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Evaluation of medicinal plant extracts against ticks and fluke

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Abstract The present study was based on assessments of the antiparasitic activities to determine the efficacies of leaf hexane, chloroform, ethyl acetate, acetone and methanol extracts of *Aegle marmelos* (Linn.) Correa ex Roxb, *Andrographis lineata* Wallich ex Nees., *Andrographis paniculata* (Burm.f.) Wallich ex Nees., *Cocculus hirsutus* (L.) Diels, *Eclipta prostrata* L., and *Tagetes erecta* L. against the adult cattle tick *Haemaphysalis bispinosa* Neumann 1897 (Acarina: Ixodidae), the larvae of *Rhipicephalus* (*Boophilus*) *microplus* Canestrini 1887 (Acari: Ixodidae) and sheep fluke *Paramphistomum cervi* Zeder 1790 (Digenea: Paramphistomatidae). All plant extracts showed moderate toxic effect on parasites after 24 h of exposure; however, the highest parasitic activity was found in leaf ethyl acetate extract of *A. lineata*, methanol extract of *A. marmelos*, *A. paniculata*, and *C. hirsutus* against *H. bispinosa* (LC_{50}=395.27, 358.45, 327.21 and 420.50 ppm); ethyl acetate extract of *A. paniculata*, *C. hirsutus*, methanol extracts of *A. marmelos*, *A. lineata*, and *E. prostrata* against the larvae of *R. microplus* (LC_{50}=207.70, 258.61, 134.09, 206.00, and 274.33 ppm); hexane extract of *A. lineata*, ethyl acetate extract of *A. paniculata*, *E. prostrata*, acetone extracts of *T. erecta*, methanol extracts of *A. marmelos* and *C. hirsutus* against *P. cervi* (LC_{50}=254.23, 451.17, 425.73, 253.60, 542.71, and 360.17 ppm), respectively. The present study is the first report on the veterinary parasitic activity of plant extracts from Southern India.

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Introduction

India accounts for a significant share of the world's livestock resources with nearly 57% of the world's buffaloes, 16.5% cattle, 16.3% goats, and 5.7% sheep (FAO 2004). In India, the damage caused by ticks and tick-born diseases (TTBDs) to livestock is considered very high (Ghosh et al. 2006). A recent estimate of US$ 498.7 million per annum has been calculated as the cost of TTBDs in India (Minjauw and McLeod 2003). TTBDs were the major constraint to cattle production and cause economic losses to farmers in terms of cattle mortality, loss of body weight, milk loss, costs of control of TTBDs through chemotherapy, infection, and treatment methods (Kivaria 2006; Homewood et al. 2006). Ghosh et al. (2007) reported the disease transmission potential for *Haemaphysalis* (theileriosis and babesiosis in sheep and goats) and *Rhipicephalus* (bovine and canine babesiosis, ehrlichiosis, and equine babesiosis). *Paramphistomum cervi* is considered one of the most important species of paramphistomes, since they are cattle parasites with cosmopolitan distribution. Paramphistomosis is one of the most important diseases in domesticated animals causing heavy economic losses to livestock industry in India (Hassan et al. 2005). *Paramphistomum* infection provokes a lower feed conversion, a loss of weight and/or a decrease in milk production that results in economic loss (Rangel-Ruiz et al. 2003).Ticks may be controlled using synthetic acaricides (Klafke et al. 2006; Li et al. 2007; Barré et al. 2008; Miller et al. 2008), but these can have undesirable effects on other organisms and the environment. Ultimately, they become ineffective as ticks develop resistance to synthetic chemicals (Solomon and Kaaya 1996; Shiferaw et al. 1997; Chagas 2004). Development of resistance to commercial acaricides by parasites has stimulated the search for new control strategies. In

recent times, many plant extracts have been tested against endo- or ectoparasites and pests, which may contaminate food and/or rooms with agents of diseases (Amer and Mehlhorn 2006; Mehlhorn et al. 2005; Athanasiadou et al. 2007; Mehlhorn et al. 2010; Bagavan et al. 2010; Kamaraj et al. 2010a).

Aegle marmelos commonly known as Bael has been widely used in indigenous systems of Indian medicine due to its various medicinal properties. The leaf acetone, chloroform, ethyl acetate, hexane, and methanol extracts of *A. marmelos*, *Andrographis lineata*, *Andrographis paniculata*, *Cocculus hirsutus*, *Eclipta prostrata*, and *Tagetes erecta* were tested against the fourth instar larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* (Elango et al. 2009a); the ethanol extract of *A. marmelos* was tested for antifilarial activity against microfilariae of *Brugia malayi* (Sahare et al. 2008a) and skimmiarepins A and C have been isolated from seeds of *A. marmelos* which exhibited moderate insecticidal activity against *Phaedon cochleariae* and *Musca domestica* (Samarasekera et al. 2004). The leaf acetone, ethyl acetate, and methanol extracts of *A. lineata* were tested for oviposition-deterrent, ovicidal, and repellent activities against *A. subpictus* (Elango et al. 2009b). Misra et al. (1992) investigated anti-malarial activity of four diterpens isolated from *A. paniculata* against *Plasmodium berghei* NK65 in *Mastomys natalensis*. Four xanthones isolated from the roots of *A. paniculata* were tested in vitro for antiprotozoal activity against *Trypanosoma brucei brucei*, *Trypanosoma cruzi*, and *Leishmania infantum* (Dua et al. 2009). *C. hirsutus* is a widely growing plant found in the plains of India in dry localities, and the aqueous extract showed significant diuretic activity and laxative effect in rats (Ganapaty et al. 2002). The compounds saponin and dasyscyphin C isolated from *E. prostrata* were tested for the leishmanicidal activity against leishmanial parasites, *Leishmania major*, *Leishmania aethiopica*, and *Leishmania tropica* (Khanna et al. 2009).

The aim of this study was to investigate the parasitic activities of the different solvent extracts of six plant species from Tamil Nadu, India. This is the first report on *Haemaphysalis bispinosa, Rhipicephalus microplus*, and *P. cervi* activity of the solvent extracts of selected plants.

Materials and methods

Collection of plant materials

The leaf of *A. marmelos* (Linn.) Correa ex Roxb (Rutaceae), *A. lineata* Wallich ex Nees. (Acanthaceae), *A. paniculata* (Burm.f.) Wall. ex Nees. (Acanthaceae), *C. hirsutus* (L.) Diels (Menispermaceae), *E. prostrata L.* (Asteraceae), and *T. erecta L.* (Compositae) were selected on the basis of aromatic smell, ethnopharmacological, and ethnobotanical literature survey. The plant materials were collected from the Tamil Nadu Medical Plant Farms and Herbal Medicine Corporation Limited, medicinal plant farm, Arumbakkam (13°13′4″ N, 79°59′7″ E Altitude 118 ft), Chennai, Tamil Nadu, and the taxonomic identification was made by Dr. C. Hema, Department of Botany, Arignar Anna Govt. Arts College for Women, Walajapet, Vellore, India. The voucher specimen was numbered and kept in our research laboratory for further reference.

Preparation of plant extracts

The leaves were dried for 7–10 days in the shade at the environmental temperatures (27–37°C). The dried leaves were powdered mechanically using a commercial electrical stainless steel blender and the powdered leaves (700 g) were extracted with hexane (1,600 ml, Fine), chloroform (1,700 ml, Fine), ethyl acetate (2,400 ml, Qualigens), acetone (1,400 ml, Qualigens), and methanol (2,800 ml, Qualigens) in a Soxhlet extractor using a sequence of solvents of increasing polarity (hexane, chloroform, ethyl acetate, acetone, and methanol) (boiling point range 60–80°C) for 8 h. The yield of extracts was hexane (5.45 g), chloroform (5.82 g), ethyl acetate (7.76 g), acetone (8.02 g), and methanol (11.43 g). The extract was concentrated under reduced pressure of 22–26 mmHg at 45°C and the residue obtained was stored at 4°C. Crude extract (1 g) was first dissolved in 100 ml of acetone (stock solution). The control was set up with acetone and polysorbate 80 (Qualigens). From the stock solution, 3,000 and 2,000 ppm were prepared with dechlorinated tap water. Polysorbate 80 was used as an emulsifier at the concentration of 0.05% in the final test solution.

Parasite collection and bioassay

Attached adult of *H. bispinosa* Neumann 1897 (Acarina: Ixodidae) were collected from October to December of 2009 from inside the ears and very rarely elsewhere on the body of cattle. *R. microplus* Canestrini 1887 (Acari: Ixodidae) adult-engorged females were collected from naturally infested cattle pastured on ranches cattle in a farm of Vellore Diary Centre, Vellore, and free of acaricidal residues for at least 30 days prior to the bioassays. Engorged females were placed into 10 ml glass vials with cotton caps and sent to the Parasitology Department of the Faculty of Veterinary Science in the Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, where they were placed on Petri dishes and incubated at laboratory conditions, at 27±2°C and a relative humidity (RH) of 75–85% under 14:10 light and dark cycles to allow oviposition. Eggs were then transferred into glass vials (10 ml) with a cotton cap. Eclosion of larvae occurred

approximately 28–30 days after collection of engorged females. In the present study, live larvae of 7–14 days of age were used. (Rodriguez-Vivas et al. 2006). *R. microplus* adults have a short, straight capitulum. The parasites were identified by Dr. A. Sangaran, Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu. The applied method in the present study to evaluate the acaricidal activity of different solvent plant extracts against adult of *H. bispinosa*, and the larvae of *R. microplus* was developed as per the method of FAO (2004), incorporating slight modifications to improve practicality and efficiency of tested materials (Fernandes 2001). From the stock solution, 2,000 ppm was prepared, and a series of filter paper envelopes (Whatman filter paper No. 1, 125 mm dia.) were treated with each concentration of extracts previously listed. Each envelope was treated with 3 ml solution uniformly distributed with a pipette on internal surfaces. Five envelopes were impregnated with each tested solution. The control papers were impregnated with solvent, polysorbate 80, and distilled water only. The opening of the five envelopes (treated and inoculated with adult ticks) was folded (10 mm) and re-sealed with a metallic clip, with its identification mark (tested solution and concentration) on the outside. The packets are placed in the BOD incubator at a temperature of 28–30°C and 80–90% RH for 24 h. The envelopes were opened 24 h after exposure and recorded, the number of live and dead parasites were also recorded (Zahir et al. 2009). The experimental media, in which 100% mortality of adult and larvae occurs alone, were selected for dose–response bioassay.

In this study, the larvae of *R. microplus* were unfed and allowed 14–21 days following eclosion. Hatching tubes with the highest larval eclosion rate (90–100%) were selected and placed in the base of a bottle, inverted in the center of a Petri dish that was subsequently filled with water, which prevented their escape. A sample of the larvae from this tube was placed in the center of a sheet of white paper, fixed to the bench with adhesive tape, and thirty or more specimens with good mobility were caught with a no. 5 paintbrush moistened in test solution, then gently transferred to each envelope. The opening of the envelopes (treated and inoculated with larval ticks) was folded (≈12 mm) and re-sealed with a metallic clip, with its identification mark (tested solution and concentration) on the outside. The packets are placed in the BOD incubator at a temperature of 27–28°C and 85–95% RH for 24 h. The envelopes were opened 24 h after exposure and inspected using a stereoscope to record the number of live larvae, and immobile larvae were scored as being dead. Five replicates were conducted at different dates. Larvae used during each replicate were from different gravid females. According to FAO (2004), the percentage mortality in all of the experimental batches of larvae was corrected by applying Abbott's formula:

Corrected percent mortality

$$\frac{\%\,\text{test mortality} - \%\,\text{control mortality}}{100 - \%\,\text{control mortality}} \times 100$$

Adult *P.* cervi Zeder 1790 (Sey 1982) were collected in 0.9% phosphate-buffered saline (pH 7–7.2) from the rumen of infected sheep killed for consumption at the local slaughterhouses and were identified. Crude extract (1 g) was first dissolved in 100 ml of acetone (stock solution). The anthelmintic assay was carried as per the method of Tandon et al. (1997) with necessary modifications. From the stock solution, 3,000 ppm was prepared with 100 ml PBS solution and 1.0 ml of the desired plant extract concentration. The adult *P. cervi* parasites were incubated at 37±28°C in media containing no extract (control) or crude extract in PBS supplemented with 0.5% dimethylsulfoxide (Qualigens). Five replicates were used for each concentration. The time required for complete death of the parasite was recorded. After being removed from the experimental medium and dipped in slightly warm water and on gentle stimulation, the death of parasite confirmed mortality. The numbers of dead parasite were counted after 24 h of incubation at 37.8°C of exposure, and the percentage mortality was reported from the average of five replicates. The experimental media, in which 100% mortality of parasite occurs alone, were selected for dose–response bioassay.

Dose–response bioassay

From the stock solution, different concentrations ranging from 23.43 to 3,000 ppm were prepared and tested for bioassay against different parasites. Based on the preliminary screening results, different crude solvent extracts prepared from the leaf of *A. marmelos*, *A. lineata*, *A. paniculata*, *C. hirsutus, E. prostrata* and *T. erecta* were subjected to dose–response bioassay against *H. bispinosa*, *R. microplus*, and *P. cervi*, respectively. The numbers of dead parasite were counted after 24 h of exposure, and the percentage mortality was reported from the average of five replicates.

Statistical analysis

The average parasite and larval mortality data were subjected to Probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and Chi-square values were calculated by using the software developed by Reddy et al. (1992). Results with $p<0.05$ were considered to be statistically significant.

Results

Mortality (100%) was obtained in methanol extracts of *A. marmelos*, *A. lineata*, *A. paniculata*, *C. hirsutus*, *E. prostrata*, ethyl acetate extracts of *A. paniculata*, *C. hirsutus*, *E. prostrata*, hexane extract of *A. lineata*, and acetone extract of *T. erecta* against *H. bispinosa*, *R. microplus*, and *P. cervi* at 3,000 and 2,000 ppm shown in Table 1. All plant extracts showed moderate toxic effect on parasites after 24 h of exposure; however, the highest mortality was found in leaf ethyl acetate extract of *A. lineata*, methanol extract of *A. marmelos*, *A. paniculata*, and *C. hirsutus* against *H. bispinosa* (LC_{50}=395.27, 358.45, 327.21, and 420.50 ppm; LC_{90}=1,949.86, 2,639.43, 1,803.26, and 1,885.69 ppm), hexane extract of *A. lineata*, ethyl acetate extract of *A. paniculata*, *E. prostrata* acetone extracts of *T. erecta*, methanol extracts of *A. marmelos* and *C. hirsutus* against the adult of *P. cervi* (LC_{50}=254.23, 451.17, 425.73, 253.60, 542.71, and 360.17 ppm; LC_{90}=2,352.32, 1,249.66, 2,102.62, 2,115.81, 2,318.93, and 1,500.60), ethyl acetate extract of *A. paniculata*, *C. hirsutus*, methanol extracts of *A. marmelos*, *A. lineata*, and *E. prostrata* against the larvae of *R. microplus* (LC_{50}=207.70, 258.61, 134.09, 206.00, and 274.33 ppm); LC_{90}=555.85, 966.80, 1,121.41, 1,465.20, and 1,212.42 ppm, respectively (Table 2).

Discussion

In this study, we evaluated the acaricidal and flukicidal activity of the leaf crude extracts of *A. marmelos*, *A. lineata*, *A. paniculata*, *C. hirsutus*, *E. prostrata*, and *T. erecta* for the control of *H. bispinosa*, *R. microplus*, and *P. cervi*. Dua et al. (2004) reported that the compound 1, 2-dihydroxy-6, 8-dimethoxy-xanthone isolated from *A. paniculata* possessed substantial anti-plasmodial activity against *Plasmodium falciparum* with its IC_{50} value of 4 μg/ml. Sahare et al. (2008b) reported that the methanol extract of *A. marmelos* at 100 ng/ml concentration showed complete loss of motility of microfilariae after 48 h of incubation against *B. malayi*. Similar studies carried out by Kamaraj et al. (2010b) shows the leaf methanol of *Rhinacanthus nasutus* and seed acetone of *Terminalia chebula* against the adult of *H. bispinosa* (LC_{50}=333.15 and 186.46 ppm; LC_{90}=1,056.07 and 590.76 ppm), and the seed acetone of *T. chebula* were tested against the adult of *P. cervi* (LC_{50}=87.08 ppm; LC_{90}=433.85 ppm). This has

Table 1 Parasitic activity of crude plant extract against *Haemaphysalis bispinosa* and *Paramphistomum cervi* at 3,000 ppm, *Rhipicephalus microplus* at 2,000 ppm

Botanical name/family (herbarium numbers) vernacular names	Species	Percent mortality[a]±SD				
		Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
Aegle marmelos (Linn.) Correa ex Roxb/Rutaceae(LE/ZB/007-05) Vilvam	*H. bispinosa*	82±1.49	83±1.56	50±1.58	61±1.62	100±0.00
	R. microplus	67±1.34	60±0.70	54±1.92	59±1.48	100±0.00
	P. cervi	82±1.14	76±0.83	56±0.83	63±1.56	100±0.00
Andrographis lineata Wallich ex Nees./Acanthaceae (ZD/AL/040–08) Siriyanangai	*H. bispinosa*	69±1.64	55±±1.00	100±0.00	75±1.41	84±2.24
	R. microplus	86±0.83	61±1.58	71±1.09	86±0.76	100±0.00
	P. cervi	100±0.00	91±.086	65±1.10	73±0.54	83±1.34
Andrographis paniculata (Burm.f.) Wall. ex Nees./Acanthaceae (ZD/AP/143–08) Periyanangai or Nilavembu	*H. bispinosa*	59±1.30	72±3.28	80±1.87	86±0.86	100±0.00
	R. microplus	60±1.41	73±0.89	100±0.00	63±1.67	90±0.70
	P. cervi	51±1.09	79±0.78	100±0.00	57±1.58	75±.063
Cocculus hirsutus (L.) Diels/Menispermaceae (ZD/CH/142–08) Vellakattukkodi	*H. bispinosa*	68±2.30	66±1.92	81±1.30	48±2.07	100±0.00
	R. microplus	79±1.51	66±1.64	100±0.00	78±1.14	70±1.58
	P. cervi	56±0.83	59±1.48	83±1.04	81±1.43	100±0.00
Eclipta prostrata L./Asteraceae (ZD/EP/114–08) Manjal Karisallankannai	*H. bispinosa*	50±1.58	59±2.48	70±1.88	46±1.42	67±1.673
	R. microplus	72±1.56	51±1.30	66±0.83	59±1.18	100±0.00
	P. cervi	60±1.32	53±1.44	100±0.00	66±1.24	82±1.140
Tagetes erecta L./Compositae (ZD/TE/156–08)Tulukkaccevvanti	*H. bispinosa*	77±3.13	62±1.81	72±1.36	84±1.724	70±2.110
	R. microplus	59±2.68	74±2.38	60±1.14	55±1.00	77±1.944
	P. cervi	60±0.70	64±1.64	58±1.14	100±1.00	79±1.303

Control—nil mortality

[a] Mean value of five replicates±SD

Table 2 LC_{50}, LC_{90}, and other statistical analysis of different solvent plant extracts against the adult of *Haemaphysalis bispinosa*, larvae of *Rhipicephalus microplus*, and adult of *Paramphistomum cervi*

Name of the plants	Species	Solvents	LC_{50}±SE (ppm)	(UCL−LCL)	LC_{90}±SE (ppm)	(UCL−LCL)	$\chi 2$ (df=4)
A. marmelos	*H. bispinosa*	Methanol	358.45±26.69	410.77−306.14	1,949.86±253.34	2,446.42–1,453.30	9.57
	R. microplus	Methanol	134.09±8.95	151.62−116.55	555.85±58.02	669.58−442.12	3.61
	P. cervi	Methanol	542.71±37.18	615.58−469.83	2,352.32±281.25	2,903.57–1,801.07	21.00
A. lineata	*H. bispinosa*	Ethyl acetate	395.27±31.91	457.83−333.71	2,639.43±394.70	3,413.04–1,865.81	14.37
	R. microplus	Methanol	206.00±14.46	234.34−177.65	966.80±112.41	1,187.13−746.48	7.97
	P. cervi	Hexane	254.23±18.14	289.79−218.68	1,249.66±151.51	1,546.62−952.70	8.78
A. paniculata	*H. bispinosa*	Methanol	327.21±24.64	375.51−278.91	1,803.26±239.32	2,272.35–1,334.18	13.63
	R. microplus	Ethyl acetate	207.70±15.50	238.08−177.33	1,121.41±142.42	1,400.56−842.25	7.97
	P. cervi	Ethyl acetate	451.17±31.76	513.33−389.01	2,102.62±257.01	2,606.36–1,598.87	14.10
C. hirsutus	*H. bispinosa*	Methanol	420.50±29.17	477.96−363.32	1,885.69±222.90	2,322.58–1,448.30	10.59
	R. microplus	Ethyl acetate	258.61±19.53	296.88−220.33	1,465.20±197.83	1,852.97–1,077.44	15.99
	P. cervi	Methanol	360.17±27.81	414.69−305.65	2,115.81±299.56	2,702.95–1,528.67	14.63
E. prostrata	*R. microplus*	Methanol	274.33±18.84	311.27−237.40	1,212.42±139.75	1,486.34−938.50	8.68
	P. cervi	Ethyl acetate	425.73±31.77	485.01−363.46	2,318.93±310.84	2,928.18–1,709.68	12.22
T. erecta	*P. cervi*	Acetone	253.60±19.94	292.70−214.52	1,500.60±199.07	1,890.77–1,110.43	9.95

Control—nil mortality. Significant at $p< 0.05$ level

LC_{50} lethal concentration that kills 50% of the exposed larvae, LC_{90} lethal concentration that kills 90% of the exposed larvae, *UCL* upper confidence limit, *LCL* lower confidence limit, x^2 Chi-square, *df* degrees of freedom

been observed earlier by Bagavan et al. (2009) that the potential of acaricidal and flukicidal activity of leaf hexane extract of *Annona squamosa,* methanol extracts of *Gloriosa superba*, and *Phyllanthus emblica* against *H. bispinosa* (LC_{50}=145.39, 225.57, and 256.08 ppm); methanol extracts of *Centella asiatica* and *G. superba* against *P. cervi* (LC_{50}=77.61and 60.16 ppm). Andrographolide (1) and 14-deoxy-11, 12-didehydroandrographolide (2) were isolated from *A. paniculata* and reported to have antiviral, antipyretic, immunostimulant, and anticancer activities (Suebsasana et al. 2009). Alkaloids isolated from *A. squamosa* have shown larvicidal growth-regulating and chemosterilant activities against *Anopheles stephensi* at concentrations of 50–200 ppm; the larvae, pupae, and adults produced about a 52–92% decrease in the laboratory experiment (Saxena et al. 1993). Bruce and Cork (2001) have reported that the benzaldehyde, (*S*)-(−)-limonene, (*R*, *S*)-(+/−)-linalool, (*E*)-myroxide, (*Z*)-beta-ocimene, phenyl-acetaldehyde, and (*R*)-(−)-piperitone isolated from *T. erecta* are used to control female *Helicoverpa armigera*. It is not yet possible to identify which of these chemicals are the active compounds in the present study, and the identities of active pure compounds of *A. paniculata, A. marmelos*, and *T. erecta* should be investigated further.

There have been few studies on the activities of Brazilian plants against tick larvae. The crude ethanol extract of soapberry, *Sapindus saponaria* (Sapindaceae), showed *R.* (*B.*) *microplus* larvicidal activity with LC_{50} and LC_{99} values of 1,258 and 6,360 ppm, respectively (Fernandes et al. 2005). The plant *S. saponaria* also demonstrated larvicidal activity for the Brown Dog tick, *Rhipicephalus sanguineus* (Acari: Ixodidae), with LC_{50} and LC_{99} values of 1,994 and 3,922 ppm, respectively (Fernandes et al. 2007). Fernandes and Freitas (2007), for instance, have reported that the oleoresinous extract (oleoresin) from *Copaifera reticulata* (Leguminosae) tested against *R.* (*B.*) *microplus* larvae showed that LC_{50} and LC_{99} values were 1,579 and 3,491 ppm, respectively. In the larval immersion test, the hexane extract of *Calea serrata* proved to be toxic, killing 100% of the larvae of both *B. microplus* and *R. sanguineus* at the concentrations of 50, 25, 12.5, and 6.25 mg/ml after 48 h (Ribeiro et al. 2008). The present result showed that the experimental plants are highly effective to control *R. microplus* compared with other plant extracts reported by earlier authors.

The chemotherapeutic value of medicinal plant extracts is also evident from an earlier study, wherein the anthelmintic activity of the aqueous and alcoholic extracts of *Ananas sativus* (Bromeliaceaea), *Embelia ribes*, *Macuna prurita* (Leguminosae), and *Melia azedarach*, which has significant activity against *P. cervi* and *M. prurita*, was especially active against trematodes (Neogi et al. 1964). Earlier authors reported that the highest parasite mortality was found in the leaf methanol extract of *Aglaia malabarica* and flower methanol extract of *G. superba* against the larvae of *R. microplus* (LC_{50}=95.97and153.73 ppm; LC_{90}= 393.88 and 1794.25 ppm); leaf chloroform extracts of *Aglaia malabarica* and *Ricinus communis* against the adult

of *P. cervi* (LC_{50}=106.69 and 69.44 ppm; LC_{90}=463.94 and 256.52 ppm) (Zahir et al. 2009). In our study, we found high efficacy observed (LC_{50}=253.60 ppm and LC_{90}= 1,500.60 ppm) using acetone extract of *T. erecta* against *P. cervi*. In the present observation, the acetone extract of *T. erecta* was more effective compared with other plant extracts reported by earlier authors.

Zahir et al. (2010) reported that the leaf acetone and methanol extracts of *Aglaia malabarica*, seed methanol of *G. superba*, and leaf methanol of *Ricinus communis* showed the LC_{50} values of 466.15, 719.78, 476.06, and 243.87 ppm and LC_{90}values of 1,837.96, 2,014.47, 1,904.36, and 2,692.15 ppm against *H. bispinosa*. In vitro screening of extracts for their acaricidal property against *B. microplus* showed that *Azadirachta indica* seed extract was most effective (80%) followed by *Prunus persica* seed (70%) and *A. indica* leaf (30%) (Srivastava et al. 2008). Schmahl et al. (2010) reported that the neem seed extract with shampoo (MiteStop®) 1:40 dilution killed the ticks of *Ixodes ricinus* and *R. sanguineus* within 5 h when sprayed on the surface or when the ticks get in contact just with their feet to the compound and the dilution of 1:66 (neem seed extract:Tre-san® killed *I. ricinus* ticks; while using this dose, *R. sanguineus* died only after direct spraying onto their backside. After 24 h of treatment, the highest acaricidal activity of 70.8% was recorded in the ticks treated with 8% extract of *A. squamosa* followed by *Nicotiana tobacum* (45.8%) and *Tamarindus indica* (41.7%) extracts, while 29.8% and 20.8% mortality, respectively, was recorded in ticks treated with *Eucalyptus globulus* and *Citrus limonum* extracts against *B. microplus* (Magadum et al. 2009)). Duarte et al. (2008) have reported that effect of six hyacinthacine analogues derived from pyrrolizidine alkaloids were toxic to the larvae of the ticks and inhibited the eggs' hatchability at 5 μg/ml, and at the lowest concentration (0.625 μg/ml), some effect in the eggs' hatchability was observed against the cattle tick *R.* (*B.*) *microplus*.

In conclusion, our screening of indigenous medicinal plants from Tamil Nadu, India showed antiparasitic activities. Some plants, such as *A. paniculata*, *A. marmelos*, and *T. erecta*, have already been reported to show parasitic and biological activities, and our study confirmed these observations. Furthermore, our study identified these with possible novel acaricidal and flukicidal activities. These plants have not been previously studied in any detail for parasitic activity of these types, and they showed useful selectivity against the target species. Our results showed that the leaf methanol extracts of *A. paniculata*, *A. marmelos*, and acetone extract of *T. erecta* is a promising biocontrol candidate for use against *H. bispinosa*, *R. microplus*, and *P. cervi*. This is the first report on the parasitic activity of selected plant extracts from Tamil Nadu, India.

The isolation and purification of crude leaf methanol extract of *A. paniculata*, *A. marmelos*, and acetone extract of *T. erecta* are in progress.

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