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Chemical composition and insecticidal activity of *Garcinia gardneriana* (Planchon & Triana) Zappi (Clusiaceae) essential oil

[Composición química y actividad insecticida del aceite esencial de Garcinia gardneriana (Planchon & Triana) Zappi (Clusiaceae)]

Carla Maria Mariano Fernandez[1], Fabiana Brusco Lorenzetti[1], Sirlene Adriana Kleinubing[1], João Paulo Pinguello de Andrade[2], Wanessa de Campos Bortolucci[3], José Eduardo Gonçalves[4,5], Ranulfo Piau Júnior[6], Diógenes Aparício Garcia Cortez[1]†, Zilda Cristiani Gazim[3] & Benedito Prado Dias Filho[1]

[1]Pós-graduação em Ciências Farmacêuticas, Universidade Estadual de Maringá, Maringá, Paraná, Brazil
[2]Pós-graduação em Engenharia Química, Universidade Estadual do Oeste do Paraná, Toledo, Paraná, Brazil
[3]Pós-graduação em Biotecnologia aplicada à Agricultura, Universidade Paranaense, Umuarama, Paraná, Brazil
[4]Pós-graduação em Tecnologias Limpas, Unicesumar, Maringá, Paraná, Brazil
[5]Unicesumar, Instituto Cesumar de Ciência, Tecnologia e Inovação– ICETI, Maringá, Paraná, Brazil
[6]Pós-graduação em Ciência Animal, Universidade Paranaense, Umuarama, Paraná, Brazil
†Deceased (1952 – 2017)

Reviewed by:
Nelson Zapata
Universidad de Concepción
Chile

Isiaka Ogunwande
Foresight Institute of Research and Translation
Nigeria

Correspondence:
Carla Maria Mariano FERNANDEZ
carla.mfernandez@hotmail.com

Section **Biological activity**

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Abstract: The present study aimed to analyze the chemical composition of the essential oil from *Garcinia gardneriana* (Planchon & Triana) Zappi leaves and fruits, and to determine its acaricidal activity on *Rhipicephalus microplus* by larval packet test and larvicidal action on *Aedes aegypti* by larval immersion test. The chemical analysis of the essential oil by gas chromatography-mass spectrometry identified sesquiterpene hydrocarbons and oxygenated sesquiterpenes in bacupari leaves and fruits, and α-cedrene, α-chamigrene, α-trans-bergamotene, and β-curcumene as major compounds. Essential oil from leaves of *G. gardneriana* presented acaricidal activity on *R. microplus* (LC_{50} = 4.8 mg/mL; LC_{99} = 10.8 mg/mL) and larvicidal effect on *A. aegypti* (LC_{50} = 5.4 mg/mL; LC99 = 11.6 mg/mL), whereas essential oil from the fruits of *G. gardneriana* showed LC_{50} = 4.6 mg/mL and LC_{99} = 8.9 mg/mL against *R. microplus* and LC_{50} = 6.4 mg/mL and LC_{99} = 13.9 mg/mL against *A. aegypti*. These results thus demonstrate the potential acaricidal and larvicidal activity of essential oil of *G. gardneriana*, offering new perspectives for the realization of bioassays from this essential oil.

Keywords: Bacupari; Essential oil; Cattle tick; *Aedes aegypti*; Sesquiterpenes.

Resumen: El presente estudio tuvo como objetivo analizar la composición química del aceite esencial de las hojas y frutos de *Garcinia gardneriana* (Planchon & Triana) Zappi, y determinar su actividad acaricida en *Rhipicephalus microplus* y larvicida en *Aedes aegypti* empleando la prueba de inmersión de larvas. El análisis químico del aceite esencial por cromatografía de gases-espectrometría de masas identificó hidrocarburos sesquiterpénicos y sesquiterpenos oxigenados en hojas y frutos de bacupari, y α-cedreno, α-chamigreno, α-trans-bergamoteno y β-curcumeno como compuestos principales. El aceite esencial obtenido de las hojas de *G. gardneriana* presentó actividad acaricida en la garrapata del ganado (LC_{50} = 4,8 mg/mL; LC_{99} = 10,8 mg/mL) y actividad larvicida en *A. aegypti* (LC_{50} = 5,4 mg/mL; LC_{99} = 11,6 mg/mL), así como, el aceite esencial obtenido de los frutos de *G. gardneriana* mostró LC_{50} = 4,6 mg/mL y LC_{99} = 8,9 mg/mL contra las larvas de garrapatas de ganado y LC_{50} = 6,4 mg/mL y LC_{99} = 13,9 mg/mL em las larvas de *A. aegypti*. Por lo tanto, estos resultados demuestran la actividad acaricida y larvicida del aceite esencial de *G. gardneriana*, ofreciendo nuevas perspectivas para la realización de bioensayos a partir de este aceite esencial.

Palabras clave: Bacupari; Aceite esencial; Garrapata del ganado; *Aedes aegypti*; Sesquiterpenos.

INTRODUCTION

Rhipicephalus microplus (Canestrini, 1887) is one of the most important ectoparasites in cattle breeding worldwide (Miguita *et al*., 2015). In Brazil, this parasite caused financial losses of approximately US$ 3 billion in 2015 (Embrapa, 2009; Grisi *et al*., 2014; Miguita *et al*., 2015). The economic impact of this tick is related to the animal's weight loss, anemia, and a decrease in milk and beef production. In addition, the inoculation of toxins and protozoa such as *Babesia bovis*, *Babesia bigemina,* and riquetsia *Anaplasma marginale*, results in the clinical state "Bovine Parasitic Sadness" (Embrapa, 2009; Chagas *et al*., 2016).

The control of cattle tick is dependent on chemical acaricides. However, these substances are toxic and can leave residues in products of animal origin and in the environment (Santiago *et al*., 2015). Moreover, the development of resistance to acaricides by the tick is a critical factor. According to many reports, there are areas in Brazil where there are no products capable of controlling this ectoparasite (Embrapa, 2009; Miguita *et al*., 2015).

Brazil and other developing countries face a major public health problem with the dissemination of viral diseases such as dengue, Zika virus, and chikungunya by mosquito *Aedes aegypti*. These diseases present high levels of morbidity and mortality with several complications and economic impacts (PAHO/WHO, 2019). Chemical insecticides are the main measures adopted by public health programs for mosquito control. However, as with chemical acaricides, the insecticides are toxic and the occurrence of resistance in several places by the mosquito has already been reported (Mendes *et al*., 2017).

The search for new substances for the control of cattle tick and *Aedes aegypti* mosquito has already led to many different researches. Essential oil (EO) from plant secondary metabolites has been a promising alternative for pest control, due to the rising demand for environmentally friendly and economically feasible products (Chagas *et al*., 2016; Braga *et al*., 2018; Fernandez *et al*., 2020).

Garcinia gardneriana (Planchon & Triana) Zappi (Clusiaceae), popularly known as *bacupari* (Lorenzi, 2002), is a native plant of Brazil distributed in Amazon and Atlantic Forests, utilized in folk medicine in the treatment of inflammation and urinary tract infections (Guimarães *et al*., 2004). Population of the floodplain of the Alto Paraná River (Porto Rico, Paraná state, Brazil) consume the bacupari *in natura* or in the form of sweets juices, whereas the fruits serve as feed to the Brown Capuchin Monkey and to rodents (Asinelli *et al*., 2011).

Phytochemical studies with leaves, branches and roots of this plant showed the presence of xanthones as 1,5-dihydroxyxanthone, 1,7-dihydroxyxanthone, 1,6-dihydroxy-5 methoxy-xanthone, 8-deoxygartanin, 7-prenyljacareubin, rheediaxanthone-A, rheediaxanthone B, rheedia-xanthone-C, isorheediaxanthone-B, macluraxanthone and xanthochymol (Braz-Filho *et al*., 1970; Delle Monache *et al*., 1983; Botta *et al*., 1984; Delle Monache *et al*., 1984); bioflavonoids as volkensiflavone, fukugetin, biflavanone GB2a, biflavanone GBla, fukugeside (Botta *et al*., 1984; Luzzi *et al*., 1997; Castardo *et al*., 2008) and I3-naringenin-II8-4'-OMe-eriodictyoI (Cechinel Filho *et al*., 2000); triterpenoids (lupeol and betulin), and steroids (β-sitosterol) (Braz-Filho *et al*., 1970). Santos *et al*. (1999) also identified in the fruits the presence of benzophenones (7-epiclusianone), sesquiterpenes (α-copaene, α-muurolene, γ-cadinene, cadinene), triterpenes (oleanolic acid), steroids (sitosterol, stigmasterol), methyl esters of palmitic, stearic, oleic, linoleic and linolenic acids, and sugars (galactose, glucose, fructose).

Moreover, extracts and compounds isolated from *G. gardneriana* showed analgesic (Luzzi *et al*., 1997; Cechinel Filho *et al*., 2000), anti-inflammatory (Castardo *et al*., 2008; Otuki *et al*., 2011), anti-HIV (Lin *et al*., 1997) and antibacterial activities (Verdia *et al*., 2004), as well as vasodilator effect (Cruz *et al*., 2006), monoamine oxidase inhibitory activity (Recalde-Gil *et al*., 2017) and effect on inhibition of melanogenesis (Campos *et al*., 2013).

To the best of our knowledge, no studies on the chemical composition and biological activity of *G. gardneriana* EO have been reported in the literature so far. Thus, considering the effort to find effective and affordable ways of controlling cattle tick and *A. aegypti* and the importance of the use of natural products for these purposes, the present work aims to analyze the chemical composition of the EO of *G. gardneriana* leaves and fruits and to determine their acaricidal activity on *Rhipicephalus microplus* and their larvicidal action on *Aedes aegypti.*

MATERIALS AND METHODS

Plant material

Leaves in the vegetative stage (500 g) and ripe fruits (500 g) of *Garcinia gardneriana* were collected in

July/2016 and December/2015, respectively, in the rural area (S23º76'38.66" e W53º65'62.96"), Xambrê, Paraná, Brazil. The exsiccate was identified by Ph.D. Lívia Godinho Temponi and deposited at the Herbarium of the State University of Western Paraná (UNOP) under the registration number 2335.

Essential oil extraction

Fresh leaves and ripe fruits (whole) of *G. gardneriana* were subjected to hydrodistillation in a Clevenger apparatus for 3 h. EO was collected, dried over sodium sulfate, filtrated, transferred to amber vials for the calculation of yield (% w/w), and kept at -20°C.

Gas chromatography-mass spectrometry analysis

The identification of the chemical components of the EO of *G. gardneriana* leaves and fruits was carried out by using a gas chromatograph (Agilent 7890 B) coupled to a mass spectrometer (Agilent 5977 A) equipped with an Agilent HP-5MS capillary column (30 m × 0.250 mm × 0.25 μm). The chromatographic conditions were: injector temperature of 250°C, injection volume 2 μL at a ratio of 1:10 (split mode), carrier gas (helium) flow was 1 mL/min and initial column temperature of 60°C, with gradual heating to 160°C at a rate of 2°C/min, after 10°C/min to 240°C. The temperatures of the transfer line, ion source, and quadrupole were 260, 230, and 150ºC, respectively. Mass spectra were obtained within a scan range of 29 to 450 *m/z* and a solvent delay time of 3 min. The identification of the compounds was based on the comparison of their retention indices (RI), obtained using various n-alkanes (C7-C30). In addition, their electron ionization (EI) mass spectra were compared with the NIST 11.0 library spectra and with the data available in the Adams (2017).

Biological activity

Acaricidal activity against Rhipicephalus microplus

Tick preparation

Engorged females of *R. microplus* were collected from naturally infected cattle at a farm in Esperança Nova, Paraná, Brazil. The animals from which the ticks were collected have not received any treatment with chemical acaricides for at least 45 before collection. Female ticks with an average weight of 0.20 g were incubated at 27 ± 1°C (70 ± 10% relative humidity) for 2 weeks until the complete deposit of eggs. Then, the eggs were collected and placed in an incubator at 27 ± 1°C (70 ± 10% RH). The 15-day-old larvae were utilized to perform the larval packet test.

Larval packet test

The larvicidal activity of EO from *G. gardneriana* leaves and fruits was realized by larval packet test (LPT), recommended by the World Food and Agriculture Organization (FAO, 1971), as previously reported by Leite (1988), with modifications. A stock solution of EO (50 mg/mL) was prepared diluted in aqueous solution with 2% polysorbate 80, and after diluted at concentrations ranging from 10.0 to 0.5 mg/mL for the bioassay. The positive control was prepared at 1.25 μL/mL by using commercial acaricide containing 150 mg/mL of cypermethrin, 250 mg/mL of chlorpyrifos, and 10 mg/mL of citronellal. The negative controls consisted of distilled water and 2% polysorbate 80 aqueous solutions. Groups of 100 larvae were placed in a dry filter paper package (2 x 2 cm) for the test. Each pack was moistened with 100 μL of a solution with EO (in different concentrations) or controls. The packages were incubated at 27 ± 1°C and 85 ± 10% relative humidity, in the absence of light. After that, the packages were opened and the number of live and dead larvae was counted. All tests were performed in triplicate. The larvae mortality was determined by the following equation: LM = [(dead larvae x 100) / (total larvae)].

Larvicidal activity on Aedes aegypti

The eggs of *A. aegypti*, which were placed into standing water for hatching, were provided by *Laboratório de Transmissores de Hematozoários of Fundação Oswaldo Cruz*, Rio de Janeiro City, Rio de Janeiro, Brazil. After 24 h, the larvae hatched and were then fed with fish feed for growth until the third stage. Third stage larvae of *A. aegypti* were then used for bio-tests.

The EO of *G. gardneriana* leaves and fruits was diluted in aqueous solution with 2% polysorbate 80 within the concentration range from 10.0 to 0.5 mg/mL. The positive control was prepared with Temephos-based organophosphorus at a concentration of 400 mg/mL. The two negative controls consisted of distilled water and 2% polysorbate 80 aqueous solutions. All tests were performed in triplicate. Ten *A. aegypti* larvae were separated by using a Pasteur pipette and placed into 250 mL vials with 10 mL of the different concentrations of EO. After 24 h, the number of dead larvae was obtained (note: the larvae were considered dead when they did not present any movement and did not respond to the stimuli) (Costa *et al.*, 2005). Larval mortality (LM) was determined as follows: LM = [(dead larvae x 100) / (total larvae)].

Statistical analysis

The data were processed and submitted to analysis of variance (ANOVA). The differences between means were determined by Tukey's test at 5% significance level. Lethal concentrations (LC) to kill 50% and 99% of larvae and their respective 95% confidence intervals (CI) were calculated by Probit Analysis by using the software Minitab Statistical Trial version.

RESULTS

The chromatograms obtained for EO of bacupari leaves and fruits by the GC/MS are shown in Figure No. 1. From these results, it was possible to identify the compounds described in Table No. 1. EO yields of bacupari leaves and fruits were of 0.33% and 0.14%, respectively. As shown in Table No. 1, the EO of *G. gardneriana* leaves and fruits presented in its composition the sesquiterpene hydrocarbons (97.11; 94.32%, respectively) and oxygenated sesquiterpenes (2.08; 4.74%, respectively).

Table No. 1
Chemical composition of the essential oil of leaves and fruits of *Garcinia gardneriana*

Peak	[A]Compound	[a]RI Literature	[b]RI Calculated	Area (%)	
				Fruits	Leaves
1	δ-Elemene	1338	1338	1.29	0.55
2	α-Cubebene	1348	1349	0.19	0.34
3	n.i.	-	1353	0.16	0.10
4	n.i.	-	1361	0.08	-
5	n.i.	-	1366	0.20	0.10
6	α-Ylangene	1375	1370	0.24	0.23
7	α-Copaene	1376	1375	0.98	0.81
8	2-*epi*-α-Funebrene	1382	1379	0.24	-
9	β-Cubebene	1388	1390	0.15	0.25
10	(-)-β-Bourbonene	1388	1392	-	0.42
11	β-Elemene	1390	1392	0.40	0.13
12	7-epi-Sesquithujene	1391	1393	-	2.14
13	n.i.	-	1402	-	0.10
14	Cycloseychellene	1407	1403	0.14	-
15	α-*cis*-Bergamotene	1407	1406	-	2.82
16	(*Z*)-Caryophyllene	1408	1409	7.02	-
17	α-Cedrene	1411	1417	3.50	19.45
18	n.i.	-	1420	-	0.12
19	α-Santalene	1417	1420	0.56	-
20	(*E*)-Caryophyllene	1419	1420	1.96	7.23
21	β-Copaene	1432	1431	-	0.75
22	β-Gurjunene	1433	1427	0.55	0.43
23	α-*trans*-Bergamotene	1434	1439	12.28	5.78
24	γ-Elemene	1436	1436	-	3.55
25	α-Guaiene	1439	1438	-	0.09
26	(*Z*)-β-Farnesene	1442	1445	0.47	t
27	α-*neo*-Clovene	1452	1448	0.78	0.46
28	α-Humulene	1454	1452	1.31	2.25
29	n.i.	-	1454	0.13	-
30	(*E*)-β-Farnesene	1456	1458	3.36	1.55
31	allo-aromadendrene	1460	1463	-	0.59

32	α-Acoradiene	1466	1462	0.46	0.42
33	n.i.	-	1468	0.14	-
34	β-Acoradiene	1470	1471	-	0.45
35	γ-Gurjunene	1479	1473	0.29	-
36	γ-Muurolene	1479	1479	3.82	5.10
37	ar-Curcumene	1480	1482	0.92	t
38	Amorpha-4,7(11)-diene	1481	1485	9.24	3.33
39	β-Selinene	1490	1485	-	4.87
40	Germacrene D	1492	1490	0.28	t
41	δ-Selinene	1492	1491	0.42	t
42	α-Zingiberene	1493	1494	-	2.38
43	Bicyclogermacrene	1498	1495	2.91	5.41
44	α-Muurolene	1500	1499	6.28	t
45	β-Himachalene	1500	1508	-	0.46
46	α-Chamigrene	1503	1508	-	10.11
47	β-Bisabolene	1505	1509	8.64	t
48	δ-Amorphene	1512	1514	0.77	t
49	β-Curcumene	1515	1519	11.22	2.02
50	(*Z*)-γ-Bisabolene	1515	1521	6.65	3.49
51	δ-Cadinene	1523	1527	5.03	3.45
52	*trans*-Cadina-1,4-diene	1534	1533	1.06	0.98
53	α-Cadinene	1538	1538	0.40	0.54
54	α-Calacorene	1545	1540	-	0.03
55	Selina-3,7(11)-diene	1546	1540	0.08	0.45
56	n.i.	-	1545	-	0.11
57	Elemol	1549	1546	0.37	t
58	Germacrene B	1561	1554	0.43	3.80
59	n.i.	-	1562	0.09	t
60	Maaliol	1567	1564	0.14	-
61	Spathulenol	1578	1573	-	0.25
62	Caryophyllene oxide	1583	1577	-	0.55
63	Globulol	1590	1581	0.29	0.14
64	n.i.	-	1584	0.08	0.10
65	Viridiflorol	1592	1588	0.23	-
66	Cubeban-11-ol	1595	1591	0.22	t
67	Rosifoliol	1600	1599	0.21	0.16
68	β-Oplopenone	1607	1610	0.06	0.09
69	Junenol	1619	1613	0.38	t
70	n.i.	-	1620	0.06	-
71	10-*epi*-γ-Eudesmol	1623	1623	0.32	-
72	1-*epi*-Cubenol	1628	1626	0.15	0.30
73	*cis*-Cadin-4-en-7-ol	1636	1630	0.07	-
74	allo-aromadendrene epoxide	1641	1632	-	0.10
75	Cubenol	1646	1640	0.56	-
76	α-Muurolol	1646	1645	0.13	0.21
77	α-Eudesmol	1653	1649	0.16	-
78	α-Cadinol	1654	1653	0.60	t
79	Selin-11-en-4-α-ol	1659	1653	-	0.22
80	n.i.	-	1665	-	0.14
81	β-Bisabolol	1675	1672	0.22	-
82	*epi*-α-Bisabolol	1684	1684	0.05	t
83	Eudesm-7(11)en-4-ol	1700	1698	-	0.06

84	n.i.	-	1698	-	0.04
85	Caryophyllene acetate	1701	1698	0.58	-
Total identified (%)				99.06	99.19
Sesquiterpene hydrocarbons (%)				94.32	97.11
Oxygenated sesquiterpenes (%)				4.74	2.08

[A]Compounds listed in order of elution in column HP-5MS. t = traces. n.i = not identified. Area (%): percentage (%) of the area occupied by compounds within the chromatogram. [a]RI literature = Retention Index found in the literature of the capillary column DB5 and comparison of Retention Indexes and/or Mass Spectra with literature (Adams, 2017). [b]RI calculated = Identification based on retention index (RI) using n-alkane C7 – C30 on an Agilent HP-5MS column

The major compounds in the EO of *G. gardneriana* leaves were α-cedrene (19.45%), α-chamigrene (10.11%), (E)-caryophyllene (7.23%), α-trans-bergamotene (5.78%), bicyclogermacrene (5.41%), γ-muurolene (5.10%), and β-selinene (4.87%), while in the EO from the fruits the major compounds were α-trans-bergamotene (12.28%), β-curcumene (11.22%), amorpha-4,7(11)-diene (9.24%), β-bisabolene (8.64%), (Z)-caryophyllene (7.02%), (Z)-γ-bisabolene (6.65%) and α-muurolene (6.28%).

The results of the acaricidal activity of EO of *G. gardneriana* leaves and fruits on *R. microplus* is presented in Table No. 2, whereas the lethal concentrations to kill 50% (LC_{50}) and 99% (LC_{99}) of cattle tick larvae is exhibited in Table No. 3. EO of leaves showed LC_{50} and LC_{99} of 4.8 mg/mL and 10.8 mg/mL, respectively, while EO of fruits presented LC_{50} and LC_{99} of 4.6 mg/mL and 8.9 mg/mL, respectively. The negative control did not show larval mortality and the positive control presented 100% mortality of cattle tick larvae at the tested concentration.

In relation to larvicidal activity on A. *aegypti*, EO of G. *gardneriana* leaves (LC_{50} = 5.4 mg/mL; LC_{99} = 11.6 mg/mL) and fruits (LC_{50} = 6.4 mg/mL; LC_{99} = 13.9 mg/mL) presented potential action as showed in Tables No. 2 and Tables No. 3. The negative control did not show larval mortality and the positive control presented 100% mortality of mosquito larvae within the tested concentration.

DISCUSSION

Sesquiterpene hydrocarbons and oxygenated sesquiterpenes were identified in the *G. gardneriana* EO. The presence of α-cedrene and α-chamigrene as major compounds in the *bacupari* EO from leaves collected in the vegetative period may be associated with the defense mechanism of the plant against numerous predators, parasites, competitors and pathogenic fungi and bacteria (Olayemi, 2017). The presence of α-*trans*-bergamotene, β-curcumene, (*Z*)-caryophyllene and others sesquiterpenes in the EO of *G. gardneriana* fruits can be related to the defense mechanism of the plant to protect the fruit against insect attack, mechanical damage or infection by fungal and bacterial pathogens (Ulubelen *et al*., 1994; Matasyoh *et al*., 2007).

Studies regarding the chemical composition of essential oil of *bacupari* are not easily found in the literature. On the other hand, sesquiterpene hydrocarbons and oxygenated sesquiterpenes were identified in other species of the family Clusiaceae of the genus *Garcinia* as described by Chagonda and Chalchat (2005), who showed sesquiterpenes (88.5-89.2%) as the majority class in EO of fruits of *Garcinia huillensis* collected in Gutu and Rusape (Zimbabwe) and the major compounds β-caryophyllene (12.6-53.9%), α-humulene (10.1-23.0%) and valencene (4.0-18.2%). In an assessment carried out by Tan *et al*. (2013) with the EO of *Garcinia atroviridis* fruits, it was reported that the sesquiterpenes were the major class obtained and the (-)-β-caryophyllene (23.8%), β-caryophyllene alcohol (15.6%) and α-humulene (10.7%) were the most abundant components, while the EO of *G. atroviridis* leaf was composed by (*E*)-β-farnesene (58.5%) and β-caryophyllene (16.9%) (Tan *et al*., 2018).

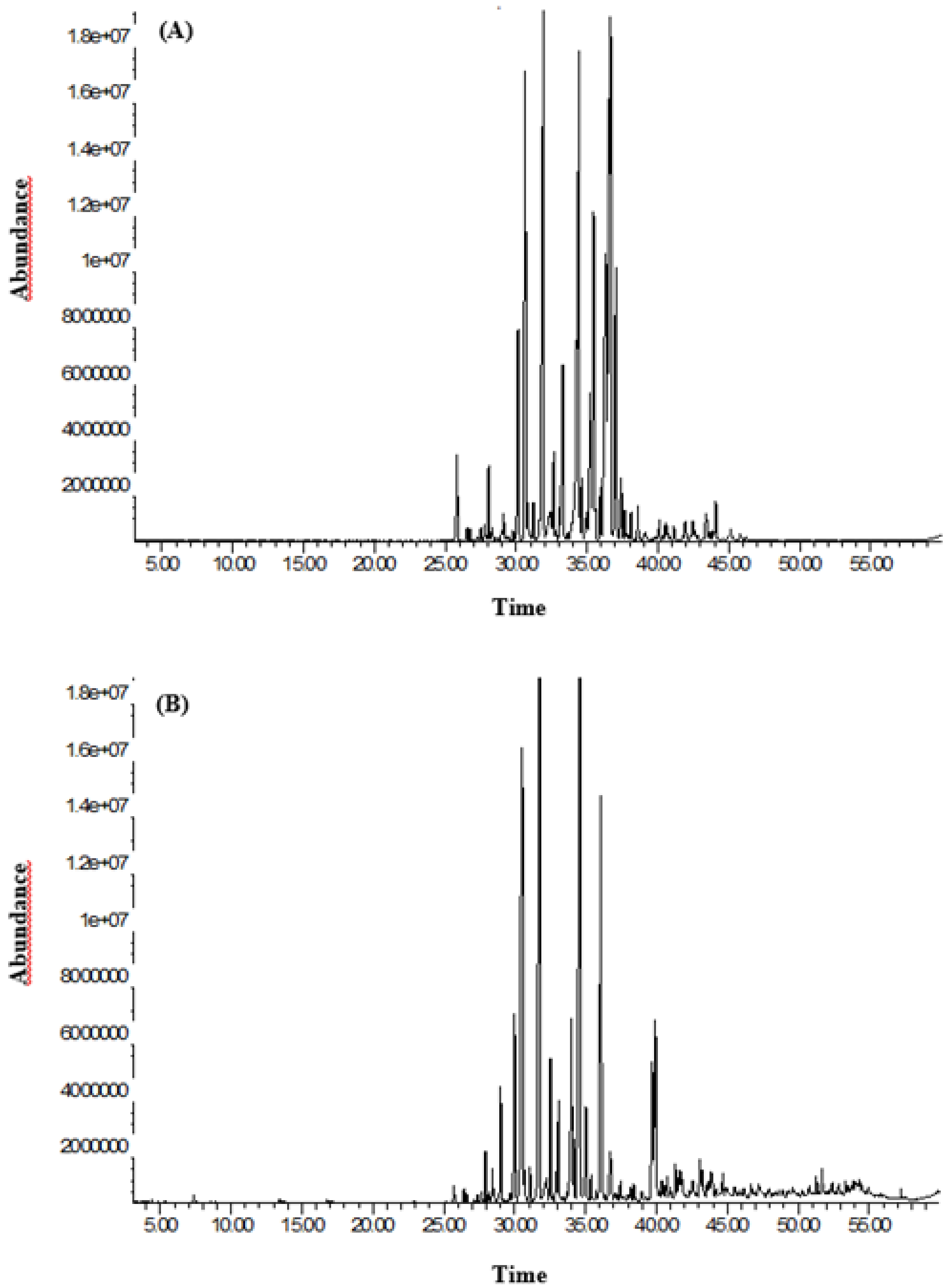

Figure No. 1

Chromatogram of the essential oil of the fruits (A) and of the leaves (B) of *Garcinia gardneriana*

Table No. 2
Mortality (%) of *Rhipicephalus microplus* and *Aedes aegypti* larvae with different concentrations of EO of leaves and fruits of *Garcinia gardneriana*

	Mortality (%)			
	Rhipicephalus microplus		*Aedes aegypti*	
Concentration mg/mL	**Leaves EO**	**Fruits EO**	**Leaves EO**	**Fruits EO**
10.0	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$
9.0	$86.4^{b} \pm 0.7$	$98.5^{a,b} \pm 2.6$	$90.0^{a,b} \pm 0.0$	$70.0^{b} \pm 0.0$
8.0	$83.4^{b} \pm 3.0$	$92.9^{a,b} \pm 1.8$	$80.0^{b} \pm 0.0$	$55.0^{c} \pm 7.1$
7.0	$76.0^{c} \pm 2.4$	$88.5^{b} \pm 1.6$	$76.4^{b} \pm 5.1$	$53.2^{c} \pm 2.3$
6.0	$69.7^{c} \pm 5.4$	$72.8^{c} \pm 4.3$	$52.3^{c} \pm 3.2$	$47.2^{c} \pm 3.9$
5.0	$63.0^{d} \pm 1.2$	$66.3^{c} \pm 7.0$	$45.0^{c} \pm 7.1$	$33.2^{d} \pm 4.5$
4.0	$58.1^{d} \pm 2.7$	$51.9^{d} \pm 3.2$	$30.0^{d} \pm 0.0$	$30.0^{d} \pm 0.0$
3.0	$34.4^{e} \pm 5.1$	$17.6^{e} \pm 7.0$	$17.1^{d,e} \pm 4.0$	$20.0^{e} \pm 0.0$
2.0	$8.9^{f} \pm 1.7$	$4.5^{f} \pm 2.1$	$14.1^{e} \pm 5.8$	$14.1^{f} \pm 5.8$
1.0	$0.0^{g} \pm 0.0$	$0.0^{f} \pm 0.0$	$10.0^{e,f} \pm 0.0$	$0.0^{g} \pm 0.0$
0.5	$0.0^{g} \pm 0.0$	$0.0^{f} \pm 0.0$	$0.0^{f} \pm 0.0$	$0.0^{g} \pm 0.0$
NC	0.0 ± 0.0		0.0 ± 0.0	
PC	100.0 ± 0.0		100.0 ± 0.0	

NC: Negative control; PC: Positive control; Averages followed by the same lower letter in the column do not differ among themselves by Tukey's test ($p \leq 0.05$)

Table No. 3
LC_{50} and LC_{99} values (mg/mL) and confidence interval of essential oil of leaves and fruits of *Garcinia gardneriana* on *Rhipicephalus microplus* and *Aedes aegypti* larvae

	Rhipicephalus microplus		*Aedes aegypti*	
	LC_{50} (CI)	**LC_{99} (CI)**	**LC_{50} (CI)**	**LC_{99} (CI)**
Leaves EO	4.8 (4.6-4.9)	10.8 (10.5-11.2)	5.4 (5.1-5.6)	11.6 (11.0-12.4)
Fruits EO	4.6 (4.5-4.7)	8.9 (8.7-9.3)	6.4 (6.1-6.7)	13.9 (13.1-15.0)

EO: essential oil; LC_{50}: lethal concentration 50%; LC_{99}: lethal concentration 99%; CI: confidence interval.

Thus, the sesquiterpenes identified in the essential oil of *bacupari* were also identified in other species of *Garcinia*, showing a chemical similarity between the essential oils of this genus.

The acaricidal and larvicidal activities of some compounds identified in the EO of bacupari leaves and fruits have been described in the literature. The (*E*)-caryophyllene, one of the major compounds in the OE of bacupari leaves, shows larvicidal activity against *A. aegypti* (LC_{50} = 88.30 µg/mL), *Culex pipiens pallens* (LC_{50} = 93.65 µg/mL) and *Ochlerotatus togoi* (LC_{50} = 97.90 µg/mL) (Perumalsamy *et al*., 2009) and acaricidal activity against *Dermatophagoides farinae* (LC_{50} = 3.13 µg/cm^{-2}), *Dermatophagoides pteronyssinus* (LC_{50} = 3.58 µg/cm^{-2}) (Oh *et al*., 2014), *Tyrophagus putrescentiae* (LC_{50} = 11.77 µg/cm^{-2}) (Kim *et al*., 2003) and *Tetranichus urticae* (LC_{50} = 0.00080 µg/mL) (Cavalcanti *et al*., 2010). The bicyclegermacrene, compound present in both fruits and leaves EO, also shows larvicidal properties on *Anopheles subpictus* (LC_{50} = 10.3 µg/mL), *Aedes albopictus* (LC_{50} = 11.1 µg/mL) and *Culex tritaeniorhynchus* (LC_{50} = 12.5 µg/mL) as reported by Govindarajan & Benelli (2016). These insecticidal actions suggest that the larvicide and acaricide activity of leaves EO is related to the presence of (*E*)-caryophyllene and bicyclogermacrene.

(Z)-γ-bisabolene, which is one of the major compounds in the OE of bacupari fruits, presents high toxicity on *A. stephensi* (LC_{50} = 2.04 µg/mL), *A. aegypti* (LC_{50} = 2.26 µg/mL), *Culex quinquefasciatus* (LC_{50} = 2.47 µg/mL), *A. subpictus* (LC_{50} = 4.09 µg/mL), *A. albopictus* (LC_{50} = 4.50 µg/mL) and

Culex tritaeniorhynchus (LC_{50} = 4.87 µg/mL) larvae (Govindarajan *et al*., 2018). Therefore, the presence of this compound in the EO of the fruits may have contributed to the larvicidal activity.

Other compounds in lower concentrations in the EO have insecticidal activity, such as the γ-elemene that also presents insecticidal activity on *Spodoptera litura* (LC_{50} = 10.64 µg/mL) and *Liposcelis bostrychophila* (LD_{50} = 102.15 µg/mL), as reported by Liang *et al*. (2016) and Benelli *et al*. (2018). The δ-cadinene presents larvicidal activity, as described by Govindarajan *et al*. (2016), on *A. aegypti* (LC_{50} = 9.03 µg/mL), *Anopheles stephensi* (LC_{50} = 8.23 µg/mL) and *Culex quinquefasciatus* (LC_{50} = 9.86 µg/mL), whereas α-humulene present in leaves and fruits EO, showed potential insecticidal activity against S. *litura* (LC_{50} = 12.89 µg/mL) and *Helicoverpa armigera* (LC_{50} = 20.86 µg/mL) (Benelli *et al*., 2018a; Benelli *et al*., 2018b). Thus, the acaricide and larvicide activity of *G. gardneriana* may be related to the presence of these sesquiterpenes in *bacupari* leaf and fruit EO.

It is worth mentioning that essential oils play an important role in the chemical defense of plants against the attack of herbivores and phytopathogens. They can be inhaled, ingested or absorbed by the cuticle of predatory insects causing chemical dysfunction and mortality by inhibiting or delaying the development of the insect, antifeedant effect, inhibition of the enzyme acetylcholinesterase, modulation of the gamma-aminobutyrate, octopaminergic, and tyramine receptors, reduced reproductive capacity or by direct toxicity (Simões *et al*., 2007; Dambolena *et al*., 2016; Said-Al Ahl *et al*., 2017).

The acaricidal activity of EOs of *Garcinia* species against *R. (B.) microplus* has not been described in the literature. However, the activity of several EOs against cattle tick has been reported. Chagas *et al*. (2016) showed that species of the Zingiberaceae and Verbenaceae families were effective against the cattle tick larvae. EO from *Curcuma longa* and *Zingiber officinale* rhizomes presented LC_{50} of 0.54 and 7.75 mg/mL, respectively. EO obtained from leaves and inflorescences of *Lippia gracilis*, *L. origanoides* and *L. alba* showed LC_{50} values of 3.21; 3.10 and 5.85 mg/mL, respectively. Comparing the larvicidal activity of EO of *G. gardneriana* leaves and fruits with other essential oils reported in the literature, it can be noticed that the larvicidal activity of the EO obtained from *G. gardneriana* was efficient against the larvae of *R. microplus*.

To date, no larvicidal activity of EOs obtained from *Garcinia* species against *A. aegypti* had been described in the literature. However, reports on the action of EOs of another species on mosquito larvae can be found, as the *Eucalyptus globulus* EO (LC_{50} = 0.11 mg/mL), EO of *Zingiber officinale* rhizome (LC_{50} = 0.15 mg/mL) and *Cymbopogon nardus* EO (LC_{50} = 1.37 mg/mL) (Khandagle *et al*., 2011; Tennyson *et al*., 2013). Confronting the results obtained in the present study with the ones achieved in previous assessments, one may notice that the EO from *G. gardneriana* leaves and fruits presented a promising larvicidal activity against *A. aegypti* larvae.

CONCLUSIONS

Essential oil from *G. gardneriana* leaves and fruits showed a predominance of sesquiterpenes compounds in its composition and exhibited potential acaricidal activity on *R. microplus* and larvicidal against *A. aegypti*. We conclude thus that the EO from *G. garderiana* leaves and fruits is an alternative source of bioactive compounds to control cattle tick and *A. aegypti* larvae. Nevertheless, further experiments involving other stages of development of the cattle tick and dengue mosquito are required.

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