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Original Article

Evaluation of the chemical composition and variability of the volatile oils from *Trembleya parviflora* leaves

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ABSTRACT

Trembleya parviflora (D. Don) Cogn., Melastomataceae, also known as "quaresmeira-branca", is a subshrub that is commonly used to treat verminosis, scabies, dermatoses, rheumatism, vaginal infections, ulcerations and wounds. The aim of this work was to perform a morphological study of *T. parviflora*, evaluate the composition and chemical variability of the volatile oils from the leaves, perform phytochemical screening of the powder from the leaves and to define parameters for quality control of the plant material. Macroscopic characterization of *T. parviflora* was carried out by naked eye in Serra dos Pireneus, Pirenópolis, Goiás for 12 months. Volatile oils were subjected to hydrodistillation with Clevenger apparatus and analyzed by gas chromatography-mass spectrometry. Phytochemical screening and ash and volatile compound content determination were performed by conventional techniques. *T. parviflora* has simple, oppositely crossed and petiolate leaves. The inflorescence of this plant is a cyme. The presence of coumarins, steroids, triterpenes, flavonoids and tannins was observed. The total ash content was 4.05 ± 0.02%; the insoluble ash content was 0.10 ± 0.03%; and the volatile compound content was 9.53 ± 0.02%. The major compounds present in the volatile oils were α-terpineol (2.7–16.5%), α-pinene (0.6–25.4%), β-pinene (2.7–23.1%), sabinene (1.2–14.1%), acetoxyeudesman-4-α-ol (0.6–6.3%) and 2,4a-8,8-tetramethyldecahydrocyclopropanaphtalene (2.4–24.4). Two clusters were identified: Cluster I represented the period with low levels of rainfall, and Cluster II represented the period with high levels of rainfall. This study provides data that can be applied for the quality control of powdered leaves and is the first description of the chemical composition and variability of the volatile oils from the leaves of *T. parviflora*.

Introduction

The genus *Trembleya*, with approximately fourteen species and characterized by non-imbricate, sessile or petiolate leaves without translucent scores, is found exclusively in Brazil. The flowers exhibit a modified dichasium, have white or pink petals and 8–10 stamens, are dimorphic, lack staminodes, and exhibit anthers with rostrate apices. The ovary has 3–5 locules free (Goldenberg et al., 2015).

Trembleya parviflora (D. Don) Cogn., Melastomataceae, popularly known as "quaresmeira-branca" (Oliveira-Filho and Flumihan-Filho, 1999), is a subshrub that is commonly used to treat verminosis, scabies, dermatoses, rheumatism, vaginal infections, ulcerations and wounds. This species is endemic to Brazil, occurring in the Distrito Federal and in the states of Goiás, Bahia, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo and Paraná in rupestrian fields, highland fields, savannahs and rainforests. Vegetative propagation of this plant occurs *via* subterranean structures, with individuals 0.4–3 m in height, flowering and fruiting during the summer season. The flowers are lightly scented (Baumgratz et al., 2007).

According to Somavilla and Graciano-Ribeiro (2011), *T. parviflora* presents glandular trichomes on both faces, with uniseriate peduncles, multicellular glandular heads and oil droplets. The deposition of phenolic compounds occurs in the palisade parenchyma, cortical parenchyma and medullar parenchyma, phloem and endodermis.

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Scientific studies have verified the leishmanicidal activity of methanolic extracts of *T. parviflora* (Antinarelli et al., 2015).

Many studies have employed multivariate analyses, such as principal component analysis (PCA) and cluster analysis (CA), to evaluate the chemical variability of various natural compounds, mainly volatile compounds. These analyses are useful methods for ordering, aiming to reduce the dimensions of the data set in order to easily understand the system based on the chemical profile of the volatile oils (Boira and Blanquer, 1998; Sampaio et al., 2016).

There have been no studies about the chemical composition of the volatile oils and other secondary metabolites of *T. parviflora*. The aim of this work was to perform a morphological study of *T. parviflora*, evaluate the composition and chemical variability of the volatile oils from the leaves, perform phytochemical screening of the powder from the leaves and to define parameters for quality control of the plant material.

Materials and methods

Plant material

The leaves of *Trembleya parviflora* (D. Don) Cogn., Melastomataceae, were collected in Serra dos Pireneus, Pirenópolis, Goiás, Brazil (15°48′15″ S to 48°52′48″ W, at an elevation of 1295 m above sea level) in the rocky fields, sandy and stony soils and dirty fields of the Cerrado region. Professor Dr. Heleno Dias Ferreira identified *T. parviflora* pecimens, and a voucher was deposited at the Herbarium of Federal University of Goiás, Brazil, Conservation Unit PRPPG, under code number UFG 50530. Climatic data for the period were obtained from the Meteorological Institute (INMET, 2017).

Morphological analysis

Macroscopic characterization of *T. parviflora* was carried out in Serra dos Pireneus (Pirenópolis, Goiás) and was performed by naked eye monthly for 12 months, and the images were recorded with a Canon EOS T4i digital camera. Samples of leaves, stems and flowers were collected and analyzed using a stereoscopic microscope at the Taxonomy Laboratory of the Department of Biology, ICB/UFG.

Physicochemical characterization of plant material

For physicochemical characterization of the plant material, the leaves were dried in a drying oven at 40 °C for 48 h and pulverized in a commercial crushing machine with a stainless steel monoblock cup (LS-08MB-N; Skymsen Metalúrgicas Siemsen LTDA, Brazil). The obtained powder was used for phytochemical screening and measurement of volatile compound content and total and insoluble ash content.

For phytochemical screening, anthraquinone, coumarins, steroids, triterpenes, digitalis glycosides, starch, alkaloids, flavonoids, saponin, tannins and methylxanthines were investigated according to methodologies described by Costa (2001) and Cunha (2005).

Measurement of the volatile compound content was performed in a moisture analyzer that produces radiation in the infrared region by means of a halogen lamp (Ohaus model MB35) (Brasil, 2010). The assays were performed in triplicate, and the values were calculated as means and coefficients of variation (CV).

The total ash and acid-insoluble content was determined as described by the Farmacopeia Brasileira (2010).

Volatile oils

For analysis of volatile oils, fresh healthy leaves were collected from ten different *T. parviflora* plants (300 g), triturated separately using a commercial crusher (Skymsen, LS-08MB-N) and subjected to hydrodistillation in a Clevenger-type apparatus for two hours. Each volatile oil sample was dried with anhydrous Na_2SO_4, measured, transferred to a vial, covered with aluminum foil and stored at −18 °C for further analysis.

The volatile oils were analyzed using a Shimadzu GC-MS QP5050A instrument 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 with the following temperature program: 60–240 °C at 3 °C/min, from 240 to 280 °C at 10 °C/min, and finally holding at 280 °C for 10 min. The carrier gas was He at a flow rate of 1 ml/min, and the split mode had a ratio of 1:20. The injection port was maintained at 225 °C. The significant quadrupole MS operating parameters were as follows: interface temperature, 240 °C; electron impact ionization, 70 eV; scan mass range, 40–350 *m*/*z*; sampling rate, 1 scan/s. Constituents were identified by a computerized search using digital libraries of mass spectral data (NIST, 1998) and by comparing the retention indices of the constituents with those found in authentic mass spectra (Adams, 2007) relative to a C_8–C_{32} *n*-alkane series in a temperature-programmed run (Van Den Dool and Kratz, 1963).

Principal component analysis (PCA) was employed to evaluate the possible interrelationships between the compounds found in the volatile oils from leaves collected in different months over one year. A hierarchical cluster analysis (HCA) was used to study the similarity among samples based on the distribution of chemical compounds, and the hierarchical clustering was performed according to Ward's minimum variance method (Ward, 1963). The chemical compounds selected for these analyses were α-pinene, β-pinene, α-terpineol, sabinene, and acetoxyeudesma-4-α-ol. Validation of the cluster analysis was performed by canonical discriminant analysis (CDA).

Results

Morphological description

Trembleya parviflora is a subshrub that is 1–2 m in height and has a cylindrical trunk that has longitudinal fissures, is gray in color, and is exfoliative (Fig. 1A). The leaves have the following features: simple; oppositely crossed; viscous; hairy; present basal acrodromous venation; ribs are imprinted on the adaxial face and protruding on the abaxial face; greenish petioles that are 4 × 1 mm – 9 × 2 mm in size; concave on the adaxial face and convex on the abaxial face. The leaves are elliptic and narrow and are 5 × 1.4 cm–5.6 × 1.8 cm in size; the base is slightly attenuated and the apex acute; the leaves are bright dark green on the adaxial face and light green on the abaxial face; and both faces are covered with trichomes, simple and glandular, with entire or revolute margins (Fig. 1B).

The flowers exhibit the following features: monochasium-type cymose inflorescence (Fig. 1C); imbricated preflowering stage (Fig. 1E); white flowers; five triangular sepals, 2 mm long and 2 mm wide at the base; five obovate free petals, 8 mm × 5 mm, with longitudinal rosy stripes and obtuse apices, glabrous or with simple trichomes; 9–10 stamens, the largest with rosy filets, vinous anthers, a rostrum, and a yellowish bifid 0.2–0.8-mm connective extension, and five smaller stamens that have yellow theca (Fig. 1F); greenish hypanthium, 3 mm × 2 mm in size; free ovary, conical to rounded, 2–3 mm in length, six carpels, six locules, and numerous ovules; capsule with numerous seeds (Fig. 1D), small seeds, 0.5 × 0.3 mm in length.

Flowering of the *T. parviflora* plants occurred between August and September, with numerous flowers in the inflorescence (Fig. 1C, E and F). In September and October, dry branches and green fruits were observed in the inflorescence, and there were

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