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Control of bovine tick [*Rhipicephalus* (*Boophilus*) *microplus*] with *Brunfelsia uniflora* leaf extract

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Abstract

Bovine tick has caused losses in livestock production profitability in Brazil. However, tick control has caused resistance of these ectoparasites against utilized acaricides. Alternative tick controls have been utilizing plants as sources of effective botanical acaricides. *Brunfelsia uniflora* is a Brazilian plant with antimicrobial and antioxidant activity; however, there are no reports on its acaricidal activity. Therefore, this study aimed to evaluate the chemical composition of *B. uniflora* leaf ethanolic extract and its efficiency to control bovine tick *in vitro* and free-living stage *ex situ*. The crude leaf extract was analyzed by gas chromatographer coupled to mass spectrometer (GC-MS) with identification of 17 compounds. The major compounds were phytol (22.96%), 9,12,15-octadecatrienoic acid, ethyl ester (Z,Z,Z) (21.18%), hexadecanoic acid, ethyl ester (12.74%) and vitamin E (8.77%). The crude extract presented acaricidal activity *in vitro* against ingurgitated adult females, larvae and eggs of bovine tick. The $LC_{99.9}$ for larvae was 103.21 mg mL^{-1} in *in vitro* tests and was 100% efficient for *ex situ* larva test (free-living stage). *B. uniflora* leaf extract is an alternative for the control of the bovine tick cycle, mainly in the free-living stage (non-parasitic stage) under field conditions.

Keywords: Manacá; Phytol; 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z); *Rhipicephalus* (*Boophilus*) *microplus*; Alternative acaricide.
Abbreviations: GC/MS_gas chromatographer coupled to mass spectrometer; LC_lethal concentration; $LC_{99.9}$_lethal concentration to eliminate 99.9% of larvae and ticks, LC_{50}_lethal concentration to eliminate 50% of larvae and ticks.

Introduction

Agribusiness has contributed to a third of the Gross Domestic Product of Brazil, and bovine production is one of its main segments with approximately 200 million heads, the largest commercial cattle herd in the world (Gomes et al., 2017). The loss caused by ticks to the livestock production profitability in Brazil is from around 3,2 to 3,4 billion dollars a year (Grisi et al., 2014). *Rhipicephalus* (*Boophilus*) *microplus* Canestrini (Acari: Ixodidae) is an ectoparasite with a 21-day parasitic life cycle in a live host and its free-living stage is up to 300 days in pastures without a live host (Abbas et al., 2014). This tick is responsible for the transmission of hemotozoas such as *Babesia bovis* and *Babesia bigemina*, and *Anaplasma marginata*, which cause diseases and may lead to the animal's death (George, 2000). The tick can cause anemia, mass reduction, milk reduction, natality decrease, mortality increase, labor and material costs, mainly for the treatment of bovine parasite sadness, besides devaluating the bovine leather due to the animal's inflammatory response to the ectoparasite (Graf et al., 2004).

The control of this ectoparasite can be done by vaccines against viruses vectored by ticks, integrated pest management practices, pheromone-based control tools, and biological control agents (Benelli et al., 2016). However, extensive employment of acaricides based on organophosphates, avermectins and pyrethroids still remains the most effective and ready-to-use strategies and the tick's resistance to these acaricides has determined the need of new products (George, 2000; Graf et al., 2004; Abbas et al., 2014, Mgocheki, 2017). Moreover, the utilization of these compounds implies in lower environmental safety with the death of non-targeted organisms and trace residues of these compounds in milk and beef (Singh et al., 2018). Alternative controls have been utilized and, therefore, plant exploration as sources of effective botanical acaricides is promising and should be encouraged (Benelli et al., 2016; Costa-Junior et al., 2016).

Extracts of 55 plants from 26 families were evaluated *in vitro* against *R.* (*B.*) *microplus* (Borges et al., 2011; Raimundo et al., 2017) and the use of tick repellents and acaricides of botanical origin was revised for 83 plant species (Benelli et al., 2016). However, few studies have evaluated the acaricidal effect *in vitro* and in the free-living stage of ticks.

The tick control in the free-living stage with plant extracts can broaden strategies to control this ectoparasite with lower environmental impact in the bovine productive chain (Singh et al., 2018).

Brunfelsia uniflora (Pohl.) D. Don (Solanaceae) is a Brazilian native bush, popularly known as manacá, with exuberant white and purple flowers (Althaus-Ottmann et al., 2006). Antimicrobial and antioxidant activities are related to the oil resin of *B. uniflora* flowers and leaves (Thiesen et al., 2017; Jorge et al., 2017), but there are not reports on its acaricidal activity. The utilization of acaricides from plants has been used such as Essentria^{TM} IC3 that is a low risk commercial pesticide for green pest management and composed of 10% rosemary, 5% geraniol, and 2% peppermint essential oils from plants (Singh et al., 2018). Essential oils are efficient in *in vitro* tick control, but there are still challenges regarding its chemical lability in open environments. On the other hand, plant extracts are more stable and remain in the environment longer, increasing the contact time and the chances of an efficient tick control during the parasitic cycle and the free-living stage. Thus, this study aimed to evaluate the chemical composition of *B. uniflora* leaf ethanolic extract and its efficiency to control bovine tick *in vitro* and in free-living stage *ex situ*.

Results

Soil characterization for** Brunfelsia uniflora **cultivation

The analyzed soil presented 1950 mg dm^{-3} of carbon and V% of 41.91% (Table 1). For Serrat et al. (2002) the values of V% below 50 represent soils with low fertility. The analyzed soil is, therefore, considered little fertile and slightly acid with pH of 5.59 (**Table 1**).

***Compounds in leaf ethanolic extract of** Brunfelsia uniflora*

The chemical analysis of the leaf extract of *B. uniflora* determined by GC-MS revealed the presence of 17 compounds (**Table 2**), and the main ones were: phytol (22.96%), 9,12,15-octadecatrienoic acid, ethyl ester (21.18%), hexadecanoic acid, ethyl ester (12.74%) and vitamin E (8.77%) (**Fig 1**). The fatty acids and fatty acid esters were the main compounds of *B. uniflora* leaf extract, totalizing 56.49%.

Acaricidal effects

B. uniflora leaf extract at concentrations at 500 or 400 mg mL^{-1} killed 90.0 and 46.7% of the female adults, respectively (**Fig 2**), and reduced egg hatchability from 13.6 to 58.2%, respectively, when compared to the negative control (**Fig 3**). The product efficiency for the crude extract at these concentrations was 92.7 and 73.3%, respectively. Therefore, a high extract concentration was necessary to kill female adults and reduce egg hatchability.

The mortality of *R.* (*B.*) *microplus* larvae was 100% when the extract concentration ranged from 500.0 to 100.0 mg mL^{-1} (**Fig 4**). At 50 and 25 mg mL^{-1} the larval mortality was 93.9 and 92.3%, respectively (**Fig 4**). From 12.5 to 1.56 mg mL^{-1} the mortality ranged from 78.4 to 31.7%, respectively. The larval mortality was zero from 0.78 to 0.19 mg mL^{-1} (**Fig 4**). From the *in vitro* mortality data obtained in LPT, the lethal concentrations to kill 50 and 99.9% (LC_{50} and $LC_{99.9}$) of the population were calculated. For the adult females, the concentration that killed 99.9% of the population was 631.89 mg mL^{-1}; however, the concentration to kill larvae was 103.21 mg mL^{-1} (**Table 3**).

The extract at 103.21 mg mL^{-1} was sprayed on *Brachiaria decumbens* leaves containing bovine tick larvae and after 24 h *B. uniflora* efficiency was observed with 100% of ectoparasite deaths (**Fig 5**). The concentration that killed 99.9% of *in vitro* larvae was also enough to kill larvae in free-living stage (*ex situ*). This result suggests that *B. uniflora* extract is efficient to combat bovine tick under similar conditions to the natural ones.

Discussion

Our study is the first report on the acaricidal activity of *B. uniflora* leaf crude extract on *R.* (*B.*) *microplus*. The crude extract concentrations that killed from 50 to 99.9% of the population of ingurgitated adult females were 364.23 mg mL^{-1} and 631.89 mg mL^{-1}, respectively. These values are considered high when compared to the lethal concentrations to kill 99.9% of the larval population which was 103.21 mg mL^{-1}. A greater value of lethal concentration for females is expected since they have thicker cuticle due to the hormonal stimulus in the parasitic phase. The cuticle thickening makes the extract penetration difficult which occurs only through the articulation and natural orifices, making the tick less vulnerable to intoxication (Reeves, 2003), and increasing the lethal concentration for engorged females when compared to larvae.

The acaricidal activity of extracts from Solanaceae has already been demonstrated in *in vitro* studies with *Nicotiana tabacum* (Araújo et al., 2016; Avinash et al., 2017) and *Capsicum frutensis* (Vasconcellos et al., 2014). However, our study is the first report on Solonaceae *B. uniflora* acaricidal activity on *R.* (*B.*) *microplus* free-living stage.

The spraying of *B. uniflora* (103.21 mg mL^{-1}) on *B. decumbens* killed 100% of *R.* (*B.*) *microplus* larvae found in *B. decumbens* leaves. The lethal concentration of the extract on *R.* (*B.*) *microplus* larvae was the same for *in vitro* test in the tick free-living stage. The control of more fragile larvae and with a lower lethal concentration is an important step to reduce the infestation of this ectoparasite. Moreover, 95% of the ticks (ingurgitated females in hatching, incubating eggs and larvae waiting for host) are in the pasture while only 5% (larvae, nymphs and adults) are parasiting bovines (Powell and Reid, 1982). Because these 5% are in the phase that causes direct losses (hematophagism and skin lesions) and indirect ones (bovine babesiosis and anaplasmosis), there is a greater concentration of studies and technologies of this parasitic stage (Chagas et al., 2001).

Borges et al. (2011) and Benelli et al. (2016) reported several extracts and plant oils against *R.* (*B.*) *microplus*. For these authors, the result comparison was difficult mainly because there are no uniform methods and some studies do not cite the lethal concentrations, there are non-uniform sizes of treated specimens, application conditions and experimental

Table 1. Analysis of pH, base saturation, micro and macro nutrients of the soil for the cultivation of *Brunfelsia uniflora*.

pH and Macronutrients								
	cmol/dm^{-3}						mg dm^{-3}	
pH($CaCl_2$)	Al^{3+}	$H^+ + Al^{3+}$	$Ca^{2+} + Mg^{2+}$	K^+	SB*	CEC*	P	C
5.59	0.00	2.29	2.00	0.13	2.13	5.08	3.80	1950
Base saturation	Micronutrients							
	(cmol/dm^{-3})							
V (%)	Fe	Cu	Mn	Zn	B	S	Ca/Mg	Ca/K	Mg/K
41.91	52.67	9.62	81.45	101.46	0.21	9.76	1.67	9.75	5.85

Sum of bases (SB); cation exchange capacity (CEC); base saturation (V), pH in $CaCl_2$ (pH), phosphorus (P), carbon (C), aluminum (Al^{3+}), potential acidity (H^++Al^{3+}), calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K^+).

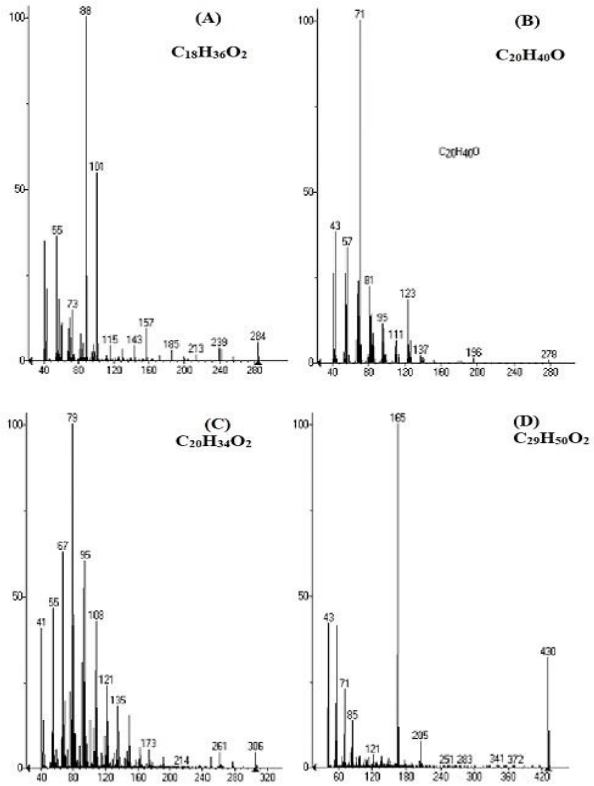


Fig 1. Mass spectra of hexadecanoic acid, ethyl ester (A), phytol (B), 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (C), and vitamin E (D) found in *Brunfelsia uniflora* crude extract obtained by gas chromatographer coupled to mass spectrometer.

Table 2. Chemical composition and area (%) of crude extract from *Brunfelsia uniflora* leaves.

Peak	Compound	RI	Area (%)	Identification Methods
1	n-hexandecanoic acid	1864	3.70	a,b
2	Hexadecanoicacid, ethyl ester	1896	12.74	a,b
3	Phytol	1948	22.96	a,b
4	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	2140	4.19	a,b
5	Oleic acid	2163	5.73	a,b
6	9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	2169	21.18	a,b
7	Linolenic acid ethyl ester	21,96	1.84	a,b
8	n.i.	2748	4.96	a,b
9	Octadecanoic acid, ethyl ester	2748	5.95	a,b
10	n.i.	2797	0.35	a,b
11	Gibberellic acid	2809	0.43	a,b
12	Tetracosanoic acid	2838	0.67	a,b
13	Stigmasterol	2910	0.67	a,b
14	β sitosterol	2974	0.98	a,b
15	Vitamin E	2981	8.77	a,b
16	β carotene	2988	4.41	a,b
17	Octadecanoicacid, 2-hydroxy-1,3-propanediyl ester	2999	0.49	a,b

[a]identification based on retention index (RI) with a homologous series of C_7-C_{28} *n*-alkanes on an Agilent HP-5MS UI; [b]identification based on comparison of mass spectra with Wiley 275 libraries; n.i.: unidentified compound; Compound: compounds listed in order of elution from an HP-5MS column.

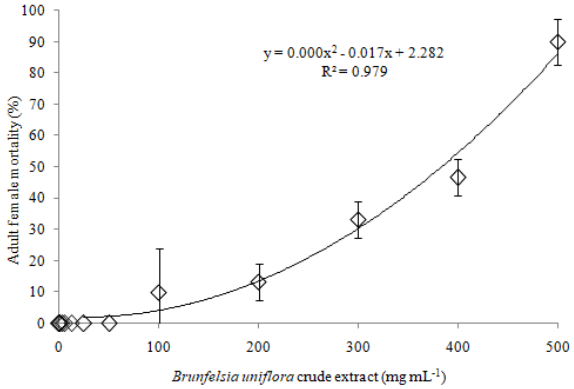


Fig 2. Ingurgitated adult female mortality (%) of *Rhipicephalus* (*Boophilus*) *microplus* subjected to adult immersion test (AIT) at different concentrations of *Brunfelsia uniflora* crude extract. Positive control (commercial solution) = 100% adult female mortality and negative control (2% polysorbate-80) = zero adult female mortality.

Table 3. Lethal concentration (LC_{50} and $LC_{99.9}$), arithmetic mean with standard deviation, and CI of *Brunfelsia uniflora* leaf crude extract on *Rhipicephalus* (*Boophilus*) *microplus* ingurgitated adult females and larvae by Probitos analysis.

Tick	LC_{50} (mg mL^{-1})	CI (mg mL^{-1})	$LC_{99.9}$ (mg mL^{-1})	CI (mg mL^{-1})
Ingurgitated adult female	364.23 ± 26.41	334.34–394.11	631.89 ± 51.61	573.49–690.29
Larva	3.96 ± 0.15	3.79–4.12	103.21 ± 8.64	90.43–112.98

LC_{50} and $LC_{99.9}$ and mg mL^{-1}; confidence interval (CI; $\alpha = 0.05$).

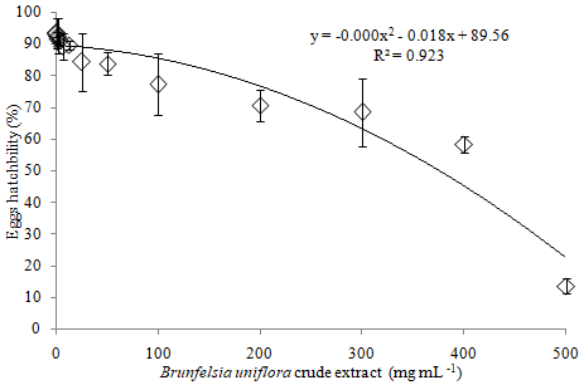


Fig 3. Egg hatchability (%) of *Rhipicephalus* (*Boophilus*) *microplus* subjected to adult immersion test (AIT) at different concentrations of *Brunfelsia uniflora* crude extract. Positive control (commercial solution) = zero egg hatchability and negative control (2% polysorbate-80) = 95.71 ± 2.17% egg hatchability.

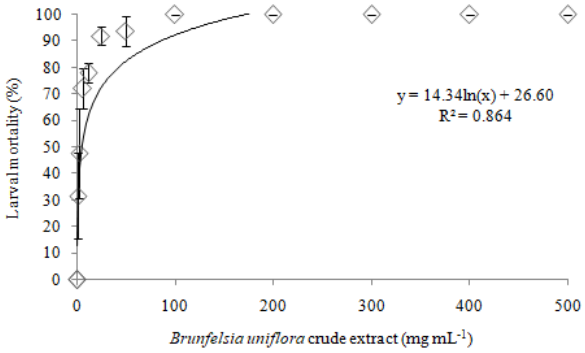
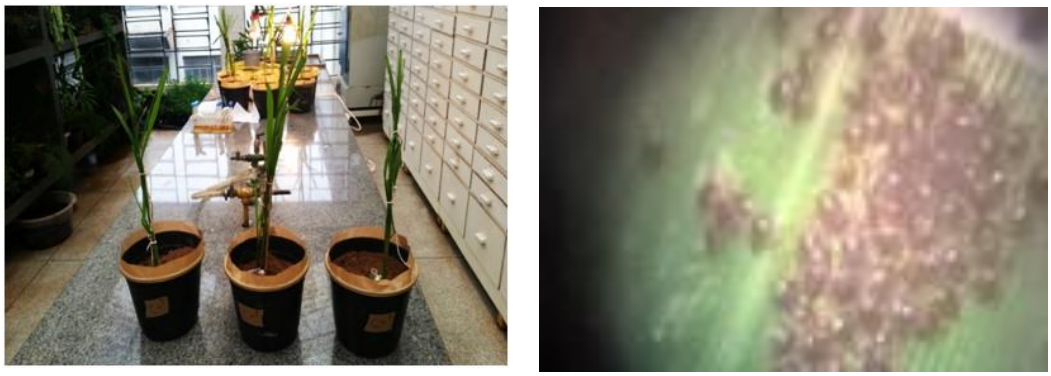


Fig 4. Larval mortality (%) of *Rhipicephalus* (*Boophilus*) *microplus* subjected to larval packet test (LPT) at different concentrations of *Brunfelsia uniflora* crude extract. Positive control (commercial solution) = 100% larval mortality and negative control (2% polysorbate-80) = zero larval mortality.



A **B**

Fig 5. Vases with *Brachiaria decumbens* and *Rhipicephalus* (*Boophilus*) *microplus* larvae sprayed with a solution of *Brunfelsia uniflora* leaf extract (103.21 mg mL^{-1}). (A) Perspective assay photo and (B) an enlarged photo of larvae on leaf edge.

conditions such as temperature and RH are not identical or standardized. Such difficulties were also found to compare the results of *B. uniflora* with extracts from other plants of the same family, besides the fact that most of the studies were done only in *in vitro* tests.

Araújo et al. (2016) reported the acaricidal action of thymol against *R.* (*B.*) *microplus* larvae in the free-living stage. For these authors, when thymol was sprayed on the top on *B. decumbens* leaves, the necessary composition for 99.87% efficiency was 20 mg mL^{-1}. However, in the *in vitro* study by Scoralik et al. (2012), the same mortality percentage was observed starting at a concentration of 2.5 mg mL^{-1}. For Araújo et al. (2016) these differences can be related to the utilized method and the greater dissipation of thymol in open environments.

In our study, the spraying of *B. uniflora* extract (103.21 mg mL^{-1}, $DL_{99.9}$ *in vitro*) on *B. decumbens* killed 100% of *R.* (*B.*) *microplus* larvae found in *B. decumbens* leaves. Essential oils may not have the same efficiency found in *in vitro* test to control ticks when they are exposed to tests in open environments due to the fact that they are volatile compounds that oxidize easily (Borges et al., 2011). On the other hand, crude plant extracts are more stable and less volatile and have better correlation between *in vitro* tests in open environments to control *R.* (*B.*) *microplus*. In this context, the application of *B. uniflora* crude extract on pastures would control infesting tick larvae.

Thus, the use of botanical acaricides as *B. uniflora* extracts is still a suitable alternative for synthetic pesticides. In general, the compounds derived from plants have low toxicity to non-target organisms, high biodegradation rates and low resistance development. Also, botanical pesticides are considered synergically acting mechanisms against ticks (Rattan, 2010; El-Wakeil, 2013).

The larvicidal activity reported in our study for *B. uniflora* leaves can be related to the presence of major compounds, 9,12,15-octadecatrienoic acid, ethyl ester (21.18%) and hexadecanoic acid, ethyl ester (12.74%). Vennila and Udayakumar (2015), Maruthupandian and Mohan (2011) and Jegadeeswari et al. (2012) described the larvicidal, insecticidal, nematicidal, fungicidal and antimicrobial activity of fatty acids as well as fatty acid esters. However, new studies should be carried out to broaden the knowledge on the action of *B. uniflora* leaf crude extract against bovine tick.

Materials and methods

Plant material and soil analysis

B. uniflora leaves were collected at coordinates S23º 07' 44" and W52º 19' 08" and altitude of 635 m. An accession was deposited in the Educational Herbarium of the Paranaense University under the registration number 2855. *B. uniflora* was cultivated in Caiuá sandy soil, classified as Red Distroferric Latosol and characterized by a sandy loam surface with low contents of clay and organic matter (Fidalski et al., 2013). The soil was collected at 0-20 cm depth in different points to obtain a composed sample. For the soil analysis, Ca, Mg, Al were extracted with 1 M KCl; P and K were extracted according to Mehlich method; H+Al was determined by Shoemaker, McLean and Pratt method; carbon by Walkley and Black method; and sum of bases and cation exchange capacity according to Embrapa (2009).

Preparation of** Brunfelsia uniflora **leaf crude ethanolic extract

B. uniflora leaves were harvested from July to August, 2014 – during the reproductive cycle – and dried in ambient temperature in the shade. The dried leaves were submitted to dynamics maceration process with solvent depletion with ethanol (96 ºGL) (Martins et al., 2009). Next, the solvent evaporation was done in a rotary evaporator to obtain the crude ethanolic extract.

Chemical characterization of** Brunfelsia uniflora **leaf crude extract

The chemical identification of the crude extract was done by gas chromatographer coupled to a mass spectrometer (GC-MS; Agilent 19091J-433). The capillary column was HP-5MS

UI 5% (30 m x 0.25 mm x 0.25 µm), with initial temperature from 60 to 250 ºC (3 ºC min^{-1}), from 250 to 300 ºC (10 ºC min^{-1}) for 5 min at 300 ºC. Helium was utilized as the carrier gas at the linear speed of 1 mL min^{-1} up to 300 °C and pressure release of 56 kPa. The injector temperature was 250 °C; the injection volume was 1 µL; the injection occurred in split mode (20:1). The transfer line was kept at 285 °C, the source of ionization and quadrupole at 230 °C and 150 °C, respectively. The EM detection system was utilized in "scan" mode, at the mass/charge rate /load (*m/z*) of 40-550, with "solvent delay" of 3 min. The compounds were identified by comparing the mass spectra found in NIST 11.0 libraries and the obtained retention indices (RI) to a homologous series of n-alkane standard (C7-C28) (Adams, 2012).

Acaricidal activity

Sensitivity of ingurgitated females in adult immersion test (AIT)

The adult immersion test (AIT) was done according to Drumond et al. (1973). Ingurgitated female adult ticks (900) from milk cattle were utilized. The ticks were washed with ultrapure water and selected according to their healthy appearance, whole body and maximum ingurgitation (Leite et al., 1995). The leaf extract was diluted in an aqueous solution of 2.00% polysorbate-80 (mass/volume) at the concentrations of: 500.00; 400.00; 300.00; 200.00; 100.00; 50.00; 25.00; 12.50; 6.25; 3.12; 1.56; 0.78; 0.39 and 0.19 mg mL^{-1}. An aqueous solution of 2.00% polysorbate-80 was utilized as negative control (mass/volume), and a broad action commercial ectoparasiticide solution containing 150.00 mg mL^{-1} of cypermethrin, 250.00 mg mL^{-1} of chlorpyrifos and 10.00 mg $mL^{-1}$of citronellal was utilized as positive control. The commercial solution was diluted in 1.25 mL L^{-1} obtaining a working solution with 0.1875 mg mL^{-1} of cypermethrin, 0.3125 mg mL^{-1} of chlorpyrifos and 0.0125 mg mL^{-1} of citronellal. After spraying the solutions on the adult ticks, the ones that presented absence of motility to touch were considered dead, and the mortality percentage of ticks was calculated. The female adult ticks that did not die after 14 days of the solution spraying continued with egg lying. The egg mass was measured and stored in assay tubes for hatching and the results of extract concentrations and positive and negative controls were recorded. The reproductive efficiency (RE) and the product efficiency (PE) were calculated from the mass of ingurgitated adult female ticks, egg mass and egg hatching rate, according to Equations 1 and 2 (Drumond et al., 1973).

RE = Egg mass (g) × Hatching rate (%) × 20000 / Female tick mass (Equation 1)

PE = RE negative control group × Retreated group × 100 / RE negative control group

(Equation 2)

The lethal concentrations that killed 50% (LC_{50}) and 99.9% ($LC_{99.9}$) of the population of ingurgitated adult female ticks with the respective Confidence Intervals (CI) were calculated by Probitos analysis (ED 50 Plus version 1.0). All the tests were done in triplicate.

Larvicidal activity of** Brunfelsia uniflora **leaf extract on Larval Packet Test (LPT)

For the Larval Packet Test (LPT), *R.* (*B.*) *microplus* larvae were placed in sealed filter paper packages (Leite et al. 1995). The extract was applied at concentrations of 500.00; 400.00; 300.00; 200.00; 100.00; 50.00; 25.00; 12.50; 6.25; 3.12; 1.56; 0.78; 0.39 and 0.19 mg mL^{-1} (mass/volume). The positive and negative controls were the same utilized in AIT. The larvae that were motionless to touch were considered dead after 24 h (Leite et al., 1995) and the percentage of larval mortality was calculated.
The lethal concentrations that killed 50% (LC_{50}) and 99.9% ($LC_{99.9}$) of the population of tick larvae with respective CI were calculated by Probitos analysis (ED 50 Plus version 1.0). All the tests were done in triplicate. The extract concentration with $LC_{99.9}$ obtained for the control of tick larvae was utilized in the subsequent test for *ex situ* control of the ectoparasite in vases.

Ex situ ***larvicidal activity (free-living stage) of** Brunfelsia uniflora **leaf extract***

Plastic vases (n = 9) with 25 cm of height and 25 cm of diameter were filled up with 2.2 kg of soil, previously autoclaved at 121 ºC for 2 h. In each vase six seeds of *B. decumbens* were planted at 2 cm of depth. The plants were kept in greenhouse for three months with irrigation. The leaves of *B. decumbens* were trimmed at 40 cm from the soil surface and adhesive tape was placed on the edge of the vases as a physical barrier to contain larvae (30 mg) deposited on the soil surface of each plant/vase. After 24 h, the larval migration to the apex of the grass leaves was observed (Araújo et al. 2015). Each treatment consisted of a group of three vases. The obtained $LC_{99.9}$ in LPT (*in vitro* test) for *B. uniflora* crude ethanolic extract was utilized for the treated group. A commercial ectoparasiticide solution was used as positive control and an aqueous solution of 2.00% polysorbate-80 (mass/volume) was utilized as negative control at the same concentrations utilized for LPT and AIT. For each treatment, 4 mL of the solution per vase was sprayed starting in the top part of the plant and going all the way down once, simulating the application of commercial acaricides in pastures. After 24 h the grass leaves were trimmed with the help of entomological lens. The larvae that did not show movement when touched were considered dead. Next, the averages of live larvae in the negative control group and live larva group treated with *B. uniflora* extract were determined. From these data, the efficiency of *B. uniflora* extract (%) on tick larvae was calculated as in Equation 3 and according to Bittencourt et al. (2003).

Efficiency of *B. uniflora* extract (%) = ((A - B) / A) × 100 (Equation 3)

where A = the average of live larvae in the negative control group and B= average of live larvae in the treated group with *B. uniflora* extract.

Statistical analysis for the acaricidal and larvicidal activity

The experiment had a completely random design. The data were submitted to analysis of variance (ANOVA) and the differences between the arithmetical averages and the standard deviation were determined by Tukey's test at 5% significance. The lethal concentrations that killed 50% (LC_{50}) and 99.9% ($LC_{99.9}$) of adults or tick larvae and the respective CI (α = 0.05) were calculated by Probitos analysis (ED 50 Plus version 1.0). All the tests were carried out in triplicate.

Conclusion

The major compounds of *B. uniflora* leaf crude extract were phytol (22.96%), 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z) (21.18%), hexadecanoic acid, ethyl ester (12.74%) and vitamin E (8.77%). The crude extract presented acaricidal activity *in vitro* against ingurgitated adult females, larvae and eggs of bovine tick. For larvae $LC_{99.9}$ was 103.21 mg mL^{-1} in *in vitro* test and presented 100% efficiency in the death of larvae in *ex situ* test (free-living stage). *B. uniflora* leaf crude extract is an alternative to control bovine tick cycle, mainly in the free-living stage (non-parasitic stage).

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Disclosure statement

No potential conflict of interest was reported by the author.

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