Variation in the essential oil composition and antimicrobial activity of *Baccharis spartioides (H. et A.) J. Rimy* from three regions of Argentina.

María M. Oliva, María P. Zunino and María L. López,
Instituto Multidisciplinario de Biología Vegetal (IMBIV). Fellowships from CONICET.

Yanina A. Soria, Flavio N. Ybarra, Liliana Sabini and Mirta S. Demo,
Dpto. de Microbiología e Inmunología. Universidad Nacional de Río Cuarto. Ruta 36, km 601. CP 5800. Río Cuarto, Argentina.

Fernando Biurrun Indellar,
(Instituto de Investigaciones para el Desarrollo Socioeconómico de los Llanos de La Rioja) Universidad Nacional de La Rioja, Sede Chamical.

Julio A. Zygadlo,*
Cátedra. de Química Orgánica Fac. Cs. Ex. Fís, y Nat. UNC – IMBIV. Av. Vélez Sarsfield 1611 X 5016 GCA Córdoba, Argentina.

Abstract

Hydrodistilled oils of *Baccharis spartioides* from three populations of Argentina (Northwest, Central and Patagonia areas) were analyzed by using GC and GC/MS. Thirty-nine compounds were identified in the oils and a relatively high variation in their contents and in their antimicrobial activity was found. The main constituents of the oils were camphor (26.5–50%), limonene (4.3–35.8%), citronellal (12%), carvone (10%) and spathulenol (2.1–11.8%). The oil was tested against ten Gram-positive and Gram-negative bacteria and against the yeast *Candida albicans*. The best antimicrobial activity of the oils of *B. spartioides* was against *S. aureus* and *B. cereus* while *P. aeruginosa* was the most resistant bacteria to all the oils.

Key Word Index

Baccharis spartioides, Asteraceae, pichana, essential oil composition, limonene, citronellal, camphor, spathulenol, antimicrobial activity, environmental conditions.

Introduction

Baccharis spartioides (H. et A.) J. Rimy (syn. Psila spartioides (H et A) Cabrera, Hetherothalamus spartioides H. et A.) is an aromatic member of the Asteraceae family. The common name in Argentina is "pichana", which was born as a pichanay verb of the keshua languages. Its branches are strongly aromatic and they are used as repellents of flies and mosquitos (1) and in the native medicine (2). The essential oil composition of *B. spartioides* was previously described as possessing a high content of citral (21.6%) and monoterpene hydrocarbons (3). However, the main components of the oil from the Central Area of Argentina were oxygen-containing monoterpenes (>50.0%), while in Patagonia and northern Chile the major constituents were sesquiterpenes (>60.7%) (3,4). The composition of the oils varies according to local climatic and environmental conditions, as a well as the area of the cultivation, the variety and the harvest season and as a consequence, they have different bioactivities including the antimicrobial activity. Thus, a different composition of *B. spartioides* oil could be causing a different bioactivity.

A study of the factors involved in the changes of the quantity, quality and biological activity would contribute to obtaining the most efficient antimicrobial activity from the oil. The purpose of this work was to study the oil composition and to evaluate the antibacterial and antifungal activity of *B. spartioides* from

*Address for correspondence

Received: January 2006
Revised: June 2006
Accepted: July 2006

1041-2905/07/0005-0501$14.00/0—© 2007 Allured Publishing Corp.

three different regions of the Argentine Republic: Northwest, Central and Patagonia areas.

Experimental

Plant especies: *Baccharis spartioides* (Asteraceae) (syn. Psila spartioides (H et A) Cabrera, Hetherothalamus spartioides H. et A.) plants were collected from the following places : -Northwest area, Monte phytogeographic province, La Rioja Province. Argentina. Altitude: 498 masl, Latitude: 29°25' South, and Longitude: 66°52' West;

-Central area, Espinal phytogeographic province, La Calera Locality, Córdoba Province. Argentina. Altitude: 469 masl, Latitude: 31°20' South, and Longitude: 64°20' West ;

-Patagonia area, Monte phytogeographic province, Villa Regina Locality, Rio Negro Province. Argentina. Altitude: 185 masl, Latitude: 39°6' South, and Longitude: 67°5' West.

Voucher specimens from the Patagonia and Central area are kept in the Herbarium of Museo Botánico de Córdoba (CORD), Universidad Nacional de Córdoba. The voucher specimen from La Rioja is kept in the Herbarium of Instituto de Investigación para el Desarrollo Socioeconómico de Los Llanos de La Rioja, Universidad Nacional de La Rioja - Sede Chamical.

Essential oil isolation: 200 g of dried leaves of *B. spartioides* from each area were hydrodistilled in Clevenger-like apparatus to yield the samples of 0.4%, 0.2% and 0.4% oil from Northwest, Patagonia and Central area, respectively. The oils obtained were dried over anhydrous sulphate and stored in a refrigerator until analysis.

Gas Chromatography analyses: Analyses were performed in a Shimadzu GC-R1A (FID) gas-chromatograph, fitted with a 30 m x 0.25 mm (0.25 µm film thickness) fused silica capillary column coated with a phase 5% phenyl 95% dimethylpolysiloxane, non polar DB-5 column. We then used a polar Supelcowax 10 capillary column, phase polyethylene glycol. The GC operating conditions were as follows: oven temperature programmed from 40°–230°C at 2°C/min, injector and detector temperatures 240°C. The carrier gas was N at a constant flow of 0.9 mL/min. The constituents of the oils were identified on the basis of their GC retention index (RI) with reference to a homologous series of n-alkanes (C_{12} - C_{25}), by comparison of their retention times with those of pure authentic samples from Sigma, Fluka and Palma Companies, peak enrichment on co-injection with authentic standards wherever possible, by GC/MS library search (Adams and NIST) and using visual inspection of the mass spectra from literature, for confirmation. GC/MS analyses were performed with a Perkin Elmer Q-700 equipped with a SE-30 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm film). The analytical conditions were: oven temperature from 40°–230°C at 2°C/min, the carrier gas was He at a constant flow of 0.9 mL/min, the source was at 70 eV.

Microorganisms: The activity of the oils was tested against the following microorganisms: *Bacillus cereus, Staphylococcus aureus ATCC 25212, Staphylococcus epidermidis, Micrococcus luteus ATCC 9341, Enterococcus faecalis ATCC 29212, Escherichia coli, Proteus mirabilis, Klebsiella sp*. and *Pseudomonas aeruginosa.* These microorganisms are the main food contaminant agents. The yeast *Candida albicans* was used in order to test the antifungal activity.

Tubes containing Triptein Soy Broth (TSB) inoculated with the microorganisms were incubated during 18 h, at 37°C. From these tubes 10 fold dilutions were made with TSB, until an OD≅0,04 (10^6 cfu/mL) was reached. The antifungal activity was tested with the same methodology but using that dilution with an OD≅0,4 (10^6 cfu/mL).

Analysis of the Antibacterial activity: The antimicrobial studies were carried out according to De Pooter et al. (5). Each experiment was made in duplicate. Disc diffusion method: 200 µl of each inoculum were spread over plates containing Mueller-Hinton Agar (MHA); paper filter discs (6 mm of diameter), impregnated with 10 µL of each oil, were placed on the surface of the media. The plates were left 30 min at room temperature to allow the oil diffusion, then they were incubated at 37°C for 24 h. After this time, the inhibition zone around the disc was measured with a calliper. Discs with gentamicine (10 µg) were used as positive control.

Analysis of the Antifungal activity: Antifungal experiments were made in the same way as those with bacteria using Sabouraud Agar (SA) for the plates. Disc with Anfotericine B (2 µg/ml) were used as positive control.

Minimun Inhibitory Concentration assay (MIC): The minimum inhibitory concentration (MIC) was performed according to the method previously described by De Feo et al. (6), only with microorganisms that showed inhibitory zones larger than 10 mm. It was determined by two-fold dilutions of the oils in dimethyl sulfoxide (DMSO), imbibing 10 µL of each dilution on a filter paper disc. The discs were placed on the surface of a TSA plate, previously inoculated with 200 µL of each inoculum, and left at room temperature to allow the diffusion of the oil. Then they were incubated at 37°C for 24 h. After this time the inhibition zone around the disc was measured with a calliper. MIC was defined as the lowest concentration that inhibited visible growth. The MIC with fungus was made in the same way as with bacteria using Sabouraud Agar (SA) in the plates.

The negative control consisted in a paper disc impregnated with 10 µL of DMSO. The positive control was a disc impregnated with the antibiotic gentamicine (10 µg) for bacteria and anfotericine B (2 µg/mL) was used for *C. albicans.*

Results and Discussion

The components of the *B. spartiodes* oil from the different areas are reported in Table I. For Central area samples, the main constituents were camphor in the highest proportion (50.5%) and limonene (4.3%). On the other hand, the oil from the Northwest area did not contain camphor but had limonene in a high percentage (35.8%). The Patagonia oil has possessed a lower amount of camphor (26.5%) compared with that of the Central area's oil. However, the Patagonia oil had a level of oxygenated monoterpenes (22.0%) higher than the other oils. The northwest oil had a level of carbonyl compounds higher than that of the Patagonia oils, but this result was obtained by high level in citronellal and carvone (12.0% and 10.0%, respectively).

Table I. Percentage composition of the oils of *Baccharis spartioides* of different areas.

Compounds	RI	Central area	Northwest area	Patagonia
camphene	953	-	-	0.4
sabinene	976 a	0.6	-	0.2
β–pinene	980	0.6	-	-
myrcene	991	1.1	-	0.1
limonene (*)	**1030 a**	**4.3**	**35.8**	**-**
β–phellandrene	1031 a	-	0.1	-
(Z)–β-ocimene	1040	-	0.1	-
γ–terpinene	1060	0.8	-	-
Hydrocarbon monoterpenes		***7.4***	***36.0***	***1.8***
1,8-cineole	1033 a	-	0.2	-
cis-linalool oxide (furanoid)	1085	-	-	0.7
cis-p-menth-2-en-1-ol	1121	-	0.1	-
trans-pinocarveol	1139	-	1.1	0.4
camphor (*)	**1143**	**50.5**	**-**	**26.5**
citronellal (*)	**1153**	**-**	**12.0**	**-**
cis-pinocarveol	1184	-	1.1	-
α–terpineol (*)	1189	1.3	8.4	-
pulegone	1237 a	0.8	-	1.4
neral (*)	1238 a	0.8	8.2	-
carvone (*)	1242	-	10.0	-
neryl acetate	1365	-	0.4	-
bornyl angelate	1566	-	-	0.7
Oxygenated monoterpenes		***53.4***	***41.5***	***29.7***
α–gurjunene	1409	4.0	-	1.2
β–caryophyllene	1418	3.5	-	6.2
β–gurjunene	1434	2.6	-	0.9
γ–elemene	1437	0.9	0.3	-
α–humulene	1454	1.5	-	6.8
γ–muurolene	1480 a	0.9	-	3.2
ar-curcumene	1483 a	-	4.9	-
germacrene D	1485 a	0.8	-	0.8
valencene	1499 a	-	-	2.6
α–muurolene	1500 a	3.3	-	4.1
viridiflorene	1501 a	1.1	0.8	3.3
bicyclogermacrene	1503 a	2.3	4.0	-
Hydrocarbons sesquiterpene		***20.9***	***10.0***	***29.1***
ledol	1569	0.9	-	2.6
germacrene D-4-ol	1576 a	0.7	-	8.6
spathulenol	**1576 a**	**2.1**	**11.8**	**7.3**
caryophyllene oxide	1583	1.6	-	3.5
santalol +	1740	0.9	0.4	-
Oxygenated sesquiterpenes		***6.2***	***12.2***	***22.0***
Total identified		***87.9***	***99.7***	***81.5***

(NI) not identified compound; (+) correct isomer not identified; (*) The identification were confirmed by comparison with standards; ([a]) Compounds also identified by Supelcowax 10; (RI) All other compounds identified by DB-5.

Table II shows the antibacterial activities of the oils. *B. spartioides* oil of the three areas studied had antimicrobial activity against *S. aureus* and *B. cereus*, while *P. aeruginosa* was not inhibited by any of the oils. Gram positive bacteria were inhibited to 80% by *B. spartioides* oil collected from Central area. The same oil was active against Gram negative bacteria to 75% and against the yeast *C. albicans*. *B. spartioides* collected in Patagonia was able to inhibit *C. albicans* and Gram positive bacteria to 80%, but it was not active against Gram negative bacteria. *Baccharis spartioides* oil from the Northwest area inhibited Gram positive bacteria (60%) only.

The Minimun Inhibitory Concentration (MIC) was made with those oils that had produced an inhibition zone larger than 10 mm (Table III). *Staphylococcus aureus, B. cereus, M. luteus, E. coli,* and *P. mirabilis* presented MIC values of 28.1 µg/µL with the oil of *B. spartioides* from the Central area. The best antimicrobial activity of this oil was against the yeast *C. albicans* (MIC=14,1 µg/µL). *Baccharis spartioides* from Patagonia shown MIC values of 112.5 and 225 µg/µL for *S. aureus* and *B. cereus*, respectively. The MIC of the oil collected from the Northwest area was 900 µg/µL with *B. cereus.* It was the only bacteria with an inhibition zone greater than 10 mm (Table II).

The oil collected from the Central area was the most effective of the three as it was able to inhibit Gram positive, Gram negative and the yeast *C. albicans* with the best MIC values.

Significant difference in concentration of the components of the three oils, especially the variation in quantities of main components e.g. camphor and limonene, might be responsible for the different antimicrobial activity. The antimicrobial activity of limonene, camphor and carvone was also studied (Table IV). Limonene showed activity against all microorganisms, except *P. aeruginosa.* It had higher activity alone than the complete fraction of the Northwest area oil, which had 35% limonene. On the other hand, camphor did not show any activity, despite that the oils from the Central and Patagonia areas had camphor as the main component. Carvone inhibited mainly *B. cereus*, *P. aeruginosa*, *M. luteus* and *C. albicans*. However, the oil of the Northwest area, which had carvone (10 %), did not show activity against *P. aeruginosa* and *C. albicans.* It is possible that the components in lower percentage might be involved in some type of synergism with the other active compounds (7,8), synergistic activity of 1,8-cineole and camphor against some bacteria and *Candida albicans* has previously been reported (9,10). Besides, carvone and camphor could be antagonistic compounds in the oil in accordance with these results.

Table II. Antimicrobial activity (inhibition zone measured in mm) of *Baccharis spartioides* essential oils from different areas

	Areas		
Microorganism	**Northwest**	**Central**	**Patagonia**
Staphylococcus aureus	8.5	11.5	20
Staphylococcus epidermidis	NI	10.5	13
Bacillus cereus	35	25	12
Micrococcus luteus	10.5	12.5	NI
Enterococcus faecalis	NI	NI	7
Escherichia coli	NI	10.5	NI
Klebsiella sp.	NI	8	NI
Proteus mirabilis	NI	14	NI
Pseudomonas aeruginosa	NI	NI	NI
Candida albicans	NI	15	7

NI: No inhibition

Table III. Minimum Inhibitory Concentration (MIC) of *Baccharis spartioides* oils with antimicrobial activity (μg/μL).

	Areas		
Microorganism	**Northwest**	**Central**	**Patagonia**
Staphylococcus aureus	ND	28.1	112.5
Staphylococcus epidermidis	ND	900	450
Bacillus cereus	900	28.1	225
Micrococcus luteus	ND	28.1	ND
Escherichia coli	ND	28.1	ND
Klebsiella sp.	ND	ND	ND
Proteus mirabilis	ND	28.1	ND
Candida albicans	ND	14.1	450

ND : Not determined

Table IV. Antimicrobial activity (inhibition zone measured in mm) of terpenes

	Monoterpenes		
Microorganism	**Camphor**	**Limonene**	**Carvone**
Staphylococcus aureus	NI	25	9
Staphylococcus epidermidis	NI	26	NI
Bacillus cereus	NI	37	22.5
Micrococcus luteus	NI	33,5	15
Enterococcus faecalis	NI	10	9.5
Escherichia coli	NI	21,5	9
Klebsiella sp.	NI	15,5	10
Proteus mirabilis	NI	18	NI
Pseudomonas aeruginosa	NI	NI	27
Candida albicans	NI	46	13.5

NI: No inhibition

In summary, the most active oil of *B. spartioides* was collected from the Central area. This activity could be linked to antagonistic or synergistic role of all compounds found in the oil.

In the future, essential oils could be used as therapeutical agents, food preservatives and/or food-born-bacteria inhibitors, but our study suggests that the genetic and environmental factors and the total composition of the oil should be taken into account in order to obtain stable quality of *B. spartioides* for extracting essential oils. Issues of safety and toxicity will need to be addressed (11,12).

Acknowledgments

The authors are grateful to Argentine government agencies CONICET, Agencia Córdoba Ciencia SE and SECyT-UNC for financial support.

References

1. A. Ruiz Leal, Flora popular mendocina., Eudeba Editorial, Buenos Aires, pp 62–73. (1975).
2. M.P. Zunino, M.L. López and J.A. Zygadlo, *Medicinal Plants of Argentina. Pharmacological Properties and Phytochemistry.* In: *Advances in Phytochemisty.* Edit., Imperato, Chapter: 7 pp. 209–245, Research Singpost Trivandrum Editorial, Kerala, India (2003).
3. J.A. Zygadlo, and H.R. Juliani (Jr.), *Study of Essential oil composition of aromatic plants from Argentina. In: Recent Progress in Medicinal Plants - Phytochemistry and Pharmacology II*. Edits., D.K. Majumdar, J.N. Govil and V.K. Singh, pp 273–293, Chapter 16, Studium Press LLC, Houston, TX (2003).
4. M. Norte, F. Cataldo, A. Sánchez and A.G. González, *Spartidienedione, a new sesquiterpene with a novel carbon skeleton, from* Baccharis spartioides. Tetrahedron Lett., **34**, 5143–5146 (1993).
5. H. De Pooter, E. Aboutabl and O. El-Shabrawy, *Chemical composition and antimicrobial activity of essential oil of leaf, stem and rhizome of* Alpinia speciosa. Flav. Fragr. J., **10**, 63–67 (1995).
6. V. De Feo, A.I. Ricciardi, D. Biscardi and F. Senatore, *Chemical composition and antimicrobial screening of the essential oil of* Minthostachys verticillata (Griseb.) Epl. (Lamiaceae). J. Essent. Oil Res., **10**, 61–65 (1998).
7. N. Didry, L. Dubreuil and M. Pinkas, *Activité antibactérienne du thymol, du carvacrol et de 1 adéhyde cinnamique seuls ou associés.* Pharmazie, **48**, 301–304 (1996).
8. M. Marino, C. Bersani and G. Comi, *Impedance measurements to study the antimicrobial activity of essential oil from Lamiaceae and Compositae.* Int. J. Food Microbiol., **67**, 187–195 (2001).
9. S. Consentino, C.I.G. Tuberoso, B. Pisano, M. Satta, V. Mascia, E. Arzedi and F. Palmas, *In-vitro antimicrobial activity and chemical composition of Sardinian* Thymus *essential oils*. Lett. Appl. Microbiol., **29**, 130–135 (1999).

10. A. Viljoen, S.V. Vuuren, E. Ernst, M. Klepser, B. Demirci, K.H.C. Baser and B.E.V. Wyk,. *Osmitopsis asteriscoides (Asteraceae)- the antimicrobial and essential oil composition of a Cape Dutch remedy*. J. Ethnopharmacol., **88**, 137–143 (2003).
11. S.G. Deans, R. Noble, R. Hiltunen, W. Wuryani and L. Pénzes, *Antimicrobial and antioxidant properties of* Syzygium aromaticum *(L): Impact upon bacteria, fungi and fatty acid levels in ageing mice*. Flav. Fragr. J., **10**, 323–328 (1995).
12. A. Smith-Palmer, J. Stewart and L. Fyfe, *Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens*. Lett. Appl. Microbiol., **26**, 118–122 (1998).