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Antiulcerogenic, Antimicrobial and Antioxidant Effects of *Melampodium camphoratum*

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SUMMARY. ***Melampodium camphoratum*** **(L.f.) Baker (Asteraceae) is a vegetal species used in Brazilian folk medicine for the treatment of gastrointestinal disorders. The freeze-dried extract (LE) obtained from its aerial parts was investigated to determine its antiulcerogenic, antioxidant and antimicrobial activities, its acute toxicity and its phytochemical profile. Oral administration of the extract at a dose of 400 mg/kg body weight was effective in preventing acid/ethanol induced gastric ulceration in rats with an ulceration index of 26.2, compared to misoprostol (25.4) and control (183.3). An acute dose of 5000 mg/kg body weight caused no mortality to mice. The *in vitro* antioxidant potential for inhibition of lipid peroxidation in brain homogenate was confirmed with the determination of thiobarbituric acid-reactive substances (Q1/2 = 4.42 µg/ml). LE was active against *S. aureus, P. aeruginosa* and *A. niger* at a concentration of 1 mg/ml. Flavonoids and terpenoids were detected in the extract.**

RESUMEN. "Efecto Antiulcerogénico, Antimicrobiano y Antioxidante de *Melampodium camphoratum"*. *Melampodium camphoratum* (L.f.) Baker (Asteraceae) es una especie vegetal usada en la medicina popular brasileña para el tratamiento de problemas gastrointestinales. El extracto seco (LE) obtenido de su parte aérea fue investigado para determinar su actividad antiulcerogénica, antioxidante y antimicrobiana, como también su toxicidad aguda y su perfil fitoquímico. La administración oral del extracto a una dosis de 400 mg/peso corporal fue efectiva en evitar la ulceración gastrica de ácido/etanol en ratas con un índice de ulceración de 26,2 comparada al misoprostol (25, 4) y al control (183,3). Una dosis aguda de 5000 mg/peso corporal no causó la muerte del animal. El potencial antioxidante *in vitro* para inhibir la peroxidación de lipídos en muestra de homogenato de cerebro fue confirmado con la determinación de substancias reactivas al ácido tiobarbitúrico (Q1/2=4,42 µg/ml). LE fue activo contra *S. aureus, P. aeruginosa* y *A. niger* a una concentración de 1mg/ml. Flavonoides y terpenoides fueron detectados en el extracto.

INTRODUCTION

Melampodium camphoratum (L.f.) Baker (Asteraceae), popularly known as *"erva-de-São-João"* or *"São- João-caá",* is an Amazonian plant species used in Brazilian folk medicine for the treatment of liver and gastrointestinal disorders. No dose is described [1]. The aerial parts, prepared as a tea, are topically used in cases of leukorrhea. Despite its use in folk medicine, few scientific studies of *Melampodium camphoratum* have been reported. Phytochemical screening carried out on the aerial parts of this plant revealed the presence of essential oil [2], acacetin, apigenin and niveusin C [3]. Acacetin was shown to inhibit proliferation in human non-small cell lung cancer [4] and apigenin may protect against colorectal cancer [5].

The purpose of the present study was to validate the medicinal use of *Melampodium camphoratum* for gastric disturbances. The toxicity study was carried out in mice to assess the safety of the extract.

Material and methods

Plant material

The aerial parts of *Melampodium camphora-*

KEY WORDS: Antimicrobial, Antioxidant, Antiulcerogenic, Medicinal plant, *Melampodium camphoratum*.
PALABRAS CLAVE: Antioxidante, Antimicrobiano, Antiulcerogénico, *Melampodium camphoratum,* Planta medicinal.

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tum, as authenticated by Prof. Dr. Fenando de Oliveira, Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, were collected in Pará State, Brazil. A voucher specimen (Sm1) is deposited at Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil.

Preparation of the hydroethanolic extract

Fresh aerial parts were dried at 40-45 °C for 72 h with air circulation, and ground before use. The dried ground plant material was extracted with 70% ethanol by percolation at room temperature [6]. The solution was concentrated at 40 °C at low pressure, and freeze-dried. The yield of this extract, called LE, was 13% (w/w) for the dried plant material.

Phytochemical studies

The LE was tested for the presence of flavonoids, tannins, terpenoids, coumarins, saponins, steroids and alkaloids by staining tests and thin layer chromatography, using various solvent systems and spray reagents [7].

Animals

Wistar rats (160-180 g) and Albino Swiss mice (30-40 g) of both sexes were housed at Faculdade de Ciências Farmacêuticas da Universidade de São Paulo. The animals were acclimatized in the experimental rooms for at least 5 days before the experiment. The environmental conditions employed for the antiulcerogenic and acute toxicity assay were a temperature of 25 ± 1 °C and 85% relative humidity in a 12 h light/dark cycle. Food and water were available ad libitum. Food was removed twenty four hours (rats) and twelve hours (mice) before each experiment. The animals continued to have unlimited access to water.

Acute gastric ulcer induced by HCl/ethanol

The experiment was performed according to Mizui & Doteuchi [8]. Groups of ten rats received LE (400 mg/kg, suspended in 7.5% Tween 80), misoprostol (Biolab-Searly, 100 µg/kg, positive control) and 7.5% Tween 80 (vehicle) by intragastric route. Thirty min afterwards, ulceration was induced by oral administration of 1.0 ml of a 0.3 M solution of HCl in 60% (v/v) ethanol. One hour later, the rats were killed and the abdomen opened. The stomachs were excised, opened along the greater curvature and washed with saline. The ulceration area was then determined. The number of ulcers was counted for each animal and the ulcers were classified as level I for ulcer area smaller than 1 mm^2, level II for ulcer area between 1 and 3 mm^2, and level III for extensive hemorrhagic ulcers larger than 3 mm^2. The ulcerative index (UI) was calculated for each stomach as follows: UI = 1 x (number of level I ulcers) + 2 x (number of level II ulcers) + 3 x (number of level III ulcers).

In vitro lipoperoxidation

The assay was performed as described by Stocks *et al.* [9]. Adult male rats were anaesthetized and the brain reperfused through the heart with 140 mM phosphate buffer, pH 7.4. They were then decapitated and the brains quickly excised for homogenization in the same buffer (1:5 w/v). This homogenate was centrifuged at 3000 rpm for 15 min at 4 °C. The solutions were evaluated for lipid peroxidation during 1 h at 37 °C. The malonildialdehyde (MDA) formed after reaction with thiobarbituric acid was measured by spectrophotometry at 535 nm. The inverse of the levels of inhibition of the lipoperoxidation (CAOx) were plotted against the inverse of concentration of LE. Q1/2 is the concentration of the extract necessary for 50% inhibition of the lipoperoxidation process.

Antimicrobial activity

The microorganisms used for the assay were *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella choleraesuis* ATCC 10708, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. The bacterial strains were maintained in casein soy broth (Difco) at 30-35 °C, while the yeasts and molds were maintained in Saboraud dextrose broth (Difco) at 20-25 °C. The following antibiotics were used: ampicillin (Sigma) - 100 µg/ml for *E. coli* and 10 µg/ml for *S.aureus;* nistatin (Bristol-Mayer) - 350 UI/ml for *C. albicans* and 600 UI/ml for *A. niger*.

LE diluted in 50% ethanol/water was added to the tubes containing culture medium and the microorganisms. Final LE concentrations were 0.1, 0.5 and 1.0 mg/ml. The tubes were incubated for 24 h or until development of the microorganisms in the control tubes, at a temperature of 30-35 ºC for bacteria or 20-25 °C for mold. The assay was developed in triplicate. Subculture was done in the tubes that presented a precipitate but did not exhibit turbidity.

Acute toxicity

For acute toxicity studies, the mice were di-

vided into groups containing five males and five females each. LE was administrated by gavage at a single dose of 5000 mg/kg body weight. The control group received 7.5% Tween 80. The animals were carefully observed during 14 days in order to record toxic manifestations. Body weight, water and food consumption were measured. On the fourteenth day, the mice were killed. The organs were macroscopically observed and the relative weights (organ/body) determined [10].

Statistical analysis

Data are presented as the mean ± SEM. Statistical analysis was performed using one way analysis of variance (ANOVA - Tukey). In all instances $P<0.05$ was considered to be significant.

RESULTS

Phytochemical screening

LE showed positive reactions for flavonoids and terpenoids.

Antiulcer activity

The pretreatment with LE produced significantly lower average lesion area after induction of gastric ulceration with HCl/ethanol (Table 1).

Antioxidant activity

The antioxidant activity of LE is shown in Table 2. The values are the mean of three determinations. LE significantly reduced lipoperoxidation levels *in vitro* with Q1/2 = 4.42 µg/ml.

Antimicrobial activity

LE was active against *S. aureus, P. aerugi - nosa* and *A. niger* only at the concentration of 1 mg/ml.

Acute toxicity study

The oral administration of LE to mice at a dose of 5000 mg/ml showed no significant difference of relative body and organ weight between the control and the animals treated with the extract (Table 3). No difference in food and water consumption between the groups was observed. Macroscopic inspection indicated no alteration of the liver, heart, lungs or kidneys, compared to control.

DISCUSSION AND CONCLUSIONS

Administration of HCl/ethanol solution to the control group produced characteristic necrotic lesions. LE was found to be more potent than misoprostol at level II and III ulcerations (Table 1). Both LE and misoprostol were significantly different from the control. It is possible that prostaglandins are involved in the antiulcer activity, as this is the mechanism of action of misoprostol. Some studies have reported that oxygen free radicals have a role in the pathogenesis of ulcers [11]. LE showed a $Q_{1/2}$ value

		Number of Ulcers			
Treatment (p.o.)	**Dose**	**I (Light ulceration)**	**II (Moderate ulceration)**	**III (Severe ulceration)**	**Lesion index**
7.5% Tween 80 (vehicle)	1.0 ml/kg	4.9 ± 2.8	11.8 ± 2.3	51.6 ± 7.6	183.3
Misoprostol	0.1 mg/kg	13.1 ± 2.7	4.4 ± 0.7*	4.5 ± 1.6*	25.4
LE	400.0 mg/kg	8.9 ± 7.8	6.4 ± 3.3**	1.5 ± 1.3**	26.2

Table 1. Effect of *Melampodium camphoratum* hydroethanol extract (LE 400 mg/kg, p.o.), misoprostol (100 mg/kg, p.o.) and 7.5% Tween 80 (vehicle) on the incidence of acute gastric lesions induced by HCl/ethanol in rats. Data are presented as mean ± S.E.M. for 10 animals. * and **Significant difference from corresponding control group (vehicle) *P<0.05; **P<0.001.

*LE (mg/ml)	**LE (mg/ml x 10^{-3})	CAOx(%) ± SEM	1/LE	1/CAOX
1.0	16.6667	97.24 ± 1.39	1	0.0103
0.5	8.3334	75.65 ± 0.75	2	0.0132
0.25	4.1667	56.21 ± 2.02	4	0.0178
0.125	2.0834	32.70 ± 1.90	8	0.0306
0.0625	1.0417	9.72 ± 0.26	16	0.1028

Table 2. Antioxidant activity (CAOx%) of *Melampodium camphoratum* extract (LE) on spontaneous oxidation of rat brain homogenate. *Initial concentration. **Final concentration with reaction medium.

	Animal weight (g)	Relative weights x 10^{-2}		
		Liver	Heart and lungs	Kidneys
Male Control	40.03±3.02	6.61±0.15	1.11±0.01	1.60±0.05
Male LE	40.29±1.89	7.30±0.37	1.17±0.03	1.70±0.05
Female Control	31.38±0.93	6.17±0.19	1.32±0.06	1.30±0.03
Female LE	31.01±1.83	6.41±0.13	1.31±0.05	1.28±0.06

Table 3. Relative weight of different mice organs 14 days after oral administration of 5000 mg/kg *Melampodium camphoratum* extract. Each value represents the mean ± SEM of 5 animals.

(4.42 µg/ml) three times lower than that for α-tocoferol (12.10 µg/ml) and similar to that for *Pothomorphe umbellata* (4.4 µg/ml) root extract, which is known for its medicinal use [12]. The results suggest that the antioxidant activity could be responsible in part for the antiulcer action.

Rios *et al.* [13] suggest that a vegetable extract can be considered to have antimicrobial activity at a maximal concentration of 1 mg/ml. The same authors suggest that the antimicrobial activity verified in 5 Asteraceae species may be related to the presence of flavonoids and sesquiterpene lactones. In our study we detected these groups of substances in LE. They were also detected by Jacobs *et al.* [3] in the same species.

Antiulcerogenic, antioxidant and antimicrobial activity are not confined to one class of compounds in plants. Phytochemical studies of *Melampodium camphoratum* LE revealed the presence of flavonoids and terpenoids. Numerous studies relate that flavonoids and terpenoids have gastroprotective, antioxidant and antimicrobial activities [14,15,16,17]. It is possible that these two classes of substances are responsible for the pharmacological activities reported in this paper.

These results suggest that *Melampodium camphoratum* LE could be used as an antiulcer phytomedicine, clinical trials being necessary to confirm the activity in humans. Further studies are necessary to elucidate the active compounds and mechanisms of action.

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