

A Comparative Chemical Study of *Maytenus ilicifolia* Mart. Reiss and *Maytenus robusta* Reiss (Celastraceae)

Rivaldo Niero^{a,*}, Renata Moser^a, Ana C. B. Busato^a, Rosendo A. Yunes^b, Ademir Reis^c and Valdir Cechinel Filho^a

^a Núcleo de Investigações Químico-Farmacêuticas (NIQFAR)/CCS, Universidade do Vale do Itajaí (UNIVALI), 88.302–202, Itajaí, SC, Brazil.

Fax: + 47–341–7601. E-mail: niero@mbbox1.univali.br

^b Departamentos de Química and

^c Botânica, Universidade Federal de Santa Catarina (UFSC), 88 040–900, Florianópolis, SC, Brazil

* Author for correspondence and reprint requests

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This work describes a comparative qualitative and quantitative chemical analysis of *Maytenus ilicifolia* and *Maytenus robusta* (Celastraceae), extracts by high-resolution gas chromatography (HRGC), using external standards as the method of determination and thin layer chromatographic (TLC). The results show that both plants have a similar chromatographic profile. However, *M. robusta* exhibited about three times higher concentration of triterpene friedelin than *M. ilicifolia*.

Introduction

M. ilicifolia is a plant belonging to the Celastraceae family which is native to the South of Brazil, being commonly known as “espinheira santa” or “cancerosa”. It is used in folk medicine in place of synthetic drugs as an anti-ulcerogenic agent (Carlini, 1988). Its chemical composition has been previously studied, showing the presence of triterpenes as the major components, as well as phenolic compounds (Oliveira *et al.* 1991; Souza-Formigoni *et al.*, 1991; Chavéz *et al.*, 1998; Itokawa *et al.*, 1994; Zhu *et al.*, 1998). Pharmacological studies have confirmed some important biological properties of this plant, such as cytotoxic and antibacterial activities (Pereira *et al.*, 1992; Corsino *et al.*, 1998; González *et al.*, 1998; Muhammad *et al.*, 2000; Kimura *et al.*, 2000). The most abundant compound in this species was identified as friedelin (**1**), which has been shown to be useful as marker for the characterization of authenticity of the crude drug plant (Vilegas *et al.*, 1994). Recently it was reported

a fast and simple method for identification of authentic and adulterated phytotherapeutics, using HPTLC-densitometry in samples of “espinheira santa” (Vilegas *et al.*, 1998). Considering that *M. ilicifolia* is presently at the extinction stage for indiscriminated use in Brasil, and that *M. robusta* has adapted very well in the South of Brazil, we report in this study a comparative analysis of the chemical composition of both species by high resolution gas chromatography (HRGC) and thin layer chromatography (TLC).

Material and Methods

Plant material

M. ilicifolia and *M. robusta* were collected in Morro do Baú Ecological Park, Ilhota, Santa Catarina, Brazil in October 1997, and identified by Dr. Ademir Reis (Department of Botany, Universidade Federal de Santa Catarina). Voucher specimens were deposited at Barbosa Rodrigues Herbarium (Itajaí – SC) under numbers V. C. Filho 015 for *M. ilicifolia* and V. C. Filho 016 for *M. robusta*.

Preparation of the samples

Dried aerial parts of these plants (100 g of each) were powdered and macerated with MeOH (50 ml) for seven days at room temperature. After evaporation of the solvent under reduced pressure, 5 g of each dry MeOH extract was suspended in 150 ml of water and successively partitioned with n-hexane and CHCl₃ affording 200 mg of the hexane fraction and 434 mg of the CHCl₃ fraction for *M. ilicifolia*, and 430 mg of the hexane fraction and 140 mg of the CHCl₃ fraction for *M. robusta*, respectively. An aliquot (10 mg) of each fraction was dissolved in 1 ml of chloroform and filtered (0.45 µm HVLP membrane) prior to analysis. All solvents were of analytical grade.

TLC analysis

The chromatographic profiles of the extracts were performed on 5x5 cm aluminum-backed silica gel 60 F₂₅₄ TLC plates, with several solvent systems. Spots were visualized by specific reagents according to the methods previously described (Marini-



Bettólo *et al.*, 1981; Block *et al.*, 1998). The specific spray reagents used included sulfuric anisaldehyde, iron (III) chloride and Dragendorff reagents.

HRGC analysis

HRGC separation was carried out using a Shimadzu model A-14 equipped with a denoted LM-1 column (25 m long, 0.25 mm i. d. with 0.33 mm liquid phase). The carrier gas was hydrogen at a flow rate of 2 ml/min which was kept constant. Aliquots of 1 μ l were injected using the split mode (split ratio 1:30), with detection using a flame ionization detector (FID). The temperature programme was increased from at 80°C to 280°C at 8°C/min, with a final isothermal of 10 min. The calibration curve was constructed using the conditions described above, with standard samples of triterpene friedelin (**1**), within the concentration range 0.5 – 1.0 mg/ml (Fig. 1).

Results and Discussion

All the experiments were performed with n-hexane and chloroform extracts, since both fractions

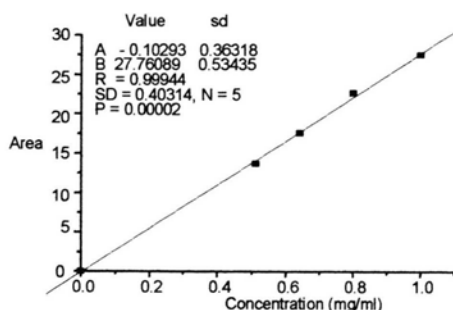


Fig. 1. Calibration curve (HRGC-FID) constructed using standard samples of friedelin within the concentration range 0.50 – 1.00 mg/ml (area in arbitrary units).

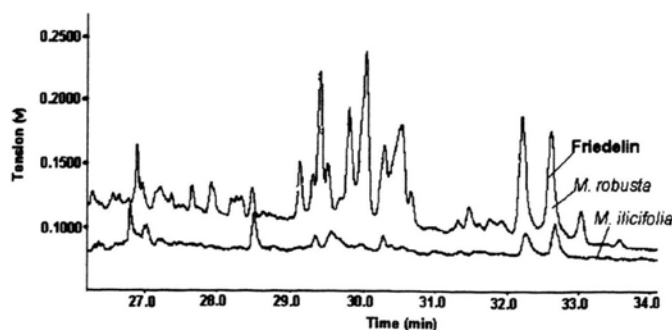


Fig. 2. HRGC-FID superposition profile (expanded) of hexane fractions of *M. ilicifolia* and *M. robusta*.

showed to be suitable for TLC and HRGC analysis. Comparative TLC of both extracts using several eluent systems with specific reagents demonstrated a similar chromatographic profile and high quantities of steroids, terpenoids and flavonoids (results not shown). However, when analyzed by HRGC, the chromatographic profile indicated a minor difference with respect to the n-hexane extract of *M. robusta* (Fig. 2) and chloroform extract (Fig. 3).

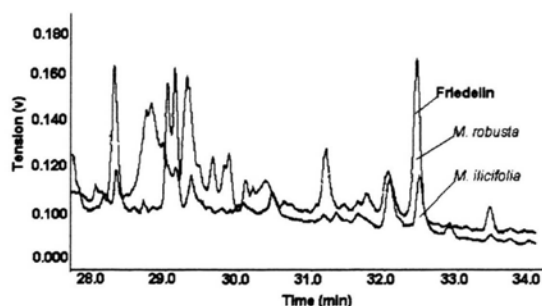


Fig. 3. HRGC-FID superposition profile (expanded) of chloroform fractions of *M. ilicifolia* (A) and *M. robusta* (B).

Considering that the triterpene friedelin (**1**) seems to be the main component responsible for antiulcerogenic action and gastritis of this plant (Pereira *et al.* 1992) we quantified it by HRGC. The quantitative analysis of compound (**1**) was performed using external calibration over a range of 0.50 – 1.0 mg/ml. The yield of (**1**) was determined as a function of the 100 g of dry plants of both species. The results indicated that the production of friedelin is about three times greater in *M. robusta* than in *M. ilicifolia* (Table I). Other terpenoids or steroids were detected by chromatographic methods, but the studies are currently in

Table I. Comparative analysis of friedelin (**1**) in aerial parts of *M. ilicifolia* and *M. robusta*.

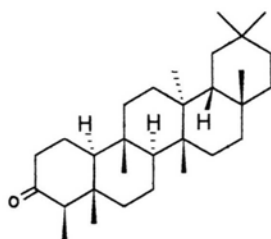
Fractions	<i>M. ilicifolia</i>		<i>M. robusta</i>	
	Hexane	Chloroform	Hexane	Chloroform
Retention time (min.)	32.67	32.67	32.63	32.63
Area	1.50	1.48	4.85	4.32
Weight of fraction Dry (mg)	0.058	0.057	0.18	0.16
Concentration (mg/10 g fraction)	200.0	434.0	430.0	140.0
Concentration (mg/100 g dry plants)	1.16	2.47	7.74	2.24

progress for their isolation, identification and pharmacological evaluation.

Although more investigations are required, our results suggest that *M. robusta* could be used in the phytotheraphic preparation instead of *M. ilicifolia*.

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