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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*.

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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

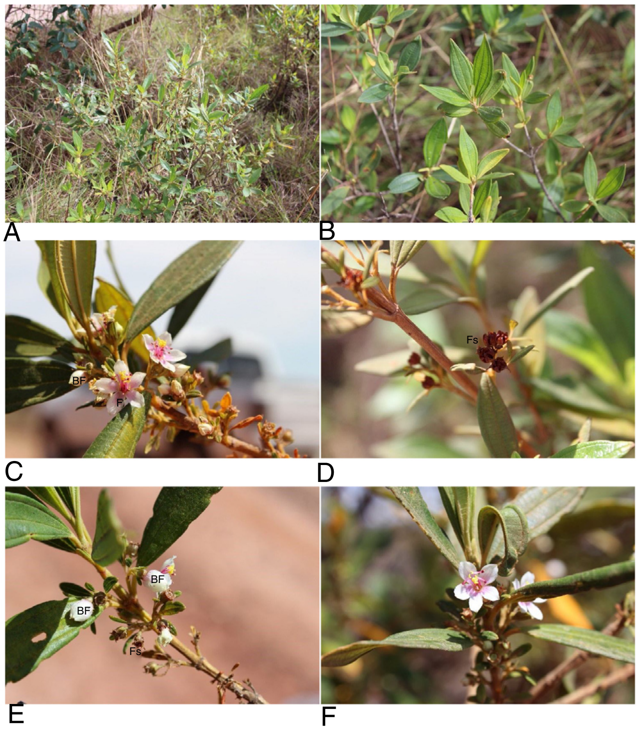

Fig. 1. *Trembleya parviflora.* (A) General appearance of the plant (November); (B) leaf detail (November); (C) detail of the inflorescence (August); (D) dried fruits (October); (E) flower buds (August); (F) flower detail (August). Legend: BF, floral button; Fs, dry fruit; F, flower.

green and yellow leaves on the branches. In November and December, greenish leaves and some green fruits were observed (Fig. 1B and D). During the months of January, February and March, there were ripe and dry fruits and rare flowers observed in the inflorescence, and there were green leaves and some yellowish leaves on the branches. During the months of March, April, May and July, the plants presented new green leaves with dried fruits and a few flower buds.

Physicochemical characterization of plant material

The presence of coumarins, steroids/triterpenes, flavonoids and tannins was observed in *T. parviflora* leaves.

The total ash content was 4.048 ± 0.017%, with a coefficient of variation of 0.0042%. The insoluble ash content was 0.10 ± 0.03%, with a coefficient of variation of 0.3%. The volatile compound content was 9.53 ± 0.015%, with a solid weight of 3.181 g and a coefficient of variation of 0.0015%.

Volatile oils

During the collection period, the months of highest rainfall were October (162 mm), November (341.9 mm), December (123.5 mm), January (141.1 mm), February (249.1 mm), March (195.1 mm) and April (303.1 mm), with average temperatures ranging from 32.7 °C to 19.2 °C. The months with low rainfall were May (0.7 mm), June (0 mm), July (0 mm), August (16.5 mm) and September (36.8 mm), with temperatures ranging from 14.0 °C to 35.2 °C (Table 1).

Among the compounds identified, 2.2–57.2% were monoterpene hydrocarbons; 5–31.1% were oxygenated monoterpenes; 0–4.3% were sesquiterpene hydrocarbons; 1.5–6.8% were oxygenated sesquiterpenes; 0.2–22.1% were oxygenated diterpenes; and 3.9–41.6% were other compounds (Table 2).

The major compounds present in the leaves were α-terpineol, ranging from 2.7 to 16.5%; α-pinene, ranging from 0.6 to 25.4%; β-pinene, ranging from 2.7 to 23.1% (absent in the January samples); sabinene, detected from August to March and ranging from 1.2%; to 14.1%; acetoxyeudesman-4-α-ol, ranging from 0.6 to 6.3% (not detected in May) and 2,4a-8,8-tetramethyldecahydrocyclopropanaphtalene detected from November to May ranging from 2.4 to 24.4% (Table 2).

The yields of volatile oils from the *T. parviflora* leaves ranged from 0.01 to 0.03% (v/w).

The results obtained from the PCA and cluster analysis showed the existence of chemical variability among samples of volatile oils obtained from leaves of *T. parviflora*. Fig. 2 indicates the relative position of the 2D axis from the PCA. This analysis suggests that Cluster I represents by the period with low levels of rainfall, discriminated by compounds α-pinene, β-pinene, sabinene and α-terpineol. Cluster II represents the period with high levels of rainfall, discriminated by the compound acetoxyeudesman-4-α-ol and 2,4a-8,8-tetramethyldecahydrocyclopropanaphtalene. The cluster analysis (Fig. 3) suggests the existence of two groups (which is consistent with the results of the PCA): Cluster I (volatile oils from leaves collected in May, June, July, August, September and October) and Cluster II (volatile oils from leaves collected in November, December, January, February, March and April). The results indicated that the classification proposed by the PCA and HCA was appropriate as the chemical profile of compounds identified from the volatile oil samples.

Canonical discriminant analysis was performed to help predict the grouping of the cluster analysis; two predictive variables were employed α-pinene and 2,4a8,8-tetramethyldecahydrocyclopropanaphtalene and the two

Table 1
Climate information for the collection period of the plant material of *Trembleya parviflora*.

Station	Date	Total rainfall	Average maximum temperature (°C)	Average minimum temperature (°C)	Relative humidity
83376	06/30/2016	0	31.3	14.9	57.05
83376	07/31/2016	0	32.4	14.0	49.91
83376	08/31/2016	16.5	33.2	16.5	48.44
83376	09/30/2016	36.8	35.2	19.1	48.33
83376	10/31/2016	162.0	32.7	19.4	64.62
83376	11/30/2016	341.9	30.3	19.9	77.36
83376	12/31/2016	123.5	31.2	19.7	73.87
83376	01/31/2017	141.1	31.4	19.2	75.43
83376	02/28/2017	249.1	30.5	19.5	79.31
83376	03/31/2017	195.1	31.3	19.2	80.13
83376	04/30/2017	303.1	31.3	19.2	77.33
83376	05/31/2017	0.7	31.0	18.0	70.97
83376	06/31/2017	0	29.8	15.1	23.0

INMET (2017).

Table 2
Percentages of the chemical constituents of the volatile oils from *Trembleya parviflora* leaves collected from Pirenópolis, Goiás.

Constituents	KI	RI	2016						2017					
			Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Mai	Jun
2E-hexenal	855	841	3.6	3.1	1.2	2.0	3.6	0.7	–	2.1	2.5	–	1.0	2.6
Bicyclo(3.1.0)hept-2-ene	911	911						2.9						
Tricyclene	926	916	–	–	–	–	–	–	0.6	0.7	–	–	–	–
Artemisia triene	929	931	–	–	8.1	6.2	–	–	–	–	–	–	–	–
α-Pinene	939	933	25.2	12.0	16.9	12.1	3.2	6.9	0.6	1.5	3.8	4.5	13.8	25.4
Camphene	954	949	0.3	0.4	–	–	–	8.2	–	–	–	–	0.4	–
Sabinene	975	975	–	13.3	5.9	5.3	6.2	14.1	1.6	1.2	2.2	–	–	
β-Pinene	979	979	19.8	14.8	13.3	12.6	6.5	5.6	–	10.2	2.7	3.4	11.5	23.1
Dehydroxy-*trans*-linalool oxide	993	988	0.4	0.3	–	–	–	0.4	–	0.8	–	–	0.7	–
Dehydroxy-*cis*-linalool oxide	1008	1006	0.3	0.2	–	0.2	–	–	–	–	–	–	0.6	–
Cyclohexene		1015	0.2											
α-Terpinene	1017	1015	–	–	0.2	–	–	–	–	–	–	–	0.3	–
o-Cymene	1026	1022	0.4	0.2	0.2	0.3	–	–	–	–	–	–	0.4	0.4
Limonene	1029	1028	2.5	3.3	3.4	4.2	0.8	–	–	0.9	–	0.9	4.1	4.4
γ-Terpinene	1059	1057	0.5	0.6	0.5	0.5	–	–	–	–	–	–	0.7	0.5
cis-Sabinene hydrate	1070	1083	–	–	–	–	1.1	0.8	–	–	–	–	–	–
p-Mentha-3.8-diene	1072	1074	1.1	1.8	1.4	1.6	1.4	0.9	–	–	0.5	0.8	2.8	1.0
Terpinolene	1088	1084	1.4	1.6	1.4	1.8	1.2	–	–	–	–	–	1.6	1.5
p-Cymenene	1091	1088	1.2	1.2	0.9	1.1	0.5	–	–	–	–	–	1.3	0.9
Linalool	1096	1098	3.4	3.3	3.1	2.6	2.6	1.8	–	1.0	0.6	–	4.1	2.3
Endo-fenchol	1116	1120	0.3	0.4	0.4	0.4	0.3	–	–	–	–	–	0.8	0.4
α-Campholenal	1126	1125	1.0	1.2	1.3	1.1	1.3	0.6	–	–	–	–	1.4	0.6
trans-Pinocarveol	1139	1138	3.1	3.6	3.8	2.5	2.1	2.1	–	1.0	0.6	1.5	2.8	2.3
Camphor	1146	1146	–	–	–	–	–	–	–	0.5	–	–	–	–
Pinocarvone	1164	1160	1.1	1.7	1.8	1.3	1.2	0.7	–	0.6	–	–	1.9	1.2
Borneol	1169	1169	0.3	0.4	0.5	0.4	–	0.5	–	–	–	–	–	–
δ-Terpineol	1166	1175	–	–	–	–	–	–	–	–	2.6	–	–	–
p-Mentha-1.5-dien-8-ol	1170	1169	–	–	–	–	6.2	3.7	–	–	–	–	–	–
Terpinen-4-ol	1177	1179	1.4	1.3	0.9	1.1	–	1.0	–	–	–	0.7	1.8	1.0
3-Cyclohexanal-methanol		1180	–	–	–	–	3.5	–	2.3	3.5	1.0	–	–	–
α-Terpineol	1188	1193	13.5	14.3	16.5	14.7	12.8	11.2	2.7	5.2	3.9	5.4	9.9	8.5
γ-Terpineol	1199	1199	–	0.2	0.2	–	–	–	–	–	–	–	–	–
Cyclohexanol		1391	0.3	0.4	0.5	0.6	0.6	–	–	0.4	0.4	–	0.5	–
Caryophyllene	1419	1416	1.2	1.3	1.4	2.7	2.1	1.4	–	0.7	0.5	1.4	2.3	2.6
α-*trans*-Bergamotene	1434	1431	–	0.3	0.3	0.4	0.4	0.5	–	–	–	–	–	0.4
Drim-8(12)-ene	1491	1491	0.8	0.8	0.9	0.8	0.7	0.8	–	0.6	0.6	1.3	1.4	–
β-Bisabolene		1505	–	–	0.2	–	–	–	–	–	–	0.7	–	–
Amorphene	1512	1515	–	–	–	0.4	–	–	–	–	–	0.5	–	–
Bergamotol		1522	0.2	–	–	–	–	–	–	–	–	–	0.9	–
E-Nerolidol	1563	1559	0.9	0.9	1.2	1.6	1.7	1.2	0.8	1.7	2.8	1.6	1.5	0.8
Caryophyllene oxide	1583	1577	1.0	0.6	0.9	1.8	1.9	1.1	0.7	1.0	2.0	2.6	2.3	1.3
2-Acorenol	1633	1624	–	–	–	–	–	–	–	–	–	–	–	–
Caryophylla-4(12).8(13)-dien-5β-ol	1640	1632	0.6	0.4	0.5	0.9	1.1	0.7	–	0.7	1.2	1.5	0.8	–
Selina-3.11-dien-6-α-ol	1644	1642	–	–	–	0.4	0.6	–	–	–	0.5	–	–	–
14-Hydroxy-*(Z)*-caryophyllene	1667	1666	–	–	–	–	–	–	–	–	–	1.1	–	–
δ-Dodecalactone	1704	1700	–	–	–	–	–	–	–	–	–	0.4	–	–
Eudesm-11-em-4-α.6-α-diol	1808	1810	0.2	0.2	0.3	0.3	0.6	0.5	–	1.1	1.0	0.5	–	–
5*Z*,9*E*-Farnesyl acetone	1889	1876	–	–	0.2	0.3	–	0.5	1.6	–	0.8	–	–	–
n-Hexadecanoic acid	1936	1936							5.6					
Acetoxyeudesman-4-α-ol	1939	1943	0.8	0.8	1.1	1.5	1.9	2.2	6.3	3.4	4.4	2.4	–	0.6
Hexadecanoic acid	1960	1959	–	–	–	–	–	–	9.0	–	–	–	–	–
Hexadecyl acetate	2003	2006	–	–	–	–	–	–	–	–	–	0.5	–	–
6*Z*,10*E*-Pseudo phytol	2031	2018	–	–	0.2	0.4	0.6	0.6	–	1.3	1.7	1.6	0.7	0.4
n-Heneicosane	2100	2108	–	–	–	–	–	–	–	0.8	0.7	1.6	–	–
E-phytol acetate	2218	2223	0.2	0.3	–	–	–	0.9	–	2.4	1.3	2.2	1.0	0.4
Diagoprogesterone	2234	2234							4.4					
7-α-Hydroxy-manool	2237	2246	0.1	0.2	–	0.2	0.4	0.5	–	2.2	1.6	1.7	0.8	0.3
Bisnor-7-desoxycholic acid	2244	2244							3.6					
5-Cholestene-3-ol,2,4 methyl	2246	2246	–	–	–	–	–	–	4.6	–	–	–	–	–
Larixol	2266	2256	0.4	0.5	–	0.8	1.7	1.5	1.9	5.4	7.3	3.0	1.9	0.7
2,4a-8,8-Tetramethyldecahydrocyclopropanaphtalene	2270	2270	–	–	–	–	2.5	2.8	5.9	13.9	12.4	24.4	2.4	–
Incensole oxide	2280	2280	–	–	–	–	1.7	–	4.8	9.7	11.3	–	2.8	–
Spiro(4,5)dec-6-en-8-one	2334	2334	–	–	–	–	–	1.0	–	6.4	5.1	–	–	–
Torulosol	2361	2352	–	–	–	–	–	–	–	0.8	1.8	7.1	–	–
Monoterpene hydrocarbons			53.3	49.7	44.1	39.7	20.9	40.7	2.2	14.6	9.2	9.6	38.2	57.2
Oxygenated monoterpenes			24.6	26.6	28.5	24.3	31.1	22.4	5	11.8	8.7	7.6	23.3	16.3
Sesquiterpenes hydrocarbons			2	2.4	2.8	4.3	3.2	2.7	0	1.3	1.1	3.9	3.7	3
Oxygenated sesquiterpenes			2.7	1.9	2.6	4.7	5.3	3	1.5	3.4	6.5	6.8	5.5	2.1
Oxygenated diterpenes			0.4	0.5	0.2	1.2	4	2.1	6.7	17.2	22.1	11.7	5.4	1.1
Other compounds			5.2	5	11.4	11.1	7.1	6.7	41.6	33.4	30.2	33.7	5.7	3.9
Total identified %			88.2	86.1	89.6	85.3	71.6	77.6	57.0	81.7	77.8	73.3	81.8	83.6
Yield			0.03	0.02	0.02	0.02	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.03

Legend: KI, Kovats index (logarithmic scale); RI, retention index; –, not identified.

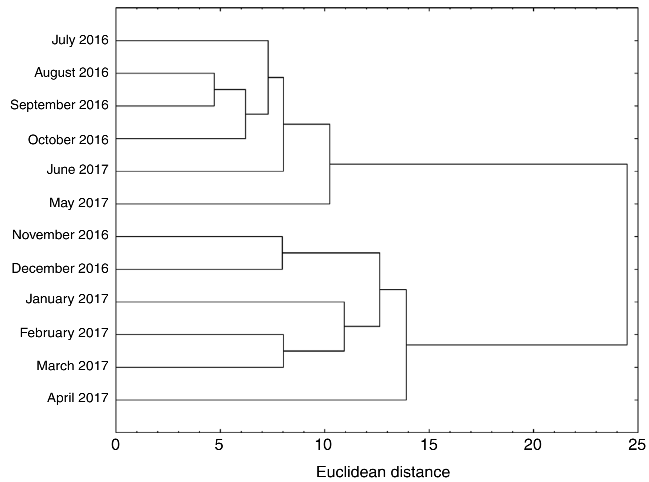

Fig. 2. Dendrogram representing the chemical composition similarity of the volatile oils from *Trembleya parviflora* leaves according to Ward's minimum variance method.

discriminant functions retained 100% of the classification in the original clusters by a cross-validation approach (Table 3). Thus, the canonical discriminant analysis revealed that the proposed classification and the variables employed were suitable for determining that the results of the HCA and PCA were consistent.

Discussion

Trembleya parviflora is a 1–2-m-tall subshrub with grayish cylindrical stems and with longitudinal fissures with single, oppositely crossed, viscous, petiolate, elliptic, oblong, hairy, ribbed leaves, as described by Somavilla and Graciano-Ribeiro (2011). These are important characteristics of Melastomataceae (Baumgratz et al., 2007). The trichomes in *T. parviflora* are, according to Goldenberg et al. (2015), simple or glandular.

The flowers observed in the inflorescence of *T. parviflora* were white with pink bases and had five petals, five sepals and loculicidal-capsule-type fruits, as described by Baumgratz et al. (2007).

In the present study, the beginning of the reproductive phase was in July, having peak flowering occurring in August and September. After this period, the plants present green fruits that then dry, open and disperse seeds. According to Goldenberg et al. (2015), in Parana, *T. parviflora* presents flowers and fruits from January to March and floral buds, flowers and fruits from March to June. This difference in behavior can be due to the seasonal differences between the South and Central-West regions of Brazil.

The flavonoids were detected during phytochemical screening of *T. parviflora*. Flavonoids have been found in other species of *Trembleya*. Bonfim-Patrício et al. (2001) isolated the flavonoids luteolin 7-*O*-glycosides, quercetin and quercetin 3-*O*-glycosides from *T. parviflora*; kaempferol 3-*O*-glycosides, and quercetin 3-*O*-glycosides from *T. laniflora* (Don) Cgn.; apigenin 7-*O*-glycosides, hispidulin (6-methoxyapigenin), luteolin 7-*O*-glycosides, kaempferol, kaempferol 3-*O*-glycosides, quercetin, quercetin 3-*O*-glycosides, and isorhamnetin (quercetin 30 methyl ether) from *Trembleya diffusa* E.; and apigenin 7-*O*-glycosides, luteolin 7-*O*-glycosides, kaempferol 3-*O*-glycosides, and quercetin 3-*O*-glycosides from *T. phlogiformis*. According to Ventura et al. (2007), flavonones generally act as phytoalexins, protecting plants against attacks by microorganisms, insects and herbivores.

In the volatile oils from *T. parviflora* leaves, the major identified compounds were monoterpene hydrocarbons, oxygenated monoterpenes and other classes of compounds. According to Fernandes et al. (2017), the major compounds identified in the leaves of *T. phlogiformis* were other classes of compounds. Therefore, although *T. parviflora* and *T. phlogiformis* are two species of the same genus, the composition of the volatile oils from the leaves are substantially different.

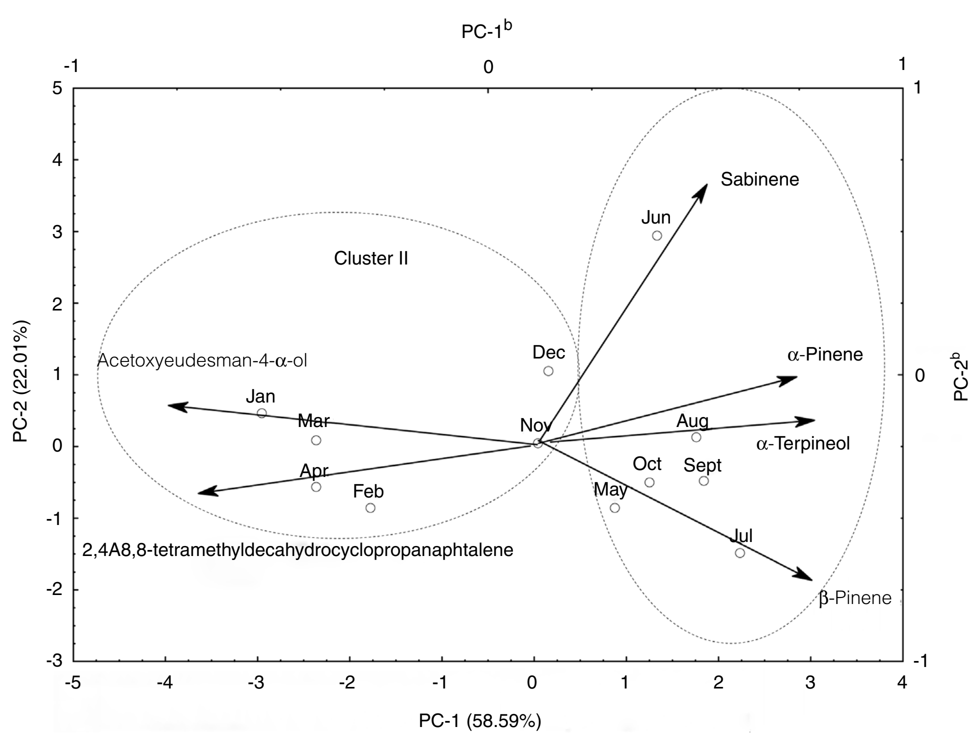

Fig. 3. Scatterplot of the PCA of *Trembleya parviflora* leaves samples collected from Pirenópolis/GO, belonging to the clusters I and II. [a]Axes refer to scores from the samples; [b]axes refer to scores from discriminant oil constituents represented as vectors from the origin.

Table 3
Summary of the canonical discriminant analysis of *Trembleya parviflora*.

	Canonical discriminant Eigenvalue functions	Canonical R	Wilk's lambda	*p*-Value
F1	3.43	0.88	0.22	0.001
	Standardized coefficients for canonical variables			
α-Pinene	−0.87			
2,4a8,8-tetramethyldecahydrocyclopropanaphtalene	0.45			
Eigenvalues	3.43			
Cumulative proportion	1			
	Percentage of total well-classified discriminants			
Cluster I	100%			
Cluster II	100%			
Total	100%			

The main compounds present in the volatile oils from the leave of *T. parviflora* in all the months were α-terpineol (2.7–16.5%) and α-pinene (0.6–25.4%). In *T. phlogiformis* leaves, the major compound present in all the months studied was oleic alcohol, ranging from 5.7 to 26.8% (Fernandes et al., 2017). Both species were collected at the same site. In *Microlicia graveolens* (Melastomataceae), three major compounds were detected in the volatile oil, namely, (+)-*trans*-pinocarvyl (78.9%), (−)-*trans*-pinocarvyl (5.5%) and β-pinene (3.8%) (Toudahl et al., 2012). Leite et al. (2007) verified the antimicrobial activity of β-pinene and α-pinene against gram-positive bacteria. Hammer et al. (2003) found that *α*-pinene had *in vitro* antifungal activity against *A. niger*, *A. flavus*, *A. fumigatus*, *Penicillium* sp. and dermatophytes. Rufino et al. (2014) the *in vitro* anti-inflammatory activity of α-pinene in human chondrocytes. Thus, these compounds might explain the common uses of *T. parviflora.*

Regarding the PCA and cluster analysis, the samples of volatile oils from *T. parviflora* were grouped in two clusters, one of them representing a dry season, discriminated by the compounds α-pinene, β-pinene, sabinene and α-terpineol, and the other cluster representing high levels of rainfall, discriminated by the compound acetoxyeudesman-4-α-ol and 2,4a-8,8-tetramethyldecahydrocyclopropanaphtalene.

This behavior was also observed with the volatile oils from *T. phlogiformis* leaves, which presented chemical variability of compounds according to the season (Fernandes et al., 2017). According to Barros et al. (2009), climatic conditions can influence enzymatic activity in a particular plant species and can consequently interfere with the biosynthesis of certain secondary metabolites, including terpene compounds. Although the chemical composition of the volatile oil is genetically determined, several abiotic factors, such as light, temperature, seasonality, nutrition and availability of water, can significantly modify the production of secondary metabolites (Lima et al., 2003; Gobbo-Neto and Lopes, 2007).

The morphological characterization of *T. parviflora* in this study will aid the correct identification of this species. 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). This study presents the first description of the chemical composition and variability of the volatile oils from *T. parviflora* leaves.

Authors' contributions

WCF contributed to collection of plant samples and laboratory experiments. HDF contributed to collection and identification of plant samples, confection of herbarium and morphological description of the species. LCC, JRON and SS contributed to chromatographic analysis. LLB contributed to the statistical analyses and critical reading of the manuscript. JRP contributed to chemical studies, chromatographic analysis and critical reading of the manuscript. TSF designed the study; supervised the laboratory work; contributed to the biological and chemical studies and the chromatographic analysis; drafted the paper; and contributed to critical reading of the manuscript. All the authors contributed to biological studies, laboratory experiments, and data analysis and have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Adams, R.P., 2007. Identification of Essential Oils Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Corporation, IL, USA.
Antinarelli, L.M., Pinto, N.C., Scio, E., Coimbra, E.S., 2015. Antileishmanial activity of some Brazilian plants, with particular reference to *Casearia sylvestris*. An. Acad. Bras. Cienc. 87, 733–742.
Barros, F.M.C., Zambarda, E.O., Heinzmann, B.M., Mallmann, C.A., 2009. Variabilidade sazonal e biossíntese de terpenóides presentes no óleo essencial de *Lippia alba* (Mill.) N. E. Brown (Verbenaceae). Quim. Nova 32, 861–867.
Baumgratz, J.F.A., Souza, M.L.R., Tavares, R.A.M., 2007. Melastomataceae na Reserva Ecológica de Macaé de Cima, Nova Friburgo, Rio de Janeiro, Brasil I – Tribos Bertolonieae. Merianieae e Microlicieae. Rodriguésia 58, 797–822.
Boira, H., Blanquer, A., 1998. Environmental factors affecting chemical variability of essential oils in *Thymus piperella* L. Biochem. Syst. Ecol. 26, 811–822.
Bonfim-Patrício, M.C., Salatino, A., Martins, A.B., Wurdack, J.J., Salatino, M.L., 2001. Flavonoids of *Lavoisiera*, *Microlicia* and *Trembleya* (Melatomataceae) and their taxonomic meaning. Biochem. Syst. Ecol. 29, 711–726.
Costa, A.F., 2001. Farmacognosia. Calouste Gulbenkian, Lisboa.
Cunha, A.P., 2005. Farmacognosia e fitoquímica. Fundação Calouste Gulbenkian, Lisboa, pp. 596–598.
Farmacopeia Brasileira, 2010. Agência Nacional de Vigilância Sanitária, Brasília, DF, 5 ed.
Fernandes, S.R., Ferreira, H.D., Sá, S., Borges, L.L., Tresvenzol, L.M.F., Ferri, P.H., Santos, P.A.d., Paula, J.R., Fiuza, T.S., 2017. Chemical composition and seasonal variation of the volatile oils from *Trembleya phlogiformis* leaves. Rev. Bras. Farmacog. 27, 419–425.
Gobbo-Neto, L., Lopes, N.P., 2007. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quim. Nova 30, 374–381.
Goldenberg, R., Bacci, L.F., Moraes, J.W., 2015. A tribo Microlicieae (Melastomataceae) no estado do Paraná. Rodriguésia 66, 155–165.
Hammer, K.A., Carson, C.F., Riley, T.V., 2003. Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. J. Appl. Microbiol. 95, 853–860.
INMET, 2017. Instituto Nacional de Meteorologia. Ministério da agricultura, pecuária e abastecimento.
Leite, A.M., Lima, E.O., Souza, E.L., Diniz, M.F.F.M., Trajano, V.N., Medeiros, I.A., 2007. Inhibitory effect of β-pinene, α-pinene and eugenol on the growth of potential

infectious endocarditis causing Gram-positive bacteria. Braz. J. Pharm. Sci. 43, 121–126.
Lima, H.R.P., Kaplan, M.A.C., Cruz, A.V.M., 2003. Influência dos fatores abióticos na produção e variabilidade de terpenóides em plantas. Floresta e Ambiente 10, 71–77.
NIST, 1998. National Institute of Standards and Technology, Version of the NIST/EPA/NIH Mass Spectral Data Base. U. S. Department of Commerce, Gaithersburg.
Oliveira-Filho, A.T., Flumihan-Filho, M., 1999. Ecologia da vegetação do parque florestal quedas do Rio Bonito. Cerne 5, 51–64.
Rufino, A.T., Ribeiro, M., Judas, F., Salgueiro, L., Lopes, M.C., Cavaleiro, C., Mendes, A.F., 2014. Anti-inflammatory and chondroprotective activity of (+)-α-pinene: structural and enantiomeric selectivity. J. Nat. Prod. 77, 264–269.
Sampaio, B.L., Edrada-Ebel, R., Costa, F.B., 2016. Effect of the environment on the secondary metabolic profile of *Tithonia diversifolia*: a model for environmental metabolomics of plants. Sci. Rep. 6, 1–11.
Somavilla, N.S., Graciano-Ribeiro, D., 2011. Análise comparativa da anatomia foliar de Melastomataceae em ambiente de vereda e cerrado sensu stricto. Acta Bot. Bras. 25, 764–775.
Toudahl, A.B., Filho, S.A.V., Souza, G.H.B., Morais, L.B., Santos, O.D.H., Jäger, A.K., 2012. Chemical composition of the essential oil from *Microlicia graveolens* growing wild in Minas Gerais. Rev. Bras. Farmacog. 22, 680–681.
Van Den Dool, H., Kratz, P.D., 1963. Generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. J. Chromatogr. A 11, 463–471.
Ventura, C.P., Dias de Souza Filho, J., Braga de Oliveira, A., Braga, F.C., 2007. A flavanone and other constituents of the Brazilian endemic species *Trembleya laniflora* (D. Don) Cogn. (Melastomataceae). Biochem. Syst. Ecol. 35, 40–41.
Ward, J.H., 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. 58, 66–103.