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Article

Chemical composition of the essential oil and hexanic fraction of *Lippia* and *Lantana* species

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RESUMO: "Composição química do óleo essencial e fração hexânica de espécies de *Lippia* e *Lantana*". Foi realizada a comparação entre dois métodos de extração dos compostos voláteis obtidos de seis espécies de Verbenaceae, coletadas na Serra do Cipó, Minas Gerais, Brasil. Os óleos essenciais e as frações hexanica obtidos das folhas de duas espécies de *Lantana* e quatro espécies de *Lippia*, coletadas em duas diferentes estações do ano, foram analisados por CG/EM. Grande número de constituintes foi identificado nas amostras oriundas dos dois métodos de extração e o componente majoritário para a maior parte das espécies foi o β-cariofileno, seguido pelo germacreno D, biciclogermacreno e α-pineno. Para os dois gêneros estudados, foram observadas pequenas diferenças na composição do óleo essencial e fração hexânica. Esses resultados sugerem que a análise da fração hexânica pode ser usada para identificar os componentes voláteis majoritários dessas espécies, além de ser uma técnica alternativa para a análise dos compostos voláteis presentes no óleo essencial, uma vez que ambos mostraram composição similar.

Unitermos: *Lippia, Lantana,* Verbenaceae, óleo essencial, fração hexânica, CG/EM.

ABSTRACT: A comparison between two extraction approaches of volatiles compounds from six species of Verbenaceae collected at Serra do Cipó, Minas Gerais, Brazil was done. The essential oil and hexanic fraction of leaves from two *Lantana* and four *Lippia* species collected in two different seasons were analyzed by GC/MS. Among various identified compounds from both extraction methods the majority of species showed major amounts of β-caryophyllene followed by germacrene D, bicyclogermacrene and α-pinene. Few differences were observed between the composition of essential oil and the hexanic fraction regarding the two studied genera. These results suggest that the analysis of hexanic fraction can be used, as an alternative way, to analyze the volatile compounds of the essential oil.

Keywords: *Lippia, Lantana,* Verbenaceae, essential oil, hexanic fraction, GC/MS.

INTRODUCTION

Lippia and *Lantana* (Verbenaceae) are two native genera from Central and South America and Tropical Africa (Mondenke, 1980) that possess many medicinal and economically important species.

Nearly 150 species can be found in the genus *Lantana* and some of them are popularly used to treatment of several diseases (Boily & Van Puyvelde, 1986; Silva et al., 2005). Some of the pharmacological properties have been studied including bronchorelaxing (Achola & Munenge, 1996), anti-inflammatory and antinociceptive (Silva et al., 2005), antimycobacterial (Jimenéz-Arellanes et al., 2007), antimicrobial and antimutagenic (Barre et al., 1997; Hernández et al., 2005) and inhibitors of human thrombin activities (O'Neill et al., 1998). Among the species of the genus, the essential oil of the *Lantana camara* L. is the most studied one (Saleh, 1974; Deena & Thoppil, 2000; Misra & Laatch, 2000; Abdel-Hady et al., 2005; Walden et al., 2009).

The genus *Lippia* comprises 250 species of herbs, shrubs and trees. Several species are used in folk medicine as an alternative to currently used drugs due to their known analgesic, anti-inflammatory, antipyretic, sedative, antifungal, antihypertensive, diuretic, larvicidal, antimicrobial, antiviral, molluscicidal, antimalarial,

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antispasmodic, anticonvulsant and stimulant activities (Valentin et al., 1995; Pascual et al., 2001; Abena et al., 1998; Monteiro et al., 2007). Concerning the chemical and pharmacological studies, *Lippia alba* (Mill.) N.E. Brown and *Lippia sidoides* Cham. are the most studied species. Recently, the composition of the essential oil from leaves and flowers of *Lippia lacunosa* Mart. and Shau. and *Lippia rotundifolia* Cham. were reported (Leitão et al., 2008).

Considering that the genera *Lippia* and *Lananta* possess many aromatic species, chemical studies of these genera reported mainly the composition of their essential oils. In addition, the volatile compounds can also be extracted using non-polar solvents, such as hexane (Simões & Spitzer, 2007). These compounds constitutes important source of biological active molecules

The present study report and compare the chemical composition of the essential oil and the hexanic fraction of *Lippia* and *Lantana* species collected in two different seasons, accessed by gas chromatography-mass spectrometry (GC/MS) analysis.

MATERIAL AND METHODS

Plant material

Leaves of *Lantana* aff. fucata Lindl., *Lantana fucata* Lindl., *Lippia aristata* Shauer, *Lippia aff. microphylla* Cham., *Lippia martiana* Shau and *Lippia salviifolia* Cham. were collected at Serra do Cipó (794 m; 19º27'47''S 43º33'10''W), Espinhaço Range, Minas Gerais State, Brazil, during the dry (August/2004 and September/2005) and rainy (March/2005 and February/2006) seasons. The specimens were identified by Dr. Fátima Regina Gonçalves Salimena (Department of Botany, UFJF) and the vouchers were deposited at the CESJ Herbarium of the Federal University of Juiz de Fora (Table 1).

Essential oil extraction

The essential oils (EO) extracted from fresh leaves collected during the dry and rainy seasons, were obtained by hydrodistillation using a Clevenger-type apparatus for 2 h. The chemical compositions of the EO were analyzed by GC⁄MS. The EO from *Lantana aff. fucata* was not analyzed due to the absent of sufficient amount of leaves found during the dry season collection.

Hexanic fraction

Fresh leaves (at about 3 g) were collected during the dry and rainy seasons, transferred to Falcon™ 50 mL conical tube containing 30 mL of ethanol P.A. The tubes were kept for one week at room temperature. After filtration, an aliquot of 100 µL of the extracts was taken, mixed with the same volume of MilliQ water and finally partitioned twice with *n*-hexane. The chemical composition of the n-hexane fractions (HF) were analyzed by GC⁄MS.

Gas chromatography-mass spectrometry analyses

The analysis was performed on a Shimadzu gas chromatograph-mass spectrometer model QP5050A equipped with a FID detector and a DB-5 fused silica capillary column (35 m×0.2 mm, film thickness 0.10 µm), using helium as a carrier gas (1.0 mLmin^{-1}). The injector temperature was 200 ºC and the column oven program was 50 ºC to 200 ºC at 4 ºC min^{-1}. The mass spectra were obtained by electronic impact 70 eV and the range from 50 to 500 *m/z* was scanned. Data acquisition and handling was done via CLASS 5000 Shimadzu software. Retention Index (RI) in the range of 900 to 3000 was generated from analysis of a standard mixture containing C_9 to C_{30} hydrocarbons. The oil constituents were identified by comparison of their mass spectra with those in a Shimadzu spectral database and RI (Adams, 1995).

RESULTS AND DISCUSSION

The results of the essential oils and hexanic fractions quantifications from leaves of *Lantana* and *Lippia* species collected during the dry and rainy seasons can be seen in Table 1. The yield of the hexanic fraction

Table 1. Percentage yields of essential oil (EO) and hexanic fraction (HF) (w/w) of *Lantana* and *Lippia* species from dry and rainy seasons.

Species	Vouchers numbers (CESJ)	EO (%)		HF (%)[b]
		Dry season	Rainy season	
Lantana aff. fucata	48.653	a	0.16	0.24
Lantana fucata	32.730	0.02	0.04	0.10
Lippia aff microphylla	47.445	0.01	0.13	0.39
Lippia aristata	48.652	0.01	0.02	0.45
Lippia martiana	47.477	0.02	0.16	0.22
Lippia salviifolia	47.444	0.20	0.28	0.20

[a] not analyzed; [b] average of dry and rainy seasons.

(w/w) was the same in both seasons and the yield of the HF was about twice higher than yield of the essential oil of *Lantana* and *Lippia* species in the rainy period, except for *Lippia salviifolia* that yields the same amount with the two methods.

Major components identified in the essential oil and hexanic fraction from leaves of *Lantana* and *Lippia* species were qualitative and quantitatively analyzed by GC/MS. The list of compounds according to their retention times can be seen in Tables 2 and 3.

The identified compounds are similar to those ones previously reported to other *Lantana* species (Muhayimana et al. 1998; Da Silva et al., 1999). The EO and HF of both *Lantana* species are mainly composed by β-cariophyllene and germacrene D (Table 2). The results obtained for *Lantana fucata*, compared with another sample collected at Northeast of Brazil, showed that the oil of this species is rich in sesquiterpenes, mainly β-cariophyllene. However, the bulnesol, the third most abundant compound reported by De Oliveira et al. (2008) were not detected in our analysis. Probably, the difference of their chemical composition can be a consequence of their geographical origin. The major compounds of HF from *Lantana aff. fucata* collected during dry and rainy seasons were β-cariophyllene, germacrene D, and bicyclogermacrene while for EO these compounds were found as major ones only for plants collected during the rainy season. Similarly, the HF and EO of *L. fucata* collected in both seasons possess germacrene D and β-cariophyllene as major compounds (Table 2). Furthermore, HF and EO observed for plants collected during the dry season showed high content of bicyclogermacrene, while the HF observed from leaves collected in rainy season was composed by ß-elemene, α-guaiene and δ-guaiene too.

Considering the Verbenaceae family, the genus *Lippia* are in outstanding position due to the great production of volatile compounds. The chemical

Table 2. Chemical composition of the essential oil (EO) and hexanic fraction (HF) of *Lantana aff. fucata* and *Lantana fucata* collected in dry and rainy seasons (RI: retention index)

Compounds	RI	*Lantana aff. fucata* (%)			*Lantana fucata* (%)			
		Dry	Rainy		Dry		Rainy	
		HF	EO	HF	EO	HF	EO	HF
α-pinene	-	-	0.68	-	-	-	-	-
sabinene	-	-	7.41	-	-	-	-	-
ß-pinene	-	-	-	-	-	-	-	15.64
limonene	1033	1.53	2.00	1.60	-	-	-	-
ß-cis-ocimene	1042	-	0.62	-	-	-	-	-
ß-trans-ocimene	1052	-	0.75	-	-	-	-	-
Γ-terpinene	1063	-	0.29	-	-	-	-	-
4-terpinol	1187	-	0.35	-	-	-	-	-
α-copaene	1385	2.50	1.07	1.89	-	-	-	-
ß-bourbonene	1394	-	1.13	2.38	1.19	-	2.10	-
ß-elemene	1401	-	0.99	-	2.06	-	0.84	15.14
ß-cariophyllene	1430	23.88	18.82	19.71	11.92	26.13	32.41	12.03
α-humulene	1465	1.75	2.50	2.26	6.38	9.75	4.17	-
aloaromadendrene	1473	2.50	1.58	2.09	1.06	-	1.25	-
Γ-muurolene	1489	-	0.51	-	-	-	-	-
germacrene D	1493	47.27	32.82	43.65	15.96	45.46	32.29	20.10
α-guaiene	1499	-	0.32	-	-	-	-	10.67
bicyclogermacrene	1510	7.09	11.57	12.22	10.46	14.27	2.48	-
germacrene A	1519	-	1.56	1.71	-	-	-	-
Δ-guaiene	1519	-	-	-	4.47	-	1.55	21.17
Γ-cadinene	1529	-	0.48	-	3.99	-	0.27	-
Δ-cadinene	1536	1.84	1.27	1.61	2.08	-	-	-
germacrene B	1573	5.33	3.09	4.27	5.62	-	9.62	5.21
germacrene D-4-ol	1593	-	-	-	-	-	0.52	-
spathulenol	1595	-	2.17	-	2.42	-	-	-
cariophyllene oxide	1601	-	1.74	-	6.08	-	6.70	-
viridiflorol	1605	1.53	2.91	2.16	3.06	-	-	-
Total identified	95.22	96.63	95.55	76.75	95.61	94.20	99.96	

Table 3. Chemical composition of essential oil (EO) and hexanic fraction (HF) of *Lippia aff. microphylla*, *L. aristata, L. martiana* and *L. salviifolia* collect in dry an rainy seasons (RI: retention index)

Compounds	RI	*Lippia aff. microphylla* (%)				*Lippia aristata* (%)				*Lippia martiana* (%)				*Lippia salviifolia* (%)			
		Dry		Rainy		Dry		Rainy		Dry		Rainy		Dry		Rainy	
		EO	HF	EO	HF	EO	HF	EO	HF	EO	HF	EO	HF	EO	HF	EO	HF
α-pinene	-	6.38	5.41	12.69	6.81	5.84	11.21	2.32	5.94	20.08	16.37	24.33	10.64	0.46	10.85	1.66	2.91
camphene	-	0.89	0.40	1.09	0.42	0.49	-	0.18	-	0.81	-	0.63	-	0.05	0.35	-	-
sabinene	-	0.60	0.39	1.10	0.92	2.79	1.71	6.83	16.01	-	-	-	-	0.15	0.76	0.59	1.02
ß-pinene	-	1.83	0.95	3.48	1.54	0.39	-	0.32	0.51	3.07	1.35	2.42	1.16	0.38	0.94	0.95	1.32
-myrcene	-	-	0.29	0.77	0.47	0.38	-	0.45	0.99	4.18	3.65	4.67	2.86	0.16	1.64	-	-
A-felandrene	1007	2.68	-	-	-	0.62	-	-	-	-	-	-	-	-	-	-	-
Δ-3-carene	1014	-	-	1.08	0.66	-	25.05	11.20	15.98	6.59	6.37	8.51	4.83	-	-	-	-
p-cymene	1028	0.94	-	0.40	-	-	-	-	-	3.19	-	0.54	-	-	-	-	-
limonene	1032	2.84	1.07	1.51	0.48	13.94	-	-	-	5.05	3.92	7.30	2.98	0.45	1.76	1.29	0.76
eucalyptol	1035	-	-	-	-	1.49	-	-	-	-	-	-	-	-	-	-	-
ß-*cis*-ocimene	1041	1.26	0.55	1.35	0.43		-	-	-	5.41	8.86	12.77	7.66	-	-	-	-
ß-*trans*-ocimene	1052	2.29	0.80	3.10	1.63	1.38	-	-	-	2.55	4.21	5.87	3.80	0.13	-	0.55	0.75
γ-terpinene	1062	-	-	0.60	-	-	-	-	-	1.81	0.31	0.48	0.34	-	-	-	-
isoterpinolene	1107	-	-	-	-	1.25	-	0.19	-	-	-	-	-	-	-	-	-
terpinolene	1093	-	-	0.86	0.47	-	-	-	-	4.19	5.97	7.36	4.66	-	-	-	-
linalool	1107	1.87	0.44	1.81	0.89	-	-	-	-	1.03	-	0.41	-	0.89	0.43	1.10	0.70
4-terpinol	1186	0.94	-	0.62	-	-	-	-	-	1.49	-	0.80	0.40	0.27	-	-	-
bornyl acetate	1295	1.78	0.31	1.22	0.61	-	-	-	-	-	-	-	-	-	-	-	-
α-copaene	1384	2.43	3.34	2.29	4.79	0.20	-	0.48	0.63	5.48	11.37	3.63	10.63	0.88	2.59	0.51	1.82
ß-bourbonene	1394	0.9	0.45	0.48	0.89	0.15	-	0.34	-	-	-	-	-	0.21	-	-	0.71
ß-cubebene	1399	-	0.32	-	1.04	-	-	0.60	0.50	-	-	-	-	-	-	-	-
ß-caryophyllene	1431	6.72	9.66	4.14	5.57	30.16	32.70	26.49	21.11	15.78	23.36	11.34	20.40	6.43	7.48	2.50	7.25
ß-gurjunene	1440	0.73	0.45	0.48	0.69	-	-	-	-	-	-	-	-	-	-	-	-
α-bergamotene	1444	-	0.20	-	0.45	-	-	-	-	1.21	1.47	0.36	1.81	-	-	-	-
α-guaiene	1449	1.52	0.73	0.79	2.35	-	-	-	-	-	-	-	-	-	-	-	-
α-humulene	1465	17.03	26.22	10.98	1.88	2.56	1.98	1.84	1.30	1.49	0.64	0.43	1.52	3.10	1.19	1.63	5.10
alloaromadendrene	1473	1.35	1.68	1.16	2.54	0.97	-	1.09	0.97	-	-	-	0.62	0.91	1.13	0.73	2.37

γ-muurolene	1489	1.73	1.07	1.33	-	-	-	-	-	0.44	0.31	-	0.88	0.43	0.42	-	0.98
germacrene D	1494	12.52	27.84	12.84	39.13	28.52	24.27	36.73	27.42	-	-	-	-	6.15	17.46	10.28	25.18
ß-selinene	1500	0.56	-	-	0.72	-	-	-	-	-	-	-	-	0.58	-	-	0.93
bicyclogermacrene	1510	8.14	11.43	9.47	11.45	3.83	3.06	6.07	3.57	0.91	-	-	-	1.34	1.34	1.53	3.08
α-muurolene	1512	-	-	-	-	-	-	-	-	0.88	0.87	0.38	1.46	0.23	-	-	-
germacrene A	1519	-	-	-	-	-	-	-	-	-	-	-	-	1.40	2.22	0.92	2.30
δ-guaiene	1519	0.94	0.43	0.50	1.15	-	-	-	-	-	-	-	-	-	-	-	-
δ-cadinene	1536	3.62	2.25	3.13	3.10	0.37	-	0.44	-	3.12	6.17	2.53	6.95	0.78	0.82	0.60	1.09
germacrene B	1574	-	-	-	-	1.33	-	1.01	-	-	-	-	-	-	-	-	-
nerolidol	1575	-	-	-	-	-	-	-	-	-	-	-	-	57.04	31.94	67.98	29.65
spathulenol	1597	-	-	2.18	0.51	-	-	-	-	1.06	-	0.93	-	2.86	-	-	0.75
caryophyllene oxide	1600	-	-	-	-	0.97	-	-	-	3.84	-	-	0.39	-	-	-	-
viridiflorol	1602	2.89	-	3.23	-	-	-	-	-	-	-	-	-	-	-	-	-
guaiol	1611	0.94	-	1.26	-	-	-	-	0.95	-	-	-	-	-	-	-	-
humulene epoxide II	1628	1.60	-	0.99	-	-	-	-	-	-	-	-	-	-	-	-	-
1,10-di-epicubenol	1633	-	-	0.34	-	-	-	-	-	-	-	-	-	-	-	-	-
1-*epi*-cubenol	1646	1.04	-	0.88	-	-	-	-	-	-	-	-	-	-	-	-	-
T-cadinol	1661	3.06	0.43	3.41	-	-	-	-	-	-	-	-	-	-	-	-	-
α-*epi*-muurolol	1661	-	-	-	1.12	-	-	-	-	-	-	-	-	-	-	-	-
δ-cadinol	1665	0.98	-	1.13	0.47	-	-	-	-	-	-	-	-	-	-	-	-
α-cadinol	1674	-	-	-	1.34	-	-	-	-	-	-	-	-	-	-	-	-
Total identified	93.59	97.09	93.08	95.00	98.12	99.98	96.78	95.88	93.66	95.20	95.69	83.99	85.28	83.82	93.34	86.35	

composition of the essential oils from many species has been investigated by gas chromatographic techniques. Limonene, β-caryophyllene, *p*-cymene, camphor, linalool, α-pinene and thymol are the components which were found in the highest frequency (Pascual et al., 2001). The OE and HF of *Lippia* species investigated in the present study were mainly composed by sesquiterpenes (Table 3). Among them, for *Lippia aff. microphylla* collected in dry and rainy periods, bicyclogermacrene and germacrene D were the main compounds, while the major monoterpene was α-pinene. Our data are in accordance with those previously reported to other *Lippia* species although thymol and 1,8-cineole were not identified by Costa et al. (2005) for *Lippia microphylla*. These differences may be explained by many factors such as soil, climatic conditions and also different genotypes (Baydar et al., 2004).

Lippia aristata, in all samples from dry and rainy seasons, showed high content of sesquiterpenes such as β-cariophyllene and germacrene D (Table 3). Among the monoterpenes, in all seasons, α-pinene and sabinene were identified both in EO and HF. The δ-3-carene was identified in the FH of plants collected in dry season and in the EO and FH of leaves collected in rainy season, while the limonene was only identified in EO from individuals collected during dry season. Indeed, sabinene, limonene, δ-3-carene, α-pinene and β-cariophyllene were also previously identified as major compounds in the essential oil of the other *Lippia* species (Craveiro et al., 1981; Terblanché & Kornelius, 1996).

The essential oil and HF from *Lippia martiana* collected in dry and rainy seasons showed higher level of the aromatic monoterpenes α-pinene and δ-3-carene (Table 3). The main sesquiterpene identified was β-cariophyllene.

The analysis of EO and HF from leaves *Lippia salviifolia* collected during dry and rainy seasons revealed that they are composed mainly by sesquiterpenes such as germacrene D and β-cariophyllene. Nerolidol was the major compound detected (Table 3). Antitrypanosomal, antifungal and antiulcer are some of the pharmacological activities already described for this oxygenated terpene (Hoet et al., 2006; Lee et al., 2007; Klopell et al., 2007).

CONCLUSIONS

For the first time, the chemical composition of the essential oil and the hexanic fraction were reported for two *Lantana* and four *Lippia species* collected at Serra do Cipó, Minas Gerais, Brazil. In general, it was possible to observe few differences between the composition of essential oil and the hexanic fraction for both genera. As the essential oil technique demands large quantities of plant material when compared to hexanic fraction, the present results offer an alternative approach to analyze the volatile compounds especially of plants that have a small amount of material.

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