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Anatomical study of the leaves and evaluation of the chemical composition of the volatile oils from *Psidium guineense* Swartz leaves and fruits

Estudo anatômico das folhas e avaliação da composição química dos óleos voláteis de folhas e frutos de *Psidium guineense* Swartz

Estudio anatómico de las hojas y evaluación de la composición química de los aceites volátiles de hojas y frutos de *Psidium guineense* Swartz

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Abstract
Psidium guineense Swartz is a bush used in urinary tract diseases, diarrhea, and dysentery. The present study aims to perform the anatomical study of the leaves and evaluation of the chemical composition of the volatile oils from *Psidium guineense* Swartz leaves and fruits. The botanical material was collected in Hidrolândia, Goiás. Anatomical characterization and phytochemical screening of the leaves were performed by conventional methods. Leaf and fruit (green fruits, immature fruits, and ripe fruits) powders were submitted to hydrodistillation in the Clevenger apparatus and the identification of the chemical components of the volatile oils obtained was done by GC-MS. The leaf blade is hypoestomatic with paracytic and anisocytic stomata. Secretory cavities are observed in the central vein, mesophyll, petiole, and young stem. The powder moisture content was 7.4%. The total ash content of the leaf powder was 6.3% and the acid-insoluble ash content was 0.8%. The presence of tannins, flavonoids, and saponins in the leaves were identified. Leaves volatile oil's majority compounds were 2Z,6E-farnesol (23.1-25.4%), α-copaene (17.7-20.3%), muurola-4,10(1.4) dien-1-β-ol (5.8-6.7%), epi-α-cadinol (5.5- 6.3%), and δ-Cadinene (5.0- 5.9%). Fruits volatile oil's majority compounds were 2Z,6E-farnesol (31.9-41.4%), α-copaene (13.3-26.6%), δ-cadinene (5.4-9.8%), γ-himachalene (3.8-6.1%), and cubenol (2.6-6.1%). This is the first report on anatomical study of the leaves, and chemical composition of volatile oils from leaves and fruits of *P. guineense* collected in Hidrolândia, Goiás.
Keywords: Cerrado; Essential oils; Medicinal plants; Myrtaceae.

Resumo

Psidium guineense Swartz é um arbusto usado em doenças do trato urinário, diarréia e disenteria. O presente estudo tem como objetivo realizar o estudo anatômico das folhas e avaliação da composição química dos óleos voláteis das folhas e frutos de *Psidium guineense* Swartz. O material botânico foi coletado em Hidrolândia, Goiás. A caracterização anatômica e a triagem fitoquímica das folhas foram realizadas por métodos convencionais. Os pós das folhas e frutos (frutos verdes, frutos imaturos e frutos maduros) foram submetidos à hidrodestilação em aparelho de Clevenger e a identificação dos componentes químicos dos óleos voláteis obtidos foi feita por CG-EM. A lâmina foliar é hipoestomática com estômatos paracíticos e anisocíticos. Cavidades secretoras são observadas na nervura principal, mesofilo, pecíolo e caule jovem. O teor de umidade do pó foi de 7,4%. O teor total de cinzas do pó da folha foi de 6,3% e o teor de cinzas insolúveis em ácido foi de 0,8%. Foi identificada a presença de taninos, flavonóides e saponinas nas folhas. Os compostos majoritários do óleo volátil das folhas foram 2Z, 6E-farnesol (23,1-25,4%) e α-copaeno (17,7-20,3%), muurola-4,10(14)-dien-1β-ol (5,8-6,7%), epi-α-Cadinol (5,5- 6.3%) e δ-Cadineno (5,0- 5,9%). Os compostos majoritários de óleo volátil dos frutos foram 2Z,6E-farnesol (31,9-41,4%), α-copaeno (13,3-26,6%), δ-cadineno (5,4-9,8%), γ-himachalene (3,8-6,1%) e cubenol (2,6-6,1%) Este é o primeiro relato de estudo anatômico das folhas e composição química dos óleos voláteis das folhas e frutos de *P. guineense* coletados em Hidrolândia, Goiás.
Palavras-chave: Cerrado; Óleos essenciais; Plantas medicinais; Myrtaceae.

Resumen

Psidium guineense Swartz es un arbusto utilizado en enfermedades del tracto urinario, diarrea y disentería. El presente estudio tiene como objetivo realizar el estudio anatómico de las hojas y evaluar la composición química de los aceites volátiles de las hojas y frutos de Psidium *guineense* Swartz. El material botánico fue recolectado en Hidrolândia, Goiás, la caracterización anatómica y cribado fitoquímico de las hojas se realizó por métodos convencionales. Los polvos de hojas y frutos (frutos verdes, frutos inmaduros y frutos maduros) fueron sometidos a hidrodestilación en aparato Clevenger y la identificación de los componentes químicos de los aceites volátiles obtenidos se realizó mediante CG-EM. La lámina de la hoja es hipoestomática con estomas paracíticos y anisocíticos. Se observan cavidades secretoras en la vina principal, mesófilo, pecíolo y tallo joven. El contenido de humedad del polvo fue del 7,4%. El contenido total de cenizas del polvo de hojas fue del 6,3% y el contenido de cenizas insolubles en ácido fue del 0,8%. Se identificó la presencia de taninos, flavonoides y saponinas en las hojas. Los compuestos principales en el aceite volátil de las hojas fueron 2Z, 6E-farnesol (23,1-25.4%), α-copaeno (17,7-20.3%), muurola-4,10(14)-dien-1β-ol (5,8-6,7%), epi-α-Cadinol (5,5- 6,3%) y δ-Cadineno (5,0- 5,9%). Los principales compuestos de aceite volátil de frutas fueron 2Z, 6E-farnesol (31,9-41,4%), α-copaene (13.3-26.6%), δ-cadinene (5.4-9.8%), γ-himachalene (3.8-6.1%) y cubenol (2.6-6.1%). Este es el primer informe de un estudio anatómico de las hojas y composición química de los aceites volátiles de las hojas y frutos de *P. guineense* recolectados en Hidrolândia, Goiás.
Palabras clave: Cerrado; Aceites esenciales; Plantas medicinales; Myrtaceae.

1. Introduction

The Cerrado is the most diverse savanna in the world, with 12,700 known vascular plants species, 35% of which are endemic (Forzza, et al., 2012; Novaes, et al., 2013). The Cerrado has 11 phytophysiognomies, divided into forest, savanna, and peasant formations (Coutinho, 2006). Myrtaceae family with 211 species occur in the Cerrado and are distributed in 14 genera, highlighting *Eugeni*a L., *Myrcia* DC. Ex. Guill., and *Psidium* L. (Novaes, et al., 2013).

Psidium guineense Swartz known as "araçá comum", "araçá-azedo", or "araçá-mirim", is distributed in Brazilian States such as Amazonas, Pará, Goiás, Minas Gerais, São Paulo, Mato Grosso, and Ceará (São Paulo, 1978). *P. guineense* is a shrub of up to 6 m, with yellowish-brown coriaceous leaves of elliptical shape and flat central rib. The flowers can be solitary or in dichásio with white petals (Silva & Mazine, 2016; Peixoto, et al., 2017). The fruit is a globular berry rich in vitamin C with high dispersion capacity, can be consumed *in natura* or as an ice cream, beverages, and liquors. The pulp is fleshy, white, mucilaginous, sweet, slightly sour, and aromatic and has numerous small seeds (Manica, 2000).

Brazilian folk medicine uses *P. guineense* to treat urinary tract diseases, diarrhea, and dysentery. Due to its high tannin content, fruit peel can be used in tanneries (Rodrigues & Carvalho, 2001; González, et al., 2005). *P. guineense* has volatile oils stored in the leaf and fruit secretory cavities (Oliveira, et al., 2014; Silva, et al., 2020).

Scientific studies performed with *P. guineense* leaves observed neuropharmacological effects in mice as the increase of sleeping time, anticonvulsant action, analgesic action (Santos, et al., 1996; 1997); anti-nociceptive, anxiolytic and antidepressant activity (Santos, et al., 2020).

The present study aims to perform the anatomical study of the leaves and evaluation of the chemical composition of the volatile oils from *Psidium guineense* Swartz leaves and fruits collected in Hidrolândia, Goiás.

2. Material and Methods

2.1 Plant material

Psidium guineense Swartz leaves, green fruits, immature fruits, and ripe fruits were collected in January and February, during the morning and the first day each month in Hidrolândia - GO (786 m, 16° 53' 59" S and 49° 13' 29" W). Professor Dr. José Realino de Paula identified the specimen, and a voucher was deposited at the UFG Herbarium, Goiás, Brazil, under code number UFG-67843. The leaves and fruits were dried in an oven with air circulation at 38 ºC by 2 days.

2.2 Anatomical study

For the anatomical study, leaves and stems were sectioned and stained with Alcian blue/safranin 9: 1 (Kraus & Arduin, 1997) and histochemical tests Steinmetz and Lugol reagents (Costa, 2001). The photographic recording of the anatomical structures was performed in a photomicroscope (Zeiss-Axiostar plus) with a coupled digital camera (Canon Power Shot G10) using the Axion Vision 4.8 software.

2.3 Phytochemical screening

The moisture analyzer (Ohaus model MB35) determined the moisture content leaf powder (Brasil, 2010). Total and insoluble ash content was determined according to the Brazilian Pharmacopoeia (Brasil, 2019). The phytochemical composition of leaf powder was screened for the presence of anthraquinone heterosides, coumarins, steroids, and triterpenes; starch (Lugol), alkaloids, flavonoid heterosides, saponins heterosides, and tannins (Costa, 2001; Cunha, 2009).

2.4 Volatile oils

Healthy leaves, green fruits, immature fruits, and ripe fruits were collected from ten different individuals in January and February, triturated immediately before volatile oil extraction, and 90g of the powder submitted to hydrodistillation in a Clevenger-type apparatus for 2 h. After drying with anhydrous Na_2SO_4, the oils were stored in glass vials at a temperature of -18 °C until further analysis. Each experiment was performed in triplicate. The composition of the volatile oils was 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% phenylmethyl polysiloxane). The following temperature program was used: the temperature was raised from 60-240 °C at a rate of 3 °C/min and then to 280 °C at a rate of 10 °C/min, ending with 10 min at 280 °C. The carrier gas (helium) had 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. The operating parameters for the quadrupole mass spectrometer were as follows: the interface temperature was set to 240 °C and the electron impact ionization to 70 eV, with a scan mass range of 40-350 m/z at a sampling rate of 1 scan/s. The components were identified by comparison of the retention indices of the components to those of C_9–C_{28} n-alkanes and comparison of the mass spectra with literature data (Van Den Dool & Kratz, 1963, Adams, 2007).

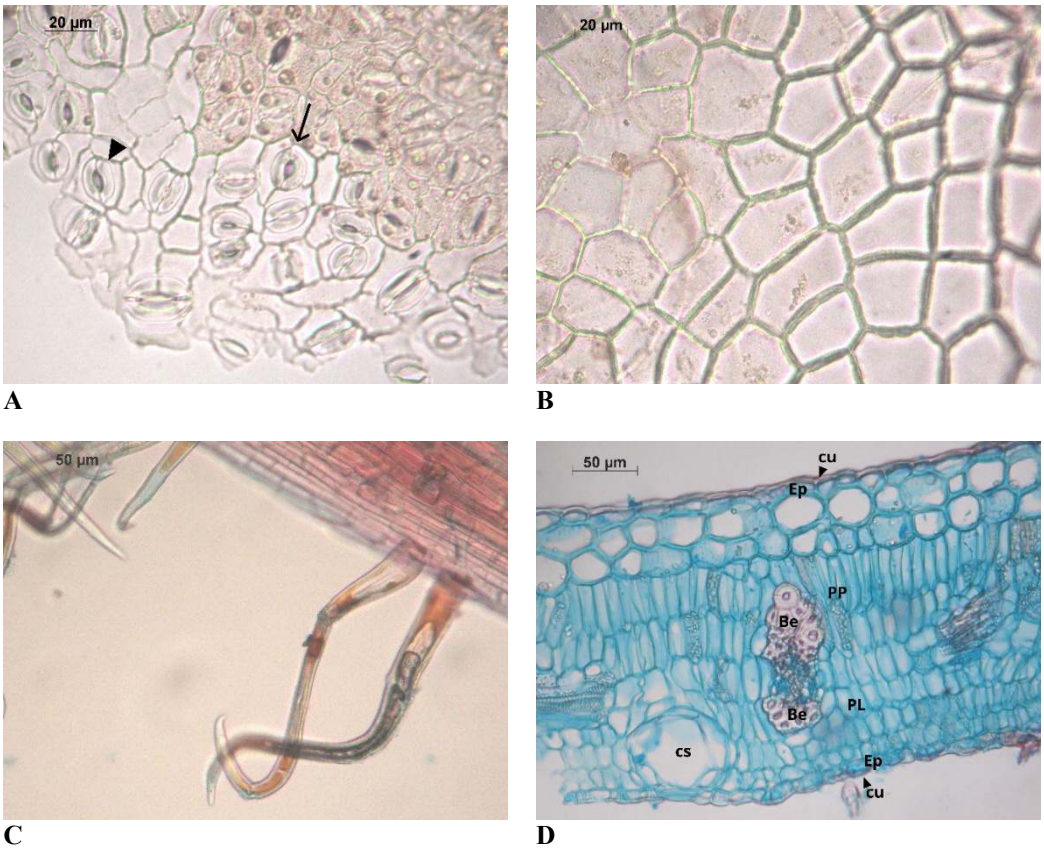
3. Results

3.1 Anatomic study

The foliar blade of *P. guineense* is hypoestomatic with paracytic and anisocytic (Figure 1A) stomata in parademic section. The epidermis on both sides present cells with straight to slightly curved walls (Figure 1B). In the transversal section, it has uniestratified epidermis on the adaxial surface and hypodermis with two layers of cells, and uniestratified epidermis covered

by thick cuticle on both sides (Figure 1D) and unicellular trichomes (Figure 1C). The mesophyll is dorsiventral, present palisade parenchyma with two cell layers, and lacunae parenchyma with four to five cell layers (Figure 1D). Secretory cavities are present in the lacunous parenchyma (Figure 1D). A vascular bundle with the extension of the sheath is observe (Figure 1D).

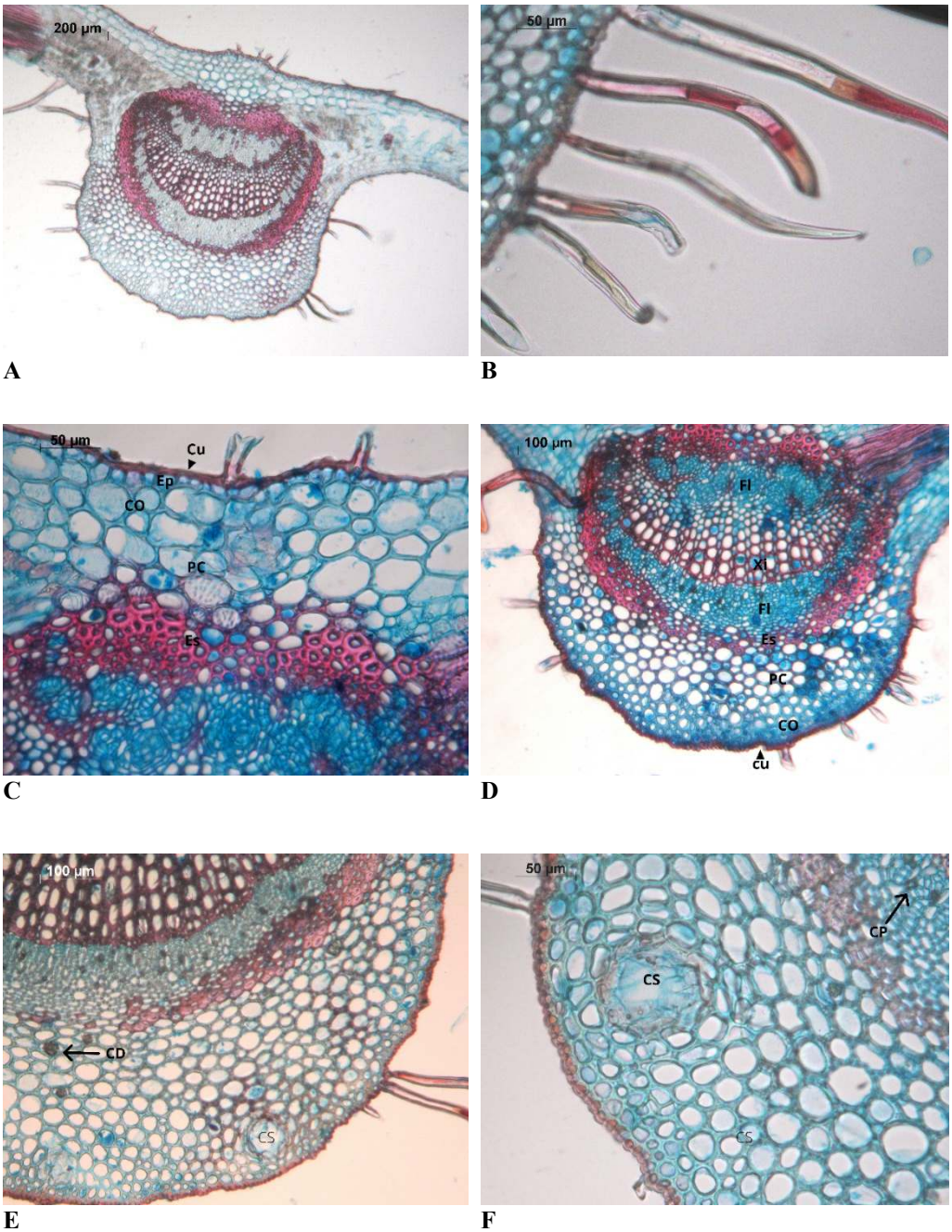
Figure 1 – A, B - Leaf blade Paradermic section. **A**- Paracytic (arrow) and anisocytic stomata (arrowhead) show in the abaxial face – B- Adaxial face. **C**-**D** - Transversal section of the mesophyll. **C**. unicellular trichomes. **D**. Secretory cavities are present in the lacunous parenchyma and cuticle on both sides (arrowhead). **Be** - Sclerenchymatous sheath. **CS** - Secretory cavity. **Cu** - Cuticle. **Ep** - Epidermis. **PL** - Lacunous parenchyma. **PP** - Palisadic parenchyma.



Source: Authors.

The main rib, in cross-section, has a plane-convex shape (Figure 2A). The epidermis is uniestratified coated by cuticle (Figure 2C), observing the presence of simple trichomes (Figure 2B). After the epidermis, there are about three to four layers of collenchyma cells (Figure 2D). The cortical parenchyma has from eight to ten layers of cells with secretory cavities (Figure 2F) and idioblasts containing druse-type crystals (Figure 2E). The vascular bundle is bicollateral with an arch shape surrounded by a range of sclerenchymatous sheath cells ranging from one to seven layers that emit projections between cells from the external and internal xylem to the phloem (Figure 2D). In the external phloem, idioblasts cells containing prismatic crystals are presented (Figure 2E).

Figure 2 - A, B, C, D, E, F- Principal rib transverse sections. **A-** General aspect. **B**. Simple trichomes. **C.** Detail of the epidermis coated by cuticle, collenchyma and cortical parenchyma. **D**. Vascular bundle detail. **E**. Idioblast cells containing prismatic crystals. **F**. Cortical parenchyma with secretory cavity **CS-** Secretory cavity. **CD -** Crystal druse. **CO -** Collenchyma cells. **CP -** Polyhedral crystal. **Cu -** Cuticle. **Ep** - Epidermis. **Es -** Sclerenchymatous sheath. **Fl -** phloem. **PC -** Cortical Parenchyma **Xi** - Xylem.

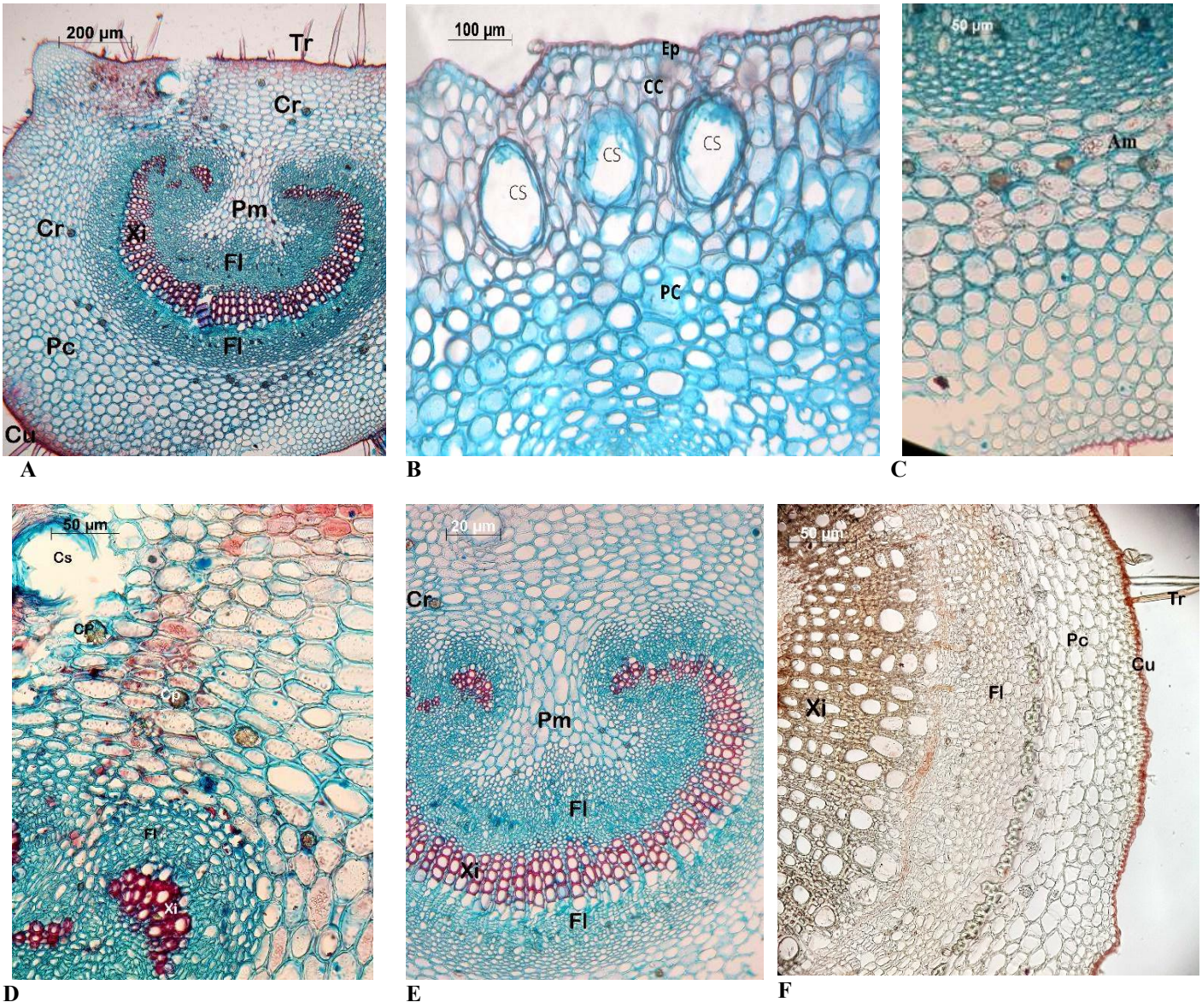


Source: Authors.

The petiole, in the transversal section, presents a plane-convex shape (Figure 3A) with uniestratified epidermis covered by a cuticle (Figure 3B and 3F). It presents a unicellular trichomas tectores. Below the epidermis, a collenchyma with three to four cell layers (Figure 3B) followed by cortical parenchyma with 10 to 12 cell layers, some presenting amyloplasts (Figure 3C). Secretory cavities (Figure 3B) and cells with points (Figure 3D) are observed in the cortical parenchyma. The vascular bundle is

bicollateral with a revolute arch shape (Figure 3E). It presents medullary parenchyma with cells of different sizes. Druse-like crystals are observed in the cortical parenchyma and medullary parenchyma and prismatic crystals in the phloem (Figure 3E).

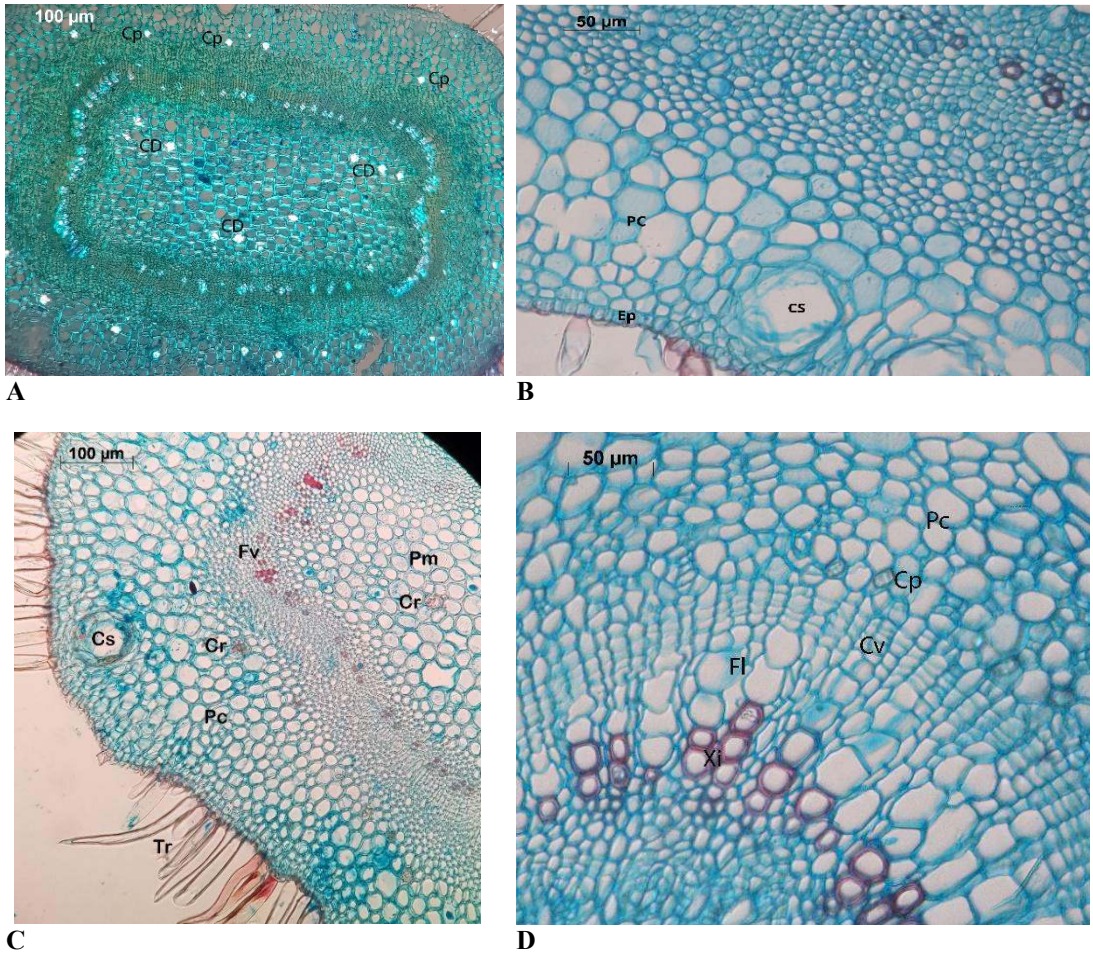
Figure 3 - **A, B, C, D, E, F** -Cross-sections of petiole. **A**. Overview of the petiole. **B**. Secretory cavities in the cortical parenchyma. **C**. Cortical parenchyma presenting amyloplasts. **D**. Secretory cavity, cells with points and druse-like crystals in the cortical parenchyma. **E-** Vascular bundle detail. **F**. Uniestratified epidermis covered by a cuticle (Steinmetz). **A, B, C, D, E-** Alcian blue/safranin. **Am -** Amiloplasts. **CS-** Secretory cavity. **CC** - Collenchyma cells. **Cr** – Crystal. **Cu** – Cuticle. **Ep -** Epidermis. **Fl –** Phloem. **PC** - Cortical parenchyma. **Pm** – Parenchyma medullary. **Tr** – Trichome **Xi**- Xylem.



Source: Authors.

The young stem, in cross-section, has an oval shape (Figure 4A) and is delimited by a uniestratified epidermis coated with cuticle (Figure 4B) and numerous unicellular trichomes (Figure 4C). The cortical parenchyma contains about eight to nine cell layers, presenting secretory cavities Figures 4B and 4C). In the vascular bundle a phloem is observed followed by medullary parenchyma with isodiametric cells of varying sizes. Presence of idioblasts containing druses in the cortical and medullary parenchyma and prismatic crystals in the phloem.

Figure 4 - A. The general appearance of the young stem in the transversal section (polarized light), **B, C, D, E** Transversal sections of young stem**. B.** Secretory cavities in the cortical parenchyma **C.** Unicellular trichomes. **D**. Vascular bundle detail. **CS-** Secretory cavity. **CD-** Crystal drusa. **Cp-** Polyhedral crystal. **Cr –** Crystal. **Cv-** Vascular exchange. **Ep -** Epidermis. **Fl-** Phloem. **Fv**- Vascular bundle **PC -** Cortical parenchyma. **Pm**- Parenchyma medullary. **Tr** – Trichome. **Xi -** Xylem



Source: Authors.

3.2 Total ash content, acid-insoluble ash content, and phytochemical screening

The powder moisture content was 7.4%. The total ash content of the leaf powder was 6.3% and the acid-insoluble ash content was 0.8%. The qualitative presence of tannins, flavonoids, and saponins in the leaves were identified.

3.3 Volatile Oil

The volatile oil yield from *P. guineense* leaves varied from 0.05 to 0.06% and 93.5 to 99.7% compounds were identified. The analysis resulted in the identification of 31 components (Table 1), which were 0.5% to 1.2% monoterpene hydrocarbons, 46.2% to 50.0% sesquiterpene hydrocarbons, 40,2% to 50.4% sesquiterpene oxygenates, 1.9% to 2.6% phenylpropanoids and 0% to 0.2% ketone. The major compounds were 2Z,6E-farnesol (23.1-25.4%), α-copaene (17.7-20.3%) (Figure 5), muurola-4,10(1.4) dien-1-β-ol (5.8-6.7%), and epi-α-cadinol (5.5- 6.3%).

For the fruits, the yield of volatile oil varied between 0.03-0.5%. and 96.8 to 100% compounds. The analysis resulted in the identification of 34 components were identified, being 0% to 0.2% monoterpene hydrocarbons, 30.6% to 56.1% sesquiterpene hydrocarbons, 43.0% to 66.2% oxygenated sesquiterpenes and 0.4% to 1.0% phenylpropanoids. The major ones

being 2Z,6E-farnesol (31.9-41.4%), α-copaene (13.3-26.6%), δ-cadinene (5.4-9.8%), γ-himachalene (3.8-6.1%), and cubenol (2.6-6.1%) (Table 1).

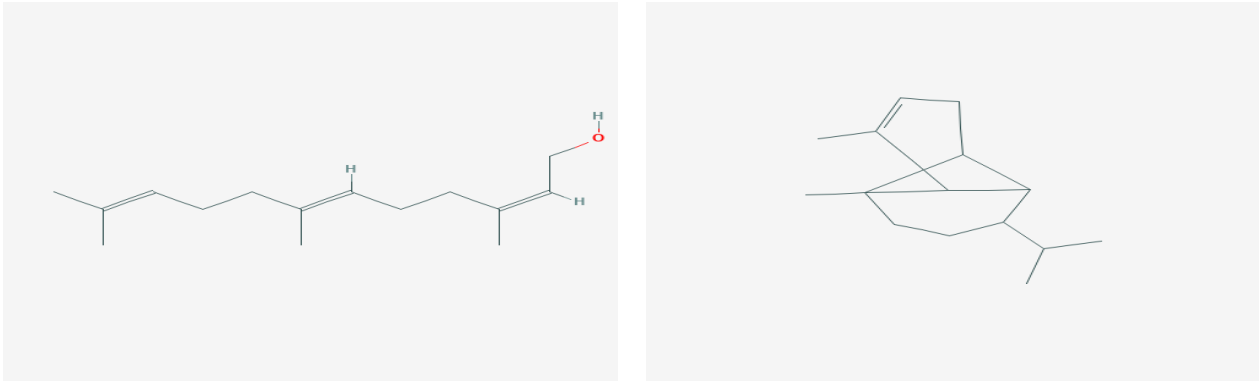
Table 1 - Percentage of chemical compounds of volatile oils from *Psidium guineense* leaves and fruits collected in Hidrolândia-Goiás, Brazil, in 2020.

			Leaves		Green fruit		Immature fruit		Ripe fruit	
Chemical compounds	**KI**	**IR**	**Jan**	**Feb**	**Jan**	**Feb**	**Jan**	**Feb**	**Jan**	**Feb**
Hepten-2-one 6-methyl-5	985	984	0.2*	0	0	0	0	0	0	0
1,8-Cineole	1031	1028	1.2	0.5	0	0	0	0	0	0.2
2-(1Z)-propenyl Phenol	1150	1158	1.1	2.1	0.9	0.9	0.9	0.6	1.0	0.4
Hydrocinnamyl acetate	1368	1367	0.8	0.5	0	0	0	0	0	0
α-Copaene	1376	1372	17.7	20.3	26.6	17.8	21.1	13.3	17.6	17.2
β-Funebrene	1414	1415	2.3	1.8	1.5	0.9	1.5	0.6	1.7	1.0
α-Humulene	1454	1449	0.8	0.7	0.6	0	0.5	0.3	0.6	0.4
E-β-Farnesene	1456	1456	0.7	0.6	0.6	0.5	0,6	0.7	0.7	0.7
9-epi-E- Caryophyllene	1466	1465	0	0	0	0	0	0.3	0	0.4
γ-Gurjunene	1477	1472	1.0	1.4	1.0	0.9	0.9	0.8	0.8	1.0
γ-Himachalene	1482	1484	4.9	5.1	6.1	4.7	4.9	3.8	4.0	4.5
β-Selinene	1490	1490	4.1	4.3	5.8	4.3	4.5	3.6	3.8	4.2
α-Muurolene	1500	1497	1.7	2.1	1.8	1.3	1.6	0	1.5	1.5
β-Bisabolene	1505	1506	0	0	0.6	0	0	0	0	0
α-Cuprenene	1505	1512	0	2.0	0	0	0	0	0	0
δ-Amorphene	1512	1510	1.5	0	1.7	1.3	1.6	1.8	1.6	0
δ-Cadinene	1523	1520	5.0	5.9	9.8	6.6	8.2	5.4	6.0	6.1
Zonarene	1529	1529	0	0.5	0	0	0	0	0	0
α-Calacorene	1545	1539	0.5	1.0	0	0	0	0	0.3	0.3
E-Nerolidol	1563	1562	3.0	2.6	1.7	3.3	2.4	3.1	2.3	3.0
β-Copaen-4-α-ol	1590	1582	1.1	0.9	0	0	0	0.2	0	0
Carotol	1599	1598	1.6	1.4	0	0	0	1.1	0.9	1.0
Globulol	1587	1590	0	0	0	0	0	0.1	0	0
Ledol	1602	1607	0.7	1.0	0	0	0	1.0	0.5	0.9

Muurola-4,10(14)-dien-1β-ol	1631	1624	6.7	5.8	0	0	0	0	0	0
α-Acorenol	1631	1624	0	0	1.7	2.8	2.4	3.4	0	0
Muurola-4,10(14)-dien-1-β-ol	1631	1624	0	0	0	0	0	0	3.6	3.4
Zonarene	1528	1529	0	0	0	0	0	0.3	0.3	0.4
Caryophylla-4(12),8(13)-dien-5α-ol	1640	1631	0.8	0.6	0	0	0	0	0.3	0
epi-α-Cadinol	1640	1637	6.3	5.5	2.7	4.7	4.0	5.1	4.8	4.8
α-Muurolol	1646	1642	2.1	1.9	0.5	1.4	0.9	1.4	1.4	1.4
Cubenol	1646	1649	0	5.0	2.6	5.5	4.7	6.1	5.4	5.5
Cadalene	1676	1670	0	0.5	0	0	0	0	0	0
2E,6Z-Farnesal	1684	1670	0	0	0	0	0	0	0	0.5
α-Bisabolol	1685	1682	0	0	0	0	0	0.3	0	0
2Z,6E-Farnesol	1723	1723	25.4	23.1	31.9	39.8	36.4	41.4	35.4	35.3
2E,6E-Farnesal	1740	1741	0.4	0.4	0	0	0	0	0	0
2E,6E-Farnesal	1740	1741	0	0	0.7	1.1	0.9	1.1	0.7	1.0
2E,6E-Farnesol	1742	1738	0	0	0	0	0	0.4	0.6	0.5
β-Bisabolenal	1767	1769	0.8	0.9	0	0	0	0	0	0
2E,6E-Methyl farnesoate	1784	1783	0	0	0	0	0	0.2	0	0
2Z,6E-Farnesyl acetate	1846	1841	1.1	1.3	1.2	1.1	1.3	1.0	1.6	1.2
Ketone			0.2	0	0	0	0	0	0	0
Phenylpropanoid			1.9	2.6	0.9	0.9	0.9	0.6	1.0	0.4
Sesquitepene hydrocarbons			50.0	46.2	56.1	38.3	45.4	30.6	38.6	37.3
Oxygenated sesquiterpenes			40.2	50.4	43.0	59.7	53.0	66.2	57.8	58.9
Hydrocarbon Monoterpenes			1.2	0.5	0	0	0	0	0	0.2
Total identified (%)			93.5	99.7	100.0	98.9	99.3	97.4	97.4	96.8
Yield (%)			0.05	0.06	0.04	0.04	0.03	0.03	0.5	0.16

KI: Kovax index**; RI:** retention index**; Jan:** January**; Feb:** February**; ∗** all the number of the constituents are expressed in percentage.
Source: Authors.

Figure 5 - Chemical structure of the major components of *Psidium guineense* volatile oil leaves **A.** 2Z,6E Farnesol**. B.** α-Copaene.



A B

Source: Pubchem (2021a, b).

4. Discussion

In the present study, uniestratified adaxial epidermis with double layer of hypodermis, palisadic parenchyma with 2 layers of cells, numerous unicellular trichomes, paracytic, and anisocytic stomata were observed while Silva, et al. (2007) observed only paracytic stomata in the abaxial epidermis of *P. guineense* and only one layer of cells in the palisadic parenchyma. According to Brewer, et al. (1991), the trichomes may be responsible for water retention on the leaf surface by retaining water droplets, improving the photosynthetic process by allowing greater opening of the stomata. Secretory cavities containing volatile oils and crystals in forms of druses described in the leaves and young stem in this study were also described by Oliveira (2015), being a common feature of Myrtaceae species (Metcalfe & Chalk 1979).

The determination of moisture in herbal drugs is important in quality evaluation. Water excess produces unwanted chemical reactions and microbial contamination. Total ash and acid-insoluble ash in high levels indicates impurities like non-organic materials and silica (Alves, et al., 2010; Brasil, 2019). Official compendia have not established parameters for *P. guineense*. However, limit values for moisture are 12.0% and total ash 9.0 % for *P. guajava* powder (Brasil, 2019). *P. guineense* powder of the leaves displayed values of 7.4% moisture, 6.3% total ash, and 0.8% acid-insoluble ash. Tannins, flavonoids, and saponins were also identified in the leaves. These compounds were also described in the methanolic extract of *P. guineense* leaves from India by Sruthi, et al. (2019), alongside coumarins, terpenoids, and quinones.

The majority of compounds in volatile oil from *P. guineense* leaves were 2Z,6E-farnesol, α-copaene, muurola-4,10(1,4),dien-1-β-ol, cubenol, epi-α-cadinol, δ-cadinene, and γ-himachalene, whereas for the fruits were 2Z,6E-farnesol, α-copaene, β-bisabolene, δ-cadinene, β-amorphene, cubenol, and epi-α-cadinol. However, Figueiredo, et al. (2018) identified α-pinene, α-copaene, and E-caryophyllene as major components in the leaf volatile oils collected in Pará, while Silva, et al. (2003) identified β-bisabolol, limonene, and epi-α-bisabolol from leaves collected in Roraima State.

In volatile oil from *P. guineense* leaves collected in Ceará State, Brazil, Neto, et al. (1994) found as major components 1.8-cineole (40.5%), β-eudesmol (19.5%) and α-pinene (13.9%). Tucker et al. (1995) determined as the main components of the leaves volatile oil in Mexico, β-bisabolene (13.18%), α-pinene (12.85%) and Z-nerolidol (5.50%). Spathulenol (80.71%) was the main component of the volatile oil, followed by 2Z,6E-farnesol (3.65%) and γ-terpineol (1.91%) in Mato Grosso do Sul (Nascimento, et al., 2018). Peralta-Bohórquezo (2011), in Mexico, identified in the fruits volatile oil the components ethyl butyrate (30.3%), ethyl hexanoate (23.8%), β-caryophyllene (3.3%) by headspace solid phase extraction and β-caryophyllene (8.6%), while butanol (7.4%) and ethyl butyrate (7.4%) when obtained by distillation extraction.

2Z,6E-farnesol belongs to the class of organic compounds known as sesquiterpenoids. These terpenes are constructed by three consecutive isoprene units (HMDB, 2021). Su, et. al. (2015) described the antimicrobial activity of 2Z-6E farnesol by microdilution broth method using minimum inhibitory concentration against *Aspergillus niger* (500 µg/mL), *Bacillus cereus* (62.25 µg/mL), *Candida albicans* (31.25 µg/mL), *Enterobacter aerogenes* (62.25 µg/mL), *Escherichia coli* (62.5 µg/mL), *Klebsiella pneumoniae* (125 µg/mL), *Pseudomonas aeruginosa* (125 µg/mL), *Staphylococcus aureus* (31.25 µg/mL), *S. epidermidis* (31.25 µg/mL), and *Vibrio parahaemolyticus* (500 µg/mL). The anticancer potential was tested on three human cancer cell lines: HT-29 (human colon), J5 (human hepatocellular carcinoma), and A549 (human lung adenocarcinoma). 2Z-6E farnesol reduced the viability 50% of HT-29, J5, and A549 cells at 10.6, 36.8, and 26.8 µg/mL, respectively.

α-Copaene belongs to the sesquiterpenoid class (HMDB, 2021b). Rodrigues, et al. (2018) observed antileishmanial activity of α-copaene *in vitro*, reducing viability by 50% for *Leishmania amazonensis* and *L. infantum* at the concentration of 17.2 and 11.4 µg/mL, respectively.

The chemical composition of the volatile oil of *P. guineense* showed component variation due to the studies being made in different regions, with different temperatures, pluviometric indexes, altitudes, type of soil, and incidence of ultraviolet rays (Gobbo-Neto & Lopes, 2007). According to Sangwan, et al. (2001) the volatile oils production depends on physiological, biochemical, metabolic and genetic aspects of the plant, and may suffer environmental and molecular modulations elucidating the chemical variations of volatile oils.

Due to the small size of the fruit, the amount of volatile oil obtained was low, a fact that represented a limitation of the study.

5. Conclusion

Anatomical studies, moisture content and total and insoluble ash in this study contribute to the quality control of plant raw material. Phytochemical screening is important to observe classes of molecules with possible biological activity. In this context qualitative presence of tannins, flavonoids, and saponins in the leaves were observed.

Leaves and fruits volatile oil's majority compounds were 2Z,6E-farnesol and α-copaene. This is the first report on anatomical study of the leaves, and chemical composition of volatile oils from *P. guineense* leaves and fruits collected in Hidrolândia, Goiás.

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