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In vitro evaluation of the susceptibility of *Echinococcus granulosus* hydatid cysts to lavender and green tea extracts

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Chemotherapy, used to treat hydatid cysts, can cause problems due to various side effects. Consequently, there is growing interest in non-chemical alternatives, such as medicinal plant extracts, as potential new treatments for hydatid cysts. This study investigated the protoscolicidal activity of green tea (*Camellia sinensis*) and lavender (*Lavandula angustifolia*) extracts at different concentrations and exposure times under laboratory conditions. Protoscolices were collected aseptically from the liver of sheep infected with hydatid cysts and exposed to three concentrations of *C. sinensis* and *L. angustifolia* extracts (10, 25, and 50 mg/mL) for 10, 20, 30, and 60 min. The viability of protoscolices was assessed using 0.1% eosin staining. The results showed that *C. sinensis* and *L. angustifolia* extracts at a concentration of 50 mg/mL effectively eliminated all protoscolices after 20 min. The scolicidal effects of *C. sinensis* and *L. angustifolia* were significant compared to the control groups. Furthermore, the results showed that these plant extracts have high protoscolicidal activity. However, further studies are needed to evaluate their *in vivo* effectiveness for treating hydatid cysts in humans and herbivorous animals.

Keywords *Echinococcus granulosus*, Green tea (*Camellia sinensis*), Hydatid cyst, *In vitro*, Lavender (*Lavandula angustifolia*), Protoscolex

Abbreviations

CE	Cystic echinococcosis
GC–MS	Gas chromatography–mass spectrometry

Cystic echinococcosis (CE), also known as echinococcosis, is a significant zoonotic disease caused by the larval stage of *Echinococcus granulosus*[1]. This disease is common in many parts of the world, including Australia, South America, the Middle East, Eastern Europe, South Africa and the Mediterranean[2] .

There are three main treatment options for the removal of hydatid cysts: surgery, chemotherapy, and percutaneous aspiration[3]. In cases that are regarded as not operable, chemotherapy with Albendazole is recommended as the best alternative to surgery[4]. Despite its effectiveness, chemotherapy can lead to various side effects, such as hepatotoxicity, severe leukopenia, thrombocytopenia, and alopecia[5]. In addition, resistance to synthetic anthelmintics in treating cystic echinococcosis has led researchers to explore the potential of medicinal herbs as an alternative scolicidal active ingredient that may have fewer side effects[3,6].

Green tea, derived from the dried leaves of the *C. sinensis* plant, contains various physiologically active compounds, including polyphenols, methylxanthines, essential oils, proteins, vitamins, and amino acids[7]. Earlier studies focused on extracting polyphenols from *C. sinensis*[8,9] and investigating its extracts' potential antioxidant, antiviral, and antitumor properties[10–13]. The most common component in *C. sinensis* is catechins[14]which are primarily responsible for the health benefits associated with this plant. These advantages include reducing the plasma lipid levels, reducing inflammation, and exhibiting antibacterial, antiparasitic, anticancer, and antioxidant properties[15–18]. Catechins that belong to the Flavan-3-ol family have attracted considerable attention due to their possible therapeutic effects. In particular, their potent antioxidants and antiviral properties can contribute to the prevention of diseases[13,19,20].

Lavender, a member of the Labiatae family (Lamiaceae), has been used for therapeutic purposes for centuries[21]. It is found in various countries and is scientifically classified as *Lavandula angustifolia*[22]. This plant is known for

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its antifungal, antimicrobial, and anti-protozoan properties[21,23–28]. Numerous studies on *L. angustifolia* have shown its antipsoriatic, antidiabetic, and antidiarrheal effects[29–32]. In addition, it has proven antiparasitic activity under both *in vivo* and *in vitro* conditions[33–37].

No research has investigated the scolicidal effects of green tea (*C. sinensis*) and lavender (*L. angustifolia*). Therefore, the present study aims to examine the *in vitro* effects of *C. sinensis* and *L. angustifolia* extracts on eliminating hydatid cysts.

Results

Gas chromatography-mass spectrometry analysis

Based on the gas chromatography-mass spectrometry (GC/MS) analysis of the extracts studied, the most significant chemical components identified in *L. angustifolia* extract were linalool (26.20%), borneol (22.70%), and alpha-pinene (14.30%) (see Table 1). In *C. sinensis* extract, the major components were methyl linoleate (24.07%), squalene (11.34%), and N-hexadecanoic acid (9.32%) (Table 1).

In vitro results

The results of our examination of the effectiveness of different concentrations of extracts from *C. sinensis* and *L. angustifolia* as protoscolicidal agents are summarized in Tables 2, 3, 4, 5, 6 and 7. Our results show that both the concentration of the extracts and exposure duration significantly influence the viability of protoscolices.

L. angustifolia extract in concentrations of 10, 25, and 50 mg/mL killed 70.75%, 93.09%, and 100% of protoscolices after 60 min, respectively. The control group, treated with a normal saline solution, showed a mortality rate of only 9.72%. Remarkably, all protoscolices were killed after only 20 min of exposure to the 50 mg/mL concentration of *L. angustifolia* (Table 4; Fig. 1). Similarly, after 60 min of exposure to varying concentrations of *C. sinensis* extract (10, 25, and 50 mg/mL), the mortality rates were 84.83%, 98.23%, and 100%, respectively, while the control group, treated with normal saline solution, had a mortality rate of 11.34% (Fig. 2).

The protoscolicidal effects of *C. sinensis* and *L. angustifolia* extracts in three different concentrations were highly significant compared to the control groups' overall exposure times ($p < 0.05$) (Figs. 1 and 2). Our study showed that both *C. sinensis* and *L. angustifolia* extracts exhibit protoscolicidal activity *in vitro*.

Discussion

Surgery is the most preferred method for removing *E. granulosus* cysts to treat hydatid disease. Various scolicidal agents have been utilized to counteract the contents of hydatid cysts, but a final strategy for exterminating this disease has not yet been determined[38]. Desired qualities for an effective scolicidal agent include low costs, potency at low concentrations, lack of adverse effects, quick effect, and non-toxicity[39].

There has recently become a growing interest in using natural anti-parasite extracts to treat various diseases. Some herbal extracts are emerging as viable alternatives to conventional medications due to their less adverse effects, lower cost, and high availability. Numerous studies have examined the scolicidal effects of various plants, such as *Nigella sative*[40]*Allium sativum*[41]*Quercus infectoria*[42] *Zingiber officinale*[43]*Salvadora persica*[44]*Ceratonia silique*[35]*Taxus baccata*[45] and *Zataria multiflora*[46]in the treatment of hydatid cysts. Additionally, Al Qaisi et al. (2021) demonstrated the *in vitro* protoscolicidal effects of methanolic extracts from Jordanian medicinal plants, including *Ruta graveolens*, *Peganum harmala*, and *Citrullus colocynthis*[47]. Furthermore, Al-khlifeh et al. (2021) reported *in vitro* protoscolicidal effects of *Juniperus phoenicea* L., *Calotropis procera* (Aiton) Dryand, and *Artemisia judaica* L. against *Echinococcus granulosus* cysts[48]. Al Qaisi et al. (2023) also explored the preventive effects of some medicinal plant extracts on the development of hydatid cyst infection[49]. Most of these studies focus more on *in vitro* evaluations than *in vivo* applications due to cost efficiency, speed, and a high throughput

Extracts	Major compounds	Percentage
L. angustifolia	Ocimene	7.82
	Camphor	4.55
	Linalyl acetate	5.60
	Borneol	22.70
	1,8-cineol	11.50
	α-pinene	14.30
	linalool	26.20
C. sinensis	Squalene	11.34
	N-hexadecanoic acid	9.32
	Methyl hexadecanoate	0.43
	Methyl linoleate	7.43
	Caffeine	24.07
	γ-muurolene	1.38
	α-bulnesene	2.52
	Naphthalene	12.36
	Eremophilene	1.22

Table 1. Major chemical compounds in *L. angustifolia* and *C. siliqua* extract identified by GC-MS.

Exposure time (min)		Experiments			Total
		1	2	3	
10	Protoscolices	450	427	424	1301
	Dead protoscolices	105	94	98	297
	Mortality rate (%)	23.33	22.01	23.11	22.82 ± 2.66
20	Protoscolices	501	438	453	1392
	Dead protoscolices	178	159	165	502
	Mortality rate (%)	35.52	36.3	36.42	36.08 ± 4.46
30	Protoscolices	434	512	410	1356
	Dead protoscolices	268	266	264	798
	Mortality rate (%)	61.75	51.95	64.39	59.36 ± 6.44
60	Protoscolices	476	457	469	1402
	Dead protoscolices	334	301	357	992
	Mortality rate (%)	70.16	65.86	76.11	70.71 ± 5.13
Control	Protoscolices	659	492	427	1378
	Dead protoscolices	46	43	45	134
	Mortality rate (%)	10.02	8.73	10.53	9.76 ± 0.91

Table 2. Scolicidal effect of *Lavandula angustifolia* extract against protoscolices of hydatid cyst at the concentration of 10 mg/mL following various exposure times.

Exposure time (min)		Experiments			Total
		1	2	3	
10	Protoscolices	398	487	400	1285
	Dead protoscolices	252	199	207	658
	Mortality rate (%)	63.31	40.86	51.75	51.97 ± 6.23
20	Protoscolices	401	399	512	1312
	Dead protoscolices	286	199	325	810
	Mortality rate (%)	71.32	49.87	63.47	61.55 ± 5.73
30	Protoscolices	469	489	387	1345
	Dead protoscolices	400	399	256	1055
	Mortality rate (%)	85.28	81.59	66.14	77.67 ± 4.57
60	Protoscolices	364	408	532	1304
	Dead protoscolices	322	390	502	1214
	Mortality rate (%)	88.46	95.58	94.36	92.80 ± 3.91
Control	Protoscolices	459	492	427	1378
	Dead protoscolices	46	43	45	134
	Mortality rate (%)	10.02	8.73	10.53	9.76 ± 0.91

Table 3. Scolicidal effect of *Lavandula angustifolia* extract against protoscolices of hydatid cyst at the concentration of 25 mg/mL following various exposure times.

of in vitro screening methods. These in vitro tests measure the anthelmintic activity directly on the hatching, development, and motility of parasites without influencing the internal physiological functions of the host[50]. Another advantage of in-vitro studies is that the extracts or compounds, as soon as reliable results are achieved, can be further evaluated *in vivo*[51]. However, it is essential to note that compounds or extracts that are effective *in vitro* may not necessarily have the same level of activity *in vivo*[52].

Several recent studies have documented the antiparasitic effects of *C. sinensis* and *L. angustifolia* extracts[53–57]. This study investigates the scolicidal activity of these extracts *in vitro*. This is the first research analyzing the effects of *C. sinensis* and *L. angustifolia* extracts on hydatid cysts under *in vitro* conditions.

Our findings demonstrate a dose-dependent and time-dependent scolicidal effect of *C. sinensis* and *L. angustifolia* extracts on the protoscolexes of hydatid cysts *in vitro.* These results align with those of Malekifard et al.[35,58]who also observed dose and time-dependent sporicidal effects of *C. silique* and *Q. infectoria olivier* extracts. In addition, a study by Moazeni et al. (2012) reported similar dose and time-dependent scolicidal effects of *Satureja khuzistanica* essential oil[59].

This study shows that the scolicidal activity of extracts from *C. sinensis* and *L. angustifolia* is significant after 20 min of exposure at a concentration of 50 mg/mL, which leads to a 100% mortality rate. The scolicidal effects observed during this concentration and exposure period are comparable to that of other active ingredients such as 95% ethyl alcohol (15 min)[60]0.5-1% cetrimide (10 min)[61]3% H2O2 (15 min)[62]and 20% hypertonic saline

Exposure time (min)		Experiments			Total
		1	2	3	
10	Protoscolices	486	395	520	1401
	Dead protoscolices	455	376	470	1301
	Mortality rate (%)	93.62	95.18	90.38	93.06 ± 2.40
20	Protoscolices	411	507	446	1364
	Dead protoscolices	411	507	446	1364
	Mortality rate (%)	100	100	100	100 ± 0
Control	Protoscolices	459	492	427	1378
	Dead protoscolices	46	43	45	134
	Mortality rate (%)	10.02	8.73	10.53	9.76 ± 0.91

Table 4. Scolicidal effect of *Lavandula angustifolia* extract against protoscolices of hydatid cyst at the concentration of 50 mg/mL following various exposure times.

Exposure time (min)		Experiments			Total
		1	2	3	
10	Protoscolices	511	456	490	1457
	Dead protoscolices	67	143	146	356
	Mortality rate (%)	13.11	31.35	29.79	24.75 ± 6.79
20	Protoscolices	480	454	463	1397
	Dead protoscolices	214	199	208	621
	Mortality rate (%)	44.58	43.83	44.92	44.44 ± 2.55
30	Protoscolices	398	444	545	1387
	Dead protoscolices	300	298	356	954
	Mortality rate (%)	75.37	67.11	65.32	69.27 ± 5.40
60	Protoscolices	485	398	416	1299
	Dead protoscolices	432	301	369	1102
	Mortality rate (%)	89.07	75.62	88.7	84.46 ± 7.24
Control	Protoscolices	627	643	598	1868
	Dead protoscolices	67	74	71	212
	Mortality rate (%)	10.68	11.5	11.87	11.35 ± 0.60

Table 5. Scolicidal effect of *Camellia sinensis* extract against protoscolices of hydatid cyst at the concentration of 10 mg/mL following various exposure times.

(15 min)[63]. Norouzi et al. (2020) examined the influence of a hydroalcoholic extract from *Taxus baccata* L. on the protoscolices of hydatid cysts. Their results showed that an extract concentration of 150 mg/mL killed 66.6% of protoscolices after 60 min[45]. Sajjadi et al. (2008) examined *Allium Sativum* extracts and found that the chloroformic extract had the highest protoscolicidal activity in a 200 mg/mL concentration[64]. Similarly, Moazeni et al. (2014) reported a strong scolicidal effect from the methanolic extract of *Zataria multiflora*, whereby concentrations of 10 and 25 mg/mL killed 100% of the protoscolices after 3 and 1 min[65]. In another study, Kavoosi and Purfard (2013) found that all protoscolices were killed within 10 min of exposure to more than 17 µg/mL essential oil concentrations by *Zataria multiflora*[66]. In addition, Mahmoudvand et al. (2014) showed that the essential oil from *Nigella sativa* eliminated 100% of protoscolices in a concentration of 10 mg/mL after 10 min of exposure[67]. In addition, Rouhani et al. (2013) examined the scolicidal effect of Barberry in different concentrations (0.5, 1, 2, and 4 mg/mL) and different exposure times (5, 15, and 30 min). They found that a 4 mg/mL concentration had a 100% effectiveness ater only 5 min[68]. The variations in the results in various studies can be attributed to the differences in the types of plants used, concentrations, and exposure times[45].

Our research provides evidence of the *in vitro* protoscolicidal activity of *L. angustifolia* extract. This study identified linalool as the primary compound, making up 26.20% of the extract. The *L. angustifolia* extract also contained small amounts of other compounds. Linalool and Linalyl acetate are known to be responsible for most biological activities of lavender[69]. Supporting our findings, Malekifard et al. (2021) demonstrated that high levels of linalool in *L. angustifolia* extract are likely the active compounds responsible for its anti-*Trichomonas gallinae* activity[36]. Several studies have focused on determining the pharmacological properties of linalool and *L. angustifolia*. Reports indicate that *L. angustifolia* extract has therapeutic effects against *Toxoplasma gondii*, *Giardia duodenalis*, *Trichomonas vaginalis*, and *Hexamita inflata* infections[31,34]. Furthermore, it is essential to note that *L. angustifolia* acts by lysing the cells of these parasites to eliminate them[34].

In this study, caffeine (24.07%) was identified as the primary component of the *C. sinensis* extract, and it has been reported to have antiparasitic effects in several studies. Findings from these studies indicated that both

Exposure time (min)		Experiments			Total
		1	2	3	
10	Protoscolices	416	462	525	1403
	Dead protoscolices	246	278	293	817
	Mortality rate (%)	59.13	60.17	55.8	58.37 ± 2.19
20	Protoscolices	437	451	467	1355
	Dead protoscolices	295	305	311	911
	Mortality rate (%)	67.5	67.62	66.59	67.24 ± 0.52
30	Protoscolices	489	402	399	1290
	Dead protoscolices	389	228	315	1042
	Mortality rate (%)	79.55	84.07	78.94	80.85 ± 2.57
60	Protoscolices	416	558	328	1302
	Dead protoscolices	409	550	320	1279
	Mortality rate (%)	98.31	98.56	97.56	98.14 ± 0.50
Control	Protoscolices	627	643	598	1868
	Dead protoscolices	67	74	71	212
	Mortality rate (%)	10.68	11.5	11.87	11.35 ± 0.60

Table 6. Scolicidal effect of *Camellia sinensis* extract against protoscolices of hydatid cyst at the concentration of 25 mg/mL following various exposure times.

Exposure time (min)		Experiments			Total
		1	2	3	
10	Protoscolices	389	511	498	1398
	Dead protoscolices	358	461	491	1310
	Mortality rate (%)	92.03	90.21	98.59	93.61 ± 4.19
20	Protoscolices	436	422	545	1403
	Dead protoscolices	436	422	545	1403
	Mortality rate (%)	100	100	100	100 ± 0
Control	Protoscolices	627	643	598	1868
	Dead protoscolices	67	74	71	212
	Mortality rate (%)	10.68	11.5	11.87	11.35 ± 0.60

Table 7. Scolicidal effect of *Camellia sinensis* extract against protoscolices of hydatid cyst at the concentration of 50 mg/mL following various exposure times.

C. sinensis extract and caffeine exhibited anti-Acanthamoeba, anti-*Trichomonas gallinae*, and antileishmanial impact[56,70,71]. Previous research[72] has shown that *C. sinensis* and its constituents demonstrated antimicrobial activity against multidrug-resistant gram-positive and gram-negative bacteria. In addition, *C. sinensis* was effective in the inhibition of *Leishmania amazonensis*[73,74]reducing exposure to *Haemonchus contortus* worms[75]and inhibiting both the promastigote and amastigote forms of *Leishmania braziliensis*[76]. Moreover, *C. sinensis* also inhibited *Babesia* spp., *Eimeria* spp., *Trichomonas gallinae*, and *Trypanosoma cruzi*[18,56,77,78]. Research conducted by Fakae et al. demonstrated that beers made from *C. sinensis* exhibited amoebic activity against Acanthamoeba trophozoites and were effective in preventing the parasite from encysting. Their findings suggested that *C. sinensis* could serve as a source of inhibitors for *A. castellanii* growth and encystation[79].

Conclusion

In conclusion, our findings demonstrated that both *C. sinensis* and *L. angustifolia* could serve as effective scolicidal agents. To fully understand and improve these plant-based compounds' pharmacological and therapeutic properties, their mechanisms of action should be further investigated[80]. In addition, further examinations are required to determine possible adverse effects of *C. sinensis* and *L. angustifolia* and confirm the scolicidal effectiveness of these herbal medications *in vivo*.

Methods

Ethical compliance

The study's ethical considerations were approved by the Animal Ethics Committee at Urmia University, Urmia, Iran (IR-UU-AEC-3/83), and conducted under its regulations.

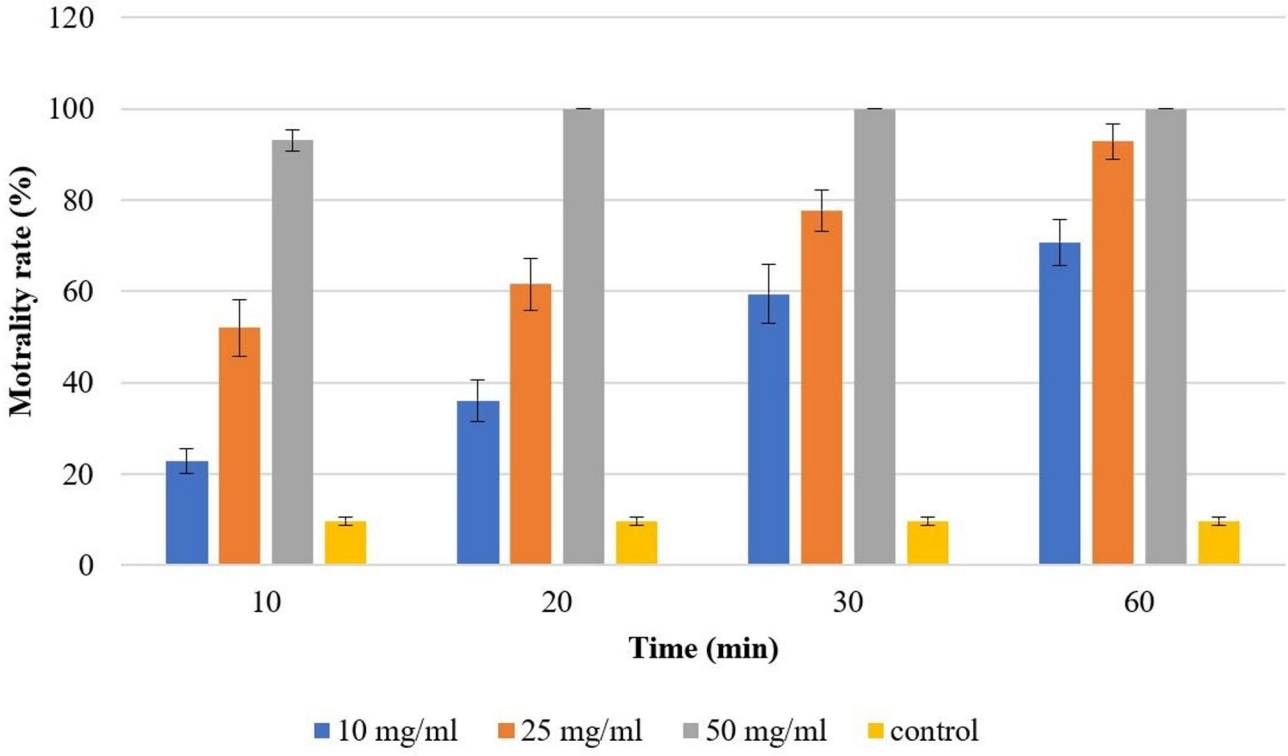

Fig. 1. Scolicidal effects of different concentrations of *Lavandula angustifolia* in various exposure times.

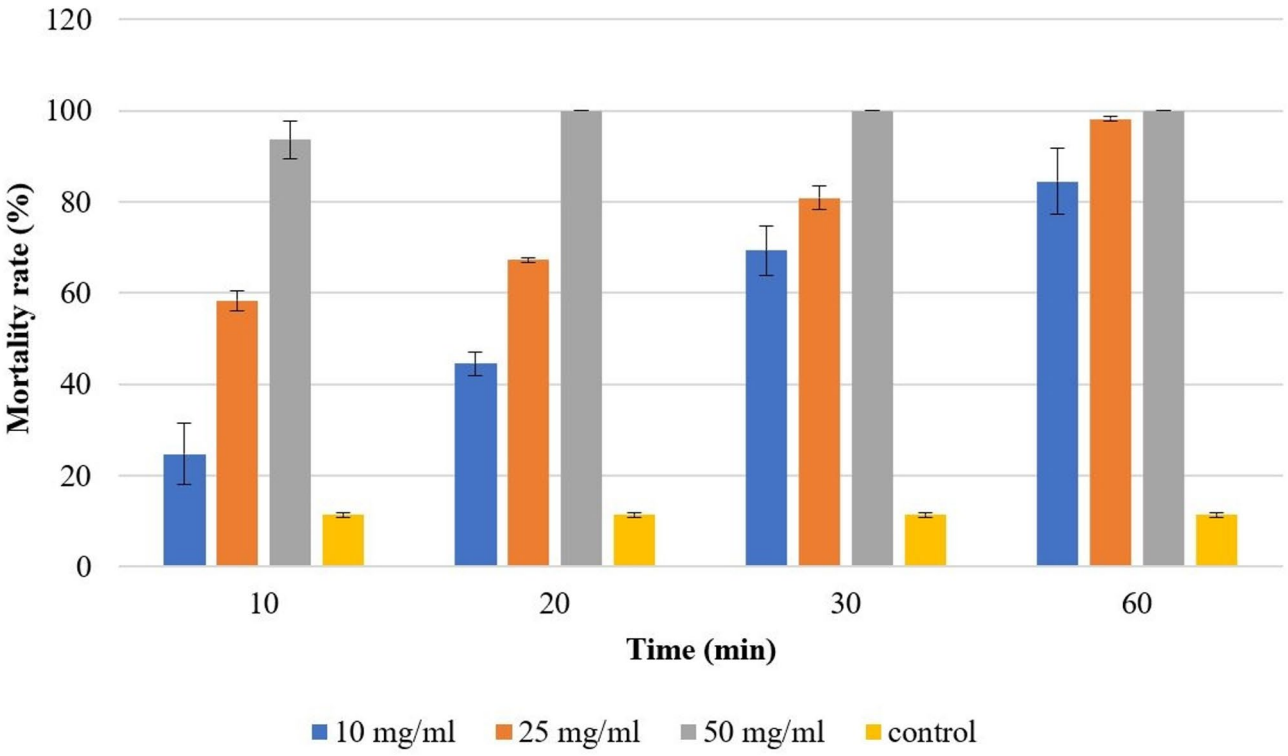

Fig. 2. Scolicidal effects of different concentrations of *Camellia sinensis* in various exposure times.

Collecting protoscolices
Protoscolices were extracted from the livers of infected sheep slaughtered at the Urmia abattoir in northwest Iran. The samples were collected postmortem, following standard abattoir procedures for meat production, with no specific anesthesia or euthanasia methods applied for this study. Under aseptic conditions, the hydatid fluid was transferred to a flask and allowed to stand for 30 min to separate the protoscolices. The supernatant was removed, and the protoscolices were washed three times with PBS solution (pH = 7.2). The viability of the protoscolices was confirmed by motility observations using a standard light microscope after staining with 0.1% Eosin[58].

Preparation of plant extract
C. sinensis and *L. angustifolia* plants were purchased from a Persian herbal market in June 2023 and verified by the Agriculture Faculty of Urmia University in Urmia, Iran[36,56]. The extraction method used was based on techniques by Baqer et al. With some modifications[81]. All dry plant materials, including the leaves of green tea and the dried branches of flowers from lavender, were ground in powder using an electrical mixer (Moulinex, Paris, France). A mixture of 500 mL of 70% ethanol and 100 g of powdered plant material was stirred with a magnetic stirrer for 2 h. The solution was left at room temperature for 24 h and filtered after another mixing round[56]. The solvent was removed using a rotary evaporator, and the semi-solid residual components were frozen for future use at 4 °C. The extraction process yielded approximately 12.5% (w/w) for *L. angustifolia* (12.5 g of dried extract from 100 g of dried flower branches) and 15.8% (w/w) for *C. sinensis* (15.8 g of dried extract from 100 g of dried leaves).

Gas chromatography-mass spectrometry (GC-MS) analysis
The chemical composition of the extracts was analyzed using gas chromatography-mass spectrometry (GC-MS) by Thermo Scientific™ in Paris, France. The helium-based carrier gas was adjusted to a 0.50 mL/min split ratio. The following GS conditions were used: an initial temperature of 40 °C, increasing to 250 °C at 80 °C per minute, and maintaining the temperature for 3 min. The injector and detector temperatures were both set at 250 °C. Individual compounds were identified by comparing their relative retention times in a capillary column with those of authentic samples and by analyzing their peak-to-peak mass spectra against data from reliable sources and published literature[56,57].

Scolicidal effects of *C. sinensis* and *L. angustifolia* extracts
C. sinensis and *L. angustifolia* extracts were tested at three different concentrations of 10, 25, and 50 mg/mL, along with various exposure times of 10, 20, 30, and 60 min, to evaluate their effectiveness in eliminating protoscolices of hydatid cysts. For each concentration, 0.5 mL was placed into a test tube, followed by the addition of 0.5 mL of protoscolices solution (containing approximately 450–550 protoscolices suspended in PBS solution). The mixtures were gently mixed and left undisturbed for the specified durations at room temperature. At the end of each time interval, the upper phase of the mixture was carefully collected using a pipette, ensuring that the protoscolices were not disturbed. Staining was performed by adding 2 mL of 0.1% eosin to the settled protoscolices in the tube, which was then gently mixed. The upper portion of the remaining solution was discarded. The remaining protoscolices were then smeared on a glass slide under a light microscope to evaluate their viability. The percentage of dead protoscolices was determined by counting at least 450, often more than 500, protoscolices. Normal saline solution was used as a control group, and all experiments were conducted in triplicate[42].

Viability test
This study utilized a 0.1% eosin solution (1 g eosin in 1000 mL distilled water) to analyze the viability of protoscolices. After 15 min of exposure to Eosin, viable protoscolices showed no significant color change, while dead protoscolices became red due to the absorption of eosin red. The mortality rate was calculated by dividing the number of protoscolices killed by the total number of protoscolices[42].

Statistical analysis
SPSS (version 26, Chicago) was used for statistical analysis. A Chi-square test was carried out to compare the differences between the test and control groups. A p-value of less than 0.05 ($P < 0.05$) was considered statistically significant.

Declaration of generative AI in scientific writing
While preparing this work the author(s) used ChatGPT to make the text antive. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the publication's content.

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

SMM, FM, and BE contributed to the conception, design, data collection, statistical analysis, and drafting of the

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The authors declare no competing interests.

Ethics approval and consent to participate
All of the protocols were approved by the Faculty of Veterinary Medicine's Committee on the Ethics of Animal Experiments at Urmia University (IR-UU-AEC-3/83). Every procedure was carried out in accordance with the relevant laws and standards. The study was conducted in compliance with the ARRIVE standards. The owner(s) of the animals gave their informed consent for us to use them in the study.

Additional information
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