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Article

HRGC-MS Analysis of Terpenoids from *Maytenus ilicifolia* and *Maytenus aquifolium* ("Espinheira Santa")

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Neste trabalho é descrita a identificação de alguns constituintes químicos minoritários da "espinheira santa" (*Maytenus ilicifolia* e *Maytenus aquifolium*, Celastraceae), planta medicinal amplamente utilizada no Brasil. Através do acoplamento entre cromatografia gasosa de alta resolução e espectrometria de massas (HRGC-MS), foi possível identificar principalmente triterpenóides e esteróides presentes na "espinheira santa", vários dos quais relatados pela primeira vez em ambas as espécies de *Maytenus*.

This work describes the identification of some of the minor chemical constituents of "espinheira santa" (*Maytenus ilicifolia* and *Maytenus aquifolium*, Celastraceae), a medicinal plant widely utilized in Brazil. By using high resolution gas chromatography coupled to mass spectrometry (HRGC-MS), it was possible to identify mainly triterpenoids and steroids in "espinheira santa", most of them reported for the first time in both *Maytenus* species.

Keywords: Maytenus aquifolium *Martius*, Maytenus ilicifolia *Martius*, *gas chromatography-mass spectrometry (HRGC-MS), triterpene analysis*

Introduction

"Espinheira santa" is the popular name of two species, *Maytenus aquifolium* Martius and *Maytenus ilicifolia* Martius (Celastraceae). These medicinal plants are also known as "cancorosa", "cancerosa", "salva-vidas", "sombra de touro", "espinho de Deus" and others[1], and are found in Brazil, Chile, Uruguay, Paraguay and Bolivia. In Brazil their utilization is mainly in stomach ulcers and gastritis treatment, due to the presence of both triterpenes friedelan-3-ol and friedelin as well as polyphenolic compounds[2,3]. In Paraguay and Bolivia, they are utilized by indian tribes and rural populations as a fertility regulating agent[4], due to the alkaloid maytensine[5].

Previous work showed by HRGC-FID (high resolution gas chromatography - flame ionization detector) the predominance of friedeline and friedelan-3-ol in terpene fraction from *Maytenus ilicifolia* and *Maytenus aquifolium* leaves[2,3], which indicates that these compounds may hide minor terpenes in conventional phytochemical study (extraction of plant material followed by purification of each individual compound, for spectroscopic and spectrometric analysis).

In this work, the characterization of further triterpenes from the extracts obtained by direct extraction with ethyl acetate from these two *Maytenus* species was done by HRGC-MS (high resolution gas chromatography-mass spectrometry), by MS fragmentation data acquisition both by SCAN and SIM mode. In the first, the mass/charge ratio scanning was changed, and in the latter the characteristic terpenoid ions were monitored, followed by molecular ion selective monitoring.

Experimental

Plant material

Leaves of *Maytenus aquifolium* Martius and *M. ilicifolia* Martius (Celastraceae) were furnished by Ana Maria Soares Pereira, UNAERP, Ribeirão Preto, SP, Brazil. The leaves were dried (*ca.* 40 °C, forced ventilation, 3 days), ground and sieved. Only plant material between 0.5-1.0 mm was utilized.

Extraction of plant material

3 g of "espinheira santa" dried leaves were extracted with ethyl acetate during 15 min at 40 °C (30 mL, three times). After filtration, the acidic compounds were extracted with aqueous KOH 5% (10 mL, three times) followed by the extraction of the basic compounds with aqueous HCl 5% (10 mL, three times). The organic fraction,

which contained the neutral compounds, was washed with 30 mL of water and concentrated in a rotatory evaporator to 30 mL, being then centrifuged during 10 min at 6000 rpm, to remove the suspended particles. The solvent was evaporated to dryness, giving a residue, which was dissolved in $CHCl_3$ for GC-MS analysis. Figure 1 summarizes the procedure of the extraction process.

Chromatographic analysis

HRGC-MS analysis was performed on a Hewlett-Packard 5890 gas chromatograph, with a split injector (1:50) at 280 °C and a Hewlett-Packard 5970 mass selective detector (MSD), with the GC-MS interface temperature at 280 °C. The injection volume was 2 µL. Hydrogen was employed as carrier gas, at a pressure of 60 kPa. A HP-1 25 m x 0.25 mm x 0.33 µm methylpolysiloxane cross-linked capillary column was employed with temperature programming from 100 °C (held for 2 min) to 280 °C (held for 30 min) at a ratio of 4 °C/min.

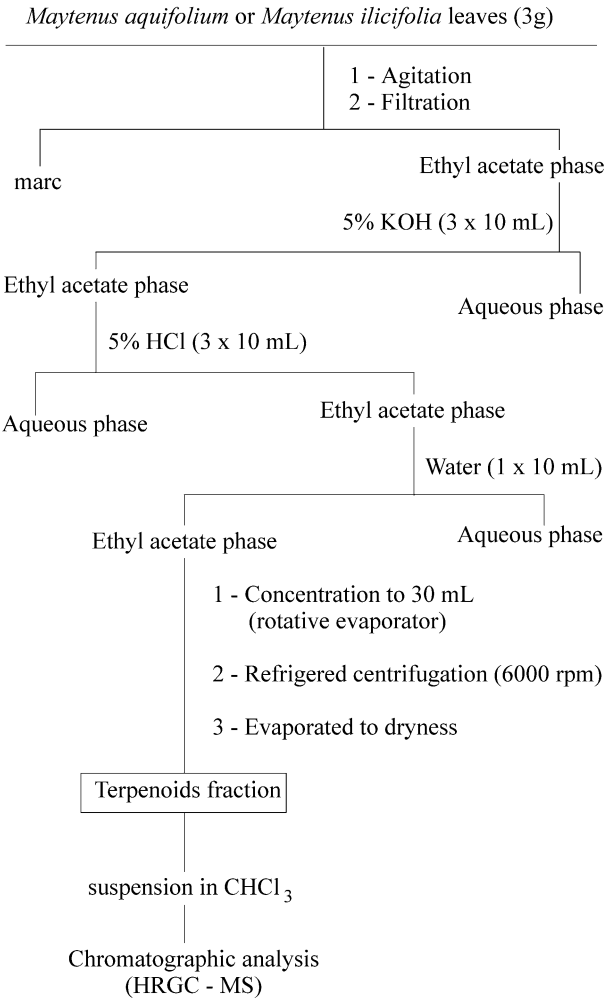

Figure 1. Extraction procedure of terpenoids of *Maytenus aquifolium* and *Maytenus ilicifolia.*

Results and Discussions

Figures 2 and 3 show the total ion chromatograms (TIC) of the leaves extracts obtained with ethyl acetate from *Maytenus aquifolium* and *Maytenus ilicifolia*, respectively. The chromatographic profile was similar for both species, differing only in the relative concentration of some compounds; this similarity had been observed in HRGC-FID analysis[2]. Most of the extracted compounds had retention times between 36 and 56 min and were identified as being terpenoids.

For the characterization of the compounds detected in TIC/HRGC-MS, a process which increased the confidence of the identification of compounds by the mass spectrometry was applied. Firstly, the fragmentogram obtained for each compound was compared with the fragmentation data base of the mass spectrometer, for obtaining a list of the 10 most probable substances (tentative identification). Sweep-

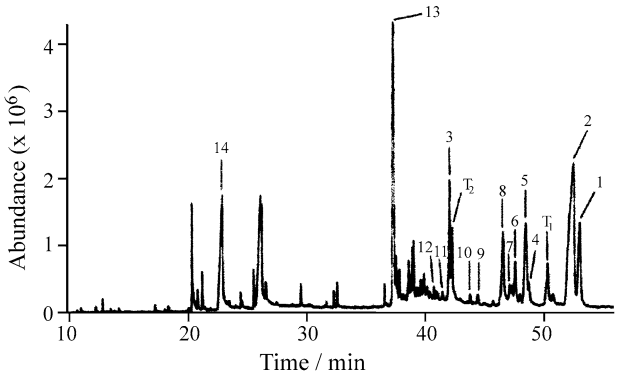

Figure 2. Total ion chromatogram (TIC-HRGC-MS) of the ethyl acetate extract obtained from *Maytenus aquifolium* leaves (peaks: 1- friedelin, 2- friedelan-3-ol, 3- α-tocopherol, 4- simiarenol, 5- lupeol, 6- lupenone, 7- β-amyrin, 8- β-sitosterol, 9- stigmasterol, 10- campesterol, 11- ergosterol, 12- brassicasterol, 13- squalene, 14- hexadecanoic acid, T_1 and T_2 - tocopherols. For chromatographic conditions, see Experimental.

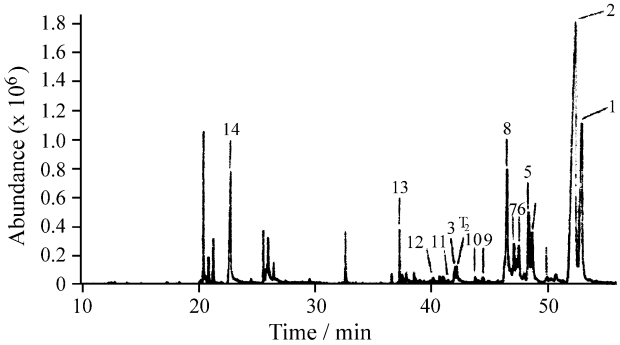

Figure 3. Total ion chromatogram (TIC-HRGC-MS) of the ethyl acetate extract obtained from *Maytenus ilicifolia* leaves (peaks: 1- friedelin, 2- friedelan-3-ol, 3- α-tocopherol, 4- simiarenol, 5- lupeol, 6- lupenone, 7- β-amyrin, 8- β-sitosterol, 9- stigmasterol, 10- campesterol, 11- ergosterol, 12- brassicasterol, 13- squalene, 14- hexadecanoic acid, T_1 and T_2 - tocopherols. For chromatographic conditions, see Experimental.

ing was done by using the SCAN mode, in which the values of m/z between 50 and 480 daltons were chosen. It was observed that, at the beginning of each probable fragmentogram, that the fragments of the compounds with t_R 40 to 56 min had the same fragmentation between 50 and 120 daltons, and that differences appeared only after 120 daltons.

The samples were analyzed again using the SCAN mode, but with the sweeping range set between m/z 120 and 550. In this new sweeping, several fragments that showed lower intensity (5 to 10% abundance) and have not been useful for comparation with standards library data, turned to 100% abundance, therefore participating in the comparison with NBS library (around 40,000 compounds). This provided alternatives for the more probable compounds, with a higher "match quality" score for the analyzed compounds.

The MS data of the compounds found in *Maytenus* samples were studied by comparison with the "Eight Peak Index" data[6]. This allowed the conclusion that, within the retention timespan from 40 to 50 min, most of the compounds belonged to the terpenoids class. Thereafter, the SIM mode (selective ion monitoring) was utilized for monitoring the terpenoids characteristic ions. Table 1 reports the monitored ions for some pentacyclic terpenoids, as indicated by Djerassis data concerning terpenoid fragmentations[7].

Table 1. List of characteristic ions of some triterpenoids, utilized for the SIM mode analysis of *Maytenus* extracts.

	ion									
m/z	189	205	218	223	313	383	411	424	426	468

Table 2. Data of standards utilized for identification of compounds in *Maytenus* extracts.

Compound	Molecular formula	Retention time (min)	Molecular ion (m/z)
Triterpenoids			
α-amyrin	$C_{30}H_{50}O$	47.41	426
β-amyrin	$C_{30}H_{50}O$	47.41	426
bauerenol acetate	$C_{32}H_{52}O_2$	54.72	468
lupeol	$C_{30}H_{50}O$	48.73	426
taraxerol	$C_{30}H_{48}O$	46.47	424
steroids; others			
campesterol	$C_{28}H_{48}O$	44.15	400
cholesterol	$C_{27}H_{46}O$	42.36	386
stigmasterol	$C_{29}H_{48}O$	45.00	412
β-sitosterol	$C_{29}H_{50}O$	46.97	414
α-tocopherol	$C_{29}H_{50}O_2$	42.42	430

For chromatographic conditions, see Experimental.

The suggested method was checked by the confirmation of the identity of some proposed structures through the injection of authentic standards. Table 2 gives the retention times and molecular ion obtained for those standards.

Combining chromatographic and MS data, the latter including search against terpenoids (2,515 compounds) and standards (9 compounds, listed at Table 2) libraries, 14 compounds were identified in both *Maytenus* extracts, including 7 substances which identity was also confirmed by direct comparison with standards: friedelin, friedelan-3-ol, α-tocopherol, simiarenol, lupeol, lupenone, β-amyrin, β-sitosterol, stigmasterol, campesterol, ergosterol, brassicasterol, squalene, and hexadecanoic acid. Two additional peaks were partially identified as tocopherol isomers (see captions of Figs. 2 and 3). The elution sequence of the triterpenoids was coherent with previous GC-MS data of these compounds in other plant extracts[2,8].

Conclusions

The utilization of mass spectrometry was effective for the identification of the minor terpenoids from *Maytenus ilicifolia* and *Maytenus aquifolium*. Most of these compounds are reported for the first time in these *Maytenus* species. Their low concentration in plant material and the similarity of skeletons justify the fact that these compounds were not previously reported in conventional phytochemical studies of *Maytenus ilicifolia* or *Maytenus aquifolium*[9].

The importance of the study of terpenoids is due to the biological activity of some of these compounds. In the case of "espinheira santa", the major triterpenoids friedelan-3-ol and friedelin were shown[9] to be related to activity against stomach ulcers and gastritis. The present study, which reveals the complexity of the terpenoidal fraction of "espinheira santa", suggest that the relative contribution of the minor components on the pharmacological activity should be evaluated.

Acknowledgments

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