Biochemical Systematics and Ecology 74 (2017) 19–29

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Essential oil chemotypes of *Aloysia citrodora* (Verbenaceae) in Northwestern Argentina

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ARTICLE INFO

Article history:
Received 25 January 2017
Received in revised form
23 June 2017
Accepted 31 July 2017

Keywords:
Aloysia citrodora
Chemotypes
Essential oil
Biodiversity

ABSTRACT

Chemical biodiversity of essential oils of natural populations of *Aloysia citrodora* Palau ("lemon verbena", "cedrón") in Northwestern Argentina was assessed by collecting in the same sites through different years. A total of 36 samples were collected in the Provinces of Salta (El Maray, La Paya, El Sunchal, El Alisal, Chorrillos), Jujuy (Chilcayo, San Roque), Catamarca (Mutquin, Colana) and Tucumán (Amaicha del Valle) in Argentina.

Essential oils were obtained by hydrodistillation (Clevenger) of naturally air-dried plant material. Yields ranged from 0.16% to 1.93% (v/w), being the highest those of the collections of Mutquin. More than 65 compounds were identified by CG-FID-MS. Only 19 of these constituents, accounting from 77.3 to 98.9% of the total oil, present in more than 4.0% in at least one sample, were considered as variables for statistical analysis. Agglomerative Hierarchical Cluster analysis was conducted, showing at 65% of similarity, five groups. This grouping was in direct accordance to the biosynthetic pathways of main compounds (chemotypes).

In the two sites of Jujuy, 21 collections evidenced four different chemotypes, named after the dominant component as follows: thujones, citronellal, carvone, and citral (neral + geranial). In the populations of Salta and Catamarca, linalool appeared as a new different chemotype.

Though cedron is considered as a citral-bearing plant, curiously, in the 36 samples collected in the NW of Argentina, only two samples contained citral as main constituent. On the other hand, a dominance of citronellal and thujones compositions were found in the bulk of the samples collected, while others had very high content of linalool or carvone and its derivatives. Northwestern Argentina has repeatedly been mentioned as the center of biodiversity of this species.

The new evidences found on the chemical biodiversity of essential oils of *Aloysia citrodora* in natural populations in this region, reinforce firmly this idea.

1. Introduction

Aloysia citrodora Palau (Verbenaceae), ("cedrón", "lemon verbena") is an aromatic species native to South America that is widely used for medicinal and aromatic purposes. It is a perennial and deciduous shrub which grows to a height of one to 3 m. It flowers in summer, exuding a powerful lemony scent on the hillsides of the Andean Mountains extending from Venezuela to Argentina, including Colombia, Peru, Bolivia and Chile, but it also grows in Brazil, Paraguay, Uruguay and Argentina.

First reports of the use of this species date from the 17th century,

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http://dx.doi.org/10.1016/j.bse.2017.07.003

showing its ethnopharmacological importance as it was widely used as medicinal by the Inca culture (Castroviejo and Coello, 1996; Siedo, 2006). The "kalawayas", medical experts of that culture, identified it with the quechua name "wari pankara" and was used as digestive, antispasmodic, against bronchitis and heart problems (Girault, 1987). It is perhaps this background that the Spanish explorers considered it as a promising species, among others, to be taken to Europe. It was recently in the 18th century that "cedron" or "lemon verbena" was introduced in Europe by the botanists Ch. Dombey and P. Commerson and since then it has been grown in Central and Eastern European countries, in Africa (Dellacassa and Bandoni, 2003) and in temperate countries such as Vietnam and Morocco.

Traditional medicine still attributes to cedron antispasmodic, diuretic, antipyretic, sedative, carminative, digestive, expectorant, against headache, for heart diseases, antihistaminic and emmenagogue properties. Some of these uses were evaluated in recent years, demonstrating various pharmacological activities (Pascual et al., 2001; Santos Gomes et al., 2005). Verbascoside is considered as the main non-volatile active compound (Nakamura et al., 1997), although many other flavonoids and phenolic derivatives have been isolated from these fractions (Valentão et al., 1999; Bilia et al., 2008). However, the most common diffusion is as an aromatic plant for the preparation of infusions, as well as for the bioactivities that were latterly confirmed in the volatile fraction. Lemon verbena is worldwide considered as a citral (neral + geranial) bearing plant species for its known main volatile component and hence is cultivated in several countries (Siedo, 2006; Gil et al., 2007).

Though qualitative and quantitative variations in the composition of the essential oils of *A. citrodora* growing in other countries was reported in the last fifteen years (Santos Gomes et al., 2005; Gomes et al., 2006; Argyropoulou et al., 2007; Escobar et al., 2010; Rojas et al., 2010), citral constituted the major component.

In Argentina, "cedron" grows in all the Central and North regions of the country, but particularly the natural populations in the NW region are characterized by a diversity of aromas at every collection site (Elechosa, 2009; López and Payo, 2010), evidencing different volatile compositions. This feature prompts us to work in collaboration with several multidisciplinary groups on this species, detecting a remarkable chemical complexity. The analyzed plant materials and reported data of our studies correspond to: A) crop samples, field trials and samples provided by local brokers from several sites of Argentina and neighboring countries; being these species mainly of the citral type (Gil et al., 2007; Di Leo Lira et al., 2008), and B) those corresponding to wild or cultivated plants collected at different locations of the NW region (Molina et al., 2003; Viturro et al., 2004; Heit et al., 2010; Juárez et al., 2012; Di Leo Lira et al., 2013).

Results obtained in our previous studies determined that the diversification of the composition of the essential oils had a genetic basis (Gil et al., 2007). This hypothesis was later reasserted by new studies indicating the existence of a great phytochemical diversity of crops at an experimental field located in Salta Province (Estación Experimental Agropecuaria Salta - INTA), using genetic materials from de same region (accessions). The chemical composition of the essential oils of the accessions remained constant for more than seven years (Di Leo Lira et al., 2013), thus indicating different chemotypes.

Northwestern Argentina has repeatedly been mentioned as the center of biodiversity of this species (Siedo, 2006); and even Parodi, in the year 1934, suggested the hypothesis that the NW region of Argentina might constitute a genetic diversification center of aromatic species.

The aim of this work was to evaluate if there were significant qualitative differences between the essential oils of *A. citrodora* collected exclusively from natural populations of the NW region, regarding the different scents detected *in situ*. New collection sites were included expanding the collection area previously considered in our preceding reports. This work is a part of a long-term project of germplasm conservation and selection of genetic materials for domestication (project INTA PNHFA064641). Such characterization is based on the yields and chemical composition of their essential oils.

2. Materials and methods

2.1. Reference compounds

The following reference compounds used for GC-FID-MS were either obtained or purchased from the following sources; namely, α-pinene, limonene, 1,8-cineole, linalool, citronellal, citronellol, carvone, citral, geranyl acetate, and β-caryophyllene (VASANA S.A.C.A.I.F. y M.; Villa Martelli, Argentina), sabinene (Dr. Ingrid Loayza, Universidad Mayor de San Simón; Cochabamba, Bolivia), 6-methyl-5-hepten-2-one (Aromática S.A.; Buenos Aires, Argentina), *cis* and *trans* thujone (Sigma-Aldrich de Argentina; Buenos Aires, Argentina), *p*-cymene (Soaljo S.A., Buenos Aires, Argentina), piperitone (R. C. Treatt & Co.; Suffolk, UK), and caryophyllene oxide (Extrasynthese; Genay, France).

2.2. Plant material

Ten wild populations of *Aloysia citrodora* located in NW Argentina in the Provinces of Salta (El Maray, La Paya, El Sunchal, El Alisal and Chorrillos), Jujuy (Chilcayo and San Roque), Catamarca (Mutquin and Colana) and Tucumán (Amaicha del Valle), were sampled for this study during November (beginning of bloom) and April (end of bloom). The upper halves of the blooming branches of each plant were collected separately because of the great diversity of scents found *in situ*. These scents were characterized as "sage", "spearmint", "citronella", "citric" and "boldo". To confirm previous results, the sampling was repeated during three years in Jujuy, at the same sites (Fig. 1).

Geographical coordinates were recorded for each population using a *Garmin Etrex Legend HCX* global positioning system (GPS). Vouchers were taken from every site and stored in the Herbarium of "Instituto de Recursos Biológicos INTA, Hurlingham, Provincia de Buenos Aires, Argentina (BAB)". Specimens were validated by one of the authors (Ana María Molina) (Fig. 2).

2.3. Essential oils isolation

Essential oils were obtained by hydrodistillation of naturally air-dried materials (*ca*. 12% moisture final content) during 3 h in a *Clevenger*-type trap (IRAM 18729, 1996). The resulting oil samples were dried over anhydrous sodium sulfate and stored at 2 °C prior to analysis. All the oil samples were analyzed within the following 40 days after the obtaining. Yields were expressed as v/w percentage of dry material.

2.4. Essential oil analysis

The essential oils analysis was performed in two chromatographic systems:

a) The samples 4 to 17, 19 and 27 to 36: were analyzed using a GC-FID-MS system Perkin Elmer Clarus 500 equipped with an

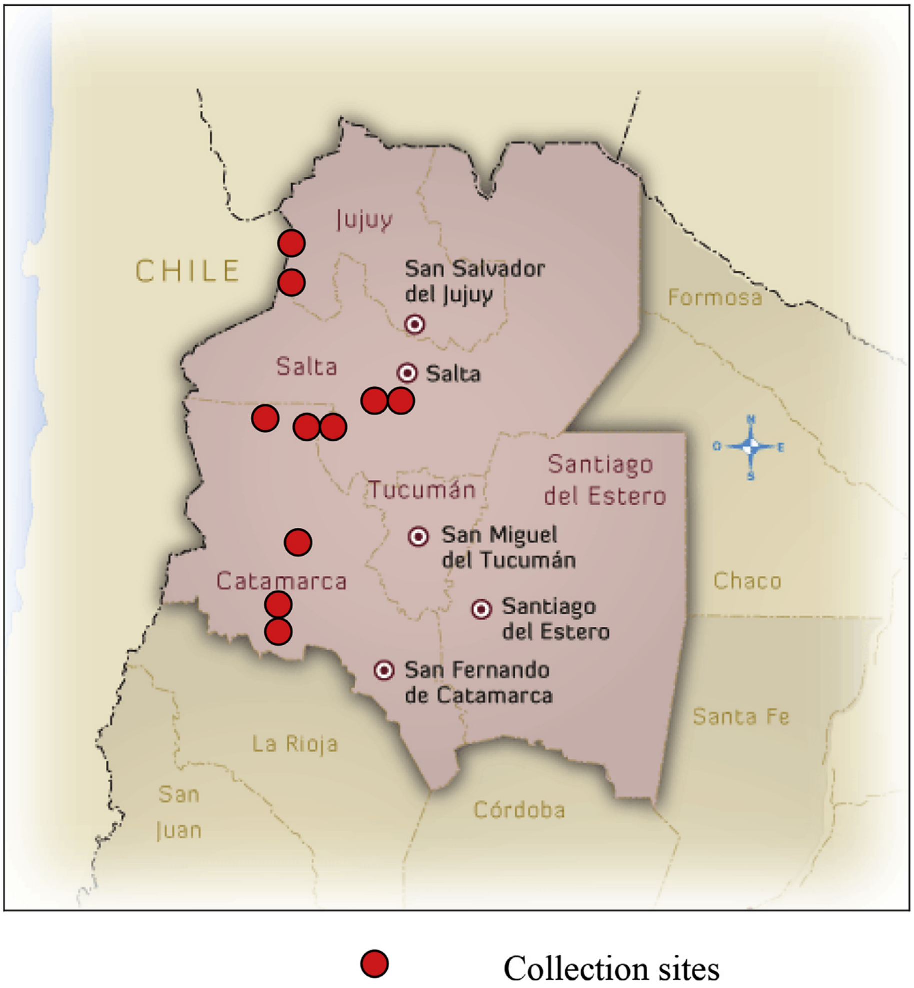


Fig. 1. Map of the Northwestern region in Argentina and collection sites.

autosampler injector (split ratio: 1:100) connected by a flow splitter to two capillary columns (*J&W*): a) polyethylene glycol MW *ca.* 20,000 and b) 5% phenyl-95% methyl silicone, both 60 m × 0.25 mm with 0.25 μm film thickness. The polar column was connected to a FID, while the non-polar column was connected to a FID and a quadrupolar mass detector (70 eV) by a vent system (*MSVent*™). Helium was used as mobile phase at a constant flow of 1.87 ml/min. The temperature was programmed using the following gradient: 90 °C-225 °C at 3°C/min, and then isothermal for 15 min. The injector and both FIDs were set at 255 °C and 275 °C, respectively. The injection volume was 0.2 μL of a 10% solution of the oil in ethanol. The temperature of the transference line and the ion source were 180 °C and 150 °C, respectively; the range of masses was 40–300 Da at 10 scan/sec.

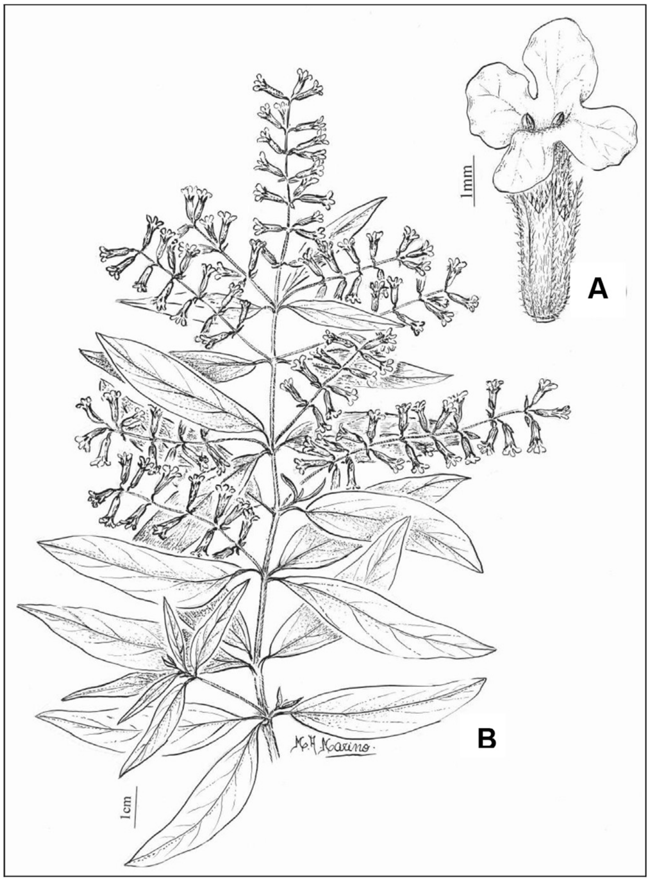
b) The samples 1 to 3, 18 and 20 to 26: were analyzed using a GC-FID *Konik KNK 3000G* equipped with a split injector was used. Column: HP-5 fused silica capillary column (30 m × 0.25 mm with 0.25 μm of film thickness). Hydrogen was used at as carrier gas at a flow rate of 1.2 ml/min. The oven temperature program was: 60 °C (5 min), 60 °C-230 °C (6 °C/min). FID temperature: 300 °C; injector: 250 °C. Meanwhile, the GC-MS analysis was carried out in an *Agilent* GC 6890/5972. Column: HP-5 MS (30 m × 0.25 mm; film thickness 0.25 μm) and a Helium carrier gas flow of 0.7 ml/min. The oven temperature program was the same as above; the temperature of the transfer line (300 °C) and the injector (250 °C) were kept constant during analyses. The range of masses was 40–300 Da at 10 scan/sec.

2.5. Compounds identification

The identification of compounds was performed by comparison of the linear retention indexes (LRI) (relative to C_8-C_{24} *n*-alkanes) obtained in each column, with those of reference compounds or chemically known essential oils. Additionally, each mass spectra obtained was compared with those from literature libraries (Adams, 2007; WILEY/NIST, 2008) and from an own laboratory-developed mass spectra library built up with standards or components of known oils. The mass spectra of the main compounds identified are shown in Supplementary Fig. S1. Sabinone was previously isolated for the first time from a natural source and identified by spectroscopic means (Di Leo Lira et al., 2013). The relative percentage composition was obtained using the single area percentage method without considering corrections for response factors. The lowest response obtained from each column for each component was considered. Two samples (3 and 19) were analyzed using both chromatographic systems described exhibiting comparable qualitative and quantitative profiles.

2.6. Statistical analysis

The Agglomerative Hierarchical Cluster analysis (AHC) (InfoStat, 2011) was conducted using only the 19 main components



A- flowering branch, B- flower

Fig. 2. Illustration of *Aloysia citrodora* Palau.

(contemplating the sums of α- + β-thujone, *cis* + *trans*-dihydrocarvone and neral + geranial), and considering as main compounds only those wich quantities were higher than 4.0% in at least one sample, choosing the Euclidean Pithagorean Coefficient of distance with the Ward's method of linkage which allowed the generation of the dendogram.

3. Results and discussion

The site references with the GPS coordinates, altitude, season, collector number and essential oil yields of thirty-six samples of *A. citrodora* collected from ten wild populations in four provinces of NW Argentina were determined. The highest yields were obtained in Mutquin (1.93%) and San Roque (1.43%) (Table 1). Though the variation in essential oil content was very high (from 0.16% to 1.93%), with most of the yields between 0.2 and 0.4%, this variability is one more hint that reflects the strong biodiversity existing in this region. Furthermore, the essential oil yield is a parameter to be considered when selecting the genetic materials useful for their multiplication.

In the collection site, the plant materials were initially selected considering the odor of each individual within a same population. In this way, it was possible to detect different aromatic profiles growing together, which reinforces the condition of different chemotypes, as each individual spreads in a same place and under the same conditions (absence of edaphoclimatic variations). For this reason, in many cases a single specimen was analyzed, while in other cases, when several plants of a population with the same odor were detected, they were grouped in a single batch. So, only a minimal sample was taken in each collection site, to avoid threatening the sustainability of the biodiversity of this species in the region. This constitutes the principle of sustainability that we just intend to preserve and highlight. For this reason, the analysis of each collection was not replicated. Nevertheless, in all cases a minimum of 100 g and a maximum of 250 g of dried material were distilled.

For the comparison of the volatile profile of the collections, only 19 components, being present in more than 4% in at least one of the samples were considered, accounting for 77.3%–98.9% of the total composition. In this way, the variations contemplated were only those that had a significant value difference. Statistical analysis of these data showed significant differences in the essential oil profiles of the samples obtained by GC-FID-MS analysis.

Fig. 3 shows the five different GC chromatographic profiles found. Table 2 shows the chemical composition of the 21 samples collected in Jujuy. In these samples, eight principal compounds were determined: β-thujone (37.9–86.5%), carvone (48.2–70.9%), *trans*-sabinol (40.3–56.7%), citronellal (33.6–54.4%), neral (19.9%), geranial (34.4%), *trans*-carveol (30.2–50.9%) and limonene (25.2–37.5%). The citral type appeared only in one collection obtained in San Roque (Tucumán). In the samples collected in Chilcayo over three years, the main components were: *trans*-sabinol (the samples 1, 8 and 14), citronellal (the samples 2, 7 and 11) and β-thujone (the samples 3, 4, 10 and 13).

Table 3 shows the results of the chemical composition of the essential oils from collections obtained in the three remaining provinces. The Salta populations displayed the same volatile profiles as those found in Jujuy (β-thujone, carvone, citronellal, limonene, sabinene), but two additional main compounds were found: linalool (79.5%) and *cis* + *trans*-dihydrocarvone (53.2%). In the samples collected in Catamarca, α-thujone (86.7%) was found, and linalool (78.2–85.1%), thus confirming the linalool chemotype. Only in a single population sampled in Tucumán, the typical profile with citral (51.0%) as main compound was found.

The mass spectra of the compounds shown in Tables 2 and 3 were included as Supplementary Fig. S1. Other compounds present in low quantities in several samples are shown in Tables 2 and 3

The AHC analysis showed at 64% similarity, a dendrogram (Fig. 4) that grouped the samples into five chemotypes. This grouping can also be explained by the biosynthetic pathways of the principal constituents of each chemotype (Fig. 5).

CHEMOTYPE 1 - named “thujones”, whose characteristic compounds belong to the biosynthetic pathway of the thujones and is represented by the collections 1, 3, 4, 6, 8, 10, 13, 14, 15, 19, 22, 24 and 32. The collections 1, 3, 6, 10, 13, 15, 19, 22, 24 and 32 were characterized by the presence of α + β thujone (31.4%–90.4%). The collections 1, 8 and 14 were characterized additionally by the high content of *trans*-sabinol (40.3%–56.7%), belonging to the same biosynthetic route.

CHEMOTYPE 2 - named “linalool” (78.2%–85.1%) is represented by the collections 29, 33, 34 and 35.

CHEMOTYPE 3 - named “citronellal” (39.0%–66.7%) is represented by the collections 2, 7, 11, 18, 20, 21, 23, 26, 28, 30 and 31.

CHEMOTYPE 4 - named “carvone”, is represented by the collections 5, 9, 12, 17, 25 and 27. The collections 9 and 27 were characterized by carvone as the principal compound (70.9% and 48.2% respectively), meanwhile the collections 5 and 12 were characterized by *trans*-carveol as the main compound (50.9%–30.2% respectively) and the collection 25 with a high content of dihydrocarvones (53.2%). All those main constituents belong to the same biosynthetic pathway. The collections 5, 12, 28 and 27

Table 1
Site references, altitude, GPS coordinates, season, voucher specimen and essential oil yield of 36 samples of *Aloysia citrodora*.

Sample N°	Province	Site/[a,b]	Month-project year of collection	Essential Oil Yields (%v/w)	Voucher specimen
1	Jujuy	Chilcayo/2290, S 23°55′16″ W 65°27′45″	Nov-I	0.48	Juárez 294 (BAB)
2	“”	“”	“”	0.34	Juárez 295 (BAB)
3	“”	“”	“”	0.69	Juárez 296 (BAB)
4	“”	“”	April-II	0.37	Juárez 524 (BAB)
5	“”	“”	“”	0.45	Juárez 521 (BAB)
6	“”	“”	“”	0.30	Juárez 523 (BAB)
7	“”	“”	“”	0.23	Juárez 522 (BAB)
8	“”	“”	“”	0.53	Juárez 520 (BAB)
9	“”	“”	“”	0.21	Juárez 519 (BAB)
10	“”	“”	“”	0.36	Juárez 518 (BAB)
11	“”	“”	April-III	0.41	Molina 6690 (BAB)
12	“”	“”	“”	0.41	Molina 6692 (BAB)
13	“”	“”	“”	0.26	Molina 6691 (BAB)
14	“”	“”	“”	0.37	Molina 6689 (BAB)
15	“”	San Roque/2916, S 23°16′08″ W 65°22′29″	April-I	0.78	Juárez 517 (BAB)
16	“”	“”	“”	0.16	Juárez 515 (BAB)
17	“”	“”	“”	0.21	Juárez 516 (BAB)
18	“”	“”	“”	0.40	Juárez 514 (BAB)
19	“”	“”	April-II	1.43	Molina 6683 (BAB)
20	“”	“”	“”	0.91	Molina 6682 (BAB)
21	“”	“”	“”	0.27	Molina 6681 (BAB)
22	Salta	El Maray/2373, S 25°11′52″ W 65°46′40″	Nov-I	0.68	Juárez 301 (BAB)
23	“”	La Paya/2428, S 25°10′45″ W 66°12′31″	Nov-I	0.76	Juárez 306 (BAB)
24	“”	El Sunchal/1922, S 25°07′44″ W 65°43′06″	Nov-I	0.50	Juárez 299 (BAB)
25	“”	“”	“”	0.65	Juárez 302 (BAB)
26	“”	“”	“”	0.36	Juárez 303 (BAB)
27	“”	El Alisal/1730, S 24°54′17″ W 65°42′44″	April-I	0.33	Juárez 495 (BAB)
28	“”	“”	“”	0.34	Juárez 494 (BAB)
29	“”	Chorrillos/2013, S 24°46′28″ W 65°42′31″	April-I	0.54	Juárez 501 (BAB)
30	“”	“”	“”	0.57	Juárez 500 (BAB)
31	“”	“”	“”	0.57	Juárez 499 (BAB)
32	Catamarca	Mutquin/1489, S 28°17′22″ W 66°08′14″	May-I	1.64	Molina 6721 (BAB)
33	“”	“”	“”	1.93	Molina 6720 (BAB)
34	“”	Colana/1694, S 28°20′31″ W 66°07′15″	May-I	0.54	Molina 6724 (BAB)
35	“”	“”	“”	0.74	Molina 6725 (BAB)
36	Tucumán	Amaicha del Valle/2650, S 26°36′03″ W 65°49′17″	April-I	0.43	Juárez 529 (BAB)

[a] Altitude (m.a.s.l.).
[b] GPS coordinates.

presented high content of limonene (18.9%–57.2%) which was not considered because this compound is known as a common early intermediate in almost all the biosynthetic pathways involved.

CHEMOTYPE 5 - named “citral” (51.0–54.3%), represented by the collections 16 and 36.

Linalool was assessed as a new chemotype which was not previously reported (Molina et al., 2003; Viturro et al., 2004; Heit et al., 2010; Juárez et al., 2012; Di Leo Lira et al., 2013) meanwhile citronellal and citral chemotypes were previously informed as a unique chemotype (Di Leo Lira et al., 2013).

4. Conclusions

The essential oil yields ranged from 0.16% to 1.93%, being the collections obtained in Mutquin, Catamarca, the ones with the highest content of essential oil. More than 65 compounds were identified by CG-FID-MS. Only 19 of these constituents, accounting from 77.3 to 98.9% of the total oil, present in more than 4.0% in at least one sample, were considered as variables for statistical analysis.

At 64% of similarity, the collections were grouped into five

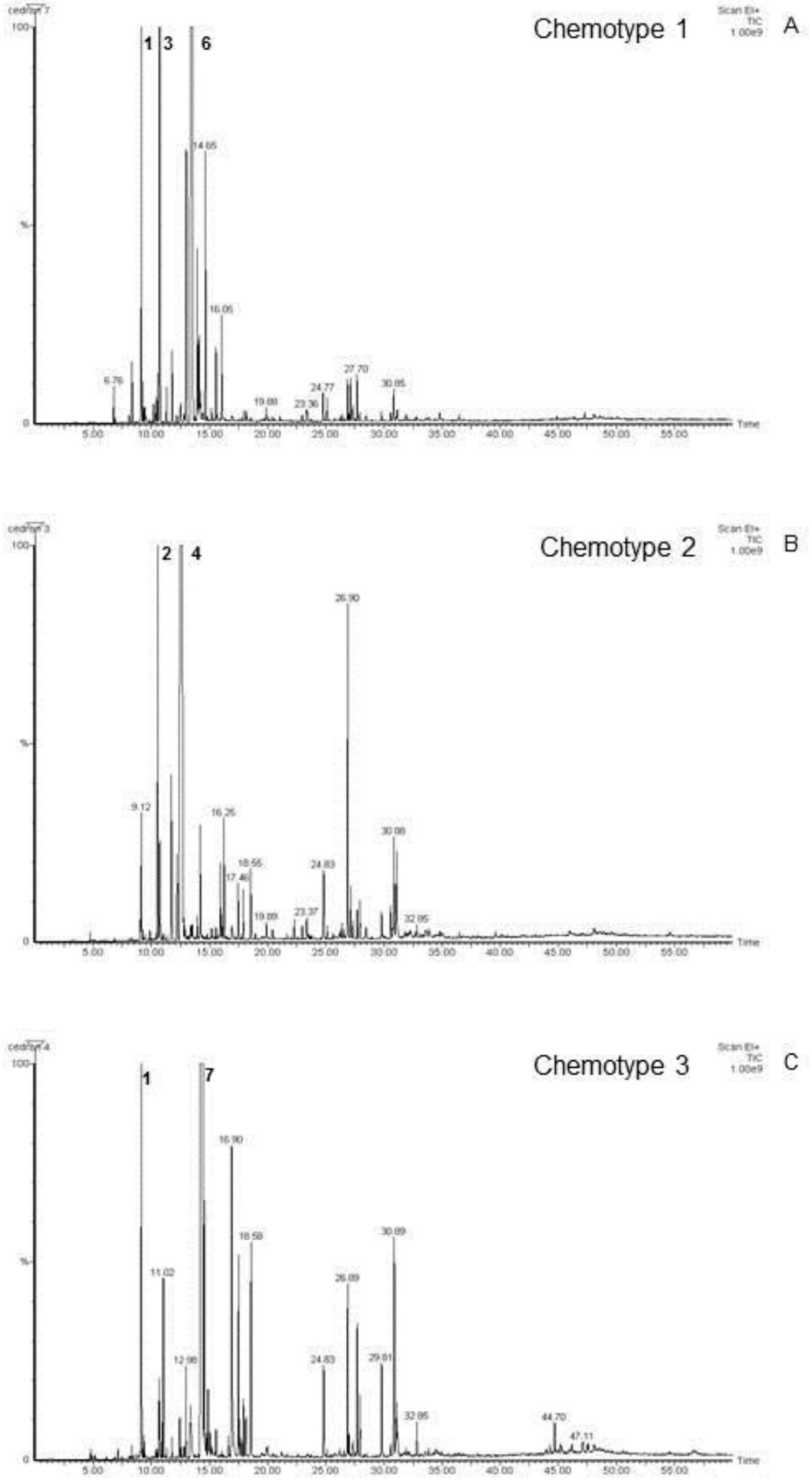


Fig. 3. Representative GC profiles of the chemotypes characterized.
A: chemotype thujones, B: chemotype linalool, C: chemotype citronellal, D: chemotype carvone, E: chemotype citral.
1.- sabinene, 2.-limonene, 3.- 1,8 cineole, 4.- linalool, 5.- *cis*-thujone, 6.- *trans*-thujone, 7.- citronellal, 8.- carvone, 9.- neral, 10.- geranial.

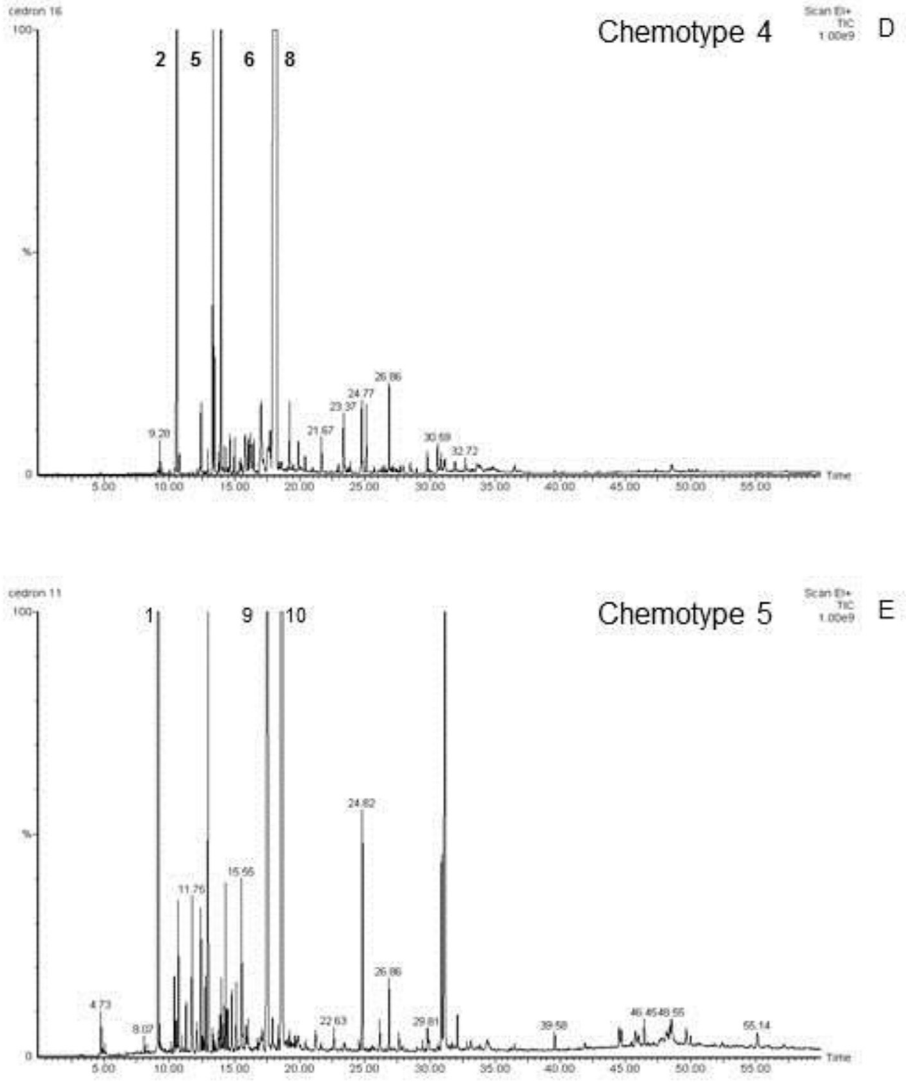


Fig. 3. (*continued*).

chemotypes, taking into account the different biosynthetic routes involved. The citronellal group was the largest one, which included 11 collections from the provinces of Jujuy and Salta.

The selection of chemotypes for industrial uses includes the sampling throughout the distribution area in different seasons, to cover all the phenological stages. In addition, the separation of those materials with different aromas *in situ* is an invaluable strategy for the conservation of the biodiversity in regions, as the herein studied, where the occurrence of very different chemotypes growing in the same poblation is found. Such samples are then subjected to standard methods of analysis. This procedure is the most important tool to discourage unsustainable practices of unrestricted wild material collection and therefore contribute to resource conservation (Elechosa, 2009).

A high degree of biodiversity was found for *A. citrodora* both within and among natural populations of this species and the frequency of the citral chemotype, which is the most cultivated and widely used, was found to be very low for only two collections out of the 36 collections analyzed belonged to this chemotype. Other types, which are very interesting for the aromatic products industry, were more frequent, such as citronellal, linalool and carvone. These results prompt researchers for further domestication work of such plant species.

The biosynthetic origin of each compound accounts for the close relationship existing among the principal markers of the different chemotypes (Fig. 5).

The results obtained herein have allowed discriminating new chemotypes within those previously proposed.

Furthermore, this study indicates that wild plants growing in Jujuy, Salta, Catamarca, Tucumán, which are chemically different from those commonly cultivated, should be in its primary gene pool.

A singular phytochemical variability in three aromatic species (*Minthostachys mollis*, *Tagetes minuta* and *Aloysia citrodora*) has been assessed previously in the NW Argentina (Gil et al., 2000, 2007; van Baren et al., 2014). Our arguments deal with the fact that the remarkable ecological variability that characterizes this region of Argentina influences the genome of the species that grow within this area generating the presence of chemotypes/genotypes. The fact that five chemotypes of *A. citrodora* were found to coexist in a reduced and uniform habitat, like the ravines of Chilcayo, and that their chemical composition remained the same for three

Table 2

Relative percentage of the main constituents of the essential oils from collections obtained in the province of Jujuy.

Compound	LRI[a]	LRI[b]	Content (%)																				
			Chilcayo														San Roque						
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Sabinene	985	1138	1.4	1.3	1.1	5.4	0.1	1.9	4.2	3.3	–	1.1	23.5	0.3	0.8	1.7	3.5	–	–	18.0	5.0	0.4	1.0
Limonene	1024	1221	t	t	2.2	0.3	25.2	t	0.2	0.3	11.5	t	1.9	37.5	0.1	4.4	0.2	3.1	11.0	–	–	7.0	..
1,8-Cineole	1026	1234	8.7	3.0	8.6	4.9	0.8	–	3.0	6.6	–	–	6.5	0.8	0.1	5.6	0.1	0.5	0.8	22.8	0.2	..	13.1
Linalool	1090	1549	0.7	–	t	0.2	0.5	t	0.5	0.4	0.3	0.2	0.4	0.2	0.5	0.2	0.1	0.5	0.5	–	0.2	–	–
α-Thujone	1100	1446	1.6	2.0	3.2	3.1	t	2.1	0.2	1.3	t	2.5	t	0.6	3.2	0.1	4.1	–	0.2	1.5	3.8	–	–
β-Thujone	1110	1464	12.1	t	65.7	75.4	t	62.6	2.1	37.9	2.5	86.2	1.9	t	83.0	1.0	86.3	1.9	4.5	–	86.5	2.3	1.7
Citronellal	1145	1491	–	54.4	–	t	–	–	47.2	–	t	–	37.3	0.3	–	–	0.0	0.7	7.0	33.6	–	39.0	41.2
trans-Sabinol	1146	1727	50.1	–	0.9	0.4	2.5	25.0	–	40.3	–	0.8	–	1.2	4.0	56.7	0.7	t	–	–	0.3	0.1	t
Sabinone	1148	1581	t	–	0.1	2.0	–	2.0	–	1.5	t	3.2	–	0.1	1.5	0.4	0.7	0.2	–	–	0.8	t	–
cis-Dihydrocarvone	1214	1623	t	0.8	0.5	t	0.1	–	–	–	0.4	–	0.1	–	–	–	–	–	0.4	–	t	–	0.1
trans-Dihydrocarvone	1226	1642	t	1.0	0.4	–	0.1	t	–	0.1	0.2	0.1	–	0.1	0.1	–	0.1	–	0.3	–	–	–	–
Citronellol	1227	1770	t	2.9	0.1	t	–	–	4.5	–	–	–	1.8	–	0.1	–	–	t	t	5.6	0.1	5.6	3.8
trans-Carveol	1227	1838	0.1	–	–	t	50.9	0.1	–	–	0.5	0.1	–	30.2	–	0.6	–	t	1.6	–	–	–	t
Neral	1232	1692	t	1.4	0.1	t	–	–	1.0	0.1	–	–	2.8	–	–	–	t	19.9	2.3	0.3	t	0.1	t
Carvone	1240	1745	0.1	–	5.0	t	0.4	–	–	t	70.9	0.1	–	12.2	0.1	11.4	t	1.4	48.2	–	–	0.1	–
Geranial	1261	1740	t	1.9	–	0.1	–	t	1.6	–	–	–	3.9	–	–	–	0.1	34.4	2.0	0.6	0.1	–	1.4
Geranyl acetate	1370	1760	0.1	–	0.1	t	–	–	0.6	–	t	–	0.2	–	–	–	t	4.7	–	–	0.1	–	–
β-Caryophyllene	1417	1614	0.6	–	–	0.2	0.5	–	0.5	0.4	–	0.5	–	–	0.2	0.2	t	–	t	0.8	t	–	–
ar-Curcumene	1472	1783	0.8	2.2	2.2	0.3	1.1	0.2	1.2	0.5	0.6	0.2	1.4	0.8	0.5	0.6	0.2	2.0	0.7	0.4	0.1	3.1	1.8
Germacrene D	1482	1721	0.4	–	0.4	0.3	1.2	0.6	0.3	0.4	–	0.5	0.2	0.5	0.2	0.3	t	–	–	–	–	–	–
Bicyclogermacrene	1512	1742	–	0.3	0.1	0.4	1.5	0.8	–	0.5	t	0.3	0.2	0.5	–	0.1	t	–	–	–	–	–	–
Spathulenol	1599	2125	–	2.2	0.2	0.2	0.6	0.3	1.8	0.1	–	0.4	0.8	0.9	0.4	0.4	0.2	5.0	1.3	1.1	0.3	3.6	2.4
TOTAL			98.3	91.9	98.9	97.1	92.4	96.1	77.3	98.3	96.4	98.2	91.3	94.1	96.4	91.1	98.6	83.6	89.2	96.2	98.9	93.0	95.8

Compounds are listed in order of their elution from a DB5 column.

t: trace, less than 0.05%.

Other minor components: α-pinene, myrcene, γ-terpinene, thujanol, camphor, *cis*-β-ocimene, isopulegol, terpinen-4-ol, α-terpineol, pulegone, carvotanacetone, thymol, carvacrol, piperitenone, α-turmerol, α-cedrene, caryophyllene oxide, Γ-cadinol.

[a] Linear retention index on DB5 column, experimentally determined using homologous series of C8-C24 alkanes.

[b] Linear retention index on DB-WAX column, experimentally determined using homologous series of C8-C24 alkanes.

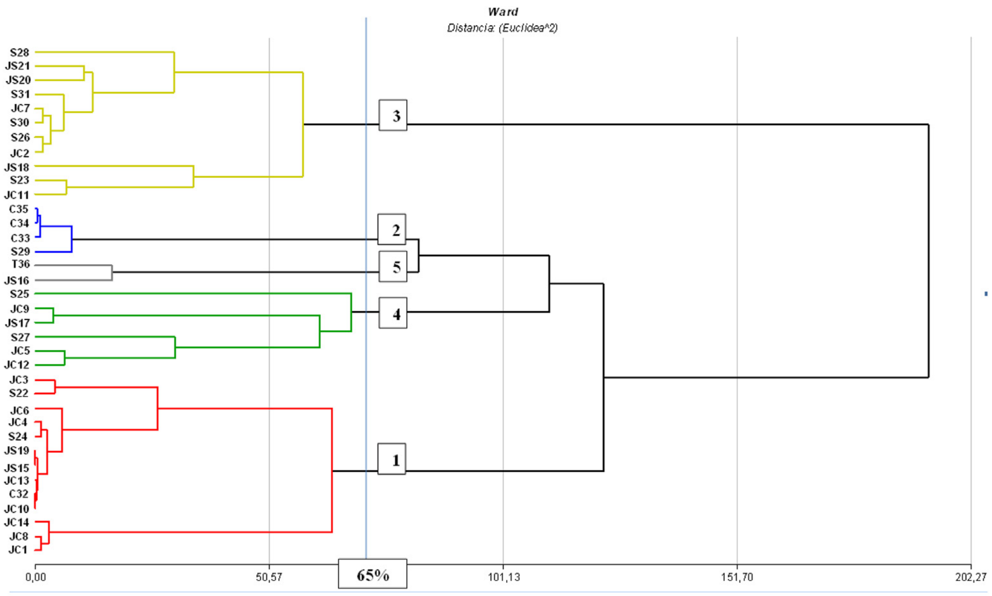
Table 3
Relative percentages of the main constituents of the essential oils from collections obtained in the provinces of Salta, Catamarca and Tucumán.

Compound	LRI[a]	LRI[b]	Content (%)														
			Salta										Catamarca				Tucumán
			22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Sabinene	985	1138	1.7	30.5	4.7	–	6.6	0.2	t	–	6.3	9.4	1.8	0.3	0.8	1.2	16.0
Limonene	1024	1221	9.0	t	t	18.9	–	57.2	18.5	5.3	0.3	–	–	4.0	1.6	3.9	0.1
1,8-Cineole	1026	1234	13.3	2.7	1.4	–	2.1	0.3	–	0.4	–	–	0.1	0.3	0.1	0.4	0.5
Linalool	1090	1549	0.2	0.4	0.2	–	0.5	0.5	0.3	79.5	0.3	0.4	–	82.3	85.1	78.2	0.7
α-Thujone	1100	1446	29.5	1.7	2.5	0.6	11.1	t	–	–	0.7	0.2	86.7	–	0.5	0.3	3.8
β-Thujone	1110	1464	1.9	–	76.8	2.6	6.7	1.2	0.3	t	0.2	1.0	1.4	t	0.1	0.1	0.2
Citronellal	1145	1491	t	35.4	–	–	41.2	0.3	46.6	0.6	66.7	48.5	–	t	t	t	0.2
trans-Sabinol	1146	1727	–	–	0.5	–	0.2	–	0.1	–	–	0.1	–	0.1	–	0.1	t
Sabinone	1148	1581	t	–	–	–	–	–	–	–	–	–	4.0	–	–	–	–
cis-Dihydrocarvone	1214	1623	1.4	1.6	–	26.1	–	6.2	t	0.1	t	–	–	–	0.1	t	0.1
trans-Dihydrocarvone	1226	1642	0.5	–	0.1	27.1	–	0.6	–	0.1	–	–	t	0.1	–		–
Citronellol	1227	1770	–	–	0.1	–	2.0	–	3.2	–	3.8	1.2	–	–	–	t	0.1
trans-Carveol	1227	1838	–	–	–	0.8	–	0.8	–	0.2	–	–	t	t	0.1	t	t
Neral	1232	1692	t	–	–	–	–	–	0.7	0.4	1.7	4.7	–	0.1	–	2.3	21.0
Carvone	1240	1745	25.0	–	0.1	1.9	–	12.8	0.4	0.4	–	–	0.1	–	–	–	–
Geranial	1261	1740	t	0.4	–	–	0.7	t	1.0	0.5	2.3	6.1	–	0.1	–	2.9	30.0
Geranyl acetate	1370	1760	0.1	t	0.1	–	0.1	t	t	0.1	0.1	0.4	–	0.1	–	–	0.1
β-Caryophyllene	1417	1614	0.2	0.1	–	0.3	–	0.5	2.7	0.4	0.8	0.6	2.0	1.9	0.9	1.2	1.6
ar-Curcumene	1472	1783	2.1	3.8	1.4	1.8	2.0	1.8	6.4	2.8	1.5	1.9	–	–	0.2	–	0.5
Germacrene D	1482	1721	0.4	0.5	0.6	0.6	0.6	0.1	0.5	0.4	–	–	0.2	0.3	–	–	0.2
Bicyclogermacrene	1512	1742	–	–	1.6	0.4	1.7	0.4	0.1	0.3	1.3	0.2	–	0.3	0.5	0.3	–
Spathulenol	1599	2125	0.2	0.2	0.1	0.7	2.3	0.5	0.4	0.8	2.2	0.9	0.3	1.2	1.9	2.3	1.5
TOTAL			98.9	98.8	98.6	97.6	96.0	93.9	88.9	95.2	92.0	84.4	97.7	93.6	93.8	95.7	83.6

Compounds are listed in order of their elution from a DB5 column.
t: trace, less than 0.05%.
Other minor components: α-pinene, myrcene, γ-terpinene, thujanol, camphor, *cis*-β-ocimene, isopulegol, terpinen-4-ol, α-terpineol, pulegone, carvotanacetone, thymol, carvacrol, piperitenone, α-turmerol, α-cedrene, caryophyllene oxide, Γ-cadinol.

[a] Linear retention index on DB5 column, experimentally determined using homologous series of C8-C24 alkanes.

[b] Linear retention index on DB-WAX column, experimentally determined using homologous series of C8-C24 alkanes.



S: Salta; JS: Jujuy-San Roque; JC: Jujuy-Chilcayo; T: Tucumán; C: Catamarca

Fig. 4. Dendrogram showing the populations clusters.

successive years, has revealed that the differences found have a genetic basis.

Therefore, our findings confirm once again the hypotheses found in the literature about the NW area of Argentina being a center of phytochemical diversity of the species analyzed. Always taking in account, as stated by Zohary (1970), centers of diversity are a biological fact, while centers of origin are a matter of interpretation.

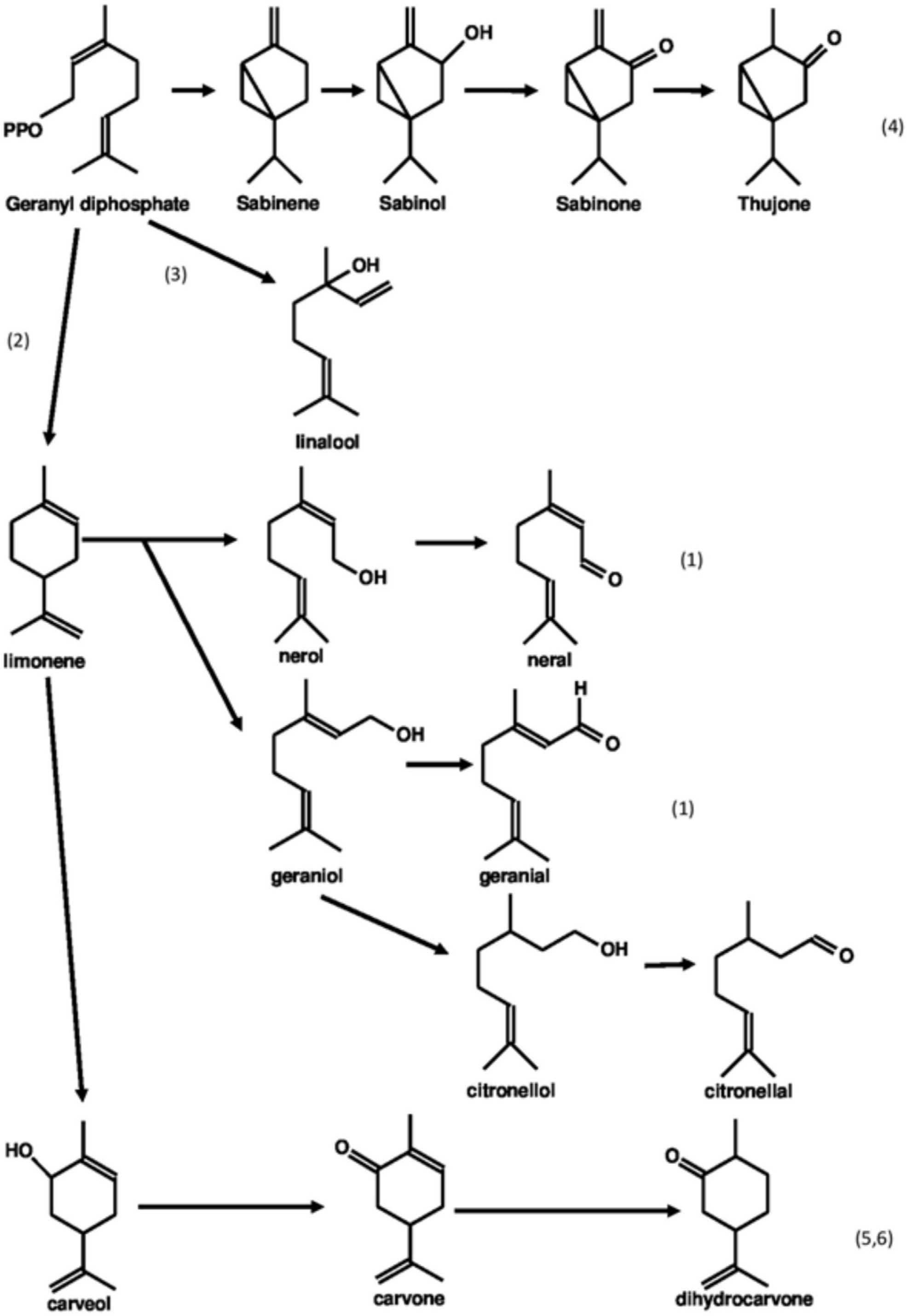


Fig. 5. Biosynthetic pathways of the main constituents from the chemotypes identified. (1):Akhila, 1985; (2): Bouwmeester et al., 1998; (3): Croteau, 1987; (4): Karp and Croteau, 1982; (5): McCaskill and Croteau, 1997; (6): van der Werf and Boot, 2000.

Acknowledgments

The authors thank Mrs. Angelica Mariño for the illustration of Fig. 2. This work was supported by grants from INTA PNHFA4164 and PNHFA064641, and from the Universidad de Buenos Aires (UBACyT 20020130200057BA and 20020130100169BA), PICTO 2008-00150, Universidad Nacional de Jujuy, Agua de los Andes (Laboratorio LanaRT).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2017.07.003.

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