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RESEARCH ARTICLE

In vitro: Effectiveness of *Laurus nobilis* extract against *Hymenolepis nana* Eggs

Mutee Murshed[1], Mohammed M. Mares[1], Hind Alzaylaee[2], Saleh Al-Quraishy[1]

[1]King Saud University, College of Science, Department of Zoology. P.O. Box 2455, Riyadh 11451, Saudi Arabia.

[2]Princess Nourah bint Abdulrahman University, College of Science, Department of Biology. P.O. Box 84428, Riyadh, 11671, Saudi Arabia

Abstract

M. Murshed, M. M. Mares, Hi. Alzaylaee, and S. Al-Quraishy. 2025. In vitro: Effectiveness of *Laurus nobilis* extract against *Hymenolepis nana* Eggs. Int. J. Agric. Nat. Resour. 138-147. Parasites are among the most widespread health threats affecting a wide range of organisms. Hymenolepis nana is a common intestinal tapeworm that infects rodents such as Mus musculus and Rattus norvegicus, posing potential complications in laboratory research where animal health is crucial for obtaining reliable results. This study aimed to investigate the ovicidal effect of Laurus nobilis leaf extract on *H. nana* eggs. Eggs were divided into six groups, each containing 500 eggs. Groups 2 to 5 were treated with L. nobilis extract at concentrations of 25, 50, 100, and 200 µg ml^{-1}, respectively, while group 6 received Albendazole at 100 mg ml^{-1} as a reference drug. A control group was maintained without treatment. All groups were incubated at 28 °C and examined after 12, 24, 36, and 48 hours. Following incubation, a mixture of the egg suspension and 0.1% eosin dye was prepared to assess egg viability under a light microscope. Results showed that the highest concentration of *L. nobilis* (200 µg ml^{-1}) killed 89% of the eggs, while Albendazole (100 mg ml^{-1}) achieved a 93% mortality rate. Non-viable (dead) eggs absorbed the red dye, while viable eggs remained unstained. Statistically significant differences ($P \leq 0.01$) were observed between treated groups and the control, as well as among the different extract concentrations. These findings demonstrate the potent ovicidal activity of *L. nobilis*, highlighting its potential as a natural antiparasitic agent and a promising candidate for the development of plant-based anthelmintic drugs.

Keywords: In vitro, *Hymenolepis nana*, *Laurus nobilis*, eggs, Albendazole.

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Corresponding author: mmurshed@ksu.edu.sa

Highlights

- Enhanced ovicidal efficacy of *Laurus nobilis* leaf extract on *Hymenolepis nana* eggs.
- *Laurus nobilis* serves as a medicinal agent and a significant source of bioactive chemicals effective against *Hymenolepis nana* eggs.
- The maximum concentration of *L. nobilis* (200 µg ml^{-1}) resulted in the mortality of 89% of the eggs.
- Emphasizing the potential of *L. nobilis* extract as a natural antiparasitic agent and a viable choice for the formulation of plant-derived anthelmintic medications.

Introduction

Hymenolepis nana is widely dispersed worldwide, particularly prevalent in temperate and tropical areas. The 2019 European Research Report indicates that from 1999 to 2016, parasites of the genus Hymenolepis are frequently found in hosts across Europe (Strand & Lundkvist, 2019). *H. nana* is the predominant zoonotic tapeworm, with an estimated infection rate of up to 75 million individuals globally. The frequency among children is as high as 25% (Crompton, 1999). In contrast to most zoonotic tapeworm infections, *H. nana* infection does not necessitate an intermediary host; this characteristic enables *H. nana* to directly consume eggs for infection and can potentially lead to autoinfection within the digestive system (Craig, 2007). Previous investigations indicate that *H. nana* can be transferred to rodents via fleas and beetles acting as intermediate hosts (Macnish et al., 2002). The transmission of *H. nana* is prevalent in poor and underdeveloped nations, particularly in rural and suburban regions, especially among children (Al-Mekhlafi et al., 2020; Murshed et al., 2025). Reports indicate that 61% of patients infected with *H. nana* are concurrently infected with other intestinal parasites, hence increasing their susceptibility to additional infections and complicating recovery. Praziquantel is highly effective in treating trematodes and cestodiasis, as a standard treatment for helminths. Besides its efficacy, praziquantel's patent has lapsed, resulting in a significantly reduced cost (Kusel & Hagan, 1999). Nonetheless, praziquantel is not an ideal medication due to several potential issues, including side effects (Liu et al., 1986; Shu-Hua & Catto, 1989; Meyer et al., 2009), which may result in drug resistance (Mwangi et al., 2014), and an unforeseen reaction following the elimination of the parasite (Woo et al., 1988). Previous trials in rats indicated that praziquantel therapy did not fully eliminate hymenolepiasis, necessitating follow-up fecal tests (Esatgil et al., 2009).

Laurus nobilis is a therapeutic plant and a notable source of bioactive compounds. The biological features of its many extracts and essential oil are detailed, especially their antibacterial and antioxidant activities, along with their wound-healing effects in animal models. Traditionally, dried bay leaves and their infusions are utilized to address digestive issues such as epigastric pain, flatulence, bloating, and belching. The leaves and fruits of the Bay plant have been utilized as an astringent, diaphoretic, stimulant, emetic, emmenagogue, abortifacient, and insect repellant. Furthermore, as an aromatic plant, its essential oil is included into cosmetic items such as soaps, creams, and fragrances (Kaurinovic et al., 2010). Phytochemical investigations of *L. nobilis* leaves and their fruits have revealed an array of secondary metabolites, including alkaloids, flavonols (kaempferol, myricetin, and quercetin), flavones (apigenin and luteolin), glycosylated flavonoids, sesquiterpene lactones, as well as monoterpene and germacrane alcohols (Fukuyama et al., 2011; Škerget et al., 2005).

There is a global concern that the use of antibiotics for bacterial, parasitic, and fungal infections may result in the emergence and dissemination of organisms resistant to broad-spectrum antibiotics, prompting the exploration of plants as natural sources

for novel antimicrobial agents with comparable efficacy. Natural medicinal plants, like *L. nobilis*, are rich in bioactive compounds. The biological properties of *L. nobilis* leaf extracts and their essential oil are well-documented, particularly their antibacterial, antifungal, and antioxidant effects. The potential antioxidant activity of *L. nobilis* leaf extract has also been reported. The methanolic extract of seed oil exhibited antioxidant activity in both the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and the β-carotene/linoleic acid test systems (Ozcan et al., 2010). A separate study evaluated the antioxidant potential of ethanolic and aqueous extracts from the leaves of *Hypericum perforatum*, *Ocimum basilicum*, and *L. nobilis* using the DPPH test. The aqueous extract of *L. nobilis* demonstrated the lowest radical scavenging capacity when compared to *H. perforatum* and *O. basilicum*. Nonetheless, the ethanolic extracts of *L. nobilis* exhibited a superior DPPH radical scavenging activity compared to their aqueous extracts (Rukhkyan et al., 2013). The current study was designed to look into the possible role that extracts of *L. nobilis* leaves might play in anthelmintic (eggs) activity when tested in vitro.

Materials and Methods

Preparation of Extracts

The *L. nobilis* extract was derived from leaves procured at spice markets in Riyadh, Saudi Arabia. The plant's botanical identity was confirmed by a taxonomist at the Department of Botany, King Saud University (No- 24649). One hundred fifty grams of leaves were air-dried at 45 °C, pulverized into a fine powder, and subsequently extracted with 70% methanolic for 24 hours at 4 °C. The extract was concentrated and dried by a rotating vacuum evaporator (Yamato RE300, Japan) at 40°C under reduced pressure. The crude extract was preserved at -20 °C until utilized in studies, as specified by Yang et al. (2014), resulting in a viscous, adhesive residue. For in vitro investigations, the powdered extract was reconstituted in distilled water.

Eggs Recovery

Infected mice were euthanized and dissected, and feces were collected from their intestines. The worm eggs were then extracted from the fecal samples utilizing immediate wet mount microscopic examination and the sedimentation technique, followed by centrifugation at 1008× g for 15 minutes (Smyth & Barrett, 1980). The eggs were retrieved from the coverslip using a 0.9% NaCl solution and stored at -20 °C.

In vitro effects of Laurus nobilis on eggs

Plant extract solutions were prepared at four concentrations: 25, 50, 100, and 200 mg ml^{-1}. Albendazole at a concentration of 100 mg ml^{-1} was used as a positive control. 1 ml of each concentration was added to the wells of a 24-well plate. Subsequently, a suspension containing 500 eggs of *H. nana* was added to each well. Mixed gently, and incubated at 28 °C for 12, 24, 36 and 48 hours. All concentrations of the extract, as well as Albendazole, were tested in triplicate to ensure the reliability and reproducibility of the results. After each incubation duration, 0.5 mL of the egg extract suspension was mixed with 0.5 mL of 0.1% eosin dye. Following a 30-minute incubation, the settled eggs were spread on a slide, covered with a cover slip, and examined by a light microscope to assess viability. Viable eggs maintained their color, while dead eggs turned red. The ratio of live to dead eggs was determined by scoring a minimum of 100 eggs. Additionally, a control group was prepared with 1 mL of distilled water containing at least 500 eggs.

Statistical analysis

A one-way ANOVA was conducted, followed by statistical comparisons between groups using the Duncan method. Results were presented as means ± standard deviations at a significance level of $p \leq 0.05$. Statistical analysis was performed utilizing

the Origin 2018 software, while Microsoft® Excel 2003 was used to calculate means and standard deviations.

Results

In Figure 1, It was observed that viable eggs did not absorb the red eosin dye, indicating that their protective shells remained intact and impermeable. In contrast, eggs that stained red showed that the extract caused damage to the eggshell, leading to its rupture. This rupture allowed the eosin dye to penetrate the egg, resulting in internal staining that reflects damage to the egg's internal structures. The use of eosin dye in this context is beneficial because it selectively stains non-viable or dead eggs, serving as an indicator of egg mortality. Therefore, the presence of red-stained eggs provides clear evidence of the extract's ovicidal effect and its ability to compromise egg viability.

When *H. nana* eggs were exposed to *Laurus nobilis* extracts, they stopped living 93% of the time in the anthelmintic bioassay, especially at a concentration of 200 µg ml^{-1}. The viability % ranged from 7 ± 2.5 -93% after 48 h. The viability rate of the *H. nana* eggs decreased with increasing exposure periods. The percentage of living cells that survived was strongly linked to exposure times (y=-12.5x + 59, R^2=0.9496, P=.0001; y=-11.7x + 57.5, R^2=0.9909, P=.0001) at 200 µg ml^{-1} and Albendazole concentrations. The current study's overall results indicate that the extract of *L. nobilis* may have potential anthelmintic properties, making it a potential tool for worm management. Phytochemical compounds had biological activity. We conclude that the *L. nobilis* extract has the potential to be useful in managing mice's anthelmintic, particularly *H. nana*.

The results obtained revealed significant differences in the mean viability at the 200 µg ml^{-1} concentration after 48 hours, compared to the Albendazole concentration, as well as in the viability inhibition means (Figure 2). Additionally, significant differences were observed between the control group and the 200-µg ml^{-1} concentration after 36 and 48 hours, respectively. The highest mortality rates were recorded after 48 hours at both the 200 µg ml^{-1} and Albendazole concentrations.

The lowest viability inhibition was observed after 12 hrs at all concentrations (Table.1). Significant differences ($P < 0.05$) were observed in the viability inhibition percentage of H. nana eggs between the plant extract concentrations (50, 100, and 200 µg ml^{-1}) and the 25 µg ml^{-1} concentration as well as the control group (Figure 3). Overall, no significant differences were found between the concentrations, except at the 200 µg ml^{-1} concentration.

The means represented by distinct lowercase letters in columns or rows are significantly different ($p<0.05$). Groups sharing identical lowercase letters exhibit no significant difference ($p>0.05$) according to Duncan's Multiple Range Test subsequent to ANOVA. All values are shown as mean ± standard error.

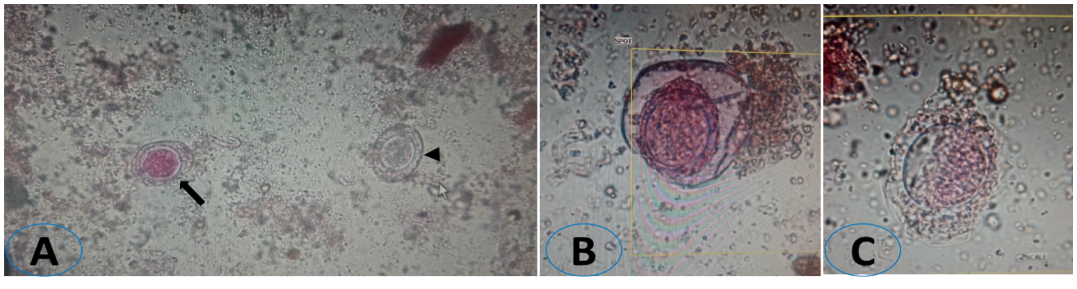


Figure 1. Effect of *L. nobilis* leaves extract on eggs viability. The non-viable eggs identified by the arrow, were treated with the extract and stained with 0.1% eosin (A, B, and C). The viable eggs remained colorless after staining with 0.1% eosin, as indicated by the arrowhead (A).

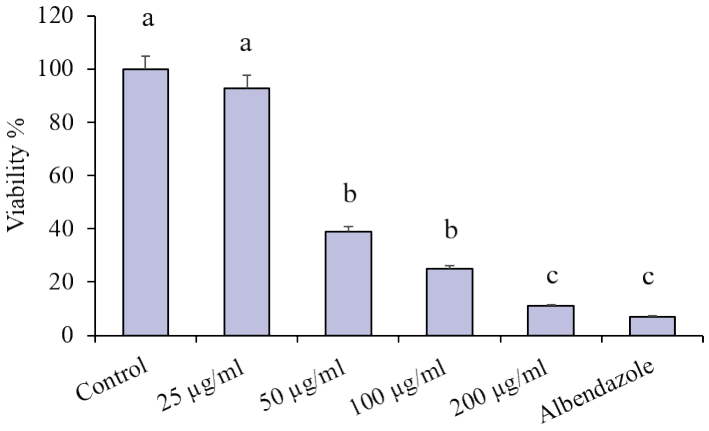


Figure 2. Mean viability percentage (± SE) of *H. nana* eggs at different concentrations. Values marked with different lowercase letters indicate significant differences ($p<0.05$), while values with the same lowercase letters are not significantly different ($p>0.05$), according to Duncan's Multiple Range Test ($p<0.05$).

Table 1. Means of viability % (± SE) of *L. nobilis* extracts at various exposure periods (12, 24, 36, and 48 hour) against *H. nana* eggs.

Concentration µg mL^{-1}	Viability (%)			
	12 hrs	24 hrs	36 hrs	48 hrs
Control	100 ± 0.0 ^{c}	100 ± 0.0 ^{c}	100 ± 0.0 ^{c}	100 ± 0.0 ^{c}
25	97 ± 1.1 ^{c}	95± 1.7 ^{ab}	94.3± 1.5 ^{bc}	93 ± 1.5 ^{c}
50	83.3± 2.7 ^{bc}	70± 2.5 ^{a}	50.4± 2.4 ^{bc}	39 ± 1.5 ^{bc}
100	74.7± 3.9 ^{bc}	59.3± 2.4 ^{ab}	38.7± 3.5 ^{ab}	25± 2.5 ^{bc}
200	45± 3.4 ^{a}	36.3± 1.3 ^{ab}	21.3± 3.9 ^{bc}	11± 2.5 ^{bc}
Albendazole	43± 3.3 ^{a}	39.3± 1.3 ^{ab}	22.3± 3.9 ^{ab}	7± 2.5 ^{bc}

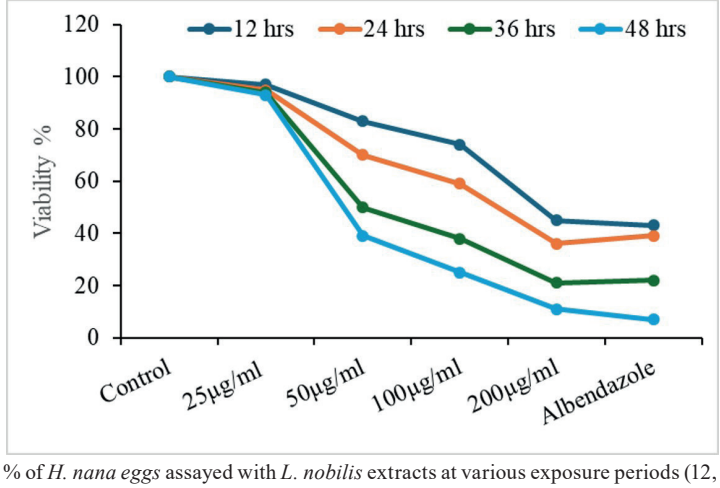


Figure 3. Viability % of *H. nana eggs* assayed with *L. nobilis* extracts at various exposure periods (12, 24, 36, and 48 min).

The viability inhibition ratio of the *H. nana* eggs increased with increasing exposure periods (Figure 4). The mortality % was highly negatively correlated with exposure times (y=-12.5x + 59, R^2=0.9496, and P=0.0001) at 200 µg mL^{-1} concentrations. Whereas the correlation coefficient at Albendazole 100 mg mL^{-1} was moderately negative (y=-12.5x + 59, R^2=0.9496, and P=0.0001) (Figure 5) and weakly negative at 25 µg mL^{-1} concentration (R=-0.2 and P=0.0001).

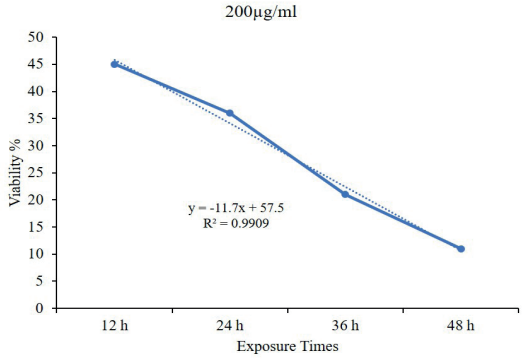


Figure 4. Mean Viability % of *H. nana* eggs at 200 µg m^{-1} concentration for various exposure times.

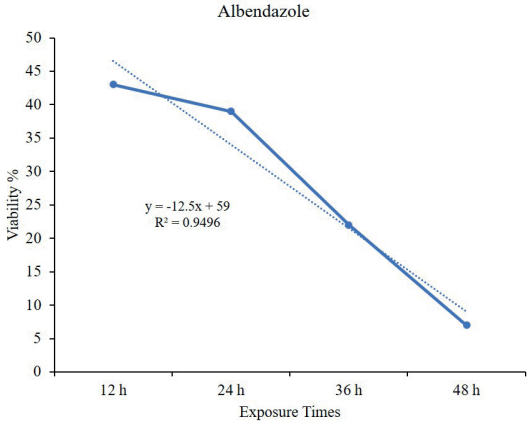


Figure 5. Mean Viability % of *H. nana* adults' eggs at Albendazole 10 µg mL^{-1} concentration for various exposure times (hrs).

Exposure of *H. nana* eggs to *L. nobilis* extracts resulted in a mortality rate of 96% throughout the bioassay, particularly at a dose of 200 µg ml^{-1}. The mortality rate varied from 7±2.5% to -97% after 48 hours. The mortality rate of H. nana eggs escalated with prolonged exposure durations. The results indicated significant disparities in the average mortality rate at the 200 µg ml^{-1} concentration of Albendazole after 48 hours as compared to the 25 µg ml^{-1} concentration. Significant disparities were seen in the means of cell viability inhibition (Figure 6). Moreover, substantial differences were noted between the control group and the 200 µg ml^{-1} concentration after 36 and 48 hours, respectively. The peak mortality rates were seen after 48 hours at both the 200 µg ml^{-1} and albendazole concentrations (Figure 6).

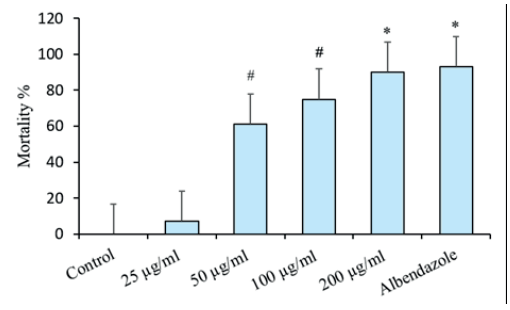


Figure 6. Mean death ratio (± SE) of *H. nana* eggs at different concentrations. (*) significant differences ($p<0.01$), (#) not significantly different ($p<0.05$), according to Duncan's Multiple Range Test.

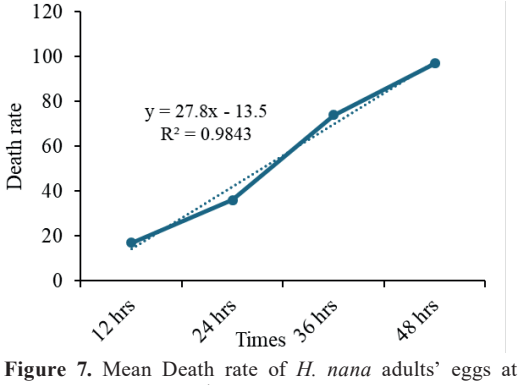


Figure 7. Mean Death rate of *H. nana* adults' eggs at Albendazole 10 µg mL^{-1} concentration for various exposure times (hrs).

Discussion

H. nana is a widespread intestinal tapeworm that affects humans, with treatment options such as praziquantel, nitazoxanide, and niclosamide. A significant obstacle in controlling helminthic infections is the growing issue of anthelmintic

resistance worldwide (Ploeger & Everts, 2018; Vineer et al., 2020). To combat this problem, incorporating natural compounds into parasite management strategies has emerged as a promising alternative to traditional medications. In this study, the effects of Laurus nobilis extract and Albendazole on *H. nana* eggs were examined. The results showed a significant impact of *L. nobilis* on Vitality *H. nana* eggs over time, with dead eggs changing to a red color. In contrast, the control group exhibited prolonged survival of eggs, with viable ones maintaining their original color. Our findings are consistent with those reported by Sebai et al. (2022), who demonstrated that *L. nobilis* essential oil exhibited strong anthelmintic activity. Specifically, it showed an ovicidal effect at a concentration of 1.72 mg mL^{-1} against *Haemonchus contortus* eggs, and caused 87.5% immobility of adult worms at 4 mg mL^{-1} after 8 hours of in vitro exposure. In vivo, at 2400 mg/kg body weight, it completely eliminated eggs output of *H. polygyrus* and reduced total worm counts by 79.2% after 7 days. Our study is consistent with the findings of Kaur et al. (2018), where GC-MS analysis of *L. nobilis* leaf essential oil identified eugenol (63.57%), α-pinene (7.68%), and 1,8-cineole (3.37%), along with isoeugenol (10.59%), spathulenol (3.74%), isospathulenol (1.29%), and caryophyllene (1.29%). These compounds have shown the ability to inhibit egg hatching of *M. incognita*, eugenol was most active as complete egg hatch inhibition was found at 1.25 mg mL^{-1}whereas α-Pinene was least effective as it showed 79 % egg hatch inhibition at same concentration. The mechanism of action of the compounds found in Laurus nobilis extract on parasites involves multiple biochemical and physiological pathways. The extract contains various bioactive compounds, including essential oils (such as 1,8-cineole, eugenol, and linalool), flavonoids, tannins, and alkaloids. These compounds are believed to exert antiparasitic effects by disrupting the integrity of the parasite's cell membrane, interfering with energy metabolism, and inducing oxidative stress through the generation of reactive oxygen species (ROS). Additionally, some compounds may inhibit key enzymes or interfere with the reproductive capacity of the parasites, particularly at the egg stage. This multifaceted mode of action contributes to the observed ovicidal and anthelmintic effects of L. nobilis extract (Caputo et al., 2017). Alimi et al. (2021) reported that the ethanolic extract of *L. nobilis* caused an 86.2% mortality rate in engorged female ticks and effectively prevented egg hatching at all tested concentrations. The study demonstrated that both the aqueous and ethanolic extracts of *L. nobilis* inhibited the growth of *Toxoplasma gondii* tachyzoites in vitro (El Faqer et al., 2024). A study by Al-Bayati and Abed, 2021 found that the extract of *L. nobilis* exhibited a potent effect against the protoscoleces of *Echinococcus granulosus* in vitro. In a study conducted by Mares et al. (2024) on the *Aspiculuris tetraptera* parasite, they observed that the extract of *L. nobilis* leaves had a significant effect on the worms, leading to their death both in vitro and in vivo. Additionally, as demonstrated by Mares et al. in their 2023 study, the methanolic extract of *L. nobilis* leaves was also effective in killing *Hyalomma dromedarii* ticks, highlighting the extract's potent antiparasitic properties. A study has shown that *Laurus nobilis* leaf oil is effective against the larvae of *Thaumetopoea pityocampa*. This suggests that the essential oil could be a viable, environmentally friendly insecticide for controlling *T. pityocampa* (Kanat & Alma, 2004). The essential oil of *L. nobilis* has shown strong activity against Enterobacter sp., *E. coli*, *P. aeruginosa*, and *Staphylococcus aureus* (Ouibrahim et al., 2013). This effect may be attributed to the presence of 1,8-cineole in the oil, as it plays a key role in its antimicrobial properties, demonstrating effectiveness against several strains, including *E. coli*, *P. aeruginosa*, and *Staphylococcus aureus* (Sivropoulou et al., 1997). Both this study and others have emphasized the potent anti-parasitic properties of *L. nobilis*, indicating its promising potential for use in parasite control initiatives.

Conclusion

The findings highlight the promising potential of plant extracts as alternative or complementary therapies to conventional anthelmintics, which face challenges such as drug resistance and environmental toxicity. Specifically, the extract of Laurus nobilis leaves was found to significantly reduce the viability and hatchability of *H. nana* eggs, demonstrating strong ovicidal properties. These results suggest that *L. nobilis* extract could be developed as a natural, eco-friendly agent in integrated parasite management programs. Further research on dosage optimization, toxicity, and in vivo efficacy is recommended to fully establish its therapeutic potential.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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Ethical Approval

The study followed the "Guide for the Care and Use of Laboratory Animals". The study followed King Saud University's institutional norms for animal usage, as well as the criteria specified by Saudi Arabia's National Committee of Bio-Ethics. According to the Royal Decree M59, which was issued on 14/9/1431H. King Saud University's Research Ethics Committee (Approval No. KSU-Se-21-86) approved all the protocols for those experiments, assuring animal rights, welfare, minimal stress, and no harm or suffering.

Author Contributions:

Mutee Murshed: Conceptualization, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation. Writing – review and editing, Visualization, Project administration.

Mohammed M. Mares: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation. Writing – review and editing, Visualization.

Hind Alzaylaee: Resources, Data Curation, Project administration, Funding.

Saleh Al-Quraishy: Conceptualization, Methodology, Visualization, Supervision.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Availability of Data and Materials: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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