© 2014
Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 13 (3): 249 - 253
ISSN 0717 7917
www.blacpma.usach.cl

tículo Original | Original Article

Volatile constituents from leaves of *Blepharocalyx salicifolius* (Kunth) O. Berg (Myrtaceae)

[Constituyentes volátiles de hojas de *Blepharocalyx salicifolius* (Kunth) O. Berg (Myrtaceae)]

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Abstract: The chemical composition of the essential oil obtained by hydrodistillation from fresh leaves of *Blepharocalyx salicifolius* (Kunth) O. Berg (Myrtaceae) was analyzed by gas chromatography/mass spectrometry (GC/MS). Twenty-two compounds were identified and a predominance of sesquiterpenes was observed (twenty sesquiterpenes - 96.53%). This study presents for the first time the qualitative chemical composition of the essential oil from the leaves of *B. salicifolius* in their native habitat of Brazilian Cerrado. The qualitative chemical profile of essential oil obtained from the specimen in Cerrado differs from those obtained from specimens collected in biomes of southern parts of South America.

Keywords: *Blepharocalyx salicifolius*, Myrtaceae, Cerrado, essential oil, GC/MS

Resumen: La composición química del aceite esencial obtenido por destilación al vapor de las hojas frescas de *Blepharocalyx salicifolius* (Kunth) O. Berg (Myrtaceae) se analizó por CG/EM (Cromatografia de Gases/ Espectrometría de Masas). Veintidós compuestos fueron identificados, predominantemente sesquiterpenos (veinte sesquiterpenos - 96.53%). Este estudio demuestra por primera vez, el análisis químico cualitativo de aceite esencial de hojas de *B. salicifolius* en su hábitat nativo del Cerrado brasileño. El perfil químico del aceite esencial obtenido de la muestra B. salicifolius del Cerrado difiere de la obtenida a partir de muestras recogidas en los biomas del sur de América del Sur.

Palabras Clave: *Blepharocalyx salicifolius*, Myrtaceae, Cerrado, aceite esencial, CG/EM

Recibido | Received: June 6, 2013
Aceptado en versión corregida | Accepted in revised form: October 25, 2013
Publicado en línea | Published online: May 30, 2014
Declaración de intereses | Declaration of interests: This work was financially supported by the Fundação de Apoio à Pesquisa de Minas Gerais (FAPEMIG -APQ-00354-08).
Este artículo puede ser citado como / This article must be cited as: WM Godinho, MM Farnezi, IM Pereira, LE Gregorio, CFF Grael. 2014. Volatile constituents from leaves of *Blepharocalyx salicifolius* (Kunth) O. Berg (Myrtaceae). **Bol Latinoam Caribe Plant Med Aromat 13 (3): 249 – 253.**

INTRODUCTION

The family of Myrtaceae comprises approximately 144 genera and 3,100 species of trees and shrubs. Some members of this family produce essential oils, which have medicinal applications. The genus *Blepharocalyx* consists of plants that are distributed throughout Latin America. *Blepharocalyx salicifolius* grows in Argentina, Bolivia, Brazil, Ecuador, Paraguay, and Uruguay (Silva Jr *et al.*, 2005; Gupta, 2008; Bruzinga *et al.*, 2009). This species is known by several synonyms, including *B. tweediei* and *B. gigantea* (Gupta, 2008; Stefanello *et al.*, 2011). In Brazilian biomes (Caatinga, Cerrado, Atlantic Forest, and Pampas), *B. salicifolius* occurs naturally and is popularly known as "maria-preta" or "murta" (Gupta, 2006; Forzza, 2010; Stefanello *et al.*, 2011). An infusion of fresh leaves of this plant is used in folk medicine for the treatment of diarrhea, urethritis, bladder infections, hypotension, rheumatism, leucorrhea, and respiratory tract infections (Tucker *et al*., 1993; Dellacassa *et al.*, 1997; Limberger *et al*., 2001; Silva Jr *et al*., 2005; Stefanello *et al.*, 2011).

Monoterpenes, sesquiterpenes, triterpenes, phenylpropanoids, and flavonoids have been reported in extracts of *B. salicifolius* (Furlan *et al.*, 2002; Siqueira *et al.*, 2011). Extracts and isolated compounds from *B. salicifolius* have been shown to possess leishmanicidal activity (Siqueira *et al*., 2010; Siqueira *et al.*, 2011). Furthermore, a study evaluating the biological potential of the essential oil obtained from this species revealed that the oil exhibits antimicrobial and antidiarrheal activity (Limberger *et al.*, 2001).

Previous analyses of the chemical composition of essential oils obtained by hydrodistillation from fresh leaves of *B. salicifolius* showed variations in quality and quantity of the components. This variation was dependent on the location and the year or season of collection (Talenti *et al.*, 1984; Tucker *et al.*, 1993; Dellacassa *et al*., 1997; Moreira *et al.*, 1999; Limberger *et al.*, 2001; Furlan *et al.*, 2002). So far, all studies of essential oil of *B. salicifolius* have been made with plant materials collected in southern part of South America (the Pampa biome). This study presents the first qualitative chemical composition of essential oil from fresh leaves of *B. salicifolius* collected in Brazilian Cerrado (Reserva Guapuruvu, Itamarandiba, Minas Gerais State, Brazil).

MATERIAL AND METHODS

Plant material

Leaves of *B. salicifolius* were collected in the legal reserve of Arcelor Mittal in Itamarandiba-MG (17°44'24.88"S and 42°46'17.72"W), in the afternoon (2 pm) of September 17, 2010. A voucher specimen was deposited in the DIAM Herbarium/UFVJM (Diamantina, MG, Brazil), under the n°1901. The identity of the species was validated by Prof. Dr. Israel Marinho Pereira (Departamento de Engenharia Florestal/FCA/UFVJM).

Extraction of essential oil and GC/MS (Gas Chromatography / Mass Spectrometry) analysis

Fresh leaves (approximately 60.0 g) were submitted to hydrodistillation for 4 h in a Clevenger apparatus. After extraction, the organic layer (essential oil) was separated from water by decantation and then desiccated by slow percolation over anhydrous Na_2SO_4 (SYNTH) in a simple filtration system. The essential oil was stored at low temperature (−20° C) in a hermetically sealed container and protected from light until its analysis.

The essential oil was analyzed by GC/MS on a Shimadzu GC-MS-QP2010 equipped with capillary column DB-5-MS AGILENT (30 m × 0.25 mm, film thickness 0.25 μm). Helium, at 65.4 kPa, was used as the carrier gas with a flow of 1.10 mL/min. The temperature in the injector was 250º C; the temperature of the oven was programmed to rise from 60 to 240º C at a rate of 3º C/min. The electron ionization of analytes was carried out at 70 eV. The quantity of each compound is reported without standardization as a raw percentage based on total ion current. Subsequently, under the same experimental conditions, the oil was co-injected with a homologous series of linear hydrocarbons (C_9–C_{25})-ALLTECH, to calculate the retention index (RI) of each constituent of the sample by applying Van den Dool and Kratz Equation (1963). The compounds were identified by comparison of RI with those reported in literature (Adams, 1995) and by mass spectral analyses (comparison with spectra in Wiley 7, NIST 62, and FFNSC 1.3 databases).

RESULTS AND DISCUSSION

The yield of essential oil obtained by extraction of fresh leaves of *B. salicifolius* was 0.70% (v/w). The constituent monoterpenes and sesquiterpenes were identified by GC/MS analysis. A qualitative predominance of sesquiterpenes was observed. Twenty-two constituents of the oil were identified, among them twenty (96.53%) were sesquiterpenes and two (1.29%) were monoterpenes. The identified terpenes are shown in Table 1.

Previous studies with leaves collected in Brazil (Rio Grande do Sul) showed 1,8-cineole (25.2%) and β-caryophyllene (22.9%) as major constituents of the essential oil (Limberger *et al.*, 2001). In another study, monoterpenes 1,8-cineole (45.8%) and β-pinene (12.3%) were identified (Moreira *et al.*, 1999). Essential oils obtained from materials collected in parts of Argentina showed monoterpenes as major constituents, for e.g., verbenone (13.5%) and limonene (10.8%) (Talenti *et al.*, 1984); 1,8-cineole (34.5%) and limonene (17.24%) (Tucker *et al.*, 1993); and 1,8-cineole (53.5%) and α-pinene (15.8%) (Furlan *et al.*, 2002). The essential oil obtained from leaves collected in Uruguay revealed the predominant presence of 1,8-cineole (55.7%) and caryophyllene oxide (4.1%) (Dellacassa *et al.*, 1997). The predominance of monoterpenes, such as 1,8-cineole (Tucker *et al.*, 1993; Dellacassa *et al.*, 1997; Moreira *et al.*, 1999; Limberger *et al.*, 2001; Furlan *et al.*, 2002) and limonene (Talenti *et al.*, 1984; Tucker *et al.*, 1993; Moreira *et al.*, 1999; Furlan *et al.*, 2002), is noted; however, these compounds were not detected in our study. Furthermore, α-pinene and linalool, monoterpenes identified in this study, were present in other samples of essential oils in concentrations ranging from 1 to 20% (Talenti *et al.*, 1984; Tucker *et al.*, 1993; Dellacassa *et al.*, 1997; Moreira *et al.*, 1999; Limberger *et al.*, 2001; Furlan *et al.*, 2002).

Table 1
Chemical constituents of the essential oil obtained from fresh leaves of *Blepharocalyx salicifolius* specimen collected in the Cerrado (Itamarandiba, MinasGerais, Brazil) identified by GC/MS.

Constituents	RI (experimental)	RI (reference)*	Percent**
α-Pinene	931	939	1.11
Linalool	1099	1098	0.18
β-Elemene	1386	1391	0.15
β-Cariophyllene	1415	1418	1.49
α-Humulene	1450	1454	0.15
Germacrene D	1476	1480	0.16
β-Selinene	1484	1485	0.13
Bicyclogermacrene	1490	1494	0.69
Elemol	1544	1549	7.29
Spathulenol	1570	1576	0.80
Cariophyllene oxide	1575	1581	0.35
Globulol	1579	1583	1.70
Viridiflorol	1587	1590	0.96
Cubeban-11-ol	1590	1599	0.46
Rosifoliol	1601	1609	1.11
10-*epi*-γ-Eudesmol	1615	1619	0.94
γ-Eudesmol	1627	1630	31.09
Hinesol	1634	1638	0.77
Agarospirol	1643	1646	0.24
α-Eudesmol	1650	1652	47.65
7-*epi*-α-Eudesmol	1655	1658	0.15
Juniper-camphor	1691	1691	0.25
TOTAL			**97.82**

GC/MS: gas chromatography/mass spectrometry; RI: retention index.
***Adams (1995).**
****** Percent based on total ion current without standardization**

As for the sesquiterpenes, β-caryophyllene, caryophyllene oxide, and viridiflorol were reported in previous studies (Dellacassa *et al.*, 1997; Limberger *et al.*, 2001; Furlan *et al.*, 2002), while low concentrations of α- and γ-eudesmol, globulol, α-humulene (Tucker *et al.*, 1993; Dellacassa *et al.*, 1997; Limberger *et al.*, 2001) were also observed. All these sesquiterpenes were observed in the essential oil analyzed in our study.

Qualitative and quantitative profile of secondary metabolites in different specimen can be influenced by environmental factors, genetic characteristics, and plant growth and development (Lopes *et al.*, 1997; Martinez *et al.*, 2005; Gobbo-Neto and Lopes, 2007, Costa *et al.*, 2009). Therefore, we observed that the qualitative chemical profile of the essential oil extracted from fresh leaves of *B. salicifolius* collected in the Cerrado differ from those observed in essential oils obtained from leaves collected from the same species elsewhere in South America. This probably results from, among other factors, because the biotic and abiotic factors of the geographic region (biome) where the specimens are found.

Terpenes α-eudesmol, γ-eudesmol, elemol, and α-pinene, when isolated or present in large amounts in the essential oils of other plant species, have been ascribed to possess antimicrobial (Tellez *et al.*, 2000, Ho *et al.*, 2009; Montenegro *et al.*, 2012; Da Silva *et al.*, 2012), antioxidant (Dar *et al.*, 2011), anti-inflammatory (Min-Jun *et al.*, 2011), and other medicinal properties (Nakatsu *et al.*, 2000; Seng-Sung *et al.*, 2012). Thus, the presence of these terpenes in the essential oil of *B. salicifolius* supports the folk-medicinal use of this plant against infections, inflammations, and other diseases.

ACKNOWLEDGEMENTS

The authors thank to Izabel Cristina Casanova Turatti and Prof. Dr. Norberto Peporine Lopes (Núcleo de Pesquisa em Produtos Naturais e Sintéticos/FCFRP-USP) for technical cooperation. Special thanks to Departamento de Farmácia/UFVJM and Arcelor Mittal. This work was financially supported by the Fundação de Apoio à Pesquisa de Minas Gerais (FAPEMIG - APQ-00354-08).

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