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Article

Volatile Constituents of Five *Baccharis Species* from Northeastern Argentina

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Os óleos essenciais obtidos por hidrodestilação das partes aéreas de *Baccharis gaudichaudiana*, *B. microcephala, B. penningtonii, B. phyteumoides e B. spicata* coletadas em diferentes regiões da Argentina foram analisados qualitativa e quantitativamente utilizando um sistema CG e CG/EM com uma configuração especial. Os rendimentos dos óleos variaram de 0,1 a 0,2% (m/v de planta seca). Diferentes compostos foram observados na composição dos óleos essenciais das espécies estudadas: *B. gaudichaudiana* apresenta espatulenol e β-pineno como constituintes principais e, *B. microcephala* e *B. spicata*, espatulenol e óxido de cariofileno em concentrações mais elevadas. Na última espécie citada, o β-pineno também foi encontrado em quantidades significativas. Germacreno D foi o principal componente presente na *B. penningtonii.* Finalmente, β-selineno e acetato de fitol E foram os principais componentes encontrados na *B. phyteumoides.*

Essential oils obtained by hydrodistillation from the aerial parts of *Baccharis gaudichaudiana, B. microcephala, B. penningtonii, B. phyteumoides* and *B. spicata* collected from different locations in Argentina were analyzed. Qualitative and quantitative analysis were performed using a GC/FID/MS system with a special configuration. Essential oil yields ranged from 0.1 to 0.2% (m/v of dry material). Different chemical patterns were observed in the essential oils composition of the species studied: spathulenol and β-pinene were the main constituents in *B. gaudichaudiana,* whereas caryophyllene oxide and spathulenol were the major constituents in *B. microcephala* and *B. spicata.* In this latter species, β-pinene has also been found in high amounts. Germacrene D was the major compound in *B. penningtonii.* Finally, β-selinene and phytol acetate E were the main components in *B. phyteumoides*.

Keywords: *Baccharis gaudichaudiana*, *B. microcephala*, *B. penningtonii*, *B. phyteumoides*, *B. spicata*, carqueja, essential oils

Introduction

The genus *Baccharis* belongs to the *Asteraceae* family. This genus comprises more than 400 species in America, mainly South America.[1] Essential oils from 38 species of this genus have previously been analyzed, accounting in many cases for the value of these plants in traditional medicine as diuretics and stomachics and in fragrance industry. Because of their bitter properties these oils are also frequently used to prepare alcoholic and non-alcoholic beverages. Ninety six species of *Baccharis* were found in Argentina, and they were classified into 15 sections. Section *Caulopterae* or *Alatae* comprises 10 species, all having stems with 2 or 3 longitudinal lateral wings.[2,3] The most common *Baccharis* species used in traditional medicine and phytotherapy of southern Brazil, Paraguay, Uruguay and Argentina belong to this section: e.g. *B. trimera, B. articulata* and *B. crispa*. Among the remaining species of this section, *B. phyteumoides*, *B. penningtonii. B. microcephala* and *B. gaudichaudiana,* were selected to be studied. *B. spicata* was also analyzed. All these aromatic species are commonly named “carqueja”. Although they have different morphological characteristics, when they are commercialized as powders they can not be well identified. The lack of chemical studies on the volatile composition of these species led us to analyze their essential oils in order

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to find a pattern that could be used to discriminate them and to identify any possible adulterations or substitutions. *B. gaudichaudiana* DC. [BG] is a shrub that grows in Brazil, Paraguay and Argentina, commonly known as "carqueja", "carqueja dulce" or "chilca melosa". It is used in folk medicine as antidiabetic, stomachic, diuretic and antipyretic. Depending on its diterpenoids composition (gaudichaudiosides), it may have different taste properties, from sweet to bitter or neutral.[4] *B. microcephala* (Less.) DC. [BM], also named "carqueija" or "carquejilla", is native to southern Brazil, Paraguay, Uruguay and northeastern Argentina.[5] The infusion of its aerial parts is used externally for the treatment of rheumatic ailments and to heal wounds. When administered orally, it is hepatic, diuretic, and antidiarrheic.[6] *B. penningtonii* Heering [BP] is a shrub native to Uruguay and northern Argentina.[5] To date, no reports about its chemical composition or local uses have been found. As for *B. phyteumoides* (Less.) DC. [BY], no ethnobotanical information has been found either. The latter species grows in Argentina, Brazil, Paraguay and Uruguay. *B. spicata* (Lam.) Baill. [BS] is also known as "chilca blanca" or "pi is í mop", and grows in Brazil, Argentina, Uruguay and Paraguay.[1] The aerial parts of *B. spicata* are used in folk medicine as diuretic and digestive. Antioxidant, trypanocidal and antibacterial activities for this species have also been reported.[7-9]

Experimental

Materials and methods

Plant materials were collected in different locations of Argentina (Table 1). The botanical identification was done by our group (Dr. M. Gattuso and Dr. S. Gattuso) and voucher specimens of wild materials were deposited in the National University of Rosario (UNR), Argentina.

The oils were obtained from the aerial parts (100 g of air dried material) by hydrodistillation for 3 h using a Clevenger apparatus.[10] The collected oil samples were dried over anhydrous sodium sulfate and stored at 2 °C until analyzed. The analysis of the oils was performed on a Perkin Elmer Clarus 500 GC/FID/MS system, with a special configuration. One injector (split ratio: 1:100) was connected by a flow splitter to two capillary columns: a) one by polyethylene glycol 20,000 and b) other by 5% phenyl-95% methyl silicone, both 60 m x 0.25 mm with 25 µm film thickness. The polar column was connected to a FID, whereas the non-polar column was connected to a FID and a quadrupolar mass detector (70 eV), split by a vent system (MS Vent™) and a restrictor, to obtain a split ratio FID/MS of 2:1. Helium was used as mobile phase at a constant flow rate of 1.87 mL min^{-1}. The temperature was programmed according to the following gradient: from 90 to 225 ºC at 3 °C min^{-1}, 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, 10 scan s^{-1}. The identification of the compounds was performed by comparison of the retention indices (relative to C_8-C_{20} *n*-alkanes) obtained in both columns, with those of reference compounds. Additionally, each mass spectra obtained was compared to those from literature libraries and from a laboratory-developed mass spectra library.[11,12] The percentage composition was determined by using the single area percentage method, without considering corrections for response factors. The lowest response obtained from each column was considered.

Results and Discussion

The hydrodistillation of the different materials yielded oils in the range from 0.1 to 0.2%. A total of 93 compounds, representing between 74-96% of each oil, were identified. The percentage composition of each sample is listed in Table 2. The composition of the essential oils analyzed in this study were qualitatively

Table 1. Collection data of analyzed samples of *Baccharis* spp

Sample	Species	Voucher	Site	Date
BG 1	*B. gaudichaudiana*	1564	Prov. Misiones	2005-10
BG 2	*B. gaudichaudiana*	1655	Prov. Misiones	2006-02
BP	*B. penningtonii*	1650	Prov. Entre Ríos	2006-03
BY	*B. phyteumoides*	1888	Prov. Santa Fe	2007-02
BM	*B. microcephala*	1657	Prov. Misiones	2006-02
SP 1	*B. spicata*	1647	Prov. Santa Fe	2006-03
SP 2	*B. spicata*	1904	Prov. Corrientes	2007-03

but not quantitatively similar. Spathulenol (8.2-38.2%) and caryophyllene oxide (3.6-22.4%) were present in all the species in high percentages, except for [BP] (1.9 and 2.9% respectively). BG 1 and BG 2 displayed a high content of β-pinene and limonene (25.1-31.0% and 4.6-7.2% respectively). BS 1 and BS 2 also showed a high content of the latter substances (7.1-26.0% and 8.1-4.4% respectively), with the addition of β-caryophyllene which was also found in a high percentage (8.3-7.7%). [BY] was characterized by a high content of β-selinene and phytol acetate E (12.9 and 16.2% respectively). The hydrocarbon monoterpene fraction was minoritary in BM, whereas humulene epoxide II and globulol were found in high amounts. Particularly in BP, the major compound identified was germacrene D with a percentage of about 29.3%.

Table 2. Chemical composition (%) of the essential oils of *Baccharis* spp

		B. gaudichaudiana		*B. penningtonii*	*B. phyteumoides*	*B. microcephala*	*B. spicata*	
RI [a]	Identification	BG 1	BG 2	BP	BY	BM	BS 1	BS 2
931	α-thujene	t	t	t	t	-	-	-
938	α-pinene	2.2	3.2	0.5	t	t	0.5	1.9
951	α-fenchene	t	t	-	t	-	-	-
955	camphene	t	t	t	-	-	-	t
960	thuja-2,4 (10)-diene	t	t	-	-	-	-	-
975	sabinene	0.4	t	0.8	0.7	-	t	t
984	myrcene	t	t	0.6	1.3	-	t	t
985	β-pinene	25.1	31.0	0.8	1.0	-	7.1	26.0
1001	δ-2-carene	t	-	-	-	-	-	-
1016	α-terpinene	t	t	t	t	-	t	t
1029	(Z)-β-ocimene	t	t	t	t	-	-	-
1030	*p*-cymene	-	-	1.1	1.6	t	-	0.4
1031	limonene	4.6	7.2	1.5	8.7	t	8.1	4.4
1032	β-phellandrene	t	t	0.2	t	-	t	0.4
1034	1,8-cineole	t	-	1.5	2.2	t	-	-
1049	(E)-β-ocimene	t	t	t	t	-	-	0.4
1062	γ-terpinene	0.1	t	t	t	-	t	t
1087	terpinolene	0.1	t	t	t	-	t	t
1092	ρ-cimenene	t	t	t	t	t	t	t
1096	6-camphene	t	t	-	-	-	-	-
1098	linalool	-	-	t	t	1.1	t	t
1101	*n*-nonanal	-	-	t	-	t	t	t
1110	1,3,8-ρ-menthatriene	t	-	-	-	-	-	-
1125	α-campholenal	0.1	t	-	-	-	-	-
1138	*trans*-pinocarveol	0.8	2.8	-	-	-	-	t
1164	pinocarvone	0.9	1.8	t	t	-	t	t
1179	terpinen-4-ol	t	0.4	t	t	t	-	t
1188	α-terpineol	t	1	t	-	t	-	t
1195	myrtenal	2.5	4.3	t	-	-	0.3	t
1207	verbenone	t	t	-	-	-	-	-
1215	*trans*-carveol	t	t	-	-	-	-	-
1217	β-cyclocitral	t	t	t	t	-	-	t
1244	carvone	0.1	0.4	t	t	-	t	t
1267	geranial	t	t	-	-	-	-	-
1272	perilla aldehyde	t	t	-	-	-	-	-
1275	ρ-menth-1-en-7-al	t	t	-	-	-	-	-
1295	perilla alcohol	t	t	-	-	-	-	-
1320	2E-,4E-decadienal	t	t	t	-	-	-	-
1326	myrtenyl acetate	t	t	-	-	-	-	-
1349	α-terpinyl acetate	t	t	t	t	-	-	t
1350	α-cubebene	t	t	t	t	-	-	t

Table 2. continuation

		B. gaudichaudiana		*B. penningtonii*	*B. phyteumoides*	*B. microcephala*	*B. spicata*	
RI [a]	Identification	BG 1	BG 2	BP	BY	BM	BS 1	BS 2
1372	α-ylangene	-	-	t	t	t	-	-
1376	isoledene	t	-	-	-	-	t	t
1379	α-copaene	0.2	t	t	t	t	1.0	0.5
1388	β-bourbonene + β-elemene	0.4	t	2.3	8.5	t	1.0	1.2
1403	methyl eugenol	t	-	-	-	-	-	t
1408	α-gurjunene	-	-	-	-	t	-	-
1409	longifolene	-	-	t	-	-	t	t
1418	β-caryophyllene	1.3	1.4	4.9	2.3	0.9	8.3	7.7
1432	β-copaene	0.2	t	0.4	t	t	-	t
1436	neryl acetone	t	t	t	t	t	-	t
1443	aromadendrene	0.1	t	t	t	1.6	t	0.3
1450	*cis*-muurola-3,5- diene	t	t	t	-	-	-	t
1458	α-humulene	0.2	1.2	0.7	1.8	1	2.8	2.5
1461	alloaromadendrene	0.1	-	0.9	t	t	0.5	0.4
1474	*trans*-cadina-1(6),4 diene	t	t	t	t	t	t	t
1482	γ-muurolene	0.3	t	1.2	t	t	0.6	0.6
1485	α-amorphene	-	-	0.9	3.7	-	t	t
1486	germacrene D	0.1	0.4	29.3	2.1	-	3.1	6.3
1490	calamenene 1,11-epoxide	-	-	-	-	t	0.3	t
1490	β-selinene	-	-	0.9	12.9	-	t	0.5
1492	*trans*-muurola-4(14), 5-diene	-	-	t	t	-	2.9	t
1500	bicyclogermacrene + α-muurolene	0.9	0.6	3.8	2.4	t	1.1	2.8
1512	γ-cadinene	-	t	0.8	t	t	t	0.6
1513	δ-amorphene	1.1	t	t	t	0.6	2.3	2.5
1531	*trans*-calamenene	t	t	t	t	t	0.3	0.2
1535	*trans*-cadina-1,4-diene	t	-	t	t	t	t	t
1540	α-cadinene	0.2	t	t	t	-	t	t
1545	α-calacorene	0.3	t	1	t	1.1	0.9	0.6
1563	E-nerolidol	-	-	1	1.6	2.6	t	t
1567	β-calacorene	t	t	t	t	t	t	t
1579	spathulenol	38.2	22.0	1.9	8.2	17.4	18.7	13.1
1582	caryophyllene oxide	8.7	14.0	2.9	5.7	22.4	15.1	3.6
1591	globulol	-	-	-	-	10.1	-	t
1592	epiglobulol (viridiflorol)	-	t	t	-	0.6	t	t
1594	salvial-4(14)-en-1-one	0.1	1.4	-	t	-	t	-
1609	humulene epoxide II	0.5	3.2	-	1.1	12.4	2.8	-
1627	cubenol-1-epi	0.2	t	1.5	t	0.8	0.7	1.2
1640	caryophylla-4(12), 8 (13)-dien-5-ol	-	t	-	t	1.2	t	t
1641	α-*epi*-cadinol	-	-	0.7	0.8	t	t	1.8
1642	α-*epi*-muurolol	0.4	t	1.1	t	t	1.1	0.7
1645	α-muurolol	-	-	1.2	t	t	0.5	0.5
1653	α-cadinol	0.3	t	2.0	t	t	0.7	1.0
1675	caladene	-	-	t	t	3.5	t	t
1672	caryophyllene 14-hydroxy-9-*epi*-(E)	t	t	t	t	t	0.6	0.2
1685	germacra-4(15),5,10(14)-trien-1α-ol [b]	-	-	2.5	t	t	t	3.0
1686	eudesma-4(15),7-dien-1β-ol (impure)	-	-	0.9	t	-	t	t
1693	cuprenen-1-ol (4-)	-	-	-	-	t	-	-
1717	curcuphenol	-	-	0.9	-	-	-	-
1769	γ-curcumen-15-al	0.3	t	-	-	-	0.4	0.8
2217	phytol acetate E	0.6	t	3.6	16.2	t	3.0	3.0
	% total	91.6	96.3	74.3	82.8	77.2	84.5	89.0

Compounds are listed in order of elution on non-polar column. t: traces (≤ 0.1%); [a]Experimental Retention Index (RI) on non-polar column. [b]Correct isomer not identified.

Regarding the industrial uses, two groups of *Baccharis* species with economic implications in South America can be defined: those named "carqueja" with widespread ethnomedicinal, phytotherapeutic and food uses, and *B. dracunculifolia*, named "vassoura", largely employed in the fragrance industry due to the high content of E-nerolidol in its essential oil. Other *Baccharis* oils have been previously described, some of them also showing a predominant content of this alcoholic sesquiterpene (*B. articulata, B. cordobensis, B. crispa, B. rufescens*).[13-16] Two species, *B. crispa* and *B. articulata*, from section *Caulopterae*, are both rich in E-nerolidol and spathulenol.

Four species belonging to section *Caulopterae,* and *Baccharis spicata,* all of them known as "carqueja", were analyzed herein. The composition of these essential oils showed the same qualitative pattern, but presented quantitative differences. Likewise in other species of this mentioned section, spathulenol or germacrene D (biosynthetic precursor of the former compound) were found as the main constituents, in agreement with other species of this section. The chemical composition of the essential oil of *Baccharis microcephala* from Brazil reported by Simoes-Pires *et al.*[16] showed a similar pattern but quantitatively different from our results, with predominance of α-cadinol, spathulenol, caryophyllene oxide and globulol. These quantitative discrepancies can be explained by differences in the response to geographic, climatic, phenological or edaphic patterns. The qualitative pattern similarities within the studied species were expected as they belong to a common genus and four of them to the same section.

B. trimera is the most common and studied species among the so-called "carquejas". For this reason it was included in the latest edition of the Brazilian Pharmacopoeia[17] and was also proposed to be included in the next edition of the Argentine Pharmacopoeia.[18] Carquejyl acetate and carquejol, which are present in its essential oil, were proposed as unequivocal chemotaxonomic marker of this species.[16] Nevertheless, some populations of *B. trimera* lacking these compounds, have been recently described suggesting the existence of a novel chemotype or chemical variations that arise as a result of different environmental conditions.[19,20] This feature emphasizes the necessity for an accurate identification of the pharmacopoeial quality of carqueja. In Brazil, the magnitude of the populations of *B. trimera* assures its sustainability.[21] However, in Argentina this species is under threat due to its great demand in the local traditional medicine, phytotherapy and food industry.[22] As a result, many adulterations with other *Baccharis* spp. have been detected in our country. For this reason, the essential oils of other species of *Baccharis* belonging to the section *Caulopterae* and growing in the same habitat were analyzed herein. *B. spicata* was also included as it is another common adulterant of carqueja. The composition of the oils of these species was studied in order to assess whether they contain the chemotaxonomic markers that have been proposed for *B. trimera.* Finally, we demonstrated that these oxygenated terpenes were not present in any of the analyzed species. These results allow us to ensure that these chemical markers could be the most suitable for the identification of the legitimate carqueja.

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