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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).