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

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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;

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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

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).

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.

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 °C min^{-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.