Industrial Crops & Products 112 (2018) 488–498

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Morphoanatomy and essential oil analysis of *Baccharis trimera* (Less.) DC. (Asteraceae) from Uruguay

Manuel Minteguiaga[a], María I. Mercado[b], Graciela I. Ponessa[b], César A.N. Catalán[c], Eduardo Dellacassa[a,*]

[a] Laboratorio de Biotecnología de Aromas, Departamento de Química Orgánica, Facultad de Química, Universidad de la República, 11800 Montevideo, Uruguay
[b] Instituto de Morfología Vegetal, Área Botánica, Fundación Miguel Lillo, T4000INI-S. M. de Tucumán, Argentina
[c] Instituto de Química Orgánica (INQUINOA-CONICET), Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán. T4000INI-S. M. de Tucumán, Argentina

ARTICLE INFO

Keywords:
Baccharis trimera
Essential oils
GC–MS
Morpho-anatomy
Histochemistry

ABSTRACT

Baccharis trimera (Less.) DC. (Asteraceae), 'carqueja', is a medicinal plant native from South America employed as decoction or infusion for the treatment of low to moderate ailments, and included in the Brazilian Pharmacopeia. This plant is also a rich source of essential oil (EO) valuable in the fragrance industry, mainly owing to carquejyl acetate, carquejol and several sesquiterpenic alcohols contributing to its characteristic aroma reminisce of rosewood. In this work, we report the EO composition, morpho-anatomical and histochemical characterization of *B. trimera* growing wild in Uruguay. 150 compounds were identified by gas chromatography-mass spectrometry (GC–MS), 79 of them not previously reported for this species, whose relative proportions varied according the season. Anatomically, the specimens analyzed did not present any leaves nor ribs between the wings. The epidermis presented polygonal cells with straight anticlinal walls, with anisocytic and less frequently anomocytic stomata. Non-glandular (multicellular, unistratified, with a characteristic elongated apex cell) and glandular (multicellular, bistratified, EO positive) trichomes were evidenced. The mesophyll at the wing level was isolateral with a central spongy parenchyma. Schyzogenous ducts with bistratified secretory epithelium (EO and polyphenols positive), were occasionally associated with the endodermis. The perimedullar parenchyma of the cladodes presented polyhedral pyramidal and prismatic crystals of calcium oxalate, according to previous reports.

1. Introduction

Baccharis trimera (Less.) DC. (synonyms: *B. genistelloides* var. *trimera*, *B. triptera* and *Molina trimera*; vernacular name 'carqueja') is a perennial dioecious shrub-like herb, of 40–50 cm tall, native from Northeastern Argentina, Bolivia, Southern and Southeastern Brazil, Paraguay and Uruguay (Barroso, 1976; Cortadi et al., 1999; Karam et al., 2013). A morphological feature of *B. trimera* is to possess three-winged cladodes, which are responsible for the photosynthesis process since this species has small or absent leaves (Barroso, 1976; Cortadi et al., 1999; Karam et al., 2013). Such cladodes are employed in folk medicine as infusions or decoctions with digestive, analgesic, diuretic, anti-rheumatic, antiseptic, anti-diabetic, anti-spasmodic and aphrodisiac properties, or as a dietary supplement to lose weight (Martínez-Crovetto, 1981; Karam et al., 2013). Several scientific works support the ethnobotanical uses of this plant as vasorelaxant (Gómez et al., 2016), antimicrobial (Quintana de Oliveira et al., 2005; Nunes et al., 2016), analgesic (Gené et al., 1996), anti-ethanol abuse detrimental effects agent (Lívero et al., 2016), anti-ophidic (Janúario et al., 2004), anti-helminthic (Nunes de Oliveira et al., 2014), anti-inflammatory (Gené et al., 1992, 1996; Paul et al., 2009; Nogueira et al., 2011; de Oliveira et al., 2012), antioxidant (Quintana de Oliveira et al., 2004; Vieir et al., 2011; de Oliveira et al., 2012, Moreira et al., 2012), gastroprotective (Biondo et al., 2011), anti-hepatotoxic (Soicke and Leng-Peschlow, 1987) and anti-cancer (de Oliveira et al., 2013). Owing to these medicinal uses, *B. trimera* was included in the 5th Brazilian Pharmacopeia as anti-inflammatory for the treatment of injuries and distensions (ANVISA, 2010). In Argentina, the medicinal 'carquejas' recognized by the 6th Argentinean Pharmacopeia are *B. crispa* Spreng. and *B. articulata* (Lam.) Pers., which are employed and commercialized indistinguishable from *B. trimera* in folk-medicine (Cortadi et al., 1999; Rodrigues et al., 2013). Morpho-anatomical studies of cladodes has been conducted to assess the quality of the plant material and to differentiate *B. trimera* from those related species (Cortadi et al., 1999; Cortadi et al., 1999; Rodrigues et al., 2008;

* Corresponding author.
E-mail address: edellac@fq.edu.uy (E. Dellacassa).

https://doi.org/10.1016/j.indcrop.2017.12.040
Received 9 July 2017; Received in revised form 30 November 2017; Accepted 16 December 2017
0926-6690/ © 2017 Elsevier B.V. All rights reserved.

Budel and Duarte, 2009; Rodrigues et al., 2013).

The phytochemical composition of aerial parts includes caffeoyl quinic acids (Biondo et al., 2011; Aboy et al., 2012), saponins (Gené et al., 1996; Borrella et al., 2006), flavonoids (Soicke and Leng-Peschlow, 1987; Gené et al., 1996; Borrella et al., 2006; Biondo et al., 2011; Gómez et al., 2016), diterpenes (Janúario et al., 2004; Biondo et al., 2011) and essential oils (Naves 1959a,b, Bauer et al., 1978; Chialva and Doglia, 1990; Ferracini et al., 1995; Simões-Pires et al., 2005; Vargas et al., 2006; Silva et al., 2006, 2007; Lago et al., 2008a,b; Alves, 2010; Nunes de Oliveira et al., 2012; Besten et al., 2013; Minteguiaga et al., 2015a,b; Suzuki et al., 2016).

Historically, Naves (1959a,b) examined *B. trimera* essential oil from Santa Catarina (Brazil) isolating, by fractional distillation, the main components: the unusual *o*-menthane skeleton compounds (namely carquejol and carquejyl acetate), nopinone, myrcene, limonene and ledol. Later, Bauer et al. (1978) applied GC-FID to analyze a population of B. trimera from Rio Grande do Sul (Brazil) and found carquejyl acetate (69.2%), β-pinene (8.4%), carquejol (6.8%), α-pinene (6.4%) and camphene (2.6%). Chialva and Doglia (1990) using GC–MS identified 45 components in the industrial oil from Rio Grande do Sul, being carquejyl acetate (42.8%), β-pinene (8.2%), palustrol (5.7%), germacrene D (4.2%) and carquejol (2.0%) the predominant constituents (Chialva and Doglia, 1990). 'Carqueja' oil has become an economic valuable aromatic resource that is obtained in large-scale and employed by the fragrance industry for it reminiscence to rosewood oil (Chialva and Doglia, 1990; Ferracini et al., 1995; Simões-Pires et al., 2005; Silva et al., 2007). This oil has been reported as anti-helminthic (Nunes de Oliveira et al., 2012) and antimicrobial (Suzuki et al., 2016). It is relevant to state that despite its importance as medicinal and aromatic resource and its potential as industrial crop, *B. trimera* is considered a not desirable weed in Uruguay and South Brazil and discarded without exploitation (Scheffer-Basso et al., 2008).

The aim of this paper is to bring up to date the information on the chemical composition of the essential oil from this species of *Baccharis* coming from Uruguay, and describe anatomical and histochemical features of their aerial parts to identify secretory structures responsible of the EO production.

2. Materials and methods

2.1. Plant material

Vegetative organs (cladodes and roots) of *B. trimera* individuals randomly selected were collected at vegetative (VE – July 2011 and September 2016) and blooming stage (BLO – March 2016) in '*Estación Porvenir*' (32°21′56,5"S; 57°54′02,1"O), Paysandú Province, Uruguay (Fig. 1). Voucher specimens were deposited at the Herbarium 'Arechavaleta' from Faculty of Chemistry (MVFQ, UdelaR): *B. trimera* (Less.) DC. M. Minteguiaga MVFQ 4420. The species was identified by Prof. Eduardo Alonso-Paz,[1] by comparing with other herbarium specimens MVFQ 4359 (Gómez et al., 2016).

Collected plant material was dried at room temperature (1 week) and fixed in formaldehyde-acetic acid-water-ethanol (FAA) for histological studies or use fresh for histochemical characterization.

2.2. Essential oil extraction

For essential oil extraction, 200 g of dried aerial parts of VE and BLO stages collections were submitted individually to hydro-distillation for 90 min using a Clevenger-type glass device (Bicchi and Maffei, 2012). To obtain greater oil volume for fractionating, 30 kg of BLO stage plant material were submitted to extraction in a pilot steam distillation unit (Eysseric Company, Nyons, France) located at Agricultural Research National Institute INIA-Las Brujas (Rincón del Colorado, Canelones Province, Uruguay) (Davies, 2004). Anhydrous sodium sulfate was added to the oil (approximately 45 mL) to eliminate remaining water being then stored in an amber flask at −4 °C until chemical analyses.

Fig. 1. *Baccharis trimera* (Less.) DC. from Uruguay.
General aspect of the wild plant at blooming stage in the place of collection (Paysandú Province, 32°21′56,5"S; 57°54′02,1"O).

2.3. Column chromatography fractionation (CCF)

A total of 43 mL of pure *B. trimera* essential oil (BLO stage) was fractionated in two-steps by column chromatography (50 cm × 5.5 cm i.d.) packed with 530 g of activated silica gel (230–400 mesh; Merck, Darmstadt, Germany). The elution was performed isocratically with hexane: AcOEt (15:1) at a flow rate of 4.5 mL min^{-1}; fractions volume collection: 40 mL. For monitoring CCF separation, TLC was performed over Si-gel commercial plates (Si-gel60F_{254}; Merck) with hexane: AcOEt (15:1) as mobile phase. Fractions 1–59 were monitored by spraying with *p*-anisaldehyde-sulphuric acid (Sigma-Aldrich, St. Louis, MI, USA) (Wagner et al., 1984) and grouped into six final fractions (1–6) based on their TLC profiles (Table 1).

2.4. GC–MS analyses

For GC–MS analyses, three cross-linked capillary columns of equal dimensions (30 m × 0.25 mm i.d. × 0.25 μm film thickness) were attached sequentially to a Shimadzu GC–MS QP2010 Ultra (Shimadzu Corporation, Kyoto, Japan). The column 1 was a non-polar HP-5MS (95%-dimethyl-5%-diphenylpolysiloxane; Agilent Technologies, Walt & Jennings Scientific, Wilmington, DE, USA); column 2 was a medium-polar OV-225 (50%-methyl-25%-cyanopropyl-25%-phenylsilicone; Quadrex Corporation, Woodbridge, CT, USA); and the column 3 was a polar Stabilwax (100%-polyethylenglycol; Restek Corporation, Bellefonte, PA, USA). The experimental conditions employed to analyze the pure essential oil (EO) or vacuum-concentrated fractions diluted (1:10) in CH_2Cl_2 (Dorwil, Buenos Aires, Argentina), were as follows. Column 1. Injector, interface and ion source, 280 °C; temperature program: 40 °C (4 min), 40–180 °C at 4 °Cmin^{-1}, 180 °C (2 min), 180–280 °C at 10 °C min^{-1}, 280 °C (10 min).

Column 2. Injector, interface and ion source, 230 °C. Temperature

[1] Paper *in memoriam* of Prof. Eduardo Alonso-Paz.

Table 1
Composition of *Baccharis trimera* essential oil from Uruguay according to GC–MS analyses.

Compound[a]	% VS[b]	% BS[c]	LRI1					Det. F.[f]
				1r[d]	2	3	3r[e]	
Hexanal	tr	–	801	801	1060	1071	1084[x]	EO
2-(E)-Hexenal	tr	–	847	846	nd	1205	1214[x]	EO
Tricyclene	0.01	tr	920	921	1032	1013	1009	1,5
α-Thujene	0.07	0.04	925	924	1036	1015	1038	EO,1,5
α-Pinene[1,2,6,7,8,11]	0.20	0.09	930	932	1037	1010	1036	EO,1,4–6
Camphene[1]	0.03	0.02	945	946	1057	1048	1083	EO,1,5
2-(E)-Heptenal	tr	–	951	947	nd	1311	1336[x]	EO
Benzaldehyde	tr	–	958	952	nd	1487	1525[x]	EO
Sabinene[1,2,11]	0.70	0.7	972	969	1088	1112	1130	EO,1–6
β-pinene[1,2,6,7,8,9,10,11]	2.60	2.90	976	974	1081	1097	1124	EO,1–3,5-6
α-Methylstyrene	0.03	tr	982	974[#]	1206	nd	1325[x]	5
6-Methyl-5-hepten-2-one	0.02	tr	987	981	1268	1329	1319[x]	2
Myrcene[1,2,6,8,9,11]	0.30	0.30	991	988	1101	1158	1156	EO,1,3,5–6
2-Octanol	–	tr	1002	994	nd	nd	1422[x]	6
Octanal	0.01	–	997	998	1246	1270	1300[x]	EO
α-Phellandrene[1,8]	0.03	0.03	1003	1002	nd	1152	1177	EO,1,4–5
3-(Z)-Hexenyl acetate	–	tr	1007	1004	nd	nd	1320[x]	2
δ-(3)-Carene	–	0.01	1008	1008	nd	1107	1141	EO,1
2,4-(E,E)-Heptadienal	–	tr	1011	1005	1359	1481	1497[x]	3
o-Cymene[1]	0.03	–	1012	1011[x]	nd	1251	1268[#]	EO
o-Isopropenyl toluene[y]	–	0.01	1012	nav	nd	nd	nav	EO,1,5–6
α-Terpinene[1]	0.05	0.01	1016	1014	1125	1167	1188	EO,1,5–6
3-Isopropenyl-4-methylene-cyclohexene (Carquejene)[y]	–	0.11	1022	nav	nd	nd	nav	EO,1,5
p-Cymene[1]	0.10	tr	1023	1022	1193	1258	1272	3–4,6
Limonene[1,2,3,8,10,11] + β-Phellandrene[1]	1.90	2.5	1028[c]	1024, 1025	1133, 1151	1188, 1151	1206, 1216	EO,1–6
1,8-Cineol [1,8]	0.05	0.02	1030	1026	1183	1197	1228	EO,2,6
(Z)-β-Ocimene[1]	0.02	0.05	1038	1032	nd	1230	1228	EO,1,3,5
β-Isophorone	tr	0.10	1039	1044	nd	1395	1416[x]	EO,2,3,6
(E)-β-Ocimene[1,2,3,10,11]	0.6	2.20	1049	1044	1158	1249	1250	EO,1–6
2-(E)-Octenal	–	tr	1056	1049	1371	1407	1434[x]	2
γ-Terpinene[1,8]	0.07	0.02	1057	1054	1176	1230	1251	EO,1,3,5–6
(Z)-Sabinene hydrate	0.04	0.01	1067	1065	1338	1446	1465[x]	EO,1–5
Terpinolene[1]	0.08	0.07	1087	1086	1209	1271	1287	EO,1,3,5
p-Cymenene	–	tr	1087	1089	nd	nd	1433[x]	6
Nonanal	0.05	–	1093	1100	1352	1384	1402[x]	EO
(E)-Sabinene hydrate	0.03	tr	1098	1098	1401	nd	1463	3
Rosefuran	–	0.01	1098	1093[x]	nd	nd	1415[x]	EO,1,5
Linalool[1,10]	0.40	tr	1101	1095	1382	1530	1555	3,4
6-Methyl-3,5-(E)-heptadien-2-one	–	tr	1106	1108[x]	nd	nd	1602[x]x	4
p-Menth-1,3,8-triene	0.20	0.01	1108	1108	nd	nd	1598[#]	EO,1,5
endo-Fenchol	–	tr	1112	1114	nd	1565	1574	2,3
4,8-dimethyl-1,3,7(E)-Nonatriene	–	0.03	1117	1116[x]	nd	nd	1304*	EO,1,5
(Z)-p-Menth-2-en-1-ol	0.09	–	1118	1118	1408	nd	1560	EO
α-Isophorone	0.20	0.03	1121	1118	1335	1395	1402[#]	EO,1–6
allo-Ocimene	0.03	–	1130	1128	1392	1355	1392[x]	EO
p-Menth-1,5,8-triene	–	0.01	1130	1135*	nd	nd	1216[x]	EO,1,5
(E)-Pinocarveol	0.30	tr	1138	1135	1451	1627	1632[x]	4
allo-neo-Ocimene	–	tr	1142	1140	nd	nd	1391[x]	1
4-Ketoisophorone	0.50	tr	1146	1140	nd	nd	1680[x]	4
Carquejol[1,2,8,10,11]	3.50	0.50	1160	1151[#]	1477	1664	nav	EO,1–5
o-Mentha-1(7),8-dien-3-ol (Dihydrocarquejol)[y]	–	tr	1167	nav	nd	nd	nav	3
Pinocarvone	0.80	0.01	1164	1160	1485	1535	1545[x]	EO,2
Terpinen-4-ol	0.20	0.04	1177	1174	1458	1576	1628	EO,1–3
2-Isopropenyl – 3-methylphenoly[z]	–	0.10	1181	nav	nd	nd	nav	EO,2–3
p-Cymen-8-ol	0.80	–	1183	1179	1609	1816	1846	EO
Cryptone[8]	–	tr	1184	1183	nd	nd	1690[x]	4
α-Terpineol[4,8,10]	1.40	0.01	1189	1186	1520	1670	1731	EO,1–4,6
Myrtenol	–	tr	1189	1194	nd	1764	1786[x]	6
Myrtenal[8]	0.30	0.01	1194	1195	1547	1596	1619[x]	EO,2
Safranal	0.2	0.01	1199	1196	1535	1615	1596[x]	EO,2
Eucarvone[1]	–	0.30	1218	1222	1622	nd	nav	EO,2,3,6
4-Methylene-isophorone	0.80	–	1220	1216	nd	nd	nav	EO
β-Citronellol	–	tr	1230	1223	nd	nd	1722	6
Cuminaldehyde	4.20	tr	1240	1238	1634	nd	1789[x]	2
Neral[11] + Carvone	2.90	tr	1246 (co)	1235, 1239	1613, nd	1657, nd	1680, 1715	2
p-Anisaldehyde	0.09	tr	1258	1247	nd	nd	2032[x]	4
(Z)-Chrysanthenyl acetate	0.08	0.10	1265	1261	1493	nd	1544[x]	EO,1,5
Geranial[11]	3.90	tr	1274	1264	1657	nd	1730	3,4
p-Cymen-7-ol	0.10	0.1	1291	1289	nd	nd	2097[x]	EO,6
Carquejyl acetate[1,2,3,8,10,11]	23.50	71.40	1300	1298	1597	1685	1669[#]	EO,1–6
2,4-(E,E)-Decadienal	–	tr	1321	1315	nd	1782	1812[x]	2,3

(*continued on next page*)

Table 1 (*continued*)

Compound[a]	% VS[b]	% BS[c]	LRI1					Det. F.[f]
				1r[d]	2	3	3r[e]	
(Z)-Pinocarvyl acetate	0.30	0.1	1322	1311	nd	nd	nav	EO,2,5
Myrtenil acetate[8]	0.06	–	1327	1324	1565	nd	1720	EO
α-Cubebene[1,8]	0.06	0.04	1352	1345	1422	1440	1458	EO,1,5–6
α-Longipinene[8]	0.05	tr	1352	1350	1428	nd	1541	6
Neryl acetate	0.05	0.02	1366	1359	1629	nd	1699	EO,2,5
(Z)-Carvyl acetate	0.08	–	1367	1365	nd	nd	1795	EO
Cyclosativene	0.30	0.05	1370	1369	1440	nd	1549	EO,5,6
Isoledene[6,7]	0.08	0.20	1370	1374	nd	nd	1559[#]	EO,1
α-Ylangene[1]	0.10	tr	1373	1373	nd	1468	1491	1,6
α-Copaene[1,5,8,9,10]	0.30	0.20	1377	1374	nd	1472	1493	EO,1,5–6
β-Bourbonene	–	0.03	1382	1387	nd	1501	1526	EO,5
2-epi-α-Funebrene	0.05	–	1385	1380	nd	nd	nav	EO
Myrtanil acetate[8]	0.10	–	1388	1385	1666	nd	1754[#]	EO
β-Cubebene[1,5,8]	0.20	0.10	1391	1387	1504	1516	1541	EO,1,4–5
β-Elemene[1,2,3,5,6,7,8,9,11]	0.60	0.30	1394	1389	nd	1569	1591	EO,1–2,4-6
Longifolene	–	0.01	1400	1407	nd	1584	1574	EO,4–5
Methyl eugenol	0.06	tr	1408	1403	nd	1976	2006[#]	2,3
β-Funebrene	–	0.04	1409	1413	nd	nd	nav	EO,1,5
α-Gurjunene[5,8,10]	0.30	0.30	1410	1409	1491	nd	1529	EO,1,4–6
β-cedrene	0.20	–	1416	1419	nd	nd	1633	EO
(E)-β-Caryophyllene[1,2,5,6,8,9,10,11]	0.40	0.50	1419	1417	1558	1572	1617	EO,1,5
γ-Elemene[10]	0.06	0.02	1432	1434	nd	nd	1642	EO,5–6
Aromadendrene[6,7,8]	0.20	tr	1437	1439	nd	1619	1650	6
α-Guaiene[5,9]	0.06	tr	1440	1437	nd	nd	1651	1,5
α-Patchulene[7]	0.20	–	1448	1454	nd	nd	1793[#]	EO
dehydro-Aromadendrene	–	0.03	1448	1460	nd	1774	nav	EO,5–6
α-Himachalene[8]	0.20	0.10	1448	1449	1574	nd	1649	EO,1,5
α-Humulene[5,6,7,9,10,11]	0.10	0.10	1453	1452	1683	1645	1672	EO,1,5
Geranyl acetone	–	tr	1454	1453	nd	1829	1848[x]	2
(E)-β-Farnesene[8]	0.30	0.30	1459	1454	1595	1643	1668	EO,2,5
allo-*Aromadendrene*[8]	0.20	0.01	1464	1458	1660	nd	1662	EO,1,4–6
β-Acoradiene	0.10	–	1470	1469	nd	nd	1693[#]	EO
γ-Gurjunene[2,5,6,8,10]	0.40	0.09	1474	1475	1601	nd	1650[#]	EO,5–6
(10-epi)-β-Acoradiene	0.20	–	1477	1474	nd	nd	nav	EO
γ-Muurolene[5,6,8,9,10]	0.20	0.20	1478	1478	1614	1671	1692	EO,6
Germacrene D[1,3,5,6,7,8,9,10,11]	0.80	1.80	1483	1484	nd	1684	1712	EO,1,4–5
(E)-β-Ionone	–	tr	1485	1487	nd	nd	1936[x]	2
γ-Himachalene	0.80	–	1487	1481	nd	nd	1723	EO
β-Selinene[5]	0.30	0.20	1487	1489	1643	nd	1756	EO,1,5–6
(E)-muurola-4(14),5-diene	0.40	0.08	1492	1493	nd	nd	1633x	EO,5
Ledene[1]	0.30	tr	1495	1496	nd	nd	1695[#]	6
β-Himachalene	–	tr	1498	1500	nd	nd	1718	4,5
Bicyclogermacrene[1,2,5,6,7,9,11]	0.40	1.20	1498	1500	1679	1707	1744	EO,1,5
α-Muurolene[1,5,6,8,9]	0.20	0.20	1502	1500	nd	1699	1730	EO,1,5
Germacrene A[6,7]	–	0.60	1503	1508	nd	nd	1757[x]	EO,1,5
α-Bulnesene[5,10]	0.40	–	1509	1509	nd	nd	1729	EO
δ-Amorphene	–	tr	1511	1511	nd	nd	1722[x]	6
δ-Cadinene[1,2,3,5,6,7,8,9,10]	0.50	0.50	1525	1522	1671	1732	1761	EO,1–2,4-6
(E)-Cadina-1,4-diene[6,7]	–	0.02	1531	1533	nd	1761	1786	EO,1,4–6
α-Cadinene[6]	0.10	0.01	1536	1537	nd	1770	1815[x]	EO,1,5–6
(E)-α-Bisabolene	–	0.01	1543	1544x	1640	nd	1766	EO,5
α-Calacorene[1,5,6,7,8]	0.30	0.04	1544	1544	1795	1882	1916	EO,1,4–6
Elemol[1,2,8,10]	0.30	0.10	1554	1548	nd	2048	2078	EO,1–6
Germacrene B[5,6,7]	–	tr	1555	1559	nd	nd	1572	1,5
(E)-Nerolidol[6,8]	0.10	tr	1565	1561	1877	2011	2044	3
Palustrol[1,3,4,8]	7.80	4.40	1574	1567	1851	1903	1931[x]	EO,1,5–6
Spathulenol[1,2,4,5,6,8,9,10,11] + Germacrene D-4-ol	3.80	0.50	1582[c]	1577, 1574	1966, 1908	2093, 2017	2153, 2038	EO,2–4
Caryophyllene oxide[6,8,10]	1.30	0.20	1587	1582	1945	1944	1966	EO,2
Viridiflorol[1,4,5,6,8,9,10] + *Globulol*[2,4,5,6,7,9,11]	3.90	0.90	1596 (co)	1592, 1590	1934, nd	2054, 2044	2112, 2104	EO,1,3–4,6
Ledol[1,2,5,8,9,10,11]	2.70	0.80	1607	1602	1928	2001	2028[x]	EO,4–5
Humulene epoxide II	0.70	–	1617	1608	1984	2007	2011	EO
α-Corocalene	0.10	–	1628	1622	nd	nd	2037[#]	EO
γ-Eudesmol[1,8,10]	0.40	0.02	1633	1630	nd	nd	2182	EO,1,4
epi-α-Cadinol[5,6]	0.20	0.02	1638	1638	nd	nd	nav	EO,1,5
δ-Cadinol[8]	0.70	0.08	1645	1644	nd	2174	2150	EO,2
α-Cadinol[2,5,6,7,8,9]	–	0.10	1653	1652	nd	2200	2224	EO,5–6
β-Eudesmol[1,2,3,8,10]	4.80	0.80	1656	1649	2059	nd	2248	EO,1–2,4-6
Cadalene	1.00	tr	1677	1675	2050	2188	2203	1,5
α-Bisabolol	0.20	tr	1687	1685	nd	nd	2022	3
Germacra-4(15),5,10(14)-trien-1-α-ol	0.30	–	1692	1685	nd	2331	nav	EO
Cyclocolorenone	0.08	–	1764	1759	nd	nd	2254[#]	EO
β-Costol	–	tr	1764	1766	nd	nd	2606x	2

(*continued on next page*)

Table 1 (*continued*)

Compound[a]	% VS[b]	% BS[c]	LRI1					Det. F.[f]
				1r[d]	2	3	3r[e]	
14-Hydroxy-δ-cadinene	0.05	–	1809	1803	nd	nd	2607[#]	EO
Neophytadiene	0.05	0.08	1837	1838[x]	nd	nd	1912[x]	EO,1,5–6
Palmitic acid	0.09	–	1967	1984[x]	nd	2829	2860[x]	EO
Total identified (%)	90.10	97.20						
Monoterpene Hydrocarbons (%)	7.00	9.10						
Oxygenated Monoterpenes (%)	43.20	72.30						
Sesquiterpene Hydrocarbons (%)	10.50	7.30						
Oxygenated Sesquiterpenes (%)	27.30	7.90						
Others (%)	2.10	0.60						

(a), compounds in italic were previously reported for the same species: (1) Chialva and Doglia (1990), (2) Simões-Pires et al. (2005), (3) Vargas et al. (2006), (4) Silva et al. (2006), (5) Silva et al. (2007), (6) Lago et al. (2008a), (7) Lago et al. (2008b), (8) Alves, 2010, (9) Nunes de Oliveira et al. (2012), (10) Besten et al. (2013), (11) Suzuki et al. (2016).
(b and c), area percentage in Full-Scan Mode.
(d), LRIs reported by Adams (2007).
(e), LRIs reported by Davies (1990); (f), identified in the essential oil (EO) or in the fractions obtained by CCF (numbers from 1 to 6).
(tr), traces (< .05%).
(co), co-elution; nd, not detected; nav, not available.
([x]) and (#), LRIs reported in electronic databases by Linstrom and Mallard (2017) and El-Sayed (2003–2017), respectively.
([y]) tentatively determined (without LRI confirmation).
([z]) this compound was identified by comparison of mass spectrum and LRI with the pure semi-synthetic product obtained and spectroscopically characterized by us. These results will be published elsewhere.

program: 60 °C (4 min), 60–160 °C at 2 °C min^{-1},160–230 °C at 20 °C min^{-1}, 230 °C (10 min). Column 3. Injector, interface and ion source, 250 °C. Temperature program: 40 °C (4 min), 40–180 °C at 5 °Cmin^{-1}, 180–220 °C at 10 °C min^{-1}, 220 °C (10 min), 180–240 °C at 20 °C min^{-1}, 240 °C (10 min). In all the cases, the ionization energy was 70 eV; injection size, 1 μL (split ratio 25:1) and helium (99.9999%; Linde, Munich, Germany) was used as carrier gas at a flow rate of 1.0 mL min^{-1}. Mass range: 35–400 *m/z*.

2.5. Identification and quantification of essential oil components

The components of the EO were identified by comparison of their mass spectra with those stored in the GC–MS databases (McLafferty and Stauffer, 1994; Adams, 2007). Linear retention indices (LRIs) were calculated for every identified compound in relation to a series of n-alkanes C_8-C_{20} (Sigma-Aldrich), and subsequently compared with databases (Davies, 1990; Adams, 2007; El-Sayed, 2003–2017; Linstrom and Mallard, 2017). For the stationary phase of the column 2, no LRI database values are available so to compare the results obtained from *B. trimera* oil in this stationary phase, a well-chemical characterized essential oil (lemon) was analyzed in the same analytical conditions.

The raw percentages of area of every compound peak in full-scan GC–MS analyses (HP-5MS) are informed without standardization, because our objective was not quantify but to identify a greater amount of oil components independently of their concentration.

2.6. Morpho-anatomical and histochemical analyses

For histological characterization of *B. trimera*, cladodes and roots of ten individuals (collection made in September 2016) were selected. Five of them were processed fresh and the remaining five were preserved in FAA solution (formaldehyde-acetic acid-water-ethanol; 1:5:0.5:3.5). For epidermis analyses, the samples were diaphanized according to Dizeo de Strittmater (1973). Fixed portions between the 3rd and the 4th internode of the cladodes and the middle section of the roots (2 cm long fragments) were selected and mounted in dental wax and then sectioned (15–20 μm film thickness) using a rotatory Microtome Microm HM 315 (GMI Inc., Ramsey, MN, USA). The sectioned material was cleared with sodium hypochlorite (50% in distilled water), then washed and double stained with cresyl violet or successively with Astra Blue and Safranin O (Sigma Aldrich). The nomenclature proposed by Dilcher (1974) was followed for stomata classification. The histochemical assays were performed in sections obtain from fresh material, according to D'Ambrogio de Argüeso (1986) and Zarlavsky (2014), employing the following reagents: ferric chloride for polyphenols (positive reaction visualized as black spots), Sudan IV solution for lipids (positive results: red color) and Nadi for essential oils (positive results for terpene compounds: dark blue). The starch granules were visualized through lugol staining and sequential observation over polarized light (Zarlavsky, 2014). After stained, the samples were mounted in a distilled water-glycerin (1:1) solution. Photographs were recorded with a Carl Zeiss Lab A1 Axiolab Optical Microscope attached to an optical camera AxioCam ERc 5 s and with the Axio Vision 4.8.2 Software for measurement of dimensions (Carl Zeiss, Oberkochen, Germany).

3. Results and discussion

3.1. General aspect of the wild plant

The general aspect of wild *B. trimera* at blooming stage in the site of collection (Paysandú province, 32°21′56,5”S; 57°54′02,1”O; Uruguay) is showed in Fig. 1. This plant occurs naturally both in native grasslands and in altered sites, i.e. sides of the roads and railways or in abandoned farmlands. According to our field observations, the blooming period of this plant in Uruguay occurs from middle summer (early January) to the early autumn (March-April). The same phenology pattern was reported for the species in South Brazil (Scheffer-Basso et al., 2008; Alves, 2010, Besten et al., 2013).

3.2. Essential oil composition

Table 1 summarizes the essential oil composition of *B. trimera* aerial parts determined by GC–MS in three capillary columns with different polarities.

Analysis of *B. trimera* essential oils obtained by hydrodistillation was performed using capillary GC–MS after fractionation on column chromatography. 150 volatile compounds were identified by GC–MS with three different capillary columns; 79 of these compounds have not previously been reported for this species. In the direct analysis of pure oil (with non-polar phase capillary column and without fractionation) only 106 compounds were identified. This fractionation approach presented here increased in 45% the peak capacity of the analysis,

detecting trace-level compounds that otherwise cannot be detected. There is a unique report in the literature that employed bidimensional GCxGC/TOFMS for the analysis of *B. trimera* oil, but it only informed the presence of 61 compounds (Alves, 2010). Meaning that, our fractionation approach was a powerful strategy for detecting trace-level compounds comparable to bidimensional chromatographic instrumental systems. In brief, our system presented two non-hyphenated separative dimensions with the first one, CCF, being not instrumental.

The main compound in the *B. trimera* oils from Uruguay was the irregular monoterpene carquejyl acetate, but with an important percentage variation between the winter vegetative (23.5%) and summer blooming (71.4%) stages. The alcohol carquejol demonstrated an opposite trend: 3.5% in winter and 0.5% in summer. In previous reports where *B. trimera* was harvested and distilled at different times, in the same collection site, showed a wide variation in the carquejyl acetate and carquejol contents between months (Simões-Pires et al., 2005; Alves 2010). Broad variation in the content of both compounds was also found when different collection sites or different plant organs were evaluated (Simões-Pires et al., 2005; Alves, 2010; Besten et al., 2013). However, no appreciable variation was found for male and female volatile profiles (Besten et al., 2013).

The role of carquejyl acetate as a chemical marker for *B. trimera* is currently in discussion, because while many authors report this compound as the main component of the oils (Bauer et al., 1978; Chialva and Doglia, 1990; Simões-Pires et al., 2005; Vargas et al., 2006; Alves, 2010; Besten et al., 2013; Minteguiaga et al., 2015a; Suzuki et al., 2016), others found absence of it (Silva et al., 2006, 2007; Lago et al., 2008a,b; Nunes de Oliveira et al., 2012; Minteguiaga et al., 2015b). Recently, we found *B. trimera* populations from Southern Brazil without carquejyl acetate in their volatile profile, but its presence as minor compound in other 'carqueja' species: *B. articulata* and *B. milleflora* (Minteguiaga et al., 2015a,b). According to our previous results, the *B. trimera* populations from Uruguay can be considered as homogeneous in their volatile metabolism since all the essential oils samples analyzed presented carquejyl acetate as major constituent (Minteguiaga et al., 2015a).

Besten et al. (2013) suggests considering the existence of two chemotypes of *B. trimera*: plants containing carquejyl acetate in their oil composition and those without it. The role of carquejol and carquejyl acetate in nature is not completely understood while the biosynthetic origin in plants requires more studies. Bohlmann and Zdero (1969) had hypothesized that the rearrangement of β-pinene could generate the *o*-menthane skeleton and sequentially, the synthesis of carquejol and piquerols A and B. Other *o*-menthane skeleton compounds (*o*-isopropenyl-toluene, 3-isopropenyl-4-methylene-cyclohexene (carquejene), dihydrocarquejol and 2-isopropenyl-3-methylphenol were found in this work showing the ability of this species to synthesize *o*-menthane derivatives. Interestingly, carquejene is an intermediate proposed by Bohlmann and Zdero (1969) for the possible biosynthetic pathway of carquejol. Thus, indicating that the results here reported could be a preliminary confirmation of the hypothesis proposed by these authors.

Other important compounds reported in this work for *B. trimera* essential oil are β-pinene (2.6% at VE stage *vs.* 2.9% at BLO stage, respectively), limonene (co-eluted with β-phellandrene; 1.9% *vs.* 2.5%), (*E*)-β-ocimene (0.6% *vs.* 2.2%), germacrene D (0.8% *vs.* 1.8%), palustrol (7.8% *vs.* 4.4%), spathulenol (co-eluted with germacrene D-4-ol; 3.8% *vs.* 0.5%), viridiflorol (co-eluted with globulol; 3.9% *vs.* 0.9%), ledol (2.7% *vs.* 0.8%) and β-eudesmol (4.8% *vs.* 0.8%). All of them have been previously reported as important components of the *B. trimera* oils (Chialva and Doglia, 1990; Simões-Pires et al., 2005; Vargas et al., 2006; Silva et al., 2006, 2007; ; Alves, 2010; Nunes de Oliveira et al., 2012; Besten et al., 2013; Minteguiaga et al., 2015a,b; Suzuki et al., 2016).

However, an interesting fact we found is the presence (in the oil obtained at VE stage) of many aldehydes that were not detected (or only detected at trace level) at BLO stage, among them, hexanal, 2-(*E*)-hexenal, 2-(*E*)-heptenal, benzaldehyde, octanal, nonanal, cuminaldehyde, neral, *p*-anisaldehyde and geranial. Besides, other aldehydes such as 2,4-(*E,E*)-heptadienal, 2-(*E*)-octenal and 2,4-(*E,E*)-decadienal, were identified only at BLO stage after a carefully identification by oil fractionation and posterior analysis by GC–MS. This metabolic behavior could be consequence of the environmental conditions or needs for attracting pollinators at blooming stage, since these plants are dioecious and pollinated by insects (Ferracini et al., 1995; Lago et al., 2008a; Besten et al., 2013).

In this work, the presence of C_9 norisoprenoids isophorone derivatives (α-isophorone, β-isophorone, 4-ketoisophorone and 4-methyleneisophorone) was reported for the first time in *B. trimera* oil. The importance of these derivatives remains in that they are the compounds responsible for saffron aroma (Condurso et al., 2017) and might be responsible for the particular organoleptic characteristics of 'carqueja' oil. In addition, it was possible the identification of the C_{13} norisoprenoid (*E*)-β-ionone, a norisoprenoid originated in the breakdown of carotenoids present in different grapes varieties where confers appreciable aromas to the wines (Mendes-Pinto, 2009). Other compounds such as 6-methyl-5-hepten-2-one (C_8, sulcatone), 6-methyl-3,5-(*E*)-heptadien-2-one (C_8), rosefuran (C_{10}) and 4,8-dimethyl-1,3,7-(*E*)-nonatriene (DMNT) (C_{11}), originated in the breakdown or rearrangement of higher terpenes, were also identified in this work.

The environmental variables in general have a great influence over the *Baccharis* plants physiology as demonstrated by Silva et al. (2006, 2007). Those findings, showing the edaphoclimatic influence on the secondary metabolism of *B. trimera*, could be the main reason to explain polymorphism in essential oil composition (Silva et al., 2007).

3.3. Morpho-anatomical and histochemical studies

B. trimera cladodes, as were previously described by Budel and Duarte (2009), did not present leaves or evident ribs and tended to be circular shaped with three equidistant wings (Fig. 2A). There is controversy with respect to the characters described for the cladodes of this species since the 5th Brazilian Pharmacopeia (ANVISA, 2010) and Barroso and Bueno (2002) reported the presence of scarce sessile leaves, and conspicuous ribs between the wings (also observed by Cortadi et al., 1999) which were not evidenced in this work.

Similarly, to other *Baccharis* species sect. Caulopterae (i.e. *B. crispa*, *B. articulata* and *B. microcephala*) and other populations of *B. trimera* (Cortadi et al., 1999; Rodrigues et al., 2008; Budel and Duarte, 2009), the epidermis of the axis and wings of *B. trimera* from Uruguay consisted of polygonal cells with straight anticlinal walls (Fig. 2B). Anomocytic and anisocytic stomata (27.54 ± 1.95 μm long. × 26.34 ± 1.41 μm width) (Fig. 2B–C) were observed in accordance to previous observations for this species (Cortadi et al., 1999; Rodrigues et al., 2008; Budel and Duarte, 2009).

Multicellular non-glandular (tector) trichomes, uniseriated with 3–4 lignified basal cells (53.57 ± 10.52 μm long.) with an erect, acute apical elongated cell, with thickened non-lignified walls (98.11 ± 12.34 μm long.) were evidenced (Fig. 2D–F). These results are in accordance with Rodrigues et al. (2013) and only with one of the three types of tector trichomes described for the same species in the 5th Brazilian Pharmacopeia (ANVISA, 2010).

Short biseriated glandular trichomes with a pair of basal cells, 2–3 pairs of body cells and a pair of terminal cells (29.20 ± 4.41 μm long. x 21.89 ± 6.44 μm width) with the cuticle slightly expanded around them were observed, as previously reported by Cortadi et al. (1999) and Budel and Duarte (2009). Glandular trichomes presented positive reactions for lipids and EOs with Sudan IV and Nadi reagents, respectively (Fig. 2G–J). In general, EOs are the content most frequently associated with glandular trichomes, whose products are synthesized by apical cells and stored in the expanded subcuticular space (Cortadi et al., 1999; Budel et al., 2012).

The non-glandular and glandular trichomes were frequently found

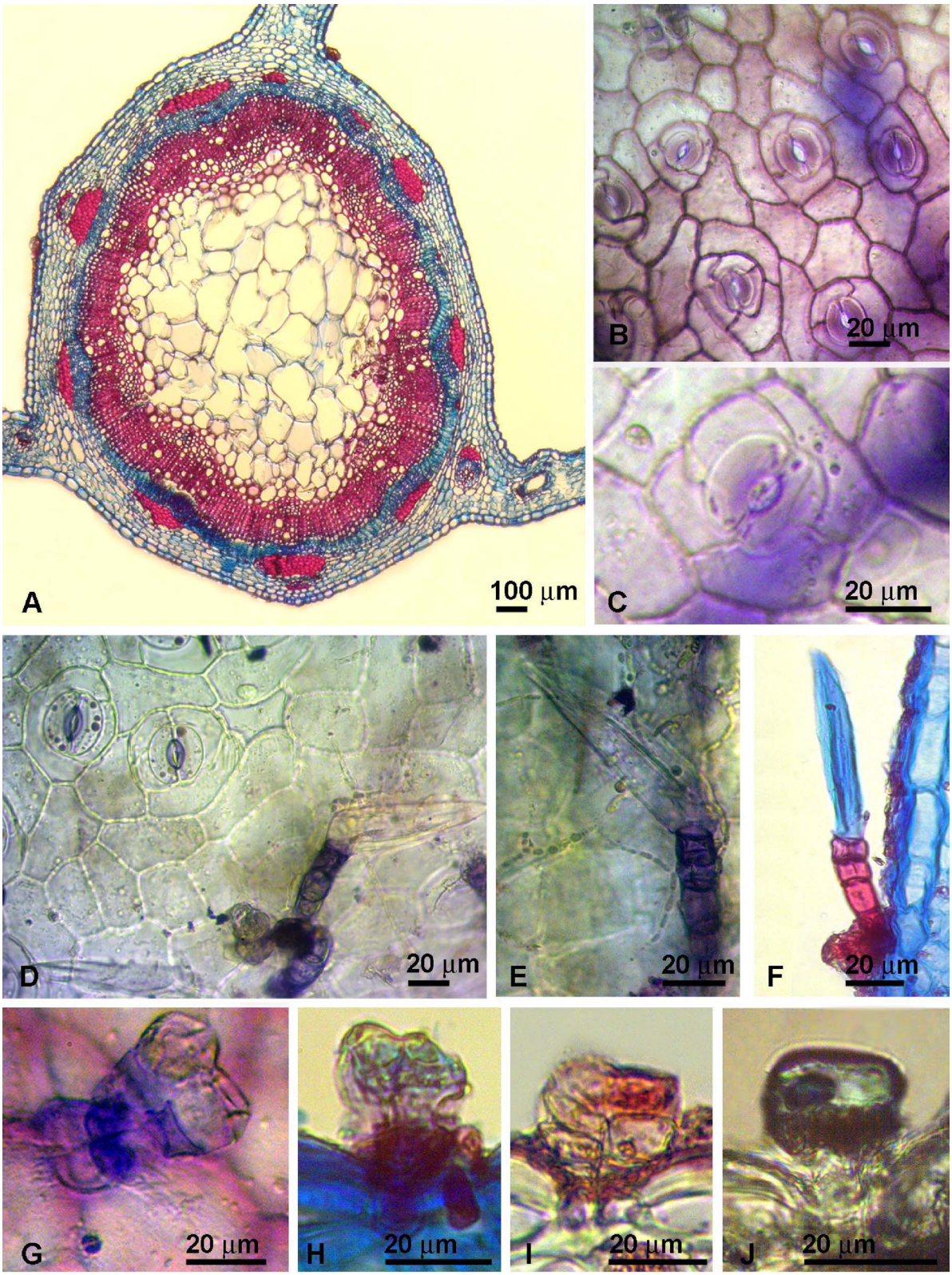

Fig. 2. *Baccharis trimera* (Less.) DC. from Uruguay.
A. Transversal section of a cladode. **B.** Paradermal view of the epidermis. **C.** Anomocytic stoma. **D.** Non-glandular and glandular trichomes in geminated groups. **E–F.** Non-glandular trichomes: multicellular, uniseriated with lignified basal cells, and a longer apical cell with thicker cellulosic non-lignified walls and an acute apex. **E.** Paradermal view stained with cresyl violet. **F.** Transection stained successively with Astra Blue and Safranin O. **G–J.** Glandular multicellular trichome, biseriated. **G.** Paradermal view stained with cresyl violet. **H.** Transection stained with Astra Blue-Safranin O. **I.** Positive result for lipids with Sudan IV reagent. **J.** Positive result for essential oils with the Nadi reagent.

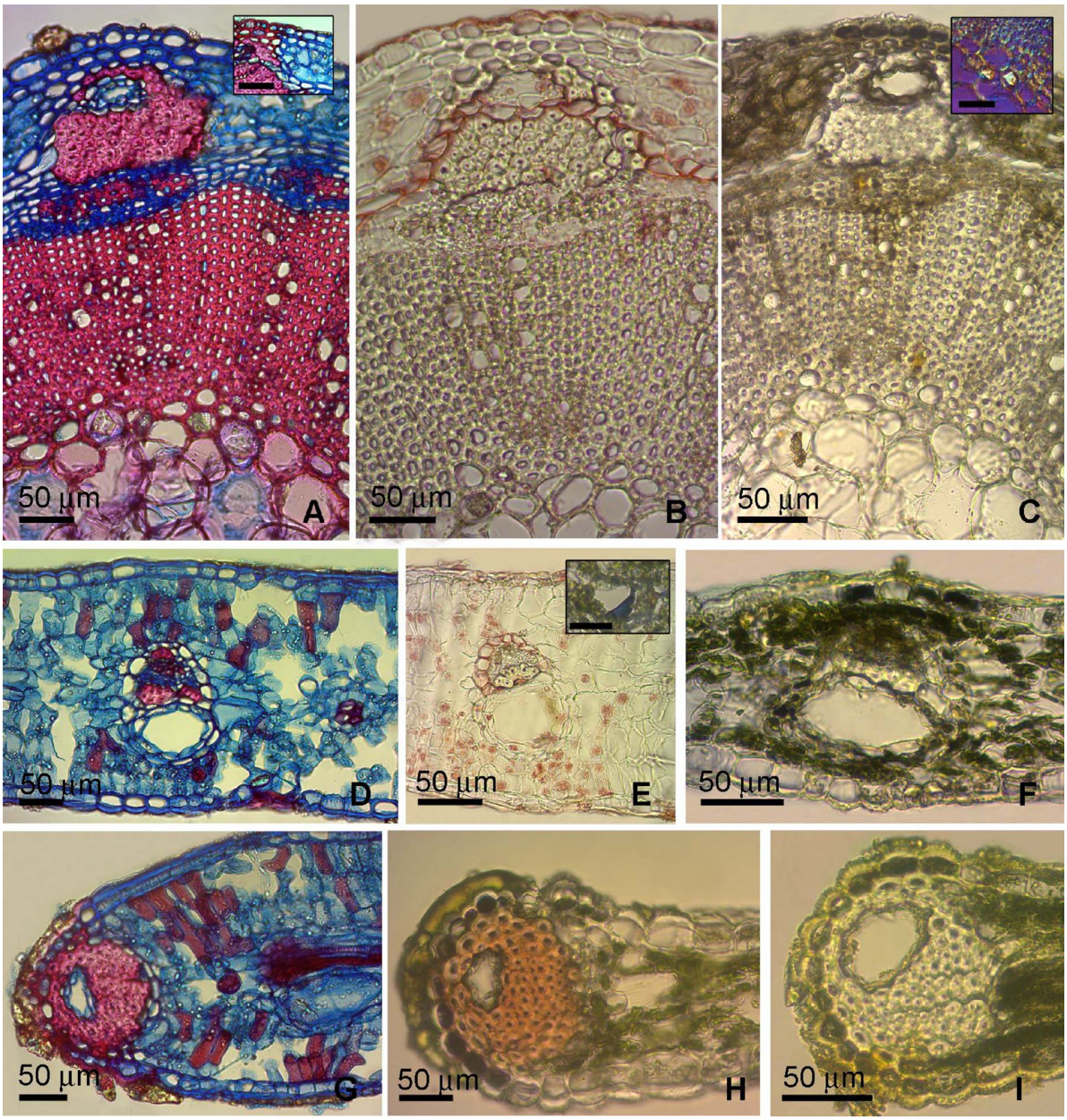

Fig. 3. *Baccharis trimera* (Less.) DC from Uruguay.

A-C. Transversal section of the stem. **A.** Astra Blue-Safranin; detail of subepidermal lignified collenchyma. **B.** Sudan IV reagent for lipids. **C.** Ferric chloride for polyphenols; detail of calcium oxalate crystals in perimedullar parenchyma over polarized light. **D-F.** Transection of the wing. **D.** Astra Blue-Safranin. **E.** Sudan IV for lipids; detail of positive results for essential oils with Nadi reagent. **F.** Ferric chloride for polyphenols. **G-I.** Transection of the wing margin. **G.** Astra Blue-Safranin O. **H.** Nadi reagent for essential oils. **I.** Ferric chloride for polyphenols.

together in geminated groups distributed all over the wing surface (Fig. 2D). This pattern of trichomes clustering was previously described for other 'carqueja' species such *B. articulata* (Cortadi et al., 1999; Rodrigues et al., 2013) and *B. crispa* (Cortadi et al., 1999; Rodrigues et al., 2008; Rodrigues et al., 2013), among others.

In cross section, the cladode axis shows one-layered epidermis with rectangular polyhedral cells (14.90 ± 2.87 µm) and a thin striated cuticle (4.25 ± 0.37 µm). The stomata were at the same level or slightly elevated than the epidermal cells. Cortex presented one or two layers of subepidermal laminar collenchyma tissue, occasionally with sclereids (between 10.15 µm and 23.17 µm thickness) and four-five layers of chlorenchyma (40.78 ± 10.34 µm thickness). Schyzogenous ducts of several dimensions disrupted the cortex, having one or two-strata of secretory epithelium with 5–10 cells in the inner cycle and 6–12 cells in the external cycle (Fig. 3A). The ducts were frequently immersed in groups of perivascular fibers. Their amber content and their epithelial cells reacted positively for lipids and EOs (Fig. 3E–F). A continuous endodermis was observed delimiting the central cylinder; in some cases, the external epithelium of schyzogenous ducts forms parts of the endodermis (Fig. 3B). In contrast with our observations, Cortadi et al. (1999), Budel and Duarte (2009) and Rodrigues et al. (2013) described unistratified secretory epithelium for the schyzogenous ducts. The vascular cylinder was constituted by an eustele with collateral vascular bundles, with caps of perivascular fibers at phloem level. The xylem presented isolated or short rows of 2–3 vessels and abundant fibers. The medulla appeared broad with polyhedral cells bigger than the ones found in the cortex. In the perimedullar parenchyma, there were abundant polyhedral pyramidal and prismatic crystals and druses of calcium oxalate (Fig. 3C). These types of crystals are common in *Baccharis* genus (Cortadi et al., 1999; Rodrigues et al., 2013; Budel and

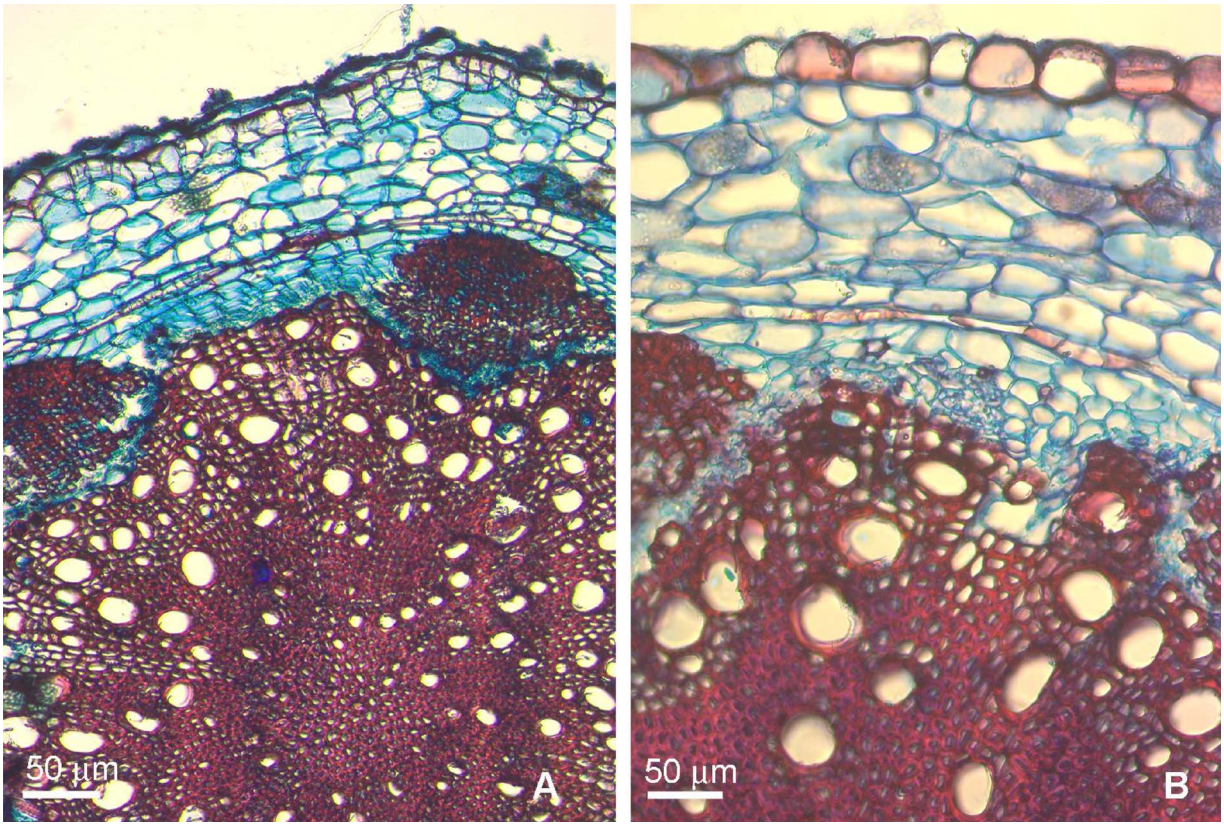

Fig. 4. *Baccharis trimera* (Less.) DC. from Uruguay.
A. Transversal cut of the root. **B.** Detail of the Casparian strip and cortical parenchyma with starch granules.

Duarte, 2009; ANVISA, 2010).

In transection, in agreement with Rodrigues et al. (2013) and Budel and Duarte (2009), the wings (184.06 ± 72.88 mm thickness) were isolateral, amphistomatic. Nevertheless, the Brazilian Pharmacopeia (ANVISA, 2010) stated a dorsiventral mesophyll, while Cortadi et al. (1999) described homogeneous or isobilateral characteristics. The mesophyll pattern found in this work has been previously reported also for *B. articulata* (Cortadi et al., 1999; Rodrigues et al., 2013) and *B. crispa* (Rodrigues et al., 2013). The wings presented adaxial and abaxial unistratified epidermis (16.00 ± 1.62 mm), two or three layers of adaxial palisade parenchyma (67.28 ± 21.72 mm), two or three layers of short cells spongy parenchyma (43.72 ± 22.00 mm) and two or three layers of abaxial palisade parenchyma (61.85 ± 22.49 mm). Abaxial position was determined by the presence of xylem in the collateral vascular bundles, sometimes accompanied by phloem and xylem fibers (the last one less developed) caps. Endodermoid sheaths surround all bundle structures (Fig. 3D–E). Schyzogenous ducts, like the previously described for the axis accompanied the more developed bundles toward to the phloem region, disrupting the adaxial palisade parenchyma. In occasions, the external epithelium of the ducts formed part of the endodermoid parenchyma sheaths (Fig. 3E).

Concerning to the anatomy of the wing margin, Cortadi et al. (1999) informed for *B. trimera* the presence of a subepidermal collenchyma. For other Caulopterae section species is frequent the presence of such kind of collenchyma tissue, i.e. *B. articulata* (Cortadi et al., 1999). Nevertheless, in this work, in accordance with Rodrigues et al. (2013) we observed a conspicuous reinforcement of fibers and one or two schyzogenous ducts immersed in such fibers, sometimes together with a small parenchyma bundle (Fig. 3G–I).

Both, the axis and the wings (specifically in the margin region) presented positive reaction with ferric chloride at epidermis, cuticle levels and at the secretory epithelium of the schyzogenous ducts, indicating the presence of polyphenols (Fig. 3C, F, I). Preliminary analyses performed by TLC in our laboratory (data not shown) have demonstrated the presence of the polyphenols quercetin, rutin, chlorogenic and gallic acids in the cladodes of *B. trimera* of VE and BLO stages. The occurrence of these compounds it has been previously published for this species (Soicke and Leng-Peschlow, 1987; Biondo et al., 2011; Aboy et al., 2012; Gené et al., 1992; Gené et al., 1996; Gómez et al., 2016). Similarly, the epidermis of the wing margin had positive results for lipids and essential oils with Sudan IV and Nadi reagents, respectively (Fig. 3H). This histochemical approach has not been previously performed for *B. trimera*, even though it was applied to other *Baccharis* species (*B. anomala*, *B. megapotamica* and *B. ochraceae*) with the reagent Sudan III (Budel et al., 2012).

Regarding to the root of *B. trimera*, in cross section shows early secondary growth with persistent unistratified rhizodermis. The cortex had 6–8 strata of assimilation parenchyma containing starch, endodermis and pericycle. The vascular system exhibited continuous phloem with prominent fiber caps. The xylem was porous and diffuse with lonely vessels elements or rarely organized in short rows (2 or 3), with abundant fibers. The medulla was solid in appearance composed by several fibers (Fig. 4A–B). No previous published works described the morpho-anatomy of such plant organ.

The anatomical differences found in this work in comparison with the results published by other authors might reflect a distinctive genotype or ecotype of *B. trimera* growing wild in Uruguay.

4. Conclusions

In this paper, it is presented a comprehensive study on chemical composition of the essential oil from aerial parts of *B. trimera* containing carquejyl acetate as main component independently of the plant phenological stage. 150 compounds were identified, 79 of which are new for *B. trimera* oil, including many isophorone and *o*-menthane derivatives. Other important compounds which characterizes the oils were β-

pinene, carquejol, palustrol, spathulenol, viridiflorol, ledol and β-eudesmol.

A series of anatomical characteristics in the cladodes of *B. trimera* growing in Uruguay were like others previously reported in the literature, i.e. the distribution of ground tissues, the presence of parenchymal veins and the type of non-glandular and glandular trichomes. In this work, schyzogenous ducts with bistratified secretory epithelium (occasionally associated with the endodermis) was evidenced. This fact has not been informed for *B. trimera* yet, which might reflect a different genotype or ecotype of this species growing wild in Uruguay. The complete absence of leaves and ribs between the wings indicated that these *B. trimera* individuals are different from the official plant described in the Brazilian Pharmacopeia, reinforcing the hypothesis of genotypes or ecotypes. The anatomy of the root of *B. trimera* is described for the first time in the literature. The results published here could benefit as start-point for the standardization of the medicinal species commercialized in Uruguay for preventing possible adulterations.

Finally, the application of Sudan IV and Nadi reagents on histochemical assays evidenced the storage of essential oils in glandular trichomes, schyzogenous ducts and in the epidermic cells located at the margin of the wings. Positive results for polyphenols compounds at secretory ducts epithelium level and at epidermal cells from the margin of the wings were also found.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors would like to thank to M. Minteguiaga, C. Camacho and N. Umpierrez for collection of plant material; P. Conde and J. Villaamil (INIA-Las Brujas) for pilot essential oil extraction assistance, and E. Lizarraga (*Fundación Miguel Lillo*, Argentina) for his support in the development of this work. Financial support was provided by Agencia Nacional de Investigación e Innovación (ANII-Uruguay) for M. Minteguiaga (POS_2011_1_3332 and POS_NAC_2013_1_11316). PEDECIBA, AUGM and National University of Tucuman gave additional financial support for this work.

References

ANVISA (Agência Nacional de Vigilância Sanitária), Fundação Oswaldo Cruz, 2010. Farmacopeia Brasileira, Volume 2-Monografias, 5ª edição. (Brasilia, Brasil).

Aboy, A.L., Apel, M.A., Debenedetti, S., Francescato, L., Rosella, M.A., Henriques, A.T., 2012. Assay of caffeoylquinic acids in *Baccharis trimera* by reversed-phase liquid chromatography. J. Chromatogr. A 1219, 147–153.

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Edition. Allured Publishing Corporation, Carol Stream, IL.

Alves, M.M., 2010. Influência sazonal no óleo essencial da *Baccharis articulata* e da *B. trimera* via cromatografia gasosa mono e bidimensional. Universidade Federal de Pelotas, Pelotas, Brazil (MSc. Thesis).

Barroso, G.M., Bueno, O., 2002. Compostas, Subtribo *Baccharidinae*. Herbário Barbosa Rodrigues, Itajaí, Brasil.

Barroso, G.M., 1976. Compositae-Subtribo *Baccharidinae* Hoffmann: Estudo das espécies ocorrentes no Brasil. Rodriguésia 40, 3–273.

Bauer, L., Silva, G.A.A.B., de Siqueira, N.C.S., Bacha, C.T.M., Sant'Ana, B.M.S., 1978. Os óleos essenciais de *Baccharis genistelloides* Pers. do Rio Grande do Sul. Rev. Centr. Cienc. Saúde 6, 7–12.

Besten, M.A., Nunes, D.S., Wisniewski Jr, A., Sens, S.L., Granato, D., Simionatto, E.L., Scharf, D.R., Dalmarco, J.B., Matzenbacher, N.I., 2013. Chemical composition of volatiles from male and female specimens of *Baccharis trimera* collected in two distant regions of Southern Brazil, a compartive study using chemometrics. Quim. Nova 36, 1096–1100.

Bicchi, C., Maffei, M., 2012. The plant volatilome, methods of analysis. In: Normanly, J. (Ed.), High-Throughput Phenotyping in Plants, Methods in Molecular Biology. Humana Press, New York, pp. 289–310.

Biondo, T.M.A., Tanae, M.M., Coletta, E.D., Lima-Landman, M.T.R., Lapa, A.J., Souccar, C., 2011. Antisecretory actions of E. D. *Baccharis trimera* (Less.) DC aqueous extracts and isolated compounds, analysis of underlying mechanisms. J. Ethnopharmacol. 136, 368–373.

Bohlmann, F., Zdero, C., 1969. Über neue terpenderivate aus *Baccharis trimera*. Tetrahedron Lett. 28, 2419–2421.

Borrella, J.C., Duarte, D.P., Novaretti, A.A.G., Menezes Jr, A, França, S.C., Rufato, C.B., Santos, P.A.S., Veneziani, R.C.S., Lopes, N.P., 2006. Variabilidade sazonal do teor de saponinas de *Baccharis trimera* (Less.) DC (Carqueja) e isolamento de flavona. Braz. J. Pharmacogn. 16, 557–561.

Budel, J.M., Duarte, M.R., 2009. Análise morfoanatômica comparativa de duas espécies de carqueja, *Baccharis microcephala* DC. e *B. trimera* (Less.) DC., Asteraceae. Braz. J. Pharm. Sci. 45, 75–85.

Budel, J.M., Duarte, M.R., Döll-Boscardin, P.M., Farago, P.V., Matzenbacher, N.I., Sartoratto, A., Sales Maia, B.H.L.N., 2012. Composition of essential oils and secretory structures of Baccharis anomala, *B. megapotamica* and *B. ochracea*. J. Essent. Oil Res. 24, 19–24.

Chialva, F., Doglia, G., 1990. Essential oil from carqueja (*Baccharis genistelloides* Pers.). J. Essent. Oil Res. 2, 173–177.

Condurso, C., Cincotta, F., Tripodi, G., Verzera, A., 2017. Bioactive volatiles in Sicilian (South Italy) saffron, safranal and its related compounds. J. Essent. Oil Res. 29, 221–227.

Cortadi, A., Di-Sapio, O., Mc Cargo, J., Scandizzi, A., Gattuso, S., Gattuso, M., 1999. Anatomical studies of *Baccharis articulata*, *Baccharis crispa* and *Baccharis trimera:* Carquejas used in folk medicine. Pharm. Biol. 37, 357–365.

D'Ambrogio de Argüeso, A., 1986. Manual de técnicas de histología vegetal. Editora Hemisferio Sur, Buenos Aires, Argentina 83 pp.

Davies, N.W., 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20 M phases. J. Chromatogr. 503, 1–24.

Davies, P., 2004. Equipo destilador para la obtención de aceites esenciales de plantas aromáticas. INIA-Uruguay (Instituto Nacional de Investigación Agropecuaria), Estudios en Domesticación y Cultivo de Especies Medicinales y Aromáticas Nativas, Serie FPTA-INIA 11, Rincón del Colorado, Canelones, Uruguay, pp. 25–28.

de Oliveira, C.B., Comunello, L.N., Lunardelli, A., Amaral, R.H., Pires, M.G.S., da Silva, G.L., Manfredini, V., Vargas, C.R., Gnoatto, S.C.B., de Oliveira, J.R., Gosmann, G., 2012. Phenolic enriched extract of *Baccharis trimera* presents anti-inflammatory and antioxidant activities. Molecules 17, 1113–1123.

de Oliveira, C.B., Comunello, L.N., Maciel, E.S., Giubel, S.R., Bruno, A.N., Chiela, E.C.F., Lenz, G., Gnoatto, S.C.B., Buffon, A., Gosmann, G., 2013. The inhibitory effects of phenolic and terpenoid compounds from *Baccharis trimera* in Siha cells, differences in their activity and mechanism of action. Molecules 18, 11022–11032.

Dilcher, D.L., 1974. Approaches to the identification of Angiosperm leaves. Bot. Rev. 40, 1–157.

Dizeo de Strittmater, C.G., 1973. Nueva técnica de diafanización. Bol. Soc. Argent. Bot. 15, 126–129.

El-Sayed, A.M., 2003–2017. The Pherobase, Database of Pheromones and Semiochemicals. Retrieved May 5, 2017, from: http://www.pherobase.com/.

Ferracini, V.L., Paraíba, L.C., Leitão Filho, H.L., da Silva, A.G., Nascimento, L.R., Marsaioli, A.J., 1995. Essential oils of seven Brazilian *Baccharis* species. J. Essent. Oil Res. 7, 355–367.

Gómez, M.A., Migues, I., Caggiani, M., Arias, X., Laprovitera, M., Blanco, F., Cesio, M.V., Migliaro, E.R., Heinzen, H., 2016. Vasorelaxant effect of a *Baccharis trimera* infusion on precontracted rat aortic rings. Nat. Prod. Comm. 11, 283–286.

Gené, R.M., Marín, E., Adzet, T., 1992. Anti-inflammatory effect of aqueous extracts of three species of the genus *Baccharis*. Planta Med. 58, 565–566.

Gené, R.M., Cartañá, C., Adzet, T., Marín, E., Parella, T., Cañigueral, S., 1996. Anti-inflammatory and analgesic activity of *Baccharis trimera:* identification of its active constituents. Planta Med. 62, 232–235.

Janúario, A.H., Santos, S.L., Marcussi, S., Mazzi, M.V., Pietro, R.C.L.R., Sato, D.N., Ellena, J., Sampaio, S.V., França, S.C., Soares, A.M., 2004. Neo-clerodane diterpenenoid, a new metalloprotease snake venom inhibitor from *Baccharis trimera* (Asteraceae), anti-proteolytic and anti-hemorrhagic properties. Chem. Biol. Int. 150, 243–251.

Karam, T.K., Dalposso, L.M., Casa, D.M., de Freitas, G.B.L., 2013. Carqueja (*Baccharis trimera*), utilização terapêutica e biossíntese. Rev. Bras. Pl. Med. 15, 280–286.

Lívero, F.A.R., Martins, G.G., Telles, J.E.Q., Beltrame, O.C., Biscaia, S.M.P., Franco, C.R.C., Elferink, R.P.J.O., Acco, A., 2016. Hydroethanolic extract of *Baccharis trimera* ameliorates alcoholic fatty liver disease in mice. Chem. Biol. Interact. 260, 22–32.

Lago, J.H.G., Romoff, P., Favero, O.A., Souza, F.O., Soares, M.G., Baraldi, P.T., Correa, A.G., 2008a. Chemical composition of male and female *Baccharis trimera* (Less.) DC. (Asteraceae) essential oils. Biochem. Syst. Ecol. 36, 737–740.

Lago, J.H.G., Romoff, P., Favero, O.A., Soares, M.G., Baraldi, P.T., Correa, A.G., Souza, F.O., 2008b. Composição química dos óleos essenciais das folhas de seis espécies do gênero *Baccharis* de Campos de altitude da Mata Atlântica Paulista. Quim. Nova 31, 727–730.

Linstrom, P.J., Mallard, W.G. (eds.). 2017. NIST Chemistry WebBook. NIST Standard Reference Database Number 69. Retrieved May 5, 2017, from: http://webbook.nist.gov.

Martínez-Crovetto, R., 1981. Plantas utilizadas en medicina en el Noroeste de Corrientes (República Argentina). Volumen 69 Miscelánea. Fundación Miguel Lillo, Tucumán, Argentina.

McLafferty, F.W., Stauffer, D.B., 1994. The Wiley/NBS Registry of Mass Spectral Data, 6th edition. Wiley-Interscience, New York.

Mendes-Pinto, M.M., 2009. Carotenoid breakdown products –the norisoprenoids- in wine aroma. Arch. Biochem. Biophys. 483, 236–245.

Minteguiaga, M., Umpiérrez, N., Fariña, L., Cassel, E., Dellacassa, E., 2015a. ¿Es el Acetato de Carquejilo un quimioindicador vñlido para las carquejas (*Baccharis* sect. Caulpoterae)? Lilloa 52 (Suppl), 62–64.

Minteguiaga, M., Umpiérrez, N., Xavier, V.B., Lucas, A., Mondin, C., Fariña, L., Cassel, E., Dellacassa, E., 2015b. Recent findings in the odorants chemistry from four *Baccharis*

species and their impact as chemical markers. Chem. Biodivers. 12, 1339–1348.
Moreira, V.E., Gasparetto, C.M., Chibli, L.A., Vieira, G.D.V., de Sousa, O.B., 2012. Teores de fenóis totais e flavonoides e avaliação da atividade antioxidante de *Baccharis trimera* (Less) DC (Asteraceae). HU Revista 38, 45–51.
Naves, Y.R., 1959a. Etudes sur les matières végétales volatiles. CLIX. Sur Íhuile essentielle de Carqueja de ÍÉtat de Santa Catarina (Brésil). Bull. Soc. Chim. France 1871–1879.
Naves, Y.R., 1959b. Etudes sur les matières végétales volatiles CLXI: Présence de lédol dans l'huile essentielle de carquéja. Helv. Chim. Acta 42, 1996–1998.
Nogueira, N.P.A., Reis, P.A., Laranja, G.A.T., Pinto, A.C., Aiub, C.A.F., Felzenswalb, I., Paes, M.C., Bastos, F.F., Sabino, K.C.C., Coelho, M.G.P., 2011. In vitro and in vivo toxicological evaluation of extracts and fractions from *Baccharis trimera* with anti-inflammatory activity. J. Ethnopharmacol. 138, 513–522.
Nunes de Oliveira, R., Rehder, V.L.G., Oliveira, A.S.S., Montanari Jr., I., de Carvalho, J.E., de Ruiz, A.L.T.G., Jeraldo, V.L.S., Linhares, A.X., Allegretti, S.M., 2012. *Schistosoma mansoni*, *in vitro* schistosomicidal activity of essential oil of *Baccharis trimera* (Less) DC. Exp. Parasitol. 132, 135–143.
Nunes de Oliveira, R., Rehder, V.L.G., Oliveira, A.S.S., Jeraldo, V.D.L.S., Linhares, A.X., Allegretti, S.M., 2014. Anthelmintic activity in vitro and in vivo of *Baccharis trimera* (Less) DC against immature and adult worms of *Schistosoma mansoni*. Exp. Parasitol. 139, 63–72.
Nunes, O.C., Jamal, C.M., Kitagawa, R.R., Gonçalves, R.C.R., 2016. Anti-*Helicobacter pylori* effect of the antioxidant extract from *Baccharis trimera* (Less.) DC. Int. J. Phytomed. 8, 472–481.
Paul, E.L., Lunardelli, A., Caberlon, E., de Oliveira, C.B., Santos, R.C.V., Biolchi, V., Bastos, C.M.A., Moreira, K.B., Nunes, F.N., Gosmann, G., de Oliveira, J.R., 2009. Anti-inflammatory and immunomodulatory effects of *Baccharis trimera* aqueous extract on induced pleurisy in rats and lymphoproliferation *in vitro*. Inflammation 32, 419–425.
Quintana de Oliveira, S., Dal-Pizzol, F., Moreira, J.C.F., Schenkel, E.P., Gosmann, G., 2004. Antioxidant activity of *Baccharis spicata*, *Baccharis trimera* and *Baccharis usterii*. Acta Farm. Bonaerense 23, 365–368.
Quintana de Oliveira, S., Trentin, V.H., Kappel, V.D., Barelli, C., Gosmann, G., Reginatto, F.H., 2005. Screening of antibacterial activity of south brazilian *Baccharis* species. Pharm. Biol. 43, 434–438.
Rodrigues, M.V., Gattuso, S., Gattuso, M., 2008. *Baccharis crispa* y *Baccharis trimera* (Asteraceae), revisión y nuevos aportes para su normalización micrográfica. Lat. Am. J. Pharm. 27, 387–397.
Rodrigues, M.V., Gattuso, S., Gattuso, M., 2013. Micrographic standarization of *Baccharis* L. species (Asteraceae). Dominguezia 29, 39–54.
Scheffer-Basso, S.M., Lubenow, R., Carneiro, C.M., Chini, S.O., 2008. Morfofisiologia da rebrota de *Baccharis trimera* (Less) DC. Asteraceae, subsídios para seu controle em pastagens naturais. Biotemas 21, 31–37.
Silva, F.G., Pinto, J.E.B.P., Cardoso, M.G., Nascimento, E.A., Nelson, D.L., Sales, J.F., Mol, D.J.S., 2006. Influence of radiation level on plant growth, yield and quality of essential oil in carqueja. Ciênc. Agrotec. 30, 52–57.
Silva, F.G., Oliveira, C.B.A., Pinto, J.E.B.P., Nascimento, V.E., Santos, S.C., Seraphin, J.C., Ferri, P.H., 2007. Seasonal variability in the essential oils of wild and cultivated *Baccharis trimera*. J. Braz. Chem. Soc. 18, 990–997.
Simões-Pires, C.A., Debenedetti, S., Spegazzini, E., Mentz, L.A., Matzenbacher, N.I., Limberger, R.P., Henriques, A.T., 2005. Investigation of the essential oil from eight species of *Baccharis* belonging to sect. Caulopterae (Asteraceae Astereae), a taxonomic approach. Plant Syst. Evol. 253, 23–32.
Soicke, H., Leng-Peschlow, E., 1987. Characterisation of Flavonoids from *Baccharis trimera* and their Anti-hepatotoxic properties. Planta Med. 53, 37–39.
Suzuki, E.Y., Caneschi, C.A., Fochat, R.C., Brandão, M.A.F., Raposo, N.R.B., 2016. Antimicrobial activity of essential oil from *Baccharis trimera* (Less.) DC. (carqueja-amarga). Rev. Cubana Plant. Med. 21, 346–358.
Vargas, R.M.F., Cassel, E., Gomes, G.M.F., Longhi, L.G.S., Atti-Serafini, L., Atti-Santos, A.C., 2006. Supercritical extraction of carqueja essential oil, experiments and modelling. Braz. J. Chem. Eng. 23, 375–382.
Vieir, T.O., Seifriz, I., Charão, C.C.T., de Oliveira, S.Q., Creczynski-Pasa, T.B., 2011. Antioxidant effects of crude extracts from *Baccharis species*, inhibition of myeloperoxidase activity, protection against lipid peroxidation, and action as oxidative species scavenger. Braz. J. Pharmacogn. 21, 601–607.
Wagner, H., Bladt, S., Zgainski, E.M., 1984. Plant Drug Analysis A Thin Layer Chromatography Atlas. Springer Verlang, Berlin Heildenberg, Germany.
Zarlavsky, G.E., 2014. Histología vegetal, Técnicas simples y complejas. Sociedad Argentina de Botánica, Buenos Aires, Argentina.