

## Phytochemical study and antiulcerogenic activity of *Syngonanthus bisulcatus* (Eriocaulaceae)

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*From the ethanol extract of the capitula and scapes of Syngonanthus bisulcatus (Koern) Ruhland the flavonoids 5-hydroxy-7,4'-dimethoxy-6-C- $\beta$ -D-glucopyranosylflavone, isovitexin (5,7,4'-trihydroxy-6-C- $\beta$ -D-glucopyranosylflavone), luteolin (5,7,3',4'-tetrahydroxyflavone), luteonarin (5,3',4'-trihydroxy-6-C-7-O- $\beta$ -D-glucopyranosylflavone) and 5,6,3',4'-tetrahydroxy-7-O- $\beta$ -D-glucopyranosylflavone were isolated. The structure of the compounds were characterized by spectroscopic methods, mainly 1D and 2D NMR experiments, as well as ESMS spectrometry. In addition we examined the effect of the ethanol extract of capitulae and scapes in the ulcer model ethanol/HCl-induced gastric mucosal lesions.*

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## INTRODUCTION

“Sempre-vivas” (everlasting plants) is the name given in Brazil to some plants whose inflorescences and scapes conserve the appearance of the living structures even after being dried when harvested out. They are sold locally and exported for ornamental purposes (Giullietti *et al.*, 1996). Most of these plants belong to the *Syngonanthus* genus (Eriocaulaceae). Until recently, the knowledge about the flavonoids chemistry of the *Syngonanthus* Ruhl. genus was limited to the paper of Ricci *et al.* (1996), who reported the presence of apigenin and luteolin derivatives in the leaves of some species.

Flavonoids encompass a large group of polyphenolic substances that has antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, and vasodilator effects. Several studies related to be potent antioxidants, capable of scavenging

hydroxyl radical, super oxide anions and lipid peroxy radicals (Miller, 1996).

The peptic ulcers are illnesses that affect a considerable number of people in the world. They are induced by stress, smoking, nutritional deficiencies and ingestion of non-steroidal-anti-inflammatory drugs (Nash *et al.*, 1984; Basil *et al.*, 1995). The etiology of ulcer is still unknown. It is generally accepted that it results from an imbalance between aggressive factors such as acid and pepsin and the maintenance of mucosal integrity through endogenous defense mechanisms (Wallace *et al.*, 1996).

Different therapeutic agents, including plant extract containing flavonoids, are used to inhibit gastric acid secretion or to boost mucosal defense mechanisms have been shown to produce promising results for the treatment of gastric ulcers (Vilegas *et al.*, 1998).

Therefore, in order to investigate the possible antiulcerogenic activity and to contribute to the chemical

study of *Syngonanthus* we have examined ethanol extracts capitula and the scapes of *S. bisulcatus* (Koern) Ruhland. This flower is known as “sempre-viva chapadeira”. It occurs mainly in Southeast region of Brazil, at Serra do Cipó and Diamantina – Minas Gerais State, and several tons are exported to many developed countries.

## MATERIAL AND METHODS

### Plant Material

The specimen were collected at Serra do Cipó – State Minas Gerais – Brazil and identified by Paulo Takeo Sano. Voucher specimen was deposited at the Herbarium of Departamento de Botânica do Instituto de Biociências – Universidade de São Paulo (SPF77735).

### Extraction and preparation of ethanolic extracts

Capitula (280 g) and scapes (300 g) were separated, dried in an oven at 45°C for 1 week and powdered. The resulting materials were separately macerated at room temperature sequentially with hexane, methylene chloride and ethanol for 1 week with each solvent. Extracts were filtered and concentrated under vacuum affording the hexane (1.2 g) methylene chloride (2.7 g) and ethanol (6.0 g) extracts of the capitula and scapes. For this work we used only ethanol extract from capitula and scapes.

### Animals

Male Swiss albino mice (25-35g) from the Central animal House of Universidade Estadual de Campinas (CEMIB/UNICAMP) were used. The animal were fed a certified Nuvilab CR-a (Nuvital) diet with free access to water under standard conditions of 12h dark 12 h light period, humidity ( $60 \pm 1.0\%$ ) and temperature ( $21.5 \pm 1.0\%$ ). The experimental protocols were approved by the institutional (UNICAMP) Animal Care and Use Committee, in accordance with the recommendations of the Canadian Council for Animal Care (Olfert *et al.*, 1993; Zimmermann, 1983), protocol number 502.

### Drugs

The following drugs were used: saline solution 0.9%, lansoprazole, ethanol 60% and HCl 0.3M. All reagents were of a high grade of purity. The substances and reagents were prepared immediately before use.

### Isolation and identification of the flavonoids (1-5)

1.5g of ethanol extracts were fractionated by gel permeation Column Chromatography (Sephadex LH-20, Pharmacia) eluted with methanol. The substances obtained were further purified by repeated Column Chromatography either on polyvinylpyrrolidone (Sigma, eluted with MeOH) or on RP 18 column (Lichroprep. RP18 – Merck, 40-63 mm) eluted with acetonitrile/water 85:15 (v/v) and 70:30 (v/v). The structures of the flavonoids **1-5** were unambiguously determined by means of spectroscopic methods (IR, ESMS,  $^1\text{H}$ ,  $^{13}\text{C}$  and 2D NMR experiments COSY, HSQC, HMBC) and compared to those previously reported (Agrawal, 1989; Harborne, 1996; Markham, 1982).

### Antiulcerogenic Activity

#### *Ethanol/HCl – Induced Gastric Mucosal Lesions*

The anti-ulcerogenic activity of ethanol extracts obtained from the capitula and scapes of *Syngonanthus bisulcatus* after ulcer induction with ethanol/HCl was performed according to the method of Mizui and Doteuchi (Mizui, Douteuchi, 1983) with some modifications. Mice were fasted for 24h, divided into groups of 7 animals and before receiving an oral dose of vehicle (saline solution 0.9%), lansoprazole (30mg/kg) and the *Syngonanthus bisulcatus* ethanolic extracts (50, 100 and 250mg/kg). Fifty minutes after the treatments, all groups received 0.2ml of 0.3 M HCl/60% ethanol solution orally.

Animals were killed 1 h after the administration of HCl/ EtOH solution; the stomachs were excised, inflated by an injection of saline (2 ml) and opened along the greater curvature. Then the stomachs were fixed and the ulcerative lesion index (ULI) calculated according to the methodology described by Szelenyi and Thieme (1978).

The lesion index was expressed as the sum of all lesions. Results are expressed as an ulcerative index (UI) as described by Szelenyi and Thieme (1978).

### Statistical analysis

Results were expressed as the mean  $\pm$  S.E. Statistical significance between groups was determined by one way analysis of variance (ANOVA) followed by Dunnett's test, with the level of significance set at  $p < 0.05$  or  $p < 0.001$ .

## RESULTS AND DISCUSSION

The effect of ethanol extracts of *Syngonanthus bisulcatus* on ethanol/HCl gastric ulcers was investigated in mice. The results of the present study are summarized in Table 1.

Oral administration of ethanol/ HCl solution to the control group clearly produced the expected characteristic zonal necrotizing mucosal lesion. The lanzoprazole and ethanolic extracts obtained from the capitula and scapes of *Syngonanthus bisulcatus* (50, 100, 250 mg/kg) significantly inhibited the ulcer formation when compared with control group.

The oral treatment of mice with ethanol, induces solubilization of mucus constituents in the stomach with a concomitant fall in the transmucosal potential difference.

This agent also increases Na<sup>+</sup> and K<sup>+</sup> flux into the lumen and increases histamine release and pepsin. It also depresses tissue levels of DNA, RNA and proteins, leading to flow, stasis in injured tissue (Szabo, 1987). The ethanol induces the formation of gastric ulcers and the presence of HCl only accelerates the process (Sun *et al.*, 1991).

The damage may be due to stasis in gastric blood flow which contributes to the development of the hemorrhage and necrotic aspects of tissue injury (Guth *et al.*, 1984). This action is direct on the gastric epithelium also causing perturbation of mast cells and release of a vasoactive mediator such as histamine (Oates *et al.*, 1988).

In order to identify some compounds present in the active fractions we have performed a chromatographic fractionation of the ethanol extracts of capitula and scapes

that led to the isolation of compounds **1-5**. When revealed with NP-PEG reagent, these compounds showed yellow or orange spots characteristic of flavonoids (Wagner *et al.*, 1984). The extract of capitulae afforded two apigenin derivatives: 5-hydroxy-7,4'-dimethoxy-6-C-β-D-glucopyranosylflavone **1** (200 mg), and 5,7,4'-trihydroxy-6-C-β-D-glucopyranosylflavone **2** (300 mg). The extract of scapes afforded three luteolin derivatives: 5,7,3',4'-tetrahydroxyflavone **3** (150 mg), 5,3',4'-trihydroxy-6-C-7-O-β-D-glucopyranosylflavone **4** (200 mg), and 5,6,3',4'-tetrahydroxy-7-O-β-D-glucopyranosylflavone **5** (250 mg) (Figure 1). These compounds were determined by comparison of their <sup>1</sup>H and <sup>13</sup>C NMR, IR, UV and ES-MS data with those previously reported (Harborne, 1996; Agrawal, 1984; Markham, 1982).

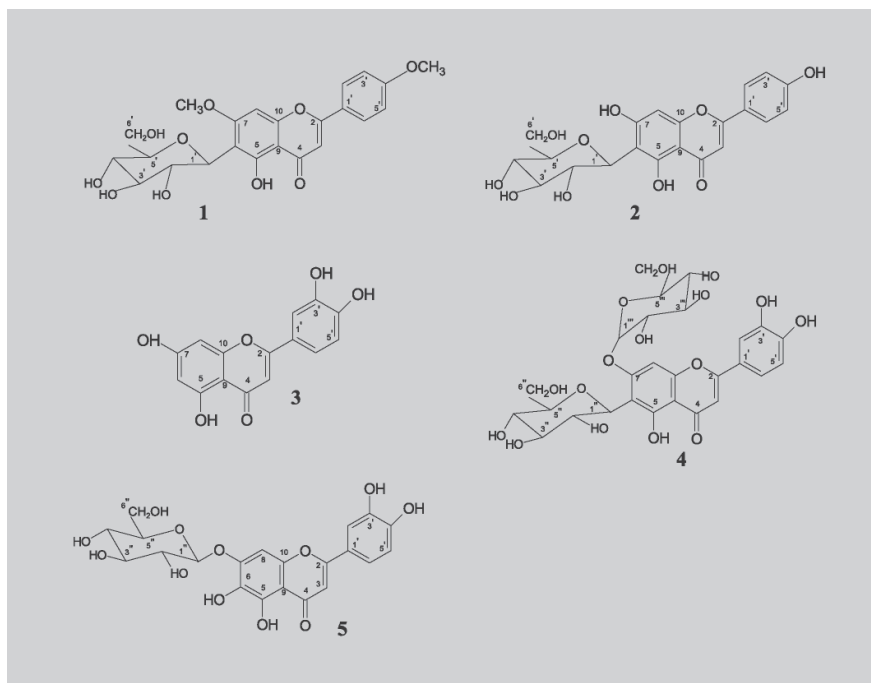
Our results suggests a more complex flavonoid pattern for *Syngonanthus bisulcatus* than that previously reported by Ricci (1996) for the leaves of *Syngonanthus xeranthemoids* and *Syngonanthus verticillatus*, since capitula present apigenin-C-glycosides whereas scapes present luteolin O- and C-glycosides (Harborne, 1996).

Ethanol-induced gastric lesion are thought to arise as a result of direct damage of gastric mucosal cells, resulting in the development of free radicals (Pihan *et al.*, 1987) and hyperoxidation of lipids (Purunen *et al.*, 1980). Literature reports that flavonoids can act as antiulcer compounds probably because of their antioxidant properties, that could prevent the formation of free radicals in the body and also minimize injuries by oxidative reactions (La Casa, 2000). Besides, compounds that have a cathecolic system, such as the ring B of luteolin

**TABLE I** - Effects of lanzoprazole and different doses of ethanolic extracts of *Syngonanthus bisulcatus* on model HCl/ethanol-induced gastric ulcer in mice.

Ulcerative Model	Treatment (p.o.)	Dose (mg/kg body wt)	Ulcerative Index (mm)	Inhibition (%)
Ethanol /HCl	Saline	-	77 ± 20	0
	Lanzoprazole	30	25 ± 13*	67
	EtOH (capitula)	50	34 ± 14*	56
	EtOH (capitula)	100	32 ± 10*	58
	EtOH (capitula)	250	30 ± 14*	61
Ethanol/HCl	Saline	-	46 ± 21	0
	Lanzoprazole	30	20 ± 8.3*	57
	EtOH (scapes)	50	17 ± 6.5*	63
	EtOH (scapes)	100	17 ± 7.1*	63
	EtOH (scapes)	-250	11 ± 4.6*	76

The results are expressed as mean ± S.E. Data were always calculated in relation to the respective control group. ANOVA:  $F_{(4,25)} = 4.2$   $p < 0.05$ . Dunnett's test \* $p < 0.05$ .



**Figure 1** - Flavonoids isolated of the ethanolic extract from the capitula and scapes of *Syngonanthus bisulcatus*.

derivatives, have presented antioxidant and antiulcer activities (Yesildada *et al.*, 2000).

Phytochemical and pharmacological studies have suggested that flavonoids present in the ethanolic extracts obtained from the *Syngonanthus bisulcatus* may be regarded as possible active compounds against gastric lesions probably due their antisecretory and cytoprotector effects. Further analyses and experiments with the isolated flavonoids will be performed in a near future to check these hypothesis.

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## RESUMO

### Estudo Fitoquímico e Atividade Antiulcerogênica de *Syngonanthus bisulcatus* (Eriocaulaceae)

Do extrato etanólico dos capítulos e dos escapos de *Syngonanthus bisulcatus* (Koern) Ruhland foram isolados os flavonóides: 5-hidroxi-7,4'-dimetoxi-6-C- $\beta$ -D-glicopiranosilflavona, isovitexina (5,7,4'-triidróxi-6-C- $\beta$ -D-glicopiranosilflavona, luteolina (5,7,3',4'-

tetraidroxiflavona), luteonarina (5,3',4'-triidróxi-6-C-7-O- $\beta$ -D-glicopiranosilflavona) e 5,6,3',4'-tetraidróxi-7-O- $\beta$ -D-glicopiranosilflavona. As estruturas dos compostos foram caracterizadas por espectroscopia (RMN mono e bi-dimensionais) e por espectrometria de massas Electrospray. Adicionalmente foram examinados os efeitos dos extratos etanólicos dos capítulos e dos escapos pelo modelo de úlcera induzida por etanol/HCl

**UNITERMOS:** *Syngonanthus bisulcatus*. Atividade antiulcerogênica. Flavonóides.

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