



Original Article

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

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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.

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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 essential 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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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 Na₂SO₄, 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).

Table 2Percentage of the chemical constituents of the essential oils from *Hyptis carpinifolia* leaves collected monthly in Nova América, Goiás during the year 2013.

Constituents	KI	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Tricyclene	926	1.5	1.8	1.5	1.2	1.5	1.3	0.9	1.2	1.4	1.1	1.1	1.0
6-Methyl-heptan-2-ol	965	0.4	0.4	0.3	0.2	2.2	0.2	–	–	–	0.1	–	–
Sabinene	975	1.1	1.2	1.0	0.9	1.2	1.2	0.9	1.1	1.2	1.0	1.0	0.9
β-Pinene	979	4.5	5.3	4.1	3.1	4.5	4.2	2.9	4.2	5.1	3.1	3.1	3.1
1,8-Cineole	1031	55.5	51.8	43.9	39.6	47.2	52.6	47.7	57.4	61.8	48.1	51.2	54.5
γ-Terpinene	1059	0.1	0.1	–	–	–	–	0.1	0.1	0.1	–	0.1	0.2
cis-Sabinene hydrate	1070	–	–	–	–	–	–	0.1	0.1	0.1	0.2	0.2	0.1
Pinocarvone	1164	0.7	–	0.1	0.3	0.5	0.8	0.9	0.7	0.4	1.3	1.3	1.4
Terpinene-4-ol	1177	0.7	0.8	0.7	0.5	0.4	0.3	0.1	0.2	0.3	0.4	0.4	0.5
α-Copaene	1376	3.4	3.7	4.1	4.4	3.6	3.3	3.1	2.7	2.4	3.9	3.6	3.2
β-Bourbonene	1388	0.4	0.4	0.4	0.4	0.7	0.7	1.0	0.8	0.8	0.8	0.7	0.5
β-Caryophyllene	1419	6.1	5.9	8.2	10.0	6.7	7.7	9.2	6.7	4.4	9.9	8.4	6.5
Pinonic acid	1442	0.7	0.7	0.9	1.3	0.9	0.8	0.8	0.6	0.4	1.2	1.0	1.0
α-Humulene	1454	0.4	–	0.2	0.4	0.2	0.5	0.9	0.6	0.2	–	0.4	0.8
trans-Cadina-1(6),4-diene	1476	3.4	2.8	10.6	17.5	5.5	4.6	5.4	3.9	2.9	7.5	6.2	4.5
β-Selinene	1490	1.1	1.1	1.2	1.0	0.9	0.9	1.1	0.8	0.7	0.9	1.0	1.0
δ-Selinene	1492	1.6	1.7	1.9	2.0	1.5	1.3	1.5	1.2	1.1	1.6	1.4	1.5
γ-Cadinene	1513	1.0	1.0	1.2	1.4	1.1	1.0	1.2	0.9	0.7	0.8	1.0	1.2
cis-Cadinene ether	1553	0.3	–	–	–	0.3	0.4	–	–	0.5	–	–	–
Prenopsan-8-ol	1577	7.0	8.5	7.6	6.0	7.5	6.9	8.3	5.7	4.2	9.6	8.5	7.0
allo-Cedrol	1589	0.2	0.3	0.3	0.3	0.4	0.4	0.4	–	0.4	0.2	0.2	0.2
allo-Hedycaryol	1589	1.7	1.7	1.7	1.4	2.2	2.4	3.4	2.9	2.9	1.7	1.9	2.0
Rosifolol	1600	0.5	0.8	0.6	0.4	0.7	0.5	0.6	0.4	0.3	0.7	0.6	0.5
epi-Cedrol	1619	0.8	0.8	0.6	0.7	0.1	–	–	–	–	0.3	0.8	–
Caryophylla-4(12),8(13)-dien-5α-ol	1640	0.3	0.5	0.3	0.2	0.5	0.4	0.5	0.3	0.3	0.3	0.2	0.3
Caryophylla-4(12),8(13)-dien-5β-ol	1640	0.7	1.1	0.9	0.6	–	1.0	1.2	0.9	0.9	0.7	0.6	0.8
Pogostol	1653	0.9	1.2	1.0	0.7	1.3	0.8	1.0	0.9	0.9	0.4	0.7	0.8
Khusinol (valerianol)	1658	1.7	2.5	1.9	1.2	2.1	1.8	1.6	1.7	2.2	1.2	1.3	1.4
Monoterpene hydrocarbons		7.5	8.9	6.9	5.3	9.4	6.9	4.7	6.6	7.9	5.4	5.3	5.1
Oxygenated monoterpenes		56.9	52.6	44.7	40.4	48.0	53.6	48.9	58.4	62.6	50.0	53.2	56.4
Sesquiterpene hydrocarbons		17.7	16.8	28.1	37.3	20.6	20.4	23.8	17.6	13.6	25.5	22.9	19.5
Oxygenated sesquiterpenes		13.9	17.1	13.6	11.2	14.7	14.1	16.6	12.8	12.3	14.8	14.5	12.7
Others		0.7	0.7	0.9	1.3	0.9	0.8	0.8	0.6	0.4	1.2	1.0	1.0
Total identified (%)		96.7	96.1	93.4	95.5	93.6	95.8	94.8	96.0	96.8	96.9	96.9	94.7
Yield (%)		1.8	2.0	2.0	1.9	2.0	1.6	1.3	1.3	1.2	1.6	1.6	1.8

KI, Kovats retention index.

January, February, March and May) characterized by pogostol (1.2 ± 0.2) and the khusinol (2.2 ± 0.3) as main components and rainfall index (92.4 ± 55.3); and cluster III (essential oils from leaves collected in the months June, July, August and September) characterized by 1,8-cineole (54.9 ± 6.1) and the lowest rainfall index (26.3 ± 12.6) (Figs. 3 and 4). The results indicated that the classification proposed by the PCA and HCA are in agreement.

Canonic discriminant analysis was performed to help to predict the grouping of the cluster analysis, and two predictive variables was employed: pogostol ($p=0.008$) and rainfall ($p=0.01$), and the discriminant function retain 92% of well - classification in the original clusters by a cross-validation approach.

Through the correlation analysis between α-copaene and pinonic acid and humidity, there are a significant correlation [$R=0.71$ ($p=0.01$); $R=0.60$ ($p=0.04$), respectively], that is, the higher the humidity the greater the amount α-copaene of and pinonic acid.

Morphological analysis

H. carpinifolia is a shrub up to 3 m high with glandular vil-lous branches. Leaves sessile or subsessile, blade oblong-ovate, oblong or sometimes rounded, usually 3–6 cm long, 2–3 cm wide, adaxial surface subglabrous, abaxial surface glandular-pubescent or tomentose, apex rounded or sometimes acute, base subcordate or rounded, serrate margin, reticulate and prominent veins in the abaxial surface. Verticillasters compact or slightly separated, pauciflorous, sessile, arranged in terminal spike, bracts 7–9 mm length, submembranous, ovate, lanceolate, linear-lanceolate, ciliated margin with branched hairs. Flowers blues or violet; calyx tube of the flower 1.5–2 mm length, glandular puberulent externally, hirsute orifice, erect and branched hairs, teeth subulate calyx, unequal or subequal, about 2 mm length, mature tube calyx 4–4.5 mm length; corolla tube with 8 mm length, slightly glandular puberulent externally, filament of the stamen with hairs superiorly, 3 mm

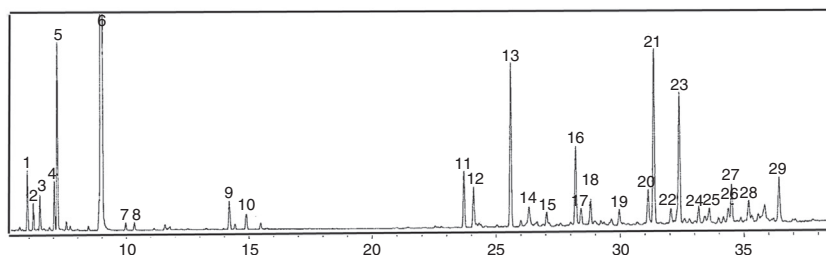
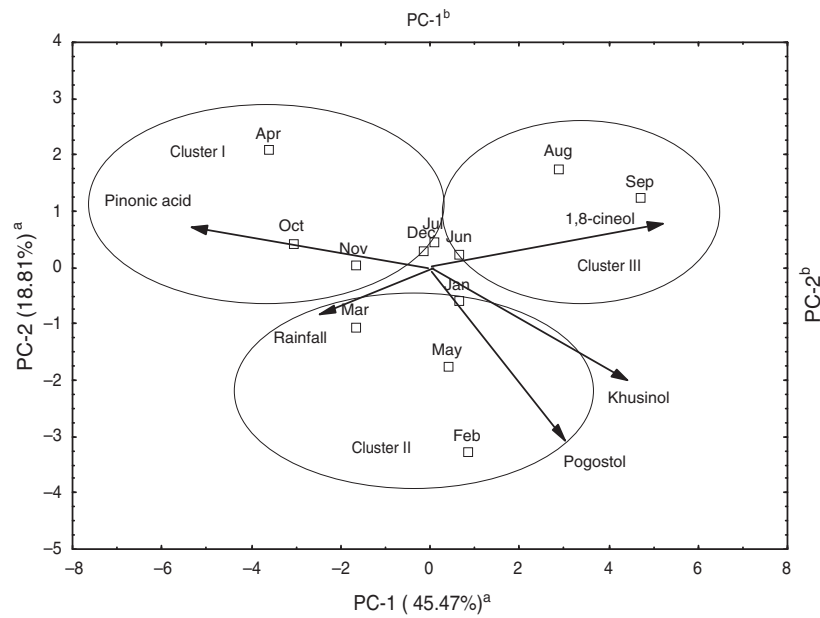


Fig. 1. GC-chromatogram of the chemical constituents of the essential oils of *Hyptis carpinifolia* leaves collected in New America, Goiás in August 2013, where are highlighted the following peaks of the major compounds: (5) β-pinene, (6) 1,8-cineole, (13) β-caryophyllene and (21) prenopsan-8-ol.



Means and standard deviation of clusters:

	Pinonic acid	Khusinol	Rainfall	Pogostol	1,8-cineole
Cluster I	1.1 ± 0.2	1.3 ± 0.2	233.6 ± 115.0	0.7 ± 0.2	49.8 ± 6.4
Cluster II	0.8 ± 0.1	2.2 ± 0.3	92.4 ± 55.2	1.2 ± 0.2	47.6 ± 3.9
Cluster III	0.6 ± 0.2	1.8 ± 0.2	26.3 ± 12.6	0.9 ± 0.1	54.9 ± 6.1

Fig. 2. Scatterplot of PCA of the essential oils from the leaves of *Hyptis carpinifolia* samples collected from Nova América/GO belonging to the clusters (I, II and III). ^aAxes referring to the scores of samples. ^bAxes referring to scores of volatile chemicals whose discriminant constituents are represented by vectors.

length; ovary glabrous, style glabrous, articulated in the ovary base, 10 mm length. The schizocarp fruit of *H. carpinifolia* presents were nutlet smooth, light brown, abruptly apiculate, 1.5 mm length.

It was found in this study that, in Goiás, *H. carpinifolia* occurs wetlands in the gallery forest margins, *stricto sensu* Cerrado. It is found at altitudes between 164 m and 1295 m with an average

elevation of 667 m. The fertile individuals were collected from May to July.

Discussion

The major compounds of the essential oils of *H. carpinifolia* leaves were 1,8-cineole, *trans*-cadin-1(6),4-diene,

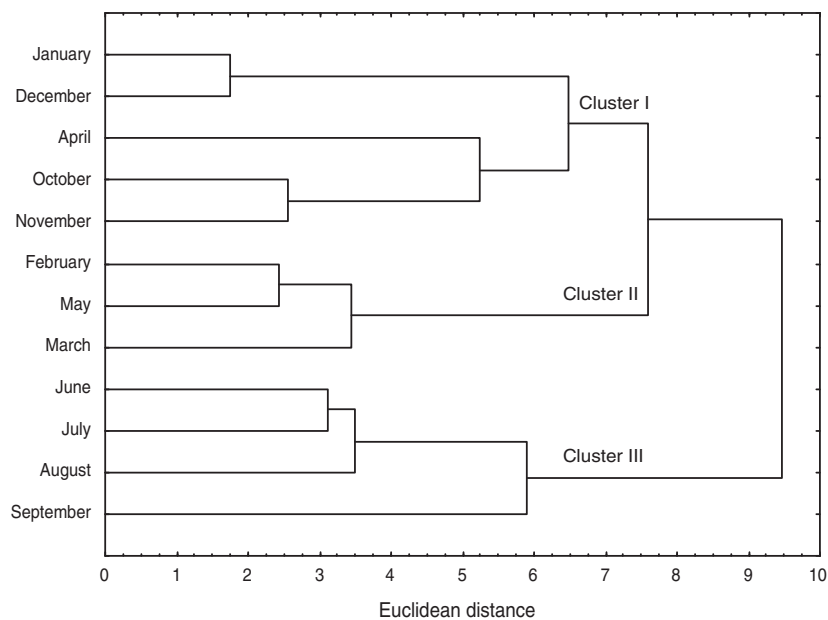


Fig. 3. Dendrogram representing the similarity relations of the chemical composition of *Hyptis carpinifolia* oils according to the method of Ward Minimization of variance. For this analysis were considered 1,8-cineole, the α -copaene, β -caryophyllene, the *trans*-cadin-1(6),4-diene, the prenopsan-8-ol, the pinonic acid, the rosifoliol, the pogostol, khusinol, precipitation, the temperature and humidity.

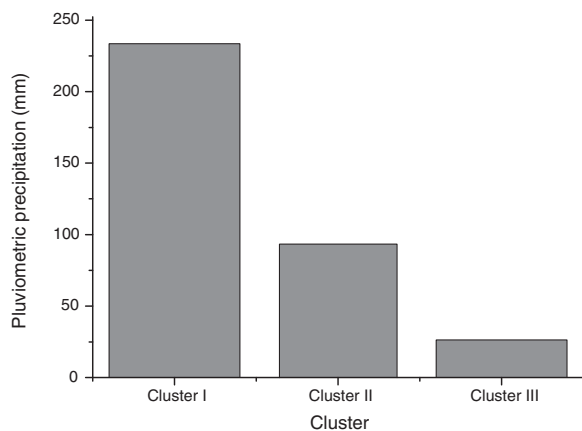


Fig. 4. Correlation between rainfall and clusters.

β -caryophyllene, prenopsan-8-ol and β -pinene. There are not data in the literature on the chemical composition of the oils of this species. However, the 1,8-cineole was described as one of the major compounds of the leaves of different species of this genus, such as, *H. crenata* Pohl ex Benth. (Rebello et al., 2009), *H. suaveolens* (L.) Poit. (Moreira et al., 2010), *H. fruticosa* Salzm. ex Benth. (Franco et al., 2011), *H. goyazensis* A.St.-Hil. ex Benth. (McNeil et al., 2011) and *H. martiusii* Benth. (Araújo et al., 2003; Caldas et al., 2014). β -Caryophyllene have been found in the essential oils of the leaves of *H. glomerata* Mart. ex Schrank (Silva and Moura, 2011), *H. martiusii* (Araújo et al., 2003), *H. marrubioides* Epling (Botrel et al., 2010; Sales et al., 2009), *H. spicigera* Lam., *H. pectinata*, *H. floribunda* Briq., *H. suaveolens* (McNeil et al., 2011); while β -pinene has been identified in the leaves of *H. crenata* (Rebello et al., 2009), *H. suaveolens* (Moreira et al., 2010) and *H. fruticosa* (Franco et al., 2011).

1,8-Cineole is a monoterpene found in many aromatic plants of the genus *Eucalyptus*, *Croton*, *Hyptis*, *Pectis*, *Rosmarinus* and *Salvia* (Araújo et al., 2003; Vilela et al., 2009; Hussain et al., 2011). Numerous biological and pharmacological proprieties were assigned to 1,8-cineole, including antimicrobial, insecticidal (Balacs, 1997), anti-allergic, anti-inflammatory (Santos and Rao, 1998), hepatoprotective (Santos et al., 2001), antitumoral (Moteki et al., 2002) and gastrointestinal activity (Santos et al., 2001). The presence of 1,8-cineole can also explain the popular use of the *H. carpinifolia* in flu, colds, and rheumatism. Cardiovascular effects were also assigned to 1,8-cineole, in reducing the blood pressure of both conscious and anesthetized rats and showing vasorelaxant effects on isolated rat aorta. This activity seems to be dependent on the integrity of the vascular endothelium and oxide nitric releasing (Lahlou et al., 2003; Pinto et al., 2009; Santos et al., 2011).

The observed variability of the chemical composition of essential oils during the year, could be correlated with rainfall. This result is in accordance with the reports of Gobbo-Neto and Lopes (2007) and also Morais (2009), which reported that the production of secondary metabolites by plants may be influenced by environmental factors such as seasonality and that these factors can change the yield and chemical composition of essential oils. Barros et al. (2009) found that climate conditions can influence the enzymatic activities in certain plant species and then influence in the biosynthesis of certain secondary metabolites, including terpenic compounds. Experiments with *H. suaveolens* showed interference of environmental factors on the chemical composition of its essential oils (Martins et al., 2006). Botrel et al. (2010) found that monoterpenes and sesquiterpenes content of *H. marrubioides*, in spring, showed very similar values, however, in winter, the monoterpenes content doubled in relation to the sesquiterpenes.

H. carpinifolia was placed by Epling (1949) in Polydesmida section, subsection Rigidae with only two species: *H. carpinifolia* and *H. violacea* Benth. *H. carpinifolia* differs from *H. violacea* for having abruptly peaked nutlets, while in *H. violacea* the nutlets are obtuse. *H. carpinifolia* has adaxial surface subglabrous, abaxial surface glandular-pubescent or tomentose, while, according with the same author. *H. violacea* has scabrous limb on the surface adaxial and almost glabra on the abaxial surface.

Epling (1949) describes *H. carpinifolia* as a shrub of 3 m high. The analyzed specimens of the herbaria and field in this study also have not found any specimens taller than 3 m. Epling (1949) also reported for *H. carpinifolia* calyx tube with 5.5–6 mm length and corolla tube 6–8 mm length. In this study, the calyx tube has smaller length (1.5–2 mm) and the corolla tube with 8 mm length.

The fertile individuals of *H. carpinifolia* were collected from May to July while in *H. suaveolens* the flowering and fruiting occurs in October to December (Jelani and Prabhakar, 1991).

This work contributed for the morphological description and for the knowledge on the chemical composition of essential oils of *H. carpinifolia*. Major compounds found in the essential oils from leaves were 1,8-cineole, *trans*-1-cadin(6),4-diene, β -caryophyllene, prenopsan-8-ol, β -pinene. The results presented herein strongly suggest that there is a seasonal variability of chemical compounds related to rainfall.

Authors' contributions

SS contributed in collecting plant sample, running the laboratory work. TSF drafted the paper and contributed to critical reading of the manuscript. HDF contributed in collecting plant sample and identification, confection of herbarium and morphological description of the species. LLB contributed to the statistical analyzes. MHR contributed in collecting plant sample and identification. LMFT contributed to critical reading of the manuscript. PHF contributed to chromatographic analysis. JRP designed the study, supervised the laboratory work contributed to biological and chemical studies, chromatographic analysis and critical reading of the manuscript. All the authors had read the final manuscript and approved the submission contributed to biological studies running the laboratory work, analysis of the data

Conflicts of interest

The authors declare no conflicts of interest.

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