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Phenolic derivatives from *Baccharis retusa* DC. (Asteraceae)

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1. Subject and source

Aerial parts of the small perennial shrub *Baccharis retusa* DC. were collected during October 2008 in Campos do Jordão, São Paulo, Brazil. A voucher specimen (SPSF44897) was deposited at the Herbario D. Bento Pickel, Instituto Florestal de São Paulo (IF/SP), São Paulo, Brazil.

2. Previous work

The genus *Baccharis* is a member of the family Asteraceae and comprises approximately 500 species. Members of this genus have been described in various regions of South and Central America, particularly in Brazil, Colombia, Argentina and Mexico (Heras, 1976). The genus is known to accumulate several classes of natural products, including flavonoids (Bohm and Stuessy, 2001; Grecco et al., 2010a; Verdi et al., 2005), diterpenes (Fullas et al., 1994; Torres et al., 2000), triterpenes (Grecco et al., 2010b; Moreira et al., 2003; da Silva Filho et al., 2009) and trichothecenes (Verdi et al., 2005). Additionally, various terpenoids have been identified in the essential oils of *Baccharis* species (Agostini et al., 2005; Biurrum et al., 2005; Garcia et al., 2005; Lago et al., 2008a; Malizia et al., 2005; Pino et al., 2006; Simões-Pires et al., 2005), and differences in the chemical profiles of male and female specimens have been reported (Ferracini et al., 1995; Lago et al., 2008b; Zunino et al., 2004).

B. retusa is distributed widely throughout Brazil and is reported to have both ethnomedicinal and therapeutic uses (Barroso, 1976). Details of the isolation of sakuranetin (Herz et al., 1977) and 5,6,7-trihydroxy-4′-methoxy flavanone (Grecco et al., 2010a) from *B. retusa* have been published, but more extensive information concerning the constituents of this species is not currently available. In this paper, we describe for the first time the phytochemical analysis of a methanolic extract derived from the aerial parts of *B. retusa* and the isolation of eight flavonoids and three chlorogenic acid derivatives.

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3. Present study

Powdered air-dried aerial parts of *B. retusa* (460 g) were defatted with hexane (4 × 500 mL) and extracted exhaustively with MeOH at room temperature. After partial removal of the solvent, the extract was resuspended in MeOH:H_2O (1:2 v/v) and extracted successively with hexane, CH_2Cl_2, EtOAc and n-BuOH. The CH_2Cl_2 phase was subjected to CC over silica gel and eluted with CH_2Cl_2 containing increasing amounts of EtOAc (up to 100%), then with EtOAc:MeOH (1:1 v/v), and finally with pure MeOH to yield eight fractions (*A1–A8*). Fraction *A1* (89 mg) comprised fatty material while fraction *A2* (736 mg) afforded **1**. Fraction *A4* was subjected to CC over silica gel with the chromatographic conditions described above to yield twelve fractions (*A4/1–A4/12*). Fractions *A4/2* (29.4 mg) and *A4/8* (6.5 mg) afforded **2** and **6**, respectively. NMR analysis revealed that fractions *A5–A8* comprised free fatty acids. The EtOAc phase was subjected to CC over Sephadex LH-20 and eluted with MeOH to yield seven fractions (*B1–B7*). Fractions *B5* (782 mg), *B6* (22.8 mg), and *B7* (5.6 mg) afforded pure compound **7**. Fractions *B3* and *B4* were subjected separately to CC over Sephadex LH-20 and eluted with MeOH to yield, respectively, five (*B3/1–B3/5*) and six (*B4/1–B4/6*) fractions. Fractions *B4/3* (94 mg) and *B4/4* (6.4 mg) afforded **7** and **6**, respectively. Fraction *B3/3* was submitted to semi-preparative reversed-phase (C_{18}) HPLC eluted with MeOH:H_2O (linear gradient from 1:1–1:0 v/v over 20 min; flow rate 2.4 mL/min; detection 280 nm) to afford **2** (9.6 mg), **4** (19.1 mg) and **5** (17.4 mg). Fraction *B4/2* was submitted to semi-preparative reversed-phase (C_{18}) HPLC using the chromatographic conditions described above to afford **3** (6.3 mg), **8** (8.1 mg) and a mixture of **7** and **8** (7.8 mg). The BuOH phase was subjected to CC over Sephadex LH-20 and eluted with MeOH to yield ten fractions (*C1–C10*). Fraction *C8* (50.3 mg) afforded a mixture of **9–11**.

The ^{1}H and ^{13}C NMR spectra, as well as the low resolution ESI-MS, of compounds **1–11** were recorded and compared with literature data to establish the following identities: **1** – sakuranetin; **2** – naringenin, **3** – eriodictyol, **4** – taxifolin, **5** – aromadendrin, **6** – apigenin, **7** – quercetin, **8** – kaempferol, **9** – 3,4-O-dicaffeoylquinic acid, **10** – 3,5-O-dicaffeoylquinic acid, and **11** – 4,5-O-dicaffeoylquinic acid.

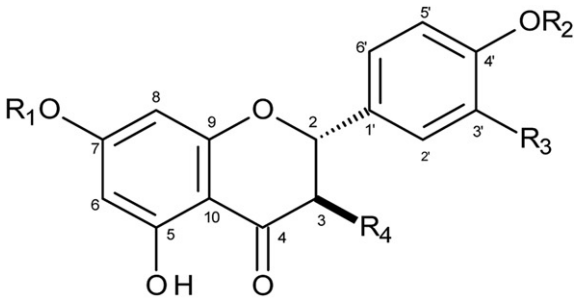
4. Chemotaxonomic significance

Details of the isolation of eight flavonoids (**1–8**) and three chlorogenic acids (**9–11**) (Fig. 1) from the aerial parts of *B. retusa* are described in the present report. Some authors have considered flavonoid derivatives to be valuable taxonomic markers for tribes and subtribes of the Asteraceae, particularly for Astereae/Baccharidinae of which the genus *Baccharis* is a member (Emerenciano et al., 2001; Grecco et al., 2010b). According to Verdi et al. (2005), species of *Baccharis* accumulate mainly flavanones and flavones, and this is in agreement with the present identification of the flavanones **1–3** and of the flavones **6–8** in *B. retusa*. Flavonoids **4** and **5** are dihydroflavonols, and the occurrence in *Baccharis* of this class of compound is more restricted. Taxifolin (**4**) and aromadendrin (**5**) have been isolated previously from *Baccharis illinita* (Verdi et al., 2004), while the former has been detected in *Baccharis petiolata* (Bohm and Stuessy, 2001) and the latter in *Baccharis pseudotenuifolia* (Moreira et al., 2003) and *Baccharis punctulata* (Jakupovic et al., 1987). The present report is the second to record the co-occurrence of **4** and **5** in a species of the genus *Baccharis*.

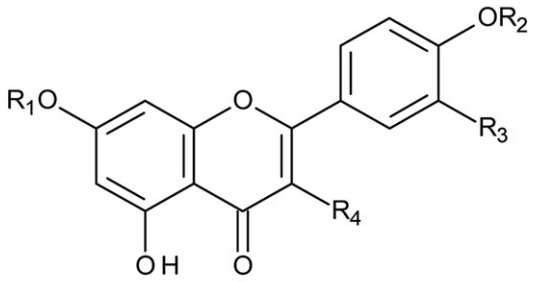
The presence of compounds **2–8** in *B. retusa* is described here for the first time. All of these flavonoids present free hydroxyl groups at C-7, C-5, C-4′ and, with the exception of **2** and **6**, at C-3 and/or C-3′. However, flavonoids that occur in species of *Baccharis* frequently possess methoxyl substituents, particularly at C-6 or C-7 of ring A or at C-4′ of ring B (Emerenciano et al., 2001). On this basis, sakuranetin (**1**) could be considered to be a typical flavonoid of the genus, and its presence has been reported in *Baccharis confertifolia* (Faini and Castillo, 1990), *Baccharis intermixta* (Bohlmann et al., 1981a), *Baccharis leptocephala* (Bohlmann et al., 1981b), *Baccharis paniculata* (Faini and Castillo, 1990), *B. petiolata* (Zdero et al., 1991), *Baccharis pteronioides* (Wollenweber et al., 1986), *Baccharis phylicoides* (Bohlmann et al., 1985), *Baccharis salicifolia* (Zdero et al., 1986), *Baccharis serrulata* (Bohlmann et al., 1981a), *Baccharis sternbergiana* (Bohlmann et al., 1984), *Baccharis thesioides* (Jakupovic et al., 1990), *Baccharis tricuneata* (Bohlmann et al., 1984), and *Baccharis trinervis* (Bohlmann et al., 1979; Jakupovic et al., 1991). According to the scheme of evolution of flavonoid characters in the Angiosperms, as proposed by Harborne (1977), the occurrence of flavone/flavanone derivatives sakuranetin (**1**), naringenin (**2**), eriodictyol (**3**) and apigenin (**6**) as the main constituents would suggest that *B. retusa* represents an advanced evolutionary state, especially when compared with other species of the subtribe Baccharidinae (Bohm and Stuessy, 2001).

The chlorogenic acids **9–11** were detected in the n-BuOH phase derived from the methanolic extract of *B. retusa*. The occurrence of all three of these compounds has been recorded previously in *Baccharis dracunculifolia* (Midorikawa et al., 2003), *Baccharis genistelloides* (Marques and Farah, 2009), *B. thesioides* (Miketova et al., 1999), *Baccharis trimera* and *Baccharis usterii* (Simões-Pires et al., 2005), while **9** has also been detected in *Baccharis gaudichaudiana* (Akaike et al., 2003). It is important to note, however, that glycosylated flavonoid derivatives, which represent an important characteristic of members of the family Asteraceae (Verdi et al., 2005), were not detected in the more polar phases derived from the methanolic extract of *B. retusa*.

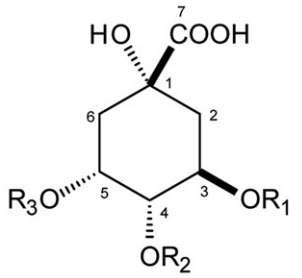
Based on the phenolic derivatives isolated from *B. retusa*, it may be concluded that the phytochemistry of this species is similar to that of other members of the genus *Baccharis* (Verdi et al., 2005). However, it is suggested that the co-occurrence of dihydroflavonols **4** and **5**, together with the presence of the C-7 methoxy flavanone **1**, may be considered as specific markers of *B. retusa*.

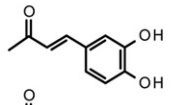


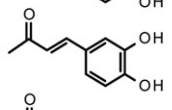
1 R_1 = Me; R_2 = R_3 = R_4 = H
2 R_1 = R_2 = R_3 = R_4 = H
3 R_1 = R_2 = R_4 = H; R_3 = OH
4 R_1 = R_2 = H; R_3 = R_4 = OH
5 R_1 = R_2 = R_3 = H; R_4 = OH



6 R_1 = R_2 = R_3 = R_4 = H
7 R_1 = R_2 = H; R_3 = R_4 = OH
8 R_1 = R_2 = R_3 = H; R_4 = OH



9 R_3 = H, R_1 = R_2 = 

10 R_2 = H, R_1 = R_3 = 

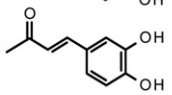
11 R_1 = H, R_2 = R_3 = 

Fig. 1. Structures of compounds isolated from the aerial parts of *B. retusa*.

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