Eurasia Specialized Veterinary Publication

International Journal of Veterinary Research and Allied Science

ISSN:3062-357X

2023, Volume 3, Issue 2, Page No: 52-62

Available online at: www.esvpub.com/

In Vitro Sporicidal Activity of Vitis vinifera Leaf Methanolic Extract Against Echinococcus granulosus Protoscolices and Eggs

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ABSTRACT

Echinococcosis is a zoonotic infection induced by Echinococcus species and is among the most significant helminthic diseases worldwide. Surgical intervention remains the preferred treatment for cystic echinococcosis. Numerous sporicidal substances have been applied to deactivate components within hydatid cysts; however, many of them trigger inflammation and adverse reactions, limiting their clinical use. This study investigated the scolicidal activity of methanolic extract from Vitis vinifera leaves against Echinococcus eggs and protoscolices, aiming to identify the optimal concentration. Protoscolices mortality and viability were examined following exposure to four V. vinifera leaf extract (VVLE) concentrations (5, 10, 30, and 50 mg/mL) for 5, 10, 20, and 30 minutes, while eggs were treated with 100, 200, and 300 mg/mL concentrations for 24 and 48 hours. Infrared spectroscopy was used to identify the major bioactive compounds within the extract. Viability testing was performed with 0.1% eosin staining. The grape leaf extract produced 100%, 91%, 60%, and 41% mortality rates after 30 minutes at 50, 30, 10, and 5 mg/mL, respectively, and caused 11% and 19% egg mortality after 24 and 48 hours at 200 mg/mL. Increasing the concentration and exposure time generally enhanced mortality. These findings indicate that V. vinifera has strong in vitro sporicidal potential. Further research is necessary to isolate the active compound, clarify its mechanism of action, and confirm efficacy through in vivo testing.

Keywords: Echinococcus, Hydatid cyst, Carnivores, Vitis vinifera leaf, Scolicidal

Received: 01 June 2023
Revised: 01 October 2023
Accepted: 05 October 2023

How to Cite This Article: Torres J, Morales P, Navarro J. In Vitro Sporicidal Activity of Vitis vinifera Leaf Methanolic Extract Against Echinococcus granulosus Protoscolices and Eggs. Int J Vet Res Allied Sci. 2023;3(2):52-62.
https://doi.org/10.51847/1LXtOsqQ5j

Introduction

Hydatidosis is a chronic zoonotic disorder of medical and veterinary relevance, caused by the larval stage of Echinococcus granulosus. Cystic echinococcosis (CE) poses serious health and economic challenges globally [1]. The parasite is endemic in extensive areas of Asia, including East Asian nations, the former Soviet territories of Central Asia, northern China, and northeastern Siberia. Its prevalence is also reported in parts of the eastern Mediterranean and Gulf-bordering Arab states [2], as well as throughout large portions of Europe [3].

Adult worms inhabit the small intestines of dogs and other carnivores, while the larval form develops in intermediate hosts such as sheep, cattle, pigs, horses, and humans [4]. Infection in humans occurs via ingestion of parasite eggs through direct contact with contaminated dog feces or polluted environments [5]. Once ingested, the embryo penetrates the intestinal wall and reaches the liver (40–70%), lungs (30–40%), or other organs via the portal circulation, forming hydatid cysts [6]. These cysts comprise two parasitic layers—an inner germinal layer and an outer laminated membrane—enclosed by a fibrous capsule produced by the host's immune reaction. They

typically enlarge by 1–5 cm annually, depending on tissue density [7]. Each cyst may contain millions of protoscolices that develop into adult worms in the small intestine of carnivores after ingestion of infected organs [8].

Liver cysts can lead to upper abdominal discomfort, hepatomegaly, cholestasis, biliary cirrhosis, portal hypertension, and ascites. Ruptured cysts may cause anaphylactic shock or cholangitis, while secondary bacterial infection can form abscesses. Pulmonary involvement results in chronic cough, sputum, dyspnea, hemoptysis, pleuritis, and abscesses, and cerebral cysts may trigger neurological symptoms [9]. Approximately 90% of cases involve hepatic or pulmonary cysts, with 2–4% detected in the kidney, spleen, peritoneum, skin, or muscle, and rare cases in the brain or heart [10].

Surgical removal remains the standard treatment for hydatidosis in many regions, including Saudi Arabia [11]. However, surgery carries the risk of intraoperative protoscolex leakage, leading to recurrence and secondary hydatid infections [12]. Common scolicidal solutions include hypertonic saline, silver nitrate, cetrimide, povidone-iodine, and ethanol, though these often cause hepatotoxicity, necrosis, or methemoglobinemia [10, 13, 14]. Thus, researchers continue exploring safer, plant-based alternatives with reduced toxicity for hydatid disease management [15, 16].

Natural plant extracts are gaining interest due to their low cost, minimal side effects, and broad accessibility [17]. Vitis vinifera, a perennial woody vine native to Asia, has been widely used in traditional medicine, especially its fruit and leaves [18]. The plant is rich in bioactive antioxidants such as flavonoids, catechins, anthocyanins, and epicatechins [19]. The aqueous leaf extract exhibits antibacterial action against E. coli, Enterococcus faecalis, and Staphylococcus aureus [20]. Moreover, grape seed extract has shown antiparasitic effects against Trichostrongylus colubriformis and Ostertagia circumcincta in sheep [21] and has been utilized in treating diarrhea [22].

The present research was designed to assess, under in vitro conditions, the scolicidal efficacy of methanolic V. vinifera leaf extract on hydatid cyst protoscolices.

Materials and Methods

Preparation of extract

Fresh Vitis vinifera leaves were purchased from a local market in Riyadh, Saudi Arabia. The species was authenticated by a taxonomist from the Botany Department, King Saud University. A total of 500 g of leaves were air-dried at 44 °C and then pulverized with an electric grinder until a uniform particle size of about 0.25–0.30 mm was achieved. Subsequently, 120 g of the powdered material was immersed in 400 mL of 70% methanol and mixed with a magnetic stirrer for one hour. The solution was kept under continuous stirring for 24 h at ambient temperature and filtered afterward. The extract was concentrated using a rotary vacuum evaporator (Yamato RE300, Tokyo, Japan) and then re-dissolved in distilled water for the planned experimental evaluations.

Infrared spectroscopy

The extract sample was blended with potassium bromide powder at a 1:99 weight ratio and compressed into pellets. Fourier-transform infrared (FT-IR) spectra were recorded using a Thermo Scientific NICOLET 6700 spectrometer (Waltham, MA, USA). Absorbance was expressed in wave numbers (cm^{-1}) covering the range of 4000–400 cm^{-1}, measured at 25 °C with a spectral resolution of 4 cm.

Collection of dog fecal samples and egg recovery

Thirty fecal specimens were obtained from multiple areas within Al-Kharj, Saudi Arabia, where both domestic and stray dogs coexist with livestock. Samples were transferred into sterile 50 mL tubes and delivered to the Parasitology Laboratory, Department of Zoology, King Saud University, where they were stored at 4 °C until analysis. Parasite eggs were isolated using direct wet mount microscopy and sedimentation, followed by centrifugation at 1008× g for 10 minutes [23]. Eggs were gently rinsed from the coverslips with a 0.9% NaCl solution and maintained at −20 °C until further use.

In vitro evaluation of extract on eggs

Extract solutions were prepared at three concentrations (100, 200, and 300 mg/mL). For each assay, 1 mL of an egg suspension (approximately 700 eggs) was placed into a test tube, then 1 mL of V. vinifera leaf extract (VVLE) was added. The mixtures were incubated for 24 and 48 h at room temperature. After incubation, 0.5 mL of each

mixture was combined with 0.5 mL of 0.1% eosin. After 30 min, samples were mounted on slides and observed microscopically. Unstained eggs were recorded as viable, while red-stained eggs indicated death. Percent viability was determined by counting at least 150 eggs. Control samples consisted of 1 mL of distilled water containing roughly 700 eggs.

Collection of protoscolices

Hydatid cysts were collected from the livers and lungs of slaughtered livestock at the Al-Kharj abattoir and transferred to the Parasitology Laboratory, King Saud University. The cysts were opened using sterile scalpels, and the hydatid fluid was drawn out with pipettes and left undisturbed for 30 minutes to allow protoscolices to settle. The supernatant was removed, and the sediment was washed three times with sterile saline. Viability was checked microscopically by observing muscular movement and by 0.1% eosin staining. Living protoscolices were kept in sterile saline at 4 °C in the dark for subsequent assays.

In vitro evaluation of extract on protoscolices

To test the scolicidal potential of V. vinifera leaf extract, concentrations of 5, 10, 30, and 50 mg/mL were prepared by dissolving 0.05, 0.1, 0.3, and 0.5 g of dried extract in 10 mL of distilled water, respectively. Each test contained 2 mL of protoscolices suspension (≈2700 protoscolices) and 2 mL of VVLE solution. Tubes were gently shaken and incubated at 37 °C for 5, 10, 20, and 30 min. After each interval, the upper liquid was removed carefully, 100 µL of 0.1% eosin was added to the residue, and after 5 minutes, slides were prepared for microscopic observation. Controls included 2 mL of distilled water containing approximately 2700 protoscolices.

Viability assessment

A 0.1% eosin solution (prepared by dissolving 1 g eosin powder in 1000 mL distilled water) was used for determining the viability of the protoscolices [23].

Statistical evaluation

Statistical comparisons were conducted using one-way ANOVA in SigmaPlot version 11.0. All analyses were two-tailed, and significance was set at $p \leq 0.001$.

Results and Discussion

The FT-IR spectral analysis of V. vinifera leaf extract revealed distinct absorption peaks at 3382.1, 2119.8, 1632.20, 1510.70, 1420.51, and 1030.32 cm^{-1} **(Figure 1)**. Functional group identification showed N–H stretching, N=C=S stretching, C=C stretching, tertiary C–O stretching, CO–O–CO stretching, and C–H bending within the 400–4000 cm^{-1} wavelength range **(Table 1)**.

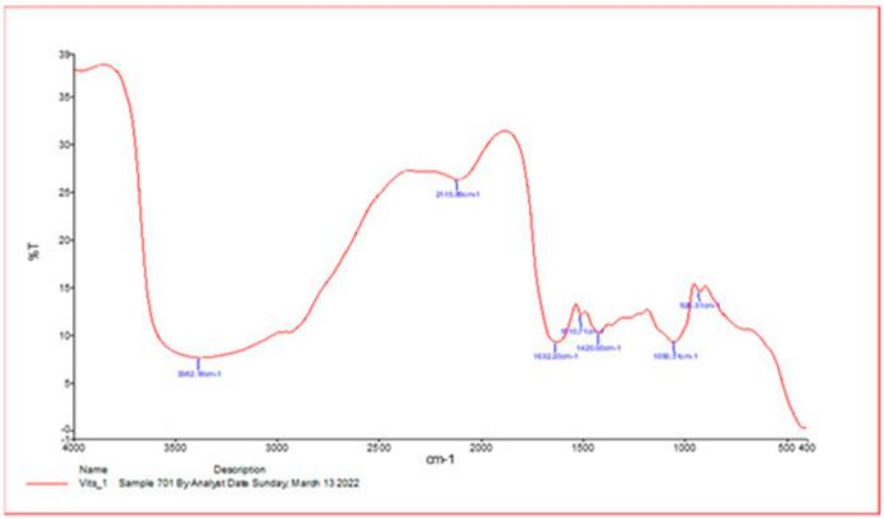
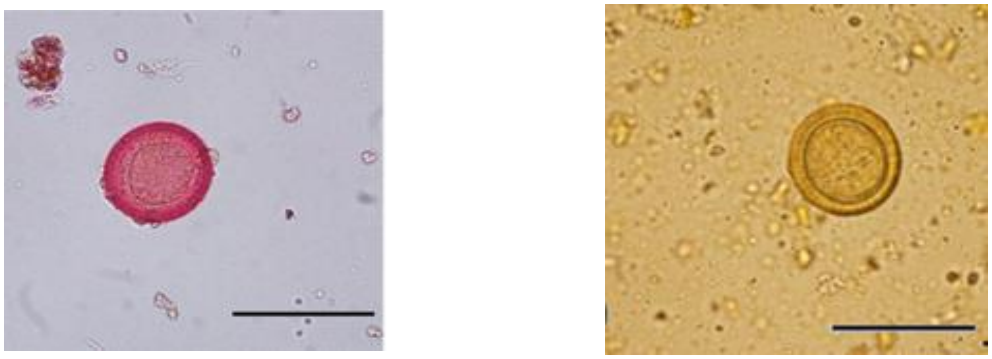


Figure 1. FT-IR spectral profile of Vitis vinifera leaf extract, showing absorption between 400–4000 cm^{-1} recorded using a Thermo Scientific NICOLET 6700 system.

Table 1. Frequency range of functional groups detected in Vitis vinifera leaf extract.

Wavenumber (cm^{-1})	Intensity	Transmittance (%)	Assignment	Functional Group / Compound Class
3425.5	Medium	12	N-H stretch	Aliphatic primary amine
2093.1	Strong	47	N=C=S stretch	Isothiocyanate
1641.4	Strong	25	C=C stretch	Alkene
1209.1	Strong	37	C-O stretch (tertiary)	Alcohol
1045.6	Strong, broad	35	CO-O-CO stretch	Anhydride
410.4	Strong	3	C-H bend	1,2-disubstituted

Unstained viable eggs retained their original appearance, whereas the nonviable ones absorbed the dye and appeared red **(Figure 2)**. The influence of different concentrations of VVLE on egg mortality is depicted in **Figures 3 and 4**. After 24 hours of exposure to Vitis vinifera leaf extract at 100, 200, and 300 mg/mL, the respective proportions of dead E. granulosus eggs were 2.6%, 11%, and 8%. Extending the exposure time to 48 hours resulted in 6%, 19%, and 13% mortality at the same concentrations. The 100 mg/mL dose showed significantly higher toxicity than the control (distilled water) for both incubation durations.



a) b)

Figure 2. Impact of V. vinifera leaf extract on egg viability. (a) Nonviable eggs stained with 0.1% eosin after exposure. (b) Viable eggs remained unstained. Scale bar = 20 µm.

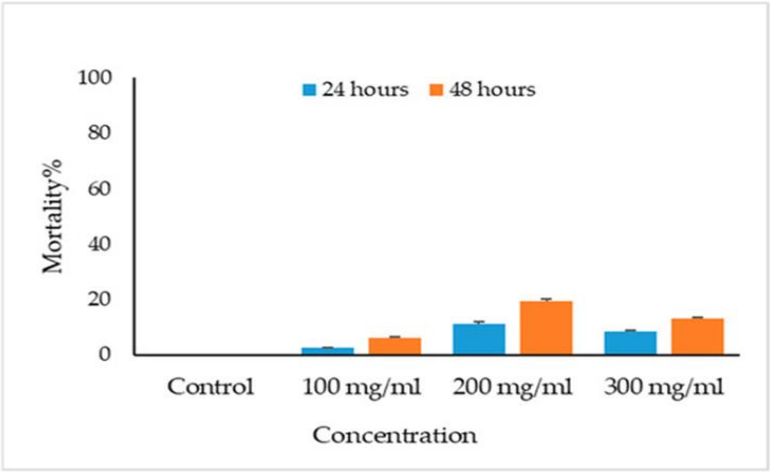


Figure 3. Mortality of E. granulosus eggs under various V. vinifera leaf extract concentrations (100, 200, and 300 mg/mL) and exposure times in vitro.

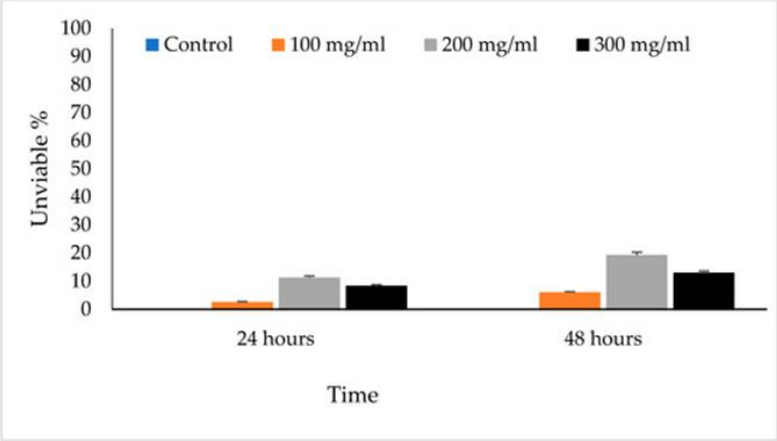
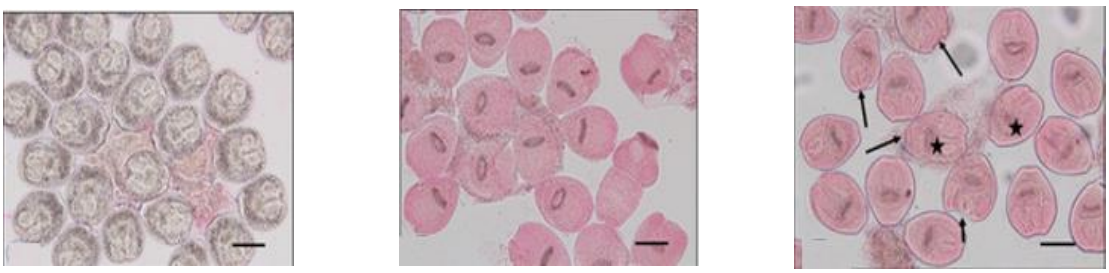


Figure 4. Comparison of viable egg percentages at 24 and 48 h following treatment with different VVLE concentrations in vitro.

When incubated for 30 min with 30 or 50 mg/mL of VVLE, mortality reached 93% and 100%, respectively, accompanied by wall disruption and hook fragmentation in the protoscolices **(Figure 5)**.



a) b) c)

Figure 5. (a) Viable protoscolices stained with 0.1% eosin. (b) Dead specimens following extract exposure. (c) Structural breakdown (arrows) and hook loss (asterisk). Scale bar = 10 µm.

A summary of the protoscolicidal effects of V. vinifera extract at varying doses revealed that after 5 minutes, 30 and 50 mg/mL caused 40% and 60% death rates, respectively, compared to ≤5% in controls. Doses of 5 and 10 mg/mL induced minimal lethality under the same conditions **(Figure 6)**.

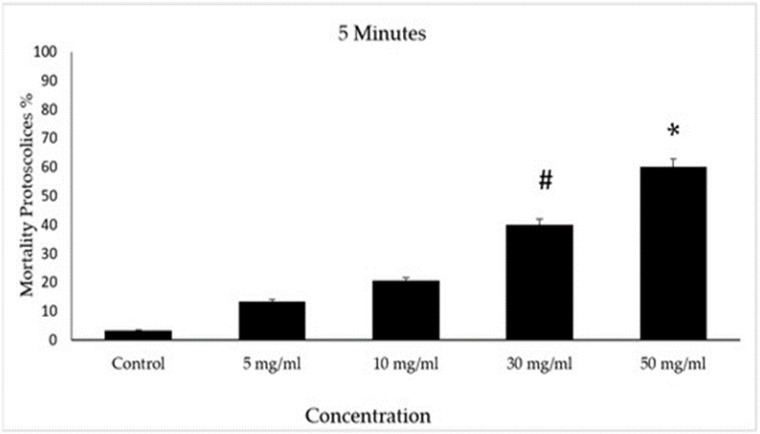


Figure 6. Mortality of protoscolices after 5 min of V. vinifera leaf extract exposure in vitro. Statistical relevance: (#) $p \leq 0.05$, (*) $p \leq 0.01$.

Following 10 minutes of incubation, the 30 and 50 mg/mL treatments resulted in about 70% and 75% mortality, while controls showed only 8%. At 5 and 10 mg/mL, the respective rates were 22% and 31% **(Figure 7)**.

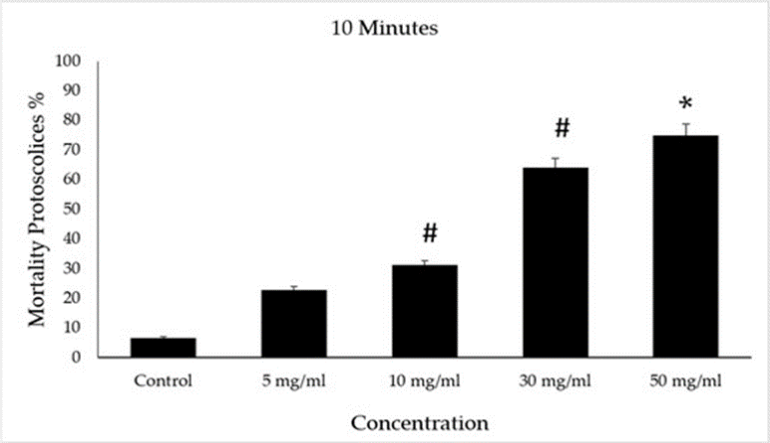


Figure 7. Effect of V. vinifera leaf extract on protoscolices after 10 min in vitro. Significance: (#) $p \leq 0.05$, (*) $p \leq 0.01$.

A 20-minute exposure increased the mortality further to roughly 83% and 95% at 30 and 50 mg/mL, respectively. Lower concentrations (5 and 10 mg/mL) showed a time-dependent increase in lethality up to 50%. The control mortality was 10% **(Figure 8)**.

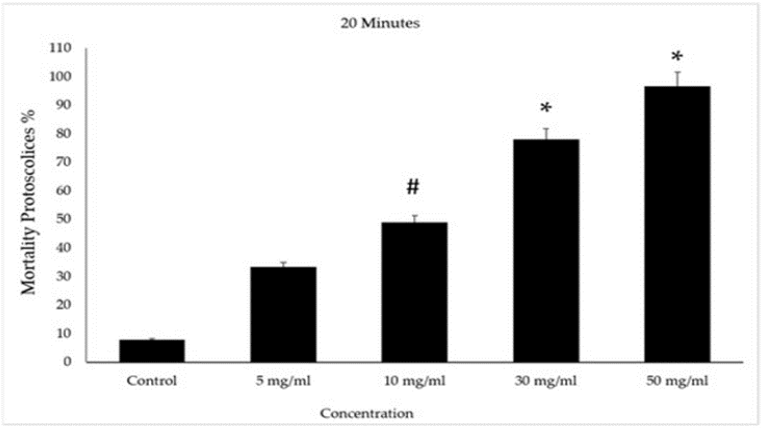


Figure 8. Mortality of protoscolices after 30 min of V. vinifera leaf extract exposure in vitro. (#): $p \leq 0.05$, (*): $p \leq 0.01$.

At 5 and 10 mg/mL, continued incubation caused mortality to reach 63%, whereas the control reached 14% **(Figure 9)**.

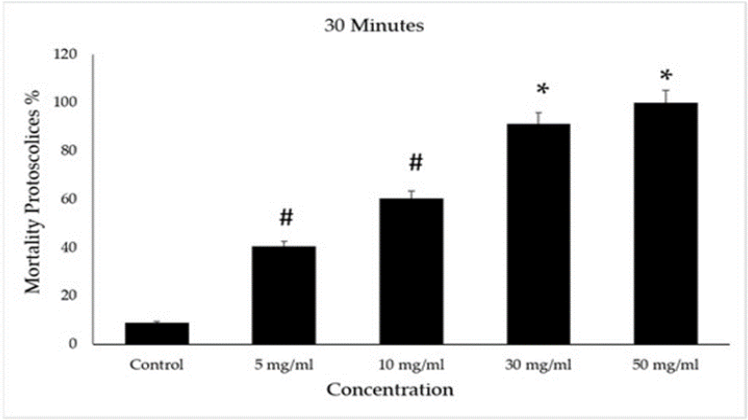


Figure 9. Mortality of protoscolices after 50 min exposure to V. vinifera leaf extract in vitro. (#): $p \leq 0.05$, (*): $p \leq 0.01$.

As illustrated in **Figures 10 and 11**, the duration of exposure and concentration both strongly influenced the proportion of dead protoscolices. Mortality rose progressively with longer incubation, whereas viability diminished. Significant differences ($p < 0.05$) were recorded up to the 20-minute mark, indicating notable variations between 5, 10, and 30 minutes.

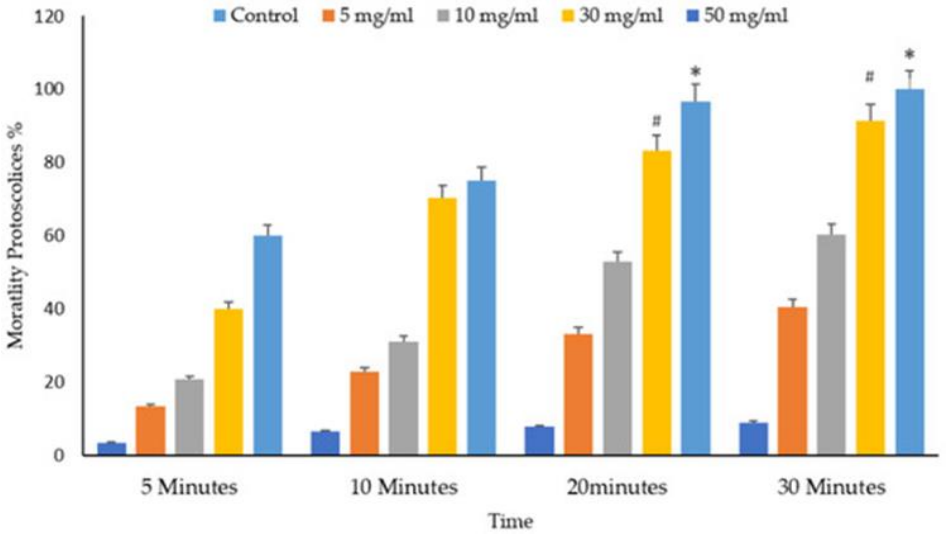


Figure 10. Mortality trends of protoscolices at various VVLE doses and contact times (5, 10, 20, and 30 min) compared with distilled water controls. Statistical notations: () $p \leq 0.01$, (#) $p \leq 0.05$.

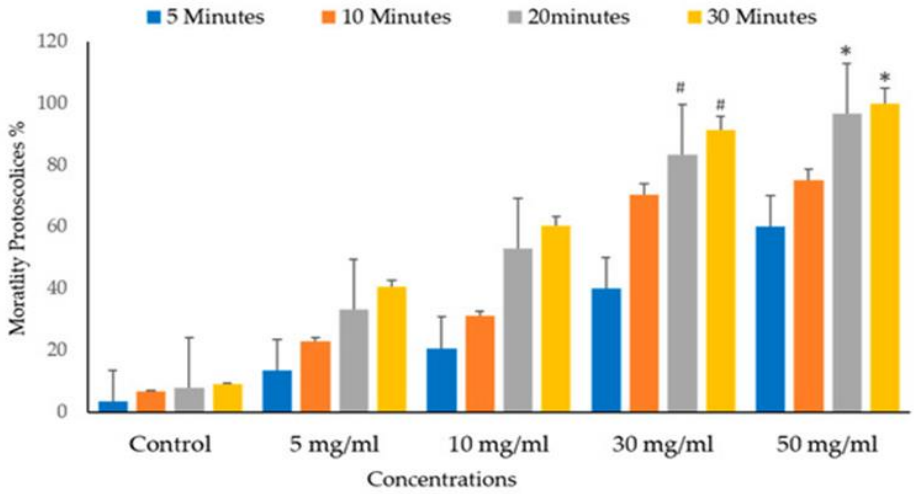


Figure 11. Mortality of protoscolices exposed to VVLE across multiple time points (5, 10, 20, and 30 mg/mL) in vitro, compared to the control. Significance: () $p \leq 0.01$, (#) $p \leq 0.05$.

The relationship between concentration, exposure time, and viability revealed that increased doses consistently enhanced mortality. Thus, higher extract levels yielded stronger scolicidal activity, with 50 mg/mL being the most effective. After 30 minutes, all concentrations displayed a dose-dependent response relative to the controls, with substantial protoscolicidal effects observed at 10, 30, and 50 mg/mL. The highest observed mortality (100%) was obtained at 50 mg/mL, while the lowest (12%) occurred at 5 mg/mL after 5 minutes **(Figure 12)**.

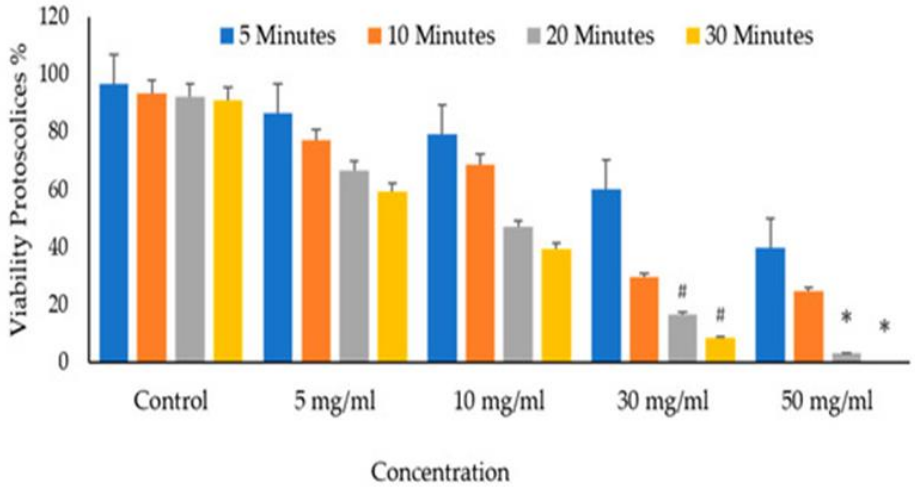


Figure 12. In vitro assessment of V. vinifera leaf extract on protoscolices viability after exposure at various concentrations for 5–30 min. Control: distilled water. Statistical significance: (*) $p \leq 0.01$, (#) $p \leq 0.05$.

The differences in death rates caused by V. vinifera leaf extract were statistically significant ($p < 0.01$) at 50 mg/mL during the 20–30 min intervals.

Although advances have been made in controlling hydatidosis, this zoonotic infection continues to pose a significant public health challenge in many endemic areas [24]. The annual rate of cystic echinococcosis varies from below one up to 200 cases per 100,000 individuals, while alveolar echinococcosis occurs at rates between 0.03 and 1.2 per 100,000 persons [25], with some localized regions showing notably higher prevalence (WHO/OIE, 2001). If left untreated, mortality exceeds 90% within 10–15 years following diagnosis [26]. Surgical intervention remains the most suitable therapy in selective patients. For those unable to undergo surgery, chemotherapeutic management using benzimidazoles and the PAIR method (puncture, aspiration, injection, and re-aspiration) is the preferred alternative [27].

Complications arising from cystic echinococcosis surgery include postoperative relapse, secondary cyst formation, and anaphylactic reactions due to accidental cyst rupture and fluid leakage, which occur in about 10% of affected individuals [13, 28]. Consequently, several adjunctive scolicidal agents—such as alcohol, povidone-iodine, and hypertonic saline—have been applied [14]. Despite the availability of various chemical scolicides aimed at neutralizing Echinococcus multilocularis cyst components, none have proven to be completely safe or highly effective [10].

In recent years, the biological potential of herbal extracts against protoscolices has received substantial attention. Certain studies have shown that plant-derived compounds can significantly influence the survival of these larval forms [29]. The current investigation assessed the scolicidal potential of Vitis vinifera methanolic leaf extract against hydatid cyst protoscolices. Traditionally, V. vinifera leaves have been used to manage ailments such as hepatitis, diarrhea, and gastric pain [10]. Moreover, the extract is known to exhibit antibacterial effects against Escherichia coli, Enterococcus faecalis, and Staphylococcus aureus [20]. Both aqueous and methanolic extracts of V. vinifera leaves and seeds have demonstrated inhibitory activity against Candida albicans, Candida glabrata, and Candida tropicalis [30, 31]. In poultry infected with Eimeria tenella, grape seed supplementation combined with organic zinc slightly enhanced growth performance and reduced lesion scores and oocyst shedding.

Effective hydatidosis prevention involves deactivating E. granulosus eggs. In this experiment, all concentrations of V. vinifera extract (VVE) significantly increased egg mortality compared to the control (distilled water). The strong efficacy of V. vinifera may be linked to its antioxidant constituents—such as flavonoids, catechins, anthocyanins, and epicatechins—that damage Echinococcus eggs in vitro [32]. Similar results were described by [26], who noted a concentration-dependent inhibitory effect of grape leaf extract on Eimeria oocyst sporulation.

Among the tested doses, 200 mg/mL of V. vinifera extract produced the highest egg mortality, potentially due to active phytochemicals functioning optimally at this concentration compared to 100 and 300 mg/mL. Previous research [4] demonstrated that methanolic V. vinifera extract exerted anti-leech effects on Limnatis nilotica in vitro, suggesting its potential as a complementary antiparasitic agent. In our findings, V. vinifera leaf extract caused marked protoscolicidal activity across all concentrations and incubation periods. Specifically, 50 mg/mL

of the methanolic extract induced 96.7% and 100% protoscolices death after 20 and 30 minutes of exposure, respectively. These results confirm that the 50 mg/mL concentration exerts the strongest lethal action within shorter exposure intervals.

Even the lowest dose (5 mg/mL) showed significantly higher lethality than the negative control throughout all treatments. Thus, V. vinifera extract demonstrates potent scolicidal efficiency at relatively low concentrations compared with other botanical preparations tested for similar purposes. The degree of scolicidal activity generally varies widely among different plant species. For instance, Nectaroscordum tripedale extract at 100 mg/mL achieved complete (100%) protoscolices death after 5 minutes of exposure [33]. Likewise, [34] observed total mortality using Olea europaea leaf extract at 300 and 150 mg/mL following 10 and 20 minutes of treatment, respectively.

In the present study, 50 mg/mL of V. vinifera extract caused severe structural damage to the protoscolices, including rupture of the walls and detachment of hooks. Correspondingly, [20] reported that aqueous and ethanolic grape extracts disrupted both the nuclear and cytoplasmic membranes of Leishmania infantum promastigotes, leading to morphological deformation. This may be attributed to alcohol content within the extract, which compromises membrane integrity. Supporting studies [35, 36] have also noted that alcohol impacts the membranes and viability of multiple cell types—such as embryonic, hepatic, intestinal, bone marrow–derived, and neuronal stem cells. To our knowledge, this is the first investigation demonstrating the scolicidal efficacy of methanolic V. vinifera leaf extract.

Conclusion

The findings of this study indicate that methanolic extracts from Vitis vinifera leaves can compromise the outer structures of both eggs and protoscolices of Echinococcus granulosus, reducing their survival under in vitro conditions. Further in vivo evaluations are recommended to determine the therapeutic potential and safety of this extract or its purified components as alternative treatments for echinococcosis.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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