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

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A B S T R A C T

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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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%,

Table 1
Volatiles (%) identified in the leaf, stem, pericarp and seed essential oils of *Copaifera langsdorffii*.

Constituents	RI[a]	R[b]	Pericarp					Leaf					Branch					Seed			
			1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	5
Methyl geranate	1326	1322	0.4	0.4	0.3	0.6	0.04	–	0.03	–	–	–	–	0.04	0.01	–	–	–	0.03	0.04	–
δ-elemene	1341	1335	0.9	1.6	2.2	7.7	3.2	–	–	3.6	0.4	0.9	–	1.6	1.6	0.7	2.5	–	–	–	–
α-cubebene	1353	1345	2.5	1.5	3.5	0.5	0.4	0.9	–	0.8	0.7	0.3	–	0.6	0.9	0.2	0.2	–	–	–	–
Cyclosativene	1370	1369	0.5	0.8	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
α-ylangene	1375	1373	–	2.0	–	–	0.7	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–
α-copaene	1380	1374	14.1	8.4	13.1	4.0	3.2	6.9	4.8	5.6	5.2	1.8	–	2.4	3.6	1.5	1.0	–	–	4.7	–
β-cubebene	1394	1387	11.1	7.5	8.9	–	–	4.7	4.6	–	3.8	2.7	1.3	2.3	1.3	0.7	–	–	–	4.8	–
β-elemene	1396	1389	–	–	–	6.4	5.3	–	–	8.4	–	–	–	–	1.3	0.7	1.7	–	–	–	–
Cyperene	1404	1398	0.7	1.2	0.7	0.6	2.5	1.0	0.9	1.3	0.5	0.6	–	0.5	0.2	0.1	0.4	–	–	–	–
(*Z*)-caryophyllene	1405	1408	–	–	–	–	–	–	–	–	–	–	5.7	–	–	–	–	–	–	–	–
(*E*)-caryophyllene[e]	1424	1417	2.7	10.3	8.1	10.5	6.2	5.7	13.5	12.2	15.7	17.5	2.4	13.9	9.7	12.2	11.9	2.1	–	–	–
β-copaene	1434	1430	0.5	0.8	0.6	–	0.7	0.8	0.6	1.6	0.6	1.0	–	1.0	0.8	0.8	1.6	–	–	–	–
γ-elemene	1437	1434	–	1.4	–	3.7	–	0.4	–	–	–	–	–	1.1	–	0.4	–	–	–	–	–
Trans–α-bergamotene	1439	1432	–	–	1.2	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–
Coumarin	1442	1432	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7.1	4.1	12.6
Aromadendrene	1444	1439	0.5	–	0.4	–	2.8	0.9	0.4	0.4	0.5	0.6	–	–	0.4	–	–	–	–	–	–
Guaia-6,9-diene	1448	1442	–	–	0.4	0.6	0.6	–	–	0.6	–	–	–	0.6	–	–	–	–	–	–	–
α-humulene	1459	1452	0.9	1.6	1.8	2.0	1.5	1.8	2.7	3.0	4.4	4.2	–	3.6	2.6	3.4	3.0	–	–	–	–
allo-aromadendrene	1466	1458	0.8	0.5	0.8	–	1.5	0.6	–	0.8	0.5	0.7	–	–	–	–	–	–	–	–	–
cis-cadina-1(6),4-diene	1468	1475	–	–	–	–	–	–	–	–	–	–	–	0.5	0.5	0.4	0.7	–	–	–	–
γ-muurolene	1481	1478	1.3	1.6	1.5	1.0	–	2.1	1.4	–	1.5	2.0	1.7	–	1.7	1.1	–	–	0.4	1.4	–
α-amorphene	1485	1483	–	–	–	–	–	0.6	–	–	–	–	–	–	–	–	–	–	–	–	–
Germacrene D	1486	1484	1.9	6.6	6.3	8.7	10.7	–	3.4	17.3	3.6	7.7	–	11.2	9.4	11.0	19.1	–	–	–	–
β-selinene	1491	1489	0.3	0.4	0.4	–	0.5	0.5	–	–	0.3	0.3	–	–	–	–	–	–	–	–	–
cis–β-guaiene	1496	1492	–	1.0	–	–	–	–	–	–	–	–	1.9	–	–	–	–	–	–	–	–
epi-cubebol	1497	1493	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	3.2	–
Viridiflorene	1498	1496	–	–	–	–	–	1.8	–	–	–	–	–	–	–	–	–	–	–	–	–
Bicyclogermacrene	1502	1500	–	–	6.3	–	5.8	–	2.4	11.5	1.8	2.7	–	4.8	7.7	2.0	8.0	–	–	–	–
α-muurolene	1505	1500	1.8	1.9	–	1.1	–	2.2	2.3	–	1.8	2.7	–	–	–	2.0	–	–	0.5	1.4	–
δ-amorphene	1511	1511	–	–	–	–	–	–	–	–	–	–	–	0.4	–	–	0.3	–	–	–	–
γ-cadinene	1519	1513	2.0	2.0	2.0	1.9	4.1	1.8	2.1	1.4	1.5	1.7	2.2	1.2	2.4	1.1	1.3	–	2.0	4.4	–
trans-calamenene	1526	1521	–	–	–	–	–	2.2	–	–	–	–	2.1	–	–	–	–	–	–	–	–
δ-cadinene	1527	1522	2.9	2.3	4.8	1.2	1.9	1.6	2.9	6.1	2.7	2.2	–	3.9	5.7	4.2	2.7	–	–	1.3	–
trans-cadina-1,4-diene	1536	1533	0.3	–	0.3	–	–	–	–	0.3	–	–	–	0.3	0.5	0.2	–	–	–	–	–
α-cadinene	1541	1537	–	–	–	–	0.4	–	–	0.4	–	0.6	–	–	–	–	0.5	–	–	–	–
α-copaen-11-ol	1544	1539	2.4	1.2	–	–	–	–	3.1	0.4	–	–	2.3	1.8	0.9	0.5	–	–	1.4	–	–
α-calacorene	1546	1544	–	–	1.2	–	0.4	1.4	–	–	1.1	0.4	–	–	–	–	–	–	–	1.7	–
Elemol	1551	1548	–	0.6	–	1.1	0.5	–	–	0.4	–	–	–	0.7	0.5	0.4	0.6	–	1.2	–	–
Germacrene B	1561	1559	–	0.5	–	1.0	–	–	–	–	–	–	–	0.7	–	–	–	–	–	–	–
(*E*)-nerolidol	1563	1561	–	–	–	–	–	1.5	–	0.9	1.2	1.0	–	–	0.8	1.0	0.3	–	–	–	–
β-calacorene	1566	1564	0.7	–	0.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Palustrol	1570	1567	–	–	–	–	–	–	–	–	–	1.2	–	–	–	–	–	–	–	–	–
Spathulenol	1580	1577	16.2	7.6	10.0	2.2	15.2	11.3	12.4	3.8	7.8	9.1	13.7	5.2	7.4	3.6	4.9	38.1	19.4	25.4	38.9
Caryophyllene oxide	1585	1582	4.6	6.9	4.0	5.1	4.0	15.1	15.6	–	13.7	11.7	13.3	7.2	9.2	8.2	4.9	–	17.6	21.8	14.0
Viridiflorol	1593	1592	–	–	–	–	–	–	1.6	–	–	2.3	–	–	1.8	1.1	2.0	–	–	–	–
Salvial-4(14)-en-1-one	1594	1594	2.0	2.5	1.8	3.2	2.6	–	–	–	–	–	3.2	1.4	–	–	–	–	3.0	1.7	–
Guaiol	1598	1600	–	–	–	–	–	–	–	–	–	–	–	1.5	–	2.3	–	–	–	–	–
Ledol	1606	1602	–	–	0.7	–	–	–	–	–	–	–	–	–	1.7	–	1.1	–	–	–	–

(*continued on next page*)

Table 1 (*continued*)

Constituents	RI[a]	R[b]	Pericarp					Leaf					Branch					Seed			
			1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	5
Humulene epoxide II	1611	1608	–	2.0	1.2	–	–	2.7	2.5	–	2.6	1.8	3.8	2.0	2.2	2.1	1.3	–	5.1	3.4	–
Junenol	1621	1618	–	–	–	–	–	1.0	–	0.5	1.3	1.9	2.9	0.8	0.5	0.7	1.0	–	1.0	–	–
Dillapiole	1625	1620	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-*epi*-cubenol	1631	1627	–	–	–	–	–	2.9	1.8	1.5	2.4	0.9	–	–	2.5	3.2	1.8	–	–	–	–
iso-spathulenol	1632	1629[c]	7.6	8.2	5.6	21.6	10.2	–	–	–	–	–	7.9	5.8	–	–	–	25.8	12.9	6.9	10.0
Caryophylla-4(12), 8(13)-dien-5-ol	1640	1639																			
epi–α-muurolol	1645	1640	3.6	2.6	2.2	–	–	6.2	5.6	4.8	5.6	5.8	7.9	3.8	6.0	5.1	3.4	5.0	3.2	1.3	3.9
α-muurolol	1651	1644	–	–	–	–	–	2.8	–	1.1	1.9	1.9	2.7	–	1.6	1.8	3.4	4.2	–	–	–
α-cadinol	1660	1652	2.6	2.5	1.4	4.7	2.5	6.8	5.4	4.9	5.8	6.2	11.5	4.9	6.2	7.2	6.4	10.4	5.0	2.0	6.5
cis-calamenen-10-ol	1664	1660	0.8	–	0.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.4	–
Mustakone	1686	1676	2.0	–	0.9	–	–	1.1	1.9	–	–	–	1.4	1.4	1.7	0.8	–	3.1	3.0	2.3	–
Eudesma-4(15), 7-dien-1β-ol	1694	1687	1.4	1.1	0.7	1.0	2.3	–	–	0.2	–	–	1.4	0.9	0.9	1.0	1.2	4.2	2.8	–	–
nor-calamenen-10-one	1711	1702	–	–	0.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Opoplanone	1746	1739	1.2	–	–	–	1.0	–	–	–	–	–	–	–	–	–	–	–	2.4	–	–
14-hydroxy-α-muurolene	1786	1779	–	1.0	–	1.1	1.1	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–
(2*E*,6*E*)-methyl farnesoate	1789	1783	–	–	–	–	–	2.4	–	0.3	2.8	–	3.6	–	0.7	0.7	5.2	–	–	–	3.6
14-hydroxy-δ-cadinene	1811	1803	–	–	–	–	–	–	–	–	–	–	–	–	0.2	–	0.4	–	–	–	–
(*E*,*E*)-geranyl linalool	2033	2026	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–	–
Kaurene	2052	2042	0.8	0.9	–	1.0	1.0	–	–	–	1.3	0.9	2.0	2.1	–	3.4	1.1	0.8	2.9	–	4.4
Kaurenal	2263	2257[d]	–	–	–	–	0.2	–	–	–	–	–	–	2.9	–	7.0	–	–	–	–	–
Kaurenol	2331	2330[d]	–	–	–	–	–	–	–	–	–	–	–	0.3	–	1.3	2.1	–	–	–	–
Total			92.0	91.4	95.3	92.5	93.0	91.7	91.9	94.1	93.2	95.3	94.9	93.7	95.4	94.1	96.0	93.7	91.9	93.2	93.9

(–) Not detected.
[a] Retention índex on HP-5MS.
[b] Adams, 2007.
[c] Pereira et al., 2008.
[d] Helliwell et al., 1999.
[e] Identified by comparison with authentic standard.

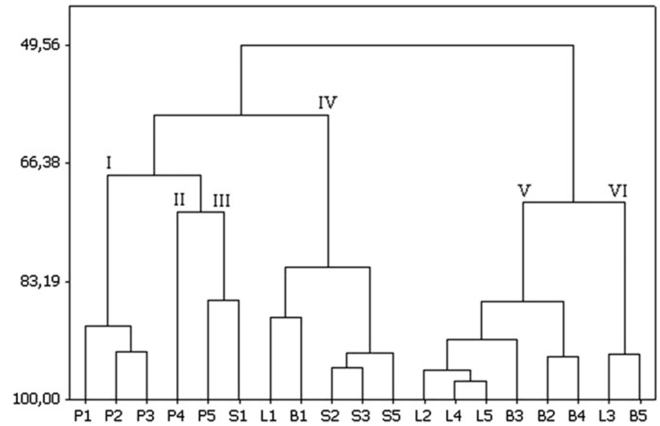

Fig. 1. Hierarchical Component Analysis (HCA) of nineteen oils of *Copaifera langsdorffii* plants collected from five individual trees from Southeastern of Brazil.

respectively) in the sample studied by Gramosa and Silveira (2005), while in all our samples this compound varied from zero to 2.1%.

In order to investigate intra-specific variability, all 69 compounds constituents of the nineteen oils studied were included in the multivariate analysis using Minitab 14 software for Hierarchical Component Analysis (HCA). The classification analysis of the 19 samples, performed with the concentrations of the different constituents in the samples, allowed to clearly differentiate two groups, Group 1 and Group 2 (Fig. 1). The Group 1 was composed of 11 samples taken from the 5 pericarp oils, all oils from the seeds, and the leaf and branch oil of the Tree 1. The Group 2 was composed by leaf and branch oils from the Trees 2, 3, 4 and 5. Higher similarity was observed between the oils obtained from the leaves of the Trees 4 and 5 (97.44%), by the presence of β-caryophyllene (15.7 and 17.5%, respectively), caryophyllene oxide (13.7 and 11.7%, respectively). These oils differ from those of the Tree 3 by the amount of germacrene D (17.3%) and bicyclogermacrene (11.5%). The oils extracted from the pericarps of Trees 1, 2 and 3 were distinguished by the presence of α-copaene (14.1/8.4/13.1%, respectively) and β-cubebene (11.1/7.5/8.9%, respectively), while spathulenol was the major compound in the oils of Tree 1 (16.2%) and 5 (15.2%). The sesquiterpene *iso*-spathulenol (21.6%) was encountered in a high amount in the pericarp of Tree 4 and in the oils from the seeds. Spathulenol and α-cadinol were present in all oils analyzed, but the percentage of spathulenol varied in a great extention (2.2% in the pericarp oil of Tree 4–38.9% in seed oil of Tree 5).

Chemical composition of copaiba oleoresin, an exudate comprising resinous acids and volatile compounds, has been extensively studied, even more than the essential oils extracted from their different organs. Analyses of the chemical composition of the oleoresin produced by several species of *Copaifera* have shown great inter- and intra-specific variability, nevertheless, few sesquiterpenes change considerably. Among the sesquiterpenes α-copaene and δ-cadinene were the major compounds in oleoresin of *Copaifera martii* (Zoghbi et al., 2007); β-caryophyllene, β-selinene and β-bisabolene in *Copaifera duckei* (Lameira et al., 2009), β-bisabolene, *trans*-α-bergamotene in *C. reticulata* from the State of Pará (Zoghbi et al., 2009a,b), β-caryophyllene β-selinene and β-bisabolene in *C. reticulata* from the State of Amapá (Zoghbi et al., 2009a,b), β-caryophyllene *trans*-α-bergamotene and β-bisabolene in *C. reticulata* from the State of Pará (Herrero-Jáuregui et al., 2011). α-Copaene was

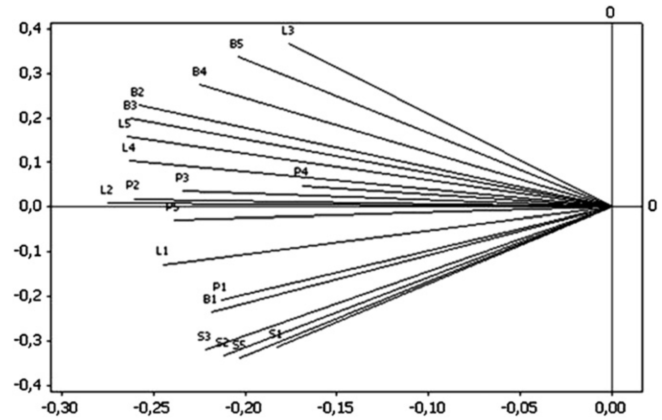

Fig. 2. Principal Component Analysis (PCA) of nineteen oils of *Copaifera langsdorffii* plants collected from five individual trees from Southeastern of Brazil.

the major sesquiterpene detected in the oleoresin form *Copaifera paupera* and *Copaifera piresii* (Zoghbi et al., 2009a,b), and β-caryophyllene was prominent in *Copaifera pubiflora* (Zoghbi et al., 2009a,b) and *Copaifera multijuga* (Cascon and Gilbert, 2000). Concerning with the leaf essential oil, besides C. *langsdorffii* β-caryophyllene also was detected in a high amount in *Copaifera trapezifolia* (Veiga et al., 2006).

Results obtained from cluster analysis showed the existence of a high variability within the essential oils of *C. langsdorffii* mostly due to the organ extracted. From those 19 oils submitted to multivariate analysis, two well-defined groups of essential oils were differentiated by cluster analysis. According to the Principal Component Analysis we can distinguish two groups: the first formed by the Tree 1 (leaf, pericarp and branch) and seed oils from Trees 1–3 and 5 and the second composed by leaf and branch oils (Fig. 2). The results obtained for *C. langsdorffii* reinforce the occurrence of high chemical polymorphism on *Copaifera* spp.

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