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Intraespecific variation in leaf oils of *Lippia junelliana* (mold.) tronc

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Abstract

The purpose of this work was to assess the essential oil variation of *Lippia junelliana* from 16 different sites of mid-west Argentina. The essential oil of *L. junelliana* was dominated by monoterpenes (70–94%) with low amount of sesquiterpenes (3–10%) and phenylpropanoids (0.2–3%). More than 80 compounds were detected in the species. However, only eight were found as major component in the oils. According to these components, four wild chemotypes were detected. The first chemotype contained high levels of ocimenone (54–76%) with lower amounts of myrcene (10%).The second was composed of large amounts of dihydrocarvone (59–80%). The main constituents found in the third chemotype were limonene (10–40%) and piperitenone (10–40%) and the fourth chemotype contained limonene (41%) and piperitenone oxide (26%).

Keywords: *Lippia junelliana*; Verbenaceae; Essential oils; Chemical diversity; Monoterpenes; Sesquiterpenes

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1. Introduction

The genus *Lippia* belongs to Verbenaceae family comprising approximately 200 species grown in South and Central America and tropical Africa (Terblanché and Kornelius, 1996). Argentina is particularly rich in species of this genus. Among them, *L. junelliana* (Mold.) Tronc., an aromatic shrub of central Argentina, 1.5 m high grows naturally in the hilly regions of Córdoba, La Rioja, San Luis, Tucuman, Jujuy (Fester et al., 1956), Catamarca, Salta and Santiago del Estero provinces (Zuloaga et al., 1999). It is locally known as "salvialora" or "salvia morada", and its leaves are claimed to have medicinal properties specially for abdominal complaints (Juliani et al., 1994).

Many years ago, it was found that the same species grown in different areas could exhibit striking variations in the composition of its essential oils (Fester et al., 1961). The Verbenaceae displays such phytochemical phenomenon and *Lippia alba* is a typical example. *Lippia alba* from Tucumán province contained piperitone, limonene and 1.8 cineole as the main constituents, whereas, material from Paraná province contained α-pinene and dihydrocarvone as the major components (Retamar, 1986).

Lippia junelliana is an endemic aromatic shrub from central Argentina. The first report of the essential oil composition detected α-pinene, phellandrene, cineol, camphor, borneol and lippiaphenol. A second assay found α-pinene, geraniol, borneol and camphor (Retamar, 1986). Using modern analytical assays, the oil from Córdoba province was found to contain myrcene (8%), ocimenone (13%), myrcenone (15%), limonene (9%) and dihydrocarvone (21%) (Velasco-Negueruela et al., 1993), while, in San Luis province, the oil contained limonene (26%), geranial (15%) and piperitenone (35%) (Juliani et al., 1994). Another oil sample from this province, contained limonene (20%) and piperitenone oxide (48%) as major components in the fall essential oil (Duschatzky et al., 1999).

In view of the chemical variation of the oils reported in literature, we decided to assess the essential oil variation of *L. junelliana* from different regions.

2. Materials and methods

Details of plant material used in this study are listed in Table 1. Voucher specimens were deposited in the herbarium of Museo Botánico (Index Herbariorum Code (IHC), CORD, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba) and in Herbario Regional del Instituto de Desarrollo de Los Llanos de La Rioja ((IHC) IZAC, Universidad Nacional de La Rioja, Sede Chamical, La Rioja). Samples were collected during summer (January– March, 1999).

Single plant leaves from each site were submitted to steam distillation. 30 g of fresh leaves were distilled (60 min) using a Clevenger-type device. Percentage yields were determined on a dry weight basis (Table 2). The oils were dried over anhydrous sodium sulphate and stored at 20°C in the dark until analyzed.

Table 1
Description of the original sources of leaf samples of *L. junelliana* from mid-west Argentina

Collection site	Province and department	Latitude (S)	Longitude (W)	Height	Voucher specimen
1—Estancia Cabeza de Novillo	Córdoba, Cruz del Eje	31°5′	64°48′	850	CORD 576
2—Agua de Oro	Córdoba, Colón	31°04′	64°18′	600	CORD 793
3—Arroyo San Antonio	Córdoba, Calamuchita	32°16′	64°40′	900	CORD 401
4—Villa Allende	Córdoba, Colón	31°16′	64°20′	650	CORD 787
5—Pan de Azúcar	Córdoba, Punilla	31°14′	64°26′	1100	CORD 788
6—Dique La Quebrada	Córdoba, Punilla	31°09′	64°20′	750	CORD 789
7—Los Chañaritos No. 2 Km. 2.5	Córdoba, Cruz del Eje	31°3′	64°57′	950	CORD 578
8—Sierra de los Llanos	La Rioja, Chamical	30°22′	66°24′	700	IZAC 5439
9—Piedras Blancas	San Luis, Junin	32°19′	65°02′	925	CORD 403
10—Río Pinto	Córdoba, Punilla	31°07′	64°42′	850	CORD 575
11—La Serranita	Córdoba, Calamuchita	31°43′	64°28′	850	CORD 740
12—La Falda	Córdoba, Punilla	31°04′	64°26′	950	CORD 786
13—Loma Bola	Córdoba, San Javier	32°10′	65°16′	950	CORD 577
14—Dique nivelador Boca del Río	Córdoba, San Alberto	31°54′	65°05′	700	CORD 792
15—Altautina	Córdoba, San Alberto	31°47′	65°11′	850	CORD 402
16—Los Chañaritos No. 2 Km. 0.5	Córdoba, Cruz del Eje	31°2′	64°57′	900	CORD 579

Table 2
Leaf essential oil content and composition of *L. junelliana* from 16 regions in mid-west Argentina

		Ocimenone chemotype						Dihydrocarvone chemotype						Limonene–Piperitenon. c.			Li–P Ox. c.
Components	KI[a]	1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
α-Pinene	937	0.2	0.4	0.2	0.2	0.12	0.2	0.3	0.4	0.1	0.6	0.2	0.3	0.7	1.3	0.4	0.4
Camphene	953	1.7	2.3	1.5	1.1	0.2	1.3	1.7	2.0	0.5	3.5	1.0	1.8	0.1	7.3	3.1	2.5
β-Pinene	980				0.1	1.1	0.1		0.1		0.3				0.4	0.2	
Myrcene	986	8.2	12.4	10	8.9	9	8.1	0.4	0.7	0.3	5.3	0.3	4.4	tr	4.5	0.2	0.3
P-Cymene	1016	tr		0.1	0.1		0.1	0.3	tr		tr		0.1				
Limonene	1031	0.1	0.8	0.7	0.9	0.6	0.9	10.8	10.0	3.5	6.3	5.3	5.0	41.2	41.4	10.0	41.0
cis-β-Ocimene	1040	0.1	0.2	0.1	0.4	0.6	0.4	0.6	1.2	0.2	0.8	0.3	0.25	0.1	3.0	0.5	0.2
Linalool	1087							0.3	0.1	0.2					0.6		
Myrcenone	1096	1.0	1.1	1.0	1.3	1.0	0.9										
Camphor	1114	0.3	0.3	0.7	0.3	0.3	0.2	4.7	2.6	0.4	3.9	2.1	8.4	2.1	11.2	20.2	6.1
trans-Tagetone	1132	0.1	0.3	0.8	0.2	0.4	0.4										
cis Tagetone	1140	tr	0.1	0.5	0.1	0.1	0.2										
Borneol	1146			0.1										0.1	2.0	4.2	
α-Terpineol	1169	0.2		0.4			0.1										
Dihydrocarvone	1172							71.3	72.1	79.2	59.3	79.8	68.8				0.4
isoDihydrocarveol	1184							0.6	0.1	0.9	0.2	0.2	0.3				
trans Carveol	1188							1.4		1.4	0.3	0.1	0.2				
neoiso Dihydrocarveol	1192							0.6	0.1	0.8	0.1	0.1	0.2				
Methylchavicol	1201													2.1	0.5		1.4
Ocimenone Z	1209	53.1	38.3	63.2	39.2	43.4	47.6								0.2		
Ocimenone E	1213	1.1	3.1	2.2	36.9	20.1	21.8										
Carvone	1218	6.7	4.6	3	tr	0.1	0.2	0.3	tr	0.3	0.1	0.2	0.5	0.3	0.2	2.9	0.2
Piperitone	1223													1.1	0.6	5.4	
Piperitenone	1304	6.6	10.2	8.5	0.1	0.5	0.4	0.2	0.8	0.5	0.3	0.4	0.1	38.5	10.1	48.2	4.4
δ-Elemene	1328	tr	8.2	0.1		1.2	0.1		0.2		0.5	0.5	0.1	1.36	0.7		
Eugenol	1327										0.1			0.2			
Piperitenone oxide	1352							3.3									26.7
Methyleugenol	1371	0.4	1.4	0.5	0.4	1.1	0.6	0.5	0.3	2.2	2.9	0.1	0.2	0.4	1.0	0.7	0.2
(E)—Caryophyllene	1419	1.1	1.1	0.7	1.7	1.8	2.7	0.8	2.3	3.6	3.5	2.2	1.5	1.9	1.2	0.3	2.6
α-Humulene	1452	tr	tr	0.1	0.9	1.0	1.2	0.2	0.3	0.1	0.5	1.2	0.7	0.6	1.5	0.9	0.4
Germacrene B	1556	0.1	1.2	2.0	2.5	3.7	2.9	1.4	3.5	1.0	1.5	2.7	2.1	1.2	2.6	1.1	1.1
Spathulenol	1576	1.8	1.2	1.9	0.2	2.8	1.2	0.3	0.1	0.6	1.2	1.3	1.1	0.3	0.3	1.1	0.3
Oil content (g/100 g dw)		1.4	3.3	2.6	2.2	1.5	1.8	2.4	2.7	2.3	2.1	1.6	2.3	2.8	2.7	4.1	3.3

[a] Kovats index on apolar column (SE 30).
[b] Collection site (Table 1); tr: traces less than 0.1%.

Essential oils were analyzed with a Shimadzu GC R1A gas chromatograph equipped with a SE 30 column (30×0.25 mm). The temperature of column was programmed from 60°C to 240°C at 4°C/min. The temperature of injector was 240°C. The gas carrier was helium at a flow rate of 1 ml/min. Peak areas were measured by electronic integration. The relative amounts of the individual components were based on the peak areas obtained without FID response factor correction. Kovats indexes (KI) were estimated by co-injection of the oil with a homologous series of *n*-alkanes (Kovats, 1965).

Next, the oils were analyzed using a Q-mass 910 Perkin Elmer mass spectrometer coupled to a gas chromatograph fitted with a capillary column (SE-30, $30\,m \times 0.25$ mm). The flow rate of helium was 1 ml/min. The oven temperature was the same as that of the GC.

Individual identifications were made by matching their 70 eV mass spectra with those from literature and from mass spectral libraries. The oil components were also identified by comparing their retention indexes with those of authentic samples and those from literature (Adams, 1995; Ahmad et al., 1999; Baratta et al., 1998; Shatar and Adams, 1998).

3. Results and discussion

The essential oil composition from different sources of *L. junelliana* was dominated by monoterpenes (70–94%), with lower amounts of sesquiterpenes (3–10%) and phenylpropanoids (0.2–3%).

More than 80 compounds were detected in the essential oils of the species. However, only seven monoterpenes were found as major components (more than 10%). These compounds were myrcene, limonene, camphor, ocimenone, dihydrocarvone, piperitenone and piperitenone oxide. According to these terpenes, four wild chemical chemotypes were detected (Table 2).

The ocimenone chemotype represented 37% of the samples. The essential oils contained high levels of ocimenone (41–72%) with low amounts of myrcene (8–12%). These oils also contained myrcenone, *cis*- and *trans*-tagetone. Another feature of this chemotype was the low concentration of limonene (less than 1%). The dihydrocarvone chemotype represented the 37% of the samples, the main constituent was dihydrocarvone (59–80%) with minor amounts of limonene (3.5–10%). These oils contained isodihydrocarveol, *trans*-carveol and neoisodihydrocarveol as minor components. The limonene–piperitenone chemotype representing 19% of the samples was composed of high amounts of limonene (40%) and piperitenone (10–40%). Camphor also occurred in high amounts (site 14 (11%) and site 15 (20%). It is worth noting the presence of piperitone which occurred in lower amounts (1%). The limonene–piperitenone oxide chemotype represented only 6% of the samples showing high levels of limonene (41%) and piperitenone oxide (26%). α-pinene, camphene, myrcene, limonene, *cis*-ocimene, camphor, carvone, piperitenone, methyleugenol, caryophyllene, α-humulene, germacrene B and spathulenol were detected in all plants (Table 2).

The essential oils from the ocimenone chemotype contained high levels of oxygenated compounds and the oils were characteristically yellow, while, the oils from the other chemotypes which contained lower levels of oxygenated compounds and appeared clear.

It seems that seasonal variations did not influence the essential oil composition. In certain sites (1, 7, 9 and 16), the essential oil composition showed non-significant variations year round (unpublished results). This agrees with those results observed in *L. junelliana* in which the essential oil composition did not vary significantly during the year (Juliani et al., 1998). This latter chemotype was cultivated in plots and its essential oil composition matched the natural population (Juliani, 1998). These findings would confirm that these chemotypes are stable. For assessment they should be cultivated in the same place.

The composition appears to be stable, whereas the oil species yields were highly variable, and ranged from 1.4% to 4.1% (Table 2). The remarkable variability of essential oil yield could be linked to monthly variations (Juliani et al., 1998) and to different environments (sun and shade) in which the plants grew. Other species such *as Origanum vulgare ssp. hirtum* have also shown great variations in their essential oil yield (Kokkini et al., 1994).

Like *Lippia junelliana*, *L. alba* also showed a great variation in its oils composition (Retamar, 1986). Therefore, it seems that this variation is a feature of the genus

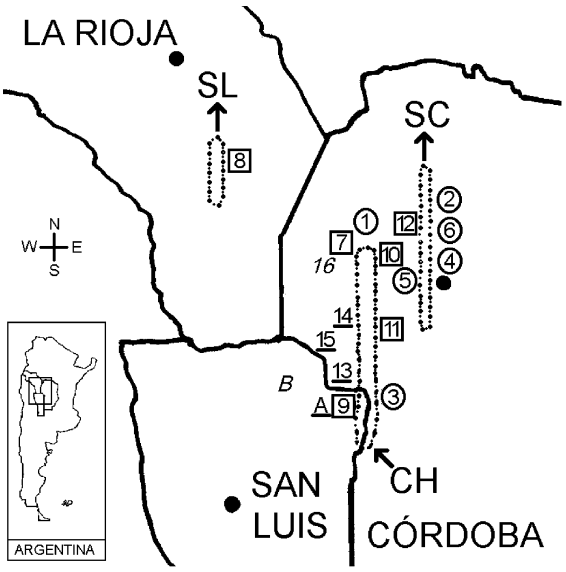

Fig. 1. Collection sites of *Lippia junelliana* from three provinces (La Rioja, San Luis and Córdoba) of Argentina (lower left). The sites 1–6 correspond to ocimenone chemotype (circle), 7–12 dehydrocarvone (square), 13–15 limonene–piperitenone (underlined) and 16, limonene–piperitenone oxide (italic). A, B, Juliani et al. (1994) and Duschatzky et al. (1999) reports. SL, SC and CH, De los llanos, Chicas and Comechingones mountains, respectively.

Lippia. The ocimenone chemotype appears to be mainly grouped in three neighboring departments (Cruz del Eje, Punilla y Colon) (Fig. 1). On the other hand, the limonene–piperitenone chemotypes were clustered south–west, off the Sierra de Comechingones (In Córdoba and San Luis provinces). Nearby, a specimen of *L. junelliana* from San Luis province was also found to contain limonene and piperitenone as leading components in the oil but with high amounts of geranial (Juliani et al., 1994) (Fig. 1). The dihydrocarvone chemotype was scattered among the collection area. While the limonene–piperitenone oxide chemotype was found only in site 16 (Córdoba, Cruz del Eje Dept.). In San Luis province was found an essential oil bearing plant belonging to this latter chemotype (Duschatzky et al., 1999) (Fig. 1).

Wild populations of medicinal and aromatic species are typically recognized by a high degree of genetic variation (Figliuolo, 1995). As a result, the survey of the chemical diversity of this endemic species from Argentina constitutes the starting point for the selection and subsequent domestication, that is now in progress.

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