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Article

Chemotaxonomy of *Marsypianthes* Mart. ex Benth. Based on Essential Oil Variability

Márcia Y. Hashimoto,[a] Deomar P. Costa,[b] Maria T. Faria,[a] Heleno D. Ferreira,[a] Suzana C. Santos,[b] José R. Paula,[c] José C. Seraphin[d] and Pedro H. Ferri*[,b]

[a]Instituto de Ciências Biológicas, [b]Instituto de Química, [c]Faculdade de Farmácia and [d]Instituto de Matemática e Estatística, Universidade Federal de Goiás, CP 131, 74001-970 Goiânia-GO, Brazil

Os óleos essenciais de quatro espécies de *Marsypianthes* (Lamiaceae) foram investigados por meio de cromatografia gasosa e análise multivariada. Cada espécie foi representada por duas a sete populações, totalizando dezessete populações. β-Elemeno, (*E*)-cariofileno, α-humuleno, germacreno D, biciclogermacreno, δ-cadineno, espatulenol, óxido de cariofileno e globulol ocorreram em todas as amostras. As análises de componentes principais e de agrupamento hierárquico evidenciaram a presença de duas seções, uma contendo *M. chamaedrys/M. montana* (seção A) e a outra contendo *M. burchellii* (seção B). *M. foliolosa* apresentou maior complexidade, dividindo-se nas duas seções. Resultados similares foram obtidos de acordo com os esqueletos carbônicos biosssintéticos. Germacranos e biciclogermacranos preponderaram na seção A, enquanto aromadendranos e guaianos caracterizaram a seção B. A análise de redundância canônica mostrou que os agrupamentos não foram influenciados por variáveis edáficas dos locais de amostragem.

Essential oils of four species of *Marsypianthes* (Lamiaceae) were investigated via gas chromatography and multivariate analysis. Each species was represented by two to seven populations, totaling seventeen populations. β-Elemene, (*E*)-caryophyllene, α-humulene, germacrene D, bicyclogermacrene, δ-cadinene, spathulenol, caryophyllene oxide, and globulol were found in all samples. Principal component and hierarchical cluster analyses revealed the presence of two sections, one containing *M. chamaedrys*/*M. montana* (section A) and the other *M. burchellii* (section B). *M. foliolosa* showed higher complexity, being divided in both sections. Similar results were obtained according to biosynthetic carbon skeletons. Germacranes and bicyclogermacranes predominated in section A, whereas aromadendranes, bourbonanes and guaianes characterized section B. Canonical redundancy analysis revealed that clusters were not influenced by edaphic factors in sampling sites.

Keywords: *Marsypianthes*, essential oils, chemical variability, chemotaxonomy

Introduction

Essential oils comprise a class of natural products whose biosynthesis involves genetic control, even though environmental factors influence a wide variety of plant species.[1] This phenotypic plasticity often occurs under conditions of biotic or abiotic stress and plays an important role in an individual's adaptation to the environment. Adaptive characteristics of essential oils affect the structure of a community in terms of chemical, genetic, and ecological aspects.[2] Such knowledge of populational structure may thus contribute to chemotaxonomy, conservation, and management of plant species.[3]

In Brazilian Cerrado areas, the family Lamiaceae is represented mainly by subtribe Hyptidinea, tribe Ocimeae, whose taxonomic and floristic patterns resulted in endemic genera, forming a large number of new species.[4] Nine genera divided into two clades are known in the subtribe, one being represented by *Eriope* Humboldt. & Bonpl. ex Benth., *Hypenia* (Mart. ex Benth.) R. Harley and *Eriopidion* Harley, and the other containing *Hyptis* Jacq., *Peltodon* Pohl, *Rhaphiodon* Schau., *Asterohyptis* Epling, *Hyptidendron* Harley, and *Marsypianthes* Mart. ex Benth. Ten new genera have recently been suggested, as well as the incorporation of *Peltodon* into genus *Hyptis* section

*e-mail: pedro_ferri@ufg.br

Peltodon, based on morphological and molecular markers.[5]

Marsypianthes contains about five species, which grow in Brazil's Cerrado regions, extending into Paraguay and Argentina. Its species have been little studied regarding botanical and chemical aspects. *M. chamaedrys* (Vahl) Kuntze, a species distributed from Mexico and the Caribbean to Argentina, is the only representative to have its chemical data reported.[6,7] This species has been the object of several past studies, which researched biologically active constituents against snake bites and analgesic and anti-inflammatory actions;[8] moreover, it has been the only species investigated on the essential oil composition of the genus.[7]

Therefore, this research investigates the chemical constituents of essential oils of four *Marsypianthes* species collected from central Brazilian Cerrado by gas chromatography (GC/FID and GC/MS). Matrices containing chemical constituents and those from soil sampling sites were subjected to multivariate statistical techniques; this led to the detection of genetic variability patterns and to the assessment of the influence of the environmental gradient as contributions to the genus' chemotaxonomic classification.

Experimental

Botanical material

Marsypianthes spp. samples at the flowering stage were collected from October 2011 to December 2012 in Goiás State, Brazil. All species were collected from different sampling sites to assess the edaphic influence on oil compositions. Specimens were identified by one of the authors (M. Y. H.) and by Dr Raymond M. Harley from the Royal Botanic Gardens, Kew. Voucher specimens were deposited at the Conservation Unit of the Herbarium of Universidade Federal de Goiás (UFG), Goiás State, Brazil. A list of the taxa investigated as well as provenance and voucher specimens is shown in Supplementary Information (SI) (Table S1).

Extraction and essential oil analysis

To assess essential oils, 2-4 individuals from each species originated from 2-7 local populations were pooled and dried at room temperature for seven days at 30 °C until constant weight. After powdering, each sample's dried aerial part (10-30 g) was submitted to hydrodistillation (3 h) using a modified Clevenger-type apparatus. At the end of each distillation, oils were collected with hexane (0.5 mL) and dried with anhydrous Na_2SO_4, then transferred to glass flasks, where they were kept at a temperature of –18 °C.

A Varian CP3900 gas chromatograph equipped with a flame ionization detector (FID) was used for the compositional analysis of the essential oils. Samples (0.4 µL in hexane 20% v/v) were injected in the split mode in a DB-5 (J&W Scientific) fused silica capillary column of 30 m × 0.25 mm; 0.25 µm film thickness (5% phenylmethylpolisiloxane). The chromatographic conditions were as follows: injector port and detector temperature were 220 °C and 240 °C, respectively; column temperature was programmed from 60 °C to 246 °C at 3 °C min^{-1}, then 10 °C min^{-1} to 260 °C. The carrier gas was N_2 at a flow of 1.0 mL min^{-1}. The relative percentages of constituents were determined from their GC peak areas without correction factors. Gas chromatography-mass spectrometry (GC/MS) analyses were performed with a Shimadzu QP505A using a CBP-5 (Shimadzu) fused silica capillary column of 30 m × 0.25 mm; 0.25 µm film thickness (5% phenylmethylpolisiloxane) and maintaining a flow rate of 1.0 mL min^{-1} (helium); injector, interface, and programmed heating temperatures were the same as above. Samples' injection volume was 0.4 µL in hexane (20% v/v) with a 1:20 ratio. The analysis was conducted in scan mode at 70 eV, mass range of 40-400 *m/z*, and speed of 1.0 scan s^{-1}.

Identifying oil constituents involved comparing mass spectra and Arithmetic Indices (AI),[9] co-injection with commercial standards, and essential oils such as ylang-ylang (*Cananga odorata* (Lam.) Hook. F. & Thoms., Annonaceae) and clary sage (*Salvia sclarea* L., Lamiaceae). Arithmetic indices were calculated by linear hydrocarbon (C_8-C_{32}) co-injection and expressed as average retention index values.[10] GC results were expressed as a matrix containing the identified compounds (17 populations × 71 constituents) and the biosynthetic carbon skeletons of oil constituents (17 × 27) which were used in subsequent chemometric analyses.

Soil analysis

Three soil samples were also collected at a 0-20 cm depth around each sample and pooled together to form a composite sample for each local population; they were subsequently air-dried, thoroughly mixed, and sieved (2 mm). The portion finer than 2 mm was kept for physical and chemical analysis, resulting in a total of 16 parameters. The pH was determined in a 1:1 soil-water volume ratio. Ca^{2+}, Mg^{2+}, and Al^{3+} were extracted with 1 mol L^{-1} KCl, and P, K^+, Zn^{2+}, Cu^{2+}, Fe^{2+}, and Mn^{2+} were extracted using Mehlich's solution. Concentrations of K^+, Ca^{2+},

Mg^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+} were measured by flame atomic absorption spectrometry (AAS, Perkin Elmer), and phosphorous was determined by spectrophotometry (DU-70 Spectrophotometer, Bekmann). Organic matter (OM), cationic exchange capacity (CEC), potential acidity ($H^+ + Al^{3+}$), Al^{3+}, and soil texture (clay, sand, and silt) were determined by applying the usual methods,[11] and were arranged in a matrix (SI, Table S2) with 17 lines (populations) and 16 columns (soil variables).

Statistical analysis

The matrix containing the chemical constituents of essential oils was submitted to principal component analysis (PCA) using the SPAD package.[12] For the variable selection, the number of residual eigenvalues (≤ 0.70) was used to determine the maximum number of variables to be removed without significant alteration to the original data (17 × 71). The eliminated variables expressed the highest loadings in residual eigenvalues and contributed with ≤ 0.30% to the chemical profiles (mean values). PCA allowed the final matrix (17 × 50) to be projected on the first factorial plan, retaining a significant variance percentage in PC1 × PC2 axes. Subsequently, hierarchical clustering analysis (HCA) was applied to the study of similarity between individuals (populations) based on the distribution of chemical constituents using scores for the first ten PCA axes according to the SPAD default option. Nearest neighbour complete linkage technique by Benzécri algorithm was used as an index of similarity and hierarchical clustering was performed according to Ward's variance minimizing method.[13] This methodology was also applied to biosynthetic carbon skeletons. Canonical discriminant analysis (CDA) was used to validate clusters. CDA was conducted in the SAS.[14] The analysis of variance (ANOVA) was used for multiple comparisons of means in clusters. Homoscedasticity of variance was verified by Hartley's test using angular or rank transformation (when violated). When the difference between means was established in ANOVA, Tukey's test at 5% probability was applied. P-values < 0.05 were considered significant.

To assess environmental influence on essential oils' chemical variability, canonical redundancy analysis (RDA) was applied to examine the relationship between chemical and environmental matrices, i.e., essential oil constituents (response variables), conditioned by the characteristics of soil samples defined as explanatory variables (16 variables). RDA employed the CANOCO 5 package.[15] Prior to the multivariate analyses, oil constituents along soil texture (clay, sand, and silt) and organic matter were converted by angular transformation. Soil macro and micronutrients were transformed by log (x +1). All variables were preprocessed by mean centering and auto-scaling.

Results and Discussion

The chemical compositions of essential oils of four *Marsypianthes* species from 17 populations were analyzed by GC/FID and GC/MS. A total of 71 chemical constituents were identified with the majority consisting of terpenes, of which 21 were monoterpenes, 43 were sesquiterpenes, and 7 included other constituents (Table 1). Among those identified, only 9 were observed for all samples: β-elemene (**29**), (*E*)-caryophyllene (**32**), α-humulene (**35**), germacrene D (**39**), bicyclogermacrene (**42**), δ-cadinene (**47**), spathulenol (**54**), caryophyllene oxide (**55**) and globulol (**57**). Germacrene D (total mean value of 18.68 ± 13.77%), spathulenol (18.54 ± 16.00%), and bicyclogermacrene (13.46 ± 13.75%) were the main constituents in the data set.

When analyzing the distribution of chemical constituents in different populations, *trans*-limonene oxide (**15**), acora-3,7(14)-diene (**31**), *allo*-aromadendrene (**37**), and α-acorenol (**63**) occurred in a single populations, whereas β-pinene (**5**), α-copaene (**26**), β-bourbonene (**28**), and α-cadinol (**67**) were absent from one population (Mmo2). These unique occurrences (absence) in terpenoid biosynthesis may be considered positive (negative) autapomorphies, and their evolution in species represents the emergence of an additional substance or the loss of a substance always present.[16] These changes may also result from alterations in terpene synthases, in which some terpenes are redirected over others, as has been suggested by some researchers.[17] Nevertheless, it is possible that low terpenoid concentrations are currently traces of substances that have functioned in the past against herbivores.[18] In this sense, essential oil chemical variability may contribute to the phylogeny and chemotaxonomy of the genus *Marsypianthes*. In fact, chemical polymorphism in essential oils has helped to identify taxonomic relationships in various Lamiaceae genera, as well as intraspecific variability when analyzing more than one population *per* taxon.[19]

To investigate chemical variability patterns, PCA followed by HCA were applied on chemical constituents of essential oils (Figure 1). Results showed that the first factorial plan retained 34.8% of total variance in the data set, which formed five natural sample clusters. In the PC1 axis, populations rich in sesquiterpene hydrocarbons (69.6 ± 12.8%, $p = 0.001$), SH (Mch1–Mch6, Mfol3, Mfol4, Mfol7 and Mmo1/Mmo2), were separated from those rich in oxygenated sesquiterpenes (58.9 ± 22.6, $p = 0.002$), SO (Mbu1/Mbu2 and Mfol1/Mfol2/Mfol5/Mfol6), whereas

Table 1. Chemical composition of essential oils from 17 populations of *Marsypianthes* Mart. ex Benth. in central Brazilian Cerrado

Constituent	AI[b]	*M. burchellii*		*M. chamaedrys*						*M. foliolosa*							*M. montana*		Identification[c]
		Mbu1	Nbu2	Mch1	Mch2	Mch3	Mch4	Mch5	Mch6	Mfol1	Mfol2	Mfol3	Mfol4	Mfol5	Mfol6	Mfol7	Mmo1	Mmo2	
1 Tricyclene	919	t	–	–	0.21	0.01	0.01	–	t	0.57	0.34	t	0.04	t	t	–	0.12	–	A
2 α-Pinene	929	0.06	1.16	0.10	–	0.02	0.05	0.07	0.21	0.90	–	0.30	0.71	0.45	–	t	–	–	A, B
3 Camphene[c]	944	t	0.61	0.10	–	–	–	0.02	–	3.57	t	0.09	1.10	0.65	0.41	–	t	–	A
4 Sabinene	969	–	0.06	0.20	–	–	–	0.03	–	–	–	0.08	–	–	–	–	0.08	–	A
5 β-Pinene[c]	973	1.76	1.68	0.81	0.47	1.60	1.52	0.15	0.80	1.59	t	3.56	3.20	2.78	0.24	t	0.37	–	A, B
6 2-Pentylfuran	986	0.01	0.99	–	–	–	–	–	–	–	0.23	–	–	–	–	–	–	–	A
7 Myrcene[c]	987	–	–	0.43	0.16	0.07	0.02	1.19	0.34	0.73	t	0.36	0.33	0.16	–	–	–	–	A, D
8 Limonene[c]	1024	t	0.46	2.35	0.21	0.38	–	2.42	2.21	0.86	0.42	0.07	t	–	–	–	0.08	–	A, B
9 1,8-Cineole	1028	t	1.46	–	–	0.17	–	–	0.06	t	–	–	–	–	–	–	–	–	A, B
10 (*Z*)-β-Ocimene[c]	1033	–	–	–	–	0.15	0.05	0.05	t	–	–	0.22	0.17	0.06	–	0.09	t	–	A
11 (*E*)-β-Ocimene[c]	1043	–	0.20	0.25	0.58	1.15	1.08	0.82	0.23	–	–	3.29	2.14	0.77	–	2.30	0.43	0.88	A
12 Linalool[c]	1096	0.42	0.43	–	0.24	0.04	0.02	–	0.06	1.03	0.48	0.18	–	0.27	0.24	t	t	–	A, D
13 *n*-Nonanal	1100	–	0.14	0.10	–	–	0.21	–	0.01	0.27	0.76	t	0.01	0.04	t	t	t	–	A
14 *trans*-Pinocarveol	1135	0.25	–	–	–	–	–	–	–	1.10	t	t	t	t	t	t	–	–	A
15 *trans*-Limonene oxide	1140	–	–	–	–	–	–	–	–	0.59	–	–	–	–	–	–	–	–	A
16 *trans*-Sabinol	1142	0.04	–	–	–	–	–	–	–	0.71	–	t	t	t	0.46	t	–	–	A
17 Pinocarvone	1158	–	–	–	–	–	–	–	–	0.27	0.06	t	t	t	t	t	–	–	A
18 Borneol[c]	1162	–	–	–	–	–	–	–	0.32	5.16	0.14	t	t	t	t	t	t	–	A
19 Naphthalene	1177	–	0.39	0.10	–	–	–	–	–	t	0.75	–	–	–	–	–	–	–	A, B
20 α-Terpineol	1188	0.08	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A, D
21 Myrtenol	1192	0.14	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A
22 Myrtenal	1194	0.06	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A
23 *cis*-hydrosabinene acetate	1224	–	–	0.27	–	–	t	t	0.72	0.86	t	0.12	0.28	0.01	t	–	t	–	A
24 Isobornyl acetate[c]	1282	–	–	t	t	t	0.02	0.01	t	2.83	t	0.06	–	0.37	t	t	t	–	A
25 δ-Elemene[c]	1334	t	t	t	1.68	0.43	2.16	0.91	0.59	0.97	t	0.10	t	0.04	t	–	2.14	–	A
26 α-Copaene[c]	1373	1.03	1.48	2.10	4.03	2.13	4.18	4.17	2.16	1.39	t	0.54	0.52	0.18	t	t	0.54	–	A, C
27 β-Cubebene[c]	1382	t	t	0.25	0.41	0.43	0.98	0.76	0.21	0.11	0.24	t	0.24	0.10	–	–	t	–	A
28 β-Bourbonene[c]	1384	1.63	3.60	3.25	0.35	3.80	2.53	2.16	1.66	4.09	11.13	2.26	4.04	5.51	2.95	0.26	1.61	–	A
29 β-Elemene[c]	1390	t	0.19	0.53	0.82	1.53	1.00	0.90	0.61	t	0.68	0.91	1.02	t	t	t	0.73	0.81	A, C
30 Longifolene[c]	1412	t	8.64	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A
31 Acora-3,7(14)-diene[c]	1412	–	7.33	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A
32 (*E*)-Caryophyllene[c]	1417	0.53	7.98	14.39	12.98	7.31	9.57	17.26	7.41	6.11	2.91	12.94	6.18	1.42	t	19.22	10.07	6.82	A, B
33 β-Copaene[c]	1427	–	–	0.49	–	0.31	0.23	0.25	0.18	t	t	0.16	0.37	0.18	t	t	t	–	A, B
34 α-*trans*-Bergamotene[c]	1434	–	–	0.51	0.42	t	0.97	0.73	0.35	t	–	–	–	–	–	0.06	t	–	A
35 α-Humulene[c]	1452	0.11	1.58	2.31	2.88	1.18	2.05	3.36	1.31	t	0.40	1.44	0.58	0.17	1.14	2.68	0.55	0.69	A, C
36 Geranylacetone	1452	–	–	t	t	0.63	t	–	–	t	1.10	–	–	0.44	–	0.18	–	–	A
37 *allo*-Aromadendrene[c]	1453	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.40	A, B
38 γ-Gurjunene[c]	1472	0.42	3.78	t	–	–	–	–	–	t	1.39	–	–	–	0.65	–	t	–	A
39 Germacrene D[c]	1483	1.29	23.49	22.52	25.90	15.74	17.35	46.06	25.45	1.05	2.13	39.57	34.92	5.56	1.67	4.83	26.53	23.52	A, C
40 (*E*)-β-Ionone	1485	–	–	–	–	–	–	–	–	t	0.65	–	–	–	–	–	–	–	A
41 β-Selinene[c]	1487	0.85	0.14	0.38	0.24	0.11	0.79	–	0.54	0.42	0.53	–	–	t	–	–	0.77	1.71	A
42 Bicyclogermacrene[c]	1497	3.11	4.33	8.54	16.95	15.40	17.45	3.17	10.47	1.15	1.60	2.35	17.60	8.73	0.92	34.23	30.33	52.50	A
43 α-Muurolene	1497	1.74	0.06	–	–	–	–	–	–	0.28	–	–	–	–	–	–	–	–	A
44 Germacrene A[c]	1505	t	1.04	1.33	2.09	1.58	1.79	2.96	1.10	1.26	t	1.40	–	–	0.21	0.70	1.25	1.50	A
45 γ-Cadinene[c]	1511	0.46	0.24	t	0.78	t	t	t	–	0.87	t	–	–	1.35	t	0.13	0.46	–	A
46 6-Methyl-α-ionone[c]	1517	–	–	t	–	–	–	–	–	6.21	t	–	–	–	–	–	t	–	A
47 δ-Cadinene[c]	1521	5.22	2.54	1.04	3.61	0.72	2.39	3.11	2.06	2.03	0.62	1.21	0.75	1.03	0.22	0.25	1.85	0.74	A, C
48 Zonarene	1535	–	–	–	1.02	–	–	–	–	t	–	–	–	–	–	0.06	–	–	A
49 *cis*-Sesquisabinene hydrate[c]	1547	–	–	1.17	0.21	–	–	–	t	1.24	0.85	1.28	t	t	–	–	–	–	A
50 Germacrene B[c]	1555	0.46	0.06	–	1.11	2.93	0.83	4.65	0.78	–	–	7.04	t	0.46	1.06	0.24	t	5.24	A
51 1-*nor*-Bourbonanone[c]	1557	0.25	0.07	0.46	t	t	–	–	–	1.15	3.22	–	t	t	3.29	–	–	–	A
52 (*E*)-Nerolidol	1565	–	–	0.46	–	–	–	–	–	t	t	–	–	–	–	t	t	–	A
53 Palustrol[c]	1566	2.34	2.07	–	–	–	–	–	–	1.53	t	–	–	–	0.52	0.16	t	–	A
54 Spathulenol[c]	1577	40.69	1.90	16.60	7.69	17.36	12.60	0.61	25.32	17.83	37.80	1.16	8.80	43.69	53.40	21.89	5.65	2.23	A, B
55 Caryophyllene oxide[c]	1582	6.14	4.04	13.24	6.48	6.65	7.34	0.75	7.35	16.90	14.44	9.47	5.15	9.29	11.80	5.20	3.37	1.49	A, B

Table 1. continuation

Constituent	AI[b]	*M. burchellii*		*M. chamaedrys*						*M. foliolosa*							*M. montana*		Identification[c]
		Mbu1	Nbu2	Mch1	Mch2	Mch3	Mch4	Mch5	Mch6	Mfol1	Mfol2	Mfol3	Mfol4	Mfol5	Mfol6	Mfol7	Mmo1	Mmo2	
56 Tujopsan-2α-ol	1586	–	–	–	–	–	–	–	–	2.35	t	–	–	–	–	–	t	–	A
57 Globulol[c]	1589	15.34	4.78	1.32	0.72	0.81	1.27	t	1.60	5.17	0.61	2.40	0.47	2.60	1.89	1.32	0.64	0.37	A
58 Ledol[c]	1601	2.63	0.02	0.18	t	–	–	–	–	0.33	0.24	–	–	0.80	0.58	–	0.26	–	A
59 β-Atlantol[c]	1602	–	–	t	t	–	–	–	–	t	t	–	–	–	–	–	0.27	–	A
60 Humulene epoxide II[c]	1607	1.50	0.23	1.96	0.63	0.50	0.92	t	1.59	1.07	1.26	0.37	0.14	1.75	2.44	0.93	t	–	A
61 1,10-di-*epi*-Cubenol[c]	1623	–	–	t	0.36	0.34	0.48	t	0.21	0.53	0.40	–	–	t	0.84	0.08	–	–	A
62 Muurola-4,10(14)-dien-1β-ol[c]	1627	0.79	–	–	1.39	1.76	4.14	0.54	0.45	t	t	t	0.33	1.09	1.01	t	1.73	–	A
63 α-Acorenol[c]	1634	–	–	–	–	11.65	–	–	–	–	–	–	–	–	–	–	–	–	A
64 *epi*-α-Cadinol[c]	1640	1.58	0.03	–	1.77	t	0.83	0.29	0.77	0.84	t	3.07	2.00	1.15	2.43	2.00	t	–	A
65 *allo*-Aromadendrene epoxide[c]	1640	–	–	–	–	–	–	–	–	t	t	–	–	t	t	–	0.42	–	A
66 α-Muurolol[c]	1640	4.12	0.31	–	t	–	–	–	–	t	t	0.58	–	0.92	t	–	0.29	–	A
67 α-Cadinol[c]	1652	2.68	0.21	0.38	0.86	0.69	0.56	1.00	0.41	1.05	1.18	0.92	1.11	0.64	1.23	0.44	0.67	–	A
68 14-Hydroxy-9-*epi*-(*E*)-caryophyllene[c]	1669	0.37	0.09	1.17	0.32	0.32	0.73	0.11	0.87	1.18	1.33	t	0.12	0.44	1.58	0.81	–	–	A
69 Mustakone[c]	1677	0.62	0.14	0.38	0.60	0.46	1.04	0.15	1.00	1.26	1.40	–	–	t	0.73	–	–	–	A
70 Germacra-4(15),5,10(14)-trien-1α-ol[c]	1683	–	t	0.25	t	–	–	–	t	t	t	–	–	–	–	–	2.90	–	A
71 Eudesma-4(15),7-dien-1β-ol[c]	1688	0.03	t	0.07	–	–	0.64	0.68	0.21	t	t	0.76	1.19	0.35	0.67	0.23	0.45	–	A
Monoterpene hydrocarbons (MH)[d]		1.82	4.17	4.24	1.63	3.38	2.73	4.75	3.79	8.22	0.76	7.97	7.69	4.87	0.65	2.39	1.08	0.88	
Oxygenated monoterpenes (OM)[d]		0.91	1.89	0.27	0.24	0.21	0.04	0.01	1.16	12.55	0.68	0.36	0.28	0.65	0.70	–	–	–	
Sesquiterpene hydrocarbons (SH)[d]		16.85	66.48	57.64	75.27	53.60	64.27	90.45	54.88	19.73	21.63	69.92	66.22	24.73	8.82	62.66	76.83	93.93	
Oxygenated sesquiterpenes (OS)[d]		79.08	13.89	37.64	21.03	40.54	30.55	4.13	39.78	52.43	62.73	20.01	19.31	62.72	82.41	33.06	16.65	4.09	
Others (OU)[d]		0.01	1.52	0.20	–	0.63	0.21	–	0.01	6.48	3.49	–	0.01	0.48	–	0.18	–	–	
Monoterpenes (M)[d]		2.73	6.06	4.51	1.87	3.59	2.77	4.76	4.95	20.77	1.44	8.33	7.97	5.52	1.35	2.39	1.08	0.88	
Sesquiterpenes (S)[d]		95.93	80.37	95.28	96.30	94.14	94.82	94.58	94.66	72.16	84.36	89.93	85.53	87.45	91.23	95.72	93.48	98.02	

[a]Percentage values; [b]average arithmetic index;[10] [c]selected for PCA/HCA; [d]supplementary variables in PCA; t = trace; – = not detected ; [c]the reliability of the identification or structural proposal is indicated by: A-mass spectrum and arithmetic index consistent with those found in literature;[9] B-mass spectrum and retention time consistent with standard; C-mass spectrum and retention time consistent with those of ylang-ylang (*Cananga odorata*) essential oil;[9] D-mass spectrum and retention time consistent with those of clare sagy (*Salvia sclarea*) essential oil.[9]

PC2 distinguished Mch1-Mch6 and Mfol1/Mfol2/Mfol6 according to the highest monoterpene content (M). Thus, five clusters were obtained by PCA/HCA: I, with all *M. chamaedrys* populations; II, incorporating populations Mfol3-Mfol5 and Mfol7 of *M. foliolosa*; III, representing *M. montana*; IV, separating *M. burchellii* and V, containing the remaining populations of *M. foliolosa* (Mfol1, Mfol2, and Mfol6).

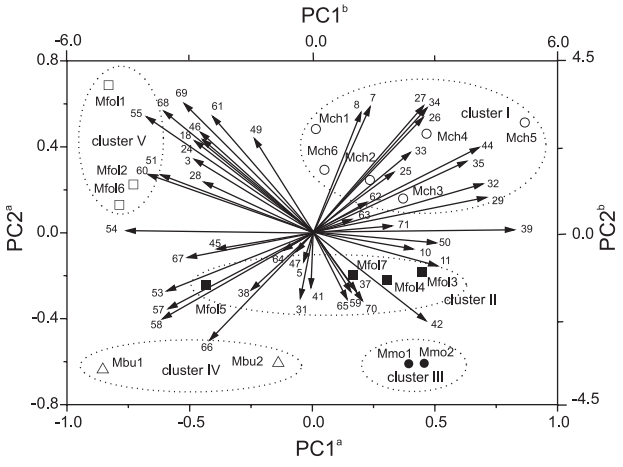

Figure 1. PCA biplot displaying chemical constituents of *Marsypianthes* essential oils according to the clusters defined by HCA: I (○), II (■), III (●), IV (△), and V (□). Oil constituents are represented by vectors starting from the origin. Essential oil constituent codes are in accordance with Table 1. *Marsypianthes* species: Mbu = *M. burchellii*, Mch = *M. chamaedrys*, Mfol = *M. foliolosa*, Mmo = *M. montana*.

The similarity between populations shown by the HCA dendrogram is represented by Figure 2. *M. burchellii* and about half of *M. foliolosa* populations showed great similarity (section B), whereas *M. chamaedrys*, *M. montana*, and other populations of *M. foliolosa* were clustered in section A. The division of *M. foliolosa* populations is consistent with the greater complexity of this species.[5]

In fact, quantitative differences in essential oil composition exist among clusters. Cluster I is mainly characterized by the accumulation of (*E*)-caryophyllene (**32**) (11.49 ± 3.69%, p = 0.048) and α-copaene (**26**) (3.13 ± 1.00%, p = 0.0001); cluster II showed the highest contents of β-pinene (**5**) (2.39 ± 1.40%, p = 0.009) and (*E*)-β-ocimene (**11**) (2.13 ± 0.90%, p = 0.001); cluster III revealed high contents of bicyclogermacrene (**42**) (41.42 ± 11.09%, p = 0.001); cluster IV had the highest

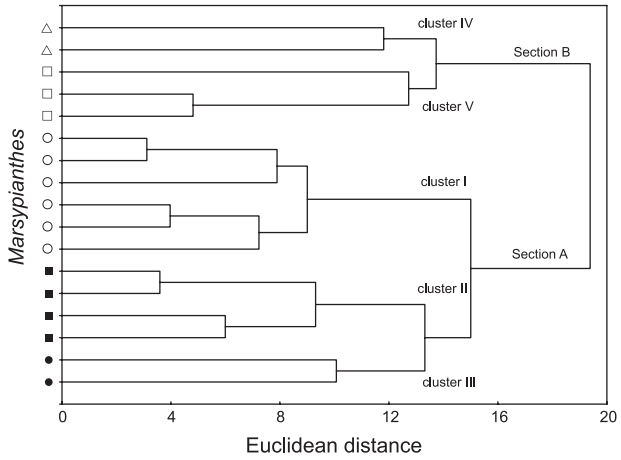

Figure 2. HCA dendrogram of similarity between *Marsypianthes* Mart. ex Benth. populations according to essential oil constituents: cluster I (○), II (■), III (●) IV (△), and V (□), and their chemical sections A and B.

levels of globulol (**57**) (10.06 ± 5.28%, p = 0.001) and δ-cadinene (**47**) (3.88 ± 1.34%, p = 0.008); cluster V featured high levels of spathulenol (**54**) (36.34 ± 14.56%, p = 0.020) and caryophyllene oxide (**55**) (14.38 ± 2.08%, p = 0.002).

The validation of the HCA results was obtained by canonical discriminant analysis (CDA). An axial representation of CDA discriminated all clusters based only on the contents of (*E*)-β-ocimene (**11**), α-copaene, β-selinene (**41**), 1-*nor*-bourbonanone (**51**) and palustrol (**53**), as predictor variables (Table 2).

CDA model showed high canonical correlation (R_{F1} = 0.992, R_{F2} = 0.930) and a low value for Wilks' lambda ($\Lambda_{(F1)}$ = 0.0002, $\Lambda_{(F2)}$ = 0.0138), thus demonstrating the excellent ability of predictor variables on clusters differentiation. Discriminant functions F1 and F2 differentiated (p < 0.0001) cluster IV due to positive palustrol scores, whereas cluster I was distinguished by its high negative (F2) α-copaene score. Cluster V was characterized by high positive (F1) and negative (F2) scores for 1-*nor*-bourbonanone and β-selinene, respectively. In turn, increasing levels of (*E*)-β-ocimene distinguished clusters II from III (SI, Figure S1). It was also possible to make an accurate prediction of 88% correct classification in the original clusters by cross-validation approach. This technique consider a slightly reduced number of samples from the parent data set, estimate parameters from each of these modified data sets, and then calculate the precision of predictions for the samples previously removed by the resulting models. Two samples belonging to clusters I and V were classified as mismatched, because they had different contents of α-copaene and 1-*nor*-bourbonanone, respectively, which is typical of such clusters. Percentages of oil constituents in clustered samples are shown in SI (Table S3).

In another analysis of sample classification, chemical constituents were reorganized according to biosynthetic carbon skeletons. This strategy reduces the uncontrolled factors affecting oil quantitative variations and may assimilate the overall trends in terpenoid biosynthesis in essential oils from *Marsypianthes* populations in a more satisfactory way. The normalized percentage of carbon skeletons (SI, Table S4) showed a preponderance of aromadendranes (mean 22.7 ± 19.3%), germacranes (22.1 ± 16.0%), caryophyllanes (17.1 ± 5.85%), and bicyclogermacranes (13.9 ± 14.0%) in *Marsypianthes* oils. The analysis of PCA/HCA applied to this matrix led to the same differences between *M. chamaedrys*/*M. montana* and *M. burchellii*, with *M. foliolosa* being divided in the two

Table 2. CDA summary for clustered *Marsypianthes* Mart. ex Benth. populations

A. Canonical function	Eigenvalue	Relative percentage	Canonical correlation	Wilks' lambda (Λ)	χ^{2a}	DF^b	P
F1	61.544	85.2	0.992	0.0002	92.62	20; 27	0.0001
F2	6.396	8.9	0.930	0.0138	47.13	12; 24	0.0001
B. Standardized coefficient	(*E*)-β-Ocimene	α-Copaene	β-Selinene	1-*nor*-Bourbonanone	Palustrol		
F1	–0.08	0.35	0.06	2.17	2.04		
F2	0.57	–1.04	–0.24	–0.33	0.33		
C. Cluster baricenter	I	II	III	IV	V		
F1	–3.49	–5.04	–4.86	10.06	10.23		
F2	–2.44	2.96	0.65	1.52	–0.51		
D. Cluster validation	Percentage of well-classification						
	I	II	III	IV	V	Total	
	100	75	100	100	67	88	

[a]Chi-square; [b]degrees of freedom; total samples = 17; cluster: I (n = 6): Mcha1-Mcha6; II (n = 4): Mfol3-Mfol5, Mfol7; III (n = 2): Mmo1, Mmo2; IV (n = 2): Mbu1, Mbu2; V (n = 3): Mfol1, Mfol2, Mfol6.

sections (SI, Figure S3), as previously defined. The latter presented a composition similar to that observed with chemical constituents as variables, although population Mfol5 did not follow the same trend.

These results support the existence of two chemical sections for *Marsypianthes*. In section A, germacranes (30.1 ± 12.8%, p = 0.003) and bicyclogermacranes (19.5 ± 14.5%, p = 0.015) were the most prevalent, whereas section B was characterized by higher values of aromadendranes (41.9 ± 18.9%, p = 0.002), bourbonanes (6.68 ± 4.47%, p = 0.008) and guaianes (1.16 ± 1.50%, p = 0.017). Elemanes, bergamotanes and camphanes, despite minor values, proved important for chemotaxonomy, leading to 94% correct classification of samples between sections A and B using CDA ($\Lambda_{(F1)}$ = 0.409, p = 0.021; canonical correlation, R_{F1} = 0.769). Section A was marked by the absence of guaianes, as well as the highest levels of elemanes (1.57%) and bergamotanes (0.28%), whereas these biosynthetic carbon skeletons showed the lowest content (elemanes) or absence (bergamotanes) in section B.

To evaluate environmental influence on essential oil variability, especially on *M. foliolosa* populations, RDA was performed assuming oil constituents as response variables, which in turn were conditioned by soil characteristics as explanatory variables. In RDA, the oil-environmental correlation equals the correlation between sampled site scores that are weighted sums of oil and site scores, which in turn are a linear combination of environmental variables.[20] RDA canonical axis is similar to PCA, but it has a restriction on sampled site scores.

RDA results indicated that edaphic factors have not been able to explain chemical variability in all *Marsypianthes* species (p = 0.663) or in the subset comprising only *M. foliolosa* populations (p = 0.728). This finding suggests the presence of two *M. foliolosa* chemotypes. However, populations in cluster I (*M. chamaedrys*) may be associated with a higher pressure of herbivory, due to the well-known defensive action of (*E*)-caryophyllene, found in higher amounts in the essential oils from this cluster's samples.[21] Contents of the main chemical constituents of *M. chamaedrys* were similar to those described for the essential oils of this species collected in northeastern Brazil.[7]

The influence of environmental and genetic factors on the chemical variability of essential oils is widely known.[1] The occurrence of chemotypes,[22] ecotypes,[23] and biotypes has been described in native central Cerrado species,[24] specially in Goiás State. Additionally, terpenes have been described as chemomarkers in other genera, such as *Helichrysum* (Asteraceae) and *Curcuma* (Zingiberaceae),[25] and have proved particularly useful for accessing the taxonomy of Lamiaceae.[3,19,26]

Results suggest the need for an anatomical study of *M. foliolosa* in view of the significant differences found in the chemical composition of essential oils between the clustered populations. These differences in essential oils also suggest a possible division of the genus into two chemical sections, which may contribute to the taxonomy of the genus, whose species have been the object of few studies as regards morphological and anatomical aspects. In addition, differences in oil composition may prove useful towards better understanding phylogenetic relationships in the subtribe Hyptidinae.

Conclusion

Essential oil chemical variability from the aerial parts of 17 populations, distributed in four *Marsypianthes* species revealed high polymorphism, which is related to genetic influences. Results indicated that clustered samples based on multivariate analyses of oil chemovariations support the division of species into two taxonomic sections. *M. burchellii* differed from *M. chamaedrys*/*M. montana*, whereas *M. foliolosa* populations were divided in the two sections, a finding which suggests that the latter species may be submitted to further botanical investigation.

Supplementary Information

Supplementary data (Figures S1-S3 and Tables S1-S4) is available free of charge at http://jbcs.sbq.org.br as a PDF file.

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