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Harvest time and geographical origin affect the essential oil of *Lippia gracilis* Schauer

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ABSTRACT

Lippia gracilis Schauer (Verbenaceae) is a promising shrubby aromatic species for the production and marketing of essential oils rich in thymol and carvacrol. The aim of this study was to evaluate the effect of harvest time and geographical origin on the essential oil of *L. gracilis* accessions. We tested the accessions LGRA106, LGRA107, LGRA108, LGRA109, LGRA110, LGRA201 and LGRA202, harvested in 2009 and 2012. The essential oils were extracted by hydrodistillation using a modified Clevenger apparatus. The chemical components were identified by gas chromatography coupled with mass spectrometry and flame ionization detection (GC/MS-FID). The essential oil contents of *L. gracilis* exhibited significant variation among accessions only in four-year-old plants. Accession LGRA202 had the highest essential oil content (4.66%), whereas the lowest content was recorded for accession LGRA107 (2.35%). When compared among harvest times, higher contents of essential oils were found in four-year-old plants. The largest variation was observed in accession LGRA202 plants, whose contents increased from 1.52% in one-year-old plants to 4.66% in four-year-old plants. In the two assessed harvest times, carvacrol was the major compound, except in accession LGRA106, whose major compound was thymol. Harvest time reduced the content of the major compound (carvacrol or thymol) of the essential oils from all accessions. Accession LGRA202 exhibited the largest reduction in carvacrol content, from 47.29% in one-year-old plants to 34.29% in four-year-old plants. The accessions exhibit chemical variability in the major compounds carvacrol or thymol. Harvest time and geographical origin affected the content and proportion of chemical constituents of essential oils of all *L. gracilis* accessions.

1. Introduction

Essential oils are one of the most important groups of raw materials for the food, pharmaceutical, and cosmetic sectors. Essential oils consist of complex mixtures of different classes of substances, including phenylpropanoids and mono- and sesquiterpenes, which belong to the secondary metabolism of plants (Moraes, 2009).

The major constituents already identified are thymol, carvacrol, geranial, linalool, ρ-cymene, carvone, neral, limonene, β-caryophyllene, caryophyllene oxide, myrcene and γ-terpinene. Due to the chemical diversity in essential oils, studies on this genus and species have confirmed the enormous potential for medicinal application, and the most promising species are *Lippia sidoides*, *Lippia gracilis* and *Lippia alba*. However, the concentrations of these chemical constituents may vary depending on various external factors, thus hindering their safe use and commercialization (Soares and Tavares-Dias, 2013).

L. gracilis Schauer (Verbenaceae) is a shrubby aromatic species that is highly branched, endemic in northeastern Brazil, commonly found in the states of Bahia, Sergipe and Piauí, and popularly known in Brazil as *alecrim de chapada* or *alecrim de tabuleiro* (Lorenzi and Matos, 2002). It is used in folk medicine to treat colds, cough, sinusitis, bronchitis and headache by traditional communities inhabiting the semi-arid region of northeastern Brazil (Albuquerque et al., 2007) and is also used externally to treat skin diseases, burns, wounds and ulcers (Pascual et al., 2001).

Thanks to studies related to pharmacological activity, *L. gracilis* has emerged as one of the most important medicinal plants of the genus. The essential oil of this species has demonstrated antimicrobial, leishmanicidal, insecticidal, acaricidal, anti-inflammatory,

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antinociceptive and cytotoxic activities, mainly due to the major presence of the monoterpenes carvacrol and thymol (Albuquerque et al., 2006; Mendes et al., 2010; Silva et al., 2008; Cruz et al., 2013; Melo et al., 2013, 2014).

The presence of these monoterpenes makes *L. gracilis* a promising species for the production and marketing of essential oils rich in thymol and carvacrol, emerging as an alternative source of income for local communities in semi-arid Northeastern Brazil.

Although essential oil biosynthesis is determined by genetic factors, it is intensely affected by factors such as harvest time, plant age, developmental stage, climate (temperature, light intensity, seasonal effect, etc.) and soil characteristics. Most aromatic species with medicinal applications are close to their wild states, maintaining strong interactions with the environment, thus making it difficult to increase the yield and to standardize the quality of essential oils (Moraes, 2009). For that reason, it is important to analyze and measure the amount of phenotypic variation determined by both genetic and edaphoclimatic factors, as such information is essential for the cultivation and future breeding of *L. gracilis*.

Harvest times has been studied as one of the factors that affects the biosynthesis of essential oils; different chemical behaviors have been observed for *L. alba* (Santos and Innecco, 2004), *L. sidoides* (Figueiredo et al., 2009), and *Hyptis suaveolens* (L.) Poit (Martins et al., 2006).

Thus, studies in this regard are vital to determine the best conditions for growing and harvest aromatic species to obtain higher essential oil contents of sufficient quality for safe use and commercialization. Hence, the aim of this study was to evaluate the effect of harvest time and geographical origin on the essential oil of *L. gracilis* Schauer accessions.

2. Materials and methods

2.1. Plant material

The Active Germplasm Bank (AGB) of *L. gracilis* Schauer was established at the Research Farm "Campus Rural da UFS" of the Federal University of Sergipe, located in the municipality of São Cristóvão, state of Sergipe, Brazil, at latitude 11°00′S and longitude 37°12′W (Table 1).

The assay was conducted in a randomized block design, in a split-plot scheme, with two replications. Each replication consists of three plants with cultivation spacing of 1.0 × 1.0 m between the plants and blocks. In the plots seven accessions were tested (accessions LGRA106, LGRA107, LGRA108, LGRA109, LGRA110, LGRA201, and LGRA202 – see Table 1 for origin) and in the split plots harvest times of plants (1 and 4 years old plants). The AGB of *L. gracilis* was established in 2008. The climate of the region is tropical semiarid, with mild and rainy winter, hot and dry summer. The plants were harvested for essential oil extraction in July of 2009 and 2012. According to Brazilian National Meteorology Institute – In MET (www.inmet.gov.br/projetos/rede/pesquisa/) the average rainfall in july, minimum and maximum temperature were 121.8 mm, 22.7 °C, 28.5 °C for 2009; and 100.6 mm, 22.3 °C, 28.3 °C for 2012, respectively.

2.2. Extraction and analysis of essential oils

When the plants were one and four years old, they were harvested for extracting essential oils. For each period, the plant material, consisting of leaves, was collected followed by manual defoliation. The leaf material was dried in a forced air oven at 40 °C for five days (Ehlert et al., 2006). The essential oils were extracted by hydrodistillation using a modified Clevenger apparatus (Guenther, 1972) coupled to a 3 L round-bottomed flask. The extraction consisted of 75 g of dried leaves to two liters of distilled water for 160 min after boiling started. After this period, the obtained essential oils were measured, collected in amber vials and stored at −20 °C until the analyses. The content (%) of essential oils was calculated by dividing the oil volume obtained by dry weight (75 g) and then multiplying by 100.

GC analyses were performed using gas chromatography coupled with mass spectrometry and flame ionization detection (GC–MS/FID; QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan); the instrument was equipped with an autosampler AOC-20i (Shimadzu). Separations were accomplished using an Rtx®-5MS Restek-fused silica capillary column (5%-diphenyl–95%-dimethyl polysiloxane; 30 m × 0.25 mm i.d., 0.25 μm film thickness) at a constant helium (99.999%) flow rate of 1.2 mL/min. The essential oils were diluted in ethyl acetate and an injection volume of 0.5 μL (5 mg/mL) was employed, with a split ratio of 1:10. The oven temperature was programmed to 50 °C (isothermal for 1.5 min), with an increase of 4 °C/min to 200 °C, then 10 °C/min to 250 °C, ending with a 5 min isothermal at 250 °C.

The MS and FID data were simultaneously acquired employing a Detector Splitting System; the split flow ratio was 4:1 (MS:FID). A 0.62 m × 0.15 mm i.d. restrictor tube (capillary column) was used to connect the splitter to the MS detector, and a 0.74 m × 0.2 mm i.d. restrictor tube was used to connect the splitter to the FID detector. The MS data (total ion chromatogram, TIC) were acquired in full scan mode (m/z of 40–350) at a scan rate of 0.3 scan/s using electron ionization (EI) with an electron energy of 70 eV. The injector temperature was 250 °C, and the ion-source temperature was 250 °C. The FID temperature was set to 250 °C, and the gas supplies for the FID were hydrogen, air, and helium at flow rates of 30, 300, and 30 mL/min, respectively. Quantification of each constituent was estimated by FID peak-area normalization (%). Compound concentrations were calculated from the GC peak areas, and they were arranged in order of GC elution.

Identification of individual components of the essential oil was performed by computerized matching of the acquired mass spectra with those stored in NIST21, NIST107 and WILEY8 mass spectral libraries of the GC–MS data system. A mixture of hydrocarbons (C9H20–C19H40) was injected under these same conditions, and the identification of constituents was then performed by comparing the spectra obtained with those of the equipment data bank and according to the Retention Index, calculated for each constituent as previously described (Adams, 2007). Retention indices were obtained using the equation proposed by Van den Dool and Kratz, (1963). Thus, it was not necessary to use any standard compound to confirm the identified compounds.

2.2.1. Statistical analysis

The data on the chemical composition of essential oils of the accessions within and between harvest times were subjected to combined analysis of variance (ANOVA), and the means were compared with the Scott-Knott test at a 5% probability, using the Sisvar 5.0 software. Multivariate analyses, principal components (PCA) were performed using Statistica version 7.0.

3. Results and discussion

The results obtained for *L. gracilis* show that the essential oil content did not vary significantly among accessions for the one-year-old plants (2009) as firstly shown by Cruz et al., (2014) (Table 2). However, there was significant variation among accessions for four-year-old plants. Accession LGRA202 had the highest essential oil content (4.66%), whereas the lowest content was detected for accession LGRA109 (2.35%) (Table 2).