

Obtaining a Dry Extract from the *Mikania laevigata* Leaves with Potential for Antiulcer Activity

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ABSTRACT

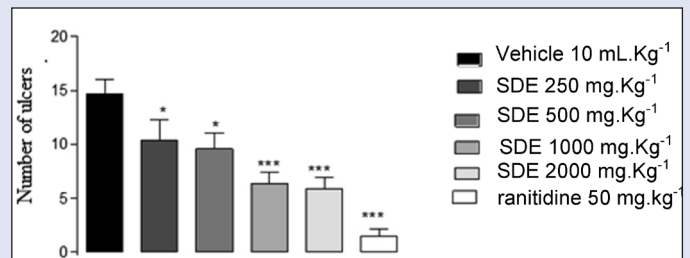
Background: *Mikania laevigata* leaves are commonly used in Brazil as a medicinal plant. **Objective:** To obtain hydroalcoholic dried extract by nebulization and evaluate its antiulcerogenic potential. **Materials and Methods:** Plant material and hydroalcoholic extract were processed and analyzed for their physicochemical characteristics. A method using HPLC was validated to quantify coumarin and o-coumaric acid. Hydroalcoholic extract was spray dried and the powder obtained was characterized in terms of its physicochemical parameters and potential for antiulcerogenic activity. **Results:** The analytical method proved to be selective, linear, precise, accurate, sensitive, and robust. *M. laevigata* spray dried extract was obtained using colloidal silicon dioxide as adjuvant and was shown to possess $1.83 \pm 0.004\%$ coumarin and $0.80 \pm 0.012\%$ o-coumaric acid. It showed significant antiulcer activity in a model of an indomethacin-induced gastric lesion in mice and also produced a gastroprotective effect. **Conclusion:** This dried extract from *M. laevigata* could be a promising intermediate phytopharmaceutical product.

Key words: Antiulcerogenic activity, medicinal plant, spray drying, quality control

SUMMARY

- Research and development of standardized dried extract of *Mikania laevigata* leaves obtained through spray drying and the production process was

monitored by the chemical profile, physicochemical properties and potential for anti-ulcerogenic activity.



Abbreviations used: DE: *M. laevigata* spray dried extract, HE: hydroalcoholic extract.

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INTRODUCTION

Mikania laevigata Sch.Bip. ex Baker is a plant used in medicine for the treatment of coughs, respiratory disorders, and other diseases.^[1] Phytochemical screening of *M. laevigata* has shown the presence of coumarins, terpenes, and organic acids,^[2,3] and also syringaldehyde.^[4]

Standardized herbal medicine, which has a known concentration of active principles,^[5,6] ensures active content uniformity in each dose and presents a longer period of stability.^[7] For that reason, the vegetal raw materials used nowadays for the production of phytomedicines are commonly presented as dried extract obtained through spray drying.^[8-14]

This is due to the adequate technological properties of dried products, their short drying time, and the high productive capacity^[12-14,16] of the technique. In this study, the spray drying technique was applied to process a hydroalcoholic extract from the *M. laevigata* leaves and coumarin and o-coumaric acid contents, physicochemical properties, and antiulcerogenic potential were evaluated.

MATERIALS AND METHODS

Plant material and characterization

The *M. laevigata* leaves were collected from the medicinal plant garden at the Faculty of Pharmacy (UFG) in Goiania, Goiás, Brazil (S16°40'33"

W49°14'39") and identified by Dr. Jose Realino de Paula of the Universidade Federal de Goiás (UFG).

The leaves were dried in an air-circulating stove (Solab Equipamentos para Laboratório Ltda, Piracicaba, SP, Brazil) at 40°C and crushed in a knife mill TE-625 (Tecnal Ltda, Piracicaba, SP, Brazil). The drug material was characterized by particle size analysis (Scanning Electron Microscopy JEOL, JSM-6610, equipped with EDS -Energy Dispersed Spectroscopy-, Thermo scientific NSS Spectral Imaging. Akishima, Japan), calculation of volatile content (moisture balance MB 35, Ohaus Inc., Pine Brook, NJ, USA), swelling index,^[17] and by the thin layer chromatographic (TLC)^[18] profile using coumarin and o-coumaric acid (Sigma, St. Louis, MO, USA) as reference substances.

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Validation of the method to quantify coumarin and o-coumaric acid by High-Performance Liquid Chromatography

Chromatographic analysis was performed using a Waters high-performance liquid chromatography (Milford, MA, USA), equipped with a diode array detector and chromatographic column XTerra RP18, 5 μ , 4.6 \times 150 mm (Waters).

A modified version of the method described by Santos *et al.*^[19] was used to quantify coumarin and o-coumaric acid. It consisted of a mobile phase of acetonitrile: acidified water (acetic acid, 0.01%, in ultrapure water; 15:85), flow rate of 1.2 mL/min, isocratic mode, oven temperature 30°C, injection volume of 20 μ L, and 275 nm wavelength.

System suitability parameters were evaluated^[20] and the following parameters were investigated in the validation study:^[21] selectivity, linearity, range, precision, accuracy, robustness, and limits of detection and quantification.

Preparation and characterization of the hydroalcoholic extract

The hydroalcoholic extract was obtained by percolating the *M. laevigata* leaf powder, using ethanol 80% (v/v; Chemis, Sao Paulo, SP, Brazil) as extractor solvent, and the process was monitored by TLC. Then the extract was concentrated by rotary evaporation (evaporator Buchi R220, Germany) at 40°C until reaching a 1:4 drug: solvent ratio and was characterized by measuring pH with a digital potentiometer PHS-3B (Labmeter, Copenhagen, Denmark), relative density, alcohol content, viscosity with a rheometer DV-III (Brookfield Engineering Laboratories Inc., (Middleboro, MA, USA), total solids content on a moisture balance MB 35 (Ohaus Inc., Pine Brook, NJ, USA), TLC, and determining coumarin and o-coumaric acid contents by HPLC (Waters high-performance liquid chromatography - Milford, MA, USA).

Preparation and characterization of the standardized dried extract

To obtain the dried extracts, a laboratory-scale spray dryer model MSD 1.0 (Labmaq do Brasil Ltda., Ribeirão Preto, SP, Brazil) with a concurrent flow regime was used. A drying pressure air spray of 60 psi, air flow sprinkler of 35 L/min, drying air flow of 1.0 m³/min, inlet temperature of 90°C, and diameter atomizing nozzle of 1.2 mm were used. The extract flow rates tested were 2 and 6 mL/min. Drying was performed without any drying adjuvants and with colloidal silicon dioxide at 15 and 30%.

The physicochemical parameters of the dried extract measured were the chemical profile using TLC and determination of coumarin and o-coumaric acid by HPLC. Process yield, volatile content, water activity (Testo 650, AG, Lenzkirch, Germany), and particle morphology (Scanning Electron Microscopy JEOL, JSM-6610, equipped with EDS -Energy Dispersed Spectroscopy-, Thermo scientific NSS Spectral Imaging. Akishima, Japan) were measured.

Antiulcerogenic activity of the standardized dry extract

Animals

The experiment, conducted with male Swiss mice (20–50 g), which were kept in controlled temperature and light conditions (light/dark cycles of 12 h) with food and water *ad libitum*, was carried out in accordance with current Brazilian College of Animal Experimentation (COBEA) requirements and approved by the Research Ethics Committee (no. 104/08) at the UFG.

Indomethacin-induced gastric lesions in mice

The animals were divided into six groups consisting of six to nine mice each. Standardized dry extracts (250, 500, 1000, and 2000 mg/kg body weight doses prepared by suspension in water and Tween 80), ranitidine (Antak - Glaxosmithkline; 50 mg/kg body weight) and vehicle (water and Tween 80, 10 mL/kg) were administered orally to the assigned groups of mice. One hour after treatment, indomethacin (Indocid - Merck Sharp and Dohme; 50 mg/kg body weight) was administered to all animals by subcutaneous injection. Then another treatment was administered by gavage with dry extract, ranitidine, and vehicle in the same doses as above. Three hours after the last treatment, the animals were sacrificed under ether anesthesia. Their stomachs were removed and opened along the lesser curvature, and then washed with a 0.9% sodium chloride solution. The number of ulcers and damage index were determined^[22] in terms of mucosal swelling, bleeding, ulceration intensity, and total number of ulcers and petechiae per cm² of gastric mucosa. The protective effect of the dry extract was compared with the results obtained from the vehicle group. The data are expressed as mean \pm SEM of six to nine animals per group and the statistical significance between groups was analyzed by one-way analysis of variance, followed by the Student–Newman–Keul's test. The differences between groups were regarded as significant at $P < 0.05$.^[23]

RESULTS AND DISCUSSION

Characterization of *M. laevigata* leaves

Particle size analysis showed that the material was classified as moderately thick powder, when almost all the particles passed through a 710 μ m mesh sieve and no more than 40% pass through a 250 μ m mesh.^[24] The use of moderately coarse powder is recommended because it prevents the compaction and formation of preferential channels during the percolation process.^[14,25] Volatile content at 9% was in accordance within the 8–14% limits.^[24] The swelling index was 3.2 in 80% ethanol, which indicated the presence of mucilage.

The chromatographic profile obtained for the plant drug shows bands of coumarin ($R_f = 0.68$) and o-coumaric acid ($R_f = 0.43$) considered *M. laevigata* markers,^[19,26] which suggests that the profile of this medicinal plant was adequate. The pharmacological effects of *M. laevigata* have been attributed to a possible synergistic interaction with coumarin and other components of the species.^[4,19,26]

Validation study

This analytical method for quantification of coumarin and o-coumaric acid by HPLC was validated and it proved to be selective, linear, precise, accurate, sensitive, and robust [Table 1]. HPLC showed that the content of coumarin in the plant drug was 0.72% (w/w) whereas that of o-coumaric acid was 0.13% (w/w) in relation to the dry basis.

Physicochemical characterization of hydroalcoholic extract

The following physicochemical parameters obtained for hydroalcoholic extract were pH 6.10, relative density 0.9256, viscosity 5.57 mPas, alcohol content 56%, and solids content 5.18%. It is essential to determine density and viscosity when planning the drying process if the spray nozzle is to be prevented from clogging. These also influence the droplet and particle size of the dried product. The solids content can significantly increase the yield of dry product to be obtained. Solid substance content in plant extracts is usually 15–40%.^[25]

The chromatographic profile of the hydroalcoholic extract obtained shows the bands of coumarin ($R_f = 0.68$) and o-coumaric acid ($R_f = 0.43$). Coumarin and o-coumaric acid levels found in hydroalcoholic extract were 4.34 and 0.76%, respectively. Thus, it can be suggested that the extraction process led to obtaining an extract in which the concentration of these substances is about six times greater than in the plant drug.

Physicochemical characterization of dried extract

Of all the drying techniques available, spray-drying is widely used for pharmaceuticals because of the adequate technological properties of dried products, short drying time, and high productive capacity.^[8-16]

The dried extracts obtained without using a drying adjuvant presented a pasty appearance and blackened coloration in about 2 days, so drying adjuvants which decisively influence increased yields are required.^[27]

The parameters coumarin and o-coumaric acid content and process yield were decisive in standardizing the dried extract. As shown in Table 2, process yield values ranged from 30 to 51% and the worst yield was with 15% colloidal silicon dioxide, at an extract feed rate of 2 mL/min. The literature reports yields ranging from 25 to 50%.^[14,25] After drying the extract, the greatest coumarin content was 1.83% whereas that of o-coumaric acid was 0.82% [Table 2]. Additional information on process adequacy is provided by volatile content and water activity analyses, which are closely related to adequateness for pharmaceutical purposes.^[28] Volatile content and water activity are crucial parameters related to chemical and microbiological stability of the product.

The coumarin and o-coumaric acid contents in the plant drug were 0.72% (w/w) and 0.13% (w/w), respectively. Their levels in hydroalcoholic extract were 4.34% (w/w) and 0.76% (w/w), respectively. In the percolation step, the concentration of coumarin and o-coumaric acid was about six times

higher. It was found that the spray drying conditions used caused a loss of coumarin when compared with the hydroalcoholic extract. This was probably due to volatilization, based on the odor of vanilla during the drying process. Nevertheless, one of the advantages of obtaining dried extracts is the possibility of getting products with a higher content of active ingredients. The results obtained in this study were adequate because the final drying process allowed for concentrations of 2.5 times greater in coumarin and about six times greater in o-coumaric acid contents than in the plant drug.

Drying condition 1 [Table 2] was selected for the production of standardized dry *M. laevigata* extract because of the higher content of coumarin (1.83%) and considerable content of o-coumaric acid (0.80%). In the particle morphology assay, the micrographs of this dry extract showed a particle size ranging from 27 to 81.4 μm . Figure 1 shows an isolated particle with a spherical shape and porous surface similar to particles in other studies.^[9,13,29] This shape is a key feature when applying the spray dried extract to obtain intermediate pharmaceutical products.^[29]

Antiulcerogenic activity of the standardized dried extract

This pharmacological activity was carried out only to monitor the process of drying the extract. Therefore, the antiulcerogenic potential of dry extract obtained in experiment 1 was investigated [Table 2].

It was noted that the number of ulcers induced by indomethacin in mice treated with standardized *M. laevigata* dried extract (SDE), at doses of 250 ($n = 6$), 500 ($n = 7$), 1000 ($n = 8$), and 2000 mg/kg - v.o. ($n = 8$) was reduced by 29.53, 34.72, 56.54, and 59.95%, respectively, whereas the control group showed 14.66 ± 1.406 ($n = 6$). The number of ulcers in animals which received ranitidine 50 mg/kg - v.o. ($n = 9$) was reduced by 90.17%. All the doses of the extract used were significantly effective in reducing the number of ulcers induced by indomethacin [Figure 2].

The injury rate in mice treated with SDE at doses of 250 ($n = 9$), 500 ($n = 8$), 1000 ($n = 9$), and 2000 mg/kg - v.o. ($n = 8$) decreased by 35.38, 34.38, 47.96, and 60.40%, respectively, whereas the injury rate in the control group was $100\% = 21.14 \pm 1.71$, with $n = 7$. The animals which received ranitidine 50 mg/kg - v.o. ($n = 9$) had their lesions reduced by 75.30%. All doses of the extract used were significantly effective in reducing the injury rate [Figure 3]. However, colloidal silicon dioxide, used as a drying adjuvant, did not show any significant antiulcerogenic activity in this model. It was demonstrated that SDE obtained by spray drying presented gastroprotective potential.

Although the test carried out in this study is not enough to define the antiulcer mechanism of action of SDE, it can be suggested that the presence of coumarin and mucilage estimated by the swelling index contributed to the activity found and corroborates the literature.^[3,30] Biguete *et al.*^[3] showed that the crude hydroalcoholic 70% extract

Table 1: Validation of the method for quantification of coumarin and o-coumaric acid

| Validation parameters | Coumarin | o-Coumaric acid |
|---------------------------------|----------------------------|------------------------------|
| Selectivity | Absence of interference | Absence of interference |
| Linearity ($\mu\text{g/mL}$) | 5–160 $\mu\text{g/mL}$ | 1.5–40 $\mu\text{g/mL}$ |
| Regression equation | $y = 72526.9949x - 941.94$ | $y = 91667.2394x - 61568.10$ |
| Correlation coefficient (r) | 0.9998 | 0.9998 |
| Limit of quantification | 4.32 $\mu\text{g/mL}$ | 1.29 $\mu\text{g/mL}$ |
| Limit of detection | 1.29 $\mu\text{g/mL}$ | 0.38 $\mu\text{g/mL}$ |
| Precision – repeatability | 4.04% | 4.24% |
| Precision – intermediate | 3.61% | 4.31% |
| Robustness – wavelength | 0.2% | 0.5% |
| Robustness – flow rate | 1.6% | 1.3% |
| Accuracy – percentage | 93.92–103.48% | 95.03–101.99% |
| Recovery | | |

Table 2: *M. laevigata* spray-dried extract obtained

| Experiment | DA | DAP(%) | EFR (mL/min) | PY (%) | VC (%) | WA | CC (%) | oCC (%) |
|------------|---------------------------|--------|--------------|--------|-----------------|-------|------------------|------------------|
| 1 | Colloidal silicon dioxide | 15% | 6 | 51 | 4.59 \pm 0.11 | 0.306 | 1.83 \pm 0.004 | 0.80 \pm 0.012 |
| 2 | Colloidal silicon dioxide | 15% | 2 | 30 | 5.62 \pm 0.11 | 0.365 | 1.11 \pm 0.017 | 0.82 \pm 0.016 |
| 3 | Colloidal silicon dioxide | 30% | 2 | 51 | 4.20 \pm 0.16 | 0.308 | 1.30 \pm 0.044 | 0.80 \pm 0.034 |
| 4 | Colloidal silicon dioxide | 30% | 6 | 51 | 4.52 \pm 0.10 | 0.310 | 1.54 \pm 0.014 | 0.79 \pm 0.007 |

CC, coumarin content; DA, drying adjuvant; DAP%, drying adjuvant proportion; EFR, extract flow rate; oCC, o-coumaric acid content; PY, process yield; VC, volatile content; WA, water activity.

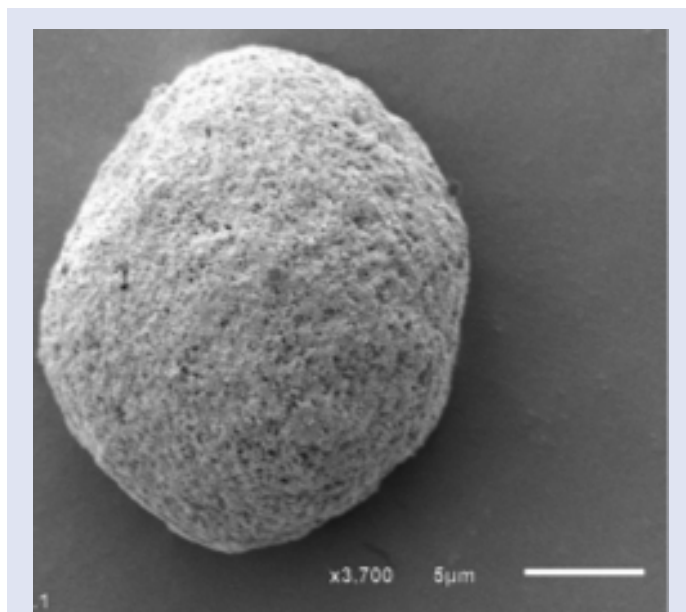


Figure 1: Scanning electron microscopy of *M. laevigata* dry extract with 15% colloidal silicon dioxide added.

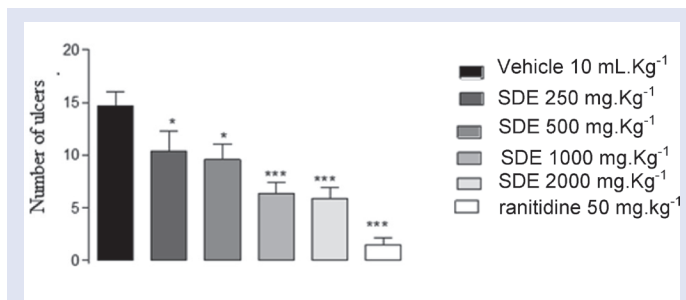


Figure 2: Indomethacin-induced ulcers in mice treated with *M. laevigata* dried extract. The vertical bars represent means \pm SEM. Posttest: Student–Newman–Keuls. *, Significant at $P < 0.05$; ***, Significant at $P < 0.001$.

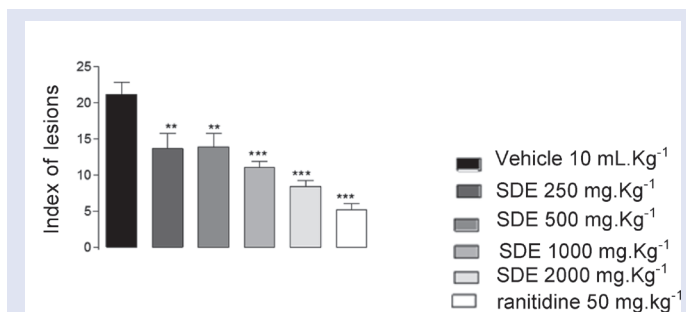


Figure 3: Index of indomethacin-induced lesions in mice treated with *M. laevigata* dried extract. The vertical bars represent means \pm SEM. Posttest: Student–Newman–Keuls. **, Significant at $P < 0.01$; ***, Significant at $P < 0.001$.

(CHE) of *M. laevigata* decreased the ulcerative lesion index produced by indomethacin, ethanol, stress, and reserpine in rats. In the pyloric ligation, model was observed a decrease of hydrogenionic concentration (53%), suggesting that the pharmacological mechanism has a relationship to antisecretory activity. The antisecretory mechanisms

of CHE and the coumarin isolated from *M. laevigata* were confirmed by acid hypersecretion induced models. Carvalho *et al.*^[30] showed that wound healing is a property commonly attributed to the mucilage and the use of *Pereskia aculeata* for this purpose was justified probably by providing energy and physical protection due to the mucilage presence, with no cytotoxic effects on fibroblasts. It should be emphasized the importance of mucilage to fibroblast growth and its presence plays a role as a growth factor and to stimulate the production of collagen and elastin by cells.^[31]

The ulcer protective potential of natural products has been investigated by other authors,^[11,32-34] and the results of this study which were promising in terms of protective potential corroborate the literature.

CONCLUSIONS

The spray drying process of hydroalcoholic extract from *M. laevigata* was suitable for maintaining the chemistry profile and antiulcerogenic activity described for the medicinal plant.

Pharmacological and toxicological studies should be performed in order to investigate the action mechanism of this extract and would thus contribute toward obtaining a safe and effective intermediate phytopharmaceutical product from *M. laevigata*.

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Conflicts of interest

There are no conflicts of interest

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