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Intraspecific variation in essential oil composition of the medicinal plant *Lippia integrifolia* (Verbenaceae). Evidence for five chemotypes

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ABSTRACT

The aerial parts of *Lippia integrifolia* (incayuyo) are widely used in northwestern and central Argentina for their medicinal and aromatic properties. The essential oil composition of thirty-one wild populations of *L. integrifolia* covering most of its natural range was analyzed by GC and GC–MS. A total of one hundred and fifty two terpenoids were identified in the essential oils. Sesquiterpenoids were the dominant components in all but one of the collections analyzed, the only exception being a sample collected in San Juan province where monoterpenoids amounted to 51%. Five clearly defined chemotypes were observed. One possessed an exquisite and delicate sweet aroma with *trans*-davanone as dominant component (usually above 80%). Another with an exotic floral odour was rich in oxygenated sesquiterpenoids based on the rare lippifoliane and africanane skeletons. The *trans*-davanone chemotype is the first report of an essential oil containing that sesquiterpene ketone as the main constituent. The absolute configuration of *trans*-davanone from *L. integrifolia* was established as 6*S*, 7*S*, 10*S*, the enantiomer of *trans*-davanone from 'davana oil' (*Artemisia pallens*). Wild plants belonging to *trans*-davanone and lippifolienone chemotypes were propagated and cultivated in the same parcel of land in Santa Maria, Catamarca. The essential oil compositions of the cultivated plants were essentially identical to the original plants in the wild, indicating that the essential oil composition is largely under genetic control. Specimens collected near the Bolivian border that initially were identified as *L. boliviana* Rusby yielded an essential oil practically identical to the *trans*-davanone chemotype of *L. integrifolia* supporting the recent view that *L. integrifolia* (Gris.) Hieron. and *L. boliviana* Rusby are synonymous.

1. Introduction

The genus *Lippia* (Verbenaceae, Lantaneae) comprises about 160 species growing in the warm and temperate regions of South America, Central America and Africa (Denham et al., 2006; Jansen-Jacobs, 1988). In the Americas, members of this genus can be found from Mexico to the province of Buenos Aires, in Argentina. An outstanding feature of this genus is the large chemical variability exhibited by the same species harvested in different places (Catalán and de Lampasona, 2002; Coronel et al., 2005; Terblanché and Kornelius, 1996). Many *Lippia* species are widely used in folk medicine and the chemical composition, pharmacological activity and traditional use of the members of this genus have been reviewed (Bassols and Gurni, 1996; Catalán and de Lampasona, 2002; Pascual et al., 2001; Terblanché and Kornelius, 1996). *L. integrifolia* (Griseb.) Hieron. is a woody aromatic shrub widely used in traditional medicine that produces ketones based on unique sesquiterpene skeletons named lippifoliane and integrifoliane (Catalán et al., 1991, 1994, 1995; Cerda-Garcia-Rojas et al., 2005; Coronel et al., 2006).

L. integrifolia, commonly known as "incayuyo" or "té del inca", is native to central and northwestern Argentina where infusions of the aerial parts are traditionally used against dyspepsia, stomach aches, indigestion, as a diuretic, emmenagogue, for cough treatment and as a sedative (Alonso and Desmarchelier, 2006; Gorzalczany et al., 2008). *L. integrifolia* has been included in the Argentine Food Code as a seasoning since it is used as an ingredient of some well known commercial non-alcoholic beverages, appetizers and teas. The anti-inflammatory effects on stomach cells and

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antiadhesive properties of the extracts of *L. integrifolia* against the main bacterial inductor of gastritis, *Helicobacter pylori*, have been demonstrated recently (Marcial et al., 2014). As stated above, *L. integrifolia* is a rich source of sesquiterpenoids with little-known skeletons called integrifoliane, lippifoliane, africanane and asteriscane (Catalán et al., 1992, 1993a,b; Coronel et al., 2006; Fricke et al., 1999). On the other hand, significant qualitative differences in the composition of oils coming from different collections of "incayuyo" have been reported (De Lampasona et al., 1999; Duschatzky et al., 1998; Velasco-Negueruela et al., 1993; Zygadlo et al., 1995).

When variations in the essential oil composition are due to environmental conditions herein described are "ecotypes" (Nurzyńska-Wierdak et al., 2012; Laribi et al., 2013) i.e. when the chemical composition is primarily under genetic control, the chemical profile remains essentially constant after several years in different environments. In this case, it is considered to be a "chemotype" (Sadeghi et al., 2015; Medina-Holguín et al., 2007).

Because of the variable composition of the essential oil of *L. integrifolia* and with the aim to evaluate the biodiversity within this species, a detailed analysis of the essential oil composition of thirty one wild populations harvested at different places of central and northwestern Argentina was undertaken (Table 1, Fig. 1 and Fig. S2) embracing most of its natural distribution area.

2. Results and discussion

The collection sites and essential oil yields from aerial parts of 31 wild populations of *L. integrifolia* harvested at different places of the central and northwestern region of Argentina (provinces of San Luis, San Juan, La Rioja, Catamarca, Salta and Tucumán) are reported in Table 1. In previous work, it was found that *L. integrifolia* produced the highest yield of oil in April and that the content of oxygenated sesquiterpenes reached its maximum in February, March and April (Coronel et al., 2006). So, 26 out of 31 collections were made in that period. It was noted though that while quantitative variations were observed for most components, the qualitative composition of the essential oil remained constant throughout the season.

The essential oil yields varied from 0.51% to 1.76% (w/w) (Table 1). Relative percentage composition of the main compounds in the 31 essential oil samples analyzed is shown in Table 2. Only compounds present in more than 3.0% of the total oil in at least one of the 31 samples analyzed were considered. Compounds with percentages below 0.1% are shown as "tr" (trace) and compounds not detected are shown as "0". A complete detail of all the compounds identified in the 31 essential oil samples is presented in Table S1 (Supplementary data).

The total percentage of monoterpenoids and sesquiterpenoids grouped according to the compound type for each essential oil sample are also included in Table 2. As can be seen, sesquiterpenoids constituted the main portion of all but one of the samples analyzed. The exception was sample No. 2 collected in San Juan province where monoterpenoids and sesquiterpenoids respectively accounted for 50.6% and 35.3% of the essential oil.

In order to establish patterns among the data shown in Table 2, an agglomerative clustering analysis was applied to determine the number of statistically significant chemotypes and to understand biogenetic relationships among the different chemotypes. Both agglomerative hierarchical cluster (AHC) analysis and principal component analysis (PCA) were applied to the oil constituent percentages as variables. The abundance of 15 different chemical compounds: α-pinene (**1**), limonene (**6**), sum of *cis*- and *trans*-sabinene hydrate (**7** and **9**), sum of african-1-ene + african-5-ene + africa-1,5-diene (**14**–**16**), β-caryophyllene (**17**), african-5-en-1-α-ol (**20**), bicyclogermacrene (**21**), lippifoli-1(6)-en-5-one (**27**) (see Table 2) – hereafter referred to as lippifolienone-, *trans*-nerolidol (**28**), *trans*-davanone (**29**), spathulenol (**30**), caryophyllene oxide (**31**), β-davanone-2-ol (**36**), integrifolian-1,5-dione (**37**) and 4,5-seco-africanan-4,5-dione (**38**) for each population were used as input variables in the AHC and PCA analysis to detect correlations among the populations, i.e. predict chemotypes. The sum of the percentages of *cis*-and *trans*-sabinene hydrate (**7** and **9**) was introduced as a single parameter given the obvious structural and biogenetic relationship between them. The same reasoning applied for the africanenes as african-1-ene (**14**), african-5-ene (**15**) and african-1,5-diene (**16**) always co-occur in *L. integrifolia* oils. It should be noted that on low polarity columns the peaks corresponding to **15** and **16** usually appear partially or completely overlapped. The result of the AHC analysis, linkage method: Ward's method, distance measure: Euclidean (Pythagorean) is outlined in Fig. 2.

The AHC analysis showed two completely different clusters having 0% similarity: a first group constituted by samples S9 and S11–S22 characterized by having *trans*-davanone (**29**) as main component, and the other one, exhibiting a more complex and diverse composition constituted by all the remaining samples, i.e. S1–S8, S10 and S23–S31. With 90% similarity, the 31 essential oil samples analyzed can be grouped into five clusters:

Cluster 1: called '*trans*-davanone chemotype', represented by samples S9, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20, S21 and S22, depicted in red in Figs. 1 and 2, which is quite homogeneous with ketone **29** as the dominant component, usually more than 75%.

Cluster 2: 'lippifolienone chemotype', represented by samples S1, S2, S3, S4, S5, S6, S7 and S8, depicted in yellow in Figs. 1 and 2, with ketone **27** as the distinctive constituent accompanied by variable amounts of several africanane derivatives, i.e.: african-5-en-1α-ol (**20**), african-1-ene (**14**), african-5-ene (**15**), african-1,5-diene (**16**) and 4,5-seco-africanan-4,5-dione (**38**), and caryophyllene oxide (**30**).

Cluster 3: 'β -davanone-2-ol chemotype' represented by sample S10, depicted in blue in Figs. 1 and 2, with keto-alcohol **36** as main component accompanied by significant amounts of *trans*-davanone (**29**).

Cluster 4: 'spathulenol/bicyclogermacrene chemotype', represented by samples S23, S24, S25, S26, S27, S28, S29 and S30, depicted in green in Figs. 1 and 2, containing significant amounts of sphatulenol (**30**), bicyclogermacrene (**21**) and β -caryophyllene (**17**).

Cluster 5: '*trans*-nerolidol chemotype', represented by sample S31, depicted in brown in Figs. 1 and 2, with sesquiterpene alcohol **28** as widely majority component.

The essential oils belonging to the lippifolienone (cluster 2) and spathulenol/bicyclogermacrene (cluster 4) chemotypes were very complex frequently displaying more than one hundred components, while the others, i.e., *trans*-davanone (**29**), β-*trans*-davanone-2-ol (**36**) and *trans*-nerolidol (**28**) chemotypes (see below), exhibited relatively simple chromatograms with a single dominant component that usually accounted for more than 75% of the oil. The correlation circle plotting obtained in the PCA was also in agreement with five chemotypes (see Fig. S1).

The stereochemistry and absolute configuration of *trans*-davanone (**29**), the distinctive component of the samples belonging to cluster 1 was established as follows. From three essential oil samples rich in davanone (**29**), *i.e.* S11, S18 and S19, the ketone was isolated by column chromatography on Si gel using hexane–ethyl acetate 97:3 as elution solvent. The isolated ketone showed to be 99.1%, 98.5% and 97.3% pure with specific optical rotation $[\alpha]_D$ (22 °C)+66.3 (c 1.9 in $CHCl_3$), +64.0 (c 0.753 in $CHCl_3$) and +63.1 (c 0.117 in $CHCl_3$) from S11, S18 and S19 respectively. The ^1H and ^{13}C NMR spectroscopic data of the isolated davanone (**29**)

Table 1
Description of the collection sites of *Lippia integrifolia* in northwestern and central Argentina.

Sample	Code	Province[a]/locality	Latitude (S)	Longitude (W)	Altitude (m. a.s.l)	Collection date	Essential oil yield (w/w,%)	Voucher specimen	Germplasm[h]
S1	LI-6	SL, Merlo	32°20′57″	65°02′01″	800	March, 1999	0.92	Dusch-11[c f]	No
S2	LI-7	SJ, Caucete	31°31′38″	68°31′17″	630	April, 1999	–	Dusch-12[c f]	No
S3	P-213	LR, Pampa de la Viuda	29°16′01,1″	67°05′50,1″	1480	April, 2008	0.79	Juárez et al. 543[d]	Yes
S4	P-210	LR, Carrizal	28°50′16,9″	66°39′16,6″	910	April, 2008	1.23	Juárez et al. 542[d]	Yes
S5	LI-1	CA, Las Pirquitas	28°16′27″	65°44′27″	800	April, 2003	1.60	LIL605837[e]	Yes
S6	TSD-DP	CA, Santa Maria[b]	26°42′42″	66°01′52″	1920	March, 2005/2009/2012	1.11/1.19/1.30 respectively	LIL607279[e]	Yes
S7	P-207	CA, Las Pirquitas	28°16′27,0″	65°44′27,0″	805	April, 2008	0.94	Juárez et al. 541[d]	Yes
S8	GEM-7	CA, Las Pirquitas	28°16′25″	65°44′28″	805	February, 2010	1.39	Incayuyo 685[f]	Yes
S9	LI-2	SA, Cachi	24°46′59″	66°12′00″	2500	March, 1997	0.79	jlgm-2[g f]	No
S10	LI-3	SA, Cachi	24°46′57″	66°12′	2500	April, 1998	0.92	jlgm-3[g f]	No
S11	CC-660	SA, Cachi	24°46′	66°12′	2500	March, 2005	0.88	LIL607276[e]	No
S12	LI-4	SA, Las Pailas	25°03′44″	66°13′15″	2690	March, 1999	–	jlgm-4[g f]	No
S13	LI-5	SA, Cachi Adentro	25°05′	66°14′	2600	April, 1999	–	jlgm-5[g f]	No
S14	CC-674	SA, Santa Victoria	22°15′01″	64°58′00″	2370	March, 2006	–	LIL Slanis et al. 311[e]	No
S15	AELI-1	SA, Molinos	25°22′05″	66°16′44″	2380	May, 2011	1.01	Incayuyo 701[f]	No
S16	P-172	SA, Chorrillos	24°47′27,8″	65°43′51,6″	2010	April, 2008	0.98	Juárez et al. 498[d]	Yes
S17	P-197	TU, Amaicha (Salas)	26°37′02,0″	65°50′17,4″	2650	April, 2008	0.51	Juárez et al. 528[d]	Yes
S18	CC-687	CA, Sierras de Quilmes	26°41′	66°05′	2150	February, 2008	0.77	Incayuyo 687[f]	Yes
S19	CC-688	TU, Ampimpa	26°36′11″	65°50′50″	2300	May, 2008	1.11	LIL606871[e]	No
S20	GEM-1	TU, Ampimpa	26°36′11″	65°50′50″	2300	February, 2009	1.40	LIL606871[e]	No
S21	CC-653	CA, Santa Maria	26°42′42″	66°01′52″	2020	February, 2005	0.80	LIL607197[e]	No
S22	GEM-2	CA, Santa Maria	26°42′42″	66°01′52″	2020	February, 2009	0.71	LIL607280[e]	No
S23	AELI-3	CA, Cuesta de la Chilca	27°38′28″	66°10′20″	1480	May, 2011	0.92	LIL32855[e]	No
S24	P-214	LR, Cuesta de Huaco	29°08′21,4″	67°02′18,9″	1305	April, 2008	1.76	Juarez et al. 546[d]	No
S25	P-192	SA, Tres Cruces	25°52′58,3″	65°42′33,3″	1470	April, 2008	0.71	Juárez et al. 526[d]	Yes
S26	P-182	SA, Cabra Corral	25°17′02,5″	65°18′02,3″	955	April, 2008	0.68	Juárez et al. 512[d]	Yes
S27	P-252	LR, Dique de Olta	30°38′25,7″	66°18′03,5″	610	December, 2008	0.68	Molina, 6420[d]	Yes
S28	GEM-4	CA, El Cajón	26°25′59″	66°16′00″	2690	March, 2010	1.28	Incayuyo 689[f]	No
S29	AELI-2	LR, Famatina	28°51′24″	67°41′16″	2400	May, 2011	0.60	Incayuyo 702[f]	No
S30	P-216	LR, Cuesta de Miranda	29°20′46,2″	67°44′02,8″	1690	April, 2008	1.29	Juárez et al. 547[d]	No
S31	P-218	LR, Puerto Alegre	29°26′40,2″	67°57′28,6″	1340	April, 2008	1.20	Molina, 6760[d]	Yes

[a] Provinces of Argentine: CA = Catamarca; LR = La Rioja; SA = Salta; SJ = San Juan; SL = San Luis; TU = Tucumán.
[b] Stem cuttings obtained in 1999 from plants of Dique Las Pirquitas (CA) and Ampimpa (TU) were cultivated and transplanted to Santa María (CA). After six, ten and thirteen years, the essential oil composition of these plants was very similar to that of the original stocks (See Table S2 and Table S3).
[c] Sample provided by Dr. C. Duschaztzky, Universidad Nacional de San Luis.
[d] A voucher specimen is on deposit in the herbarium of Jardín Botánico Arturo E. Ragonese (BAB), province of Buenos Aires, Argentina.
[e] A voucher specimen is on deposit in the herbarium of the Instituto Miguel Lillo (LIL), Tucumán, Argentina.
[f] A voucher specimen has been deposited at Laboratorio de Química Orgánica II, Facultad de Bioquímica Química y Farmacia, Universidad Nacional de Tucumán, Argentina.
[g] Aerial parts provided by Ing. Agr. José Luis Giménez Monge, INTA Cerrillos, SA, Argentina.
[h] Germplasm available at Instituto de Recursos Biológicos, INTA Castelar, Buenos Aires.

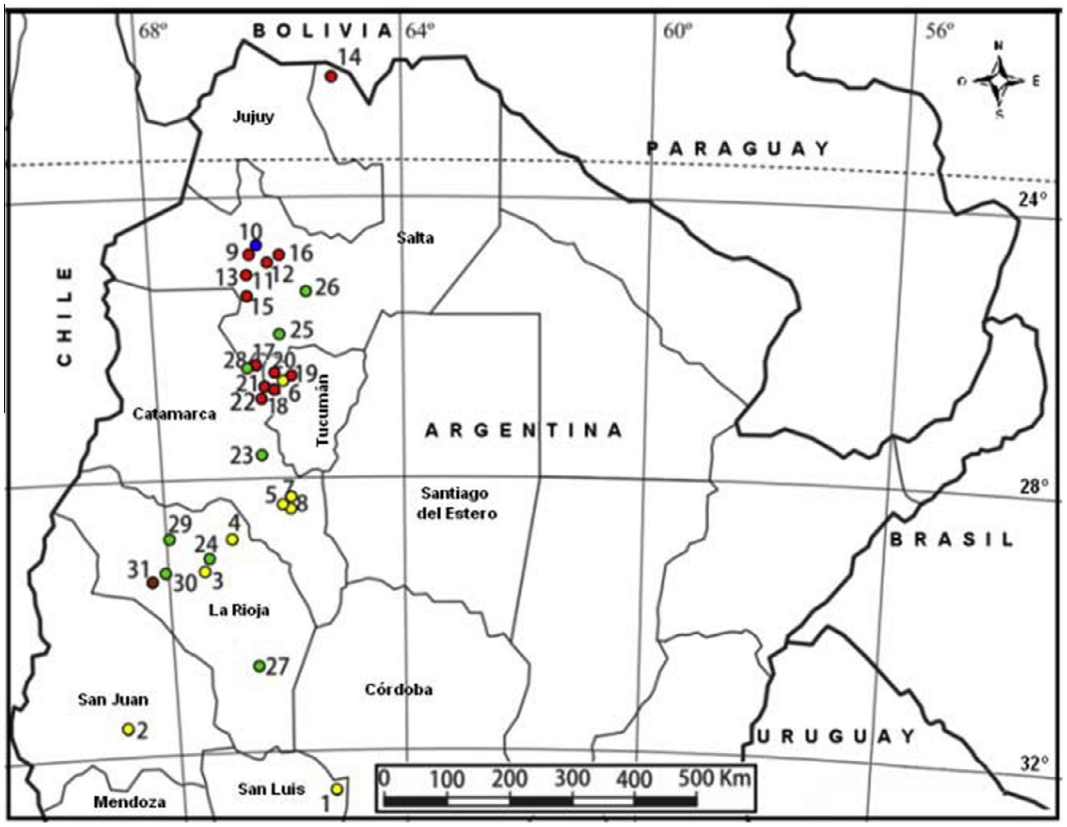


Fig. 1. Geographic location of the sampled populations of *Lippia integrifolia.* Approximate sites of collection are identified on the map as colored dots (red, yellow, blue, green and brown) corresponding to the five chemotype determined in this study.

are summarized in Table 3 where the data reported for (6*S*,7*S*,10*R*)-*cis*- and (6*R*,7*R*,10*R*)-*trans*-davanone (**39** and **29**), both isolated from *Artemisia pallens* (Thomas et al., 1974; Weyerstahl et al., 1997) are included for comparison. As can be seen, the spectroscopic data for the davanone (**29**) from *L. integrifolia* match perfectly with those of the *trans*-stereoisomer (**29**) isolated from davana oil. In addition, in Table 3 reported are the chemical shifts for C-2 (δ 135.1 ppm) and C-5 (δ211.8 ppm) of *trans*-davanone (**29**) that were missed in a previous work (Thomas et al., 1974). Of particular note, *trans*-davanone (**29**) shows a significant downfield shift for C-5 (carbonyl) (δ_C 211.8) in comparison with the *cis*-substituted isomer (δ_C 208.6) and this parameter can be used to rapidly characterize the geometric isomers.

cis-Davanone (**39**) (see Fig. 3) is the main constituent of the essential oil from *Artemisia pallens* (davana oil) and its stereochemistry and absolute configuration was established as (6*S*,7*S*,10*R*) (Fig. 3) by Thomas et al. (1974). Equilibration with potassium *t*-butoxide in *t*-butyl alcohol produced a mixture of the four possible davanones with *cis*-davanone (**39**) (*threo-cis* isomer) and *trans*-davanone (enantiomer form of **29**) (*threo-trans* isomer) as dominant stereoisomers. After purification, *cis*-davanone (**39**) showed an $[\alpha]_D$+81 and *trans*-davanone (the enantiomer of structure **29** shown in Fig. 3), $[\alpha]_D$−66 (Thomas et al., 1974). Since the treatment with base produces epimerization at C-6 and C-7, the absolute configuration of *trans*-davanone from davana oil showing an optical rotation $[\alpha]_D$−66° must be (6*R*,7*R*,10*R*), i.e., the enantiomer form of structure **29** shown in Fig. 3.

trans-Davanone (**29**) from *L. integrifolia* shows exactly the same optical rotation but dextrorotatory, $[\alpha]_D$+66.3° (22 °C) (see Table 3) and consequently it must correspond to the (6*S*,7*S*,10*S*)-stereoisomer as shown in Fig. 3.

Plants belonging to the *trans*-davanone (**29**) and *trans*-β-davanone-2-ol (**36**) chemotypes exhale an exquisite fresh, sweet, floral aroma with great potential to be used in perfume compositions and for flavoring foods and beverages. It is noted that also in the essential oils belonging to cluster 1, *trans*-davanone (**29**) content varies between 59.3% (S22) and 94.8% (S11), these being the highest values reported so far for that ketone in any other essential oil.

trans-Davanone (**29**) and β-davanone-2-ol (**36**) chemotypes are biogenetically closely related. Presumably, β-davanone-2-ol (**36**) is biosynthesized by a process simulated by reaction of **29** with singlet oxygen, a reaction which has in fact been duplicated in the laboratory (Appendino et al., 1984; Thomas and Dubini, 1974). Biogenetic considerations also dictate a *trans*-stereochemistry at the tetrahydrofuran ring of β-davanone-2-ol (**36**) from *L. integrifolia*. Indeed, β-davanone-2-ol (**36**) isolated from *Artemisa lobelii* var. *conescens* exhibits potent antifugal activity (Vajs et al., 2004), comparable to that of the commercial agent bifonazole.

The absolute configuration at C-10 of *trans*-davanone (**29**) from *L. integrifolia* is the same as that of the chiral carbon atom of *trans*-nerolidol (**28**) isolated from the same plant which showed to be the (*S*)-(+)-form (Catalán et al. 1995). This fact and the co-occurrence of both compounds in the essential oil samples S1 and S10 strongly suggest they are biogenetically related. A likely biogenetic pathway for *trans*-davanone (**29**) and *trans*-β-davanone-2-ol (**36**) from *trans*-nerolidol (**28**) is shown in Fig. 4. It is worth noting that the essential oil samples rich in *trans*-davanone (**29**) (cluster 1) decompose rapidly in the presence of air. Thus, samples S9, S11, S12 and S15 kept two months in the refrigerator at 5 °C in either clear or amber borosilicate screw cap vials showed less than a half of the amount of *trans*-davanone (**29**) found in the freshly distilled oil and the emergence of davanone peroxide (Thomas and Dubini, 1974), *trans*-β-davanone-2-ol (**36**) and several degradation

Table 2
Percentage of the main components in the essential oil from 31 different samples of *L. Integrifolia* collected in northwestern and central Argentina.

No.	Sample # Sample code Compound	AI*	S1 LI6 %	S2 LI7 %	S3 P213 %	S4 P210 %	S5 L11 %	S6 TPD-DP %	S7 P207 %	S8 GEM7 %	S9 LI2 %	S10 LI3 %	S11 CC660 %	S12 LI4 %	S13 LI5 %	S14 CC674 %	S15 AELI1 %	S16 P172 %	S17 P197 %	S18 CC687 %	S19 CC688 %
1	α-Pinene	**929**	5.1	4.2	5.4	4.9	0.4	0.1	0.2	0.3	0.7	0.5	0	0.5	0.7	0	tr	tr	0.2	0.1	0.5
2	2(5H)-furanone-5,5-dimethyl	**933**	tr	0	0	0	tr	0	0	0	1.1	5.5	0	0.4	0.2	1.4	tr	0.7	0.4	0	0
3	Sabinene	**966**	0.1	0.4	3.3	2.4	tr	0	0	tr	0.2	0	0	0.2	0.3	0	tr	0	0.2	0.2	1.5
4	α-Phellandrene	**1002**	0.1	tr	0.5	0	tr	0	0	0	0	0	0	0	3.0	0	0	0	0	0	tr
5	*p*-Cymene	**1018**	4.0	3.5	2.3	2.7	0.2	0	0	0.1	0.6	4.5	0	0.2	0.2	0	tr	tr	0.6	0.4	0.8
6	Limonene	**1024**	11.8	4.1	7.5	6.3	1.3	1.4	1.3	0.8	3.9	1.6	0.2	1.9	2.5	0.1	0.1	0.6	0.8	0.6	1.6
7	*cis*-Sabinene hydrate	**1064**	tr	13.2	1.0	0.4	0	0	0	0	tr	0	0	0	0	0	0	0	0.2	0	0.7
8	4-Methyl-4-vinylbutyrolactone	**1090**	0	0	0	0	0	0	0	0	0.4	3.1	0	0	0	0	0	0	0	0	0
9	*trans*-Sabinene hydrate	**1097**	0	3.6	1.2	0.6	0	0	0	0	0	0	0	0	0	0	0	0	0.3	tr	0.9
10	Camphor	**1141**	0.4	0	0	0	tr	0	0	0	1.0	0.9	0	1.0	0.8	0.8	0	1.6	0.3	0.2	0.7
11	Borneol	**1163**	tr	3.8	0.5	0.7	0.2	0.7	1.1	0.1	0.2	0.5	0	0.1	0.2	tr	0	0.8	0	0.1	1.1
12	Terpinen-4-ol	**1174**	1.0	5.9	3.9	2.6	tr	tr	0	0	0.2	0.4	0	0.1	tr	0	0	0	0.8	0.3	1
13	*trans*-Piperitol	**1206**	0	0	tr	tr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	African-1-ene	**1333**	1.0	0.8	0.5	0.9	0.7	0.6	0.7	1.4	0	0	0	0	0	0	0	0	0	0	0
15	African-5-ene	**1345**	4.1	2.0	0.5	3.3	3.9	11.6	7.3	12.5	0	0	0	0	0	0	0	0	0	0	0
16	African-1,5-diene	**1346**			5.9	1.1	3.1				0	0	0	0	0	0	0	0	0	0	0
17	β-Caryophyllene	**1419**	2.1	0.4	4.3	4.0	2.9	4.4	5.9	5.1	0.8	0	1.0	1.4	1.5	0.4	1.3	0.1	0.4	1.1	1.4
18	α-Humulene	**1449**	0.8	0.2	0.6	1.6	1.3	2.3	3.1	2.7	0.3	0	0	0.5	0	0	0.3	0	0	tr	0.6
19	Germacrene-D	**1480**	0	0	1.1	0.9	0	0	0	0	0	0	0	0	0	0	0.2	0	0	0	0
20	African-5-en-1α-ol	**1484**	1.1	6.0	6.8	6.0	10.8	21.0	12.1	9.2	0	0	0	0	0	0	0	0	0	0	0
21	Bicyclogermacrene	**1497**	0	0	1.2	1.0	0	0.9	0.9	0.9	0	tr	0	0	0	0	0.4	0	0	tr	0.2
22	β-Bisabolene	**1504**	0	0	2.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	Davana ether isomer	**1507**	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2	0	0	0.7	0.8	1.7
24	*cis*-Calamenene	**1529**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0.4
25	Davana ether isomer	**1529**	1.2	0	0	0	0	0	0	0	0	0	0	1.0	1.1	0	6.4	0	0	tr.	0.5
26	Elemol	**1550**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	Lippifoli-1(6)-en-5-one	**1551**	23.7	12.1	17.3	26.5	24.1	17.2	29.9	25.7	0	0	0	0	0	0	0	0	0	0	0
28	*trans*-Nerolidol	**1560**	5.2	0	3.5	0	0	0	0	0	0	2.2	0	0	0	0	0	0	0	0	0
29	*trans*-Davanone	**1564**	4.6	tr.	0	0	0	0	0	0	67.3	16.1	94.8	82.8	84.4	80.6	85.8	78.4	82.7	84.0	78.1
30	Spathulenol	**1576**	0	0	2.1	1.6	6.0	0	0	0	0	0	0	0	0	0	0.8	0	0	0	0
31	Caryophyllene oxide	**1580**	4.7	5.3	5.4	7.2	7.1	2.9	7.6	8.0	2.1	0	0	0	0	1.9	0	1.2	2.1	1.3	1.1
32	Fokienol	**1598**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0
33	Guaiol	**1600**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	Lippifoli-1(6)-en-4β-ol-5-one	**1609**	0	0	1.0	0	1.6	2.7	0	0	0	0	0	0	0	0	0	0	0	0	0
35	Bulnesol	**1671**	0	0	0.4	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	β-Davanone-2-ol (trans)	**1693**	0	0	0	0	0	0.1	0.2	0.2	9.5	39.1	0	1.2	0.7	tr.	0.1	5.6	2.3	0	0
37	Integrifolian-1,5-dione	**1732**	1.1	1.4	1.1	1.3	1.7	0.5	1.0	1.2	0	0	0	0	0	0	0	0	0	0	0
38	4,5-Seco-africanan-4,5-dione	**1747**	0.6	1.5	1.1	1.1	10.0	0.5	2.5	2.7	0	0	0	0	0	0	0	0	0	0	0
	Monoterpene hydrocarbons▽		25.5	14.8	23.7	17.8	2.0	1.5	1.7	1.2	6.1	7.0	0.2	3.7	7.8	0.3	0.1	0.6	2.3	2.3	6.8
	Oxygenated monoterpenes▽		2.1	35.8	8.5	6.4	0.3	1.2	1.3	0.2	4.1	4.1	tr	1.7	1.8	3.7	tr	2.7	2.5	1.0	4.9
	Total monoterpenoids %		27.6	50.6	32.2	24.2	2.3	2.7	3.0	1.4	10.2	11.1	0.2	5.4	9.6	4.0	0.1	3.3	4.8	3.3	11.7
	Sesquiterpene hydrocarbons▽		9.3	5.4	19.4	14.2	14.2	23.9	21.1	26.5	1.3	tr	1.0	1.9	1.5	0.7	3.1	0.1	0.4	1.1	2.6
	Oxygenated sesquiterpenes▽		47.9	29.9	41.3	49.9	69.5	56.9	61.3	55.9	78.9	66.7	95.1	85.4	86.4	86.9	94.9	89.1	91.0	88.4	84.1
	Total sesquiterpenoids %		57.2	35.3	60.7	64.1	83.7	80.8	82.4	82.4	80.2	66.7	96.1	87.3	87.9	87.6	98.0	89.2	91.4	89.5	86.7
	Others		–	–	–	–	0.1	–	–	0.2	–	1.5	–	0.4	0.2	–	–	–	0.8	0.6	–
	Total identified %		84.8	85.9	92.9	88.3	86.1	83.5	85.4	84.0	90.4	79.3	96.3	92.7	97.5	91.6	98.1	92.5	96.2	92.8	98.4

No.	S20 GEM1 %	S21 CC653 %	S22 GEM2 %	S23 AELI3 %	S24 P214 %	S25 P192 %	S26 P182 %	S27 P252 %	S28 GEM4 %	S29 AELI2 %	S30 P216 %	S31 P218 %	Identification
1	0.2	0.2	0.2	0.3	2.2	1.7	tr	0.7	1.2	0.4	1.4	1.0	a,c
2	0	1.0	0.1	0	0	0	0	0	0	0	0	0	a,c
3	0.3	0.2	0.3	1.9	4.7	1.2	Tr	1.3	0.3	1.5	6.0	0.5	a,c

(*continued on next page*)

Table 2 (*continued*)

No.	S20 GEM1 %	S21 CC653 %	S22 GEM2 %	S23 AELI3 %	S24 P214 %	S25 P192 %	S26 P182 %	S27 P252 %	S28 GEM4 %	S29 AELI2 %	S30 P216 %	S31 P218 %	Identification
4	0	0	0	tr	0.3	0.6	0.4	0	0.2	0.2	0.1	0.1	a,c
5	0.4	0.6	1.4	0.4	2.1	3.0	0.4	1.4	0.7	0.4	1.5	0.6	a,c
6	1.0	1.1	1.2	5.3	6.4	7.8	0.3	5.6	11.2	2.6	4.1	4.5	a,c
7	0.1	0	0.2	1.7	3.8	2.0	0	0.4	0.2	1.9	4.1	0.6	a,c
8	0	0	0	0	0	0	0	0	0	0	0	0	a,c
9	0.2	0	0.2	2.5	3.8	1.0	0	0.2	0.3	3.2	7.4	0.4	a,c
10	0	0	0	1.2	0.6	2.2	0	0	0	0.4	0	0.9	a,c
11	0.2	0	0.1	2.6	0.3	0.7	1.3	0.8	1.2	0.1	0.3	0	a,c
12	0.3	0.2	0.6	1.1	4.3	6.1	0	4.8	0	0.8	4.0	1.0	a,c
13	0	0	0	0	0	1.3	8.4	0	0	0	0	0	a,c
14	0	0	0	tr	0	0	0	0	0	0	0	0	b,c
15	0	0	0	tr	0	0	0	0	0	0	0	0	b,c
16	0	0	0	1.7	2.0	1.3	0	0	0	0	0	0	b,c
17	1.7	1.0	0.2	12.2	7.0	9.7	26.0	22.9	12.3	14.1	8.1	1.0	a,c
18	0.6	0.2	0	0.8	1.2	5.0	1.4	13.4	2.2	1.7	0.7	0.3	a,c
19	0	0	0	2.8	1.2	0	1.1	0.7	0.6	6.2	2.0	0	a,c
20	0	0	0	3.0	3.6	1.0	0	0	0	0	0.7	0	b,c
21	0	0	0	8.4	4.8	1.5	4.5	3.4	3.5	14.6	8.3	0.1	a,c
22	0	0	0	4.1	5.3	0	0	0	1.4	2.6	2.4	0	a,c
23	4.5	1.7	6.6	0	0.4	0	0	0	0	0	0	0	b,c
24	0	0.7	0	0	1.6	0	0	6.5	7.8	0	0	0.4	a,c
25	1.9	0	3.2	0	1.6	0	0	0	0	0	0	0	b,c
26	0	0	0	tr	1.5	0	1.3	0	0	4.1	0.8	0	a,c
27	0	0	0	3.0	4.7	1.3	0	0	0	0	0.5	0	b,c
28	0	0	0	0	0	0	0	0	10.5	1.4	0	75.0	a,c
29	74.0	78.8	59.3	1.6	8.2	17.8	0	0	0	0	0	0	b,c
30	0	0	0	6.6	4.4	1.6	2.9	5.2	4.8	9.2	9.2	0	a,c
31	2.9	4.0	3.2	6.5	3.8	6.2	4.8	4.9	3.2	1.9	2.9	1.5	a,c
32	0	0	1.8	0	0	1.2	0	0	5.7	0	0	2.6	a,c
33	0	0	0	0	0.6	0	3.0	1.0	0	0	1.9	0	a,c
34	0	0	0	0	0	0	0	0	0	0	0	0	b,c
35	0	0	0	0	2.0	0.7	11.5	1.6	0	0.6	5.8	0.9	a,c
36	0	1.7	0	0	0	0	0	0	0	0	0	0	b,c
37	0	0	0	1.6	1.0	0.5	0	0	0	0	0.4	0	b,c
38	0	0	0	0.5	0.6	0	0	0	0	0	0	0	b,c
	2.8	2.1	3.9	9.0	20.0	18.8	1.4	11.7	14.7	10.3	16.8	7.3	
	1.2	0.2	1.4	9.1	13.3	20.2	15.7	8.8	2.7	6.6	19.1	3.7	
	4.0	2.3	5.3	18.1	33.3	39.0	17.1	20.5	17.4	16.9	35.9	11.0	
	2.5	1.9	0.3	37.2	26.1	20.2	36.9	51.0	42.7	47.7	26.1	1.8	
	89.0	88.4	79.5	30.3	36.1	34.5	29.8	22.0	29.8	28.1	29.4	80.0	
	91.5	90.3	79.8	67.5	62.2	54.7	66.7	73.0	72.5	75.8	55.5	81.8	
	0.3	0.2	1.3	7.3	-	-	0.5	1.3	1.6	0.3	-	-	
	95.8	92.8	86.4	92.9	95.5	93.7	84.3	94.8	91.5	93.0	91.4	92.8	

[*]AI = Arithmetic Index (as defined in Adams (2007)) on a HP-5 column; [v]The total percentage was calculated with Table S1 where all the compounds identified in each sample are listed; 0: not detected; tr = less than 0.1%. Identification: a, b and c, see Section 4.4 GC–MS analysis.

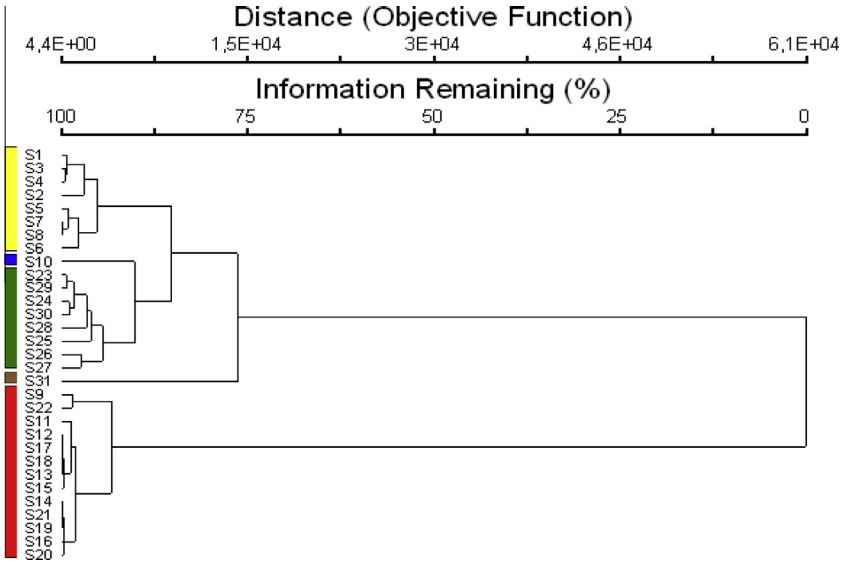


Fig. 2. Agglomerative hierarchical clustering (AHC) based on the composition of the essential oils of 31 samples of *Lippia integrifolia*. The percentages of 15 essential oil components were submitted for analysis. Dendogram obtained by Euclidean Pythagorean distance dissimilarity (Linkage method: Ward's method).

Table 3
^{1}H and ^{13}C NMR spectroscopic data of *cis*- and *trans*-davanone (**39** and **29**) in $CDCl_3$.

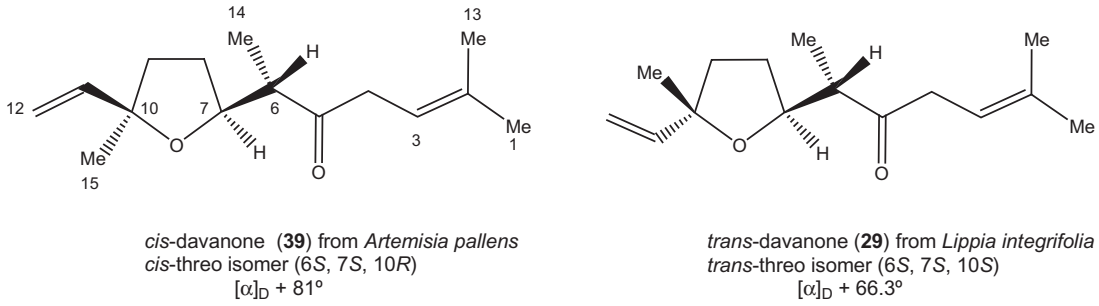
	(6*S*,7*S*,10*S*)-*trans*-davanone		(6*R*,7*R*,10*R*)-*trans*-davanone		(6*S*,7*S*,10*R*)-*cis*-davanone	
	Lippia integrifolia		*Artemisia pallens*		*Artemisia pallens*	
	$[\alpha]_D$+66.3		$[\alpha]_D$−66°		$[\alpha]_D$+81°	
	H[d]	C	H[b]	C[c]	H[b e]	C[c]
1	1.75 d	25.5 q	1.74 s br	25.5 q	1.74 s br	25.6 q
2	–	135.1 s	–	nd	–	134.9 s
3	5.33 m	115.9 d	5.33 m	116.1 d	5.34 m	116.0 d
4a	3.22 dd	42.6 t	3.21 dd br	42.6 t	3.22 dd br	42.4 t
4b	3.28 dd		3.28 dd br		3.31 dd br	
5	–	211.8 s	–	nd	–	208.6 s
6	2.70 dq	51.0 d	2.69 dq	51.0 d	2.70 dq	51.0 d
7	4.05 ddd	80.4 d	4.04 ddd	80.5 d	4.09 ddd	80.8 d
8a	1.6–2.0 m (4H)	29.1 t	1.6–2.0 m	29.7 t	2.00 dddd 1.58 dddd	29.7 t
8b			(4H)			
9a	1.6–2.0 m (4H)	36.6 t	1.6–2.0 m	36.8 t	1.89 ddd	37.3 t
9b			(4H)		1.73 ddd	
10	–	82.8 s	–	82.9 s	–	82.8 s
11	5.81 dd	143.4 d	5.81 dd	143.4 d	5.89 dd	144.0 d
12a	4.95 dd	111.1 t	4.94 dd	111.2 t	4.98 dd	111.1 t
12b	5.13 dd		5.14 dd		5.19 dd	
13[a]	1.63 d	17.9 q	1.61 s br	17.8 q	1.61 s br	17.8 q
14[a]	1.01 d	12.7 q	0.99 d	12.7 q	0.99 d	13.0 q
15[a]	1.29 s	27.0 q	1.28 s	27.1 q	1.25 s	26.4 q

In ppm from TMS as internal reference.
[a] Intensity of three protons; **nd**: not detected.
[b] From Weyerstahl et al., Flavour & Fragrance J. **12**:315–325, 1997.
[c] From Thomas et al., Helv. Chim. Acta **57**:2055–2061, 1974.
[d] Couplings in Hz: 1,3 = 3,13 = 1.4; 3,4a = 3,4b = 7; 4a,4b = 18; 6,7 = 7,8b = 8.5; 6,14 = 7; 7,8a = 6; 11, 12a = 10.5; 11,12b = 17.1; 12a,12b = 1.5.
[e] Couplings in Hz: 1,3 = 3,13 = 1.0; 3,4a = 3,4b = 7; 4a,4b = 18; 6,7 = 7,8b = 8.5; 6,14 = 7; 7,8a = 6; 8a,8b = 12; 8a,9a = 3.5; 8a,9b = 7; 8b,9a = 8; 8b,9b = 10; 9a,9b = 12; 11, 12a = 10.5; 11,12b = 17;12a,12b = 1.5.

products. The freshly distilled oil can be preserved from oxidation by adding 500 ppm of butylated hydroxy toluene (BHT) or butylated hydroxy anisole (BHA), flushing with argon and keeping it at −20 °C in a tightly closed vial. Accordingly, sample S10 containing β-davanone-2-ol (**36**) (39.1%) along with *trans*-davanone (**29**) (16.1%) was considered a true chemotype and not an artifact because davanone peroxide (Thomas and Dubini, 1974) – the primary air-oxidation of davanone (**29**) – was not detected in the oil.

The stereochemistry, absolute configuration and biogenetic pathway for lippifolienone and africanane derivatives belonging to group II chemotype were discussed elsewhere (Catalán et al., 1991, 1992, 1993a,b, 1994, 1995; Cerda-Garcia-Rojas et al., 2005, 2008; Coronel et al., 2006).

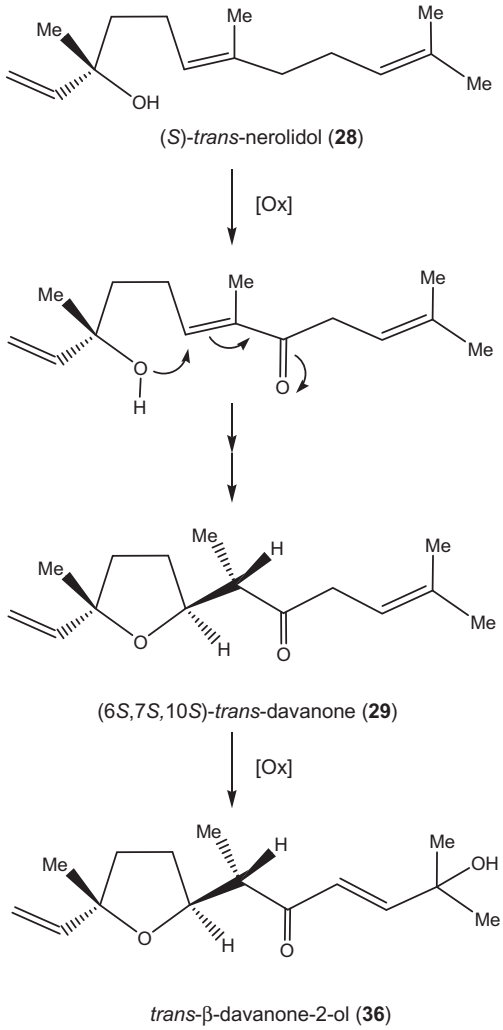
The essential oil of *L. integrifolia* from different provinces of Argentina has been studied by various authors although in several cases the number of identified components is small or account for less than 70% of the total oil. Thus, the essential oil from aerial parts of plants collected in Department of Colón, Córdoba province (Velasco-Negueruela et al., 1993), contained lippifolienone (**27**), camphor (**10**) and an unidentified sesquiterpene MW 220 as main



cis-davanone (**39**) from *Artemisia pallens*
cis-threo isomer (6*S*, 7*S*, 10*R*)
$[\alpha]_D$ + 81°

trans-davanone (**29**) from *Lippia integrifolia*
trans-threo isomer (6*S*, 7*S*, 10*S*)
$[\alpha]_D$ + 66.3°

Fig. 3. Structure and absolute configuration of *cis*-davanone (**39**) from davana oil and *trans* davanone (**29**) from *Lippia integrifolia*.



(6*S*,7*S*,10*S*)-*trans*-davanone (**29**)

trans-β-davanone-2-ol (**36**)

Fig. 4. Proposed biogenetic pathway for (6*S*,7*S*,10*S*)-*trans*-davanone (**29**) and *trans*-β-davanone-2-ol (**36**) from (S)-*trans*-nerolidol (**28**).

constituents while the flower oil from plants collected at 'La Calera', also in Colón Department, exhibited a similar composition with lippifolienone (**27**), camphor (**10**) and limonene (**6**) as major components (Zygadlo et al., 1995). It is worth to note here that the mass spectrum and retention index of the unidentified sesquiterpene with a MW 220 reported by Velasco-Negueruela et al. (1993) are in agreement with those reported for african-5-en-1α-ol (**20**) by Coronel et al. (2006). The essential oil analyzed by Fricke et al. (1999), also from the province of Córdoba, contained lippifolienone (**27**), african-5-en-1α-ol (**20**) and africanenes (**14**–**16**) but percentages were not given. Also, aerial parts of *L. integrifolia* bought at a local market in the city of Tucuman (De Lampasona et al., 1999) and wild plants collected in the provinces of San Juan (Lima et al., 2011) and La Rioja (Juliani et al., 2004) produced, in all three cases, oils with lippifolienone (**27**) as the dominant component. In the latter case, lippifolienone (**27**) (22.4%) was accompanied by similar amounts of 4,5-*seco*-africanan-4,5-dione (**38**) (23.4%) and spathulenol (**30**) (17.0%). All the collections described above along with the population from Dique Las Pirquitas, province of Catamarca (Coronel et al., 2006), clearly belong to cluster 2 (lippifolienone chemotype) and the collection sites informed fit the regional distribution of the chemotypes shown in Fig.1 (yellow dots).

On the other hand, the oil from plants gathered at Lujan, province of San Luis (Duschatzky et al., 1998), contained β-caryophyllene (**17**), α-humulene (**18**) and limonene (**6**) as major constituents. Plants collected in Valle Antinaco-Los Colorados, province of La Rioja, also yielded an oil containing β-caryophyllene (**17**) as the dominant component accompanied by significant amounts of spathulenol (**30**), bicyclogermacrene (**21**), caryophyllene oxide (**31**) and terpinen-4-ol (**12**) (Barbieri et al., 2015). Although, in these last two papers, less than 70% of the essential oil was identified, the reported composition suggests that they could be related to the spathulenol/bicyclogermacrene chemotype (cluster 4) and their collection sites also fit with the regional distribution of this chemotype (Fig. 1, green dots). More recently, a collection from an undisclosed location of the Córdoba province produced an essential oil with camphor (**10**), methylheptenone and limonene (**6**) as the dominant components (Gleiser et al., 2011) and thus seems to be a new chemotype different to those described in this work.

As can be seen in Fig. 1, the *trans*-davanone (**29**) chemotype (red dots) is concentrated in the northern part of the natural range of *L. integrifolia*, *i.e.*, from the north of the province of Catamarca until the south of Bolivia. The closely related β-davanone-2-ol (**36**) chemotype (blue dots) is also found in this region. The remaining chemotypes (yellow, green and brown dots) grow in the southern part of the natural range, with some overlapping of the *trans*-davanone (**29**) and lippifolienone (**27**) chemotypes in the center. In all cases, the reported collection areas fit well with the regional distribution of the chemotypes shown in Fig. 1. Thus, excepting the work of Gleiser et al. (2011) that describes what seems to be a new chemotype different to those described here, all the collections from the provinces of Cordoba, La Rioja, San Juan and San Luis, which are located in the southern part of the distribution range, belong to the lippifolienone (**27**), *trans*-nerolidol (**28**) or spathulenol (**30**)/bicyclogermacrene (**21**) chemotypes as discussed above. In general, the essential oils from these last chemotypes show a composition much more complex and variable than the oils belonging to the *trans*-davanone chemotype.

In 1999 stocks from wild plants belonging to '*trans*-davanone (**29**) chemotype' and 'lippifolienone (**27**) chemotype' were propa-

gated by stem cutting and cultivated in the same garden in the city of Santa Maria city, province of Catamarca. After six, ten and thirteen years, the essential oil composition of the cultivated plants were essentially identical to the wild stocks as shown in Tables S2 and S3. The stabil**ity** of the chemical profile of plants cultivated at the same place during several years strongly supports that our collections represent true chemotypes and that the putative intraspecific variations are inherited (Medina-Holguín et al., 2007).

The plant to plant variation was investigated in a wild population near Dam "Las Pirquitas", province of Catamarca (lippifolienone chemotype). In April 2012, five healthy plants were selected randomly and their essential oils analyzed by GC–MS. The results are summarized in Table S4. As can be seen, all the plants showed identical qualitative composition with lippifoli-1(6)-en-5-one (**27**) and african-5-en-1α-ol (**20**) as the two main components. The variations in the amounts of the main components among plants I, III, IV and V showed to be relatively small while the plant II exhibited some significant differences, namely, a substantial increase in the content of limonene (**6**), β-caryophyllene (**17**) and spathulenol (**30**) and an important decrease in the amounts of african-5-en-1α-ol (**20**) and lippifoli-1(6)-en-5-one (**27**) that still remained dominant in the oil.

3. Conclusions

The results presented here indicate that *L. integrifolia* possesses a rich genetic diversity. The AHC and PCA analysis showed the presence of two completely different clusters with 0% similarity: one group constituted by the '*trans*-davanone (**29**) chemotype' is characterized by having that ketone as main component (usually above 80%); and the other, comprising the four remaining chemotypes which generally display very complex oils with more than one hundred compounds. The '*trans*-davanone (**29**)', 'lippifolienone (**27**)' and 'spathulenol (**30**)/bicyclogermacrene (**21**)' chemotypes are widely distributed in the wild whilst the '*trans*-nerolidol (**28**)' and 'β-davanone-2-ol (**36**)' chemotypes are rarely found. Besides, the absolute configuration of *trans*-davanone (**29**) from *L. integrifolia* is the enantiomeric form of the same compound isolated from davana oil. It is demonstrated also that the chemical composition of the oils obtained from the same plant stock remain essentially constant after several years indicating that the chemical profile is largely under genetic control, a trait that seems to be characteristic of the genus *Lippia* (Souto-Bachiller et al., 1996).

In view of the lack of quality markers in the Argentine Food Code and the remarkable chemical diversity described here, there is an urgent need to investigate the properties and pharmacological activities of the different chemotypes as well as to promote actions for protection of the genetic diversity of 'incayuyo' in northwestern and central Argentina

4. Experimental

4.1. Plant material

Aerial parts from thirty one wild populations of *L. integrifolia* were collected in the months of February, March or April, except sample No. 27 (Table 1) which was collected in December. Plants were collected in six provinces from central and north-western Argentina at altitudes between 610 and 2690 m above sea level covering most of the distribution area of this species (Zuluaga and Morrone, 1999) (Fig. 1). Data for each sample (collection site, collection date and essential oil yield) are summarized in Table 1. All materials were authenticated by one of the authors (A.S.) or Dr. Ana María Molina (INTA Castelar, Buenos Aires province, Argentina)

4.2. Essential oil extraction

To obtain the essential oil, some 20 healthy individuals from each population were harvested conservatively (*ca.* 10% of the aerial part from each plant). Leaves and inflorescences from each sample were submitted to hydrodistillation in a Clevenger apparatus for 4 h. The yield (w/w) of the obtained essential oils ranged from 0.51% to 1.76%, based on dry wt. (Table 1). The essential oils were stored at −18 °C until their analysis by gas chromatography–mass spectrometry (GC–MS). All the oil samples were analyzed in no longer than 40 days after its obtaining.

4.3. Chemical analysis

The analysis of the essential oil samples was carried out using a Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector (FID) and a capillary HP-5 column (30 m × 0.32 mm; 0.25 μm film thickness); injector and detector temperature were maintained at 250 °C and 270 °C respectively. Injection size: 0.5 μL of a 10% solution of the oil in CH_2Cl_2, split mode, He was used as carrier gas at a flow rate of 1 mL min^{-1}. The oven was programmed as follows: 60 °C for 5 min, rose at 0.8 °C/min to 100 °C, rose at 0.4 °C min^{-1} to 140 °C, rose at 10 °C min^{-1} to 275 °C and then kept for 5 min. This program with three temperature ramps gave the best resolution for the very complex sesquiterpene region of the essential oils belonging to lippifolienone chemotype. For arithmetic index (AI) measurements, the oven temperature program suggested by Adams (2007) (60–246 °C at 3 °C min^{-1}) was used. Percentages reported in Table 2 were obtained from electronic integration measurements using FID.

4.4. GC–MS analysis

Mass spectra were recorded on a 5973 Hewlett Packard selective mass detector coupled to a Hewlett Packard 6890 GC using HP-5MS (5% phenylmethylsiloxane) capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness). The injector, GC–MS interphase, ion source and selective mass detector temperatures were maintained at 250 °C, 275 °C, 280 °C and 150 °C, respectively; ionization energy, 70 eV; injection size: 0.1 μL (10% solution in ethyl acetate) (split mode). He was used as carrier gas at a flow rate of 1.0 mL min^{-1}. The oven was programmed as follows: 50 °C for 10 min, rose at 1.0 °C min^{-1} to 100 °C, rose at 0.5 °C min^{-1} to 150 °C, rose at 10 °C min^{-1} to 270 °C and then kept for 5 min.

Identification: The components percentage was taken from capillary GC traces with FID using an integrator HP 3395 without FID response factor correction. The identification of the individual components was based on (a) computer matching with commercial mass spectra libraries (NBS75K, NIST 98, WILEY275) and published data (Adams, 2007); (b) comparison with spectra available in our files and authentic compounds isolated in previous works (Catalán et al., 1991; 1992; 1993a,b; 1994; 1995; Coronel et al., 2006); (c) comparison of their GC AI on a HP-5 column.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2015.11.004.

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectrometry, fourth ed. Allured Publishing Corporation, Carol Stream, Illinois, USA.

Alonso, J., Desmarchelier, C., 2006. Plantas Medicinales Autóctonas de la Argentina. Ediciones Fitociencia, Buenos Aires, Argentina, pp. 267–270.

Appendino, G., Garibold, P., Nano, G.M., Tetenyi, P., 1984. Tetrahydrofuran-type terpenoids from Tanacetum vulgare. Phytochemistry 23, 2545–2551.

Barbieri, N., Costamagna, M., Gilabert, M., Perotti, M., Schuff, C., Isla, M., Benavente, A., 2015. Antioxidant activity and chemical composition of essential oils of three aromatic plants from La Rioja province. Pharm. Biol.. http://dx.doi.org/10.3109/13880209.2015.1028077.

Bassols, G.B., Gurni, A.A., 1996. Especies del género *Lippia* utilizadas en medicina popular latinoamericana. Dominguezia 13, 7–25.

Catalán, C.A.N., de Lampasona, M.E.P., 2002. The chemistry of the genus Lippia (Verbenaceae). In: Kintzios, S.E. (Ed.), Oregano. The genera Origanum and Lippia. Taylor & Francis Inc., New York, pp. 127–149.

Catalán, C.A.N., de Fenik, I.J.S., Dartayet, G.H., Gros, E.G., 1991. Integrifolian-1,5-dione and a revised structure for 'africanone', biogenetically related sesquiterpene ketones from *Lippia integrifolia*. Phytochemistry 30, 1323–1326.

Catalán, C.A.N., de Fenik, I.J.S., Arriazu, P.J., Kokke, W.C.M.C., 1992. 4,5-Seco-africandione from *Lippia integrifolia*. Phytochemistry 31, 4025–4026.

Catalán, C.A.N., de Lampasona, M.E.P., de Fenik, I.J.S., Cerda-García-Rojas, C.M., Joseph-Nathan, P., 1993a. Structure and conformation of a humulendione from *Lippia integrifolia*. J. Nat. Prod. 56, 381–385.

Catalán, C.A.N., de Fenik, I.J.S., Cerda-García-Rojas, C.M., Mora-Pérez, Y., Joseph-Nathan, P., 1993b. Total assignment of the 13C NMR spectra of integrifolian-1,5-dione and derivatives by 2D spectroscopy. Spectroscopy 11, 1–8.

Catalán, C.A.N., de Lampasona, M.E.P., de Fenik, I.J.S., Cerda-García-Rojas, C.M., Mora-Pérez, Y., Joseph-Nathan, P., 1994. Minor constituents of *Lippia integrifolia*. J. Nat. Prod. 57, 206–210.

Catalán, C.A.N., de Lampasona, M.E.P., Cerda-García-Rojas, C.M., Joseph-Nathan, P., 1995. Trace constituents of *Lippia integrifolia*. J. Nat. Prod. 58, 1713–1717.

Cerda-Garcia-Rojas, C.M., Coronel, A.C., de Lampasona, M.E., Catalán, C.A.N., Joseph-Nathan, P., 2005. Absolute configuration of Lippifoliane and africanane derivatives. J. Nat. Prod. 68, 659–665.

Cerda-García-Rojas, C., Catalán, C.A.N., Muro, C., Joseph-Nathan, P., 2008. Vibrational circular dichroism of africanane and lippiafoliane sesquiterpenes from *Lippia integrifolia*. J. Nat. Prod. 71, 967–971.

Coronel, A.C., de Lampasona, M.E., Catalán, C.A.N., 2005. Variability of essential oils of *Lippia fissycalix* and *Lippia turbinata* from Argentina. Rev. Latinoamer. Quim. 33, 40–48.

Coronel, A.C., Cerda-García-Rojas, C.M., Joseph-Nathan, P., Catalán, C.A.N., 2006. Chemical composition, seasonal variation and a new sesquiterpene alcohol from the essential oil of *Lippia integrifolia*. Flavour Frag. J. 21, 839–847.

De Lampasona, M.E.P., de Fenik, I.S., Catalán, C.A.N., Dartayet, G.H., Gros, E.G., Cerda-García-Rojas, C.M., Mora-Pérez, Y., Joseph-Nathan, P., 1999. Constituents of the essential oil of *Lippia integrifolia* (Griseb.) Hieron. (Verbenaceae). Acta Hort. 500, 81–88.

Denham, S., Múlgura de Romero, M.E., Slanis, A., Bulacio, E., 2006. *Lippia integrifolia* versus *L. boliviana* (Verbenaceae). Darwiniana 44, 363–364.

Duschatzky, C., Bailac, P., Firpo, N., Ponzi, M., 1998. Composición de los Aceites Esenciales de *Lippia juneilliana, L. integrifolia* y *L. turbinata* de la provincia de San Luis (Argentina). Rev. Colomb. Quím. 27, 9–16.

Fricke, C., Hardt, I.H., König, W., Joulian, D., Zygadlo, J.A., Guzmán, C.A., 1999. Sesquiterpenes from *Lippia integrifolia* essential oil. J. Nat. Prod. 62, 694–696.

Gleiser, R.M., Bonino, M.A., Zygadlo, J.A., 2011. Repellence of essential oils of aromatic plants growing in Argentina against *Aedes aegypti* (Diptera: Culicidae). Parasitol. Res. 108, 69–78.

Gorzalczany, S., Sülsen, V., Redko, F., Vescina, C., Muschietti, L., Martino, V., Acevedo, C., 2008. Choleretic and antispasmodic effects of *Lippia integrifolia* aqueous extract. Braz. J. Pharmacog. 18, 16–20.

Jansen-Jacobs, M.J., 1988. Verbenaceae. In: Rijn, A.R.A. (Ed.), Flora of the Guianas. Series A: Phanerogams, Fascicle 4, vol. 148. Görts-Van Hoetz Scientific Books, Koenigstein, p. 116.

Juliani, H.R., Koroch, A., Simon, J.E., Biurrum, F.N., Castellano, V., Zygadlo, J.A., 2004. Essential oils from Argentinean aromatic plants. Acta Hort. 629, 491–498.

Laribi, B., Kouki, K., Bettaieb, T., Mougou, A., Marzouk, B., 2013. Essential oils and fatty acids composition of Tunisian, German and Egyptian caraway (*Carum carvi* L.) seed ecotypes: a comparative study. Ind. Crop Prod. 41, 312–318.

Lima, B., López, S., Luna, L., Agüero, M.B., Aragon, L., Tapia, A., Zacchino, S., Lopez, M. L., Zygadlo, J., Feresin, G.E., 2011. The essential oils of medicinal plants of the Central Andes of Argentina; chemical composition, antifungal, antibacterial and insect repellent activities. Chem. Biodivers. 8, 924–936.

Marcial, G., Sendker, J., Brandt, S., de Lampasona, M.P., Catalán, C.A.N., de Valdez, G. F., Hensel, A., 2014. Gastroprotection as an example: antiadhesion against *Helicobacter pylori*, anti-inflammatory and antioxidant activities of aqueous extracts from the aerial parts of *Lippia integrifolia* Hieron. J. Ethnopharmacol. 155, 1125–1133.

Medina-Holguín, A.L., Micheletto, S., Holguín, F.O., Rodriguez, J., O'Conell, M.A., Martin, C., 2007. Environmental influences on essential oils in roots of *Anemopsis californica*. HortScience 42, 1578–1583.

Nurzyńska-Wierdak, R., Bogucka-Kocka, A., Sowa, I., Szymczak, G., 2012. The composition of essential oil from three ecotypes of *Origanum vulgare* L. ssp. *vulgare* cultivated in Poland. Farmacia 60, 571-57.

Pascual, M.E., Slowing, K., Carretero, E., Sánchez Mata, D., Villar, A., 2001. *Lippia*: traditional uses, chemistry and pharmacology: a review. J. Ethnopharmacol. 76, 201–214.

Sadeghi, H., Robati, Z., Saharkhiz, M.J., 2015. Variability in *Zataria multiflora* Bioss. essential oil of twelve populations from Fars province. Iran. Ind. Crop Prod. 67, 221–226.

Souto-Bachiller, F.A., de Jesus-Echevarria, M., Cárdenas-Gonzalez, O., 1996. Hernandulcin is the major constituent of *Lippia dulcis* Trev. (Verbenaceae). Nat. Prod. Lett. 8, 151–158.

Terblanché, F.C., Kornelius, G., 1996. Essential oil constituents of the genus *Lippia* (Verbenaceae) – a literature review. J. Essent. Oil Res. 8, 471–485.

Thomas, A.F., Dubini, R., 1974. Terpenoids derived from linalyl oxide, Part 4. The oxidation of davanone. Isolation and synthesis of the davana ethers, sesquiterpenes of *Artemisia pallens*. Helv. Chim. Acta 57, 2076–2081.

Thomas, A.F., Thommen, W., Willhalm, B., Hagaman, E.W., Wenkert, E., 1974. Terpenoids derived from linalyl oxide, part 1. The stereochemistry of the davanones. Helv. Chim. Acta 57, 2055–2061.

Vajs, V., Trifunović, S., Janaćković, P., Soković, M., Milosavljević, S., Tešević, V., 2004. Antifungal activity of davanone-type sesquiterpenes from *Artemisia lobelii* var. *conescens*. J. Serb. Chem. Soc. 69, 969–972.

Velasco-Negueruela, J.A., Perez-Alonso, M.J., Guzmán, C.A., Zygadlo, J.A., Ariza Espinar, L., Sanz, J., García Vallejo, M.C., 1993. Volatile constituents of four *Lippia* species from Córdoba. Argentina. J. Essent. Oil Res. 5, 513–524.

Weyerstahl, P., Marschall, H., Schröder, M., Wahlburg, H.-C., Vkaul, V.K., 1997. The sesquiterpene fraction of the essential oil of *Artemisia laciniata* Willd. Flavour Fragrance J. 12, 315–325.

Zuluaga, F.O., Morrone, O. (Eds), 1999. Catálogo de las Plantas Vasculares de la República Argentina II. Fabaceae-Zygophyllaceae (Dicotyledoneae). Monographs in Systematic Botany from the Missouri Botanical Garden, vol. 74, St. Louis, Missouri, pp. 1153–1158.

Zygadlo, J.A., Lamarque, A.L., Guzmán, C.A., Grosso, N.R., 1995. Composition of the flowers oils of some *Lippia* and *Aloysia* species from Argentina. J. Essent. Oil Res. 7, 593–595.