SUPPLEMENTARY MATERIAL

Chemical composition and evaluation of the antimicrobial activity of essential oil from leaves of *Eugenia platysema*.

Adrielli Tenfen^a, Diogo Alexandre Siebert^a, Celina Noriko Yamanaka^a,
Caio Maurício Mendes de Córdova^a, Dilamara Riva Scharf^b, Edésio Luiz
Simionatto^b, Michele Debiasi Alberton^{a,*}

^aDepartamento de Ciências Farmacêuticas, Universidade Regional de Blumenau, Brazil; ^bInstituto de Pesquisas Tecnológicas, Universidade Regional de Blumenau, Brazil

*Corresponding author. Address: Rua São Paulo, 2171. Blumenau-SC, Brazil. CEP 89030-000. E-mail: michele@furb.br. Telephone: +55 47 33217318.

Abstract

This study describes the qualitative and quantitative chemical composition and evaluates the antibacterial activity of essential oil from *Eugenia platysema* leaves. Analysis by GC-FID and GC-MS allowed the identification of 22 compounds. Different from the other species of the *Eugenia* genus, the major compound found in the essential oil was the diterpene phytol (66.05 %), being this the first report of the presence of this compound in the essential oils from *Eugenia* genus. The sesquiterpene elixene was the second most concentrated compound in the studied essential oil (9.16%). The essential oil from *E. platysema* was tested for its antibacterial activity against cell-wall bacteria and mollicute strains of clinical interest using the microdilution broth assay. The results showed that the essential oil of *Eugenia platysema* was inactive until 1,000 $\mu\text{g.mL}^{-1}$ against tested bacteria.

Keywords: *Eugenia platysema*; essential oil; antibacterial activity; phytol.

Experimental

Plant material

Leaves of *E. platysema* were collected in June 2013 in Blumenau (26°54'26.8"S 49°04'52.4" O), Santa Catarina State, Southern Brazil. Professor Dr. Marcos Sobral from the Botany Department of the University of São João Del-Rey and Msc. André Luis Gasper from the Department of Natural Sciences of the University of Blumenau identified the samples. A voucher specimen was deposited at the herbarium Roberto Miguel Klein from Univerdade Regional de Blumenau under number FURB 42336.

Extraction procedure

Essential oils of fresh leaves were obtained by hydrodistillation for 4 h in a modified Clevenger-type apparatus (Houghton & Raman 1998), in the proportion of 1g of leaves to 10 mL of distilled and deionized water. After extraction, the essential oils were dried with sodium sulfate and stored at a low temperature.

GC-FID and GC-MS Analysis

All reagents were of analytical grade. The qualitative analysis were performed by GC-MS in a chromatograph Shimadzu® GCMS-QP2010 Plus (nonpolar capillary column RTX®-5MS: 30 m x 0.25 mm x 0.25 µm), and the quantitative analysis by GC-FID in a chromatograph Shimadzu® GC-FID 2010 (nonpolar capillary column OV®-5: 30 m x 0.25 mm x 0.25 µm), being this the quantitative analysis performed in triplicate. The oven temperature program was 60°C for 5 min, with increase of 3°C per each min until 240°C remaining in this temperature for 5 min. Helium was used as carrier gas (flow rate of 1 mL.min⁻¹), with the temperature of the injector of 250°C (1:20 split) and an ion source of 70 eV on the MS, with interface at 280°C. Identification of the essential oil components was based upon their retention indexes (in comparison with an homologous series of alkanes from C8 to C19), and by comparison of these and their mass spectral patterns with those reported in the literature (Adams 2007) and stored in the MS library NIST 2008 (National Institute for Standards and Technology) database. Since the standard for the positive confirmation were not used in the identification of the compounds, they must be considered only tentative identified.

Evaluation of antibacterial activity

Microorganisms and medium

The Laboratory of Clinical Microbiology from Universidade Regional de Blumenau (FURB) provided the bacterial strains. Tests were evaluated against gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *S. saprophyticus* (ATCC 15305) and against gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Mollicutes strains *Mycoplasma mycoides* subsp. *capri* (NCTC 10137); *Mycoplasma genitalium* (ATCC 33530), and *Ureaplasma urealyticum* (ATCC 27618) were also assessed.

For the growth of bacterial strain, Müller-Hinton broth was used for *S. aureus*, *S. saprophyticus*, *E. coli* and *P. aeruginosa*, U10 broth (Shepard 1974) was used for *U. urealyticum*, SP4 broth to *M. mycoides* subsp. *capri* and *M. genitalium* (Velleca et al. 1979).

Determination of minimum inhibitory concentration (MIC)

The antibacterial activity of the essential oil was evaluated by determination of the minimum inhibitory concentration (MIC). The microdilution broth assay was performed in sterile 96-well microplates, as recommended by the Clinical and Laboratory Standards Institute (CLSI 2012) for cell-wall bacteria and Bebear & Robertson (1996) for mollicutes.

The essential oil was properly prepared and transferred to each microplate well with the appropriate culture medium, in order to obtain a twofold serial dilution of the original extract in a 10 % H₂O/dimethyl sulfoxide (DMSO) solution, obtaining sample concentrations ranging between 1000 µg.mL⁻¹ to 7.81 µg.mL⁻¹. The inoculum containing 10⁴ to 10⁵ microorganisms per mL were then added to each well. A number of wells were reserved in each plate to test for sterility control (no inoculum added), positive control (gentamycin or ciprofloxacin), inoculum viability (no extract added), and the DMSO inhibitory effect.

The microplates were incubated at 37 ± 1°C for 24 or 48 h (depending on the bacterium). Thereafter, growth of mollicutes strains was detected by observing the colour change in the medium. For cell-wall bacteria, a methanolic solution of triphenyltetrazolium chloride (5 mg.mL⁻¹) was added into each well, and the presence of a reddish bacterial “button” observed at the bottom of each well. The MIC was defined as the lowest concentration of the essential oil able to inhibit bacterial growth.

Table S1. Compounds identified (%) in the essential oil of leaves from *E. platysema*.

Compound	Rt ^a (min)	Chromatographic area (%)	AI (E) ^b	AI (L) ^c
δ-elemene	24.531	0.38 ± 0.04	1328	1335
β-elemene	26.899	1.50 ± 0.12	1383	1389
α-gurjunene	27.619	0.52 ± 0.02	1400	1409
β-cariophyllene	27.974	0.56 ± 0.03	1409	1417
Aromadendrene	28.793	1.26 ± 0.18	1429	1439
α-humulene	29.379	0.49 ± 0.07	1443	1452
Allo-aromadendrene	29.678	1.02 ± 0.06	1451	1458
Elixene	31.154	9.16 ± 0.50	1487	1505
α-farnesene	31.783	1.18 ± 0.13	1497	1505
Trans-dauca-4(11),7-diene	33.921	0.53 ± 0.08	1557	1556
Spathulenol	34.310	1.40 ± 0.09	1567	1577
Globulol	34.590	5.71 ± 0.18	1573	1590
Viridiflorol	34.866	1.25 ± 0.03	1581	1592
Rosifoliol	36.045	0.65 ± 0.01	1611	1600
β-himachalene-oxide	36.623	0.93 ± 0.26	1627	1615
γ – eudesmol	37.183	0.64 ± 0.15	1643	1630
Allo-himachalol	38.994	0.25 ± 0.06	1659	1661
2-Z, 6-E- farnesal	40.207	0.93 ± 0.04	1728	1715
2-E, 6-E-farnesyl acid	40.298	0.02 ± 0.02	1821	1816
2-E, - 6-E- farnesyl acetate	46.941	3.15 ± 0.07	1846	1845
2-Z, 6-Z –farnesyl acetate	47.976	1.42 ± 0.12	1863	1860
Phytol	52.522	66.05 ± 0.81	-	1942
Monoterpenes		0.00 %		
Oxygenated sesquiterpenes		16.60 %		
Sesquiterpene hydrocarbon		16.35 %		
Oxygenated diterpenes		66.05 %		
Total		99.00%		

Identified compounds listed by order of elution in nonpolar column (RTx[®]-5MS).^aRt = Retention time in minutes.^bAI (E) =Arithmetic index (experimental date)^cAI= Arihmeticindex (Adams, 2007)

Table S2. Antibacterial activity ($\mu\text{g.mL}^{-1}$) of essential oil from *E. platysema*.

Strains	MIC*	
	<i>E. platysema</i> essential oil	Positive control**
<i>Staphylococcus aureus</i>	>1000	20.0
<i>Pseudomonas aeruginosa</i>	>1000	20.0
<i>Escherichia coli</i>	>1000	20.0
<i>Ureaplasma urealyticum</i>	>1000	7.3
<i>Mycoplasma mycoides</i> sub. <i>capri</i> (MLC)	>1000	7.3
<i>Mycoplasma genitalium</i>	>1000	7.3

* MIC: Minimum inhibitory concentration, $\mu\text{g.mL}^{-1}$

**Positive control: Gentamicin, for cell-wall bacteria; Ciprofloxacin, for mollicute strains.

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