

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS FROM VERBENACEAE SPECIES: ALTERNATIVE SOURCES OF (*E*)-CARYOPHYLLENE AND GERMACRENE-D

Ricardo M. Montanari, Luiz C. A. Barbosa,* Antonio J. Demuner e Cleber J. Silva

Departamento de Química, Universidade Federal de Viçosa, Av. PH Rolfs, s/n, 36570-000 Viçosa – MG, Brasil

Larissa S. Carvalho e Nélio J. Andrade

Departamento de Tecnologia de Alimentos, Universidade Federal de Viçosa, Av. PH Rolfs, s/n, 36570-000 Viçosa – MG, Brasil

Recebido em 7/1/11; aceito em 4/5/11; publicado na web em 29/6/11

Volatile oils from the leaves of Verbenaceae species *Aloysia virgata*, *Lantana camara*, *Lantana trifolia*, *Lantana montevidensis*, *Lippia brasiliensis* and *Lippia sericea* were investigated for its chemical composition and antibacterial activity. The volatile oils were characterized by a high content of sesquiterpenes of which (*E*)-caryophyllene (10-35%), germacrene-D (5-46%) and bicylogermacrene (7-17%) were the major components for all studied species. For the flowers, a higher concentration of monoterpenes was observed for the species *L. camara*, *L. trifolia* and *L. brasiliensis*. These compounds probably act as attractive to specific pollinators. The volatile oils from *A. virgata* was the most active, exhibiting moderate antimicrobial activity against the bacteria *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*.

Keywords: *Lippia* spp; *Aloysia virgata*; *Lantana* spp.

INTRODUCTION

The Verbenaceae comprises several species with medicinal and ornamental uses, especially those of the genera *Aloysia* Palau, *Lantana* L., *Lippia* L. and *Stachytarpheta* Vahl. This family occurs in virtually all lands ecosystems, and it is one of the five most important dicotyledons families of the Brazilian altitude grassland.¹ The family includes 2600 species grouped into 100 genera with pantropical distribution.

Cavalcanti *et al.*² described the acaricidal activity for the *Lippia sidoides* Cham volatile oils and its major compounds (thymol, carvacrol and (*E*)-caryophyllene). The volatile oils from *L. multiflora* Moldenke was more active than the one from *L. chevalieri* Moldenke against Gram-negative and Gram-positive bacteria.³ The infusion from leaves of *L. javanica* (Burm f.) Spreng is commonly used as coughs suppressant and decongestant and its essential oil has been shown to possess antimicrobial activity.⁴ The non-polar extracts of *L. adoensis* Hochst had an inhibitory effect on growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and the fungus *Trichophyton mentagrophytes*.⁵

The essential oils from *Aloysia triphylla* Royle and *Lippia alba* (Mill.) N.E. Br has been shown to present antifungal activity against *Candida albicans*.⁶ Essential oils from *Aloysia virgata* collected in Cuba⁷ and Argentina⁸ were rich in sesquiterpenes such (*E*)-caryophyllene (16-19%), germacrene-D (16-27%) and bicylogermacrene (18-29%). The chemical composition and the antibacterial activity were also demonstrated by the volatile oils from *Lantana camara* L.⁹ and *Lantana montevidensis*.¹⁰ However, the geographic origin and genetic variability of the plants can influence the chemical composition. Therefore, studies on the occurrence and chemical composition of these plants in different regions are needed.

It is known that the occurrence of secondary metabolites with similar biological activities can be expected in phylogenetically

related plants,¹¹ which may contribute to the implementation of more rational approaches for the search of new substances with potential economic interest. This hypothesis supports the search for antimicrobial substances among Verbenaceae species. In addition, the Verbenaceae family is widely spread in Brazil. The Cadeia do Espinhaço region, in Minas Gerais and Goiás states, represent one of the major centers of diversity of *Lippia* genera. Most species from this genus at this region are at risk of extinction due to the vulnerability of this area and there are few phytochemical studies to date on such plants.¹²

Therefore, in line with our studies on the biological activities and composition of volatile oils from several plant species,¹³ this work describes the chemical composition of essential oils from *Aloysia virgata* (Ruiz & Pav.) Pers., *Lippia brasiliensis* (Link) T. Silva, *Lippia sericea* Sham., *Lantana camara* L., *Lantana trifolia* L. and *Lantana montevidensis* (Spreng.) Briq. and the antibacterial activity of oil from five of these species. Also, we report on the seasonal variation of volatiles from *Lippia brasiliensis* since, to the best of our knowledge, its chemical composition has not been previously reported.

EXPERIMENTAL

Plant material

Aerial parts of the following species were collected in Atlantic Rainforest fragments of different cities of the Minas Gerais state, Brazil, the voucher specimens have been deposited in the VIC Herbarium of the Departamento de Biologia Vegetal, Universidade Federal de Viçosa (UFV): *Aloysia virgata* (Ruiz & Pav.) Pers., Viçosa, VIC 7633; *Lippia brasiliensis* (Link) T. Silva, Viçosa, VIC 31852; *Lippia sericea*, Ouro Branco, VIC 21365; *Lantana camara* L., Tocantins, VIC 30159; *Lantana trifolia* L., Tocantins, VIC 21550; and *Lantana montevidensis* (Spreng.) Briq., Viçosa, VIC 21551. The samples were handling along March 2008 to January 2009.

*e-mail: lcab@ufv.br

Essential oil extraction

Leaves and flowers were collected separately, in triplicate and completely randomized way among individuals of the studied populations. Each sample (100 g) was chopped and subjected to three hours hydrodistillation in a Clevenger-type apparatus. The resulting oils were separated, weighed and their yields calculated with respect to dry matter mass. Oils were stored under nitrogen atmosphere and maintained at -4 °C, until they were analyzed by gas chromatography or used in the bioassays.

Gas chromatography (GC)

GC analyses were carried out with a GC-17A Series instrument (Shimadzu, Japan) equipped with a flame ionization detector (FID). Chromatographic conditions were as follows: fused silica capillary column (30 m x 0.22 mm) with a DB-5 bonded phase (0.25 µm film thickness); carrier gas, N₂ at a flow rate of 1.8 mL min⁻¹; injector temperature 220 °C, detector temperature 240 °C; column temperature was programmed to start at 55 °C (isothermal for 2 min), with an increase of 3 °C min⁻¹, to 240 °C, isothermal at 240 °C for 15 min; injection of 1.0 mL (1% w/v in dichloromethane); split ratio 1:10; column pressure of 115 kPa.

The analyses were carried out in triplicate and the amount of each compound was expressed as a relative percentage of the total area of the chromatograms.

Gas chromatography-mass spectrometry (GC-MS)

The GC-MS unit (model GCMS-QP5050A, from Shimadzu, Japan) was equipped with a DB-5 fused silica column (30 m x 0.22 mm i.d., film thickness 0.25 µm) and interfaced with an ion trap detector. Transfer line temperature, 240 °C; ion trap temperature, 220 °C; carrier gas, He at a flow rate of 1.8 mL min⁻¹; injector temperature 220 °C, detector temperature 240 °C; column temperature was programmed to start at 55 °C (isothermal for 2 min), with an increase of 3 °C min⁻¹, to 240 °C, isothermal at 240 °C for 15 min; injection of 1.0 µL (1% w/v in dichloromethane); split ratio 1:10; column pressure of 100 kPa; ionization energy, 70 eV; scan range, 29-450 u; scan time, 1 s. The identity of each component was assigned by comparison of their retention indexes (RRI), relative to a standard alkane series (C₉-C₂₇) and also by comparison of its mass spectrum with either reference data from the equipment database (Wiley 330 000) and from the literature.¹⁴

Biological assays

Bacterial strains were obtained from the collections of the Department of Microbiology, Federal University of Viçosa, Viçosa, Minas Gerais state, Brazil. Microorganisms used were Gram-positive *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus*, Ribotype 1 222-173-S4 isolated from post-pasteurization equipment surfaces,¹⁵ and Gram-negative *Escherichia coli* (ATCC 11229).

The agar disc diffusion method was employed to determine the antimicrobial activity of the essential oils, as previously described.¹⁶ Briefly, a suspension of the tested microorganism (2 x 10⁸ CFU mL⁻¹) was spread on Petri plates with Mueller Hinton agar. Filter paper discs (6 mm diameter) were individually impregnated with 5 µL of the essential oils and placed on the inoculated plates. The plates were incubated for 48 h at 37 °C in the cases of *S. aureus* and *E. coli* and at 32 °C for *B. cereus*. The diameters of the inhibition zones were measured using a paquimeter and expressed in millimeters. The antibiotic chloramphenicol (30 µg) and sterile water were included in each experiment as positive and negative controls. Each test was performed

in triplicates and repeated three times. The results were analyzed by ANOVA and Scott-Knott's multiple-range tests at P ≤ 0.05 by using the software GENES (Genetics and Statistical Analysis. Version 2007.0.0 - Federal University of Viçosa, Viçosa – MG, Brazil).

Determinations of minimum inhibitory concentration (MIC)

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC).¹⁷ A serial doubling dilution of each essential oil was prepared in a 96-well microtiter plate over the range 0.0156-2%. The broth was supplemented with Tween 80 (Merck, Germany) at a concentration of 0.5% (v/v) in order to enhance essential oils solubility. Overnight broth cultures of each strain were prepared in Brain Heart Infusion Broth (Himedia) and the final concentration in each well was adjusted to 2 x 10⁵ CFU/mL following inoculation. The concentration of each inoculum was confirmed by viable count on Plate Count Agar (Himedia). Positive and negative growth controls were included in every test. The plates were incubated aerobically at 30 °C (Gram-negative) or 37 °C (Gram-positive) according to strain. The MIC is defined as the lowest concentration of the essential oil at which the microorganism tested does not demonstrate visible growth. The bacteria growth was indicated by the turbidity, accessed in a spectrophotometer at 625 nm.

RESULTS AND DISCUSSION

Chemical composition

The yields of volatile oils from the leaves and flowers ranged from 0.04 to 0.7% depending on the species and the part of the plant analyzed (Table 1). The highest yields were found in the flowers from *L. brasiliensis* (0.7%), *L. camara* (0.6%) and *L. trifolia* (0.7%). For the leaves, the lowest yields were in the species *Lippia sericea* (0.04%).

The major constituents identified from the volatile oils of the species investigated are listed in Table 1.

It was found that essential oils from *Lippia* species contains greater amount of (*E*)-caryophyllene than germacrene-D, whereas the oils from *Aloysia* and *Lantana* species have more germacrene-D than (*E*)-caryophyllene.

Sesquiterpene skeletal diversity arises from the action of a number of sesquiterpene synthases enzymes.¹⁸ The reaction mechanism of all sesquiterpene synthases starts with the ionization of farnesyl diphosphate. The resulting carbocation can undergoes a range of cyclizations. The 11,1-closure yields the 11-membered ring humulyl cation. Then, deprotonation followed by cyclobutane ring formation leads to (*E*)-caryophyllene production. Otherwise, the 10,1-closure originates the germacrene cation, which can be converted to germacrene-D. Thus, the precursors flow toward one of these constituents can reduce the amounts of the other.¹⁹

Lantana camara is the most widespread species of *Lantana* genus. The volatiles oils of some varieties of *L. camara* have been studied and they differ in composition according to geographic origin of the plants.²⁰ In spite of geographic differences, the major constituents observed for some samples obtained from different locations in Brazil were limonene (16.5%), α-phellandrene (16.4%), germacrene-D (13.2%), (*E*)-caryophyllene (10.8%) and sabinene (8.9%).²¹

The species *L. trifolia* is a small shrub that occurs in all regions of Brazil and is used in folk medicine in the form of infusions and syrups for the treatment of respiratory disorders and as sedative. Muhayimana *et al.*²² observed that (*E*)-caryophyllene and germacrene-D were also the major volatile oil constituents in this plant.

In general, for all studied species, the content of monoterpenes was higher in oils extracted from flowers compared with the oils

Table 1. Constituents and yields of essential oils (%) from species *Aloysia virgata* (ALVG), *Lippia sericea* (LPSR), *Lantana camara* (LTCM), *L. trifolia* (LTTF) and *L. montevidensis* (LTMT)

Constituents	IR ^a	ALVG	LPSR	LTCM		LTTF		LTMT
		Leaves	Leaves	Leaves	Flowers	Leaves	Flowers	Leaves
Hydrocarbon Monoterpenes		8.8	0.5	-	17.8	-	12.3	-
α-Thujene	931	-	-	-	0.5±0.1	-	-	-
α-Pinene	939	1.2±0.0	-	-	1.5±0.2	-	-	-
Sabinene	976	0.4±0.1	-	-	2.2±0.3	-	7.9±0.6	-
β-Pinene	980	-	-	-	1.4±0.2	-	0.2±0.0	-
p-Cymene	1026	-	-	-	0.3±0.0	-	-	-
Limonene	1031	1.4±0.1	-	-	7.8±0.4	-	2.8±0.2	-
(Z)-β-Ocimene	1040	0.8±0.2	-	-	-	-	1.4±0.1	-
(E)-β-Ocimene	1050	5.0±0.5	0.5±0.2	-	0.9±0.1	-	-	-
γ-Terpinene	1062	-	-	-	3.2±0.2	-	-	-
Oxygenated Monoterpenes		-	2.9	-	1.8	-	1.4	-
Linalol	1098	-	1.4±0.3	-	1.8±0.1	-	0.8±0.0	-
Terpinen-4-ol	1177	-	-	-	-	-	0.6±0.1	-
Piperitone	1252	-	0.4±0.2	-	-	-	-	-
Bornyl acetate	1285	-	1.1±0.3	-	-	-	-	-
Hydrocarbon Sesquiterpenes		81.4	48.3	91.0	73.0	64.3	79.9	80.7
δ-Elemene	1339	0.7±0.1	0.6±0.1	1.5±0.2	1.8±0.2	-	-	-
α-Cubebene	1351	-	0.2±0.1	-	-	-	-	-
α-Copaene	1376	0.4±0.0	0.6±0.2	3.3±0.1	4.7±0.3	3.8±0.3	3.0±0.2	1.3±0.2
β-Bourbonene	1384	-	-	1.1±0.2	0.3±0.1	-	-	0.8±0.1
β-Cubebene	1390	-	0.3±0.2	-	-	-	-	-
β-Elemene	1391	1.8±0.1	1.3±0.6	-	0.8±0.2	1.4±0.1	2.9±0.2	1.8±0.3
α-Gurjunene	1409	-	-	-	-	-	-	-
(E)-Caryophyllene	1418	8.9±0.8	16.8±1.2	17.0±1.1	13.6±0.9	10.2±1.2	16.1±0.7	9.2±1.2
Aromadendrene	1439	-	1.0±0.5	-	-	-	-	-
α-Guaiene	1439	-	1.5±0.3	-	-	-	-	-
α-Humulene	1454	1.6±0.1	-	1.7±0.2	1.6±0.2	-	1.5±0.1	1.3±0.1
(E)-β-Farnesene	1458	-	-	-	0.5±0.1	-	-	-
Alloaromadendrene	1461	0.2±0.1	-	2.3±0.3	1.9±0.2	2.4±0.2	1.9±0.2	2.1±0.5
Germacrene-D	1480	46.3±1.1	4.6±0.7	46.1±1.4	32.4±1.6	29.5±1.4	38.1±1.2	42.7±1.2
β-Selinene	1485	-	0.8±0.4	-	-	-	-	-
Valecene	1491	-	-	-	-	-	-	0.7±0.1
Bicyclgermacrene	1494	17.4±1.0	7.1±1.5	8.5±0.7	5.8±0.6	9.1±0.3	11.0±0.6	16.1±0.9
α-Murolene	1499	-	0.5±0.3	-	0.4±0.1	2.5±0.1	1.0±0.1	1.0±0.2
Germacrene-A	1503	1.1±0.3	2.9±0.6	-	0.5±0.2	1.8±0.1	2.5±0.2	1.7±0.3
γ-Cadinene	1513	0.5±0.1	0.4±0.2	1.7±0.2	0.4±0.0	-	-	-
(Z)-Calamenene	1521	-	-	-	-	-	-	-
δ-Cadinene	1524	1.3±0.3	2.8±0.9	3.6±0.3	3.3±0.1	3.6±0.2	1.9±0.1	2.0±0.2
Germacrene-B	1556	1.2±0.2	6.9±1.1	4.2±0.5	5.0±0.4	-	-	-
Oxygenated Sesquiterpenes		6.8	32.9	1.5	1.3	8.7	2.8	14.3
(E)-β-Damascenone	1380	-	0.3±0.1	-	-	-	-	-
Geranyl-acetone	1453	-	5.0±1.3	-	-	-	-	-
Elemol	1549	0.7±0.1	-	-	-	-	-	-
(E)-Nerolidol	1564	-	0.4±0.2	-	-	5.3±0.4	-	6.1±0.5
Germacrene-D-4-ol	1574	1.2±0.2	-	0.7±0.1	0.5±0.1	-	0.6±0.1	-
Spathulenol	1576	-	6.4±0.7	-	-	-	0.9±0.0	3.2±0.4
Caryophyllene oxide	1581	-	8.1±0.7	0.8±0.1	-	2.3±0.1	-	1.7±0.3
Viridiflorol	1590	4.2±0.3	1.4±0.2	-	-	-	-	-
α-Murolol	1645	-	5.3±0.5	-	-	-	-	1.8±0.1
α-Cadinol	1653	0.7±0.1	2.9±0.9	-	0.8±0.2	1.1±0.0	1.3±0.3	1.5±0.1
α-Bisabolol	1653	-	3.1±0.2	-	-	-	-	-
Others		-	10.5	-	1.6	19.3	1.8	2.4
Nonane	899	-	1.1±0.2	-	1.6±0.2	-	-	-
Decane	999	-	0.5±0.1	-	-	-	-	-
Dodecane	1199	-	1.0±0.2	-	-	2.1±0.1	-	-
Tetradecane	1399	-	6.3±0.4	-	-	17.5±1.3	1.8±0.1	2.4±0.4
Hexadecane	1599	-	1.6±0.3	-	-	1.8±0.1	-	-
Total		97.0	95.1	92.5	95.5	92.3	98.2	97.4
Yields (%)		0.4±0.0	0.04±0.0	0.3±0.1	0.6±0.1	0.2±0.0	0.7±0.1	0.3±0.1

^a Retention index relative to C₉-C₂₇ n-alkanes on a DB-5 column.

from the leaves of the same species. Among the sesquiterpenes, the oxygenated ones presented low concentrations, slightly higher for the oils from *A. virgata*, *L. brasiliensis* and *L. montevidensis*. The major constituents for all species were the sesquiterpenes (*E*)-caryophyllene (10-35%), germacrene-D (5-46%) and bicyclogermacrene (7-17%).

Although the *Lippia* genus has been investigated, this work is the first report on the chemical composition of volatile oils from *L. brasiliensis* and *L. sericea*. For the species *L. brasiliensis*, due to its availability during the year, samples were collected during one year and it was observed that the oil composition varied slightly with the seasons (Table 2). There were no significant differences in the contents of major constituents; however, in the cold and dry months (May-September), there was increase in the oxygenated compounds concentrations. Similar results were found by Barros *et al.*²³ for the species *L. alba* grown in São Luiz Gonzaga city, RS, Brazil. An increase in the content of oxygenated monoterpenes and sesquiter-

pene during autumn (April) and winter (July) was observed. The phenotypic variations of morphology and volatiles production have been demonstrated to plants of *Lippia* genus in response to seasonal,²³ phenological²⁴ and soil²⁵ variations.

According to Tavares *et al.*²⁴ the contents and composition of essential oil from *L. alba* varied within the phenological cycle, with higher oil production outside the flowering period. Barros *et al.*²³ reported that during the spring, when the plants are flowering, greatest chemical diversity in the volatile oils from *L. alba* is observed. In this work, the *L. brasiliensis* flowering period occurred during the winter (July) (Table 2). For the oils extracted from the leaves at this time, there was no increase in the number of constituents. However, the oil extracted from the inflorescences revealed a higher variety of compounds, mainly with the presence of monoterpenes.

Higher concentrations of monoterpenes can also be observed for the oils from *L. camara* and *L. trifolia* flowers. In general, it is obser-

Table 2. Constituents and yields of essential oils (%) from species *Lippia brasiliensis*

Constituents	RI ^a	March	May	July		September	November	January
		Leaves	Leaves	Leaves	Flowers	Leaves	Leaves	Leaves
Hydrocarbon Monoterpenes		2.8	-	-	9.9	-	2.6	6.1
α-Pinene	939	-	-	-	3.1±0.0	-	-	-
Sabinene	976	1.4±0.1	-	-	2.6±0.1	-	1.7±0.1	4.1±0.2
Limonene	1031	1.4±0.0	-	-	4.2±0.1	-	0.9±0.0	2.0±0.1
Oxygenated Monoterpenes		-	-	-	5.8	-	-	-
Linalol	1098	-	-	-	3.4±0.2	-	-	-
Terpinen-4-ol	1177	-	-	-	2.4±0.1	-	-	-
Hydrocarbons Sesquiterpenes		84.5	82.7	78.7	59.2	81.0	82.8	82.0
δ-Elementene	1339	4.8±0.1	4.9±0.1	3.6±0.0	2.0±0.0	5.3±0.1	4.6±0.2	4.1±0.1
α-Cubebene	1351	2.5±0.1	1.1±0.0	1.4±0.0	0.8±0.0	1.1±0.1	1.1±0.1	0.9±0.0
α-Copaene	1376	5.9±0.1	2.1±0.1	2.7±0.1	1.3±0.0	2.3±0.2	2.3±0.2	1.8±0.1
β-Bourbonene	1384	-	0.5±0.0	0.5±0.0	-	0.7±0.0	0.8±0.0	1.4±0.1
β-Cubebene	1390	3.2±0.1	1.6±0.1	1.7±0.1	0.8±0.0	1.8±0.1	1.8±0.1	-
β-Elementene	1391	-	0.6±0.0	0.8±0.0	1.1±0.0	0.5±0.0	0.7±0.0	0.7±0.0
α-Gurjunene	1409	1.1±0.0	0.4±0.0	0.7±0.0	-	0.5±0.0	0.6±0.0	0.4±0.0
(<i>E</i>)-Caryophyllene	1418	33.2±1.8	31.4±2.2	30.7±1.3	24.3±2.1	29.1±2.4	34.4±2.1	35.6±1.8
α-Humulene	1454	2.0±0.1	2.7±0.1	2.8±0.1	2.5±0.1	2.8±0.2	2.4±0.1	2.8±0.2
Alloaromadendrene	1461	2.4±0.1	1.8±0.0	1.7±0.1	1.2±0.1	1.7±0.1	-	1.6±0.1
Germacrene-D	1480	16.4±0.8	15.5±0.9	13.9±0.8	11.9±0.6	16.1±0.9	16.5±0.7	19.3±0.9
Bicyclogermacrene	1494	2.0±0.1	6.8±0.1	4.2±0.1	3.2±0.1	7.1±0.4	4.1±0.1	2.4±0.1
α-Muurolene	1499	2.8±0.1	1.6±0.1	0.5±0.0	-	0.5±0.0	1.1±0.1	1.8±0.1
α-Farnesene	-	-	0.3±0.0	-	-	-	0.2±0.0	0.2±0.0
γ-Cadinene	1513	0.8±0.0	1.1±0.0	1.1±0.0	0.5±0.0	1.1±0.2	1.0±0.0	0.7±0.0
(<i>Z</i>)-Calamenene	1521	4.3±0.1	4.0±0.1	6.1±0.1	5.6±0.1	3.4±0.3	4.3±0.3	4.1±0.3
δ-Cadinene	1524	-	1.1±0.1	1.0±0.0	-	1.0±0.1	1.5±0.1	1.0±0.1
4-isopropyl-1,6-dimethyl-1,2,3,4,4a,7-hexahydronaphthalene	1532	3.1±0.1	4.2±0.1	4.1±0.1	3.1±0.1	5.0±0.3	4.3±0.2	3.2±0.2
Germacrene-B	1556	-	1.0±0.0	1.2±0.1	0.9±0.0	1.0±0.1	1.1±0.1	-
Oxygenated Sesquiterpenes		3.5	8.6	13.6	12.0	7.3	4.4	4.3
Caryophyllene oxide	1581	1.4±0.1	1.3±0.1	1.2±0.0	0.9±0.0	1.1±0.0	1.4±0.2	1.3±0.1
Viridiflorol	1590	0.9±0.0	1.1±0.0	2.6±0.1	2.1±0.1	1.4±0.1	1.1±0.0	1.3±0.0
α-Muurolol	1645	0.6±0.0	2.5±0.1	3.9±0.1	3.4±0.2	2.2±0.2	1.1±0.1	1.1±0.1
α-Cadinol	1653	0.6±0.0	3.7±0.1	5.9±0.4	5.6±0.3	2.6±0.1	0.8±0.0	0.6±0.0
Others		6.8	7.5	5.8	9.5	7.2	7.7	5.9
oct-1-en-3-ol	978	-	-	-	2.1±0.1	-	-	-
ni ^b	1454	6.8±0.2	7.5±0.5	5.8±0.2	7.4±0.3	7.2±0.6	7.7±0.3	5.9±0.4
Total		97.6	98.8	98.1	96.4	95.5	97.5	98.3
Yields (%)		0.4±0.0	0.5±0.1	0.3±0.1	0.7±0.1	0.5±0.1	0.3±0.0	0.4±0.1

^a Retention index relative to C₉-C₂₇ *n*-alkanes on a DB-5 column. ^b Not identified.

ved that floral volatiles serve as attractions for specific pollinators, whereas the volatiles emitted by vegetative parts, appear to protect plants by deterring the herbivores from attacking.²⁶

Therefore, the different composition of essential oils from flowers of these species may be associated to certain ecological interactions, such as attracting species-specific pollinators.

(*E*)-Caryophyllene has been commonly used as a fragrance chemical since the 1930s.²⁷ It is used in spice blends, citrus flavors, soaps, detergents, creams and lotions, and in a variety of food products and beverages. (*E*)-Caryophyllene is also known for its anti-inflammatory and local anesthetic properties.²⁸ Recently, Gertsch et al.²⁹ showed that this compound selectively binds to the cannabinoid receptor type 2 (CB2), but not to type 1 (CB1), leading to cellular activation and anti-inflammatory effects. Although the CB1 receptor is responsible for the psychomodulatory effects of cannabinoids agonists, activation of the CB2 receptor is a potential therapeutic strategy for the treatment of inflammation, pain, atherosclerosis, and osteoporosis. Since the essential oils constituents, such as (*E*)-caryophyllene and germacrene-D can be purified,³⁰ the investigated plants become potential alternative sources of these important bioactive compounds.

Antibacterial activity

The evaluation of antibacterial activity by the Agar disc diffusion method showed that the volatile oils isolated from *L. brasiliensis*, *A. virgata*, *L. camara*, *L. montevidensis* and *L. trifolia* have different activity levels against each bacterial strain (Table 3). The quantity available of *Lippia sericea* was reduced and it did not produce enough oil for the bioassays.

Table 3. Diameter of the inhibition zones of bacterial growth for the essential oils from leaves of the species *Lippia brasiliensis*, *Aloysia virgata*, *Lantana camara*, *L. trifolia* and *L. montevidensis* and the minimum inhibitory concentration (MIC) of bacterial growth for the essential oils extracted from leaves of the species *Lippia brasiliensis* and *Aloysia virgata*

Essential Oils	Inhibition zones diameter (mm) ^a		
	G (-)	G (+)	
	<i>E. coli</i>	<i>B. cereus</i>	<i>S. aureus</i>
<i>Lippia brasiliensis</i>	6 C c	19 A b	15 B b
<i>Aloysia virgata</i>	11 B b	16 A b	16 A b
<i>Lantana camara</i>	6 A c	8 A d	6 A d
<i>Lantana trifolia</i>	6 C c	13 A c	10 B c
<i>Lantana montevidensis</i>	6 C c	18 A b	13 B b
Control (H ₂ O)	6 A c	6 A d	6 A d
Chloramphenicol 30 µg	29 B a	29 B a	32 A a
Essential Oils	Minimum inhibitory concentration (% v/v)		
<i>Lippia brasiliensis</i>	0.5	0.125	0.125
<i>Aloysia virgata</i>	0.125	0.125	0.125

^a Means followed by same capital letter in the lines and small letter in the columns are not different for the Scott-Knott's test at P ≤ 0.05.

The oils of all tested species, except *L. camara*, showed moderate activity against Gram-positive bacteria (*B. cereus* and *S. aureus*). Only the oil isolated from *A. virgata* was active against Gram-negative *E. coli*.

Deena and Thoppil³¹ observed antibacterial activity to the essential oils from *L. camara* collected in Calicut, India. The major compounds found by these authors were (*E*)-caryophyllene (35%), geranyl acetate (22%), terpenyl acetate (6%) and bornyl acetate

(4%). In our work, the absence of these oxygenated compounds in *L. camara* oil may be co-related to the lack of antibacterial activity for the plant from Brazil.

From the leaves of *L. trifolia* were isolated umhengerin, a pentamethoxyflavone with antibacterial and antifungal activities.³² More recently, it has been reported anti-inflammatory and antinociceptive activities of non-polar extracts from the leaves of *L. trifolia*.³³ However, no reports on biological activities of essential oils from this species were found in the literature. Our results indicate a low antibacterial activity for the volatile oils produced by *L. trifolia* against the tested bacterial strains.

Essential oils from *L. achyranthifolia*, mainly composed by carvacrol (31%), -bisabolol (11%) and 1,8-cineole (5%), showed moderate antibacterial activity, with MIC values between 1.0 and 0.25 mg L⁻¹.³⁴ Those substances have known antimicrobial activity. Additionally, it has been demonstrated that the presence of phenolic hydroxyl group in carvacrol is essential for its activity against *B. cereus*.³⁵

The mechanism of action of essential oils from *Melaleuca alternifolia* (Myrtaceae) (tea tree oil – TTO) against the bacteria *E. coli* and *S. aureus*, and the yeast *Candida albicans* has been investigated. It has been shown that the main effects on bacteria and yeast were respiration inhibition and increase of cell membrane permeability. The disruption of cell membrane integrity leads to a K⁺ ion leakage and consequent loss of chemiosmotic cell control.³⁶ This mode of action is similar to that of others broad-spectrum membrane-active disinfectants and preservatives, such as phenol derivatives, chlorhexidine and parabenoic acid derivatives.³⁷

The MIC value obtained (Table 3) confirms that the oil from *L. brasiliensis* have very low activity against *E. coli* (0.5%), whereas the oil from *A. virgata* inhibit the growth of this bacteria at the concentration of 0.125%. The oils from both plants showed the same MIC values (0.125%) against *B. cereus* and *S. aureus*.

Although the volatile oils obtained from this Verbenaceae have very similar composition, they showed different levels of activity against different bacterial strains. Some studies have shown that whole essential oils have greater antibacterial activity than the major components mixed.³⁸ This suggests that the minor components are critical to the activity and may have a synergistic effect or potentiating influence. Besides, the mode of action of the essential oil seems depending on the bacterial strain. Furthermore, it is also noteworthy that synergistic and, or antagonistic effects might be taken into account for the activity observed in complex mixtures, such as essential oils.

CONCLUSIONS

In conclusion, the essential oils from *A. virgata*, *L. brasiliensis*, *L. sericea*, *L. camara*, *L. montevidensis* and *L. trifolia*, collected in Minas Gerais, Brazil, are chemically similar, and this species are potential alternative source of (*E*)-caryophyllene and germacrene-D. The results described also demonstrate the promising possibility of using of the essential oils from *A. virgata*, *L. brasiliensis*, *L. montevidensis* and *L. trifolia* as an alternative to some disinfectants and preservatives against Gram-positive and Gram-negative bacteria.

ACKNOWLEDGEMENTS

The authors are grateful the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support and research fellowships (L. C. A. Barbosa and A. J. Demuner). We are also grateful to Dr. A. Rathi (Department of Chemistry, Oxford University, UK) for suggestions and corrections on the manuscript. We also thank Prof. R. Meira (UFV) for collecting a sample of *Lippia sericea*.

REFERENCES

1. Giulietti, A. M.; Menezes, N. L.; Pirani, J. R.; Meguro, M.; Wanderley, M. L.; *Bol. Bot. Univ.* **1987**, *9*, 1.
2. Cavalcanti, S. C. H.; Niculau, E. S.; Blank, A. F.; Câmara, C. A. G.; Araújo, I. N.; Alves, P. B.; *Bioresour. Technol.* **2010**, *101*, 829.
3. Bassole, I. H. N.; Ouattara, A. S.; Nebie, R.; Ouattara C. A. T.; Kabore, Z. I.; Traore, A. S.; *Phytochemistry* **2003**, *62*, 209.
4. Manenzhe, N. J.; Potgieter, N.; Ree, R.; *Phytochemistry* **2004**, *65*, 2333.
5. Tadeu, H.; Mohammed, E.; Asres, K.; Gebre-Mariam, T.; *J. Ethnopharmacol.* **2005**, *100*, 168.
6. Duarte, M. C. T.; Figueira, G. M.; Sartoratto, A.; Rehder, V. L. G.; Delarmelina, C.; *J. Ethnopharmacol.* **2005**, *97*, 305.
7. Pino, J. A.; Marbot, R.; Fuentes, V.; *J. Essent. Oil Res.* **2004**, *16*, 44.
8. Ricciardi, G. A. L.; Torres, A. M.; Baren, C. V.; Lira, P. D. L.; Ricciardi, A. I. A.; Dellacassa, E.; Lorenzo, D.; Bandoni, A. L.; *Flav. Frag. J.* **2005**, *20*, 645.
9. Padalia, R. C.; Verma, R. S.; Sundaresan, V.; *Rec. Nat. Prod.* **2010**, *4*, 109; Costa, J. G. M.; Sousa, E. O.; Rodrigues, F. F. G.; Lima, S. G.; Braz-Filho, R.; *Braz. J. Pharmacogn.* **2009**, *19*, 710; Oliveira, J. C. S.; Neves, I. A.; Camara, C. A. G.; Schwartz, M. O. E.; *J. Essent. Oil Res.* **2008**, *20*, 530.
10. Sousa, E. O.; Rodrigues, F. F. G.; Coutinho, H. D. M.; Campos, A. R.; Lima, S. G.; Costa, F. G. M.; *Rec. Nat. Prod.* **2011**, *5*, 60.
11. Gottlieb, O. R.; Kaplan, M. A. C.; Borin, M. R. M. B.; *Biodiversidade – um enfoque químico-biológico*, Editora da UFRJ: Rio de Janeiro, 1996.
12. Viccini, L. F.; Pierre, P. M. O.; Praça, M. M.; Costa, D. C. S.; Romanel, E. C.; Sousa, S. M.; Peixoto, P. H. P.; Salimena, F. R. G.; *Plant Syst. Evol.* **2006**, *256*, 171; Silva, C. J.; Barbosa, L. C. A.; Demuner, A. J.; Montanari, R. M.; Pinheiro, A. L.; Dias, I.; Andrade, N. J.; *Quim. Nova* **2010**, *33*, 104.
13. Barbosa, L. C. A.; Paula, V. F.; Azevedo, A. S.; Silva, E. M. M.; Nascimento, E. A.; *Flav. Fragr. J.* **2005**, *20*, 39; Fonseca, M. C. M.; Barbosa, L. C. A.; Nascimento, E. A.; Casali, V. W. D.; *J. Essent. Oil Res.* **2006**, *18*, 345; Martins, F. T.; Santos, M. H.; Pólo, M.; Barbosa, L. C. A.; *Quim. Nova* **2006**, *29*, 1203; Barbosa, F. F.; Barbosa, L. C. A.; Melo, E. C.; Botelho, F. M.; Santos, R. H. S.; *Quim. Nova* **2006**, *29*, 122; Martins, F. T.; Dorigueto, A. C.; Souza, T. C.; Souza, K. R. D.; Santos, M. H.; Moreira, M. E. C.; Barbosa, L. C. A.; *Chem. Biodivers* **2008**, *5*, 251.
14. Adams R. P.; *Identification of essential oil components by gas chromatography/mass spectroscopy*, Allured Publishing Corporation: Illinois, 1995.
15. Salustiano, V. C.; Andrade, N. J.; Soares, N. F. F.; Lima, J. C.; Bernardes, P. C.; Luiz, L. M. P.; Fernandes, P. E.; *Food Control* **2009**, *20*, 439.
16. National Committee for Clinical Laboratory Standards; *Performance standards for antimicrobial susceptibility test. Ninth International Supplement, M100-S9*, NCCLS: Wayne, 1997.
17. National Committee for Clinical Laboratory Standards; *Performance standards for antimicrobial susceptibility test. Ninth International Supplement. M100-S9*, Wayne, PA, 1999.
18. Bülow, N.; König, W. A.; *Phytochemistry* **2000**, *55*, 141.
19. Degenhardt, J.; Köllner, T. G.; Gershenzon, J.; *Phytochemistry* **2009**, *70*, 1621; Barbosa, L. C. A.; Demuner, A. J.; Clemente, A. D.; Paula, V. F.; Ismail, F. M. D.; *Quim. Nova* **2007**, *30*, 1959.
20. Ghisalberti, E. L.; *Fitoterapia* **2000**, *71*, 467.
21. Silva, M. H. L.; Andrade, E. H. A.; Zoghbi, M. G. B.; Luz, A. I. R.; Silva, J. D.; Maia, J. G. S.; *Flav. Fragr. J.* **1999**, *14*, 208.
22. Muhayimana, A.; Chalchat, J. C.; Garry, R. P.; *J. Essent. Oil Res.* **1998**, *10*, 547.
23. Barros, F. M. C.; Zambarda, E. O.; Heinzmann, B. M.; Mallmann, C. A.; *Quim. Nova* **2009**, *32*, 861.
24. Tavares, E. S.; Julião, L. S.; Lopes, D.; Bizzo, H. R.; Lage, C. L. S.; Leitão, S. G.; *Rev. Bras. Farmacogn.* **2005**, *15*, 1.
25. Montanari, R. M.; Sousa, L. A.; Leite, M. N.; Coelho, A. D. F.; Viccini, L. F.; Stefanini, M. B.; *Rev. Bras. Pl. Med.* **2004**, *6*, 96.
26. Pichersky, E.; Gershenzon, J.; *Curr. Opin. Plant Biol.* **2002**, *5*, 237.
27. Opdyke, D. L. J.; *Food and Cosmetics Toxicol.* **1973**, *11*, 1059.
28. Ghelardini, C.; Galeotti, N.; Di Cesare, M. L.; Mazzanti, G.; Bartolini, A.; *Il Farmaco* **2001**, *56*, 387.
29. Gertsch, J.; Leonti, M.; Raduner, S.; Racz, I.; Chen, J. Z.; Xie, C. Q.; Altmann, K. H.; Karsak, M.; Zimmer, A.; *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 9099.
30. Brochini, C. B.; Núñez, C. V.; Moreira, I. C.; Roque, N. F.; Chaves, M. H.; Martins, D.; *Quim. Nova* **1999**, *22*, 37.
31. Deena, M. J.; Thoppil, J. E.; *Fitoterapia* **2000**, *71*, 453.
32. Rwangabo, P. C.; Claeys, M.; Pieters, L.; Corthout, J.; Vanden-Berghe, D. A.; Vlietinck, A. J.; *J. Nat. Prod.* **1988**, *51*, 966.
33. Silva, G. N.; Martins, F. R.; Matheus, M. E.; Leitão, S. G.; Fernandes, P. D.; *J. Ethnopharmacol.* **2005**, *100*, 254.
34. Hernández, T.; Canales, M.; Avila, J. G.; García, A. M.; Martínez, A.; Caballero, J.; Vivar, A. R.; Lira, R.; *J. Ethnopharmacol.* **2005**, *96*, 551.
35. Ultee, A.; Bennik, M. H. J.; Moezelaar, R.; *Appl. Environ. Microbiol.* **2002**, *68*, 1561.
36. Cox, S. D.; Mann, C. M.; Karkham, J. L.; Bell, H. C.; Gustafson, J. E.; Warmington, J. R.; Wyllie, S. G.; *J. Appl. Microbiol.* **2000**, *88*, 170.
37. McDonnell, G.; Russell, A. D.; *Clinical Microbiol. Rev.* **1999**, *12*, 147.
38. Al-Bayat, F. A.; *J. Ethnopharmacol.* **2008**, *116*, 403.