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NOTE

POLYPHENOLS FROM *BALBISIA CALYCINA*

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ABSTRACT

The aerial parts of Balbisia calycina (Gris.) A.T. Hunziker et Ariza (Ledocarpaceae), widely used in South American folk medicine, have been investigated for the first time. From the dichloromethane extract, scopoletin was obtained, while apigenin, caffeic and ferulic acids were isolated from the methanol extract.

INTRODUCTION

The Ledocarpaceae was proposed by Meyen (1834) including the genera *Balbisia* and *Wendtia*. The exomorphology, the anatomy of the leaves, and the palynology of the genera *Wendtia* Meyen and *Balbisia* Cav., was critically reviewed by Hunziker and Ariza Espinar (1973). These authors concluded that the 11 species involved should be treated as pertaining to one genus with 3 sections: *Balbisia* Sect. *Balbisia* (6 species), *Balbisia* Sect. *Tricarpellatae* (2 species), and *Balbisia* Sect. *Wendtia* (3 species), including *B. calycina*.

Balbisia calycina (Gris.) A.T. Hunziker et Ariza (Ledocarpaceae) (= *Wendtia calycina* Gris.) (= *Viviana calycina* Gris.), commonly known as “té andino” or “té del burro”, is a widely distributed plant

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in Northwestern Argentina growing at an altitude of 2,000 to 3,000 meters above sea level (Hunziker & Ariza Espinar, 1973; Amorín, 1980; Boelcke, 1992).

In folk medicine, the infusions of the aerial parts of *B. calycina* are used in the Andean region of South America and its efficacy, either as hepatoprotective and carminative, or for the treatment of gastrointestinal disorders, has been repeatedly remarked upon (Hieronymus, 1882; Saggese, 1959). These medicinal properties and the fact that no phytochemical reports on this genus are available, prompted us to investigate this plant for its chemical constituents.

EXPERIMENTAL

Plant Material

The aerial parts (leaves and stems) were collected in March 1994 in Tafí del Valle, Tucumán, Argentina at the flowering period and identified as *Balbisia calycina* (Gris.) A.T. Hunziker et Ariza by Ing. Gustavo Giberti. A voucher specimen is deposited at the University Herbarium, Museo de Farmacobotánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

Extraction

The aerial parts of *B. calycina* were dried in an oven between 50 and 60°C under air flow and powdered mechanically. Powdered plant material (1250 kg) was sequentially extracted in a Soxhlet apparatus with dichloromethane, methanol and water. After evaporation of the solvents under reduced pressure, a dichloromethane extract (25.0 g; 2%), a methanol extract (100.0 g; 8%) and an aqueous extract (50.0 g; 4%) were obtained.

A portion of the dichloromethane extract (5 g) was chromatographed on a silica gel (75 g) (MN-Kiesel-gel 60, 70–230 mesh) column packed with dichloromethane. The column was eluted successively with mixtures of increasing polarity of dichloromethane and methanol. The eluate from dichloromethane-methanol (8:2) afforded scopoletin (20 mg; 0.008%).

A portion of the methanol extract (5 g) was chromatographed on a polyamide (90 g) (MN-Polyamid-SC6, grain size < 0.16 mm) column and eluted with dichloromethane-methanol mixtures of increasing polarity. Fractions eluted with dichloromethane-methanol (9:1) were purified on Sephadex LH-20 eluted with hexane-ethyl acetate (6:4). Apigenin (25 mg; 0.04%) was obtained upon recrystallization from methanol.

Spectrophotometric Determination of the Phenylpropanoid Content of the Infusion

The determination of the phenylpropanoid content of the infusion was carried out according to the method reported by Martino et al. (1989). The infusion was prepared as described in the Argentine Pharmacopoeia 6 th Edn. adding 100 ml of boiling water to 5 g of plant material. One ml of this 5% infusion was appropriately diluted with methanol. Spectrophotometric measurements at 330 nm were performed on a Shimadzu UV240, using an automatic calculation program and chlorogenic acid as a standard ranging from 0.12 to 2.50 mg%.

HPLC Analysis

For HPLC analysis, dried powdered aerial parts (3 g) were extracted with 70% methanol in a Soxhlet apparatus for 2 h. The aqueous-methanol extract was concentrated, redissolved in methanol-water (1:1) and washed with petroleum ether-ethyl ether (1:2). This aqueous-methanol extract was examined by HPLC using Spherisorb 5 ODS, 250 mm × 4.60 mm, Phenomenex column; Solvent A: H₂O/HCOOH (98:2), Solvent B: MeOH/HCOOH (98:2) as eluent, gradient: 85–50% A in 0–30 min, flow rate: 1.2 ml/min, UV detection: 325 nm, to give caffeic acid t_R 11.1 min (0.17%), ferulic acid t_R 16.4 min (0.08%). Identification of the isolated compounds was performed by comparison with t_R of the authentic samples (Art. 5869 and Art. 9936, Carl Roth GmbH+Co).

RESULTS AND DISCUSSION

Scopoletin (0.008%) was isolated from the dichloromethane extract of aerial parts of *B. calycina*.

The identification of the isolated compound was performed by comparison of the spectroscopic data (UV, IR, ¹H-NMR and MS) with those reported in the literature (Hnatyszyn et al., 1993).

Apigenin (0.04%) was obtained from the methanol extract. The isolated compound was identified by direct comparison with authentic material (Art. 5640, Carl Roth GmbH+Co) by mixed melting points, UV spectral analysis with diagnostic shift reagents and MS analysis.

Other compounds, caffeic acid (0.17%) and ferulic acid (0.08%), were obtained from the aqueous-methanol extract and identified by HPLC analysis using commercial compounds as standards.

The phenylpropanoid derivatives are considered an important group of pharmacological compounds, mainly because of their hepatoprotective effects (Adzet et al., 1987). Caffeic, ferulic and *p*-coumaric acids are claimed to possess cholagogue activities (Czok et al., 1971); caffeic acid has been shown to exhibit spasmolytic activity (Ortiz de Urbina et al., 1990).

The spectrophotometric determination of the total phenylpropanoid content of the infusion, prepared according to Argentine Pharmacopoeia (5%), yielded 108 mg% w/v (corresponding to 2.17 g% w/w in the dried plant material). Similar amounts have been reported by Martino et al. (1989) for other species such as *Achyrocline satureioides*, *A. alata*, *Baccharis crispa*, and *B. notoserigila*, widely used in Argentine traditional medicine as hepatoprotective agents.

In consequence, the presence of caffeic and ferulic acids in *B. calycina* and the considerable amount of total phenylpropanoids present in the infusion seem to validate the folk medicinal use of this plant.

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