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Short communication

Chemical constituents from *Casearia* spp. (Flacourtiaceae/Salicaceae *sensu lato*)

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ABSTRACT

Chemical investigation of the leaves of *Casearia gossypiosperma* Briq., Salicaceae, led to the isolation of two known flavonoids, (+)-taxifolin and quercetin, the leaves of *Casearia decandra* Jacq. have afforded hydroquinone, the leaves of *Casearia rupestris* Eichler and *Casearia lasiophylla* Eichler have afforded a diterpene, (*E*)-phytol, and the leaves of *C. rupestris* and *Casearia obliqua* Spreng. have afforded sitosterol. The twigs of *Casearia lasiophylla* Eichler led to the isolation of two compounds (+)-pinoresinol, and *N-trans*-feruloyltyramine, and the twigs of *C. obliqua* have afforded *N-trans*-feruloyltyramine, *N-trans*-cumaroyltyramine, and cinamic acid. This is the first report of the compounds (+)-taxifolin, quercetin, hydroquinone, (+)-pinoresinol and *N-trans*-cumaroyltyramine from the *Casearia* genus.

Introduction

Recent phylogenetic and chemical studies, besides ecological and morphological observations from the Angiosperm Phylogeny Group (APG) showed that the Flacourtiaceae family was separated into two clades (Achariaceae and Salicaceae), with the genus *Casearia* belonging to the Salicaceae (APG, 2003). *Casearia* genus is found in Brazil and contains ca. of 180 species, 70 belonging to the American continent and 37 present in Brazil (Shen et al., 2004). No chemical studies have been previously reported for *C. gossypiosperma* Briq. and *C. decandra* Jacq. However, previous studies of our group with *C. sylvestris* Sw. (Santos et al., 2010), *C. obliqua* Spreng. (Vieira-Júnior et al., 2009), and *C. rupestris* Eichler (Vieira-Júnior et al., 2011) related the presence of clerodane diterpenes as well as in many other *Casearia* species (Kanokmedhakul et al., 2007). Previous studies of the *C. lasiopylla* Eichler species described the chemical composition of the essential oils from leaves (Salvador et al., 2011). In the present short communication, the occurrence of nine known compounds from *Casearia* spp. is described.

Materials and methods

The aerial parts, twigs and leaves of the *Casearia* species were collected in May 2007 at Campinas municipality, São Paulo State, Brazil. The plant material was identified by Dr. Roseli B. Torres, Instituto Agronômico de Campinas, Brazil. A voucher specimens [IAC38438 (*C. gossypiosperma* Briq.), IAC42645 (*C. decandra* Jacq.), IAC41542 (*C. rupestris* Eichler), IAC42645 (*C. lasiophylla* Eichler), and IAC46529 (*C. obliqua* Spreng.)] have been deposited in the herbarium of the Instituto Agronômico de Campinas (São Paulo State, Brazil). Optical rotation was measured on a Perkin-Elmer 341 LC polarimeter. IR spectra was measured on a Nicolet Impact 400 spectrometer, using KBr disks. The 1D and 2D NMR experiments were recorded on a Varian INOVA 500 spectrometer (11.7 T) at 500 MHz (^{1}H) and 125 MHz (^{13}C) with pulse field gradient and a Varian INOVA 300 spectrometer (7.4 T) at 300 MHz (^{1}H) and 75 MHz (^{13}C), using CD_3OD, DMSO-d_6 or $CDCl_3$. Positive-ion HRMS spectra were recorded on an UltrOTOFq (Bruker Daltonics) ESI-qTOF mass spectrometer, using TFANa as the internal standard. Open column chromatography was performed over silica gel (40–63 μm, Merck), silica C18 (40 μm, J. T. Baker), or on Sephadex LH-20 (Pharmacia Biotech). TLC was performed using Merck silica gel 60 (>230 mesh) and precoated silica gel 60 PF_{254} plates. Spots on TLC plates were observed under UV light and by spraying the plates with anisaldehyde-H_2SO_4 reagent, followed by heating at 120 °C. All solvents were purchased from Sigma–Aldrich (St. Louis, MO, USA). The

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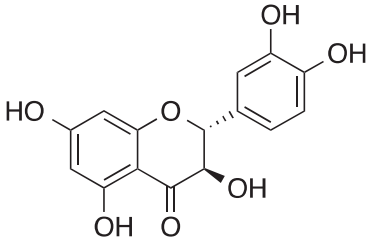
air-dried and powdered aerial parts of *C. gossypiosperma* (leaves 1.4 kg), *C. decandra* (leaves 0.3 kg), *C. rupestris* (leaves 0.4 kg), *C. lasiophylla* (leaves 0.6 kg, and twigs 1.1 kg), and *C. obliqua* (twigs 0.5 kg) were exhaustively extracted by maceration successively with hexane, ethanol and water (3 l × 3) at room temperature. The aqueous extract (20 g) of the leaves from *C. gossypiosperma* was subjected to chromatographic column on Amberlite XAD-4 using water, water/MeOH (1:1) and MeOH, to afford (+)-taxifolin (**1**, 581.0 mg, 0.04% – eluted with MeOH). The hexane extract (4 g) of the leaves from *C. lasiophylla* was chromatographed over silica gel using hexane containing increasing amounts of EtOAc. The subfraction 3 (223 mg) was subjected to thin-layer preparative chromatography with hexane/EtOAc (7:3), resulting in the isolation of (*E*)-phytol (13 mg, 0.002%). The hexane extract (14 g) of the leaves from *C. rupestris* was chromatographed over silica gel using hexane containing increasing amounts of EtOAc. The subfraction 3 (1 g) was rechromatographed in a C-18 column using water containing increasing amounts of MeOH afforded a mixture of (*E*)-phytol + sitosterol (362 mg, 0.09%, eluted with MeOH). The hexane extract (1.5 g) of the leaves from *C. obliqua* was chromatographed over silica gel using hexane containing increasing amounts of EtOAc and MeOH. The subfraction 3 (350 mg) was rechromatographed over silica gel using hexane containing increasing amounts of EtOAc afforded sitosterol (27.2 mg, 0.005%), eluted with hexane/EtOAc (9:1). All ethanolic extracts were diluted with MeOH/H_2O (3:1) and partitioned with hexane, Et_2O, and EtOAc, successively. The MeOH–H_2O phase (7.7 g) of the leaves from *C. gossypiosperma* was subjected to chromatographic column on silica gel using hexane containing increasing amounts of EtOAc to afford (+)-taxifolin (**1**, 1 g, 0.07%), eluted with hexane/EtOAc (1:1). The Et_2O phase (9.6 g) of the leaves from *C. gossypiosperma* was subjected to gel filtration (Sephadex LH-20 with MeOH) to afford (+)-taxifolin (**1**, 2.8 g, 0.2%) and quercetin (37.4 mg, 0.002%). The EtOAc phase (6.1 g) of the leaves from *C. decandra* was subjected to chromatographic column on silica gel using hexane containing increasing amounts of EtOAc, the subfraction 21 (146.1 mg) was subjected to gel filtration (Sephadex LH-20 with MeOH). The subfraction 21–4 (115 mg) was rechromatographed in EFS-C18 (reverse phase) column using MeOH afforded hydroquinone (110.5 mg, 0.03%). The EtOAc phase (6 g) of the twigs from *C. lasiophylla* was subjected to gel filtration (Sephadex LH-20 with MeOH). The subfraction 20 (332 mg) was chromatographed over silica gel using hexane containing increasing amounts of EtOAc to provide *N-trans*-feruloyltyramine (**2**, 19.5 mg, 0.001%), eluted with hexane/EtOAc (3:7). The subfraction 20–10 (41 mg) was rechromatographed over silica gel using hexane containing increasing amounts of AcOEt to provide (+)-pinoresinol (**3** 19 mg, 0.001%), eluted with hexane/EtOAc (5:5). The EtO_2 phase (1 g) of the leaves from *C. obliqua* was subjected to gel filtration (Sephadex LH-20 with MeOH). The subfraction 60 (32 mg) was submited to preparative RP-HPLC on a C18 column eluted with H_2O/MeOH 20:80 (v/v) at a flow rate of 15 ml/min and UV detection at 254 nm, affording the cinnamic acid (9 mg, 0.001%). The subfraction 73 (11 mg) was submitted to preparative RP-HPLC on a C18 column eluted with H_2O/MeOH/HOAc 33:67:0.01 (v/v/v) at a flow rate of 11 ml/min and UV detection at 254 nm, affording the *N-trans*-cumaroyltyramine (**4**, 2.2 mg, 0.0004%), and *N-trans*-feruloyltyramine (**2**, 4.5 mg, 0.0009%).

Results and discussion

The structures of the nine compounds were identified by comparison of their optical rotation, spectroscopic and spectrometric data (UV, IR, HRESIMS or ESIMS and ^1H and ^{13}C NMR) with those reported in the literature (see Supporting Information).

This work reported the isolation of two flavonoids ((+)-taxifolin (**1**) and quercetin), one simple phenolic compound (hydroquinone), one diterpene ((*E*)-phytol), one steroid (sitosterol), one lignan ((+)-pinoresinol (**3**)), two amides (*N-trans*-feruloyltyramine (**2**) and *N-trans*-cumaroyltyramine (**4**)), and cinnamic acid from five *Casearia* species. All compounds were isolated from these species for the first time. Except for (*E*)-phytol, sitosterol, *N-trans*-feruloyltyramine (**2**), and cinnamic acid, all other compounds were isolated from the genus *Casearia* for the first time. Sitosterol has



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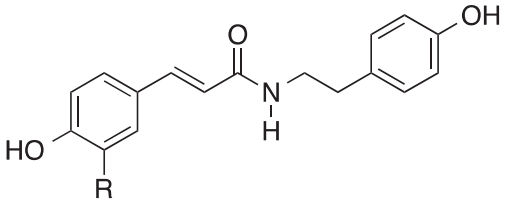
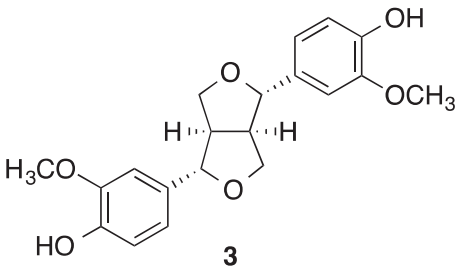


2 R=OCH_3
4 R=H



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been isolated previously from *C. grewiifolia* (Rayanil et al., 2012), *C. sylvestris* (Wang et al., 2009), *C. membranacea* (Chang et al., 2003), and *C. graveolens* (Talapatra et al., 1983), *N-trans*-feruloyltyramine (**2**) from *C. membranacea* (Chang et al., 2003) and *C. grewiifolia* (Rayanil et al., 2012), (*E*)-phytol from *C. balansae* (Wang et al., 2013), and cinnamic acid from *C. sylvestris* (Wang et al., 2009). *Casearia* species contains flavonoids (Raslan et al., 2002), lignans (Wang et al., 2010), steroids (Wang et al., 2009; Chang et al., 2003), coumarins (Talapatra et al., 1983), amides (Chang et al., 2003), terpenoids (Bou et al., 2014), phenolic compounds (Chai et al., 2010), and mainly clerodane diterpenes (Vieira-Júnior et al., 2009; Vieira-Júnior et al., 2011; Bou et al., 2014).

The compound (+)-taxifolin (**1**) was isolated in a significant amount (3.8 g) from leaves of *C. gossypiosperma*, but it can be found in larger quantities in conifers, e.g., the *Siberian larch*, *Larix sibirica*, *Pinus roxburghii* and *Cedrus deodara* (Willför et al., 2009). The clerodane diterpenes are considered chemosystematic markers of this genus, but the literature does not describe the presence of these substances in *C. lasiophylla*, *C. gossypiosperma* and *C. decandra*.

The best of our knowledge, this is the first report of the chemical constituents of *C. gossypiosperma* and *C. decandra*. The compounds (+)-taxifolin (**1**), quercetin, hydroquinone, (+)-pinoresinol (**3**) and *N-trans*-cumaroyltyramine (**4**) are reported for the first time in the *Casearia* genus. From 1.4 kg of dried leaves from *C. gossypiosperma*, an amount of 3.8 g of (+)-taxifolin (**1**) was obtained. This extract is a rich source of (+)-taxifolin (**1**), which may offer interesting potential applications in the food, cosmetic and/or pharmaceutical industries.

Author contributions

LAD and GMVJ carried out laboratory work as part of her final year research project. NB and GMVJ obtained the NMR and MS data and contributed to compound identification. RBT identified and deposited the specimens in the herbarium of the Instituto Agronômico de Campinas (São Paulo State, Brazil). GMVJ, MHC, VSB, DHSS, and AJC supervised this project, provided intellectual input and prepared the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjp.2017.10.003.

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