



Acaricidal activity of *Alstonia scholaris* and *Sida cordifolia* leaf extracts against *Hyalomma anatolicum* ticks

R GODARA¹✉, S I RAFIQI¹, R SHARMA², R KATOCH¹, A YADAV¹, P K PILANIA¹ and P K VERMA¹

Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir 181 102 India

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ABSTRACT

Development of acaricidal resistance and environmental pollution has driven need for eco-friendly pesticides and herbal acaricides. Leaf extracts of *Alstonia scholaris* and *Sida cordifolia* in four different solvents were used in the range of 0.25–8.0% for larval immersion test against unfed larvae of *Hyalomma anatolicum*. Mortality rates of larval ticks were in dose-dependent manner ranged from 0.5 to 100% for *A. scholaris* and from 0.8 to 100% for *S. cordifolia*. Acetone extract of *A. scholaris* and methanolic extract of *S. cordifolia* were most effective against larval ticks with LC₅₀ values of 0.71% and 0.42%, respectively. Significant larvicidal activity of *A. scholaris* and *S. cordifolia* leaf extracts against unfed larvae of *H. anatolicum* qualify them as green pesticides which could be combined with other tools for integrated pest management.

Keywords: *Alstonia scholaris*, *Hyalomma anatolicum*, Larval immersion test, *Sida cordifolia*

Hyalomma anatolicum is an economically important ixodid tick infesting dairy animals in central and north-western plain regions of India and is responsible for transmitting theileriosis in dairy animals with an estimated annual loss of US\$ 239.5 million (Ghosh and Nagar 2014). The advent of cheap and easily available acaricides has led to upsurge in their use for tick control resulting in development of resistance. The emergence of acaricidal resistance ultimately initiated the quest for newer compounds. Acaricides of herbal origin are a viable alternative against the challenges of resistance development and environmental contamination. Many plants can synthesize secondary metabolites to protect them from pathogens and pests (Chhabra and Saxena 1998). The structural diversity of natural compounds of plant origin provides the advantage of wide range of target sensitivity which can be explored to combat resistance. The pesticidal effects of plant substances have been used against arthropods and initially, people used to crush parts of the plants to obtain a fine powder, which was then applied against various ectoparasites (Khare *et al.* 2019).

Alstonia scholaris is a tree belonging to Apocynaceae family and is widely used in traditional system of medicine in Asia, America and Africa. It is a rich source of flavonoidal glycosides, steroids, terpenoids and other alkaloids (Dung *et al.* 2001). Various phytoconstituents such as cycloeucalenol, cycloartanol, lupeol, lupeol acetate and

botulin have been reported from the leaves of *A. scholaris* (Ragasa *et al.* 2016) and have been evaluated for their bactericidal, fungicidal and anthelmintic properties (Kulkarni 2013). *Sida cordifolia* (Family: Malvaceae), a perennial shrub is widely distributed throughout the tropical and sub-tropical plains of India and Sri Lanka (Sutradhar *et al.* 2006). It has been proven experimentally that the areal parts of plant possess analgesic (Sutradhar *et al.* 2006), hepato-protective (Rao and Mishra 1998) and anthelmintic properties (Pawar *et al.* 2011). However, no published report is available regarding acaricidal activities of *A. scholaris* and *S. cordifolia* against ixodid ticks. Thus, the aim of present study was to evaluate the acaricidal properties of *A. scholaris* and *S. cordifolia* leaf extracts against unfed larvae of *H. anatolicum*.

MATERIALS AND METHODS

Collection of plant materials and extract preparation: The leaves of *A. scholaris* and *S. cordifolia* were collected from University Campus at R.S. Pura (Jammu, India) and Meerut city (Uttar Pradesh, India), respectively. The plant samples were identified by Taxonomist Dr. Harish Chander, Department of Botany, University of Jammu with voucher specimen number AU-2875 and AU-2893, respectively. Sufficient fresh leaves were collected and air-dried in shade (temperature not exceeding 40°C) for 3–4 weeks. Air dried leaves were pre-crushed and later pulverized into fine powder using electric blender. The aqueous extracts were prepared by soaking dry powder in 1:10 ratio in distilled water for 72 h with intermittent shaking. After 72 h of soaking, the contents were filtered through filter paper (0.45

Present address: ¹SKUAST-Jammu; ²Animal Husbandry Department, Jammu and Kashmir. ✉Corresponding author e-mail: godara1972@gmail.com

Table 1. Effect of various *A. scholaris* (leaf) extracts on unfed larvae of *H. anatolicum*

Extract	Conc. (%) (mean±SE)	Mortality (%) (95% CI)	Slope±SE	R ² (95% CI)	LC50 (%) (95% CI)	LC95 (%)
Methanolic	0.25	6.2±2.1 ^{ab}	3.39±0.51 (1.95–4.83)	0.91	0.91 (0.88–0.93)	2.76 (2.61–2.91)
	0.5	17.2±6.1 ^c				
	1.0	40.5±8.9 ^e				
	2.0	65.2±6.4 ^f				
	4.0	100.0±0.0 ^h				
Acetone	8.0	100.0±0.0 ^h	3.11±0.39 (1.99–4.21)	0.93	0.71 (0.69–0.73)	2.37 (2.23–2.51)
	0.25	12.5±3.2 ^{bc}				
	0.5	28.0±2.3 ^d				
	1.0	57.0±2.1 ^f				
	2.0	82.2±3.1 ^g				
Ethanolic	4.0	100.0±0.0 ^h	3.43±0.51 (2.04–4.82)	0.92	0.91 (0.88–0.93)	2.71 (2.55–2.85)
	8.0	100.0±0.0 ^h				
	0.25	4.2±1.1 ^{ab}				
	0.5	19.0±3.7 ^{cd}				
	1.5	51.5±7.4 ^f				
Aqueous	2.0	61.7±4.1 ^f	3.22±0.8 (0.92–5.52)	2 0.7	9 2.3 (2.23–2.36)	7.42 (7.01–7.86)
	4.0	100.0±0.0 ^h				
	8.0	100.0±0.0 ^h				
	0.25	0.5±0.1 ^a				
	0.5	1.7±0.5 ^{ab}				
Distilled water	1.0	3.7±1.1 ^{ab}	2 0.7	2 0.7	9 2.3 (2.23–2.36)	7.42 (7.01–7.86)
	2.0	10.2±3.5 ^{abc}				
	4.0	55.8±3.9 ^f				
DMSO	2.0	0.0±0.0 ^a				
Deltamethrin	0.0025	40.0±4.6 ^e				

DMSO, Dimethyl sulphoxide; CI, Confidence interval. Mean followed by same letters do not differ statistically at a significance level of 5%.

µm) and filtrate was concentrated under reduced pressure using rotatory evaporator (temp 40–45°C, 10–15 rpm). Methanolic, acetone and ethanolic extracts were prepared by using methanol, acetone and ethyl alcohol, respectively in extract container of Soxhlet apparatus according to method described by Harborne (1984) and the dried extracts were stored in air tight containers at –20°C till subsequent use.

Preparation of test concentrations: Dimethylsulphoxide (DMSO) was used to dissolve the leaf extracts obtained from *A. scholaris* and *S. cordifolia*. After preparing stock solutions, different working concentrations (0.25, 0.5, 1.0, 2.0, 4.0 and 8.0%) were made in distilled water to conduct Larval Immersion Test (LIT).

Collection of ticks: Adult female ticks which dropped off from the host after full engorgement were collected from cattle sheds located at Bikaner, Rajasthan (India). Ticks placed into plastic boxes covered with muslin cloths to allow exchange of air were transported to Division of Veterinary Parasitology, Sher-e-Kashmir University of Agriculture Sciences and Technology of Jammu (SKUAST-J), India. After thorough washing and drying with paper towels, ticks were placed in individual vials in a desiccator which was kept in incubator maintained at 28±1°C and 85±5% relative humidity to obtain eggs. After 14 days, ticks were discarded

and the eggs were kept in similar conditions of incubation to obtain larvae. About 14–21 days old unfed larvae were used in LIT.

Larval immersion test: For the evaluation of acaricidal activity of *A. scholaris* and *S. cordifolia* leaf extracts against 14–21 days old unfed larvae of *H. anatolicum* ticks LIT (Shaw 1966) was used with minor modifications as described by Singh *et al.* (2017). For each concentration of extract, the test was repeated four times. Dimethylsulphoxide (2%) and distilled water were used as negative controls and the larvae in positive control group were treated with deltamethrin (0.0025%).

Statistical analyses: Dose-response data were analysed by probit method (Finney 1962) using Graph Pad Prism 4 software (La Jolla, CA, USA). The lethal concentrations (LC50 and LC95) and their respective 95% confidence intervals (CI) were determined by applying regression equation analysis to the probit transformed data of mortality.

RESULTS AND DISCUSSION

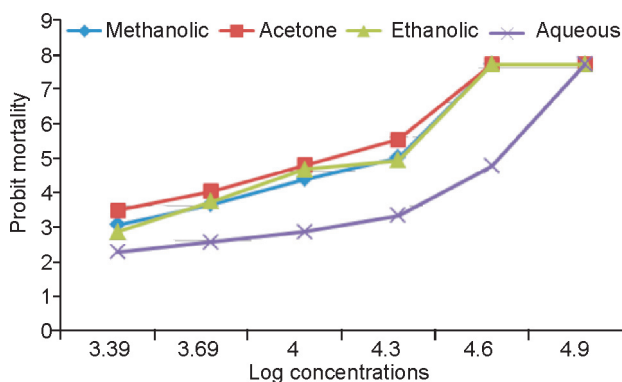
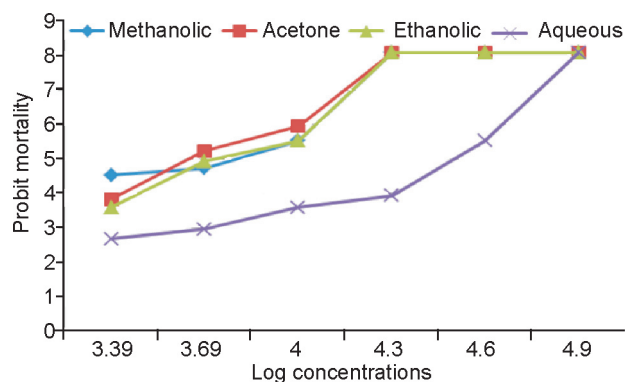
The per cent mean larval mortality (Figs. 1 and 2), slope (95% CI), R², LC50 and LC95 (95% CI) values for different extracts of *A. scholaris* and *S. cordifolia* are presented in Tables 1 and 2, respectively. The mortality rates of larval ticks were in dose-dependent manner ranged from 0.5 to

Table 2. Effect of various *S. cordifolia* (leaf) extracts on unfed larvae of *H. anatolicum*

Extract	Conc. (%) (mean±SE)	Mortality (%) (95% CI)	Slope±SE (95% CI)	R ² (95% CI)	LC50 (%) Methanolic	LC95 (%) 0.25 32.2±5.1 ^b
Methanolic	0.5	39.0±2.8 ^{bc}	2.88±0.61 (1.22–4.54)	0.85	0.42 (0.41–0.43)	1.55 (1.45–1.65)
	1.0	69.7±3.3 ^{ef}				
	2.0	100.0±0.0 ^g				
	4.0	100.0±0.0 ^g				
	8.0	100.0±0.0 ^g				
Acetone	0.25	12.2±3.4 ^a	3.03±0.55 (1.49–4.356)	0.88	0.43 (0.41–0.44)	1.56 (1.42–1.61)
	0.5	59.0±1.6 ^{de}				
	1.0	82.5±4.9 ^e				
	2.0	100.0±0.0 ^g				
	4.0	100.0±0.0 ^g				
Ethanollic	0.25	8.0±1.1 ^a	3.27±0.61 (1.59–4.94)	0.88	0.52 (0.51–0.54)	1.67 (1.57–1.76)
	0.5	46.7±4.6 ^{cd}				
	1.0	67.9±3.7 ^e				
	2.0	100.0±0.0 ^g				
	4.0	100.0±0.0 ^g				
Aqueous	0.25	0.8±0.1 ^a	3.32±0.71 (1.34–5.29)	0.84	2.1 (1.98–2.09)	6.38 (6.03–6.75)
	0.5	2.2±0.5 ^a				
	1.0	7.5±0.7 ^a				
	2.0	13.5±2.2 ^a				
	4.0	70.2±4.6 ^{ef}				
Distilled water	-	0.0±0.0 ^a				
DMSO	2.0	0.0±0.0 ^a				
Deltamethrin	0.0025	40.0±4.6 ^{bc}				

DMSO, Dimethyl sulphoxide; CI, Confidence interval.

Mean followed by same letters do not differ statistically at a significance level of 5%.

Fig. 1. Probit mortality of larvae of *H. anatolicum* against log concentrations of *A. scholaris*.Fig. 2. Probit mortality of larvae of *H. anatolicum* against log concentrations of *S. cordifolia*.

100% for *A. scholaris* and from 0.8 to 100% for *S. cordifolia*. Acetone extract of *A. scholaris* and methanolic extract of *S. cordifolia* were most effective against larval ticks with LC50 values of 0.71% and 0.42%, respectively. Mortality slope values ranged from 3.11 to 3.43 with R² values of 0.79 to 0.93 for various extracts of *A. scholaris* whereas, mortality slope values of *S. cordifolia* extracts ranged from 2.88 to 3.32 with R² values of 0.84 to 0.88, indicating that the statistical model was a good fit. No mortality of larval ticks was seen in controls exposed to distilled water and DMSO (2%) whereas, deltamethrin

caused 40.0% mortality of larval ticks at recommended dose (0.0025%) for field use.

In the current study, the minimum LC50 and LC95 (95% CI) values of 0.71% (0.69–0.73) and 2.37% (2.23–2.51), respectively were recorded for acetone extract of *A. scholaris*. For *S. cordifolia*, the minimum LC50 and LC95 (95% CI) values of 0.42% (0.41–0.43) and 1.55% (1.45–1.65), respectively were recorded for methanolic extract. In a previous study, the leaf extract of *A. scholaris* showed significant insecticidal activity against *Aedes aegypti* with LC50 value of 239.9 ppm (Kaushik and Saini 2009). Earlier,

evaluation of ethanolic extract of aerial parts of *Artemisia absinthium* against adults of *H. anatolicum* revealed maximum mortality of 86.7% at 20% concentration with LC50 and LC95 values of 6.51 and 55.43%, respectively. However, in larval packet test (LPT) the extract caused 100% mortality at all the concentrations (2.5, 5.0, 10.0 and 20.0%) used (Godara *et al.* 2015).

Similarly, Singh *et al.* (2017) studied acaricidal activities of alcoholic and various aqueous extracts of *Piper longum*, *P. nigrum* and *Zingiber officinale* against unfed larvae of *H. anatolicum* using LIT. They observed the highest acaricidal activity with the alcoholic extract of *P. longum* seeds with the minimum LC50 and LC95 values of 0.071 and 0.135%, respectively. However, the extracts prepared from the rhizome of *Z. officinale* had shown no acaricidal activity. In another study, Singh *et al.* (2014) observed a concentration dependent increase in larval tick mortality while evaluating the aqueous and ethanolic extracts of leaves of *Cymbopogon winterianus*, *Vitex negundo* and *Withania somnifera* along with roots of *V. negundo* for their acaricidal activity against larvae of deltamethrin resistant *H. anatolicum*. They observed the highest mortality of 93.7% at the 5% concentration of *C. winterianus* leaves extract. The extract of neem (*Azadirachta indica*) seed caused significant mortalities of newly hatched larvae, unfed larvae and unfed adults of *H. a. excavatum*, reaching 100% mortality on day 15, 3 and 15 post-treatment, respectively (Abdel-Shafy and Zayed 2002). The crude ethanolic extract prepared from the leaves of *Annona squamosa* showed high acaricidal activity against *H. anatolicum* larvae with LC50 and LC99 values of 1.36 and 10.17%, respectively (Ilham *et al.* 2014).

The present study is an attempt towards exploring tick control potential of *A. scholaris* and *S. cordifolia*. From the above findings it can be concluded that the leaf extracts of *A. scholaris* and *S. cordifolia* possess acaricidal properties and may be a promising alternative to control *H. anatolicum* ticks.

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