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# *In vivo* inhibition of gastric acid secretion by the aqueous extract of *Scoparia dulcis* L. in rodents

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**Abstract**

The freeze-dried aqueous extract (AE) from the aerial parts of *Scoparia dulcis* was tested for its effects on experimental gastric hypersecretion and ulcer in rodents. Administration of AE to animals with 4 h pylorus ligature potently reduced the gastric secretion with $ED_{50}$s of 195 mg/kg (rats) and 306 mg/kg (mice). The AE also inhibited the histamine- or bethanechol-stimulated gastric secretion in pylorus-ligated mice with similar potency suggesting inhibition of the proton pump. Bio-guided purification of the AE yielded a flavonoid-rich fraction (BuF), with a specific activity 4–8 times higher than the AE in the pylorus ligature model. BuF also inhibited the hydrolysis of ATP by $H^+$,$K^+$-ATPase with an $IC_{50}$ of 500 μg/ml, indicating that the inhibition of gastric acid secretion of *Scoparia dulcis* is related to the inhibition of the proton pump. Furthermore, the AE inhibited the establishment of acute gastric lesions induced in rats by indomethacin ($ED_{50}$ = 313 mg/kg, p.o.) and ethanol ($ED_{50}$ = 490 mg/kg, p.o.). No influence of the AE on gastrointestinal transit allowed discarding a possible CNS or a cholinergic interaction in the inhibition of gastric secretion by the AE. Collectively, the present data pharmacologically validates the popular use of *Scoparia dulcis* in gastric disturbances.


*Keywords:* *Scoparia dulcis*; Scrophulariaceae; Antiacid; Medicinal plant; $H^+$,$K^+$-ATPase; Antiulcer

## 1. Introduction

*Scoparia dulcis* (Scrophulariaceae), known in Brazil as *vassourinha*, is a widely distributed species in tropical and subtropical regions of South America and Asia. In Brazil it is used in folk medicine to treat respiratory, gastric and hepatic disturbances (Gonzales-Torres, 1986; Kawasaki et al., 1987) and as an anti-inflammatory (Freire et al., 1993). *Scoparia dulcis* is also used in India for the treatment of diabetes and hypertension (Perry, 1980). The chemistry and biological activities of *Scoparia dulcis* have been studied in the past, leading to the isolation of friedelin, glutinol, α-amyrin and ifflocionic, dulcionic and betulinic acids (Mahato et al., 1981). Amelin, a compound with anti-diabetic properties (Nath, 1943) and 6-metoxibenzoxazolinone, a depressor of arterial blood pressure (Chen and Chen, 1976; Kawasaki et al., 1987) have also been identified in this species. Inhibition of β-glucuronidase by scoparic acid A (Hayashi et al., 1992) and by 8-hydroxytricetin-7-glucuronide (Kawasaki et al., 1988), antiviral activity by scopadulin (Hayashi et al., 1990a) and scopaducilic acid B (Hayashi et al., 1988; Hayashi et al., 1992) have been also reported. Previous studies from our laboratory demonstrated analgesic (Freire et al., 1991) and anti-inflammatory (Freire et al., 1993) activities due to the presence of the triterpene glutinol isolated from its ethanolic crude extract. A simpaticomimetic activity has been attributed to the presence of catecholamines (adrenaline and noradrenaline) in *Scoparia dulcis* (Freire et al., 1996). Scopaducilic acid B, scopadulciol and diacetyl scopadol isolated from the paraguayan *Scoparia dulcis* (Hayashi et al., 1987) reversibly inhibited the activity of gastric enzyme, $H^+$,$K^+$-ATPase (Asano et al., 1990; Hayashi et al., 1990b; Hayashi et al., 1991a) showing the potential gastroprotective effect of the plant as reported by the traditional medicine.

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The aim of the present study was to assess the efficacy of aqueous extracts of *Scoparia dulcis* as a gastroprotective agent using *in vivo* rodent models.

## 2. Materials and methods

### 2.1. Plant material, extractions fractionation and identification by HPLC of cirsitakaoside and quercetin from aqueous extract (AE)

Sample of *Scoparia dulcis* was collected in Maranhão-Brazil and was identified by Dr. Terezinha Rego from Universidade Federal do Maranhão. A voucher of this specimen is kept at the Herbário Ático Seabra under acquisition number 114. The aerial parts of the plant (fruits, flowers, leaves and haste) were dried on the shade, ground and extracted (20 g/l, w/v) with hot water (73 °C) for 30 min. The aqueous extract was concentrated under vacuum to 1/4 of the original volume and freeze-dried with yield of 15% (3 g). This powder was re-taken up in water and extracted four times with 250 ml of *n*-butanol. The resulting aqueous phase-1 (AF, yield 9.6%, 1.92 g of original material) and the butanolic phase (BuF, yield 4.3%, 0.86 g of original material) were concentrated under vacuum and freeze-dried. The activity of the AE extract and all fractions obtained was determined using the *in vivo* model of pylorus ligature as described below. All extracts were dissolved in water/saline prior tests. The AE and fractions were screened by thin layer chromatography (TLC) on silicagel 60 F254 (0.2 μm, Merck and Co., USA) plates eluted with *n*-butanol:acetic acid:water (40:10:15). The plates were scanned with short and long wave UV light and developed with iodine, Liebermann–Burchard reagent, ninhydrin and sulfuric vanillin. The fractions were also tested with 1% $FeCl_3$ or Shinoda reagent to detect presence of flavonoids and phenolic compounds. The HPLC fingerprint of fraction BuF (stock solution 4.0 mg/ml,) was performed by HPLC (Pro Star Varian, data acquisition workstation multi instrument Version 6.4 1). Column: C-18 reverse phase (Varian) 150 mm × 4.6 mm i.d.; mobile phase: A = 0.1% trifluoroacetic acid (TFA) (pump A) and acetonitrile (ACN) (pump C); gradient: 0–10 min 100% A; 10–20 min 80% A; 20–30 min 60% A and 30–40 min 40% A; detection: 350 nm, flow rate: 1 ml/min; run time 40 min, BuF sample 20 μl. Calibration curve was made using standard curves for quercetin (Sigma and Co., USA) and pure cirsitakaoside (5-hydroxy-6,7-dimethoxyflavone 4-*O*-β-D-glucopyranoside previously isolated, purified and identified at our laboratory from *Scoparia dulcis* leaves (Pereira-Martins et al., 1998).

### 2.2. Animals

F1 male mice (25–30 g), a hybrid from a cross between inbred C57Bl/6 female and Balb/C male, Male Wistar rats (250–300 g) or rabbits (1–2 kg) of either sex were used in this study. All animals were maintained at (22 ± 2 °C) on 12-h light/12-h dark period (lights on at 07:00 h) with food and water available *ad libitum* in accordance to The Guide for the Care and Use of Laboratory Animal, National Research Council, USA (1996). The experimental protocols used in this study were approved by UNIFESP/EMP Ethics Committee. Animals were deprived from food 6–12 h before the experiments.

### 2.3. Drugs

Bethanechol chloride, histamine dihydrochloride, adenosine 5′-triphosphate (ATP), atropine, Tris base, potassium chloride, magnesium chloride, ethylene glycol tetraacetic acid (EGTA), HEPES and indomethacin were purchased from Sigma Chem. Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

### 2.4. Determination of intestinal transit

Mice were treated with AE (0.5 to 2 g/kg, p.o., $n = 5$) or vehicle (water 0.1 ml/10 g, p.o., $n = 5$) 1 h prior to the administration of a charcoal suspension (10%, 0.1 ml/10 g body weight, p.o.). After 45 min, the animals were killed and their stomach and small intestine were removed. The distance from the pylorus, reached by the marker in the intestine, was measured and expressed as a percentage of the total intestinal length. Atropine-treated (1 mg/kg, s.c., $n = 5$) animals were used as positive control (Stickney and Northup, 1959).

### 2.5. Antiulcer activity

Fasted rats were treated orally with AE (0.5 and 1 g/kg, $n = 5$) or vehicle (10 ml/kg, $n = 5$). After 1 h, gastric lesions were induced by either 75% ethanol (1.5 ml/200 g, p.o., $n = 5$) or indomethacin (10 mg/kg, s.c., $n = 5$). After 1 and 5 h, respectively, the animals were killed under ether anesthesia. The stomach was dissected, washed and inspected under magnification to determine the number of ulcers and to score the index of mucosal damage (IMD). The quantification of lesions was done by two different persons on the same preparation, considering the color, the presence of edema and of hemorrhage of gastric folds, the number of pethechyae, the number and size of necrosis regions considered as ulcers (Vela et al., 1997).

### 2.6. Pylorus ligature in rodents

A pylorus ligature was carefully done in mice or rats under ether anesthesia according to Shay et al. (1945). The AE (0.01 to 1 g/kg for rats or 0.5 to 1 g/kg for mice, $n = 5$) or vehicle (water, 0.1 ml/10 g body weight, p.o., $n = 5$) was injected into the duodenal lumen (i.d.). After 4 h, the animals were killed by deep ether anesthesia, the stomach was opened and the gastric secretion collected. The final volume and pH were directly determined after washing the mucosal side of the stomach with 2 ml of distilled water. Total acidity of the gastric juice was titrated with 0.01N or 0.1N NaOH (for mice and rats, respectively) with 2% phenolphtalein as an indicator. BuF (320 mg/kg, i.d., $n = 5$) and $AF_1$ (640 mg/kg, i.d., $n = 5$) fractions, separated by solvent partition from AE, were also tested in rats. In addition, effect of AE (1 g/kg, i.d., $n = 6$) was tested on gastric acid secretion induced in pylorus-ligated (4 h)