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Evaluation of the anti-ulcerogenic activity of a dry extract of *Maytenus ilicifolia* Martius ex. Reiss produced by a jet spouted bed dryer

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We undertook the present study to evaluate the activity of the dried extract *of Maytenus ilicifolia* against stomach ulcers and in the increase of the volume and pH of the gastric juice of Wistar rats, as a model to evaluate the viability of the Jet Spouted Bed Dryer for the production of dry-extracts of medicinal Brazilian plants. The extract was obtained from the drying of a hydro-alcoholic extract with drying aid (40% of colloidal SiO_2 related to solid content in the concentrated extract, at a concentration of 15.82% in water). The drying conditions employed were: Temperature of the spouting gas 150 °C, feed flow-rate of hydro-alcoholic extract 16.0 g/min, feed flow rate of the spouting gas 1.67 m^3/min and static bed height 7.0 cm. Wistar rats received three different doses (140, 280 and 420 mg/kg) of the dried extract by intraperitoneal way and, after 60 minutes, were immobilized with wire screen and placed at temperature of 4 °C for two hours (cold-restraint stress). The animals were sacrificed and the stomach removed, examined and the volume and pH of the gastric secretion determined. A significant reduction in the ulceration index, was observed as well as a significant increase of the volume and of the pH of the gastric secretion for all doses administered. This is an indication that the preparation of dried extracts by the Jet spouted bed technique does not alter the biological activity of *Maytenus ilicifolia*.

1. Introduction

Maytenus ilicifolia Martius ex Reiss (Celastraceae) is a small shrubby evergreen tree native to many parts of South America and southern Brazil. In southern Brazil it is know by the population as Espinheira-Santa (PR, RS), cancorosa (PR), erva-cancrosa, cancerosa, erva-santa and espinheira divina. The Espinheira Santa (*Maytenus ilicifolia*) has a long and well-documented history of use in urban areas and in South American herbal medicine practices in particular the treatment of ulcers, indigestion, chronic gastritis, and dyspepsia. In Brazilian traditional medicine, Espinheira Santa is described as an excellent analgesic, disinfectant, tonic and cicatrizant and as the leading remedy for gastritis, ulcers and several other complaints [1].

The antiulcerogenic potential of a boiling water extract of equal parts of *Maytenus aquifolium* and *Maytenus ilicifolia* leaves against ulcer lesions induced by indomethacin and cold-restraint stress in rats was evaluated by Souza-Formigoni et al. [2], using ranitidine and cimetidine as reference drugs. The results showed that the oral and intraperitoneal administration of the extract of Espinheira Santa had a potent antiulcerogenic effect against both types of ulcers. The extract was shown to cause an increase in volume and pH of gastric juice of the animals with the pH effects comparable to those of cimetidine. Oliveira et al. [3], examined the effects of a boiling water extract of equal parts of *Maytenus aquifolium* and *Maytenus ilicifolia* leaves on acute administration in rats and mice, in an attempt to detect any general depressant, hypnotic, anticonvulsant and analgesic effects. General depressant and hypnotic effects were seen only after intraperitoneal administration. After chronic administration, the overall behaviour of animals did not change and they continued to gain weight at the same rate as controls. Several biochemical and haematological parameters as well as a pathological examination of different organs did not show any significant alterations after 3 months of treatment. A search for the potential effects of the extract on the fertility of female and male rats and on the course of pregnancy as well as a search for potential teratogenic effects did not reveal any significant differences from controls. Taken together, the results indicate that these *Maytenus* species may be safe for human use.

Currently, Bersani-Amado et al. [4], investigated the antiulcer activity of spray dried extracts of *Maytenus aquifolium*. These authors concluded that the spray-drying process did not alter the pharmacologic properties of the dried product.

Dry extracts, if adequately prepared, show numerous advantages over the conventional fluid form including higher stability and concentration of active substances, facility of standardization and quality control increasing the product

value. Different drying processes can be used for the production of dried extracts, such as freeze drying, vacuum band dryers, spray drying and, more recently, the conventional and jet spouted beds [4–7].
The spouted beds with inert particles have been extensively used for the drying of liquid materials such as pastes, solutions and suspensions, including chemical and biological products [8]. The suitability of this process for heat-sensitive materials is related to the short residence times of the material in the bed and also to the low bed annulus temperatures compared to the inlet air temperature [9]. This study was carried out to investigate the activity of the dried extract *of Maytenus ilicifolia* as a model to evaluate the viability of the Jet Spouted Bed Dryer for the production of dry-extracts of medicinal Brazilian plants.

2. Investigations, results and discussion

2.1. Characteristics of the hydro-alcoholic and dried extract

The solid content of the hydro-alcoholic extract with drying aid was 8.8%. The spectrophotometric analysis of the hydro-alcoholic extract with drying aids showed a total flavonoid content of 21.5 mg of total flavonoid/g of solid. The dried extract obtained under the drying conditions used had a total flavonoid concentration of 18.8 mg of total flavonoid/g of solid, moisture content less than 3% (dry base), and a mean particle size of 9.33 ± 4.8 μm. This clearly shows little degradation of the flavonoid compounds induced by the drying process (approximately 12.5%) and indicates the viability of the Jet spouted bed technique for the production of dry-extracts of *Maytenus ilicifolia* and of other medicinal Brazilian Plants.

2.2. Determination of the anti-ulcerogenic activity of the dry-extract

The pharmacologic results indicated that the administration of the dried extract of *Maytenus ilicifolia* had a protective effect against the experimental development of gastric ulcers in rats (Table 1 and 2). The percentage of reduction is similar and even higher than the results obtained with cimetidine [3, 4, 10].

Table 1: Effect of the dried extract of *Maytenus ilicifolia* against ulceration index induced by cold restraint stress in rats

Treatment	N	Way	Ulceration Index (x ± dp)	Reduction (%)
Control (water)	12	Ip	16.5 ± 3.2	–
Maytenus (280 mg/kg)	12	Ip	7.5 ± 3.1	54.5*

* $p < 0.05$ (significantly different from control group)

Table 2: Effect of the dried extract of *Maytenus ilicifolia* against ulceration index induced by cold restraint stress in rats

Treatment	N	Way	Ulceration Index (x ± dp)	Reduction (%)
Control (water)	12	Ip	20.6 ± 2.9	–
Maytenus (140 mg/kg)	6	Ip	12.3 ± 2.1	40.3*
Maytenus (420 mg/kg)	6	Ip	11.5 ± 3.5	44.4*

* $p < 0.05$ (significantly different from control group)

Table 3: Effect of different doses of dried extract of *Maytenus ilicifolia* in the volume of the gastric secretion

Treatment	N	Way	Gastric volume (ml) (x ± dp)	Increase (%)
Control (water)	12	Ip	0.06 ± 0.02	–
Maytenus (140 mg/kg)	12	Ip	0.70 ± 0.21	1167*
Maytenus (280 mg/kg)	6	Ip	0.88 ± 0.42	1467*
Maytenus (420 mg/kg)	6	Ip	0.55 ± 0.15	917*

* $p < 0.05$ (significantly different from control group)

Table 4: Effect of different doses of dried extract of *Maytenus ilicifolia* in the pH of the gastric secretion

Treatment	N	Way	Gastric secretion (ml) (x ± dp)	Increase (%)
Control (water)	12	Ip	2.16 ± 0.38	–
Maytenus (140 mg/kg)	12	Ip	3.70 ± 0.54	171*
Maytenus (280 mg/kg)	6	Ip	3.75 ± 0.74	173*
Maytenus (420 mg/kg)	6	Ip	4.08 ± 0.30	188*

* $p < 0.05$ (significantly different from control group)

2.3. Effect of the dried extract of Maytenus ilicifolia against the volume and pH of the gastric secretion

From the data presented in Table 3 and 4, it can concluded that the dried extract of *Maytenus ilicifolia* produced a significant increase ($p < 0.05$) in the volume and in the pH of the gastric secretion, at all doses administered.

3. Discussion

The study of the anti-ulcerogenic effect of a preparation of *Maytenus ilicifolia* obtained by a new production process showed promising results, permitting the systematic evaluation of the therapeutic efficacy of the developed product even in industrial scale.
The use of spouted beds for the production of dry extracts of medicinal plants is not very common, due to possible thermal degradation of the active compounds. However, in this work, spectrophotometric analysis indicated low variation in the concentration of the thermo-sensitive substances (total flavonoids), compared with the original composition in the concentrated crude extract.
In relation to the anti-ulcerogenic activity, the dry extracts of *Maytenus ilicifolia* produced by the "Jet Spouted Bed Process", protected the gastric mucosa in all doses administered. However, the anti-ulcerogenic activity of this preparation was not dose dependent. An explanation for this behaviour may be associated with the not yet completely explained pathogenesis of ulcer formation induced by immobilization and low temperatures. Additionally, stress has a high importance in the etiology of the gastric duodenal ulcers. Moreover, the integrity of gastric mucosa is related to a complex balance between aggressive and defensive factors. Disturbances in the gastric microcirculation [11], alteration in the gastric secretion [12], damages in the stomach musculature [13], mast cell degranulation [14], and release of several biogenic amines and a stimulus of the lipoperoxioxidation are some of the factors inducing lesions of the gastric mucosa [15]**.** According to Eryn et al. [16], the immobilization in low temperature results not only in the development of the gastric ulcers, but also produces important morphological and biochemical alterations in the liver as well, which can be prevented or, at least, attenuated by the administration of calcitonine.

The administration of the dried extract of *Maytenus ilicifolia* significantly increased volume and in the pH of the gastric fluid, corroborating previous results reported by Carlini [10] and by Oliveira et al. [3] with lyophilised extracts, and by Bersani-Amado et al. [4] using spray dried extracts. Therefore, it can be concluded that the production of dried extracts of *Maytenus ilicifolia* by jet spouted bed dryer does not alter its biological activity, indicating the viability of the process.

4. Experimental

4.1. Materials and equipment

Dry and powdered leaves of *Maytenus ilicifolia* Martius ex. Reiss, with a mean diameter of 0.3 mm, acquired from Elly Martins S.A. (Ribeirão Preto, SP, Brazil), and hydro-alcoholic solutions were used in the preparation of the crude extract.

The drying operations were carried out in a jet spouted bed consisting of a conical base with included angle of 38º, inlet orifice diameter of 60 mm and upper diameter of 340 mm. All parts were made of stainless steel. The Fig. shows a schematic diagram of this equipment. Teflon® beads with a concave cylindrical shape were used as inert material. The main physical properties of the inert particles are presented in Table 5. In Table 5, m_{p0} is the mean mass, d_{p0} is the mean diameter, ϱ_{p0} is the density, S is the specific surface and φ is the shape factor of the inert material. These properties were determined according to Oliveira [9].

4.2. Preparation of the dried extract

The extract was prepared following procedure presented in Cordeiro [7]. First, the dried and powdered leaves were placed in contact with a hydro-ethanolic solution at 66% and temperature of 60.2 °C, and maintained under agitation for 2 h in an extraction system, composed by a jacketed agitated vessel connected to a heating circulation bath. Next, the crude extracts were filtered through a vacuum filtration system using filter paper as the filter medium (grade 80 G). Then, the filtered product was concentrated approximately 3 times in a rotary evaporator at a vacuum pressure of 600 N/m^2 and at a temperature of 55 °C. The concentrated crude extract prepared in this way was submitted to drying in the jet spouted bed. The drying operation started with the introduction of a given load of inert material into the equipment in order to maintain the static bed height, H, at 7 cm. Spouting occurred by injecting air at the base of the bed. The feed flow rate of the spouting gas employed was of 1.52 m^3/min. With the establishment of the spout, the inlet air was heated to 150 °C. Then, the feeding of the concentrated extract, with a flow rate of 16.0 g/min, and of the atomizing air (15 l/min at 1.5 kgf/cm^2), were started. The drying aid, 40% (w/w) of colloidal silica (related to solid content in the concentrated crude extract), at a concentration of 15.82% in water, were added in order to improve the pharmaco-technical properties of the obtained product. Measurements of the outlet gas temperature were made at regular intervals in order to determine when the process reached the steady state (±5 minutes). Thereafter the samples of dried product used in the pharmacologic tests were withdrawn.

Table 5: Main physical properties of the inert material

$\bar{m}_{p0}$ (mg)	$\bar{d}_{p0}$ (mm)	$\bar{\varrho}_{p0}$ (g/cm^3)	S (cm^2/g)	φ (−)
183.0	5.45	2.16	5.27	0.96

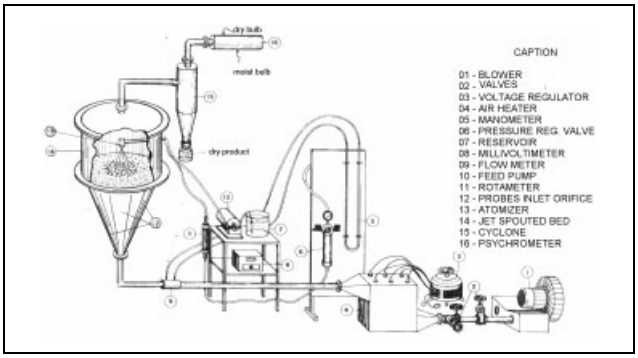

Fig.: Schematic Diagram of the Jet Spouted Bed Dryer

4.3. Determination of physical and chemical properties of the concentrated crude extract and of the dried product

Samples of the concentrated extracts were placed in an oven in order to determine the solid concentration and chemically analysed for the determination of the total flavonoid content. The procedure used for the quantification of total flavonoid content in the concentrated and dried extracts are based on the reaction between the flavonoids and aluminium chloride forming a complex with yellow colour that was measured in a spectrophotometer (HP8545 UV) at a wavelength of 420 nm. A complete description of this methodology is presented by Runha et al. [6].

The dry extracts were also characterized by the moisture content and by the particle size distribution. The moisture content was determined by the oven method. The product size distribution was determined by optical microscopy and image analysis. A powder sample was dispersed on a glass sheet. Images of the powder were obtained with the aid of an Olympus microscope (Model BX60MIV), connected to an analogue camera. The images obtained were analysed with an Image Analysis System [17].

4.4. Evaluation of the anti-ulcerogenic activity of the dried extract

4.4.1. Animals

Albino rats, males, Wistar, with age of 3–4 months, from Department of Psicobiology of UNIFESP – EPM, São Paulo, Brazil.

4.4.2. Ulcer induction

The rats were divided in four groups, A, B, C and D, being one the control (A) and three the experimental ones (B, C and D). Then, the rats were maintained for 24 h in individual cages with food privation. Six hours after starting the "fast" the animals received a solution of condensed milk diluted in water (1 : 2). After 24 h, water (control) or the following doses of the dried extract of *Maytenus ilicifolia* were administered intraperitoneally: 140 mg/kg (group B), 280 mg/kg (group C) and 420 mg/kg (group D). After 60 min, the rats were immobilized with wire screen and placed at a temperature of 4 °C for 2 h. Then, the animals were sacrificed and the stomach opened along the great curvature. The mucosa was removed, smoothly washed with water and stretched, with the aid of pins, on a polystyrene platform. Then the ulceration index was quantified, being attributed values to the gray discoloration of mucosa (1 point), loss of normal mucosa morphology (1 point), mucosal edema (1 point), hemorrhage, (1 point), petechiae (<10, 2 points; ≥10, 3 points) and number/size of the ulcers (number of ulcers until 1 mm × 2; larger than 1 mm × 3).

4.4.3. Volume and pH of gastric juice

The rats were divided in four groups, A, B, C and D, using the same procedure described above. After 24 h, water (control group) or different doses of the dried extract of *Maytenus ilicifolia* were administered intraperitoneally: 140 mg/kg (group B), 280 mg/kg (group C) and 420 mg/kg (group D). After 60 min the stomach was removed and, through a small incision, the gastric secretion was extracted and the volume and pH were measured.

4.5. Statistical analysis

The individual data were grouped following each treatment. The mean with the respective standard deviation was used as a measure of the central tendency, for the ulceration index as well as for the volume and for the pH of the gastric secretion. The results were analysed through a two-way analysis of variance, followed by the Ducan test.

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References

1 Martins, A. G.: Influência de fatores tecnológicos na avaliação analítica e farmacológica de extratos secos de *Maytenus ilicifolia* Martius ex Reiss, M. Sc. Thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil 1998
2 Souza-Formigoni, M. L. O.; Oliveira, M. G. M.; Monteiro, M. G.; Siveira Filho, N. G.; Braz, S.; Carlini, E. A.: J. Ethnopharmacol. **34**, 21 (1991)
3 Oliveira, M. G. M.; Monteiro, M. G.; Macaubas, C.; Barbosa, V. P. ; Carlini, E. A.: J. Ethnopharmacol. **34**, 29 (1991)
4 Bersani-Amado, C. A.; Massao, L. B.; Baggio, S. R., Johanson, l.; Albiero, A. L. M; Kimura, E.: Phytoter. Res. **14**, 543 (2000)
5 Senna E. I.; Petrovick P. R.; Ortega G. G.; Bassani V. L.: Phytother. Res. **11**, 123 (1997)
6 Runha, F. P.; Cordeiro, D. S.; Pereira, C. A. M.; Vilegas, J.; Oliveira, W. P. : Trans IchemE **79**, part C, 160 (2001)

7 Cordeiro, D. S.: Produção de extrato seco de *Maytenus ilicifolia* Martius ex Reiss pelo processo leito de jorro. MSc thesis. Faculdade de Ciências Farmacêuticas de Ribeirão Preto/USP, Ribeirão Preto, Brazil 2000
8 Passos, M. L.; Massarani, G.; Freire, J. T.; Mujumdar, A. S.: Drying Tech. **14**, 605 (1997)
9 Oliveira, W. P.: Estudo da secagem de pastas em leito de jorro cônico. Ph.D. Thesis PPG-EQ/UFSCar, São Carlos, Brazil 1996
10 Carlini, E. L. A.: Estudos da ação antiúlcera gástrica de plantas brasileiras: Maytenus ilicifolia (Espinheira Santa) e outras. CEME/AFIP, Brasilia, Brazil 1998
11 Murakami, M.; Lam, S. K.; Inada, M.; Miyake, Y.: Gastroenterology **88**, 141 (1985)
12 Hayase, M.; Takeuchi, K., Digest. Dis. Sci. **41**, 166 (1996)
13 Sairam, K.; Rao, C. H. V.; Babu, M. D.; Goel, R. K.: Phytomedicine **8**, 423 (2001)
14 Garg, G. P.; Cho, C. H.; Ogle, C. W.: Pharmacology **44**, 177 (1992)
15 Yegen, B. C.; Dedeoglu, A.; Aykac, I.; Oktay, S.; Yalcin, A. S. Pharmacol. Res. **22**, 45 (1990)
16 Eryn, N.; Okari, I.; Oktay, S.; Ercan, F.; Arbak, S.; Yegen, B. C.: Diges. Dis. Sci. **40**, 55 (1996)
17 Image Pro-Plus – Reference Guide, Medya Cybernetics, Georgia EUA, 1999