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# Infraspecific chemical variability in the essential oils of *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (Myrtaceae)

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ABSTRACT

Leaf essential oils of *Pimenta pseudocaryophyllus* from the central Brazilian Cerrado were obtained by hydrodistillation and investigated by GC and GC–MS. A total of 57 constituents were identified, accounting for 96–100% of the volatile constituents. Principal component and cluster analysis identified three chemotypes: cluster I, characterized by high percentages of geranial (41.2 +/− 3.9%), neral (26.8 +/− 1.3%), caryophyllene oxide (3.8 +/− 2.5%), and spathulenol (3.7 +/− 1.8%); cluster II, with high contents of (E)-asarone (21.8 +/− 30.9%), (E)-caryophyllene (16.2 +/− 7.7%), and elemicin (8.8 +/− 2.4%); and cluster III, with high amounts of (E)-methyl isoeugenol (93.2 +/− 1%). The occurrence of these chemotypes at the same site indicates that chemovariation is genetically determined.



## 1. Introduction

The Myrtaceae family, which consists of approximately 130 genera and 4000 species, is distributed throughout pantropical and subtropical regions. In Brazil, 23 genera with about 1000 species are known (Landrum and Kawasaki, 1997; Souza and Lorenzi, 2005). Among the representatives of this family include species of the *Pimenta* genus, mostly native to the Caribbean and Central America, which are well known because of the economic importance of "allspice", *Pimenta dioica* (L.) Merrill (Suárez et al., 1997).

*Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum is the only species of the *Pimenta* genus native to Brazil (Landrum, 1986; Landrum and Kawasaki, 1997). It consists of three varieties, *P. pseudocaryophyllus* var. *pseudocaryophyllus* (Gomes) Landrum, *P. pseudocaryophyllus* var. *fulvescens* (DC.) Landrum, and *P. pseudocaryophyllus* var. *hoehnei* (Burret) Landrum. This plant is found in high-altitude regions of the Atlantic forests and Cerrado regions in Brazil (Landrum, 1986; Landrum and Kawasaki, 1997). It is popularly known as "pau-cravo", "louro-cravo", "louro", "craveiro", "craveiro-do-mato", "chá-de-bugre", and "cataia". In folk medicine, the leaf tea has been used to produce a refreshing drink with sedative, diuretic, and aphrodisiac properties, and to treat colds as well as digestive and menstrual problems (Landrum, 1986; Nakaoka-Sakita et al., 1994; Landrum and Kawasaki, 1997; Lima et al., 2006; Paula et al., 2008; Santos et al., 2009). Previous studies have demonstrated the antimicrobial activities of crude ethanol extract (Paula et al., 2009) and essential oils from *P. pseudocaryophyllus* leaves (Lima et al., 2006).

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**Table 1**
Percentages and yields of volatiles of twelve specimens of *P. pseudocaryophyllus* from the Brazilian Cerrado.

| Constituent | RI[a] | Origins | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SGA1[b] | SGA2[b] | SGA3[b] | SGA4[b] | SGA5[b] | SGA6[b] | SGA7[b] | SGA8[b] | SJB1[c] | SJB2[c] | SJB3[c] | BRA[d] |
| α-Thujene | 930 | | | | | | 0.8 | | | | | | |
| β-Pinene | 973 | 0.9 | | 0.8 | | 1.3 | 1.9 | | 1.0 | | | | |
| 6-Methyl-5-hepten-2-one | 980 | | | | | | 0.2 | | 0.4 | | | | |
| Dehydro-1,8-cineole | 987 | | | | | | | | 0.2 | | | | |
| Limonene | 1024 | | | | 0.2 | | | | | 0.3 | 0.3 | | |
| 1,8-Cineole | 1028 | | | | | | | | | 0.2 | 0.1 | 0.1 | |
| (E)-β-Ocimene | 1042 | | | 0.7 | | 1.2 | | | 0.2 | | | 0.3 | |
| Linalool | 1096 | 0.4 | 0.2 | 1.3 | | 1.4 | 6.6 | 1.1 | 0.5 | 0.6 | 0.8 | 4.9 | |
| n-Nonanal | 1100 | | 0.1 | 0.8 | | | | | | | | | |
| 2-Methylbutyl-2-methylbutyrate | 1100 | | | | | | | | | | | 0.1 | |
| *exo*-Isocitral | 1139 | | | | | | | | 0.3 | | | | |
| (Z)-Isocitral | 1160 | 0.6 | | | | | 0.8 | | 1.0 | | | | |
| (E)-Isocitral | 1178 | 1.0 | | | | | 1.3 | | 1.8 | | | | |
| α-Terpineol | 1188 | | | | | | | | 0.2 | | | 0.1 | |
| Citronellol | 1224 | | | | | | | 0.4 | | | | | |
| Nerol | 1225 | 0.9 | | | | | 1.0 | | 0.9 | | | | |
| Neral | 1237 | 25.9 | | | | | 25.8 | 0.8 | 28.7 | | | 0.1 | |
| Geraniol | 1251 | 2.3 | | | | | 2.8 | | 2.2 | | | 0.1 | |
| Geranial | 1267 | 46.6 | | | | | 39.6 | 1.0 | 37.3 | | | 0.1 | |
| Methyl geranate | 1320 | | | | | | | | 0.1 | | | | |
| α-Cubebene | 1347 | | | | | 0.8 | | | | | | | |
| α-Copaene | 1373 | 0.6 | 0.1 | | 0.3 | 5.7 | 1.8 | 0.2 | 0.6 | | | 0.3 | 0.4 |
| Geranyl acetate | 1379 | | | | | | 0.3 | | | | | | |
| β-Bourbonene | 1382 | | | | | | | | 0.2 | | | | |
| β-Elemene | 1389 | | | | | | | | 0.2 | | 0.3 | | |
| Methyleugenol | 1399 | | 2.4 | | 1.3 | | | 1.8 | | | 0.2 | | 3.1 |
| α-Gurjunene | 1406 | | | 1.0 | | | | | | | | | |
| (E)-Caryophyllene | 1416 | 1.7 | 0.6 | 13.3 | 1.3 | 26.6 | 0.7 | 1.7 | 8.0 | 1.9 | 2.2 | 8.5 | 1.6 |
| β-Copaene | 1426 | | | | | 1.2 | | | 0.3 | | | 0.1 | |
| Aromadendrene | 1436 | | | 3.9 | | | | | 0.3 | | | | |
| (Z)-Methyl isoeugenol | 1451 | | 2.5 | 2.2 | 3.3 | | | 1.4 | | 2.3 | 1.8 | | 0.9 |
| α-Humulene | 1451 | 0.8 | | | | 4.9 | | | 1.5 | | | 1.4 | |
| 9-*epi*-(E)-Caryophyllene | 1458 | | | | | | 0.3 | | | | | | |
| *allo*-Aromadendrene | 1458 | | | 1.8 | | | | | 0.5 | | | | |
| *trans*-cadina-1(6),4-diene | 1470 | | | | | 0.6 | | | | | | | |
| γ-Muurolene | 1473 | | | | | 4.5 | 0.3 | | 4.2 | | | 1.6 | |
| *trans*-Muurola-4(14),5-diene | 1477 | | | | | 4.7 | | | | | | | |
| γ-Himachalene | 1482 | | | | | | | | | 0.5 | 0.5 | | |
| (E)-Methyl isoeugenol | 1492 | 1.8 | 93.7 | 61.3 | 92.5 | 22.7 | 0.4 | 91.5 | | 94.3 | 93.8 | 5.0 | 93.6 |
| Bicyclogermacrene | 1494 | | | | | | | | 5.7 | | | | |
| α-Muurolene | 1497 | | | | | 1.4 | | | 0.2 | | | 0.2 | |
| γ-Cadinene | 1510 | | | | | 1.9 | | | 0.2 | | | 0.2 | |
| δ-Cadinene | 1520 | 0.5 | 0.2 | 1.1 | 0.4 | 9.2 | 0.6 | | 0.9 | | | 0.9 | 0.4 |
| Elemicin | 1550 | | | 11.7 | | 8.8 | | | | | | 5.8 | |
| Spathulenol | 1574 | 6.1 | 0.3 | | 0.4 | | 3.0 | | 1.9 | | | 0.3 | |
| Caryophyllene oxide | 1580 | 5.5 | | | | | 5.5 | | 0.3 | | | 0.8 | |
| Globulol | 1589 | | | | | | | | | | | 0.3 | |
| (Z)-Asarone | 1595 | | | | | | | | | | | 1.3 | |
| Humulene epoxide II | 1606 | 0.8 | | | | | 0.8 | | | | | | |
| 5-*epi*−7−*epi*-α-Eudesmol | 1619 | | | | | | | | | | | 0.1 | |
| 1-*epi*-Cubenol | 1624 | | | | | | | | | | | 0.1 | |
| 1,10-di-*epi*-Cubenol | 1628 | | | | | | | | | | | 0.1 | |
| Muurola-4,10(14)-dien-1-β-ol | 1634 | 0.7 | | | | | 0.3 | | | | | | |
| *epi*-α-Muurolol | 1638 | | | | | | | | | | | 0.7 | |
| α-Muurolol | 1650 | | | | | | 0.3 | | | | | 0.2 | |
| 14-hydroxy-(Z)-Caryophyllene | 1667 | 0.4 | | | | | 0.7 | | | | | | |
| (E)-Asarone | 1671 | | | | | | | | | | | 65.5 | |
| Monoterpene hydrocarbons | | 1.0 | | 1.5 | 0.2 | 2.5 | 2.7 | | 1.4 | 0.3 | 0.3 | 0.3 | |
| Oxygenated monoterpenes | | 77.7 | 0.2 | 1.3 | | 1.4 | 77.9 | 3.4 | 73.0 | 0.8 | 0.9 | 5.4 | |
| Sesquiterpene hydrocarbons | | 3.6 | 0.9 | 21.2 | 2.0 | 61.6 | 3.8 | 1.9 | 22.3 | 2.4 | 3.0 | 13.3 | 2.4 |
| Oxygenated sesquiterpenes | | 13.6 | 0.3 | | 0.4 | | 10.7 | | 2.2 | | | 2.5 | |
| Phenols | | 1.9 | 98.5 | 75.2 | 97.1 | 31.5 | 0.4 | 94.8 | | 96.6 | 95.9 | 77.6 | 97.6 |
| Others | | | 0.1 | 0.8 | | | 0.6 | | 0.5 | | | 0.1 | |
| Yield (%, v/w) | | 1.4 | 1.2 | 0.3 | 1.2 | 0.6 | 1.6 | 0.8 | 1.3 | 1.1 | 1.5 | 0.8 | 0.8 |

[a] Calculated linear retention indices.
[b] São Gonçalo do Abaeté.
[c] São José do Barreiro.
[d] Brasília.

Infraspecific chemical variability in essential oils has been described, particularly in the Lamiaceae (Grayer et al., 1996; Azevedo et al., 2002; Echeverrigaray et al., 2003; Thompson et al., 2003; Miguel et al., 2004; Loziene and Venskutonis, 2005; Carvalho-Filho et al., 2006) and Apiaceae families (Barazani et al., 2002). In the Myrtaceae family, chemical variability has been observed in several species, including *Melaleuca quinquenervia* (Cav.) S. T. Blake, *Melaleuca alternifolia* Cheel, *Eugenia dysenterica* DC., and *Pimenta racemosa* var. *racemosa* (P. Miller) J. W. Moore (Abaul et al., 1995; Homer et al., 2000; Wheeler et al., 2003; Duarte et al., 2009). Olfactorial differences in essential oils were found in *P. pseudocaryophyllus* specimens collected from two sites in the central Brazilian Cerrado (Paula et al., 2010). Differences in essential oil constituents were observed between specimens collected in the Brazilian Cerrado and those collected in the southeastern region of Brazil (Nakaoka-Sakita et al., 1994; Lima et al., 2006; Santos et al., 2009). These differences indicate the possibility of chemical polymorphism in this plant species.

It is crucial to consider the chemical variations in essential oils caused by genetic, physiological or environmental factors when domesticating and improving species of medicinal interest. Therefore, it is necessary to characterize and identify the existence of chemotypes, especially when referring to plant material used in chemical, pharmacological and agronomic studies that aim to produce herbal medicines, because pharmacological activities of the same species can differ due to differences in essential oil composition (Lima et al., 2003; Potzernheim et al., 2006).

In this work, we report on the chemical variability observed in leaf essential oils of *P. pseudocaryophyllus*. Qualitative and quantitative analysis of the volatile oils of twelve specimens that occur naturally in three different locations in the central Brazilian Cerrado were performed by GC and GC–MS. The chemical constituents were submitted to principal component and cluster analysis to study the infraspecific variability patterns in individuals.

## 2. Material and methods

### 2.1. Plant material

Leaves of twelve specimens of *P. pseudocaryophyllus* were collected from mature trees in São Gonçalo do Abaeté in the state of Minas Gerais (18°20′58″ S/45°55′23″ W, 864 m) in February, 2006 (samples SGA1–SGA6), and February, 2008 (samples SGA7 and SGA8); São José do Barreiro in the state of Minas Gerais (20°20′16″ S/46°29′9″ W, 864 m) in July, 2007 (samples SJB1–SJB3); and Brasília in the Federal District of Brazil (15°51′51″ S/47°49′43″ W, 767 m) in January, 2006 (sample BRA). The plants were identified by Dr. Carolyn E. B. Proença of the Brasília Botanical Garden, Federal District, Brazil. Voucher specimens were deposited at the Ezechias Paulo Heringer Herbarium (EPH), Brasília, Federal District, Brazil, and at the Herbarium of Universidade Federal de Goiás (UFG), Goiás State, Brazil.

### 2.2. Essential oil extraction

Leaf samples (50 g) were air-dried in a chamber at 40 °C for three days, ground into a powder and submitted to hydrodistillation in a modified Clevenger-type apparatus (2 h). Each essential oil was dried over anhydrous sodium sulfate and stored at −20 °C for further analysis.

### 2.3. Essential oil analyses

Oil samples were analyzed on a Varian 3900 gas chromatograph (FID) equipped with a CB-SIL-5CB fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness). The temperature program used was as follows: ramp up from 60 °C to 240 °C at 3 °C $min^{-1}$, increase to 280 °C at 10 °C $min^{-1}$, and end with 10 min at 280 °C. The carrier gas nitrogen was injected at a flow rate of 1.0 mL $min^{-1}$; the injector port and detector temperature were 220 °C and 240 °C, respectively. Samples were injected by splitting; the split ratio was 1:20. GC–MS analysis was performed on a Shimadzu QP5050A instrument. The column, a CBP-5 (Shimadzu) fused silica capillary column (30 m long × 0.25 mm i.d. × 0.25 μm film thickness composed of 5% phenylmethylpolysiloxane), was connected to a quadrupole detector operating in EI mode at 70 eV. Helium was used as the carrier gas at a flow rate of 1 ml $min^{-1}$. The injector and interface temperatures were 220 °C and 240 °C, respectively, with a split ratio of 1:5. The injection volume was 0.5 μl (10% in hexane), and the oven temperature program consisted of ramping up from 60 °C to 240 °C at 3 °C $min^{-1}$, followed by an increase to 280 °C and 10 °C $min^{-1}$, and ending with 5 min at 280 °C.

Essential oil constituents were identified by comparing their mass spectra with those from the *National Institute of Standards and Technology* (NIST, 1998), and by comparing the mass spectra and calculated linear retention indices (RI) with values in the literature (Adams, 2007). Retention indices were obtained by co-injection with a mixture of linear hydrocarbons, $C_8$–$C_{32}$ (Sigma, USA), and by the equation of Van Den Dool and Kratz (1963).

### 2.4. Chemical variability

Principal component analysis (PCA) using SPAD data mining (Coheris Corp., 2002) was applied to examine the interrelationships between different populations and chemical constituents. Cluster analysis was also applied to study the similarity of samples on the basis of constituent distribution. The nearest neighbor complete linkage technique using the Benzécri

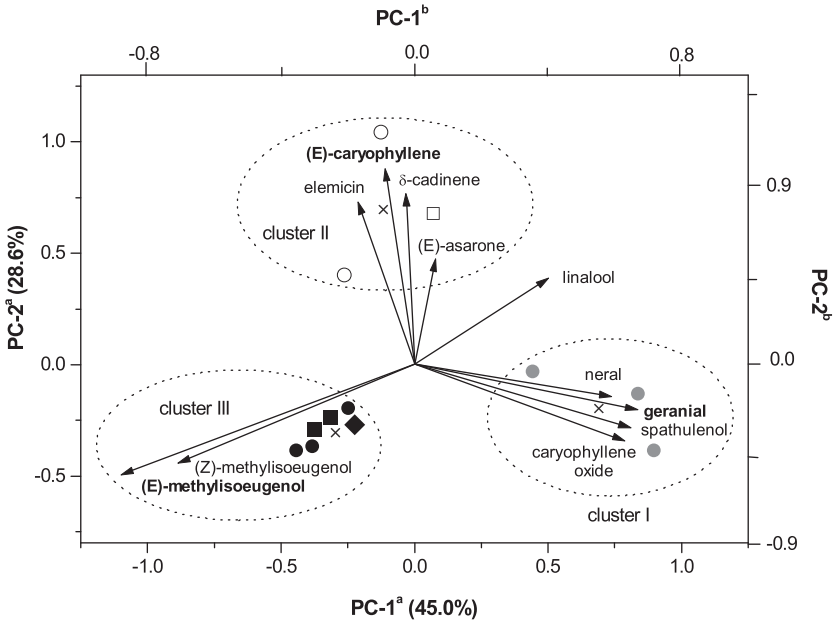


**Fig. 1.** PCA biplot of *P. pseudocaryophyllus* leaf oil samples from SJB (square symbols), SGA (circle symbols) and BRA (triangle symbols). The cluster is denoted as follows: I (gray shaded symbols), II (unshaded symbols), and III (black shaded symbols). [a] Axes refer to scores from the samples. [b] Axes refer to loadings from oil constituents (Table 1) with selected variables represented as vectors from the origin. Crosses represent cluster centroids, and values in parentheses refer to the variance of each principal component.

algorithm (Benzécri, 1980) was used as an index of similarity, and hierarchical clustering was performed according to the Ward's variance minimization method (Ward, 1963). For variable selection, the threshold of residual eigenvalues ($\leq$0.70) in the original data matrix (12 samples $\times$ 57 variables = 684 data points) was used to establish the maximum number of variables that could be removed (48 variables) (Mardia et al., 1980). The 46 variables that were effectively eliminated revealed the highest loadings in the lowest residual eigenvalues and contributed $\leq$1% to the chemical profiles (average values).

Canonical discriminant analysis was performed by SAS CANDISC and SAS DISCRIM (SAS Inc., 1996) procedures and used to differentiate populations and clusters on the basis of oil composition. The predictive ability of discriminant functions was

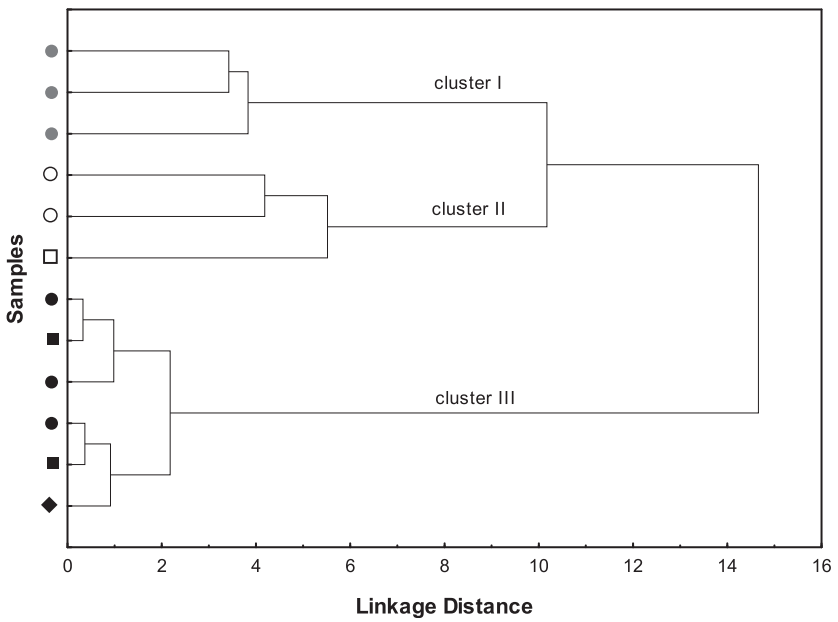


**Fig. 2.** Dendrogram representing chemical composition similarity relationships among *P. pseudocaryophyllus* leaf oil samples from SJB (square symbols), SGA (circle symbols) and BRA (triangle symbols). Clusters are denoted as follows: I (gray shaded symbols); II (unshaded symbols); and III (black shaded symbols).

**Table 2**
Percentage averages[a] in volatiles of clustered leaf oils from *P. pseudocaryophyllus* collected in the central Brazilian Cerrado.

| Constituent | Clusters | | |
|---|---|---|---|
| | I | II | III |
| α-Thujene | 0.3 | – | – |
| β-Pinene | 1.3 a | 0.7 a | – |
| 6-Methyl-5-hepten-2-ona | 0.2 | – | – |
| Dehydro-1,8-cineole | 0.1 | – | – |
| Limonene[b] | – | 0.01 a | 0.1 a |
| 1,8-Cineole | – | 0.04 a | 0.1 a |
| (E)-β-Ocimene[b] | 0.1 a | 0.7 a | – |
| Linalool[c,d] | 2.5 a | 2.5 a | 0.4 a |
| n-Nonanal | – | 0.3 | – |
| 2-Methylbutyl-2-methylbutyrate | – | 0.04 | – |
| *exo*-Isocitral | 0.1 | – | – |
| (Z)-Isocitral | 0.8 | – | – |
| (E)-Isocitral | 1.4 | – | – |
| α-Terpineol | 0.1 a | – | – |
| Citronellol | – | – | 0.1 |
| Nerol | 0.9 | – | – |
| Neral[b,d] | 26.8 a | – | 0.1 b |
| Geraniol | 2.4 a | – | – |
| Geranial[b,d] | 41.2 a | – | 0.2 b |
| Methyl geranate | – | – | – |
| α-Cubebene | – | 0.3 | – |
| α-Copaene[b] | 1.0 a | 2.0 a | 0.2 a |
| Geranyl acetate | 0.1 | – | – |
| β-Bourbonene | 0.1 | – | – |
| β-Elemene | 0.1 a | – | 0.1 a |
| Methyleugenol | – | – | 1.5 |
| α-Gurjunene | – | 0.3 | – |
| (E)-Caryophyllene[b,d] | 3.5 b | 16.2 a | 1.5 b |
| β-Copaene | 0.1 a | 0.4 a | – |
| Aromadendrene[b] | 0.1 a | 1.3 a | – |
| (Z)-Methyl isoeugenol[d] | – | 0.7 a | 2.0 a |
| α-Humulene | 0.8 a | 2.1 a | – |
| 9-*epi*-(E)-Caryophyllene | 0.1 | – | – |
| *allo*-Aromadendrene | 0.2 a | 0.6 a | – |
| *trans*-cadina-1(6)4-diene | – | 0.21 | – |
| γ-Muurolene | 1.5 a | 2.0 a | – |
| *trans*-Muurola-4(14)5-diene | – | 1.6 | – |
| γ-Himachalene | – | – | 0.2 |
| (E)-Methyl isoeugenol[c,d] | 0.7 b | 29.7 b | 93.2 a |
| Bicyclogermacrene | 1.9 | – | – |
| α-Muurolene | 0.1 a | 0.5 a | – |
| γ-Cadinene[b] | 0.1 a | 0.7 a | – |
| δ-Cadinene[c,d] | 0.7 a | 3.8 a | 0.2 b |
| Elemicin[b,d] | – | 8.8 | – |
| Spathulenol[b,d] | 3.7 a | 0.1 b | 0.1 b |
| Caryophyllene oxide[b,d] | 3.8 a | 0.3 a | – |
| Globulol | – | 0.1 | – |
| (Z)-Asarone | – | 0.4 | – |
| Humulene epoxide II | 0.6 | – | – |
| 5-*epi*−7−*epi*-α-Eudesmol | – | – | – |
| 1-*epi*-Cubenol | – | – | – |
| 1,10-di-*epi*-Cubenol | – | – | – |
| Muurola-4,10(14)-dien-1β-ol | 0.4 | – | – |
| *epi*-α-Muurolol | – | 0.2 | – |
| α-Muurolol | 0.1 a | 0.1 a | – |
| 14-hydroxy-(Z)-Caryophyllene | 0.4 | – | – |
| (E)-Asarone[d] | – | 21.8 | – |
| Monoterpene hydrocarbons[b] | 1.6 a | 1.5 a | 0.1 b |
| Oxygenated monoterpenes | 76.3 a | 2.7 b | 0.9 b |
| Sesquiterpene hydrocarbons[c] | 10.1 a | 32.0 a | 2.1 b |
| Oxygenated sesquiterpenes[c] | 8.9 a | 23.1 a | 0.1 a |
| Others[b] | 0.4 a | 0.3 a | – |
| Phenols[b] | 0.7 c | 39.2 b | 96.8 a |
| Yield (%, v/w) | 1.4 a | 0.6 b | 1.1 a |

[a] Data based on original values.
[b] Arcsine.
[c] Rank-transformed in ANOVA (see experimental).
[d] Selected for PCA. Averages followed by the same letter in a row were not significantly different at 5% probability by Tukey's test.

determined by cross-validation implemented in the SAS. Prior to the multivariate analysis, the data were preprocessed by means of auto-scaling and mean centering.

Multiple comparisons of means were established by one-way analysis of variance (ANOVA) using the PROC GLM procedure in SAS. All data were checked for homoscedasticity of variances with the Hartley test. The Tukey test was applied when a difference between means was established. *P*-values below 0.05 were regarded as significant.

## 3. Results and discussion

A total of 57 constituents were identified, accounting for 96–100% of the volatile constituents. The yields in percentage (v/w) and the qualitative and quantitative analytical results of twelve samples of *P. pseudocaryophyllus* essential oils are shown in Table 1 with oil constituents listed in order of elution on a CBP-5 column.

Phenols (0.2–94.3%), oxygenated monoterpenes (0.1–46.6%), and sesquiterpene hydrocarbons (0.2–26.6%) were the major constituents in most samples. Samples SGA1, SGA6 and SGA8 contained geranial (46.6%, 39.6%, and 37.3%, respectively) and neral (25.9%, 25.8%, and 28.7%, respectively) as the main constituents. Traces of these constituents were detected in SGA7 (1.0% and 0.8%, respectively) and SJB3 (0.1% for both). (E)-methyl isoeugenol was a major constituent in samples SGA2 (93.7%), SGA3 (61.3%), SGA4 (92.5%), SGA7 (91.5%), SJB1 (94.3%), SJB2 (93.8%), and BRA (93.6%). However, this constituent was only detected in small quantities in SGA1 (1.8%), SGA6 (0.4%) and SJB3 (5.0%). SGA5 contained a high percentage of (E)-caryophyllene (26.6%) and (E)-methyl isoeugenol (22.7%). (E)-Caryophyllene was the only constituent present in all samples and was the second most abundant constituent in samples SGA3 (13.3%) and SJB3 (8.5%). (E)-Asarone was the most abundant constituent (65.5%) in sample SJB3. Significant levels of elemicin were found in SGA3 (11.7%), SGA5 (8.8%) and SJB3 (5.8%).

Literature data have indicated citral and (E)-methyl isoeugenol are the major constituents of *P. pseudocaryophyllus* (Nakaoka-Sakita et al., 1994; Paula et al., 2010), but (E)-caryophyllene and (E)-asarone are listed for the first time as the major constituents in samples of this species. Eugenol and methyleugenol were the major constituents in *P. pseudocaryophyllus* samples collected from Cardoso Island and Paranapiacaba, both in São Paulo State, Brazil (Lima et al., 2006). Furthermore, chavibetol and methyleugenol have been cited as the main constituents in essential oils of this species in samples collected in the Ribeira Valley, in the southeastern region of Brazil (Santos et al., 2009).

The results obtained by principal component analysis (PCA) (Fig. 1) and cluster analysis (CA) using Ward's technique (Fig. 2) indicate large variability in the essential oil chemical composition, with 73.6% cumulative variance in the first factorial plane. PC-1 separated the group of terpenes, observed mainly on the right side of the Fig. 1, which were present in higher amounts in samples SGA1, SGA6 and SGA8 (cluster I) from phenolic constituents, observed mainly on the left side and detected at high levels in samples SJB and BRA (cluster II). On the other hand, PC-2 separated samples SGA3, SGA5 and SJB3 by high levels of elemicin, (E)-caryophyllene, δ-cadinene, and (E)-asarone (cluster III).

Thus, three clusters were obtained (Table 2): cluster I ($P < 0.001$; 25% of the samples: SGA1, SGA6 and SGA8) was characterized by high amounts of oxygenated monoterpenes (76.3 ± 2.2%) ($P < 0.0001$), geranial (41.2 ± 3.9%) ($P < 0.0001$), neral (26.8 ± 1.3%) ($P < 0.0001$), caryophyllene oxide (3.8 ± 2.5%) ($P < 0.004$), and spathulenol (3.7 ± 1.8%) ($P < 0.002$); cluster II ($P < 0.001$; 25% of the samples: SGA3, SGA5 and SJB3) was characterized by a high content of sesquiterpene hydrocarbons (32.0 ± 21.2%) ($P < 0.01$), (E)-asarone (21.8 ± 30.9%) ($P < 0.042$), (E)-caryophyllene (16.2 ± 7.7%) ($P < 0.003$), and elemicin (8.8 ± 2.4%) ($P < 0.001$); and cluster III ($P < 0.017$; 50% of the samples: SGA2, SGA4, SGA7, SJB1, SJB2 and BRA) was characterized by a substantial amount of phenols (96.8 ± 1.2%) ($P < 0.001$) and (E)-methyl isoeugenol (93.2 ± 1.0%) ($P < 0.001$).

To validate the clustered samples and assess the importance of chemical constituents as discriminating variables for clusters I-III, the canonical discriminant analysis (Wold and Eriksson, 1995) was performed using only geranial, (E)-caryophyllene and (E)-methyl isoeugenol as predictive variables. The first discriminant function (*F*1) was able to describe 93.6% of the total variance of the original data and was highly significant ($P < 0.0001$). *F*1 (degrees of freedom, $DF = 6$ and 14; Fischer value, $F = 59.1$) distinguished cluster I by a positive high score of geranial (1.0), whereas the second discriminant function (*F*2) ($P < 0.0003$; $DF = 2$ and 8; $F = 25.4$) discriminated cluster II by a high negative score of (E)-caryophyllene (−0.6). The results also indicate that it is possible to classify the samples with 89% accuracy by cross-validation with only these three constituents. The only misclassified sample (cluster II: SGA3) was misclassified due to its high content of (E)-methyl isoeugenol, a characteristic of the samples in cluster III.

Although edaphic and physiological factors are important for determining the chemical composition of essential oils (Cunha et al., 2005; Martins et al., 2006; Souza, 2009; Paula et al., 2010), the chemical variability observed in this study clearly indicates that genetic factors contribute to chemical polymorphism in this plant species. In the present work, the specimens from São Gonçalo do Abaeté were exposed to similar edaphoclimatic factors due to their geographical proximity and the three chemotypes were found concomitantly in this location. However, chemotype I (with a predominance of citral) was also detected in Campos do Jordão, São Paulo State, Brazil by Nakaoka-Sakita et al. (1994). It is therefore possible that genetic differences within species determine the expression of different metabolic pathways regardless of geographic location.

It is also necessary to highlight the occurrence of small quantities of the major component of a chemotype in other *P. pseudocaryophyllus* chemotypes. For example, (E)-metilisoeugenol, a major component of cluster III, was found in 11 of 12 samples analyzed, including SGA1 (1.8%) and SGA6 (0.4%), which belong to chemotype I. Likewise, traces of geranial and neral (citral), major components of chemotype I, were detected in SJB3 and SGA7, which belong to chemotypes II and III, respectively. Similarly, Abaul et al. (1995) observed that the estragole concentration in essential oils of *P. racemosa* var. *racemosa* of the chemotype "anise" was 33%, whereas its concentration was less than 0.2% in chemotypes "clove" and "lemon".

The genetic information for the synthesis of the main constituents of essential oils, especially phenylpropanoids, seems to be present in most samples, almost independently of chemotype. However, it is possible that a chemotype is characterized by differential expression of certain genes likely involved in encoding specific enzymes regulated by as yet unknown mechanisms. In this regard, Keszei et al. (2010) proposed the existence of three specific terpene synthases to explain the major chemotypes of *M. alternifolia*. However, according to Pichersky et al. (2006), specialized enzymes in secondary metabolism, including volatile oils, have a common property, the propensity to act on various substrates. Thus, the rate of volatile compound biosynthesis can be correlated with the concentration of substrates for these enzymes and/or with the levels of transcripts of the genes that encode the final enzymes.

There are probably other chemotypes of *P. pseudocaryophyllus* because chavibetol and methyleugenol (Santos et al., 2009), as well as eugenol and methyleugenol (Lima et al., 2006), have been reported as major constituents in essential oils. These data highlight the fact that most of the major constituents found in essential oils of *P. pseudocaryophyllus* are derived from phenylpropanoids, which are formed from shikimic acid. This suggests the preponderance of enzymes involved in this metabolic pathway.

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## References

Abaul, J., Bourgeois, P., Bessiere, J.M., 1995. Chemical composition of the essential oils of chemotypes of *Pimenta racemosa* var. *racemosa* (P. Miller) J. W. Moore (Bois d'Inde) of Guadaloupe (F. W. I.). Flavour Fragrance J. 10, 319–321.

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, fourth ed. Allured Publishing Corporation, Carol Stream, Illinois.

Azevedo, N.R., Campos, I.F.P., Ferreira, H.D., Portes, T.A., Seraphin, J.C., Paula, J.R., Santos, S.C., Ferri, P.H., 2002. Essential oil chemotypes in *Hyptis suaveolens* from Brazilian Cerrado. Biochem. Syst. Ecol. 30, 205–216.

Barazani, O., Cohen, Y., Fait, A., Diminshtein, S., Dudai, N., Ravid, U., Putievsky, E., Friedman, J., 2002. Chemotypic differentiation in indigenous populations of *Foeniculum vulgare* var. *vulgare* in Israel. Biochem. Syst. Ecol. 30, 721–731.

Benzécri, J.P., 1980. L'Analyse des Données: la Taxinomie, Tome 1. Dunod, Paris.

Carvalho-Filho, J.L.S., Blank, A.F., Alves, P.B., Ehlert, P.A.D., Melo, A.S., Cavalcanti, S.C.H., Arrigoni-Blank, M.F., Silva-Mann, R., 2006. Influence of the harvesting time, temperature and drying period on basil (*Ocimum basilicum* L.) essential oil. Braz. J. Pharmacogn. 16, 24–30.

Coheris Corp., 2002. SPAD Data Mining, v. 5.5 Software Program. Coheris Corp., Suresnes, France.

Cunha, A.P., Cavaleiro, C., Salgueiro, L., 2005. Fármacos aromáticos (plantas aromáticas e óleos essenciais). In: Cunha, A.P. (Ed.), Farmacognosia e Fitoquímica. Fundação Calouste Gulbenkian, Lisboa, pp. 339–401.

Duarte, A.R., Naves, R.R., Santos, S.C., Seraphin, J.C., Ferri, P.H., 2009. Seasonal influence on the essential oil variability of *Eugenia dysenterica*. J. Braz. Chem. Soc. 20, 967–974.

Echeverrigaray, S., Fracaro, F., Santos, A.C.A., Paroul, N., Wasum, R., Serafini, L.A., 2003. Essential oil composition of south Brazilian populations of *Cunila galioides* and its relation with the geographic distribution. Biochem. Syst. Ecol. 31, 467–475.

Grayer, R.J., Kite, G.C., Goldstone, F.J., Bryan, S.E., Paton, A., Putievsky, E., 1996. Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. Phytochemistry 43, 1033–1039.

Homer, L.E., Leach, D.N., Lea, D., Lee, L.S., Henry, R.J., Baverstock, P.R., 2000. Natural variation in the essential oil content of *Melaleuca alternifolia* Cheel (Myrtaceae). Biochem. Syst. Ecol. 28, 367–382.

Keszei, A., Hassan, Y., Foley, W.J., 2010. A biochemical interpretation of terpene chemotypes in *Melaleuca alternifolia*. J. Chem. Ecol. 36, 652–661.

Landrum, L.R., 1986. Flora Neotropica: Monograph 45 *Campomanesia*, *Pimenta*, *Blepharocalyx*, *Legrandia*, *Acca*, *Myrrhinium*, and *Luma* (Myrtaceae). Organization for Flora Neotropica, New York.

Landrum, L.R., Kawasaki, M.L., 1997. The genera of Myrtaceae in Brazil: an illustrated synoptic treatment and identification keys. Brittonia 49, 508–536.

Lima, H.R.P., Kaplan, M.A.C., Cruz, A.V.M., 2003. Influência dos fatores abióticos na produção e variabilidade de terpenóides em plantas. Floresta e. Ambiente 10, 71–77.

Lima, M.E.L., Cordeiro, I., Young, M.C.M., Sobra, M.E.G., Moreno, P.R.H., 2006. Antimicrobial activity of the essential oil from two specimens of *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (Myrtaceae) native from São Paulo State – Brazil. Pharmacologyonline 3, 589–593.

Loziene, K., Venskutonis, P.R., 2005. Influence of environmental and genetic factors on the stability of essential oil composition of *Thymus pulegioides*. Biochem. Syst. Ecol. 33, 517–525.

Mardia, K.V., Kent, J.T., Bibby, J.M., 1980. Multivariate Analysis. Academic Press, London.

Martins, F.T., Santos, M.H., Polo, M., Barbosa, L.C.A., 2006. Variação química do óleo essencial de *Hyptis suaveolens* (L.) Poit., sob condições de cultivo. Quim. Nova 29, 1203–1209.

Miguel, M.G., Duarte, F.L., Venâncio, F., Tavares, R., 2004. Comparison of the main components of the essential oils from flowers and leaves of *Thymus mastichina* (L.) L. ssp. *mastichina* collected at different regions of Portugal. J. Essent. Oil Res. 16, 323–327.

Nakaoka-Sakita, M., Aguiar, O.T., Yatagai, M., Igarashi, T., 1994. Óleo essencial de *Pimenta pseudocaryophyllus* var. *pseudocaryophyllus* (Gomes) Landrum (Myrtaceae) I: cromatografia a gás/espectrometria de massa (CC/EM). Rev. Inst. Flor. 6, 53–61.

National Institute of Standards and Technology, 1998. PC Version of the NIST/EPA/NIH Mass Spectral Data Base. U. S. Department of Commerce, Gaithersburg.

Paula, J.A.M., Paula, J.R., Bara, M.T.F., Ferri, P.H., Santos, S.C., Silva, L.H.S., 2010. Chemical differences in the essential oil of *Pimenta pseudocaryophyllus* (Gomes) L.R.Landrum leaves from Brazil. J. Essent. Oil Res. 22, 555–557.

Paula, J.A.M., Paula, J.R., Bara, M.T.F., Rezende, M.H., Ferreira, H.D., 2008. Estudo farmacognóstico das folhas de *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum – Myrtaceae. Rev. Bras. Farmacogn. 18, 265–278.

Paula, J.A.M., Paula, J.R., Pimenta, F.C., Rezende, M.H., Bara, M.T.F., 2009. Antimicrobial activity of the crude ethanol extract from *Pimenta pseudocaryophyllus*. Pharm. Biol. 47, 987–993.

Pichersky, E., Noel, J.P., Dudareva, N., 2006. Biosynthesis of plant volatiles: nature's diversity and ingenuity. Science 311, 808–811.

Potzernheim, M.C.L., Bizzo, H.R., Vieira, R.F., 2006. Análise dos óleos essenciais de três espécies de *Piper* coletadas na região do Distrito Federal (Cerrado) e comparação com óleos de plantas procedentes da região de Paraty, RJ (Mata Atlântica). Rev. Bras. Farmacogn. 16, 246–251.

Santos, B.C.B., Silva, J.C.T., Guerrero Júnior, P.G., Leitão, G.G., Barata, L.E.S., 2009. Isolation of chavibetol from essential oil of *Pimenta pseudocaryophyllus* leaf by high-speed counter-current chromatography. J. Chromatogr. A 1216, 4303–4306.

SAS Inc., 1996. Statistical Analysis System. Cary, NC, USA.

Souza, A., 2009. Variabilidade dos óleos voláteis de espécies de Myrtaceae nativas da Mata Atlântica. PhD. thesis, Universidade de São Paulo, São Paulo.
Souza, V.C., Lorenzi, H., 2005. Botânica Sistemática: Guia Ilustrado para a Identificação das Famílias de Angiospermas da Flora Brasileira, Baseado em APG II. Insituto Plantarum de Estudos da Flora, Nova Odessa.
Suárez, A., Ulate, G., Ciccio, J.F., 1997. Efectos de la administración aguda y subaguda de extractos de *Pimenta dioica* (Myrtaceae) en ratas albinas normotensas e hipertensas. Rev. Biol. Trop. 44, 39–45.
Thompson, J.D., Chalchat, J., Michet, A., Linhart, Y.B., Ehlers, B., 2003. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. J. Chem. Ecol. 29, 859–880.
Van Den Dool, H., Kratz, P.D., 1963. Generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. 11, 463–471.
Ward, J.H., 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. 58, 238–244.
Wheeler, G.S., Massey, L.M., Southwell, I.A., 2003. Dietary influences on terpenoids sequestered by the biological control agent *Oxyops vitiosa*: effect of plant volatiles from different *Melaleuca quinquenervia* chemotypes and laboratory host species. J. Chem. Ecol. 29, 189–208.
Wold, S., Eriksson, L., 1995. Statistical validation of QSAR results. In: Waterbeemd, H. (Ed.), Chemometric Methods in Molecular Design. VCH Publishers, Weinheim, pp. 309–318.