In Vitro Scolicidal Potential of Citrus bergamia Essential Oil Against Echinococcus granulosus Compared to Albendazole

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Received: 28/5/2025 **Accepted:**25/5/2025 **Published:** 15/6/2025

***Abstract*— Cystic echinococcosis, caused by *Echinococcus granulosus*, poses significant health and economic challenges globally due to limited treatment options, including recurrence, drug resistance, and toxicity. This study evaluates the in vitro scolicidal potential of *Citrus bergamia* (bergamot) essential oil against *E. granulosus* protoscoleces compared to albendazole and a DMSO control. Hydatid cysts were collected from infected sheep livers in Karbala, Iraq, and protoscoleces were exposed to four concentrations (1,250, 2,500, 5,000, and 10,000 ppm) of each treatment. Viability was assessed using eosin staining over 1–116 hours. Bergamot oil at 10,000 ppm achieved 100% protoscolex mortality within 1 hour, compared to 36 hours for albendazole at the same concentration. Lower concentrations showed comparable efficacy by 48 hours. One-way ANOVA revealed significant differences ($p<0.05$). Bergamot oil's rapid action positions it as a promising natural alternative for cystic echinococcosis treatment, though in vivo studies are needed to confirm its efficacy and safety..**

***Keywords* — Citrus bergamia, Echinococcus granulosus, protoscoleces, bergamot essential oil, albendazole, protoscolicidal activity, in vitro.**

I. INTRODUCTION

THE 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, primarily affecting the liver and lungs, can lead to severe complications, such as cyst rupture and anaphylactic shock, if untreated [2]. Current treatment strategies, including surgical removal of cysts and chemotherapy with benzimidazoles like albendazole, are hindered by challenges such as secondary cyst formation, drug resistance, and toxicity (3). These limitations underscore the urgent need for safer, more effective alternatives to manage this neglected tropical disease.

To address these challenges, natural compounds, particularly essential oils, have emerged as promising antiparasitic agents due to their bioactive constituents, including terpenoids, phenolics, and flavonoids (4). Previous studies have demonstrated the scolicidal potential of essential oils from plants like Zataria multiflora and Cuminum cyminum, which achieved complete protoscolex mortality at specific concentrations and exposure times (5,6). In Iraq, research on locally sourced essential oils highlights their potential in combating CE, especially in endemic regions with limited access to advanced medical treatments (7)]. Among these, *Citrus bergamia* (bergamot), a member of the Rutaceae family, is renowned for its antimicrobial, antifungal, and anti-inflammatory properties, attributed to bioactive compounds such as limonene, linalool, and bergapten (8,9).

While bergamot's efficacy against various pathogens is well-documented, its scolicidal potential against *E. granulosus* protoscoleces remains underexplored. In contrast, albendazole, a standard chemotherapeutic agent for CE, requires prolonged exposure to achieve complete protoscolex mortality, often exceeding 120 hours, limiting its intraoperative applicability (10). This study aims to: (1) evaluate the in vitro scolicidal potential of *Citrus bergamia* essential oil against *E. granulosus* protoscoleces, (2) compare its efficacy with albendazole, and (3) explore its potential as a natural alternative for CE treatment. These findings could pave the way for cost-effective, nature-based therapies in endemic regions like Iraq.

Figure 1. Photograph of the *Citrus bergamia* .

II. Materials and Methods

Study Design

This in vitro study investigated the scolicidal potential of *Citrus bergamia* essential oil and albendazole against *Echinococcus granulosus* protoscoleces. Four concentrations (10,000, 5,000, 2,500, and 1,250 ppm) of each treatment were tested, with DMSO as the control. Protoscolices viability was assessed at multiple time points (1, 12, 24, 36, and 48 hours for both treatments, extending to 116 hours for bergamot oil) using eosin staining.

Source of Hydatid Cysts

Hydatid cysts were collected from naturally infected sheep livers obtained from slaughterhouses in Karbala, Iraq. The livers were transported in ice-filled containers to the Parasitology Laboratory, Department of Medical Laboratory Techniques, AlSafwa University College, Karbala, and processed on the same day to ensure protoscolex viability, as recommended for optimal survival under controlled conditions (11).

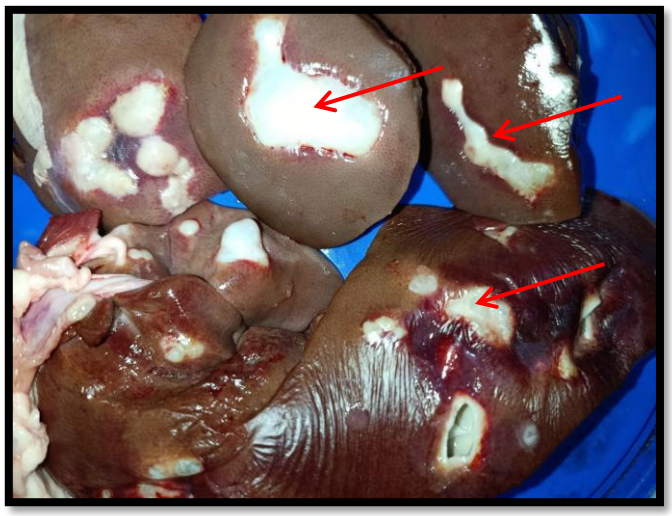

Figure 2. Photograph of a sheep liver infected with hydatid cysts.

Collection of Protoscolices

Protoscolices were isolated using a modified Smith method (12). Cyst surfaces were disinfected with a 1% alcoholic iodine solution, and hydatid fluid was aspirated using a 10 mL syringe with a 21G needle. Cysts were then incised, and the germinal layer was washed with a Pasteur pipette to collect protoscoleces. The fluid was allowed to settle for 10 minutes, and excess fluid was discarded to concentrate the protoscoleces.

Essential Oil Extraction and Albendazole Preparation

Citrus bergamia essential oil was extracted from dried peels of Citrus bergamia sourced from a certified supplier in Karbala, Iraq, ensuring consistency in fruit variety and quality. The extraction was performed using a Clevenger steam distillation apparatus at the Department of Life Sciences, College of Science, University of Baghdad (13). 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. Albendazole (Sigma-Aldrich, purity ≥ 98%) was procured and prepared as a stock solution in DMSO for subsequent dilution.

Preparation of Concentrations

Stock solutions (100,000 ppm) of bergamot oil and albendazole were prepared by dissolving 10 µL of the substance in 90 µL of DMSO. Serial dilutions were made with hydatid fluid to achieve test 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 (14,15). A 5 µL sample of the 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 (unstained). The number of protoscoleces per mL was calculated by multiplying the count in 5 µL by 100, with three replicates per sample.

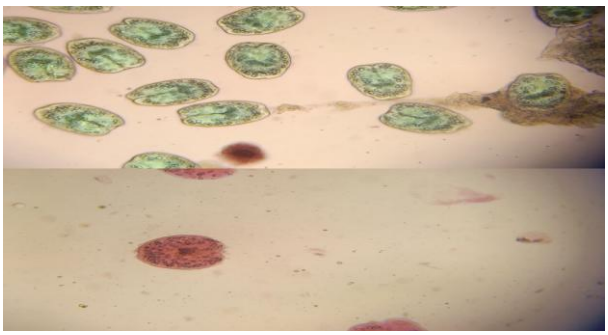

Figure 3. Microscopic image (400x) showing viability staining of hydatid cyst protoscoleces: live (green) and dead (red, stained with eosin).

Treatment and Data Collection

Protoscolices were incubated with bergamot essential oil, albendazole, or DMSO at 37°C in a water bath to simulate physiological conditions. Viability was assessed at multiple time points: 1, 12, 24, 36, and 48 hours for all treatments, with additional time points for bergamot oil at 64, 72, 92, and 116 hours to evaluate long-term effects at lower concentrations. For each concentration and time point, three 5 µL samples

were examined, and the percentage of dead protoscoleces was recorded by counting at least 100 protoscoleces per replicate under a microscope. The mean mortality percentage was calculated from the three replicates.

Statistical Analysis

Data were analyzed using SPSS version 22. One-way analysis of variance (ANOVA) was performed to compare mortality rates across concentrations and treatments at each time point, with a significance level of $p < 0.05$. A unified least significant difference (LSD) value was calculated to identify significant differences between concentrations, assuming an average mean square error (MSW) across time points. Results are presented as mean mortality percentages ± standard deviation (SD). Linear regression analysis was used to assess the relationship between concentration and mortality rate over time for both treatments.

III. Result & Discussion

The scolicidal potential of Citrus bergamia essential oil and albendazole against Echinococcus granulosus protoscoleces was concentration- and time-dependent, as shown in Table 1. Bergamot oil exhibited rapid lethality, particularly at higher concentrations, while albendazole required longer exposure to achieve comparable effects. The DMSO control showed minimal antiparasitic activity, with mortality rates increasing gradually over time.

Table 1. Mean Mortality Percentages (%) of *E. granulosus* Protoscolices Exposed to *C. bergamia* Essential Oil, Albendazole, and DMSO Control Across Different Time Points .

Time	Bergamot 10,000	Bergamot 5,000	Bergamot 2,500	Bergamot 1,250	Albendazole 10,000	Albendazole 5,000	Albendazole 2,500	Albendazole 1,250	DMSO	LSD
1 hr	100.0 ± 0.0	100.0 ± 0.0	2.0 ± 1.0	2.3 ± 1.2	5.0 ± 1.5	5.3 ± 1.0	5.3 ± 1.2	5.0 ± 1.0	1.7 ±0.6	
12 hr	100.0 ± 0.0	100.0 ± 0.0	17.3 ± 2.1	30.7 ± 3.5	25.0 ± 2.0	15.0 ± 1.7	9.0 ± 1.0	9.0 ± 1.5	4.3 ±0.6	
24 hr	100.0 ± 0.0	100.0 ± 0.0	46.7 ± 3.2	41.0 ± 2.6	63.3 ± 3.1	44.0 ± 2.6	36.7 ± 2.1	25.0 ± 2.0	7.3 ±1.2	
36 hr	100.0 ± 0.0	100.0 ± 0.0	71.7 ± 4.0	52.3 ± 3.8	100.0 ± 0.0	72.0 ± 3.6	71.0 ± 3.0	70.0 ± 2.8	22.0 ±2.5	
48 hr	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	73.0 ± 4.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	98.0 ± 1.0	87.7 ±3.8	4.40
64 hr	100.0 ± 0.0	100.0 ± 0.0	71.7 ± 4.0	52.3 ± 3.8	-	-	-	-	-	
72 hr	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	73.0 ± 4.5	-	-	-	-	-	
92 hr	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	73.0 ± 4.5	-	-	-	-	-	
116 hr	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	-	-	-	-	-	

Data represent mean ± SD of three replicates. LSD: Unified least significant difference ($p < 0.05$) for comparing concentrations. '-' indicates no data available.

At 1 hour, bergamot oil at 10,000 and 5,000 ppm achieved 100% protoscolex mortality, significantly outperforming albendazole, which recorded only 5.0-5.3% mortality across all concentrations ($p<0.05$). By 12 hours, bergamot oil at 1,250 ppm reached 30.7% mortality, surpassing albendazole at 10,000 ppm (25.0%). At 36 hours, albendazole at 10,000 ppm achieved 100% mortality, aligning with bergamot oil at higher concentrations, while lower concentrations of both treatments showed comparable effects (e.g., 71.7% for bergamot oil at 2,500 ppm vs. 70.0-72.0% for albendazole at 5,000-1,250 ppm). By 48 hours, most concentrations of both treatments reached 100% mortality, except bergamot oil at 1,250 ppm (73.0%) and albendazole at 1,250 ppm (98.0%). Bergamot oil at 1,250 ppm achieved 100% mortality by 116 hours. The DMSO control reached 87.7% mortality at 48 hours.

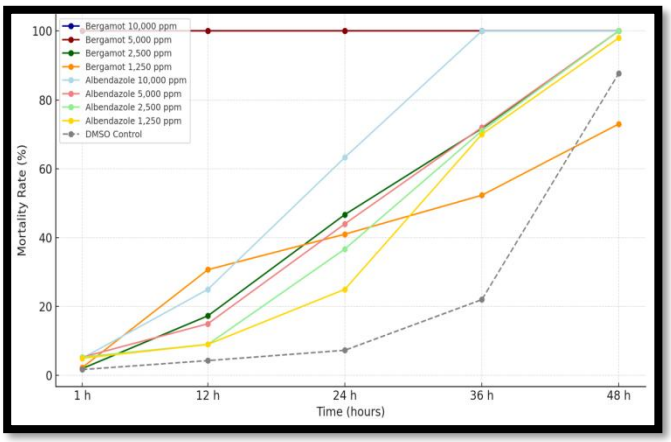

Figure 4. Line graph comparing the mortality percentages (%) of *E. granulosus* protoscoleces exposed to *C. bergamia* essential oil, albendazole, and DMSO control over time (1 to 48 hours).

Linear regression analysis revealed a strong positive correlation between concentration and mortality for both treatments. For bergamot oil, R^2 values were 0.92 at 1 hour and 0.97 at 48 hours ($p < 0.001$); for albendazole, R^2 values were 0.88 at 1 hour and 0.95 at 48 hours ($p < 0.001$).

This study demonstrates the superior in vitro scolicidal potential of *Citrus bergamia* essential oil against *Echinococcus granulosus* protoscoleces compared to albendazole, particularly in terms of speed and efficacy at higher concentrations. At 10,000 ppm, bergamot oil achieved 100% mortality within 1 hour, whereas albendazole required 36 hours, consistent with reports of its slower action (10,16). Compared to other essential oils, bergamot oil outperforms Zataria multiflora, which achieves 100% mortality at 6–8 mg/mL in 7–60 minutes due to thymol and carvacrol disrupting membrane integrity and enzymatic activity (5), and Cuminum cyminum, which reaches 100% mortality at 50 µL/mL in 20 minutes via cuminaldehyde-induced oxidative stress and mitochondrial dysfunction (6). Bergamot oil's faster lethality at lower concentrations (10 mg/mL in 1 hour) likely results from the synergistic effects of limonene, linalool, and bergapten, which disrupt cellular membranes, induce oxidative stress, and impair mitochondrial function(8,17).

At lower concentrations (1,250 and 2,500 ppm), bergamot oil initially prolonged protoscolex survival compared to DMSO (e.g., 2.0–2.3% mortality at 1 hour vs. 5.0–5.3% for albendazole), possibly due to its antioxidant and antimicrobial properties creating a protective microenvironment (20). This effect diminished over time, with 100% mortality achieved at 116 hours for 1,250 ppm. Albendazole's slower action, driven by inhibition of tubulin polymerization and glucose uptake (21), limits its intraoperative use, whereas bergamot oil's rapid effect suggests potential for surgical sterilization to prevent secondary cyst formation (23).

The DMSO control exhibited mild antiparasitic activity (87.7% mortality at 48 hours), likely due to osmotic stress and

membrane penetration (22). This highlights the need for future studies to isolate bergamot oil's specific effects using alternative solvents. Compared to other natural agents, bergamot oil's efficacy aligns with Thymus vulgaris and Origanum vulgare, which achieve 100% mortality at 10–20 mg/mL in 30–60 minutes (24,25). However, bergamot oil's lower effective concentration (10 mg/mL) indicates higher potency, possibly due to its multifaceted bioactive compounds (17,26).

The convergence of bergamot oil and albendazole at 36–48 hours for lower concentrations suggests potential synergistic effects, warranting further investigation (27,28). The in vitro design limits clinical applicability, as DMSO's mild effects may influence results, and bergamot oil's in vivo bioavailability, toxicity, and pharmacokinetics remain unknown (29). Encapsulation techniques, such as liposomes or nanoparticles, could enhance delivery and stability, as shown with Melaleuca alternifolia (30). Bergamot oil's rapid action and natural origin position it as a promising candidate for intraoperative use in CE-endemic regions like Iraq, where cost-effective solutions are critical (7).

IV. Conclusion

This study demonstrates that *Citrus bergamia* essential oil exhibits superior in vitro scolicidal potential against *Echinococcus granulosus* protoscoleces compared to albendazole, achieving 100% mortality at 10,000 ppm within 1 hour and at 1,250 ppm by 116 hours. Albendazole, while effective, required longer exposure times, highlighting bergamot oil's potential as a rapid-acting natural alternative for cystic echinococcosis (CE) treatment. Statistical analysis confirmed significant differences ($p<0.05$) between treatments, with bergamot oil outperforming albendazole at early time points. These findings position bergamot oil as a promising candidate for intraoperative use and long-term CE management, particularly in endemic regions like Iraq.

Future research should focus on in vivo studies to validate bergamot oil's efficacy, assess its toxicity, and determine its bioavailability. Synergistic studies combining bergamot oil with albendazole could explore ways to enhance efficacy and reduce resistance risks. Additionally, developing stable clinical formulations, such as emulsions or nanoparticle-based delivery systems, could improve the oil's applicability. These findings warrant integration into public health strategies in endemic regions, pending further validation.

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