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NOTE

Chemical composition and antibacterial activity of essential oils of *Eugenia* species

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Abstract The essential oils of the leaves of *Eugenia brasiliensis*, *Eugenia beaurepaireana*, and *Eugenia umbelliflora* were analyzed by GC–MS. The major compounds found in the oil of *E. brasiliensis* were spathulenol (12.6%) and τ-cadinol (8.7%), of *E. beaurepaireana* were β-caryophyllene (8.0%) and bicyclogermacrene (7.2%), and of *E. umbelliflora* were viridiflorol (17.7%) and β-pinene (13.2%). These oils were assayed to determine their antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. All of the oils analyzed showed antibacterial activity, ranging from moderate to strong, which was most accentuated for the *E. umbelliflora* and *E. brasiliensis* oils, which strongly inhibited the growth of *S. aureus* giving values of MIC = 119.2 and 156.2 μg/mL, respectively.

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Introduction

Eugenia is one of the largest genera of the Myrtaceae family and comprises around 350 native species [1]. Several *Eugenia* species are appreciated for their edible fruits, such as *Eugenia uniflora* [2] and *Eugenia edulis* [3]. Also, some species are used in folk medicine for their antidiarrheic, antifebrile, antirheumatic (*E. uniflora*) [4–6], and antidiabetic (*E. jambolana*) properties [7].

Eugenia brasiliensis Lamarck is a tree that grows in the coastal Brazilian forests and is commonly known as "grumixama" or Brazilian cherry [1]. Traditionally, the leaves, fruits, and bark wood of *E. brasiliensis* are used for rheumatism, diarrhea, and as a diuretic [8]. *Eugenia beaurepaireana* (Kiaerskou) Legrand is a tree popularly called "ingabaú" or "guamirim-ferro". The use of this species in South America is recommended for the treatment of inflammatory and ulcerative diseases and also as an astringent [8]. *Eugenia umbelliflora* (Berg.), known as "baguaçu", is a tree that grows in the southern Brazil, and the fruits are similar to sweet cherries [9]. A previous study has demonstrated the antibacterial activity of baguaçu leaf extracts [10].

This paper describes the chemical composition of essential oils from *E. brasiliensis*, *E. beaurepaireana*, and *E. umbelliflora* collected in southern Brazil and evaluates their antimicrobial properties toward Gram-positive and Gram-negative microorganisms of medical importance.

Materials and methods

Plant material

Leaves of *E. brasiliensis*, *E. beaurepaireana*, and *E. umbelliflora* were collected in October 2004, in Santo Amaro da Imperatriz (27.4029°S, 48.4705°W), Santa Catarina State, southern Brazil. Samples were identified by Prof. Dr. Daniel de Barcellos Falkenberg of the Botany Department, Universidade Federal de Santa Catarina. Voucher specimens were deposited at the Herbarium FLOR under numbers FLOR 34675, 34674, and 17890, respectively.

Extraction procedure

Essential oils of leaves from the species studied were obtained by hydrodistillation for 4 h in a modified Clevenger-type apparatus. After extraction, the oils were dried with sodium sulfate and stored at a low temperature.

GC–MS analysis of essential oils

All reagents were of analytical grade. Mass spectra were collected using a Varian 3800 Series GC-EIMS (Varian SATURN 2000), 70 eV, capillary column CP-SIL 8 CB (30 m × 0.25 mm i.d. × 0.25 μm film thickness) with helium as the carrier gas at a flow rate of 1.0 mL/min; the temperature program was 60°C (3 min) increasing at a rate of 5°C/min to 220°C which was held for 15 min; injection in the split mode at an injector temperature of 250°C; detector temperature of 240°C; and injection volume, 1.0 μL. Individual components were identified by comparison of both their mass spectra and experimental retention indices (RI), which were calculated for all volatile constituents by using a homologous series (C9 to C25) recorded under the same operating conditions, and comparison with literature data [11]. The NIST 1998 (National Institute for Standards and Technology) was also used for comparison of mass spectra. Since the standards for the positive confirmation were not used in the identification of the compounds, they must be considered only tentatively identified. The quantitative data were obtained by electronic integration of the GC–FID peak areas.

Microorganisms and medium

The bacterial strains were provided by the Laboratory of Clinical Microbiology, Universidade Regional de Blumenau (FURB) and acquired from The American Type Culture Collection (ATCC). Tests were carried out in duplicate with strains of *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922).

The identification of strains was confirmed by the use of biochemical profiles according to the recommendations of the *Manual of Clinical Microbiology* [12]. All organisms were maintained in brain–heart infusion (BHI) medium containing 30% (v/v) glycerol at 20°C. Before testing, the suspensions were transferred to trypticase soy agar supplemented with 5% sheep blood (Difco) and grown aerobically overnight at 35°C. The inocula were prepared by adjusting the turbidity of the suspension to match the 0.5 McFarland standard in saline solution (0.9%). Gentamycin was used as the reference antibiotic control.

Minimum inhibitory concentration determination

The broth microdilution method was used to determine the MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of the *Eugenia* species oils against the test organisms as recommended by the National Committee for Clinical Laboratory Standards [13]. This test was performed in sterile 96-well microplates. The oils were properly prepared and transferred to each microplate well in order to obtain a twofold serial dilution of the original extract in DMSO.

The inocula (10 μL) containing 5×10^5 CFU of each microorganism were added to each well. A number of wells were reserved in each plate to test for sterility control (no inocula added), inocula viability (no extract added), and the DMSO inhibitory effect. Plates were aerobically incubated at 35°C. After incubation for 18–24 h, bacterial growth was evaluated by the presence of turbidity and a pellet on the well bottom. MIC was defined as the lowest concentration of oils that had no macroscopically visible growth.

Minimum bactericidal concentration determination

To determine the MBC values, 1 μL of each well medium with no visible growth, along with those of the next two higher and lower concentrations, was removed and inoculated in blood agar plates. After 18–24 h of aerobic incubation at 35°C, the number of surviving organisms was determined. MBC was defined as the lowest extract concentration at which 99% of the bacteria were killed. Each experiment was repeated at least twice.

Results and discussion

Essential oil composition

After the extraction, the yields (v/w) of the essential oils of the three *Eugenia* species were calculated as follows: *Eugenia brasiliensis*, 0.07%; *Eugenia beaurepaireana*, 0.20%; and *Eugenia umbelliflora*, 0.33%.

A total of 65 compounds were identified, accounting for 83.0–93.3% of the constituents detected by GC. Table 1 shows the qualitative and quantitative chemical composition of the samples studied.

All oils showed some similarity in the qualitative composition, but they differed significantly from a quantitative point of view, showing some differences in their main constituents. The sesquiterpenoids constituted the dominant fraction of these oils. The oils had low amounts of monoterpenes, 14.6% in *E. brasiliensis* oil and 6.9% in *E. beaurepaireana*, with the exception of *E. umbelliflora* with 34.2%. This profile was the same as that previously reported for most essential oils of this genus. The exceptions are *E. caryophyllata* and *E. stigmatosa* whose major compounds were eugenol and (*Z*)-tetradec-5-enoic acid, respectively [14].

The major compounds found in *E. brasiliensis* were spathulenol (12.6%), τ-cadinol (8.7%), viridiflorol (7.1%), α-cadinol (6.6%), and 1-*epi*-cubenol (6.3%), all of which are oxygenated sesquiterpenes. In the monoterpene fraction, the most significant constituents were 1,8-cineole (3.2%) and α-pinene (2.9%). A previous study on the essential oil of the leaves of *E. brasiliensis* from southeastern Brazil found that the main compounds were α- and β-selinene (14.8 and 12.6%, respectively), not found in the oils studied here, and β-caryophyllene (12.6%) [1]. In fact, there are often large differences in the qualitative and quantitative composition of oils from the same plant species. The reasons for this variability may be the different geographical sources, the harvesting seasons, the genotype, the climate, the drying procedure, and the part of the plant distilled. These variables influence the relative concentration of each constituent in the oil [15].

In *E. beaurepaireana* essential oil, the major constituents were the sesquiterpenes β-caryophyllene (8.0%), bicyclogermacrene (7.2%), valencene (5.5%), viridiflorol (4.9%), and Δ-cadinene (4.9%). Among the more abundant monoterpenes were α-pinene (4.1%) and limonene (1.3%). Previous studies on specimens of *E. beaurepaireana* collected in southeastern Brazil have shown the prevalence of sesquiterpenic hydrocarbons, and, as the major substances, bicyclogermacrene (14.3%), Δ-cadinene (7.2%), and caryophyllene (6.4%), along with alcohols with a cadinane skeleton, such as τ-cadinol (6.5%) and α-cadinol (6.1%) [16]. Compounds with a cadinane skeleton were also found in the oil of *E. beaurepaireana* in the present study, and there was a similarity in relation to the presence of caryophyllene and bicyclogermacrene as compounds of greatest relevance in these oils.

Finally, the major compounds found in the *E. umbelliflora* oil were viridiflorol (17.7%), β-pinene (13.2%), α-pinene (11.2%), aromadendrene (6.9%), and ledol (4.7%). It is noteworthy that monoterpenes were among the most significant compounds in this oil. In a previous study, oil samples of *E. umbelliflora* from southern Brazil contained α-pinene and β-pinene as the major compounds in concentrations of 24.7% and 23.5% [17], respectively, differing from the oil studied here in which viridiflorol was the compound found in greatest amount. The amounts of α- and β-pinene found in the oil of the present study were also significantly lower.

Of all the compounds found in these oils, α-pinene (a monoterpene) and viridiflorol (a sesquiterpene) were present in greatest quantities in all of the species analyzed. Another eighteen sesquiterpenes (β-caryophyllene, aromadendrene, valencene, bicyclogermacrene, Δ-cadinene, spathulenol, 1-*epi*-cubenol, isoledene, α-*neo*-clovene, α-humulene, alloaromadendrene, β-*cis*-guaiene, β-*trans*-guaiene, γ-cadinene, cadina-1,4-diene, guaiol, 1,10-di-*epi*-cubenol, and 10-*epi*-γ-eudesmol) and one monoterpene (limonene) were common to three oils of the *Eugenia* species investigated in this study. The observation of differences in the mixture of compounds in the essential oils of the different *Eugenia* species suggests that they may provide characteristics useful for understanding the phylogenetic relationships in this large genus whose species are notoriously difficult to classify and identify [18].

Antimicrobial assays

Table 2 shows the results of the antimicrobial assays with essential oils of three species of *Eugenia*. Aligianis et al. [19] proposed a classification for the antimicrobial activity of plant products, based on the MIC results as follows: strong inhibitors—MIC below 500 μg/mL; moderate inhibitors—MIC between 600 and 1,500 μg/mL; weak inhibitors—MIC above 1,600 μg/mL. Thus, based on the MIC results obtained in the microplate assays, *Eugenia* essential oils have interesting antibacterial potential, since all samples tested showed positive activity, with MIC values below 1,600 μg/mL.

Essential oils of *E. umbelliflora* and *E. brasiliensis* showed potent antibacterial activity against *S. aureus*, with MIC values of 119 and 156 μg/mL, respectively. The bacteria *Staphylococcus aureus* is a major human pathogen, which can colonize many different tissues and organs, thereby causing a wide variety of diseases. The resulting complexity of staphylococcal pathogenesis poses an urgent challenge, especially in light of increasing resistance to antibiotics and the emergence of severe invasive infections [20].

The essential oil of *E. beaurepaireana* was the most active oil against Gram-negative bacteria (*P. aeruginosa*, MIC = 278 μg/mL; *E. coli*, MIC = 477 μg/mL). This oil showed better activity against Gram-negative than Gram-positive bacteria. This finding is significant because the

Table 1 Composition (%) of essential oils from the leaves of *Eugenia* species

	Compounds[a]	Rt (min) (RI(E))[b]	*E. brasiliensis*	*E. beaurepaireana*	*E. umbelliflora*
1	α-Tujene	6.317 (936)	–	–	0.3
2	α-Pinene	6.551 (944)	2.9	4.1	11.2
3	Sabinene	7.795 (984)	–	0.3	–
4	β-Pinene	7.814 (984)	2.2	–	13.2
5	Myrcene	8.068 (991)	0.4	–	0.5
6	α-Phellandrene	8.598 (1,008)	–	0.4	1.5
7	Δ-3-Carene	8.674 (1,011)	–	–	0.2
8	α-Terpinene	8.908 (1,020)	–	–	0.5
9	*o*-Cymene	9.142 (1,028)	–	0.2	0.2
10	Limonene	9.282 (1,033)	2.3	1.3	1.4
11	β-Phellandrene	9.343 (1,035)	–	–	0.6
12	1,8-Cineole	9.394 (1,037)	3.2	–	–
13	β-(*E*)-Ocimene	9.742 (1,049)	–	–	0.2
14	γ-Terpinene	10.140 (1,062)	–	–	0.4
15	Terpinolene	10.968 (1,088)	–	0.4	2.9
16	Myrcenol	14.254 (1,119)	–	0.2	–
17	Terpin-4-ol	13.828 (1,185)	0.4	–	0.2
18	α-Terpineol	14.251 (1,198)	2.0	–	0.9
19	Myrtenyl acetate	17.793 (1,250)	1.2	–	–
20	Δ-Elemene	18.040 (1,336)	–	0.3	–
21	α-Cubebene	18.444 (1,351)	–	1.4	0.2
22	Isoledene	19.225 (1,380)	0.4	0.2	0.2
23	α-Copaene	19.239 (1,381)	–	2.3	–
24	β-Bourbonene	19.461 (1,389)	–	0.2	–
25	β-Elemene	19.580 (1,393)	–	3.1	–
26	α-Gurjunene	20.070 (1,411)	–	0.3	1.0
27	β-Caryophyllene	20.417 (1,426)	3.6	8.0	4.3
28	β-Gurjunene	20.598 (1,436)	–	–	1.2
29	γ-Elemene	20.693 (1,437)	–	0.8	–
30	Aromadendrene	20.851 (1,445)	0.7	0.6	6.9
31	α-Guaiene	20.896 (1,445)	–	1.0	–
32	α-*neo*-Clovene	21.107 (1,455)	0.3	0.6	0.2
33	α-Humulene	21.318 (1,462)	0.7	2.3	0.4
34	Alloaromadendrene	21.436 (1,467)	0.5	0.9	0.8
35	β-Chamigrene	21.691 (1,479)	–	0.7	–
36	γ-Muurolene	21.744 (1,477)	0.5	–	0.7
37	Germacrene D	21.858 (1,483)	0.3	1.6	–
38	β-*cis*-Guaiene	22.030 (1,489)	0.3	0.6	0.1
39	Valencene	22.187 (1,496)	1.1	5.5	1.5
40	Bicyclogermacrene	22.323 (1,501)	2.4	7.2	1.4
41	β-*trans*-Guaiene	22.458 (1,507)	0.3	0.4	0.2
42	γ-Cadinene	22.723 (1,518)	0.4	1.6	0.3
43	Δ-Cadinene	22.850 (1,524)	2.7	4.9	2.6
44	*trans*-Calamene	22.928 (1,527)	1.0	–	–
45	Cadina-1,4-diene	23.161 (1,537)	0.2	0.7	0.3
46	α-Cadinene	23.257 (1,539)	–	1.6	–
47	α-Calacorene	23.406 (1,548)	0.4	–	–
48	Selina-3,7-(11)-diene	23.436 (1,549)	–	0.3	–

Table 1 continued

	Compounds[a]	Rt (min) (RI(E))[b]	*E. brasiliensis*	*E. beaurepaireana*	*E. umbelliflora*
49	Germacrene B	23.843 (1,566)	–	–	0.1
50	Ledol	23.910 (1,569)	–	2.1	4.7
51	Spathulenol	24.312 (1,586)	12.6	4.9	3.1
52	Caryophyllene oxide	24.436 (1,590)	3.1	–	0.9
53	Viridiflorol	24.540 (1,595)	7.1	4.9	17.7
54	Guaiol	24.732 (1,603)	5.4	3.7	2.5
55	1,10-di-*epi*-Cubenol	24.988 (1,615)	1.1	1.8	0.2
56	10-*epi*-γ-Eudesmol	25.250 (1,626)	3.1	1.6	1.6
57	1-*epi*-Cubenol	25.450 (1,636)	6.3	1.5	1.7
58	γ-Eudesmol	25.578 (1,640)	2.7	–	–
59	τ-Cadinol	25.783 (1,650)	8.7	2.0	–
60	Cubenol	25.795 (1,651)	–	3.2	2.5
61	α-Muurolol	25.870 (1,654)	2.8	–	0.4
62	α-Cadinol	26.083 (1,663)	6.6	–	1.3
63	7-*epi*-α-Eudesmol	26.102 (1,665)	–	3.3	–
64	Khusinol	26.352 (1,679)	0.3	–	0.1
65	Khusimol	27.733 (1,739)	0.2	–	–
Total identified			90.4	83.0	93.3
Monoterpene hydrocarbons			7.8	6.7	33.1
Oxygenated monoterpenes			6.8	0.2	1.1
Sesquiterpene hydrocarbons			15.8	47.1	22.4
Oxygenated sesquiterpenes			60.0	29.0	37.3

Rt retention time (min), *RI(E)* retention index (experimental data), – not detected

[a] The identified constituents are listed in their order of elution from a nonpolar column (CP-SIL 8 CB)

[b] $RI(E) = 100.Z + 100\ (\log t'_{RX} - \log t'_{RZ})/(\log t'_{RZ+1} - \log t'_{RZ})$, where Z = number of carbons of the hydrocarbon with previous retention time; t'_{RX} = retention time of the sample; t'_{RZ} = retention time of the previous hydrocarbon (in relation to the sample); and t'_{RZ+1} = retention time of the later hydrocarbon (in relation to the sample)

Table 2 Antimicrobial activity of essential oils from *Eugenia* species

Essential oils	*S. aureus* (ATCC 25923)		*E. coli* (ATCC 25922)		*P. aeruginosa* (ATCC 27853)	
	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)
Eugenia brasiliensis	156.2	624.9	624.9	624.9	624.9	1,250.0
Eugenia beaurepaireana	1,110.0	2,200.0	556.6	1,110.0	278.3	1,110.0
Eugenia umbelliflora	119.2	477.0	477.0	477.0	477.0	954.0
Gentamycin	5.0	–	7.5	–	1.0	–

MIC minimum inhibitory concentration, *MBC* minimum bactericidal concentration

great majority of plant extracts are more active against Gram-positive bacteria. The greater resistance of Gram-negative bacteria can be explained through the fact that the outer membrane of Gram-negative bacteria presents a barrier to many substances, including antibiotics [21], and the periplasmic space contains enzymes that are able to break down foreign molecules [22]. Moreover, Gram-negative bacteria have efflux pumps that reduce the cellular levels of antibiotics [23]. Essential oil of *E. umbelliflora* also showed significant activity against *P. aeruginosa* and *E. coli.*

The results obtained revealed the potential in vitro antibacterial properties of essential oils of the three species of *Eugenia* investigated, indicating the importance of further studies related to their application in the antibiotic treatment of infectious diseases.

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