

*Full Length Research Paper*

## **Structural organization and phytochemical analysis of *Pimenta dioica* (L.) Merrill (Myrtaceae) leaves collected from Goiás State, Brazil**

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Several biological properties are attributed to the *Pimenta dioica* plants that are native to Central America. Given the possible variabilities in both the morpho-anatomical and chemical aspects of the species that grow in different locations, this study aimed to contribute to the pharmacognostic study of *P. dioica* leaves from Brazil. Therefore, we morpho-anatomically described the plants, performed a phytochemical screening, analyzed the chemical composition of the essential oil and determined the quality parameters of *P. dioica* leaves that were collected in Goiás State. The leaves are simple and exhibit a camptodromous-brochidodromous venation pattern. The blades exhibit anomocytic stomata on the abaxial side, a dorsiventral mesophyll, secretory cavities on both sides, and a uniseriate epidermis. Trichomes can be observed in the petiole. The tests revealed the presence of phytochemical saponins, tannins and flavonoids in the samples. The major components of the essential oil were eugenol (60.8%) and myrcene (19.3%). The results regarding the moisture content (7.87% w/w), total ash (8.84% w/w), HCl-insoluble ash (1.74% w/w) and total flavonoids (1.88% w/w) can be used as parameters for quality control of botanical material. Additionally, the results of this study indicates strong similarities between the chemical compositions of the leaves of *P. dioica* plants that were grown in Goiás, Brazil and those from other parts of Brazil and the world.

**Key words:** Myrtaceae, essential oils, eugenol, morpho-anatomy, *Pimenta dioica*.

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### **INTRODUCTION**

The Myrtaceae family is one of the most important plant families worldwide, contains approximately 130 genera and 4000 species, has pantropical and subtropical distributions, and contains species of great medical

interest (Landrum and Kawasaki, 1997; Judd et al., 1999; Joly, 2002; Souza and Lorenzi, 2005). Among this family is the genus *Pimenta*, which contains 14 species that are native to Central America and a single species that is

native to Brazil, *Pimenta pseudocaryophyllus* (Gomes) LR Landrum (Landrum and Kawasaki, 1997).

Among the species that are native to Central America, *Pimenta dioica* (L.) Merrill (Figure 1a and b) was introduced to Brazil, where it can be found as an ornamental tree (Lorenzi et al., 2003). Popularly known as "pimenta-da-jamaica", "malaqueta", and "pimento", *P. dioica* is an evergreen tree that can reach 7 to 10 m in height and is native to Mexico, Guatemala, Belize, Honduras, Nicaragua, El Salvador, Cuba and Jamaica (Landrum, 1986; Lorenzi et al., 2003). The fruits of this species are used in confectionery because they express an aroma and flavor similar to the combination of clove, cinnamon and bay. For this reason, the fruits are known as "allspice" (Lorenzi et al., 2003). In addition to the use of *P. dioica* as a condiment or spice, the people of Central America and the Caribbean use preparations made from *P. dioica* leaves for the treatment of hypertension, diabetes, obesity, digestive disorders, dysmenorrhea and abdominal pain (Zhang and Lokeshwar, 2012). Scientific studies conducted using crude extracts, fractions or essential oils from the leaves of specimens from Central America have uncovered several biological characteristics, including antinociceptive, antipyretic, antidermatophyte, antimicrobial, hypotensive, central nervous system depressant, anti-hemorrhagic, anticancer and antioxidant activities (Benítez et al., 1998; Oussalah et al., 2006, 2007; Marzouk et al., 2007; Khandelwal et al., 2012; Zhang and Lokeshwar, 2012; Shamaladevi et al., 2013).

The scientific literature has shown that the essential oils from the leaves have contributed to many of the biological activities of *P. dioica*; the eugenol of *P. dioica* is a major component that has frequently been identified (Hernández et al., 2003; Oussalah et al., 2006, 2007; Zhang and Lokeshwar, 2012). In addition to these substances, some authors have identified flavonoids and tannins in the leaves of *P. dioica* (Castro et al., 1999; Marzouk et al., 2007; Nitta et al., 2009). A recent search of scientific databases revealed a lack of studies of Brazilian specimens of this important medicinal species. As demonstrated earlier, research has evaluated specimens that are native to Central America. Variability, both morpho-anatomical and chemical, between the species from different locations, is possible due to adaptive responses (Dickison, 2000; Simões and Spitzer, 2010; Cunha et al., 2005) that might have resulted in distinct or even unique biological activities of the exotic species.

According to Jorge et al. (2005), research and

microscopic analyses are essential for the identification of plant products and allow for the identification of fraud and possible adulterations. The data from anatomical analyses have unique values in taxonomic studies as stated by Johnson (1980) and Van Wik et al. (1982). The existence of morphological and anatomical references is extremely important, particularly for plants that are used in popular medicine, so that the samples can be confirmed and to enable tests of authenticity that are needed because confusion between morphologically similar species which are very common and can lead to the improper use of a particular plant. Thus, the anatomical characteristics add to the set of information that allows for the correct identification of plant species.

This study aimed to conduct a pharmacognostic analysis of the leaves of *P. dioica* from Brazil. This analysis included a description of the petiole and leaf blade structures, a phytochemical screening, an analysis of the chemical composition of the essential oil using gas chromatography coupled to mass spectrometry (GC-MS) and the determination of parameters that can be used for identification and plant material quality control.

## MATERIALS AND METHODS

### Plant

The plant material was collected from *P. dioica* specimens that were cultivated as ornamental trees in the cities of Goiânia, Goiás State (16° 40' 21.6" S and 49° 14' 30.9" W, 731 m) in October, 2007 and inhumas, Goiás State (16° 35' 33.9" S and 49° 49' 39.8" W, 780 m) in September, 2011 on public roads. The plants were identified by Professor Dr. José Realino de Paula of the Universidade Federal de Goiás, and the voucher specimens were deposited, respectively at the Herbarium of the Universidade Federal de Goiás (UFG-40.112) and at the Herbarium of the Universidade Estadual de Goiás (HUEG-6779). The leaf samples were air-dried in a chamber at 40°C, ground into a powder, packaged, labeled and stored until use in the phytochemical assays. The morpho-anatomical study was performed using fresh adult, fully expanded leaves that were collected below the third node.

### Morpho-anatomical study

The macroscopic leaf characterization was performed by observation with the naked eye and a stereoscopic microscope when necessary according to the parameters that were described by Oliveira et al. (1998) and Oliveira and Akisue (2003). The leaf venation pattern was determined based on the parameters described by Cardoso and Sajo (2006) after leaf diaphanization. The fresh material was diaphanized according to the method of Kraus and Arduin (1997) and visualized using a stereomicroscope

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(Leica model EZ4D). Using an average of 5 leaves, sections of approximately 1.0 × 0.5 cm of fresh leaf were excised by hand from the following regions of the leaf blade: the midrib segments, the internodal regions and the edges. In the middle region of the petiole sections, sections of approximately 0.5 cm were excised. These sections were fixed in 37% formaldehyde, propionic acid and 70% ethanol (FPA) at a ratio of 1:1:18 (V/V) for 3 days (Kraus and Arduin, 1997) and then stored in 70% ethanol. To mount the slides, transverse sections were cut by hand, clarified with 30% sodium hypochlorite (V/V), washed with distilled water, neutralized with 5% acetic acid, washed again with distilled water, double-stained with Alcian Blue:Safranin (9:1) and mounted onto slides with 50% glycerol (V/V) according to a technique adapted from Bukatsch (Kraus and Arduin, 1997). The fresh paradermal leaf sections were treated as described. Approximately, three slides, each containing three sections, were prepared from each leaf region and mounted in a colorless varnish. Photomicrographs of the anatomical structures were obtained using a photomicroscope (ZEISS-Axioskop) with the photographic film Kodacolor ASA 100. The illustration scales were obtained under the same optical conditions. Scanning electron microscopy (SEM) was performed at the LabMic of the Universidade Federal de Goiás. The fresh leaf sections were fixed in Karnovisk (Kraus and Arduin, 1997), dehydrated in an ethanol series and subjected to a CO<sub>2</sub> critical-point evaporator (Autosamdri). The specimens were mounted on aluminum ("stubs") and metalized with gold (Denton vacuum). The leaves were examined in a frontal and transverse scanning electronic microscope (Jeol, JSM – 6610, equipped with EDS, Thermo scientific NSS Spectral Imaging).

#### Determination of the plant material quality parameters

The tests for determining the humidity, total ash and HCl-insoluble ash, which were used as the plant material quality parameters, were performed in triplicate according to the Brazilian Pharmacopoeia V (Brazil, 2010). The volumetric method (Karl Fisher) was used to quantify the humidity content. The results are expressed as the mean of three replicates, and the standard deviations were calculated.

#### Phytochemical screening

The qualitative analysis of the major classes of secondary metabolites was performed according to methods that were adapted from Costa (2001), Matos (2009) and Matos and Matos (1989). Tests were performed to detect the presence of anthraquinone heterosides, digitalis heterosides, flavonoids, saponins, tannins, alkaloids and coumarins. The total flavonoid content was determined by the method described in the calendula monograph as stated by the Brazilian Pharmacopoeia V (Brazil, 2010) in triplicate, and the results are expressed as the means and the standard deviations.

#### Analysis of the essential oil

The leaf samples were ground into a powder and submitted to hydrodistillation in a modified Clevenger-type apparatus (2 h). The essential oils were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, the yields were measured, and the oils were stored at -18°C for further analysis. The GC-MS analysis was performed at the Institute of Chemistry of the Universidade Federal de Goiás on a Shimadzu QP5050A instrument. The column, a CBP-5 (Shimadzu)-fused silica capillary

column (30 m long × 0.25 mm i.d. × 0.25 µm film thickness, composed of 5% phenyl methylpolysiloxane), was connected to a quadrupole detector operating in EI mode at 70 eV. Helium was used as the carrier gas at a flow of 1 ml min<sup>-1</sup>. The injector and interface temperatures were 220 and 240°C, respectively, and the split ratio was 1:5. The injection volume was 0.5 µl (10% in hexane), and the oven temperature program consisted a ramp from 60 to 240°C at 3°C min<sup>-1</sup> followed by an increase to 280°C at 10°C min<sup>-1</sup>, and ending with 5 min at 280°C. The analysis was conducted in the scan mode with a mass range of 40 to 400 m/z. The quantitative result was obtained by integrating the total ion chromatogram (TIC). The essential oil constituents were identified by comparing their mass spectra to those from the National Institute of Standards and Technology (NIST, 1998) and by comparing the mass spectra and the calculated linear retention indices (RI) with the corresponding values in the literature (Adams, 2007). The retention indices were obtained by co-injection with a C8-C32 mixture of linear hydrocarbons (Sigma, USA) and by the equation of Van den Dool and Kratz (1963).

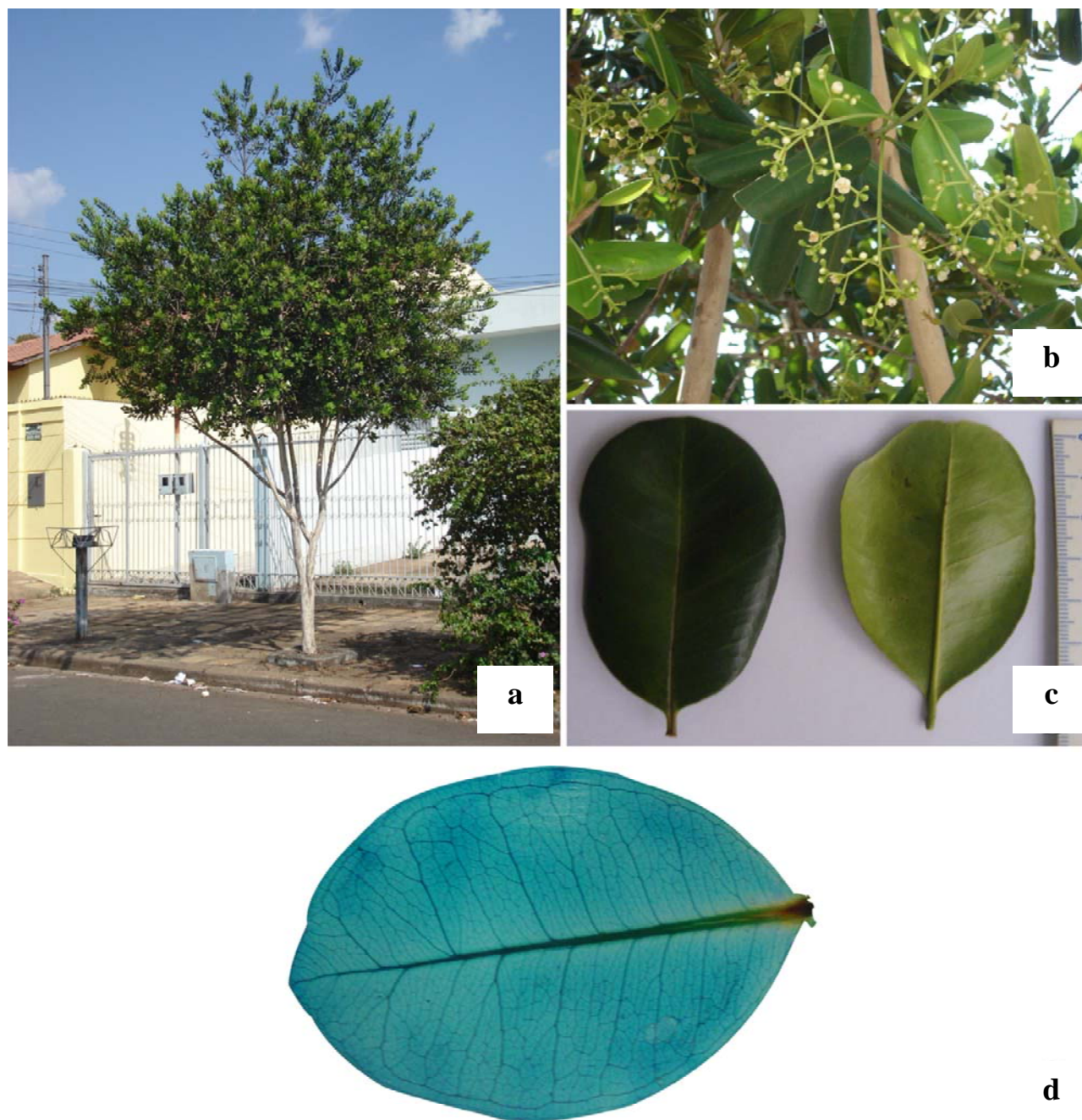
## RESULTS AND DISCUSSION

### Morpho-anatomical study

The *P. dioica* leaves are simple, oblong-elliptic or elliptic-lanceolate and shortly petiolate (Figure 1c). The leaf blade is intact and varies from coriaceous to subcoriaceous. The blade measures 3.3 to 7.3 cm × 2.6 to 5.3 cm. The apex is obtuse to slightly emarginate. The leaf base is a symmetrical cuneata. The leaves are glabrous and overwrought on the adaxial surface with a glossy dark green coloration. There are no trichomes on either the adaxial or abaxial surfaces. The leaves exhibit a pinnate venation with the primary and secondary veins prominent on the abaxial surface, and the adaxial surface is printed on. The venation pattern (Figure 1d) is a pinned type camptodromus-brochidodromus in which the ribs diverge from the midrib at different angles and bend and arc (Figure 2a and b) before reaching the leaf margin. A similar venation pattern have also been recorded by Donato and Morretes (2009) for *Eugenia florida* DC, by Paula et al. (2008) for *P. pseudocaryophyllus* (Gomes) LR Landrum and by Cardoso and Sajo (2006) for other species of Myrtaceae. The petiole is curved and varies from circular to biconvex, measures 0.5 to 0.7 cm in length, and its insertion and surface are marginal and striated, respectively.

The morphological characteristics of the leaves that were examined in this work are in accordance with those described by Joly (2002) and Judd et al. (1999) for Myrtaceae. The characteristics also accord with the morphology described by Landrum (1986) and are highly similar to the descriptions of Donato and Morretes (2005) for *Psidium widgrenianum* Berg., Paula et al. (2008) and Farias et al. (2009) for *P. pseudocaryophyllus* and Donato and Morretes (2011) for *Myrcia multiflora* (Lam.) DC.

From the front view, the adaxial epidermal cells have



**Figure 1.** *Pimenta dioica* (L.) Merrill (Myrtaceae). (a) The general aspects of the plant; (b) the general aspects of the inflorescence; (c) the general aspects of the adaxial and abaxial surfaces of the leaves; (d) stereomicroscopy after clearing the leaf surface showing the details of the venation pattern.

irregular shapes and sizes, thick anticlinal walls that are predominantly punctuated, straight to slightly sinuous and lack stomata (Figure 2c). Secretory cavities are present in large numbers and are covered by pair of reniform cells that are surrounded by approximately 10 cells that are

smaller than the other epidermal cells (Figure 2c and d). Using scanning electronic microscopy (Figure 2d), it was possible to observe the corresponding holes in the tops of the secretory cavities, but the contours could not be observed due to a thick cuticle layer covering the entire

epidermal surface that assumed aspects in the granular cuticle along the entire length and exhibited some flat regions. According to Vannucci and Rezende (2003), the cuticle is formed by cutin (a lipid complex substance) and cuticular wax, and various strata or layers are formed by cutin and cuticular cellulose. Variable thicknesses can result in different patterns of ornamentation that are thicker in plants in dry conditions that receive sunlight radiation and have an important function in reducing plant water loss (Cutler et al., 2011).

From the front view, the cells of the abaxial epidermis (Figure 2e) also have irregular shapes and sizes and anticlinal punctuated walls, but these walls are thinner than those of the adaxial epidermis. The anticlinal walls are straight or slightly winding. There are many anomocytic stomatas surrounded by 3 to 5 epidermal cells (Figures 2e to 3a). Notably, secretory cavities are rarely present (Figures 2e and f). The pairs of kidney-shaped cells that overlap the secretory cavities are surrounded by approximately 7 smaller cells that have a characteristic shape and size that differs from those of the epidermal cells. The contours of the epidermal cells are thin (Figures 2f and 3a) due to the thick layers of the cuticle coats, but it was possible to observe numerous stomata and some secretory cavities. The cuticle on the surface exhibits aspects that range from smooth to rough, and some sparse protrusions are present (Figures 2f and 3a). In both epidermises, the cells have thick anticlinal walls that are similar to those described by Paula et al. (2008) for *P. pseudocaryophyllus*. According to Gomes and Neves (1993/1997), the outlines of the anticlinal walls of the epidermal cells can display variations within the Myrtaceae family.

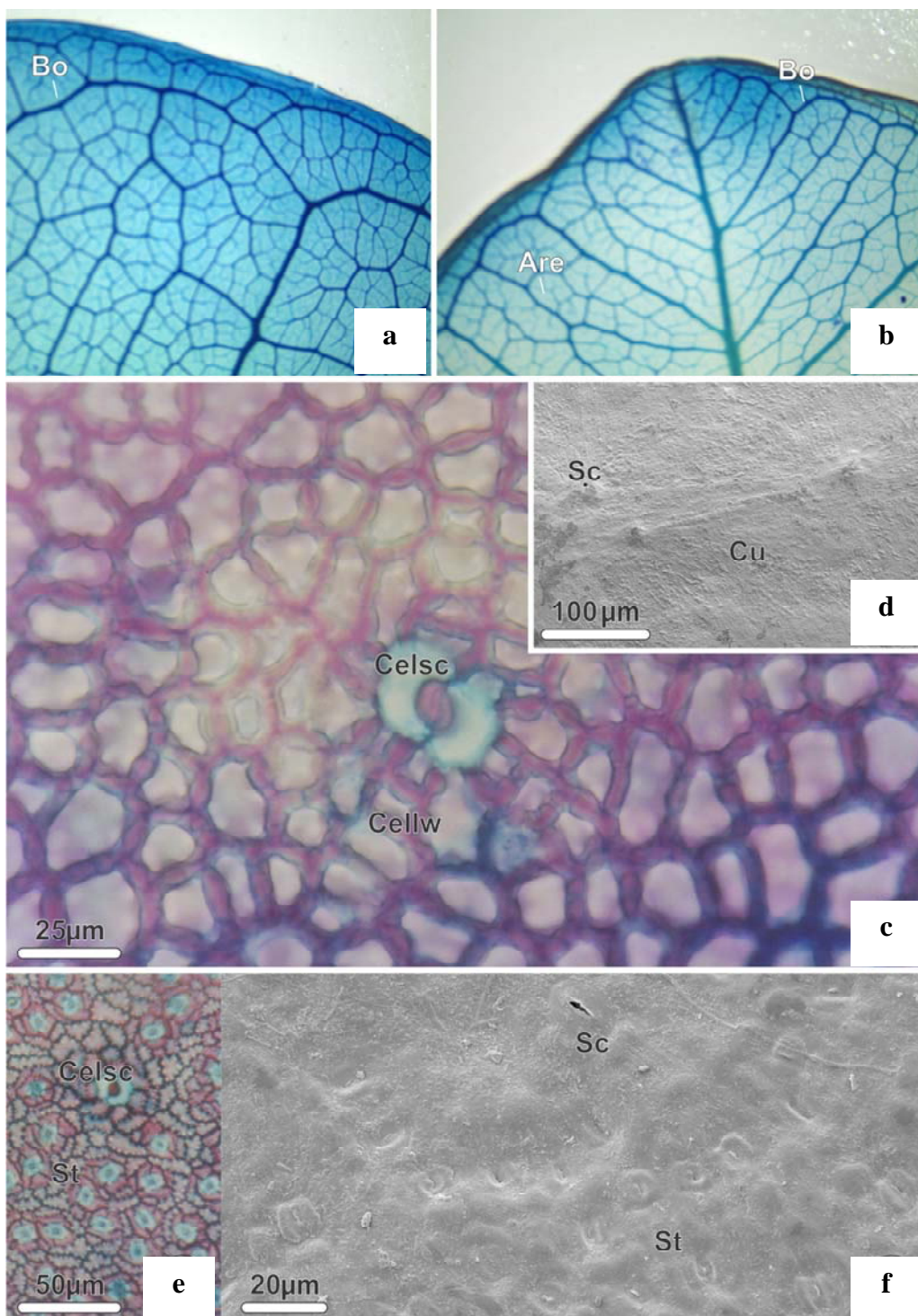
In transverse sections, the epidermis is unistratified and typically has a dorsiventral mesophyll (Figures 3b to d). The epidermal cells of the adaxial surface are larger than those of the abaxial surface. The abaxial epidermal cells are aligned such that they are more compact than the adaxial epidermal cells (Figures 3b to d). Both epidermises are coated on their external anticlinal walls with a cuticle layer, and the adaxial surface cuticle seems to be thicker than the abaxial surface and extends toward the anticlinal walls to form cuticular flanges (Figure 3e). The mesophyll consists of 2 to 3 layers of palisade parenchyma. The first layer is formed by cells that are very compactly arranged in a rectangular palisade, and the second layer has smaller cells that are followed by spongy parenchyma (Figures 3b to d). The spongy parenchyma is formed by cells that are loosely distributed in approximately 8 strata in which the conductive fabric that can extend the palisade parenchyma is located (Figure 3b). In the sub-epidermal region at the time of the observations of the vascular bundle, 2 to 4 layers of angular collenchyma were observed on both sides. The rounded parenchymal cells were of varying dimensions

and surrounded the vascular bundle side, which was surrounded by sclerenchymal fibers.

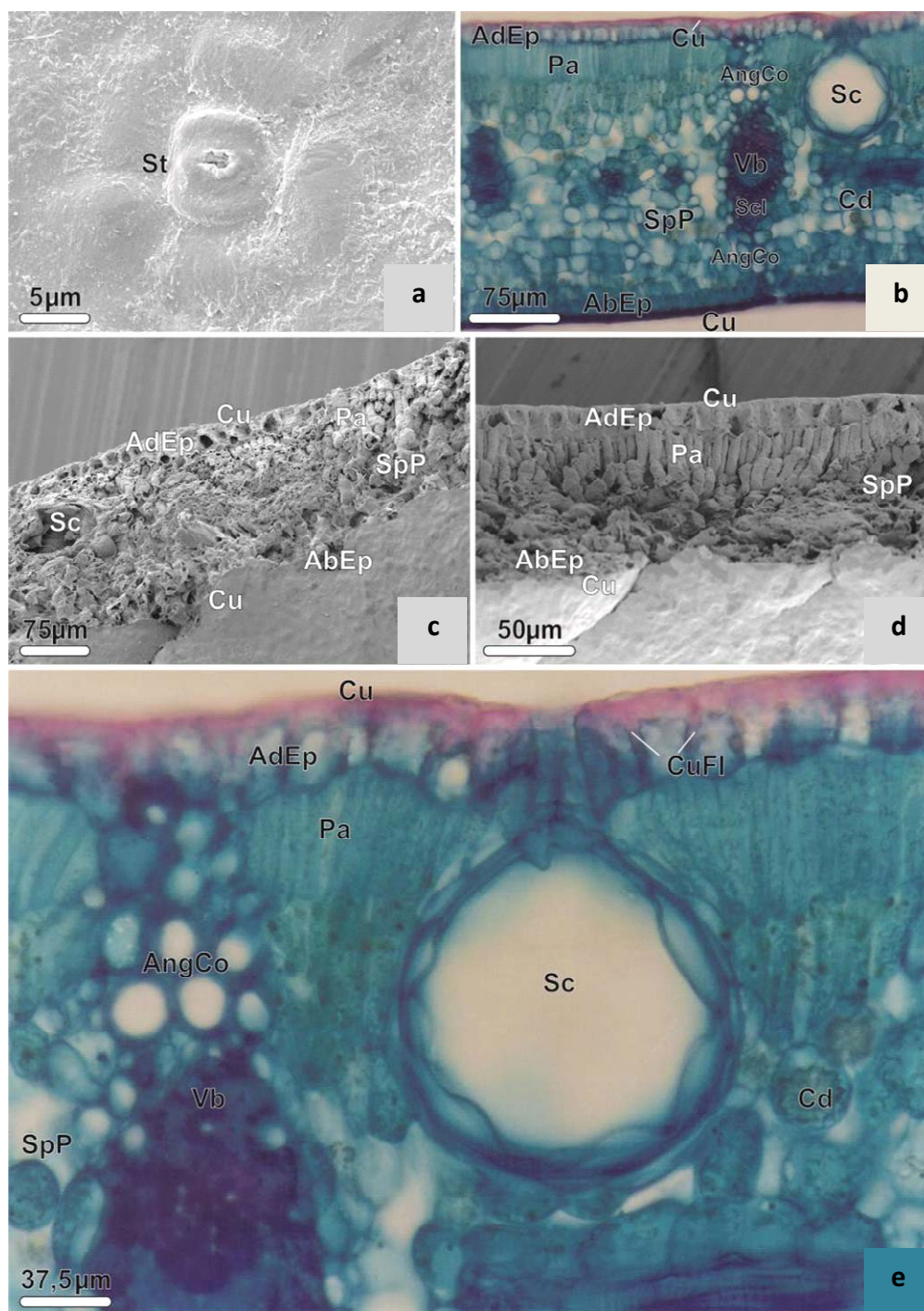
The schizogenous secretory cavities are spherical and internally bound by flattened cells that are found more frequently below the adaxial epidermis (Figures 3b, c, e and 4). The lumen of the secretory cavity communicates with the outside through 4 to 5 pairs of cells that are immersed in the epidermis and palisade (Figures 3e and 4a). Although in the mesophyll it is possible to observe a large number of idioblasts, carriers of calcium oxalate crystals in the form of drusen can also be observed (Figure 4b). In the leaf margin (Figure 4c), the mesophyll cells are gradually replaced by the angular collenchyma to end in approximately 5 layers of sub-epidermal cells. There, the secretory cavities and epidermal cells are covered by a thick cuticle layer. This presence of this cuticle travelling to the leaf margin has also been noted in the studies of Gomes and Neves (1993/1997).

In transverse sections, along the main vein (Figure 4d) from the periphery to the center, a thick cuticular layer of epidermis covering the adaxial and abaxial surfaces is present, and it is unistratified. Cuticular flange can also be observed. In the sub-epidermal region on both sides but with greater frequency on the abaxial surface, secretory cavities and a variable number of layers of lacunar collenchyma can be observed. The parenchyma cells surround the vascular bundle core. The cortical parenchyma is formed by round cells that often contain idioblasts with calcium oxalate crystals in the form of druse. Similar observations have been made in the genus *Gomidesia* (Myrtaceae) by Gomes and Neves (1993/1997) regarding the provision of crystals throughout the parenchyma and the presence of secretory cavities on both sides.

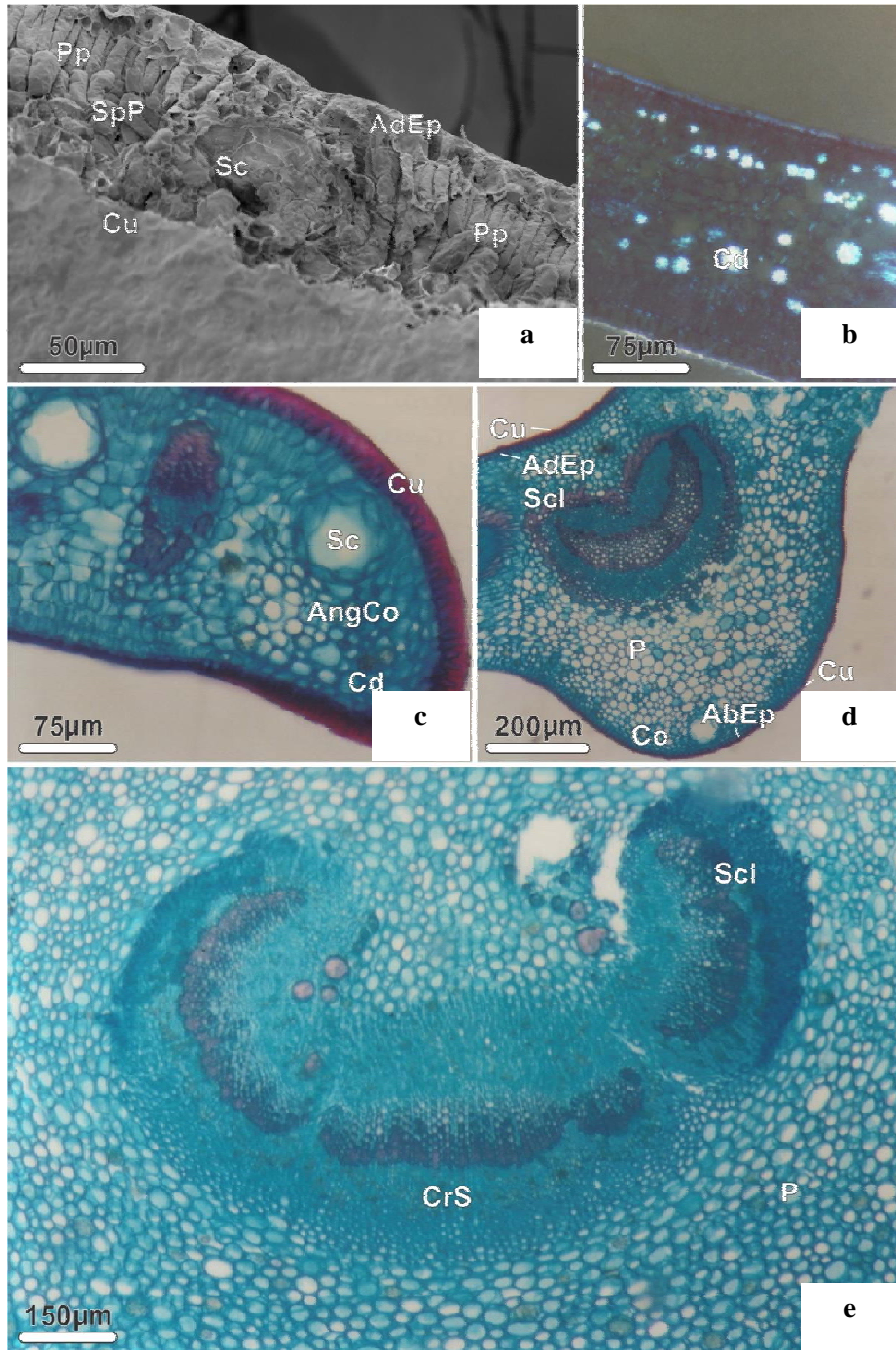
The central vascular bundle is arranged in the form of a continuous bicollateral arc in which the xylem appears slightly compressed and is surrounded by the phloem internally and externally; this characteristic was also described by Paula et al. (2008) for *P. pseudocaryophyllus* and Siqueira-Nunes and Martins (2010) for *Syzygium cumini*. Near the adaxial surface, the vascular system is thickly surrounded by a sclerenchymatic sheath. There are still a number of crystals scattered throughout the parenchyma and phloem that might, in some regions, constitute a crystal series (the most common condition in the phloem). In cross-section, the petiole presents itself as a circular, biconvex (Figure 4e), slightly thick cuticle coupled with a layer of epidermal cells. There are some trichomes. The vascular system is arranged in a bicollateral arc. The cortex is collenchymatous and filled with petiole parenchymal cells with thick walls. There are also numerous idioblasts that contain calcium oxalate crystals in the form of drusen. In the subepidermal region, secretory cavities were present as in the leaf blade.



**Figure 2.** *Pimenta dioica* (L.) Merrill. (a and b) Stereoscopic microscopy showing details of the leaf venation pattern; (c) photomicrograph from the front view showing the adaxial epidermal cells that cover the secretory cavity; (d) electron micrograph from the front view showing the adaxial epidermis, the anomocytic stomata and the cell wall; (e) front-view photomicrograph showing the abaxial epidermis, the anomocytic stomata and the cell wall; (f) front-view electron micrograph of the abaxial epidermis. Bo – bows , Are – areolas, Celsc– cells that cover the secretory cavity, Sc – secretory cavity, Cu – cuticle, St – stomata, Cellw – cell wall.

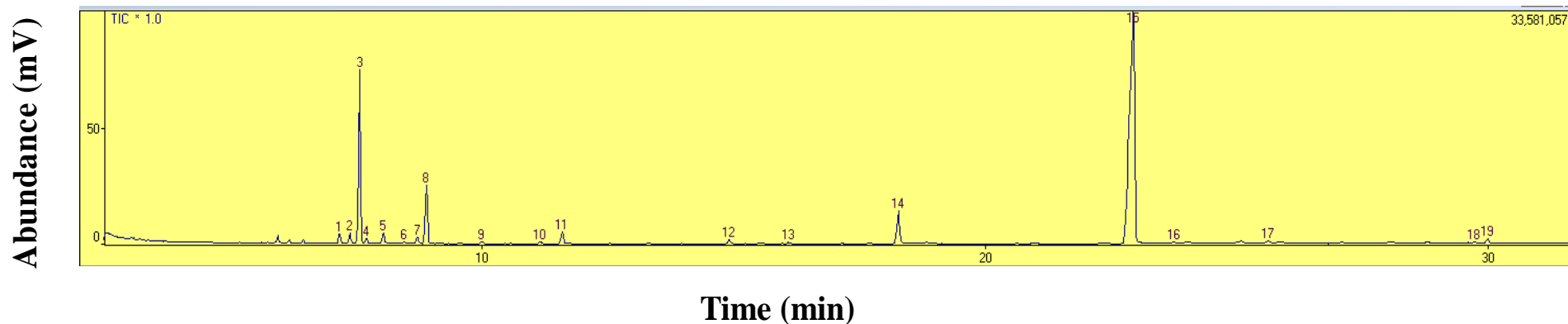


**Figure 3.** Leaf blade of *Pimenta doica* (L.) Merrill. (a) Electron micrograph from the front view showing details of the adaxial epidermis of an anomocytic stoma; (b) photomicrograph of a cross section showing the interval region; (c) electron micrograph of a cross section showing the mesophyll; (d) electron micrograph of a cross section showing the organization of the parenchyma along the leaf lamina; (e) photomicrograph of a cross section showing details of the secretory cavity cells, cuticular flanges on the walls of the adaxial epidermal cells and collenchyma. Cd – crystal (druze), AngCo – angular collenchyma, Sc – secretory cavity, Cu – cuticle, AbEp – abaxial epidermis, AdEp – adaxial epidermis, S – stoma, Scl – sclerenchyma, CuFl – cuticular flange, Vb – vascular bundle, SpP – spongy parenchyma, Pa – palisade.



**Figure 4.** Leaf blade of *Pimenta dioica* (L.) Merrill. (a) Electron micrograph of a cross section showing the internal cavity lining and secretory cells that form the lumen; (b) photomicrograph of a cross section under polarized light showing the internodal region; (c) photomicrograph of a cross section showing the leaf margin; (d) photomicrograph of a cross section showing the midrib of the leaf; (e) photomicrograph of a cross section showing the vascular system of the petiole. Co – collenchyma, AngCo – angular collenchyma, Cd – crystal (druze), Sc – secretory cavity, Cu – cuticle, AbEp – abaxial epidermis, AdEp – adaxial epidermis, Scl – sclerenchyma, SpP – spongy parenchyma, P – parenchyma, Pp – palisade parenchyma, CrS – crystal series.





**Figure 5.** Gas chromatogram of the essential oil of the *Pimenta dioica* leaves from Goiás State, Brazil. For details of the chromatographic conditions, see the Materials and Methods section.

### Plant material quality parameters

The samples of *P. dioica* exhibited a moisture content of  $7.87 \pm 2.11\%$  (w/w). This value is between the limits that are recommended by the Brazilian Pharmacopoeia V (Brazil, 2010), which established values between 8 and 14% for the moisture content of raw vegetable material. Moisture content tests are important because they reduce errors in estimating the actual weight of the plant material, and low humidity is suggestive of better stability against product degradation. The samples exhibited  $8.84 \pm 0.125\%$  (w/w) total ash. The content of HCl-insoluble ash was  $1.74 \pm 0.233\%$  (w/w). The ash value is an important parameter for judging the identity and purity of crude drugs (Brazil, 2010).

### Phytochemical screening

In addition to the essential oils, the secondary metabolites that were found in the phytochemical

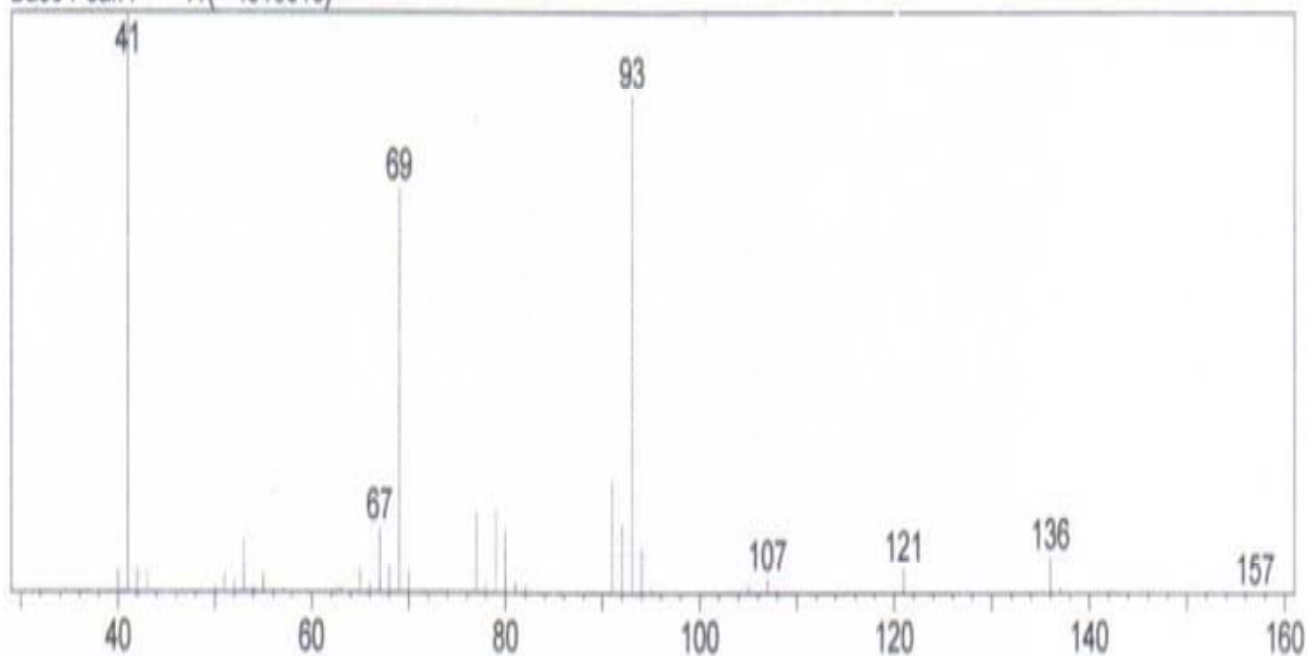
screening of the *P. dioica* leaves included saponins, tannins and flavonoids (Table 1). The total flavonoid found in the samples was  $1.88 \pm 0.133\%$  (w/w) as assayed by the method described in the monograph of “Calêndula” (Brazil, 2010). This value is similar to that for *P. pseudocaryophyllus* (Paula et al., 2008). According to Vadlapudi and Kaladhar (2012), *P. dioica* leaves collected from Vijayawada of the Krishna district of India exhibit a flavonoid content of  $116.25 \mu\text{g/g}$ . These authors attributed the antioxidant and antimicrobial activities that have been observed for *P. dioica* leaves to the high contents of phenolic compounds, including flavonoids, and to the main constituents found in the essential oils. Marzouk et al. (2007) verified the anticancer and antioxidant properties of tannins isolated from *Pimenta dioica* leaves.

### Analysis of the essential oils

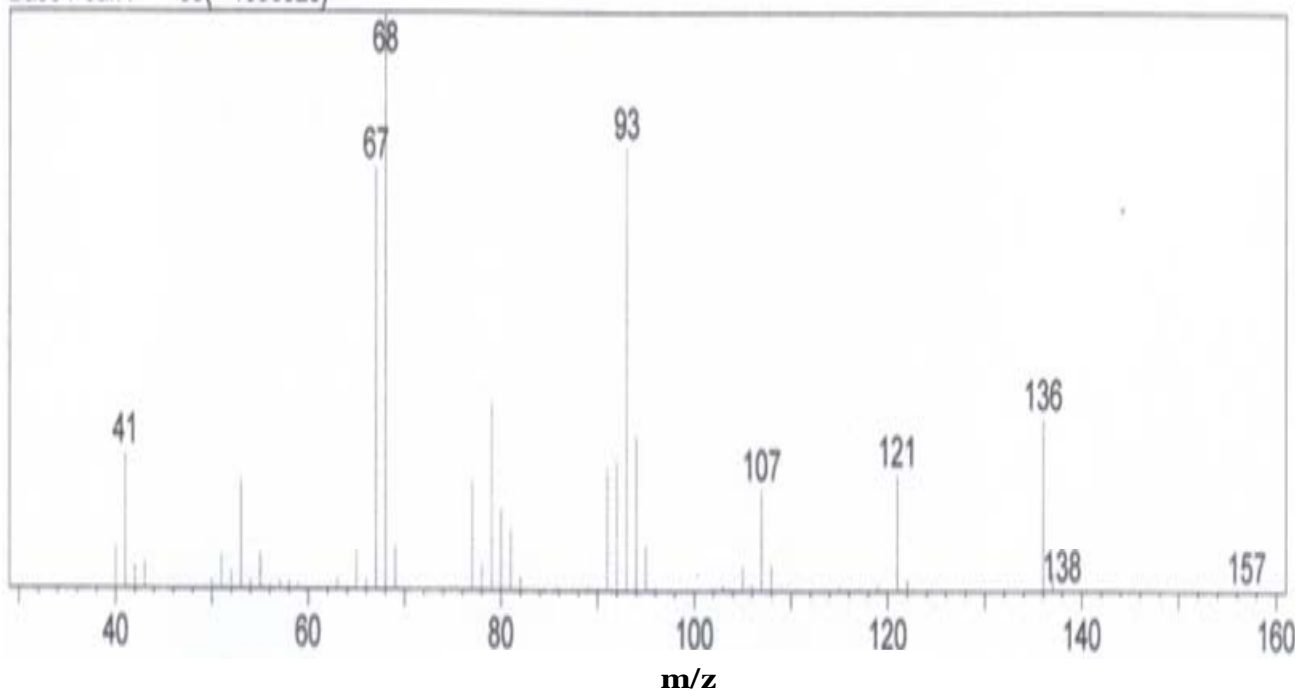
The essential oil yield was 1.3% (v/v), which is

higher than that found in a study of the leaves of sun-dried *P. dioica* specimens that were grown in the southern region of Bahia (Oliveira et al., 2009). The GC/MS analysis of the *P. dioica* leaf-extracted oil identified 19 compounds, and the major components were the phenylpropanoids eugenol (60.8%) and chavicol (4.8%) and the non-oxygenated monoterpenes myrcene (19.3%) and limonene (6.5%; Table 2). The chromatogram is presented in Figure 5, and the mass spectra of the main constituents are represented in Figure 6. These analyses revealed that this botanical material is rich in eugenol and chavicol, which agrees with the report of Mendes-Ferrão (1993). Similar findings have been reported for samples from the southern region of Bahia state in Brazil in which 26 compounds were identified, and the major components were found to be eugenol (78.5%), followed by myrcene (7.3%),  $\beta$ -phellandrene (2.2%) and chavicol (5.5%) (Oliveira et al., 2009). Other authors have identified eugenol as the major component of *P. dioica*

PKNO : 3 Peak #: 86 Ret. Time : 7.583  
 Scan # : ( 305 - 307 ) B.G. Scan # : ( 945 - 1201 )  
 Base Peak : 41( 4510016)

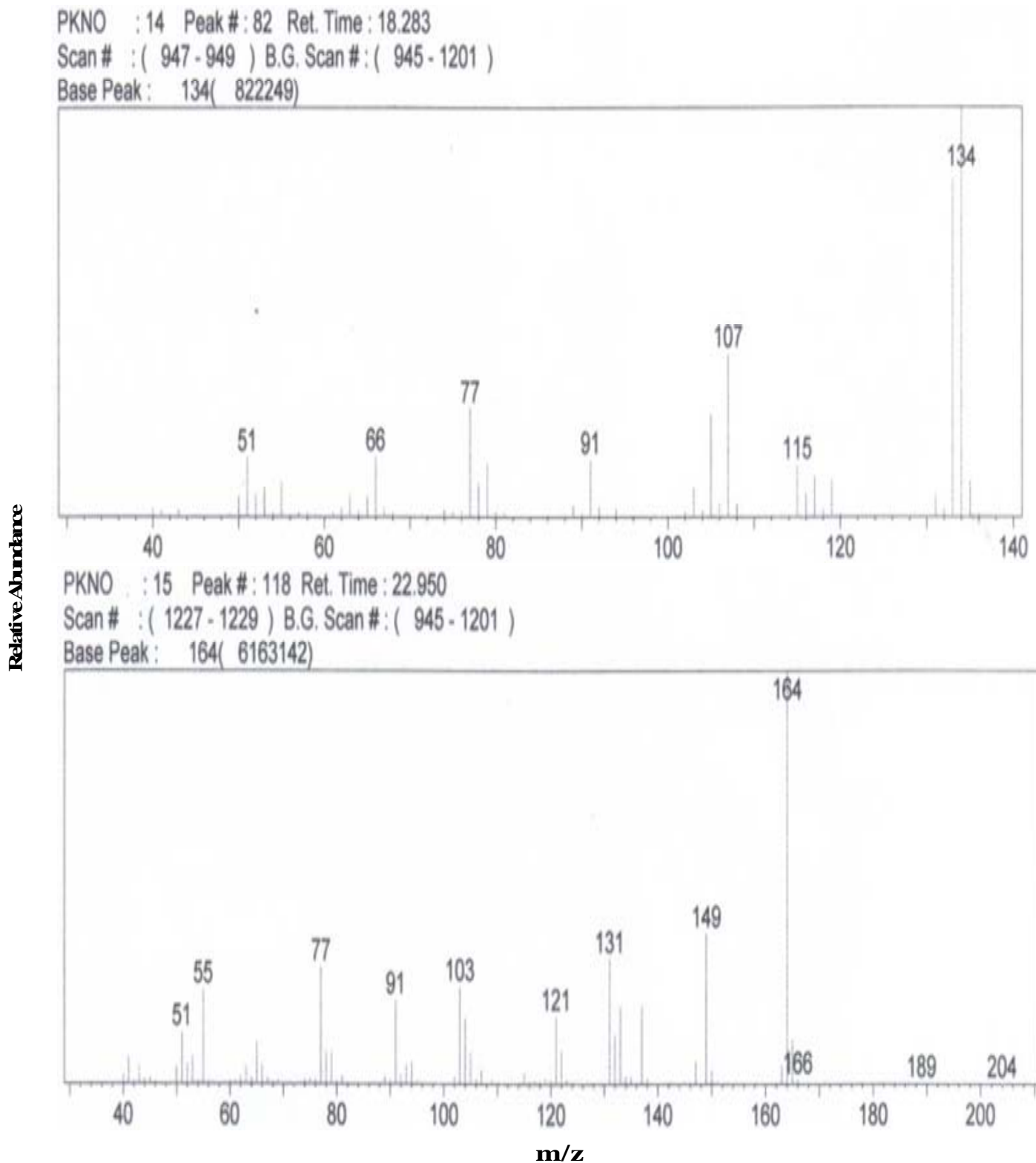


PKNO : 8 Peak #: 82 Ret. Time : 8.900  
 Scan # : ( 384 - 386 ) B.G. Scan # : ( 945 - 1201 )  
 Base Peak : 68( 1096928)



Relative Abundance

m/z



**Figure 6.** Mass spectra of peaks 3, 8, 14, and 15 of the chromatogram, which indicate the major compounds of the essential oil of the *Pimenta dioica* leaves, which were myrcene, limonene, chavicol, and eugenol, respectively.

**Table 1.** The phytochemical components of the *P. dioica* leaves based on the preliminary screening.

Phytochemical compound	Occurrence	Total Content (% w/w)
Anthraquinone heterosides	-	-
Digitalis heterosides	-	-
Flavonoids	+	1.88 ± 0.133%
Saponins	+	NP
Tannins	+	NP
Alkaloids	-	-
Coumarins	-	-

+ = presence, - = absence, NP = not performed.

**Table 2.** Chemical composition of the essential oil from the *Pimenta dioica* (L.) Merrill leaves that were collected in an urban area of Goiás State, Brazil.

Component	KI*	Percentage
1-Octen-3-ol	972	0.97
3-Octanone	979	0.96
Myrcene	986	19.3
3-Octanol	990	0.59
α-Phellandrene	1002	1.19
α-Terpinene	1013	0.17
p-Cymene	1019	0.72
Limonene	1024	6.48
γ-Terpinene	1053	0.20
Terpinolene	1083	0.25
Linalool	1095	1.39
Terpinen-4-ol	1173	0.59
n-Decanal	1200	0.15
Chavicol	1249	4.78
Eugenol	1354	60.8
α-Copaene	1372	0.21
(E)-Caryophyllene	1415	0.35
Myrac aldehyde	1512	0.20
δ-Cadinene	1518	0.68

KI\* - Kovats indices.

specimens that were collected in other countries. Marongiu et al. (2005) found that the essential oil of *P. dioica* leaves from Australia is 77.9% eugenol. The leaf oils from fruiting female and non-fruiting male *P. dioica* from Shawbury, St. Ann, Jamaica have been analyzed, and 79.8 to 84.0% eugenol was found (Minott and Brown, 2007). Oussalah et al. (2006, 2007) found that the essential oil of *P. dioica* leaves from Antilles is rich in eugenol (47.78%), myrcene (26.76%) and geraniol (10.40%) and

exhibits antibacterial activity against a *Pseudomonas putida* strain isolated from meat, *E. coli* O157:H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. Vadlapudi and Kaladhar (2012) verified the antioxidant properties of the essential oil of *P. dioica* leaves collected in India and attributed those properties to the high eugenol, methyl eugenol and β-caryophyllene contents (the percentages of each of the constituents was not reported by the authors).

## Conclusion

The anatomy of the leaves of the *P. dioica* plants that were grown in Goiás, Brazil exhibited characteristics that were common to other members of the Myrtaceae family and the genus *Pimenta*. The consideration of the anatomical characteristics in conjunction with the morphological characteristics might contribute to the identification of the species and quality control of the medicinal plant. The moisture (7.87%, w/w), total ash (8.84%, w/w), HCl-insoluble ash (1.74%, w/w) and total flavonoids (1.88%, w/w) contents found in the analyzed plant material are important parameters for the quality control of materials which can enable the detection of fraud and tampering. The detection of flavonoids, tannins, eugenol and chavicol in the analyzed botanical materials indicates strong similarities between the chemical compositions of the leaves of *P. dioica* plants that were grown in Goiás, Brazil and those from other parts of Brazil and the world. Additional qualitative and quantitative analyses are necessary to verify the correspondence between the chemical composition of our samples and the biological properties mentioned in the literature.

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## Conflict of Interest

Authors have not declared any conflict of interest.

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