolicidal agent, while the translucent organisms remain viable

with Tukey's post-hoc analysis represented no statistically significant difference ( $P=0.894$ ) in protoscolicidal mortality between the vehicle control and the negative control across all evaluated exposure times. Consequently, the surfactant medium negligibly contributed to protoscolex lethality, confirming that the potent dose-dependent and time-dependent scolicidal effects observed in the experimental treatments were exclusively driven by the botanical extract (Table 1).

Comparative Analysis with Silver Nitrate

AgNO<sub>3</sub>, serving as the positive reference control, displayed superior kinetic potency and an accelerated onset of scolicidal action compared to the botanical fractions ( $P<0.001$ ). At experimental concentrations of 0.1% and 0.01%, AgNO<sub>3</sub> achieved immediate, absolute mortality ( $100\pm0.0\%$ ) within the initial 5 minutes of fluid contact. Even when diluted down to an extremely low concentration of 0.0001%, AgNO<sub>3</sub> statistically outperformed the highest dose of the plant extract during the early phases of incubation, achieving  $100\pm0.0\%$  parasitic lethality by the 20-minute interval ( $P<0.05$ ).

Concurrently, the vehicle control group consisting of 5% (v/v) Tween 20 alone yielded a baseline mean mortality rate of  $1.8\pm0.4\%$  at 30 minutes, confirming that the surfactant vehicle possessed no independent confounding toxic effect on the parasite.

Qualitative Microscopic Evaluation

The eosin exclusion test provided qualitative confirmation of scolicidal efficacy. In addition, viable protoscoleces successfully excluded the dye, appearing translucent and colorless under light microscope. In contrast, non-viable (dead) protoscoleces exhibited compromised membrane integrity, absorbing the eosin dye and appearing distinctively red. This qualitative increase in the proportion of stained protoscoleces was directly correlated with higher extract concentrations and longer exposure times (Figure 1).

Discussion

CE, also known as hydatid disease, remains a serious public health and economic challenge globally, particularly in regions where livestock farming involves close contact

between humans, domestic animals, and dogs. The World Health Organization has classified CE as a neglected tropical disease, emphasizing its substantial economic burden on health systems and the livestock industry (22). Recent studies based on Global Burden of Disease data indicate that echinococcosis continues to significantly contribute to global morbidity and disability-adjusted life years, requiring a “One Health” approach for effective control (23,24). In Iran, epidemiological evidence and slaughterhouse surveys confirm the persistence of the transmission cycle and the critical role of intermediate hosts in maintaining the disease (25,26). While surgical intervention and minimally invasive techniques such as puncture, aspiration, instillation, and re-aspiration technique, is the primary treatment modalities, the intraoperative leakage of viable protoscoleces remains a critical risk factor for secondary recurrence. The risk of such leakage, leading to recurrence in approximately 10% of cases, necessitates the use of effective scolicidal agents during surgery (7,27). However, common chemical scolicides (e.g., hypertonic saline, AgNO<sub>3</sub>, and povidone-iodine) are associated with serious complications, including biliary tract damage and sclerosing cholangitis, especially if there is a cyst-biliary communication (9,21,28). Consequently, identifying highly effective scolicidal agents with minimal host toxicity remains a priority in parasitological research. The current study evaluated the scolicidal efficacy of the purified organic extract of *A. dracunculus* (Tarragon) against *E. granulosus* protoscoleces *in vitro*. Our findings demonstrated that the inhibitory effect of the extract is strictly time-dependent and concentration-dependent. Specifically, at a 0.1% concentration, the mortality rate reached 100% within 30 minutes, whereas lower concentrations (0.01%, 0.001%, and 0.0001%) failed to achieve complete lethality within the same timeframe. This “dose-time dependency” is consistent with the findings of various studies on botanical extracts, which often require optimal concentrations and exposure durations to achieve maximum biological impact (19,27). In a comparative context, a previous study reported that while *A. dracunculus* exhibits significant scolicidal potential, its performance may vary based on the extraction method, dosage, and geographical origin of the plant (29). Our observation that the 0.1% concentration reached 100% mortality suggests that the extraction conditions or the specific phytochemical profile of the samples used in this study provided a high concentration of active constituents.

From a phytochemical perspective, Tarragon contains a diverse array of bioactive compounds, including essential oil components (e.g., estragole and methyl eugenol) and polyphenols. While these active constituents are hypothesized to potentially induce parasite death by disrupting membrane permeability or compromising internal organelle stability (3,30). It should be noted that the current study exclusively focused on macro-level phenotypic viability endpoints. Previous literature on alternative botanical scolicides indicates that certain plant

extracts can cause severe ultrastructural damage to the parasite’s tegument and rostellum, occasionally triggering underlying apoptotic pathways, intracellular oxidative stress, or direct DNA damage (31,32). However, because our present experimental framework did not include direct biochemical tracking of cellular oxidation or nucleic acid degradation, these specific molecular modes of action must be cautiously interpreted as speculative pathways. Whether the purified organic extract of *A. dracunculus* directly initiates these internal cascades in *E. granulosus* protoscoleces remains a compelling hypothesis that warrants future empirical verification through targeted molecular screens, such as reactive oxygen species quantification assays and genomic fragmentation analyses. This aligns with previous reports identifying AgNO<sub>3</sub> as one of the most potent scolicides in experimental settings (8,33). However, as emphasized in clinical literature, the choice of a scolicidal agent must balance efficacy with tissue safety. Given the risks of liver necrosis and sclerosing cholangitis associated with traditional chemicals, the 100% efficacy achieved by the 0.1% Tarragon extract presents it as a promising, potentially safer biological alternative. Moreover, it must be noted that *in vitro* results do not always translate directly to *in vivo* efficacy or surgical conditions, where factors such as host proteins, cyst fluid composition, pH variations, and tissue sensitivity may alter the outcome (27,33). A primary limitation of this study was the 30-minute exposure required to achieve 100% *in vitro* scolicidal efficacy, which is slower than the ideal intraoperative surgical window of 5–10 minutes. Accordingly, future investigations should focus on nano-formulations to accelerate the penetration and parasitocidal kinetics of these purified fractions. In conclusion, our study provided robust preliminary evidence that the purified organic extract of *A. dracunculus* is a potent scolicidal agent *in vitro*. Given its efficacy at 0.1% concentration, it serves as a promising biological candidate for reducing the risk of recurrence during hydatid cyst surgery. Likewise, future research should focus on the standardization of the extract’s bioactive components through gas chromatography-mass spectrometry or high-performance liquid chromatography analysis and the evaluation of its safety and efficacy in *in vivo* models in order to determine its true clinical potential.

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### Competing Interests

The authors declare no conflict of interest in this study.

### Ethical Approval

The study protocol was approved by the Ethics Committee of Birjand University of Medical Sciences (Ethical Code: IR.BUMS.REC.1401.446).

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