

Artículo Original / Original Article

**Antimicrobial activity of essential oil from
Psidium cattleianum Afzel. ex Sabine leaves**[Actividad antimicrobiana del aceite esencial de hojas de *Psidium cattleianum* Afzel. ex Sabine]**Thais Lorana Savoldi¹, Jasmina Glamočlija², Marina Soković², José Eduardo Gonçalves³, Suelen Pereira Ruiz¹,
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Abstract: The search for natural sources to control microorganisms is of interest in food production. This study evaluated the chemical composition and antimicrobial activity of the essential oil from *Psidium cattleianum* leaves. The essential oil was extracted by hydrodistillation, and identified by GC-MS. The predominant class of compounds was sesquiterpenes (47.6%) and the major compounds were trans- β -caryophyllene (14.7%), 1,8-cineole (11.7%) and γ -muurolene (5.6%). The antimicrobial activity was carried out by microdilution technique against eight fungi and eight bacteria. The minimum inhibitory concentration ranged from 0.17 to 11.25 mg mL⁻¹ for fungi, and from 1.40 to 16.87 mg mL⁻¹ for bacteria. The highest activities were against fungi *Aspergillus fumigatus* (ATCC 1022), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC 11730), and *Trichoderma viride* (IAM 5061), and bacteria *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (clinical isolate), and *Staphylococcus aureus* (ATCC 6538) with potential to prevent foodborne diseases.

Keywords: 1,8-cineole; *Psidium cattleianum* essential oil; Bactericide; Fungicide; trans- β -caryophyllene; γ -muurolene.

Resumen: La búsqueda de fuentes naturales para controlar los microorganismos es de interés en la producción de alimentos. Este estudio evaluó la composición química y la actividad antimicrobiana del aceite esencial de las hojas de *Psidium cattleianum*. El aceite esencial se extrajo por hidrodestilación y se identificó por GC-MS. La clase predominante de compuestos fueron los sesquiterpenos (47,6%) y los principales fueron trans- β -cariofileno (14,7%), 1,8-cineol (11,7%) y γ -muuroleno (5,6%). Actividad antimicrobiana se realizó mediante la técnica de microdilución contra ocho hongos y ocho bacterias. Concentración inhibitoria mínima varió de 0,17 a 11,25 mg mL⁻¹ para hongos y de 1,40 a 16,87 mg mL⁻¹ para bacterias. Principales actividades fueron contra hongos *Aspergillus fumigatus* (ATCC 1022), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC 11730) y *Trichoderma viride* (IAM 5061), y bacterias *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (clinical isolate) y *Staphylococcus aureus* (ATCC 6538) con potencial para prevenir enfermedades transmitidas por alimentos.

Palabras clave: 1,8-cineol; *Psidium cattleianum* aceite esencial; Bactericida; Fungicida; trans- β -cariofileno; γ -muuroleno.

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INTRODUCTION

Microorganisms are considered one of the principal responsible sources of food deterioration which produces mycotoxins that can be fatal if eaten. The losses in food processing, due to contamination by microorganisms, are hard to be estimated (Rawat, 2015; Pandey *et al.*, 2016). Besides these losses, foodborne diseases affect approximately 10% of the world population yearly, and the most frequent etiologic agent is bacteria (WHO, 2015). In order to control microorganisms, antimicrobial substances are used in human and animal health (FAO, 2017). The massive use of these compounds resulted in the development of microorganisms that are resistant to several antibiotics, and become a public health problem (Andreotti & Nicodemo, 2004). It has been estimated that infections resistant to medications will risk around 10 million human lives a year if no action is taken by 2050; the generated economic impact will be US\$ 100 trillion dollars in the economic production (O'Neill, 2016). Stefanakis *et al.* (2013) reported that essential oils could be used as a tool to reduce bacterial dissemination and to control bacterial resistance. Therefore, it has become important to search for natural compounds from plants to control microorganisms (Guimarães *et al.*, 2010).

Brazil has approximately 20% of the world plant diversity (Modolo & Foglio, 2019), which represents a great potential to obtain new biomolecules. These molecules can aggregate value to the food, cosmetic, and pharmaceutical industries, mainly due to their antioxidant and antimicrobial properties (Pandey *et al.*, 2016; Salen *et al.*, 2018). It is estimated that out of 300,000 plant species in the world, only 15% has been evaluated to determine their biological activities, showing that the development of new products from natural sources is still a field of opportunities to be studied (De Lucas *et al.*, 2012).

Psidium cattleianum Afzel. ex Sabine (Myrtaceae) has 21 synonyms (Hassler, 2020) and presents reddish or yellowish fruits. It is popularly known in Brazil as *little guava*, red araçá and yellow araçá to name just a few. It is natively distributed in Brazil, Oceania, the Caribbean Basin, and North America (McCook-Russell *et al.*, 2012). The species is characterized as a small perennial fruit tree or bush that can vary from 1 to 4 m in height. The fruits are 2.2 to 5 cm long, have oval or oblong shape, and present mass smaller than 20 g with many seeds (Dos Santos Pereira *et al.*, 2018).

The essential oil from *P. cattleianum* (red fruit) leaves was reported to have antifungal activity against several *Candida* spp. (Castro *et al.*, 2015), but it was inactive against *Aspergillus flavus* (ATCC 15517) (Soliman *et al.*, 2016). In preliminary tests in our laboratory, *P. cattleianum* essential oil showed antifungal activity against *Aspergillus* spp. (locally isolated microorganism; data not published). In addition, it has shown antibacterial activity against *Staphylococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 10145), and *Neisseria gonorrhoeae* (ATCC 19424) in a qualitative assay with ampicillin as control (Soliman *et al.*, 2016). Dias *et al.* (2018) reported that the essential oil from *Psidium myrtilodes* Berg (purple araçá) leaves presented antibacterial activity against several *Streptococcus* spp.

Despite some reports on the antimicrobial activity of the essential oil from *P. cattleianum* leaves, the spectrum of antimicrobial activity against agents that cause deterioration and foodborne diseases has been little explored. Thus, this study aimed to evaluate the chemical composition and antimicrobial activity of the essential oil from *P. cattleianum* (red fruit) dried leaves against a broad spectrum of fungi and bacteria that cause foodborne diseases.

MATERIAL AND METHODS

Plant material

Psidium cattleianum Afzel. ex Sabine (red fruit) leaves were collected in the vegetative phenological phase at the coordinates of latitude 25°56'28"S and longitude 52°10'32"W, altitude of 921 m, from January to February 2015. A voucher was authenticated, and deposited in the Herbarium of Maringá State University under the number HUEM-30716. This species is registered in the National System of Genetic Heritage Management and Associated Traditional Knowledge (SisGen, in Portuguese) under the registration number A344082.

Essential oil extraction

For the essential oil extraction, the leaves were dried at room temperature, and submitted to hydrodistillation process for 2 h in a modified Clevenger apparatus (Marques *et al.*, 2008). The essential oil was withdrawn from the apparatus with *n*-hexane, filtered in anhydrous sodium sulfate (Na₂SO₄), stored in an amber flask and kept at -20°C until complete solvent evaporation (Castro *et al.*, 2015). The essential oil yield was calculated by the

essential oil mass divided by the plant dried leaf mass, multiplied by 100, and expressed in percentage.

Chemical composition of the essential oil

The essential oil chemical identification was carried out by a gas chromatographer (Agilent 7890B) coupled to a mass spectrometer (Agilent 5977A MSD) (GC-MS). The capillary column was HP5-MS UI 5% (30 × 250 μm × 0.25 μm; Agilent Technologies) with initial oven temperature from 60°C to 280°C (3°C min⁻¹ and maintained for 1 min). Helium was utilized as the carrier gas at the linear speed of 1 mL min⁻¹ up to 300°C, and pressure release of 8.23 psi. The injector temperature was 220°C; the injection volume was 1 μL; the injection occurred in split mode (20:1) with injector temperature kept at 220°C. The temperatures of the transfer line, ion source, and quadrupole were 260, 230 and 150°C, respectively. The mass spectrometry detection system was utilized in “scan” mode, in the mass/charge range of 40 to 500 *m/z* with 3-min solvent delay. The compounds were identified by comparing the mass spectra found in NIST 11.0 libraries and by comparing the retention indices (RI) obtained by a homologous series of *n*-alkane standard (C7-C28) (Adams, 2017).

Antifungal activity

For the antifungal bioassays, eight fungi of the following species were used: *Aspergillus fumigatus* Fresenius (ATCC 1022), *Aspergillus ochraceus* Batista et Maia (ATCC 12066), *Aspergillus niger* van Tieghem (ATCC 6275), *Aspergillus versicolor* (Vuillemin) Tiraboschi (ATCC 11730), *Penicillium funiculosum* Thom (ATCC 8725), *Penicillium ochrochloron* Biourge (ATCC 90288), *Penicillium verrucosum* var. *cyclopium* (Westling) Samson, Stolk & Hadlok (food isolate), and *Trichoderma viride* Pers. (IAM 5061). The microorganisms were kept in malt extract agar at 4 °C, and subcultivated every 30 days (Booth, 1971). For the evaluation of the antifungal activity of the essential oil, the modified microdilution technique was used (Hänel & Raether, 1988; Espinel-Ingroff, 2001). The fungal spores were washed from the agar plate surfaces with an aqueous sterile solution containing 8.5 mg mL⁻¹ NaCl and 1 mg mL⁻¹ polysorbate-80. The spore suspension was adjusted with solution up to 1.0 × 10⁵ spores for 100 μL per well. The inoculum dilutions were cultivated in malt extract agar to verify the absence of contamination and the inoculum validity. The

minimum inhibitory concentration (MIC) was determined by serial dilution, utilizing microtiter plates with 96 wells. The essential oil was diluted (0.1-100 mg mL⁻¹) in aqueous solution containing 50 mL L⁻¹ dimethyl sulfoxide (DMSO) with 1 mg mL⁻¹ polysorbate-80, and added in malt extract broth cultivation medium (MA) with inoculum. The microplates were incubated in a rotary agitator (160 rpm) for 72 h at 28°C. The smallest concentrations without visible growth in optical microscope were defined as MIC. The minimum fungicidal concentration (MFC) was determined by serial subcultivation, utilizing 2 μL of broth per well into microplates containing 100 μL MA for 72 h at 28 °C. The lowest concentration without visible growth was defined as MFC, indicating 99.5% of death of the original inoculum. The negative control was an aqueous solution of 50 mL L⁻¹ DMSO, and the positive controls were the commercial fungicides bifonazole (Srbolek, Belgrade, Serbia) and ketoconazole (Zorkapharma, Šabac, Serbia) (Gupta *et al.*, 1994), and the food additives sodium sulphite (E221) and potassium metabisulphite (E224) (Garcia-Fuentes *et al.*, 2015) from 0.001 to 3.5 mg mL⁻¹.

Antibacterial activity

The antibacterial activity of the essential oil was tested in eight bacterial strains such as Gram-positive *Bacillus cereus* Frankland and Frankland (clinical isolate), *Listeria monocytogenes* (Murray *et al.*) Pirie (NCTC 7973), *Micrococcus luteus* (Schroeter) Cohn (ATCC 10240), and *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC 6538), and Gram-negative *Enterobacter cloacae* (Jordan) Hormaeche and Edwards (clinical isolate), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC 35218), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC 27853), and *Salmonella enterica* subsp. *enterica* (ex Kauffmann and Edwards) Le Minor and Popoff serovar Typhimurium (ATCC 13311).

The method of microdilution in broth (CLSI, 2015) using 96-well microplates was utilized for the assays. The bacterial suspensions were standardized with sterile saline solution up to the concentrations of 1.0 × 10⁵ CFU mL⁻¹ (Hänel & Raether, 1988; Espinel-Ingroff, 2001). The inoculum was prepared daily and stored at 4°C until its utilization, and their dilutions were cultivated in solid medium to verify the absence of contamination and inoculum growth. The essential oil was dissolved (0.1-100 mg mL⁻¹) in aqueous solution of 50 mL L⁻¹ DMSO with 1 mg mL⁻¹ polysorbate-80 and added to Luria-Bertani (LB)

cultivation medium (100 μL) with bacterial inoculum (1.0×10^4 CFU per well) to obtain the desired concentrations. The microplates were incubated in a rotary agitator (160 rpm) for 24 h at 37°C. The MIC was defined as the lowest concentration without visible growth of the microbial biomass under optic microscope. The minimum bactericidal concentration (MBC) was determined by a serial subcultivation of 2 μL in microtiter plates containing 100 μL LB per well, and incubated for 24 h. The lowest essential oil concentration, without visible growth under optical microscope, was defined as MBC, indicating 99.5% of death of the original inoculum. The optical density of each well was measured at 655 nm in a Microplate manager 4.0 (Bio-Rad Laboratories) and compared to positive controls. The negative control was an aqueous solution of 50 mL L⁻¹ DMSO, and the positive controls (1 mg mL⁻¹ in sterile saline solution) were the commercial antibiotics streptomycin (Sigma P7794) and ampicillin (Panfarma, Belgrade, Serbia), and the food additives E221 and E224 (Garcia-Fuentes *et al.*, 2015) from 0.001 to 3.5 mg mL⁻¹.

Statistical analysis

The antimicrobial assays were carried out in duplicate with three replications. The results were expressed in arithmetical average \pm standard deviation, and analyzed by unidirectional analysis of variance (ANOVA) followed by Tukey's HSD (honestly significant difference) test with $\alpha = 0.05$ to determine the statistical significance of the results. The analysis was done using Statistica® 8.0 software.

RESULTS

Essential oil yield and chemical composition

The essential oil yield from *P. cattleianum* dried leaves was 0.83%. A gas chromatography analysis showed 68 compounds, and 60 out of them were identified. The major class was hydrocarbon sesquiterpenes (47.6%) and the major identified were *trans*- β -caryophyllene (14.7%), 1,8-cineole (11.7%), γ -muurolene (5.6%), α -santalol (4.7%), globulol (4.7%), δ -selinene (4.5%), and δ -cadinene (4.1%) (Table No. 1).

Table No. 1
GC-MS chemical composition from the essential oil of *Psidium cattleianum* leaves

Peak	^a Compounds	Relative area (%)	^b RI calculate d	^c RI theoretic al	Methods of identification
1	2-thujene	0.18	925	924	a, b, c
2	α -pinene	3.13	933	932	a, b, c
3	β -pinene	0.25	974	974	a, b, c
4	β -myrcene	0.71	987	988	a, b, c
5	α -phellandrene	0.12	1001	1002	a, b, c
6	δ -3-carene	0.07	1007	1001	a, b, c
7	α -terpinene	0.21	1014	1014	a, b, c
8	<i>o</i> -cymene	1.40	1026	1022	a, b, c
9	1,8-cineole	11.70	1035	1026	a, b, c
10	<i>Trans</i> - β -ocimene	1.15	1038	1044	a, b, c
11	<i>Cis</i> - β -ocimene	0.32	1046	1032	a, b, c
12	γ -terpinene	1.41	1058	1054	a, b, c
13	n.i.	0.33	-	-	a
14	Terpinolene	0.15	1085	1086	a, b, c
15	Linalool	2.03	1098	1095	a, b, c
16	n.i.	0.17	-	-	a
17	n.i.	0.06	-	-	a
18	<i>Trans</i> - β -terpineol	0.71	1174	1159	a, b, c
19	α -terpineol	2.78	1189	1186	a, b, c
20	n.i.	0.38	-	-	a
21	α -ylangene	0.14	1364	1373	a, b, c

22	α -copaene	1.91	1370	1374	a, b, c
23	α -farnesene	0.10	1380	-	a, b, c
24	β -cubebene	0.15	1384	1387	a, b, c
25	α -gurjunene	0.15	1401	1409	a, b, c
26	<i>Trans</i> - β -caryophyllene	14.7	1421	1419	a, b, c
27	β -gurjunene	0.18	1423	1431	a, b, c
28	γ -elemene	0.04	1426	1434	a, b, c
29	α -guaiene	0.08	1429	1437	a, b, c
30	Aromadendrene	1.40	1435	1439	a, b, c
31	Eremophilene	0.07	1438	1439	a, b, c
32	α -humulene	1.61	1449	1452	a, b, c
33	<i>Allo</i> -aromadendrene	0.44	1454	1458	a, b, c
34	n.i.	0.07	-	-	a
35	4,5-di- <i>epi</i> -aristolochene	0.13	1463	1471	a, b, c
36	γ -gurjunene	2.65	1471	1475	a, b, c
37	γ -muurolene	5.62	1483	1478	a, b, c
38	δ -selinene	4.54	1491	1492	a, b, c
39	β -guaiene	0.67	1494	1492	a, b, c
40	Valencene	0.27	1497	1496	a, b, c
41	α -selinene	0.69	1501	1498	a, b, c
42	α -muurolene	0.48	1505	1500	a, b, c
43	β -bisabolene	1.40	1510	1505	a, b, c
44	<i>Cis</i> - α -bisabolene	2.54	1517	1506	a, b, c
45	γ -cadinene	3.75	1530	1513	a, b, c
46	δ -cadinene	4.12	1537	1522	a, b, c
47	Selina-3,7(11)-dieno	0.12	1545	1545	a, b, c
48	<i>Cis</i> -sabinene hidrate	0.32	1550	-	a, b, c
49	<i>Trans</i> -nerolidol	0.24	1553	1561	a, b, c
50	Caryophyllene oxide	0.21	1556	1582	a, b, c
51	Epiglobulol	2.03	1564	-	a, b, c
52	Globulol	4.67	1580	1590	a, b, c
53	Viridiflorol	0.32	1586	1592	a, b, c
54	Guaiol	0.36	1592	1600	a, b, c
55	7- <i>epi</i> - β -eudesmol	0.21	1595	-	a, b, c
56	Humulene epoxide II	0.31	1602	1608	a, b, c
57	γ -eudesmol	0.40	1613	1630	a, b, c
58	α -acorenol	0.53	1611	1632	a, b, c
59	n.i.	0.24	-	-	a
60	Isoaromadendrene epoxide	2.16	1624	-	a, b, c
61	α -muurolol	0.78	1627	1644	a, b, c
62	Cubenol	2.40	1639	1645	a, b, c
63	β -eudesmol	3.13	1646	1649	a, b, c
64	α -santalol	4.72	1667	1674	a, b, c
65	n.i.	0.58	-	-	a
66	<i>Cis</i> -bisabolene epoxide	0.19	1676	-	a
67	n.i.	0.11	-	-	a, b, c
68	Juniper camphor	0.66	1705	1700	a, b, c
Total identified		97.98			
Hydrocarbon monoterpenes		9.10			

Oxygenated monoterpenes	17.97
Hydrocarbon sesquiterpenes	47.61
Oxygenated sesquiterpenes	23.30
n.i.	1.94
Total	99.92

^aCompounds listed in elution order in HP-5MS UI column; ^bRI = identification based on the calculated retention index (RI) utilizing a standard homologous series of *n*-alkanes C₇-C₂₈ in Agilent HP-5MS UI column; ^cRI theoretical, identification based on the comparison of mass spectra found in NIST 11.0 libraries (Adams, 2017); Relative area (%): percentage of the area occupied by the compounds in the chromatogram; n.i. = non-identified; (-) not found

Antifungal activity of the essential oil

The MIC values for the essential oil varied from 0.17 to 11.25 mg mL⁻¹; for bifonazole and ketoconazole – widely used fungicides (Gupta *et al.*, 1994) – the values ranged from 0.10 to 0.20 mg mL⁻¹ and from 0.20 to 2.50 mg mL⁻¹, respectively, whereas for E221 and E224 – main used food additives to control microorganisms (Garcia-Fuentes *et al.*, 2015) – the MIC values ranged from 1.00 to 2.00 mg mL⁻¹ and from 0.50 to 1.00 mg mL⁻¹, respectively (Table No. 2). The MIC value of the essential oil for *A. fumigatus* was similar ($p > 0.05$), and 1.2-fold lower ($p \leq 0.05$) than ketoconazole. The MIC values of the essential oil for *A. ochraceus*, *A. versicolor*, and *T. viride* were 3-, 6-, and 4-fold lower ($p \leq 0.05$) than E221, respectively, and 3-, 3-, and 4-fold lower than E224, respectively (Table No. 2). The fungistatic activity of the essential oil against *A. fumigatus*, *A. ochraceus*, *A. versicolor*, and *T. viride* indicates high antimicrobial activity of the essential oil. Although the essential oil showed fungistatic activity against *A. niger*, *P. funiculosum*, *P. ochrochloron*, and *P. verrucosum*, the MIC values were higher than for those obtained for all positive controls (Table No. 2). It indicates that the first group of fungi was more sensitive than the second group to low concentrations of the essential oil, mainly *A. fumigatus*, and it could be used as a selective fungicide for the former.

The fungicidal activity of the essential oil had MFC values ranging from 0.23 to 22.50 mg mL⁻¹; for bifonazole they ranged from 0.20 to 0.25 mg mL⁻¹; for ketoconazole the values were from 0.30 to 3.50 mg mL⁻¹ whereas for E221 and E224 they were from 1.00 to 4.00 mg mL⁻¹, and from 0.50 to 2.00 mg mL⁻¹, respectively (Table No. 2). The essential oil MFC value for *A. fumigatus* was similar ($p > 0.05$) to bifonazole, and 2.2-fold lower ($p \leq 0.05$) than ketoconazole while it was 8.7- and 4.3-fold lower ($p \leq 0.05$) than E221 and E224, respectively (Table No. 2). In addition, the MFC values of the essential oil for *A. ochraceus*, *A. versicolor*, and *T. viride* were

higher ($p \leq 0.05$) than bifonazole, but still lower than ($p \leq 0.05$) or similar to ($p > 0.05$) ketoconazole, E221, and E224 (Table No. 2). The MFC values of the essential oil for *A. ochraceus* and *A. versicolor* ranged from 2.2- to 4.3-fold lower than the food additive controls, and for *T. viride* the values were 1.1-fold lower than E221, and 0.5 higher than E224. It indicates that the essential oil was a very effective and selective fungicide to control *A. fumigatus*, *A. ochraceus*, *A. versicolor*, and *T. viride* at low concentrations compared to all positive controls.

Antibacterial activity of the essential oil

The MIC values for the essential oil ranged from 1.40 to 16.87 mg mL⁻¹; for streptomycin and ampicillin they ranged from 0.04 to 0.25 mg mL⁻¹ and from 0.25 to 0.75 mg mL⁻¹, respectively; while the MIC values ranged from 1.00 to 4.00 mg mL⁻¹ and from 0.5 to 1.0 mg mL⁻¹ for E221 and E224, respectively (Table No. 3). The lowest MIC values obtained for the essential oil were against *P. aeruginosa*, *B. cereus*, and *S. aureus* (Table No. 3). The MIC values of the essential oil were in general higher than the controls, except for E221 that were 1.4- and 1.9-fold lower for *P. aeruginosa* and *S. aureus*, respectively. It suggests that the essential oil has potential use to control these bacteria. Although the essential oil showed bacteriostatic activity against the other bacteria, the MIC values were much higher than the controls, indicating that this essential oil was less promising to control *E. cloacae*, *E. coli*, *L. monocytogenes*, *M. luteus*, and *S. enterica*.

The bactericidal activity of the essential oil ranged from 2.81 to 22.50 mg mL⁻¹; the MBC values for streptomycin and ampicillin varied from 0.10 to 0.50 mg mL⁻¹ and from 0.40 to 1.20 mg mL⁻¹, respectively; while for E221 and E224 these values ranged from 2.00 to 4.00 mg mL⁻¹ and from 0.50 to 2.00 mg mL⁻¹, respectively (Table No. 3). The lowest MBC values obtained for the essential oil were also against *P. aeruginosa*, *B. cereus*, and *S. aureus* (Table No. 3). The MBC values of the essential oil

were in general higher than controls, except for E221 that were 1.4-fold lower for *P. aeruginosa* and *S. aureus*, respectively. It suggests that the essential oil has potential use to strongly inhibit these bacteria. Although the essential oil showed bactericidal

activity against the other bacteria, the MBC values were much higher than the controls, indicating that this essential oil was less promising to inhibit *E. cloacae*, *E. coli*, *L. monocytogenes*, *M. luteus*, and *S. enterica*.

Table No. 2
Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) from *Psidium cattleianum* (red fruit) leaf essential oil and positive controls bifonazole, ketoconazole, sodium sulphite (E221), and potassium metabisulphite (E224)

Fungus	essential oil	bifonazole	ketoconazole	E221	E224
	(mg mL ⁻¹)	(mg mL ⁻¹)	(mg mL ⁻¹)	(mg mL ⁻¹)	(mg mL ⁻¹)
	MIC	MIC	MIC	MIC	MIC
	MFC	MFC	MFC	MFC	MFC
<i>A. fumigatus</i>	0.17 ± 0.02 ^a	0.15 ± 0.02 ^a	0.20 ± 0.02 ^b	1.00 ± 0.02 ^c	1.00 ± 0.02 ^c
	0.23 ± 0.03 ^a	0.20 ± < 0.01 ^a	0.50 ± 0.03 ^b	2.00 ± 0.01 ^d	1.00 ± 0.03 ^c
<i>A. ochraceus</i>	0.34 ± 0.03 ^b	0.15 ± 0.03 ^a	1.50 ± 0.20 ^d	1.00 ± 0.03 ^c	1.00 ± 0.20 ^c
	0.46 ± 0.03 ^b	0.20 ± 0.02 ^a	2.00 ± 0.30 ^d	1.00 ± 0.02 ^c	2.00 ± 0.30 ^d
<i>A. niger</i>	11.25 ± 0.10 ^c	0.15 ± 0.04 ^a	0.20 ± 0.02 ^a	1.00 ± 0.04 ^b	1.00 ± 0.02 ^b
	22.50 ± 0.10 ^e	0.20 ± 0.06 ^a	0.50 ± < 0.01 ^b	2.00 ± 0.06 ^d	1.00 ± 0.01 ^c
<i>A. versicolor</i>	0.34 ± 0.02 ^c	0.10 ± < 0.01 ^a	0.20 ± 0.06 ^b	2.00 ± 0.01 ^e	1.00 ± 0.06 ^d
	0.46 ± 0.06 ^b	0.20 ± 0.03 ^a	0.50 ± 0.02 ^b	2.00 ± 0.03 ^d	1.00 ± 0.02 ^c
<i>P. funiculosum</i>	11.25 ± 0.10 ^d	0.20 ± 0.02 ^a	0.20 ± 0.02 ^a	1.00 ± 0.02 ^c	0.50 ± 0.02 ^b
	22.50 ± 0.20 ^d	0.25 ± 0.06 ^a	0.50 ± 0.06 ^b	2.00 ± 0.06 ^c	0.50 ± 0.06 ^b
<i>P. ochrochloron</i>	11.25 ± 0.10 ^d	0.20 ± 0.01 ^a	2.50 ± 0.30 ^c	2.00 ± 0.10 ^c	0.50 ± 0.30 ^b
	22.50 ± 0.06 ^e	0.25 ± 0.06 ^a	3.50 ± 0.60 ^d	2.00 ± 0.06 ^c	1.00 ± 0.06 ^b
<i>P. verrucosum</i>	8.40 ± 0.60 ^e	0.10 ± 0.02 ^a	0.20 ± 0.03 ^b	2.00 ± 0.02 ^d	1.00 ± 0.03 ^c
	11.25 ± 0.03 ^e	0.20 ± 0.03 ^a	0.30 ± 0.02 ^b	4.00 ± 0.03 ^d	1.00 ± 0.02 ^c
<i>T. viride</i>	0.23 ± 0.03 ^b	0.15 ± 0.03 ^a	1.00 ± 0.20 ^c	1.00 ± 0.03 ^c	1.00 ± 0.20 ^c
	1.87 ± 0.06 ^c	0.20 ± < 0.01 ^a	1.00 ± 0.20 ^b	2.00 ± 0.01 ^d	1.00 ± 0.20 ^b

*Averages followed by different letters in the same row for MIC or for MFC differ by Tukey's HSD (honestly significant difference) test ($p \leq 0.05$). *Aspergillus fumigatus*; *Aspergillus niger*; *Aspergillus ochraceus*; *Aspergillus versicolor*; *Penicillium funiculosum*; *Penicillium ochrochloron*; *Penicillium verrucosum* var. *cyclopium*; *Trichoderma viride*

Overall, the essential oil from *P. cattleianum* leaves showed fungistatic, fungicidal, bacteriostatic, and bactericidal activities. In addition, the essential oil had inhibitive selective action among microorganisms at different concentrations, and it

could be used in studies where it is necessary to inhibit one microorganism at the expense of another, by adding the essential oil in the cultivation medium to make it a selective medium.

Table No. 3

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) from *Psidium cattleianum* (red fruit) leaf essential oil and positive controls streptomycin, ampicillin, sodium sulphite (E221), and potassium metabisulphite (E224)

Bacterium	essential oil (mg mL ⁻¹)	streptomycin (mg mL ⁻¹)	ampicillin (mg mL ⁻¹)	E221 (mg mL ⁻¹)	E224 (mg mL ⁻¹)
	MIC	MIC	MIC	MIC	MIC
	MBC	MBC	MBC	MBC	MBC
<i>B. cereus</i>	1.40 ± 0.03 ^d	0.10 ± < 0.01 ^a	0.25 ± 0.04 ^b	1.00 ± 0.01 ^c	1.00 ± 0.04 ^c
	2.81 ± 0.06 ^d	0.20 ± 0.06 ^a	0.40 ± 0.03 ^b	2.00 ± 0.06 ^c	2.00 ± 0.03 ^c
<i>E. cloacae</i>	11.25 ± 0.30 ^d	0.20 ± 0.02 ^a	0.25 ± 0.03 ^a	2.00 ± 0.02 ^c	0.50 ± 0.03 ^b
	22.50 ± 0.60 ^d	0.30 ± 0.04 ^a	0.50 ± 0.04 ^b	4.00 ± 0.04 ^c	0.50 ± 0.04 ^b
<i>E. coli</i>	8.43 ± 0.06 ^e	0.20 ± 0.02 ^a	0.40 ± 0.02 ^b	2.00 ± 0.02 ^d	0.50 ± 0.02 ^c
	11.25 ± 0.10 ^e	0.30 ± 0.01 ^a	0.50 ± 0.06 ^b	2.00 ± 0.01 ^d	1.00 ± 0.06 ^c
<i>L. monocytogenes</i>	16.87 ± 0.10 ^e	0.20 ± 0.03 ^a	0.40 ± < 0.01 ^b	1.00 ± 0.03 ^d	0.50 ± 0.01 ^c
	22.50 ± 0.30 ^e	0.30 ± < 0.01 ^a	0.50 ± 0.02 ^b	2.00 ± 0.01 ^d	1.00 ± 0.02 ^c
<i>M. luteus</i>	16.87 ± 0.90 ^c	0.20 ± 0.03 ^a	0.25 ± 0.06 ^a	1.00 ± 0.03 ^b	1.00 ± 0.06 ^b
	22.50 ± 0.30 ^d	0.30 ± 0.01 ^a	0.40 ± < 0.01 ^b	2.00 ± 0.01 ^c	2.00 ± 0.01 ^c
<i>P. aeruginosa</i>	1.40 ± 0.06 ^e	0.20 ± < 0.01 ^a	0.75 ± 0.03 ^b	2.00 ± 0.01 ^d	1.00 ± 0.03 ^c
	2.81 ± 0.03 ^d	0.30 ± < 0.01 ^a	1.20 ± 0.20 ^b	4.00 ± 0.01 ^e	2.00 ± 0.20 ^c
<i>S. enterica</i>	5.62 ± 0.30 ^e	0.25 ± 0.02 ^a	0.40 ± 0.02 ^b	1.00 ± 0.02 ^d	0.50 ± 0.02 ^c
	22.50 ± 0.60 ^e	0.50 ± 0.02 ^a	0.75 ± 0.02 ^b	2.00 ± 0.02 ^d	1.00 ± 0.02 ^c
<i>S. aureus</i>	2.10 ± 0.10 ^d	0.04 ± < 0.01 ^a	0.25 ± 0.06 ^b	4.00 ± 0.01 ^e	1.00 ± 0.06 ^c
	2.81 ± 0.20 ^d	0.10 ± < 0.01 ^a	0.40 ± 0.01 ^b	4.00 ± 0.01 ^e	1.00 ± 0.01 ^c

*Averages followed by different letters in the same row for MIC or for MFC differ by Tukey's HSD (honestly significant difference) test ($p \leq 0.05$). *Bacillus cereus*; *Enterobacter cloacae*; *Escherichia coli*; *Listeria monocytogenes*; *Micrococcus luteus*; *Pseudomonas aeruginosa*; *Salmonella enterica* subsp. *enterica*; *Staphylococcus aureus*

DISCUSSION

Essential oil yields can vary in function of the genetic, regional, and climatic characteristics of the plant (Baser & Buchbauer, 2010). The essential oil yield from *P. cattleianum* dried leaves has been reported to range from 0.51 to 0.76% (Apel et al., 2006; Chalannavar et al., 2013; Oh et al., 2014; Pino et al., 2004). This range is close to the yield obtained in our study (0.83%). The essential oil yield obtained in our study was 8.3 mL kg⁻¹ (dry material), a much greater value than the reference of 2 mL kg⁻¹ (dry material) considered necessary to develop an industrial application according to the European Pharmacopoeia (2013).

The chemical variation of the compounds and their concentrations in plants can be related to factors such as genetic variability, environmental aspects, growth conditions, and soil type (Morais & Castanha, 2012). The characterization of the chemical compounds depends on the response to the

conditions in each ecosystem, and therefore, *P. cattleianum* could produce different volatile metabolites (Table No. 4). Chalannavar et al. (2013) observed that the dried-leaf essential oil of *P. cattleianum* in the southern region of Africa presented oxygenated sesquiterpenes (36.8%) as the major class, and caryophyllene oxide (12.4%) as the major compound. Soliman et al. (2016) reported that the essential oil from *P. cattleianum* leaves in Egypt presented β -caryophyllene (28.8%) as the major compound of the sesquiterpene class. This result is in accordance to the ones found in our study in which the major compounds were *trans*- β -caryophyllene (14.7%) and 1,8-cineole (11.7%). For the dried-leaf essential oil of *P. cattleianum* in Cuba, Pino et al. (2004) found as the major compounds *epi*- α -muurolol (21.9%) and α -cadinol (20.0%). The chemical characterization of essential oils from different sources may well serve as a good reference for the analysis of biological activities of *P. cattleianum*.

Table No. 4
Major compounds from leaf essential oil of *Psidium cattleianum* (red fruit) obtained by hydrodistillation

Compound	Amount (%)	Source
<i>trans</i> - β -caryophyllene	14.7	(Data from our study)
1,8-cineole	11.7	
γ -muurolene	5.6	
Caryophyllene oxide	12.4	(Chalannavar <i>et al.</i> , 2012)
Bicyclo(4.4.0)dec-I-ene	6.6	
2,3-butadnediol diacetate	4.8	
Patchoulene	4.7	
Caryophyllene oxide	29.6	(Chalannavar <i>et al.</i> , 2013)
Alloaromadendrene oxide-(1)	6.8	
12-oxabicyclo[9.1.0] dodeca-3,7-diene	5.8	
1H-cycloprop[e]azulene	3.5	
Isocaryophyllene	59.6	(Castro <i>et al.</i> , 2015)
Caryophyllene oxide	18.2	
α -caryophyllene	6.4	
Cadimol	4.6	
Epi- α -muurolol	21.9	(Pino <i>et al.</i> , 2004)
α -cadinol	20.0	
Epi- α -cadinol	16.7	
Caryophyllene oxide	13.6	
α -thujene	25.2	(Marques <i>et al.</i> , 2008)
1,8-cineole	16.4	
β -caryophyllene	10.2	
α -copaene	22.0	(Scur <i>et al.</i> , 2016)
Eucalyptol	15.0	
δ -cadinene	9.6	
α -selinene	6.5	

The factors that interfere in the chemical composition and major compounds of *P. cattleianum* essential oil also affect the antimicrobial activity. Scur *et al.* (2016) reported that *P. cattleianum* leaf essential oil presented α -copaene (22%) and eucalyptol (15%) as major compounds, and the antibacterial activity against *P. aeruginosa* and *S. aureus* showed a MBC value of 200 mg mL⁻¹ for both strains. In our study, *trans*- β -caryophyllene (14.7%) and 1,8-cineole (11.7%) were the major compounds, and the MBC values were approximately 70 times lower for the same microorganisms, suggesting greater antibacterial activity efficacy of the essential oil at lower concentration.

The major compounds observed in our study were also described for other plants of the *Psidium*

genus and the Myrtaceae family. Essential oils from *Psidium guajava* leaves showed great variation of β -caryophyllene concentration, ranging from 16.1% (Silva *et al.*, 2019) to 20.3% (Arain *et al.*, 2019). Chen *et al.*, (2007) reported β -caryophyllene (27.7%) followed by 1,8-cineole (12.4%) from the essential oil of *P. guajava* leaves. The chemical composition of the essential oil from *P. myrtilodes* leaves had 1,8-cineole as the major compound, varying from 29.8 to 48.1%, due to the effect of the sazonal harvest period (Macêdo *et al.* 2020). Also, γ -muuroleno has been reported as a minor compound (0.7%) in *Psidium myrsinites* (Medeiros *et al.*, 2015).

Trans- β -caryophyllene is a bicyclic hydrocarbon sesquiterpene that presents several biological properties against diseases like cancer (Di

Giacomo *et al.*, 2017), cardio protective characteristics against myocardium infarction (Younis *et al.*, 2019), and anti-inflammatory activity (Benitez *et al.*, 2009). The Research Institute for Fragrance Materials (RIFM) evaluated it as safe, and the Food and Drug Administration (FDA) in the USA approved the use of *trans*- β -caryophyllene as an aromatic agent in cosmetics products and in food additives (Api *et al.*, 2018). Also, β -caryophyllene isolated from *Murraya paniculata* has shown antibacterial activity against *S. aureus* (ATCC 25923), *Salmonella typhimurium* (ATCC 14028), *E. coli* (ATCC 8739), and *Enterococcus faecalis* (ATCC 14506) with MBC ranging from 2.0 to 4.0 mg mL⁻¹, and antifungal activity against *A. niger* (ATCC 40067), *A. fumigatus* (ATCC 40014), *A. parasiticum* (ATCC 40100), and *Fusarium solani* (ATCC 40099) with MFC ranging from 0.5 to 4.0 mg mL⁻¹ (Neta *et al.*, 2017). In our study, the essential oil showed a similar MBC value of 2.18 mg mL⁻¹ against *S. aureus*, and a lower MFC value of 0.23 mg mL⁻¹ against *A. fumigatus*, indicating greater efficacy of the essential oil.

Among the oxygenated monoterpenes, 1,8-cineole, the second major compound in our study, also known as eucalyptol, is a bicyclic monoterpene and the main compound in the essential oils from *Eucalyptus*, *Rosmarinus*, *Psidium*, *Croton*, and *Salvia* genera (Kovar *et al.*, 1987; Manoel *et al.*, 1994; Farhat *et al.*, 2001). Several biological and pharmacological activities have been reported for this compound such as anti-inflammatory (Santos & Rao, 2000), anticancer (Rodenak-Kladniew *et al.*, 2020), and antifungal (Vilela *et al.*, 2009) activities. Vuuren *et al.* (2007) reported that 1,8-cineole (Sigma-Aldrich) presented antibacterial activity against *S. aureus* (MIC = 8 mg mL⁻¹), *B. cereus* (MIC = 2 mg mL⁻¹), *E. coli* (MIC = 8 mg mL⁻¹), and *P. aeruginosa* (MIC = 4 mg mL⁻¹). These values are similar or lower than the ones obtained in our study for the same species with MIC ranging from 1.40 to 8.43 mg mL⁻¹. This indicates that the essential oil shows more antibacterial activity than the isolated compounds, suggesting the synergistic effect among the essential oil compounds in the present study. In addition, Sun *et al.* (2018) studied the antibacterial mechanism of 1,8-cineole in the genus *Salmonella*, and observed that 2.5 mg mL⁻¹ of this compound in ethanol modified the metabolism of carbohydrates and the genes related to ompF, ompD, yidC, lpp1, mraY, murD and skp of membrane proteins. The antifungal activity of 1,8-cineole (Sigma-Aldrich) was also

reported against *Candida albicans* with MIC of 8 mg mL⁻¹ and MFC of 64 mg mL⁻¹ (Hendry *et al.*, 2009). This suggests that, despite the antifungal activity found in our study at high concentrations, it was more efficient than the isolated compound. In our study, the compounds *trans*- β -caryophyllene and 1,8-cineole found in the leaves represents 26.4% of the essential oil mixture, and are probably the main antimicrobial agents, showing that the essential oil with this composition is a promising alternative to control microorganisms.

In the website of PubChem (Open Chemistry Database at the National Institutes of Health, USA), γ -muurolene and α -santalol are cited as general flavoring agents used in foods, including condiments, and seasonings; globulol is cited as anxiolytic, antipsychotic, neuroleptic, and a drug for schizophrenia, whereas δ -selinene and δ -cadinene are cited as surfactants and emulsifiers (PubChem, 2020a; PubChem, 2020b; PubChem, 2020c; PubChem, 2020d; PubChem, 2020e). These sesquiterpenes can potentialize the antimicrobial activity of the essential oil promoting the permeability of the microbial membranes (Bakkali *et al.*, 2008).

Our studies showed that *P. cattleianum* essential oil presents bactericidal activity, mainly against *B. cereus*, *S. aureus*, and *P. aeruginosa*, important pathogenic bacteria in public health. *S. aureus* is one of responsible for the most common pathogenic infections of Gram-positive bacteria in humans (Tong *et al.*, 2015), with resistance to several antibiotics around the world (Widianingrum *et al.*, 2019). *P. aeruginosa* is a pathogenic Gram-negative agent, and a common foodborne pathogen that can develop resistance to conventional antimicrobials and represents a serious threat to public health, mainly because it could cause bloodstream infections (Paramythiotou & Routsis, 2016). *B. cereus* is a Gram-positive bacterium that causes foodborne outbreaks, frequently associated with diarrheal and emetic syndromes (Zhu *et al.*, 2016).

Sivakumar & Bautista-Baños (2014) and Burt (2004) recommended that the use of essential oils to preserve food must be at concentrations from 0.1 to 6% (mass/mass). The fungicide concentrations of the leaf essential oil of *P. cattleianum* in our study ranged from 0.23 to 22.50 mg mL⁻¹ (equivalent to 0.2 to 2.2%, respectively). Therefore, the essential oil concentrations that showed antimicrobial activity in our study are in the range suggested as food preservative, making the essential oil from *P.*

cattleianum leaves an alternative for utilization in foods, as long as it has no cytotoxicity. Castro *et al.* (2015) evaluated the acute toxicity potential of the leaf essential oil of *P. cattleianum* on mice and showed that 500 mg kg⁻¹ (body mass) was not lethal or caused body mass reduction. Although the LD₅₀ is not documented in this study, given the variations in chemical composition of the essential oils obtained from different locations, toxicity studies must be evaluated for each mixture and their safety established, prior to its development to prevent foodborne diseases.

CONCLUSION

The essential oil yield from *P. cattleianum* leaves was 0.83%, and showed 60 compounds, mainly hydrocarbon sesquiterpenes (47.6%). The major compounds identified were *trans*- β -caryophyllene (14.7%) and 1,8-cineole (11.7%), followed by γ -muurolene (5.6%), α -santalol (4.7%), globulol (4.7%), δ -selinene (4.5%), and δ -cadinene (4.1%).

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The essential oil showed antimicrobial activity against all tested fungi and bacteria. The most promising fungicidal activities were against *A. fumigatus*, *A. ochraceus*, *A. versicolor*, and *T. viride*, and the bactericidal activities against *P. aeruginosa*, *B. cereus*, and *S. aureus*. The toxicity studies of the essential oil will support its development as a food preservative to control foodborne diseases.

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