Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

# PHCOG MAG.: Research Article
# Antiulcer and antimicrobial activities of *Stryphnodendron rotundifolium* Mart.

**Fabiola Fernandes Galvão Rodrigues, Brisa do Svadesvich Cabral, Henrique Douglas Melo Coutinho, André Luiz Herzog Cardoso, Adriana Rolim Campos, and José Galberto Martins da Costa*.**

*Laboratório de Pesquisas de Produtos Naturais, Departamento de Química Biológica, Universidade Regional do Cariri, Rua Cel. Antônio Luiz, 1161, CEP 63105-000, Crato – Ceará, Brazil.*

**Correspondence to: galberto@urca.br*

**ABSTRACT**

The ethanolic extract of *Stryphnodendron rotundifolium* stem bark (EESR) was investigated for therapeutic properties using ethanol-induced ulceration in mice. Antimicrobial and preliminary phytochemical screening of the extract was also investigated. The extract (200-400 mg/kg p.o.) dose dependently reduced ethanol (0.2 mL/animal p.o.) - induced ulceration in mice. EESR showed antimicrobial activity against five bacterial species (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Shigella flexineri, Staphylococcus epidermidis*) but not against the fungi *Aspergillus niger*. The above results show that *Stryphnodendron rotundifolium* bark probably contains some active ingredients that could be developed for health problems as have been claimed by traditional popular use.

**KEY WORDS.** *Stryphnodendron rotundifolium*; ethanolic extract; antiulcer; antimicrobial activity.

## INTRODUCTION

Plants are potent biochemists and have been components of phytomedicine since times immemorial (1). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct (2).

The genus *Stryphnodendron* Mart., family Leguminosae, includes about 48 species, all native to central savannas of Brazil, including *Stryphnodendron obovatum* Benth. (3). The stem bark of several species of *Stryphnodendron,* which contains about 20% tannins, is used by the local population for wound healing and treatment of leukorrhea and diarrhoea (4). Tannin-rich plants are used in folk medicine because of their antimicrobial properties, and act as scavengers of free radicals (4).

*Stryphnodendron rotundifoloium* (Leguminoseae) popularly know as "Barbatimão" is a typical tree of Cariri Region, Ceará State, Brazil (5). Phytochemical analysis of the ethanol extract of stem bark of "barbatimão" allowed the identification and isolation of tannins and flavonoids. Their barks have been used in folk medicine as cicatrizant and for that reason your biological activity probably can be attributed to the high content of tannins (c.a. 40%) (6,7).

In view of the popular use of extracts of *Stryphnodendron rotundifoloium,* the present study was undertaken to investigate the effect of the ethanolic extract of "barbatimão" stem bark (EESR) on ethanol-induced gastric ulcers and its antimicrobial activity.

## MATERIAL AND METHODS

### *Plant material*

Plant material of *S. rotundifolium* was collected in September 2005, in line D of the National Forest Araripe, Crato, Ceará State, Brazil. A voucher specimen (# 33621) is deposited at the Herbarium Prisco Bezerra, Universidade Federal do Ceará.

### *Ethanolic bark extract*

Dried and powdered stem bark of *S. rotundifolium* (1.7 Kg) was exhaustively extracted with EtOH at room temperature. The EtOH stem bark extract was dried under reduced pressure to yield (183 g).

### *Animals*

Experiments were performed in male Swiss mice (20 – 25 g) obtained from the Central Animal House of this University. They were housed at 22 ± 2° C under a 12 h light/12 h dark cycle and had free access to standard pellet diet (purina chow) and tap water. For experiments, the animals were deprived of food for 24

h but allowed free access to water. The experimental protocol was approved by the Animal Care and Use Committee of this University in accordance with the guidelines for Care and Use of Laboratory Animals.

***Gastric lesions induced by ethanol***

The method of Robert et al, 1979 (8) was followed. After 24 h food deprivation, groups of animals (n = 6) were given orally EESR (200 and 400 mg/kg) or vehicle (tap water 10 mL/kg, control). One hour after, each animal was given orally 0.2 mL of ethanol (96 %) and the animals were killed 30 min later, the stomachs were excised, opened along the greater curvature, rinsed with saline (0.9 %) and the mucosal lesion area ($mm^2$) was measured by planimetry with a transparent grid (1 $mm^2$ area) placed on the glandular mucosal surface (9) and was expressed in percentage (%) in relation to total area of stomach.

***Antimicrobial assay***

Experiments were done using know procedure (10). The paper disc-agar diffusion technique was used for the determination of the antimicrobial and antifungal activities of the EESR using the following microorganisms: American Type Culture Collection (ATCC) Gram positive: *Staphylococcus aureus* (ATCC 12692), *Staphylococcus epidermidis* (ATCC 12228) and Gram negative: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442) e *Shigella flexineri* (ATCC 12022) and *Aspergillus niger* (ATCC 16404). For bacteria the culture medium used was Mueller-Hilton agar and PDA (potato dextrose agar dextrose). After incubation for 24 h at 37 °C, plates were examined for detection of inhibition zone and measured in millimeters.

Ampicillin (50 μg), Amikacin (50 μg) and Ketoconazol (50 μg) was used as positive control. The minimal inhibitory concentration (MIC) was determined using 20 μL of EESR in concentrations 10 – 1000 μg / mL. Experiments were done in triplicate.

***Statistical analysis***

All values are expressed as the mean ± SEM. ANOVA and Student Newman Keul´s tests were used to verify the statistical significance of the differences between groups. Differences were considered to be significant when $p < 0.05$.

**RESULTS AND DISCUSSION**

The effect of EESR is presented in Figure 1. Vehicle-treated control mice showed extensive gastric mucosal lesions in the glandular segments only. EESR at the doses tested, significantly suppressed the gastric mucosal hemorrhagic erosions induced by ethanol. The results of this study showed that the stem bark extract of *Stryphnodendron rotundifolium* prevents gastric damage caused by ethanol, the most commonly utilized experimental model in the evaluation of anticulcer activity (8). Phytochemical analysis showed that barbatimão extract contains saponins, flavonoids and tannins, substances that have proven anticulcer activity (10, 11, 12). The mechanism underlying the protective action of barbatimão extract against ethanol-induced gastric mucosal lesions can be relationated to the presence of tannins and flavonoids which are known to possess antioxidant and cytoprotective properties (13).

Tannins are polyphenolic compounds, produced by a wide variety of plants, that can be separated into two structural groups. Hydrolysable tannins consist of a central polyol,

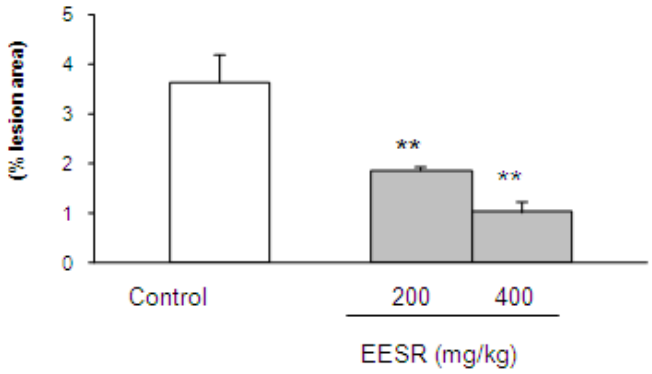


*Figure 1. Effect of EESR on the ethanol-induced gastric mucosal lesions. Each column represents the mean ± SEM (n=6/group). ANOVA, Student-Newman-Keul's test; **p < 0.01 compared x control)*

***Table 1. Antimicrobial activity of the ethanolic bark extract of S. rotundifolium*.***
**** Used concentration: 1000 μg/mL of extract; Ampicilin, Amicacin 50 μg;***

| Zone of inhibition (mm) | | | Control | | |
|---|---|---|---|---|---|
| Microrganisms | 24 h | 48 h | Ampicillin | Amikacin | Ketoconazol |
| *Escherichia coli* | 25 | 23 | 20 | 18 | - |
| *Staphylococcus aureus* | 20 | 20 | 18 | 15 | - |
| *Pseudomonas aeruginosa* | 17 | 15 | 20 | 20 | - |
| *Shigella flexineri* | 10 | - | 19 | 18 | - |
| *Staphylococcus epidermidis* | 18 | 15 | 18 | 15 | - |
| *Aspergillus niger* | - | - | - | - | 25 |

- No active.

***Table 2. Minimum inhibitory concentrations (μg/mL) of the ethanolic bark extract of S. rotundifolium *.***
****Value are the diameter of inhibitory zone (mm)***

| MIC (μg/mL) | | | | | Control | | |
|---|---|---|---|---|---|---|---|
| Microrganisms | 500 | 100 | 50 | 10 | Ampicillin | Amikacin | Ketoconazol |
| *Escherichia coli* | 20 | 13 | 9 | 6 | + | + | - |
| *Staphylococcus aureus* | 13 | 10 | 6 | - | + | + | - |
| *Pseudomonas aeruginosa* | 11 | 8 | 6 | - | + | + | - |
| *Shigella flexineri* | 6 | - | - | - | + | + | - |
| *Staphylococcus epidermidis* | 9 | - | - | - | + | + | - |
| *Aspergillus niger* | | | | | - | - | + |

- No active. ; + Active.

e.g., glucose, surrounded by several gallic acid units (14). Their antimicrobial activity has been the focus of research in many fields: food science, wood science, soil science, plant pathology, pharmacology, and human and animal nutrition (15). The mechanism(s) by which tannins inhibit bacteria have not been clearly elucidated, but the biological activity of tannins is probably determined to a great extent by the molar content and spatial configuration of the *ortho*-phenolic hydroxyl groups (16).

All the bacterial species and the fungi specie were inhibited by EESR, as show in Table 1. The EESR showed a broad spectrum of activity against at the tested concentration of 10-500 μg/disc, as summarized in Table 2.

The antimicrobial and antifungal effects of ESSR may be relationated of the tannin content. The tannins are toxic to microorganism because they generate H(2)O(2) and cause oxidative stress (14). Other mechanisms proposed so far to explain tannin antimicrobial activity include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation (15).

In addition such results justify the traditional use of *Stryphnodendron rotundifolium*. Further it also supports some of the phytochemical and pharmacological investigation of this plant carried by many researchers. The results suggest that traditional folk medicine could be used as a guide in our continuing search for new natural products with potential medicinal properties.

**ACKNOWLEDGMENTS**

The authors are grateful to the Brazilian Agencies FUNCAP, CAPES, CNPq, for financial support

**REFERENCES**

1. Parekh J, Karathia N and Chanda S Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* L. bark *African Journal of Biomedical Research,* **9(1):** 53-56, 2006.
2. M. Wink. M *Introduction Biochemistry, role and biotechnology of secondary products*. In: M Wink, Ed, Biochemistry of Secondary Product Metabolism. CRC Press, Boca Raton,FL, 1-16, (1999).
3. A. Cronquist. *The evolution and classification of flowering plants*. 2nd.ed., New York: The New York Botanical Garden, 261-449, (1988).
4. S.C. Santos, J.C.P, Mello, J.C.P. Mello. In: C.M.O. Simões, E.P. Schenkel, G. Gosmann, J.C.P. Mello, L.A. Mentz, P.R. Petrovick. *Farmacognosia da planta ao medicamento*. 5.ed. Porto Alegre/Florianópolis: Editora da URFGS/UFSC, 615-656 (2004).
5. P. Correia. Dicionário das plantas úteis do Brasil e exóticas cultivadas, Imprensa nacional, Ministério da agricultura, Rio de Janeiro, vol. III, pp. 499-501, (1984).
6. F.J.A. Matos. *Introdução à Fitoquímica Experimental*, EDUFC, Fortaleza, (1988).
7. Ferreira, S. H., *Medicamentos a partir de Plantas Medicinais do Brasil*, Academia Brasileira de Ciências, (1998).
8. A. Robert, J.E. Nezamis, C. Lancaster. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced

by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. *Gastroenterology*, **77**: 433 – 443, (1979).

9. A. Robert, J.E. Nezamis, C. Lancaster. Mild irritants prevent gastric necrosis through adaptive cytoprotection mediated by protaglandisns. *American Journal of Physiology,* **245**, G113-G121, (1983).
10. A.W. Bauer, M.M. Kirby, J.C. Sherris. Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Pathol* , **45**: 493-496, (1966).
11. T. Morikawa, N. Li, A. Nagatomo, H. Matsuda, X. Li, M. Yoshikawa. Triterpene saponins with gastroprotective effects from tea seed (the seeds of *Camellia sinensis*). *Journal of Natural Products,* **69(2):** 185-190, (2006).
12. V.S. Rao, F.A. Santos, T.T. Sobreira, M.F. Souza, C.L. Melo, E.R. Silveira. Investigations on the gastroprotective and antidiarrhoeal properties of ternatin, a tetramethoxyflavone from *Egletes viscosa. Planta Medica***, 63(2):**146-149, (1997).
13. A.R. Campos, A.I. Barros, F.A. Santos, V.S. Rao. Guarana (*Paullinia cupana* Mart.) offers protection against gastric lesions induced by ethanol and indomethacin in rats. *Phytotherapy Research,* **17(10),** 1199-1202, (2003).
14. A.H. Smith, J.A. Imlay, R.I. Makie. Increasing the oxidative stress response allows Escherichia coli to overcome inhibitory effects of condensed tannins. *Appl Environ Microbiol*. **69(6):**3406-11, (2003).
15. A. Scalberto. A. Antimicrobial properties of tannins. *Phytochemistry* **30:**3875-3883, (1991).
16. P.D. Schofield, M. Mbugua and A.N. Pell. 2001. Analysis of condensed tannins: a review. *Anim. Feed Sci. Technol*. **91:**21-40, (2001).