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## Evaluation of the essential oil and tea produced from *Baccharis myriocephala* leaves

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### ABSTRACT

*Baccharis myriocephala* is a carqueja species less studied than other *Baccharis* species. This study evaluated the chemical composition, toxicological and antioxidant potentials of the essential oils and infusions of *B. myriocephala*. Essential oils (hydrodistillation) and the volatile fractions of infusions (SPE) were analyzed by chromatographic techniques. The TPC and TFC of the infusions, and the antioxidant activities of essential oils and infusions were measured by spectrophotometry; their toxicological potentials were evaluated by *Artemia salina* bioassay (ASB). Twenty-eight terpenic compounds were identified in the essential oils, fourteen of them for the first time. Nine compounds were identified in the volatile fraction of the infusions, and seven of them were absent in essential oils. Infusions [ $IC_{50(DPPH)} = 2.21 \text{ mg mL}^{-1}$ ] are better antioxidants than the essential oils [ $IC_{50(DPPH)} = 40.37 \text{ mg mL}^{-1}$ ]. The essential oils were considered highly cytotoxic ( $LD_{50} = 26.64 \text{ } \mu\text{g mL}^{-1}$ ), according to the ASB.

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## Introduction

*Baccharis* (Asteraceae family) is a monophyletic genus (1) which comprises 442 species native to the Americas, from Southeastern Canada to Southern Argentina and Chile (2). In Brazil, there are 179 species, 114 of them endemic to the country (3), from which the species known as ‘carqueja’ generally present winged stems and are classified into two sections: *Baccharis* sect. *Aphyllae* Baker (33 species) and *Baccharis* sect. *Caulopterae* DC. (15 species) (2), being used frequently in traditional medicine to treat gastrointestinal diseases (4). Spasmolytic, diuretic, anti-inflammatory and healing properties in the treatment of bacterial and fungal infections can also be associated to several members of these species (5). Due to the morphological similarity of these species, it’s not easy to discriminate one from another, even by taxonomists. So, they are often collected and marketed indiscriminately (4). In this context, a deeper knowledge of the chemical composition of these plants could be an useful tool to differentiate them, allowing a more efficacious and conscious employment of the medicinal properties of each one. However, the researches on the chemical composition and biological properties of these plants have focused on few species, like *Baccharis trimera* and *B. dracunculifolia*, that have high commercial value (5). On the other hand, there are relatively few

studies, for instance, about *B. myriocephala* (*Baccharis Genistelloides* var. *myriocephala* Baker ex G.M.Barroso) species (5–11). Histochemical and phytochemical studies indicated the presence of essential oil, starch, fatty and cyanogenic compounds, alkaloids, anthocyanins and saponins, as well as gums, mucilages and tannins (7,8). So far, the chemical composition of the essential oils of this species was evaluated only by five research groups (5,6,9–11). *B. myriocephala* is considered a morphologically close taxon of *B. trimera* and, according to Dutra et al. (10), these species have similar chemical compositions, which could explain the failure to discriminate one from the other using high-resolution magic angle spinning nuclear magnetic resonance spectroscopy ( $^1\text{H}$  HR-MAS NMR) combined with chemometric tools. This kind of result brings up the need for a closer look at *B. myriocephala* (10).

Thus, the aim of this study was to characterize the chemical composition, toxicological and antioxidant potentials of the essential oils and infusions of natural *B. myriocephala* samples. According to our knowledge, it’s the first time that the volatile fraction composition of this kind of infusion is being evaluated. There is also no information available in the scientific literature about the antioxidant and toxicological potentials of the essential oils and infusions of this herb.

## Material and methods

### Samples

The fresh samples of this carqueja species were collected during phenological stage by Mrs. Vera Fróes Fernandes in Teresópolis, Rio de Janeiro State, Brazil (22.407270° S; 42.84890° W). These samples were identified by Dr. Gustavo Heiden from Embrapa Temperate Agriculture (RS, Brazil) as *Baccharis myriocephala* DC. A voucher specimen of this plant material was deposited in the ECT Herbarium Embrapa (RS, Brazil) under the ECT0004300 code. The remained samples, divided in three groups, were dried in an oven (FANEM, SP, Brazil) during 24 hours at a temperature range of 55–65°C and, then, milled with the aid of an analytical bench mill (Quimis, model 0298A21, Brazil). Each group was treated as a distinct sample to be investigated during the development of the present study.

### Chemicals

The extraction solvents acetone (purity grade = 99.9%) and ethyl acetate (99.9%), methanol (99.9%),  $\beta$ -caryophyllene (98.5%), hexadecanoic acid (98%), methyl linoleate ( $\geq 98\%$ ), nerolidol (98%), 2-nonanone (99%), and vanillin (99%) were purchased from Aldrich (Milwaukee, WI, USA). The adsorbent known as Porapak Q (50/80 mesh) and the C<sub>9</sub>-C<sub>26</sub> alkane mixture, used as a retention index marker probe, were obtained from Supelco (Bellefonte, PA). All other reagents used in this study were of analytical grade.

### Essential oil extraction from natural *Baccharis myriocephala* samples

The isolation of the essential oil was based on a previous work with some few modifications (12). The essential oils were isolated from 70 g of dried and milled leaves by hydrodistillation (Clevenger apparatus) using a 2000 mL flask with 700 mL of distilled water. This isolation process was carried out during 2 hours at a temperature of 100°C. At the end, the water was eluted from the Clevenger apparatus, and the essential oil was captured by washing the system with 10 mL of ethyl acetate. This organic phase was filtered over anhydrous sodium sulfate and, then, the solvent was eliminated with a N<sub>2</sub> flow. Finally, the essential oil was stored at -4°C until the development of the analyses.

### Isolation of the volatile fraction of *Baccharis myriocephala* infusions

The isolation of the volatile fraction of infusions was carried out according to a previous work (13). First of all, sample (2,0 g) was infused in boiling water (50 mL) with shaking (10 minutes). This extract was cooled with tap water, filtered by gravity and adjusted to a final volume of 100 mL with distilled water. A glass column (14.0 cm x 1.0 cm i.d.) packed with 700 mg of Porapak Q was activated by heating at 220°C during 3 hours under a N<sub>2</sub> flow of 0.9–1.0 L min<sup>-1</sup>. The carqueja infusion was, then, passed through the column by means of a peristaltic pump (Model p-3, Pharmacia, Swiss) at a flow rate of 1.5 mL min<sup>-1</sup>. After that, the column was inverted and washed with 20 mL of water. Adsorbed volatiles were eluted with 100 mL of acetone and concentrated with a vacuum rotary evaporator system (20°C) and nitrogen to a final volume of 50  $\mu$ L. This acetone extract was stored under N<sub>2</sub> at -4°C until the development of the chromatographic analyses.

### Capillary gas chromatography combined with flame ionization detection (GC-FID)

The GC/FID analyses were performed in a GC-2010Plus (Shimadzu, Japan) coupled to a flame ionization detector (FID). The volatile compounds contained in the essential oils or in the acetone extracts obtained from infusions were separated in a fused silica capillary column (30 m x 0.25 mm i.d.) coated with dimethyl polysiloxane (100%) with a film thickness of 0.25  $\mu$ m (SPB-1, Supelco, USA). The temperature of the chromatographic oven was initially programmed to stay during 5 minutes at 60°C and, then, to increase from 60°C to 120°C at a rate of 2°C/minute. This last temperature was maintained during 10 minutes. Finally, the temperature increased at a 10°C/minute rate until it reached 230°C, where it was kept during 30 minutes. The injector temperature was kept at 230°C, while the detector temperature was kept at 240°C. Helium was used as the carrier gas at a flow rate of 1.0 mL minute<sup>-1</sup>. Injections of the undiluted essential oils and acetone extracts (1  $\mu$ L) were performed in splitless mode. Retention rates of the compounds in the column were estimated by the modified Kovats method (14), with the aid of a mixture of saturated alkanes (1,000  $\mu$ g mL<sup>-1</sup> of each component in hexane). The concentrations of the volatile compounds in the essential oils were estimated based on the relative percentage area of their chromatographic peaks in relation to the total area of the chromatogram (normalization technique). The concentrations of the volatile compounds in infusions were estimated by

the external standardization method. Calibration curves were constructed by analyzing standard solutions at three different concentrations under identical experimental conditions. When a reference compound was not available, the semi-quantification process was carried out with the most structurally similar reference compound found in the laboratory. So, in the *Baccharis myrioccephala* infusions, the concentration of 2-methylcyclohexanone was estimated with regard to 2-nonanone; the concentrations of triacetin and methyl (9Z,15Z)-octadeca-9,15-dienoate were estimated with regard to methyl linoleate; the concentrations of palustrol, ledol, spathulenol and  $\beta$ -eudesmol were estimated with regard to nerolidol.

### Capillary gas chromatography combined with mass spectrometry (GC/MS)

Electron impact mass spectrometry analysis was developed in a GC/MS system of the type GC-2010Plus/GCMS-QP2010 of Shimadzu (Japan). The column and chromatographic conditions were the same as described for the GC/FID analysis. The mass spectrometer operated at an ionization voltage of 70 eV, scanning the fragments in the range of 30 to 400  $m/z$ , in cycles of 3 tenths of a second. The temperatures of the ion source and the GC interface were maintained at 240°C. During the analysis of the acetone extracts, the solvent delay used in the GC/MS apparatus was 5 minutes. The identification of the mass spectra of the compounds under analysis was based on comparison with the data available in the NIST12.lib and NIST62.lib libraries, contained in the management software of the GC/MS system. Identification was complemented by the coelution with available external standards and by comparison of the calculated Kovats indexes with those available in literature.

### Total phenols assay

The total phenolic content of the essential oils and infusions was evaluated by the spectrophotometric method of Folin-Ciocalteu, using gallic acid as reference compound (15). The essential oils were analyzed as methanol solutions (2.5 mg mL<sup>-1</sup>), while the infusions were analyzed as the original infusions (see item 2.4). The total phenolic content was calculated from a calibration curve of gallic acid (1, 5, 10, 25, 50 and 100  $\mu$ g mL<sup>-1</sup>) and results were expressed as mg of gallic acid equivalents (GAE) per g of the dry sample. Absorbance was recorded at 760 nm.

### Total flavonoids assay

The total flavonoid content of the infusions was evaluated by the spectrophotometric method of aluminum chloride, using rutin as reference compound (16). Again, the infusions were analyzed as the original beverages (see item 2.4). The total flavonoid content was calculated from a calibration curve of rutin (20, 60, 100, 150, 200, 300, 600 and 1,200  $\mu$ g mL<sup>-1</sup>) and results were expressed as mg of rutin equivalents per g of the dry sample. Absorbance was recorded at 510 nm.

### DPPH assay

Antioxidant activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (17). In the case of the essential oils, four methanol solutions of each oil (1.0 mg mL<sup>-1</sup>, 4.0 mg mL<sup>-1</sup>, 8.0 mg mL<sup>-1</sup> and 12.0 mg mL<sup>-1</sup>) were used to make the antioxidant curve that was employed to calculate the IC<sub>50</sub>. In the case of the aqueous extracts, the original infusions (item 2.4) were used to allow the preparation of four new solutions of each sample (0.10 mg mL<sup>-1</sup>, 0.15 mg mL<sup>-1</sup>, 0.30 mg mL<sup>-1</sup> and 0.60 mg mL<sup>-1</sup>) to be used in the DPPH assay. Absorbance was recorded at 515 nm.

### Brine shrimp lethality assay

This toxicological evaluation was carried out to determine the lethal dose (LD<sub>50</sub>) of the essential oils and infusions by the *Artemia salina* bioassay (ASB) (18). The essential oils were first diluted in methanol, and these methanolic solutions of the essential oils were added in sea water to produce final solutions of 5, 10, 30, 60, 100 and 150 ppm, containing 1.43% (v/v) of methanol. The blank test was carried out with pure methanol (100  $\mu$ L). In the case of infusions, its aliquots were directly added to the sea water to produce final solutions of 100, 200, 400, 600 and 1,000 ppm. In this case, the blank test was done with distilled water. Ten Nauplii (second instar) of brine shrimp were added in each flask and maintained in direct contact with the solutions during 24 hours. A magnifying glass, a light focus and a dark background were used to conduct the readings. These readings consisted in determining how many subjects have died at the end of the experiment. This information allowed the production of mortality curves that were used to establish the LD<sub>50</sub> of the oils and infusions, that is, their ability to kill 50% of the study population. Experiments were conducted with each concentration in triplicate. No deaths were registered when the tubes of the blank tests were analyzed.

## Statistical analysis

All statistical analyses were performed using Graph Pad Prism 6.0 software. The existence of significant statistical differences ( $p < 0.05$ ) between the essential oils and infusions were evaluated by the use of the parametric t test.

## Results and discussion

The volatile compounds found in the essential oils of *Baccharis myriocephala* leaves are listed in Table 1. These essential oils were isolated by hydrodistillation from the leaves with a mean yield of  $(0.18 \pm 0.04)$  g per 100 g of dry sample. Twenty-eight compounds were identified in the essential oils of *Baccharis myriocephala*, and all of them were classified as terpenic compounds: four monoterpenes, twelve sesquiterpenes, eleven oxygenated sesquiterpenes and one oxygenated diterpene. These 28 compounds represent 89.10% of the total content of the essential oils analyzed.  $\beta$ -Copaene  $[(36.06 \pm 5.32)\%]$ ,  $\beta$ -caryophyllene  $[(13.8 \pm 1.81)\%]$ ,  $\beta$ -myrcene  $[(7.17 \pm 2.05)\%]$ ,  $\gamma$ -elemene  $[(3.83 \pm 1.65)\%]$ ,  $\delta$ -guaiene  $[(3.65 \pm 0.64)\%]$ , and  $\beta$ -elemene  $[(3.61 \pm 0.29)\%]$  were the major compounds found in these *Baccharis myriocephala* essential oils. At least in

part, these results differed from those presented by Trombin-Souza et al. (5). In this scientific research, the main constituents of the essential oil of *Baccharis myriocephala* were limonene (41.9%),  $\beta$ -pinene (16.7%), myrcene and caryophyllene oxide (both 4.9%), and (E)-caryophyllene and spathulenol (both 2.2%). According to Simões-Pires et al. (9) the main constituents were the following: epiglobulol (45.8%), globulol (14.5%),  $\beta$ -eudesmol (9.8%), caryophyllene oxide (6.6%), spathulenol (5.6%), and  $\beta$ -pinene (4.8%). On the other hand, Ferracini et al. (6) listed the following major compounds of *B. myriocephala* essential oils:  $\delta$ -cadinene and spathulenol (both 9.44%), (E)-nerolidol (9.01%),  $\beta$ -caryophyllene (4.52%),  $\alpha$ -copaene (2.86%), and globulol (2.45%). These differences could be explained by variations in the soil and climate conditions to which the plants were submitted during their development (19).

The study of Struiving et al. (11) was carried out without separating the samples of *Baccharis myriocephala* from those of *Baccharis trimera*. Therefore, the chemical profiles of the volatile fractions analyzed in that study cannot be compared with the data presented in this one. The study of Dutra et al. (10) identified by the first time carquejyl acetate as a *B. myriocephala* constituent. This compound is commonly reported as

**Table 1.** Chemical Composition of Leaf Essential Oil of *Baccharis myriocephala*.

Compounds	LRI	LRI*	Concentration (%)	Classification
$\beta$ -pinene <sup>b,c</sup>	957	964 <sup>Ph</sup>	0.17 $\pm$ 0.02	M
$\beta$ -Myrcene <sup>b,c</sup>	978	981 <sup>N</sup>	7.17 $\pm$ 2.05	M
Cis- $\beta$ -Ocimene <sup>b,c</sup>	1042	1043 <sup>Ph</sup>	0.97 $\pm$ 0.18	M
$\alpha$ -Cubebene <sup>b,c</sup>	1334	1336 <sup>N</sup>	0.42 $\pm$ 0.31	M
$\alpha$ -Copaene <sup>b,c</sup>	1357	1359 <sup>N</sup>	0.87 $\pm$ 0.01	S
$\beta$ -Elemene <sup>b,c</sup>	1374	1382 <sup>P</sup>	3.61 $\pm$ 0.29	S
$\beta$ -Caryophyllene <sup>a,b,c</sup>	1403	1405 <sup>N</sup>	13.8 $\pm$ 1.81	S
$\alpha$ -Guaiene <sup>b,c</sup>	1418	1418 <sup>N</sup>	0.34 $\pm$ 0.11	S
$\alpha$ -Humulene = $\alpha$ -Caryophyllene <sup>b,c</sup>	1428	1428 <sup>P</sup>	1.94 $\pm$ 0.43	S
$\beta$ -Copaene <sup>b,c</sup>	1466	1460 <sup>N</sup>	36.06 $\pm$ 5.32	S
$\beta$ -Selinene <sup>b,c</sup>	1467	1467 <sup>P</sup>	2.72 $\pm$ 1.60	S
Epi-Bicyclosquisphellandrene <sup>b,c</sup>	1469	1469 <sup>P</sup>	0.82 $\pm$ 0.80	S
Elixene = $\gamma$ -Elemene <sup>b,c</sup>	1478	1478 <sup>P</sup>	3.83 $\pm$ 1.65	S
$\alpha$ -Bulnesene = $\delta$ -Guaiene <sup>b,c</sup>	1485	1489 <sup>P</sup>	3.65 $\pm$ 0.64	S
Dihydro-beta-Agarofuran <sup>b,c</sup>	1493	1493 <sup>P</sup>	0.50 $\pm$ 0.04	OS
$\delta$ -Cadinene <sup>b,c</sup>	1504	1509 <sup>P</sup>	3.43 $\pm$ 1.42	S
Germacrene B <sup>b,c</sup>	1533	1533 <sup>P</sup>	0.71 $\pm$ 0.32	S
Caryophyllene oxide <sup>b,c</sup>	1552	1552 <sup>N</sup>	0.82 $\pm$ 0.17	OS
Palustrol <sup>b,c</sup>	1557	1557 <sup>P</sup>	1.42 $\pm$ 0.79	OS
Globulol <sup>b,c</sup>	1558	1560 <sup>N</sup>	0.29 $\pm$ 0.01	OS
Epiglobulol <sup>b,c</sup>	1563	1564 <sup>P</sup>	0.25 $\pm$ 0.02	OS
Ledol <sup>b,c</sup>	1574	1574 <sup>N</sup>	0.61 $\pm$ 0.08	OS
Cubeno <sup>b,c</sup>	1576	1592 <sup>N</sup>	0.65 $\pm$ 0.04	OS
Carotol <sup>b,c</sup>	1610	1614 <sup>N</sup>	0.96 $\pm$ 0.70	OS
$\delta$ -Cadinol <sup>b,c</sup>	1613	1620 <sup>N</sup>	0.53 $\pm$ 0.20	OS
$\alpha$ -Acorenol <sup>b,c</sup>	1618	1621 <sup>N</sup>	0.45 $\pm$ 0.01	OS
$\alpha$ -Cadinol <sup>b,c</sup>	1623	1624 <sup>P</sup>	0.89 $\pm$ 0.58	OS
Phytol <sup>b,c</sup>	2098	2098 <sup>N</sup>	1.22 $\pm$ 0.15	OD
Total	—	—	89.10	—

LRI – modified Kovats index calculated using C<sub>9</sub>-C<sub>26</sub> alkanes (12); LRI\* – LRI obtained from literature; Monoterpene (M); Sesquiterpene (S); Oxygenated sesquiterpene (OS); oxygenated diterpene (OD); <sup>a</sup> - Identified by coelution with standard volatile compounds; <sup>b</sup> - Identified by comparing the calculated LRI with the LRI from literature (LRI\*); <sup>c</sup> - Identified by mass spectra data; <sup>P</sup> - PubChem, <sup>N</sup> - NIST, <sup>Ph</sup> - Pherobase.

a major constituent of the essential oils of *B. trimera*, being considered the chemical marker of this species. Carquejyl acetate was not found in the samples analyzed in the present work.

In the present study, 14 terpenic compounds were identified for the first time as *Baccharis myriocephala* essential oil constituents:  $\alpha$ -guaiene,  $\beta$ -copaene,  $\beta$ -selinene, *epi*-bicyclosesquiphellandrene,  $\gamma$ -elemene,  $\delta$ -guaiene, dihydro- $\beta$ -agarofuran, germacrene-B, palustrol, cubenol, carotol,  $\delta$ -cadinol,  $\alpha$ -acorenol, and phytol.

Table 2 and 3 shows the volatile compounds identified in *Baccharis myriocephala* infusions. To our knowledge, this is the first time that this volatile fraction is evaluated and nine compounds were found in this kind of beverage: one ketone, one triglyceride, four terpenic compounds, one phenolic aldehyde, one fatty acid and one fatty acid derivative. The selectivity of the solid phase extraction method used to isolate the volatile fraction of the infusions allowed the identification of other compounds not found in the essential oils of these samples: 2-methylcyclohexanone, triacetin, vanillin, spathulenol,  $\beta$ -eudesmol, hexadecanoic acid, and methyl (9Z,15Z)-octadeca-9,15-dienoate. Although present in this group, spathulenol and  $\beta$ -eudesmol had yet been identified as *Baccharis myriocephala* constituents (5,6,9). The aroma of vanillin is associated with sweet, vanilla-like, delightful, hard candy and sucrose notes

(20). It presents an extremely low odor threshold in air (0.6–1.2 ng/L) and also a low odor threshold in water (30  $\mu$ g/L) (20,21). Nonetheless, this compound cannot be classified as an odor-active compound of this kind of infusion, since its odor activity value (OAV) was estimated as 0.05, being lower than a unit.

The mean value for the TPC of the *Baccharis myriocephala* infusions was (57.42  $\pm$  0.14) mg of GAE L<sup>-1</sup> of beverage. This value was lower than the mean TPC value (806.1 mg of GAE L<sup>-1</sup>) found for commercial Chilean boldo infusions (18). The TPC of the *Baccharis myriocephala* infusions analyzed in the present study [(25.26  $\pm$  1.72) mg GAE g<sup>-1</sup> of herb] was also lower than the TPC [(89.81  $\pm$  13.96) mg of GAE g<sup>-1</sup>] of fresh infusions produced from the flour of *Pereskia aculeata* Miller stems (22). Concerning the TFC of the *Baccharis myriocephala* infusions, the estimated mean value [(11.58  $\pm$  1.25) mg of RE g<sup>-1</sup> of herb] was close to that found for the TFC of fresh stem infusions of *Pereskia aculeata* Miller [(14.80  $\pm$  3.28) mg of RE g<sup>-1</sup>] (22), but lower than the mean TFC value (755.92 mg of RE L<sup>-1</sup>) estimated for Chilean boldo infusions (18).

The mean IC<sub>50(DPPH)</sub> value found for the *Baccharis myriocephala* essential oils was (40.37  $\pm$  4.69) mg mL<sup>-1</sup>. This value was 18.3 times bigger ( $p < 0.05$ ) than that found for infusions [IC<sub>50(DPPH)</sub> = (2.21  $\pm$  0.27) mg mL<sup>-1</sup>], indicating that the infusions of this herb are more powerful antioxidant agents than its essential oils. These essential

**Table 2.** Chemical Characterization of the Volatile Fraction of *Baccharis Myriocephala* Infusion.

Compounds	LRI	LRI*	Concentration ( $\mu$ g L <sup>-1</sup> )	Classification
2-Methylcyclohexanone <sup>c</sup>	984	—	20.75 $\pm$ 1.06 <sup>d</sup>	ketone
Triacetin <sup>b,c</sup>	1310	1313 <sup>N</sup>	10.00 $\pm$ 0.01 <sup>e</sup>	Triglyceride
Vanillin <sup>a, b, c</sup>	1337	1347 <sup>N</sup>	1.50 $\pm$ 0.71	Phenolic aldehyde
$\beta$ -Caryophyllene <sup>a,b,c</sup>	1403	1405 <sup>N</sup>	17.5 $\pm$ 3.54	Sesquiterpene
Palustrol <sup>b,c</sup>	1551	1557 <sup>P</sup>	20.00 $\pm$ 7.07 <sup>f</sup>	Oxygenated sesquiterpene
Spathulenol <sup>b,c</sup>	1564	1572 <sup>N</sup>	18.0 $\pm$ 3.46 <sup>f</sup>	Oxygenated sesquiterpene
$\beta$ -Eudesmol <sup>b,c</sup>	1620	1622 <sup>P</sup>	20.58 $\pm$ 6.05 <sup>f</sup>	Oxygenated sesquiterpene
Hexadecanoic acid <sup>a,b,c</sup>	1941	1942 <sup>N</sup>	117.5 $\pm$ 34.25	Fatty acid
Methyl (9Z,15Z)-octadeca-9,15-dienoate <sup>c</sup>	2101	—	5.75 $\pm$ 2.01 <sup>e</sup>	Fatty acid derivative

LRI – modified Kovats index calculated using C<sub>9</sub>-C<sub>26</sub> alkanes (12); LRI\* – LRI obtained from literature; <sup>P</sup> – PubChem, <sup>N</sup> – NIST, <sup>a</sup> – Identified by coelution with standard volatile compounds; <sup>b</sup> – Identified by comparing the calculated LRI with the LRI from literature (LRI\*); <sup>c</sup> – Identified by mass spectra data; <sup>d</sup> – Concentration given in ppb 2-nonanone equivalent; <sup>e</sup> – Concentration given in ppb methyl linoleate equivalent; <sup>f</sup> – Concentration given in ppb nerolidol equivalent.

**Table 3.** Antioxidant Activity (IC<sub>50(DPPH)</sub>), Toxicological Potential, Total Phenolic Content, and Total Flavonoid Content of *Baccharis Myriocephala* Infusions and Essential Oils.

Samples	IC <sub>50(DPPH)</sub> (mg mL <sup>-1</sup> )	LD <sub>50(ASB)</sub> ( $\mu$ g mL <sup>-1</sup> )	TPC (*mg GAE g <sup>-1</sup> ) or (#mg GAE L <sup>-1</sup> )	TFC ( <sup>§</sup> mg RE g <sup>-1</sup> ) or ( <sup>®</sup> mg RE L <sup>-1</sup> )
Infusions (Avg $\pm$ SD)	2.21 $\pm$ 0.27 <sup>a</sup>	>1000 <sup>a</sup>	*25.26 $\pm$ 1.72 #57.42 $\pm$ 0.14	<sup>§</sup> 11.58 $\pm$ 1.25 <sup>®</sup> 525.56 $\pm$ 19.64
Essential oils (Avg $\pm$ SD)	40.37 $\pm$ 4.69 <sup>b</sup>	26.64 $\pm$ 0.90 <sup>b</sup>	—	—

IC<sub>50(DPPH)</sub> – the concentration of an antioxidant which reduces the free radical DPPH• about 50%; LD<sub>50(ASB)</sub> – lethal dose capable to kill 50% of the tested population by the *Artemia salina* bioassay; TPC – total phenolic content; GAE – gallic acid equivalent; TFC – total flavonoid content; RE – rutin equivalent; \* – TPC expressed as mg GAE g<sup>-1</sup> of herb; # – TPC expressed as mg GAE L<sup>-1</sup> of beverage; <sup>§</sup> – TFC expressed as mg RE g<sup>-1</sup> of herb; <sup>®</sup> – TFC expressed as mg RE L<sup>-1</sup> of beverage; Avg – average value; SD – standard deviation. In a specific column of this table, when infusions were compared with essential oils in relation to a parameter that was measured for both, the values marked with different letters were statistically different ( $p < .05$ ).

oils are still weaker antioxidant than those obtained from *Baccharis trimera* ( $IC_{50(DPPH)} = 6.193 \text{ mg mL}^{-1}$ ) and *Baccharis dracunculifolia* ( $IC_{50(DPPH)} = 3.521 \text{ mg mL}^{-1}$ ) (23). The antioxidant potentials of the infusions of both herbs (*Baccharis trimera* –  $IC_{50(DPPH)} = 0.049 \text{ mg mL}^{-1}$ ; *Baccharis dracunculifolia* –  $IC_{50(DPPH)} = 0.037 \text{ mg mL}^{-1}$ ) are also stronger than the antioxidant capacity of the *Baccharis myriocephala* infusions (23). It can be explained by the variability of the chemical composition of these different *Baccharis* species and also by differences in the preparation methods employed to produce the aqueous extracts. The *Baccharis myriocephala* infusions presented a higher  $IC_{50(DPPH)}$  mean value than the fresh infusions prepared from the *Pereskia aculeata* Miller stems [ $IC_{50(DPPH)} = (0.50334 \pm 0.03155) \text{ mg mL}^{-1}$ ], being weaker antioxidants than the last ones (22). This behavior was also noted when the essential oils and infusions of the *Baccharis myriocephala* samples were compared with the same products prepared from commercial Chilean boldo samples. The mean  $IC_{50(DPPH)}$  value of the essential oils of this last herb was estimated as  $12.53 \text{ mg mL}^{-1}$  (18). For the infusions, the mean  $IC_{50(DPPH)}$  value found was  $0.43 \text{ mg mL}^{-1}$  (18).

The  $LD_{50(ASB)}$  estimated for the *B. myriocephala* essential oils was  $(26.64 \pm 0.90 \mu\text{g mL}^{-1})$ . This  $LD_{50(ASB)}$  value is similar to that found for the essential oils of commercial Chilean boldo samples [ $(26.20 \pm 5.59) \text{ ppm}$ ] sold in sachets (18). This value shows a high cytotoxic potential ( $LD_{50} < 200 \text{ ppm}$ ) for this kind of essential oil, encouraging future studies to evaluate its application, for instance, as an antibacterial or antifungal agent. On the other hand, the *Baccharis myriocephala* infusions had no toxicity ( $LD_{50} > 1000 \text{ ppm}$ ) according to the *Artemia salina* bioassay. However, this kind of infusion must still be consumed with care. Further toxicological tests must be carried out, and the composition of all its fractions must be thoroughly clarified to allow a safe consumption of this plant.

## Conclusions

In the present study, 13 compounds ( $\alpha$ -guaiene,  $\beta$ -copaene,  $\beta$ -selinene, *epi*-bicyclosesquiphellandrene,  $\gamma$ -elemene,  $\delta$ -guaiene, dihydro- $\beta$ -agarofuran, germa-crene-B, cubenol, carotol,  $\delta$ -cadinol,  $\alpha$ -acorenol, phytol) were identified by the first time as *Baccharis myriocephala* essential oil constituents. Additionally, the same happen with five volatile compounds (2-methylcyclohexanone, triacetin, vanillin, hexadecanoic acid, and methyl (9Z,15Z)-octadeca-9,15-dienoate) detected in the infusions of this herb.

Palustrol, identified in both fractions, was also identified for the first time in the leaves of this plant. Measurable amounts of phenolic and flavonoid compounds were detected in the *B. myriocephala* infusions by the spectrophotometric methods employed in the present study. Infusions of this herb [ $IC_{50(DPPH)} = (2.21 \pm 0.27) \text{ mg mL}^{-1}$ ] are better antioxidants than its essential oils [ $IC_{50(DPPH)} = (40.37 \pm 4.69) \text{ mg mL}^{-1}$ ], thereby indicating that antioxidant activity is concentrated in the non-volatile fraction of this kind of herb. The  $LD_{50(ASB)}$  estimated for the *B. myriocephala* essential oils was  $(26.64 \pm 0.90 \mu\text{g mL}^{-1})$ . This value shows a high cytotoxic potential ( $LD_{50} < 200 \text{ ppm}$ ) for this kind of essential oil. On the other hand, *Baccharis myriocephala* infusions had no toxicity ( $LD_{50} > 1000 \text{ ppm}$ ) according to the *Artemia salina* bioassay.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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