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Essential Oils from two *Lantana* species with Antimycobacterial Activity

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Lantana trifolia L. and *L. fucata* Lindl. are two Brazilian species used in folk medicine for the treatment of respiratory disorders. The composition of the essential oils from the leaves was investigated, as well as their *in vitro* activity against *Mycobacterium tuberculosis*. *L. trifolia* yielded an oil (0.2%) rich in sesquiterpenes. The major substances found were germacrene D (45.1%), (*E*)-caryophyllene (12.8%), bicyclogermacrene (12.7%) and α-humulene (4.4%). Sesquiterpenes were also the main components of the oil of *L. fucata* (0.3% yield), the principal ones being β-elemene (27.1%), germacrene D (11.6%), (*E*)-caryophyllene (7.7%), valencene (5.7%) and germacrene A (4.6%). Both oils exhibited *in vitro* antimycobacterial activity by the MABA assay with MICs of 80 μg/mL for *L. trifolia* and 100 μg/mL for *L. fucata*.

Keywords: *Lantana trifolia* L., *Lantana fucata* Lindl., essential oil, MABA, *Mycobacterium tuberculosis*, tuberculosis, Verbenaceae.

Lantana, family Verbenaceae, has approximately 150 species distributed in the tropics and subtropics of America, Africa and Asia [1]. *L. camara* L. is the most widely known species of the genus, occurring in tropical, sub-tropical and temperate regions. Many works concerning the chemical composition and pharmacological activity of this species are described, including the essential oil composition of plants growing in different regions of the globe [1-6]. For *L. trifolia* L. and *L. fucata* Lindl., however, there are only a few reports on essential oil composition.

L. trifolia (syn. *L. celtidifolia* H.B. & K.) is a small shrub that occurs in all regions of Brazil and is extensively used in folk medicine in the form of infusions and syrups for the treatment of respiratory disorders and as a calming agent [7,8]. *L. fucata* (syn. *L. lilacina* Desf.) is also a small shrub, with pink or purple flowers, found in tropical and subtropical temperate regions of the Americas. The leaves have been used in the traditional medicine of Brazil as an anti-inflammatory, for stomach affections, and as infusions to treat cold and bronchitis [7-9]. The essential oil of both species has been analysed. Germacrene D and caryophyllene were described as the major components of the essential oil of *L. trifolia* from Rwanda [10], while caryophyllene oxide and gossonorol were the most abundant substances in the oil of *L. fucata* from Pernambuco-Brazil, where it is used as an antiseptic for wounds [9].

Table 1: Chemical composition (relative % peak area) of the essential oils from *Lantana trifolia* and *L. fucata* (leaves) obtained by GC and GC-MS on a HP-5 column.

	Compound	RI*	RI $_{lit}$**	Leaf oil (%)	
				Lantana trifolia	*Lantana fucata*
1	(*Z*)-3-Hexenol	888	859	0.5	0.1
2	Sabinene	975	975	0.2	0.3
3	1-Octen-3-ol	994	979	-	0.1
4	Limonene	1031	1029	0.1	-
5	Linalool	1100	1097	0.2	-
6	δ-Elemene	1338	1338	-	0.6
7	β-Bourborene	1384	1388	1.7	-
8	β-Cubebene	1390	1388	0.5	-
9	β-Elemene	1391	1391	2.4	27.1
10	(*E*)- Caryophyllene	1418	1419	12.8	7.6
11	β-Gurjunene	1428	1434	0.3	0.2
12	γ-Elemene	1433	1437	-	0.7
13	Aromadendrene	1448	1441	-	0.2
14	α-Humulene	1453	1454	4.4	2.3
15	*allo*-Aromadendrene	1460	1460	2.1	0.7
16	α-Amorphene	1483	1485	-	0.4
17	Germacrene D	1485	1485	45.1	11.6
18	Valencene	1496	1496	-	5.7
19	α-Muurolene	1498	1500	0.8	-
20	Bicyclogermacrene	1500	1500	12.7	0.6
21	Germacrene A	1505	1509	1.2	4.5
22	γ-Cadinene	1514	1514	1.3	-
23	Cubebol	1522	1515	1.2	-
24	δ-Cadinene	1564	1523	1.3	-
25	10-*epi*-Cubebol	1523	1535	-	0.3
26	Sesquisabinene-hydrate	1527	1544	-	0.6
27	Elemol	1550	1550	-	0.7
28	Germacrene-B	1557	1561	-	2.8
29	(*E*)-Nerolidol	1561	1563	-	0.1
30	Longicamphenylone	1565	1564	-	0.8
31	Spathulenol	1577	1578	-	0.8
32	Caryophyllene oxide	1581	1583	0.3	1.0
33	β-Atlantol	1598	1608	-	0.4
34	Humulene epoxide II	1605	1608	2.0	0.4
35	β-Oplopenona	1606	1608	0.4	-
36	*epi*-α-Cubebol	1619	1640	0.5	0.5
37	Cadinol	1641	1640	1.1	-
38	α-Muurolol	1653	1646	0.9	-
39	Cubenol	1645	1647	0.4	-
40	Himachalol	1639	1654	-	1.5
41	α-Eudesmol	1642	1654	-	0.4
42	α-Cadinol	1684	1654	1.2	0.9
43	Khusinol	1682	1680	-	0.6
44	α-*trans*-Bujanol	1705	1690	-	0.3
45	Curcumenol	1734	1734	-	0.2
46	Phytol	2113	1943	2.3	2.6
	Sesquiterpenes			94.6	74.5
	Monoterpenes			0.5	0.3
	Total Identified Compounds (%)			97.9	77.6

*RI= Retention index values are calculated from retention times in relation to *n*-alkanes. **RI from reference [27].

In the course of our continuous search for Brazilian plant extracts active against *Mycobacterium tuberculosis*, we have investigated the essential oils from *Lantana trifolia* and *L. fucata* due to their traditional uses against respiratory disorders. In this work we describe for the first time the antimycobacterial activity of both essential oils, as well as their chemical composition.

The essential oils from the leaves of *L. trifolia* and *L. fucata* were obtained in yields of 0.2% (light yellow) and 0.3% (light green-yellow), respectively. The main identified compounds are listed in Table 1. The essential oils of both species presented a high content of sesquiterpenes: 94.6% for *L. trifolia* and 74.5% for *L. fucata*, and a very low content of monoterpenes (0.5% and 0.3%, respectively). This has already been observed for the oils of other *Lantana* species [4,6,9-11], as well as their small yields [11].

Twenty-seven compounds were identified in the essential oil of *L. trifolia*, where the major components were germacrene D (45.1%), (*E*)-caryophyllene (12.8%), bicyclogermacrene (12.7%) and α-humulene (4.4%). In the essential oil of *L. fucata*, 37 compounds were detected. Among them, β-elemene (27.1%), germacrene D (11.6%), (*E*)-caryophyllene (7.7%), valencene (5.7%) and germacrene A (4.6%) were the major components. Caryophyllene is an important component of the essential oils of most *Lantana* species with high contents of sesquiterpenes, being frequently cited as their major sesquiterpene [2-6, 9,10]. Germacrene D, one of the main components in the samples analyzed in this work, was not detected in the oil of *L. fucata* samples from Pernambuco, Brazil [9].

There are few reports in the literature on the antimicrobial activity of essential oils against mycobacteria [12-19]. Among them, essential oils from *Canella winterana*, *Trachyspermum ammi* and *Heliotropium indicum* presented activity against some mycobacteria, including *M. tuberculosis* in a range that varied between 12.5 mg/L to 100 μg/mL [12-14]. These essential oils presented myrcene, thymol and phytol as the major compounds, respectively. One very interesting report in the literature is the successful inhalational use of the essential oil of *Eucalyptus globulus* to treat pulmonary tuberculosis [15]. Ten days post-inhalation of the oil, the patient was tuberculosis negative (via sputum culture), with no clinical symptoms. The traditional uses of *L. trifolia* and *L. fucata* against respiratory disorders prompted us to evaluate their essential oils against *M. tuberculosis*.

Both oils, when assayed against *M. tuberculosis* by the MABA assay [20], presented MIC values of

80 μg/mL and 100 μg/mL, respectively. Germacrene D, (*E*)-caryophyllene, valencene and β-elemene have been identified in essential oils from *Salvia tomentosa*, *Origanum minutiflorum*, *O. syriacum* and *Thymus revolutus*, which displayed *in vitro* antimycobacterial activity [16-19]. However, the microorganism assayed [16-19] was *M. smegmatis,* which is a rapidly growing environmental species not considered to be a human pathogen [21]. α-Humulene and phytol have been assayed as isolated compounds and presented MIC values of 6.3 μg/mL and of 2 μg/mL, respectively, against *M. tuberculosis* by the MABA assay [22,23]. Phytol is not a very common component of essential oils and has also been described in other works of bioassay-guided isolation of antimycobacterial active principles from plant extracts [24].

Terpene derivatives present moderate to high lipophilicity, which would aid their penetration into the mycobacterial cell wall [25]. It has been demonstrated that for each series of terpenes the activity improves with lipophilicity of a given substance when compared with their more polar analogues [23]. In the case of the studied species, some terpenoids from active antimycobacterial essential oils have been detected, including α-humulene, germacrene D and (*E*)-caryophyllene. However, the contribution of minor components to the antimycobacterial activity of theses oils cannot be ruled out.

This is the first report of the *in vitro* activity of the essential oils from *L. trifolia* and *L. fucata* against *M. tuberculosis*. These results corroborate, at least in part, the traditional use of these plants in Brazil.

Experimental

Plant material: Fresh leaves of *L. trifolia* and *L. fucata,* were collected at Mendes-RJ (22º 32' 00'' S, 43º 42' 00''W) and at the campus of the Federal University of Juiz de Fora, Juiz de Fora, Brazil, (22°46'48.6"S, 43°22'24.5"W), in January 2005 and April 2007, respectively. Plants were authenticated by Dr Fatima Regina Gonçalves Salimena, and voucher specimens were deposited at the Herbarium of the Botanical Department, Federal University of Juiz de Fora (CESJ 30801 for *L. trifolia* and CESJ 48653 for *L. fucata*).

Essential oil extraction: The essential oils from fresh leaves of *L. trifolia* and *L. fucata* were obtained by hydrodistillation in a Clevenger-type apparatus for 4 h.

GC and GC-MS analyses: Gas chromatographic analyses were performed using a HP 5890 series II gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a HP-5 (5% phenyl/95% dimethylpolysiloxane) fused silica capillary column (30 m x 0.25 mm x 0.25 μm). Hydrogen was the carrier gas (1.0 mL min^{-1}). The injector temperature was kept at 250°C and the oven temperature program was from 60° to 240°C at a rate of 3°C min^{-1}. The detector (FID) was operated at 280°C. Pure oils (0.03 μL) were injected in split mode (100:1). The GC-MS analyses were performed in an Agilent 5973N mass selective detector coupled to an Agilent 6890 gas chromatograph (Palo Alto, CA), equipped with a HP5-MS capillary column (30 m X 0.25 mm X 0.25μm), operating in electronic ionization mode at 70 eV, with the transfer line maintained at 260°C, while mass analyzer and ion source temperatures were held at 150°C and 230°C, respectively. Helium (1.0 mL min^{-1}) was used as carrier gas. Oven temperature program, injector temperature and split rate were the same as stated for GC analyses. A standard solution of *n*-alkanes (C_7-C_{26}) was used to obtain the retention indices [26]. Individual volatile components were identified by comparison of their mass spectra (MS) and retention indices (RI) with those reported in literature [27] and also in the Wiley Registry of Mass Spectral Data, 6th Edition (Wiley Interscience, New York).

Antimycobacterial tests: MABA (Microplate Alamar Blue Assay) susceptibility testing was performed at FIOCRUZ according to the method described by Franzblau [16]. Final concentration of essential oils was 100 μg/mL. Media plus bacteria with and without rifampicin were used as controls. The H37Rv (ATCC - 27294) strain was used. The minimal inhibitory concentration (MIC) was determined (starting from 100 μg/mL in 1:2 serial dilutions).

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References

[1] Ghisalberti EL. (**2000**) *Lantana camara* L. (Verbenaceae). *Fitoterapia*, ***71***, 467-486.

[2] Khan M, Srivastava SK, Syamasundar KV, Singh M, Naqvi AA. (**2002**) Chemical composition of leaf and flower essential oil of *Lantana camara* from India. *Flavour and Fragance Journal*, ***17***, 75-77.

[3] Sefidkon F. (**2002**) Essential oil of *Lantana camara* L. occurring in Iran. *Flavour and Fragance Journal*, ***17***, 78-80.

[4] Ngassoum MB, Yonkeu S, Jirovetz L, Buchbauer G, Schmaus G, Hammerschmidt FJ. (**1999**) Chemical composition of essential oils of *Lantana camara* leaves and flowers from Cameroon and Madagascar. *Flavour and Fragance Journal*, ***14***, 245-250.

[5] da Silva MHL, Andrade EHA, Zoghbi MDB, Luz AIR, da Silva JD, Maia JGS. (**1999**) The essential oils of *Lantana camara* L. occurring in north Brazil. *Flavour and Fragance Journal*, ***14***, 208-210.

[6] Walden AB, Haber WA, Setzer WN. (**2009**) Essential oil compositions of three *Lantana* species from Monteverde, Costa Rica. *Natural Product Communications*, ***4***, 105-108.

[7] Correa MP. (**1984**) *Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas.* (Reimpressão sem alterações edição 1926). Ministério da Agricultura / IBDF, Brasília, DF.

[8] Lorenzi H. (**2000**) *Plantas Daninhas do Brasil*, 3rd Ed., Instituto Plantarum de Estudos da Flora, Nova Odessa, SP.

[9] de Oliveira JCS, Neves IA, da Camara CAG, Schwartz MOE. (**2008**) Essential oil composition of two *Lantana* species from mountain forests of Pernambuco (northeast of Brazil). *Journal of Essential Oil Research*, ***20***, 530-532.

[10] Muhayimana A, Chalchat JC, Garry RP. (**1998**) Chemical composition of essential oils of *Lantana trifolia* L. from Rwanda. *Journal of Essential Oil Research*, ***10***, 547-549.

[11] Deena MJ, Thoppil JE. (**2000**) Antimicrobial activity of the essential oil of *Lantana camara*. *Fitoterapia*, ***71***, 453-455.

[12] Rastogi N, Abaul J, Goh KS, Devallois A, Philogeéne E, Bourgeois P. (**1998**) Antimycobacterial activity of chemically defined natural substances from the Caribbean flora in Guadeloupe. *Immunology and Medical Microbiology*, ***20***, 267-273.

[13] Sinha P, Santha Kumar TR, Darokar MP, Khanuja SPS. (**2006**) Isolation of oxidative stress response mutants in *Escherichia coli* for their use as a genetic screen to identify new anti-mycobacterials as functional analogues of isoniazid. *World Journal of Microbiology & Biotechnology*, ***22***, 791–798.

[14] Machan T, Korth J, Liawruangrath B, Liawruangrath S, Pyne SG. (**2006**) Composition and antituberculosis activity of the volatile oil of *Heliotropium indicum* Linn. growing in Phitsanulok, Thailand. *Flavour and Fragrance Journal*, ***21***, 265–267.

[15] Sherry E, Warnke PH. (**2004**) Successful use of an inhalational phytochemical to treat pulmonary tuberculosis: A case report. *Phytomedicine*, ***11***, 95-97.

[16] Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M. (**2005**) Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chemistry*, ***90***, 333–340.

[17] Vardar-Ünlü G, Ünlü M, Dönmez E, Vural N. (**2007**) Chemical composition and *in vitro* antimicrobial activity of the essential oil of *Origanum minutiflorum* O Schwarz & PH Davis. *Journal of the Science of Food and Agriculture*, ***87***, 255–259.

[18] Alma MH, Mavi A, Yildirim A, Digrak M, Hirata T. (**2003**) Screening chemical composition and *in vitro* antioxidant and antimicrobial activities of the essential oils from *Origanum syriacum* L. growing in Turkey. *Biological and Pharmaceutical Bulletin*, ***26***, 1725-1729.

[19] Karaman S, Digrak M, Ravid U, Ilcim A. (**2001**) Antibacterial and antifungal activity of the essential oils of *Thymus revolutus* Celak from Turkey. *Journal of Ethnopharmacology*, ***76***, 183–186.

[20] Franzblau SG, Witzig RS, Mclaughlin JC, Torres P, Madico G, Hernandez A, Degnan MT, Cook MB, Quenzer VK, Ferguson RM, Gilman RH. (**1998**) Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue Assay. *Journal of Clinical Microbiology*, ***36***, 362-366.

[21] Wallace RJJr, Nash DR, Tsukamura M, Blacklock ZM, Silcox VA. (**1988**) Human disease due to *Mycobacterium smegmatis*. *Journal of Infectious Disease*, ***158***, 52-59.

[22] Kanokmedhakul S, Kanokmedhakul K, Lekphrom R. (**2007**) Bioactive constituents of the roots of *Polyalthia cerasoides*. *Journal of Natural Products*, ***70***, 1536–1538.

[23] Cantrell CL, Franzblau SG, Fischer NH. (**2001**) Antimycobacterial plant terpenoids. *Planta Medica*, ***67***, 685-694.

[24] Rajab MS, Cantrell CL, Franzblau SG, Fischer NH. (**1998**) Antimycobacterial activity of (*E*)-phytol and derivatives. A preliminary structure-activity study. *Planta Medica*, ***64***, 2-4.

[25] Copp BR. (**2003**) Antimycobacterial natural products. *Natural Products Report*, ***20***, 535-557.

[26] Van Den Dool H, Kratz PD. **(1963**) A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. *Journal of Chromatography A*, ***11***, 463-471.

[27] Adams RP. (**2001**) *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectrometry.* Allured Publishing Corp, Carol Stream, IL.