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# Chemical compositions of essential oil from the aerial parts of male and female plants of *Baccharis tridentata* Vahl. (Asteraceae)

Manuel Minteguiaga [ID][a], Laura Fariña[a], Eduardo Cassel [ID][b], Stephanie Fiedler[a], César A.N. Catalán[c] and Eduardo Dellacassa [ID][a]

[a]Laboratorio de Biotecnología de Aromas (LaBiotA). Facultad de Química, Universidad de la República (UdelaR). Av. Gral. Flores 2124, CP: 11800. Montevideo, Uruguay; [b]Laboratório de Operações Unitárias (LOPE). Escola Politécnica, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS). Av. Ipiranga 6681, CEP: 90619-900. Porto Alegre, Brasil; [c]INQUINOA-CONICET, Instituto de Química Orgánica. Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán (UNT). Ayacucho 471, CPA: T4000INI. San Miguel de Tucumán, Argentina

## ABSTRACT

Differences in female/male plant metabolism for dioecious species are poorly understood but relevant if the economic exploitation of such resources is the objective. In this work, the composition of *Baccharis tridentata* Vahl essential oil from the flowering aerial parts of male and female plants was analysed separately by GC-MS (gas chromatography-mass spectrometry; area percentage and internal standard), and CPGC-MS (chiral phase gas chromatography analysis using modified β-cyclodextrin), reporting the enantiomeric distribution of monoterpene hydrocarbons as a genuineness criterion. The results confirmed high qualitative similarities between the essential oils from both genders, but quantitative differences in the main components α-pinene, β-pinene, limonene and bornyl acetate. Regarding the CPGC-MS analysis of monoterpene hydrocarbons, both sexes presented both enantiomers in the same ratios, which is in contrast to the values reported in the literature for related plants. However, natural bornyl acetate (isolated from *B. tridentata* essential oil) demonstrated a 100% enantiomeric excess of the levogyre enantiomer, a fact that has chemotaxonomic relevance.

## Introduction

The genus *Baccharis* (Asteraceae), comprising estimated 350 species, is distributed mainly in South America. According to the number of species found in Brazil and the Andes, this region has been proposed as the probable centre of distribution of the genus. *Baccharis* has also been demonstrated to be able to express a wide phytochemistry diversity (1–5), meaning the evolutionary success of the taxon could be attributed to its array of secondary metabolites. In particular, in recent years, the analysis of *Baccharis* volatile compounds has increased for two main reasons: the search for new key aromas that can be applied in the food and fragrance industries, and improving our understanding of the role of volatile organic compounds in plant metabolism and in ecosystem relationships (6,7). Several papers have reported on the analysis of *Baccharis* essential oils and volatile extracts from male and female plants (8–12). The composition data have been compared, in general, by reporting the area percentage of compounds obtained from GC-FID and/or GC-MS analysis. The main limitation associated with such an approach is the impossibility of performing a reliable comparison in order to

understand female/male differences and, in general, the role of the plant volatilome in biological interactions (13). In fact, for comparison purposes, the evaluation of quantifiable differences in volatile compounds can only be carried out by using an internal standard (13,14).

*Baccharis tridentata* Vahl (popular name 'vassoura' or 'carqueja folhuda' in Portuguese) is a perennial shrub species that grows in southeastern and southern Brazil, Paraguay, Uruguay, and northern and central Argentina (15). In Brazil, the infusions of aerial parts are employed traditionally by the indigenous population as antipyretics and diuretics (16). This species has a pleasant aroma and, because of its aesthetic anatomical features (long stems, lush leaves and outstanding green colour and rustic aspect), is considered a promising ornamental species (15). There are only four reports dealing with the volatile composition of the aerial parts of this species, but none of them considered female/male chemical differences (8,17–19). The study of those published results suggests that *B. tridentata* could show broad plasticity, leading us to hypothesise the existence of chemotypes according to the variability observed in its essential oil composition, that might indicate a great

genetic diversity among the populations studied. Moreover, such essential oil showed promising inhibition of the mycelial growth of several phytopathogenic fungi of agricultural importance (19).

Moreover, Ferracini et al. (1995, 8) described essential oils with a high proportion of sesquiterpenes (spathulenol, δ-cadinene and globulol) and a near absence of monoterpene hydrocarbons in plants collected in the Brazilian state of Minas Gerais. Souza et al. (2011, 19) also described, in the same region, an essential oil rich in monoterpene hydrocarbons such as α-thujene, β-pinene and β-phellandrene. Recently, we have described in southern Brazil (Rio Grande do Sul) (17) a new possible chemical race (chemotype) of this species characterised by a high proportion of α-pinene (46.7%), limonene (14.1%), β-pinene (11.4%) and bornyl acetate (6.0%). Due to the fresh, sweet, camphoraceous and piney-wood sensory characters of bornyl acetate, together with its low odour threshold ranging from 75 ppb to 1.38 ppm (20), it is reasonable to propose that this compound is a key component responsible for the pleasant aroma of B. tridentata essential oil. Found in many essential oils distilled from the leaves of plants belonging to the family Pinaceae (Pinus densiflora, Abies balsamea and Abies sibirica, among others), bornyl acetate is employed in soap and bath products. The levogyre enantiomer has been reported to induce relaxation of the autonomic nervous system (21).

In this work, we characterised the particularities of essential oil composition in male and female genders from a Southern Brazilian B. tridentata population through the application of gas chromatography and mass spectrometry. Moreover, the enantiomeric distribution of the main monoterpene hydrocarbons was evaluated by chiral gas chromatography coupled to mass spectrometry (CPGC-MS). Due to its potential relevance in the composition and aroma profile, a major compound (bornyl acetate) was isolated from the individual essential oils and its optical activity was measured by circular dichroism.

## Experimental

### Plant material

Aerial parts (300 g) of Baccharis tridentata Vahl at the early full flowering stage were collected at 'Centro de Conservação e Pesquisa da Natureza Pró-Mata (CPCN-Pró Mata-PUCRS)' (São Francisco de Paula, Rio Grande do Sul, Brazil) during two seasons (2016 and 2017). The individual gender samples were carefully separated at the place of collection into male and female branches. Taxonomical identification was performed by Prof.

Pedro M. Abreu (PUCRS) and a sample was deposited at the PUCRS herbarium with the accession number M. Minteguiaga MPUC 19926.

### Extraction of the essential oils

The plant material (composite sample of male and female branches separately) was maintained in the shade at room temperature for 2 days and then extracted for 90 minutes in a Clevenger type apparatus, yielding clear yellow and sweet essential oils. The essential oils were stored (−20°C) in 10 mL brown flasks until chemical analysis.

### Essential oil analysis

### Gas chromatography coupled with mass-spectrometry (GC-MS)

The analyses were carried out in duplicate on a Shimadzu GC2010 coupled to Shimadzu QP2010 Ultra mass spectrometer (Shimadzu Corporation, Kyoto, Japan). The capillary column employed was a cross-linked fused silica 95%-dimethyl-5%-diphenylpolysiloxane HP-5 MS (Agilent Technologies, Walt & Jennings Scientific, Wilmington, DE, USA; 30 m × 0.25 mm i.d. × 0.25 μm film thickness). The experimental conditions were the same as those reported by Minteguiaga et al. (17). The temperature programme was as follows: 60°C (8 min), 60–180°C at 3°C/min, then to 230°C at 20°C/min; injector temperature, 250°C; carrier gas, helium at 122.2 kPa (51.6 cm/s); injection mode, split; split ratio, 1:40; the sample volume injected was 0.2 μL; the interface temperature, 250°C and the acquisition mass range was 40–400 a.m.u.

Linear retention indices (LRIs) were calculated analysing a homologous n-alkane series (alkane analytical standard solution C8-C20 from Sigma-Aldrich) in the same experimental conditions of the samples. These values were compared with data obtained from literature (22) and with previous work by our group for the same species (17). Compounds were considered tentatively identified when experimental and reported LRI did not differ by more than 10 units and when the similarity between the mass spectrum of each chromatographic peak and spectrum of the Adams mass spectral library was at least 80% (22).

GC-MS only allows to obtain semi-quantitative results using peak area percentages, so the relative content of each compound was performed on the basis of both their peak areas without corrections for response factors and, in parallel, by using n-tetradecane (analytical standard available from Sigma-Aldrich; 20 mg/mL for the pure essential oil) as an internal standard.

*n*-Tetradecane was selected after a careful revision of the eluting times of all the components of the essential oils. All compound peak areas were normalised to internal standard without any correction factor for different chemical classes present, and then female and male compositions were compared. % obtained using GC-MS is "semi-quantitative". The samples were injected in triplicate, demonstrating a standard deviation of peaks areas of less than 5%.

### Chiral phase gas chromatography analysis (CPGC-MS)

Identification and chiral separation of the main monoterpenes of the essential oil (α-thujene, α-pinene, camphene, sabinene, β-pinene, limonene and bornyl acetate) was performed on an HP6890 gas chromatograph coupled to an HP5973 mass spectrometer (Hewlett-Packard, Palo Alto, CA, USA) equipped with a CycloSil-B chiral selector capillary column composed of 30% Heptakis (2,3-di-*O*-methyl-6-*O*-t-butyl dimethylsilyl)-β-cyclodextrin in DB-1701 (Agilent Technologies, Walt & Jennings Scientific; 30 m × 0.25 mm i.d. × 0.25 μm film thickness). The temperature program optimised to separate monoterpene enantiomers was as follows: 65°C (1 min), 65–100°C at 1°C/min, 100°C (1 min), 100–150°C at 2°C/min, 150–220°C at 10°C/min, 220°C (3 min). All other experimental conditions for GC-MS were identical to those described in the GC-MS analysis section. Male and female essential oils were analysed in duplicate. The elution order for the monoterpene hydrocarbon enantiomers was obtained by comparison with the literature that employed the same chiral stationary phase (23–26) and with results previously obtained in our laboratory (27).

### Bornyl acetate isolation

*B. tridentata* essential oil (0.856 g, composite sample of female and male essential oils) was separated by column chromatography (CC) over 30.09 g of Si-Gel (230–400 mesh; Merck, Darmstadt, Germany) as the stationary phase. All the solvents employed for CC, and in the next sections were purchased from Cicarelli (San Lorenzo, Santa Fe, Argentina) and distilled prior to use. The CC elution was performed isocratically with a mobile phase mixture of petroleum ether-AcOEt (20:1) at a flow rate of 2.4 mL/min. To monitor CC separation, TLC was employed (Si-gel60F$_{254}$; Merck) using the same mobile phase and *p*-anisaldehyde-sulphuric acid (Sigma-Aldrich) as a spray reagent. The chromatographic separation yielded 30 mg of pure bornyl acetate.

### Preparation of borneol by the saponification of natural bornyl acetate

An aliquot of 15 mg of bornyl acetate isolated from *B. tridentata* oil was submitted to hydrolysis to obtain borneol. To proceed under very mild conditions, the procedure described by Theodorou *et al.* (2007, 28) was followed, with slight modifications. For comparative purposes, enantiomerically impure borneol standard was purchased from Merck (Darmstadt, Germany).

Briefly, bornyl acetate (15 mg) in 2.25 mL of recently distilled $CH_2Cl_2$ was mixed at room temperature with 0.25 mL of 2.2 N methanolic NaOH in a covered flask. The mixture was allowed to react at room temperature for 2 hours and the reaction progress was monitored by TLC. Once the reaction was complete (2 hours), the mixture was diluted with $CH_2Cl_2$ (15 mL) and washed in a separatory funnel with 5% HCl (2 x 5 mL) and distilled water (2 x 3 mL). The organic layer was dried with anhydrous $Na_2SO_4$, then filtered and evaporated in rotavap prior to CPGC-MS analyses.

### Circular dichroism (CD) and optical rotation measurements

The measurements were performed according to Cerda-García-Rojas *et al.* (2005, 29) employing pure integrifolian-1,5-dione previously isolated from *Lippia integrifolia* (Verbenaceae) (30) as the standard for CD spectra acquisition. Solutions of natural isolated bornyl acetate and integrifolian-1,5-dione (0.5 mg/mL in EtOH) were measured for their CD spectra in a Jasco J-815 CD spectrometer (Jasco Inc., Easton, MD, USA) in the range of 200–400 nm.

### Statistical analysis

Mean rating and least-significant differences between values were calculated from an analysis of variance. The analyses were performed with Statistica v. 7.0 software (Statsoft, Tulsa, USA)

## Results and discussion

### Chemical compositions of the essential oils

The *B. tridentata* essential oil obtained had a pale yellow colour with a yield ranged from 0.28% to 0.34% w/w. Table 1 shows the chemical compositions of the *B. tridentata* male and female essential oils, whereas Table 2 shows the enantiomeric ratios for selected monoterpene hydrocarbons obtained by CPGC-MS (with the exception of bornyl acetate, as discussed below).

**Table 1.** Chemical compositions of *B. tridentata* female (F) and male (M) essential oils.

| | | LRI | | %Area GC-MS[4] | | | Conc. GC-MS (µg/L)[5] | | p |
|---|---|---|---|---|---|---|---|---|---|
| | Compound[1] | Lit.[2] | Exp.[3] | N | F | M | F | M | |
| 1 | hexanal | 801 | 801 | - | tr | tr | 92 ± 8 | 158 ± 13 | * |
| 2 | 3-(Z)-hexen-1-ol | 850 | 847 | 0.1[6] | tr | 0.1 | 19 ± 2 | 16 ± 2 | n.s. |
| 3 | 1-hexanol | 863 | 862 | - | tr | tr | 420 ± 34 | 26 ± 2 | * |
| 4 | tricyclene | 921 | 917 | tr | 0.1 | 0.1 | 37 ± 4 | 470 ± 39 | * |
| **5** | **α-thujene**[a] | 924 | 921 | 1.3 | 0.8 | 1.0 | 9200 ± 500 | 15800 ± 800 | * |
| **6** | **α-pinene**[a] | 932 | 928 | 46.7 | 29.5 | 30.8 | 794000 ± 40000 | 1173000 ± 60000 | * |
| 7 | camphene | 946 | 941 | 1.4 | 2.7 | 2.6 | 27000 ± 1000 | 35000 ± 2000 | * |
| 8 | thuja-2,4-(10) diene | 953 | 947 | - | tr | tr | 28 ± 2 | 32 ± 2 | n.s. |
| 9 | sabinene | 969 | 968 | 3.1 | 1.7 | 1.6 | 36 ± 4 | 38 ± 3 | n.s. |
| **10** | **β-pinene**[a] | 974 | 971 | 11.4 | 17.9 | 17.0 | 287000 ± 14000 | 362000 ± 18000 | * |
| **11** | **myrcene**[a] | 988 | 989 | 1.8 | 3.1 | 2.9 | 27000 ± 1000 | 37000 ± 2000 | * |
| 12 | α-phellandrene | 1002 | 1000 | - | 0.1 | 0.1 | 1900 ± 100 | 1600 ± 100 | * |
| 13 | 3-(Z)-hexen-1-ol acetate | 1004 | 1009 | - | 0.1 | 0.1 | 39700 ± 3176 | 58650 ± 4692 | * |
| 14 | α-terpinene | 1014 | 1015 | 0.2 | tr | tr | 3700 ± 200 | 2600 ± 100 | * |
| **15** | **p-cymene**[a] | 1020 | 1023 | 0.1 | 0.1 | 0.1 | 1350 ± 108 | 1850 ± 148 | * |
| **16** | **limonene**[a] | 1024 | 1023 | 14.1 | 16.4 | 16.7 | 204000 ± 10000 | 273000 ± 14000 | * |
| 17 | (Z)-β-ocimene | 1032 | 1034 | 0.1 | 0.2 | 0.2 | 2100 ± 100 | 1400 ± 100 | * |
| 18 | benzene acetaldehyde | 1036 | 1040 | - | tr | tr | 185 ± 15 | 130 ± 10 | * |
| **19** | **(E)-β-ocimene**[a] | 1044 | 1046 | 3.6 | 4.6 | 4.1 | 42000 ± 2000 | 47000 ± 2000 | * |
| **20** | **γ-terpinene**[a] | 1054 | 1057 | 0.2 | 0.4 | 0.4 | 3600 ± 200 | 3800 ± 200 | n.s. |
| 21 | cis-sabinene hydrate | 1065 | 1066 | 0.1 | 0.1 | 0.1 | 105 ± 8 | 800 ± 100 | * |
| **22** | **terpinolene**[a,b] | 1086 | 1086 | 0.2 | 0.4 | 0.4 | 230 ± 18 | 3200 ± 200 | * |
| 23 | trans-sabinene hydrate | 1098 | 1097 | 0.1 | tr | 0.1 | 2100 ± 168 | 2350 ± 188 | n.s. |
| 24 | linalool | 1095 | 1099 | 0.1 | 0.2 | 0.3 | 2800 ± 100 | 3200 ± 200 | * |
| 25 | nonanal | 1100 | 1104 | - | tr | 0.1 | 525 ± 42 | 40 ± 4 | * |
| 26 | 1,3,8-p-menthatriene | 1108 | 1109 | - | tr | tr | 115 ± 12 | 160 ± 13 | n.s. |
| **27** | **endo-fenchol**[a] | 1114 | 1112 | - | tr | tr | 800 ± 64 | 450 ± 36 | * |
| 28 | cis-p-menth-2-en-1-ol | 1118 | 1119 | 0.1 | 0.1 | 0.1 | 140 ± 11 | 2000 ± 100 | * |
| 29 | α-campholenal | 1122 | 1124 | - | tr | tr | 180 ± 14 | 190 ± 15 | n.s. |
| 30 | allo-ocimene | 1128 | 1128 | - | tr | tr | 215 ± 21 | 40 ± 3 | * |
| 31 | trans-pinocarveol | 1135 | 1138 | tr | tr | tr | 400 ± 32 | 225 ± 18 | * |
| 32 | trans-p-menth-2-en-1-ol | 1136 | 1138 | - | tr | tr | 75 ± 6 | 100 ± 8 | * |
| 33 | cis-verbenol | 1137 | 1136 | - | tr | tr | 90 ± 8 | 95 ± 8 | n.s. |
| 34 | trans-verbenol | 1140 | 1143 | - | 0.1 | 0.1 | 4700 ± 200 | 1800 ± 100 | * |
| 35 | pinocarvone | 1160 | 1162 | - | tr | tr | 20 ± 2 | 1125 ± 90 | * |
| 36 | borneol | 1165 | 1165 | tr | 0.1 | 0.1 | 375 ± 30 | 1200 ± 100 | * |
| 37 | p-mentha-1,5-dien-8-ol | 1166 | 1166 | tr | tr | 0.1 | 450 ± 36 | 95 ± 8 | * |
| **38** | **terpinen-4-ol**[a] | 1174 | 1177 | 0.5 | 0.9 | 0.9 | 6100 ± 300 | 6500 ± 300 | n.s. |
| 39 | p-cymen-8-ol | 1179 | 1183 | - | tr | tr | 105 ± 14 | 250 ± 20 | * |
| **40** | **α-terpineol**[a,b] | 1186 | 1191 | 0.2 | 0.3 | 0.3 | 2200 ± 100 | 2400 ± 100 | n.s. |
| 41 | myrtenol | 1194 | 1194 | - | tr | tr | 225 ± 18 | 47 ± 5 | * |
| 42 | myrtenal | 1195 | 1196 | - | 0.1 | tr | 305 ± 24 | 325 ± 26 | n.s. |
| 43 | cis-carveol | 1226 | 1231 | - | tr | tr | 56 ± 5 | 77 ± 6 | * |
| 44 | carvone | 1239 | 1242 | - | tr | tr | 110 ± 32 | 120 ± 9 | n.s. |
| **45** | **bornyl acetate**[a] | 1284 | 1287 | 6.1 | 10.1 | 9.1 | 69000 ± 3000 | 60000 ± 3000 | * |
| 46 | δ-elemene | 1335 | 1338 | - | 0.6 | 0.5 | 1525 ± 122 | 1625 ± 130 | n.s. |
| **47** | **α-cubebene**[b] | 1345 | 1348 | - | tr | 0.1 | 280 ± 27 | 885 ± 71 | * |
| **48** | **α-copaene**[b] | 1375 | 1374 | tr | - | - | - | - | n.s. |
| **49** | **β-bourbonene**[a] | 1387 | 1389 | - | tr | tr | 3450 ± 276 | 3000 ± 240 | n.s. |
| **50** | **β-elemene**[a,b] | 1389 | 1391 | tr | tr | tr | 76 ± 8 | 82 ± 7 | n.s. |
| 51 | β-cubebene | 1387 | 1391 | - | tr | tr | 14 ± 5 | 45 ± 5 | * |
| 52 | methyl eugenol | 1403 | 1407 | 0.1 | 0.1 | 0.1 | 615 ± 56 | 460 ± 38 | * |
| **53** | **(E)-β-caryophyllene**[a,b] | 1417 | 1420 | 0.8 | 0.9 | 0.9 | 8000 ± 400 | 24000 ± 1000 | * |
| 54 | β-gurjunene | 1431 | 1437 | - | 0.1 | 0.1 | 147 ± 12 | 110 ± 9 | * |
| **55** | **aromadendrene**[b] | 1437 | 1440 | tr | - | - | - | - | n.s. |
| **56** | **allo-aromadendrene**[b] | 1458 | 1462 | - | 0.1 | 0.1 | 276 ± 35 | 277 ± 26 | n.s. |
| **57** | **α-humulene**[a,b] | 1452 | 1452 | tr | 0.1 | 0.1 | 214 ± 17 | 1217 ± 112 | * |
| 58 | 9-epi-caryophyllene | 1464 | 1464 | - | 0.1 | 0.1 | 138 ± 11 | 660 ± 53 | * |
| 59 | γ-muurolene | 1478 | 1484 | tr | tr | 0.1 | 185 ± 14 | 85 ± 6 | * |
| **60** | **germacrene D**[a] | 1484 | 1484 | 1.5 | 2. 1 | 2.1 | 12300 ± 600 | 9200 ± 500 | * |
| **61** | **β-selinene**[b] | 1489 | 1490 | 0.1 | 0.1 | 0.1 | 368 ± 32 | 176 ± 12 | * |
| 62 | (E)-muurola-4,(14), 5-diene | 1493 | 1494 | - | tr | tr | 230 ± 18 | 231 ± 17 | * |
| **63** | **bicyclogermacrene**[a] | 1500 | 1500 | 3.4 | 2.2 | 2.3 | 17800 ± 900 | 13100 ± 700 | * |
| **64** | **α-muurolene**[a,b] | 1500 | 1502 | 0.1 | 0.1 | 0.1 | 115 ± 9 | 55 ± 4 | * |
| 65 | (E,E)-α-farnesene | 1505 | 1506 | tr | - | - | - | - | n.s. |
| **66** | **γ-cadinene**[a] | 1513 | 1520 | 0.1 | 0.1 | 0.1 | 395 ± 32 | 700 ± 100 | * |
| **67** | **δ-cadinene**[a,b] | 1522 | 1527 | 0.3 | 0.4 | 0.4 | 3700 ± 200 | 1700 ± 100 | * |
| 68 | elemol | 1548 | 1552 | - | 0.1 | 0.1 | 369 ± 56 | 276 ± 23 | * |
| **69** | **spathulenol**[a,b] | 1577 | 1584 | 0.9 | 0.8 | 0.8 | 5600 ± 300 | 3500 ± 200 | * |

(Continued)

**Table 1. (Continued).**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 70 | caryophyllene oxide[a] | 1582 | 1584 | 0.2 | 0.2 | 0.2 | 111 ± 14 | 51 ± 3 | * |
| 71 | viridiflorol[b] | 1592 | 1598 | - | 0.3 | 0.4 | 4700 ± 200 | 1700 ± 100 | * |
| 72 | 1-*epi*-cubenol | 1627 | 1629 | - | tr | tr | 168 ± 13 | 105 ± 8 | * |
| 73 | ζ-cadinol | 1640 | 1649 | 0.1 | 0.1 | 0.2 | 1200 ± 100 | 700 ± 100 | * |
| 74 | α-muurolol | 1648 | 1654 | 0.3 | - | - | - | - | n.s. |
| 75 | α-cadinol[a,b] | 1652 | 1663 | - | 0.1 | 0.2 | 168 ± 15 | 800 ± 100 | * |
| | Total identified (%) | | | 99.4 | 96.6 | 98.7 | | | |
| | Monoterpene hydrocarbons (%) | | | 84.3 | 78.1 | 78.2 | | | |
| | Oxygenated monoterpenes (%) | | | 7.1 | 12.0 | 11.2 | | | |
| | Sesquiterpene hydrocarbons (%) | | | 6.2 | 4.7 | 7.0 | | | |
| | Oxygenated sesquiterpenes (%) | | | 1.6 | 1.7 | 2.0 | | | |
| | Others (%) | | | 0.1 | 0.1 | 0.3 | | | |

(1) Compounds in bold were previously reported for the same species by (a) Souza *et al.*, 2011 and (b) Ferracini *et al.*, 1995 (8). (2) LRIs literature values from Adams, 2007 (22). (3) Experimental LRIs. (4) Average quantitative composition by peak area normalization considering response factors for each component as equal to 1; (N) indicates compounds found in *B. tridentata* plant material not differentiated by gender (Minteguiaga *et al.*, 2015) (17). (5) Concentration (relative to internal standard *n*-tetradecane) in μg/L of compound in the pure oil. (6) (tr) trace-level compounds (<0.1%). (*) values differ statistically (p ≤ 0.05); n.s., not significant.

**Table 2.** Enantiomeric distribution of selected monoterpene hydrocarbons of *B. tridentata female* (F) and male (M) essential oils.

| | %  | | | | | |
|---|---|---|---|---|---|---|
| Compound | Ba (+)[1] | (+) F | (+) M | Ba (-)[1] | (-) F | (-) M |
| α-thujene | - | 5 ± 2 | 3 ± 1 | - | 95 ± 2 | 97 ± 1 |
| α-pinene | 51.5 | 16 ± 4 | 15.3 ± 0.6 | 48.5 | 84 ± 4 | 84.7 ± 0.6 |
| camphene | - | 3 ± 1 | 4 ± 3 | - | 97 ± 1 | 96 ± 3 |
| sabinene* | - | 24 ± 14 | 23 ± 17 | - | 76 ± 14 | 77 ± 17 |
| β-pinene* | 38.4 | 73 ± 4 | 73 ± 2 | 61.6 | 27 ± 4 | 27 ± 2 |
| limonene | 77.3 | 66.0 ± 0.3 | 66.3 ± 0.3 | 22.7 | 34.0 ± 0.3 | 33.7 ± 0.3 |

The order of elution of monoterpenes was obtained from the literature, working in the same stationary phase. (1) Enantiomeric distribution for *B. articulata* essential oil reported by Simionatto *et al.* (2008) (31). (*) (-)-sabinene and (+)-β-pinene partially co-eluted, resulting in a high standard deviation in the sabinene enantiomeric distribution.
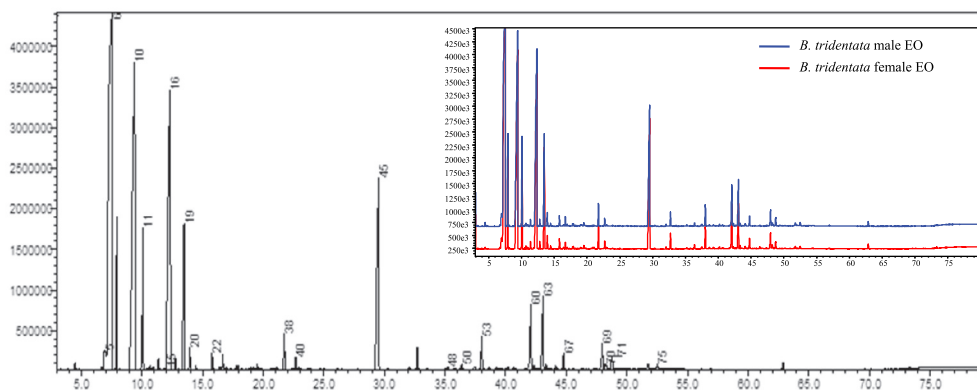


**Figure 1.** Chromatogram of *B. tridentata* male essential oil and comparison of the chromatographic profiles for both genera. For peak identification, see Table 1.

For both sexes, 75 compounds were identified, of which the monoterpene hydrocarbons (α-pinene, β-pinene, limonene) and bornyl acetate were the major components, in agreement with previous results (17). The essential oil components of the female and male oils were essentially identical with no relevant differences between the genders for GC-MS peak percentage areas (Figure 1). From Table 1, it is evident that several compounds of the essential oil were present at trace levels (less than 0.1% of the peak area). A few compounds were responsible for more than 90% of the essential oil composition: α-thujene, α-pinene, camphene, sabinene, β-pinene, myrcene, limonene, (*E*)-β-ocimene, bornyl acetate, germacrene D and bicyclogermacrene. Moreover, the

composition reported in Table 1 is almost the same as that of our previous study, having only small to medium differences in area percentages of the chromatogram peaks. For comparative purposes, we have included in Table 1 the peak area percentage of late-flowering plant material not differentiated by gender collected in our previous work and the compounds found by other authors in the same essential oil (8,17,19). More extreme variations were found in α-pinene, which was less abundant (29.5% in female and 30.8% in male compared to 46.7% in unsexed plant material) and in bornyl acetate, which had increased its proportion in the essential oils (10.1% in female and 9.1% in male against 6.0% in unsexed plant material) (17).

Previously, we found that the volatile composition at the late full flowering stage (September to November) remained almost constant, and with the present results from early blooming, we can ensure that this chemotype of *B. tridentata* has a relatively constant volatile profile during its reproductive period. This information could be of value for the possible exploitation of this aromatic plant by the fragrance industry.

When comparing the quantification results relative to *n*-tetradecane as the internal standard with those obtained using the area normalisation method, large differences were found (Table 1). This was expected considering that an MS detector in full scan mode is not suitable for normalising areas because the analyte structure influences the formation and abundance of the ion fragments (13). Despite this, a great number of papers in the literature have reported normalised areas of essential oil components in full scan mode (13,32).

As can be seen for both genders, there were quantitative differences in the concentrations of the main compounds of the essential oil: in general, most monoterpene hydrocarbons were present in higher levels in the male essential oil. The main compound, α-pinene, was present at 794 ± 40 µg/L in the female essential oil while it was at 1173 ± 60 µg/L in the male essential oil. Compounds such α-phellandrene, α-terpinene, (*Z*)-β-ocimene, *trans*-verbenol, bornyl acetate, germacrene D, bicyclogermacrene, δ-cadinene, spathulenol, viridiflorol and ζ-cadinol appeared to be more concentrated in female essential oil than in male essential oil (see Table 1). These female/male differences could be of ecological importance in the attraction of insect pollinators or maybe in the deterrence of phytophagous insects. For instance, different concentrations in the aromatic profiles render different aromas and different attractiveness of visitors to the flowers (8,12).

The enantiomeric distribution of selected monoterpenes for female and male *B. tridentata* essential oils is presented in Figure 2 and Table 2. The results show no appreciable differences in the enantiomeric distribution between female and male *B. tridentata* essential oils for the studied monoterpene hydrocarbons. To the best of our knowledge, no previous enantiomeric ratios for male/female essential oil components have been published for *Baccharis* species.

The distribution data shown in Table 2 could be useful for chemotaxonomic determinations, since the enantiomeric distribution of essential oil components is a proper characteristic of the plant, reflecting the enzymes engaged in the synthesis of such compounds (24). Therefore, it can be asserted there are significant differences in the enantiomeric distribution obtained here with those shown by Simionatto *et al.* (2008, 31), the only report found dealing with this subject in *Baccharis* genus. These authors reported a majority of dextrogyre α-pinene and levogyre β-pinene in the
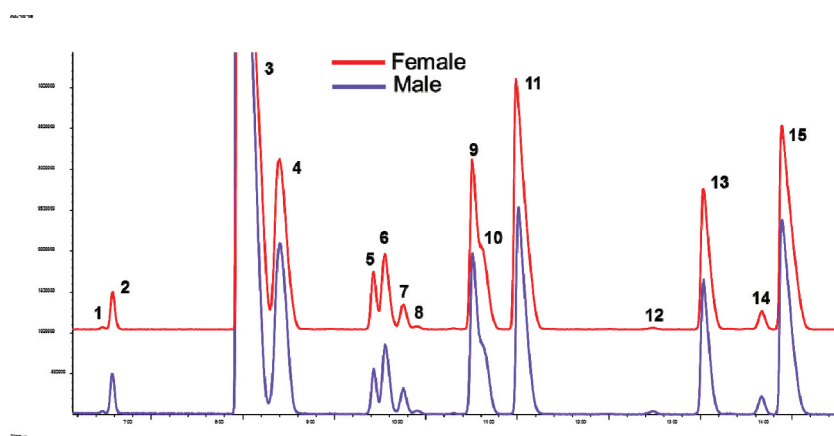


**Figure 2.** CPGC-MS chromatogram of female/male *B. tridentata* essential oil that shows the same enantiomeric distribution for both sexes.

essential oil of unsexed *B. articulata*, while we found the opposite trend. Moreover, both studies found a predominance of limonene dextrogyre enantiomers, but in different ratios (Table 2). These results highlight the importance of enantioselective gas chromatography as a tool to assess the genuineness of essential oils and for detecting adulterations (24).

The same pattern of distribution for monoterpene hydrocarbons was observed for the monoterpene alcohols linalool, terpinen-4-ol and α-terpineol (results not shown), with high standard deviations surely owing to the fact that they are minor components of the essential oils and no exact measure of the peak area could be made. For the case of all sesquiterpenes, only one peak was obtained for every chiral compound. In general, this is the case for almost all aromatic plants as a consequence of the terpene synthase machinery: an enantiomeric mixture of monoterpenes and only one enantiomer for sesquiterpenes (rarely two) and diterpenes (24). In general, the biosynthesis of both sesquiterpene and diterpene enantiomers involves a high metabolic cost to the plant and mixtures have only been observed in hybrid materials with different genetic information (24).

In the case of the enantiomers of minor and trace level compounds present in the essential oils, no generalisation can be made as many peaks were not resolved in the chiral selector or co-eluted with other major peaks.

### Isolation of bornyl acetate from B. tridentata essential oil

The CC isolation of bornyl acetate from *B. tridentata* essential oil (a composite sample of both genders) resulted in four enriched fractions of this compound (F4-F7) which rendered 30 mg of 84% pure bornyl acetate (according to GC-MS analysis). When it was analysed by CPGC-MS, a single peak at the same retention time as enantiomerically pure (-)-bornyl acetate [*endo*-(1*S*)-1,7,7-trimethylbicyclo(2.2.1)hept-2-yl acetate] was observed.

### Analysis of semi-synthetic borneol by CPGC-MS

An impure borneol standard was analysed by CPGC-MS, obtaining good resolution for both enantiomers (together with the resolution of minor impurities camphor and isoborneol), similar to the results reported by Ravid *et al.* (1996, 26). In that analyses, (-)-borneol corresponded to 4% and (+)-borneol amounted to 86% of the total standard area in CPGC-MS.

However, bornyl acetate is one of the worst guest molecules for β-cyclodextrin complexation, similar to limonene and other hydrocarbons, so the selectivity of

inclusion is highly dependent on the experimental conditions, i.e. solid and solution state and the presence of co-substrates (33). In fact, when Donze and Coleman (1993, 33) put together borneol and bornyl acetate in a 1:1 ratio and allowed complexation with β-cyclodextrin to take place, the complex formed had a proportion of 70% borneol and 30% bornyl acetate, demonstrating a preference for the former. The reason for such behaviour remains in the differential settling interactions between chiral molecules and chiral selectors: polar compounds have a greater possibility of interacting with the hydroxyl groups of cyclodextrins through H binding (as donors and acceptors) or dipole-dipole bonding, while non-polar compounds (hydrocarbons and esters) can only interact through Van der Waals forces (24,33). In the case of bornyl and isobornyl acetates, additional esteric effects of the acetyl group and the *gem*-dimethyl bridge could disturb effective interactions with the cyclodextrin structure.

Finally, to determine the chirality of natural bornyl acetate, a borneol sample obtained by saponification of the natural ester was analysed by CPGC-MS. Only one peak was detected, corresponding to the retention time of (-)-borneol. Additional analysis of *B. tridentata* essential oils (female and male) also showed a single peak corresponding to the levogyre enantiomer of natural borneol. This result demonstrated that, independently of the gender, *B. tridentata* synthesizes enantiospecifically (-)-borneol and (-)-bornyl acetate, a common finding in aromatic plants, i.e. *Coridothymus capitatus, Artemisia herba alba, Origanum vulgare, Ocimum canum* and *Tanacethum partenium* (feverfew) (26,27). Conversely, high enantiomeric purities of (+)-borneol are reported in lavender and lavandin commercial essences, while cultivars of rosemary (*Rosmarinus officinalis*) present wide variation in the enantiomeric distribution of borneol and bornyl acetate (26,27).

### Analyses by circular dichroism and optical rotation measurements

To confirm the results obtained by CPGC-MS, natural isolated bornyl acetate was analysed by circular dichroism (Figure 3). The minimum of the spectrum was at 237 nm (−0.64) for bornyl acetate and at 233 nm (−0.59) for borneol. These results are in agreement with published literature results (34) and confirm that natural bornyl acetate is the levogyre enantiomer.

### Conclusion

In this study, we demonstrated a differential volatile profile in the essential oils from early flowering female and male
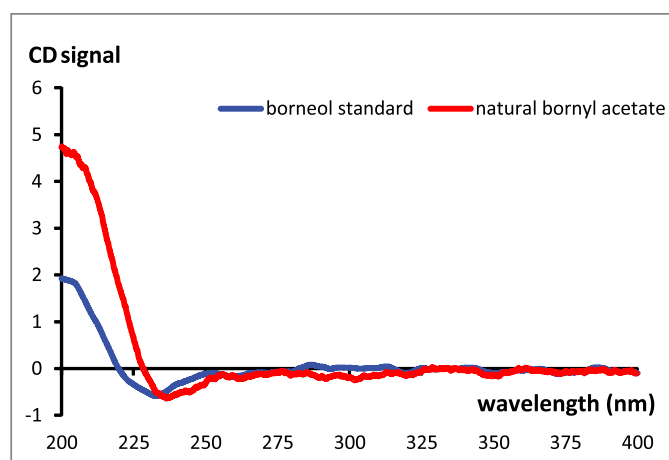
**Figure 3.** CD spectra of borneol standard (mainly dextrogyre enantiomers) and natural bornyl acetate.

*Baccharis tridentata* Vahl, regarding the concentration of individual compounds assessed by GC-MS internal standard quantification. Huge differences were found when comparing these results with those obtained by GC-MS area percentage standardisation in full scan mode, demonstrating the importance of the employment of the former methodology for the accurate quantification of essential oil components. Despite those differences, equal enantiomeric distributions of chiral monoterpene hydrocarbons and enantiospecific biosynthesis of (-)-borneol and (-)-bornyl acetate was found for both genders.

Differential aromatic profiles might be clues for insect pollinators (or possibly phytophagous insects), as we previously hypothesised for the differential gender aromas of *B. articulata* (12). Further chemical ecology research is advised to shed light on female/male metabolic differences in *B. tridentata* Vahl.

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## ORCID

Manuel Minteguiaga http://orcid.org/0000-0002-3177-7272
Eduardo Cassel http://orcid.org/0000-0001-8843-0694
Eduardo Dellacassa http://orcid.org/0000-0002-4764-4212

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