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Chemical variability of the essential oils from fruits of *Pterodon emarginatus* in the Brazilian Cerrado

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Abstract: *Pterodon emarginatus* Vogel, Fabaceae, is a tree species commonly known as "sucupira-branca". It is a popular medicinal plant in the Brazilian cerrado (Savanna). This study investigates the chemical variability of the essential oils from fruits of *P. emarginatus*. The fruits were collected from five sites in the Brazilian Cerrado and their essential oils were analyzed by GC/MS. The results obtained by Principal Component and Cluster Analysis identified two groups: cluster I containing β-caryophyllene and δ-elemene and cluster II containing α-copaene, β-cubebene, allo-aromadendrene, α-cubebene and γ-muurulene. The Canonical Discriminant Analysis was used to differentiate between clusters on the basis of essential oil composition. The results showed high variability in the composition of the essential oils from fruits of *P. emarginatus*, contributing to studies of domestication of this species.

Introduction

The Fabaceae family, also known Leguminoseae, is one of the largest families among dicotyledons in Brazil with 3200 species distributed in three subfamilies: Papilionoideae, Caesalpinoideae and Mimosoideae. It is well represented in all major Brazilian biomes (Giulietti et al., 2005), being the most representative family in the Cerrado with around 777 species from 101 genera (Mendonça et al., 1998).

According to the Brazilian flora list, the genus *Pterodon* consists of four species distributed in Brazil: *Pterodon abruptus* Vogel, *P. apparicioi* Pederdoli, *P. emarginatus* Vogel (synonym *Pterodon polygalaeflorus* Benth) and *Pterodon pubescens* Benth (Lima, 2012). Although these species have been extensively studied from the phytochemical aspect (Spindola et al., 2009; 2010), there are few studies in the literature on essential oils yield and their chemical variability from species growing in different localities.

Pterodon emarginatus Vogel, Fabaceae, is popularly known in Brazil as "sucupira-branca" or "faveiro". It is a native tree reaching 5-10 m in height (Lorenzi & Matos, 2002), easily found in the Brazilian Cerrado, blooms between April and May, fruit between May and June and releases its fruits between June and August (Mascaro et al., 2004). Fruits of *P. emarginatus* are widely used in folk medicine for the treatment of rheumatism, sore throat, respiratory dysfunction (bronchitis and tonsillitis), beyond from their activities anti-inflammatory and analgesic (Agra et al., 2008; Dutra et al., 2009a; 2009b; Leite de Almeida & Gottlieb, 1975). Previous studies have demonstrated antimicrobial activity of crude ethanolic extract (Santos et al., 2010) and of essential oils (Alves, 2012; Dutra et al., 2009a; 2009b), as well as anti-inflammatory and antinociceptive effects of the ethanolic extract (Moraes et al., 2009; 2012; Oliveira et al., 2012).

Environmental factors can affect the development of plants; particularly of aromatic species, influencing on the percentage and composition of essential oils (Botrel et al., 2010). Genetic factors also have an important contribution to the occurrence of chemical polymorphism in plant species. A same population of plants undifferentiated morphologically and sexually compatible may present individuals producers of essential oils with different chemical composition and distinguishable scent (Gobbo Neto & Lopes, 2007; Paula et al., 2011). Essential oils extracted may change qualitative and quantitative the chemical composition according to the climate factors, soil composition, plant organ, age, seasonality and phase of the circadian cycle (Angioni et al., 2003; Masotti et al., 2003; Bakkali et al., 2008). These variations influence directly the quality of the medicinal plant.

In this work, we report on the chemical variability of essential oils observed in *P. emarginatus* fruits. Qualitative and quantitative analysis of the volatile oils of eleven individuals that naturally occur in five different sites in the central Brazilian Cerrado were performed by GC/MS. The chemical constituents were submitted to multivariate analysis, in order to study its intra-specific variation.

Material and Methods

Plant material

Fruits of eleven individuals of *Pterodon emarginatus* Vogel, Fabaceae, were collected after spontaneous fall in Correntina in the state of Bahia (13º 22’ 50” S/ 44º 37’ 02” W, 561 m) in September, 2007 (sample PES-1); Campestre in the state of Goiás (16º 46’ 01” S/ 49º 42’ 06” W, 612 m) in September, 2009 (sample PES-2); Jussara in the state of Goiás (15º 43’ 11” S/ 50º 52’ 11” W, 319 m) in September, 2007 (samples JUS-1, JUS-2, JUS-3); Nova América in the state of Goiás (15º 00’ 50” S/ 49º 59’ 03” W, 712 m) in September, 2009 (samples NA-1, NA-2, NA-3, NA-4, NA-5); and Jaciara in the state of Mato Grosso-Brazil (15º 58’ 03” S/ 54º 54’ 51” W, 367 m) in September, 2007 (sample MTOE). The plants were identified by Dr. José Realino de Paula of the Universidade Federal de Goiás. Voucher specimens were deposited in the Herbarium of Universidade Federal de Goiás (UFG), Goiás State, Brazil.

Essential oil extraction

Fruits of *P. emarginatus* (60 g) were pulverized in a knife mill and submitted to hydrodistillation in a modified Clevenger-type apparatus (4 h). Each essential oil was dried over anhydrous sodium sulfate and stored at -20 ºC for further analysis.

Essential oil analyses

The GC/MS analysis was performed on Shimadzu QP5050A instrument. The column, a CBP-5 (Shimadzu) fused silica capillary column (30 m long x 0.25 mm i.d. x 0.25 mm 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 ºC and 240 ºC, respectively, with a split ratio of 1:5. The injection volume was 0.5 mL (10% in hexane), and the oven temperature program consisted of ramping up from 60 ºC to 240 ºC at 3 ºC min^{-1}, followed by an increase to 280 ºC and 10 ºC min^{-1}, and ending with 5 min at 280 ºC. 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 & Kratz (1963).

Chemical variability/Multivariate analyses

Statistical analysis was performed to examine the chemical variability between populations and their chemical constituents using SPAD.N software package for Principal Component Analysis (PCA) and Hierarchical Cluster (HCA). Canonical Discriminant Analysis (CDA) was performed by SYSTAT software for Windows, version 10, SPSS Inc., Chicago, IL, USA, 2000. Principal Component Analysis (PCA) showed that the whole data set could be projected in the space defined by the first two principal components retaining significant percentage of the total variance. For the selection of variables, the number of eigenvalues residual of the original matrix (11 samples x 34 variables = 374 data points) was used to establish the maximum number of variables to be removed. Hierarchical Cluster Analysis (HCA) was performed to study the similarities of the samples based on the distribution of chemical components of oil. The hierarchical clusters were formed according to the method of Minimum Variance of Ward (Ward, 1963). Canonical Discriminant Analysis (CDA) was used to propose the classification of groups and the percentage of correct classification was determined by Jackknife test. The significance for the Canonical Redundancy Analysis was determined by a Monte Carlo test (with 999 permutations under a reduced model). *p*-Values below 0.05 were considered significant.

Results and Discussion

A total of 34 components of the essential oil of *Pterodon emarginatus* Vogel, Fabaceae, were identified. The yields in percentage (v/w) and the qualitative and quantitative analytical results of eleven samples of

P. emarginatus essential oils are shown in Table 1. β-caryophyllene appears as the major constituent in samples PES-01 (20.30%), PES-02 (39.80%), NA-05 (68.10%), JUS-01 (49.80%) and MTOE (49.80%). However, a smaller percentage of this constituent was found in NA-01 (2.32%), NA-02 (17.7%), NA-03 (4.64%), NA-04 (3.17%) and JUS-03 (17.2%). This compound was not only found in JUS-02.

For samples NA-02, NA-03, NA-04 and JUS-03 the major compound was γ–muurolene (36.4%, 41.3%, 48.2% and 41.9%, respectively), as the sample NA-01 major compound was spathulenol (37.1%) and the sample JUS-02 the compound β-elemene (13.0%). The samples NA-04, NA-05 and PES-01 (3.48%, 3.10% and 1.66%, respectively) showed the best essential oil yield (Table 1).

The component α-humulene was found in species of most regions with significant percentages for the characterization of the essential oils, varying from 0.63 to 16.9%. Legaut et al. (2003) conducted a study on the antitumor activity of the compounds β-caryophyllene and α-humulene against strains of cells from solid tumors, however suggest that this activity is related to α-humulene, which was highly active against several tumor cell lines, while β-caryophyllene proved to be inactive.

The Principal Component Analysis (PCA) and Cluster Analysis (CA) resulted in 64.8% retaining the information in the first factorial plane (Figure 1). Using the first three factorial axes the cumulative variance was 77.6%. It was obtained two main groupings by sequential HCA from scores of PCA. Therefore, samples were grouped by similarity of the chemical components (Cluster Analysis) into two Clusters, Cluster I formed by the samples of JUS, PES, MTOE and NA-5 and the Cluster II composed only by others the samples of NA-1, NA-2, NA-3 and NA-4 (Figure 2).

Hierarchical Cluster Analysis (HCA) allowed a classification in two main clusters (Table 2), which to assess the importance of following variables: β-caryophyllene and α-copaene for the definition of Cluster I and II, respectively. Canonical discriminant analysis (CDA) data set, suggests that only the variable α-copaene is sufficient to correct classification of all samples of the data.

These results suggest the presence of two classes primarily characterized by high percentage of β-caryophyllene (35.0±21.8%; Cluster I) and of α-copaene (11.2±2.73%; Cluster II), with Cluster II comprising only samples from NA (Figure 2).

Samples from Nova America showed higher similarity among individual (Cluster II), unlike samples from Cluster I, which probably due the influence of environmental factors. The samples of Cluster I have a different location. However, it is possible to observe that even the Nova América samples presenting a cluster apart, excepting NA-5, with others locations in Goiás state, Bahia state and Mato Grosso state also showed similarities in the chemical composition of essential oils of *P. emarginatus* (Figure 2). Canonical Discriminant Analysis using the technique of cross-validation discriminated so distinctive cluster I and II with 100% of hit. The compounds β-caryophyllene and α-copaene were responsible for the characterization of two clusters due to their high percentage values, which suggests they are the major components of essential oils of *P. emarginatus*.

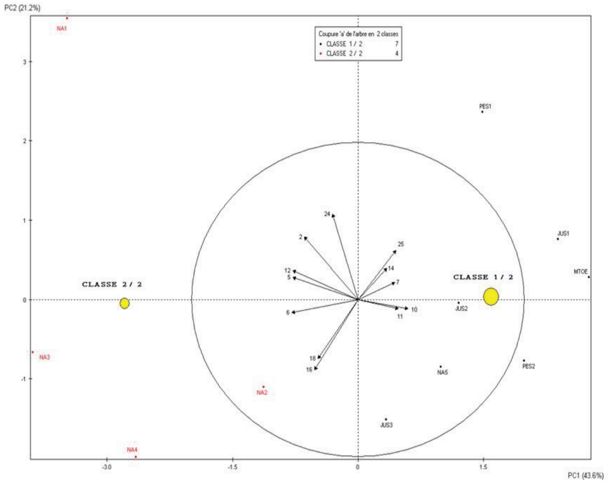

Figure 1. First factorial plane of the PCA showing the two clusters obtained by HCA sequence from the scores of PCA of the constituents of the essential oils of the fruits of *Pterodon emarginatus*.

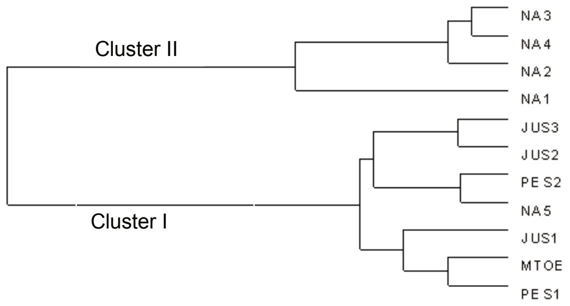

Figure 2. Similarity dendrogram based on Euclidean distance from the scores of the PCA with two groups of constituents of essential oils from fruits of *Pterodon emarginatus*.

Nascimento et al. (2012) reported a study in which the essential oils of *Copaifera langsdorffi*, Leguminoseae, presented a high chemical variability, with formation of two clusters based in PCA and agreeing with the high morphological polymorphism in *Copaifera* sp. It is quite frequent in the literature the presence of chemotypes when studying wild aromatic plants, such as mentioned for *Pimenta* *pseudocaryophyllus* (Gomes) L.R. Landrum (Paula et al., 2011). However, considering that this is a first study of chemical variability in the essential oils of *P. emarginatus*, and that there is not enough individuals sampled in each population, it is not possible to confirm the existence of two chemotypes in this species.

Potzernheim et al. (2012) observed that the chemical caracterization of essential oil constituents of four populations of *Piper aduncum* L. from Federal District of Brazil presented chemotypes from different biosynthetic pathways in populations from the Brazilian Cerrado, showing that they both can occur in a same

Table 1. Chemical composition and yield percentage of essential oils of eleven individuals of *P. emarginatus* of the Brazilian Cerrado.

Constituents	RI[a]	Sampling sites[b]										
		PES-1	PES-2	NA-1	NA-2	NA-3	NA-4	NA-5	JUS-1	JUS-2	JUS-3	MTOE
6-methyl-2-heptanol	958								1.32	3.18	0.45	
α-cubebene	1345	0.90		1.59	0.30	1.04	0.43	0.16				
citronelila acetate	1350		0.65									
α-ylangene	1373		1.66						1.78		2.48	
α-copaene	1374	4.74		13.2	10.9	13.9	6.92	2.09				
β-cubebene	1387		1.00	4.64	4.61	8.61	5.32	1.61	0.76			
β-elemene	1389	16.6	0.38							13.0	13.8	16.7
longifolene	1407					0.82		0.14				
α-gurjunene	1409						0.47					
β-caryophyllene	1417	20.3	39.8	2.32	17.7	4.64	3.17	68.1	49.8		17.2	49.8
α-humulene	1452	4.63	16.9	0.88	5.47	0.94	0.63	4.97	5.01		1.58	7.65
allo-aromadendrene	1458			11.1	1.00	8.04	5.10	1.49			1.81	
9-*epi*-(*E*)-caryophyllene	1464	2.99	1.04						1.38			
dauca-5,8-diene	1471	9.81							26.1			
γ-gurjunene	1475			0.99	0.90	0.60		0.17				
γ-muurolene	1478	1.20	6.59	1.59	36.4	41.3	48.2	14.9			41.9	1.93
α-amorphene	1483					0.61	0.45	0.13				
biciclogermacrene	1500	3.89	0.74		5.91	13.5	22.1	4.17	3.13		12.4	
α-muurolene	1500	1.21										
germacrene A	1508										5.30	4.93
δ-amorphene	1511			1.49	2.53		0.68	0.19			0.45	
γ-cadinene	1513	1.98										
δ-cadinene	1522	3.82										
sphatulenol	1577	13.8		37.1					4.72		1.86	
caryophyllene oxide	1582	8.33							4.18		0.45	4.72
globulol	1590	0.52										
salvial-4(14)-en-1-one	1594			1.87	0.42							
humulene epoxide II	1608	1.86	5.16									
α-cadinol	1652	1.16										
14-hidroxi (*E*)-caryophyllene	1668	0.51										
(2*E*,6*Z*)-farnesol	1714	0.96										
(*E*)-nerolidila acetate	1716			3.87	3.33	0.47	0.91					
n-hexadecanol	1874		0.61							6.03		
(*E*,*E*)-geranil linalool	2026									11.54		
sesquiterpene hydrocarbons		72.1	68.1	37.8	85.7	94.0	93.5	98.2	87.9	13.0	96.9	81.1
oxygenated sesquiterpenes		27.1	5.2	38.9	0.42				8.90		2.31	4.72
diterpenes										11.5		
other			1.26	3.87	3.33	0.47	0.91		1.32	9.21	0.45	
Total identified		99.2	74.5	80.6	89.4	94.5	94.4	98.2	98.2	33.7	99.6	85.8

[a]Retention índex; [b]PES-1: Correntina/Bahia; NA: Nova América/Goiás; JUS: Jussara/Goiás; PES-2: Campestre/Goiás and MTOE: Jaciara/Mato Grosso.

Table 2. Analysis between Classes I and II, indicating the variables of interclass discriminatory power.

p*	Averages		Standard deviation		Number	Variable
	Cluster	Geral	Cluster	Geral		
For Cluster I						
0.028	35.00	24.81	21.76	22.31	10	β-caryophyllene
0.035	8.63	5.49	7.47	7.27	7	β-elemene
For Cluster II						
0.002	11.24	4.71	2.73	5.38	5	α-copaene
0.002	5.80	2.41	1.65	2.78	6	β-cubebene
0.007	6.30	2.59	3.71	3.64	12	*allo*-aromadendrene
0.021	0.84	0.40	0.52	0.52	2	α-cubebene
0.036	31.87	17.64	17.98	18.97	16	γ-muurulene

*Probability

location. Therefore, this aspect seems to be more dependent on the gene flow of alleles than of abiotic factors.

Although *P. emarginatus* is widely distributed on the Cerrado vegetation, few morphological variations are found. The large chemical variation found on volatile oils of *P. emarginatus* is in agreement with most of the literature on chemical polymorphism of native tree species, supporting the results reported (Lindroth et al., 1987; Hartmann, 1996).

To our knowledge, the data reported in this paper has not been published elsewhere. It is relevant and innovative, since *P. emarginatus* is an important brazilian medicnal plant, consider in the list of species priority for conservation (Vieira & Silva, 2002). Further studies on seasonality, genetics and growing conditions of *P. emarginatus* should be performed in order to minimize the chemical variability observed in the essential oils. The results obtained for *P. emarginatus* showed the occurrence of chemical polymorphism on essential oils from fruits of Pterodon sp.

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Authors contributions

SFA: Development study; LLB: English reviewer; JAMP: Contribution in the interpretation of mass spectra; RFV: Statistical analysis and discussion; PHF: Statistical analysis; ROC: Collection and availability of fruits "sucupira"; JRP: Botanical identification; MTFB: Guiding this work and revising the text.

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