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Original Article

Chemical composition and seasonal variation of the volatile oils from *Trembleya phlogiformis* leaves

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ABSTRACT

Trembleya phlogiformis DC., Melastomataceae, is a shrub whose leaves are used as a dye for dyeing wool and cotton. The present article aimed to carry out the morphological description of the species, to study the chemical composition of volatile oils from the leaves and flowers and the seasonal variability from the leaves during a year. Macroscopic characterization was carried out with the naked eye and with a stereoscopic microscope. Volatile oils were isolated by hydrodistillation in Clevenger apparatus and analyzed by gas chromatography/mass spectrometry. The major components of the volatile oil of *T. phlogiformis* flowers were: *n*-heneicosane (33.5%), phytol (12.3%), *n*-tricosane (8.4%) and linoleic acid (6.1%). It was verified the existence of a large chemical variability of the volatile oils from the leaves of *T. phlogiformis* over the months, with the majority compound (oleic alcohol, ranging from 5.7 to 26.8%) present in all samples. A combination of Cluster Analysis and Principal Component Analysis showed the existence of three main clusters, probably related to the seasons. The results suggested that the volatile oils of *T. phlogiformis* leaves possess high chemical variability, probably related to variation associated with rainfall and the variation in the behavior of specimens throughout the year. This research provides insights for future studies on the volatile oils obtained from the *T. phlogiformis* leaves and flowers, mainly related to biological markers of applications monitored in the leaves and flowers of this species.

Introduction

Melastomataceae Juss. family has around 150–166 genera. In Brazil can be found 66 genera and about 1360 species (Goldenberg et al., 2012; Baumgratz et al., 2014). These plants present strategies and adaptations as a large seed production, high germination rates and rapid growth, with great importance in environmental restoration (Albuquerque et al., 2013).

The genus *Trembleya* DC. belongs to the Melastomataceae family, subfamily Melastomatoideae Seringe and tribe Microlicieae Triana. This tribe consists of six genera: *Stenodon* Naudin, *Chaetostoma* DC., *Lavoisiera* DC., *Microlicia* D. Don, *Rhynchanthera* DC. and *Trembleya* DC, characterized by reniform, ellipsoids or elongated seeds (Fritsch et al., 2004). The most species of this tribe contains dimorphic stamens, with prolonged connective below anther with rostrate apex except *Stenodon* Naudin (Goldenberg et al., 2015).

Trembleya is an exclusively Brazilian genus, with about fourteen species, and most of these occur in rock formations in Minas Gerais State (Martins, 1997; Martins, 2009; Goldenberg et al., 2015). *Trembleya* is characterized by herbs to erect shrubs, non-imbricated, sessile or petiolate leaves, without translucent scores, modified dichasium flowers with white or pink petals and 5-locular ovary (Goldenberg et al., 2015).

Cota et al. (2002) verified antimicrobial activity of the *Trembleya laniflora* (D. Don) Cogn. leaves against different strains of bacteria and fungi. Ventura et al. (2007) observed antimicrobial activity of the crude extract of the leaves and stems of *T. laniflora* against *Micrococcus luteus* and *Staphylococcus aureus*.

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Trembleya phlogiformis DC., popularly known as "quaresminha-do-campo", is a shrub whose leaves are used in communities as a natural dye to wool and cotton (Sá et al., 2007). No papers in the scientific literature have been found regarding chemical profile and biological activity of this species.

Several authors employed multivariate techniques such as principal component analysis (PCA) and cluster analysis (CA) to verify the chemical variability and the interrelationships between samples and its similarities, regarding various secondary metabolites, including volatile oils. These approaches are ordering methods, aiming the reducing the data set dimension to easily the explanation the behavior of the system according the chemical profile (Boira and Blanquer, 1998; Santos et al., 2006; Lei et al., 2010; Sampaio et al., 2016).

In search of new plants with pharmacological potential, the purposes of this work were: study of the development *in loco* and the morphological behavior of *T. phlogiformis* specimens over a period of 12 months; study of the chemical composition of volatile oil of leaves and flowers and analyze the seasonal variability of the volatile oil of leaves for 12 months.

Materials and methods

Localization

The plant material was collected in Pirenópolis, Goiás, Brazil (15°48′15″ S to 48°52′48″ W, at an elevation of 1295 m above sea level). Climatic data for the period were obtained from the Meteorological Institute (INMET, 2014).

Plant material and morphological analysis

The characterization of *Trembleya phlogiformis* DC., Melastomataceae, external morphology was made with the naked eye, every month for 12 months, *in loco*, and in the Plant Taxonomy Laboratory of the Department of Botany, Institute of Biological Sciences, Federal University of Goiás, using the stereoscopic microscope Olympus SZ-ST.

To study the volatile oil *T. phlogiformis* DC., the leaves were collected monthly for 12 months and the flowers in March 2014.

During field visits, the external morphological data and the behavior of specimens were recorded in a field book. The images were registered with a digital camera Canon EOS T4i. The specimens of *T. phlogiformis* were identified by Professor Dr. Heleno Dias Ferreira and a voucher specimen deposited at the Herbarium of Federal University of Goiás, Brazil, Conservation Unit PRPPG, under code number UFG-47868.

Volatile oils

For analysis of volatile oils, healthy leaves and flowers were collected of ten different individuals of *T. phlogiformis* and dried at room temperature. The plant material (300 g) were triturated separately and submitted to hydrodistillation in a Clevenger-type apparatus for 2 h. 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 volatile 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 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 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).

PCA was applied to examine the interrelationships between the chemical constituents of the volatile oils from leaves collected in different months using the software Statistica (Stat Soft, 2004). A hierarchical cluster analysis (HCA) 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 (DCA).

Results

Morphological description

Trembleya phlogiformis is a subshrub or shrub up to 2 m tall. Stem cylindrical, woody, slightly exfoliative, sub-ligneous branches, yellowish-green, slightly alates (angular), with tectores and glandular trichomes. Sticky leaves, simple, opposite cross, short-petiolate, 3–4 mm long, ovate blade, 2.5–5 cm long, 1–1.5 cm wide, membranous, margin entire to serrate, apex acute, base cuneate or sub cordiform, acrodrome both sides with large amount of glandular trichomes, serous surface. Inflorescence terminal or lateral with flowers arranged in dichasia. 5-mere flowers, hypanthium campanulate, green, thick hair with glandular trichomes, 3–4 mm long, about 1.8 mm wide, presence of a pair of bracts; pedicel 3–4 mm long, with glandular trichomes; calyx 5-laciniate, triangular laciniae, 1.2–3 mm long with glandular trichomes externally; 5-corolla petals, obovate, glabrous, free, white to pink, about 9 mm long, 4.5–5 mm wide; 10 stamens 10, free, dimorphic, white anthers, wine color, poricidal, prolonged connective ventrally; ovary glabrous, 5-carpels, gamo-carpels, 5-locular, placentation axillary, terminal stylus, curved, white. Fruit glabrous capsule type or with glandular trichomes, 4–6 mm long, 3 mm wide, 5-locular. Small seeds numerous, brown-clear, elongated and stooped, 1 mm long, 0.2–0.3 mm wide.

Behavior

Trembleya phlogiformis showed different behavior during their development cycle, over a period of 12 months. In the months of December and January most of the individuals was in the vegetative state with green and large leaves with sticky substance on the leaf surface and absence of insects and fungi, probably due to the presence of this sticky substance, as were observed some stuck insects and dead in the leaves. The flower buds began to appear in February and in early May the flowers emerge gradually, and may be found throughout this month, inflorescences with many buds and open flowers with petals whose colors range from white to pink-dark. They found individuals 60 cm high with inflorescences. The fruiting began in May, green-brownish capsules can be found, which become brownish when ripe in June. During the dry season, which usually goes from July to October, the aerial branches of *T. phlogiformis* started to get dry, yellowish or brownish and even completely dry subjects. The yellowing and leaf fall were observed from the month of August with the majority of individuals arrested carrying fruit on their branches. This month, none of the subjects had flowers. From September to November the specimens still have dry fruits attached to their branches. From September to October, the individuals were leafless or with small leaves at the ends of

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

Station	Date	Rainfall total	Average maximum temperature (°C)	Average minimum temperature (°C)
83376	07/31/2013	0	31	14.6
83376	08/31/2013	4.4	32.5	15.8
83376	09/30/2013	14.5	33.7	18.5
83376	10/31/2013	144.2	32	19.4
83376	11/30/2013	264.6	30.6	19.1
83376	12/31/2013	343.9	30	20
83376	01/31/2014	270.1	30.9	18.3
83376	02/28/2014	252.3	30.7	19.2
83376	03/31/2014	277.1	30.2	19.1
83376	04/30/2014	298.5	30.35	19.2
83376	05/31/2014	4.3	30.9	16.1
83376	06/30/2014	0.1	30.1	14.7

Source: INMET (2014).

branches. In the rainy season, in November, observed the presence of new leaves, restarting another cycle.

Volatile oils

During the collection period the months of highest rainfall were October (144.2 mm), November (264.6 mm), December (343.9 mm), January (270.1 mm), February (252.3 mm), March (277.1 mm) and April (298.5 mm), with average temperatures ranging from 32 °C to 18.3 °C. The months with less rainfall were May (4.3 mm), June (0.1 mm), July (0 mm), August (4.4 mm) and September (14.5 mm), with temperatures ranging from 14.6 °C to 33.7 °C (Table 1).

The yield of volatile oil of flowers was 0.008% (w/v) and leaves varied 0.01 to 0.03% (w/v) (Table 2).

In the flowers were identified 82.7% of the substances: 4.8% monoterpenes oxygenates, 0.17% sesquiterpenes hydrocarbons, 0.76% sesquiterpenes oxygenates and 76.9% other compounds. The major compounds found in volatile oils of flowers were *n*-heneicosane (33.5%), phytol (12.3%), *n*-tricosane (8.4%) and linoleic acid (6.1%) (Table 2).

In the monthly analysis, they identified 68.9–94.2% of the chemical compounds of volatile oils of *T. phlogiformis* leaves. Among them, 0.2–2.2% were monoterpene hydrocarbons, 4.8–29.9% oxygenated monoterpenes, 2.8–24.5% sesquiterpene hydrocarbons, 1.2–12.6% oxygenated sesquiterpenes and 37.9–66.7% of other classes of compounds. The major compound present in all leaf samples was oleic alcohol ranging from 5.7 to 26.8%. The neryl acetone was majority in the leaves in January (9.2%), March (11.4%), April (13.4%) and November (7.8%), the hexenal in February (9.7%), butylated hydroxytoluene in February (13.6%), March (20.6%) and November (6.7%); acetate β-ment-1-en-9-ol in March (7.3%), dictamnol in April (7.5%), heneicosane in May (12.6%), the 1,1,6-trimethyl-1, 2-dihydronaphthalene in June (8.2%), the hexadecanoic acid in July (6.4%) and December (8.3%), 6,10,14-trimethyl-2-pentadecanone in August (8.4%), the 2-pentadecanone in October (19.3%), linalool (7.7%) and α-terpineol (8.5%) in November, geranyl acetone in December (7.6%) (Table 2).

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. phlogiformis*. Fig. 1 indicates that the relative position of the axis 2D originated in the PCA. This analysis suggests that the cluster I is discriminated by compounds butylated hydroxytoluene, demothoxyageratochromene and neryl acetone, the samples present in the cluster I are characterized by the period with higher levels of rainfall. Cluster II observed in Fig. 1 suggests that oleic acid is the compound capable of discriminate this group characterized in samples collected in months of low rainfall, except for the sample obtained in December. Cluster III is composed of three months also with less precipitation and was not discriminated by any of the selected compounds, but coincides with the period in which the *T. phlogiformis* specimens have few leaves and dry fruits. The cluster analysis (Fig. 2) suggests the existence of three groups (which showed agreement with the PCA): cluster I (volatile oils from leaves collected in November, January, March and April); cluster II (volatile oils from leaves collected in October, December, February, May and July), cluster III (volatile oils from leaves collected in June, August and September). The results indicate that the classification proposed by the PCA and HCA was appropriate for the classification of samples as the chemical profile of volatile oils.

Canonic discriminant analysis was performed to help to predict the grouping of the cluster analysis, and two predictive variables were employed: oleic alcohol and demothoxyageratochromene, and the two discriminant functions retain 100% of well – classification in the original clusters by a cross-validation approach. Thus, the canonic discriminant analysis revealed that the classification proposed and their variables employed are suitable to show that the findings of the HCA and the PCA were consistent (Table 3).

Discussion

It was observed that *T phlogiformis* has simple, opposite, cross leaves with inflorescences dichasia type with poricidal anthers, typical characteristics of Melastomataceae (Cogniaux, 1891; Wurdack et al., 1993; Goldenberg and Reginato, 2007). It's a shrub about two meters tall with pyramid aspect, with the wider base of the leaves that found at the apex; the leaves are simple, short, opposite, petiolate crusades, ovate, with acute apex, sub condiform base, palmate-parallel leaf venation and serrated; the flowers have a calyx with 5 sepals and five petals free with 10 stamens, which features within the genre *Trembleya* (Don, 1823; Martins, 1997). It was observed that *T. phlogiformis* petals vary from white to pink and classified as dialipetal with acute apex as described by Martins (1997). The seeds showed great similarities as to the format of species of the genus by the presence of elongated and curved seeds, but with differences when compared to the size, since the *T. phlogiformis* seeds have about 1 mm in length and described in the genre have up to 0.57 mm (Martins, 1997).

Regarding the development cycle, the specimens were in a vegetative state from December to January. The flower buds begin to emerge in February, extending to May, where inflorescence with open buds, flowers and fruiting from May to June. The fruits at the beginning of development, are brownish-green, and in June, they mature and become brownish. From July (dry season) individuals are in a vegetative state. From September to October, the individuals were leafless or with small leaves at the ends of branches. This development cycle led to different composition of the volatiles oils of leaves.

Table 2
Percentage of the chemical constituents of the volatile oils from *Trembleya phlogiformis* leaves and flowers collected in Pirenópolis, Goiás.

			Leaves												Flowers
Constituents	KI	RI	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	–
Hexenal	855	832	–	9.7	–	–	–	–	–	–	–	–	–	–	–
Heptanal	902	888	–	0.6											
2-Pentyl furan	988	986	0.8	–	–	–	–	–	–	–	–	–	–	–	–
Hepta-2,4-dienal	1007	1002	2.6	–	–	–	–	–	–	–	–	0.7	–	–	–
p-Cymene	1024	1014	–	1.2	–	–	–	–	–	–	–	0.2	–	–	–
Limonene	1029	1019	–	0.7	–	–	–	–	–	–	–	–	–	–	–
1,8-cineole	1031	1022	–	1.6	–	–	–	–	–	–	–	–	–	–	–
Benzene acetaldehyde	1042	1031	0.7	0.9	–	–	–	–	–	–	–	–	1.8	–	–
Octen-1-al	1054	1046	0.3	3.7	–	2.2	2.2	3.0	1.1	1.7	2.7	0.9	–	–	–
n-Octanol	1068	1061	–	1.0	–	2.0	–	–	–	0.7	2.2	1.0	1.5	–	0.1
Linalool oxide B	1072	1063	0.4	–	–	2.4	0.6	–	–	–	1.7	0.7	1.2	2.2	–
m-Cymene	1085	1079	–	–	–	–	–	2.2	–	0.3	–	–	–	–	–
Linalool	1096	1091	1.1	4.6	–	5.6	5.2	1.4	1.2	0.9	2.8	2.2	7.7	–	0.5
n-Nonanal	1100	1094	0.9	2.0	–	4.7	2.8	5.4	1.4	1.8	2.8	1.6	2.5	1.2	2.2
Nonen-1-al <(2Z)>	1144	1150	–	0.5	–	–	–	–	–	–	–	–	–	–	–
Camphor	1146	1134	–	–	–	–	0.8	–	–	–	–	–	–	–	–
2*Z*-Nonenal	1149	1143	0.6	–	–	–	–	0.9	–	0.63	0.7	0.6	1.3	–	–
2*E*,6*Z*-Nonadienal	1154	1150	1.4	–	–	–	0.4	–	–	–	–	0.8	1.9	1.1	–
Terpinen-4-ol	1177	1182	–	–	–	–	–	–	–	–	–	0.3	–	–	–
α-Terpineol	1188	1182	1.6	0.8	–	4.0	3.6	–	–	0.9	1.6	2.0	8.5	2.0	0.2
Safranal	1196	1191	0.8	–	–	–	–	–	–	0.9	1.5	–	–	–	–
n-Decanal	1201	1196	0.5	–	–	2.9	0.8	2.2	1.2	1.4	1.5	1.0	–	–	0.2
β-Cyclocitral	1219	1211	1.5	1.6	–	2.1	1.0	2.6	–	1.4	2.9	0.9	2.5	1.6	–
2*E*-decenal	1263	1251	0.5	0.9	–	3.2	1.3	3.5	1.9	1.7	2.0	1.0	1.2	–	0.2
Thymol	1290	1284	–	–	0.6	–	–	–	1.8	–	–	–	–	–	–
2*E*,4*Z*-Decadienal	1293	12844	0.4	–	–	–	–	–	–	–	–	0.5	–	1.1	0.2
Undecan-2-one	1294	1284	–	–	–	–	–	–	–	0.5	0.7	–	–	–	–
n-Tridecane	1300	1291	–	–	–	–	–	–	–	–	–	2.4	–	–	–
Undecanal	1306	1296	–	–	–	1.5	–	2.7	1.6	1.3	1.6	0.6	–	–	–
2*E*,4*E*-Decadienal	1316	1307	1.7	–	–	–	–	–	–	–	–	0.7	1.9	–	–
1,1,6-Trimethyl-1,2-dihydronaphthalene sno	1355	1342	1.6	1.8	–	1.8	1.2	8.2	2.6	3.4	2.7	1.1	3.6	1.9	–
2*E*-Undecenal	1360	1353	–	–	–	1.9	–	1.6	2.0	0.8	0.7	0.5	–	–	–
β-(2*Z*)-Damascenona	1364	1375	1.1	–	–	1.8	–	–	–	0.7	1.6	0.9	–	1.2	–
α-Copaene	1376	1368	–	–	–	–	–	–	–	0.8	–	–	1.4	–	–
Dodecanal	1408	1398	–	–	–	–	–	–	–	–	–	–	–	–	0.2
α-Santalene	1417	1409	1.6	1.0	1.2	2.6	1.3	5.9	4.7	3.7	3.7	1.4	2.5	1.1	–
β-Caryophyllene	1419	1410	–	–	–	4.0	–	3.6	1.8	5.8	1.6	0.6	2.0	–	0.2
β-menth-1-en-9-ol acetate	1423	1420	4.1	–	7.3	–	1.0	–	–	2.4	3.1	3.2	2.0	–	–
Dictamnol	1429	1423	2.6	–	2.3	7.5	–	–	–	–	–	–	5.5	5.8	–
Neryl acetone	1436	1442	9.2	1.1	11.4	13.4	–	4.2	–	–	3.9	4.0	7.8	–	0.6
Geranyl acetone	1455	1442	–	–	–	–	4.7	–	1.8	3.4	–	–	–	7.6	–
allo-aromadendrene	1460	1451	–	–	–	–	–	–	–	1.4	–	–	–	–	–
6-Demothoxy-ageratochromene	1463	1472	3.4	1.5	5.2	5.0	–	–	–	–	0.7	0.9	3.2	–	–
trans-cadina-1-(6),4-diene	1476	1467	–		–	–	–	–	–	0.9	–	–	–	–	–
γ-Muurolene	1478	1479	–		–	–	1.2	–	–	2.3	–	–	–	–	–
trans-β-Ionone	1488	1476	3.2	1.6	3.1	3.9	1.9	1.9	–	1.0	1.5	1.5	5.2	3.4	0.2
β-Selinene	1490	1486	–	–	–	–	–	2.0	–	2.8	1.1	–	–	–	–
2-Tridecanone	1496	1485	–	–	–	–	–	–	–	–	–	1.2	–	–	–
Tridecanal	1510	1498	2.5	1.2	–	2.8	–	3.7	5.6	4.2	2.4	1.6	1.6	–	0.2
Butylated hydroxytoluene	1515	1502	2.0	13.6	20.6	5.9	–	–	2.7	1.0	–	2.2	6.7	–	3.3
δ-Cadinene	1523	1513	0.6	–	2.5	–	1.6	–	–	3.5	1.3	0.6	5.8	1.3	–
E-Nerolidol	1563	1554	–	–	–	–	–	–	–	–	–	4.9	–	–	0.4
Dodecanoic acid	1566	1567	1.8	0.6	7.9	–	–	4.9	5.4	2.7	4.0	–	–	–	1.1
n-Hexadecane	1600	1589	–	–	–	–	–	–	–	–	0.9	0.5	–	–	–
Tetradecanal	1612	1600	0.6	–	–	–	–	1.8	3.4	1.6	–	0.9	–	–	0.2
Pentadecanone	1697	1686	–	–	–	–	–	–	–	–	0.9	19.3	–	–	–
n-Heptadecane	1700	1689	–	–	1.2	–	–	–	0.8	–	2.0	–	–	–	–
n-Octadecane	1800	1788	–	–	2.0	–	–	–	1.6	–	2.1	0.5	–	–	–
6,10,14-trimethyl-2-pentadecanone	1833	1826	–	–	3.2	–	1.9	–	–	8.4	–	1.2	3.1	5.8	–
Farnesyl acetate	1822	1825	3.8	–	1.3	3.3	–	1.8	–	–	–	0.8	–	1.6	0.1
n-Nonadecane	1900	1887	0.4	–	1.3	–	1.4	1.2	2.0	–	1.4	0.5	–	–	1.2
5E, 9E, 13E-Farnesyl-2-one	1913	1905	3.4	–	2.1	–	1.2	1.4	2.1	1.1	1.6	1.3	–	2.0	0.4

Table 2 (*Continued*)

			Leaves											Flowers
Constituents	KI	RI	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	–
Phytol	1943	1935	2.8	5.3	0.8	–	1.6	–	2.1	1.4	1.2	1.8	–	2.1	12.3
Hexadeconoic acid	1960	1957	5.7		1.0	–	2.4	1.9	6.4	0.6	3.1	2.8	–	8.3	–
ethyl hexadecanoate	1993	1981	–	–	–	–	0.7	–	–	–	–	0.7	5.6	2.8	0.8
n-Eicosane	2000	1987	–	–	1.87	–	–	–	–	–	–	–	–	–	1.3
E,E-geranyl linalool	2027	2016	–	–	–	–	–	–	–	–	–	–	–	–	0.1 0.11
Kaurene	2043	2027	–	–	–	–	–	–	–	–	–	–	–	–	0.6
Methyl linoleate	2085	2128	–	–	–	–	–	–	–	–	–	–	–	–	2.7
n-Heneicosane	2100	2091	0.6	1.3	1.0	1.8	12.6	0.7	4.6	0.7	1.0	1.2	–	0.6	33.5
Oleic alcohol	2101	2101	16.8	16.7	7.2	7.9	14.4	9.0	16.4	5.7	7.8	17.0	6.1	26.8	–
Linoleic acid	2133	2131	3.0	–	–	–	1.4	–	2.0	–	–	0.6	–	3.2	6.1
n-Docosane	2200	2186	–	1.2	–	–	–	–	–	–	–	–	–	–	2.8
n-Tricosane	2300	2287	–	1.4	–	–	–	–	–	–	–	–	–	–	8.4
n-Tretacosane	2400	2384	–	–	–	–	–	–	–	–	–	–	–	–	0.8
n-Pentacosane	2500	2484	–	–	–	–	–	–	–	–	–	–	–	–	1.7
Hydrocarbons monoterpenes			–	1.9	–	–	–	2.2	–	0.3	–	0.20	–	–	–
Monoterpenes oxygenates			18.6	9.7	19.3	27.5	16.8	8.1	4.8	9.9	17.6	13.3	29.9	13.8	4.8
Hydrocarbons sesquiterpenes			3.8	2.8	3.7	8.4	5.3	19.7	9.1	24.5	10.5	3.1	15.4	4.2	0.2
Sesquiterpenes oxygenates			10.8	0	5.7	12.6	1.2	3.2	2.1	1.9	3.1	8.5	5.5	10.6	0.8
Others			53.3	63.7	56.4	45.7	45.7	44.5	62.2	37.9	44.0	66.7	43.6	56.4	77.0
Total identified			86.5	78.1	85.2	94.1	69.0	77.7	78.1	74.5	75.2	91.8	94.2	84.9	82.7
Yield			0.022	0.024	0.028	0.022	0.020	0.015	0.010	0.013	0.016	0.024	0.031	0.033	0.008

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

The major components of the volatile oil of *T. phlogiformis* flowers were: *n*-heneicosane (33.5%), the phytol (12.3%), *n*-tricosane (8.4%) and linoleic acid (6.1%). It was verified the existence of a large chemical variability of the volatile oils from the leaves of *T. phlogiformis* over the months, with the majority compound (oleic alcohol) present in all samples, which differentiates it from other species of the family. Studies of volatile oils of Melastomataceae species from the Amazon found as major compounds in *Bellucia grossularioides* (L.) Triana the pentadecanone (10.0%), palmitic acid (13.1%); in *Miconia ciliata* (Rich.) DC the hexyl acetate (8.4%), *p*-cymene (10.3%) (E, E)-α-farnesene (14.7%); in *Miconia minutiflora* (Bonpl.) DC the α-copaene (22.8%), β-caryophyllene (14.5%), α-humulene (12.2%); in *Miconia rubiginosa* (Bonpl.) DC. The nonanal (18.5%), α-copaene (32.9%); in *Tibouchina stenocarpa* (DC.) Cogn. Type A: terpinen-4-ol (11.2%), palmitic acid (22.9%) type B: (E)-β-ionone (17.2%), palmitic acid (22.9%) (Zoghbi et al., 2001). Studies carried out by Maya and Andrade (2009) with Melastomataceae species using various parts of plants (leaves, wood, bark, fruit, flowers) revealed as major compounds of the *Bellucia grossularioides* (L.) pentadecanone (10.0%), palmitic acid (13.1%); the *Miconia ciliata* (Rich.) DC-hexyl acetate (8.4%), *p*-cymene (10.3%) (E, E)-α-farnesene (14.7%); of the *Miconia minutiflora* (Bonpl.) DC-α-copaene (22.8%), β-caryophyllene (14.5%), α-humulene (12.2%), β-curcumene (7.9%); of the *Miconia rubiginosa* (Bonpl.) DC-nonanal (18.5%), α-copaene (32.9%); of the

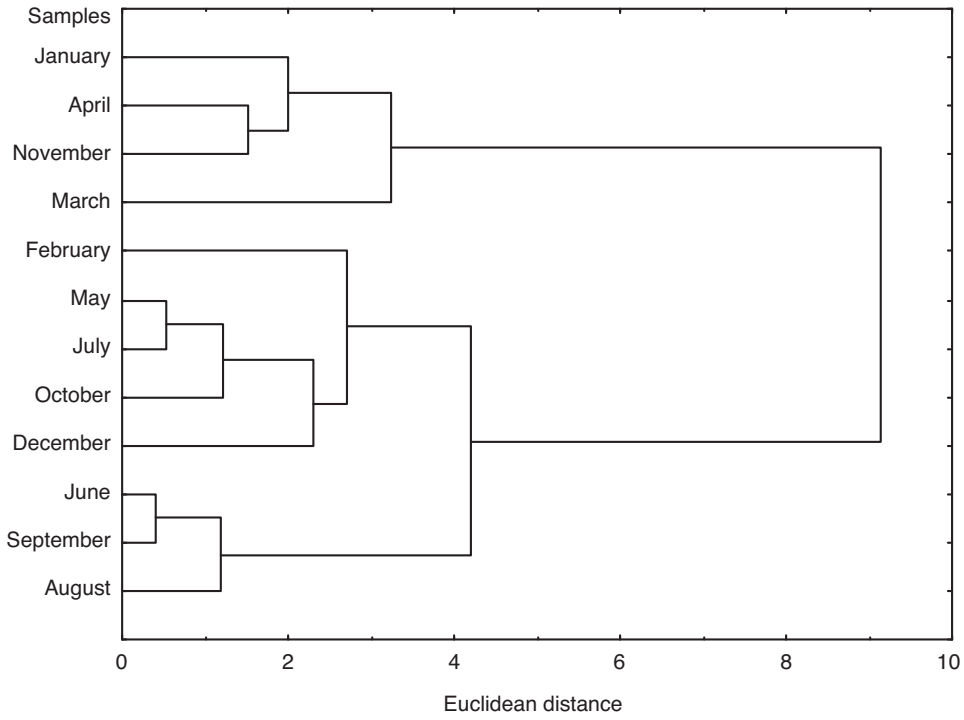

Fig. 1. Dendrogram representing the chemical composition similarity relationships of *Trembleya phlogiformis* leaves volatiles oils according to Ward's variance minimization method.

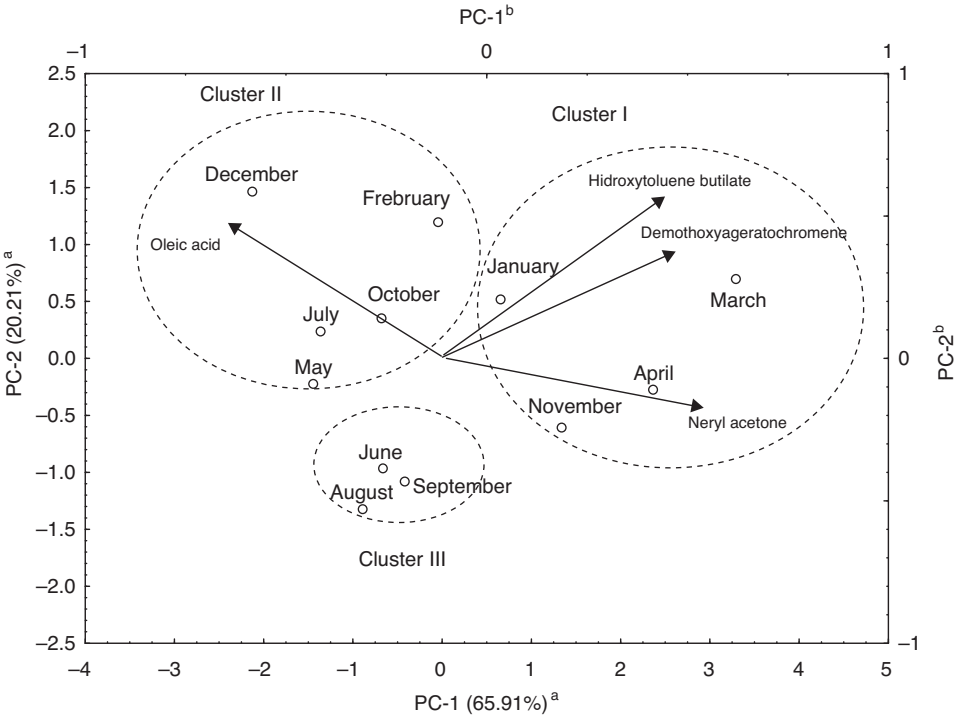

Fig. 2. Scatterplot from PCA of leaves of *Trembleya phlogiformis*, samples collected from Pirenópolis, GO belonging to the clusters I, II and II. [a]Axes refer to scores from the samples; [b]Axes refer to scores from discriminant oil constituents represented as vectors from the origin.

Tibouchina stenocarpa (DC.) – Type A: terpinen-4-ol (11.2%), germacrene D (7.9%), palmitic acid (22.9%); type B: (E)-β-ionone (17.2%). Toudahl et al. (2012) identified in the volatile oil of *Microlicia graveolense* thirteen compounds, with the majority *trans*-pinocarvila acetate (78.9%). It can be observed the occurrence of constituents β-pinene, limonene, pinocarvone, terpinen-4-ol, *trans*-α-terpineol pinocarveole in the species *M. crenulata* e *M. graveolens* (Pereira, 2013). Komalavalli et al. (2014) identified as major compounds in *Sonerila tinneveliensis* Fischer (Melastomataceae) tetrahydrospirilloxanthin (18:50%), ethyl iso-allocate (18:27%).

It was verified a relationship between the phenology and variation in chemical composition of volatile oils of *T. phlogiformis*. The samples were classified into three clusters: Cluster I (volatile oils from leaves collected in the months of November, January, March and April), consisting of samples collected in the period with the highest rainfall. In January specimens are mostly in a vegetative state. Cluster II (volatile oils from leaves collected in October, December, February, May and July). In February there were the first flower buds that develop through the month of May, forming inflorescences with many flower buds and open flowers. The month of December had the highest rainfall rate (343.9 mm) and probably due to this increased rainfall, the samples collected in December showed a different chemical profile to the volatile oil, with similarity to other months of other seasons. Cluster III (volatile oils from leaves collected in June, August and September) is to highlight the low level of rainfall and during this period the dry plant and the leaves fall, a very common feature among the plants of the Cerrado.

Seasonal differences in the chemical composition of the leaf volatile oils have also been described for the volatile oils of *Hortia oreadica* leaves (Santos et al., 2015). Despite the volatile oil chemical composition is genetically determined, many abiotic factors such as light, temperature, seasonality, nutrition and water availability

Table 3
Canonical discriminant analysis summary of *Trembleya phlogiformis*.

	Canonical discriminanant			
	Eingenvalues functions	Canonical *R*	Wilk's Lambda	*p*-Level
F1	7.071305	0.936004	0.051284	0.000045
F2	1.415894	0.765555	0.413925	0.006178
		Standardized coefficients		
Oleic acid	−0.12459	−1.03697		
Demothoxyageratochromene	−1.02881	−0.17993		
Eigenvalues	7.07130	1.41589		
Cumulative Proportion	0.83317	1.00000		
	Percentage of total well-classification	Cluster I $p=0.33$	Cluster II $p=0.42$	Cluster III $p=0.25$
Cluster I	100	4	0	0
Cluster II	100	0	5	0
Cluster III	100	0	0	3
Total	100	4	5	3

can significantly change the production of secondary metabolites. Environmental stimuli can alter the metabolic pathways for production of these compounds, leading to the biosynthesis of different compounds (Gobbo-Neto and Lopes, 2007; Lima et al., 2003).

Thus, the results reported herein may suggest that the volatile oils of *T. phlogiformis* leaves possess high chemical variability, probably related to variation associated with rainfall and the variation in the behavior of specimens throughout the year. The majority compounds of volatile oils of *T. phlogiformis* leaves and flowers were not found in literature as major compounds of the volatile oils in other species of Melastomataceae, may be typical of their species. This research provides insights for future studies on the volatile oils obtained from the *T. phlogiformis* leaves and flowers, mainly related to biological markers of applications monitored in the leaves and flowers of this species.

Authors' contributions

SRF contributed in collecting plant sample, running the laboratory work. HDF contributed in collecting plant sample and identification, confection of herbarium and morphological description of the species. SS contributed in collecting plant sample, running the laboratory work. LLB contributed to the statistical analyzes. LMFT contributed to critical reading of the manuscript. PHF contributed to chromatographic analysis. JRP contributed to chemical studies, chromatographic analysis and critical reading of the manuscript. TSF designed the study, supervised the laboratory work contributed to biological and chemical studies, chromatographic analysis and drafted the paper and contributed to critical reading of the manuscript. All the authors had 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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