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Original Article

Chemical composition of essential oils of leaves, flowers and fruits of *Hortia oreadica*

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ABSTRACT

Hortia oreadica Groppo, Kallunki & Pirani, Rutaceae, known as "para-tudo", "quina", and "quina-do-campo", is used in traditional medicine locally to treat stomach pain and fevers. The aims of this study were: analyze the chemical composition of essential oils from leaves, flowers and fruits of *H. oreadica* and verify the seasonal variation of the chemical components of essential oils from leaves. The essential oils were obtained by hydrodistillation using a Clevenger type apparatus and analyzed by GC/MS. The major components found in the samples of the essential oils were the amorpha-4,7(11)-diene (29.27% – flowers, 20.26% – fruits, 27.66–37.89% – leaves), bicyclogermacrene (23.28% – flowers, 20.64% – fruits, 14.71% to 31.37% – leaves). This work represents the first study of the chemical composition of essential oils from leaves, flowers and fruits and seasonal variation in the essential oils from leaves of *H. oreadica*.

Introduction

Hortia is a neotropical genus of the Rutaceae family with only ten species, nine of them occur in Brazil (Groppo et al., 2010). In general, species of Rutaceae are strongly aromatic and have considerable importance as source of citrus fruits and as ornamentals plants (Perveen and Qaiserm, 2005). Many essential oils of species of this family are used in the pharmaceutical and cosmetic industries, nutritional supplements and aromatherapy (Kondo et al., 2000; Misharina and Samusenki, 2008). *Hortia oreadica* Groppo, Kallunki & Pirani is a shrub with about 1 m tall, well-developed underground system, forming cloned individuals. The leaves are subsessile, leathery and glossy; the flowers have pink petals (Groppo et al., 2010).

H. oreadica is popularly known as "para-tudo", "quina", "quina-do-campo" and its bitter bark is used to treat stomach pain and fever, as a substitute for quinine alkaloid extracted from *Cinchona*, Rubiaceae (Pio-Corrêa, 1984).

Phytochemical studies of *Hortia oreadica* led to the identification from dichloromethane extract of taproots: six limonoids (Severino et al., 2012), the dihydrocinnamic acid derivatives (Braga et al., 2012) and three new limonoids (9α-hydroxyhortiolide A, 11β-hydroxyhortiolide C and 1(*S**)-acetoxy-7(*R**)-hydroxy-7-deoxoinchangin) (Severino et al., 2014); and from dichloromethane extract from stems, two limonoids (9,11-dehydro-12α-hydroxyhortiolide A and 6-hydroxyhortiolide C) (Severino et al., 2012).

Severino et al. (2009) verified antimicrobial activity of the hexane extract of *H. oreadica* roots and the dictamnine alkaloid isolated of this extract against oral pathogens *Enterococcus faecalis* (ATCC 4082), *Streptococcus salivarius* (ATCC 25975), *S. mitis* (ATCC 49456), *S. mutans* (ATCC 25275), *S. sobrinus* (ATCC 33478), *S. sanguinis* (ATCC 10556) and *Lactobacillus casei* (ATCC 11578). Severino et al. (2015) demonstrated an inhibitory effect of the dichloromethane extract of leaves of *H. oreadica* (MIC 31.25 μg/ml), indolequinazoline (15.62 μg/ml) and furoquinoline (31.25 μg/ml) alkaloids, and dihydrocinnamic acid derivatives (62.50 μg/ml) on the growth of *M. tuberculosis*.

The use of essential oils requires detailed chemical characterization and evaluation of possible changes regarding to different climatic conditions and/or geographical origins and genetic factors that can lead to the formation of different chemotypes. The principal pharmacological activities of the essential oils are antimicrobial, anti-inflammatory and the antioxidant (Yunes and Cechinel Filho, 2009).

This study aimed to analyze the chemical composition of essential oils from leaves, flowers and fruits of *H. oreadica* and verify the

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seasonal variability of the chemical components of essential oils from leaves during 12 months.

Material and methods

Plant material

The plant material (300 g) was collected in Pirenópolis, Goiás, during 12-month period (15° 48′ 15″ S, 48° 52′ 48″ W, at an elevation of 1295 m above sea level) and received botanic identification by Dr. Heleno Dias Ferreira, of the Institute of Biological Sciences, Federal University of Goiás (UFG). A voucher specimen of *Hortia oreadica* Groppo, Kallunki & Pirani, Rutaceae, has been deposited at the Herbarium of Federal University of Goiás, Brazil, Conservation Unit PRPPG, under code number UFG-47798. Climatic data of the collection period were obtained from the National Institute of Meteorology site (INMET, 2014).

Essential oils

For analysis of essential oils, healthy leaves, flowers and fruits were collected of ten different individuals of *H. oreadica*. The flowers were collected in November and fruits in January. The leaves were collected monthly for one year. Fresh plant material was triturated separately and submitted to hydrodistillation in a Clevenger-type apparatus for two hours. At the end of each distillation the oils were collected, dried with anhydrous Na_2SO_4, measured, and transferred to glass flasks and kept at a temperature of −18 °C for further analysis.

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 He at a flow rate of 1.0 ml/min and the split mode had a ratio of 1:20. The injection port was set at 225 °C. Significant quadrupole MS 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 1.0 scan/s. Constituents were identified by computer search using digital libraries of mass spectral data (NIST, 1998) and by comparison of their retention indices and authentic mass spectra (Adams, 2007), relative to C_8–C_{32} *n*-alkane series in a temperature-programmed run (Van Den Dool and Kratz, 1963).

Principal Component Analysis (PCA) was applied to examine the interrelationships between the chemical constituents of the essential oils from flowers, fruits and 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 and Hotteling t^2 test.

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

During the leaf collection period, the months of highest precipitation of rain were November/2012 (289.1 mm), December/2012 (202.5 mm), January/2013 (501 mm), February/2013 (231 mm) and March/2013 (312.1 mm), where the temperature ranged from 19 to 31 °C. The months with less precipitation of rain were July/2012 (8.6 mm), August/2012 (0 mm), September/2012 (9 mm) and July/2013 (0 mm) where the temperature ranged from 14 to 34 °C (Table 1).

H. oreadica grows in the mountain range Pireneus on rocky-sandy soil and at altitudes in the range of 1100–1295 m. Regarding the adult plant behavior, initially has the vegetative state subsequently formed green buds that with the development they acquire color that varies from light pink to dark pink. The flowers produce lots of nectar/resin and are visited by various insects such as ants, bees (*Trigonas*, *Apis*), wasps, butterflies, grasshoppers and beetles. The flowering was observed from September to December 2012. Fruit production (November and December 2012) was much lower than the flowers (about 580 flowers per inflorescence) and ranged from 3 to 37 per inflorescence.

Essential oils

The yields of essential oil were 0.09% for the flowers, 0.12% for the fruits and ranged from 0.25 to 0.50% for the leaves. It was verified the presence of sesquiterpene hydrocarbons (73.72%, flowers; 75.17%, fruits; 81.87–95.12%, leaves); oxygenated sesquiterpene (25.84%, flowers; 17.83%, fruits; 4.88–17.04%, leaves). Twenty nine constituents were identified in the essential oil of flowers of *H. oreadica*, being the major components the amorpha-4,7-(11)-diene (29.27%) bicyclogermacrene (23.28%) and pogostol (20.68%); thirty constituents were identified in the essential oil of the fruit and the major components were the same of flowers (20.26; 20.64 and 9.95% respectively) and γ-muurolene (9.24%); 21–28 constituents were identified in the essential oils of the leaves and the major

Table 1
Climate information of collection period of the plant material of *Hortia oreadica*.

Station	Date	Number of days of rainfall	Rainfall total	Average maximum temperature (°C)	Average minimum temperature (°C)
83376	31/07/2012	2	8.6	30.9	14.3
83376	31/08/2012	0	0	31.0	15.3
83376	30/09/2012	5	9	34.4	18.3
83376	31/10/2012	8	84.4	34.5	19.7
83376	30/11/2012	22	289.1	29.4	20.0
83376	31/12/2012	20	202.5	31.2	19.4
83376	31/01/2013	28	501	28.9	19.8
83376	28/02/2013	19	231	31.4	19.2
83376	31/03/2013	23	312.1	30.6	19.9
83376	30/04/2013	10	75.5	30.4	18.7
83376	31/05/2013	3	51.2	30.8	16.2
83376	30/06/2013	7	23.9	30.1	16.3
83376	31/07/2013		0	31.0	14.6

Source: INMET (2014).

Table 2
Percentage of chemical constituents of *Hortia oreadica* leaves, flowers and fruits essential oils.

Constituent	KI	Leaves													Flowers	Fruits
		2012						2013							2012	2013
		July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Nov	Jan
δ-Elemene	1331	–	–	–	–	0.46	–	–	–	–	–	–	–	–	0.3	0.33
α-Cubebene	1348	–	0.23	0.17	–	–	–	–	–	–	0.13	0.13	–	–	–	–
cyclosativene	1371	0.34	0.61	0.55	0.43	0.66	0.49	0.27	0.76	0.72	0.76	0.64	0.41	0.46	0.36	0.66
α-Yalangene	1375	3.62	8.46	6.37	3.2	2.32	3.53	4.59	5.53	4.25	5.75	5.03	4.37	5.25	2.23	4.35
β-Bourbonene	1388	0.56	1.3	1.9	1.36	1.97	0.96	–	1.37	1.41	1.06	0.93	1.17	0.53	0.52	1.51
β-Cubebene	1384	0.78	1.11	1.03	–	0.75	0.69	0.58	1.07	0.87	0.93	0.86	0.63	0.81	0.48	0.95
β-Elemene	1390	1.38	0.89	1.08	2.84	2.28	2.16	0.97	1.43	1.74	1.51	1.31	1.59	0.71	1.38	1.92
(*E*)-caryophyllene	1419	3.0	2.8	2.23	1.73	1.57	1.39	3.42	3.83	1.94	1.77	1.62	3.34	1.95	2.13	1.92
β-Copaene	1432	0.16	0.33	0.37	0.26	0.4	0.25	–	0.33	0.37	0.32	0.3	0.23	–	–	0.32
γ-Elemene	1436	–	–	0.29	–	–	–	–	–	–	0.32	0.32	–	–	–	0.39
α-Guaiene	1439	–	–	–	–	0.15	0.14	–	–	–	–	0.13	–	–	–	–
α-Humulene	1454	0.5	0.36	0.31	0.31	0.3	0.29	0.54	0.59	0.35	0.3	0.28	0.44	0.28	0.4	0.4
allo-Aromadendrene	1460	0.94	1.49	1.07	0.37	0.31	0.57	0.6	0.86	0.64	0.91	0.83	0.68	0.81	0.3	0.79
cis-cadina-1(6),4-diene	1463	–	–	–	–	0.35	–	–	–	–	–	–	–	–	–	–
γ-Muurolene	1479	7.23	9.14	8.41	9.12	8.7	10.61	6.26	9.22	8.57	9.92	8.75	8.82	8.16	5.21	9.24
Amorpha-4,7(11)-diene	1481	33.93	37.89	35.27	32.11	31	27.66	35.2	28.61	28.04	30.8	35.44	28.54	37.58	29.27	20.26
β-Selinene	1490	0.9	0.18	0.53	1.7	2.01	1.91	0.93	1.2	1.3	1.17	1.07	1.25	0.69	1.31	1.55
trans-Muurola-4(14),5-diene	1493	0.56	0.42	0.3	–	–	0.35	–	–	–	0.33	0.35	0.38	0.35	0.25	–
Bicyclogermacrene	1500	21.24	14.71	16.47	29.7	31.37	31.26	20.82	19.41	21.85	20.2	20.84	22.16	17.93	23.28	20.64
α-Muurolene	1500	1.48	1.73	1.44	0.91	–	–	0.84	1.63	1.1	1.39	1.23	1.39	1.4	–	1.39
Epizonarene	1501	0.47	0.31	–	–	–	–	–	–	–	–	–	–	–	–	–
γ-Pachoulene	1502	–	–	–	–	–	–	–	–	–	–	–	–	––	2.67	–
Germacrene A	1509	1.61	1.1	1.38	2.37	3.01	2.71	1.87	1.91	1.99	1.91	1.95	2.06	1.49	0	2.11
γ-Cadinene	1513	2.27	2.1	2.1	0.78	0.47	0.94	1.34	1.9	2.12	1.39	1.26	2.75	2.22	0.91	1.61
7-*epi*-α-Selinene	1522	–	–	–	0.15	0.21	0.19	–	–	–	–	–	–	–	–	–
δ-Cadinene	1523	–	4.4	3.12	1.82	1.34	2.23	1.77	1.98	1.74	2.16	2.38	2.81	3.09	–	1.48
Zonarene	1529	–	–	–	–	–	–	–	–	–	–	–	–	–	0.12	–
Germacrene B	1561	1.72	5	9.22	0.35	1	0.73	1.87	1.61	4.1	9.54	9.47	2.44	6.13	0.44	4.01
Spatulenol	1578	0.7	0.47	1.07	0.49	0.78	0.78	–	2.51	1.98	1.17	0.54	1.23	0.35	0.67	2.46
Globulol	1590	–	–	–	–	–	–	–	–	–	–	–	–	–	0.21	0.25
Carotol	1594	1.3	1.32	1.15	0.42	–	0.62	1.06	1.02	0.92	0.71	0.58	1.19	1.54	0.74	0.87
7-α-Olisolongifolan-	1619	–	–	–	–	0.9	–	–	1.18	1.15	–	–	–	–	1.71	2.14
1,10-di-*epi*-Cubenol	1619	0.33	0.33	0.26	–	–	0.17	0.3	–	0.66	0.2	0.15	–	0.38	0.34	–
Muurola-4,10(14)-dien-1-β-ol	–	–	–	–	–	0.5	–	–	–	–	–	–	–	–	–	–
cis-Cadin-4-en-7-ol	1636	0.65	0.52	0.33	–	–	0.27	0.39	0.4	0.33	0.25	0.23	0.33	0.75	0.44	0.38
α-Muurol	1646	1.15	0.97	0.78	0.32	–	0.37	0.93	0.89	0.78	0.47	0.37	0.91	1.18	0.76	0.9
Cubenol	1648	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pogostol	1653	8.22	1.81	2.78	9.26	7.18	8.09	14.36	5.68	8.86	3.54	3.01	10.85	5.79	20.68	9.95
Occidentalol acetate	1682	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.19
5-*neo*-Cedranol	1685	–	–	–	–	–	–	–	–	–	–	–	–	–	0.29	–
Isobicyclogermacrenal	1734	–	–	–		–	–	–	–	–	–	–	–	–	–	0.64
α,α-Hidroxy-amorpha-4,7(11)-diene	1776	–	–	–		–	–	–	–	–	–	–	–	–	–	0.24
Monoterpene hydrocarbons		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxygenated monoterpenes		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sesquiterpene hydrocarbons		82.69	94.56	93.61	89.51	90.63	89.06	81.87	83.24	83.1	92.6	95.12	85.46	89.84	73.72	75.17
Oxygenated sesquiterpenes		12.35	5.42	6.37	10.49	9.36	10.3	17.04	11.68	14.68	6.34	4.88	14.51	9.99	25.84	17.83
Others		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.19
Total identified (%)		95.4	99.98	99.98	100	99.99	99.36	98.91	94.92	97.78	98.9	100	99.97	99.83	99.56	93.19
Yield (%)		0.49	0.29	0.27	0.25	0.30	0.31	0.42	0.50	0.34	0.32	0.26	0.31	0.4	0.09	0.12

(–) = not detected. KI = Kovats retention index (values from literature).

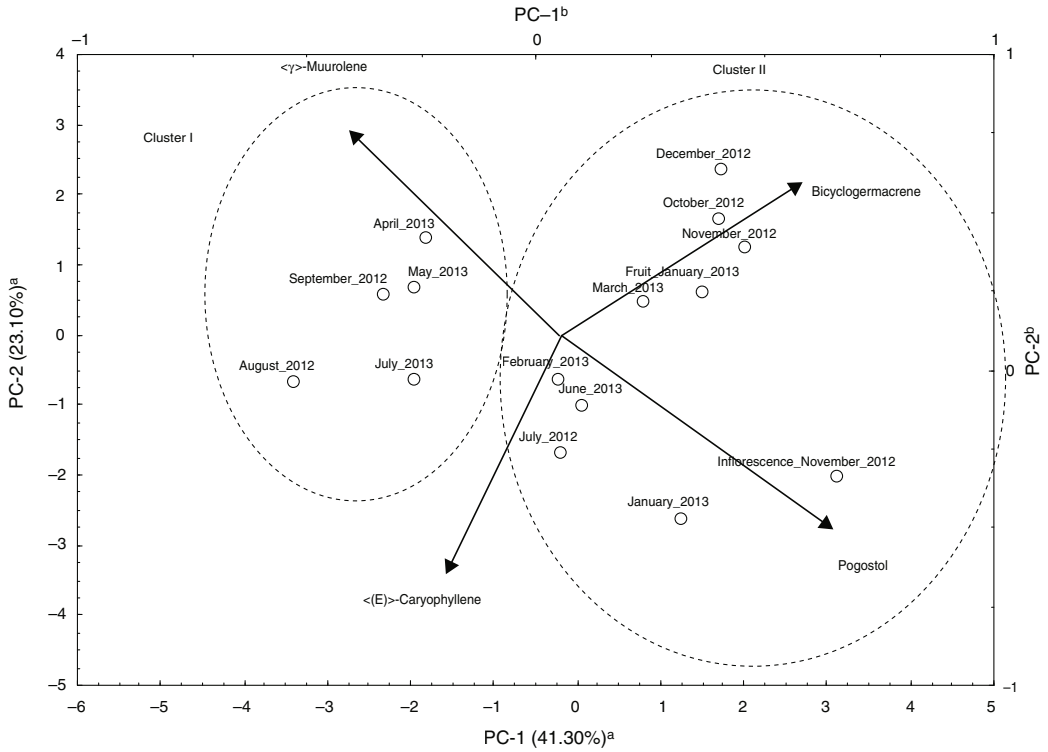

Figure 1. Scatterplot from PCA of leaves, flowers and fruits of *Hortia oreadica* samples collected from Pirenópolis/GO belonging to the clusters I and II. [a]Axes refer to scores from the samples; [b]Axes refer to scores from discriminant oil constituents represented as vectors from the origin.

components were amorpha-4,7-(11)-diene (ranging from 27.66% to 37.89%), bicyclogermacrene (14.71% to 31.37%) and γ-muurolene (6.26% to 10.61%) (Table 2).

The results obtained by analysis of the principal component (PCA) and cluster analysis showed a chemical variability among the *H. oreadica* oils. Fig. 1 shows the relative position of the samples according to the 2D-axis originated in the PCA. Cluster analysis suggests that there are two main types of oils: cluster I (essential oil of leaves collected in the months of August, September 2012, April, May and July 2013) characterized by γ-muurolene (8.876 ± 0.69%) as main constituent; cluster II (essential oils from leaves collected in the months July, October, November, December 2012, January, February, March and June 2013; inflorescences collected in November 2012 and fruits collected in January 2013)

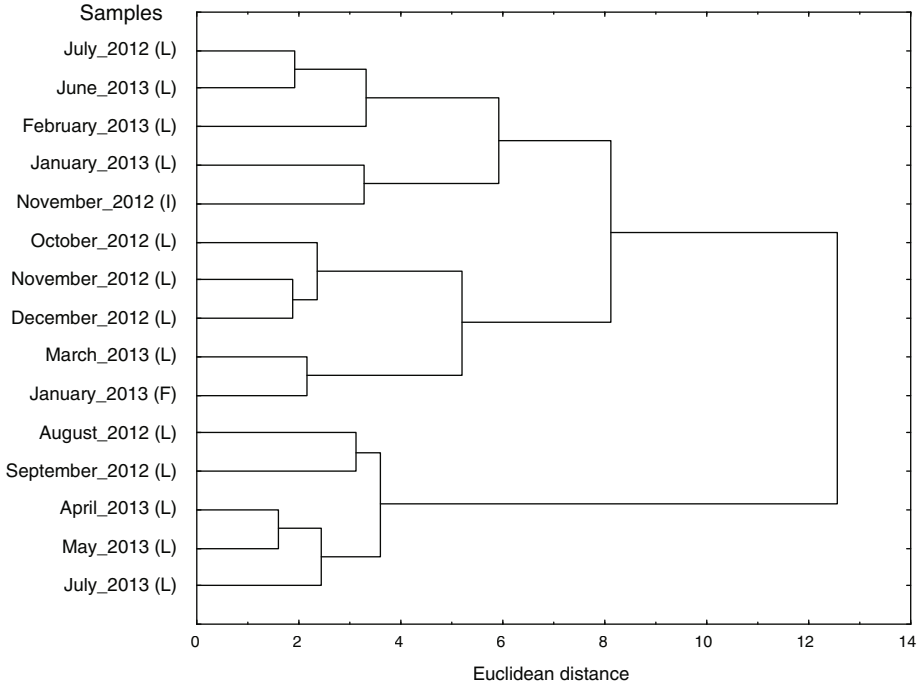

Figure 2. Dendrogram representing the chemical composition similarity relationships of *Hortia oreadica* essentials oils according to Ward's variance minimization method. L = Leaves, I = flowers, F = fruits.

characterized by bicyclogermacrene (24.173 ± 4.69%) and pogostol (10.31 ± 4.32%) as main constituent (Fig. 2). Through discriminant analysis Hotteling t^2 ($p = 0.024$) was also observed that the separation into two clusters is correct for 86.7% of the samples. Canonic Discriminant Analysis has also been performed to help predict cluster by using only two predictive: γ-muurolene and bicyclogermacrene and there was a significant difference between the proposed classes ($p = 0.0248$). The results indicated that the classification proposed by PCA and cluster has 80% of correct classification.

Through the Pearson linear correlation analysis it was found that the pogostol is present in greater amounts in the rainiest months, due to the moderate value ($0.3 < r < 0.6$) of the correlation coefficient $r = 0.56824$, significant at 5%. The α-yalangene constituent appears in smaller proportions in the rainiest months and the value of $r = -0.39684$ also reveals a moderate correlation ($0.3 < r < 0.6$). The temperature presented a weak association ($r < 0.3$) with α-yalangene constituent. There was a negative association between the percentage of α-yalangene and the percentages of bicyclogermacrene and pogostol ($p < 0.05$) (strong negative correlation, $r > 0.6$), the correlation coefficients were −0.79846, −0.68858 and, both significant at level of 5%) (Callegari-Jacques, 2003). The α-yalangene constituent correlated very strongly positively with the constituent germacrene B, with the correlation coefficient $r = 0.63209$.

Discussion

At flowering time, due to the copious production of nectar, it was observed the presence of various insects visitors. It was found that the pogostol is present in greater amounts in rainy months. The α-yalangene constituent, unlike appears in smaller proportions in the rainiest months. Borges et al. (2013) noted that the rainfall is able to influence both positively and negatively in the concentration of sesquiterpenes constituents present in essential oils from *Myrcia tomentosa* leaves, which is species also found in the Brazilian Cerrado. The temperature presented a weak association with the constituent α-yalangene, which indicates that other environmental factors are associated with changes in concentration of the substance. There was no correlation between constituents of essential oils and the periods of flowering and fruiting for *H. oreadica* species.

The constituents identified in *H. oreadica* essential oil from leaves, flowers and fruits are sesquiterpene hydrocarbons and oxygenated sesquiterpene. Among the major constituents identified in the essential oils of the flowers, leaves and fruits of *H. oreadica*, we highlight the amorpha-4,7(11)-diene and bicyclogermacrene, which chemically differentiates this species of *H. brasiliana* that has the nonacosane, eicosane and guaiol as the major constituents in the essential oil from leaves (Magalhães et al., 2013). The amorpha-4,7(11)-diene was a differential for this species, it has not been found as major constituents in Rutaceae literature. Moreover, bicyclogermacrene has also been reported as a major constituent of the essential oil *Spiranthera odoratissima* A. St. Hil. leaves (Chaibub et al., 2013), the aerial parts of *Haplophyllum linifolium* (L.) G. Don fil. (Iñigo et al., 2002), of *Zanthoxylum hyemule* A. St. Hil. and of *Zanthoxylum naranjillo* Griseb (Guy et al., 2001).

Although the amorpha-4,7-(11)-diene is the major compound found in the samples of essential oils of *H. oreadica* leaves, when the PCA was generated with this compound, the classification was not suitable, because this compound presented low eigenvectors in the first principal components axes, which present higher amounts of explicability of the system. So, the amorpha-4,7-(11)-diene was not selected for the classification of the oil samples.

Results obtained from de principal component and cluster analyses showed a great chemical variability in the oils of *H. oeradica*. The cluster II is characterized by the bicyclogermacrene (18.0 ± 2.5), which is the discriminant factor mainly of the wet season, besides this substance is present in fruit samples of *H. oreadica*. The pogostol is related to the cluster II, mainly in the inflorescence sample and in the sample of leaves collected in January. The compound γ-muurolene (8.3 ± 1.6) has a relationship with cluster I, characterized by leaves collected in months with lower levels of rainfall (dry season). In discriminant analysis the combination of the compounds showed that γ-muurolene and bicyclogermacrene were the suitable predicted variables to the validation of the cluster obtained. With these two essential oils compounds was possible confirm the percentage of well-classification at the level of 5% ($p = 0.0248$), and the two functions retain 80% of the overall variability.

In conclusion, the study of the constituents of the essential oils of *H. oreadica* leaves for 12 months contributed to the understanding of the behavior of chemical constituents in relation to seasonal variations. The major constituents of the essential oils of the flowers, the leaves and fruits of *H. oreadica* were the amorpha-4,7(11)-diene and bicyclogermacrene, the first being a differential for this species. This work represents the first study of essential oils from leaves, flowers and fruits of *H. oreadica* collected in Pirenópolis, Goiás. The knowledge gained from this study should be useful for further exploitation and application of the resource.

Authors' contributions

DLS (student) contributed in collecting plant sample, running the laboratory work. HDF contributed in collecting plant sample and identification, confection of herbarium. LLB contributed to the statistical analyzes. JRP contributed to biological and chemical studies, chromatographic analysis and critical reading of the manuscript. LMFT contributed to critical reading of the manuscript. PAS contributed to chemical studies. PHF contributed to chromatographic analysis. SS contributed to evaluate the antimicrobial activity. TSF designed the study, supervised the laboratory work, drafted the paper and contributed to critical reading of the manuscript. All the authors have 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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