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Essential Oil Against Echinococcus Granulosus, Protoscolicidal Activity of C. Bergamia

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ABSTRACT

Cystic echinococcosis, caused by Echinococcus granulosus, is a zoonotic disease with significant global health and economic impacts. Current treatments, including surgery and chemotherapy, face challenges such as recurrence and toxicity. This study evaluated the in vitro protoscolicidal activity of C. bergamia essential oil against E. granulosus protoscoleces. Hydatid cysts were collected from sheep livers in Karbala, Iraq, and protoscoleces were exposed to four concentrations (1,250, 2,500, 5,000, and 10,000 ppm) of the oil. Viability was assessed using eosin staining at 0 and 10 minutes post-exposure. The oil exhibited concentration- and time-dependent lethality, with 10,000 ppm achieving 100% mortality instantly and lower concentrations reaching complete lethality within 10 minutes. These findings highlight C. bergamia essential oil as a potent natural protoscolicidal agent, offering a promising alternative to conventional therapies. Further in vivo studies are needed to validate its efficacy and safety

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INTRODUCTION

Cystic echinococcosis (CE), caused by the larval stage of Echinococcus granulosus, is a zoonotic parasitic disease prevalent in pastoral regions, including the Middle East and Iraq [1]. Hydatid cysts, commonly found in the liver and lungs, can cause severe complications, such as cyst rupture and anaphylactic shock, if untreated [2]. Current treatments, including surgical cyst removal and chemotherapy with benzimidazoles like albendazole, face challenges such as secondary cyst formation, drug resistance, and toxicity [3]. Thus, there is an urgent need for safer and more effective alternatives.

Essential oils from medicinal plants have emerged as promising antiparasitic agents due to their bioactive compounds, such as terpenoids and phenolics [4]. Previous studies have reported protoscolicidal effects of essential oils from Zataria multiflora and Cuminum cyminum [5,6]. In Iraq, research on locally sourced essential oils has further highlighted their potential in combating CE, particularly in endemic regions [7]. Citrus bergamia (bergamot orange), a member of the Rutaceae family, is recognized for its antimicrobial and antifungal properties, attributed to compounds like limonene, linalool, and bergapten [8,9]. However, its efficacy against E. granulosus protoscoleces remains largely unexplored.

This study aimed to: (1) evaluate the in vitro protoscolicidal activity of C. bergamia essential oil against E. granulosus protoscoleces and (2) explore its potential as a natural alternative for CE treatment. The findings could inform the development of cost-effective therapies for endemic regions.

LITERATURE REVIEW

Cystic echinococcosis (CE), caused by the larval stage of the cestode *Echinococcus granulosus*, is a significant zoonotic parasitic disease. It is particularly prevalent in pastoral communities worldwide, including the Middle East and Iraq, where it poses considerable public health and economic challenges [10, 11]. The disease pathology is characterized by the development of hydatid cysts, which grow predominantly in vital organs such as the liver and lungs. If left untreated, these cysts can lead to severe and life-threatening complications, including cyst rupture and anaphylactic shock [12].

Current treatment modalities for CE primarily involve surgical intervention to remove the cysts and chemotherapy with benzimidazole derivatives, most notably albendazole. However, these established treatments are fraught with limitations. Surgical procedures carry the risk of intraoperative spillage of protoscoleces, potentially leading to secondary cyst formation and disease recurrence. Furthermore, chemotherapy regimens are often long, associated with significant host toxicity, and challenged by the emergence of drug-resistant parasite strains [12, 13]. These drawbacks underscore an urgent need for the development of safer, more effective, and readily available therapeutic alternatives.

In recent years, essential oils derived from medicinal plants have garnered attention as promising sources of novel antiparasitic agents, owing to their rich composition of bioactive phytochemicals, including terpenoids and phenolic compounds [14]. Several studies have demonstrated the potent *in vitro* protoscolicidal effects of various essential oils. For instance, essential oils from *Zataria multiflora* and *Cuminum cyminum* have shown high efficacy in killing *E. granulosus* protoscoleces [15, 16]. Research conducted within Iraq has further corroborated these findings, highlighting the potential of locally sourced medicinal plants in managing CE [17].

Citrus bergamia (bergamot), a fruit-bearing tree from the Rutaceae family, is well-documented for its potent antimicrobial and antifungal properties. These effects are largely credited to its primary chemical constituents, such as limonene, linalool, and bergapten [18, 19]. The proposed mechanism of action for these compounds involves the disruption of cellular membrane integrity, induction of oxidative stress, and impairment of mitochondrial function, which can culminate in apoptosis-like cell death in parasites [20]. Despite its established bioactivity, the specific efficacy of *C. bergamia* essential oil against the protoscoleces of *E. granulosus* remains largely unexplored. This knowledge gap forms the basis of the present study, which aims to investigate its potential as a novel natural protoscolicidal agent.

METHODOLOGY

Study Design

This in vitro study investigated the protoscolicidal activity of *C. bergamia* essential oil at four concentrations (1,250, 2,500, 5,000, and 10,000 ppm) against *E. granulosus* protoscoleces. Viability was assessed at 0 and 10 minutes post-exposure using eosin staining

Source of Hydatid Cysts

Hydatid cysts were collected from naturally infected sheep livers obtained from slaughterhouses in Karbala, Iraq. Livers were transported in ice-filled containers to the Parasitology Laboratory, Department of Medical Laboratory Techniques, Al-Safwa University College, and processed on the same day to ensure protoscolex viability, as recommended for optimal survival under controlled conditions [21].

Figure 1. Sheep Liver Infected with Hydatid Cysts

Collection of Protoscolices

Protoscolices were isolated using a modified Smith method [22]. Cyst surfaces were disinfected with 1% alcoholic iodine solution, and hydatid fluid was aspirated using a 10 mL syringe with a 21G needle. Cysts were incised, and the germinal layer was washed with a Pasteur pipette to collect protoscoleces. The fluid was left to settle for 10 minutes, and excess fluid was discarded to concentrate the protoscoleces.

Essential Oil Extraction

C. bergamia essential oil was extracted from dried peels purchased from a local market. Extraction was performed using a Clevenger steam distillation apparatus at the Department of Life Sciences, College of Science, University of Baghdad [23]. Briefly, 250 g of dried peels were boiled with 1.2 L of distilled water for 3 hours. The extracted oil was stored at 4°C until use

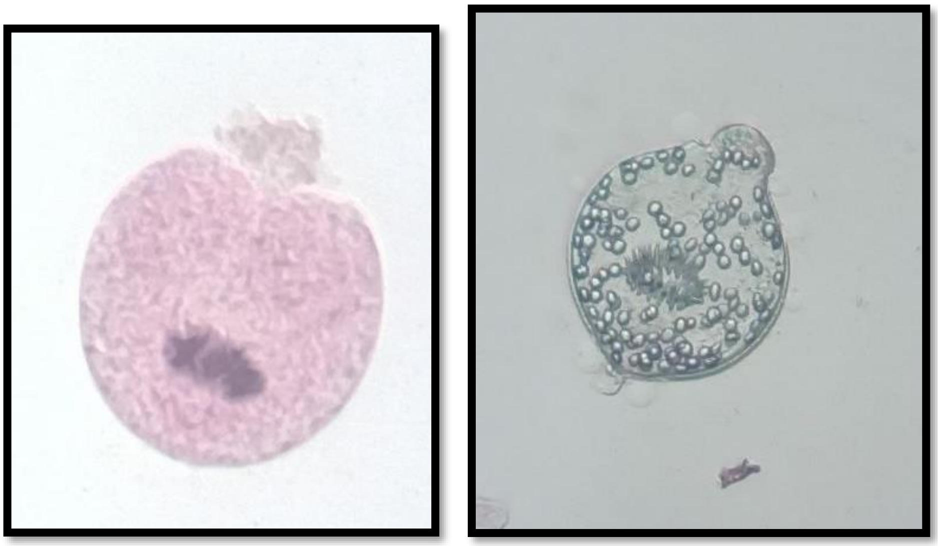
Figure 2. Clevenger Steam Distillation Apparatus Used or Extracting *C. Bergamia* Essential Oil [12]

Preparation of Concentrations

A stock solution (100,000 ppm) was prepared by dissolving 10 µL of essential oil in 90 µL of dimethyl sulfoxide (DMSO). Dilutions were made with hydatid fluid to achieve concentrations of 10,000, 5,000, 2,500, and 1,250 ppm. DMSO alone served as a control to account for its potential antiparasitic effects.

Viability Assessment

Protoscolices viability was determined using the eosin staining method, a reliable technique for assessing in vitro protoscolex survival [24,25]. A 5 µL sample of protoscoleces suspension was mixed with an equal volume of 0.1% aqueous eosin stain and examined under a compound microscope at 400x magnification. Dead protoscoleces stained red, while viable ones remained green. The number of protoscoleces per mL was calculated by multiplying the count in 5 µL by 100, with three replicates per sample.



Figure(3): Microscopic Image (400x) Showing Viability Staining of Hydatid Cyst Protoscoleces: Live (Green) Vs. Dead

Treatment and Data Collection

Protoscolices were incubated with the essential oil at 37°C in a water bath. Viability was assessed at 0 and 10 minutes post-treatment. Three 5 µL samples were examined per concentration and time point, and the percentage of dead protoscoleces was recorded.

Statistical Analysis

Data were analyzed using SPSS v22. One-way ANOVA was used to compare mortality rates across concentrations, with a significance level of $p < 0.05$. Results are presented as mean ± standard deviation.

RESULT

The protoscolicidal activity of *C. bergamia* essential oil was concentration- and time-dependent (Table 1). At 10,000 ppm, 100% mortality was observed immediately (0 min). Lower concentrations (5,000, 2,500, and 1,250 ppm) achieved 100% mortality after 10 minutes, with partial mortality at 0 min (Table 1). Linear regression analysis showed a strong positive correlation between oil concentration and mortality rate ($R^2 = 0.95$ at 0 min, $R^2 = 0.98$ at 10 min), with higher concentrations yielding faster effects ($p < 0.001$) (Figure 4).

Table 1. Percentage of *E. Granulosus* Protoscoleces Mortality at Different Concentrations of *C. Bergamia* Essential Oil.

Concentration (ppm(	**Mortality at 0 min(%)**	**Mortality at 10 min(%)**
10,000	**0 ± 100**	**0 ± 100**
5,000	**4.2 ± 85**	**0 ± 100**
2,500	**5.1 ± 60**	**0 ± 100**
1,250	**6.3 ± 45**	**0 ± 100**
Control (DMSO(	**2.1 ± 5**	**2.5 ± 7**
Data represent mean ± SD of three replicates.		

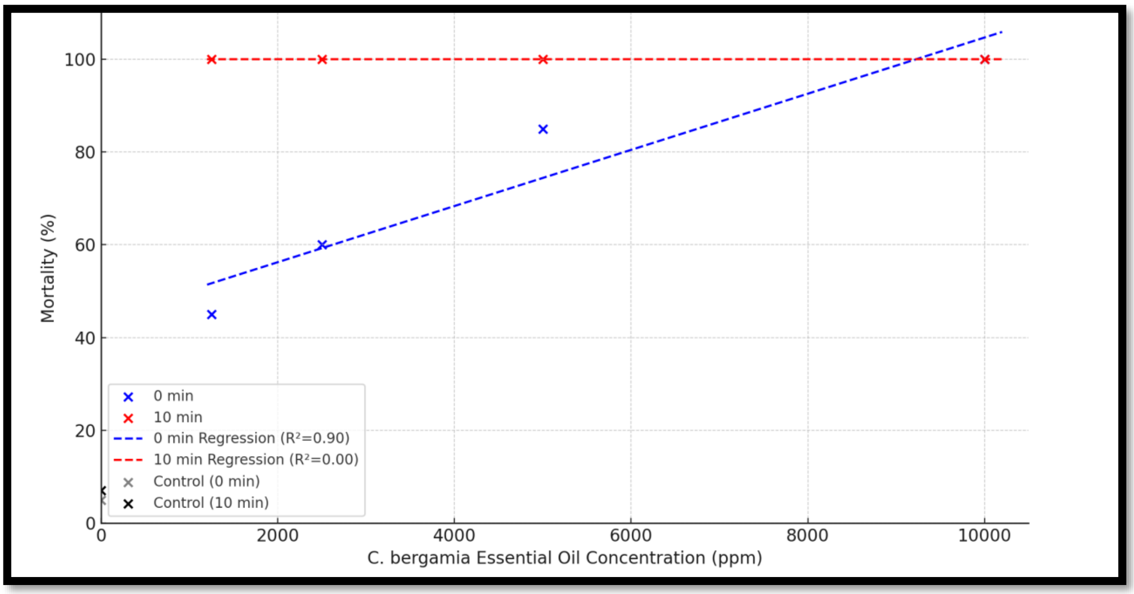


Figure 4. Linear Regression Analysis of *E. Granulosus* Protoscoleces Mortality (%) Versus C. *Bergamia* Essential Oil Concentration (Ppm) at 0 Min (Blue) and 10 Min (Red) [X]

DISCUSSION

This study demonstrates that C. bergamia essential oil possesses potent in vitro protoscolicidal activity against E. granulosus protoscoleces. At 10,000 ppm, the oil achieved 100% mortality instantly, outperforming conventional drugs like albendazole, which require over 120 hours for complete lethality [26]. Compared to other essential oils, such as Zataria multiflora (100% mortality at 6-8 mg/mL in 7-60 min) [5] and Cuminum cyminum (100% at 50 µL/mL in 20 min) [6], C. bergamia oil is effective at lower concentrations and shorter exposure times. These findings align with local studies in Iraq, which have reported protoscolicidal effects of essential oils from regional medicinal plants, highlighting their potential in CE management [7].

The protoscolicidal effect is likely due to bioactive compounds like limonene, linalool, and bergapten, which disrupt cellular membranes, induce oxidative stress, and impair mitochondrial function [27]. These mechanisms are consistent with reports of essential oils triggering apoptosis-like cell death in parasites [28]. The concentration-dependent effect suggests that lower doses may require extended exposure to overcome protective mechanisms in the cyst fluid, such as inhibitory proteins [29].

The oil's rapid action and natural origin make it a promising candidate for intraoperative sterilization of hydatid cysts, particularly in resource-limited settings like Iraq, where CE is endemic. However, the in vitro design limits immediate clinical applicability. The mild antiparasitic effect of DMSO, evidenced by 5-7% mortality in the control group (Table 1), necessitates further studies to isolate the oil's specific effects. Future research should focus on in vivo efficacy, bioavailability, toxicity, and potential synergies with drugs like albendazole to mitigate resistance risks.

CONCLUSIONS AND RECOMMENDATIONS

C. bergamia essential oil exhibits significant in vitro protoscolicidal activity, achieving 100% mortality at 10,000 ppm instantly and at 1,250 ppm within 10 minutes. These findings position the oil as a promising natural alternative for cystic echinococcosis treatment. Future studies should validate in vivo efficacy, assess toxicity, and develop stable clinical formulations. Synergistic studies with antiparasitic drugs and public health initiatives in endemic regions are recommended to translate these findings into practical solutions..

Conflicts of Interest

The authors declare no conflicts of interest.

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