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## Chemical constituents from *Spermacoce verticillata* (Rubiaceae)

Jesu C. Ferreira Júnior<sup>a</sup>, Rosangela P. Lyra Lemos<sup>b</sup>, Lucia M. Conserva<sup>a,\*</sup><sup>a</sup> Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, 57072-970, Maceió-AL, Brazil<sup>b</sup> Instituto do Meio Ambiente do Estado de Alagoas, 57017-020, Maceió-AL, Brazil

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### 1. Subject and source

The genera *Spermacoce* and *Borreria* (Rubiaceae: tribe Spermacoceae) comprise each about 250–300 species (Groeninckx et al., 2009) distributed in the tropical and subtropical regions (Dessein et al., 2006). Based on the peculiarities of the fruit dehiscence, they are considered by many authors to be distinct genera and most others, however, prefer to combine the two taxa under the generic name *Spermacoce* (Chaw and Sivarajan, 1989; Dessein et al., 2002). Whereas the discussion is still unclear, there are studies that consider the species *Spermacoce verticillata* L. as *B. verticillata* G.F.W. Mey (Burger and Taylor, 1993; Fosberg et al., 1993; Govaerts, 1996; Mori et al., 2002). This species, known in Brazil as “poaia, poaia preta, poaia miúda, cordão-de-frade, and vassourinha”, is a small herb distributed from the Southern United States to South America (Burger and Taylor, 1993; Chiquieri et al., 2004). It is used in traditional medicine of Latin America (Vieira et al., 1999; Lorenzi and Matos, 2002; Moreira et al., 2010), Africa (Asprey and Thornton, 1955; Benjamin, 1979; Maynard et al., 1980) and West Indies (Ayensu, 1978) for various purposes. The aerial parts, stems and roots of this plant were collected from Reserva de Proteção Ambiental de Santa Rita (Mucuri), Marechal Deodoro-AL, Brazil, in April 2007 and identified by Rosangela P. Lyra Lemos of the Instituto do Meio Ambiente de Alagoas (IMA/AL), where a voucher specimen (MAC-22949) was deposited.

### 2. Previous work

Some species of the genera *Borreria* and *Spermacoce* have been studied and more than 60 compounds distributed in different classes have been isolated. Alkaloids, iridoids (Damak and Riche, 1977; Jossang et al., 1977a,b; 1981), flavonoids (Purushothaman and Kalyani, 1979; Bhadoria and Gupta, 1981; Sharma and Gupta, 1987; Noiarsa et al., 2007), and terpenoids

\* Corresponding author. Tel.: + 55 82 32141390; fax: + 55 82 32141384.

E-mail addresses: [lmc@qui.ufal.br](mailto:lmc@qui.ufal.br), [lucia.conserva@pq.cnpq.br](mailto:lucia.conserva@pq.cnpq.br) (L.M. Conserva).

(Kapoor et al., 1969; Benjamin, 1980; Bhadoria and Gupta, 1981; Sharma and Gupta, 1987; Mukherjee et al., 1993, 1994; 2004) are the main classes of constituents. Besides these, other compounds, such as benzyl, (Z)-3-hexenyl, phenethyl glycosides derivatives, megastigman glycoside (Noiarsa et al., 2007), alcohol, and carboxylic acids (Bhadoria and Gupta, 1981; Sharma and Gupta, 1987; Mukherjee et al., 1993; Sultana et al., 2008) were also found. Previous phytochemical investigations on *B. verticillata* have revealed the occurrence of indole [borrerine, verticillatines A and B (Poussett et al., 1973, 1977; Ferreira et al., 1978; Baldé et al., 1991; Moreira et al., 2010)], bis-indole [borreverine, isoborreverine and spermacoceine (Poussett et al., 1977; Maynard et al., 1980; Baldé et al., 1991)] and tetrahydroisoquinoline alkaloids [(–)-emetine (Willaman and Schubert, 1961; Moreira, 1964)], in addition to iridoids [asperuloside, asperulosidic acid, borriagenin, daphylloside deacetylasperuloside, deacetyl-asperulosidic acid, feretoside (= scandoside methyl ester), methyl deacetylasperulosidate, and 6'-O-(2-glyceryl)scandoside methyl ester (Sainty et al., 1981; Vieira et al., 1999; Moreira et al., 2010)], terpenoids [cariophyllene, guaiane (Benjamin, 1979, 1980), campesterol,  $\beta$ -sitosterol, and stigmasterol (Andre et al., 1976)], and mixtures of aliphatic acids, tri-O-acylglycerols and sucrose (Moreira et al., 2010). Phytol, 1,8-cineole,  $\alpha$ -pinene, and *p*-cymene were identified by GC–MS from the aerial parts (Ogunwande et al., 2010).

### 3. Present study

The air-dried and powdered aerial parts (AP; 226.5 g), stems (S; 7.700 g) and roots (R; 485.3 g) were exhaustively extracted with 90% EtOH at room temperature. After removal of solvents under vacuum, these extracts [AP (28.7 g), S (21.2 g) and R (7.6 g)] were suspended in MeOH–H<sub>2</sub>O solution and extracted successively with hexane, CHCl<sub>3</sub> and EtOAc [hexane (AP: 6.1 g; S: 4.3 g; R: 1.5 g), CHCl<sub>3</sub> (AP: 6.5 g; S: 4.3 g; R: 1.8 g), EtOAc (AP: 1.6 g; S: 3.1 g; R: 2.7 g) and MeOH–H<sub>2</sub>O (AP: 10.9 g; S: 7.8 g; R: 0.3 g)]. The CHCl<sub>3</sub> fraction from aerial parts (6.5 g) was subjected to chromatographic column on silica gel using CHCl<sub>3</sub> containing increasing amounts of MeOH, followed by gel filtration (Sephadex LH-20 with MeOH) and successive recrystallizations with acetone to afford morolic acid (**1**, 16 mg; Mahato and Kundu, 1994; Zhang et al., 2009), ursolic acid (**2**, 190 mg; Connolly and Hill, 1991) and oleanolic acid (**3**, 21 mg; Mahato and Kundu, 1994). EtOAc fraction from aerial parts (1.6 g) after gel filtration (Sephadex LH-20 with MeOH) and chromatographic fractionations over silica gel column with hexane containing increasing amounts of EtOAc afforded quercetin (**4**, 13 mg; Kim, 2005) and 3-O- $\alpha$ -L-rhamnopyranosylquercetin (**5**, 14 mg; Arot and Williams, 1997). The hexane fraction from stems (4.3 g) after successive chromatographic fractionations over silica gel column with hexane containing increasing amounts of EtOAc afforded 3,5-dioxo-friedelane (**6**, 32 mg; Mahato and Kundu, 1994). CHCl<sub>3</sub> fraction from stems (4.0 g) was also fractionated on silica gel column using CHCl<sub>3</sub>–EtOAc mixtures of increasing polarity proportions. The sub-fraction 58–62 (0.032 g) was subjected to gel filtration (Sephadex LH-20 with MeOH) to provide 2-hydroxy-3-methylanthraquinone (**7**, 25 mg; Kitajima, 1998). The hexane fraction from roots (1.5 g) was also chromatographed on silica gel column with hexane containing increasing amounts of EtOAc. The remaining sub-fraction 9–11 (0.069 g) after TLC preparative [silica gel PF<sub>254</sub> and hexane–EtOAc (9:1)] yielded a mixture of sitostenone (**8**) and stigmatenone (**9**) (30 mg, Della-Greca et al., 1990).

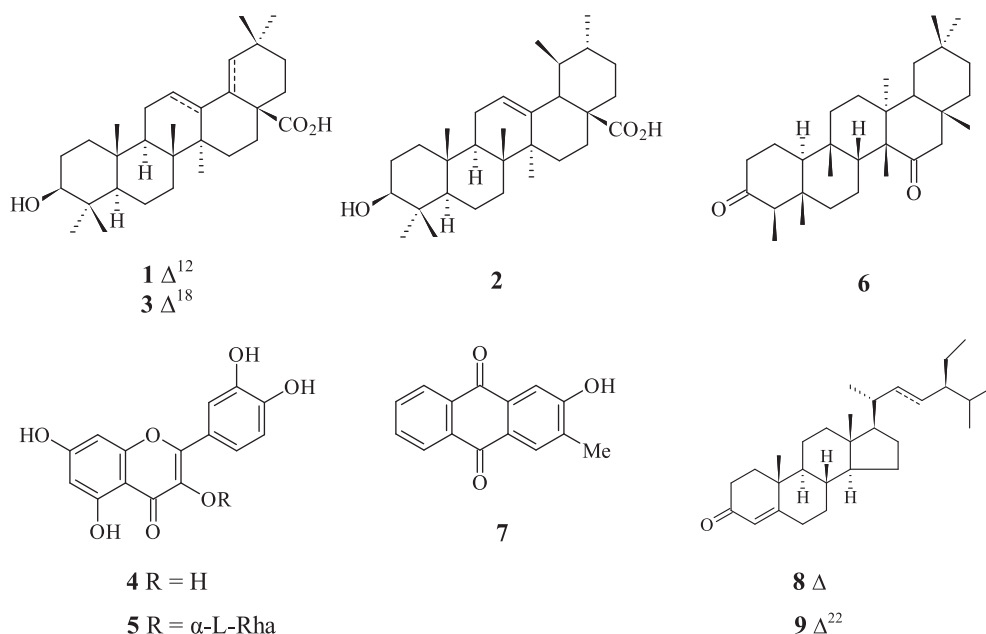


Fig. 1. Isolated compounds from *S. verticillata*.

The structures of the compounds (**1–9**) (Fig. 1) were identified by interpretation of their spectral data (IR, EIMS and  $^1\text{H}$  and  $^{13}\text{C}$  NMR, including DEPT, HSQC and HMBC experiments) and by the comparison with those reported in literature.

#### 4. Chemotaxonomic significance

This work reports for the first time the isolation of four triterpenes (**1–3** and **6**), two flavonoids (**4–5**), one anthraquinone (**7**), and two phytosteroids (**8–9**) in the investigated species. Species of *Spermacoce* or *Borreria* are known to contain alkaloids (Poussett et al., 1973, 1977; Damak and Riche, 1977; Joessang et al., 1977a; 1977b; 1981; Ferreira et al., 1978; Maynard et al., 1980; Baldé et al., 1991; Moreira et al., 2010), iridoids (Sainty et al., 1981; Vieira et al., 1999; Kamiya et al., 2002; Moreira et al., 2010), flavonoids (Purushothaman and Kalyani, 1979; Bhadoria and Gupta, 1981; Sharma and Gupta, 1987; Noiarsa et al., 2007), triterpenoids (Kapoor et al., 1969; Andre et al., 1976; Benjamin, 1979, 1980; Sharma and Gupta, 1987; Mukherjee et al., 1993, 1994; 2004; Kamiya et al., 2002; Vadivelan et al., 2007; Sultana et al., 2008), and other compounds (Noiarsa et al., 2007). Flavonoids and triterpenoids are common constituents in members of the family Rubiaceae as well as other higher plants. In this work, among flavonoids and triterpenoids isolated, compounds **1**, **3**, **5** and **6** are being reported for the first time from this genus. Anthraquinones are frequently found in several genera of the tribe Spermacoceae, which belongs to the genus *Spermacoce* or *Borreria*. In this tribe, the compound 2-hydroxy-3-methyl-anthraquinone (**7**) have been found in the genera *Hedyotis* (Si et al., 2006; Zhang and Luo, 2008; Shi et al., 2008; Huang et al., 2009), *Oldenlandia* (Zhou et al., 2007), *Morinda* (Rath et al., 1995; Zhang et al., 2010), *Rennellia* (Osman et al., 2010), *Ophiorrhiza* (Kitajima et al., 1998), and *Heterophyllaea* (Montoya et al., 2003). So, this is the first report of the occurrence of anthraquinone in *Spermacoce* or *Borreria* species. Therefore, the occurrence of this class of compound in this genus may be considered an important finding and may be useful as a chemotaxonomic marker of the tribe Spermacoceae.

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