Phytochemical and Pharmacognostic Investigation of *Bauhinia forficata* Link (Leguminosae)

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We have isolated two phytoconstituents present in the *B. forficata* leaves, a medicinal plant employed in folk medicine specially for the treatment of diabetes. These compounds were isolated by column chromatography and identified as β-sitosterol and kaempferol-3,7-dirhamnoside (kaempferitrin) by spectroscopical data and comparison with authentic samples. A comparative study with different parts of the plant indicated that the latter is present only in the leaves, suggesting that it might be useful for a suitable quality control of phytotherapeutics which contain this organ of *B. forficata* in its composition.

Introduction

The plants belonging to the genus *Bauhinia* (Leguminosae) are commonly known in Brazil as "Pata-de-vaca" or "Unha-de boi", being frequently employed in folk medicine to treat several ailments, specially diabetes (Costa, 1975).

We have previously investigated a plant of this genus, *B. splendens* HBK, which exhibited antinociceptive (Cechinel Filho *et al.*, 1995; Willain Filho *et al.*, 1997) and antibacterial properties (Savi *et al.*, 1997). Such effects appeared to be related to the presence of steroids or flavonoids (Cechinel Filho *et al.*, 1995).

In this work we have extended our studies with the genus *Bauhinia* and selected the *B. forficata*, which is used in Brazilian pharmacopoeia, for phytochemical analysis and characterization of some botanicals and physico-chemicals parameters for quality control of this species.

Material and Methods

Plant material

Young leaves of *B. forficata* were collected in Itajaí, state of Santa Catarina, Brazil, in October 1997, and identified by Dr. Haroldo Cavalcante de Lima (Jardim Botânico/Rio de Janeiro), and a voucher specimen was deposited at the Barbosa Rodrigues Herbarium (BRH, Itajaí) under number VC Filho 013.

Phytochemical and pharmacognostic analysis

For microscopical analysis, an optical microscope INALH-MBS coupled to a Minolta X-300s photographic camera was used. Chromatographic analysis was carried on TLC using Merck silica pre-coated aluminum plates of 200 μm in thickness with several solvent systems. NP-PEG (Natural Products – Polyethylene glycol reagents) and $FeCl_3$ were employed as specific reagents for visualization of flavonoids.

Leaves of *B. forficata* (500 g) were dried at room temperature for approx. 6 days, powdered, and macerated with methanol for one week. After solvent removal under reduced pressure, the extract was then suspended in water and successively partitioned with solvents of increasing polarity, such as hexane, dichloromethane and ethyl acetate, respectively (Cechinel Filho and Yunes, 1998). During the latter procedure, a yellow solid was formed (145 mg). The aqueous fraction was cooled over several weeks and more yellow solid was formed (177 mg), and proved to be identical to that above by TLC. It was identified as the glycoside flavonoid, kaempferol 3,7-dirhamnoside (kaempferitrin) (**1**) which was compared with an authentic sample. The spectroscopic data (IR, ^{1}H-NMR and ^{13}C-NMR) are identical to those reported in the literature (Luo and Jin, 1988; Aragão *et al.*, 1990).

The hexane (4.21 g) and dichloromethane (4.37 g) fractions were combined since they showed a similar phytochemical profile, and chromatographed on a silica gel column eluted with

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hexane/acetone with increasing amounts of acetone. Fractions which showed a positive reaction with anisaldehyde/sulfuric acid reagent were combined and rechromatographed as described above, furnishing a white crystalline solid (150 mg), which was identified as pure β-sitosterol on basis of its spectral data and co-injection (HRGC) with an authentic sample.

Results and Discussion

Leaf transverse sections of *B. forficata* showed abundant cytoplasmatic inclusions of calcium oxalate as druse formate as well as multicellular (2–3 cells) long hair, with rough aspect. Stomata were found in both epidermis consisting of a stomata with four guard cells. Crystalliferous sheath cells around veins composed of prismatic crystals were observed.

The presence of druses in *B. forficata* is an important anatomic factor to differentiate *B. forficata* from *B. variegata* since the second one had little or nothing of inorganic structures (results not shown). Both species seem to be macroscopically very similar leading to misuse or fraud.

In order to determine the constituents present in *B. forficata*, we have initially investigated its leaves, since this part is employed in folk medicine (Almeida, 1993).

Thus, the methanolic extract was successively partitioned with hexane, dichloromethane and ethyl acetate, respectively, according to a previously described method (Cechinel Filho and Yunes, 1998). The use of conventional chromatographic procedures allows us to determine a mixture of aliphatic alcohols as well as pure β-sitosterol in the hexane fraction.

This sterol is a common compound present in natural products, and important pharmacological effects have been demonstrated, such as antiinflammatory (Handa *et al.*, 1992), analgesic (Santos *et al.*, 1995), and has been used for the treatment of benign prostatic hyperplasia (BPH) (Lowe and Ku, 1996). Other steroids or terpenoids were detected, but in small quantities.

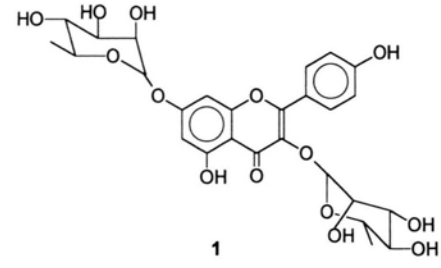
On the other hand, during the partition with ethyl acetate a yellow solid was formed which gave a positive reaction with $FeCl_3$, suggesting to be a phenolic compound (Marini-Bettólo *et al.*, 1981). This same compound was further separated from the aqueous fraction (see experimental part).

The spectral data (IR, ^1H and ^{13}C-NMR) were identical to those reported for kaempferol 3,7-dirhamnoside (kaempferitrin) (**1**) (Luo and Jin, 1988; Aragão *et al.*, 1990). Its molecular structure was confirmed by Co-TLC with an authentic sample and by acidic hydrolysis, which furnished kaempferol and rhamnose. This compound was previously isolated from *Holocalilyx glaziovii* (Aragão *et al.*, 1990) and *Hedyotis verticillata* (Hamzah *et al.*, 1994) showing antiinflammatory activity (Pathak *et al.*, 1991).

A comparative study of different parts of the plant (leaves, stems and barks), indicated that steroids and terpenes are present in all parts predominantly in the leaves. However, kaempferitrin (**1**) was only detected in the leaves of *B. forficata*.

Kaempferitrin can be considered a chemical marker for quality control, since it is absent in other parts of the plant. In addition, (**1**) was not observed on *B. variegata* leaves by TLC analysis. Thus, this finding may be useful for quality control of phytotherapics containing leaves of *B. forficata* in its composition.

Further studies are needed to determine whether both compounds, β-sitosterol and kaempferitrin, exhibit hypoglycemic effects to confirm and justify its use as a hypoglycemic agent.



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