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In vitro Assessment of the Acaricidal Activity of *Laurus nobilis* and *Croton tiglium* Seeds Extract against *Hyalomma dromedarii* Ticks

Mohammed M. Mares[1], Rewaida Abdel-Gaber[1], Saleh Al Quraishy[1]

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ABSTRACT

Background: Ticks are destructive ectoparasites that feed on the blood of domestic animals and the spread of ticks causes significant losses in meat, milk and leather production. About 800 species of ticks are known around the world, *Hyalomma dromedarii* is one of the ticks that attack camels as their main host. The objective of this study was to identify the acaricidal activity of *Laurus nobilis* and *Croton tiglium* seeds extract against *H. dromedarii* ticks and comparison with some drugs used against external parasites.

Methods: A study was performed to evaluate the acaricidal activities of methanolic extracts of two medicinal plants, namely the seeds of *C. tiglium* and *L. nobilis*, against *H. dromedarii* using an adult immersion test and larval bundle test. Five graduated concentrations of extracts, 6.25, 12.5, 25, 50 and 100 mg/ml, were tested at different periods and changes over time in the viability of ticks were registered for 24 hr. Distilled water and cypermethrin (0.1%) were used as a negative and positive control, respectively.

Result: From 30 min after exposure, a concentration of 100 mg/ml of *C. tiglium* seed extract resulted in higher mortality ($p<0.05$) compared with cypermethrin. A significant rise in tick mortality began 2 hr after exposure to a concentration of 100 mg/ml of *C. tiglium* seed extract and cypermethrin. At 24 hr after exposure, cypermethrin and concentrations of 50 and 100 mg/ml of *C. tiglium* extract induced significantly higher tick mortality compared to the rest of the concentrations. A significant increase in tick mortality began 3 hr after exposure to cypermethrin and concentrations of 50 and 100 mg/ml of *Laurus nobilis* extract and 12 hr after exposure to concentrations of 6.25, 12.5 and 25 mg/ml *L. nobilis* extract. At 24 hr after the exposure time, concentrations of 50 and 100 mg/ml of the extract and cypermethrin had a comparable higher tick mortality effect compared to the remaining concentrations below 25 mg/ml ($p<0.05$). The lower concentration (6.25 mg/ml) resulted in notably higher mortality of adult ticks and larvae compared to the negative control (distilled water) at 24 hr exposure to both extracts. At 24 hr after the exposure period, the tick mortality of all estimated plant extracts also increased with raised exposure time and concentration. Therefore, studied plants can be used against *H. dromedarii* as a potential alternative to commercially available medicines. Further studies should include more research on separating each component and validating the materials.

Key words: *Croton tiglium, Hyalomma dromedarii*, *Laurus nobilis*, Ticks.

INTRODUCTION

Ticks are destructive ectoparasites that feed on the blood of domestic animals and wild causing great economic losses (Habeeb, 2010). About 800 species of ticks are known around the world, some of which can carry pathogens such as bacteria, viruses, or other organisms that cause health problems (Thorsell *et al*., 2006). *Hyalomma dromedarii* is one of the ticks that attack camels as their main host; this kind is known to be a major hurdle to camel production in some parts of the Middle East (Klafke *et al*., 2006). The spread of ticks causes significant losses in meat, milk and leather production (Eskezia and Desta, 2016).

Currently, global tick control is largely based on the reiterated use of acaricides, leading to problems concerning environmental pollution, contamination of milk and meat and the development of drug resistance leading to raise control costs (Pavela *et al*., 2016). Therefore, there is an urgent need for new tick control strategies to defeat the disadvantages related to the use of synthetic drugs. An alternative management strategy could be phytotherapy because it is safer for public health and the environment (Madzimure *et al*., 2011).

[1]Department of Zoology, College of Sciences, King Saud University, Riyadh, Saudi Arabia.

Corresponding Author: Mohammed M. Mares, Department of Zoology, College of Sciences, King Saud University, Riyadh, Saudi Arabia. Email: mmares@ksu.edu.sa

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Laurus nobilis of the camphor family (Lauraceae), commonly known as laurel, is a species of fragrant angiosperm native to the southern Mediterranean region and widely grown in Europe and the United States. It is grown commercially for its fragrant leaves. It is especially distinguished by the fact that it exhibits biological activity (Caputo *et al*., 2017). It is associated with its extract and essential oils as an antifungal agent (Simi *et al*., 2004), antibacterial (Siriken *et al*., 2018), acaricidal

activity (Fernandez *et al*., 2020) and insecticidal activity (Jemaa *et al*., 2012).

Croton tiglium belongs to the family Euphorbiaceae in equatorial and moderate regions of the world (Hecker, 1968). It is widely used in folk medicine to treat certain cancers (Nath *et al*., 2013). The seeds, leaves, roots and bark of *C. tiglium* are used in conventional medicine to treat constipation, dyspepsia, dysentery, digestive disorders, enteritis, diarrhea, peptic ulcers, fever and snake poisoning (Tsai *et al*., 2004). *C. tiglium* seeds have been reported to be famous for their toxicity. This is because seed oil contains phorbol esters and crotonic acid in addition to fatty acids, also to the existence of active plant components (Hu *et al*., 2010).

The present study aimed to evaluate the acaricidal activity of *L. nobilis* and *C. tiglium* seeds extract against *H. dromedarii* ticks.

MATERIALS AND METHODS

Collection of ticks, eggs and larvae

Adult engorged females of *H. dromedarii* (Fig 1) were collected from naturally infected camels on different farms in Al-Kharj city, Saudi Arabia. To collect ticks, the entire body surface of the animal is carefully examined and adult ticks are collected from the animal's body if present. Collected ticks are placed in vials and wrapped in cotton mesh gauze to provide oxygen. The collected ticks were transported to Parasitology Lab at the Department of Zoology, College of Science, King Saud University. Ticks were identified according to Estrada-Peña *et al*. (2004). A portion of these ticks was utilized for the adult immersion test while the remainder was incubated under laboratory conditions at 27±1.5°C and 70-80% relative humidity (Drummond *et al*., 1973) to obtain eggs and then larvae (Fig 1) that used in the further bioassays.

Preparation of extracts

L. nobilis and *C. tiglium* seeds were collected from a local market in Riyadh, Saudi Arabia. Powder totaling 500 g from each plant was extracted separately with 70% methanol as follows: 100 g of dry powder was added to 400 ml of 70% methanol and mixed gently for one hour using a magnetic stirrer. The obtained solution was left at room temperature for 24 hr, then stirred again and filtered. Then the solvent was evaporated on a rotary evaporator.

Acaricidal activity evaluation

Preparation of concentrations of methanolic extracts

The dry extracts were diluted in distilled water to the concentrations coveted for biological assays (6.25, 12.5, 25, 50 and 100 mg/ml) for the tested plants. The concentrations were used to test the acaricidal effect. Distilled water and cypermethrin (0.1%) were used as negative and positive control. The positive control, 0.1% cypermethrin was diluted in water according to the manufacturer's recommendation (1:1000) before being utilized for the further experiment (Heukelbach *et al*., 2006).

Adult immersion test (AIT)

In vitro testing commenced within 6 hr of tick collection. Ten adult ticks active in three replicates were placed in a petri dish and 3 mL of each concentration was added directly to three repeat Petri-dishes for 2 min exposure. After soaking, the ticks were filtered through filter paper and placed in separate Petri-dishes (Zaman *et al*., 2012). 3 ml of distilled water and 0.1% cypermethrin 60 EC were used as negative and positive controls. Petri dishes were incubated at 28°C with 80% relative humidity and all tick in each petri dish was closely spotted for death under a stereomicroscope at 30 min, 1 hr, 2 hr, 3 hr, 6 hr, 12 hr and 24 hr periods (Du *et al*., 2008). The survival rate of the tick was regularly checked by acupuncture and if there was no response, the tick was recorded as dead. Mortality was calculated using the formula given by Krishnaveni and Venkatalakshmi (2014), as follows:

$$\text{Mortality \%} = \frac{\text{Number of dead ticks}}{\text{Total number of ticks}} \times 100$$

Larval packet test (LPT)

H. dromedarii larval pack assay was used for each treatment according to Stone and Haydock (1962). Filter paper sheets (2 × 2 cm) were impregnated with 1 ml extracts of *L. nobilis* and *C. tiglium* seed at different concentrations of 6.25, 12.5, 25, 50 and 100 mg/ml, respectively. One hundred larvae, 15 to 20 days old, were deposited on each leaf impregnated with the solution. After 24 hr of impregnation, larvae were placed in packets and then incubated at 28°C with 80% relative humidity (Figueiredo *et al*., 2018). After 24 hr, a mortality assessment was performed. Larvae that do not

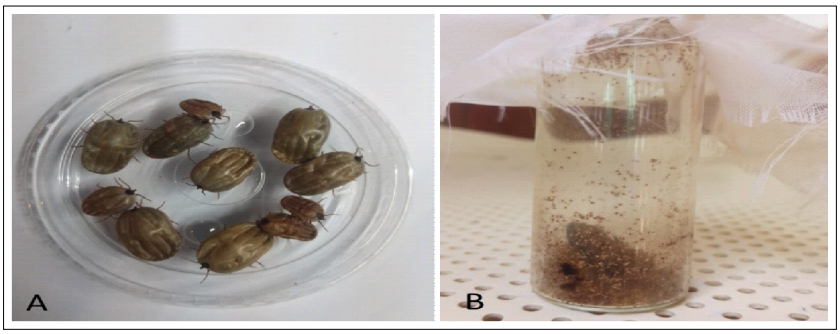

Fig 1: (A): Adult engorged female *Hyalomma dromedarii* (before application of any treatment); (B): Larvae *Hyalomma dromedarii* (before application of any treatment).

move are considered dead. Three replicates were performed for each concentration, as well as for distilled water and 0.1% cypermethrin 60 EC was used as negative and positive controls. Mortality was calculated using the formula given by Krishnaveni and Venkatalakshmi (2014), as follows:

$$\text{Mortality \%} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

Statistics

Statistical analysis of the data was performed utilizing the Statistical package for the social sciences (SPSS for Windows (IBM), version 22, Chicago, USA). ANOVA tests and subsequent Duncan's multiple range tests were applied to determine the differences between means. Data were presented as averages and the values were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

In vitro* acaricidal activity of the *C. tiglium* seeds extracts against adult and larval *H. dromedarii

A significant rise in tick mortality beginning 2 hr post-exposure with 100 mg/ml concentration of *C. tiglium* seeds extract and cypermethrin. From 30 min after exposure, a concentration of 100 mg/ml of *C. tiglium* seed extract resulted in significantly higher mortality than cypermethrin ($p<0.05$). At 24 hr after the exposure time, cypermethrin and concentrations of 50 and 100 mg/ml of the extract resulted in significantly higher tick mortality compared to the remaining concentrations below 50 mg/ml ($p<0.05$). The lower concentration (6.25 mg/ml) was significantly more lethal than the negative control (distilled water) at 24 hr of exposure (Table 1). At 24 hr after exposure, concentrations of 50 and 100 mg/ml of *C. tiglium* seed extract and cypermethrin (Fig 2, 3) were more effective against larvae than the remaining concentrations below 50 mg/ml ($p<0.05$). The lower concentration (6.25 mg/ml) was significantly more lethal than the negative control (distilled H_2O) at 24 hr of exposure (Table 3). *C. tiglium* seeds extract showed a good *in vitro* tick lethal effect. As the concentration and duration of exposure increased, the mortality of *H. dromedarii* adults and larvae also increased. The present result is comparable to those obtained utilizing different kinds of parasites reported by some researchers. Bodas *et al*. (2014) reported that the *C. tiglium* extracts showed paralysis and death of Indian earthworms than the reference drug albendazole. Abon (2021) reported the ability of *C. tiglium* seeds in native chickens (*Gallus domesticus*) particularly against *Ascaridia galli* and *Heterakis gallinarum* as alternative anti-worms. Liu (2014) reported the ability of *C. tiglium* extract caused 100% mortalities of the root-knot nematode at 1000 µg/ml for 72 hr. Dohutia *et al*. (2015) reported that the extract of *C. tiglium* seeds had remarkable mosquito larvicidal activity *Anopheles stephensi*. This may be attributed to the fact that the *C. tiglium* seeds extracts are considered poisonous plants and can eliminate ticks and their larvae, as well as many kinds of parasitic worms.

In vitro* acaricidal activity of the *L. nobilis* extracts against adult and larval *H. dromedarii

Tick mortality was significantly increased beginning 3 hr after exposure to cypermethrin and concentrations of 50 and 100 mg/ml of *L. nobilis* extract and 12 hr after exposure to concentrations of 6.25, 12.5 and 25 mg/ml of *L. nobilis* extract. At 24 hr after the exposure time, concentrations of 50 and 100 mg/ml of the extract and cypermethrin were equally effective against the ticks compared to the remaining concentrations under 25 mg/ml ($p<0.05$). The lower

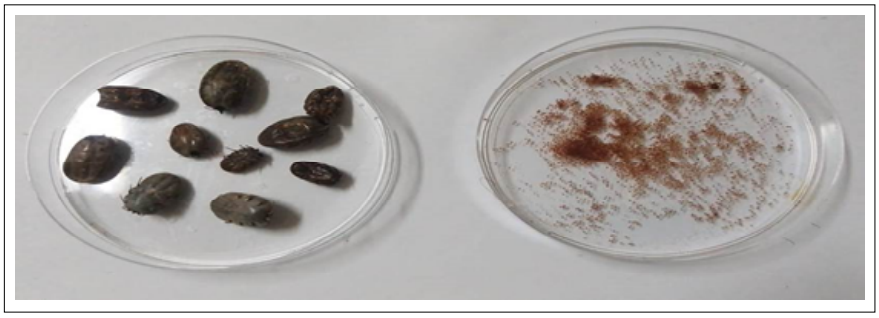

Fig 2: Adult *Hyalomma dromedarii* ticks and larva treated with two extracts (24 hr after treatment).

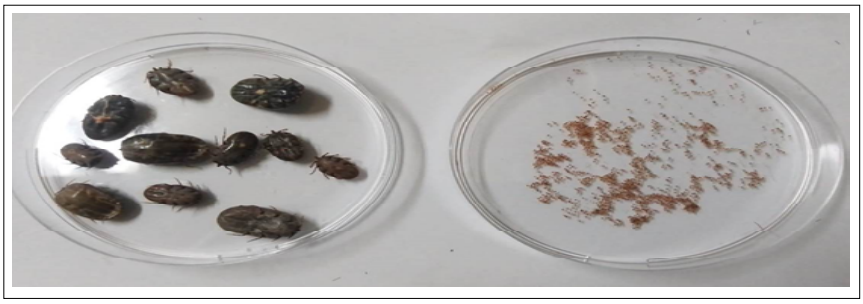

Fig 3: Adult *Hyalomma dromedarii* ticks and larva treated with cypermethrin (24 hr after treatment).

Table 1: *In vitro* tick lethal effect of *C. tiglium* seeds extract against *H. dromedarii.*

Extract concentration (mg/ml)	Average number of ticks dead (average of mortality±SD) at minute/hour after exposure						
	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
100	0.33±0.57	1.33±0.00	2.33±0.00	3.33±0.00	5.00±0.57	6.66±057	9.00±0.57
50	0.00±0.00	0.66±0.57	1.66±0.00	2.66±0.00	4.00±0.57	5.66±0.57	8.00±0.57
25	0.00±0.00	0.00±0.00	0.66±0.57	1.66±0.00	3.00±0.57	4.66±0.57	6.66±0.00
12.5	0.00±0.00	0.00±0.00	0.00±0.00	1.00±0.00	2.33±0.57	3.66±0.57	5.33±0.57
6.25	0.00±0.00	0.00±0.00	0.00±0.00	0.33±0.57	1.00±0.57	1.66±0.57	3.00±0.57
0.1% cypermethrin	0.00±0.00	0.66±0.57	2.33±0.57	3.66±0.57	5.33±0.57	7.00±0.00	9.66±0.57
Distilled water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 2: *In vitro* tick lethal effect of *L. nobilis* extract against *H. dromedarii.*

Extract concentration (mg/ml)	Average number of ticks dead (average of mortality±SD) at minute/hour after exposure						
	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
100	0.00±0.00	0.66±0.57	1.66±0.00	3.00±0.57	4.33±0.57	6.00±0.57	8.00±0.00
50	0.00±0.00	0.00±0.00	0.33±0.57	1.66±0.57	3.00±0.57	4.66±0.57	6.66±0.00
25	0.00±0.00	0.00±0.00	0.00±0.00	0.66±0.57	1.66±0.00	3.00±0.57	5.00±0.00
12.5	0.00±0.00	0.00±0.00	0.00±0.00	0.33±0.57	1.00±0.57	2.33±0.57	4.00±0.57
6.25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.66±0.57	2.00±0.57	3.33±0.57
0.1% cypermethrin	0.00±0.00	0.66±0.57	2.00±0.57	3.33±0.57	5.00±0.57	7.00±0.00	9.66±0.57
Distilled water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 3: *In vitro* larval lethal effect of *C. tiglium* seeds and *L. nobilis* extract against larval *H. dromedarii.*

Extract concentration (mg/ml)	Average number of Larval dead (average of mortality±SD) an hour after exposure 24 hr	
	C. tiglium seeds	*L. nobilis*
100	70.00±7	63.33±11.54
50	50.66±4.04	37.00±11.26
25	35.33±4.61	28.33±2.88
12.5	21.66±2.88	17.66±2.08
6.25	18.33±1.52	13.66±5.50
0.1% cypermethrin	81.66±2.88	81.66±2.88
Distilled water	0.00±0.00	0.00±0.00

concentration (6.25 mg/ml) was significantly more lethal than the negative control (distilled H_2O) at 24 hr of exposure (Table 2). At 24 hr after the exposure time, concentrations of 50 and 100 mg/ml of *L. nobilis* extract and cypermethrin (Fig 2,3) were more effective against larvae than the remaining concentrations below 50 mg/ml ($p<0.05$). The least concentration (6.25 mg/ml) was significantly more lethal than the negative control (distilled H_2O) at 24 hr of exposure (Table 3). All concentrations of *L. nobilis* showed a lethal effect on ticks and larvae at different concentrations and exposure times compared with the negative control. The present results are comparable with those obtained using different tick species reported by several researchers. Fernandez *et al*. (2020) studied the effect of essential oils and isolated fractions of *L. nobilis* on the tick *Rhipicephalus microplus*; *in vitro* testing showed mortality in engorged females at a concentration of 200 µl/mL. Alimi *et al*. (2021) reported that the ethanolic extract of *L. nobilis* induced higher mortality in engorged females (86.2%) and eggs hatched at all tested concentrations. The acaricidal activity of *L. nobilis* oil was maximum (100%) on egg hatching at 50 and 100 mg/mL concentrations with 90.67% mortality of *H. scupense* larvae. According to Vinturelle *et al*. (2021), the adult immersion test (AIT) revealed that *L. nobilis* essential oil at 5% or 10% caused 80.5% mortality of engorged females after 24 hr and 96.9% and 100% mortality on the third day after treatment, respectively. Based on the above-tested parameters, the methanol and ethanol extracts of leaves and essential oils of *L. nobilis* showed higher acaricidal activity. The differences between these studies may be due to differences in the solvents utilized for extraction.

CONCLUSION

Extracts of *L. nobilis* and *C. tiglium* seeds were tested against *H. dromedarii* ticks and their larvae for their lethal efficacy at different concentrations and periods. It was observed that *L. nobilis* and *C. tiglium* seeds had strong acaricidal activity greatly comparable to the effect of 0.1% cypermethrin at higher concentrations. The current study concluded that the medicinal plants tested showed a promising lethal effect against *H. dromedarii* ticks and their larvae that could be utilized as a possible alternative to replace commercially available drugs. More *in vivo* and *in vitro* studies are needed to better evaluate the possibility of these extracts, or some of their pure components, as useful alternatives for the treatment of external and internal parasites.

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Conflict of interest: None.

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