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Acantholippia seriphioides: Chemical biodiversity of wild populations from the Cuyo region in Argentina

[*Acantholippia seriphioides*: Biodiversidad química de poblaciones silvestres de la región de Cuyo de la Argentina]

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Abstract: Nineteen samples from the aerial parts in bloom of *Acantholippia seriphioides* (Verbenaceae) were collected in three provinces belonging to the Cuyo region of Argentina. The essential oils were obtained by hydrodistillation, yielding between 0.57 and 2.46% (v/w). On the basis of the 13 main compounds determined by GC-FID-MS, representing the 87.2 and 95.3% of the total identified, four chemotypes were determined: carvacrol, thymol, cis and trans-dihydrocarvone and linalool-geraniol. The co-occurrence of different chemotypes in a same population indicates that the sample collection must be carefully done with the aid of an in situ olfactory testing of the plants, since no morphological differences at first sight were observed among them. The results showed in this study suggest that the pattern of variations for this species exists throughout its natural distribution area.

Keywords: *Acantholippia seriphioides,* Verbenaceae, essential oil, chemotypes.

Resumen: Se colectaron 19 muestras de partes aéreas en floración de *Acantholippia seriphioides* (Verbenaceae) de tres provincias de la región de Cuyo de la Argentina. Los aceites esenciales fueron obtenidos por hidrodestilación, arrojando rendimientos entre 0.57 y 2.46% (v/p). A partir de la determinación por GC-FID-MS de los 13 compuestos principales, que representaban entre el 87.2 y 95.3% del total de los identificados, fueron determinados cuatro quimiotipos: carvacrol, timol, cis y trans-dihidrocarvona, y linalol-geraniol. La co-ocurrencia de diferentes quimiotipos en una misma población indica que la colecta debe ser realizada con la ayuda de un examen olfatorio in situ de la planta, ya que, a primera vista no se observan diferencias morfológicas entre ellas. Los resultados sugieren que el patrón de variación para esta especie existe en toda su área de distribución natural.

Palabras clave: *Acantholippia seriphioides*, Verbenaceae, aceite esencial, quimiotipos

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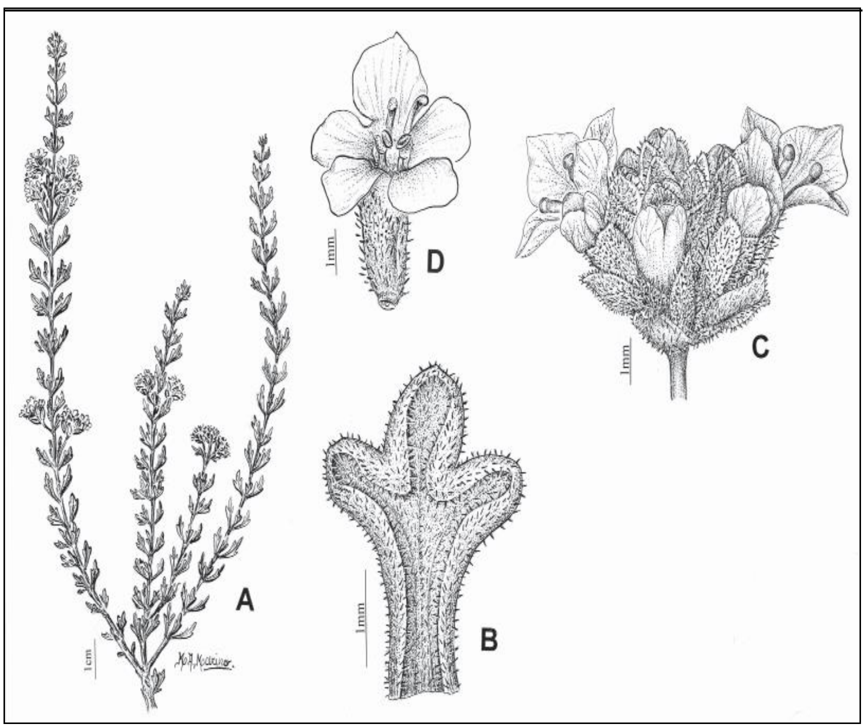
INTRODUCTION

A great richness of native species grows in Argentina including aromatic, medicinal and ornamental species with potential value. Most of them are endemic and grow in marginal fragile ecosystems (Zuloaga & Morrone, 1996). *Acantholippia seriphioides* (A. Gray) Moldenke (Verbenaceae) is a stunt aromatic bush 30-60 cm height, branched from the basis, with revolute sessile leaves, 3-5 lobed in the apex, narrow toward the base, puberule, with reddish spherical glands more abundant on the underside, inflorescence in clusters of white flowers grouped at the end of the branches (Figure 1). It is found in Chile, Bolivia, and in Argentina, in the provinces of Buenos Aires, Chubut, La Pampa, Mendoza, Neuquén, Río Negro, Santa Cruz, San Juan and San Luis, growing in arid environments characterized by stony or silty soils, from sea levels up to 1000 meters a.s.l. In Argentina, it is mainly known as "tomillo andino" or "tomillo de la sierra" and infusions of the leaves and flowers are used for their medicinal properties, against gastrointestinal disorders (Toursarkissian, 1980; Ratera & Ratera, 1980), for the treatment of influenza (Gonzalez & Molares, 2004), and also as a condiment in replacement of the authentic thyme (*Thymus vulgaris* L.) for its typical high content of thymol and carvacrol (Ruiz Leal, 1972; Roig, 2001). Some reports on this species have shown that its essential oil presents acaricidal, antifungal, antibacterial and repellent properties (Ruffinengo *et al.*, 2005; Fuselli *et al.*, 2007; Gillij *et al.*, 2008, Lima *et al.*, 2011).

Figure 1
***Acantholippia seriphioides* (A. Gray) Moldenke.**



A, flowering branch; B, leaf detail; C, inflorescence; D, flower.

The intense commerce of medicinal and aromatic species generated an irrational overexploitation that threatens biodiversity in central and western Argentina and has put this species at risk (Delucchi, 2006; Elechosa *et al.*, 2009). Its propagation could be achieved uneasily through seeds or cuttings, but the most common way in nature seems to be the spontaneous layering (Strasser *et al.*, 2002; (Strasser *et al.*, 2006). The introduction to cultivation could be a useful strategy to reduce the harvest pressure on natural areas and therefore contribute to their conservation. Previous reports on the chemical composition of essential oils from natural populations of *A. seriphioides* from Patagonia in Southern Argentina: Auca Mahuida (Neuquén province), Chasicó (Rio Negro province) and Sierra Chata and Comodoro Rivadavia (Chubut province), showed several chemotypes (Bandoni *et al.*, 1998; Mazzuca *et al.*, 2011).

The aim of this study was to determine the variations in yields and chemical composition of the essential oils of native populations from the Cuyo region in Argentina, particularly in the provinces of San Luis (SL), Mendoza (MZ) and San Juan (SJ) during several years (2006-2009). In addition, the confirmation of the correspondence of the scents detected *in situ* with the chemical compositions would assess this practice as a useful tool to detect different compositions/chemotypes in a same site of collection (Fester *et al.*, 1961; Di Leo Lira *et al*., 2007).

MATERIALS AND METHODS

Sampling

Nineteen samples of the aerial parts in bloom were collected in different seasons of consecutive years (2006-2009) from two populations of SL (Zanjitas and Paso de las Carretas), seven of MZ (Las Horquetas, San Jose, La Carrera, Los Arboles, Pareditas, Yaucha and General Alvarado), and one of SJ (Pachaco). 30-50 plants per population were sampled, attaining a total fresh weight of 0.5-2.0 kg (Figure 2). In some collection sites, distinct scents were detected among the same population; in these cases, separate samples were collected.

Geographical coordinates were taken for each population using a Garmin Etrex Legend HCX. Plant materials were identified by one of the authors (AM) and vouchers specimens from each site were deposited in the "Instituto de Recursos Biológicos-INTA", Hurlingham, Argentina (BAB) (Table 1).

Figure 2

Collection sites in the Cuyo region in Argentina

 Collection sites.

Table 1
Site references and essential oil yields of the studied populations

Population Nº	Province	Site Collection	Collection date	Phenology	Essential oils yields (% v/w)	Herbarium specimen Nº
1	San Luis	**Zanjitas**	14/11/06	1	1.05	Juárez, 264
			26/11/07	2	1.21	Juárez, 400
			25/3/09	3	1.26	Molina, 6564
2 A		**Paso de las Carretas**	14/12/06	3	1.99	Juárez, 329
			23/3/07	2	1.75	Juárez, 381
			27/11/07	3	1.81	Juárez, 401
			16/3/08	3	1.75	Juárez, 455
2 B		**Paso de las Carretas**	14/12/06	3	2.46	Juárez, 330
			23/3/07	2	1.82	Juárez, 380
			16/3/08	3	2.29	Juárez, 456
3	Mendoza	**Las Horquetas**	14/11/06	1	1.44	Juárez, 263
4		**San Jose**	4/12/08	3	0.71	Molina, 6353
5		**La Carrera**	4/12/08	3	0.58	Molina, 6358
6		**Los Arboles**	4/12/08	3	1.08	Molina, 6362
7		**Pareditas**	5/12/08	3	0.95	Molina, 6368
8 A		**Yaucha**	5/12/08	3	1.07	Molina, 6369
8 B		**Yaucha**	5/12/08	3	0.67	Molina, 6370
9		**General Alvarado**	5/12/08	3	0.57	Molina, 6375
10	San Juan	**Pachaco**	8/12/08	3	0.67	Molina, 6400

Phenology: 1, beginning of bloom; 2, mid bloom (50%); 3, full bloom

Essential oils isolation

The essential oils were obtained from natural air-dried material (12-15% final moisture) by hydrodistillation for 3 hours using a Clevenger-type trap (IRAM, 1996). Oils were desiccated with anhydrous sodium sulfate and were stored at -5 °C prior to analysis. The yields were expressed as v/w ratios.

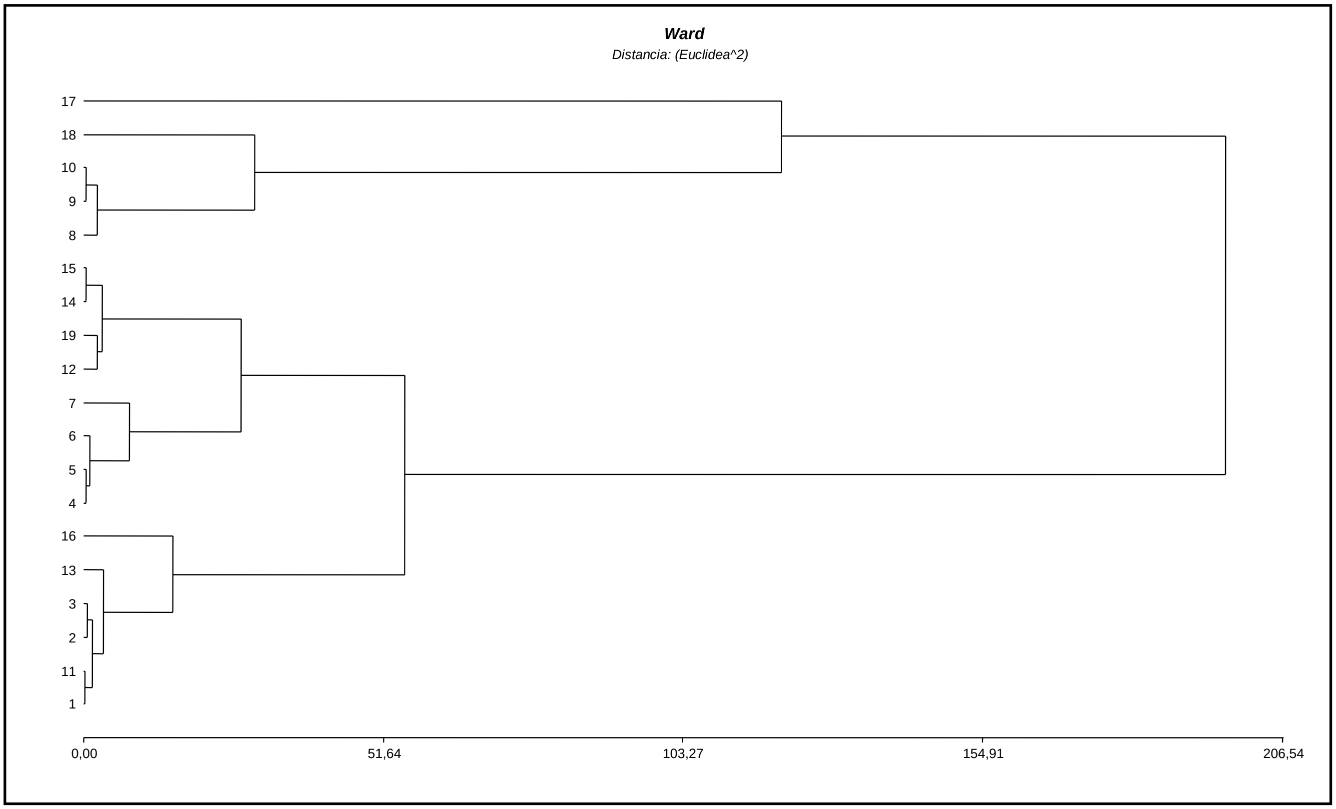
Essential oils identification

A GC-FID-MS system Perkin Elmer Clarus 500 was used with an 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 x 0.25 mm with 0.25 μm film thickness. The polar column was connected to a FID, while the apolar column was connected to a FID and a quadrupolar mass detector (70 eV), by a vent system (MSVent™). Helium was used as carrier gas at a constant flow of 1.87 ml/min. The temperature was programmed according to the following gradient: 90º- 240º at 3º/min, and then isothermal for 15 min. The injector and both FIDs were set at 255º and 275º, respectively. The injection volume was 0.5 μl of a 30% solution of oil in hexane. The temperature of the transference line and the ion source were 180º and 150º, respectively; the range of masses was 40-300 Da, 10 scan/sec. Identification of the compounds was performed by comparison of the lineal retention indices (relative to C8-C24 n-alkanes) obtained in both columns, with those of reference compounds or those reported in literature; in some cases, co-cromatography was also applied for unequivocal identification. Additionally, each mass spectra obtained was compared with those from usual libraries and from a laboratory-developed mass spectra library built up from standards or components of known oils (Adams, 2007; Wiley/NIST, 2008). The relative percentage composition was achieved using the single area percentage method, without considering corrections for response factors. The lowest response obtained from each column for each component was considered.

Statistical analysis

Agglomerative hierarchical cluster (AHC) analysis using the 13 main components as variables was carried out (InfoStat, 2011). Euclidean Pithagorean coefficient of distance and Ward's method of linkage were applied which resulted in a dendrogram (Fig. 3).

Figure 3
Dendrogram showing populations grouping



RESULTS AND DISCUSSION

Site references and essentials oil yields are stated in Table 1. The essential oils contents ranged between 0.57 and 2.46%, being the yields of Paso de Las Carretas (SL) the highest. In the three-four years repetitions of populations 1 and 2, some differences were observed that were attributed to the phenological stage. Table 2 shows the linear retention indices (LRI) obtained and identification methods used. Percentage composition of the essential oils of samples from San Luis and San Juan determined by GC-FID-MS are shown in Table 3. Samples from Zanjitas fits on the thymol type (49.9-60.1%) with p-cymene (17.1-21.2%), γ-terpinene (4.3-8.5%) and minimum carvacrol content (0.3-0.6%). In Paso de las Carretas, two compositions were found: one (2 A) with high carvacrol percentage (36.5-50.8%), followed by p-cymene (12.3-20.3%), γ-terpinene (12.1-20.5%) and little thymol (4.2-8.5%); the other type (2B) showed *cis* and *trans*-dihydrocarvone (72.2-74.3%) and limonene (14.5-20.2%) as main compounds, being phenols almost absent (1.0-2.6%). San Juan sample presented an intermediate phenols type, composed of carvacrol (37.5%) and thymol (23.5%), along with p-cymene (13.7%) and γ-terpinene (11.8%).

Meanwhile, the percentage composition of the essential oils of seven populations of Mendoza is showed in Table 4. Thymol type (52.6-55.6%) predominated in Las Horquetas and La Carrera; carvacrol type (56.6%) in San José, and a phenols intermediate type in Los Árboles and Pareditas. On the other hand, in Yaucha population, two different compositions were detected: 8A with high thymol content (78.0%) and 8B containing mainly linalool-geraniol (78.0%). In a neighboring population, General Alvarado, the main components in the essential oil were *cis* and *trans*-dihydrocarvone (71.4%) and limonene (16.9%). The organoleptic characteristic “sweet-citric” of this composition (9) was very similar to that of Paso de las Carretas (2B) population.

Table 2
LRI of the main compounds identified.

Components	LRI non polar	Published, non polar (1)	LRI polar	Published, polar	Method used
Myrcene	992	988	1170	1161 (2)	A
α-Terpinene	1010	1014	1195	1178 (2)	B
p-Cymene	1017	1020	1286	1270 (2)	A
Limonene	1024	1024	1221	1198 (2)	A
γ-Terpinene	1051	1054	1264	1245 (2)	B
Linalool	1090	1095	1549	1543 (2)	A
cis-Dihydrocarvone	1188	1191	1623	1606 (3)	B
trans-Dihydrocarvone	1201	1200	1642	1623 (2)	B
Carvone	1240	1239	1745	1734 (2)	A
Geraniol	1247	1249	1852	1839 (2)	A
Thymol	1291	1289	2188	2164 (2)	A
Carvacrol	1293	1298	2216	2211 (2)	A
Geranyl acetate	1375	1379	1760	1751 (2)	A

(1): Adams (2007). (2): Babushok *et al.* (2011). (3): Lee *et al*. (2005). A: identified by two LRI (apolar and polar columns), mass spectrum and co-chromatography with standards. B: identified by two LRI (apolar and polar columns), mass spectrum and comparison with compounds detected in authentic well known essential oils.

Table 3
Percentage composition of main components of the essential oils from collections of San Luis and San Juan provinces

PROVINCE	SAN LUIS										SAN JUAN
Population Nº	1			2 A				2 B			10
Year	2006	2007	2009	2006	2007	2007	2008	2006	2007	2008	2008
Sample Nº	1	2	3	4	5	6	7	8	9	10	19
Components											
Myrcene	2.7	3.1	3.8	3.2	3.4	2.5	4.4	0.5	0.6	0.4	1.6
α-Terpinene	2.1	2.5	3.1	4.0	4.1	3.1	5.9	t	t	t	2.2
p-Cymene	21.2	17.1	20.5	13.0	15.7	12.3	20.3	0.9	1.1	1.0	13.7
Limonene	0.9	0.8	1.1	0.8	0.5	0.6	1.0	14.3	16.4	20.2	0.8
γ-Terpinene	4.3	8.0	8.5	12.3	14.5	12.1	20.5	0.6	0.7	0.5	11.8
cis-Dihydrocarvone	--	--	--	--	--	--	--	16.5	10.2	11.1	---
trans-Dihydrocarvone	--	--	--	--	--	--	---	57.8	62.3	61.1	---
Thymol	60.1	59.1	49.9	7.2	5.1	8.5	4.2	1.5	0.3	0.7	23.5
Carvacrol	0.6	0.3	0.3	49.2	44.5	50.8	36.5	1.1	1.5	0.3	37.5
Subtotal identified	91.9	90.9	87.2	89.7	87.8	89.9	92.8	92.7	92.5	95.3	91.1

t: traces (< 0.05%). Other minor components: α-thuyene, α- and β-pinene, α and β-phellandrene, *trans*- β-ocimene, α–terpinolene, carvone, linalool, geraniol, β-caryophyllene, α-humulene.

The dendrogram obtained by the AHC analysis (Figure 3) showed that the oil samples can be grouped in the following four clusters with 85% of similarity:

- Group 1, Chemotype linalol-geraniol: sample 17.
- Group 2, Chemotype *cis* and *trans*-dihydrocarvone: samples 8, 9, 10 and 18.
- Group 3, Chemotype carvacrol: samples 4, 5, 6, 7, 12, 14, 15 and 19.
- Group 4, Chemotype thymol: samples 1, 2, 3, 11, 13 and 16.

Table 4
Percentage composition of main components of the essential oils from collections of Mendoza province

PROVINCE	MENDOZA							
Population Nº	3	4	5	6	7	8 A	8 B	9
Sample Nº	11	12	13	14	15	16	17	18
Components								
Myrcene	3.2	1.2	1.5	1.6	2.1	t	t	--
α-Terpinene	2.2	1.5	1.5	t	--	t	--	t
p-Cymene	20.9	14.3	17.5	15.8	18.5	7.8	2.2	1.1
Limonene	0.6	0.6	0.7	0.7	0.8	0.4	0.4	16.9
γ-Terpinene	5.3	6.1	5.5	7.5	7.3	3.1	0.8	t
cis-Dihydrocarvone	--	--	t	t	--	--	t	8.9
trans-Dihydrocarvone	t	--	--	t	t	t	--	62.5
Linalool	0.2	0.2	0.2	0.1	0.3	2.2	53.5	--
Carvone	t	--	t	--	t	t	--	3.8
Geraniol	--	t	t	--	--	t	24.5	t
Thymol	55.6	7.8	52.6	20.6	23.1	78.0	t	--
Carvacrol	0.8	56.6	9.9	41.9	35.8	0.5	t	--
Geranyl acetate	t	t	--	--	--	t	10.5	--
Subtotal identified	88.8	88.3	89.4	88.2	87.9	92.0	91.9	93.2

t: traces (<0.0.5%). Other minor components: α-thuyene, α and β-pinene, α and β-phellandrene, *trans*-β-ocimene, terpinolene, terpinen-4-ol, carvacryl methyl ether, β-caryophyllene, α-humulene.

CONCLUSIONS

Essential oil yields of Paso de las Carretas populations (SL) were outstanding, although most of them showed values over 1.0% which might be considered notable, especially for natural populations. The co-occurrence of different chemotypes in a same population - as in Yaucha and Paso de las Carretas- indicates that the sample collection must be carefully done with the aid of an *in situ* olfactory testing of the plants, since no morphological differences among them can be observed at first sight. The best collection sites for each chemotype were, Yaucha (MZA), for thymol (78.0%) and linalool-geraniol (78.0%), San José, (MZA) for carvacrol (56.6%), and Paso de las Carretas (SL) and General Alvarado (MZA) for *cis* and *trans*-dihydrocarvone (71.4-74.3%).

Previous studies on collections from three provinces of the Patagonian region revealed three of the herein described chemotypes: thymol in Auca Mahuida (Neuquén), geraniol-linalool in Chasicó (Rio Negro) and carvacrol in Sierra Chata (Chubut) (Mazzuca *et al.*, 2011). Other authors have also reported essential oils with high contents of thymol and p-cymene (Gilij *et al.*, 2008) and thymol, carvacol, γ-terpinene and p-cymene (Ruffinengo *et al.*, 2005, Fuselli *et al.*, 2007, Lima *et al.*, 2011).

The results obtained in San Luis confirmed former reports (Di Leo Lira *et al.*, 2007), showing the dominance of the phenols chemotypes in these populations. Interestingly, the co-occurrence in Mendoza of all the chemotypes identified, showed that the pattern of variation for this species exists throughout its natural distribution area.

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