Revista Brasileira de Farmacognosia xxx (2016) xxx–xxx

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Chemical composition and seasonal variability of the essential oils of leaves and morphological analysis of *Hyptis carpinifolia*

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ARTICLE INFO

Article history:
Received 25 January 2016
Accepted 5 May 2016
Available online xxx

Keywords:
Hyptis carpinifolia
Sesquiterpenes
Essential oil composition
1,8-Cineole

ABSTRACT

Hyptis carpinifolia Benth., Lamiaceae, is a species known popularly as "rosmaninho" and "mata-pasto", and leaves are employed in Brazilian folk medicine to treat colds, flu, and rheumatism. The aim of this study was to perform a morphological description of *H. carpinifolia* and to evaluate the seasonal chemical variability of the leaf essential oils during 12 months. Macroscopic characterization of *H. carpinifolia* was carried out with the naked eye and with a stereoscopic microscope. Essential oils were isolated from leaves by hydrodistillation in Clevenger apparatus and analyzed by gas chromatography/mass spectrometry. Major compounds were found to be 1,8-cineole (39.6–61.8%), *trans*-cadina-1(6),4-diene (2.8–17.5%), β-caryophyllene (4.4–10.0%), prenopsan-8-ol (4.2–9.6%) and β-pinene (2.9–5.3%). Results of essential oils compositions were processed by cluster analysis and principal component analysis. Data showed high variability in the concentration of the components. Besides, there was a seasonal variability of chemical composition, probably related mainly to the rainfall regime.

Introduction

Hyptis genus, belongs to the Lamiaceae family, subfamily Nepetoideae, tribe Ocimeae and subtribe Hyptidinae. It is one of the largest and most widely distributed plant genera in the world with more than 300 species (Harley, 1988). It is composed of herbs, subshrubs, shrubs and more rarely small trees. The stems of the species of this genus are often square in cross-section; the leaves are usually opposite, occasionally whorled, simple or rarely with slits, petiolate, shortly petiolate or sessile and aromatic, hairs gland-headed with essentials oils, simple, non-glandular, usually multicellular or both multicellular and unicellular (Bordignon, 1990).

Species of this genus are commonly used in traditional folk medicine to treat a variety several diseases such as gastrointestinal infections, cramps, pains and skin infections (Corrêa, 1931).

The essential oils found in the genus *Hyptis* have a great importance as a source of bioactive constituents, especially due to its biological properties such as antimicrobial, cytotoxic and insecticide (Kuhnt et al., 1995).

Hyptis carpinifolia Benth., commonly known in Brazil as "rosmaninho" and "mata-pasto", has a strong rosemary aroma and is used in folk medicine to treat colds, flu (Silva et al., 2000), rheumatism (decoction or infusion of the leaves) (Rodrigues and Carvalho, 2001) and for baths (Brandão et al., 2012).

It is a branched shrub up to 3 m high, glabrous leaves, sessile, elliptic to oblong-ovate with cordate base (Epling, 1949; Harley, 2012); inflorescences large, in terminal spike with 1–2 cm in diameter; violet flowers; linear-lanceolate bracts among the flowers (Harley, 2012; Pignal et al., 2013). Xylematic rays and prismatic crystals were visualized in the calix (Rabei and El-Gazzar, 2007). *H. carpinifolia* is distributed in Brazil (Mato Grosso, Mato Grosso do Sul, Piauí, São Paulo, Roraima, Tocantins), Bolivia (Harley, 2012) and Peru (Mobot, 2016).

No references were found in the literature regarding the chemical profile and biological activities of *H. carpinifolia*. Thus, this study aimed to perform the morphological description of *H. carpinifolia* and to evaluate the chemical constituents of the essential oil of leaves and its seasonal variability.

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http://dx.doi.org/10.1016/j.bjp.2016.05.011

Materials and methods

Plant material

For the morphological description, specimens of *Hyptis carpinifolia* Benth., Lamiaceae, deposited in the herbaria of: Município Alvorada do Norte, Goiás, 02.VII. 1964, J.M. Pires 58142 (UB); Nova Colina, Goiás, Estrada Belém-Brasília, 5–10 km of Nova Colina, 31.VII.1964, G.T. Prance & N.T. Silva 58495 (UB); Mineiros, Goiás, Parque Nacional das Emas, 03.VII.1983, 05.VII.1983, H.D. Ferreira 225 (UFG); specimens collected in Parque Nacional das Emas, in Mineiros and in Nova América, Goiás, Brazil, were examined.

Morphological analysis

Macroscopic characterization of *H. carpinifolia* was carried out with the naked eye and with a stereoscopic microscope Olympus SZ-ST.

Essential oils isolation and GC–MS analysis

For isolation of essential oils, *H. carpinifolia* leaves were collected from ten different individuals, monthly, for one year (2013), in the city of Nova América/Goiás (15°02′29.5″ S and 49°59′0.05″ W, at an elevation of 1295 m above sea level). Plant material was identified by Dr. Heleno Dias Ferreira and a voucher specimen was deposited at the Herbarium of the Federal University of Goiás, Brazil, under code UFG 43.833.

The leaves were dried at room temperature and ground in a knife mill. Different batches (100 g) of powdered leaves were submitted to hydrodistillation in a Clevenger-type apparatus for 2 h. After dried over anhydrous Na_2SO_4, oils were kept in glass vials at a temperature of −18 °C prior to further analysis. The essential 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 essential oils were analyzed using a Shimadzu GC-MS QP5050A fitted with a fused silica SBP-5 (30 m × 0.25 mm I.D.; 0.25 μm film thickness) capillary column (composed of 5% phenylmethylpolysiloxane) 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 C8–C32 n-alkanes and mass spectra with literature data (Adams, 2007).

Statistical analysis

Principal Component Analysis (PCA) was applied to examine the interrelationships between the chemical constituents of the essential oils from leaves collected in different months using the software Statistica 7 (Stat Soft, 2004). A cluster analysis was used to study the similarity of samples based on the distribution of the constituents, and hierarchical clustering was performed according to the method of minimum variance Ward (Ward, 1963). To validate the cluster analysis was carried out using the canonic discriminant analysis (DCA).

To verify the possible association between the essential oil components selected along with climatic variables (temperature and rainfall) was used the Pearson's correlation analysis (Callegari-Jacques, 2003).

Results

Essential oils

Within the collection period, the highest rainfall regimens were registered in the months of October, November and December. In this period, the maximum temperature ranged from 32 to 35 °C and the minimum temperature ranged from 20 to 22 °C. Lowest precipitation was observed in the months of June, July, August and September and the maximum temperature ranged from 33 to 36 °C and the minimum temperature ranges from 18 to 22 °C (INMET, 2014) (Table 1).

The loss of weight of leaves after drying was 72%. The yields of the essential oils of *H. carpinifolia* leaves ranged from 1.2 to 2.0%. The oils are mainly composed of oxygenated monoterpenes (40.4–62.6%), followed by sesquiterpene hydrocarbons (13.6–37.3%), oxygenated sesquiterpenes (11.2–17.1%) and monoterpene hydrocarbons (4.7–9.4%). Major compounds were found to be 1,8-cineole (39.6–61.8%), *trans*-1-cadina-(6),4-diene (2.8–17.5%), β-caryophyllene (4.4–10.0%), prenopsan-8-ol (4.2–9.6%), β-pinene (2.9–5.3%) (Table 2, Fig. 1).

The results obtained by the PCA and cluster analysis showed a chemical variability within the *H. carpinifolia* oils. Fig. 2 shows the relative position of the 2D-axis originated in the PCA. Cluster analysis suggests that there are three groups: cluster I (essential oils from leaves collected in the months of April, October, November and December) characterized by pinonic acid (mean = 1.05 ± 0.21), as the main component and the highest rainfall index (233.6 ± 115.1); cluster II (essential oils from leaves collected in the months of

Table 1
Climate information of the collect period of the plant material of *Hyptis carpinifolia*.

Station	Date (2013)	Rainfall number of days	Total rainfall	Maximum temperature average	Minimum temperature average	Relative humidity average
83374	01/31	26	376.3	31	21	78.00
83374	02/28	19	117.1	34	21	74.75
83374	03/31	22	131	34	21	77.05
83374	04/30	6	99.4	33	21	72.26
83374	05/31	2	29.1	34	19	61.42
83374	06/30	4	8.3	33	19	64.25
83374	07/31	0	34.11	34	18	51.16
83374	08/31	0	35.78	35	19	42.39
83374	09/30	4	26.9	36	22	44.05
83374	10/31	13	114.9	35	22	65.66
83374	11/30	20	232	33	20	77.50
83374	12/31	27	315.5	32	21	80.36

Source: INMET (2014).