## **RESEARCH ARTICLE**

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# The Assessment of the Anthelmintic Activity of *Laurus nobilis* Extract in Mice Naturally Infected with *Aspiculuris tetraptera*

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## **ABSTRACT**

**Background:** The safety of laboratory mice plays an important role in the success of laboratory experiments for correct and accurate results. Parasites are one of the most common diseases that affect most organisms. The worm *Aspiculuris tetraptera* is a common intestinal parasite of Mus musculus and rats, it is spread around the world. *Aspiculuris tetraptera* infection remains a problem for modern research groups; They should be excluded and monitored in rat populations due to their effects on animal health.

**Methods:** The present study was conducted to assess the anthelmintic activity of *Laurus nobilis* extract in mice naturally infected with *Aspiculuris tetraptera*. Used was Thirty-six adult male and female (C57BL/6) mice, naturally infected with *Aspiculuris tetraptera*, were divided into six groups, each comprising six mice: Group 1, was the negative control (infected, untreated), and Groups 2, 3, 4, and 5 were treated with 50, 100, 200 and 400 μg/ml of *L. nobilis* for 5 days respectively. Group 6 was treated with 10 mg/mL Albendazole for 3 days as a positive control. Different six treatments were used to test *Aspiculuris tetraptera* worms *in vitro*, Containing 4 concentrations (50, 100, 200 and 400 mg/ml) of *L. nobilis* extract. Ten actively moving adult worms were then placed in each petri dish at room temperature. A saline solution and 10 mg/ml of Albendazole were prepared and used as negative and positive controls. After treatment, observations were made by recording the death time for worms at 20, 40, 80, 120 and 180 minutes. Worms are considered dead if they do not move for 30 seconds after touching their body parts using a surgical needle and the petri dish is shaken.

Result: The analysis of phytochemicals by FT-IR for alcoholic extracts of *L. nobilis* extracts revealed the presence of 15% effective chemical ingredients responsible for killing worms' activity. *In vitro*, worms died in 20, 40, 80, 120 and 180 minutes. Attained 96% and 100% after 180 minutes at the highest concentrations (200 and 400 mg/ml). At the same time, the untreated group lasted for long hours. The effects of the plant extract (*Laurus nobilis*) on *Aspiculuris tetraptera* worms were studied *in vivo* at therapeutic doses of 50, 100, 200 and 400 mg/mL. and the concentration of 400 ml/kg showed the most lethal effects for worms, infected mice that did not receive drugs were compared with the highest concentration of the extract and the reference treatment. The attention showed significant differences P≤0.05. The results showed that the mortality rate of worms taken from the intestines of the treated mice that were slaughtered three and six sdays after giving the treatment reached 96% and 100% in the concentration of 400 ml/kg of the extract and 89% and 97% for the mice that treated in 10 ml/kg of met Albendazole respectively. This research showed that herbal remedies could lead to new parasitic disease drugs, and their derivatives can be used for medication production and bioactivity improvement.

Key words: Aspiculuris tetraptera, Flavonoids, Laurus nobilis, Mice, Phenolics, Phytochemicals.

# INTRODUCTION

The health and safety of experimental mice play an essential role in the success of Laboratory experiments to obtain correct and accurate results. Parasitic worms are one of the most common diseases that infect most organisms. Despite advancements in veterinary medicine, gastrointestinal (GI) helminths continue to pose a serious threat and cause major public health problems, which can result in morbidity and even death sometimes, particularly in less developed nations (Weinstock and Elliot, 2009; Ranasinghe et al., 2023). Infections caused by gastrointestinal helminths and protozoa can have an impact on a host's ability to survive and reproduce both directly, they can cause pathological effects such as blood loss and tissue damage and indirectly (Scantlebury et al., 2007; Taylor et al., 2015). To date, 20 species of the genus Aspiculuris have been recognized, including the recently described A. huascaensis and A. tianjinensis (Falcón-Ordaz et al., 2010). A. tetraptera

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worms are common intestinal parasites of the Mus musculus and mice, which happen all over the world. *A. tetraptera* infections remain a problem for contemporary research colonies; they should be excluded from and monitored for in mouse colonies due to their effects on animal health and

research, including behavior, gastrointestinal physiology, immunology, growth and hematopoiesis (Gerwin et al., 2017). The life cycle of A. tetraptera is direct and infection occurs after the host ingests the eggs, with a preparation time of 21-35 days (Pritchett-Corning and Clifford, 2012) Larvae in the first stage survive for a week in the submucosa of the colon and develop into third-stage larvae that return to the colonic lumen to develop into adult larvae (Pritchett-Corning and Clifford, 2012). The large intestine is home to mature females, who spend 45 to 50 days there before laying their eggs. The eggs are released at night and coat fecal pellets with a mucous layer. They can persist for weeks outside the host and take 6-7 days at 24°C to become contagious (Stepek et al., 2006). Observations on naturally acquired and experimental oxyuroid infection with A. tetraptera clearly show significant differences in drug resistance and susceptibility between mouse strains (Omer et al., 2020). So, infection in laboratory mice cannot be prevented and animals will remain infected if left untreated. Although infected animals may not show clinical signs in immunocompetent experimental mice, numerous studies have shown the effects of several parasites, including A. tetraptera, associated with decreased immune response, reduced hemoglobin, red blood cell count and serum albumin, which may adversely affect the results of experiments (Moulia et al., 1995).

Helminth control strategies rely on the use of dewormed drugs. Due to growing evidence that many parasites are resistant to medications and that there are adverse effects associated with the synthetic therapies that are accessible, the currently used anthelmintic drugs are insufficient to control these organisms. This has led to increased research on alternative medicines that can treat parasitic diseases (Gaherwal *et al.*, 2012). In this setting, nature has always been the source of drug development, investigating possible antiparasitic agents derived from plants, cheap and readily available and a promising alternative. So, studying the anthelmintic abilities of traditionally used plants may provide better options for treatment (Amirmohammadi *et al.*, 2014).

Laurus nobilis is a plant species commonly known as "sweet bay" or "bay laurel." The camphor family (Lauraceae), widely known as laurel, is a species of fragrant angiosperm native (Oliveira et al., 2009). It is an evergreen tree or large shrub with smooth, green leaves that are aromatic and used in cooking. It is native to the Mediterranean region and is widely cultivated in many areas worldwide for its ornamental, culinary and medicinal uses (Paparella et al., 2022). The fact that Laurus nobilis plant displays biological activity sets it apart the most from plant others (Begum et al., 2013). It is associated with its extract and essential oils as an antifungal agent (Caputo et al., 2017), an antiviral agent, an antibacterial agent (Simić et al., 2004), an acaricidal agent (Sırıken et al., 2018) and an insecticidal agent (Fernandez et al., 2020). Knowledge of the disposal methods for helminths infected by plant extracts in laboratory animals is essential because parasites may act as variables affecting experimental outcomes. This study aimed to evaluate the anthelmintic efficacy of *Laurus nobilis* in mice naturally infected with *Aspiculuris tetraptera*; this type of worm was also assessed *in vitro*. Hence, the results obtained from this trial could be extrapolated to humans and other animal species.

#### **MATERIALS AND METHODS**

## Preparation of extracts

The *L. nobilis* extract was prepared using leaves obtained from the spice markets of Riyadh, Saudi Arabia and the botanical identity of the plant was confirmed by a taxonomist at the Department of Botany, University of King Saud, No. 24649. The leaves (100 g) were air dried at 40°C, ground into a powder and then extracted with 70% methanol for 24 h at 4°C. After this, the extract that was produced was concentrated and dried using a rotating vacuum evaporator (Yamato RE300, Japan) at 40°C and under decreased pressure. When the crude extracts were created, they were kept at -20°C until they were employed in an experiment, as previously reported by (Yang *et al.*, 2014), until a thick, sticky material formed. Distilled water was used to dissolve the powder for the *in vitro* experiments.

## Infrared spectroscopy

A small amount of the material was processed and then homogenized by adding excessive potassium bromide powder (1:99 wt%). After a rough mashing, the material was placed in a pellet-forming die. Thermo Scientific's NICOLET 6700 optical spectrometer with Fourier-transform infrared spectroscopy was utilized to analyze infrared (I.R.) data and identify potential compound classes. The number of waves (cm<sup>-1</sup>) used to describe the most outstanding absorption at 25°C, spectra with a resolution of 4 cm and a range of 4000 cm<sup>-1</sup> were captured (Abu Hawsah *et al.*, 2023).

#### Identification of total phenolics

The method described in Singleton *et al.* (1999), with a few adjustments, was used to determine the total amount of phenolic content in LNLE. Gallic acid solutions (25-150 g/mL) were utilized to create a standard curve. Briefly, 0.1 mL of Folin-Ciocalteu reagent, 1.5 mL of Milli-Q ultrapure water, 0.1 mL of plant extract (1 mg/mL) and 0.3 mL of 20% sodium carbonate solution were blended and combined by a vortex in complete darkness for two hours and the combination was then incubated. A UV-visible spectrophotometer was used to measure the absorbance of the ensuing blue color at 765 nm. The overall phenolic content of the extracts was determined as gallic acid equivalent (mg/g DW), where (y) absorbance and (x) gallic acid equivalent concentration (mg/g), using the equation based on the calibration of the curve (y =  $0.005 \times 0.0088$ ).

# Identification of total phenolics

A technique described by (Ordonez *et al.*, 2006) was used to calculate the total flavonoids in AMLE. In a nutshell, 1.0 mL of plant extract (1 mg/mL) was combined with 2% AICI3

water solution. After one hour at room temperature, absorbance at 420 nm was measured. The standard solution was prepared using a 50-800 g/mL quercetin solution and a standard curve (R2 = 0.9996) was produced. The flavonoids in the extracts were represented as quercetin (mg/g DW) using the equation for the calibration curve,

y = 0.0011x + 0.0928.

Where,

y = Absorbance.

x = Quercetin equivalent concentration (mg/g).

### Collection of adult worms

Selected 90 mice randomly, similar in age and weight; each mouse was isolated in a cage to obtain feces and we examined them and found 36 mice infected with pinworms of the species *Aspiculus tetraptera*. The intestines of mice were opened and washed with a saline solution (0.9% NaCl). Adult worms were collected from the cecum and colon of infected mice. Worms were cultured in approximately 7 ml of sterile physiological saline at 23°C. The worms were identified under a stereomicroscope (BX51TF, O.L.Y.M.P.U.S., Tokyo, Japan). Healthy worms with standard microscopic structure and good motility were selected. Then the experiment started immediately after the worms were collected.

## Adult worm motility test in vitro

The larval or adult motility test is the method of choice to investigate the medication effects of various worm species. The subculture method was used in in vitro sensibility analyses (Alimi et al., 2021). Can be characterized as an extraordinarily rigorous and sensitive approach to evaluating the antiparasitic effects of a substance. The vitality of the treated worms was determined by calculating the % inhibition and motility in treated worms (Merghni et al., 2016) with a total of 15 worms (in replicates of 5 worms) for the motility assay per test concentration. After test times of exposure to all doses, parasite motility was examined under a microscope and the findings were rated. Loss of motility is defined as the worms' inability to regain pretreatment level motility after incubating in fresh medium minus the test agent at 37°C for 10-20 minutes and it is reported as a percentage (%) of control. 100% suppression of worm adult motility of adult parasites was deemed appropriate anti-A. tetraptera activity. A calculation was made to determine the percentage of viable or surviving organisms in each Petri dish (Koike et al., 2015). The mortality % of the parasite for each extract's activity was carried out according to the following formula:

Mortality of parasites (%) =

 $\frac{Control - tested \ sample \ with \ extract}{Control} \times 100\%$ 

# Concentration processing

Different six treatments were used to test *A. tetraptera* worms *in vitro*, Containing 4 concentrations (50, 100, 200 and 400 mg/ml) of *L. nobilis* extract. Ten actively moving adult worms were then placed in each petri dish at room temperature. A

saline solution and 10 mg/ml of Albendazole were prepared and used as negative and positive controls. The number of test worms in each petri dish, 10 worms (5 males and 5 females), The test was repeated three times for all concentrations. After treatment, observations were made by recording the death time for worms at 20, 40, 80, 120 and 180 minutes. Worms are considered dead if they do not move for 30 seconds after touching their body parts using a surgical needle and the petri dish is shaken.

#### **Experimental animals**

This experiment used thirty-six mice, adult male and female C57BL/6 between 10-12 weeks old and weighed an average of 21 g/mouse, naturally infected with *A. tetraptera*. They were housed in clean cages and maintained under standard laboratory conditions at an ambient temperature of 20°C and a 12-hour light-dark cycle. They were allowed free access to a normal pellet diet and water *ad libitum*. Parasitological examinations of animals were made by centrifugal sedimentation techniques prepared with salt water. The mice naturally infected by *A. tetraptera* were identified and used in the study.

# Experimental design and treatment strategy

The thirty-six mice naturally infected with *A. tetraptera* were divided into six groups (G1, G2, G3, G4, G5 and G6) according to the egg burden of the animals and each group comprised six mice. These groups were treated as Group 1, Negative control (infected, untreated). Group 2 was treated with 50  $\mu$ g/ml. Group 3 was treated with 100  $\mu$ g/ml. Group 4 was treated with 200  $\mu$ g/ml. Group 5 was treated with 400  $\mu$ g/ml of *L. nobilis* for 5 days. Group 6 was treated with 10 mg/mL Albendazole (Veterinary Agriculture Products Company (V.A.P.C.O.) for 3 days as a positive control. The doses of treatment and extract were given to the mice according to the weights of the mice, where the average weight of the mice was 22 grams.

# Adult worm motility test in vivo

Post-treatment examination, the mice were divided into two phases: The first was to collect the feces from each group after treatment for about three days. The eighth day after the treatment for five days. Each mouse in a cage was separated from each group and 1 gm of feces was taken. The worm count was examined, then 3 mice from each group were slaughtered and the intestines of mice in all groups were opened and washed with saline solution. Parasites were collected and identified under a stereomicroscope. Worm burden was compared between groups.

The second, a week after the first stage and in the same way, the stools were collected, examined and the worms counted, then the rest of the mice were slaughtered in all a group. The worm burden was compared between the groups and between the two stages.

#### Statistical analysis

The ANOVA analysis was performed in one direction and statistical comparisons between the groups were then made

using the Duncan technique. At a significance level of p≤0.05, values have been reported as the mean minus the standard deviation. The origin 2018 program was applied for statistical analysis. Using Microsoft ® Excel 2003, we were able to determine the means as well as the standard deviations. The rates of worm expulsion were determined by dividing the number of worms expelled from a treatment group by the total number of worms that the group carried.

#### **RESULTS AND DISCUSSION**

### Infrared spectroscopy

The results of infrared spectroscopy reveal several active chemical compounds in *Laurus nobilis* extract with different leading bands at 3409.71 Cm<sup>-1</sup>, 2927.1 Cm<sup>-1</sup>, 1712.53 Cm<sup>-1</sup>, 1606.44 Cm<sup>-1</sup>, 1515.18 Cm<sup>-1</sup>, 1450.96 Cm<sup>-1</sup>, 1368.61 Cm<sup>-1</sup>, 1270.36 Cm<sup>-1</sup>, 1152.72 Cm<sup>-1</sup>, 1122.52 Cm<sup>-1</sup>, 1066.69 Cm<sup>-1</sup>,

1033.40 Cm<sup>-1</sup>, 818.35 Cm<sup>-1</sup>, 795.06 Cm<sup>-1</sup> and 596.17 Cm<sup>-1</sup>, respectively (Fig 1). However, compounds were also detected (Table 1).

# Total flavonoids and phenolics

The amounts of some secondary metabolites in the LNE were measured, like phenolics and flavonoids (Fig 2) showing that the amount of phenols concentration 141.76±0.4 was high compared to the flavonoids concentration 12.08±0.2.

## In vitro

The effects of the plant extract (*Laurus nobilis*) on *A. tetraptera* worms were studied in vitro at therapeutic doses of 50, 100, 200 and 400 mg/mL. They compared it to a positive control (Distilled water) and controlled negative 10 ml/ml Albendazole. Results showed that thorough worm

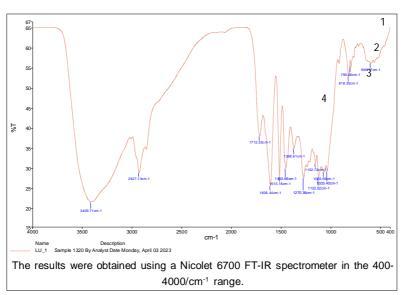


Fig 1: Infrared spectroscopy results of L. nobilis extract.

Table 1: FT-IR spectrum of L. nobilis extracts based on the frequency range.

Absorption (cm <sup>-1</sup> )	Appearance	Transmittance (%)	Group	Compound class	
3409.71	Medium	22	N-H stretching	Aliphatic primary amine	
2927.1	Strong, broad	28	N-H stretching	Amine salt	
1712.53	Strong	35	C=C stretching	Aliphatic ketone	
1606.44	Medium	25	C=C stretching	Conjugated alkene	
1515.18	Strong	30	N-O stretching	Nitro compound	
1450.96	Medium	29	C-H bending	Alkane	
1368.61	Strong	39	S=O stretching	Sulfonamide	
1270.36	Strong	36	C-O stretching	Alkyl aryl ether	
1152.72	Strong	31	C-O stretching	Aliphatic ether	
1122.52	Strong	25	C-O stretching	Secondary alcohol	
1066.69	Strong	29	C-O stretching	Primary alcohol	
1033.40	Strong	24	S=O stretching	Sulfoxide	
818.35	Medium	53	C=C bending	Alkene	
795.06	Medium	57	C=C bending	Alkene	
596.17	Strong	62	C-CI stretching	Halo compound	

examination after 20, 40, 80, 120 and 180 minutes of drug doses were given to test the anti-parasitical effects and see if it was still alive or dead. The worms exposed to a low concentration of extracts (50 and 100 mg/mL) had lower mortality rates during the periods (20, 40 and 80 min), respectively. (Fig 3A). While the worms that were exposed to a high concentration of extracts (200 and 400 mg/mL), the mortality rate was (96 and 100%) had a high mortality rate during the periods (120 and 180 min), respectively. (Fig 3B). Compared to the negative control, the mortality rate was (80 and 90%) respectively. While the positive control was not exposed to drugs, the death rate is almost zero.

In general, we notice an increased mortality rate of the *A. tetraptera* worms with more extended periods and high-concentration doses of the *L. nobilis* extract, which is inversely proportional to the time, the higher the concentration, the parasite dies in a shorter period and vice versa, with the increase in the worm's period with low

concentration doses of the extract, it needs a more extended period until it is dead.

Mortality rates of 82% and 100% were observed following treatment periods of 180 minutes for A. tetraptera worms with concentrations of 200 and 400 mg/mL of L. nobilis extract and 90% at 10 mL/mL Albendazole, compared to the other group, not exceeding a mortality rate of 2%, after 80 minutes of exposure. The 400 and 200 mg/mL concentrations and the reference drug (10 mg/mL) showed a higher mortality rate than the control group (distilled water). While at the other concentrations (50 and 100 mg/mL), mortality was low during the periods after 20 and 40 minutes, which showed low levels of motility for all periods. At the same time, they were common in other concentrations. While the A. tetraptera worm experienced 97% mortality after 120 minutes of L. nobilis extract treatment at a concentration of 400 mg/mL, the albendazole dose had a death rate of 80% and low death in other concentrations absent in the

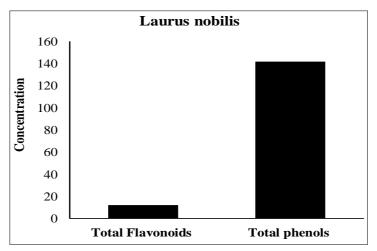


Fig 2: Flavonoids and total polyphenols in the leaves methanolic extract of the Laurus nobilis extracts.

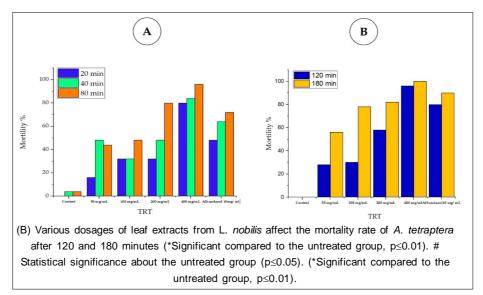


Fig 3: (A) The influence of various dosages of leaf extracts from L. nobilis on the mortality rate of A. tetraptera after 20, 40 and 80 minutes.

control group. The main impacts of the mortality rate and experimental groups on the survival and death of A. tetraptera worms in vitro are shown in Fig 3. In contrast, as treatment times were extended, the death proportion increased. For treated periods up to 160 min (p $\leq$ 0.01) and 120 min (p $\leq$ 0.05), the mortality of the A. tetraptera worms considerably increased, demonstrating statistically different viability between 20, 40 and 80 min exposure (Fig 4).

The present result is comparable to those obtained utilizing different kinds of parasites reported by some researchers (Monforte et al., 1995), found that Laurus nobilis essential oil had an inhibitory effect against H. contortus egg hatching with an inhibition value of 1.72 mg/mL and 87.5% immobility of adult worms after 8 h of exposure to 4 mg/mL of L. nobilis essential oil in vitro. L. nobilis essential oil extracted from the leaves of L. nobilis has shown the presence of

monterpenese 1,8-cineol, D-limonene and linalool. Previous studies have demonstrated that plant *linalool-rich* extracts exhibit promising biological activities, including cytotoxic, antimicrobial and antiparasitic properties. also, found that in leaf extracts of *Cinnamomum camphora*, linalool was the most abundant constituent. Linalool effects were exhibited on snails and cercaria of *Schistosoma japonicum in vitro* (Cedillo Rivera *et al.*, 2002). It was observed that *L. nobilis* extract has acaricidal activity and larval efficacy at different concentrations and periods (Mares *et al.*, 2022).

#### In vivo

In Fig 5, the Concentrations of the plant extract were tested to obtain the best concentration that leads to the death of worms and the concentration of 400 ml/kg showed the most lethal effects for worms (Fig 6).

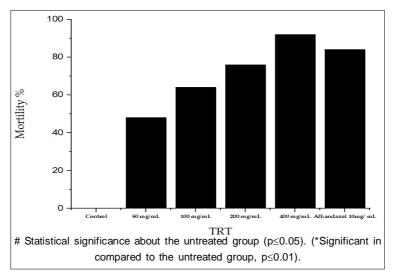


Fig 4: The general influences of various dosages of leaf extracts (50, 100, 400 mg/ml) of L. *nobilis* and reference drug (10 mg/ml Albendazole) on the mortality rate of A. tetraptera of 20 to 180 minutes.

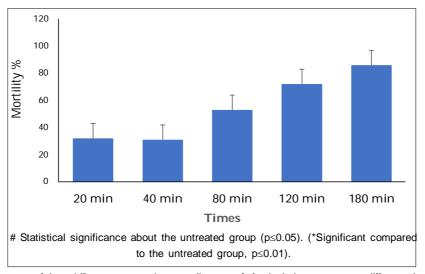


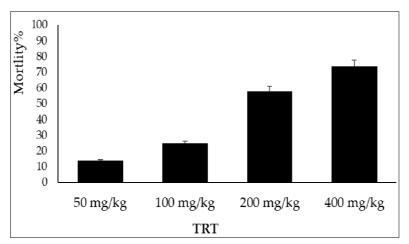
Fig 5: Main times influences of *L. nobilis* extracts on the mortality rate of *Aspiculuris tetraptera* at different dosages during 20, 40, 80, 120 and 180 minutes

In Fig 7, infected mice that did not receive drugs were compared with the highest concentration of the extract and the reference treatment. The attention showed significant differences  $P \le 0.05$ .

In Fig 8, the results showed that the mortality rate of worms taken from the intestines of the treated mice that were slaughtered three days after giving the treatment reached 96% in the concentration of 400 ml/kg of the extract and 89% for the mice that treated in 10 ml/kg of met albendazole. In comparison, the mortality rate of worms taken from the intestines of treated mice that were slaughtered six days after administration of the treatment reached 100% in the concentration of 400 ml/kg of extract and 97% for the treated mice in 10 ml/kg of Albendazole (Fig 8). It is clear from Table 2 that the group treated with 400 mg/kg of plant extract and Albandazole 10 mg/kg reduced the number of eggs in the feces until it reached

zero on the sixth day, as well as when slaughtering in the intestines (Table 2).

This study is consistent with many other studies that verified the effectiveness of *L. nobilis* leaves against various parasites. Sebai *et al.* (2022), found *in vivo* anthelmintic potential in *L. nobilis* extract eliminated the egg output of *Heligmosomoides polygyrus* after 7 days of treatment and with a 79.2% reduction in total worm counts. In addition, the presence of linalool in the *Cinnamomum camphora* plant markedly reduced the recovered schistosomulum from mouse skin after challenge infection. It decreased the worm burden in infected animals (Batiha *et al.*, 2018). The *L. nobilis* plant was rich in linalool, which plays a vital role in eliminating worms in the intestines of infected mice (Sebai *et al.*, 2022). Reported that *L. nobilis* has potent antioxidant, antimicrobial and antibacterial activity (Kivçak and Mert, 2002). Traditionally, *L. nobilis* leaves have been used to treat gastrointestinal



**Fig 6:** Comparison of dose best to influences of *L. nobilis* extracts on the mortality rate of *Aspiculuris tetraptera* at different concentrations *in vivo*.

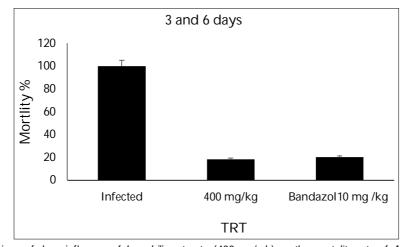


Fig 7: Comparison of dose influence of *L. nobilis* extracts (400 mg/mL) on the mortality rate of *Aspiculuris tetraptera* with the untreated group and reference drug (10 mg/kg albendazole) *in vivo*.

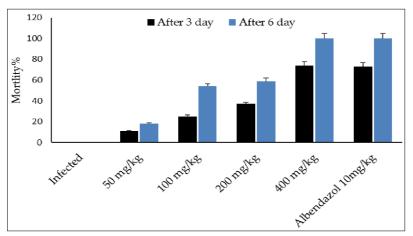


Fig 8: In vivo effects of L. nobilis extracts on the mortality rate of Aspiculuris tetraptera at different dosages after 3 and 6 days of treatment.

**Table 2:** The average number of *A. tetraptera* worms obtained by fecal and intestinal centrifugation flotation per cage per mouse at necropsy in a strain of mice treated with *L. nobilis* extract and the anthelmintic drug.

	n	Average no. of worms after 3 days of treatment		Average no. of worms after 6 days of treatment	
Drugs					
		In feces	In intestinal	In feces	In intestinal
Infectad	6	332±30	445±77	295±25	419±75
50 mg/kg	6	250±10	299±15	220±5	177±5
100 mg/kg	6	80±10	130±15	110±5	125±5
200 mg/kg	6	50±10	90±15	20±5	25±5
400 mg/kg	6	3±1	7±1	00	00
Albandazole 10 mg/kg	6	5±2	12±2	00	00

symptoms, such as eructation, epigastric bloating, impaired digestion and flatulence (Jemâa et al., 2012).

This study and other studies revealed the appropriate anti-parasite effects of L. nobilis, which indicate that they might be used instead of chemical drugs in parasite control programs.

## CONCLUSION

This article summarizes medicinal plants' in vitro and in vivo antiparasitic efficacy and their constituents against gastrointestinal parasites. According to the findings of these studies, herbal medicines have a significant amount of potential for developing novel drugs to combat parasitic disorders and the derivatives of these plants are helpful structures for the synthesis of drugs and the optimization of their bioactivities. In addition, herbal medicines have a significant amount of potential for developing novel drugs to combat parasitic disorders and the derivatives of these plants are helpful structures for the synthesis of drugs and the optimization of their bioactivities.

# Ethics approval and consent to participate

The research complied with the ethical standards for using animals set forth by the Kingdom of Saudi Arabia (Ethics Committee, King Saud University, Ethics Agreement ID: KSU-SE-21-86).

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No found.

## **Conflict of interest**

The authors declare no conflict of interest.

## REFERENCES

Abu Hawsah, M., Al-Otaibi, T., Alojayri, G., Al-Shaebi, E.M., Dkhil, M.A., Elkhadragy, M.F., Abdel-Gaber, R. (2023). In vitro studies for the antiparasitic activities of Azadirachta indica extract. Food Science and Technology. 43(2). DOI: 10.1590/ fst.117122.

Alimi, D., Hajri, A., Jallouli, S., Sebai, H. (2021). *In vitro* acaricidal activity of essential oil and crude extracts of *Laurus nobilis*, (Lauraceae) grown in Tunisia, against arthropod ectoparasites of livestock and poultry: Hyalomma scupense and Dermanyssus gallinae. Veterinary Parasitology. 298: 109507. https://doi.org/10.1016/j.vetpar.2021.109507.

- Amirmohammadi, M., Khajoenia, S., Bahmani, M., Rafieian-Kopaei, M., Eftekhari, Z., Qorbani, M. (2014). In vivo evaluation of antiparasitic effects of Artemisia abrotanum and Salvia officinalis extracts on Syphacia obvelata, aspiculoris tetrapetra and Hymenolepis nana parasites. Asian Pacific Journal of Tropical Disease. 4: S250-S254.
- Batiha, G.E.S., Beshbishy, A.M., Tayebwa, D.S., Shaheen, H.M., Yokoyama, N., Igarashi, I. (2018). Inhibitory effects of uncaria tomentosa bark, myrtus communis roots, *Origanum* vulgare leaves and cuminum cyminum seeds extracts against the growth of babesia and theileria in vitro. Jpn. J. Vet. Parasitol. 17(1).
- Begum, A., Sandhya, S., Vinod, K. R., Reddy, S., Banji, D. (2013).

  An in-depth review on the medicinal flora *Rosmarinus* officinalis (Lamiaceae). Acta Scientiarum Polonorum Technologia Alimentaria. 12(1): 61-74.
- Caputo, L., Nazzaro, F., Souza, L. F., Aliberti, L., De Martino, L., Fratianni, F., De Feo, V. (2017). *Laurus nobilis*: Composition of essential oil and its biological activities. Molecules. 22(6): 930. doi: 10.3390/molecules22060930.
- Cedillo Rivera, R.O.B.E.R.T.O., Chávez, B., González Robles, A.R.T.U.R.O., Tapia, A., Yépez Mulia, L.I.L.I.Á.N. (2002). *In vitro* effect of nitazoxanide against entamoeba histolytica, giardia intestinalis and trichomonas vaginalis trophozoites. Journal of Eukaryotic Microbiology. 49(3): 201-208.
- Falcón-Ordaz, J., Pulido-Flores, G., Monks, S. (2010). Una nueva especie de Aspiculuris (Nematoda: Heteroxynematidae), parásito de Mus musculus (Rodentia: Muridae), de Hidalgo, México. Revista mexicana de biodiversidad. 81(3): 669-676.
- Fernandez, C.M.M., da Rosa, M.F., Fernandez, A.C.A.M., Bortolucci, W.D.C., Ferreira, F.B.P., Linde, G.A., Gazim, Z.C. (2020). Essential oil and fractions isolated of laurel to control adults and larvae of cattle ticks. Natural Product Research. 34(5): 731-735.
- Gaherwal, S., Solanki, S., Prakash, M.M., Wast, N. (2012). Aspicularis tetraptera induced hematological parameters in infected and vaccinated mice. Iranian Journal of Parasitology. 7(2): 61-66.
- Gerwin, P.M., Ricart Arbona, R.J., Riedel, E.R., Lepherd, M.L., Henderson, K.S., Lipman, N.S. (2017). Evaluation of traditional and contemporary methods for detecting Syphacia obvelata and aspiculuris tetraptera in laboratory mice. Journal of the American Association for Laboratory Animal Science. 56(1): 32-41.p8.
- Jemâa, J.M.B., Tersim, N., Toudert, K.T., Khouja, M.L. (2012). Insecticidal activities of essential oils from leaves of Laurus nobilis L. from Tunisia, Algeria and Morocco and comparative chemical composition. Journal of Stored Products Research. 48: 97-104.
- Kivçak, B., Mert, T. (2002). Preliminary evaluation of cytotoxic properties of laurus nobilis leaf extracts. Fitoterapia. 73(3): 242-243.
- Koike, A., Barreira, J. C., Barros, L., Santos-Buelga, C., Villavicencio, A.L., Ferreira, I.C. (2015). Edible flowers of Viola tricolor L. as a new functional food: Antioxidant activity, individual phenolics and effects of gamma and electron-beam irradiation. Food Chemistry. 179: 6-14.

- Mares, M.M., Abdel-Gaber, R., Quraishy, S.A. (2022). In vitro assessment of the acaricidal activity of laurus nobilis and croton tiglium seeds extract against *Hyalomma dromedarii* ticks. Indian Journal of Animal Research. 57(5): 626-631. DOI:10.18805/IJAR.BF-1546.
- Merghni, A., Marzouki, H., Hentati, H., Aouni, M., Mastouri, M. (2016). Antibacterial and antibiofilm activities of *Laurus nobilis* L. essential oil against *Staphylococcus aureus* strains associated with oral infections. Current Research in Translational Medicine. 64(1): 29-34.
- Monforte, M.T., Trovato, A., Kirjavainen, S., Forestieri, A.M., Galati, E.M., RB, L.C. (1995). Biological effects of hesperidin, a citrus flavonoid. (note II): Hypolipidemic activity on experimental hypercholesterolemia in rat. Farmaco (Societ\( \tilde{A} \) chimica italiana: 1989). 50(9): 595-599.b
- Moulia, C., Brun, N. L., Loubes, C., Marin, R., Renaud, F. (1995).
  Hybrid vigour against parasites in interspecific crosses between two mice species. Heredity. 74(1): 48-52.
- Oliveira, L.M.B., Bevilaqua, C.M.L., Costa, C.T.C., Macedo, I.T.F., Barros, R.S., Rodrigues, A.C.M., Navarro, A.M.C. (2009). Anthelmintic activity of *Cocos nucifera* L. against sheep gastrointestinal nematodes. Veterinary Parasitology. 159(1): 55-59.b
- Omer, S.A., Alghamdi, J.M., Alrajeh, A.H., Aldamigh, M., Mohammed, O.B. (2020). Morphological and molecular characterization of aspiculuris tetraptera (Nematoda: Heteroxynematidae) from Mus musculus (Rodentia: Muridae) in Saudi Arabia. Bioscience Reports. 40(12): BSR20203265. doi: 10.1042/BSR20203265.
- Ordonez, A.A.L., Gomez, J.D., Vattuone, M.A. (2006). Antioxidant activities of sechium edule (Jacq.) swartz extracts. Food Chemistry. 97(3): 452-458.þ
- Paparella, A., Nawade, B., Shaltiel-Harpaz, L., Ibdah, M. (2022). A review of the botany, volatile composition, biochemical and molecular aspects and traditional uses of *Laurus* nobilis. Plants. 11(9): 1209. https://doi.org/10.3390/ plants11091209.
- Pritchett-Corning, K.R., Clifford, C.B. (2012). Parasitic infections of laboratory mice. The Laboratory Mouse. 503-518.
- Ranasinghe, S., Armson, A., Lymbery, A. J., Zahedi, A., Ash, A. (2023). Medicinal plants as a source of antiparasitics: An overview of experimental studies. Pathogens and Global Health. 1-19.
- Scantlebury, M., Waterman, J.M., Hillegass, M., Speakman, J.R., Bennett, N.C. (2007). Energetic costs of parasitism in the Cape ground squirrel Xerus inauris. Proceedings of the Royal Society B: Biological Sciences. 274(1622): 2169-2177.
- Sebai, E., Abidi, A., Benyedem, H., Dhibi, M., Hammemi, I., Akkari, H. (2022). Phytochemical profile and anthelmintic effects of *Laurus nobilis* essential oil against the ovine nematode *Haemonchus contortus* and the murine helminth model heligmosomoides polygyrus. Veterinary Parasitology. 312: 109835.b doi: 10.1016/j.vetpar.2022.109835.
- Simić, A., Soković, M.D., Ristić, M., Grujiæ Jovanović, S., Vukojević, J., Marin, P.D. (2004). The chemical composition of some Lauraceae essential oils and their antifungal activities. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 18(9): 713-717.

- Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M. (1999). [14]

  Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-ciocalteu Reagent.

  In Methods in Enzymology. Academic Press. 299: 152-178.
- Sırıken, B., Yavuz, C., Güler, A. (2018). Antibacterial activity of Laurus nobilis: A review of literature. Medical Science and Discovery. 5(11): 374-379.
- Stepek, G., Lowe, A.E., Buttle, D.J., Duce, I.R., Behnke, J.M. (2006). *In vitro* and *in vivo* anthelmintic efficacy of plant cysteine proteinases against the rodent gastrointestinal nematode, Trichuris muris. Parasitology. 132(5): 681-689.
- Taylor, M.A., Coop, R.L., Wall, R.L. (2015). Veterinary Parasitology. John Wiley Sons.
- Weinstock, J.V., Elliott, D.E. (2009). Helminths and the IBD hygiene hypothesis. Inflammatory Bowel Diseases. 15: 1-128-133.
- Yang, F., Long, E., Wen, J., Cao, L., Zhu, C., Hu, H., Lv, Z. (2014). Linalool, derived from *Cinnamomum camphora* L. presl leaf extracts, possesses molluscicidal activity against *Oncomelania hupensis* and inhibits infection of Schistosoma japonicum. Parasites Vectors. 7(1): 1-13.