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Original Article

Chemical composition and seasonality variability of the *Spiranthera odoratissima* volatile oils leaves

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ABSTRACT

Spiranthera odoratissima A. St.-Hil., Rutaceae, known as "manacá" is a shrub native of the Brazilian Cerrado. Their leaves and roots are popularly used to treat rheumatism, infection and abdominal pain. This study analyzed the chemical composition of volatile oils from leaves of *S. odoratissima* and verified the seasonal variability of its chemical composition. The volatile oils were obtained by hydrodistillation using a Clevenger type apparatus and analyzed by gas chromatography coupled to mass spectrometry. The main chemical components found in samples of volatile oils were β-caryophyllene, bicyclogermacrene, δ-cadinene, amorphous-4,7(11)-diene, α-epi-muurolol, α-cadinol, α-muurolol and γ-cadinene. The hierarchical clustering identified three groups: the first was characterized by α-epi-muurolol, the second by amorphous-4,7(11)-diene and the third group was characterized by α-muurolol. The discriminant canonical analysis was used to differentiate between clusters on the basis of oil composition. The results suggest that the rainfall presented a relationship with the chemical composition of the volatile oil. This is the first study conducted on the seasonal behavior of the chemical constituents in volatile oil from leaves of *S. odoratissima*.

Introduction

Spiranthera odoratissima A. St.-Hil., Rutaceae, is a shrub known as "manacá" with erect stems that come together to form clumps very aromatic. It is native to Central Brazil (Cerrado), occurring mainly in Goiás, Mato Grosso and Bahia states. The leaves and roots are popularly used for treatment of various diseases such as syphilis, rheumatism, renal infections and urinary retention, abdominal pain, gout, furuncles and acne (Matos et al., 2003, 2014; Albernaz et al., 2010; Barbosa et al., 2012). *Spiranthera* sp. volatile oils and their components possess anti-inflammatory, analgesic (Silva et al., 2010; Matos et al., 2004, 2014), anxiolytic (Galdino et al., 2012) and antiprotozoal activities (Albernaz et al., 2012).

The chemical composition of secondary metabolites could be related to climate and atmospheric parameters. The temperature and precipitation were identified as factors that might influence the chemical composition of volatile oil (Cruz et al., 2014). Studies showed that the chemical patterns of the Cerrado species are directly related to the seasonality (Sá et al., 2016).

Water availability in the Cerrado defines two seasons: dry (April to September) and wet (October to March) (Santos et al., 2006). Thus, it is assumed that the secondary metabolism responds in two ways, depending on environmental conditions (Gobbo-Neto and Lopes, 2007; Oliveira et al., 2012; Cruz et al., 2014; Amaral et al., 2015).

The objective of this study was to analyze the chemical composition and seasonal variability volatile oil leaves of *S. odoratissima* over a seasonal cycle from November 2014 to October 2015.

Materials and methods

Plant material

Leaves of about fifty individuals of *Spiranthera odoratissima* A. St.-Hil., Rutaceae, were collected at 8 and 9 am, monthly, in the city of Aparecida de Goiânia, Goiás State,

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Brasil (16°45′45.2″ S/49°07′06.8″ W, 762 m), in the period between November 2014 to October 2015. Plant material was identified by Prof. Dr. José Realino de Paula and a voucher specimen was deposited at the Herbarium of the Federal University of Goiás, Brazil, under code UFG 60010. The leaves were dried at room temperature. Meteorological data of Aparecida de Goiânia, GO (November 2014 to October 2015) were obtained from the online climate database of the National Institute of Meteorology (INMET, 2013). For data analysis, monthly averages of temperature and accumulated rainfall and average daylength for each month were used.

Volatile oils extraction and GC–MS analysis

For the extraction of the volatile oil, leaves (115 g) were dried at room temperature for three days, triturated using commercial crusher (Skymsen, LS-08MB-N) immediately prior to the extraction of the volatile oil, avoiding loss by volatilization, and submitted to hydrodistillation in a Clevenger-type apparatus for 3 h. After dried over anhydrous Na_2SO_4, oils were stored in sealed brown vials and at −18 °C. The volatile oil volume was measured in the graduated tube of the apparatus and was calculated as percentage relative to the initial amount of dry plant material used in the extraction. Each experiment was performed in triplicate.

The volatile oil were analyzed using a gas chromatography coupled to mass spectrometry Shimadzu GC-MSQP5050A fitted with a fused silica SBP-5 (30 m × 0.25 mm I.D.; 0.25 m film thickness) capillary column (composed of 5% phenyl-methylpolysiloxane) and temperature programmed as follow: 60–240 °C at 3 °C/min, then to 280 °C at 10 °C/min, ending with 10 min at 280 °C. The carrier gas was a flow rate of 1 ml/min and the split mode had a ratio of 1:20. The injection port was set at 225 °C. Significant quadrupole mass spectrometer operating parameters: interface temperature 240 °C; electron impact ionization at 70 eV with scan mass range of 40–350 *m*/*z* at a sampling rate of 1 scan/s. Constituents were identified by computer search using digital libraries of mass spectral data (NIST, 1998) and also by comparison of their retention indices (Van Den Dool and Kratz, 1963) relative to C_8–C_{32} *n*-alkanes and mass spectra with literature data (Adams, 2007).

Statistical analysis

The Principal Component Analysis (PCA) was applied to analyze the interrelationships between the chemical constituents of leaves of volatile oil collected in different months using Statistica 7.0 software (StatSoft Inc., Oklahoma, USA). The hierarchical cluster analysis (HCA) was used to study the similarity between the samples according to the distribution of the constituents, and this analysis was performed by Ward's method (Ward, 1963). To validate the cluster analysis was performed the canonical discriminant analysis (CDA). The predictive ability of linear discriminant functions was evaluated by cross-validation. The p values less than 0.01 were considered significant. Prior to the multivariate analysis, the data were preprocessed by means of auto-scaling and mean centering. The Pearson's correlation between β-caryophyllene and daylength average was performed to verify their possible association.

Results

Climate data collection of plant material period of *S. odoratissima* is described in Table 1. In November 2014 to April 2015, there was high rainfall with values ranging from 170.5 to 337.9 mm, respectively, except for January 2015 that had an atypical behavior recording a value of 73.6 mm. The months of June 2015 July 2015 and August 2015 is presented as the months of extreme drought, reaching the highest rainfall value of 3.6 mm in August, September 2015 (30.4 mm) and October 2015 (18.2 mm). May stands out with an intermediate rainfall value of 70.7 mm. The same behavior is observed for the relative humidity (%), higher humidity during the rainy season (March 2015) and lower in the dry season (August 2015), with values of 75.9 and 38.4%, respectively.

Table 1
Climatic information of the period of collection of plant material of *Spiranthera odoratissima*.

Date	Rainfall precipitation (mm)	Relative humidity (%)	Daylight (h)	Average temperature (°C)	
				Maximum	Minimum
11/30/2014	170.5	67.3	163.5	31.9	20.8
12/31/2014	337.9	72.4	144.1	30.2	20.2
01/31/2015	73.6	56.5	249.9	34.0	21.0
02/28/2015	225.2	70.2	165.7	31.0	20.4
03/31/2015	312.3	75.9	148.5	30.0	20.1
04/30/2015	204.2	72.5	188.2	31.0	20.6
05/31/2015	70.7	66.0	225.2	29.7	18.4
06/30/2015	0.0	56.3	250.8	30.2	17.0
07/31/2015	2.7	50.9	252.5	31.4	17.0
08/31/2015	3.6	38.4	283.7	33.1	17.4
09/30/2015	30.4	42.5	251.0	36.0	20.4
10/31/2015	18.2	43.6	242.5	36.8	22.2

Source: INMET (Goiânia Station – OMM: 83423). 2013.

Temperature variations during the collection period were not significant, with temperatures ranging from 17 °C to 36.8 °C, respectively (Table 1).

Volatile oil

The volatile oils yield ranges from 2.3% in November (rainy) to 3.4% in July (low rainfall).

Through the GC–MS analysis identified 41 chemical compounds. The highest percentages of identified chemical compounds occurred in the rainy months, especially the month of March 2015 to 99.42% while the lowest percentages identified, occurred in the dry season, reaching a minimum of 81.13% in July 2015 (Table 2). The class of sesquiterpene hydrocarbons showed more highlight with 26 compounds (from 67.95 to 86.17%), with higher values during the rainy season, followed by oxygenated sesquiterpenes (12 compounds) in small percentages (5.73–19.00%) and a minority of monoterpenes hydrocarbons with quantities below 2%.

The major chemical components of the samples along the seasonal cycle were β-caryophyllene (6.78–12.15%), bicyclogermacrene (17.61–23.08%), δ-cadinene (12.31–16.55%) and amorphous-4,7(11)-diene (10.71–19.87%).

The major compounds α-epi-muurolol and α-cadinol were not identified in the June to August and June to October (dry months), respectively. These compounds were produced in similar amounts in December 2014, when the highest rainfall occurred in the period, with the lowest percentage of 4.34% (α-epi-muurolol) and 4.89% (α-cadinol), while the highest values were 6.25% (November 2014) and 8.35% (May 2015), respectively. α-Muurolol presented a slight percentage increase in the dry months of July (7.65%) and August (5.10%). The compound γ-cadinene was identified in all months with a percentage ranging of 2.37% (May/2015) and 5.68% (September 2015), respectively. It was verified that there was no correlation between beta caryophyllene and daylength ($R = 0.057$, $p = 0.86$).

The results obtained from the Principal Component Analysis (PCA) and Cluster analysis (CA) (Figs. 1 and 2) indicate large chemical variability in the samples of *S. odoratissima* EO. The majority of the data could be represented in two main axes, which contained 87.4% of total variance (PC1 = 62.5, and PC2 = 24.88%; Fig. 1). The two-dimensional representation of the first two axes of the PCA