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

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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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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