Coumarin Content and Physicochemical Profile of *Mikania laevigata* Extracts

Maique W. Biavatti*, Cesar A. Koerich, Carlos H. Henck, Enderson Zucatelli, Fernanda H. Martineli, Tania B. Bresolin, and Silvana N. Leite

Universidade do Vale do Itajaí (UNIVALI), Centro de Ciências da Saúde (CCS), Curso de Farmácia, CP 360, 88303-202, Itajaí, Santa Catarina (SC), Brazil. Fax: +55 47 3417 6600. E-mail: maique@ccs.univali.br

* Author for correspondence and reprint requests

Z. Naturforsch **59 c**, 197–200 (2004); received July 15/August 21, 2003

The 'guaco' lianous herb *Mikania laevigata*, which is widespread in Southern Brazil, is traditionally used to treat bronchitis, asthma and cough.

This work investigates the influence of the extraction method, solvent:drug ratio, ethanol proportion, harvest season (summer or winter) and solvent heating on the physicochemical profile of the extracts (dry weight, density, pH) and the coumarin (1,2-benzopyrone) content determined by LC. Among the results obtained, it is observed that higher ethanol content increases the amount of coumarin in the extract. Leaves harvested in summer also produce an extract with a high coumarin yield. The most efficient method of extraction is percolation, independent of the solvent used.

Key words: Mikania laevigata, Coumarin, Extract Analysis

Introduction

Mikania laevigata Sch. Bip. ex Baker (Asteraceae) and also a number of *Mikania* (*Globosae* section Robinson) are widely known in Brazil as 'guaco' species. The 'guaco' leaves are traditionally used as extract, syrup or infusion to treat bronchitis, asthma and cough (Oliveira *et al*., 1994).

The analogous species *M. glomerata* is considered the official drug in the Brazilian Pharmacopoeia (Farmacopeia Brasileira, 1929). The morphological similarity of *M. glomerata* and *M. laevigata* often leads to confusion between the species. In Southern Brazil, *M. laevigata* is more commonly harvested than *M. glomerata*, due to its local abundance (Moraes, 1997).

Phytochemical studies of the leaves of these species indicate a similar composition; both present coumarin (1,2-benzopyrone) in large yields, together with kaurenoic and grandifloric acid, compounds which are, at least partially, responsible for the pharmacological activity attributed to the 'guaco' extracts (Oliveira *et al*., 1984; Davino *et al*., 1989; Santos *et al*., 1999). *M. laevigata* can be considered a succedaneum of *M. glomerata* (Oliveira *et al*., 1994).

This paper describes the physicochemical analysis and LC determination of coumarin contents in fluid extracts of *M. laevigata* leaves harvested in winter and summer and prepared by different methods (maceration and percolation) using ethanol in different proportions (70, 60 and 36%) as solvent.

Results and Discussion

Quantitative analysis of coumarin

Fig. 1 presents the chromatographic profile of standard (coumarin) and the extract material. No interference of current components is observed in

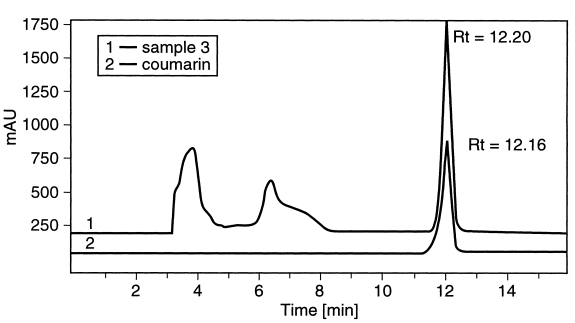

Fig. 1. Chromatograms of guaco extract 3 (1) and the standard coumarin (2). Mobile phase: 1:1 (v/v) acetonitrile/water. Coumarin retention time: 12.2 min. Extracts were diluted in MeOH (1:10, v/v), filtered over regenerated cellulose membrane [pore diameter 0.45 μm (Schleicher & Schuell, Dassel, Germany)] and injected (20 μl). All extracts were analysed in triplicate.

the elution window corresponding to this target compound.

The calibration curve using the standard coumarin showed a good linearity of the detector over the tested range (25–350 μg/ml), as shown by the correlation coefficient of the regression line (R^2 = 0.997437, y = 357,884.4+ 168,093,397.3x). Also, the y-intercept of the curve is 5% less than the response obtained for the analyte at the target level of 100 μg/ml (data not shown) (Green, 1996).

In order to evaluate the accuracy of the method, a recovery experiment was carried out, spiking the standard coumarin in the appropriated diluted matrix extracts, taking into consideration the linearity of the method. Table I shows the results of the coumarin recovery. The recovery average indicated the accuracy of the method, which was 104.1%. In the target level (100 μg/ml) the recovery was 102.9%, which is close to the desirable 100 ± 2.0% (Green, 1996). Comparing the recovery experiments in the presence and absence of matrix, no interference of the extract matrix on the coumarin determination was observed, which demonstrates the specificity of the method. The coefficient variation was less than 2.0%. The limit of quantification (LQ) of the method was 6.9 μg/ml, demonstrating good sensitivity (ICH, 1996).

Table II shows the coumarin concentration in extracts of *Mikania laevigata*, determined by liquid chromatography and extracted by different methods, collected in summer (except extracts 2[a] and 3[a], which were collected in winter). All the extracts presented statistical differences when evaluated by the F test, with good reproducibility as observed by a coefficient of variation (CV) < 2%. The traditional Pharmacopoeia method (Farmacopeia Brasileira, 1929) yielded extract 1, which presented an excellent macroscopic aspect; sweet taste, brown color, and free of the resinous feature found in the extracts prepared with higher ethanol concentrations (extracts 2, 3, 4 and 6). In contrast, extract 1 included a lower percentage of coumarin than extracts 2 and 3. The most efficient method (which yielded higher coumarin concentration and lower CV) for extracting coumarin was used to obtain extract 5 (with similar organoleptic characteristics to extract 1), using ethanol 70% at 50 °C. The coumarin content in extract 5 was higher than

Table I. Recovery of standard coumarin added to *M. laevigata* extracts.

Coumarin added [μg/ml]	Coumarin found[a] [μg/ml] (mean ± SD)	Coefficient of variation (%)	Recovery (%)
50.0	52.01 ± 0.70	0.99	104.0
100.0	102.96 ± 0.67	0.55	102.9
200.0	210.88 ± 1.77	0.77	105.4
100.0[b]	103.76 ± 1.14	1.09	103.7
0.0[c]	18.23 ± 0.28	1.54	–

[a] Average of three determinations.
[b] In absence of matrix.
[c] Coumarin found in the matrix.

Table II. Coumarin concentration and dry weight of extracts 1–6.

Extract	Coumarin concentration [mg/ml] (mean ± SD)	Coefficient of variation[b] (%)	Dry weight (%, mean ± SD)
1	1.01 ± 0.016	1.55	7.73 ± 0.002
2	1.58 ± 0.003	0.19	7.25 ± 0.16
2[a]	1.07 ± 0.001	0.12	5.71 ± 0.02
3	1.77 ± 0.012	0.64	5.04 ± 0.002
3[a]	1.71 ± 0.011	0.64	4.20 ± 0.32
4	1.48 ± 0.017	1.62	5.09 ± 0.002
5	2.45 ± 0.001	0.05	5.94 ± 0.45
6	0.27 ± 0.003	1.19	2.64 ± 0.05

[a] Plant collected in winter; for details see experimental.
[b] The coefficient of variation is a measure of relative dispersion and is given by the standard deviation divided by the mean, as a percentage.

that found by Celeghini *et al*. (2001) for leaves of the same species (0.696, 0.656 and 0.394 mg/ml for maceration, ultra-sonicated maceration and infusion, respectively). The amount of coumarin can vary drastically according to the extraction method used (Vilegas *et al*., 1997). A comparison of coumarin content between extracts 4 and 5 indicates, as expected, that maceration is less efficient than the percolation method. Extract 6 is a tincture extract, usually used for the production of 'guaco' syrup. The concentration of coumarin is very low in this form of pharmaceutical dosage, and may be neglected in favor of fluid extract.

As stated in previous papers on *M. glomerata*, the summer-harvested plant offers a high yield of coumarin (Celeghini *et al*., 2001; Pereira *et al*., 2000). Extracts 2^a and 3^a showed a lower coumarin content using the same extraction method like for the summer extracts, with a statistical difference.

Physicochemical analysis of extracts

In addition to the coumarin content of 'guaco' extracts, the physicochemical profile is also relevant for quality detection and the formulation of the extracts.

All the extracts analysed presented a statistical difference in the dry weight (%), with a CV of 10.6% between samples (Table II). In general, the lower the ethanol proportion, the more dry weight increased. This indicates a significant influence of the amount of water on the content of extractible substances (Aboy *et al*., 2000). This characteristic is mainly demonstrated in extract 1, which shows the highest dry weight. In addition to the lower coumarin content, winter extracts 2^a and 3^a presented lower dry weight in comparison with summer extracts 2 and 3, which demonstrates that it is advantageous to collect 'guaco' leaves in summer for 'guaco' extract preparation.

The relative density of summer extracts showed statistical differences, with a low CV (3.3%), while the winter extracts presented no differences for this parameter. Extract 5, with its high coumarin content, presented the highest relative density.

Some differences in pH were observed between the samples, with low variation (CV = 1.3%). However, all the values were within the range (pH 4.6 to 5.0) specified elsewhere (Aboy *et al*., 2000).

Other than the higher coumarin content in more ethanolic extracts (advantageous for more effective microbiological growth control in stored extracts), the lower ethanol content tested presented a satisfactory physicochemical profile. It may be interesting to develop a fluid extract that combines lower ethanol content with solvent heating, to generate an improved 'guaco' extract.

Experimental

Plant material

Leaves of *M. laevigata* were collected in mid January/July (2001) from cultivated specimens obtained from vegetative propagation of an authentic specimen of *M. laevigata*, identified by Pedro M. Magalhães (CPQBA – Universidade Estadual de Campinas), Ilhota, in the State of Santa Catarina, Brazil. Voucher specimens [M. Biavatti 30 (08/30/01)] were deposited at the Herbário Barbosa Rodrigues (HBR) in Itajaí, in the State of Santa Catarina, Brazil.

Extract preparation

The leaves (1 kg) were air dried (40 °C, forced ventilation, 3 d), powdered and sieved. Only particles between 0.5–1.0 mm were used. The extracts were prepared by percolation and maceration (British Pharmacopoeia, 2000) in concentrations of 1:1 and 1:10 (w/v) with ethanol 36%, 60% and 70% (v/v), heated to 50 °C or room temperature, resulting in six extracts as described below. These are numbered 1–6 for the samples harvested in summer and 2^a–3^a for the winter samples.

Extract 1: plant/solvent ratio 1:1, prepared by percolation with ethanol 36%; extract 2: plant/solvent ratio 1:1, prepared by percolation with ethanol 60%; extract 3: plant/solvent ratio 1:1, prepared by percolation with ethanol 70%; extract 4: plant/solvent ratio 1:1, prepared by maceration with ethanol 70% for 7 d; extract 5: plant/solvent ratio 1:1, prepared by maceration with ethanol 70% at 50 °C; extract 6: plant/solvent ratio 1:10, prepared by percolation with ethanol 70%.

LC apparatus and reagents

Liquid Chromatographic separations were performed using a Shimadzu LC-10AD (Tokyo, Japan) pump [0.5 ml/min flow, Luna RPC18 (5 μm), a (4.6 mm × 250 mm i.d.) Phenomenex column (Torrance, CA, USA)], a Shimadzu SPD-M10A photodiode array detector monitoring 274 nm and a Shimadzu CTO-10A column oven fit to 30 °C.

A Rheodyne manual injector (Rohnert Park, CA, USA) model 7725i was used to inject the sample. All reagents used were LC grade.

The standard coumarin was determined in plant material using the external standard method (Snyder *et al.*, 1997). Coumarin (1,2-benzopyrone, Sigma-Aldrich, St. Louis, MO, USA) was used as the external standard. This was dissolved in MeOH at a concentration of 500 μg/ml and diluted in triplicate to 25, 50, 100, 250 and 350 μg/ml. The software Shimadzu Class-VP 5.03 was used to fit the regression curve and to calculate the corresponding correlation coefficient.

The accuracy of the method was determined through an analyte recovery test (ICH, 1996), adding known standard concentrations from 50, 100 and 200 μg/ml to the matrix sample, appropriately diluted in triplicate, regarding the linearity of the method. The analyte recovery in the presence and absence of the extract matrix was compared, in order to analyse the specificity of the method.

Coumarin analysis

The extraction yield was determined by measuring the content of the extractive substances (soluble solids) in the extractive solutions. Samples of the extracts (20 g) were dried at 105 °C until they were at constant weight. The percentage of extractive substances in each extract was determined in triplicate.

The pH (alcoholic solutions electrode) and extract densities were evaluated according to the Brazilian Pharmacopoeia (Farmacopeia Brasileira, ed. 1988) procedure, in triplicate.

Acknowledgements

We wish to thank Dr. Pedro M. Magalhães for identifying the plant material, the Laboratory for Production and Analysis of Medicines (LAPAM-UNIVALI) for the LC measurements, the Center for Chemical-Pharmaceutical Investigations (NIQFAR), MEC-BNDS and UNIVALI for its financial support.

Aboy L. A., Ortega G. G., Petrovick R. P., Langeloh A., and Bassani V. L. (2000), Desenvolvimento tecnológico de soluções extrativas de *Mikania glomerata* Sprengel (Asteraceae), Guaco. Braz. J. Pharm. Sci. **36**, 165–172.

British Pharmacopoeia (2000), Vol. II. The Stationery Office, London, p. 1661.

Celeghini R. M. S., Vilegas J. H. Y., and Lanças F. M. (2001), Extraction and quantitative HPLC analysis of coumarin in hydroalcoholic extracts of *Mikania glomerata* Spreng. ('guaco') leaves. J. Braz. Chem. Soc. **12**, 706–709.

Davino S. C., Giesbrecht A. M., and Roque N. F. (1989), Antimicrobial activity of kaurenoic acid derivatives substituted on carbon-15. Braz. J. Med. Biol. Res. **22**, 1127–1129.

Farmacopeia Brasileira (1929), 1st ed. Companhia Editora Nacional, São Paulo.

Farmacopeia Brasileira (1988), 4th ed., Parte I. Atheneu, São Paulo.

Green M. J. (1996), A practical guide to analytical method validation. Anal. Chem. **68**, 305A.

ICH. Q2B (1996), Validation of Analytical Procedures: Methodology (http://www.fda.gov/cder/guidance/index.htm).

Moraes M. D. (1997), A Família Asteraceae na planície litorânea de Picinguaba – Município de Ubatuba. Master Thesis, Instituto de Biologia, Universidade Estadual de Campinas, São Paulo, Brazil.

Oliveira F., Alvarenga M. A., Akisue G., and Akisue M. K. (1984), Isolation and identification of chemical compounds of *Mikania glomerata* Sprengel and *Mikania laevigata* Schultz Bip. ex Baker. Rev. Farm. Bioquim. Univ. S. Paulo **20**, 169–183.

Oliveira F., Saito M. L., and Garcia L. O. (1994), Morfologia externa das partes aéreas e anatomia foliais das espécies brasileiras de *Mikania* Willdenow secção *Globosae* Robinson – visão farmacognóstica. Lecta **12**, 23–65.

Pereira A. M. S., Câmara F. L. A., Celeghini R. M. S., Vilegas J. H. Y., Lanças F. M., and França S. C. J. (2000), Seasonal variation in coumarin content of *Mikania glomerata*. Herbs Spices Med. Plants **7**, 1–10.

Santos P. A., Pereira A. M. S., França S. C., and Lopes N. P. (1999), Esteroides e cumarina em calos de *Mikania glomerata* Sprengel. Braz. J. Pharm. Sci **35**, 231–235.

Snyder L. R., Kirkland J. J., and Glajch J. L. (1997), Practical LC Method Development, 2nd ed. John Wiley & Sons, New York, pp. 655–656.

Vilegas J. H. Y., Marchi E., and Lanças F. M. (1997), Extraction of low-polarity compounds (with emphasis on coumarin and kaurenoic acid) from *Mikania glomerata* ('guaco') leaves. Phytochem. Anal. **8**, 266–270.