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Artigo

Chemical composition and seasonal variation of the volatile oils from leaves of *Michelia champaca* L., Magnoliaceae

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RESUMO: "Composição química e variação sazonal dos óleos voláteis das folhas de *Michelia champaca* L., Magnoliaceae". Os óleos voláteis das folhas de *Michelia champaca* L., coletadas bimestralmente ao longo de um ano (quatro vezes no décimo quinto dia de janeiro, março, maio, julho, setembro e novembro de 2004), foram submetidos à análise por CG/DIC e CG-EM, de onde foram identificados treze componentes. Adicionalmente, parte do óleo obtido na coleta de janeiro foi submetida a fracionamento em gel de sílica impregnada com $AgNO_3$ fornecendo cinco dos principais sesquiterpenos (β-elemeno, β-cariofileno, α-humuleno, β-selineno e α-cadinol). Os dados obtidos mostram uma variação significativa na proporção dos componentes, a qual pode estar associada a parâmetros microclimáticos em cada período de coleta.

Unitermos: *Michelia champaca,* terpenoides, variação sazonal.

ABSTRACT: The volatile oils from leaves of *Michelia champaca* L. collected bimonthly during one year (four times on the fifteenth day of January, March, May, July, September, and November - 2004) were subjected to GC/FID and GC-MS analysis, from which thirteen components were identified. Additionally, part of the oil obtained from January collection was subjected to fractionation over silica gel soaked with $AgNO_3$ to afford five of the main sesquiterpenes (β-elemene, β-caryophyllene, α-humulene, β-selinene, and α-cadinol). The obtained data showed a significative variation in the proportions of the components, which could be associated to climatic parameters in each collection periods.

Keywords: *Michelia champaca,* terpenoids, seasonal variation.

INTRODUCTION

Michelia champaca L., Magnoliaceae, is a tree native in Taiwan, Malasya and China which is also cultivated in Brazil (Corrêa, 1984). This specie has been used in folk medicine to the treatment for rheumatism (Sharma & Mehta, 1998; Monteiro et al., 2007). Several phytochemical studies reported the occurrence of sesquiterpene lactones, alkaloids, flavonoids, tannins, and saponins in leaves, stems and roots of *M. champaca* (Monteiro et al., 2007; Jacobsson et al., 1995; Khan et al., 2002). The volatile oil from leaves of *M. champaca* was composed by several compounds, mainly benzyl acetate, linalool, isoeugenol and has been used as a starting material for perfums (Lai & Lee, 1994).

However, there is no information in the literature about the dynamics in the proportions of the components of the oil from leaves of Brazilian *M. champaca,* Magnoliaceae. Thus, in this report we describe the identification and the variation of the chemical constituents in these oils obtained bimonthly during one year (four collections at fifteenth day at January, March, May, July, September, and November 2004).

MATERIAL AND METHODS

Plant material

Leaves were collected bimonthly (fifteenth day, in four times during a day (8 am, 12 am, 4 pm, 8 pm), from January/2004 to November/2004 from a specimen which is growing in the center of São Paulo city (Mackenzie University Campus). Identification of the plant material was performed by Dr. Lucia Rossi. Voucher specimen was deposited in the Herbarium of Instituto Florestal, São Paulo-SP, Brazil.

Volatile oil isolation

Leaves of each collection (approximately 200 g) were subjected to hydrodistillation for four hours using a Clevenger-type apparatus. The oils were

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separated from water using CH_2Cl_2 as solvent, dried over anhydrous Na_2SO_4 and stored at 4 ºC in the dark. After solvent evaporation, the oil from *Michelia champaca* L. (Magnoliaceae) was submitted to analysis by GC/FID and GC-MS (Lago et al., 2003). The yield of crude volatile oils from *M. champaca* was approximately constant (0.04%) in all different seasons.

Volatile oil fractionation

Part of the crude oil from collection of January 2004 (approximately 150 mg) was submitted to column chromatography over silica gel soaked with $AgNO_3$ (15%) (50 g, 55 x 2 cm) eluted with pentane (100 mL), CH2Cl2 (150 mL) and mixtures of CH_2Cl_2:Me_2CO 9:1 (100 mL) and 8:2 (50 mL), to afford fifty four fractions, which were analyzed by GC/FID and pooled together in eleven groups (A-1 to A-11) (Brochini et al., 1999; Brochini & Lago, 2007). This analysis indicated that some groups were composed by one major derivative and had their NMR spectra recorded. Therefore, the compounds β-elemene (group A-3), β-caryophyllene (group A-4), α-humulene (group A-6), β-selinene (group A-7) and α-cadinol (group A-9) were identified after comparison of those reported in the literature (Adio et al., 2004; Kanokmedhakul et al., 2007; Rivero-Cruz et al., 2006; Momin et al., 2000).

GC

A Hewlet-Packard 5890 series II equipped with FID detector and a capillary column HP-5, crosslinked 5% phenyl in methyl silicone (30 m x 0.32 mm; film thickness 0.25 µm), an automatic injector (HP 7673) and electronic integrator (HP 3396A) were used. The temperature programming was performed from 50 ºC, isothermal from 2 min, 50 º-280 ºC at 5 ºC/min, then isothermal at 280 ºC for 5 min. The injector and detector temperatures were 180 ºC and 260 ºC, respectively. Helium was used as the carrier gas (1 $mL.min^{-1}$) and the split range was 1:50. Quantitative data were obtained from electronic integration of the area percent data without the use of internal standard or correction factors.

GC-MS

The GC-MS analysis were carried out in as EIMS 70 eV Hewlet-Packard HP-5973 (mass range from 40 to 350) coupled with a Hewlet-Packard HP-6890 with DB-5 column (30 m x 0.25 mm, film thickness 0.25 µm) using the same temperature programming conditions described above. The identification of the compounds was performed by comparing the mass spectra with those of authentic samples (Adams, 2001) and the retention indices, which were determined relatively to the retention time of a series of *n*-alkanes.

NMR

NMR spectra were recorded on a Bruker DPX-300 spectrometer operating at 75 MHz to ^{13}C and 300 MHz to ^{1}H, using $CDCl_3$ (Tedia Brazil) as solvent and TMS as internal standard.

RESULTS AND DISCUSSION

The present article describes the chemical composition and variation on the relative amounts of constituents of the volatile oils from leaves of *Michelia champaca* L. (Magnoliaceae), which was carried out on six bimonthly collections over the period from January to November 2004 (four collections at fifteenth day). The essential oils were obtained by steam distillation and analyzed by GC-FID and GC-MS. The medium values of the relative amount of each compound identified in the crude oils are presented in Table I. Additionally, part of the oil obtained from leaves of January collection was subjected to fractionation over silica gel soaked with $AgNO_3$ (15%). Analysis of the fractions composed by one major derivative using GC-FID, ^{1}H and ^{13}C NMR spectroscopy allowed the identification of the sesquiterpenes β-elemene, β-caryophyllene, α-humulene, β-selinene, and α-cadinol.

In a qualitative point of view, the oils contained thirteen identified compounds, corresponding to one monoterpene (α-terpinolene), six sesquiterpene hydrocarbons (β-elemene, β-caryophyllene, α-humulene, β-selinene, α-selinene, and γ-cadinene), four oxygenated sesquiterpenes [(*E*)-nerolidol, α-cadinol, β-bisabolol, and (*Z,E*)-farnesol], and two aliphatic alcohols (pentadecanol and hexadecanol).

Quantitatively, the relative amount of hydrocarbon sesquiterpenes (β-elemene, β-caryophyllene, and α-humulene the main derivatives) was higher that the content of oxygenated derivatives during all collections (47-69%). Otherwise, the amount of oxygenated sesquiterpenes showed to be constant (5-7%) during January, March, May, July and December, increasing up to 21% in September. Despite of the occurrence of terpenoid derivatives as main derivatives, aliphatic C_{15} and C_{16} alcohols were also detected in all collection, being their relative amount constant during all year (14-17%).

Finally, it is important mentioned that the variations in the chemicals of the oils could be associated to microclimatic parameters such as precipitation, temperature or phenological state, which showed to be different in the collections of leaves from *M. champaca* and are known to affect the oil chemical compositions (Vallat et al., 2005; Lago et al., 2006).

Table 1. Relative percentage composition of the leaf oils of *Michelia champaca* (January/2004-November/2004).

Compounds	IK	January	March	May	July	September	November
α-terpinolene	1088	2.1±0.2	1.9±0.1	2.4±0.3	2.7±0.2	2.5±0.3	1.8±0.2
β-elemene*	1391	13.0±2.0	12.0±3.0	16.0±1.0	8.8±0.9	7.0±2.0	15.0±2.0
β-caryophyllene*	1418	21.0±4.0	23.0±3.0	19.6±0.9	25.0±5.0	16.0±4.0	24.0±3.0
α -humulene*	1454	11.1±0.9	10.0±2.0	13.6±0.6	10.0±2.0	9.4±0.9	14.0±1.0
β-selinene*	1485	8.0±1.0	8.2±0.7	7.8±0.4	8.1±0.6	8.2±0.7	9.2±0.9
α-selinene	1494	6.9±0.6	5.2±0.5	6.2±0.4	5.9±0.7	5.3±0.8	5.3±0.5
γ-cadinene	1513	1.42±0.07	0.95±0.07	1.2±0.1	1.6±0.2	1.31±0.08	1.56±0.06
(*E*)-nerolidol	1564	1.5±0.2	-	-	2.6±0.4	9.5±0.9	3.7±0.7
α-cadinol*	1653	1.3±0.7	2.5±0.5	-	-	7.3±0.7	-
β-bisabolol	1671	3.2±0.4	2.9±0.6	3.7±0.4	3.3±0.5	2.7±0.4	3.0±0.6
(*Z,E*)-farnesol	1697	1.06±0.01	-	0.98±0.02	-	1.29±0.07	-
pentadecanol	1778	7.2±0.9	7.5±0.8	8.9±0.8	8.0±2.0	9.0±2.0	7.2±0.8
hexadecanol	1879	6.4±0.3	7.1±0.7	8.0±3.0	5.8±0.9	8.2±0.7	6.9±0.7
Monoterpenes		2.1±0.2	1.9±0.1	2.4±0.3	2.7±0.2	2.5±0.3	1.8±0.2
Sesquiterpene hydrocarbons		61.0±4.0	59.0±3.0	64.0±1.0	59.0±5.0	47.0±4.0	69.0±3.0
Oxygenated sesquiterpenes		7.0±0.7	5.4±0.6	4.7±0.4	5.9±0.5	20.8±0.9	6.7±0.7
Other metabolites		13.6±0.9	14.6±0.8	17.0±3.0	14.0±2.0	17.0±2.0	14.1±0.8
TOTAL		84.0±4.0	81.0±3.0	88.0±3.0	82.0±5.0	87.0±4.0	92.0±3.0

*also identified by NMR after chromatographic separation.

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