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Comparative analysis of scolicidal properties of two ecotypes of *Leonurus cardiaca*: implications for combating *Echinococcus granulosus*

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Abstract

In this study, leaf and flower samples from Khansar and Kerman ecotypes of *Leonurus cardiaca* were extracted with methanol and ethanol to analyze their phenolic content, antioxidant activity, and scolicidal efficacy against *Echinococcus granulosus* protoscoleces. Total phenolic content analysis revealed higher values in methanolic extracts, with Khansar leaf extracts yielding 37.6 ±1.2 mg GAE/g compared to Kerman's 31.1 ±1.4 mg GAL/g. Total flavonoid content showed a similar trend, with Khansar leaf methanolic extracts producing 21.6 ± 1.1 mg QE/g versus Kerman's 15.6 ± 1.1 mg QE/g. Total flavonol content analysis corroborated these findings, with Khansar leaf methanolic extracts yielding 7.5 ± 1.0 mg QE/g compared to Kerman's 6.6 ± 1.1 mg QE/g. DPPH radical scavenging assays demonstrated concentration-dependent antioxidant activity, with Khansar leaf methanolic extracts showing 62.2 ± 1.0% inhibition at 120 µg/ml, surpassing Kerman's 59.0 ± 1.2%. Ferric-reducing power assays further supported these results, with Khansar leaf methanolic extracts exhibiting an absorbance of 0.437 ± 0.005 at 400 µg/ml. Scolicidal activity against *E. granulosus* protoscoleces increased with concentration, with the Khansar ecotype achieving 100% mortality at 90 mg/ml after 30 minutes, and at 120 mg/ml after 20 minutes. The Kerman ecotype did not reach 100% mortality at 90 mg/ml concentration, but at 120 mg/ml, it achieved 100% mortality after 30 minutes. These results indicated that *L. cardiaca* extracts, particularly from Khansar and Kerman ecotypes, show promise as a potential treatment for *E. granulosus* infections.

Introduction

In recent years, there has been a growing interest in the potential use of plant extracts for the treatment of parasitic infections, including cystic echinococcosis (CE). *Echinococcus granulosus* is a parasitic tapeworm responsible for CE in humans and livestock. The disease is prevalent in numerous regions worldwide, including the Mediterranean, Central Asia, and South America, primarily affecting sheep and goats (Tamarozzi *et al.,* 2020). Current CE treatment involves surgical cyst removal, followed by medical therapy using benzimidazole compounds. However, benzimidazole drugs come with adverse side effects, and the emergence of drug-resistant strains poses a significant challenge (Mahmoudvand *et al.,* 2014; Mahmoudvand *et al.,* 2017). As a result, alternative approaches for CE treatment are needed, particularly

from natural sources such as medicinal plants (Ali *et al.,* 2020).

Iran is known for its diverse array of plant species and countless natural habitats, characterized by many unique plants and centers of local endemism (Khourang *et al.,* 2014; Kiani *et al.,* 2017). One plant genus that contributes significantly to this botanical richness is *Leonurus*, which is widely distributed across certain parts of Iran.

Leonurus cardiaca (Lamiaceae), commonly known as lion's tail or motherwort, is a perennial herb found from Europe through southwest and central Asia to parts of China (Fierascu *et al.,* 2019). The genus comprises around 20 species, with *L. cardiaca* being the sole representative of the Iranian flora (Zamani *et al.,* 2018). The genus name *Leonurus* derives from the Greek words "Leon," meaning lion, and "oura," meaning tail, alluding to the plant's inflorescence. The specific epithet, *cardiaca*, stems from the Latin word denoting its historical use in heart-related ailments. It also enjoys a historical reputation for addressing various gynecological afflictions (Fierascu *et al.,* 2019), hence the common name "motherwort" (Fig. 1).

L. cardiaca is known to harbor a range of bioactive compounds, including terpenes, phenylpropanoids, phenolics, nitrogen-containing compounds, and iridoids (Wojtyniak *et al.,* 2013; Flemmig *et al.,* 2015). These compounds have been reported to possess diverse biological activities, encompassing antioxidant and antimicrobial properties (Jafari *et al.,* 2010; Wojtyniak *et al.,* 2013; Tahmouzi and Ghodsi, 2014). The plant has found applications in treating various ailments, including cardiovascular disorders, anxiety, and female medical issues. Its potential health benefits encompass anti-cancer properties (Tao *et al.,* 2009), anti-inflammatory effects (Ali *et al.,* 2007), and anti-blood-clotting properties. Additionally, it has been employed in managing nervous disorders (Miłkowska-Leyck *et al.,* 2002), cramping, insomnia, and hypertension. Given the diverse range of medicinal properties of *L. cardiaca*, it emerges as a promising candidate for the development of new therapeutic agents for various diseases.

This study aimed to investigate the potential of *L. cardiaca* as a natural anthelmintic against *E. granulosus* protoscoleces. Specifically, the study compared the phenolic content, antioxidant activity, and scolicidal efficacy of leaf and flower extracts from Khansar and Kerman ecotypes of *L. cardiaca*. The findings of this research contribute to the exploration of alternative therapeutic options for cystic echinococcosis, potentially reducing reliance on conventional benzimidazole drugs and addressing the challenges associated with drug resistance.

Fig. 1. Morphology of *Leonurus cardiaca.*

Materials and Methods

Plant material

In 2021, leaf and flower samples of *L. cardiaca* were collected from two geographically distinct ecotypes located in the Khansar and Kerman regions of Iran. At each location, mature *L. cardiaca* plants were selected based on established botanical identification criteria. Voucher specimens were deposited at the herbarium of the Agricultural and Natural Resources Center of Mazandaran province in Iran for future reference.

Isolation of plant extracts

The plant materials, flowers and leaves, were washed, dried, and powdered at room temperature. Each powdered sample (3 g) was suspended and extracted in 80 ml of 80% methanol and ethanol (v/v) and kept for 2 days on a shaker (90 rpm) at room temperature. The methanolic and ethanolic extracts were then dried using a vacuum rotary evaporator in a water bath at 40°C. The dried samples were weighed and kept at 4°C until use (Ghimire *et al.,* 2011).

Phenolics measurement

The total phenolic content (TPC) was measured using the Folin-Ciocalteu reagent, according to the method of Kim *et al*. (2007), with slight modifications using gallic acid as a standard. Briefly, 100 µl of extract solution (1 mg/ml) was added to a 15 ml volumetric flask containing about 6 ml of distilled water. Then, 500 µl of Folin-Ciocalteu reagent was added, and the contents of the flask were allowed to stand for 5

minutes after stirring. Next, 1500 µl of Na_2CO_3 (20% in water, v/v) was added and vortexed, and the final volume (10 ml) was obtained by adding 3.9 ml of distilled deionized water. The final mixture was vortexed and then incubated for 90 minutes in the dark at 25°C. The absorbance was measured at 765 nm using a UV-VIS spectrophotometer. Total phenolic values were expressed in terms of gallic acid equivalents (GAE) in milligrams per gram of plant extract.

The total flavonoid content (TFC) in the extracts was determined according to Moreno *et al*. (2000). Briefly, a 500 µl solution of sample extracts (1 mg/ml) in methanol was separately mixed with 100 µl of 10% aluminum chloride, 100 µl of 1 M potassium acetate, and 4.3 ml of distilled water, and left at room temperature for 40 minutes. The absorbance of the reaction mixture was measured at 415 nm. The total flavonoid concentration was calculated using quercetin as a standard.

The total flavonol content (TFLC) in the extracts was determined according to Loziene *et al*. (2007). Briefly, a 400 µl solution of sample extracts (1 mg/ml) in 80% methanol was mixed with 400 µl of 2% aluminum chloride and 1200 µl of 5% sodium acetate solution, and left at room temperature for 2.5 hours. The absorbance of the reaction mixture was measured at 440 nm. The total flavonol concentration was calculated using quercetin as a standard.

Antioxidant assays

The antioxidant activity of the methanolic and ethanolic extracts was determined using the DPPH free radical scavenging assay and reducing power assay methods, as previously described by Singh *et al* (2009), respectively. Briefly for the DPPH free radical scavenging assay, a 1 ml solution of 90 µM DPPH in methanol was mixed with 1 ml of sample solutions (15, 60, and 120 µg/ml), and then kept at room temperature in the dark for 30 minutes. The absorbance was measured at 517 nm. Percent inhibition of DPPH radical scavenging activity was calculated using the following equation: (% Inhibition) = ((A control - A sample)/A control)× 100

For reducing power assay, three concentrations of the ethanolic and methanolic extracts (100, 200, and 400 µg/ml) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1% w/v). This mixture was kept at 50°C in a water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 minutes (if necessary) to precipitate any remaining reactants. The upper layer of the solution (2.5 ml) containing the reduced Prussian blue complex was then mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance of the final solution was measured at 700 nm. Higher absorbance values indicated greater reducing power (Benzie and Strain, 1999).

Scolicidal activity

Hydatid cyst protoscoleces were collected from the livers of infected sheep in Mazandaran province, Amol, Northern Iran. The viability of protoscoleces was assessed by 0.1% eosin staining under light microscopy. To determine the scolicidal activity of the methanolic and ethanolic extracts against the protoscoleces of hydatid cysts, five dilutions (20, 40, 60, 90, and 120 mg/ml) of the extracts were used for 1, 3, 5, 10, 20, and 30 minutes. A 0.5 ml aliquot of protoscolex suspension was added to each test tube, followed by the addition of 0.5 ml of each extract concentration or control solution. The contents of the tubes were mixed gently and incubated at 37°C for varying time periods (1, 3, 5, 10, 20, and 30 minutes). After incubation, the upper parts of the solutions were removed with a pipette. The remaining protoscoleces were stained with 1 ml of 0.1% eosin solution, and the number of motile (viable) and non-motile (dead) protoscoleces were counted under a light microscope from a sample of 250 protoscoleces per treatment (Mahmoudvand *et al.,* 2014). The scolicidal activity of each extract concentration and incubation time was expressed as the percentage of dead protoscoleces.

Statistical analysis

All analytical experiments were carried out in three replicates, and the results were presented as the mean of the obtained values with the standard deviation (SD). The analysis of variance (ANOVA) procedure followed by Duncan's test using SPSS 16 (SPSS Inc., USA) software was applied to determine the significant difference ($p < 0.05$) between treatment means.

Results and discussion

Medicinal plants are a valuable source of bioactive compounds that are essential to the biomaterial industry. The industrial utilization of these plants is closely related to the composition of their active substance, which is subject to a complex interplay of

intrinsic and extrinsic factors (Kiani *et al.,* 2015). This variation can be observed even within the same plant species, with different ecotypes and plant parts exhibiting distinct metabolic profiles. Geographically distinct ecotypes may have adapted to their environments, resulting in variations in their chemical makeup (Kiani *et al.,* 2017). In this study, the Khansar and Kerman ecotypes were collected from different regions of Iran, which may explain some of the differences observed in their chemical compositions and biological activities. Additionally, leaves and flowers have specialized functions, leading to differences in the types and amounts of bioactive compounds they produce (Mazooji *et al.,* 2012). These variations can have implications for the functional roles and properties of active substances.

Phenolics measurement

Phenolic compounds are crucial plant metabolites known for their antioxidant capacity and potential health benefits. They have also been shown to exhibit biocidal and anthelmintic properties. Therefore, quantifying these phenolics in *L. cardiaca* extracts can provide insights into their potential biological activities. TPC, TFC, and TFLC were comparatively quantified in methanolic and ethanolic extracts derived from leaf and flower samples of two *L. cardiaca* ecotypes, Khansar and Kerman (Table 1).

Total phenolic content (TPC)

The TPCis a widely recognized marker of the antioxidant potential of plant extracts. It refers to the concentration of all phenolic compounds present in a given sample (Benzie and Strain, 1999). These compounds are known for their ability to scavenge free radicals and prevent oxidative damage in the body. As such, the TPC is often used as an indicator of the potential health benefits of consuming plant extracts, as higher TPC values are associated with greater antioxidant activity.

Analysis of TPC revealed a consistent trend across both Khansar and Kerman ecotypes. For the Khansar ecotype, methanolic extracts yielded 37.6 ± 1.2 mg GAE/g for leaves compared to 32.2 ± 1.5 mg GAE/g for flowers. Ethanolic extracts from Khansar produced 32.2 ± 1.0 mg GAE/g for leaves versus 26.9 ± 1.3 mg GAE/g for flowers. Regarding the Kerman ecotype, methanolic extracts resulted in 31.1 ± 1.4 mg GAL/g for leaves compared to 28.9 ± 1.2 mg GAL/g for flowers. Kerman's ethanolic extracts showed 29.7 ±1.2 mg GAL/g for leaves versus 26.7 ± 1.0 mg GAL/g for flowers.

Total flavonoid content (TFC)

The analysis of TFC revealed patterns consistent with those observed for TPC. Leaf extracts consistently showed significantly higher TFC compared to flower extracts across both Khansar and Kerman ecotypes, irrespective of the extraction solvent employed. Methanolic leaf extracts exhibited the highest TFC within each ecotype. For the Khansar ecotype, leaf methanolic extracts yielded a TFC of 21.6 ± 1.1 mg QE/g, substantially higher than the 9.1 ± 1.0 mg QE/g observed in flower extracts. This trend persisted in ethanolic extracts, with leaf extracts showing 12.2 ± 1.2 mg QE/g compared to 8.2 ± 1.1 mg QE/g in flower extracts. The Kerman ecotype displayed a similar pattern; with leaf methanolic extracts produced a TFC of 15.6 ± 1.1 mg QE/g, exceeding the 9.6 ± 1.2 mg QE/g found in flower extracts. Ethanolic extracts of Kerman echoed this trend, with leaf extracts yielding 13.7 ± 1.3 mg QE/g compared to 8.0 ± 1.0 mg QE/g in flower extracts. Notably, the leaf methanolic extract of the Khansar ecotype demonstrated the highest overall TFC (21.6 ± 1.1 mg QE/g), surpassing that of the Kerman ecotype (15.6 ± 1.1 mg QE/g). These findings underscore the superior flavonoid content in leaf tissues and highlight the potential of the Khansar ecotype as a rich source of flavonoids.

Total flavonol content (TFLC)

In both ecotypes, the leaf extracts showed significantly higher compared to the flower extracts, regardless of whether methanol or ethanol was used as the solvent. Specifically, the methanolic leaf extracts of the Khansar ecotype had a TFLC of 7.5 ± 1.0 mg QE/g, while the flower extracts had a TFLC of 4.5 ± 1.0 mg QE/g. Similarly, the ethanolic leaf extracts had a TFLC of 5.1 ± 1.0 mg QE/g, while the flower extracts had a TFLC of 4.1 ± 1.1 mg QE/g. For the Kerman ecotype, the methanolic leaf extracts had a TFLC of 6.6 ± 1.1 mg QE/g, whereas the flower extracts had a TFLC of 4.5 ± 1.0 mg QE/g. The ethanolic leaf extracts had a TFLC of 4.6 ± 1.2 mg QE/g, while the flower extracts had a TFLC of 4.1 ± 1.1 mg QE/g. Among all the samples, the methanolic leaf extract of the Khansar ecotype exhibited the highest TFLC, measuring 7.5 ± 1.0 mg QE/g, while the Kerman ecotype had a slightly lower TFLC of 6.6 ± 1.1 mg QE/g.

A comprehensive analysis of TPC, TFC, and TFLC revealed consistent patterns across various experimental parameters. Foliar extracts

demonstrated significantly higher concentrations of these phytochemicals compared to floral extracts, irrespective of ecotype or solvent used. This observation suggests that leaves serve as a more abundant source of phenolics, flavonoids, and flavonols. The Khansar ecotype consistently outperformed the Kerman ecotype in all three measures, indicating a potentially higher antioxidant capacity. Regarding extraction solvents, methanolic extracts generally yielded higher concentrations than ethanolic extracts, with this trend being particularly evident for TPC and TFC. However, for TFLC, while methanolic extracts showed higher concentrations in leaf samples, no significant difference was observed between solvents for flower extracts.

These findings highlight the importance of optimizing extraction parameters, including plant organ selection, ecotype consideration, and solvent choice, to maximize the yield of these bioactive compounds. The consistently higher values in leaf extracts suggest their potential as a preferable source for these phytochemicals in both ecotypes. The superior performance of the Khansar ecotype implies its potential for cultivation in antioxidant-focused applications. The general efficacy of methanol as an extraction solvent, particularly for phenolics and flavonoids, provides valuable insight for future phytochemical isolation protocols.

The analysis of TPC, TFC, and TFLC in *L. cardiaca* revealed a clear trend: TPC values were consistently higher than TFC, which in turn were higher than TFLC. This pattern suggests that phenolic compounds are the most abundant antioxidant group in *L. cardiaca*, with flavonoids and flavonols being subgroups within the larger category of phenolics. The intermediate values for TFC indicate that not all phenolics in *L. cardiaca* are flavonoids. However, flavonoids still represent a significant portion of the plant's antioxidant profile.

Antioxidant activity

Building upon the observed trends in TPC, TFC, and TFLC, two complementary antioxidant assays were employed to validate the potential antioxidant activity of *L. cardiaca* extracts.

DPPH radical scavenging capacity assay

The DPPH assay was used to assess the ability of plant extracts to donate hydrogen atoms and scavenge DPPH free radicals. Higher DPPH radical scavenging activity indicates greater antioxidant potential (Singh *et al.,* 2009). Tables 2 presented the DPPH radical scavenging activity of different concentrations (15, 60, and 120 µg/ml) of the leaf and flower extracts of *L. cardiaca* ecotypes from Khansar and Kerman using methanol and ethanol as solvents.

For the methanolic extracts, at a concentration of 15 µg/ml, the leaf extract had a radical scavenging activity of 15.0 ± 0.9% and 14.7±1.1% for Khansar and Kerman ecotypes, respectively, while the flower extract exhibited 10.1 ± 1.3% and 10.1 ± 1.0% activity. At higher concentrations of 60 and 120 µg/ml, the leaf extract had higher activity than the flower extract, with values ranging from 58.9 ± 1.1% to 62.2 ± 1.0% and 53.0 ± 1.2% to 59.0 ± 1.2% for the leaf extract and 48.1 ± 1.1% to 55.6 ± 1.0% and 40.7 ± 1.2% to 48.4 ± 1.3% for the flower extract for Khansar and Kerman ecotypes, respectively.

Based on the data in Table 3, only the flower extracts of the two ecotypes tested at 15 µg/ml failed to exhibit a statistically significant difference in DPPH radical scavenging activity, with 10.1 ± 1.3% and 10.1 ± 1.0% inhibition for Khansar and Kerman flowers, respectively. All other comparisons between leaf/flower extracts within and between the ecotypes demonstrated significant divergences in antioxidant activity.

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was employed to further investigate the antioxidant potential of *L. cardiaca extracts*. This assay assesses a plant extract's ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), with a higher absorbance value indicating greater reducing power and, consequently, stronger antioxidant activity (Benzie and Strain, 1999). Table 4 presented the ferric reducing power of different concentrations (100 to 400 µg/ml) of leaf and flower extracts obtained from two ecotypes of *L. cardiaca*, Khansar and Kerman, using two solvents, methanol and ethanol. The results indicated statistically significant differences in ferric-reducing power in all comparisons: within the Khansar ecotype, increasing the concentration of the methanolic extract led to a significant enhancement in ferric-reducing power for both leaf and flower extracts (Table 4). For instance, the leaf extract exhibited an absorbance value of 0.228 ± 0.004 at 100 µg/ml, which increased to 0.437 ± 0.005 at 400 µg/ml. A similar trend was observed for the flower extracts, with values rising from 0.192 ± 0.008 to 0.358 ± 0.006 as the concentration increased to 400 µg/ml (Table 4).

Similarly, the ethanolic extracts also exhibited a concentration-dependent increase in ferric reducing power, although the overall reducing power remained lower compared to the methanolic extracts at each concentration tested (Table 3). For example, the leaf extract of the Khansar ecotype exhibited a value of 0.026 ± 0.003 at 100 µg/ml, which increased to 0.313 ± 0.008 at 400 µg/ml. The flower extracts followed a similar trend, with values rising from 0.019 ± 0.002 at 100 µg/ml to 0.263 ± 0.005 at 400 µg/ml.

In line with the findings from the analyses of TPC, TFC, and TFLC, the results of the DPPH and FRAP assays further reinforce the link between the observed antioxidant potential and various factors (Table 4). Generally, plant extracts with higher levels of phenolic compounds, particularly flavonoids and flavonols, displayed stronger antioxidant potential as evidenced by greater DPPH radical scavenging activity and higher FRAPS values. This finding aligns with the observation that the methanolic extract of the Khansar ecotype, which exhibited the highest content of flavonoids and flavonols, also demonstrated the strongest antioxidant activity in both assays. This strongly suggested that phenolic compounds, especially flavonoids and flavonols, play a significant role in the antioxidant capacity of *L. cardiaca* leaves and flowers.

Furthermore, a clear correlation between plant part and antioxidant potential was observed. Leaf extracts, consistently containing higher levels of TPC, TFC, and TFLC, displayed the strongest DPPH radical scavenging activity and ferric reducing power across all tested concentrations. Conversely, flower extracts with lower phenolic compound content exhibited weaker antioxidant activity. This observation strengthens the link between phenolic content and antioxidant potential.

Finally, the solvent employed for extraction significantly impacted the observed antioxidant activity. Within both leaf and flower extract groups of each ecotype, methanolic extracts with higher levels of phenolic compounds generally displayed a superior ability to scavenge free radicals and reduce ferric ions compared to their ethanolic counterparts with lower phenolic content. This preferential extraction by methanol might be attributed to its higher polarity compared to ethanol. Polar solvents, like methanol, are more efficient at extracting polar compounds, potentially including the phenolic antioxidants responsible for the observed antioxidant activity in *L. cardiaca* extracts (Atwi-Ghaddar *et al.,* 2023). This combined analysis from the phytochemical profiling, DPPH and FRAP assays highlights the importance of phenolic compounds, particularly flavonoids and flavonols, in the antioxidant potential of *L. cardiaca*. Additionally, it emphasizes the influence of ecotype, plant part, and solvent selection on the overall antioxidant activity of the extracts.

Scolicidal effects

The growing interest in natural alternatives to conventional chemotherapeutics has driven research efforts towards identifying effective anti-*Echinococcus* compounds from plants. Several recent studies have demonstrated promising scolicidal activity of various herbal extracts (Gholami *et al.,* 2013; Mahmoudvand *et al.,* 2016; Mahmoudvand *et al.,* 2016; Mahmoudvand *et al.,* 2016; Mahmoudvand *et al.,* 2017), including *Pistacia atlantica* and *Sambucus ebulus*, against *E. granulosus* protoscoleces. Notably, Mahmoudvand et al. (2016) reported complete elimination (100%) of protoscoleces using *Pistacia atlantica* extract at 50 mg/ml after just 10 minutes of exposure. Similarly, Gholami *et al*. (2013) observed significant scolicidal activity of methanolic *Sambucus ebulus* extract at different concentrations and exposure times, with up to 98.6% protoscolicidal effect at 100 mg/ml after 60 minutes. These findings highlighted the potential of herbal medicines as a source for developing novel, natural anti-*Echinococcus* treatments.

Building upon the rationale for exploring natural anti-*Echinococcus* agents and the absence of prior research on *L. cardiaca*, this study investigated its scolicidal potential against *E. granulosus* protoscoleces. Given the established correlation between phenolic content and bioactivity observed in the phytochemical analysis, methanolic leaf extracts from both Khansar and Kerman ecotypes were selected for their demonstrably higher levels of TPC, TFC, and TFLC compared to their ethanolic counterparts. Our findings revealed a concentration- and exposure time-dependent efficacy, with higher concentrations and longer exposure times leading to a greater scolicidal effect on the *E. granulosus* protoscoleces (Table 4).

Both Khansar and Kerman ecotypes exhibited concentration-dependent scolicidal activity against *E. granulosus* protoscoleces. At the lowest concentration tested (20 mg/ml), the effects were minimal. The Khansar ecotype showed a mortality rate ranging from 7.3% after 1 minute to a maximum of 28.5% after 30 minutes of exposure. Similarly, the Kerman ecotype displayed limited activity at 20 mg/ml, with a mortality rate between 6.6% at 1 minute and 24% after 30

minutes. As the concentration increased to 40 mg/ml, a significant rise in scolicidal activity was observed for both ecotypes. At 40 mg/ml, the Khansar ecotype demonstrated its lowest efficacy after a 1-minute exposure, with a mortality rate of 13.6%. However, the efficacy increased with longer exposure times, reaching a maximum mortality rate of 55.3% after 30 minutes. Similarly, the Kerman ecotype displayed a minimum efficacy of 12.8% at the 1-minute mark. The scolicidal activity also rose with extended exposure time, achieving a maximum mortality rate of 46.8% after 30 minutes. This concentration-dependent trend continued at 60 mg/ml. The Khansar ecotype again showed its least effectiveness initially, with only 16.3% mortality after 1 minute. However, its efficacy significantly improved over time, reaching 72.9% after 30 minutes. Likewise, the Kerman ecotype displayed its lowest efficacy at 60 mg/ml after 1 minute (13%), but this value increased to 59.9% after 30 minutes. This trend became even more pronounced at 90 mg/ml. The Khansar ecotype demonstrated a marked rise in scolicidal activity compared to lower concentrations. While its lowest efficacy was still observed after 1 minute (51.9%), it achieved a significantly faster and more potent scolicidal effect, reaching a maximum mortality rate of 100% after only 20 minutes of exposure. Similarly, the Kerman ecotype displayed a minimum efficacy of 49.2% after 1 minute at 90 mg/ml, but its efficacy rose considerably after 30 minutes, reaching a maximum of 82.6%. These findings suggest a threshold concentration for the Khansar ecotype, where scolicidal activity becomes significantly faster and more potent at 90 mg/ml compared to lower concentrations.

The concentration-dependent trend in scolicidal efficacy exhibited a complex pattern at the highest concentration tested (120 mg/ml). While both ecotypes achieved their highest efficacy at this concentration, the most effective time point differed. For the Khansar ecotype, the lowest efficacy was observed after 1 minute (88.8%), but it achieved complete scolicidal activity (100% mortality) after only 20 minutes of exposure. This suggests a rapid increase in scolicidal effect between 1 and 20 minutes at this concentration. In contrast, the Kerman ecotype displayed a minimum efficacy of 62.6% after 1 minute, and its efficacy continued to rise with longer exposure times, reaching a maximum of 100% mortality after 30 minutes. This observation suggests a more gradual increase in scolicidal activity for the Kerman ecotype even at the highest concentration. An effective scolicidal agent should have high potency at lower concentrations, exhibit high efficacy in a shorter time of exposure, be readily available and easy to prepare, and have low toxicity (Organization, 1988; Norouzi *et al.,* 2020). Our findings suggested that *L. cardiac* ecotypes have potent scolicidal activity comparable to existing agents such as 20% silver nitrate (20 minutes), 3% H_2O_2 (15 minutes), and 95% ethyl alcohol (15 minutes) (Mahmoudvand *et al.,* 2014).

The scolicidal activity of methanolic extracts from Khansar and Kerman ecotypes of *L. cardiaca* against *E. granulosus* protoscoleces exhibited concentration-dependent effects with distinct kinetic profiles. The Khansar ecotype extract demonstrated superior efficacy at high concentrations, achieving 100% protoscolex mortality after 20 minutes of exposure at 120 mg/ml, compared to 30 minutes for the Kerman ecotype. This observation aligns with the higher phenolic content previously noted in the Khansar ecotype. However, at lower concentrations (20 and 40 mg/ml), the Khansar extract exhibited a slower initial scolicidal effect, with mortality rates increasing gradually over 20-30 minutes. This suggests the possibility of a threshold concentration required for optimal scolicidal activity in the Khansar ecotype. Conversely, the Kerman ecotype extract displayed a more rapid initial scolicidal effect at lower concentrations, with a significant proportion of its overall activity occurring quickly. These divergent kinetic profiles indicate the potential influence of ecotype-specific factors on the scolicidal mechanisms.

Leonurus cardiaca extracts have demonstrated scolicidal activity against *E. granulosus* protoscoleces, likely due to the presence of various bioactive compounds, particularly phenolic compounds. These compounds can exert their anthelmintic effects through multiple mechanisms. One potential mechanism involves the antioxidant properties of phenolic compounds. While antioxidants are generally beneficial in reducing oxidative stress, excessive concentrations can lead to pro-oxidant effects, generating reactive oxygen species (ROS). These ROS can damage cellular components and ultimately lead to parasite death (Dai and Mumper, 2010). Additionally, phenolic compounds can interact with the parasite's cell membrane, disrupting its integrity and causing leakage of intracellular contents (Verma *et al.,* 2014). Furthermore, they may interfere with the parasite's metabolic processes, inhibiting essential enzymes or pathways required for survival (Dai and Mumper, 2010). Taking all these into consideration, main mechanisms involved in scolicidal effects of phytochemicals remain poorly understood and elucidation of these mechanisms awaits further

studies.

In conclusion, this study demonstrated a positive correlation between phenolic content and scolicidal activity against *E. granulosus* protoscoleces in *L. cardiaca* extracts. The Khansar ecotype, characterized by higher levels of TPC, TFC, and TFLC, exhibited greater scolicidal potency, suggesting the involvement of these phenolic compounds in the antiparasitic effect. Additionally, the higher phenolic content in the Khansar ecotype implies potentially enhanced antioxidant activity, which may contribute to the observed scolicidal effect, although further investigation is warranted. To elucidate the specific bioactive compounds responsible for the scolicidal activity and their underlying mechanisms of action, future research should focus on compound identification, quantification, and in-depth mechanistic studies. A comprehensive understanding of these aspects can significantly contribute to optimizing *L. cardiaca* extracts as a potential therapeutic strategy for cystic echinococcosis.

Table 1. Total phenolic (TPC), total flavonoid content (TFC), and total flavonol content (TFLC) of the *Leonurus cardiaca* ecotypes.

		TPC (mg GAL/g)		TFC (mg QE/g)		TFLC (mg QE/g)	
Sample	**Extracts**	**Leaf**	**Flower**	**Leaf**	**Flower**	**Leaf**	**Flower**
Khansar	Methanolic	37.6 ±1.2^{a}	32.2 ±1.5^{b}	21.6 ±1.1^{a}	9.1 ±1.0^{e}	7.5 ±1.0^{a}	4.5 ±1.0^{d}
	Ethanolic	32.2 ±1.0^{b}	26.9 ±1.3^{e}	12.2 ±1.2^{d}	8. 2 ±1.1^{f}	5.1 ±1.0^{c}	4.1 ±1.1^{d}
Kerman	Methanolic	31.1 ±1.4^{c}	28.9±1.2^{d}	15.6 ±1.1^{b}	9.6 ±1.2^{e}	6.6 ±1.1^{b}	4.5 ±1.0^{d}
	Ethanolic	29.7 ±1.2^{d}	26.7 ±1.0^{e}	13.7 ±1.3^{c}	8.0 ±1.0^{f}	4.6 ±1.2^{cd}	4.1 ±1.1^{d}

*Mean ± standard deviation. Values with the same letter have no significant difference. GAL = gallic acid, QE = quercetin equivalents

Table 2. DPPH radical scavenging activity of leaf and flower of *Leonurus cardiaca* ecotypes.

Extract	**Concentration (µg/ml)**	**Khansar**		**Kerman**	
		Leaf	**Flower**	**Leaf**	**Flower**
Methanolic	15	15.0 ±0.9^{p}	10.1±1.3^{u}	14.7±1.1^{r}	10.1±1.0^{u}
	60	58.9±1.1^{c}	48.1±1.1^{g}	53.0 ±1.2^{e}	40.7±1.2^{l}
	120	62.2±1.0^{a}	55.6±1.0^{d}	59.0 ±1.2^{b}	48.4±1.3^{f}
Ethanolic	15	14.8±1.0^{q}	10.2±1.0^{t}	13.1±1.0^{s}	9.9±1.0^{v}
	60	40.8±0.9^{k}	36.8±1.4^{n}	39.7±1.1^{m}	33.1±1.1^{o}
	120	48.4±1.2^{f}	46.0 ±1.3^{i}	46.8±1.2^{h}	43.0 ±1.2^{j}

* Values with same letter have no significant difference

Table 3. Ferric reducing power of leaf and flower extracts of *Leonurus cardiac* ecotypes.

Extract	**Concentration (µg/ml)**	**Khansar**		**Kerman**	
		Leaf	**Flower**	**Leaf**	**Flower**
Methanol	100	0.228±0.004^{j}	0.192±0.008^{o}	0.226±0.006^{m}	0.165±0.003^{p}
	200	0.351±.007^{d}	0.250±0.007^{k}	0.308±.008^{f}	0.199±0.004^{n}
	400	0.437±0.005^{a}	0.358±006^{c}	0.427±0.005^{b}	0.267±0.006^{i}
Ethanol	100	0.026±0.003^{u}	0.019±0.002^{v}	0.017±0.002^{w}	0.015±0.003^{x}
	200	0.132±.006^{q}	0.130±0.004^{r}	0.105±0.003^{s}	0.098±0.004^{t}
	400	0.313±0.008^{e}	0.263±0.005^{j}	0.293±0.002^{g}	0.281±0.007^{h}

Note: Values with the same letter have no significant difference

Table 4. Scolicidal effect of *Leonurus cardiaca* ecotypes against protoscoleces of *Echinococcus granulosus* at various concentrations after various exposure times.

Ecotype	Concentration mg/ml	Mean of mortality rate (%)					
		Exposure time (min)					
		1	3	5	10	20	30
Khansar	20	7.3	17.8	19	21.5	25.9	28.5
	40	13.6	18.7	23.2	31.2	39.3	55.3
	60	16.3	29.6	33.8	44.6	59.9	72.9
	90	51.9	54.7	60.9	73.4	88.3	100
	120	88.8	89.5	92.4	97.5	100	100
Kerman	20	6.6	15.8	16	18.4	23.7	24
	40	12.8	17.4	18.2	26.3	33.4	46.8
	60	13	20	26.5	35.6	45.2	59.9
	90	49.2	49.6	56.2	57.2	71.5	82.6
	120	62.6	83	89.2	91.3	94.5	100

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Conflict of Interest

The authors declare no conflict of interest.

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