

Phytochemical and Pharmacological Analysis of *Bauhinia microstachya* (Raddi) Macbr. (Leguminosae)

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Bauhinia microstachya, Phenolic Compounds, Analgesic Activity

This paper describes the isolation of four phytoconstituents from the leaves of *Bauhinia microstachya*, a Brazilian medicinal plant used in folk medicine for the treatment of several ailments. Based on spectroscopic evidence, these compounds were identified as methyl galate (1), kaempferol 3-*O*-rhamnosyl (2), quercitrin (3) and myricitrin (4). The crude methanolic extract and two compounds (3 and 4) were tested as analgesic using the writhing test in mice. The extract and compound 3 caused potent and dose-related analgesic effects, confirming the popular use of this plant for the treatment of painful processes.

Introduction

Bauhinia microstachya is a native plant, known as cipó-escada, escada-de-jabutí or escada-de-macaco, widely-distributed in the South of Brazil. Its leaves and bark are used in folk medicine against several disorders including infections, inflammations, diabetes, affections of the urinary tract and respiratory and painful processes (Pio Correia, 1984; Cirilo, 1993). Preliminary phytochemical studies have revealed that the plants of genus *Bauhinia* are mainly constituted of steroidal glycosides, triterpenes, lactones and flavonoids (Iribarren and Pomilio, 1989; Da Silva *et al.*, 2000). Previous studies carried out in our laboratories with the genus *Bauhinia* have shown that some species exhibit an interesting chemical and biological profile. *B. splendens* HBK exhibited antinociceptive (Cechinel Filho *et al.*, 1995; Willian Filho *et al.*, 1997) and antibacterial properties (Savi *et al.*, 1997) and such effects appeared to be related to the presence of steroids or flavonoids (Cechinel Filho *et al.*, 1995). *B. forficata* revealed the presence of β -sitosterol and kaempferitrin and the pharmacognostic investigation indicated that the

latter might be useful for suitable quality control of phytotherapeutics (Da Silva *et al.*, 2000).

The biological significance and the fact that no previous chemical or biological studies have been reported concerning this species encouraged us to study the *B. microstachya*. The present paper describes the isolation of phenolic compounds from *B. microstachya* leaves and the evaluation of the possible analgesic effect of the crude methanolic extract and some compounds obtained from the ethyl acetate fraction of this plant.

Material and Methods

Plant material

Leaves of *B. microstachya* were collected in Urussanga (State of Santa Catarina, Brazil), in February 2000 and classified by Dr. Ademir Reis (Department of Botany/UFSC, Florianópolis). A voucher specimen was deposited at the Barbosa Rodrigues Herbarium (Itajaí), under number VC Filho 021.



Isolation of constituents

Air-dried leaves (600 g) were powdered and extracted with methanol (5 l) at room temperature for approximately two weeks. After solvent removal, the extract was then concentrated under reduced pressure and successively partitioned with n-hexane, dichloromethane (DCM), ethyl acetate (EA) and butanol (BuOH) (Cechinel Filho and Yunes, 1998), respectively, to give the following yields for each fraction: n-hexane (15.2 g), DCM (7.1 g), EA (16.7 g) and n-BuOH (5.4 g). A part of the EA fraction (2.83 g), which showed the most suitable phytochemical profile and good analgesic activity in preliminary analysis (results not shown), was chromatographed using a silica gel column eluted with a mixture of CHCl_3 :MeOH with increasing polarity. Elution with CHCl_3 :MeOH 9:1 v/v gave a phenolic compound, identified as methyl gallate (**1**) (41 mg) and elution with CHCl_3 :MeOH 8:2 v/v furnished a glycoside flavonoid, identified as kampferol 3-*O*-rhamnoside (12 mg) (**2**), which were directly compared with authentic samples. The spectroscopic data, especially ^1H - and ^{13}C -NMR, are identical to those reported in the literature (Binutu and Cordell, 2000; Fossen *et al.*, 1999). Other similar fractions were combined and rechromatographed over a silica gel column (1.36 g) and elution with CHCl_3 :MeOH 7:3 v/v yielded compound **3** (184 mg), identified as quercetin 3-*O*-rhamnoside (quercitrin) by physical and spectral data comparison with those of published values (Majinda *et al.*, 1997) and compound **4** (41 mg), identified as myricetin 3-*O*-rhamnoside (myricitrin), whose spectral data are identical to those of the literature (Martin *et al.*, 1999). Both compounds were confirmed by TLC with authentic samples.

Animals

Male Swiss mice, 25–35 g, were kept in a temperature-controlled environment ($23 \pm 2^\circ\text{C}$) with a 12 h light-dark cycle. Food and water were freely available.

Pharmacological analysis: Abdominal constriction response caused by intraperitoneal injection of dilute acetic acid

Abdominal constriction was induced by intraperitoneal injection of acetic acid (0.6%) according to the procedure described previously (Collier *et al.*, 1968). The animals were pre-treated intraperitoneally (30 min before) with the methanolic extract and compounds obtained from *B. microstachya* before injection of acetic acid. The control animals received the same volume of 0.9% NaCl solution (10 ml/kg) and all experiments were performed at $20\text{--}22^\circ\text{C}$. After the challenge, pairs of mice were placed in separate boxes and the number of abdominal constrictions was cumulatively counted over a period of 20 min. Antinociceptive activity was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with methanolic extract and pure compounds.

Statistical analysis

The results are presented as mean \pm s.e.m., and statistical significance between groups was determined by analysis of variance by Dunnett's multiple comparison test. *P* values less than 0.05 ($P < 0.05$) were considered as indicative of significance. When appropriate, the mean ID_{50} values (i.e. the dose of extracts or compounds, which reduced responses by 50% relative to control values) were estimated by linear regression from individual experiments using "GraphPad Software".

Results and Discussion

The pharmacological analysis of the crude methanolic extract of *B. microstachya* leaves indicated that it causes considerable and dose-dependent inhibition of abdominal constrictions when administered intraperitoneally, with calculated ID_{50} (mg/kg) of 7.9 (and confidence interval between 4.8 and 12.8) and maximum inhibition of $94 \pm 4\%$. The phytochemical and pharmacological analysis with the respective fractions suggested that the ethyl acetate fraction exhibits the best profile for a more detailed investigation. This fraction was then successively chromatographed over silica gel eluted with chloroform and methanol, four compounds being isolated, which were identified as methyl gallate (**1**) (0.007%), kaempferol 3-*O*-rhamnosyl (**2**) (0.002%), quercitrin (**3**) (0.03%) and myricitrin (**4**) (0.007%). These were directly compared with authentic samples and their

spectral data are identical to those described in the literature (see experimental part).

Compound **1** consists of one of the active principles of the plants of the genus *Phyllanthus*, showing interesting analgesic action (Calixto *et al.*, 1998; 2000) and for this reason it was not included in our pharmacological study. Compound **2** was not studied because of its limited quantity.

Quercitrin (**3**) dose-dependently inhibited abdominal constrictions in mice, with calculated ID_{50} value of 2.4 (with confidence interval between 0.9 and 6.4) mg/kg (5.4 (with confidence interval between 2.0 and 14.3) μ mol/kg), being more active than the methanolic extract itself. When compared with some non-steroidal antiinflammatory and analgesic drugs, such acetyl salicylic acid (ID_{50} = 133 μ mol/kg) or 4-hydroxy-acetanilide (acetaminophen) (ID_{50} = 125 μ mol/kg), compound **3** was approximately 24 fold more active in the same experimental procedure.

The analgesic effect of this compound against the writhing test does not enable its mechanism of action to be elucidated, further pharmacological studies being necessary. However, recent studies carried by Ribeiro and co-workers (2000) have demonstrated that the nociceptive activity of acetic acid in the writhing model is due to the release of $TNF-\alpha$ (Tumour Necrosis Factor), interleukin 1 β and interleukin 8 by resident peritoneal macrophages and mast cells.

Although compound **3** is a well-known natural product, to our best knowledge this is the first report concerning its analgesic action. Previous works have demonstrated important biological effects for quercitrin, including anti-inflammatory activity (Taguchi *et al.*, 1993; Fermin *et al.*, 1996), hypotensive activity (Novoa *et al.*, 1985), antiviral activity against herpesviruses (Mucsi *et al.*, 1992). On the other hand, myricitrin (**4**) caused only a moderate analgesic activity against the writhing test, causing approximately 30% of inhibition of abdominal constrictions at 10 mg/kg, given intraperitoneally. Since the difference between compounds **3** and **4** consists of the presence of one additional hydroxyl group in compound **4**, the activity may be related to the polarity or molar volume of these molecules, but other complementary studies are required to confirm this hypotheses.

Due to the presence of the flavonoids described here, plus others that have been detected but not

yet identified, this plant might be used as a beneficial agent to treat a great number of pathologies. Some of the recent advances in flavonoid research have been reviewed, showing that they are capable of modulating the activity of enzymes and affecting the behaviour of many systems, suggesting that this class possess important therapeutic potential (Wang, 2000; Harborne and Willians, 2000).

In summary, our results suggest that *B. microstachya* leaves produce active principles, especially phenolic compounds, which exert analgesic effects in mice, justifying the popular use of this plant to treat dolorous processes. The potent analgesic effect of quercitrin (**3**) encourages additional studies of structural modification in order to obtain new analgesic agents.

Phytochemical and pharmacological investigation are in progress in our laboratories in order to confirm the analgesic effects shown here in other experimental models and also to verify other active principles present in other parts of *B. microstachya*.

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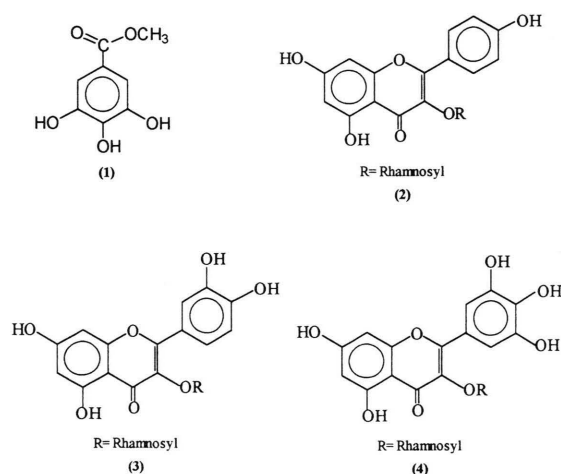


Fig. 1. Molecular structures of methyl gallate (**1**), kaempferol-3-*O*-rhamnosyl (**2**), quercitrin (**3**) and myricitrin (**4**) isolated from *B. microstachya* leaves.

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