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Artigo

Antiulcer effect of the pepper trees *Schinus terebinthifolius* Raddi (aroeira-da-praia) and *Myracrodruon urundeuva* Allemão, Anacardiaceae (aroeira-do-sertão)

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RESUMO: "Ação antiúlcera das aroeiras *Schinus terebinthifolius* Raddi (aroeira-da-praia) e *Myracrodruon urundeuva* Allemão, Anacardiaceae (aroeira-do-sertão)". Foram avaliados, em ratos e camundongos, os efeitos antiúlcera de duas plantas usadas popularmente no Brasil para o tratamento de "males gástricos": a *Schinus terebinthifolius* Raddi (aroeira-da-praia) e a *Myracrodruon urundeuva* Allemão (aroeira-do-sertão). Os decoctos de ambas as plantas apresentaram um marcante efeito protetor da mucosa gástrica contra as ulcerações induzidas por estresse de imobilização em baixa temperatura em ratos. Ambas as plantas apresentaram, ainda: elevação do pH, do volume do conteúdo gástrico, redução das hemorragias gástricas e do trânsito intestinal em camundongos, mesmo em doses tão reduzidas quanto 3,4 mg/kg (1/4 da dose utilizada pelo homem).

Unitermos: *Schinus terebenthifolius, Myracrodruon urundeuva,* pH, trânsito intestinal; úlcera gástrica; hemorragia gástrica.

ABSTRACT: *Schinus terebinthifolius* Raddi and the *Myracrodruon urundeuva* Allemão were evaluated in rats and mice for antiulcer effects, as these two plants are widely used in Brazil for gastric ulcer treatment. Extracts of the plants showed a marked protective effect against gastric ulcerations induced by immobilization stress at low temperature in rats. They also showed an increase in the pH and volume of the gastric contents, and reduction in gastric hemorrhage in rats, and decrease in intestinal transit in mice, even at the low doses of 3.4 mg/kg (1/4 of the dose used by humans).

Keywords: *Schinus terebenthifolius, Myracrodruon urundeuva,* pH, intestinal transit, gastric ulcer, gastric hemorrhage.

INTRODUCTION

In the last years, some Brazilian plants have been studied in order to determine their possible antiulcerogenic effect, such as *Maytenus ilicifolia* (Schrad) Planch. (Formigoni et al., 1991; Tabach & Oliveira, 2003), *Croton cajucara* Benth. (Brito et al., 1998; Albino de Almeida et al., 2003), *Turnera ulmifiolia* L. (Gracioso et al., 2002), *Quassia amara* L. (Toma et al., 2002), *Sedum* sp., *Bryophyllum calycinum* Salisb. and *Brassica oleracea* L. (Macaubas et al., 1988) and *Astronium urundeuva* (Allemão) Engl, currently named *Myracrodruon urundeuva* Allemão (Menezes et al., 1986; Rao et al., 1987).

The present work presents data on two plants (pepper trees) in popular use in Brazil for the treatment of "gastric maladies": *S. terebinthifolius* (aroeira-da-praia) and *M. urundeuva* (aroeira-do-sertão), utilizing the method of ulcer induction by immobilization stress at low temperature, and measure of pH and volume of gastric contents in rats.

S. terebinthifolius is a pepper tree with a wide geographic distribution, covering various continents, including the Americas, Africa and Oceania (Lorenzi, 1992). This plant is known by the popular name "aroeira-da-praia". In Brazil, in 1889, its medicinal properties, antidiarrheic and anti-inflammatory, were described by Peckolt (1939). Pio Correa (1926) reported *S. terebinthifolius* as having astringent, tonic, stimulant and antineuralgic effects. Little attention has been given to this species from a pharmacologic and phytochemical point of view, only some works on its antimicrobial (Martinez et al., 1996; Melo Jr et al., 2002; Lima et al. 2006) and mutagenic (Carvalho et al., 2003) effects. Phytochemical studies have demonstrated its composition of essential oils (Santos et al., 2000) and flavonoids (Heringer et al., 2004). To the best of our knowledge, there are no studies in the literature

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on the antiulcerogenic effects of *S. terebinthifolius*.

M. urundeuva, Anacardiaceae, is a pepper tree native to South America (Lorenzi & Matos 2002). Phytochemical studies conducted on this species have shown the presence of essential oils (Maia et al., 2002) and tannins (Cunha et al., 2004). The antiulcerogenic effects of alcoholic and aqueous extracts of *M. urundeuva* against experimental ulcers in rats were observed by Menezes et al., (1986, 1988), Rao et al., (1987) and more recently by Souza et al. (2007). Other studies have demonstrated antioxidant (Desmarchelier et al., 1999), antidiarrheic (Chaves et al., 1994), antiinflammatory and analgesic (Viana et al., 2003; Deharo et al., 2004) effects.

MATERIAL AND METHODS

Plant material

The collections and experiments described here were carried out in 1988 and 1989; portions of the data were presented in the form of abstracts in the X Symposium of Medicinal Plants of Brazil (Formigoni & Carlini, 1988). The samples of *S. terebinthifolius* (**S**) and *M. urundeuva* (**M**) were collected and identified by Dr. Abreu Matos of the Universidade Federal do Ceará, and vouchers were deposited in this university's herbarium.

Preparation of extracts

Based on popular recipes, 10 to 12 g of bark from the pepper tree are boiled in water and the decoction obtained, after filtration, is taken at a dose corresponding more or less to a tea cup (140-150 mL), which would correspond to approximately 2 mL per kg of body weight (for human of 70 kg). Volumes of 2 mL of the decoctions **S** and **M** were lyophilized, yielding respectively 17.6 mg and 13.8 mg of powder, which equals the human dose per kg of weight (dose C1).

Analysis of phenolic compounds

The phenolic compounds of the lyophilized extracts (P and S) were extracted according to Swain & Hillis (1959) and determined by the Folin-Denis method (AOAC, 1965). The characterization of gallic, ellagic and condensed tannins was performed according to Bate Smith (1972) and Swain & Hillis (1959), and the analyses were carried out in the Institute of Chemistry of USP by Dr. Eudes Velozo.

Animals

The animals used in the study were male Wistar rats (3-4 months of age), and male Swiss Albino mice (3-4 months of age), bred in the Department of Psychobiology in an animal facility with air conditioning (23±2 ºC), where they were housed in plastic cages with water and food provided *ad libitum.*

Ulcer induction by immobilization stress at low temperature

Rats received for 18 h as the only food a solution of condensed milk diluted in water (1:2). At the end of this period, they were treated with water (control group) or lyophilized extract. After 30 min, they were slightly anesthetized with sulfuric ether and wrapped with wire screen, at the same time that the hindlegs and forelegs were bound with adhesive tape, making it impossible for the animal to move. The rats were then placed in a cold room (4 °C) for two hours. Afterward, they were sacrificed to determine the presence or not of gastric ulcers, and to examine the gastric juice with regard to pH, volume and presence of blood.

Influence of route of administration

Initially, the effects of the lyophilized extracts administered by gavage or intraperitoneal injection (*i.p.*) were determined. Two groups of six rats each received by both routes, doses of 440 mg/kg of lyophilized extract **P** or 330 mg/kg of lyophilized extract **M**, which corresponds to a dose 25 times greater than that used by humans (C25).

Dose-effect relationship

The dose-effect relationship of the extracts **S** and **M** was determined utilizing nine groups of eight rats each that received *i.p*. respectively 0 (water), 3, 6, 12, 25, 50, 100, 200 and 300 mg/kg of lyophilized material of each plant. In this experiment, the lower doses were chosen because of the results obtained in an earlier study.

Evaluation of ulcerogenesis

The quantification of the ulcers was carried out with an entomologic magnifying glass (Nikon, Japan). After the rats were killed with excess ether, the stomachs were opened along the greater curvature, and the mucosa was washed gently with water and stretched out on a polycarbonate platform. The magnitude of ulceration was determined using an ulceration index, calculated by the number of ulcers and the size of each lesion, along with other parameters such as the presence of edema, petechiae and hemorrhage, according to Formigoni et al (1991).

Measure of volume and pH of gastric contents

The volume and pH of the gastric juice were performed in two experiments. In the first, three groups of six rats each, fasted for 18 h and without being submitted to ulcer induction, received by the *i.p.* route respectively

water and doses of 50 mg/kg of the extracts **S** and **M**, where they were sacrificed one hour later. In the second, another eighteen animals were treated by the *i.p*. route respectively with water and 50 mg/kg of the extracts **S** and **M**. They were then submitted to the method of immobilization at low temperature for two hours. Later, they were sacrificed by cervical dislocation and the stomachs tied off at the esophagus and at 2 cm below the pylorus, in order to facilitate the collection and measurement of the gastric contents in small assay tubes. Due to the small volume, pH was measured with indicator strips (Spezialindikator, pH 0-2.5; 2.5-4.0 and 4.0-7.0, E. Merck, Darmstadt). However, the pH of the total volume of gastric contents of the stomachs of each experimental group was measured using a pH meter (PH1400 - Incibras). It was not possible to measure the total volume of gastric juice of animals treated with water (control group) due to the small volume.

Measure of intestinal transit in mice

Groups of ten mice each, fasted for 24 h, received by the *i.p.* route various doses of the extracts **P** and **M**. Thirty minutes later, 0.35 mL of a suspension of 10% activated charcoal (Merck) prepared in a 5% solution of gum arabic, was introduced by gavage into the stomach of each animal. Fifteen minutes later, the animals were sacrificed by cervical dislocation, and the small intestine was removed; the total length was measured along with the distance up to the point of dislocation of the charcoal, where transit was expressed a percentage of distance (Turner, 1965; Carlini et al. 1986).

Statistical analyses

In the experiments where interval values were utilized (ulcer index, number of ulcers per rat, centimeters and pH), the data were grouped according to each treatment, utilizing as measure of central tendency the arithmetic mean and standard deviation. Analysis of variance (*F test*) was used for comparison among three or more groups followed by *Student's t-test*, two-tailed, when necessary. In experiments where ordinal measurements were used, the data were grouped in accordance with treatment utilizing the median as the measure of central tendency. In these cases, the *Kruskal-Wallis* test was utilized followed by the *Mann-Whitney* test for comparisons between each two groups. In the case of nominal measurements (with or without gastric hemorrhage), statistical significance was evaluated by the chi-squared test for independent samples. In all cases, the level of statistical significance was set at 5% ($p<0.05$).

RESULTS

Analysis of phenolic compounds

The lyophilized extract of *S. terebenthifolius* (**S**) showed 43.5% total phenols, of which 0.9% corresponded to gallic tannins. The lyophilized extract of *M. urundeuva* (**M**) showed 41.3% total phenols of which 1.0% was gallic tannins. Neither extract showed a reaction for condensed or ellagic tannins, where their UV-visible spectra indicated maximal absorbances in regions very different from those observed for condensed tannins.

Ulcer induction by stress due to immobilization at low temperature in rats.

Influence of administration route

Table 1 shows that both pepper tree extracts have a protective effect, statistically significant in relation to the control group. This held true for extracts given by gavage as well *i.p*., whether evaluated by an ulceration index or the number of ulcers/rat, where *S. terebinthifolius* (**S**) was slightly more active. Since it was found that the decoctions were active by both administration routes, the rest of the experiments were performed using only *i.p.* injection, because it was an easier form of administration.

Table 1. Protective effect of *Schinus terebenthifolius* (**S**) and *Myracrodruon urundeuva* (**M**), administered by oral or intraperitoneal route, on gastric ulcers induced in rats by the method of immobilization at low temperature (n=6).

Route	Drug	Dose* mg/kg	Ulceration index (X±sd)	Nº of ulcers per rat (X±sd)
	water	-	21.3±15.5	9.8±7.2
oral	S	440	4.6±2.5*	1.0±1.7*
	M	330	9.6±9.3*	3.8±4.6*
	water	-	12.2±8.8	4.5±4.3
i.p.	S	440	3.9±0.0*	0.0±0.0*
	M	330	3.3±1.3*	0.2±0.4*

Results are expressed as means±standard deviation. The doses represent 25 times (C25) that ingested by humans. *$p\leq 0.05$ in relation to the control group.

Dose-effect relationship

The results obtained with *S. terebinthifolius* (**S**) with regard to the ulceration index and number of ulcers/rat are displayed in Figure 1A. An antiulcer effect was seen starting at 50 mg/kg, where animals treated with this and higher doses showed no gastric lesions. On the other hand, the protective effect of *M. urundeuva* (**M**) (Figure 1B) showed an optimal dose range and decreased at higher doses. The bloody appearance of the gastric secretion was also checked; Table 2 shows that starting at 12 mg/kg, the gastric juice appeared clean with no traces of blood.

Table 2. Presence of bloody secretion in the stomachs of rats treated *i.p*. with various doses of lyophilized extract of *Schinus terebenthifolius* (**S**) and of *Myracrodruon urundeuva* (**M**) and submitted to ulcer induction by immobilization at low temperature (n=8).

Drug	Dose (mg/kg)	Nº and % of animals with bloody gastric secretion	
		N	%
Water	0	8	100.0
	3	6	75.0
	6	4	50.0
	12	0*	0.0
S	25	0*	0.0
	50	0*	0.0
	100	0*	0.0
	200	2*	25.0
	300	1*	12.5
Water	0	8	100.0
	3	7	82.5
	6	7	82.5
	12	3	37.5
M	25	2*	25.0
	50	0*	0.0
	100	0*	0.0
	200	1*	12.5
	300	0*	0.0

*$p \leq 0.05$ in relation to control group.

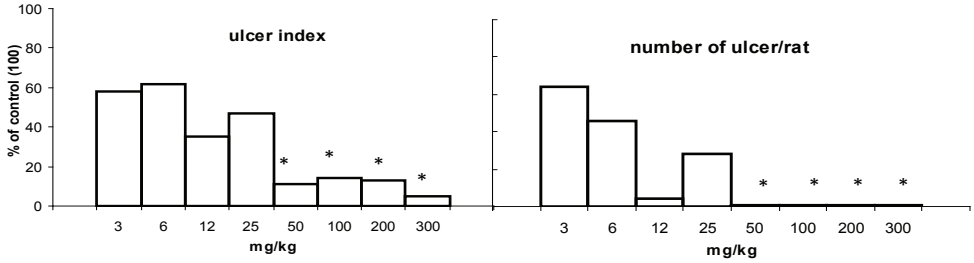
The dose-effect relationship of *M. urundeuva* (**M**) is shown in Figure 1B. Doses of 12 to 100 mg/kg exerted a significant protective effect with respect to the ulceration index; in relation to the number of ulcers/rat, the protective effect was observed up to a dose of 200 mg/kg, where at doses of 50 and 100 mg/kg no ulcers were detected in the stomach of the rats. Figure 1B also shows that at 50 mg/kg the protective effect of *M. urundeuva* begins to diminish.

Measure of volume and pH of gastric contents

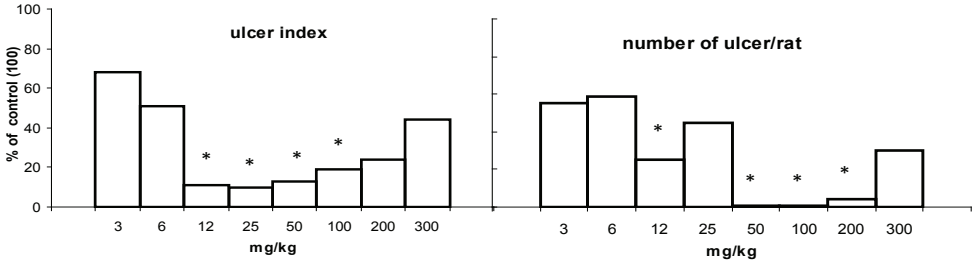
As can be seen in Table 3, only the treatment of the animals with the pepper tree extracts, without submitting them to immobilization + cold, was sufficient to increase the volume of the gastric juice, where *S. terebinthifolius* (**S**) caused a greater increase in volume in comparison to *M. urundeuva* (**M**). On the other hand, the extracts were also capable of increasing significantly the pH of gastric juice, in animals whether or not submitted to previous stress.

Intestinal transit in mice

Table 4 shows the results obtained with the extracts S and M given by either the oral or i.p. route. Oral administration of *S. terebinthifolius* extract (**S**) was shown be most active; at doses of 704 and 1408 mg/kg, corresponding respectively to 40 and 80 times the human dose, there was a significant, albeit small, decrease in transit. However, the *M. urundeuva* extract (**M**) was



Part **A** – *S. Terebenthifolius*



Part **B** – *M. urundeuva*

Figure 1. Antiulcer effect of extracts of *Schinus terebenthifolius* (Part A) and of *Myracrodruon urundeuva* (Part B), administered *i.p*. in rats submitted to immobilization stress and cold. The ulcer index and number of ulcer/rat for animals treated are expressed in percentages in relation to the values of the control group (100%; respectively 25.7 ± 10.1 and 12.0 ± 5.7). *$p \leq 0.05$ in relation to control group.

incapable of altering intestinal transit, even at a high dose of 1104 mg/kg, corresponding to 80 times the dose ingested by humans. However, both plant extracts were found to be much more active with *i.p*. administration. Thus, even at a dose corresponding to ¼ of the human dose (4.4 mg/kg for **S** and 3.4 mg/kg for **M**), there was a reduction of about 40-50% in intestinal transit, and at a dose corresponding to about half the human dose, the effect reached a plateau with a reduction of about 70%.

Table 3. pH and gastric volume of rats pre-treated i.p. with lyophilized extract of *Schinus terebenthifolius* (**S**) or *Myracrodruon urundeuva* (**M**) (n=6).

Method of ulcer induction	Extract	Dose (mg/kg)	Volume (mL)	pH
None	Water	-	0.00 (a)	1.71(a)
	S	50	0.59 (a)	5.92(a)
	M	50	0.26 (a)	5.94(a)
Immobilization	Water	-	0.64 (a)	1.60±0.07
	S	50	4.53 (a)	5.85±1.23(b)*
	M	50	5.0 (a)	6.57±1.19 (b)*

(a) Values obtained for total volume, pooling the gastric juice of all animals of each group. (b) Values obtained for the mean pH of the gastric juice of each specimen, using pH strips (Merck). *$p \leq$ 0.05 in relation to control group.

Table 4. Effects of lyophilized extract of *Schinus terebenthifolius* (**S**) and *Myracrodruon urundeuva* (**M**) on the intestinal transit of mice treated by the oral or intraperitoneal route (n=10).

Route of Administration	Extract	Dose (mg/kg)	Intestinal transit
oral	S	water	68.4±106
		342	57.7±17.5
		704	40.7±5.4*
		1408	36.2±10.4*
	M	water	68.4±10.6
		276	64.2±11.7
		552	52.0±15.5
		1104	52.0±9.0
i.p.	S	water	58.8±10.8
		4.4	35.6±14.1*
		8.8	22.6±7.5*
		17.6	17.4±8.8*
		176	31.1±3.4*
	M	water	58.8±10.8
		3.4	29.2±13.5*
		6.9	17.9±5.1*
		13.8	18.0±5.9*
		138	23.4±6.7*

* $p \leq$ 0.05 in relation to control group.

DISCUSSION

The results of the present work show that the lyophilized extracts of *S. terebinthifolius* (**S**) and *M. urundeuva* (**M**) possess a marked protective effect of the gastric mucosa in rats against ulcerations induced by immobilization stress at low temperature. The antiulcer effect was accompanied by an increase in volume and pH of the gastric juice, and by reduction in bleeding. Besides, both pepper trees reduced intestinal transit.

Literature reviews of plants with antiulcerogenic activity point out the actions of phenolic compounds (Borrelli & Izzo, 2000; Falcão et al., 2008). These actions are related to antioxidant and protective effects in the gastric mucosae, especially the tannins. The presence of high levels of tannins in our samples reinforces this notion. This result is also corroborated by studies of Viana et al. (1997) and Souza et al. (2007), carried out with *M. urundeuva.*

Souza et al. (2007) showed that extracts of *M. urundeuva* administered orally produced antiulcer activity utilizing the model of ulcer induction in rats with indomethacin and ethanol. In our study, this effect was observed with oral as well as *i.p*. administration. *M. urundeuva* was shown to be more active, since a dose of 12 mg/kg of lyophilized decoction (Figure 1B) conferred protection, whereas the protective effect of *S. terebinthifolius* was seen starting at 50 mg/kg (Figure 1A). However, *M. urundeuva* begins to lose its protective activity at 50 mg/kg, which does not occur with *S. terebinthifolius* at least up to a dose of 300 mg/kg (Figure 1B).

Menezes et al. (1986) described the properties of the ethanolic extract of *M. urundeuva* in protecting rats against ulcers induced by histamine or by the Shay method. The same group (Rao et al., 1987) also showed a protective effect in rats of extracts of *M. urundeuva* against ulcers induced by aspirin and by containment + histamine. Thus, our data corroborate the above findings, where we used another method, *i.e*., immobilization stress in the cold, demonstrating that the extract of *M. urundeuva* has protective activity against four different types of ulcers in rats (by histamine, by aspirin, using the Shay method and through immobilization at low temperature).

Our results also showed that the lyophilized extracts of both plants have a marked effect by increasing the pH and volume of the gastric juice, in addition to diminishing gastric hemorrhage. Menezes et al., (1986) also demonstrated the decrease in acid production in the stomach of rats submitted to the Shay method; however, these authors described a diminution of the volume of the gastric contents, data in contrast with our findings. The difference in methods used could explain this discrepancy in effects between the two groups.

The effects of pepper tree extracts on the digestive tract are not limited to protection at the level of the stomach. Rao et al. (1986) described a potent antiinflammatory effect

of the ethanolic extracts of *M. urundeuva* in experimental colitis induced with acetic acid in rats; Menezes and Rao (1988) demonstrated that the oral administration of the alcoholic extract of *M. urundeuva* did not affect intestinal transit in mice but significantly reduced gastrointestinal propulsion induced by physostigmine. Our results confirm and extend those findings, because although the aqueous extracts of both pepper tree extracts also showed little activity with oral administration at the highest doses, *i.p.* doses as low as 3.4 mg/kg (about ¼ the dose commonly used by humans), reduced transit markedly. On the other hand, based on indications that both pepper tree extracts had a certain potential for toxicity with acute as well as chronic administration, and that they can even induce skeletal malformations in the offspring of rats that are treated during pregnancy, it is recommended that these plants should not be used indiscriminately (Carlini and coworkers - manuscript in preparation).

In summary, the results of this work show for the first time the antiulcerogenic activity of *S. terebenthifolius* and reinforce the popular allegations that the decoctions of both *S. terebinthifolius* and *M. urundeuva* are useful for the treatment of gastric maladies.

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