



Chemical diversity of the volatiles of *Lippia rotundifolia* Cham. (Verbenaceae) in Minas Gerais, Brazil

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Abstract: *Lippia rotundifolia* is an aromatic species, native and endemic to rocky fields, which are isolated by small mountains. It is little known about their chemical composition. Because of that we aimed to study the chemical diversity of volatiles released from rosemary leaves (*Lippia rotundifolia* Cham.) coming from 11 populations of the Minas Gerais, Brazil. The material for chemical analysis was obtained from the accessions in the active germplasm bank of the Instituto de Ciências agrárias of the Universidade Federal de Minas Gerais. A total of 67 compounds were detected by HS-CG-MS. Most volatiles were detected in the São Gonçalo do Rio das Pedras accession, with 40 compounds, whereas in the Santana do Riacho accession only 5 compounds were detected. Volatiles myrcene, linalool and caryophyllene were detected in the majority of the accessions. We concluded that *Lippia rotundifolia* presents high chemical variability. The volatiles myrcene, linalool and caryophyllene are the most frequent, detected in most of the accessions. Due to some accession to present predominance of monoterpenes, others sesquiterpenes, studies of molecular analysis are necessary for associate the chemotypes, since environmental variation influence the gene expression of the secondary metabolites.

Key words: chemical variability, medicinal plant, rosemary, static headspace.

INTRODUCTION

The Cerrado concentrates one of the highest endemism rates of Brazilian flora. With approximately 30% of the local vegetation intact, it is considered one of the conservation hotspots (Gastauer et al. 2012). In Minas Gerais these environments are located in rupestrian fields, one of the phytophysiognomies of the Brazilian Cerrado. The rupestrian fields are characterized by altitudes

above 800 m, xeromorphism and presence of rocky outcrops (Rapini et al. 2008).

In the floristic composition of this phytophysiognomy, there is a predominance of species of the Verbenaceae family (Carvalho et al. 2012). The genus *Lippia* Linn. is the second largest in this family, where most endemic species are concentrated at these altitudes (Fernandes et al. 2011, Jacobi et al. 2011, Carvalho et al. 2012). Among the endemic species of rupestrian fields is *Lippia rotundifolia* Cham. Popularly known as pedestrian tea and rosemary, it is a shrub from 0.5 to 2 m tall (Salimena and Silva 2009), in restricted

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populations with few individuals (Martens 2008). Its flowers are grouped in large bunches ranging from pink-lilac to magenta or false pink (Salimena and Silva 2009).

In the natural environment *Lippia rotundifolia* is confused with others of the same genus due to the great morphological similarity and reproductive synchronism, making it difficult to botanical identification and collect of plant material. For this reason, chemical composition analysis studies of native species are scarce (Salimena and Mulgura 2015).

This plant is aromatic and produces volatile compounds, as a product of the secondary metabolism, which are released by the leaves and flowers. Studies of essential oils report mircene (22.3 %), limonene (26 %) as majorities (Leitão 2008). The hydrodistillation method to obtaining the chemical compounds is the most common and widely used method (Council of Europe 2010). The volatile fraction, the emitted fragrance, is established by the equilibrium between the plant and its vapor phase, but there is no record of volatile fraction studies for this species. The headspace method of extracting volatiles is the most appropriate for studies of these fragrances, since this method allows to obtain the composition of the genuine odor of the plant (Stashenko et al. 2002).

Therefore the knowledge of the chemical profile starting from the study of the volatile compounds of this species is important to the systematic and botanical contribution. In addition to distinguishing chemotypes of commercial interest that deserve to be maintained in the natural environment, aiming at the conservation of their existing genetic resources (Stashenko et al. 2002). Due to endemism and aromatic potential of the species, the aim of this research was to study the chemical diversity of volatiles released from rosemary leaves (*Lippia rotundifolia* Cham.) coming from 11 populations of the Minas Gerais, Brazil.

MATERIALS AND METHODS

SAMPLES ACQUISITION

Samples of *Lippia rotundifolia* were collected in 11 naturally occurring populations in the state of Minas Gerais. The specimens were identified by Dr. Rúbia Santos Fonseca and deposited in the EPAMIG herbarium. The propagation material was grown in conditions of the greenhouse of the Institute of Agrarian Sciences of the Federal University of Minas Gerais (ICA / UFMG). The location and climatic characteristics of each accession are presented in Table I.

The samples were taken from the apical meristem and leaf primordia of individuals from each place of species occurrence at 7:00 h in the morning. Approximately 1.5 g were weighed on an analytical balance and were transferred to 20 mL vials where were kept for 1 hour in a freezer at -20 °. Subsequently, the identified fresh material was sealed with silicone septum / PTFE and transported to the Analytical Chemistry Laboratory of the ICA / UFMG.

EXTRACTION OF VOLATILES AND CHROMATOGRAPHIC ANALYSIS VIA STATIC HEADSPACE (HS CG-EM)

The volatiles extraction was through static headspace using an automatic sampler system (HS Combi-PAL). Each sample was homogenized at 500 rpm and incubated at 75 °C for 5 minutes and immediately analyzed by HS-CG-MS. After this period, a 1000 µL volume of the sample in steam phase was injected in splitless mode with a gastight 2.5 mL-HS syringe. The injection rate was 500 µL/s at 220 °C.

Analyzes were performed on an Agilent® 7890A gas chromatograph coupled to an Agilent® MSD 5975C mass spectrometer (Agilent Technologies, California, USA). A DB-5 MS fused silica capillary column (Agilent Technologies, California, USA) with stationary phase of 5%

TABLE I
Location of eleven naturally occurring populations of *Lippia rotundifolia* in Minas Gerais, Brazil.

Code	City	Coordinates		Climate conditions			Herbal depot
		Latitude	Longitude	Alt. (m)	Precip. (mm)	Temp. (C°)	
PVP	Cônego Marinho	-14°55'S	-44°38'W	729	700	23±1	PAMG 58090
SNO	Rio Pardo de Minas	-15°36'S	-42°44'W	790	700	20±1	PAMG 58096
ABO	Montes Claros	-16°56'S	-43°55'W	700	1100	22.5±1	PAMG 58101
GIG	Botumirim	-16°35'S	-42°55'W	726	1350	22.5±2	PAMG 58097
RPE	Botumirim	-16°52'S	-43°28'W	722	1100	22.5±2	PAMG 58094
ODA	Olhos D'água	-17°26'S	-43°37'W	691	1100	22.5±2	PAMG 58094
JFE	Joaquim Felício	-17°44'S	-44°11'W	1010	1350	22.5±3	PAMG58093
PRP	São Gonçalo do Rio Preto	-18°06'S	-43°20'W	901	1350	< 19	PAMG 58091
SGS	Serro	-18°25'S	-43°28'W	1020	1350	18±2	PAMG 58100
RTI	Gouveia	-18°33'S	-43°49'W	1020	1350	20±2	PAMG 58092
SRI	Lapinha	-19°16'S	-43°37'W	756	>1500	20±2	PAMG 58099

SNO: Serra Nova State Park; PVP: Veredas do Peruçu State Park; ABO: Abóboras Community; GIG: Gigante Community; RPE: Margins of the Rio do Peixe; ODA: APA de Olhos d'água; JFE: Private Property in Joaquim Felício; PRP: Rio Preto State Park; SGS: São Gonçalo do Rio das Pedras; RTI: Stream of Rio Tigre; SRI: Santana do Riacho. Alt.: Altitude in meters; Precip.: Average annual precipitation in millimeters; Temp.: Average annual temperature in degrees.

phenyl and 95% polymethylsiloxane (30 m length x 250 µm internal diameter x 0.25 µm internal film thickness) (California, USA). Helium (99.9995 % purity) was used as drag gas with flow of 1 mL.min⁻¹.

The heating ramp started at 35 °C, maintaining it in an isothermal condition for 2 min, increasing at 3 °C.min⁻¹ to 150 °C, holding for 10 min and ending at 300 °C for one minute. Injector and transfer line temperatures for MS were maintained at 220 °C and 240 °C, respectively. The mass spectrometer was operated by electron impact ionization (70 eV), in a range of 35-550 m/z.

The concentrations of the constituents of the chemical analysis were expressed by the percentage of relative area of the total chromatogram ions ± standard deviation (n = 3). The identification of each constituent was by comparison of their retention times relative to the co-determination of a hydrocarbon standard solution (C8-C20, Sigma-Aldrich®, St. Louis, USA) and by comparison of the mass spectra of the (NIST/EPA/NHI (Nist

2008) library database and literature. The retention indices were calculated using the equation proposed by Van Den Dool and Kratz (1963).

MULTIVARIATE STATISTICAL ANALYSIS

The Cluster analysis was performed using the closest neighbor pairing methods (UPGMA). For this analysis, the data were arranged in a binary matrix of presence and absence of the identified volatiles. This grouping was based on the Jaccard (j) similarity coefficient and the Euclidean distance between the compounds, adopting SAHN (Sequential Agglomeration Hierarchy and Nested Clustering). For greater efficiency in the results, the data were submitted to the Pearson correlation analysis (r) (P ≤ 0.5), and were also autoscaled to verify if had the minimum correlation to justify its use in the data matrix as well as to avoid the effects produced by the variable size. Following conducted to principal component analysis (PCA). The retention of PCA axes to be interpreted was obtained by reducing the set of in linear combinations,

which generated scores identification of the most relevant chemical compounds in the discrimination of the different sites of occurrence. All statistical analysis were performed with the help of the Ntsys PC program. 2.11 (Rohlf 2000).

RESULTS AND DISCUSSION

In the chemical analysis by the HS-GC-MS method, 67 peaks were detected and of these 57 were identified. The percentages of volatiles are expressed in relative area of the peak for each analyte in Table II. The number of compounds identified were higher than the reported by Gomide et al. (2013) that identified 24 compounds in leaves and lower than the reported by Leitão (2008) that identified 108 compounds in leaves and 54 in flowers. Of the compounds identified by the latter author, 18 were identified in the present study. The chemical composition of the volatiles of the plant was quite varied, both in composition and in percentage, from one place to another. As for chemical classes in five populations (GIG, RPE, RTI, SGS, PRP), there was a predominance of monoterpenes, ranging from 42.4 % in the RPE to 61 % in RTI. In the other six populations (JFE, ABO, SRI, SNO, ODA, PVP), there was a predominance of sesquiterpenes, with 43.7 % in ABO and 65.5 % in JFE, respectively (Table II). This percentage was higher than that in the results obtained in previous studies with essential oils for the same species, in which the monoterpenes were predominant, with 52.5 %, even considering the loss of the most volatile fraction by the method (Leitão 2008).

The chemical compounds identified were well distributed among the individuals analyzed for the 11 environments. The chemical composition varied both in the number of volatile compounds as well as in the percentage of relative area of the total chromatogram of each accession. The results showed that the accession are heterogeneous in

relation to the production of these volatiles. Of the 67 compounds detected, ten stood out regarding higher production. The most important compounds were α -pinene, myrcene, α -phellandrene, limonene, linalool, tagetone, β -elemene, caryophyllene, germacren and α -selinene. Of these compounds, β -elemene, caryophyllene and α -selinene, stood out in production in two populations, the others stood out as majorities for all accessions in relation to the percentage of the relative area of the peak.

The chemotypes identified in the present study were mircene (11) that was highlighted as majority in five accessions: (GIG 30.5 %), (RPE 14.5 %), (JFE 13.5 %), (ODA 26.9 %) and (RTI 15.3 %), linalool (22), was the majority in two accessions (PRP 18.3 %) and (SRI 33.7 %). Gomide et al (2013) recorded the mircene as majority with 18.48 %, but the linalool was lower than the percentage of this study, with only 1.03 % for the accession collected in Juíz de Fora (Zona da Mata). However, Leitão (2008) did not record the production of this compound in the accession collected in Diamantina (Vale Jequitinhonha). The α -pinene (5) was the majority in SNO with 15.7 % and limonene (15) in ABO (28.6 %), in which the latter compound was higher than that reported by Leitão (2008) with 8.26 % for the same species. Lower percentages were also obtained by hydrodistillation for *Lippia thymoides* Mart.; Shauer, with contents between 1.67 and 3.75 % and by headspace for *Lippia citriodora* kunth and *Lippia alba* Mill. With 13.4 % and 4.7 %, respectively (Meshkatalasadat et al. 2010, Parra-Garcés et al. 2010).

The monoterpene, tagetone (26) 18 %, was detected only in SGS, being the first reported for the species. This compound was observed in *Lippia javanica* (Burm.f.) Spreng. at concentrations ranging from 0.3 to 4.93 % (Viljoen 2005). In *Lippia triplinervis* Gardner, they obtained an isomeric form (Z) that also varied with seasonality, with 0.1 % in April and 19.4 % in September (Leitão et al. 2011). The (Z) isomer has also been reported in small

amounts in *Lippia lacunosa* Mart. & Schauer with 0.5 %, whose species is considered synonymous of *Lippia rotundifolia* (Leitão 2008). The principal volatiles obtained in the present study diverged from the compounds presented by Leitão (2008), which reported myrtenal as majority compound of *Lippia rotundifolia*, with production of 16.7 %. In the analysis, this compound was recorded in small concentrations only in two accessions, (GIG 0.6 %) and (RTI 8.8 %). Gomide et al. (2013) didn't register its production, however they obtained (E/Z) farnesol in good quantity (16.47 %), being this the second most abundant compound reported by the authors for the Juíz de Fora access, whose compound, were not recorded in any of the 11 accessions analyzed.

The chemical polymorphism in the species is confirmed by the clustering of the environments as a function of volatile production. The dendrogram by the UPGMA method grouped the accessions into three groups (Figure 1).

The first group is formed by the SRI and PVP accessions, although they have different chemical compounds, they present the highest Caryophyllene production (49), (SRI = 24 % and PVP = 14.1 %), whose percentages were higher than those reported in the literature for the accession of Diamantina (7.9 %) and of Juíz de Fora (2.02 %) (Leitão 2008, Gomide et al. 2013). Another majority compound for these two accessions was germacrene in SRI, a secondary marker with (57 = 24.4 %) and in PVP was the majority with (57 = 21.5 %). These values were considered high since they were not detected in other accesses studied (Gomide et al. 2013). The compounds β -elemene (45 = 17.2 %) and α -selinene in PVP (60 = 13.8 %) were the secondary constituents. The β -elemene, although it was not the majority in any accession, was higher than the percentage reported in the literature for Diamantina (10.9 %) and Juíz de fora (3.45 %) accessions (Leitão 2008, Gomide et al. 2013). In

addition to these characteristics, the plants of these environments were those that produced the least amount of volatile compounds (n = 5 and 14) and are the two most distant accessions geographically (Table II and Figure 1).

The second group was formed by accessions ODA, GIG, RPE, ABO, PRP, JFE and SNO. In these environments, the individuals analyzed produce the same volatiles; myrcene, limonene, α -pinene and linalool with good representativeness. However, the JFE accession did not produce limonene and β -ocimene (15 to 17), and SNO did not produce germacrene (57), this being the main chemical difference observed among the seven accessions belonging to this group. The third group was formed by RTI and SGS accessions, where they grouped by chemical proximity, since both produced the main volatiles common to all environments, with limonene (15 = 14.6 %) as the secondary compound of RTI and linalool (22 = 7.7 %) as SGG secondary (Table II and Figure 1).

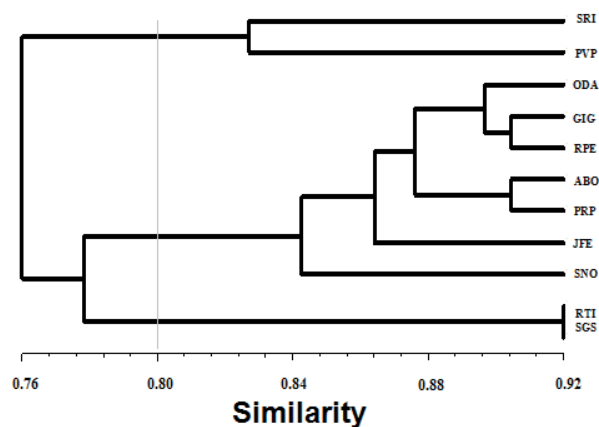


Figure 1 - Analysis of grouping by the UPGMA method of accessions of *Lippia rotundifolia* Cham. from eleven naturally occurring populations in Minas Gerais, Brazil. SRI: Santana do Riacho in Lapinha; PVP: Veredas do Peruáçu State Park; ODA: APA de Olhos D'água; GIG: Gigante Community in Botumirim; RPE: Rio do Peixe in Botumirim; ABO: Abóboras Community in Montes Claros; PRP: Rio Preto State Park; JFE: Serra Geral in Joaquim Felício; SNO: Serra Nova State Park; RTI: Rio Tigre in Gouveia and SGS: São Gonçalo do Rio das Pedras in the Serro district.

TABLE II
Chemical composition of volatiles of *Lippia rotundifolia* in eleven naturally occurring populations in Minas Gerais, Brazil.

N°	Compounds	LRI _{Lit.}	RI _{Cal}	Collection Sites												
				GIG	RPE	JFE	RTI	SGS	ABO	SRI	SNO	ODA	PVP	PRP		
1	hexanal	801	800	0.4	9.6	1.0	-	-	-	-	-	-	-	-	-	-
2	2-hexenal	854	850	0.3	1.5	6.7	-	-	-	-	-	-	-	-	-	-
3	3-hexen-1-ol	857	855	0.2	1.4	-	-	-	-	-	-	-	-	-	-	-
4	α -thujene	924	926	-	-	-	1.0	-	-	-	-	-	-	-	-	0.7
5	α -pinene	939	939	2.3	7.7	1.6	4.3	-	1.9	-	-	15.7	1.6	0.4	0.2	-
6	unknown	-	-	-	4.1	1.7	4.0	-	3.6	-	-	-	1.3	-	-	-
7	camphen	946	946	0.1	0.4	-	-	-	-	-	-	-	-	-	-	-
8	sabinene	969	972	0.4	5.7	-	1.7	0.3	0.1	7.8	2.0	1.0	1.0	-	2.9	-
9	β -pinene	974	973	2.4	7.5	1.5	4.5	0.5	1.4	-	-	-	1.7	0.4	3.0	-
10	1-wooten-3-ol	978	976	0.1	0.3	-	-	0.6	-	-	-	-	-	0.3	0.4	-
11	mircene	991	989	30.5	14.5	13.5	15.3	3.6	10.7	-	1.5	26.9	-	-	2.2	-
12	α -felandren	1005	1006	5.0	3.6	-	-	5.9	-	-	15.5	11.3	-	0.6	2.6	-
13	o-cimene	1022	1023	-	1.7	-	-	-	-	-	2.49	-	-	-	-	-
14	m-cimene	1026	1025	4.4	1.4	-	-	0.4	-	-	-	1.4	-	-	0.4	-
15	limonene	1027	1029	5.7	2.8	-	14.6	2.5	28.6	-	2.7	6.4	0.3	0.3	2.5	-
16	z-ocimene	1032	1033	0.6	1.0	-	-	-	-	-	-	0.3	-	-	0.2	-
17	β -ocimene	1044	1045	4.3	2.4	-	7.4	1.8	-	-	1.1	4.7	-	0.4	1.3	-
18	y-terpinene	1054	1056	2.7	-	-	5.9	-	-	-	-	-	-	-	-	-
19	linalyl oxide	1067	1067	-	-	-	-	0.4	-	-	-	-	-	-	0.3	-
20	terpinolene	1086	1085	0.2	-	-	-	5.0	-	-	-	-	-	-	0.4	-
21	unknown	-	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-
22	linalool	1098	1099	3.6	2.8	13.3	1.4	7.7	17.7	33.7	5.0	1.9	-	-	18.3	-
23	1,3,8-p-mentatriene	1110	1110	-	-	-	-	0.4	-	-	-	-	-	-	1.0	-
24	unknown	-	-	-	-	-	-	0.2	-	-	-	-	-	-	2.9	-
25	cosmene	1130	1132	0.1	-	0.6	-	1.1	-	-	-	0.2	-	-	0.5	-
26	tagetone	1139	1139	-	-	-	-	18.0	-	-	-	-	-	-	-	-
27	pinocarvone	1164	1162	0.4	-	-	1.8	0.4	-	-	0.7	-	-	-	-	-
28	unknown	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-
29	borneol	1165	1167	-	1.3	-	-	-	-	-	-	-	-	-	-	-

TABLE II (continuation)

N°	Compounds	LRI _{Lit.}	RI _{Cal.}	Collection Sites												
				GIG	RPE	JFE	RTI	SGS	ABO	SRI	SNO	ODA	PVP	PRP		
30	unknown	-	-	0.2	0.8	-	3.2	-	-	-	-	-	-	-	-	-
31	butanoate-(Z)-3-hexenyla	1186	1185	-	-	-	-	0.2	-	-	-	-	-	-	-	0.2
32	α -terpineol	1186	1187	-	-	-	-	0.1	-	-	-	-	-	-	-	-
33	butanoate-2-hexenyl	1191	1190	-	-	-	-	3.5	-	-	-	-	-	-	-	0.1
34	myrtenal	1195	1193	0.6	-	-	8.8	-	-	-	-	-	-	-	-	-
35	myrtenol	1196	1196	0.6	-	-	-	-	-	-	-	-	-	-	-	-
36	unknown	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-
37	<i>p-mentha-1,8-dien-3-one</i>	1285	1285	-	-	-	-	0.3	-	-	-	-	-	-	-	-
38	tridecane	1300	1302	-	-	-	-	-	0.1	-	-	-	-	-	-	-
39	myrtenyl acetate	1324	1321	0.2	-	-	-	-	-	-	-	-	-	-	-	-
40	δ -elemene	1335	1335	0.1	0.8	0.4	-	0.4	-	-	-	8.0	-	-	2.1	0.8
41	α -cubebene	1351	1353	0.2	0.7	0.2	-	0.8	-	-	-	0.6	1.0	-	-	1.2
42	2-methyl-tridecane	1365	1364	0.1	-	-	-	-	-	-	-	-	-	-	-	-
43	α -copaene	1376	1373	0.2	0.6	2.0	1.5	0.8	1.1	10.0	0.6	0.6	0.5	-	-	3.0
44	β -cubebene	1387	1388	-	0.7	1.3	2.0	0.5	0.7	-	-	-	3.6	-	-	-
45	β -elemene	1390	1390	3.6	-	8.1	1.8	-	-	-	-	-	-	-	17.2	3.0
46	unknown	-	-	-	1.7	2.6	-	1.0	-	-	-	-	4.4	-	-	-
47	α -gujunene	1409	1406	0.2	1.00	0.2	-	1.0	-	-	0.6	0.6	0.2	-	-	0.4
48	α -cedrene	1413	1413	-	-	0.4	-	-	-	-	-	-	-	-	-	-
49	caryophyllene	1418	1416	3.6	3.8	4.6	3.7	4.1	4.6	24.0	13.0	3.9	3.9	14.1	14.1	6.7
50	β -cedrene	1419	1419	-	-	0.3	-	0.2	-	-	-	-	-	-	-	-
51	γ -elemene	1434	1434	-	-	0.7	-	-	-	-	5.8	-	-	3.0	0.1	0.1
52	α -bergamotene	1436	1436	-	5.6	0.3	-	1.0	-	-	-	-	4.4	-	-	-

GIG: Gigante Community in Botumirim; RPE: Rio do Peixe Margins in Botumirim; JFE: Serra Geral in Joaquim Felício; RTI: Rio Tigre in Gouveia; SGS: São Gonçalo do Rio das Pedras in the Serro district; ABO: Aboboras Community in Montes Claros; SRI: Santana do Riacho in Lapinha; SNO: Serra Nova State Park; ODA: APA de Olhos D'água; PVP: Veredas do Peruaçu State Park and PRP: Rio Preto State Park. LRI_{Lit.}: Linear retention index of the literature. RI_{Cal.}: Retention index calculated. (-):Compound not detected.

The distribution of the chemical compounds was confirmed by analysis of the principal component (PCA) of the relative area of each compound detected for the 11 accessions. This analysis summarized the chemical variables in the first two main components (PC). These were retained for interpretation, as they presented cumulative eigenvalues of 96.58 % of the total variance among volatile compounds. The first component explained 90.175 % of the variability for all the samples, in which it showed clear separation of the volatile attributes of the species. The second component explains only 6.405 % of the total variance. The attributes with the highest factor load in the first component are presented in Table III.

The distribution of the ten most abundant volatile compounds in the two main components corroborates their detection for the 11 accessions, in which monoterpene, tagetone (26), is the compound that is farthest from center, presenting higher standard deviation, which confirms its exclusivity in the SGS accession (Figure 2).

The graphical projection of the discriminant analysis of the 11 accessions corroborates the results presented in Table II, which it shows that the species has chemotypes belonging to the two main chemical classes. The accessions with a greater predominance of monoterpenes were grouped in the upper plane (GIG, RPE, RTI, SGS, PRP), whereas accessions with predominance of sesquiterpenes were grouped in the lower plane (JFE, ABO, SRI, ODA, PVP). This analysis also corroborates the in loco observation, in which the accessions that were farther away in the graphic dispersion of the discriminant analysis were those that produced the lowest number of volatiles $n = 5$ for SRI and $n = 14$ for PVP (Figure 2).

These two accessions are the most chemically and geographically distant. The small amount of volatiles produced in SRI and PVP can be explained by anthropic interference, since in PVP there was

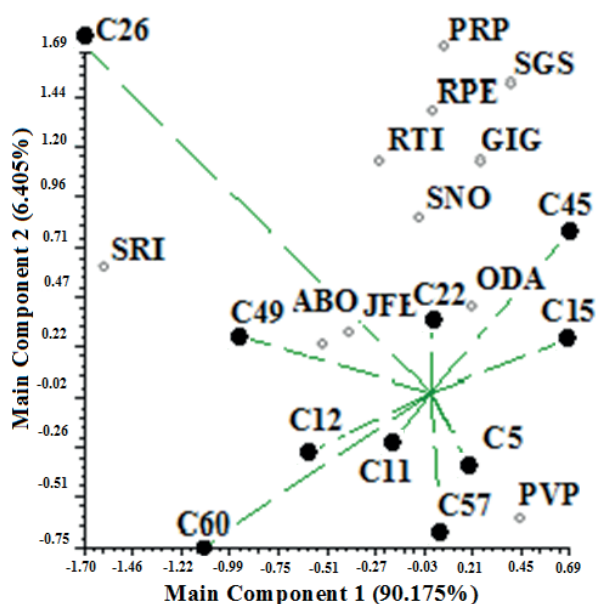


Figure 2 - Principal component analysis and two-dimensional scale dispersion of the ten major volatiles (a) of *Lippia rotundifolia* Cham. of 11 accessions belonging naturally occurring populations in Minas Gerais, Brazil. (5) α -pinene; (11) myrcene; (12) α -felandrene; (15) limonene; (22) linalool; (26) tagetone; (45) β - elemene; (49) caryophyllene; (57) germacrene; (60) α -selinene. SRI: Santana do Riacho in Lapinha; PVP: Veredas do Peruaçu State Park; ODA: APA Olhos D'água; GIG: Gigante Community in Botumirim; RPE: Rio do Peixe in Botumirim; ABO: Abóboras Community in Montes Claros; PRP: Rio Preto State Park; JFE: Serra Geral in Joaquim Felício; SNO: Serra Nova State Park; RTI: Rio Tigre in Gouveia and SGS: São Gonçalo do Rio das Pedras in the Serro district.

occurrence of fires, while in SRI, although the environment is close to a water course, suitable for the establishment of the species, there was the presence of domestic animals foraging on the site. In these two environments, individuals presented leathery leaves with no apparent odor. These observations evidence the phytochemical and morphological differences of the individuals in response to the environmental conditions to which they are exposed (Osorno-Sanchez et al. 2012, Calvo-Irabién et al. 2014).

The variation in the chemical profile is also explained by the genetic interaction with the environment, since the secondary metabolites are genetically controlled, probably polygenic, and

TABLE III
Chemical attributes of volatiles of *Lippia rotundifolia* with the eigenvalues of the main components from the eleven naturally occurring populations in Minas Gerais, Brazil.

Components of variance		Volatile compound index	
		CPI	CP2
Variability (%)		90.175	6.405
Accumulated variability (%)		90.175	96.58
Variables		Factorial load (Scores)	
15	limonene	0.987	0.024
49	caryophyllene	0.959	0.167
57	germacrene	0.959	0.189
22	β -linalool	0.998	0.016
26	tagetone	0.978	0.025
12	α - felandren	0.941	0.003
45	β -elemene	0.949	0.014
59	eremophilene	0.659	0.105
48	α -cedrene	0.545	0.089

are associated with the plant defense mechanism, in which it causes significant alterations to the chemical composition of the volatiles. Thus, environmental conditions may influence the expression of the gene responsible for the activation of the terpene synthase enzymes involved in the biosynthesis of terpene volatiles (Pinto-Zevallos et al. 2013, Winska et al. 2014, Pavarini and Lopes 2016).

In order to conserve native aromatic plants with economic potential, the first step is to identify the best adapted genotypes and, from these, to work on their genetic improvement in order to standardize the crop to meet the market demand. Within this idea, the influence of the environment on the variation of the chemical profile of *Lippia rotundifolia*, is a positive characteristic as a survival mechanism. This factor also shows the potential of the species to be explored in the natural products market, since each environment expresses the genes responsible for the production of a certain chemical compound, which can be a source of income for the region in which it occurs (Yamamoto et al. 2008, Winska et al. 2014).

From the results presented, the highest number of compounds was obtained in SGS (n= 40), GIG (n = 38) and PRP (n = 35) and the lowest number of compounds was obtained in SRI (n = 5) and PVP N = 14). However, within the chemical polymorphism presented by the species, it is proposed that the chemical variation of the species is distributed in six potential chemotypes which are attributed by the volatiles myrcene, linalool, α -pinene, limonene, tagetone and germacrene, whose chemical compounds are the most abundant for the species in Minas Gerais. However, studies of molecular analysis are necessary for support this hypothesis.

CONCLUSIONS

Lippia rotundifolia presents high chemical variability. The SGS accession has the highest number of chemical compounds and SRI accession has smaller number. PVP accession holds the greatest chemical distance. The volatiles mircene, linalool and caryophyllene are the most frequent, detected in most of the accessions. Due to some accession to present predominance of monoterpenes, others sesquiterpenes, studies of

molecular analysis are necessary for associate the chemotypes, since environmental variation influence the gene expression of the secondary metabolites.

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First author: chapter thesis of Doctorate; Second author: research coordinator; Third author: Chemical Technique responsible for chemical analysis.

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