



## RESEARCH ARTICLE

### Ethnopharmacological Screening of Promising Medicinal Plants with *in vitro* Acaricidal Effect on *Rhipicephalus microplus* in the State of Meta, Colombia

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#### ARTICLE HISTORY (25-1176)

Received: December 06, 2025  
Revised: March 13, 2026  
Accepted: March 17, 2026  
Published online: March 23, 2026

#### Key words:

Acaricidal activity  
Ethnobotany  
Parasites  
Phytotherapy  
*Rhipicephalus microplus*  
Tick Control.

#### ABSTRACT

Given the increasing resistance to conventional acaricides, the search for plant-based therapeutic alternatives to control the cattle tick *Rhipicephalus (R.) microplus* has been driven. This tick is responsible for transmitting zoonotic hemoparasites and causing significant economic losses. This study aimed to validate ethnopharmacological reports on the acaricidal effects of plants traditionally used in the state of Meta, Colombia, on *R. microplus* teleogines and larvae, and to identify groups of secondary metabolites. Ethnopharmacological surveys were conducted, finding seventeen plant species. Eighteen ethanolic extracts were prepared from these species for *in vitro* evaluation of their acaricidal effect at concentrations of 10, 20, 40, 80, and 160mg/mL. In addition to performing a preliminary phytochemical analysis, adult immersion tests (AIT) and larval immersion tests (LIT) were performed observing mortality and impact on the reproductive cycle. Probit analysis was used to determine LC<sub>50</sub> and LC<sub>90</sub> values, and ANOVA and Tukey's post-hoc test were used to determine differences between the concentrations of the extracts and the negative (Tween-80 2%) and positive (Cypermethrin 0.015%) controls (P<0.05). When mortality in AIT and LIT was compared between the highest concentration (160mg/mL) of the extracts and cypermethrin, five and three extracts, respectively, showed higher mortality rates (P<0.05). Similarly, eight extracts had significant adverse effects on the reproductive cycle in AIT (P<0.05). The best LC<sub>50</sub> and LC<sub>90</sub> values were obtained in *Annona muricata* (AIT=20.74mg/mL and 106.04mg/mL) and *Dioclea virgata* (LIT=26.80mg/mL and 116.78mg/mL). Of the twelve groups of secondary metabolites evaluated in the phytochemical analysis, alkaloids, coumarins, cardiotoxic glycosides, and sapogenins were the most prevalent. Seven promising plants were found for the control of *R. microplus*. These results confirm the value of traditional ethnopharmacological knowledge and provide evidence of the potential of plant extracts as a sustainable alternative for tick management in cattle.

**To Cite This Article:** Velasquez-Peña MA, Lesmes-Rodríguez LC and Jaramillo-Hernández DA, 2026. Ethnopharmacological screening of promising medicinal plants with *in vitro* acaricidal effect on *Rhipicephalus microplus* in the state of Meta, Colombia. Pak Vet J, 46(4): 910-919. <http://dx.doi.org/10.29261/pakvetj/2026.071>

#### INTRODUCTION

The tick *Rhipicephalus (R.) microplus* (Murrell and Barker, 2003) is the new taxonomic classification assigned to *Boophilus microplus* (Canestrini, 1887). It is an ixodid tick characterized as monoxenous, completing its life cycle on a single host, feeding on blood for 21 days. Its different developmental stages include larva, metalarva, nymph, metanymph, neogyne, parthenogyn, and teleogine; the last two being partially regurgitated and

totally regurgitated females, respectively (Estrela *et al.*, 2010). *R. microplus* is widely distributed in tropical and subtropical regions located between latitudes S30 and N40, in the Americas, Africa, Asia, and Europa (Luciano *et al.*, 2025).

Infestation by *R. microplus* ticks is one of the main problems in cattle farming in tropical countries (Mollong *et al.*, 2025). Worldwide, tick-borne diseases cause annual losses estimated at US\$ 14 to 19 billion. Specifically, *R. microplus* represents annual losses of US\$ 168 million in

Colombia (Shahzad *et al.*, 2025). Ticks also have a significant impact on public health, as *R. microplus*, transmit multiple zoonotic diseases such as Crimean-Congo hemorrhagic fever virus and tick-borne encephalitis; bacterial diseases such as Q fever (*Coxiella*) and borreliosis (*Borrelia*); protozoal diseases such as theileriosis (*Theileria*) and babesiosis (*Babesia*); and rickettsial diseases such as anaplasmosis (*Anaplasma*) and ehrlichiosis (*Ehrlichia*, *Rickettsia*) (Johansson *et al.*, 2020; Luciano *et al.*, 2025).

Tick control in cattle has been performed worldwide since the 19<sup>th</sup> century using chemical agents (allopathic drugs). However, due to the irresponsible handling of these products, these organisms have undergone a process of selection pressure (Rajput *et al.*, 2006), with *R. microplus* being the tick that has most rapidly developed resistance to conventional acaricides worldwide (Dzemo *et al.*, 2022). Colombia is no exception to this phenomenon, with reports of antiparasitic resistance in *R. microplus* from various regions of the country (López-Arias *et al.*, 2015; Villar *et al.*, 2020).

Considering these inferences, the FAO (2004) recommends developing sustainable pest control strategies in animal production systems, from an environmental perspective and effective from an animal health approach. This way, the use of medicinal plant extracts for parasite control, based on ethnopharmacological reports, is a widely used scientific practice today that has demonstrated good results in integrated pest management (Jaramillo-Hernández *et al.*, 2020; Aponte-Forero *et al.*, 2024). Furthermore, these extracts are very convenient for production systems, because they are environmentally sustainable due to their short biodegradation time in the soil (Pereira *et al.*, 2025). Although vaccination exists as a fundamental pillar of preventive plans for *R. microplus* infestation in cattle, its commercialization and use are highly limited in Colombia (Jaramillo-Hernández, 2022).

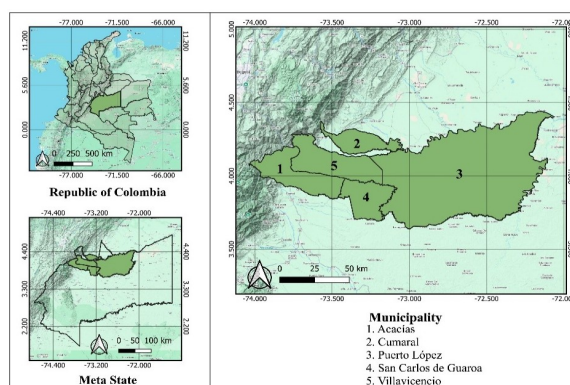
The most studied plants with acaricidal and repellent potential against various tick species belong to the Asteraceae, Euphorbiaceae, and Solanaceae families (Bisht *et al.*, 2025). Among the groups of secondary metabolites isolated from plant extracts with acaricidal potential, coumarins stand out. These compounds are distinguished for inhibiting serine proteases involved in coagulation, such as trypsin and chymotrypsin, which could compromise the parasite's hemoglobin digestion (Estrela *et al.*, 2007; 2010). Terpenoids could also play a significant role as acaricides against *R. microplus*, thanks to their biocidal properties, potential repellent effects, and mechanisms such as acetylcholinesterase inhibition. In fact, compounds such as thymol, carvacrol, p-cymene, and citral have exhibited acaricidal activity in both susceptible and resistant strains (dos Santos Cardoso *et al.*, 2020). Taken together, this evidence supports the idea that secondary metabolites such as coumarins and terpenoids have convergent mechanisms of action by interfering with hemoglobin digestion or the reproductive physiology of the parasite, thus supporting their potential as sustainable alternatives to synthetic acaricides.

In the state of Meta (Colombia), experimental validation, both *in vitro* and *in situ*, of plant extracts with

acaricidal activity and effects on the reproductive biology of *R. microplus* remains limited. To date, only one study conducted in the city of Villavicencio has been documented (Jaramillo-Hernández *et al.*, 2020). This represents a significant knowledge gap and highlights the need for further research to scientifically support the traditional use of medicinal plants with potential acaricidal properties in the region. Therefore, the present study aimed to validate ethnopharmacological reports on the use of medicinal plants in the state of Meta (Colombia) for their acaricidal activity against engorged adult females (teleogines) and larvae of *R. microplus*, as well as to identify the secondary metabolite groups present in these plant extracts.

## MATERIALS AND METHODS

**Collection, identification of plants, and preparation of plant extracts:** Fifty (50) cattle production systems were randomly selected from five (05) municipalities in the state of Meta, Colombia (Fig. 1). The database of the Colombian Agricultural Institute (ICA, 2024) was used to identify the cattle production systems registered in each selected municipality. Agricultural workers at these sites completed an ethnopharmacological survey validated by Gallegos-Zurita *et al.* (2016). Most of the medicinal plants were collected *in situ* and taxonomically classified at the National Herbarium of Colombia (COL); the remaining plants were purchased at local markets according to the instructions of agricultural workers. Their botanical identity was verified using taxonomic keys, literature, and the database of the National Herbarium of Colombia, without depositing specimens (N/A) (Table 1).



**Fig. 1:** Municipalities in the state of Meta where surveys were conducted in the cattle livestock.

Five kilograms (5kg) of fresh plant material were collected in accordance with the part used as indicated in the surveys (Table 1). Extraction was performed according to Jaramillo-Hernández *et al.* (2020), using 96% ethanol (Sigma-Aldrich, St. Louis, MO, USA), and the yield percentages for each ethanolic extract were determined. The resulting extract was reconstituted in distilled water/Tween-80 (2%) at concentrations of 10, 20, 40, 80, and 160mg/mL (Jaramillo-Hernández *et al.*, 2020; Silva *et al.*, 2009).

**Table 1:** Botanical data for the plants used in the present study.

Botanical name	Popular name	Family	Part of plant	Mode of preparation	Method of application on the animal	Voucher specimen	Ethanol extraction yield (%)
<i>Allium sativum</i>	Ajo	Amaryllidaceae	Fruit	Molienda	Dietary inclusion	N/A	8.5
<i>Annona muricata</i>	Guanabana	Annonaceae	Leaves	Decoction	Spray bath	COL632419	0.12
<i>Azadirachta indica</i>	Neem	Meliaceae	Leaves	Decoction	Spray bath	COL632459	5.8
<i>Capsicum annuum</i>	Aji	Solanaceae	Fruit	Maceration	Spray bath	N/A	9.12
<i>Carica papaya</i>	Papaya	Caricaceae	Leaves/Seeds	Decoction/ Maceration	Spray bath	COL632465	6.1/3.45
<i>Curcubita maxima</i>	Ahuyama	Cucurbitaceae	Seeds	Maceration	Spray bath	N/A	2.35
<i>Diolea virgata</i>	Kudzu	Fabaceae	Leaves	Decoction	Spray bath	COL632464	5.34
<i>Eugenia stipitata</i>	Arazá	Myrtaceae	Seeds	Maceration	Spray bath	N/A	0.04
<i>Gliricidia sepium</i>	Matarratón	Fabaceae	Leaves and Branch	Decoction	Spray bath	COL576461	10.27
<i>Guazuma ulmifolia</i>	Guásimo	Malvaceae	Leaves and bark	Decoction	Spray bath	N/A	5.11
<i>Ipomea quamoclit</i>	Enredadera	Convolvulaceae	Leaves and Branch	Decoction	Spray bath	COL632462	0.04
<i>Jacaranda obtusifolia</i>	Gualanday	Bignoniaceae	Leaves	Decoction	Spray bath	COL632420	8.5
<i>Lantana cujabensis</i>	Lantana	Verbenaceae	Leaves and Branch	Maceration	Spray bath	COL632461	2.26
<i>Miconia dolichorrhyncha</i>	Barratón	Melastomataceae	Leaves	Decoction	Spray bath	COL632421	8.53
<i>Morinda citrifolia</i>	Noni	Rubiaceae	Fruit	Maceration	Spray bath	N/A	7.6
<i>Trichanthera gigantea</i>	Cajeto	Acanthaceae	Leaves	Decoction	Spray bath	COL632463	0.13
<i>Urera baccifera</i>	Ortiga	Urticaceae	Leaves	Decoction	Spray bath	COL632418	1.52

COL: Initial de identification code of the National Herbarium of Colombia. N/A: Not applicable.

### Ticks, adult immersion tests and larval immersion tests:

Teleogines of *R. microplus* were collected from naturally infested cattle. According to official records from 2025, 295472 cattle were reported in the selected municipalities (ICA, 2025), of which approximately 7.9% correspond to dairy systems (FEDEGAN, 2024), with an estimated population of 23342 animals. Considering a prevalence of *R. microplus* ticks of 79.3% (Valencia *et al.*, 2017), a sample size of 250 cattle was calculated with a 95% confidence level using OpenEpi software. In each cattle production system, between 5 and 6 animals were sampled, collecting between 15 and 20 teleogines from each bovine. This research was endorsed by the Bioethics Committee of the University of the Llanos, according to Act 006 by consensus on November 24, 2022, and was carried out in accordance with Law 1774 of 2016 on Animal Protection in Colombia. The tick species was confirmed by stereoscopy using the taxonomic keys of Walker *et al.* (2003). They were then placed in Petri dishes for teleogines –adult- immersion tests (AIT), whereas others were incubated at 27-28°C and 85-95% RH for oviposition and hatching of larvae, which were then used for larval immersion tests (LIT).

The AIT was performed according to Drummond *et al.* (1973), modified by Jaramillo-Hernández *et al.* (2020). Each replicate (three per concentration and control) consisted of a group of 10 teleogines of uniform weight, immersed for 10min in 10mL of the extract solution at each of the evaluated concentrations (10, 20, 40, 80, 160mg/mL), a negative control (distilled water/Tween-80 2%), and a positive control (Cypermethrin 0.015%, AGROZ®, AGROZ S.A., Colombia). The teleogines were then placed in Petri dishes and incubated under the conditions described above. The number of dead ticks was determined by stereoscopy after 24h, 7 days, and 14 days post-treatment. After 14 days of post-immersion, the eggs laid on the slides were collected and weighed. Subsequently, 100 eggs from each experimental unit were placed in glass vials sealed with a gauze stopper and incubated for 21 days until hatching. Post-hatching larval mortality was also assessed over a 6h period. The visual estimation technique for mortality and hatching rate was performed by a single observer (Drummond *et al.*, 1973), who was unaware of the treatment of each Petri dish or

vial to avoid biased estimation. The following formulas were used with the data obtained to calculate the oviposition index (OI), the percentage of oviposition inhibition (RO%), the percentage of larval hatching (E%), the reproductive efficiency (REI), and the reproductive control (EP%):

$$IO = \frac{\text{Weight of oviposited eggs (g)}}{\text{Initial weight of the teleogines}}$$

$$IO\% = \frac{IO \text{ Negative control} - IO \text{ extracts}}{IO \text{ Negative control}} \times 100$$

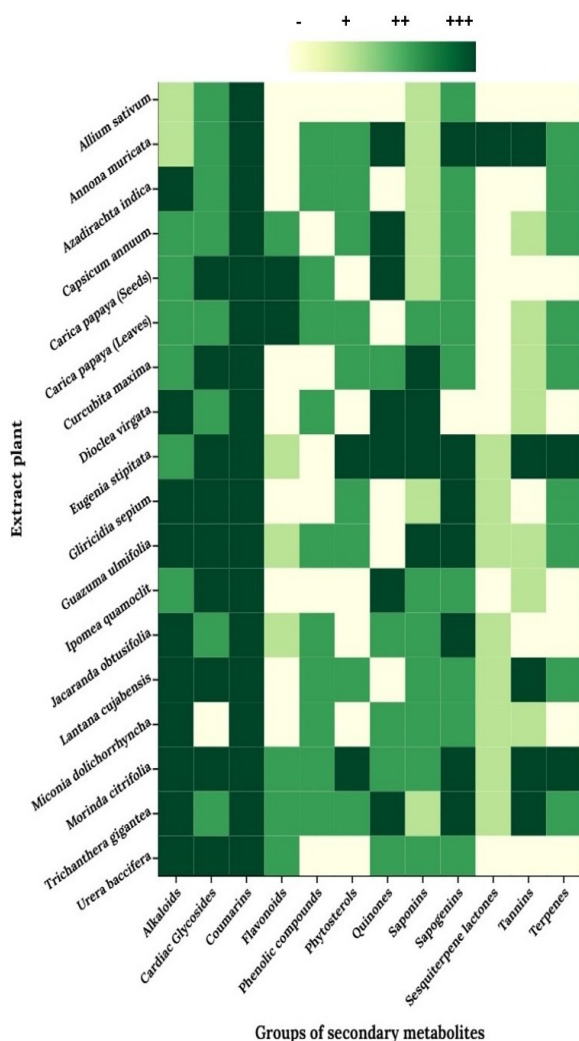
$$ER = IO \times E\%$$

$$CR\% = \frac{ER \text{ Negative control} - ER \text{ extracts}}{ER \text{ Negative control}} \times 100$$

LIT was performed according to Shaw (1966) modified by Jaramillo-Hernández *et al.* (2020). Approximately 100 fourteen-day-old larvae were used for each concentration of the extract and controls described in the AIT test. The larvae were immersed for 10min, and the number of dead larvae per treatment and control was determined after 24h of incubation, with three replicates. Larvae were considered dead if they were immobile, exhibited ataxia, or showed appendicular movements only upon stereoscopic examination. The percentage of mortality was calculated according to the FAO “Plan Protection Bulletin” recommendations (1971):

$$\% \text{ mortality} = \frac{\text{number of dead larvae}}{\text{Total larvae}} \times 100$$

**Phytochemical analysis:** A preliminary phytochemical analysis of the collected plant parts was performed (Table 1) following the methodology proposed by Sanabria (1983) and Maheshwaran *et al.* (2024) using colorimetric methods in order to determine the presence or absence of secondary metabolites groups (alkaloids, phenolic compounds, coumarins, cardiogenic glycosides, flavonoids, phytosterols, sesquiterpene lactones, quinones, saponins, saponinins, tannins, and terpenes) (Fig. 2).



**Fig. 2:** Preliminary phytochemical analysis of ethanolic plant extracts tested against *Rhipicephalus microplus*. Color intensity represents the presence of secondary metabolite groups detected by colorimetric assays (- = not detected; += low presence; ++= moderate presence; +++= high presence).

**Table 2:** Mortality (%) of *Rhipicephalus microplus* telegines exposed to different concentrations of ethanolic plant extracts.

Treatment	Concentration (mg/mL)				
	10	20	40	80	160
Tween-80 2% <sup>(-)</sup>	6.67± 5.77 <sup>a</sup>	6.67± 5.77 <sup>a</sup>	6.67± 5.77 <sup>a</sup>	6.67± 5.77 <sup>a</sup>	6.67± 5.77 <sup>a</sup>
Cypermethrin 0.015% <sup>(+)</sup>	76.67±5.77 <sup>b</sup>	76.67±5.77 <sup>b</sup>	76.67±5.77 <sup>b</sup>	76.67±5.77 <sup>b</sup>	76.67±5.77 <sup>b</sup>
<i>Allium sativum</i>	43.33±5.77 <sup>c</sup>	46.67±5.77 <sup>c</sup>	60.00±0.00 <sup>c</sup>	63.33±5.77 <sup>b</sup>	66.67±5.77 <sup>b</sup>
<i>Annona muricata</i>	26.67±5.77 <sup>c</sup>	56.67±5.77 <sup>c</sup>	66.67±5.77 <sup>b</sup>	76.67±5.77 <sup>b</sup>	100.0±0.0 <sup>c</sup>
<i>Azadirachta indica</i>	33.33±5.77 <sup>c</sup>	40.0±0.0 <sup>c</sup>	56.67±5.77 <sup>c</sup>	70.0±0.0 <sup>b</sup>	76.67±5.77 <sup>b</sup>
<i>Capsicum annuum</i>	13.33±5.77 <sup>a</sup>	33.33±5.77 <sup>c</sup>	53.33±5.77 <sup>c</sup>	60.0±0.0 <sup>c</sup>	76.67±5.77 <sup>b</sup>
<i>Carica papaya S.</i>	33.33±11.55 <sup>c</sup>	36.67±5.77 <sup>c</sup>	53.33±5.77 <sup>c</sup>	63.33±5.77 <sup>b</sup>	76.67±11.55 <sup>b</sup>
<i>Carica papaya L.</i>	16.67±5.77 <sup>a</sup>	23.33±5.77 <sup>c</sup>	26.67±5.77 <sup>c</sup>	33.33±5.77 <sup>c</sup>	43.33±5.77 <sup>c</sup>
<i>Cucurbita maxima</i>	20.0±0.0 <sup>c</sup>	33.33±5.77 <sup>c</sup>	40.0±0.0 <sup>c</sup>	100.0±0.0 <sup>c</sup>	100.00±0.00 <sup>c</sup>
<i>Dioclea virgata</i>	13.33±5.77 <sup>a</sup>	20.0±0.0 <sup>c</sup>	50.0±0.0 <sup>c</sup>	63.33±5.77 <sup>b</sup>	76.67±11.55 <sup>b</sup>
<i>Eugenia stipitata</i>	36.67±5.77 <sup>c</sup>	40.0±0.0 <sup>c</sup>	53.33±5.77 <sup>c</sup>	73.33±5.77 <sup>b</sup>	100.00±0.00 <sup>c</sup>
<i>Gliricidia sepium</i>	10.0±0.0 <sup>a</sup>	10.0±0.0 <sup>a</sup>	10.0±0.0 <sup>a</sup>	20.0±0.0 <sup>c</sup>	26.67±5.77 <sup>c</sup>
<i>Guazuma ulmifolia</i>	10.0±0.0 <sup>a</sup>	16.67±5.77 <sup>a</sup>	23.33±5.77 <sup>c</sup>	23.33±5.77 <sup>c</sup>	40±10.0 <sup>c</sup>
<i>Ipomea quamoclit</i>	30.0±0.0 <sup>c</sup>	50.0±0.0 <sup>c</sup>	63.33±5.77 <sup>b</sup>	63.33±5.77 <sup>b</sup>	76.67±5.77 <sup>b</sup>
<i>Jacaranda obtusifolia</i>	16.67±5.77 <sup>a</sup>	20.0±0.0 <sup>c</sup>	33.33±5.77 <sup>c</sup>	50.0±0.0 <sup>c</sup>	63.33±5.77 <sup>b</sup>
<i>Lantana cujabensis</i>	36.67±5.77 <sup>c</sup>	40.0±0.0 <sup>c</sup>	43.33±5.77 <sup>c</sup>	53.33±5.77 <sup>c</sup>	60.0±0.0 <sup>c</sup>
<i>Miconia dolichorrhyncha</i>	23.33±5.77 <sup>c</sup>	50.0±0.0 <sup>c</sup>	53.33±5.77 <sup>c</sup>	70.0±0.0 <sup>b</sup>	100.0±0.0 <sup>c</sup>
<i>Morinda citrifolia</i>	10.0±0.0 <sup>a</sup>	23.33±5.77 <sup>c</sup>	30.0±0.0 <sup>c</sup>	36.67±5.77 <sup>c</sup>	43.33±5.77 <sup>c</sup>
<i>Trichanthera gigantea</i>	23.33±5.77 <sup>c</sup>	50.0±0.0 <sup>c</sup>	73.33±5.77 <sup>b</sup>	80.0±0.0 <sup>b</sup>	100.00±0.00 <sup>c</sup>
<i>Urera baccifera</i>	0.0±0.0 <sup>a</sup>	10.0±0.0 <sup>a</sup>	23.33±5.77 <sup>c</sup>	33.33±5.77 <sup>c</sup>	33.33±5.77 <sup>c</sup>

Means within a column followed by different letters are significantly different (ANOVA, HSD Tukey; P<0.05). \*: Significant differences greater than the positive control (P<0.05). (-): Negative control. (+): Positive control.

**Statistical analysis:** The mortality rates of telegines and larvae, as well as reproductive parameters of telegines, were analyzed using ANOVA, followed by a post-hoc analysis with Tukey's test for multiple comparisons of means. Proportional data were transformed to arcsine-square root before analysis, and data were presented as percentages for presentation purposes in the study. Additionally, a probit analysis was performed to calculate LC<sub>50</sub> and LC<sub>90</sub> with a 95% confidence level (P<0.05). The data were organized and analyzed using SPSS Statistics version 25 (IBM Corporation, Chicago, IL, USA).

## RESULTS

Seventeen plants indicated in the surveys were collected, and eighteen extracts were prepared (*Carica papaya* was the subject of two extracts, one from its seeds [S] and the other from its leaves [L]). It was observed that, of the plants used in the different productions, 76.5% used the aerial parts of the plants (leaves), 63.2% used the decoction to prepare the extract, and 94.7% of those extracts were applied by spraying on cattle for tick control. The percentage yields of plant ethanolic extract are shown in Table 1.

The acaricidal activity as observed by AIT test is shown in Table 2. Four extracts (*Annona muricata*, *Cucurbita maxima*, *Eugenia stipitata* and *Miconia dolichorrhyncha*) showed 100% mortality at a concentration of 160mg/mL. One extract (*Cucurbita maxima*) showed 100% mortality at a concentration of 80mg/mL, which was statistically significant compared to the positive control (P<0.05). Seven extracts at 160mg/mL and nine extracts at 80mg/mL showed activity similar to that of the positive control (P>0.05). In the LIT test (Table 3), it was observed that, at a concentration of 160mg/mL, two extracts (*Carica papaya* seeds and *Dioclea virgata*) had higher mortality rates than the positive control (P<0.05). Three extracts (*Azadirachta indica*, *Cucurbita maxima* and *Eugenia stipitata*) showed acaricidal activity similar to that of the positive control (P>0.05).

The effect of the plant extracts on the reproductive parameters measured in teleogines have been depicted in Table 4. Eight extracts showed a reduction in the oviposition index (RO%), similar to the positive control (P>0.05), and four extracts inhibited oviposition (100%) at concentrations of 80 to 160mg/mL (P<0.05). Regarding the reproductive efficiency index (REI), three extracts at 80mg/mL and two extracts at 160mg/mL showed a reproductive efficiency of 0%, demonstrating complete inhibition of egg hatching (P<0.05). Regarding the efficacy of the ethanolic extracts of the different plants (EP%), it ranged from 2.76 to 100%, depending on the concentrations evaluated. Nine extracts achieved efficacy greater than 90% EP. On the other hand, with respect to the extracts of *Azadirachta indica*, *Capsicum annum*, and *Miconia*

*dolichorrhyncha*, early larval mortalities were observed less than six hours post-hatching (P<0.05) (Table 5).

In the probit analysis for teleogines, the best LC<sub>50</sub> and LC<sub>90</sub> values were recorded for *Annona muricata* and *Cucurbita maxima* (Table 6), and for larvae, they were *Dioclea virgata* and *Carica papaya* (seeds); the ethanol extract of *Lantana cujabensis* was not included in the Probit analysis, as it did not cause mortality at any of the concentrations evaluated (Table 7). According to the phytochemical analysis by colorimetry of the evaluated secondary metabolites groups, all plants contained coumarins. The other metabolites varied among the extracts, with alkaloids, coumarins, cardiotonic glycosides, and sapogenins being the most prevalent in the different plant extracts (Fig. 2).

**Table 3:** Mortality (%) of *Rhipicephalus microplus* larvae exposed to different concentrations of ethanolic plant extracts.

Treatment	Concentration (mg/mL)				
	10	20	40	80	160
Tween-80 2% (-)	0.0±0.0 <sup>a</sup>	0.0±0.0 <sup>a</sup>	0.0±0.0 <sup>a</sup>	0.0±0.0 <sup>a</sup>	0.0±0.0 <sup>a</sup>
Cypermethrin 0.015% (+)	85.94±5.73 <sup>b</sup>	85.94±5.73 <sup>b</sup>	85.94±5.73 <sup>b</sup>	85.94±5.73 <sup>b</sup>	85.94±5.73 <sup>b</sup>
<i>Allium sativum</i>	10.44±1.62 <sup>c</sup>	11.39±1.43 <sup>c</sup>	12.60±1.65 <sup>c</sup>	15.58±1.52 <sup>c</sup>	20.65±0.82 <sup>c</sup>
<i>Annona muricata</i>	5.94±1.20 <sup>a</sup>	16.17±1.06 <sup>c</sup>	30.47±2.04 <sup>c</sup>	43.30±3.52 <sup>c</sup>	62.71±2.81 <sup>c</sup>
<i>Azadirachta indica</i>	3.21±0.35 <sup>a</sup>	17.06±3.68 <sup>c</sup>	35.01±4.00 <sup>c</sup>	66.06±3.25 <sup>c</sup>	84.19±3.30 <sup>b</sup>
<i>Capsicum annum</i>	1.96±0.66 <sup>a</sup>	4.54±1.52 <sup>a</sup>	5.87±2.99 <sup>a</sup>	8.71±3.59 <sup>a</sup>	9.19±3.36 <sup>a</sup>
<i>Carica papaya S.</i>	13.39±2.90 <sup>c</sup>	23.85±2.93 <sup>c</sup>	32.29±5.71 <sup>c</sup>	73.90±7.28 <sup>b</sup>	97.31±1.56 <sup>c</sup>
<i>Carica papaya L.</i>	9.88±1.97 <sup>c</sup>	18.37±3.2 <sup>c</sup>	24.91±5.82 <sup>c</sup>	31.06±1.62 <sup>c</sup>	43.20±5.89 <sup>c</sup>
<i>Curcubita maxima</i>	21.40±0.36 <sup>c</sup>	30.55±9.94 <sup>c</sup>	45.56±2.01 <sup>c</sup>	60.07±8.73 <sup>c</sup>	71.99±8.52 <sup>b</sup>
<i>Dioclea virgata</i>	20.10±1.42 <sup>c</sup>	44.85±6.63 <sup>c</sup>	58.57±5.18 <sup>c</sup>	80.96±2.18 <sup>b</sup>	97.96±0.08 <sup>c</sup>
<i>Eugenia stipitata</i>	6.98±3.91 <sup>a</sup>	14.59±1.47 <sup>c</sup>	26.09±4.44 <sup>c</sup>	58.47±9.86 <sup>c</sup>	92.63±2.28 <sup>b</sup>
<i>Gliricidia sepium</i>	4.30±0.81 <sup>a</sup>	11.75±1.99 <sup>c</sup>	26.94±4.75 <sup>c</sup>	39.66±4.82 <sup>c</sup>	44.32±3.99 <sup>c</sup>
<i>Guazuma ulmifolia</i>	0.60±1.03 <sup>a</sup>	4.33±0.20 <sup>a</sup>	7.35±0.64 <sup>a</sup>	10.45±3.16 <sup>c</sup>	43.07±3.57 <sup>c</sup>
<i>Ipomea quamoclit</i>	9.90±2.65 <sup>c</sup>	12.08±0.44 <sup>c</sup>	21.66±1.52 <sup>c</sup>	25.60±6.18 <sup>c</sup>	57.78±7.00 <sup>c</sup>
<i>Jacaranda obtusifolia</i>	5.85±0.26 <sup>a</sup>	8.55±1.39 <sup>c</sup>	9.33±0.21 <sup>c</sup>	10.72±0.38 <sup>c</sup>	15.02±0.27 <sup>c</sup>
<i>Lantana cujabensis</i>	0.0 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>a</sup>
<i>Miconia dolichorrhyncha</i>	2.07±1.14 <sup>a</sup>	3.34±1.75 <sup>a</sup>	6.10±0.48 <sup>a</sup>	6.80±0.61 <sup>a</sup>	8.95±0.97 <sup>c</sup>
<i>Morinda citrifolia</i>	11.34±0.38 <sup>c</sup>	15.29±2.96 <sup>c</sup>	18.13±5.24 <sup>c</sup>	35.03±11.69 <sup>c</sup>	55.62±4.91 <sup>c</sup>
<i>Trichanthera gigantea</i>	22.99±6.84 <sup>c</sup>	25.75±3.76 <sup>c</sup>	41.03±6.86 <sup>c</sup>	45.34±3.69 <sup>c</sup>	71.39±1.94 <sup>c</sup>
<i>Urera baccifera</i>	5.62 ± 4.11 <sup>a</sup>	8.95 ± 2.55 <sup>a</sup>	12.20 ± 0.26 <sup>c</sup>	23.06 ± 3.84 <sup>c</sup>	24.67 ± 4.32 <sup>c</sup>

Means within a column followed by different letters are significantly different (HSD Tukey: P<0.05). \*: Significant differences greater than the positive control (P<0.05). (-): Negative control. (+): Positive control.

**Table 4:** Effect of ethanolic plant extracts on egg production index (EPI%), reduction in oviposition (RO%), reproduction efficiency index (REI) and efficacy of the extracted product (EP%) in engorged females (teleogines) of *Rhipicephalus microplus*.

Treatment	Concentration (mg/mL)	EPI	RO (%)	REI	EP (%)
Tween-80 2% (-)	20	0.520±0.03 <sup>a</sup>	N/A	52.04± 3.23 <sup>a</sup>	N/A
Cypermethrin 0.015% (+)	0.15	0.102±0.05 <sup>b</sup>	80.32±9.19 <sup>a</sup>	5.90±3.11 <sup>b</sup>	88.67±5.98 <sup>a</sup>
<i>Allium sativum</i>	10	0.314±0.03 <sup>c</sup>	39.63±5.36 <sup>b</sup>	29.85±2.65 <sup>c</sup>	42.65±5.09 <sup>b</sup>
	20	0.309±0.0 <sup>c</sup>	40.55±0.73 <sup>b</sup>	27.84±0.34 <sup>c</sup>	46.50±0.66 <sup>b</sup>
	40	0.247±0.01 <sup>c</sup>	52.49±2.00 <sup>b</sup>	19.78±0.83 <sup>c</sup>	62.0±1.60 <sup>b</sup>
	80	0.216±0.01 <sup>c</sup>	58.44±2.35 <sup>b</sup>	16.22±0.92 <sup>c</sup>	68.83±1.76 <sup>b</sup>
	160	0.193±0.0 <sup>c</sup>	62.99±0.75 <sup>b</sup>	13.48±0.27 <sup>c</sup>	74.09±0.53 <sup>b</sup>
<i>Annona muricata</i>	10	0.224±0.0 <sup>c</sup>	57.03±0.70 <sup>b</sup>	21.24±0.35 <sup>c</sup>	59.18±0.66 <sup>b</sup>
	20	0.138±0.03 <sup>b</sup>	73.51±6.28 <sup>a</sup>	11.03±2.61 <sup>b</sup>	78.81±5.02 <sup>a</sup>
	40	0.069±0.02 <sup>b</sup>	86.68±2.92 <sup>a</sup>	1.39±0.30 <sup>b</sup>	97.34±0.58 <sup>a</sup>
	80	0.034±0.0 <sup>b</sup>	93.44±0.63 <sup>a</sup>	0.0 ± 0.0 <sup>b</sup>	100.0±0.0 <sup>b</sup>
	160	0.0±0.0 <sup>a</sup>	100.0±0.0 <sup>a</sup>	0.0 ± 0.0 <sup>b</sup>	100.0±0.0 <sup>b</sup>
<i>Azadirachta indica</i>	10	0.187±0.01 <sup>b</sup>	64.06±2.45 <sup>b</sup>	16.83±1.15 <sup>c</sup>	67.65±2.21 <sup>b</sup>
	20	0.179±0.01 <sup>b</sup>	65.57±1.05 <sup>b</sup>	10.75±0.33 <sup>b</sup>	79.34±0.63 <sup>a</sup>
	40	0.141±0.01 <sup>b</sup>	72.82±2.83 <sup>a</sup>	5.66±0.59 <sup>b</sup>	89.13±1.13 <sup>a</sup>
	80	0.073±0.01 <sup>b</sup>	86.04±1.18 <sup>a</sup>	2.18±0.18 <sup>b</sup>	95.81±0.35 <sup>a</sup>
	160	0.067±0.01 <sup>b</sup>	87.05±1.25 <sup>a</sup>	0.67±0.07 <sup>b</sup>	98.70±0.13 <sup>b</sup>
<i>Capsicum annum</i>	10	0.226±0.01 <sup>c</sup>	56.49±1.61 <sup>b</sup>	21.51±0.80 <sup>c</sup>	58.67±1.53 <sup>b</sup>
	20	0.211±0.03 <sup>c</sup>	59.38±4.91 <sup>b</sup>	19.02±2.30 <sup>c</sup>	63.45±4.42 <sup>b</sup>
	40	0.172±0.01 <sup>b</sup>	66.96±1.54 <sup>a</sup>	10.32±0.48 <sup>b</sup>	80.17±0.92 <sup>b</sup>
	80	0.119±0.01 <sup>b</sup>	77.12±1.22 <sup>a</sup>	5.95±0.32 <sup>b</sup>	88.56±0.61 <sup>a</sup>
	160	0.029±0.01 <sup>b</sup>	94.34±1.15 <sup>b</sup>	0.88±0.18 <sup>b</sup>	98.30±0.35 <sup>b</sup>
<i>Carica papaya S.</i>	10	0.188±0.01 <sup>c</sup>	63.86±1.68 <sup>b</sup>	17.87±0.83 <sup>c</sup>	65.67±1.60 <sup>b</sup>
	20	0.165±0.02 <sup>b</sup>	68.30±2.88 <sup>a</sup>	14.85±1.35 <sup>c</sup>	71.47±2.59 <sup>b</sup>
	40	0.143±0.0 <sup>b</sup>	72.51±0.77 <sup>a</sup>	10.73±0.30 <sup>b</sup>	79.38±0.58 <sup>b</sup>
	80	0.109±0.02 <sup>b</sup>	79.12±3.77 <sup>a</sup>	7.61±1.37 <sup>b</sup>	85.38±2.64 <sup>a</sup>
	160	0.041±0.0 <sup>b</sup>	92.09±0.95 <sup>a</sup>	2.06±0.25 <sup>b</sup>	96.05±0.47 <sup>a</sup>
<i>Carica papaya L.</i>	10	0.363±0.01 <sup>c</sup>	30.28±1.43 <sup>b</sup>	34.47±0.71 <sup>c</sup>	33.77±1.36 <sup>b</sup>
	20	0.360±0.01 <sup>c</sup>	30.85±2.10 <sup>b</sup>	32.39±0.98 <sup>c</sup>	37.76±1.89 <sup>b</sup>
	40	0.354±0.01 <sup>c</sup>	31.99±1.31 <sup>b</sup>	28.32±0.55 <sup>c</sup>	45.59±1.05 <sup>b</sup>
	80	0.227±0.03 <sup>c</sup>	56.37±5.38 <sup>b</sup>	18.17±2.24 <sup>c</sup>	65.09±4.31 <sup>b</sup>
	160	0.219±0.01 <sup>c</sup>	57.93±2.61 <sup>b</sup>	17.52±1.09 <sup>c</sup>	66.34±2.09 <sup>b</sup>
<i>Curcubita maxima</i>	10	0.197±0.01 <sup>c</sup>	62.08±1.54 <sup>b</sup>	15.79±0.64 <sup>c</sup>	69.66±1.23 <sup>b</sup>
	20	0.140±0.01 <sup>b</sup>	73.19±0.97 <sup>a</sup>	5.58±0.20 <sup>b</sup>	89.28±0.39 <sup>b</sup>

	40	0.127±0.01 <sup>b</sup>	75.55±1.32 <sup>a</sup>	2.54±0.14 <sup>b</sup>	95.11±0.26 <sup>b</sup>
	80	0.0±0.0 <sup>ce</sup>	100.0±0.0 <sup>be</sup>	0.0±0.0 <sup>b</sup>	100.0±0.0 <sup>be</sup>
	160	0.0±0.0 <sup>ce</sup>	100.0±0.0 <sup>be</sup>	0.0±0.0 <sup>b</sup>	100.0±0.0 <sup>be</sup>
<i>Dioclea virgata</i>	10	0.256±0.0 <sup>c</sup>	50.76±0.25 <sup>b</sup>	23.06±0.12 <sup>c</sup>	55.69±0.22 <sup>b</sup>
	20	0.173±0.01 <sup>b</sup>	66.73±2.42 <sup>a</sup>	13.85±1.01 <sup>c</sup>	73.38±1.94 <sup>b</sup>
	40	0.109±0.0 <sup>b</sup>	79.13±0.62 <sup>a</sup>	7.60±0.23 <sup>b</sup>	85.39±0.43 <sup>a</sup>
	80	0.080±0.01 <sup>b</sup>	79.89±8.30 <sup>a</sup>	4.81±0.63 <sup>b</sup>	90.76±1.21 <sup>a</sup>
	160	0.040±0.0 <sup>b</sup>	92.34±0.95 <sup>a</sup>	1.20±0.15 <sup>b</sup>	97.70±0.28 <sup>bc</sup>
<i>Eugenia stipitata</i>	10	0.254±0.01 <sup>c</sup>	51.23±1.12 <sup>b</sup>	22.84±0.53 <sup>c</sup>	56.11±1.01 <sup>b</sup>
	20	0.203±0.01 <sup>c</sup>	60.97±2.77 <sup>b</sup>	16.25±1.15 <sup>c</sup>	68.77±2.22 <sup>b</sup>
	40	0.167±0.01 <sup>b</sup>	67.88±2.70 <sup>a</sup>	11.70±0.98 <sup>b</sup>	77.52±1.89 <sup>b</sup>
	80	0.165±0.01 <sup>b</sup>	68.31±2.28 <sup>a</sup>	10.72±0.77 <sup>b</sup>	79.40±1.48 <sup>a</sup>
	160	0.0±0.0 <sup>ce</sup>	100.0±0.0 <sup>be</sup>	0.0±0.0 <sup>b</sup>	100.0±0.0 <sup>be</sup>
<i>Gliricidia sepium</i>	10	0.516±0.03 <sup>a</sup>	0.77±4.89 <sup>b</sup>	50.61±2.49 <sup>a</sup>	2.76±4.79 <sup>b</sup>
	20	0.472±0.0 <sup>a</sup>	9.24±0.75 <sup>b</sup>	46.29±0.38 <sup>a</sup>	11.06±0.73 <sup>b</sup>
	40	0.385±0.02 <sup>c</sup>	26.07±4.03 <sup>b</sup>	37.70±2.05 <sup>c</sup>	27.55±3.95 <sup>b</sup>
	80	0.363±0.03 <sup>c</sup>	30.17±4.82 <sup>b</sup>	35.25±2.43 <sup>c</sup>	32.26±4.68 <sup>b</sup>
	160	0.321±0.0 <sup>c</sup>	38.40±0.86 <sup>b</sup>	30.46±0.42 <sup>c</sup>	41.48±0.82 <sup>b</sup>
<i>Guazuma ulmifolia</i>	10	0.474±0.01 <sup>a</sup>	8.95±2.85 <sup>b</sup>	46.91±1.47 <sup>a</sup>	9.86±2.82 <sup>b</sup>
	20	0.437±0.0 <sup>c</sup>	16.09±0.83 <sup>b</sup>	42.79±0.42 <sup>c</sup>	17.77±0.81 <sup>b</sup>
	40	0.344±0.02 <sup>c</sup>	33.96±4.45 <sup>b</sup>	33.34±2.25 <sup>c</sup>	35.94±4.32 <sup>b</sup>
	80	0.312±0.01 <sup>c</sup>	40.14±1.28 <sup>b</sup>	29.60±0.64 <sup>c</sup>	43.13±1.22 <sup>b</sup>
	160	0.282±0.01 <sup>c</sup>	45.78±1.42 <sup>b</sup>	25.40±0.66 <sup>c</sup>	51.20±1.27 <sup>b</sup>
<i>Ipomea quamoclit</i>	10	0.372±0.01 <sup>c</sup>	28.60±2.72 <sup>b</sup>	29.73±1.13 <sup>c</sup>	42.88±2.18 <sup>b</sup>
	20	0.313±0.02 <sup>c</sup>	39.91±3.71 <sup>b</sup>	23.45±1.45 <sup>c</sup>	54.93±2.78 <sup>b</sup>
	40	0.222±0.04 <sup>c</sup>	57.43±8.60 <sup>b</sup>	15.51±3.13 <sup>c</sup>	70.20±6.02 <sup>b</sup>
	80	0.121±0.02 <sup>b</sup>	76.72±3.99 <sup>a</sup>	7.27±1.25 <sup>b</sup>	86.03±2.39 <sup>a</sup>
	160	0.106±0.01 <sup>b</sup>	79.56±2.72 <sup>a</sup>	5.32±0.71 <sup>b</sup>	89.78±1.36 <sup>a</sup>
<i>Jacaranda obtusifolia</i>	10	0.446±0.0 <sup>a</sup>	14.28±0.56 <sup>b</sup>	43.72±0.29 <sup>c</sup>	16.0±0.55 <sup>b</sup>
	20	0.332±0.01 <sup>c</sup>	36.24±2.57 <sup>b</sup>	31.52±1.27 <sup>c</sup>	39.43±2.44 <sup>b</sup>
	40	0.328±0.01 <sup>c</sup>	36.98±2.74 <sup>b</sup>	31.16±1.35 <sup>c</sup>	40.14±2.60 <sup>b</sup>
	80	0.305±0.02 <sup>c</sup>	41.37±3.18 <sup>b</sup>	28.38±1.54 <sup>c</sup>	45.47±2.96 <sup>b</sup>
	160	0.197±0.02 <sup>c</sup>	62.10±3.86 <sup>b</sup>	17.75±1.81 <sup>c</sup>	65.89±3.47 <sup>b</sup>
<i>Lantana cujabensis</i>	10	0.304±0.01 <sup>c</sup>	41.64±2.40 <sup>b</sup>	27.34±1.12 <sup>c</sup>	47.48±2.16 <sup>b</sup>
	20	0.280±0.0 <sup>c</sup>	46.29±0.24 <sup>b</sup>	23.76±0.11 <sup>c</sup>	54.34±0.21 <sup>b</sup>
	40	0.211±0.02 <sup>c</sup>	59.44±3.45 <sup>b</sup>	16.89±1.43 <sup>c</sup>	67.55±2.76 <sup>b</sup>
	80	0.143±0.01 <sup>b</sup>	72.60±1.69 <sup>a</sup>	9.98±0.62 <sup>b</sup>	80.82±1.18 <sup>a</sup>
	160	0.116±0.01 <sup>b</sup>	77.62±2.14 <sup>a</sup>	6.99±0.67 <sup>b</sup>	86.57±1.28 <sup>a</sup>
<i>Miconia dolichorrhyncha</i>	10	0.188±0.01 <sup>c</sup>	63.83±2.23 <sup>b</sup>	16.94±1.04 <sup>c</sup>	67.45±2.0 <sup>b</sup>
	20	0.108±0.01 <sup>b</sup>	79.33±1.96 <sup>a</sup>	7.53±0.71 <sup>b</sup>	85.53±1.37 <sup>a</sup>
	40	0.094±0.0 <sup>b</sup>	81.94±0.72 <sup>a</sup>	4.70±0.19 <sup>b</sup>	90.97±0.36 <sup>a</sup>
	80	0.059±0.01 <sup>b</sup>	88.60±1.19 <sup>a</sup>	0.0±0.0 <sup>b</sup>	100.0±0.0 <sup>be</sup>
	160	0.0±0.0 <sup>ce</sup>	100.0±0.0 <sup>be</sup>	0.0±0.0 <sup>b</sup>	100.0±0.0 <sup>be</sup>
<i>Morinda citrifolia</i>	10	0.278±0.01 <sup>c</sup>	46.66±1.45 <sup>b</sup>	26.37±0.72 <sup>c</sup>	49.32±1.38 <sup>b</sup>
	20	0.270±0.02 <sup>c</sup>	48.05±4.19 <sup>b</sup>	24.33±1.96 <sup>c</sup>	53.25±3.77 <sup>b</sup>
	40	0.264±0.01 <sup>c</sup>	49.21±2.21 <sup>b</sup>	22.47±0.98 <sup>c</sup>	56.83±1.88 <sup>b</sup>
	80	0.178±0.01 <sup>b</sup>	65.79±2.32 <sup>b</sup>	12.46±0.85 <sup>b</sup>	76.06±1.63 <sup>b</sup>
	160	0.145±0.01 <sup>b</sup>	72.21±2.64 <sup>a</sup>	8.68±0.83 <sup>b</sup>	83.33±1.59 <sup>a</sup>
<i>Trichanthera gigantea</i>	10	0.396±0.01 <sup>c</sup>	23.97±1.46 <sup>b</sup>	35.61±0.68 <sup>c</sup>	31.57±1.31 <sup>b</sup>
	20	0.172±0.0 <sup>b</sup>	66.96±0.27 <sup>b</sup>	12.90±0.10 <sup>c</sup>	75.22±0.20 <sup>b</sup>
	40	0.124±0.02 <sup>b</sup>	76.22±3.79 <sup>a</sup>	8.66±1.38 <sup>b</sup>	83.35±2.65 <sup>a</sup>
	80	0.089±0.02 <sup>b</sup>	82.93±3.16 <sup>a</sup>	5.33±0.99 <sup>b</sup>	89.76±1.90 <sup>a</sup>
	160	0.034±0.01 <sup>b</sup>	93.47±1.05 <sup>a</sup>	0.0±0.0 <sup>b</sup>	100.0±0.0 <sup>be</sup>
<i>Urera baccifera</i>	10	0.471±0.0 <sup>a</sup>	9.57±0.80 <sup>b</sup>	46.12±0.41 <sup>a</sup>	11.38±0.79 <sup>b</sup>
	20	0.424±0.01 <sup>c</sup>	18.51±1.95 <sup>b</sup>	41.56±1.00 <sup>c</sup>	20.14±1.92 <sup>b</sup>
	40	0.321±0.01 <sup>c</sup>	38.38±2.21 <sup>b</sup>	31.43±1.13 <sup>c</sup>	39.61±2.17 <sup>b</sup>
	80	0.315±0.01 <sup>c</sup>	39.46±6.20 <sup>b</sup>	29.93±3.06 <sup>c</sup>	42.48±5.89 <sup>b</sup>
	160	0.310±0.01 <sup>c</sup>	40.52±1.50 <sup>b</sup>	29.41±0.74 <sup>c</sup>	43.49±1.42 <sup>b</sup>

Means within a column followed by different letters are significantly different (ANOVA, HSD Tukey: P<0.05). \*: Significant differences greater than the positive control (P<0.05). (-): Negative control. (+): Positive control.

**Table 5:** Mortality (%) of *Rhipicephalus microplus* larvae post-hatching (Concentration 160mg/mL).

Treatment	% Larval mortality post-hatching (<6 hours)
Tween-80 2% (+)	0.0±0.0 <sup>a</sup>
Cypermethrin 0.015% (+)	0.0±0.0 <sup>a</sup>
<i>Allium sativum</i>	0.0±0.0 <sup>a</sup>
<i>Annona muricata</i>	0.0±0.0 <sup>a</sup>
<i>Azadirachta indica</i>	33.33±5.77 <sup>b</sup>
<i>Capsicum annuum</i>	73.33±5.77 <sup>c</sup>
<i>Carica papaya S.</i>	0.0±0.0 <sup>a</sup>
<i>Carica papaya L.</i>	0.0±0.0 <sup>a</sup>
<i>Curcubita maxima</i>	0.0±0.0 <sup>a</sup>
<i>Dioclea virgata</i>	0.0±0.0 <sup>a</sup>
<i>Eugenia stipitata</i>	0.0±0.0 <sup>a</sup>
<i>Gliricidia sepium</i>	0.0±0.0 <sup>a</sup>
<i>Guazuma ulmifolia</i>	0.0±0.0 <sup>a</sup>
<i>Ipomea quamoclit</i>	0.0±0.0 <sup>a</sup>
<i>Jacaranda obtusifolia</i>	0.0±0.0 <sup>a</sup>
<i>Lantana cujabensis</i>	0.0±0.0 <sup>a</sup>
<i>Miconia dolichorrhyncha</i>	86.67±5.77 <sup>d</sup>
<i>Morinda citrifolia</i>	0.0±0.0 <sup>a</sup>
<i>Trichanthera gigantea</i>	0.0±0.0 <sup>a</sup>
<i>Urera baccifera</i>	0.0±0.0 <sup>a</sup>

Means within a column followed by different letters are significantly different (ANOVA, HSD Tukey: P<0.05). (-): Negative control. (+): Positive control.

**Table 6:** LC<sub>50</sub> and LC<sub>90</sub> values for engorged females of *Rhipicephalus microplus* exposed to ethanolic plant extracts.

Treatment	LC <sub>50</sub> (CI 95%) mg/mL	LC <sub>90</sub> (CI 95%) mg/mL	Slope±SE	X <sup>2</sup> (df)	R <sup>2</sup>
<i>Allium sativum</i>	20.68 (4.59-93.14)	4968.43 (1102.53-22389.59)	0.31±0.24	1.47 (13)	0.770
<i>Annona muricata</i>	20.74 (13.98-27.85)	106.04 (71.10-213.73)	1.80±0.30	5.99 (13)	0.861
<i>Azadirachta indica</i>	28.80 (15.04-46.26)	501.73 (198.70-6245.18)	1.03±0.26	1.26 (13)	0.934
<i>Capsicum annuum</i>	45.39 (32.72-64.47)	294.18 (166.04-880.60)	1.57±0.28	2.40 (13)	0.918
<i>Carica papaya S.</i>	33.70 (18.26-56.39)	656.62 (236.08-12167.94)	0.84±0.25	2.74 (13)	0.779
<i>Carica papaya L.</i>	330 (80-1320)	36520 (9320-1.43x10 <sup>5</sup> )	0.63±0.26	1.87 (13)	0.751
<i>Curcubita maxima</i>	28.27 (22.41-35.09)	84.72 (63.64-131.78)	2.69±0.37	15.89 (13)	0.874
<i>Dioclea virgata</i>	50.87 (37.12-72.75)	314.44 (177.56-932.65)	1.62±0.28	3.66 (13)	0.910
<i>Eugenia stipitata</i>	24.30 (16.11-33.36)	152.37 (94.78-369.03)	1.60±0.28	8.04 (13)	0.835
<i>Gliricidia sepium</i>	2680 (490-14600)	4.45 x10 <sup>5</sup> (81640-2.43 x10 <sup>6</sup> )	0.61±0.30	1.18 (13)	0.755
<i>Guazuma ulmifolia</i>	420 (130-1410)	20330 (6170-66900)	0.76±0.28	2.74 (13)	0.752
<i>Ipomea quamodit</i>	25.75 (11.41-42.94)	590.05 (209.57-13961.36)	0.94±0.25	2.24 (13)	0.867
<i>Jacaranda obtusifolia</i>	85.13 (55.23-180.16)	1072.82 (378.44-15060.54)	1.16±0.27	1.56 (13)	0.924
<i>Lantana cujabensis</i>	58.84 (11.95-289.77)	20010.94 (4063.17-98553.03)	0.50±0.24	1.03 (13)	0.808
<i>Miconia dolichorrhyncha</i>	26.61 (18.70-35.64)	141.65 (91.96-304.35)	1.76±0.29	7.29 (13)	0.872
<i>Morinda citrifolia</i>	210 (90-3870)	7680 (990-7.43x10 <sup>7</sup> )	0.73±0.27	1.68 (13)	0.864
<i>Trichanthera gigantea</i>	22.22 (14.57-30.41)	133.33 (84.88-305.78)	1.64±0.29	2.04 (13)	0.944
<i>Urera baccifera</i>	230 (120-1120)	2530 (670-1.16 x10 <sup>5</sup> )	1.24±0.32	4.69 (13)	0.757

CI 95%: 95% confidence intervals

**Table 7:** LC<sub>50</sub> and LC<sub>90</sub> values for *Rhipicephalus microplus* larvae exposed to ethanolic plant extracts.

Treatment	LC <sub>50</sub> (CI 95%) mg/mL	LC <sub>90</sub> (CI 95%) mg/mL	Slope±SE	X <sup>2</sup> (df)	R <sup>2</sup>
<i>Allium sativum</i>	6020 (3070-11830)	4.26x10 <sup>6</sup> (2.17x10 <sup>6</sup> -8.37 x10 <sup>6</sup> )	0.36±0.08	4.03 (13)	0.817
<i>Annona muricata</i>	96.19 (85.75-109.48)	686.07 (523.58-956.28)	1.50±0.08	5.18 (13)	0.984
<i>Azadirachta indica</i>	56.11 (52.62-59.94)	199.83 (177.38-229.03)	2.32±0.09	11.87 (13)	0.987
<i>Capsicum annuum</i>	3840 (2320-6350)	182440 (110280-301810)	0.52±0.10	20.19 (13)	0.647
<i>Carica papaya S.</i>	42.68 (35.17-52.03)	158.86 (116.79-251.70)	2.24±0.08	97.37 (13)	0.889
<i>Carica papaya L.</i>	250 (180-380)	7300 (3360-21950)	0.88±0.07	13.17 (13)	0.913
<i>Curcubita maxima</i>	51.42 (42.49-63.65)	694.90 (418.43-1461.03)	1.13±0.06	30.74 (13)	0.898
<i>Dioclea virgata</i>	26.80 (23.78-30.06)	116.78 (96.75-147.56)	2.00±0.08	25.32 (13)	0.954
<i>Eugenia stipitata</i>	57.25 (48.43-68.53)	193.70 (146.84-287.33)	2.42±0.09	74.98 (13)	0.920
<i>Gliricidia sepium</i>	160 (120-230)	1700 (900-4540)	1.23±0.07	36.08 (13)	0.928
<i>Guazuma ulmifolia</i>	250 (190-410)	1310 (700-3710)	1.80±0.12	36.84 (13)	0.843
<i>Ipomea quamodit</i>	160 (120-260)	1760 (850-5870)	1.23±0.08	41.26 (13)	0.846
<i>Jacaranda obtusifolia</i>	80650 (8990-1.54x10 <sup>7</sup> )	9.69x10 <sup>6</sup> (4.71x10 <sup>6</sup> -1.99x10 <sup>7</sup> )	0.40±0.08	2.47 (13)	0.914
<i>Miconia dolichorrhyncha</i>	20360 (4770-307890)	2.44x10 <sup>6</sup> (187860-3.04x10 <sup>6</sup> )	0.61±0.09	11.80 (13)	0.733
<i>Morinda citrifolia</i>	150 (110-240)	1820 (860-6100)	1.18±0.07	43.60 (13)	0.831
<i>Trichanthera gigantea</i>	70.90 (55.13-98.71)	1341 (625.76-4924.35)	1.00±0.07	39.33 (13)	0.860
<i>Urera baccifera</i>	1060 (520-3870)	46330 (9680-829800)	0.78±0.07	24.23 (13)	0.759

## DISCUSSION

The ethnopharmacological survey of promising acaricidal plants conducted in the present study allowed identifying and collecting seventeen plant species, adapted and traditionally used in small-scale cattle production in the state of Meta, Colombia. Of these, seven plants showed acaricidal activity *in vitro* against *R. microplus*, achieving mortalities  $\geq 90\%$  (Tables 2 and 3). This criterion was considered in accordance with the guidelines of the World Association for the Advancement of Veterinary Parasitology (Holdsworth *et al.*, 2022), which establishes that therapeutic efficacy against single host ticks should be at least 90%, given that, to date, there are no standardized guidelines for evaluating the *in vitro* efficacy of plant extracts for tick control (Adenubi *et al.*, 2018). This approach is consistent with previous studies that highlighted the importance of local knowledge for selecting species with acaricidal potential against *R. microplus* (dos Santos *et al.*, 2014; Azokou *et al.*, 2016; Molina *et al.*, 2025).

The ethnopharmacological survey applied in the present study revealed that the most commonly used part of the plants for preparing the extracts was the leaves, which is in line with a recent review which highlighted that most studies implemented the use of leaves because they are easier to collect and process and may contain higher proportions of the active ingredients responsible

for the acaricidal effect (Bisht *et al.*, 2025). Decoction was the most commonly used method for preparing the extracts, and the main method of application was spray baths on the animals (Table 1). These findings are consistent with recent reports, which found that cattle livestock in tropical regions implemented decoction because it was associated with better efficiency in the extraction of polar metabolites. Likewise, the preference for applying the extracts through spray baths is considered the most viable practice for tick control (Paucar-Quishpe *et al.*, 2024; Mollong *et al.*, 2025). The percentage yield of the eighteen plant ethanol extracts reflects the ratio between the dry biomass used and the crude extract obtained from the extraction process, rather than being a direct indicator of acaricidal activity in this study. The differences in extraction yield percentage among the different plant species can be attributed to the chemical composition and fraction of ethanol-soluble compounds; this solvent has been widely used for its effectiveness in recovering bioactive secondary metabolites when compared to other solvents (Gonfa *et al.*, 2020; El Mannoubi, 2023).

The AIT results indicated that the ethanolic extracts of *Annona muricata*, *Curcubita maxima*, *Eugenia stipitata* and *Miconia dolichorrhyncha* achieved 100% mortality at the highest concentration evaluated (160 mg/mL), and *Curcubita maxima* exhibited this effect even at 80 mg/mL (Table 2). The *Trichanthera gigantea* extract, along with

the aforementioned extracts, showed a REI of 0% and EP of 100% (Table 4). For LIT, the extracts of *Carica papaya* (seeds) and *Dioclea virgata* exhibited higher mortality than the positive control ( $P < 0.05$ ), and similar mortality to the positive control for the extracts of *Azadirachta indica*, *Cucurbita maxima* and *Eugenia stipitata* at a concentration of 160mg/mL ( $P > 0.05$ ). These findings indicate a relevant biological potential, comparable to the effect of 0.015% cypermethrin under *in vitro* conditions. In the present study, as in the one conducted by Bravo-Ramos *et al.* (2021), the acaricidal activity of the plant extracts was dose dependent (Tables 2, 3, and 4). Within this group, the *Annona muricata* plant extract showed promising acaricidal activity, similar to findings in other studies investigating the activity of the leaves of this plant with different solvents, primarily the effect of its seed extract (Lopera *et al.*, 2017; Vijay *et al.*, 2025). Additionally, the *Azadirachta indica* extract has been extensively studied, yielding results similar to those of the present study for both teleogines and larvae mortality (Dalei *et al.*, 2022; Gareh *et al.*, 2022).

To the authors' knowledge, the present study is the first to report acaricidal activity of ethanolic extracts of *Capsicum annuum*, *Cucurbita maxima*, *Dioclea virgata*, *Eugenia stipitata*, *Guazuma ulmifolia*, *Ipomoea quamoclit*, *Jacaranda obtusifolia*, *Lantana cujabensis*, *Miconia dolichorrhyncha*, *Trichanthera gigantea* and *Urera baccifera* against *R. microplus*. Some of these extracts have been studied for other biological purposes (Benvenuti *et al.*, 2020; Serra *et al.*, 2020; Tilaqza *et al.*, 2021). *Capsicum annuum* extract has previously been tested against the camel tick *Hyalomma dromedarii* using a 17% aqueous extract, showing a mortality rate of 87.5% in teleogines (Khater *et al.*, 2024), similar to that found in this study with a concentration of 16%. However, plants of the same genus have been tested against both *R. microplus* and other parasites (Khater, 2014; de Carvalho *et al.*, 2023; Ramirez *et al.*, 2025). For example, the ethanolic extract of *Capsicum frutescens* showed mortality and altered reproductive parameters of *R. microplus* (Vasconcelos *et al.*, 2014), similar to the *Capsicum annuum* extract tested in the present study.

In contrast, *Allium sativum* is among other ethanolic extracts that showed lower mortality than 0.015% cypermethrin in the present study. This plant has been evaluated in different solvents, showing acaricidal activity superior to that reported here (Nasreen *et al.*, 2020; Lima *et al.*, 2022). However, to the authors' knowledge, this is the first report of this extract tested on *R. microplus* in Colombia. Likewise, the *Gliricidia sepium* extract showed lower acaricidal activity than that reported by Jaramillo-Hernández *et al.* (2020) and higher than that described by Ravindran *et al.* (2017), confirming that this plant shows promising potential in various biological applications (Punyashree *et al.*, 2025). Additionally, the *Morinda citrifolia* extract evaluated in the present study showed higher mortality rates against *R. microplus* than those found by Nápoles *et al.* (2016). Although its acaricidal activity against the ectoparasites *Amblyomma cajennense* and *Dermatobia hominis* have been previously tested, the biocidal activity found in those studies was lower than that reported in the present study (de Menezes *et al.*, 2021; Quevedo *et al.*, 2024).

It was observed that the LC<sub>50</sub> and LC<sub>90</sub> values of teleogines were lower than the values of larvae (Tables 6 and 7). This result may indicate greater sensitivity of teleogines compared to immature stages. This finding coincides with that reported by Chagas *et al.* (2003), suggesting that this factor may be due to variations in cuticle formation and the presence of wax, which acts as a physiological barrier. The lipid layer of the cuticle appears later in larval development, and it is likely that the use of Tween-80 as a diluent for the extract in the immersion tests facilitated the penetration of secondary plant metabolites into the larvae (Gonçalves *et al.*, 2007).

Early larval mortality (<6 hours post-hatching) was detected in three extracts (*Azadirachta indica*, *Capsicum annuum*, and *Miconia dolichorrhyncha*) (Table 5). This phenomenon could be associated with a residual effect of secondary metabolites transferred from teleogines to eggs, affecting embryonic development and larval viability. A previous study with *Azadirachta indica* on *Hyalomma anatolicum excavatum* showed a significant decrease in the hatching rate and the production of larvae that died a few hours after hatching (Abdel-Shafy and Zayed, 2002). Although the acaricidal effect of *Azadirachta indica* has been attributed mainly to azadirachtin A, other compounds have also been found, such as 3-O-butyl(-)-epigallocatechin, 3-O-butyl(-)-epicatechin, and octadecanoic acid-tetrahydrofuran-3,4-diyl ester, which have an acaricidal effect on *R. microplus* (Sosa-Rueda *et al.*, 2023). Similarly, phytochemically evaluated plant extracts rich in tannins have been directly implicated in the acaricidal and ovicidal effect on *R. microplus* (Fernández-Salas *et al.*, 2011).

The phytochemical results indicated that all extracts contained coumarins, and several extracts presented a variety of secondary metabolites groups (Fig. 2). Metabolites such as coumarins are known to inhibit serine-dependent proteases, interfering with digestive enzymes and affecting nutrition and vitellogenesis in *R. microplus* (Estrela *et al.*, 2007; 2010).

Tannins have been directly linked to acaricidal and ovicidal effects (Fernández-Salas *et al.*, 2011), a phenomenon that may be related to the early larval mortality observed with the use of some extracts (*Azadirachta indica*, *Capsicum annuum*, and *Miconia dolichorrhyncha*) in the present study (Table 5). Saponins and saponinins have the capacity to alter membrane integrity (Negash *et al.*, 2024), and alkaloids, phenolic compounds, and terpenoids have neurotoxic properties. The latter have been extensively studied, determining their potential to generate synergism with conventional acaricides such as cypermethrin, conferred by their acetylcholinesterase-inhibiting activity (dos Santos Cardoso *et al.*, 2020; Alvares *et al.*, 2025). The lower LC<sub>50</sub> and LC<sub>90</sub> values observed in some of the ethanolic extracts in the present study likely reflect a multifactorial action of secondary metabolites that act synergistically, affecting the physiological conditions of the tick *R. microplus*, however, this interaction was not evaluated experimentally. Qualitative phytochemical analysis identified the presence of different groups of secondary metabolites in the plant extracts evaluated, providing initial information on their chemical composition. However, further analysis is required to isolate, identify,

and quantify the bioactive compounds responsible for the acaricidal activity (Bisht *et al.*, 2025).

Among the limitations of this study is the absence of determination of the degree of susceptibility or resistance to cypermethrin (positive control) of *R. microplus* ticks collected in the field. Although, the positive control did not achieve 100% mortality, no discriminatory doses were implemented, nor was the resistance factor calculated by comparison with a susceptible reference strain (FAO, 2025). The mortality observed in the positive control could be associated with resistance to conventional acaricides (López-Arias *et al.*, 2014; Villar *et al.*, 2020), an aspect that was not specifically evaluated within the objectives of this study. In the Probit analysis, some lethal concentrations presented wide values and confidence intervals, influenced by the variability of mortality response and a limited adjustment of the model recorded in the different concentrations evaluated in the different plant extracts. Future studies should consider a greater number of replicates or additional dilutions to obtain more robust estimates of LC<sub>50</sub> and LC<sub>90</sub> (Pum, 2019).

**Conclusions:** Ethnopharmacological screening highlighted the importance of traditional knowledge in the selection of plants used in bovine production systems for the control of *R. microplus*. Seven ethanolic extracts (*Annona muricata*, *Carica papaya* (Seeds), *Cucurbita maxima*, *Dioclea virgata*, *Eugenia stipitata*, *Miconia dolichorrhyncha* and *Trichanthera gigantea*) showed promise, exhibiting high mortality and reproductive effects *in vitro*, demonstrating their potential as sustainable alternatives for managing this ectoparasite. These alternatives contribute to reducing dependence on conventional acaricides and strengthen the integration of local knowledge and scientific validation. However, *in situ* studies are necessary to confirm their efficacy, and practical applicability under field conditions.

**Acknowledgements:** To veterinarians, veterinary zootechnicians, zootechnicians, and workers in each cattle livestock. To young researcher Carmen María Rocha Mora and students Jessica Alejandra Aponte Forero and Julieth Viviana Rodríguez Rincón.

**Authors contribution:** MV and LL conducted the experiments and drafted the original manuscript. DJ designed and supervised the entire study. All authors interpreted the data and approved the final version.

## REFERENCES

- Abdel-Shafy S and Zayed AA, 2002. In vitro acaricidal effect of plant extract of neem seed oil (*Azadirachta indica*) on egg, immature and adult stages of *Hyalomma anatolicum excavatum* (Ixodoidea: Ixodidae). *Veterinary Parasitology* 106:89-96.
- Adenubi OT, Ahmed AS, Fasina FO, *et al.*, 2018. Pesticidal plants as a possible alternative to synthetic acaricides in tick control: A systematic review and meta-analysis. *Industrial Crops and Products* 123:779-806.
- Alvares F, Ferreira L, Silva J, *et al.*, 2025. Acaricidal efficacy of the monoterpene linalool against the cattle tick *Rhipicephalus microplus* and its synergistic potential with cypermethrin. *Experimental and Applied Acarology* 94:45.
- Aponte-Forero J, Rodríguez-Rincón J, Velasquez-Peña M, *et al.*, 2024. Revisión sistemática de extractos de plantas y sus metabolitos secundarios de interés garrapaticida para *Rhipicephalus microplus* en bovinos. *Revista Sistemas de Producción Agroecológicos* 15(2):e1180.
- Azokou A, Achi Y and Koné M, 2016. Control of cattle ticks in Côte d'Ivoire using traditional methods. *Livestock Research for Rural Development* 28:52.
- Benvenuti R, Dalla C, Locatelli G, *et al.*, 2020. Gastroprotective activity of hydroalcoholic extract of the leaves of *Urera baccifera* in rodents. *Journal of Ethnopharmacology* 250:112473.
- Bisht N, Fular A, Ghosh S, *et al.*, 2025. Systematic review and meta-analysis of acaricidal properties of plant derived products against ixodid ticks population. *Discover Applied Sciences* 7:1-31.
- Bravo-Ramos J, Flores-Primo A, Paniagua-Vega D, *et al.*, 2021. Acaricidal activity of the hexanic and hydroethanolic extracts of three medicinal plants against southern cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *Experimental and Applied Acarology* 85:113-129.
- Chagas A, Mascarenhas W, Teixeira H, *et al.*, 2003. Sensibilidade do carrapato *Boophilus microplus* a solventes. *Ciência Rural* 33:109-114.
- Dalei M, Dehuri M, Palai S, *et al.*, 2022. Phytochemical analysis of ethanolic extract of leaf of *Azadirachta indica* and in vitro acaricidal effect against *Rhipicephalus microplus*. *Indian Journal of Veterinary Sciences and Biotechnology* 18(3):30-34.
- de Carvalho V, Marchesini P, Duque L, *et al.*, 2023. Acaricidal activity of a 2-methoxy-clovan-9-ol rich fraction from *Eugenia copacabanensis* (Myrtaceae) extract on *Rhipicephalus microplus* (Acari: Ixodidae). *Experimental Parasitology* 253:108604.
- de Menezes V, Lima R, da Silva I, *et al.*, 2021. Eficácia de extratos vegetais no controle de *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae). *Revista de Ciências Agroveterinárias* 15:1-11.
- dos Santos Cardoso A, Santos E, da Silva A, *et al.*, 2020. Terpenes on *Rhipicephalus (Boophilus) microplus*: acaricidal activity and acetylcholinesterase inhibition. *Veterinary Parasitology* 280:109090.
- dos Santos F, Albuquerque U, Júnior L, *et al.*, 2014. An ethnopharmacological assessment of the use of plants against parasitic diseases in humans and animals. *Journal of Ethnopharmacology* 155:1332-1341.
- Drummond R, de Ernest S, Trevino J, *et al.*, 1973. *Boophilus annulatus* and *B. microplus*: laboratory tests of insecticides. *Journal of Economic Entomology* 66:130-133.
- Dzemo W, Thekisoe O and Vudriko P, 2022. Development of acaricide resistance in tick populations of cattle: a systematic review and meta-analysis. *Heliyon* 8:e08718.
- El Mannoubi I, 2023. Impact of different solvents on extraction yield, phenolic composition, in vitro antioxidant and antibacterial activities of deseeded *Opuntia stricta* fruit. *Journal of Umm Al-Qura University for Applied Sciences* 9:176-184.
- Estrela A, Seixas A, Teixeira V, *et al.*, 2010. Vitellin- and hemoglobin-digesting enzymes in *Rhipicephalus (Boophilus) microplus* larvae and females. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 157:326-335.
- Estrela A, Seixas A and Termignoni C, 2007. A cysteine endopeptidase from *Rhipicephalus (Boophilus) microplus* larvae with vitellin digestion activity. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 148:410-416.
- FAO, 1971. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative methods for larvae of cattle tick *Boophilus microplus*. Food and Agriculture Organization of the United Nations, Rome, pp:15-18.
- FAO, 2004. Resistance management and integrated parasite control in ruminants: guidelines. Food and Agriculture Organization of the United Nations, Rome, pp:25-77.
- FAO, 2025. Guidelines for sustainable tick control and acaricide resistance management in livestock. Food and Agriculture Organization of the United Nations, Rome.
- FEDEGAN, 2024. Inventario ganadero. Federación Colombiana de Ganaderos. <https://www.fedegan.org.co/estadisticas/inventario-ganadero>
- Fernández-Salas A, Alonso-Díaz M, Acosta-Rodríguez R, *et al.*, 2011. In vitro acaricidal effect of tannin-rich plants against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *Veterinary Parasitology* 175:113-118.
- Gallegos-Zurita M, Mazacon B and Troncoso L, 2016. Diseño y validación del cuestionario U-PlanMed para identificación del uso de plantas medicinales en Babahoyo, Ecuador. *Anales de la Facultad de Medicina* 77:207-212.

- Gareh A, Hassan D, Essa A, et al., 2022. Acaricidal properties of four neem seed extracts (*Azadirachta indica*) on the camel tick *Hyalomma dromedarii* (Acari: Ixodidae). *Frontiers in Veterinary Science* 9:946702.
- Gonfa T, Teketle S and Kiros T, 2020. Effect of extraction solvent on qualitative and quantitative analysis of major phyto-constituents and in vitro antioxidant activity evaluation of *Cadaba rotundifolia* leaf extracts. *Cogent Food and Agriculture* 6:1853867.
- Gonçalves K, Toigo E, Ascoli B, et al., 2007. Effects of solvents and surfactant agents on female and larvae of cattle tick *Boophilus microplus*. *Parasitology Research* 100:1267-1270.
- Holdsworth P, Rehbein S, Jonsson N, et al., 2022. World Association for the Advancement of Veterinary Parasitology (WAAVP): guideline for evaluating the efficacy of parasiticides against ectoparasites of ruminants. *Veterinary Parasitology* 302:109613.
- ICA, 2024. Censo pecuario año 2024. Instituto Colombiano Agropecuario. <https://www.ica.gov.co/areas/pecuaria/servicios/epidemiologia-veterinaria/censos-2016/censo-2018>
- ICA, 2025. Censo pecuario año 2025. Instituto Colombiano Agropecuario. <https://www.ica.gov.co/areas/pecuaria/servicios/epidemiologia-veterinaria/censos-2016/censo-2018>
- Jaramillo-Hernández D, 2022. Importance of vaccination within the integrated management of *Rhipicephalus microplus* in cattle. *Revista Sistemas de Producción Agroecológicos* 13:47-63.
- Jaramillo-Hernández D, González A, Pedraza N, et al., 2020. Evaluación del efecto acaricida de *Momordica charantia*, *Megaskepasma erythroclamy* y *Gliricidia sepium* sobre *Rhipicephalus microplus*. *Journal of MVZ Córdoba* 25:1951.
- Johansson M, Mysterud A and Flykt A, 2020. Livestock owners' worry and fear of tick-borne diseases. *Parasites and Vectors* 13:331.
- Khater H, 2014. Bioactivities of some essential oils against the camel nasal botfly, *Cephalopina titillator*. *Parasitology Research* 113:593-605.
- Khater H, Bazh E, Gawad S, et al., 2024. Acaricidal efficacy of thirty-five Egyptian plants against the camel tick, *Hyalomma dromedarii*. *Acta Parasitologica* 69:1231-1243.
- Lima E, Abrão D, Vieira D, et al., 2022. *Allium sativum* extract against free-living larvae of *Rhipicephalus microplus*: a potential environmental biocontrol. *Veterinary Parasitology: Regional Studies and Reports* 34:100769.
- Lopera J, León G, Guzmán P, et al., 2017. Efecto de los extractos vegetales de *Jatropha curcas* y *Annona muricata* sobre teleoginas de *Rhipicephalus (Boophilus) microplus* bajo condiciones de laboratorio. *CES Medicina Veterinaria y Zootecnia* 12:21-32.
- López-Arias A, Villar-Argaiz D, Chaparro-Gutiérrez J, et al., 2015. Reduced efficacy of commercial acaricides against populations of resistant cattle tick *Rhipicephalus microplus* from Antioquia, Colombia. *Environmental Health Insights* 8(Suppl 2):71-80.
- Luciano A, Jallow B, Liu M, et al., 2025. Distribution of *Rhipicephalus microplus* and *Hyalomma lusitanicum*, and the pathogens they carry: a systematic review. *Parasite Epidemiology and Control* e00437.
- Maheshwaran L, Nadarajah L, Senadeera S, et al., 2024. Phytochemical testing methodologies and principles for preliminary screening and qualitative testing. *Asian Plant Research Journal* 12:11-38.
- Molina W, Diaz-Rivas I and Serrato-Hurtado C, 2025. Evaluación de susceptibilidad de *Rhipicephalus microplus* a extractos de *Nicotiana tabacum* y *Couroupita guianensis* en condiciones de laboratorio. *Revista Científica Amazonia* 8:23-38.
- Mollong E, Lébrí M, Marie-Magdeleine C, et al., 2025. Sustainable management of tick infestations in cattle: a tropical perspective. *Parasites and Vectors* 18:62.
- Murrell A and Barker S, 2003. Synonymy of *Boophilus* Curtice, 1891 with *Rhipicephalus* Koch, 1844 (Acari: Ixodidae). *Systematic Parasitology* 56:169-172.
- Nápoles D, Sebasco K, Colas M, et al., 2016. Eficacia in vitro de *Morinda citrifolia* para el control de *Rhipicephalus (Boophilus) microplus*. *Revista de Investigaciones Veterinarias del Perú* 27:833-839.
- Nasreen N, Niaz S, Khan A, et al., 2020. Anti-tick activity of *Allium sativum* and *Cannabis sativa* extracts against *Rhipicephalus microplus*. *Experimental and Applied Acarology* 82:281-294.
- Negash N, Andualem D and Mandefro B, 2024. Phytochemical screening and in vitro efficacy of *Calpurnia aurea* against *Amblyomma variegatum* and *Rhipicephalus microplus*. *Journal of Experimental Pharmacology* 16:243-255.
- Paucar-Quishpe V, Pérez-Otáñez X, Rodríguez-Hidalgo R, et al., 2024. Farmers' adoption, knowledge and perceptions of tick control measures on dairy farms in subtropical Ecuador. *Transboundary and Emerging Diseases* 2024:5023240.
- Pereira V, Castilho P and Pereira J, 2025. Analysis of the environmental impact of botanical pesticides in soil. *Agriculture* 15:1053.
- Pum J, 2019. A practical guide to validation and verification of analytical methods in the clinical laboratory. *Advances in Clinical Chemistry* 90:215-281.
- Punyashree S, Mani T, Pavithra T, et al., 2025. Phytochemicals, pharmacological properties and traditional uses of *Gliricidia sepium*: a comprehensive review. *International Journal of Pharmacognosy* 12:85-93.
- Quevedo R, Cadena D, González A, et al., 2024. In vitro effect of methanolic extract of *Morinda citrifolia* against the life cycle of *Dermatobia hominis*. *Vitae* 31.
- Rajput Z, Hu S, Chen W, et al., 2006. Importance of ticks and their chemical and immunological control in livestock. *Journal of Zhejiang University Science B* 7:912-921.
- Ramírez J, Balcázar K, López J, et al., 2025. Chemical composition and acaricidal activity of *Lantana camara* essential oils against *Rhipicephalus microplus*. *Plants* 14:2336.
- Ravindran R, Juliet S, Ramankutty S, et al., 2017. Effects of ethanolic extract of *Pongamia glabra* and *Gliricidia sepium* against *Rhipicephalus annulatus*. *Advances in Animal and Veterinary Sciences* 5:1-6.
- Sanabria A, 1983. Análisis fitoquímico preliminar: metodología y su aplicación en la evaluación de 40 plantas de la familia Compositae. Universidad Nacional de Colombia, Bogotá, Colombia, pp:1-61.
- Serra M, Barroso W, Filho F, et al., 2020. Pharmacological evidence from plants of genus *Jacaranda*. *Biomedical Journal of Scientific and Technical Research* 28:21730-21734.
- Shahzad S, Akinsulie O, Idris I, et al., 2025. Ticks and tick-borne diseases in Global South countries: impact and implications of environmental changes. *Frontiers in Tropical Diseases* 6:1597236.
- Shaw R, 1966. Culture of an organophosphorus strain of *Boophilus microplus* and assessment of its resistance spectrum. *Bulletin of Entomological Research* 56:389-405.
- Silva W, de Souza J, de Souza H, et al., 2009. Toxicity of *Piper aduncum* from the Amazon forest for the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *Veterinary Parasitology* 164:267-274.
- Sosa-Rueda J, Villaruz F, Domínguez-Meléndez V, et al., 2023. Ixodicide action of natural products from native Mexican plants. *Revista Mexicana de Ciencias Pecuarias* 14:292-308.
- Tilaqza A and Herbani M, 2021. In silico study of antihypertensive potential and pharmacokinetic profile of *Guazuma ulmifolia*. *Jurnal Kesehatan Islam* 10:45-52.
- Vasconcelos V, Martins M, de Oliveira N, et al., 2014. Effect of ethanolic extract of *Capsicum frutescens* on adult females of *Rhipicephalus microplus* (Ixodidae). *Parasitology Research* 113:1389-1394.
- Valencia CE, Appel VA, Ruiz GA, et al., 2017. Identification and prevalence of ticks (Acari: Ixodidae) in bovines in municipalities of Popayán plateau, Colombia. *Revista Colombiana de Ciencias Pecuarias* 30:239-248.
- Vijay M, Rajitha M, Krishnagaanth M, et al., 2025. Controlling deltamethrin-resistant *Rhipicephalus microplus* with a phytoformulation of *Annona muricata* and *Piper longum*. *Acta Tropica* 107644.
- Villar D, Klafke G, Rodríguez-Durán A, et al., 2020. Resistance profile and molecular characterization of pyrethroid resistance in *Rhipicephalus microplus* from Colombia. *Medical and Veterinary Entomology* 34:105-115.
- Walker A, 2003. Ticks of domestic animals in Africa: a guide to identification of species. *Bioscience Reports, Edinburgh, UK*.