CHEMICAL COMPOSITION OF VOLATILE OILS FROM LEAVES OF *Nectandra megapotamica* SPRENG. (LAURACEAE)

Paulete Romoff * e Marcelo J. P. Ferreira
Centro de Ciências e Humanidades, Universidade Presbiteriana Mackenzie, Rua da Consolação, 930, 01302-907 São Paulo - SP, Brasil
Ricardo Padilla, Daniela O. Toyama e Oriana A. Fávero
Centro de Ciências Biológicas e da Saúde, Universidade Presbiteriana Mackenzie, Rua da Consolação, 930, 01302-907 São Paulo - SP, Brasil
João Henrique G. Lago
Departamento de Ciências Exatas e da Terra, Universidade Federal de São Paulo, Rua Prof. Artur Riedel, 275, 09972-270 Diadema - SP, Brasil

The volatile oils from *Nectandra megapotamica* Spreng. leaves, collected in February and August of 2007 and at 7:00 and 12:00 h (samples A – D), were extracted by hydrodistillation and the chemical composition was analyzed by GC-FID and GC/MS. A total of nineteen compounds were identified with predominance of oxygenated sesquiterpenes, among them, α-bisabolol, was the main constituent (62.3–69.4 %). After chromatographic separation procedures, this compound was purified from crude oil and its structure was confirmed by analysis of NMR data. This paper describes for the first time the composition of the leaves volatile oil from *N. megapotamica*.

Keywords: *Nectandra megapotamica*; volatile oil composition; α-bisabolol.

INTRODUCTION

As current circumscribed, the Lauraceae family consist of 52 genera and about 2500-3000 species. This group is widely distributed in the tropical and subtropical regions of the planet, predominantly in Southeast Asia and in Brazil.[1] *Nectandra* genus is one of the most representatives of the family and some species have been used in folk medicine as antifungal, antidiarrheal, analgesic and antirheumatic agents. Several investigations have demonstrated the biological activity of certain species of this family, *e.g.*, antitumor activity of *N. rigida* (H.B.K.) Nees,[2] anti-inflammatory activity of *N. falcifolia* (Nees) Castiglioni ex Martinez C. & Piccinini[3] and antimalarial activity of *N. cuspidate* Nees & Mart. and *N. salicifolia* (H.B.K.) Nees.[4,5] Five species have been studied regarding the volatile oil composition: *N. falcifolia* (syn. *N. angustifolia*),[6] *N. coriácea* (Sw.) Griseb.,[7] *N. elaiophora* Barb. Rodr.,[8] and *N. salicina* C. K. Allen.[9]

Nectandra megapotamica Spreng., popularly known as "canela-lora" or "canela-preta", is a species found in Brazil and used in folk medicine as anti-rheumatic and analgesic.[10,11] Earlier phytochemical studies have shown the presence of indole alkaloids[10] as well as anti-inflammatory and antichagasic activities of lignoids.[12,13] Furthermore, recent chemical studies reported the isolation of four phenylpropanoids, one gallic acid derivative, one flavan and one sesquiterpene.[14]

The antitumoral, anti-inflammatory and antimicrobial activities of the volatile oil obtained from *N. megapotamica* also had been previously evaluated, but, the compounds presented in these samples were not reported.[15] Thus, in continuation of our studies about volatile oils from São Paulo city species,[16] in this work we report, at first time, the chemical composition of the volatile oils from *N. megapotamica* leaves collected in two distinct months at different hours of a day. Compounds were characterized by GC-FID and GC-MS. Additionally, the main constituent present in the volatile oil, the sesquiterpene α-bisabolol, through NMR analysis after chromatographic separation procedure.

*e-mail: romoff@mackenzie.br

EXPERIMENTAL

Plant material

Leaves of *N. megapotamica* were collected in São Paulo (São Paulo State, Brazil) in February 15th, 2007 and August 15th, 2007 at 7:00 a.m. and 12:00 a.m., totalizing 4 samples (Samples A – D). The botanical identification of the plant was made by Profa. Dra. Oriana A. Fávero and a voucher sample (No. IF-SPSF 39648) was deposited in the Herbarium of Florestal Institute of São Paulo, São Paulo, Brazil.

Volatile oil extraction

For each collection, the fresh leaves of *N. megapotamica* were submitted to hydrodestillation extraction in Clevenger apparatus during 4 h. The volatile oils were extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4, and filtered. After evaporation of solvent, the yield of each oil was calculated based in weight of fresh leaves.

Volatile oil analysis and identification of constituents

The volatile oils were analyzed by GC (Hewlett-Packard 5890 – series II) equipped with a FID-detector, using a HP-5 capillary column (30 m x 0.32 mm x 0.25 µm), an automatic injector (HP 7673) and an electronic integrator (HP 3396A). These analyses were done injecting 1.0 µL of a solution at 1.0 mg/mL of volatile oil in CH_2Cl_2 in a split mode (1:30). Injector and detector temperatures were set at 220 and 280 °C, respectively. The oven temperature was programmed from 60-280 °C at 3 °C/min, keeping 10 min at 280 °C in the end of analysis and helium was employed as carrier gas (1 mL/min) for all analyses. The percentage compositions of the oil samples were computed from the GC peak areas without using correction for response factors.

The sample was analyzed by GC-MS, using a Hewlett-Packard 6890 chromatograph and coupled with a Hewlett-Packard 5973 spectrometer operating at 70 eV, at the same conditions as described above. The identification of the individual compounds was performed by comparison of both Kovat's retention indexes (determined relati-

vely to the retention times of a series of n-alkanes) and mass spectra with those available in the system.[17]

Main component isolation and identification

The volatile oil (1.62 g) from *N. megapotamica* leaves (Sample C) was subjected to CC on silica gel eluted with CH_2Cl_2 (100 mL) and CH_2Cl_2/MeOH mixtures of increasing polarities [99:1 (100 mL), 98:2 (50 mL) and 9:1 (50 mL), respectively] to afford 44 fractions. These fractions were analyzed by TLC and pooled into four groups (G_1-G_4). From G_2 1.18 g of α-bisabolol was obtained with 99.7% purity (GC).

The ^{13}C and DEPT 135° NMR spectra of this compound were registered at 75 MHz into a Bruker spectrometer (DPX-300 model) using $CDCl_3$ as solvent.

RESULTS AND DISCUSSION

The yields, the chemical composition, and the relative percentages of constituents found in the volatile oils obtained from the samples A, B, C, and D of *N. megapotamica* leaves are shown in Table 1. The yields, calculated on basis of weight of fresh leaves, were determined as 0.11-0.18%, similar to the volatile oil obtained from fresh leaves of *N. salicina*.[9]

Table 1. Chemical composition yields of volatile oil from *Nectandra megapotamica* leaves in each collection period

Components	KI	Sample A	Sample B	Sample C	Sample D
α-Pinene	939	0.2	0.1	3.3	2.0
Camphene	953	-	-	0.3	0.2
β-Pinene	980	0.9	0.4	4.2	3.1
β-Myrcene	991	-	-	0.1	0.1
Limonene	1031	0.9	0.5	1.4	0.1
Safrole	1285	1.6	1.3	0.4	0.4
δ-Elemene	1339	16.2	8.2	22.6	22.5
β-Elemene	1391	-	1.6	-	-
γ-Elemene	1433	-	0.2	-	-
β-Farnesene	1458	0.1	0.2	-	-
Allo-aromadendrene	1461	0.2	0.4	-	-
Germacrene D	1480	0.3	-	-	0.1
Bicyclogermacrene	1494	0.8	0.9	-	-
α-Muurolene	1499	-	0.4	-	-
β-Bisabolene	1504	0.1	0.5	-	0.2
Elemol	1549	1.5	-	-	0.9
Nerolidol	1564	0.9	0.9	0.3	0.5
Spathulenol	1576	0.2	-	-	0.2
α–Bisabolol	1683	67.7	69.4	64.8	62.3
Monoterpenoids		2.0	1.0	9.3	5.5
Hydrocarbon sesquiterpenoids		17.7	12.4	22.6	22.8
Oxygenated sesquiterpenoids		70.3	70.3	65.1	63.9
Terpenoids		90.0	83.7	97.0	92.2
Phenylpropanoid		1.6	1.3	0.4	0.4
TOTAL		91.6	85.0	97.4	92.6
Yield / %		0.11	0.18	0.14	0.17

Sample A (February 7:00 a.m.), Sample B (February 12:00 a.m.), Sample C (August 7:00 a.m.), Sample D (August 12:00 a.m.).

The identification of volatile derivatives was carried out by automated interpretation of mass spectra of constituents in each analyzed oil and also by retention index. A total of nineteen derivatives – mono-terpenes, sesquiterpenes and phenylpropanoid – were characterized, corresponding to 85.0-97.4% of the identified derivatives.

The occurrence of phenylpropanoids in *N. megapotamica* was reported previously,[14] in which elemicin and isoelemicin were detected from stem bark. However, in the present work, only a reduced relative amount (1.3-1.6%) of one C_6C_3 derivative, safrole, was found in the volatile oil from leaves in February, being lower in August samples (0.4%).

Other eighteen components, among them mono and sesquiterpenes, were identified in the volatile oils corresponding to 83.7-97.0% of the samples (Table 1). In the August collections, five monoterpenes (α-pinene, camphene, β-pinene, β-myrcene and limonene) were detected corresponding to 5.5-9.3% of the volatile oils. Otherwise, in February collections only the monoterpenes α-pinene, β-pinene and limonene were identified corresponding to 1.0-2.0% of the oils. However, if a higher diversification in the skeleton of monoterpenes was detected in August in comparison to February collections, the opposite was observed to sesquiterpenes, since only seven C_{15} derivatives were identified in the samples C and D.

Regarding the percentage of sesquiterpenes, was observed a small variation in the ratio of these components (82.7-88.0%) being δ-elemene (8.2-22.6%) and α-bisabolol (62.3-69.4%) the main constituents in all samples. However, when comparing the content of hydrocarbon x oxygenated sesquiterpenes, there is a greater accumulation (70.3%) of oxygenated derivatives in both February samples A and B (7:00 and 12:00 a.m.) in comparison to August collections C and D (63.9-65.1%). In August, a decrease in the content of oxygenated derivatives is observed with consequent increase in total of sesquiterpene hydrocarbons. The highest percentage of non-oxygenated sesquiterpenes has not been accompanied by structural diversification once only δ-elemene, β-bisabolene, and germacrene D were detected in the samples. Comparatively, the volatile oils from other *Nectandra* species showed to be composed mainly by monoterpenes [*N. falcifolia*[6] – mentha-1(7),8-diene (25.2%), α-terpinolene (20.9%), and α-pinene (10.0%); *N. elaiophora*[8] – α-pinene (35%), β-pinene (20%), and α-terpineol (12%)] and sesquiterpenes [*N. coriacea*[7] – valerianol (16.7%) and γ-eudesmol (12.3%); *N. salicina*[9] – atractylone (leaves: 14.6%, stems: 21.1%), viridiflorene (leaves: 10.1%, stems: 7.9%), and germacrene D (leaves: 5.8%, stems: 10.7%)]. However, the occurrence of α-bisabolol as major derivative was not reported in the previous volatile oils from *Nectandra* species.

Finally, the high concentration of α-bisabolol in the volatile oils from *N. megapotamica* leaves had led us to the isolation of the compound by chromatographic column separation, aiming the identification of this constituent by NMR techniques. The ^{13}C and DEPT 135° NMR spectra of pure derivative showed four signals corresponding to sp[2] carbons at δ 133.7 (C), 131.1 (C), 124.5 (CH_2), and 120.4 (CH_2), one carbinolic carbon at δ 74.0 (C), as well as four methyl groups at δ 25.5 (2 X CH_3), 17.4 (CH_3), and 23.1 (CH_3). The comparison of the obtained spectral data with those reported in the literature, confirmed the structure of α-bisabolol.[18]

CONCLUSION

This work reports, at first time, the chemical composition of volatile oils obtained from leaves of *N. megapotamica*. It was observed that α-bisabolol was the major component encountered, without seasonal and circadian variation in all analyzed samples. Considering the studied volatile oils from other *Nectandra* species it can be observed a dichotomy in relation to production of chemical constituents, i.e. monoterpenes or sesquiterpenes enriched oils. *N. megapotamica* belongs to the second group because the major components are sesquiterpenoids. However, a comparison with literature data does not allow verifies a preferential accumulation of a specific C_{15} skeleton carbonic in the genus. Further investigations should be carried out

with other specimen to verify the influence of environmental conditions and/or microclimatic factors in the production/accumulation of volatile derivatives.[19]

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