Acta Farm. Bonaerense **21** (4): 279-82 (2002)
Recibido el 09 de febrero de 2002
Aceptado el 14 de septiembre de 2002

Effect on Gastrointestinal Propulsion and Preliminary Phytochemical Analysis of *Aster squamatus* (Asteraceae)

Joceana S. SPEROTTO [1], Nediana BIALESKI [1], Lucielli SAVEGNAGO [1], Berta M. HEINZMANN [2], Margô G.O. KARNIKOWSKI [1] & Bernardo BALDISSEROTTO [1]*

[1] *Depto. de Fisiologia, UFSM, Prédio 21, Campus Universitário 97105.900 - Santa Maria, RS, Brazil.*
[2] *Depto. de Farmácia Industrial, UFSM, Prédio 26, Campus Universitário 97105.900, Santa Maria, RS, Brazil.*

SUMMARY. The aim of this study was to determine the effect of *Aster squamatus* on gastrointestinal propulsion. Phytochemical screening suggested that stalks and roots contain steroids, terpenes, flavonoids, phenols, substances that contain amino-groups, saponins and hydrolysable and condensed tannins. The decoctions of leaves, stalks and roots indicated the presence of caffeic, cinnamic, and sinapic acids. All *A. squamatus* infusions significantly reduced gastrointestinal propulsion compared to control, whereas the decoctions and ethanolic extracts did not alter this parameter. The effect of the infusions and the lack of activity of the decoctions and ethanolic extracts may be linked to the presence of unstable or volatile constituents in the plant.

RESUMEN. "Efecto sobre la propulsión intestinal y análisis fitoquímico preliminar de *Aster squamatus* (Asteraceae)". El objetivo de este trabajo fue determinar el efecto de extractos de *Aster squamatus* en la propulsión gastrointestinal. El screening fitoquímico sugirió que los tallos y raíces contienen esteroides, terpenos, flavonoides, fenoles, grupos amino, saponinas y taninos condensados e hidrolizables. La decocción de las hojas, tallos y raíces indicaron la presencia de cafeína, ácidos cinámico y sinápico. Todas las infusiones redujeron significativamente la propulsión gastrointestinal en comparación con el grupo control, en tanto que las decocciones y extractos etanólicos no alteraron tal parámetro. El efecto de las infusiones y la ausencia de actividad de las decocciones y extractos etanólicos puede estar relacionado con la presencia de constituyentes volátiles o inestables en la planta.

INTRODUCTION

Diarrheal diseases continue to cause million deaths per year among young infants, mainly in non-developed countries. This disease also results in thousands hospitalizations per year in developed countries such as the United States [1]. The usual treatment consists of the use of oral rehydrating solutions to preserve homeostasis [2], and administration of an antimicrobial drug to remove the infectious agent [1]. However, medicinal plants can also be a valuable alternative, since poor nations, where diarrheas are an important cause of children death, lack the ability to produce and distribute oral rehydrating solutions [3].

The infusion of the aerial parts of *Aster squamatus* (Spreng.) Hieron. is considered to have an antidiarrhoeic effect because it increases the intestinal absorption of water and reduces gastrointestinal propulsion [4]. Complementary studies showed that the infusion of stalks and roots altered ion transport in the colon [5]. In addition, the aqueous and ethanolic extract of leaves, stalks, and roots of this plant have low acute toxicity [6] and the use of infusions for one month induced only minor changes in some serum biochemical parameters [5]. Therefore, it is important to determine the effect of the different parts of this plant on gastrointestinal propulsion, to define which parts could be used as antidiarrhoeic agent.

KEYWORDS: Antidiarrhoeal, *Aster squamatus*, Gastrointestinal propulsion.
PALABRAS CLAVE: Antidiarréico, *Aster squamatus*, Propulsión gastrointestinal.

* Author to whom correspondence should be addressed.

MATERIAL AND METHODS

A. squamatus was collected on January 1994, in Santa Maria, Southern Brazil. A voucher specimen was prepared and registered in the herbarium of the Department of Biology of the Federal University of Santa Maria (SMDB N° 4895). The leaves, stalks, and roots were separated and maintained in a ventilated oven (50 °C) for drying and stabilization of the plant, and the material was then pulverized in a Willye mill. To prepare the crude extracts 50 g of leaves, stalks, or roots were boiled in 500 ml of water (aqueous extract) or ethanol (ethanolic extract) for 15 min. This process was repeated three times. The concentrate was dried in a water-bath (60 °C), and maintained in an oven at the same temperature until the weight remained constant. The dry residue was scraped and stored under refrigeration in previously labeled brown glass bottles. Just before to use, the infusions of leaves, stalks, or roots were prepared by the addition of 2.4 g of the pulverized plant to 40 mL of boiling destilled water. The mixture remained covered for 30 min and was then filtered.

After a 24 h fast, mice (17-33 g) received by gavage 600 mg/kg of aqueous or ethanolic extracts, or infusions. Control animals were similary treated with distilled water only. All animals received a constant volume of 0.1 mL/10 g live weight. Gastrointestinal propulsion was investigated using a charcoal suspension, according to the method of Almeida *et al.* [4]. All values are expressed as the mean ± SEM, and statistical differences among the experimental groups were assessed by one-way analysis of variance and the Duncan test, with the aid of the SPSS program (version 1986).

Preliminary phytochemical screening was undertaken using described methods [7-9]. The screening covered steroids, terpenes, alkaloids, flavonoids, terpenes, coumarins, quinones, phenols, amino-groups, cyanogenic glycosides, saponins, anthocyanic glycosides and hydrolysable and condensed tannins.

The aqueous extracts were fractionated, yielding ether, EtOAc and, MeOH soluble fractions. These fractions were analysed by TLC using quercetin, cinnamic, caffeic, vanillic, and sinapic acids as reference substances, cellulose sheets 0.1 mm (Merck) as adsorbent and $CHCl_3$–acetic acid–H_2O (50:45:5) and benzene–MeOH–acetic acid (90:16:8) as eluents. The constituents were detected using UV light at 254 nm. Saponins and terpenes were analysed using silicagel 60 sheets 0.25 mm (Merck), $CHCl_3$–MeOH–H–O (65:50:10) and n-hexane–EtOH (85:15) as eluents, respectively. Both classes of chemical constituents were detected by anysaldehyde –H_2SO_4.

RESULTS AND DISCUSSION

The phytochemical screening suggested that stalks and roots contain steroid, terpenes, flavonoids, phenols, amino-groups, saponins, hydrolysable and condensed tannins as chemical contituents. TLC of the EtOAc, ether, and methanolic soluble fractions of the aqueous extracts of leaves, stalks, and roots indicated the presence of caffeic, cinnamic, and sinapic acids in all fractions. Vanillic acid seems to be a constituent only of the AcOEt soluble fraction obtained from the roots. Rfs values of the reference substances in benzene–MeOH–acetic acid (90:16:8) are 0.55 for cinnamic acid, 0.13 for caffeic acid, 0.47 for sinapic acid, and 0.41 for vanillic acid.

The infusions of leaves, stalks, and roots of *A. squamatus* reduced significantly the gastrointestinal propulsion compared to control, whereas the aqueous and ethanolic extracts did not alter this parameter (Table 1).

The inibitory effect of all the studied infusions of *A. squamatus* on gastrointestinal propulsion in mice encourages additional clinical studies of this plant as an antidiarrhoeic agent. The effects of the infuses of *A. squamatus* on the gastrointestinal propulsion and the lack of this activity by ethanolic and aqueous extracts indicated that the active compounds of *A. squamatus* must be soluble in water and are unstable.

Several flavonoids (squamatin, ternatin, ramnetim, kaempferol, baicalein, luteolin-7-metileter, and quercetin) were isolated from the flowers of *A. squamatus* from Egypt [10]. Quercetin is known as an antidiarrhoeic agent [11] and inhibits the acetylcholine response in the guinea pig ileum and the synthesis of prostaglandins [12]. However, this substance was not responsible for the reduction of gastrointestinal propulsion in our experiments because flavonoids are stable substances and would not be destroyed by a decoction [8]. In addition, quercetin could not be detected by TLC of the studied extracts.

The phytochemical screening results reveal the contituents of the extract to include tannins. It is possible that the astrigent properties of tannins may be responsible for the antidiarrhoeal

Infusions/Extracts	Gastrointestinal propulsion (%)
control	67.91 ± 1.37 (10)
Infusions of leaves	48.50 ± 7.18 (9) *
Infusions of stalks	57.44 ± 2.67 (9) *
Infusions of roots	54.56 ± 2.81 (10) *
aqueous extract of leaves	62.74 ± 5.07 (10)
aqueous extract of stalks	65.57 ± 4.59 (10)
aqueous extract of roots	62.62 ± 2.44 (10)
ethanolic extract of leaves	63.05 ± 5.67 (10)
ethanolic extract of stalks	65.17 ± 3.93 (9)
ethanolic extract of roots	**65.15 ± 3.66 (10)**

Table 1. Effect of the infusions and aqueous and ethanolic extracts of leaves, stalks, and roots of *Aster squamatus* on gastrointestinal propulsion in mice. Gastrointestinal propulsion expressed as percentage of the distance travelled by the charcoal with respect to the total length of the intestine. Number of animals is given parenthesis. Data analysed by one-way analysis of variance and Duncan test. * Different from control (p<0.05).

effect of the infusions. Tannins are soluble in water and can polimerize and hydrolyse rapidly at high temperatures [13]. Most frequently tannin–containing plants are used to treat diarrhoea and dysentery [14]. Other unstable compounds may also be responsible for the activity of *A. squamatus*. Potential candidates include phenols, glycosides or other esthers and mono- and sesquiterpenes. Their instability can be explained by phenol oxidation, esther hydrolisis and terpene evaporation when submitted to high temperatures for long period of time [8]. Consequently, more detailed studies are necessary to identify the active principle(s) of this plant and its mechanism of action.

The lack of activity of *A. squamatus* decoctions does not fully rule out the possibility of their use as antidiarrhoeic agents, since all of them contain caffeic and cinnamic acids. Both acids were shown to reduce the growth of *Escherichia coli* [15] and caffeic acid inhibited arylamine N-acetyltransferase activities in human gastrointestinal microflora [16]. Therefore, experiments carried out to determine the antimicrobial effects of these decoctions could give good results.

Acknowledgements. The authors are grateful to Prof. Dr. Eloir P. Schenkel, Universidade Federal de Santa Catarina, for furnishing the authentic samples for CCD. This work was supported by FAPERGS, and J. S. Sperotto and L. Savegnago received CAPES and CNPq fellowships, respectively.

REFERENCES

1. Sack, R.B. (1996) *Infect. Med.* 13: 301-13.
2. Martinez, C.A., D. Barua & M.H. Merson (1988) *World Health Stat. Q.* **41**: 74-81.
3. Lutterodt, G.D. (1992) *J. Ethnopharmacol.* **37**: 151-7.
4. Almeida, C.E., M.G.O. Karnikowski, R. Foleto & B. Baldisserotto (1995) *Rev. Saúde Pública* **29**: 428-33.
5. Meneghetti, B.H. (1997) *Avaliação da atividade antidiarréica e toxicidade ubaguda de Aster squamatus (Spreng.) Hieron. (Asteraceae).* Universidade Federal de Santa Maria, Santa Maria, Master Dissertation in Farmaceutical Science and Technology, 54 p.
6. Karnikowski, M.G.O., R.S. Gioda, M. Karnikowski, M.A.M. Bortoluzzi & B. Baldisserotto (1998) *Rev. Bras. Toxicol.* **11**: 24-6.
7. Moreira, E.A. (1979) *Trib. Farm.* **47**: 455-63.
8. Harborne, J.B. (1984) *"Phytochemical methods"* 2 ed. Ed. Chapman & Hall, New York, 288 p.
9. Stahl, E. & W. Schild (1981) *"Drogenanalyse II:*

Inhaltsstoffe und Isolierung" en "*Pharmazeutische Biologie*", Gustav Fischer, Stuttgart, Vol. 4, 461 p.
10. Sayed, H.M. & S.A. Ross (1987) *Bull. Pharm. Sci.* **9**: 149-63.
11. Lutterodt, G.D. (1989) *J. Ethnopharmacol.* **25**: 235-47.
12. Sweis, J., J. Robak, L. Dabrowski, S. Dunicz, Z. Michalska & R.J. Gryglenski (1984) *Pol. J. Pharmacol. Pharm.* **36**: 455-63.
13. Hänsel, R., O. Sticher & E. Steinegger (1999) "*Pharmakognosi- Phytopharmazie*" 6 Aufl. Springer, Berlin, 1403 p.
14. Otshudi, A.L., A. Vercruysse & A. Foriers (2000) *J. Ethnopharmacol.* **71**: 411-23.
15. Bernkop-Schnurch, A., S. Krist, M. Vehabovic & C. Valenta (1998) *Eur. J. Pharm. Sci.* **6**: 303-9.
16. Lo, H. H. & J. G. Schung (1999) *Anticancer Res.* **19**: 133-9.