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Efficacy of resin and ethanol extract from leaves of *Protium spruceanum* (Bent) Engl. combined with Cypermethrin to control *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887)

Eficácia do extrato de resina e etanol a partir de folhas de *Protium spruceanum* (Bent) Engl. combinado com Cypermetrina para controlar *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887)

Eficacia del extracto de resina y etanol de las hojas de *Protium spruceanum* (Bent) Engl. combinado con cipermetrina para controlar *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887)

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Abstract
Rhipicephalus microplus is one of the most important ectoparasites in cattle because it affects animal production and is a vector of bovine babesiosis. Although conventional control of the tick depends mainly on the use of chemical acaricides, the intensive use of these drugs favors the selection of resistant populations. Thus, the objective of this study was to evaluate the *in vitro* acaricide activity of ethanolic extract (EE) of leaves and crude resin of *Protium spruceanum* (Benth) in association with a synthetic acaricidal product in two strains of *R. microplus* using larval package test (LPT) and biocarrapaticidogram test. The qualitative characterization was performed by colorimetric analyses. EE and resin combined with cypermethrin showed efficacy above 90% on *R. microplus* larvae at concentrations of 50.96 mg ml^{-1} + 75 µg ml^{-1} (EE + cypermethrin) and 5.08 mg ml^{-1} + 15 µg ml^{-1} (resin + cypermethrin) for both as strains used. High efficacy was not observed in females when the combination was used. However, the values were significantly higher than the efficacy for cypermethrin. The results of phytochemical analysis showed the presence of secondary metabolites of the following classes: phenolic compounds, tannins, alkaloids, terpenes, flavonoids and saponins for EE of *P. spruceanum* leaves. Only tannins, alkaloids and flavonoids were detected for the crude resin. The association between EE, crude resin and cypermethrin has implications for the control of immature and female stages engendered with *R. microplus*, which can reduce concentration and cost.
Keywords: Synergism; Family burseraceae; Biological acaricide; Scrubland.

Resumo

Rhipicephalus microplus é um dos mais importantes ectoparasitas da pecuária porque afeta a produção animal e é um vetor de babesiose bovina. Embora o controle convencional do carrapato dependa principalmente do uso de acaricidas químicos, o uso intensivo desses fármacos favorece a seleção de populações resistentes. Por isso, o objetivo deste estudo foi avaliar a atividade acaricida *in vitro* do extrato etanólico (EE) de folhas e resina bruta de *Protium spruceanum* (Benth) em associação com um produto de acaricida sintético em duas cepas de *R. microplus* utilizando o teste de pacote de larvas (LPT) e o teste de biocarrapaticidograma. A caracterização qualitativa foi realizada por meio de análises colorimétricas. A associação do EE e/ou resina com cipermetrina mostraram eficácia superior a 90% em larvas *R. microplus* em concentrações de 50,96 mg ml^{-1} + 75 µg ml^{-1} (EE + cipermetrina) e 5,08 mg ml^{-1} + 15 µg ml^{-1} (resina + cipermetrina) para ambas as cepas utilizadas. Não foi observada alta eficácia nas fêmeas ingurgitadas quando utilizada a associação. No entanto, os valores foram significativamente maiores do que a eficácia da cipermetrina sozinha. Os resultados da análise fitoquímica mostraram a presença de metabólitos secundários das seguintes classes: compostos fenólicos, taninos, alcaloides, terpenos, flavonoides e saponinas para EE a partir de folhas de *P. spruceanum*. Apenas taninos, alcaloides e flavonoides foram detectados para a resina bruta. A associação entre EE, resina bruta e cipermetrina tem implicações para o controle de estágios imaturos e fêmeas ingurgitadas de *R. microplus*, o que pode reduzir a concentração e o custo.

Palavras-chave: Sinergismo; Família burseraceae; Acaricida biológico; Cerrado.

Resumen

Rhipicephalus microplus es uno de los ectoparásitos más importantes en el ganado porque afecta la producción animal y es un vector de babesiosis bovina. Aunque el control convencional de la garrapata depende principalmente del uso de acaricidas químicos, el uso intensivo de estos fármacos favorece la selección de poblaciones resistentes. Por lo tanto, el objetivo de este estudio fue evaluar la actividad acaricida *in vitro* del extracto etanólico (EE) de hojas y resina cruda de *Protium spruceanum* (Benth) en asociación con un producto acaricida sintético en dos cepas de *R. microplus* utilizando la prueba de paquete larval (LPT) y la prueba de biocarrapaticidograma. La caracterización cualitativa se realizó mediante análisis colorimétricos. La EE y la resina combinadas con cipermetrina mostraron una eficacia superior al 90% en larvas de *R. microplus* a concentraciones de 50,96 mg ml^{-1} + 75 µg ml^{-1} (EE + cipermetrina) y 5,08 mg ml^{-1} + 15 µg ml^{-1} (resina + cipermetrina) para las cepas utilizadas. No se observó una alta eficacia en las hembras hinchadas cuando se utilizó la combinación. Sin embargo, los valores fueron significativamente más altos que la eficacia de la cipermetrina. Los resultados del análisis fitoquímico mostraron la presencia de metabolitos secundarios de las siguientes clases: compuestos fenólicos, taninos, alcaloides, terpenos, flavonoides y saponinas para EE de hojas de *P. spruceanum*. Solo se detectaron taninos, alcaloides y flavonoides para la resina cruda. La asociación entre EE, resina cruda y cipermetrina tiene implicaciones para el control de las etapas inmaduras y hembras hinchadas de *R. microplus*, lo que puede reducir la concentración y el costo.

Palabras clave: Sinergismo; Familia burseraceae; Acaricida biológico; Matorral.

1. Introduction

The tick *Rhipicephalus microplus* causes economic losses in several continents, and infests cattle in tropical and subtropical countries (Khan et al., 2019). It is not only a vector of microbial pathogens responsible for serious diseases such as babesiosis and anaplasmosis, but also directly promotes weight loss, significant decrease in milk and meat production, and affects the quality of leather (Feder et al., 2019). Control of *R. microplus* is often accomplished using synthetic acaricides. However, indiscriminate use of these drugs can select resistant tick populations, cause poisoning of animals and humans, and promote environmental contamination (Lazcano Díaz et al., 2019). Multidrug resistance in ticks has been reported in several continents (Fular et al., 2018; Bisht et al., 2021). Santo et al. (2021) studied nine acaricides of different brands and observed multidrug resistance to cypermethrin and amitraz in tick strains in southern Bahia.

Studies have shown that the combination of compounds of plant origin can enhance the activity of these compounds and can be synergistic, additive, or antagonistic (Berthoud, 2013; Geary, 2013; Lederer et al., 2019). The synergism of these compounds helps improves efficiency, reduces the accumulation of waste in the environment, avoids poisoning of animals, and reduces the concentrations of chemical compounds used, which consequently reduce the cost of acaricide application (Gallardo et al., 2012; Vale et al., 2021). The use of these combinations also reduces the selection of ticks resistant to synthetic products (Greco et al., 1996; Ma & Motsinger-Reif, 2019).

Protium spruceanum (Benth) Engler is locally known as breu branco, almecega, almescla, almecegueira, or almecega-vermelha. (Vieira et al., 2010). It is traditionally used as an expectorant and anti-inflammatory agent in topical treatments in folk medicine. (Amparo et al., 2019). In addition, ethanol extract (EE) and ethyl acetate (EA) from leaves of *P. spruceanum* showed acaricidal activity against females and larvae of *R. microplus* in vitro (Figuereido et al., 2019). The aim of this study was to demonstrate the efficacy of the combination of EE from leaves and resin of *P. spruceanum* with cypermethrin.

2. Methodology

2.1 Plant collection and identification

Plant material was collected from the Água Doce River Basin, Bonito de Minas municipality, Minas Gerais, Brazil (15°13′18.7″S 44°55′21.2″W). The leaves of *P. spruceanum* were collected, identified by specialists, and deposited in the Montes Claros Herbarium (Herbário Montes Claros (HMCMG)) of the State University of Montes Claros (Universidade Estadual de Montes Claros (UNIMONTES)) under the voucher number 5,060. All plant material used was registered on the platform of the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGEN)) under the number AE6B119.

The leaves of adult plants were washed under running water and inspected, and those with lesions and damage were sorted out. They were then dehydrated in an oven with forced air circulation (TE 394/4, Equipamentos Técnicos Científicos, Tecnal, Piracicaba, SP, Brazil) at 40 °C for 72 h, ground in an industrial blender to obtain the dry powder, and stored in paper bags under light protection until use. The resin was collected by randomly selecting adult individuals after cutting the bark of the stems using a stainless steel knife. The crude material was stored in an airtight plastic bottle and cooled to approximately 4 °C in an isothermal box.

2.2 Extract preparation

Ethanolic extract (EE) was prepared by adding 100 g of dry leaf powder to 1000 mL of absolute ethyl alcohol PA 99.5° GL. The material was then stored in an amber jar at room temperature for 10 days. Subsequently, it was filtered in a glass funnel with gauze and cotton and dehydrated in a forced-air oven at 40 ± 5 °C. The dry extract was then separated and stored in dark bottles. The dry matter of three subsamples containing 2 g each were determined by the AOAC (Association of Official Analytical Chemists) technique for standardization and calculation of the tested concentrations.

2.3 Assessed tick populations

The first population evaluated was from a farm in the municipality of São João da Ponte, Minas Gerais. Infested females were collected from crossbred cattle (Gyr × Holstein) that were naturally infested and had no contact with acaricides for at least 60 days. After collection, the ticks were placed in plastic containers and transported to the laboratory, where they were incubated in an oven (BOD) at 28 °C and relative humidity of approximately 80% until use.

The other strain studied, Santa Rita, was kindly provided by Prof. Lívio Martins Costa Júnior of the Federal University of Maranhão (Universidade Federal do Maranhão (UFMA)). This strain is resistant to amidines and synthetic pyrethroids and sensitive to organophosphates. Larvae were maintained in artificially infested cattle at the experimental farm of the Institute of Agrarian Sciences of the Federal University of Minas Gerais (Instituto de Ciências Agrárias da Universidade Federal de Minas Gerais (ICA/UFMG)), Montes Claros, Minas Gerais, Brazil. The engorged females were morphologically selected and used for immersion tests of engorged females and kept in BOD ovens at 28 °C and relative humidity of approximately 80% for 15 days until hatching to obtain larvae. Both strains were approved for use by the Ethics Committee for

the use of animals - UFMA Ethics Committee, Santa Rita strain under protocol 23115.005443/2017-51 and São João da Ponte strain under protocol 265/2017.

2.4 Effects of association between EE and *P. spruceanum* resin and cypermethrin on *R. microplus* larvae

Larval Packet Test (LPT)

EE LC90 concentrations were estimated at 101.92 mg ml^{-1} and resin at 50.18 mg ml^{-1} for the calculations in the formulations (Figueiredo et al., 2019). Larvae aged between 15–28 days post-hatch were used. The tests were carried out according to the methodology proposed by Stone and Haydock (1962) with modifications. Approximately 100 larvae were inserted into filter paper packages (Whatmann No 1) measuring 6 × 6 cm. Subsequently, the filter paper packets were sealed with staplers and separately impregnated with 300 µL of the binary combination (Table 1). The entire experiment consisted of four replicates per treatment.

Table 1. Concentrations of *Protium spruceanum* ethanolic extract (EE) and crude resin combined with synthetic acaricide cypermethrin (Barrage®, Zoetis, Brazil).

EE + Cypermethrin	Resin + Cypermethrin
101.92 mg ml^{-1} + 150 µg ml^{-1}	50.15 mg ml^{-1} + 150 µg ml^{-1}
71.13 mg ml^{-1} + 105 µg ml^{-1}	35.12 mg ml^{-1} + 105 µg ml^{-1}
50.96 mg ml^{-1} + 75 µg ml^{-1}	25.09 mg ml^{-1} + 75 µg ml^{-1}
30.57 mg ml^{-1} + 45 µg ml^{-1}	15.05 mg ml^{-1} + 45 µg ml^{-1}
10.19 mg ml^{-1} + 15 µg ml^{-1}	5.08 mg ml^{-1} + 15 µg ml^{-1}
150 µg ml^{-1} (cypermethrin alone)	150 µg ml^{-1} (cypermethrin alone)
101.92 mg ml^{-1} (EE alone)	50.15 mg ml^{-1} (crude resin alone)

Source: Authors.

The filter paper packets containing larvae were kept in petri dishes and placed in an oven (BOD) at 27 °C and 80% humidity for 24 h. The filter paper packets were then placed in the oven. Subsequently, the filter paper packets were opened on a white surface and the live and dead larvae were counted. The relative number of dead larvae compared to the total number of larvae was used for analysis of variance, and the means were compared using the Scott–Knott test at the 5% significance level in a completely randomized design. Lethal concentrations killing 90% of infectious larvae (LC90) were estimated using probit regression analysis of the SAEG 9.1 statistical package (SAEG 2007).

2.5 Immersion test in engorged females

Efficacy of the combination of *P. spruceanum* resin or EE with cypermethrin was evaluated at the concentrations shown in Table 1. The efficacy of the formulations was evaluated using the acaricide resistance test as described by

Drummond et al. (1973). Engorged adult females 4–6 mm in size were selected, washed in distilled water, and dried on paper towels. They were then divided into homogeneous groups of five (05) adult females according to size and weight and immersed in 5 ml of the combination solution for 5 min. The excess solution was removed with a paper towel, placed in a Petri dish, and maintained at 28 °C and 70% relative humidity in a BOD incubator. All procedures were repeated five times (Leite, 1995).

After 15 days of incubation, egg masses from each group of adult females were weighed on an analytical balance and then transferred to 3 ml disposable syringes, sealed with hydrophilic cotton, and stored at 28 °C and 70% relative humidity (Drummond et al., 1973). Thirty days after the onset of larval hatching, the contents of the syringe were transferred to petri dishes containing 3 ml of a solution of water and detergent (50:50). Then, three 200 µl aliquots of this suspension were placed on glass slides to count the eggs, larval eggs, and larvae under an optical microscope with a 10x objective and to determine the hatching rate of each group. All procedures related to hatching tests were triplicated (Vasconcelos et al., 2018).

A modified version of the formula described by Bennett (1974) was used to determine egg laying capacity (ELC):

ELC = (egg mass weight/initial female weight) × 100

Treatment efficacy (product efficacy) was estimated using the method of Drummond et al. (1973):

RE (reproductive efficiency) = (egg mass weight × % incubation × 20,000)/initial female weight,

PE (product efficiency) = ((RE control group − RE treated group)/RE control group)) × 100

Tests were repeated five times, data were transformed and submitted to analysis of variance, and means for each group were compared using the Scott–Knott test ($\alpha = 0.05$). The concentration of EE sufficient to inhibit 90% of hatching (IC90) was estimated by probit analysis using the SAEG 9.1 statistical package.

2.6 Phytochemical analysis

The protocols presented by Royo et al. (2015) were applied for phytochemical analysis. Qualitative characterization was performed with colorimetric and precipitation analyses of the classes of phenolic compounds, tannins, saponins, flavonoids, and terpenes. The reagents used for the photochemical tests were ferric chloride, 10% sodium hydroxide, Bertrand reagent, Dragendorff reagent, Mayer reagent, Lieberman–Burchard reagent, aluminum chloride, and distilled water. The intensity of the reaction was classified as (+) weak positive, (++) moderate positive and (+++) strong positive, and (−) negative.

3. Results and Discussion

3.1 Effects on larval mortality

The combinations containing EE or resin combined with cypermethrin showed efficacy of over 90% against *R. microplus* larvae (Tables 2 and 3).

Table 2 Larval mortality of *Rhipicephalus microplus* strains (São Joao da Ponte (SJP) and Santa Rita) treated with the combination of ethanol extract (EE) from *Protium spruceanum* leaves with cypermethrin.

Concentrations (EE + Cypermethrin)	SJP strain Mortality (%)	Santa Rita strain Mortality (%)
101.92 mg ml^{-1} (EE)	99.31 a	100.0 a
101.92 mg ml^{-1} + 150 µg ml^{-1}	90.45 a	100.0 a
71.13 mg ml^{-1} + 105 µg ml^{-1}	99.19 a	100.0 a
50.96 mg ml^{-1} + 75 µg ml^{-1}	90.19 a	93.38 a
30.57 mg ml^{-1} + 45 µg ml^{-1}	93.52 a	65.22 b
10.19 mg ml^{-1} + 15 µg ml^{-1}	38.18 b	24.35 c
Cypermethrin (150 µg ml^{-1})	98.2 a	99.27 a
Distilled water	0.00 c	0.0 c
CV	8.49	9.81

*Means followed by the same letter are statistically similar by Scott–Knott test at 5% significance level
**CV: Coefficient of variation Source: Authors.

The combination of EE from *P. spruceanum* leaf with cypermethrin at different concentrations showed satisfactory results with efficacy above 90% for both tick strains SJP and Santa Rita ($p < 0.05$).

Table 3 Larval mortality of *Rhipicephalus microplus* strains (São Joao da Ponte (SJP) and Santa Rita) treated with a combination of crude resin extracted from the stem of *Protium spruceanum* and cypermethrin

Concentrations (Resin + Cypermethrin)	SJP strain Mortality (%)	Santa Rita strain Mortality (%)
50.15 mg ml^{-1} (Resin)	61.43 b	93.83 a
50.15 mg ml^{-1} + 150 µg ml^{-1}	100 a	100 a
35.12 mg ml^{-1} + 105 µg ml^{-1}	100 a	100 a
25.09 mg ml^{-1} + 75 µg ml^{-1}	99.48 a	100 a
15.05 mg ml^{-1} + 45 µg ml^{-1}	93.91 a	99.02 a
5.08 mg ml^{-1} + 15 µg ml^{-1}	93.05 a	96.69 a
Cypermethrin (150 µg ml^{-1})	99.27 a	99.27 a
Distilled water	0	0
CV	11.51	4.85

*Means followed by the same letter are statistically similar by Scott–Knott test at 5% significance level
**CV: Coefficient of variation. Source: Authors.

The combination of crude resin with cypermethrin used against SJP and Santa Rita strains showed efficacy above 93% at all concentrations tested, which is similar to the results observed for cypermethrin in the control with cypermethrin ($p < 0.05$). To the best of our knowledge, to date, no study has evaluated the effect of the combination of a plant extract with an acaricidal compound on the control of *R. microplus*. However, essential oil combinations have already been analyzed. The combination of the monoterpenes thymol and carvacrol with the phenylpropanoid eugenol was synergistic and effective in controlling larvae of *R. microplus* and *R. sanguinius* (Araujo et al., 2016). A moderate synergistic effect was observed when controlling larvae of *Amblyomma sculptum* with the combinations carvacrol + thymol and carvacrol + (E)-cinnamaldehyde (Novato et al., 2015).

3.2 Effects on reproductive parameters of females of *Rhipicephalus microplus*

High efficacy was not observed when the association of EE or resin with cypermethrin was applied to engorged females of *R. microplus* at any of the concentrations evaluated (Tables 4 and 5). However, a higher efficacy of the combination of EE or resin with cypermethrin was observed when comparing the efficacy of cypermethrin alone on engorged females ($p < 0.05$).

Table 4. Effect of the combination of crude resin extracted from *Protium spruceanum* stem with cypermethrin on the reproductive parameters of engorged females of *Rhipicephalus microplus* collected in the municipality of São João da Ponte, Minas Gerais.

Resin + Cypermethrin combination	ELC*	Hatching (%)	RE** (%)	Efficiency ** (%)
50.15 mg ml^{-1} + 150 µg ml^{-1}	24.26 c	95.62 a	46.30 c	44.57 a
25.09 mg ml^{-1} + 75 µg ml^{-1}	28.22 c	90.58 a	51.36 c	38.50 a
15.05 mg ml^{-1} + 45 µg ml^{-1}	25.52 c	89.93 a	49.23 c	37.56 a
5.08 mg ml^{-1} + 15 µg ml^{-1}	22.87 c	85.81 a	39.14 c	53.14 a
Crude resin	29.87 c	90.03 a	54.54 c	34.69 a
Distilled water	44.82 a	99.57 a	89.25 a	-
Cypermethrin	35.73 b	99.34 a	71.00 b	14.99 b
CV	12.45	9.27	14.79	33.75

Means followed by the same letter are statistically similar by Scott–Knott test at the 5% significance level.
* Egg laying capacity (ELC) = (weight of egg mass/initial weight of females) × 100
** Product Efficiency (PE) = (Control RE − Product RE/Control RE) × 100
CV: Coefficient of variation. Source: Authors.

Table 5. Effect of the combination of crude resin extracted from the stem of *Protium spruceanum* with cypermethrin on the reproductive parameters of engorged females of Santa Rita *Rhipicephalus microplus* .

resin + cypermethrin combination	ELC*	Hatching (%)	RE** (%)	Efficiency ** (%)
50.15 mg ml^{-1} + 150 µg ml^{-1}	35.78 b	95.29 a	47.98 b	29.67 b
25.09 mg ml^{-1} + 75 µg ml^{-1}	19.77 c	96.63 a	33.43 b	65.47 a
15.05 mg ml^{-1} + 45 µg ml^{-1}	43.26 a	93.21 a	80.63 a	16.71 b
5.08 mg ml^{-1} + 15 µg ml^{-1}	39.41 b	93.11 a	54.63 b	24.57 b
Crude resin	36.10 b	98.46 a	70.07 a	26.58 b
Distilled water	48.40 a	100 a	96.81 a	-
Cypermethrin	50.53 a	89.21 a	89.47 a	07.58 c
CV	11.89	5.788	29.21	62,71

Means followed by the same letter are statistically similar by Scott–Knott test at the 5% significance level.
* Egg laying Capacity (PC) = (weight of egg mass/initial weight of females) × 100
** Product Efficiency (PE) = (Control RE − Product RE/Control RE) × 100
CV: Coefficient of variation .Source: Authors.

According to Novato et al. (2019), combinations (1:1) of thymol + carvacrol (3.125 mg/mL), carvacol + eugenol, and thymol + eugenol (6.25 mg/mL) were ineffective against engorged females of *R. microplus*. Lazcano et al. (2019) used essential oil combinations against engorged females of *R. microplus* containing 66% *Cinnamomum zeylanicum*, 17% *Cuminum cyminum*, and 17% *Pimenta dioica* with efficacy greater than 90%.

In this study, combinations of plant extract and synthetic acaricide at low concentrations retained acaricidal activity in *R. microplus* larvae; this is in contrast to what was observed in engorged females, although the associations increased efficacy levels compared to cypermethrin alone. Larvae are the first stage of contact with the ruminant and initiate the parasitic phase.

Thus, the use of formulations in ruminants that eliminate larvae greatly reduces tick infestation and consequent damage caused by these ectoparasites.

The differences between the results obtained for larvae and mature females of *R. microplus* obtained in this study could be attributed to the morphological and physiological characteristics of the larvae. The larval body surface area is small, thereby making it easier for the products to penetrate the larval cuticle (Sonenshine and Roe, 2014; Vasconcelos et al, 2014).

3.3 Photochemical characterization of *Protium spruceanum* extract and resin

The results of phytochemical analysis showed the presence of secondary metabolites of the following classes: phenolic compounds, tannins, alkaloids, terpenes, flavonoids, and saponins in the EE from leaves (Table 6). However, only tannins, alkaloids, and flavonoids were detected in the crude resin.

Table 6. Phytochemical characterization of *Protium spruceanum* (Benth.) Engl., Burseraceae, leaf extract and crude resin.

Metabolites	Leaf Extract	Crude Resin
Phenolic Compounds	+++	+
Tannins	+++	-
Alkaloids	+	-
Terpenes	++	+++
Flavonoids	+++	-
Saponins	++	++

Source: Authors.

The results to the present study are consistent with those of the study by Menezes Filho et al. (2019) on the hydroethanolic extract of *P. spruceanum* resin, indicating the presence of secondary metabolites of different classes, including tannins, alkaloids, and flavonoids. Another study using the EE from *Protium* sp. leaves indicated the presence of phenolic compounds, flavonoids, and saponins. (Barata et al., 2020). Additionally, Ruedigera et al. (2007) found substances such as terpenes and flavonoids in the essential oil extracted from the leaves and resin of *P. spruceanum* (Ruedigera et al., 2007).

Flavonoids and terpenoids have considerable antiparasitic activities. Figueiredo et al. (2019) attributed the acaricidal activity of ethyl acetate extract from *P. spruceanum* leaves on *R. microplus* to the presence of flavonoids such as catechin and terpenoids such as β-amyrin. Binary triterpene compounds were identified in another study using chloroform extract from *P. heptaphyllum* resin (Maia et al., 2009). Fernández–Salas et al. (2011) demonstrated that tannins had acaricidal activity in *R. microplus*. However, Vasconcelos et al. (2018) tested ethanolic extracts of cerrado plants with low tannin content against the tick *Dermacentor nitens* but did not observe the acaricidal potential of tannins. Although the presence of tannin was not observed in the crude resin from *P. spruceanum*, the presence of other secondary metabolites could contribute to the acaricidal effect on *R. microplus* larvae here demonstrated.

In this study, acaricidal compounds were found both in the EE and crude resin from *P. spruceanum* leaves. The efficacy of these bioproducts was observed in larvae and adult females of *R. microplus* when combinations of these compounds were used. In this way, substances of plant origin can act synergistically with drugs or chemicals already used in tick control, thereby allowing the reduction of doses required for the desired effect.

4. Conclusion

The association between ethanol extract, crude resin from *P. spruceanum* leaves and cypermethrin has implications

for the control of immature stages and engorged females of *R. microplus*. The results of phytochemical analysis showed the presence of phenolic compounds, tannins, alkaloids, terpenes, flavonoids and saponins in the ethanol extract of *P. spruceanum* leaves and for raw resin only tannins, alkaloids and flavonoids were detected.

Finally, it is suggested that these association be tested in non-target organisms, to have more reliability in their use and advance to *in vivo* experiments.

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