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COMPARATIVE ACARICIDAL BIOEFFICACY OF *SPILANTHES ACMELLA* AND *CALOTROPIS PROCERA* OF EASTERN HIMALAYAN REGION AGAINST CATTLE TICK, *RHIPICEPHALUS MICROPLUS*

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Abstract: In this study, relative toxicity of *Spilanthes acmella* and *Calotropis procera* was evaluated against adults and larvae of *Rhipicephalus (Boophilus) microplus*. The aerial part of both plants materials were collected from Eastern Himalayan Region (West Bengal) of India. Plant materials were washed, shade dried, coarsely ground, methanol extracted and dried by rotary evaporator and collected proper yield of extracts. The crude methanolic extracts were further fractionated using solvents (hexane, ethyl acetate, chloroform) of different polarity and finally aqueous fraction was collected and dried. Methanolic crude extracts and their fractions (hexane, ethyl acetate, chloroform and aqueous) concentrations of both the plants were tested against the engorged adult females and cultured larvae of *Rhipicephalus (Boophilus) microplus.* The bioefficacy observations are shown in table 3 and mentioned LC_{50}, LC_{90} and their related statistics. Adult and larval stages were significantly affected by the chloroform extract of both the plants selected and observed the most potent with LC_{50} 50.22 and 13.86 mg/ml of *Calotropis procera* and LC_{50} 60.94 and 25.82 mg/ml of *Spilanthes acmella*.

Keywords: *Spilanthes acmella, Calotropis procera, Rhipicephalus (Boophilus) microplus,* Acaricidal bioefficacy.

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INTRODUCTION

Ticks are the main problem of the livestock industry in tropical and subtropical regions of the world and they are considered as vectors of many infectious diseases to animals and man. It has been reported that approximately 75% of livestock animals are at risk for ticks and tick-borne diseases causing a global annual loss of 7000 million American dollars [1, 2, 3]. The severe tick infestation may lead to anaemia, reduction in weight gain, direct damage to cattle skins and related production losses in addition to transfer of various tick borne diseases in animals and human beings viz. Lyme disease, Rocky mountain, Spotted fever, Relapsing fever, Tularemia, Meningoencephalitis, Colorado tick fever, Crimean-congo hemorrhagic fever, Bovine Babesiosis, Anaplasmosis, Cytauxzoonosis, etc. [4, 5].

Control of ticks is usually based on chemical acaricidal applications including synthetic pyrethroids, organophosphates, amitraz, pyrethroids and macrocyclic lactones and Ivermectin, Moxidectin, Doramectin and Abamectin etc. Most of these acaricides possess harmful effects on animal health and environment and their uses have caused great concern in society and government [6, 7]. Due to indiscriminate use of these conventional pesticides, number of incidences of resistance have been reported worldwide [8, 9, 10, 11, 12, 13, 14]. Now a days, 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 effects on environment, mainly human beings are the indirect target [15].

The time is, therefore, demanding to develop sustainable, ecofriendly and cost effective alternative, especially for the marginal farmers with limited capital who constitutes the majority of animal rearers in developing countries. The use of phytoextracts is a sustainable option to synthetics as they are biodegradable, cost effective and efficient. Several plant extracts with acaricidal effects have reported worldwide [16, 17, 18, 19]. Among the natural products, plant extracts and essential oils have shown significant activity against all the stages of economically important tick species resistible as compared to synthetic pesticides [20, 21, 22, 23, 24, 25, 26]. Several Indian plants have been mentioned in literature to show promising efficacy against ticks. The present study is, therefore, intended to evaluate the acaricidal properties of two traditional used medicinal plants, *Calotropis procera* and *Spilanthes acmella* against *Rhipicephalus microplus*.

MATERIALSAND METHODS

Collection extraction of plant materials

Fresh aerial parts of *Calotropis procera* and *Spilanthes acmella* (Figure 1) were collected from Eastern Himalayan region of India and washed, shade dried at room temperature and grinded by stainless steel grinder as coarse powder. The coarse powders were extracted in soxhlet assembly in methanol solvent and dried in rotary evaporator to get proper yield. *Calotropis procera* and *Spilanthes acmella* plants methanolic crude extract were separated in 3 different solvents and distilled water. 10 g methanolic crude extract was dissolved in 40 ml distilled water into 100 ml glass beaker. The solution was transferred into separating flask and added 60 ml hexane, mixed well and left until 30 to 60 minutes for separation of hexane layer. It was collected in 100 ml glass bottle (upper layer was hexane) and rest amount of 40 ml aqueous part transferred into separating flask and added 60 ml ethyl acetate, mixed it and left 30 to 60 minutes untouched, ethyl acetate layer collected in 100 ml glass bottle (upper layer was ethyl acetate) and rest amount of 40 ml aqueous part transferred into separating flask and added 60 ml chloroform, mixed it and left 30 to 60 minutes undisturbed for separation of two layers. After that, chloroform layer and aqueous part collected in 100 ml two glass bottles (lower layer was chloroform). Hexane, ethyl acetate and chloroform layers dried at room temperature to remove the solvents and distilled water and to get the fractions.

Bioassay

The dropped ticks after taking full blood meal from the animal's body during night were collected early in the morning. Ticks were collected from farmer's house from Salempur and Makhdoom village in plastic containers and covered with cotton cloth for proper oxygen supply. Ticks were washed in running tap water to remove dust and other impurity on the body surface and dried by filter paper after then keep in refrigerator (4^0C) for further study. In adult immersion test (AIT), 105 ticks were divided into seven groups, six groups treated one kept as control and 15 ticks in each group having weight approximately 2 to 3g. Ticks were taken in petri dishes of 9 cm in diameter containing bed of filter paper (Whatman No.1) and treated group immersed into the respective test concentrations of methanolic crude extract and fractionations (hexane, ethyl acetate, chloroform and aqueous parts) and the control group immersed in distilled water and time of test approximately 2-3 minutes. Treated ticks placed on petri dishes and incubated into BOD incubator at 28±2.0ºC temperature and 70-80 % relative humidity, mortality data observed after 24 hours and died ticks removed from petri dishes and observed in the same way up to 15 days until complete oviposition [27]. 5 days old larvae were used [28] in larval immersion test (LIT) test and larvae were divided to seven groups (n=100-150). Larvae were picked with a help of paint brush No. 4 and gently transferred to clean 1 ml tubes covered with muslin cotton clothes and tied with rubber band. Each group was immersed into the methanolic crude extract and fractionations concentrations while the control group was immersed in distilled water and test time for 2-3 minutes. The treated tubes were placed in 100 ml glass beaker and incubated for 24 hours in BOD at 28±2.0ºC temperature and 70-80% relative humidity. Mortality data was observed after 24 hours [27]. LC_{50} and LC_{90} values were calculated by using to Probit Analysis [29] and other statistical parameters by using software developed by Indian Institute of Chemical Technology, Hyderabad [30].

Figure 1: *Spilanthes acmella* and *Calotropis procera*

RESULTS AND DISCUSSION

The bioefficacy observations of methanolic crude extracts and its fractions of *Calotropis procera* and *Spilanthes acmella* are shown in table 1 and mentioned LC_{50}, LC_{90} and their related statistics. Chloroform extract of *Calotropis procera* was found the most effective against both adult and larval stages with LC_{50} 50.22 mg/ml and LC_{90} 156.48 mg/ml (adult) and LC_{50} 13.86 mg/ml and LC_{90} 47.68 mg/ml (larvae). In case of *Spilanthes acmella,* chloroform fraction was again observed the most potent against both adults and larvae with LC_{50} 60.94 mg/ml and LC_{90} 195.08 mg/ml (adult) and LC_{50} 25.82 mg/ml and LC_{90} 74.50 mg/ml (larvae). In present study methanolic crude extracts and their fractions hexane, ethyl acetate, chloroform and aqueous concentrations both of plants were tested against the engorged adult females and cultured larvae of *Rhipicephalus (Boophilus) microplus* and chloroform fractions of both the plants selected were observed to be the most effective.

Table 1: Relative acaricidal toxicity of *Calotropis procera* and *Spilanthes acmella* against different stages of *Rhipicephalus (Boophilus) microplus* after 24 hours of treatment.

Plants name	Target stage	Extracts	χ2	Regression equation Y=Intercept X + Reg. Coeff.	LC_{50}±SE (UFL-LFL) (mg/ml)	RT	LC_{90}±SE (UFL-LFL) (mg/ml)	RT
Calotropis procera	Adult	Methanol crude	5.70	Y=0.99X+1.93	129.82±21.53 (172.03-87.62)	2.59	600.71±293.92 (1176.79-24.62)	3.84
		Hexane fraction	4.75	Y=5.25X+3.44	95.39±10.77 (116.50-74.28)	1.89	224.89±35.55 (294.56-	1.44

							155.21)	
		Ethyl acetate fraction	2.37	Y=3.77X+3.14	61.78±9.59 (80.57-42.99)	1.23	157.96±24.26 (205.51-110.40)	1.01
		Chloroform fraction	3.04	Y=2.01X+2.59	**50.22±10.69** (71.64-29.27)	1	156.48±28.60 (212.53-100.42)	1
		Aqueous fraction	1.22	Y=0.89X+1.97	96.99±17.04 (130.39-63.59)	1.93	432.34±170.77 (767.04-197.64)	2.76
	Larvae	Methanol crude	12.16	Y=0.98X+1.61	30.79±1.85 (34.43-27.16)	2.22	191.57±18.17 (227.19-155.95)	4.02
		Hexane fraction	38.74	Y=0.94X+2.29	38.99±1.47 (41.88-36.11)	2.81	141.28±8.04 (157.04-125.52)	2.96
		Ethyl acetate fraction	39.59	Y=0.95X+1.83	16.50±1.44 (19.33-13.67)	1.19	83.10±4.55 (92.01-74.19)	1.74
		Chloroform fraction	25.09	Y=0.12X+2.39	**13.86±1.11** (16.03-11.69)	1	47.68±1.90 (51.41-43.95)	1
		Aqueous fraction	17.07	Y=0.71X+2.12	49.33±1.75 (52.75-45.90)	3.56	198.22±15.09 (227.81-168.64)	4.16
Spilanthes acmella	Adult	Methanol crude	9.58	Y=5.99X+3.21	259.67±29.91 (318.29-201.04)	4.26	649.60±119.22 (883.27-415.94)	3.33
		Hexane fraction	2.07	Y=1.39X2.27	65.16±10.96 (86.65-43.68)	1.07	238.63±56.78 (349.92-127.35)	1.22
		Ethyl acetate fraction	2.15	Y=2.49X+2.65	66.83±11.38 (89.15-44.52)	1.09	203.11±39.46 (280.45-125.77)	1.04

		Chloroform fraction	2.30	Y=2.06X+2.54	**60.94±11.44** (83.37-38.52)	1	195.08±38.69 (270.93-119.23)	1
		Aqueous fraction	4.27	Y=1.52X+2.31	65.27±12.76 (90.28-40.25)	1.07	233.19±54.52 (340.05-126.34)	1.19
	Larvae	Methanol crude	70.82	Y=0.74X+2.34	28.61±1.32 (31.20-26.01)	1.39	101.12±4.76 (110.44-91.79)	1.36
		Hexane fraction	101.98	Y=1.24X+2.57	26.46±1.12 (28.64-24.27)	1.29	83.23±3.12 (89.35-77.12)	1.12
		Ethyl acetate fraction	65.69	Y=0.08X+2.19	20.56±1.26 (23.02-18.09)	1	78.74±3.49 (85.57-71.91)	1.06
		Chloroform fraction	48.58	Y=1.72X+2.78	**25.82±1.04** (27.85-23.78)	1.26	74.50±2.58 (79.56-69.45)	1
		Aqueous fraction	24.04	Y=0.81X+1.63	37.11±1.96 (40.96-33.27)	1.80	226.93±23.86 (273.69-180.16)	3.05

RT: Relative toxicity

Acaricidal properties of different botanical derivative have been reported against the different species of the ticks by different researcher from different parts of the world. The acaricidal effect of crude ethanolic extract of *Acmella oleracea* (L.) R.K. Jansen aerials parts at different concentrations on *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae) ticks was reported by [31, 32] studied the acaricidal effect of *Synadenium glaucescens* (Euphorbiaceae) extracts on *Boophilus decoloratus* and *Boophilus microplus*. *In Vitro* studies on *Rhipicephalus sanguineus* in Abomey-Calavi has been reported the effect of ethanolic extract of *Tephrosia Vogelii* [33]. The relative toxicity against adult ticks and larvae of *Rhipicephalus (Boophilus) microplus* has been reported for methanolic extract of *Adhatoda vasica* roots by [34]. [35] reported anti-tick activity against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) and identification of potential plant extracts. Observations on the crude extracts of *Cymbopogan wintericmus Jowett* and *Azadirachta Indica* efficacy against *Rhipicephalus (Boophilus) microplus* and inhibited of oviposition 94.29 % mortality were made by [36]. Acaricidal property of hexane extract of *Adhatoda vasica* roots against *Rhipicephalus (Boophilus) microplus,* has been reported by [37]. The extract of *Carapa guianensis* seed oil, leaf essential oil of *Cymbopogon* *martini* and crude extract of *piper tuberculatum* [38]. [39] were reported 10% (100000 ppm) and 20% (200000 ppm) as effective concentrations of leaf and stem for AIT and oviposition inhibition, respectively of methanol extract of *Petiveria alliacea* against *Rhipicephalus (Boophilus) microplus.*

The results of the present work showing the promising acaricidal activities against the target organism and might be used as an potential alternative to control the ticks. The efficacy of *Calotropis procera and Spilanthes acmella* methanolic crude extracts and its fractionation have been observed against *Rhipicephalus (Boophilus) microplus*. The target organism revealed a dose dependent pattern as expected and the study indicates that the plants *Calotropis procera* and *Spilanthes acmella* have a great potential as an efficacious bioacaricide and needs to be explored for avoiding indiscriminate pesticide use in animals. So, further study should be carried out in future to isolate the specific chemicals constituents and pharmacological activity will be carried out in proper scientific way.

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