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Antioxidant and antibacterial activity of *Myrcianthes pungens* leaf essential oil

[Actividad antioxidante y antibacteriana del aceite esencial de hoja de *Myrcianthes pungens*]

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Section **Biological activity**

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Abstract: *Myrcianthes pungens* (Myrtaceae) is a native tree to Brazil known as guabijú. In our study, we described the chemical composition of the essential oil from *M. pungens* dried leaves, the antioxidant activity by different methods and antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*. The chemical identification was done by gas chromatograph coupled to mass spectrometer and antibacterial activity was evaluated by broth microdilution method. The leaf essential oil presented hydrocarbon sesquiterpenes (42.6%) as its main chemical class whose major compounds were β-caryophyllene (11.7%) and 1,8-cineole (10.1%). The best antioxidant protection was from 57.5 to 63.3% of β-carotene. The highest antibacterial activity was against *S. aureus* (MIC of 78.12 µg/mL). This value was 2.56-fold lower than the positive streptomycin control. *M. pungens* is a promising source of natural molecules with potential study for the development of pharmacological, cosmetic and food products.

Keywords: β-caryophyllene; 1,8-cineole; β-carotene/linoleic acid co-oxidation; Sesquiterpenes; Guabijú; Myrtaceae.

Resumen: *Myrcianthes pungens* (Myrtaceae) es un árbol nativo de Brasil conocido como guabijú. En nuestro estudio, describimos la composición química del aceite esencial de las hojas secas de *M. pungens*, la actividad antioxidante por diferentes métodos y la actividad antibacteriana contra *Staphylococcus aureus*, *Bacillus cereus* y *Pseudomonas aeruginosa*. La identificación química se realizó mediante un cromatógrafo de gases acoplado a un espectrómetro de masas y la actividad antibacteriana se evaluó mediante un método de microdilución en caldo. El aceite esencial de la hoja presentó sesquiterpenos de hidrocarburos (42,6%) como su principal clase química cuyos compuestos principales fueron β-cariofileno (11,7%) y 1,8-cineol (10,1%). La mayor protección antioxidante fue de 57.5 a 63.3% de β-caroteno. La mayor actividad antibacteriana fue contra *S. aureus* (MIC de 78.12 µg/mL). Este valor fue 2,56 veces menor que el control positivo de estreptomicina. *M. pungens* es una fuente prometedora de moléculas naturales con potencial estudio para el desarrollo de productos farmacológicos, cosméticos y alimenticios.

Palabras clave: β-Cariofileno; 1,8-Cineol; Co-oxidación de β-Caroteno/Ácido linoleico; Sesquiterpenos; Guabijú; Myrtaceae.

INTRODUCTION

The most utilized synthetic antioxidants such as BHA (butyl hydroxyl anisole), BHT (butylated hydroxyl toluene), and TBHQ (tertiary butyl hydroquinone) have molecules that are capable of stabilizing free radicals and preventing the initiation of oxidation chains (Yehye *et al*., 2015). However, they can also exhibit a pro-oxidant behavior at high concentrations, causing molecule reduction and inducing the oxidative process (Marin *et al*., 2008; Škrovánková *et al*., 2012). These compounds have also been associated to adverse effects such as carcinogenesis and damages to the liver and lungs (Suh *et al*., 2005). Food and cosmetics consumers have valued products without the addition of synthetic preservatives. Food spoilage is a serious widely neglected problem, mainly in grain due to poor harvesting practices, inappropriate drying, handling, packaging, storage, and transport conditions. The utilization of synthetic antibiotics or chemicals has increased microorganism resistance, mostly against antibiotics and the search for new antimicrobials is an alternative to reduce synthetic chemicals uses to extend shelf life and prevent foodborne pathogens (Anyanwu & Okoye, 2017). Plant essential oils are a source of new molecules and from 1981 to 2010 approximately one third of the drugs approved by FDA (*Food and Drug Administration from United States of America*) were from plant molecules (Newman & Cragg, 2012).

Myrcianthes pungens (Berg) C. D. Legrand and its synonyms *Acreugenia pungens* (Berg) Kausel, *Eugenia pungens* Berg, *Eugenia ybaviyu* Parodi, and *Luma pungens* (Berg) Herter (Hassler, 2019), popularly known as *guabijú* and *guabirá* (in Portuguese) is geographically distributed in Brazil, Paraguay, Bolivia, Uruguay, and Argentina. It is an evergreen tree with up to 25 m height and round canopy, light reddish and smooth trunk. Its bright dark green petiolate leaves have pointed end and rounder base, measuring from 3.5 to 7.6 cm length and from 1.4 to 3.4 cm width. Its fruits are sweet, round and smooth with 1-cm diameter average, and they have dark purplish color when ripe (Marin et al., 2008). *M. pungens* leaves have essential oil (Zygadlo *et al*., 1997; Apel *et al*., 2006), but they have been little studied, differently from fruits which are edible and present antioxidant activity (Andrade *et al*., 2011), reduce hypercholesterolemia induced by cisplatin in rats (Nora *et al*., 2014), and antichemotactic activity (Andrade *et al*., 2011).

Moreover, the utilization of leaves is more sustainable and economically viable than fruits due to the possibility of collecting them throughout the year instead of a single yearly fruit harvest. Thus, this study aimed to characterize the chemical composition of the essential oil from *M. pungens* leaves and evaluate its antioxidant and antibacterial activity for potential applications in the food, pharmaceutical and cosmetic industries.

MATERIAL AND METHODS

Biological material

Myrcianthes pungens can be found at latitude 24°59'17.2"S and longitude 53°28'00.4"W; its leaves were collected in April 2017, from 7h to 9h o'clock in the morning, in the vegetative phase, and dried in the shade at ambient temperature (~30°C) for 10 days. The plant was identified and deposited in the herbarium of Maringá State University under the registration HUEM-32695, and registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen, acronym in Portuguese) under the registration AA3F7DB.

Essential oil extraction

The essential oil was extracted from leaves that were dried and ground in a mixer with ultrapure water and submitted to hydrodistillation in a modified Clevenger for 2 h (Apel *et al*., 2006). The essential oil was dehydrated by filtration in anhydrous sodium sulfate, stored in an amber flask and kept under refrigeration.

The leaf essential oil yield was calculated by the essential oil mass divided by the dried leaf mass, multiplied by 100 and expressed in percentage. The essential oil density was determined at 20°C in 5.0 µL graduated capillaries (mass/volume). The refraction index was determined by refractometer (Abbe RL3 model) calibrated with ultrapure water (refraction index of 1.3330) at 20°C (Farmacopeia Brasileira, 2010).

Identification of chemical compounds

Sample of 1 µL dichloromethane (HPLC degree) with essential oil (20 mg/mL) was used for each analysis. The chemical identification carried out by gas chromatograph (Agilent 7890B) coupled to a mass spectrometer (GC-MS) (Agilent 5977A MSD) and a HP5-MS UI Agilent fused silica capillary column (30 × 250 × 0.25 µm; Agilent Technologies), with initial oven temperature from 80°C (1 min), followed by increased to 185°C at 2°C/min and maintained for 1 min, followed by an increase to 275°C at 9°C/min and maintained for 2 min and finally increased to 300°C at 25°C/min and

maintained for 1 min. Helium was utilized as the carrier gas at the linear speed of 1 mL/min up to 300°C, and pressure release of 56 kPa. The injector temperature was 280ºC; the injection volume was 1 µL; the injection occurred in split mode (2:1). The temperatures of the transfer line, ion source and quadrupole were 280ºC, 230ºC and 150ºC. The EM detection system was utilized in "scan" mode, at the mass/charge rate/load (*m/z*) of 40-600, with "solvent delay" of 3 min. The compounds were identified by comparing the mass spectra found in NIST 11.0 libraries and by comparing the retention index (RI) obtained by a homologous series of *n*-alkane standard (C_7-C_{28}) (Adams, 2012).

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH•) method

The essential oil sequestration capability of free radicals by 2,2-diphenyl-1-picrylhydrazyl (DPPH•) was made according Rufino *et al*. (2007). The essential oil was prepared in methanol at 1.00, 0.75, 0.50, and 0.25 mg/mL and 0.1 mL this mixture was added to 3.9 mL DPPH• methanolic solution (60 µM). The negative control had 0.1 mL methanol in DPPH• solution (60 µM). The mixture was kept in the dark at room temperature for 30 min and the absorbance reduction was measured in 515 nm in a UV/VIS spectrophotometer. The total antioxidant capacity was calculated utilizing a standard quercetin solution (60 µM) as a 100% reference. From the correlation between absorbance and concentration of the antioxidant sample, the inhibitory concentration to reduce 50% of the free radicals (IC_{50}) was determined.

β-carotene/linoleic acid (BCLA) method

The antioxidant activity of the essential oil was evaluated using the method based on *β*-carotene/linoleic acid (BCLA) co-oxidation system. A 1.0-mL solution (20 mg BCLA in 1 mL chloroform) was mixed to 40 µL linoleic acid and 530 µL polisorbate-40 emulsifier. Chloroform was removed in a rotary evaporator at 50°C, and 450 mL of ultrapurified water (previously saturated with oxygen for 30 min) was added under vigorous agitation until reaching absorbance of 0.7 at 470 nm. Aliquots (280 µL) of this emulsion were transferred to microplates with 96 wells. In each well 20 µL ethanolic solution with essential oil at 1.00, 0.75, 0.50, or 0.25 mg/mL was poured. The reaction was kept at 40°C for 120 min and the absorbance was measured at 470 nm in spectrophotometer (Spectra Max Plus^{384} Microplate Reader) every 5 min of zero time until 120 min. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was utilized as a reference at 100 µg/mL. The results were expressed in absorbance reduction along the reaction time. The *β*-carotene bleaching rate was calculated according to Equation No. 1.

$$\mathbf{R = \ln (a/b)/t}$$

where R = bleaching rate of *β*-carotene in the mixture; ln = natural log; a = absorbance in zero time; b= absorbance in t time (t = 0, 5, 10, ... 120 min).

The antioxidant activity was calculated according to the percentage of inhibition in relation to the control, using Equation No. 2.

$$\mathbf{AA = [(R_{control} - R_{sample})/R_{control}] \times 100}$$

where AA = Antioxidant activity; $R_{control}$ and R_{sample} were the bleaching rates of *β*-carotene in the mixture without the antioxidant ($R_{control}$) and with essential oil (R_{sample}), respectively (Velioglu *et al*., 1998)

Ferric reducing antioxidant power (FRAP)

For the ferric reducing antioxidant power (FRAP), 25 mL acetate buffer (0.3 M), 2.5 mL TPTZ (2,4,6-Tris (2-piridil)-triazine) aqueous solution (10 mM) and 2.5 mL ferrous chloride aqueous solution (20 mM) were mixed according to Rufino *et al*. (2006). Essential oil (90 µL), previously prepared in methanol at 1.00, 0.75, 0.50, or 0.25 mg/mL, was mixed to 2.7 mL FRAP reagent for the antioxidant activity reaction. The mixture was vigorously homogenized and kept at 37°C for 30 min. The absorbance variance was read at 595 nm. A standard curve of ferrous sulfate (0–2000 µM) was used to calculate the antioxidant activity. The antioxidant activity was expressed in µM of ferrous sulfate per mg of sample.

Antibacterial activity

The antibacterial activity of essential oil was evaluated by broth microdilution method (adapted CLSI method) (CLSI, 2006) with three bacteria: *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 14458™), *Bacillus cereus* Frankland and Frankland (ATCC® 14579™), and *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™). Each bacterium was cultivated in nutrient broth (DIFCOR®) at 37ºC and kept in sloped nutrient agar at room temperature.

Standardization of bacterial suspension

From a 24 h bacterial culture, a new cultivation in Mueller-Hinton (DIFCOR®) broth for 8-10 h was done. After that, in a tube containing sterile saline solution (0.9% NaCl), the bacterial culture was added until obtaining turbidity identical to the one compared to the tube with 0.5 McFarland standard ($BaSO_4$ suspension which corresponds to 1.0 10^8 CFU/mL). A dilution of 1:10 in Mueller-Hinton broth was done to obtain 10^7 CFU/mL bacterial suspension, which was utilized as inoculum in the assay to determine the minimum inhibitory concentration (MIC) (CLSI, 2006).

Broth dilution method

The essential oil was diluted in ultrapure water at 40 mg/mL utilizing 2% polisorbate-80 as solubilizing agent. In a microplate with 96 wells, 90 µL Mueller-Hinton broth was added to each well except for the ones in column 1, and 100 µL diluted essential oil were added to the wells of columns 1 and 2. Serial dilutions were done from column 2 and, then, 10 µL inoculum were added to each well, totaling a final volume of 100 µL. The microplates were incubated at 37°C for 24 h and streptomycin (200.0 mg/mL) was used as positive control. After that, 10 µL 2,3,5-triphenyltetrazolium chloride was added to each well and in 10 min at 37°C the microbial growth inhibition was observed to determine MIC. The absence of pink color indicated microbial growth inhibition (CLSI, 2006).

Statistical analysis

The results were submitted to analysis of variance (Anova) and the differences between the arithmetical means and standard deviation were determined by Tukey test ($p \leq 0.05$). All tests were done in triplicates.

RESULTS

The essential oil yield of *M. pungens* dried leaves was 0.19 ± 0.04% with density of 0.98 g/mL and refraction index of 1.4980. The essential oil presented sesquiterpenes as the main chemical class: 42.6% hydrocarbon sesquiterpenes and 30.7% oxygenated sesquiterpenes (Table No. 1). The major compounds (greater or equal to 6%) were *β*-caryophyllene (11.7%), 1,8-cineole (10.1%), bicyclogermacrene (7.9%), spathulenol (7.8%), and 5-*epi*-neointermedeol (6.0%). The essential oil chromatogram is represented in Figure No. 1 and the mass spectra of the major compounds can be seen in Figures No. 2, No. 3, No. 4, No. 5 and No. 6.

Table No. 1
Chemical composition of essential oil from *Myrcianthes pungens* leaves obtained by gas chromatograph coupled to mass spectrometer

Peak	[a]Compound	Relative area (%)	[b]RI	Identification methods
1	*α*-thujene	0.12	929	a,b,c
2	*α*-pinene	0.46	936	a,b,c
3	Canphene	t	952	a,b,c
4	n.i.	0.13	965	a,b,c
5	Sabinene	0.11	976	a,b,c
6	*β*-pinene	0.48	979	a,b,c
7	Myrcene	0.72	992	a,b,c
8	*α*-phellandrene	0.74	1001	a,b,c
9	*δ*-3-carene	0.16	1012	a,b,c
10	*α*-terpinene	0.38	1018	a,b,c
11	*p*-cymene	1.39	1027	a,b,c
12	Limonene	3.52	1031	a,b,c
13	1,8-cineole	10.10	1035	a,b,c
14	*Cis*-ocimene	t	1040	a,b,c
15	*Trans*-*β*-ocimene	3.28	1051	a,b,c
16	*γ*-terpinene	1.15	1061	a,b,c
17	Terpinolene	0.60	1089	a,b,c
18	Linalool	t	1100	a,b,c
19	*p*-menth-2-en-1-ol	t	1123	a,b,c
20	Terpinen-4-ol	1.58	1178	a,b,c
21	*α*-terpineol	1.55	1191	a,b,c
22	*α*-cubebene	2.82	1351	a,b,c

23	*α*-copaene	0.22	1375	a,b,c
24	*β*-elemene	3.01	1391	a,b,c
25	*α*-gurjunene	0.33	1407	a,b,c
26	*β*-caryophyllene	11.70	1419	a,b,c
27	*γ*-elemene	0.14	1433	a,b,c
28	Aromadendrene	0.58	1437	a,b,c
29	*α*-humulene	1.69	1449	a,b,c
30	*Allo*-aromadendrene	0.32	1452	a,b,c
31	*α*-amorphene	0.19	1453	a,b,c
32	Germacrene D	0.36	1467	a,b,c
33	*β*-selinene	3.44	1471	a,b,c
34	*γ*-selinene	0.86	1476	a,b,c
35	Valencene	t	1481	a,b,c
36	*α*-selinene	t	1484	a,b,c
37	Bicyclogermacrene	7.92	1485	a,b,c
38	*α*-muurolene	0.25	1488	a,b,c
39	Germacrene A	2.27	1502	a,b,c
40	*γ*-cadinene	0.17	1512	a,b,c
41	*δ*-cadinene	2.99	1522	a,b,c
42	cadina-1-4-diene	0.53	1531	a,b,c
43	Germacrene B	2.21	1555	a,b,c
44	Aristolene	0.60	1565	a,b,c
45	Spathulenol	7.81	1577	a,b,c
46	Caryophyllene oxide	5.17	1582	a,b,c
47	Globulol	1.31	1589	a,b,c
48	Viridiflorol	0.95	1591	a,b,c
49	Guaiol	0.62	1599	a,b,c
50	Humulene oxide	0.38	1606	a,b,c
51	Guaia-4,11-diene	0.97	1610	a,b,c
52	n.i.	0.86	1620	a,b,c
53	Caryophilla-4(12),8(13)-dien-5β-ol	0.23	1626	a,b,c
54	*Iso*-spathulenol	1.25	1634	a,b,c
55	*α*-muurolol	1.72	1637	a,b,c
56	*β*-eudesmol	0.74	1641	a,b,c
57	*α*-cadinol	0.45	1646	a,b,c
58	5-*epi*-neointermedeol	6.03	1654	a,b,c
59	Valerenol	0.33	1668	a,b,c
60	Caryophylla-3,8(13)-dien-5-β-ol	0.48	1670	a,b,c
61	Aristol-9-en-3-ol	0.27	1696	a,b,c
62	Calarene epoxide	0.28	1714	a,b,c
63	γ-costol	0.88	1718	a,b,c
	Total identified	**98.81**		
	Hydrocarbon monoterpenes	**13.11**		
	Oxygenated monoterpenes	**13.23**		
	Hydrocarbon sesquiterpenes	**42.60**		
	Oxygenated sesquiterpenes	**29.87**		
	Non-identified	**0.99**		

aCompounds listed according to the elution order in HP5MS column; bretention index (RI) calculated utilizing n-alkanes C7 to C28 in a capillary column (HP5-MS UI); cIdentification based on the comparison with mass spectrum from NIST 11.0 libraries; Relative area (%): percentage of the area occupied by the compounds in the chromatogram. n.i. = not identified. t = traces.

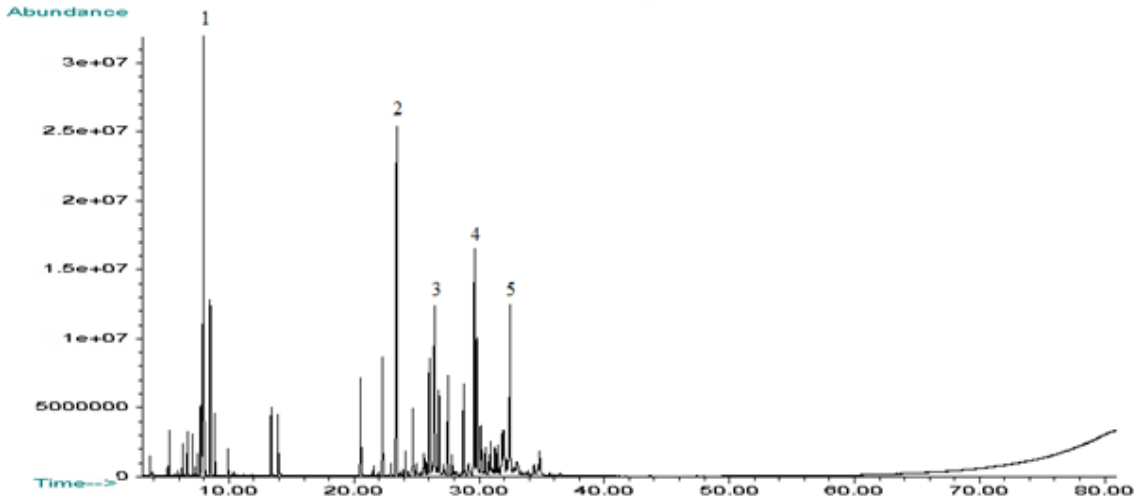

Figure No. 1
Chromatogram of Myrcianthes pungens leaf essential oil obtained by gas chromatograph coupled to mass spectrometer. (1) 1,8-cineole; (2) β-caryophyllene; (3) bicyclogermacrene; (4) spathulenol; (5) 5-epi-neointermedeol

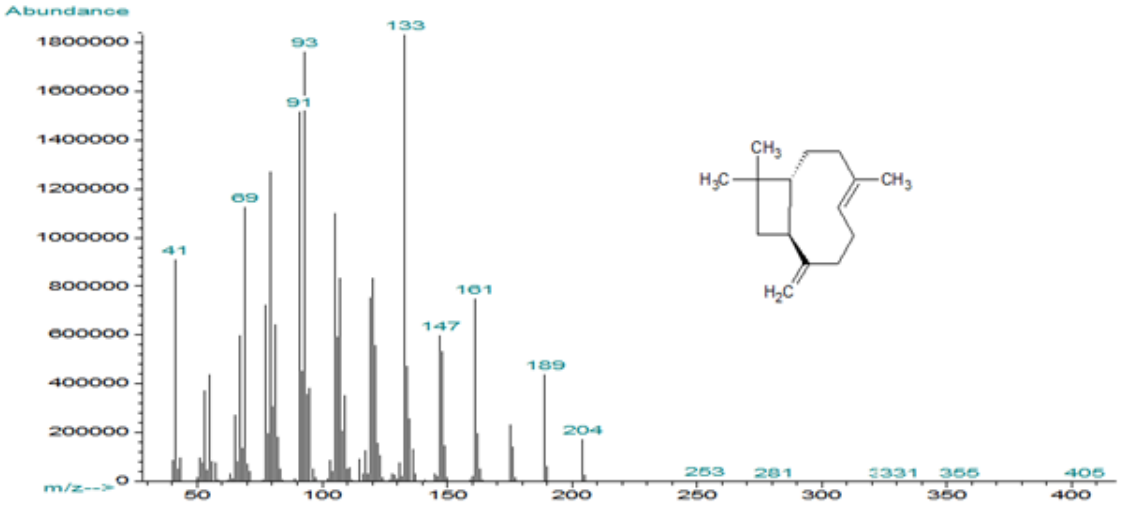

Figure No. 2
Mass spectrum of β-caryophyllene (m/z = 204) found in Myrcianthes pungens leaf essential oil obtained by gas chromatograph coupled to mass spectrometer

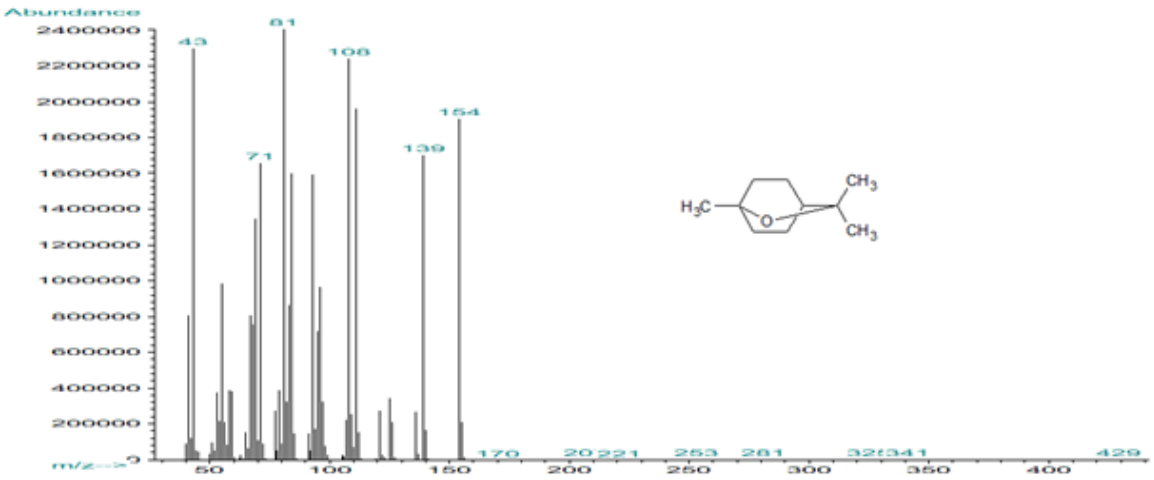

Figure No. 3
Mass spectrum of 1,8-cineole (m/z = 154) found in *Myrcianthes pungens* leaf essential oil obtained by gas chromatograph coupled to mass spectrometer

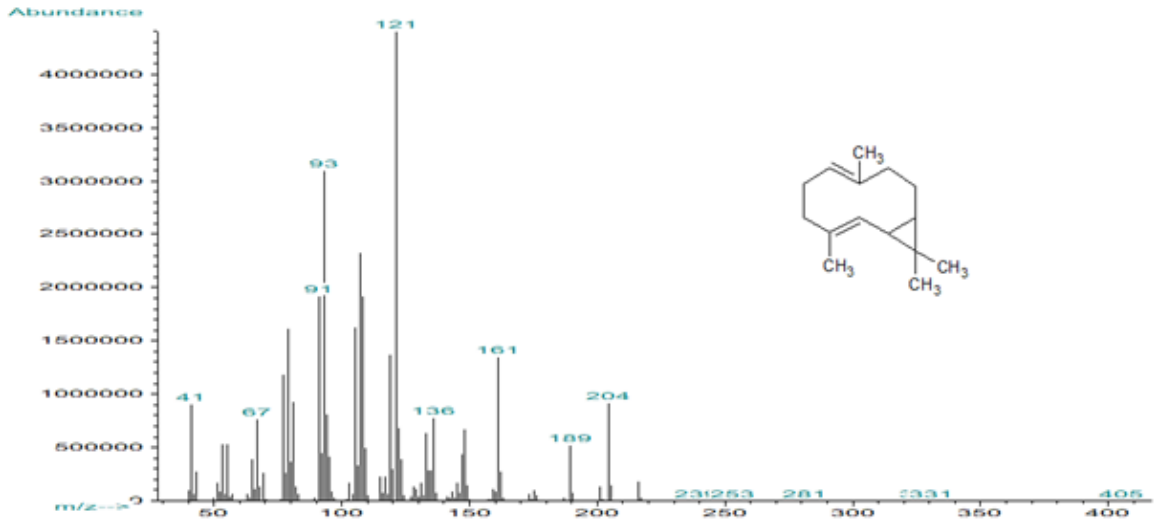

Figure No. 4
Mass spectrum of bicyclogermacrene (m/z = 204) found in *Myrcianthes pungens* leaf essential oil obtained by gas chromatograph coupled to mass spectrometer

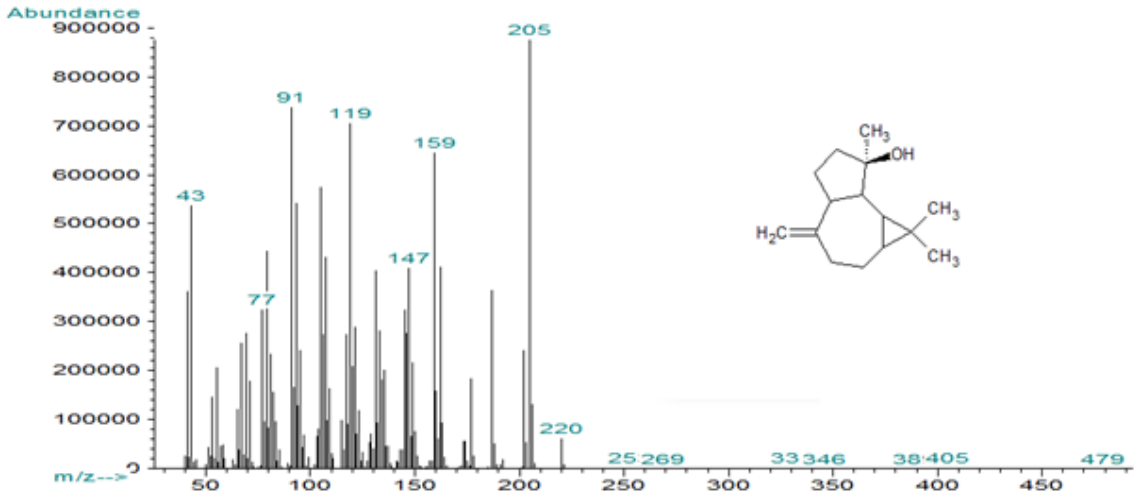

Figure No. 5
Mass spectrum of spathulenol (m/z = 220) found in *Myrcianthes pungens* leaf essential oil obtained by gas chromatograph coupled to mass spectrometry

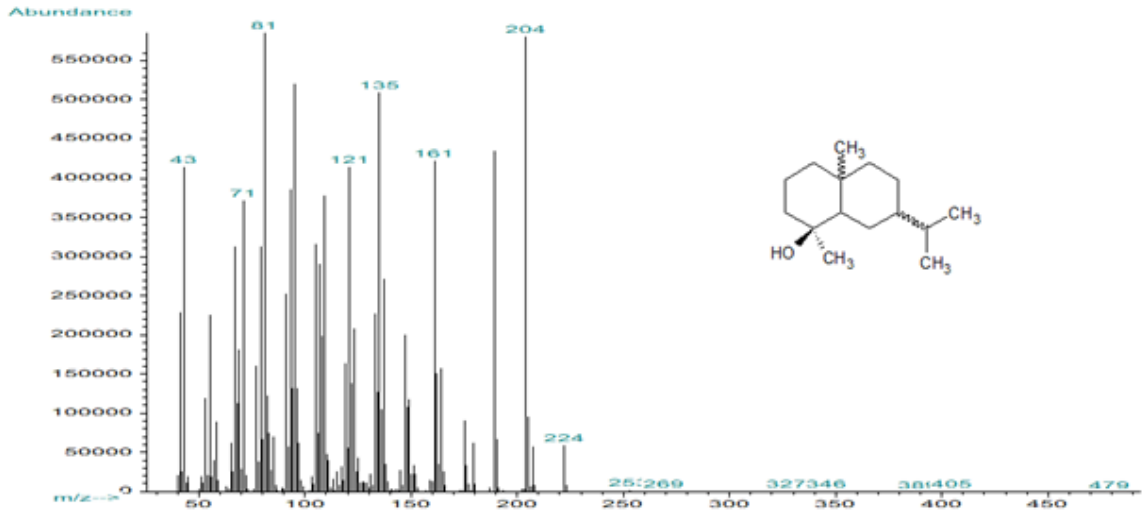

Figure No. 6
Mass spectrum of 5-epi-neointermedeol (m/z = 224) found in *Myrcianthes pungens* leaf essential oil obtained by gas chromatograph coupled to mass spectrometer

Essential oil antioxidant activity by DPPH• method had IC_{50} value of 24.47 ± 2.03 mg/mL. This value was 2447-fold higher than quercetin, positive control (Table No. 2), indicating an essential oil with reduced antioxidant activity by DPPH•. Through FRAP method, the reducing capacity was 0.27 µM Fe^{2+}/mg of sample and comparing it to the positive control trolox it was 9.17 µM Fe^{2+}/mg of the sample (Table No. 2), indicating an essential oil with reduced capacity to decrease Fe^{+3} ions. However, by BCLA method, the protecting action of the essential oil was of 57.5% (0.25 mg/mL essential oil) (Table No. 2). The antioxidant activity of the essential oil, a mixture of compounds, at 0.25 mg/mL was just 1.26-fold lower than the positive control trolox (0.2 mg/mL) (Table No. 2). The absorbance reduction, which shows β-carotene oxidation by fat peroxidation chain, was lower for the positive control followed by different essential oil concentrations (Figure No. 7). This suggests that the essential oil have intermediate antioxidant activity to protect β-carotene against free radicals.

Table No. 2
Antioxidant activity (arithmetical means ± standard deviation; n = 3) of the essential oil from *Myrcianthes pungens* dried leaves

Antioxidant activity	*DPPH• IC_{50} (mg/mL)	FRAP (µM Fe^{2+}/mg of sample)	BCLA β-carotene protection (%)
Essential oil	$24.47 \pm 2.03^{b**}$	0.27 ± 0.01^{b}	57.48 ± 4.21^{b}
Quercetin	0.01 ± 0.01^{a}	--	--
Trolox	--	9.17 ± 0.01^{a}	72.39 ± 4.55^{a}

***DPPH• method (2,2-diphenyl-1-picrylhydrazyl) using quercetin as positive control (IC50 = inhibitory concentration to reduce 50% of free radical by DPPH•); FRAP method (ferric ion reducing antioxidant power) using trolox (0.2 mg/mL) as positive control; BCLA method (β-carotene/linoleic acid co-oxidation system) of essential oil at 0.25 mg/mL and trolox (0.2 mg/mL) as positive control. **Different letters in the same column indicate significant differences by Tukey test ($p \leq 0.05$)**

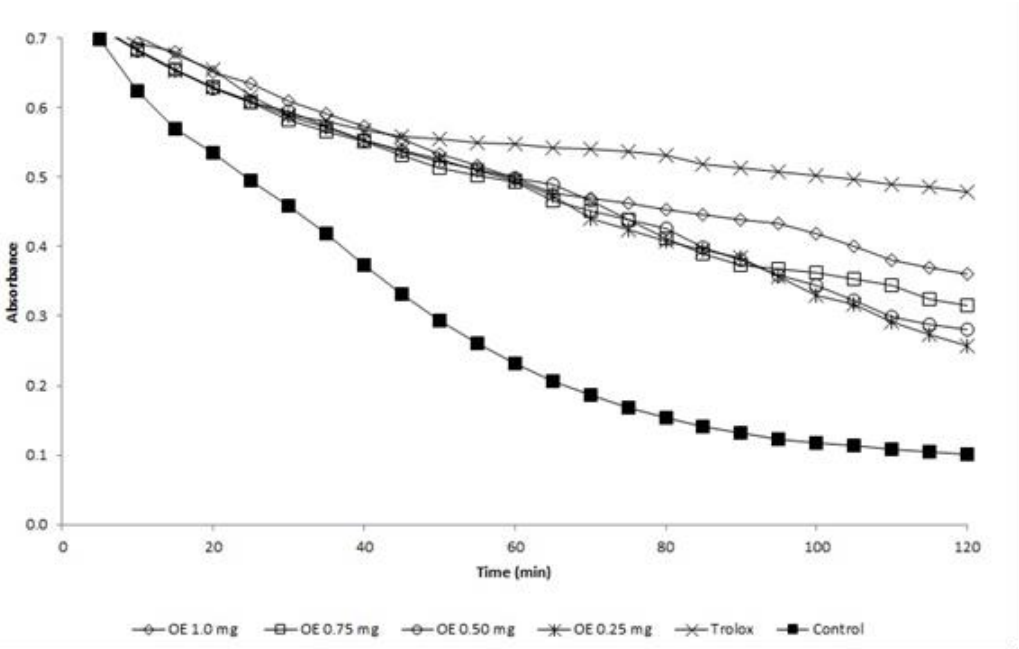

Figure No. 7
Absorbance by time through BCLA method (β-carotene/linoleic acid co-oxidation system) of the essential oil from *Myrcianthes pungens* dried leaves

The antibacterial activity of the essential oil was effective and specific for *S. aureus* (78.12 µg/mL), with activity 2.56–fold greater than the control streptomycin (200.0 µg/mL), which indicates essential oil high efficiency against this bacterium (Table No. 3). The essential oil did not have bacterial activity against *B. cereus* and *P. aeruginosa*, showing efficiency 801 and 33003-fold lower than the control streptomycin, respectively, and, therefore, low efficiency for the control of these bacteria (Table No. 3).

Table No. 3
Minimum inhibitory concentration (MIC) of the essential oil from *Myrcianthes pungens* dried leaves (arithmetical mean ± standard deviation; n = 3) and streptomycin (control) against different bacteria

Bacteria	Essential oil (µg/mL)	Streptomycin (µg/mL)
Staphylococcus aureus	$78.12 \pm < 0.01^{aA}$	$200.00 \pm < 0.01^{cB}$
Bacillus cereus	416.67 ± 180.42^{bB}	$0.52 \pm < 0.01^{aA}$
Pseudomonas aeruginosa	$33333.33 \pm 11547.00^{cB}$	$1.01 \pm < 0.01^{bA}$

Different small letters in the same column and capital letters in the same row indicate significant differences by Tukey test ($p \leq 0.05$)

DISCUSSION

Essential oil characteristics

Myrcianthes pungens essential oil yield of 0.19%, obtained in our study, is in accordance with the values in the literature for this plant (Table No. 4). Zygadlo *et al*. (1997) reported that in the province of Catamarca, Argentina, the essential oil yield from dried leaves was 0.10%, and Apel *et al*. (2006) reported that in Rio Grande do Sul, Brazil, the essential oil yield from fresh leaves was 0.20%, collected in the plant vegetative phase. According to the European Pharmacopoeia, the minimum extraction essential oil yield of 2 mL/kg is recommended for the continuity of application development studies (Nemeth & Bernath, 2008). The essential oil yield in our study was 1.8 mL/kg (dry basis) indicating that it is a little lower than the recommended by European Pharmacopoeia (European Pharmacopoeia, 2013), limiting the potential use of the leaf essential oil of this plant. Notwithstanding, this is a wild plant that can still be the target of genetic breeding programs to increase the essential oil yield.

Myrtaceae family has one of the highest concentrations of foliar terpenes in the Plant Kingdom. For Padovan *et al*. (2014) and Zygadlo *et al*. (1997), 1,8-cineole is the most representative and abundant compound in the profile of foliar terpenes of this family. However, in *M. pungens* essential oil, the major compounds reported are β-caryophyllene (10.1%), bicyclogermacrene (6.9%), and 1,8-cineole (45.8%) (Zygadlo *et al*., 1997; Apel *et al*., 2006; Marin *et al*., 2008). In our study, 1,8-cineole (10.1%), β-caryophyllene (11.7%), and bicyclogermacrene (7.9%) were the main major compounds found. The chemical composition and the amount of essential oil compounds are modulated by the vegetative phase, chemotype, and plant extrinsic factors such as soil, temperature, rainfall among others (Figueiredo *et al*., 2008), which allow certain variation within the species.

β-caryophyllene has been reported as a non-steroidal anti-inflammatory agent and it has also analgesic, antipyretic, and platelet-inhibitory actions by blocking the synthesis of prostaglandins. It is found β-caryophyllene in the essential oil of *Syzygium aromaticum* (L.) Merr. & Perry, *Cannabis sativa* L., *Rosmarinus officinalis* L., and *Humulus lupulus* L. In addition, it is reported that 99% β-caryophyllene may be fatal if swallowed and enters airways and if at 72% may cause allergic skin reaction (PubChem, 2020). 1,8-cineole, also known as eucalyptol, has a camphor-like odor and spicy cooling taste. It has mucolytic, bronchodilating, anti-inflammatory, and is used as anti-infective, mouthwashes, antitussive, and insect repellent. It is found 1,8-cineole in rhizomes of *Curcuma zedoaria* (Christm.) Roscoe, and leaves of *Eucalyptus* spp (PubChem, 2020).

Bicyclogermacrene has cytotoxic activity and may be fatal if swallowed and enters airways. It is reported as antimicrobial, acaricidal, insecticidal, antitumor, and antiprotozoal. It is found bicyclogermacrene in the essential oil of *Kundmannia sicula* (L.) DC. (81.2%) and *Torilis japonica* (Houtt.) DC. (57.9-71.8%) (PubChem, 2020). Spathulenol has cytotoxic activity and also antimycotic, antitumor, acaricide, antibiotic, antiseptic, contraceptive, and is used as antidepressant to treat neurodegenerative disorders of the central nervous system. It is found spathulenol in the essential oil of *Campomanesia adamantium* (Cambessedes) Berg, *Cardiopetalum calophyllum* Schltdl., *Salvia sclarea* L., *Artemisia vulgaris* L., and *Artemisia dracunculus* L. (PubChem, 2020). 5-epi-neointermedeol has been little studied, but intermedeol, which is the backbone structure of 5-epi-neointermedeol, is an acaricide against several ticks such as *Amblyomma cajennense* Fabricius, *Ixodes scapularis* (current name *Rhipicephalus bursa* Canastrini & Fanzago), and *Amblyomma americanum* Linnaeus, and repellent against arthropods such as fire ants. Intermedeol has been found in the essential oil of *Cymbopogon nardus* (L.) Rendle, *Callicarpa*

americana L., *Callicarpa americana* L., *Callicarpa japonica* Thunb., and *Ligularia fischeri* (Ledeb.) Turcz. (PubChem, 2020).

Table No. 4
Yield, utilized plant part, hydrodistillation time and major compounds of *Myrcianthes* spp essential oil

Plant	Utilized part/hydrodistillation (h)	Yield (%)	Major compounds	Reference
M. cisplatensis	Fresh leaf /(4 h)	0.20	1,8-cineole (29.8%), limonene (10.9%), *β*-caryophyllene (10.8%) and *α*-pinene (8.9%)	Apel *et al*. (2006)
M. gigantea	Fresh leaf /(4 h)	0.10	spathulenol (28.9%), *iso*-spathulenol (9.5%), *α*-cadinol (7.0%) and cariophellene oxide (6.7%)	Apel *et al*. (2006)
M. pungens	Fresh leaf /(4 h)	0.10	*β*-caryophyllene (10.1%). spathulenol (9.7%), *β*-elemene (9.1%), *α*-cadinol (8.0%), bicyclogermacrene (6.9%) and globulol (6.2%)	Apel *et al*. (2006)
M. osteomeloides	Fresh leaf /(ni)	0.60	1,8-cineole (55.7%), *α*-pinene (17.9%) and *α*-terpineol (8.5%)	Arze *et al*. (2005)
M. pseudomato	Fresh leaf /(ni)	0.10	1,8-cineole (24.4%), *α*-pinene (17.1%), linalol (11.7%), limonene (8.5%) and *γ*-terpinene (7.3%)	Arze *et al*. (2005)
M. pseudomato	Dried leaf/(ni)	0.30	1,8-cineole (32.5%), *β*-caryophyllene (18.9%), sabinene (6.6%), *α*-pinene (6.5%).	Demo *et al*. (2002)
M. pungens	Fresh fruit/(4 h)	0.10	*β*-caryophyllene (32.7%), germacrene D (14.2%) and bicyclogermacrene (11.2%)	Marin *et al*. (2008)
M. cisplatensis	Dried leaf/(1 h)	0.15	1,8-cineole (40.72%), limonene (22.14%), *α*-terpineol (7.71%)	Zygadlo *et al*. (1997)
M. pungens	Dried leaf/(1 h)	0.20	1,8-cineole (45.85%), limonene (17.31%) and *α*-terpineol (8.14%)	Zygadlo *et al*. (1997)

ni: not informed

Antioxidant activity

Most of terpenes present antioxidant properties (Škrovánková *et al*., 2012), however, the essential oil of M. pungens leaves showed in our study high antioxidant activity by BCLA method and lower antioxidant activity by DPPH• and FRAP methods. Essential oils are generally poorly soluble in aqueous and methanolic solutions such as those used in the FRAP and DPPH• methods and high soluble in nonpolar media such as BCLA. Thus, chemical interactions among essential oil and solvents may explain the lower antioxidant activity of *M. pungens* essential oil by FRAP and DPPH• methods and the high antioxidant activity by BCLA.

β-caryophyllene hydrocarbon sesquiterpene, one of the major compounds in our study, was reported for DPPH• method with IC_{50} ranging from 0.13 mg/mL (Calleja *et al*., 2013) to 0.28 mg/mL (Selestino Neta *et al*., 2017), but these authors used an isolated compound instead of crude essential oil. According to Selestino Neta *et al*. (2017) essential oils with great concentration of monoterpenes with aromatic rings attached to hydroxyl groups have high antioxidant activity but conversely essential oils with great concentration of sesquiterpene hydrocarbons, such as in our study, have low antioxidant activity to scavenge free radical as DPPH•.

Wannes *et al*. (2010) reported that stem essential oil of Myrtus communis var. italica (Myrtaceae), which had as major compound 1,8-

cineole (32.8%), had a reduced capacity to chelate iron ions. Lee & Shibamoto (2001) reported that 1,8-cineole had small antioxidant activity in polar condition and high activity in organic and lipid systems. These reports indicate that this compound has reduced antioxidant activity in polar systems and high antioxidant activity in lipid systems, as found in our study.

Jayasena & Jo (2013) reported that the use of natural products against the lipid oxidation in food products. In addition, Falowo *et al*. (2014) carried out extensive literature revision on the use of extracts of medicinal plants as phytoremedy against lipid–protein oxidation and synergistic antimicrobial activity of these natural antioxidants were reported against oxidative deterioration with addition from 0.0005 to 2.0% in meat and meat products, enhancing their functional properties and meat conservation. Therefore, it suggests that *M. pungens* essential oil obtained in our study has potential application to inhibit the oxidation in food susceptible to lipid oxidation at 1.0 mg/mL, equivalent to 0.1% in food.

Antibacterial activity

Non-polar compounds such as cyclic hydrocarbons can penetrate the bacterial lipid bilayer by diffusion transfer. These compounds accumulate in the cell membrane, causing their integrity loss (Sikkema *et al*., 1995) and/or increasing their permeability, favoring ion overflow, ATP intracellular depletion, and death (Burt, 2004). Terpenes are molecules that mimic effects of other toxins and act as cellular membrane solvents (Gershenzon & Dudareva, 2007). In our study, the major compound class was hydrocarbon sesquiterpenes and oxygenated sesquiterpenes that act on the bacterial lipid bilayer and may be the main mechanism of action of the antibacterial activity of M. pungens essential oil.

Hendry *et al*. (2009) reported antibacterial activity of 1,8-cineole against *S. aureus* and *P. aeruginosa*, but at a 64-fold higher concentration, indicating that the antimicrobial activity of *M. pungens* oil against *S. aureus* was already expected. *S. aureus* is a Gram-positive bacterium responsible for several infectious processes with different degrees of virulence and resistance to antibiotics (Plata *et al*., 2009). *M. pungens* essential oil may be a new alternative to control this microorganism.

However, in our study, *M. pungens* essential oil was little effective against *P. aeruginosa*, a Gram-negative bacterium capable of adapting to several conditions of pH and temperature, and is related to serious infections in patients with a weak immune system. Gram-negative bacteria have a hydrophobic polysaccharide layer for non-polar compounds (Sikkema *et al*., 1995), which can explain the lower effectiveness of *M. pungens* essential oil against *P. aeruginosa*. Also, the cellular membrane of P. aeruginosa has an efflux pump such as MexAB-OprM, and porins that increased the microorganism tolerance against terpene compounds such as 1,8-cineole (Papadopoulos *et al*., 2008).

M. pungens essential oil was not effective against *B. cereus*, a Gram-positive and facultative aerobic bacterium that can produce endospores, which make it more resistant to antibiotics and/or form biofilms that physically protect the microorganisms and prevent antibacterial activity (Valero *et al*., 2006). It has been shown that B. cereus has one or more plasmids, which contributes to its virulence and pathogenicity (Helgason *et al*., 2000). Although it is a Gram-positive bacterium, such as *S. aureus*. Although it is a Gram-positive bacterium, such as *S. aureus*, used strain of *B. cereus* showed resistance to the crude essential oil of *M. pungens*. The 1,8-cineole is reported as effective against S. aureus with MIC values ranging from 8 to 64 mg/mL (Mulyaningsih *et al*., 2010). β-caryophyllene is also reported as effective against *S. aureus* with MIC value of 1 mg/mL, and other Gram-positive and Gram-negative bacteria (Selestino Neta, 2017). In our study, the crude essential oil, containing 1,8-cineole and β-caryophyllene as major compounds, was effective against S. aureus with MIC value ranging from 102 to 819-fold lower than the isolated 1,8-cineole (Mulyaningsih *et al*., 2010). Further tests would clarify a possible synergistic effect between these essential oil compounds.

CONCLUSION

The yield of essential oil from *M. pungens* dried leaves is 0.2% with 0.98 g/mL density, and 1.4980 refraction index. Essential oil major compounds are β-caryophyllene (11.7%), 1,8-cineole (10.1%), bicyclogermacrene (7.9%), spathulenol (7.8%), and 5-epi-neointermedeol (6.0%). From the total identified compounds, 42.6% are hydrocarbon sesquiterpenes and 30.7% are oxygenated sesquiterpenes. The essential oil has high antioxidant activity by BCLA method with protective action of β-carotene of 57.5% at 0.25 mg/mL but it has low antioxidant activity by DPPH• and FRAP methods. The crude essential oil has antibacterial activity against *S. aureus* but it is not effective against *B. cereus* and *P. aeruginosa*, indicating selective antibacterial activity.

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