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Chemical variability of the volatiles of *Copaifera langsdorffii* growing wild in the Southeastern part of Brazil

Manoel Euclides do Nascimento [a,*], Maria das Graças Bichara Zoghbi [b], José Eduardo Brasil Pereira Pinto [c], Suzan Kelly Vilela Bertolucci [c]

[a] *Instituto de Ciências Agrárias, Universidade Federal Rural da Amazônia, Av. Presidente Tancredo Neves, 2501, 66077-901 Belém, PA, Brazil*
[b] *Coordenação de Botânica, Museu Paraense Emílio Goeldi, Av. Perimetral, 1901, 66077-901 Belém, PA, Brazil*
[c] *Departamento de Agricultura, Universidade Federal de Lavras, Campus Universitário, C.P. 3037, 37-200-000 Lavras, MG, Brazil*

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ABSTRACT

Volatiles obtained from leaves, branches, pericarps and seeds of five specimens (Tree 1–5) of *Copaifera langsdorffii* growing wild in the municipality of Lavras, Southeastern part of Brazil were obtained by hydrodistillation (HD) and investigated by gas chromatography/flame ionization detector (GC/FID) and gas/chromatography/mass spectrometry (GC/MS). The results of the oil compositions were processed by Hierarchical Component Analysis (HCA) allowing establish two main groups and further divided in six subgroups, which were defined by different concentrations of the six main compounds. The results showed high intra-population variability in the composition and concentration of the compounds. Major compounds were β-caryophyllene, germacrene D, spathulenol, caryophyllene oxide and *iso*-spathulenol. Coumarin was encountered only in the seeds of *C. langsdorffii*.

1. Introduction

As current circumscribed, the genus *Copaifera* belonging to the Leguminosae–Caesalpinioideae, consists of 43 species, and is largely distributed in South America (37 species, 28 species in Brazil), Central America (4 species), and Africa (4 species) (Dwyer, 1951; Martins-da-Silva et al., 2008; Costa, 2007). In Brazil, *Copaifera langsdorffii* Desf. is widely distributed, occurring in "cerrado", gallery forests and dry forests, from the State of Tocantins to Paraná (Almeida et al., 1998). The oleoresin of copaiba (*Copaifera* spp.) has been characterized mostly by the presence of sesquiterpenes (Zoghbi et al., 2009a,b; Lameira et al., 2009; Cascon, and Gilbert, 2000) and diterpenes (Pinto et al., 2000; Veiga-Junior and Pinto, 2002). Analytical methods validation of commercial oleoresin of copaiba have been described (Veiga-Junior et al., 1997; Vasconcelos and Godinho, 2002; Tappin et al., 2004; Biavatti et al., 2006; Sousa et al., 2011). The volatiles of *C. langsdorffii* identified in oleoresin and in essential oils of some organs of the plant have been previously reported: in one specimen from the State of Ceará, Brazil, β-caryophyllene was encountered as the major constituent of oleoresin, while γ-muurolene was encountered in high amount in the essential oil of leaves and fruits, caryopyhllene oxide was the principal constituent present in fruit peel, root wood, root bark oil and trunk wood essential oils, β-bisabolol (trunk bark oil), kaurene (trunk wood) and kaurenal (trunk bark) (Gramosa and Silveira, 2005).

The oleoresin of *C. langsdorffii* from Brazil have been found to contain the diterpenes polyalthic acid, (-)-kaur-16-en-19-oic, (-)-16β-kauran-19-oic acid and eperu-8(20)-en-15,18-dioic acid, and the sesquiterpene hydrocarbons caryophyllene, copaene and β-bisabolene (Ferrari et al., 1971). The essential oils obtained from pericarp of the one specimen of *C. langsdorffii* from the

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[*] Corresponding author.
E-mail address: nascimento-15@hotmail.com (M.E. do Nascimento).

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State of Minas Gerais, Brazil, in nature and maintained in water for 4 h and 240 h were dominated by caryophyllene oxide (33.72/38.70 and 38.98%, respectively) (Pereira et al., 2008). Phytochemical investigation of the hexane extract of fruit shells of one specimen of *C. langsdorffii* from the State of Ceará, Brazil, afforded *ent*-kaur-16-en-19-oic acid, polyalthic acid, nivenolide and caryophyllene oxide (Lima Neto et al., 2008). Chemical analysis of the oleoresin and essential oils of *Copaifera* spp. has shown great variability among species and locations. However, except Zoghbi et al. (2009b) and Herrero-Jáuregui et al. (2011), who studied the oleoresins extracted from 12 to 24 trees of *Copaifera reticulata* Ducke, respectively, most of the researchers analyse samples from one individual tree, and little is therefore known of the intra-specific variation of the oleoresin components or of the factors that might be influencing this variability. To our knowledge, there are no references in the literature regarding to the comparative study of the volatiles of *C. langsdorffii* obtained from more them one specimen of *C. langsdorffii*. The aim of this study was to investigate the occurrence of chemical variability of the volatiles of *C. langsdorffii* from five specimens growing wild in the State of Minas Gerais, Brazil.

2. Material and methods

2.1. Material and distillation of the volatile constituents

Samples of leaves (L1–L5), branches (B1–B5), pericarps (P1–P5) and seeds (S1–S5) of (September, 2008) were taken from five specimens of *C. langsdorffii* growing wild in the campus of the Universidade Federal de Lavras (UFLA), in fruit dispersion stage. The relation of height × CBH of the trees were: Tree 1 (8 m × 74 cm), Tree 2 (15 m × 262 cm), Tree 3 (16 m × 237 cm), Tree 4 (12 m × 125 cm) and Tree 5 (16 m × 280 cm). Voucher specimen (Tree 1: 24,946; Tree 2: 24,947; Tree 3: 24,952; Tree 4: 24,954 and Tree 5: 24,956) were deposited in the Herbarium ESAL of the UFLA. The samples were dried for 15 days at room temperature (medium temperature: 20.7 °C and medium relative humidity: 74.5%) and then ground.

2.1.1. Extraction of volatiles

The dry plant material was hydrodistilled during 4 h using a Clevenger-type apparatus; the oils extracted were separated by washing with CH_2Cl_2 as solvent, and dried with magnesium sulphate. The solutions containing 2 μL of the oil in 1 mL of hexane were immediately prepared to gas chromatography analysis.

2.2. Analysis of the volatiles

2.2.1. GC/MS

The oils were analyzed using a Shimadzu GC/MS Model QP 2010 Plus, equipped with a Rtx-5MS (30 m × 0.25 mm; 0.25 μm film thickness) fused silica capillary column. Helium was used as carrier gas adjusted to 1.2 mL/min at 57 KPa; splitless injection of 1 μL, of a hexane solution; injector and interface temperature were 250 °C; oven temperature programmed was 60–240 °C at 3 °C/min. EIMS: electron energy, 70 eV; ion source temperature was 200 °C. Identification of β-caryophyllene was carried out by comparison of its retention index and mass spectrum with those of an authentic compound (available from Robertet do Brasil). Other components were identified by comparison of their GC retention data with NIST-05 library and cited in the literature (Adams, 2007; Pereira et al., 2008; Helliwell et al., 1999). Retention indices were calculated using *n*-alkane standard solutions (C8–C26) available from Fluka S. A, in the same chromatographic conditions.

2.2.2. GC

This was performed on a Shimadzu QP-2010 instrument, equipped with FID, in the same conditions, except hydrogen was used as the carrier gas. The percentage composition of the oil samples were computed from the GC peak areas without using correction for response factors.

3. Results and discussion

In Table 1, the percentage of the compounds identified in the nineteen essential oils from the leaves, branches, pericarps and seeds of *C. langsdorffii* are listed in sequence of their retention indices. The medium value of yield (%) of the oils isolated from the leaves, branches, pericarps and seeds accounted for 0.63, 0.11, 2.33 and <0.05%, respectively. Table 1 shows the volatiles identified in the oils of five specimens of *C. langsdorffii*. In total, 69 compounds were detected, among them α-copaene, β-caryophyllene, germacrene D, spathulenol, caryophyllene oxide, *iso*-spathulenol and α-cadinol appears, at least in one oil, as the major constituent. The oils from all samples were terpenoid in nature, with the predominance of sesquiterpene hydrocarbons (leaf, branch and pericarp) and oxygenated sesquiterpenes (seed). Diterpenes were detected in a minor percentage, among them, *ent*-kaurenoids were the principal, mostly in the oils extracted from the branches of the Tree 2 (kaurene: 2.1%, kaurenal: 2.9%, kaurenol: 0.3%), and Tree 4 (kaurene: 3.4%, kaurenal: 7.0%, kaurenol: 1.3%). Kaurenal also was detected only in the pericarp of the Tree 5 (0.2%). Comparison of our results with the data presented by Gramosa and Silveira (2005) and (Pereira et al., 2008) reveals a great quantitative difference between the pericarp oils: these researchers found a high amount of caryophyllene oxide (47.30% and 33.75%, respectively), while we found this compound varying from 4.0% to 6.9%. Other important difference was the presence of γ-muurolene in high amounts in the leaf and fruit oils (25.2% and 29.8%,