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Novel Acaricidal Activity of *Vitex castus* and *Zingiber officinale* Extracts against the Camel Tick, *Hyalomma dromedarii*

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ABSTRACT

Hyalomma dromedarii is one of the most important tick species in the Middle East. Acaricides of chemical origin are used for controlling ticks, but because of their harmful effect, there is a need to find out some natural alternatives such as the promising plant-based pesticides. This work is aimed to study the novel efficacy of ethanol extracts of castus, *Vitex castus*, and ginger, *Zingiber officinale* against the camel tick, *Hyalomma dromedarii* through adult immersion technique and determine their lethal time and concentration values and their toxicity indices. Five days post-treatment (PT), the mortality (MO)% reached 53.9 and 53.8%, respectively. Meanwhile, 15 days PT, MO% were 80.8 and 84.7%, respectively. Three days PT, the LC_{50} values were 12.2 and 11.8%, respectively. Such values PT for five days were 10.5 and 9.6%, respectively. The toxicity indices three days PT with *V. castus* and *Z. officinale* were 96.72 and 100.00%, respectively, and the corresponding values five days PT were 91.43 and 100.00, respectively. LT_{50} values PT with 25% of *V. castus* and *Z. officinale* were 2.6 and 2.5 days, respectively. According to LT_{50} values and PT with 25, 12, and 3%, *Z. officinale* killed ticks 1.04, 1.24, and 1.77 times faster than *V. castus,* whereas the corresponding values according to the LT_{90} values were 1.46, 1.58, and 1.18 times, respectively. In conclusion, the ethanolic extract of *V. castus* and *Z. officinale* extracts were highly effective candidates and could be applied as eco-friendly acaricides after revealing their ecotoxicological profile.

Key words: Costus, Chaste tree, Ginger, Asteraceae, Zingiberaceae, Adult immersion test.

INTRODUCTION

Ticks are blood-feeding ectoparasites infecting wild and domesticated animals and transmit severe infectious diseases (Farooq et al. 2020; Selim and Khater 2020; Selim et al. 2022). The camel tick, *Hyalomma dromedarii* is widely distribution throughout North Africa, Asia and the Middle East infesting mainly camels, but could infest a wide range of domestic species and wildlife (Apanaskevich et al. 2008a). *H. dromedarii* is the vector of the Crimean–Congo hemorrhagic fever virus, *Theileria annulata*, *Theileria camelensis*, and *Coxiella burnetii* (Hoogstraal et al. 1981). Acaricide resistance against conventional acaricides has been reported which opened the door for searching for effective and safe alternative products. Vaccination, botanical and biological control by pathogens or predators, pheromone- assisted acaricides are promising methods used for tick control (Khater 2012).

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Botanicals have long been used for parasite control and are characterized by high efficacy (Seddiek et al. 2011, 2013; Abbas et al. 2020), rapid biodegradation, low toxicity, and prevention of development of resistance against active substances because of various mechanisms of action (Khater 2012). Botanicals controlled pests through ovicidal, larvicidal, and adulticidal effects (Khater and Geden 2019; Alkenani et al. 2021; Baz et al. 2022a, b). There are many studies evaluated with botanical acaricides, but there are very few studies dealing with their adulticidal effects against *H. dromedarii*. Therefore, this work is aimed to study the novel efficacy of ethanol extracts of costus and ginger against the camel tick, *H. dromedarii* and determine their lethal time and concentration values and their toxicity indices.

MATERIALS AND METHODS

Ticks Collection

Adult males of *H. dromedarii* were collected from sites around camels at the domestic slaughterhouse of Toukh, Qalyubia Governorate, Egypt (30° 21′ 11.6″ N, 31° 11′ 31.5″ E). Collected ticks were transported to the laboratory and morphologically identified (Apanaskevich et al. 2008b).

Plant Extraction

Plants were identified at the National Research Centre, Egypt. The ethanol extracts of chaste tree (costus), *Vitex castus* (Asteraceae) and ginger, *Zingiber officinale* (Zingiberaceae) were prepared. Plants were washed twice with distilled water; after dryness at 500°C in vacuum oven for three days, the plants were ground to fine powder and washed with distilled water. About 250g of each plant were placed in one-liter beaker containing about 600ml of 10% ethanol v/v. The beaker was then transferred to a hotplate, and the temperature was raised to 50°C for 3hr with occasional mixing using a glass rod (every 10 min) with flipping the flakes up and down to achieve good extraction. The beaker was attained to cool to the room temperature and left at the temperature of 5-10°C for 2 hr. The cooled beaker was filtered several times using cotton tissue, then filtered using a Whattman filter paper, and the supernatant was concentrated using a vacuum rotary evaporator to 50ml, which was placed in a plastic bottle and kept at the temperature of 5-10°C. About 5ml (weighted before) was re-concentrated until solvent evaporation to evaluate the solid content and concentration by measuring the difference in weight before and after evaporation.

Adult Tick Immersion Tests

In-vitro adult immersion test (AIT) was used to evaluate the toxicity of plant extracts against *H. dromedarii* (Khater et al. 2016). For each plant extract, five concentrations were diluted in distilled water. Three replicates were used for each concentration (30 ticks/concentration). Ten males were immersed for 60's in 100ml solution of each concentration. Distilled water was used to treat the control group. After immersion, the ticks were added to filter paper in a Petri dish (Whatman N. 1). Tick mortalities were checked up to 15 days' post-treatment (PT) and recorded as dead if no reaction was shown after stimulation (Khater and Hendawy 2014).

Data Analyses

The data were analyzed through SPSS V23 (IBM, USA) to compare the significant difference within and between groups using the one-way analysis of variance (ANOVA) (Post Hoc/Turkey's HSD test). The significant levels were set at $P<0.05$. The Probit analyses was used to calculate the lethal concentration (LC) and time (LT) values.

The corrected mortality data was calculated according to the following equation (Abbott 1925):

Corrected Mortality%= (MT%-MC%)/ (100-MC%) * 100

MT: mortality of the tested group MC: mortality of control group.

The toxicity indices were determined (Baz et al. 2022c) for a comparison of the tested extracts where the most toxic plant extract has given 100 units on the toxicity index scale

Toxicity index = LC_{50} of the most toxic plant extract ×100/ LC_{50} of each tested plant extract. Relative toxicities were also calculated (Khater and Geden 2018).

Relative toxicity = LC_{50} (or LC_{90}) of the least toxic plant extract/LC_{50} (or LC_{90}) of each tested plant extract.

Times potency= LT_{50} of the least toxic plant extract/LT_{50} of each tested plant extract.

RESULTS

Acaricidal Efficacy of the Plant Extracts against *H. dromedarii* Tick

The present study investigated the *in vitro* efficacy of plant extracts against *H. dromedarii*. The results showed that the mortality (MO)% depended on dose and time of exposure. Post- treatment (PT) for three days with 25%, MO% reached 42.9 and 42.8% for *V. castus* and *Z. officinale*, respectively. Five days PT, MO% reached 53.9 and 53.8%, respectively. Meanwhile, 15 days PT, MO% were 80.8 and 84.7%, respectively (Table 1). Three days PT, the LC_{50} values were 12.2 and 11.8%, respectively (Table 2). Such values for five days PT were 10.5 and 9.6%, respectively (Table 3). The toxicity indices three days PT with *V. castus* and *Z. officinale* were 96.72 and 100.00%, respectively, and the corresponding values five days PT were 91.43 and 100.00, respectively (Table 2 and 3). LT_{50} values PT with 25% of *V. castus* and *Z. officinale* were 2.6 and 2.5 days, respectively. According to LT_{50} values and post-treatment with 25, 12, and 3%, *Z. officinale* killed ticks 1.04, 1.24, and 1.77 times, respectively, faster than *V. castus,* whereas the corresponding values according to the LT_{90} values were 1.46, 1.58, and 1.18 times, respectively (Table 4).

DISCUSSION

Ticks have economic importance; therefore, their control with eco-friendly acaricides is required. Botanicals have fungicidal, bactericidal, pesticidal, and antioxidant properties and are used in medicine and cosmetics (Khater 2012a,b; Khater et al. 2020).

There are a few studies on the efficacy of plant extracts against *H. dromedarii* ticks. This study evaluated the acaricidal effect of ethanol extracts of *V. castus* and *Z. officinale* against *H. dromedarii* and showed a time and concentration- dependent relationship. Alike finding was recorded in previous studies (Singh et al. 2017; Godara et al. 2020).

Table 1: The Effect of ethanol extracts against male *Hyalomma dromedarii* tick

		Mortality%*							
Plant extracts	Concentration%	Day 1	Day 2	Day 3	Day 5	Day 7	Day 9	Day 12	Day 15
Control	0	00.0e	00.e	00.0e	00.0e	00.0e	00.0e	00.0e	00.0e
Vitex	1.5	13.3±0.6d	16.7±0.6d	20.0d	26.7±0.6cd	36.7±0.6cb	43.3±0.6ab	46.7±0.6ab	53.3±0.6a
castus	3	16.7±0.6d	20.0±.6d	23.3±.6cd	30.0cd	40.0±0.6bc	53.3±.6ab	56.7±0.6ab	63.3±.6 a
	6	23.3±0.6d	26.7±0.6cd	36.7±1.2cd	40.0±.6bcd	46.7±0.6abc	60±.6ab	63.3±0.6a	66.7±0.6a
	12	26.7±0.6d	33.3±0.6d	43.3±0.6dcd	50.0±0.6bcd	60.0±0.6cba	66.7±1.15ab	70.0ab	73.3±0.6a
	25	34.5±0.6d	39.3±0.6cd	42.9±1.2cd	53.9±0.6bc	65.4ab	69.4±.6ab	73.2±0.6a	80.8±0.6a
Zingiber	1.5	13.3±0.6cd	20.0±0.6d	23.3±0.6cd	30.0±1bcd	40.0abc	46.7±0.6ab	50.0a	56.7±.6a
officinale	3	16.7±0.6d	23.3±1d	26.7±0.6d	30.0cd	43.3±1bd	56.7±0.6ab	60.0a	66.7±0.6a
	6	26.7±0.6d	30.0±1d.c	40.0±1dc	43.3±1.15d	50±1bcd	63.3±0.6ab	66.7±0.6a	70.0a
	12	30.0±0.6d	36.7±0.6cd	43.3±0.6bc	53.3±1.15ab	63.3±0.6d	73.3±0.6a	76.7±0.6a	80.0a
	25	34.4±0.6d	39.2±.6cd	42.8±1.2cd	53.8±0.6bc	69.2±0.6ab	73.1±0.6ab	80.8±0.6a	84.7±0.6a

Means followed by the same letter on the same row are not significantly different by ANOVA (P > 0:05): *corrected morality%.

Table 2: Toxicity of the ethanol plant extracts of *Vitex castus* and *Zingiber officinale* against *Hyalomma dromedarii* tick, three days' post-exposure

Plant extract	*Vitex castus*			*Zingiber officinale*		
	LC_{50} ppm	LC_{90} ppm	LC_{95} ppm	LC_{50} ppm	LC_{90} ppm	LC_{95} ppm
	Lower	Lower	Lower	Lower	Lower	Lower
	Upper	upper	Upper	upper	upper	Upper
	12.2	25.8	29.6	11.8	25.9	29.9
	9.0	20.7	20.9	8.7	15.1	21.0
			56.1			58.7
	18.4	48.2		20.7	39.9	
X^2	14.3			6.4		
Df	4			4		
Significance	.014a			.171		
Regression equation	Y=1.26+0.11xX			Y=1.2+0.11xX		
R^2	0.686			0.621		
Relative toxicity	1.00			1.03		
(LC50), (LC90)	1.00			1.00		
Toxicity index	96.72			100.00		

UCL: Confidence limit, LCL: Lower confidence limits**;** X^2: Chi-squared; df: degree of freedom; R^2: *R-squared*, a goodness-of-fit measure for linear *regression* models: Relative toxicity=LC_{50} (or LC_{90}) of the least toxic plant extract/LC_{50} (or LC_{90}) of each tested plant extract: Toxicity index=LC_{50} of the most toxic compound ×100/LC_{50} of the tested compound.

Table 3: Toxicity of the tested ethanol plant extracts of *Vitex castus* and *Zingiber officinale* against *Hyalomma dromedarii* tick five days post- exposure

Plant extract	*Vitex castus*			*Zingiber officinale*		
	LC_{50}	LC_{90}	LC_{95}	LC_{50}	LC_{90}	LC_{95}
	LCL–UCL	LCL–UCL	LCL–UCL	LCL–UCL	LCL–UCL	LCL–UCL
Chi	10.5	23.8	27.6	9.6	22.2	25.8
	7.8-16.9	17.2-42.9	19.8- 50.4	7.2-16.3	14.9- 38.0	18.8- 44.7
	6.2			7.1		
df	4			4		
Significance	0.188			0.130		
Regression equation	Y=1.14+0.11xX			Y=1.12+0.12xX		
R^2	0.628			0.645		
Relative toxicity	1.00			1.09		
(LC_{50}), (LC_{90})	1.00			1.07		
Toxicity index (%)	91.43			100.00		

UCL: Confidence limit, LCL: Lower confidence limits; X^2: Chi-squared; df: degree of freedom; R^2: *R-squared*, a goodness-of-fit measure for linear *regression* models: Relative toxicity = LC_{50} (or LC_{90}) of the least toxic plant extract/LC_{50} (or LC_{90}) of each tested plant extract: Toxicity index = LC_{50} of the most toxic compound ×100 / LC_{50} of the tested compound.

Table 4: Median lethal time (LT_{50}) value (per day) and time potency of *Vitex castus* and *Zingiber officinale* against *Hyalomma dromedarii*

Plant extracts	Concentration (%)					
	25		12		3	
	LT_{50} (LCL–UCL)	LT_{90} (LCL–UCL)	LT_{50} (LCL–UCL)	LT_{90} (LCL–UCL)	LT_{50} (LCL–UCL)	LT_{90} (LCL–UCL)
Vitex castus	2.6 (1.5-3.7)	35.6 (18.7-142.8)	4.2 (2.8-5.9)	58.3 (27.4-309.1)	9.4 (6.8-15.8)	106.4 (44. 7-713.9)
Zingiber officinale	2.5 (1.6-3.5)	24.4 (14.7-65.2	3.4 (2.3-4.7)	36.8 (20.1-123.6)	5.3 (93.6-8.0)	89.9 (36.2-783.2)
Times potency	1.04	1.46	1.24	1.58	1.77	1.18

UCL: Confidence limit, LCL: Lower confidence limits; Reference material (lowest toxicity): *Vitex castus*: Times potency = LT_{50} of the least toxic plant extract/ LT_{50} of each tested plant extract.

In this study, the least concentration (1.5%) induced a significantly higher mortality when compared with the control. The ethanol extract of *Z. officinale* showed acaricidal effect against *H. dromedarii* inducing 73.1 and 84.7% MO nine- and 15-days PT with 25%. It's LC_{50} PT for three and five days were 11.8 and 9.6% with toxicity index reaching 193 100% and its LT50 values were 2.5- and 5.3-days PT with 25 and 3%, respectively.

Similar studies revealed the effect of its aqueous extract on the brown dog tick. *Rhipicephalus sanguineus*, inducing 100% acaricidal effect at a 5% concentration on females, males and larvae (Jeyathilakan et al. 2019). Moreover, *Z. officinale* induced repellent, insecticide, and antioviposition effect against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae) (Chaubey 2013; Zaki et al. 2021). Our finding disagrees with another study indicated no acaricidal effect of the ethanol extract of *Z. officinale* against *H. anatolicum* ticks; whereas the alcoholic extract of *Piper longum* seeds induced a high acaricidal effect as its LC50 and LC95 values were 0.071and 0.135%, respectively (Singh et al. 2017)

Against Rhipicephalus (Boophilus) microplus treated through Larval Packet Test, essential oils from Zingiberaceae and Verbenaceae families showed 100% mortality PT with 25 mg/mL; the LC_{50} and LC_{90} of *C. longa*, *L. gracilis*, *L. origanoides*, *L. alba*, and *Z. officinale* were 0.54 and 1.80 mg/mL, 3.21 and 7.03 mg/mL, 3.10 and 8.44 mg/mL, 5.85 and 11.14 mg/mL, and 7.75 and 13.62 mg/mL, respectively (de Souza Chagas et al. 2016). Brazilian essential oils, including ginger, adversely affected the reproduction potential of the cattle tick, *Rhipicephalus microplus* (Pazinato et al. 2016).

The data of the present study indicated that *V. castus* showed lethality against *H. dromedarii* reaching 69.4 and 80.8% PT with 25% for nine and 15 days, respectively. Its LC_{50} three- and five-days PT were 12.2 and 10.5% with toxicity indices reached 96.72 and 91.43, respectively. Its LT_{50} value PT with 25% was 2.6 days. A comparable finding was reported as essential oils from different parts of *V. agnus-castus* harvested in the Atlantic forest in Brazil, have acaricidal effects against the two-spotted spider mite, *Tetranychus urticae* (Neves and Da Camara 2016).

Similar studies revealed the acaricidal effect of some other plant extracts like *Protium spruceanum* against the resistant strains of *R. annulatus* inducing > 80 and 90% PT with 100 and 50 mg/ml ethanolic and ethyl acetate extracts, respectively (Figueiredo et al. 2019); *Melia azedarach* and *Artemisia herba-alba* (ethyl alcohol and petroleum ether extracts) were effective acaricides against egg, nymph, larva, and adult of *H. dromedarii* when compared to Butox®5.0 (Deltamethrin) (Abdel-Ghany et al. 2021).

A related study showed that the methanol extract of neem leaves (*Azadirachta Indica*) was more toxic against eggs, larvae, and females of *H. dromedarii* than *Citrullus colocynthis* extract (Mahran et al. 2020).

Acetone extract of *Alstonia scholaris* and methanolic extract of *Sida cordifolia* were highly effective larvicides against *Hyalomma anatolicum* (LC_{50}= 0.71% and 0.42%, respectively) (Godara et al. 2020). The hexane extract of the gum Haggar, *Commiphora holtziana*, resin repelled the cattle tick, *Boophilus microplus* for up to 5 h (Birkett et al. 2008).

It could be concluded that both ethanolic extracts of *V. castus* and *Z. officinale* were effective and could be used as eco-friendly acaricides to prevent tick bites and their associated diseases. Further studies could be directed toward the phytochemical analysis of both plants and in vivo and ecotoxicological studies.

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Author's Contribution

RE, HK: writing the manuscript; MB, HK, and HA: doing bioassays, IR and HT: preparation of the extracts: KH, MY, and AS, AF: data collection and editing the manuscript.

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