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Chemical constituents from *Kielmeyera rugosa* Choisy (Clusiaceae)

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1. Subject and source

The genus *Kielmeyera* (family Clusiaceae, subfamily: Kielmeyeroideae) is endemic to South America, with the vast majority of the 47 species occurring exclusively in Brazil (Saddi, 1982). Some species of this genus are popularly known in Brazil as "pau-santo", "rosa-do-campo" and "malva-do-campo" (Gottlieb et al., 1971; Pinheiro et al., 2003). Plant parts, especially leaves of such species have been frequently used in folk medicine to treat several tropical diseases, including schistosomiasis, leishmaniasis and malaria as well as bacterial and fungal infections (Alves et al., 2000). For the present study, leaves, stems and fruits of *Kielmeyera rugosa* Choisy were collected in a "restinga" (the vegetation mosaic of Brazilians coastal sandy plains) near Pomonga River in the Municipality of Santo Amaro das Brotas, Sergipe state, Brazil. Voucher specimens (M.C.E. Amaral, V. Bittrich, A.S. Ribeiro and P.C.L. Nogueira, No. 206) were deposited in the herbaria LEEP, UFS and UEC.

2. Previous work

Up to now, only one phytochemical study on the essential oil composition of *K. rugosa* Choisy has been published (Andrade et al., 2007). Previous chemical reports on the genus *Kielmeyera* include the isolation of xanthones, coumarins, anthraquinones, triterpenes and sterols (Antonaccio et al., 1965; Correa et al., 1966; Cortez et al., 1998; Cruz et al., 1998a,b, 2001, 2002; Duarte et al., 1968; Gottlieb et al., 1965, 1966, 1969, 1971; Gottlieb and Stefani, 1970; Gramacho et al., 1999; Lopes et al., 1977; Nagem and Silva, 1988; Pimenta et al., 1964; Pinheiro et al., 2003; Pinto et al., 1987; Scio et al., 2003; Silva et al., 1968).

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3. Present study

The air-dried and powdered fruits (504.7 g), leaves (163.4 g), and stems (397.4 g) of *K. rugosa* were extracted at room temperature with methanol. The solvent was removed under reduced pressure to give the correspondent crude extracts of fruits (52.4 g), leaves (31.5 g), and stems (20.9 g). Each extract was suspended in MeOH:H_2O (9:1) solution and extracted successively with hexane (leaves: 4.38 g, stems: 1.13 g and fruits: 4.44 g), $CHCl_3$ (leaves: 7.19 g, stems: 1.70 g and fruits: 7.32 g) and EtOAc (leaves: 1.50 g, stems: 2.21 g and fruits: 2.28 g).

A part of the hexane extract from fruits (2.04 g) was subjected to silica gel column eluting with hexane, EtOAc and MeOH as binary mixtures of increasing polarity to yield a mixture of compounds **1** and **2** (67.1 mg, yellowish oil) (Cruz et al., 2001; Games and Haskins, 1971), and compound **3** (95.2 mg, yellowish amorphous solid) (Chakraborty and Chatterji, 1969; Gramacho et al., 1999; López-Pérez et al., 2005; Nagem and Silva, 1988; Verotta et al., 2004). A part of the hexane extract from leaves (1.5 g) was purified in the same way and afforded 20 fractions. Fraction 8 (73.0 mg) was further purified by TLC preparative over silica gel eluted with hexane:CH_2Cl_2:MeOH (38:38:24) to yield a mixture of lupeol **4** and α-amyrin **5** (37.4 mg) (Mahato and Kundu, 1994; Olea and Roque, 1990; López-Pérez et al., 2007).

A part of the chloroform extract from leaves (1.2 g) and stems (623.6 mg) were each separately purified using column chromatography over silica gel eluting with hexane, EtOAc and MeOH as binary mixtures in a gradient manner to yield, respectively, compound **6** (38.9 mg, yellowish amorphous solid) (Crombie et al., 1972; Reyes-Chilpa et al., 2004), and compound **7** (53.4 mg, yellowish amorphous solid) (Cruz et al., 1998a,b). A part of the $CHCl_3$ extract from fruits (1.38 g) was purified in the same way and afforded 26 fractions. Fraction 15 (26.5 mg) was purified by TLC preparative over silica gel eluted with hexane:EtOAc (90:10) to yield compound **8** [4.2 mg, $[\alpha]_D^{25} = -2.75^\circ$ ($c = 0.004$, $CHCl_3$)]. To our best knowledge, this is the first report of the isolation of compound **8** [5,7-dihydroxy-8-(2-hydroxy-3-methylbut-3-enyl)-6-(3-methylbutanoyl)-4-propyl-2H-chromen-2-one, called disprorinol A] from a natural source. Till now, it was known only as a synthetic product (Helesbeux et al., 2004), but its 6-(2-methylbutanoyl) isomer has been isolated from *Calophyllum dispar* P.F. Stevens (Guilet et al., 2001).

Structures of all isolated compounds were identified on the basis of their spectral data (IR, MS, NMR, including DEPT 135° and 90°, HSQC and HMBC experiments), and by comparison of their spectral data with reported data. For compounds **1** (Mammea B/AA Cyclo D) and **6** (Mammea B/BB Cyclo F), to the best of our knowledge, only ^{1}H NMR data were reported (Crombie et al., 1972, 1987; Reyes-Chilpa et al., 2004). Therefore, its ^{13}C NMR data are presented here.

15.39 (*s*, 1H, 5-OH), 6.83 (*d*, 1H, 10 Hz, H-4‴), 5.95 (*s*, 1H, H-3), 5.64 (*d*, 1H, 10 Hz, H-5‴), 2.97 (*d*, 2H, 6.8 Hz, H-2″), 2.94 (*t*, 2H, 7.5 Hz, H-1′), 2.27 (*m*, 1H, H-3″), 1.64 (*m*, 2H, H-2′), 1.54 (*s*, 6H, H-7‴ and 8‴), 0.98 (*d*, 6H, 6.8 Hz, H-4″ and 5″), 0.92 (*t*, 3H, 7.5 Hz, H-3′). ^{13}C NMR (11.7 T, $CDCl_3$, *δ*): 207.1 (C-1″), 165.2 (C-5), 160.0 (C-2), 159.4 (C-4), 157.2 (C-7), 155.0 (C-10), 126.1 (C-5‴), 115.7 (C-4‴), 110.3 (C-3), 107.1 (C-6), 103.3 (C-9), 101.5 (C-8), 79.6 (C-6‴), 53.6 (C-2″), 38.4 (C-1′), 28.2 (C-7‴ and C-8‴), 25.1 (C-3″), 22.7 (C-2′), 22.6 (C-4″ and C-5″), 13.9 (C-3′). Correlations observed in the HMBC spectrum: *δ* 15.39 (C-9, C-6 and C-5), *δ* 5.95 (C-1′, C-9, C-2 and C-4), *δ* 5.64 (C-6‴ and C-8), *δ* 6.83 (C-6‴, C-10 and C-7), *δ* 2.97 (C-3″, C-4″, C-5″ and C-1″), *δ* 2.94 (C-3′, C-2′, C-9, C-3 and C-4), *δ* 1.64 (C-3′, C-1′ and C-4), *δ* 1.54 (C-7‴, C-8‴, C-6‴ and C-5‴), *δ* 0.98 (C-2″, C-3″ and C-4″), *δ* 0.92 (C-2′ and C-1′).

Mammea B/BB Cyclo F **(6)** ^{13}C NMR (9.4 T, $CDCl_3$, *δ*): 210.4 (C-1‴), 163.1 (C-5 and C-7), 162.0 (C-4), 159.5 (C-2), 157.3 (C-10), 110.1 (C-6), 109.3 (C-3), 104.6 (C-8), 99.3 (C-9), 92.8 (C-2″), 71.4 (C-4″), 46.6 (C-2‴), 37.6 (C-1′), 27.1 (C-3‴), 26.5 (C-3″), 26.1 (C-5″), 24.7 (C-6″), 22.6 (C-2′), 17.0 (C-5‴), 13.9 (C-3′), 11.6 (C-4‴).

4. Chemotaxonomic significance

Previous phytochemical studies with Brazilian *Kielmeyera* species showed mainly xanthones and 4-alkyl and 4-phenyl coumarins, which are regarded as the characteristic constituents of plants belonging to this genus. It is worth noting that the xanthones were identified as the principal constituents of some species of this genus that occur mainly in the "cerrado" (savanna) of the Central Brazilian plateau (Cortez et al., 1998; Gottlieb et al., 1966, 1969, 1971; Gottlieb and Stefani, 1970; Pimenta et al., 1964; Pinto et al., 1987). Studies with *Kielmeyera* species collected in "restinga" (sandy coastal vegetation), however, reported mainly 4-phenyl and 4-propylcoumarins (Cruz et al., 1998a,b, 2002). In agreement with these results, in the present work, we report the isolation and structure identification of 4-phenyl and 4-n-propylcoumarins from *K. rugosa* fruits, leaves, and stems collected in a "restinga" of Sergipe state, Brazil. Xanthones are known as antioxidants and also show activities as ovicides and larvicides (Steiner and Summerland, 1943), as well as molluscicides (Pinheiro et al., 2003) among others, while coumarins are toxic for higher animals due to their anticoagulant properties, but also deter feeding of certain herbivorous insects. Due to their different activities and biosynthetic pathways, both xanthones and coumarines might occur in the same plant, but this was rarely observed in *Kielmeyera*. Using material of a plant collected in "campo rupestre" vegetation (rocky savannas above 1000 m alt. often intermingled with cerrado patches) of a species identified as *Kielmeyera lathrophyton* Saddi, Cruz et al. (2001) found xanthones as well as 4-alkyl and 4-phenylcoumarins. According to Saddi (1982), however, *K. lathrophyton* occurs mainly in the cerrado savanna of Central Brazil and in the inland forests of southeastern Brazil and the analyzed plant material might belong to a closely related species. As already emphasized by Saddi (1989) for *Kielmeyera*, species names used in the literature need to be treated with some caution as misidentifications are not rare. Due to the still grossly incomplete taxonomic knowledge of the Brazilian flora such misidentifications are unavoidable and this will