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Research paper

Acaricidal activity of *Acmella oleracea* (Asteraceae) extract against *Rhipicephalus microplus*: What is the influence of spilanthol?

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ABSTRACT

The present study was carried out to evaluate and compare the acaricidal activity of different fractions of *Acmella oleracea* methanolic extract, containing 0.0 % (F1), 24.5 % (F2), 48.0 % (F3) and 100 % (F4) of spilanthol, on unfed larvae and engorged females from the same *Rhipicephalus microplus* population. To obtain these fractions, the crude extract was subjected to different extraction procedures using increasingly polarized solvents to isolate the spilanthol compound. The Larval Packet Test was used to evaluate acaricidal activity in unfed larvae at concentrations ranging from 0.2 to 25.0 mg/mL, while for engorged females, the Adult Immersion Test was performed at concentrations from 3.1 to 25.0 mg/mL. The F1 fraction showed no activity on unfed larvae, while a control percentage of 44.6 % was observed at a concentration of 25.0 mg/mL for engorged females. For unfed larvae, the F2 fraction resulted in 95.7 % mortality at a concentration of 1.6 mg/mL, with a control percentage of 92.7 % for engorged females at a concentration of 12.5 mg/mL. Fractions F3 and F4 had similar activity against unfed larvae, with mortality > 84.0 % from the concentration of 0.8 mg/mL. This similarity between the fractions was also observed for engorged females from a concentration of 12.5 mg/mL, resulting a control percentage > 94.0 %. These results demonstrate that the presence of spilanthol is an important factor for the acaricidal activity of *A. oleracea* extract. Fraction extracts with 24.5, 48 and 100 % of spilanthol have similar acaricidal activity on *R. microplus*.

1. Introduction

Rhipicephalus microplus (Canestrini, 1888) is a hematophagous ectoparasite of the family Ixodidae with wide geographic distribution. This tick, which has cattle as its preferred host, is of great economic importance as it limits successful cattle production, given that parasitized animals show reduced weight gain, decreased milk production, and leather depreciation (Graf et al., 2004; Ghosh et al., 2007; Grisi et al., 2014).

Currently, *R. microplus* is controlled using synthetic acaricides belonging to different chemical groups; however, the continuous use of these products has led to the selection of resistant populations (Furlong et al., 2007; Pereira, 2008). There are records of populations resistant to pyrethroids, amidines, organophosphates, macrocyclic lactones, phenylpyrazols and benzoylphenylureas (Reck et al., 2014), as well as of multidrug-resistant populations (Klafke et al., 2016).

Natural products obtained from plant secondary metabolism have been studied in order to select compounds with potential for the development of new acaricides. These compounds have proved to be an interesting alternative in controlling this ectoparasite, because they have varied mechanisms of action and, generally, little mammalian toxicity (Koul et al., 2008; Pavela and Benelli., 2016; Jankowska et al.,

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2017; Khater et al., 2018; Abbas et al., 2018; Fayaz et al., 2019). Studies conducted with *Acmella oleracea* (L.) RK Jansen (Astereacea) (synonym - *Spilanthes acmella* and *Acmella ciliata*), commonly known as Jambu, have shown that active compounds found in extracts of this plant present potential for the development of botanical acaricides (Marchesini et al., 2018).

A. oleracea is a plant native to the Amazon region, and may also occur in other tropical and subtropical regions of the world. In Brazil, Jambu is widely used in local cuisine as a spice for traditional dishes from the North region of the country, and in traditional medicine, as an analgesic, skin anesthetic and antiviral drug (Torres and Chávez, 2001;). Studies have shown that *A. oleracea* extract presents antibacterial (Alcantara et al., 2014; Sudevan et al., 2015), antifungal (Rani and Murty, 2006; Khatoon et al., 2014), antimalarial (Pandey and Agrawal, 2009), insecticide (Amer and Melhorn, 2006; Benelli et al., 2019) and anesthetic (Chakraborty et al., 2010; Barbas et al., 2016) activities. Regarding ticks, it has been shown that different types of *A. oleracea* extracts (ethanolic, hexane, and methanolic) show activity on *R. microplus* (Cruz et al., 2016; Oliveira et al., 2016), *Dermacentor nitens*, Neuman, 1897 (Cruz et al., 2016), *Amblyomma sculptum* Berlese, 1888 (Marchesini et al., 2018) and *Rhipicephalus sanguineus* sensu lato (sl) (Oliveira et al., 2018).

Phytochemical analyses have shown that *A. oleracea* extracts contain alkylamides, coumarin, triterpenoids, and phenolic compounds. Among these compounds, spilanthol, an *N*-alkylamide, is believed to be the major secondary metabolite responsible for biological activity on bacteria, protozoa, helminths, and arthropods (Prachayasittikul et al., 2013). In some studies, addressing the activity of *A. oleracea* extract on ticks, no quantification of spilanthol was performed (Anholeto et al., 2017a, 2017b; 2018; Oliveira et al., 2016, 2018). In other studies, with *R. microplus*, in which the percentage of this *N*-alkylamide was quantified, a large variation in the percentage of spilanthol (0.19–14.8 %) was observed (Castro et al., 2014; Cruz et al., 2016).

In these studies, there are also variations in the extract type (ethanolic, hexane, or methanolic), target tick species and populations, test methodologies, and solvents used as a vehicle. This hinders data comparison between the studies, which would allow a better understanding of the importance of spilanthol for acaricidal activity of *A. oleracea* extracts. Therefore, the present study aimed to evaluate the acaricidal activity of different fractions of *A. oleracea* methanolic extract, containing different percentages of spilanthol, on unfed larvae and engorged females from the same *R. microplus* population.

2. Material and methods

2.1. Collection and identification of the plant material

Aerial parts of *Acmella oleracea* (Jambu) were collected in the municipality of Igarapé-Açu, located in the Bragantina region of the state of Pará, Brazil (01°07'33" S; 47°37'27" W). A sample of the plant was identified as *Acmella oleracea* and deposited (MG205534) in the herbarium of the Emílio Goeldi Museum, in the city of Belém, state of Pará, Brazil.

2.2. Preparation of the fractions

The process of obtaining *A. oleracea* fractions was performed according to Barbosa et al. (2016). Four fractions were obtained and classified as: F1, F2, F3 and F4. *A. oleracea* methanolic extract obtained from leaves, stems and inflorescences was first solubilized in MeOH/H_2O (8:2), and the solution was then subjected to successive extractions in a separatory funnel with the solvents: hexane, dichloromethane and ethyl acetate. For each solvent, four successive and sequential extractions (1, 2, 3, and 4) were performed, and the chemical profile of each fraction was analyzed by Gas Chromatography Mass Spectrometry (GC–MS). Regarding hexane, extractions 1 and 2 were pooled, forming fraction F2, while extractions 3 and 4 were pooled, to form fraction F3 (Fig. 1). The contents of the four dichloromethane extractions (1, 2, 3, and 4) were pooled into a single fraction (F4). The final residue obtained after ethyl acetate extractions was also pooled into a single fraction (F1) (Fig. 1).

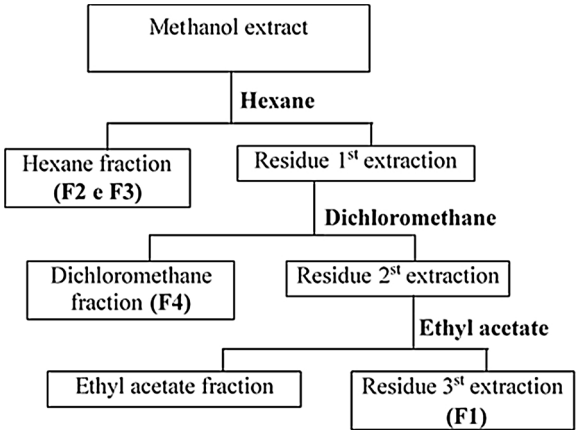

Fig. 1. Fractionation of the *Acmella oleracea* methanol extract.

2.3. Chemical analyses

The fractions obtained with hexane (F2 and F3) and dichloromethane (F4) were analyzed using a Gas Chromatograph coupled to a Mass Spectrometer (CG-MS Shimadzu, QP-2010 model) and by Nuclear Magnetic Resonance (NMR ^{1}H and ^{13}C, Bruker, 500 MHz). The residual fraction (F1) was analyzed only by the NMR ^{1}He ^{13}C technique. The percentage of each component of fractions F2, F3 and F4 was calculated by the integral area under the respective peaks in comparison to the total area of all sample components presented in the chromatograms, according to Barbosa et al. (2016).

2.4. Dilution of the samples

The samples were diluted in 50 % ethanol + 1% DMSO (dimethyl sulfoxide) using an ultrasonic washer (UNIQUE - USC 1400). For the unfed larvae, fractions F1, F2, F3 and F4 were tested at concentrations of 0.2, 0.4, 0.8, 1.6, 3.1, 6.2, 12.5 and 25.0 mg/mL, whereas for engorged females, the concentrations were 3.1, 6.2, 12.5 and 25.0 mg/mL.

2.5. Ticks

Ticks were obtained from naturally infested cattle in the municipality of Lima Duarte, state of Minas Gerais, Brazil. Larvae between 15 and 21 days after hatching and engorged females freshly detached from the host were used in the experiments.

2.6. Larval packet test (LPT)

The larval packet test (LPT) proposed by Stone and Haydock (1962) and modified by Monteiro et al. (2012) was used. In this method, approximately 50–100 unfed larvae were placed in the center of a filter paper (6cm × 6cm - Whatman n°01). The filter paper was then folded in half and closed on the sides with binder clips. Subsequently, each side of the filter paper was moistened with 90 µL, totaling 180 µL of the tested concentrations (one treatment per concentration). A control group was also formed, in which the ticks were treated with solvent (50 % ethanol + 1% DMSO), and 10 replicates were realized for each treatment. After that, the packets containing the ticks were stored in a B.O.D (Biological Oxygen Demand) incubator at 27 ± 1 °C and RH >

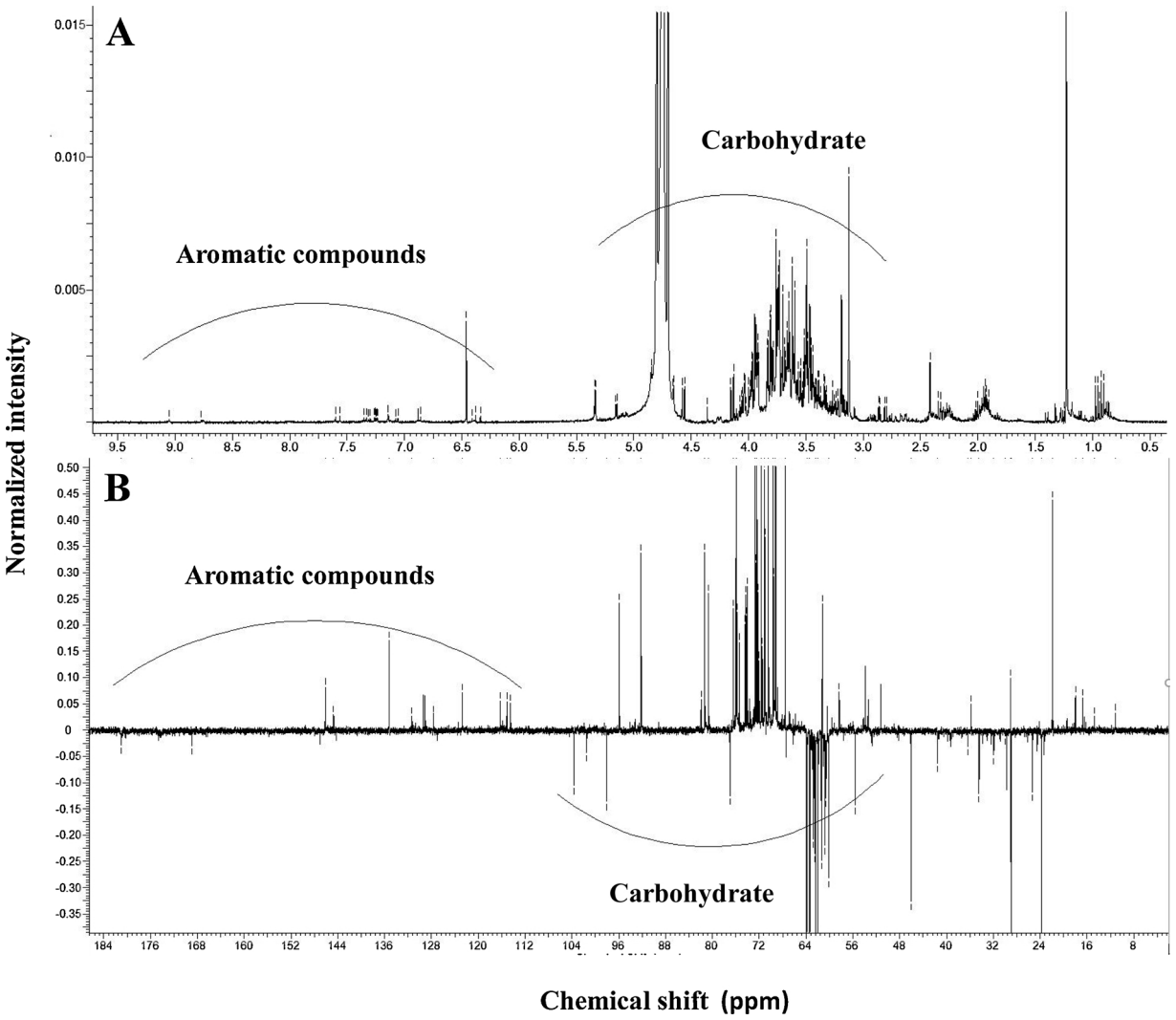

Fig. 2. Residual fraction spectrum (F1) of *Acmella oleracea*. A: RMN ^{1}H (500 MHz, D_2O); B: RMN ^{13}C (125 MHz, D_2O).

80 ± 10 % for 24 h. After this period, the dead and live larvae were counted to obtain the mortality percentage.

2.7. Adult immersion test (AIT)

In the adult immersion test (AIT), 17 groups with 15 engorged females with statistically homogeneous weights ($p > 0.05$) were immersed for five minutes in the solutions tested. After immersion, each female was weighed individually and kept on a Petri dish (6 × 6 cm) for oviposition. The groups were kept in a B.O.D incubator under the previously mentioned conditions to evaluate the reproductive biology. The following biological parameters were evaluated: female weight before oviposition, egg mass weight, and larval hatching. From these values, the control percentage was calculated according to Drummond et al. (1973).

2.8. Statistical analysis

The statistical analyses used in both tests were carried out using Biostat 5.0 software (Ayres et al., 2007). The treatments were compared by analysis of variance (ANOVA) followed by the Tukey test. In the case of nonparametric distribution, values were compared by the Kruskal-Wallis test followed by the Student-Newman-Keuls test. Probit analysis was performed to calculate the lethal concentration 50 (LC_{50}) using the POLO-PC® software (LeOra Software, 1987, Berkeley, CA, EUA).

3. Results

3.1. Chemical analyses

Fraction F1 showed no spilanthol in its chemical composition. In this fraction, polar substances such as carbohydrates and phenolic compounds were observed, according to NMR ^{1}H and ^{13}C spectral data (Fig. 2). The GC–MS chromatograms showed spilanthol peak retention times t_R (x) of 12,867, 12,892 and 12,094 (in min.), and percentages of 24.5, 48.0 and 100.0 % in fractions F2 (Hexane), F3 (Hexane) and F4 (Dichloromethane), respectively (Fig. 3). The mass spectrum of these fractions showed as main peaks in *m/z* (%): ($M^{\cdot +}$, 2) 221, 141 (M-C_6H_8, 50), 126 ($[C_7H_{12}NO]^+$, 40), 98 ($[C_5H_8NO]^+$, 35), 81 ($C_6H_9^+$, 100), which are in accordance with the structure of this *N*-alkylamide. The structural proposal was confirmed both through NMR ^{1}H and ^{13}C spectral analysis and through comparison with the literature (Nakatani and Nagashima, 1992; Barbosa, 2016).

3.2. Larval packet test (LPT)

Fraction F1 (= 0% spilanthol), even at the highest concentration (25 mg/mL), showed no activity against *R. microplus* unfed larvae, whereas fraction F2 (= 24.5 % spilanthol) resulted in 54.8 % mortality at a concentration of 0.8 mg/mL, with differences ($p < 0.01$) compared with the control group, in which no larval mortality occurred. In the

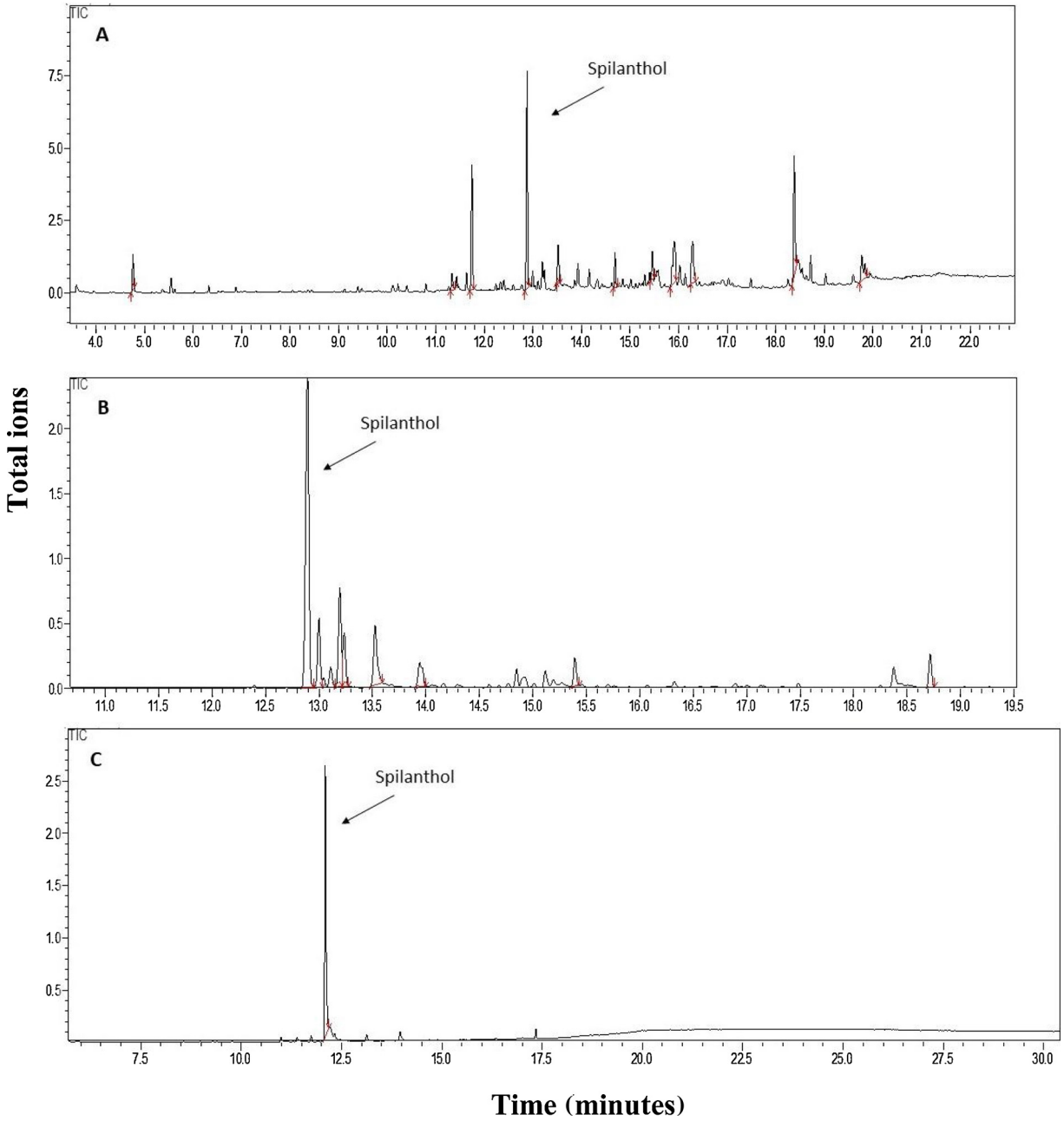

Fig. 3. Gas chromatography coupled to mass spectrometry chromatograms of the *Acmella oleracea* fractions. A - F2 (Hexane fraction); B - F3 (Hexane fraction); C - F4 (Dichloromethane fraction).

other concentrations (1.6–25 mg/mL) with fraction F2, the mortality percentage ranged from 95.7 to 100%. For fractions F3 (= 48 % spilanthol) and F4 (= 100 % spilanthol), differences ($p < 0.05$) in larval mortality were observed in relation to the control group, from the concentration of 0.4 mg/mL, with values of 25.9 and 84.8, respectively. In the other concentrations, for these two fractions, the mortality percentage ranged from 84.8 to 100.0% (Table 1).

For unfed larvae, the LC_{50} values of fractions F2 (= 24.5 % spilanthol), F3 (48.0 % spilanthol) and F4 (100.0 % spilanthol) were 0.7; 0.5, and 0.3 mg/mL, respectively. The absence of overlap in the confidence intervals reveals significant differences between the LC_{50} of the tested fractions, with greater activity for F4, followed by F3 and F2 (Table 3).

3.3. Adult immersion test (AIT)

Female weight before oviposition did not differ statistically between treatments ($p = 0.98$). No statistically significant differences ($p > 0.05$) were observed for egg mass weight and hatching percentage of groups treated with fraction F1 (= 0.0 spilanthol) compared with the control group. In these treatments, the egg mass weight of females ranged from 70.3 to 89.0 mg and larval hatching from 60.4 to 72.1 %, whereas in the control group, values of 94.5 mg (egg mass weight) and 82.7 % (larval hatch percentage) were observed. The control percentage at the highest concentration (25 mg/mL) was 44.6 % (Table 2).

In fraction F2 (= 24.5 % spilanthol), was observed difference ($p < 0.05$) in the egg mass weight of the group treated with the concentration of 25 mg/mL (32.7 mg), compared with the control (94.5 mg). For larval hatching, all extract concentrations (3.1–25.0 mg/mL) resulted in values lower (0.0–47.0 %; $p < 0.01$) than that observed in

Table 1
Mortality (Mean ± standard deviation) of unfed *Rhipicephalus microplus* larvae treated with different concentrations of *Acmella oleracea* fractions (F1, F2, F3 and F4), with different spilanthol percentages, under laboratory conditions (27 ± °C and 80 ± 10 % RH).

Treatments	Fraction F1 0.0 % of spilanthol	Fraction F2 24.5 % of spilanthol	Fraction F3 48 % of spilanthol	Fraction F4 100% of spilanthol
Control	0.0^{a} ± 0.0	0.0^{a} ± 0.0	0.0^{a} ± 0.0	0.0^{a} ± 0.0
0.2 mg/mL	0.0^{a} ± 0.0	0.0^{a} ± 0.0	0.6^{a} ± 1.0	11.0^{a} ± 5.4
0.4 mg/mL	0.0^{a} ± 0.0	5.7^{a} ± 2.5	25.9^{b} ± 7.1	72.0^{b} ± 8.2
0.8 mg/mL	0.0^{a} ± 0.0	54.8^{bc} ± 2.6	84.8^{c} ± 4.0	84.0^{bc} ± 5.4
1.6 mg/mL	0.0^{a} ± 0.0	95.7^{c} ± 1.8	100.0^{c} ± 0.0	100.0^{c} ± 0.0
3.1 mg/mL	0.0^{a} ± 0.0	100.0^{c} ± 0.0	100.0^{c} ± 0.0	100.0^{c} ± 0.0
6.2 mg/mL	0.0^{a} ± 0.0	100.0^{c} ± 0.0	100.0^{c} ± 0.0	100.0^{c} ± 0.0
12.5 mg/mL	0.0^{a} ± 0.0	100.0^{c} ± 0.0	100.0^{c} ± 0.0	100.0^{c} ± 0.0
25.0 mg/mL	0.0^{a} ± 0.0	100.0^{c} ± 0.0	100.0^{c} ± 0.0	100.0^{c} ± 0.0

Different letters in the same column mean significant differences at the level of 5%.
Control = ethanol 50 % + DMSO 1%.
F1: Residual fraction; F2 and F3: Hexane fraction; F4: Dichloromethane fraction.

the control group (82; 7 %). Control percentages at concentrations of 3.1 and 6.2 mg/mL were 45.6 and 54.0 %, while the highest concentrations (12.5 and 25.0 mg/mL) were 92.7 and 98.9 %, respectively (Table 2).

All concentrations of fraction F3 (= 48 % spilanthol) resulted in lower values ($p < 0.05$) for egg mass weight and hatching percentage, ranging from 33.6 to 53.9 mg (egg mass weight) and 13.0 to 47.5 % (larval hatching). No oviposition was observed at the highest concentration, because all females had died before starting the process. The percent control ranged from 66.6 to 100 % (Table 2).

In tests performed with fraction F4 (= 100.0 % spilanthol), values lower than those of the control group ($p < 0.01$) for egg mass weight (28.9 and 5, 7 mg) were observed only at the two highest concentrations (12.5 and 25.0 mg/mL), whereas for larval hatching, all concentrations caused reductions ($p < 0.05$), with values ranging from 13.5 to 20.6%. At the highest concentration (25.0 mg/mL), no larvae hatched. The control percentages were 79.6, 86.1, and 94.9 % at the first three concentrations (3.1, 6.2, and 12.5 mg/mL), respectively, reaching 100 % at concentration of 25 mg/mL (Table 2).

The LC_{50} values of fractions F1, F2, F3 and F4 on engorged females were 48.5, 4.4, 2.1, and 1.1 mg/mL, respectively. Considering the overlap of the confidence intervals (Table 3), fraction F1 (= 0% spilanthol) differed from the others (F2, F3, and F4), showing lower acaricidal activity.

4. Discussion

Currently, ecofriendly alternatives for the control of ticks and other arthropods have been widely investigated, seeking to develop sustainable pest control strategies with low environmental toxicity (Pavela and Benelli, 2016). Results from studies conducted with extracts of *A. oleracea*, which is considered the most important native plant of the Amazon region (Rebello and Homma, 2005), have shown the potential of this plant for development of botanical acaricides (Castro et al., 2014; Oliveira et al., 2018; Marchesini et al., 2018). In the present study, we present results for the influence of spilanthol on the acaricidal activity of *A. oleracea* extract on ticks.

Phytochemical studies conducted with *A. oleracea* have revealed some diversity in the identification of the compounds found in extracts of this plant. In extraction processes, where the F1 (residual), F2 (hexane) and F4 (dichloromethane) fractions were obtained, the spilanthol percentages were 0.0, 24.5, and 100 %, respectively, with this variability possibly being associated with the solvents used at each extraction stage. The present study used the liquid-liquid extraction process, in which the plant components are carried according to solvent affinity, using solvents with increasing polarities. Spilanthol has an amphiphilic character, with one hydrophobic pole and one hydrophilic pole, and can be extracted using solvents with different polarities such as ethanol, hexane, and methanol (Ramsewak et al., 1999; Boonen et al., 2010a, 2010b). In this study, a crude extract was obtained, which was diluted in methanol/water, followed by a more apolar solvent (hexane – F2), and by solvents with intermediate polarity (dichloromethane and ethyl acetate), generating a final residue (fraction F1) rich in polar compounds (carbohydrates and aromatic compounds). These successive stages applying different solvents enabled the complete removal of spilanthol, which was initially present in the crude extract.

Comparison between fractions F2 and F3, which were obtained

Table 2
Weight of females before laying (mg), egg mass weight (mg), larval hatch (%) and percent control of *Rhipicephalus microplus,* treated with different concentrations of *Acmella oleracea* fractions, with different spilanthol percentages, under laboratory conditions (27 ± °C and 80 ± 10 % RH) (Mean ± standard deviation).

Fraction and spilanthol percentage	Treatments - Concentrations	Female weight before oviposition (mg)	Egg mass weight (mg)	Larval hatching (%)	Percent control (%)
	Control(n)	211.2^{a} ± 36.7(15)	94.5^{a} ± 24.5 (15)	82.7^{a} ± 7.8 (15)	…
F1 0.0 %	3.1 mg/mL (n)	208.4^{a} ± 17.8 (15)	89.0^{a} ± 18.8 (15)	72.1^{a} ± 16.4 (15)	16.9
	6.2 mg/mL (n)	208.3^{a} ± 18.9 (15)	85.5^{a} ± 26.3 (15)	73.7^{a} ± 15.1 (15)	18.3
	12.5 mg/mL (n)	207.1^{a} ± 11.2 (15)	84.0^{a} ± 19.2 (15)	70.0^{a} ± 18.4 (04)	23.3
	25.0 mg/mL (n)	207.1^{a} ± 26.1 (15)	70.3^{a} ± 17.16 (15)	60.4^{a} ± 22.5 (15)	44.6
F2 24.5%	3.1 mg/mL (n)	207.0^{a} ± 18.2 (15)	88.7^{a} ± 10.2 (15)	47.0^{b} ± 16.0 (15)	45.6
	6.2 mg/mL (n)	207.7^{a} ± 14.8 (15)	87.4^{a} ± 12.6 (15)	40.5^{b} ± 16.9 (15)	54.0
	12.5 mg/mL (n)	207.6^{a} ± 20.4 (15)	68.0^{ab} ± 32.6 (15)	8.2^{c} ± 5.4 (09)	92.7
	25.0 mg/mL (n)	208.2^{a} ± 19.1 (15)	32.7^{b} ± 27.5 (15)	2.5^{c} ± 2.2 (06)	98.9
F3 48 %	3.1 mg/mL (n)	207.5^{a} ± 20.2 (15)	53.9^{b} ± 36.6 (15)	47.5^{b} ± 14.8 (08)	66.6
	6.2 mg/mL (n)	208.6^{a} ± 20.7 (15)	52.8^{b} ± 27.8 (15)	28.3^{ab} ± 15.4 (09)	80.6
	12.5 mg/mL (n)	207.8^{a} ± 20.3 (15)	33.6^{b} ± 39.2 (15)	13.0^{b} ± 5.7 (05)	94.3
	25.0 mg/mL (n)	207.6^{a} ± 17.7 (15)	… (15)	… (0)	100.0
F4 100 %	3.1 mg/mL (n)	208.3^{a} ± 18.4 (15)	75.9^{a} ± 30.6 (15)	20.6^{b} ± 6.7 (09)	79.6
	6.2 mg/mL (n)	208.8^{a} ± 21.2 (15)	61.7^{a} ± 38.9 (15)	17.3^{b} ± 11.7 (08)	86.1
	12.5 mg/mL (n)	209.1^{a} ± 24.9 (15)	28.9^{b} ± 25.7 (15)	13.5^{b} ± 9.1 (07)	94.9
	25.0 mg/mL (n)	209.0^{a} ± 24.8 (15)	5.7^{b} ± 18.25 (15)	0.0^{b} ± 0.0 (3)	100.0

Different letters in the same column mean, for the same fraction, significant differences at the level of 5%. Treatments with different concentrations of the same fraction was compared with control group.
(n) Sample size.
Control = ethanol 50 % + DMSO 1%.
F1: Residual Fraction; F2 and F3: Hexane Fraction; F4: Dichloromethane fraction.

Table 3
Lethal concentration 50 % (LC_{50}) of *Acmella oleracea* fractions with different spilanthol percentages, against unfed larvae and engorged females of *Rhipicephalus microplus*.

Tick	*Acmella oleracea* fraction/ spilanthol concentration	LC50	Confidence interval	*p* value
Unfed larvae	Fraction F1 – 0.0 % of spilanthol	…	…	…
	Fraction F2 – 24.5 % of spilanthol	0.74[a]	0.62–0.89	< 0.01
	Fraction F3 – 48.0 % of spilanthol	0.52[b]	0.46–0.61	< 0.01
	Fraction F4 – 100.0 % of spilanthol	0.35[c]	0.28–0.44	< 0.01
Engorged female	Fraction F1 – 0.0 % of spilanthol	48.58[a]	38.67–272.15	< 0.01
	Fraction F2 – 24.5 % of spilanthol	4.40[b]	2.88–5.84	< 0.01
	Fraction F3 – 48.0 % of spilanthol	2.10[b]	1.00–4.39	< 0.01
	Fraction F4 – 100.0 % of spilanthol	1.09[b]	0.24–4.94	< 0.01

LC50 values followed by different letters in the column show significant differences, due to the absence of confidence interval overlap.
F1: Residual Fraction; F2 and F3: Hexane Fraction; F4: Dichloromethane fraction.

using hexane, showed a difference in the percentages of spilanthol (F2 = 24.5 % / F3 = 48.0 %). The difference in the percentages of spilanthol present in hexane fractions may be associated with the majority of fatty compounds that come out in the first extractions (1 and 2) with hexane, thus reducing the spilanthol content in these initial samples that generated fraction F2 (Barbosa et al., 2016).

All fractions of Jambu extract containing spilanthol (F2, F3, and F4) showed high acaricidal activity on unfed *R. microplus* larvae and engorged females, whereas the fraction without spilanthol (F1) showed no activity on larvae and low activity on females. These results reinforce data available in the literature, indicating that spilanthol is the main compound responsible for the biological activities of *A. oleracea* extracts (Cruz et al., 2016). Also regarding this aspect, it is worth mentioning that in the treatment with fraction F1, no larvae died at the highest concentration (25.0 mg/mL), whereas in the study conducted by Cruz et al. (2016), using a fraction of *A. oleracea* with 0.19 % spilanthol, 100 % mortality at the concentration of 3.1 mg/mL was observed. This not only reinforces the importance of spilanthol, but also demonstrates that the presence of this amide in small quantities results in high acaricidal activity. One of the action mechanisms described for spilanthol in arthropods is associated with its ability to act on the nervous system, modifying muscle activity, as well as disrupting histogenic and histolytic processes (Saraf and Dixit, 2002).

In contrast, comparison of the activity of the *A. oleracea* extracts with different spilanthol percentages (F2, F3, and F4), considering the LC_{50} values, showed that higher percentages of spilanthol did not increase the acaricidal activity on engorged females, and led to slightly increased efficacy on unfed larvae. These data show that the variation in spilanthol percentage in the fractions of *A. oleracea* extract used in the present study had little influence on the increase of acaricidal activity. Cruz et al. (2016) found that pure spilanthol presented higher activity than the *A. olereacea* extract with 0.19 % spilanthol on *R. microplus* engorged females and unfed larvae. This difference is possibly related to the variation of spilanthol in the tested samples, which was smaller in the present study (24–100 %), while in the research conducted by Cruz et al. (2016), the variation was 0.19–100 %.

In *in vitro* studies conducted with plant extracts and essential oils for *R. microplus* control, the results have generally shown that unfed larvae are more susceptible than engorged females. However, regarding the tests with fraction F1, which was rich in carbohydrates such as monosaccharides, oligosaccharides, polysaccharides and phenolic compounds, interestingly, the opposite was observed, with better activity on engorged females (44 % efficacy) compared with that on unfed larvae (0 % efficacy). These results indicate that there are compounds present in fraction F1 that have different action mechanisms that act on the reproductive biology of engorged females, but do not cause larval mortality. As previously mentioned, this is a fraction (F1) rich in carbohydrates and phenolic compounds, for which data from the literature on biological activities are still scarce. One of the few relevant studies was conducted by Nascimento (2012), who reported antinociceptive action of a extract without the presence of spilanthol or another amide. Nascimento suggested that the antinociceptive activity would be associated with the presence of these other compounds. Finally, it is interesting to mention that, in some studies carried out with essential oils of *Maesosphaerum suaveolens* (Castro et al., 2018), *Psidium guajava* and *Cordia verbenacea* (Castro et al., 2018), higher sensitivity of engorged females was also verified in comparison with unfed larvae.

It was observed that fractions F2, F3 and F4 caused reduction in the biotic potential of *R. microplus*, with a smaller number and lower viability of eggs produced by engorged females, resulting in a control percentage between 44.5–100 %. This reduction was more pronounced at the highest concentration, in some cases with total reduction of oviposition or larval hatching. This action on the reproductive biology of engorged females correlates with the cytotoxic effect of *A. oleracea* extract on the reproductive system of female ticks (Oliveira et al., 2016, 2018). For semi-engorged *R. microplus* females, it was shown that Jambu extract caused changes in oocyte size and shape, reduction in granule number, increased cytoplasm vacuoles, and nuclear damage (Oliveira et al., 2016). In a study conducted with semi-engorged *R. sanguineus* s.l. females, similar morphological changes were also observed (Oliveira et al., 2018). In addition, data from the study by Oliveira et al. (2018) showed that treatments with *A. oleracea* extract also affected protein and polysaccharide synthesis and storage in *R. sanguineus* s.l germ cells.

Fractions F2, F3 and F4 presented similar activity, showing LC_{50} with overlap of confidence intervals and control percentage > 95 % from the concentration of 12.5 mg/mL, reaching 98 (F2), 100 (F3) and 100 % (F4), respectively, at the highest concentration (25 mg/mL). This is important when considering the extraction processes for spilanthol using organic solvents (Barbosa et al., 2016). If the extract containing 24.5 % spilanthol has activity similar to that of the extracts with 48 and 100 %, there is no need for additional -refinement to obtain a fraction with 100 % purity. This reduces costs with solvents and obtaining plant material.

In conclusion, *A. oleracea* extracts containing different spilanthol percentages (24.5, 48, and 100 %) have similar acaricidal activity on both unfed *R. microplus* larvae and engorged females. However, the extract without this amide shows no activity on larvae and low activity on females, suggesting that the presence of spilanthol is fundamental to obtain strong acaricidal activity.

CRediT authorship contribution statement

1. Paula Marchesini - Responsible for conducting the study (experiments and writing), during the development of the doctoral Thesis.
2. Alan Franco Barbosa - Collaboration in production and identification different extracts.
3. Mirza Nalesso Gomes Sanches - Collaboration in production and identification different extracts.
4. Rafael Moreira do Nascimento - Participation in the study design

and text review.

5. Francisca Leticia Vale - Participation in the study design and text review.

6. Rodrigo Luiz Fabri - Participation in the study design and text review.

7. Ralph Maturano - Participation in the study design and text review.

8. Mário Geraldo de Carvalho - Participation in the study design and text review.

9. Caio Márcio Oliveira Monteiro - Study supervisor. Participation in the study design and text review.

Declaration of Competing Interest

The authors declared no conflict of interest.

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