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Anti-Tick Activity of Some Methanol-Extracted Plants Indigenous in Saudi Arabia

Khaled B. Al-Harbi, Ibrahim M. El-Ashmawy, Hussein M. Omar and Naser A. Al-Wabel
Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine,
Qassim University, Buraidah, 51452 P.O. 6622, Al-Qassim, Kingdom of Saudi Arabia

Abstract: An in vitro screening of the effect of whole plant extracts was carried out. Twenty-one indigenous plant species were extracted in methanol and tried against the most prevalent tick species; *Hyalomma dromederi* of camels. Fully engorged female ticks of the same weight were immersed in the different extracts for 5, 15, 30 and 60 min. Treated ticks were revived and incubated with the control groups for daily observation of their activity and egg production. While some plant extracts have killing effects after 30-60 min exposure (*Rhanterium epapposum, Achillea fragrantissima* and *Eragrostis poaeodes*) others have stopped oviposition (*Artemisia herba alba, Haloxylon salicornicum, Plantago coronopus, Moltkiopsis ciliate* and *Lasiurus hirsutus*). Marked reduction of the egg masses associated with delayed oviposition was recorded on exposure to *Lepidium sativum seeds* extract. Most of the extracts have reducing effect on the egg mass that can be attributed to synergistic effect of flavonoids and phenolics. Tannins and non-tannin phenolics, flavonoids, alkaloids and saponins were determined by a standard methods. Further studies are going on to support such ant-tick effects of the bioactive components.

Key words: Plant extracts, *Hyalomma dromederi*, acaricidal, oviposition, flavonoids, phenolics

INTRODUCTION

Ticks are economically important pests all over the world. Beside their stressful, cutaneous and systemic pathological effects, tick species are vectors of different animal and human disease agents such as bacteria, protozoa, rickettsia and viruses (Wall and Shearer, 1997; Shammery and Fetoh, 2011; Alrasheed *et al*., 2015). Ectoparasitism caused by hard ticks in camels has a big concern in Saudi Arabia (El-Bahy *et al*., 2008) where an infestation rate had reached 20.7%. In general, *Hyaloma dromedarii* is one of the most important ectoparasites of livestock, both in the tropics and subtropics. Tick control is globally based on chemical acaricides but the developing tick resistance to most of the currently used synthetic acaricides encouraged to look for alternative ways to control.

Acaricides of botanical origin represent the promising natural products that have low mammalian toxicity, short environmental persistence and complex chemistries that should limit development of resistance (Miresmailli *et al*., 2006). Saudi Arabia has been looked upon as a natural reservoir of a variety of medicinal plants (Sher *et al*., 2010) but in spite of that a few studies have been carried out in this country to investigate the acaricidal activity or to determine phytoconstituents of medicinal plants and very scarce studies in Al Gassim region which is considered to have high ecological and economic significance and possibly have own biological diversity and productivity (Ghazanfar, 2006). Therefore, this study was carried out to screen the anti-tick effect of some native plant extracts collected from different places in Saudi Arabia.

MATERIALS AND METHODS

Plant identification and extraction-Plants collected from different families (Table 1) and localities in Saudi Arabia were identified (Al-Yahya *et al*.,1990), cleaned under running tap water, shade-dried, powdered and stored in airtight containers. The powdered plant materials were extracted with methanol in a Soxhlet apparatus according to Harborne (1998). Extracts were then passed through Whatman filter paper No. 1. Filtrates were then evaporated under reduced pressure using rotary evaporator (Butchi, Switzerland) at a temperature not >40°C and the dry residues were stored at 4°C until used for acaricidal and phytochemical testing.

Phytochemical testing: The residue (methanolic extract) was subjected to qualitative and quantitative analyses.

Qualitative analysis: Qualitative analysis was done to identify the presence of the phytoconstituents; alkaloids, flavonoids, tannins, phenols and saponins using standard procedures (Tiwari *et al*., 2011; Habrone 1998).

Corresponding Author: Khaled B. Al-Harbi, Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Buraidah, 51452 P.O. 6622, Al-Qassim, Kingdom of Saudi Arabia

Table 1: List of the tested plants and their families

Plant species	Family	Plant species	Family
Rhazya stricta	Apocynaceae	*Hordeum distichon*	Poaceae alt. Gramineae
Heliotropium bacciferum	Boraginaceae	Eragrostis poaeoides	
Tribulus longipetalus	Zygophyllaceae	*Deverra triradiata*	Apiaceae
Rhanterium epapposum	Asteraceae	*Lycium shawii*	Solanaceae
Achillea fragrantissima		*Echinops spinosus*	Asteraceae
Artemisia herba-alba		*Echinops hussoni*	
Haloxylon salicornicum	Chenopodiaceae	*Prosopis farcta*	Minosaceae
Plantago coronopus	Plantaginaceae	*Cyperus conglomeratus*	Cyperaceae
Moltkiopsis ciliate	Boraginaceae	*Astragalus spinosus*	Leguminosae
Lasiurus scindicus	Poaceae alt. Gramineae	*Lepidium sativum*	Cruciferae
Lasiurus hirsutus			

Quantitative analysis: Total phenolics and total Tannins content-Total phenolic content was determined using Folin-Ciocalteus reagent (Singleton and Rossi, 1965) with some modifications. Few amount of residue (50 mg) was mixed with 2.5 mL of deionized water followed by 0.25 mL of Folin-Ciocalteu's reagent and allowed to react 6 min. Then 2.5 mL of sodium carbonate 7% was added and allowed to stand for 1 h, then absorption at 765 nm was measured. Measurements were calibrated to a standard curve of prepared gallic acid solution and the total phenolic was expressed as mg gallic acid equivalent per g of residue. Total tannin in the extracts was determined by a modification of the Folin-Ciocalteu method using Poly Vinyl Poly Pyrrolidone (PVPP) to separate tannin phenols from non-tannin phenols (Osoro *et al.*, 2007). About 100 mg of PVPP was added to 1ml sample extract diluted with 1 mL water and left 15 min at 4°C. After centrifugation, PVPP forms a precipitate with tannins and the supernatant has only simple phenols. Simple phenols were determined using the Folin-Ciocalteu reagent as previously mentioned. The difference between total and simple phenol values represents the total tannin content, expressed as mg gallic acid equivalents g residue.

Total flavonoids: The flavonoid content was measured using a colorimetric assay (Zhishen *et al.*, 1999). A known weight of extract residue was dissolved in 1 mL methanol was added to a 10 mL volumetric flask. Distilled water was added to make a volume of 5 mL. At zero time, 0.3 mL of 5% w/v sodium nitrite was added to the flask. After 5 min, 0.6 mL of 10% (w/v) $AlCl_3$ was added and, after 6 min, 2 mL of 1M NaOH were added to the mixture, followed by the addition of 2.1 mL distilled water. Absorbance was read at 510 nm against the blank (water) and flavonoid content was expressed as mg quercetin equivalents/g residue.

Collection of hard ticks: Engorged female ticks were manually collected from heavily infested camels admitted to the Veterinary Teaching Hospital of the college of Agriculture and Veterinary Medicine, Qassim University.

Fig. 1: Adult immersion test of camels ticks: a) Before immersion; b) After immersion

Camels have neither previously parasitized with ticks nor sprayed with any acaricide. Ticks were immediately brought to the Parasitology laboratory in the Department of Veterinary Medicine to be identified. *Hyalomma dromedarii* female ticks from the same camel were separately exposed to the different plant extracts.

Experimental design: Based on the recommended method (FAO, 1984 report; Fernandez-Salas *et al.*, 2011) an Adult Immersion Test (AIT), Fig. 1, was applied on two treated groups; Extract treated and Tween-80 treated group. A third control non-treated tick group was incubated at 28°C and relative humidity 85% to allow for oviposition.

Ten female ticks of the same size and weight, were immersed in 60 mL of 1% crude methanolic plant extract dissolved in Tween-80 (2%, w/v). The immersed ticks were closely observed for moving around or leg movement by using dissecting Stereomicroscope, over 5, 15, 30 and 60 min. Viability was confirmed also by using a desk light which caused the not affected ticks to move away. Revived ticks were individually transferred into rearing containers under 28°C/85% RH.

The incubated revived and control ticks were checked daily for activity and oviposition for 22 day (Fig. 1). Reduction percentage of the egg mass was calculated (Ghosh *et al.*, 2005).

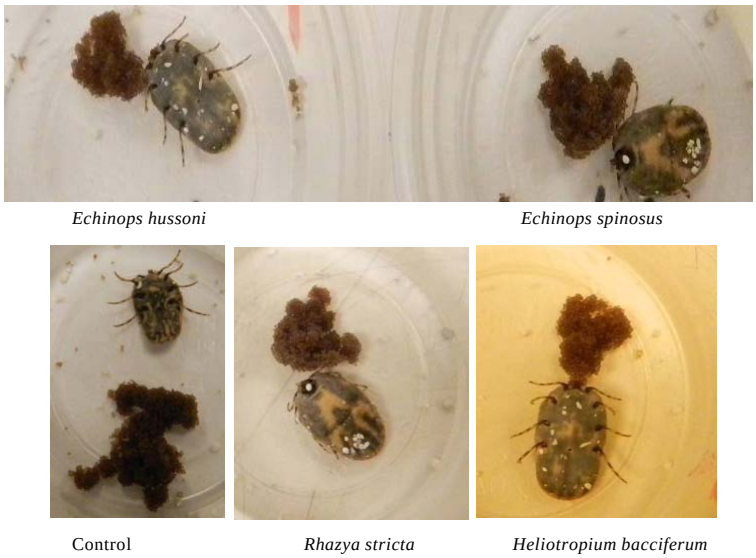

Fig. 2: Effect of methanol extract of some plants on the oviposition of ticks after incubation

Table 2: Mortality and sterility effects of 13 plant extracts on female *Hyalomma dromedarii* ticks (n = 10 ticks)

	Exposure period (min)				
Plant name	5	15	30	60	Revival
Rhanterium epapposum	VS	VS	NO	NO	NR
Achillea fragrantissima	S	VS	NO	NO	NR
Artemisia herba alba	S	S	S	A	R (0/10)
Haloxylon salicornicum	VS	S	S	A	R (0/10)
Plantago coronopus	VS	S	S	A	R (0/10)
Moltkiopsis ciliate	S	S	S	A	R (0/10)
Lasiurus hirsutus	S	VS	A	A	R (0/10)
Hordeum distichon	VS	S	S	A	R (0/10)
Eragrostis poaeoides	VS	S	S	NO	NR
Echinops spinosus	VS	S	NO	NO	RE (1/10) died
Egg mass 0.427 gm					
Echinops hussoni	S	S	NO	NO	RE (1/10) died
Egg mass 0.266 gm					
Prosopis farcta	VS	S	S	NO	RE (1/10) died
Egg mass 0.306 gm					
Cyperus conglomerates	VS	S	NO	NO	RE (2/10) died
					Egg mass 0.285 and 0.199 gm

VS: Very Sluggish movement, S: Sluggish, NO: Not moving and apparently dead, A: Active, R: Revived without egg laying, NR: Not revived, RE: Revived but died after oviposition

RESULTS AND DISCUSSION

Immersed female *Hyalomma dromedarii* ticks showed variable reactions based on the plant species and the time of exposure. Generally the treated ticks were similar in response to immersion for 5 and 15 min. Between 30-60 min. some of them were not moving and apparently dead while others were active all over the exposure period (Table 2). Three plant extracts (*Rhanterium epapposum, Achillea fragrantissima* and *Eragrostis poaeoides*) showed a killing effect on the immersed ticks. The apparently dead treated females started moving slowly but died at different incubation days .However, only one or two females from groups (treated with extracts of *Echinopis, Prosopis* and *Cyperus* species) were found dead with egg mass of 0.427, 0.266, 0.306, 0.285 and 0.199 gm, respectively. Females remained active over 60 min didn't lay eggs on revival and incubation. Three to nine revived females started to lay eggs on the 4th day of incubation (Table 3). Markedly delayed oviposition associated with high reduction percentage (83.3%) was observed in the group immersed in *Lepidium sativum* extract. *Lasiurus scindicus* has the least effect (35.9%). Effects of the other extracts were variable (Fig. 2) as compared with control groups. Six extracts; *Artmesia, Haloxylon, Plantago, Moltkiopsis, Lasiurus hirsutus* and *Hordeum* seem to have sterility effect where none of the revived females gave eggs. Where *Lasiurus hirsutus* has no effect, *Lasiurus scindicus* reduced the oviposition at a rate of 35.9% (Table 3).

Table 3: Effect of 8 plant extracts on revived female *Hyalomma dromedarii* ticks after 60 minutes exposure (n = 10 females)

Plant name	Revived and lay eggs	Oviposition day(s)	Mean egg mass	Egg reduction (%)
Rhazya stricta	RE (6)	6th	0.306	56.4
Heliotropium bacciferum	RE (3)	4th, 6th, 10th	0.298	57.5
Tribulus longipetalus	RE (3)	4th	0.327	53.4
Lasiurus scindicus	RE (9)	4th , 5th, 7th	0.450	35.9
Deverra triradiata	RE (3)	4th	0.364	48.2
Lycium shawii	RE (6)	4th	0.309	55.9
Astragalus spinosus	RE (6)	7th , 8th, 10th	0.259	63.1
Lepidium sativum seeds	RE (6)	19th , 21th	0.168	83.3
Control ,Tween 80 (2 %)	RE (9)	6th, 8th, 9th.	0.693	1.28
Control	4th, 8th, 12th	0.702		-

Table 4: Qualitative analysis of methanolic extracts of the tested plants

Plant name	Phenolics	Alkaloids	Flavonoids	Saponins
Rhazya stricta	++	+++	+	+++
Heliotropium bacciferum	++	-	++	
Tribulus longipetalus	+	-	+	++
Rhanterium epapposum	+++	-	++	-
Achillea fragrantissima	+	-	+	-
Artemisia herba alba	++	-	+	-
Haloxylon salicornicum	+	-	+	+
Plantago coronopus	++	-	+	+
Moltkiopsis ciliate	++	-	+	++
Lasiurus scindicus	+	-	++	-
Lasiurus hirsutus	+	-	+	-
Hordeum distichon	+	-	++	-
Eragrostis poaeoides	++	-	++	-
Deverra triradiata	+	-	+++	-
Lycium shawii	+++	-	++	-
Echinops spinosus	++	-	+++	-
Echinops hussoni	+	-	+	+
Prosopis farcta	+++	-	+++	+++
Cyperus conglomerates	+++	-	+++	++
Astragalus spinosus	+	-	++	++
Lepidium sativum seeds	+	-	+	-

- = Not detected, += low concentration, ++ = moderate concentration, +++ = high concentration

Qualitative and quanitative analyses (Table 4 and 5) showed that all the extracts contain flavonoids and phenolics. *Rhazya stricta* was the only extract that contain alkaloids. Different concentrations of saponins were detected from nine extracts.

Ticks causes severe economic losses by blood loss, reduction in weight gain, direct damage to camels skins and hides for the manufacture of leather and also serving as a vector of infectious diseases such as babesiosis and anaplasmosis (Ghosh *et al*., 2006). Today, tick control has become a challenge to researchers around the world, who seek a sustainable way to do it. Synthetic acaricides have been used widely and extensively, however, the high cost, the hazardous effect on environment make their use a concern, mainly because human beings are the indirect target (Ghosh *et al*., 2006).

In search for low-cost, safe and eco-friendly botanical derivatives alternative to chemical acaricides. The present study is the first report on the acaricidal activity of most these plant extracts from Saudi Arabia. Twenty-one native plant extracts were found to have different efficacies.

Where some have killed female ticks, others completely or partially affected the fecundity in term of oviposition. Severe reduction of eggs percentage by *Lepidium sativum* extract may be due to high content of tannins which have potential acaricidal effect against *Rhipicephalus (Boophilus) microplus* (Fernendez *et al*., 2011). The different effects of two *Lasiurus* species may due to concentration of the bioactive materials. An oviposition reduction of 21.5% due to glcosylated flavonoids identified from the aerial parts of *Tagetes patula* (Politi *et al*., 2012).

Concerning the bioactive material based on chemical analyses of each extract, all extracts were found containing phenolics and flavonoides, only *Rhyaza stricta* contains alkaloids and 9 extracts contain saponins. Alkaloids, phenolics and flavonoids were found to have destructive effect on reproductive functions of ticks besides their killing effects due to the neurotoxic properties (Gosh *et al*., 2015; Wink, 2012; Juliet *et al*., 2012). Spectroscopic analyses revealed occurrence of saponins in *Artemesia sphaerocephala* (Li *et al*., 2008). Concentrations of the different phytoconstituents depend upon to many factors such as parts of the plant, geographic locales, seasonality, time of harvest, storage and method of extraction (George *et al*., 2014; Subaei, 2015).

Table 5: Quantitative analysis of methanolic extracts of the tested plants

Plant	Total phenolics	Tannin phenolics	Non-tannins phenolics	Total flavonoids
Rhazya stricta	52.59	26.55	26.04	18.6
Heliotropium bacciferum	60.52	13.85	46.67	58.1
Tribulus longipetalus	30.93	19.16	11.77	24.0
Rhanterium epapposum	124.26	-	124.26	30.2
Achillea fragrantissima	55.57	36.62	18.95	80.4
Artemisia herba alba	94.19	71.60	22.59	99.7
Haloxylon salicornicum	45.83	30.63	15.20	36.1
Plantago coronopus	64.46	29.43	35.03	38.5
Moltkiopsis ciliata	59.48	11.40	48.08	31.9
Lasiurus scindicus	47.42	22.96	24.46	51.2
Lasiurus hirsutus	29.28	14.15	15.13	39.6
Hordeum distichon	37.71	15.17	23.54	54.1
Eragrostis poaeoides	52.94	28.87	24.07	59.5
Deverra triradiata	41.30	15.92	25.38	110.3
Lycium shawii	101.70	47.66	54.04	59.8
Echinops spinosus L.	66.54	20.28	46.26	44.6
Echinops hussoni	46.86	22.90	23.96	23.8
Prosopis farcta	271.92	81.24	190.68	135.0
Cyperus conglomeratus	164.29	-	164.30	123.5
Astragalus spinosus	43.46	-	43.40	86.6
Lepidium sativum seeds	45.10	26.70	18.40	19.8

CONCLUSION

It could be concluded that three of 21 extracts having an *in vitro* acaricidal effect against engorged female *Hyalomma dromederii*. Other extracts were found to have different effects on the fecundity in terms of sterility or reduction of laying egg capacity. More detailed studies are going on support the obtained findings.

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