

Morphoanatomical and Histochemical Description of Vegetative Organs and Phytochemical Characterization of Leaf Extracts of *Plantago major*

Auygna Pamyda GOMES da SILVA¹, Cledson dos SANTOS MAGALHÃES², Marina Maria BARBOSA de OLIVEIRA³, Karina PERRELLI RANDAU^{4*}

¹Universidade Federal de Pernambuco, Departamento de Ciências Farmacêuticas, Laboratório de Farmacognosia, Pós-Graduação em Ciências Farmacêuticas, Av. Prof. Arthur de Sá, s/n, Cidade Universitária, 50740-521 Recife, Pernambuco, BRAZIL

²Universidade Federal de Pernambuco, Departamento de Ciências Farmacêuticas, Laboratório de Farmacognosia, Pós-Graduação em Inovação Terapêutica, Av. Prof. Arthur de Sá, s/n, Cidade Universitária, 50740-521 Recife, Pernambuco, BRAZIL

*Corresponding Author: karina.prandau@ufpe.br

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Abstract

Aim of study: To perform morphoanatomical, histochemical, and phytochemical analyses of *Plantago major*.

Area of study: Samples were collected in Muribeca, Jaboatão dos Guararapes, Pernambuco, Brazil.

Material and method: Root, stem, petiole, leaf blade, and floral scape were analyzed through macroscopic, anatomical, and histochemical techniques using optical microscopy. Methanolic leaf extracts were evaluated by thin-layer chromatography.

Main results: Anatomical analyses characterized the vegetative organs and identified structures such as hypodermis, collenchyma, sclerenchyma, and glandular and non-glandular trichomes. Histochemical and phytochemical analyses detected different metabolites.

Research highlights: The findings provide relevant morphoanatomical, histochemical, and phytochemical data for the pharmacobotanical characterization and standardization of *P. major*.

Keywords: Plantagenaceae, Microscopy, Morphoanatomy, Chemistry, Taxonomy

Plantago major Bitkisinin Vejetatif Organlarının Morfoanatomik ve Histokimyasal Tanımlaması ve Yaprak Özütlerinin Fitokimyasal Karakterizasyonu

Öz

Çalışmanın amacı: *Plantago major*'un morfoanatomik, histokimyasal ve fitokimyasal analizlerini gerçekleştirmek.

Çalışma alanı: Örnekler, Brezilya'nın Pernambuco eyaletine bağlı Jaboatão dos Guararapes kentinin Muribeca bölgesinden toplanmıştır. Belediyesi'ne bağlı Muribeca'dan toplanmıştır.

Materyal ve yöntem: Kök, gövde, yaprak sapı, yaprak ayası ve çiçek sapı; ışık mikroskobu kullanılarak makroskopik, anatomik ve histokimyasal tekniklerle analiz edilmiştir. Metanolik yaprak özütleri ince tabaka kromatografisi ile değerlendirilmiştir.

Temel sonuçlar: Anatomik analizler, vejetatif organların karakterizasyonunu sağlamış ve hipodermis, kollenkima, sklerenkima ile salgı yapan ve yapmayan tüyler gibi yapıları ortaya koymuştur. Histokimyasal ve fitokimyasal analizlerde farklı metabolitler tespit edilmiştir.

Araştırma vurguları: Bulgular, *P. major*'un farmakobotanik karakterizasyonu ve standardizasyonu için önemli morfoanatomik, histokimyasal ve fitokimyasal veriler sağlamaktadır.

Anahtar kelimeler: Plantagenaceae, Mikroskopi, Morfoanatomik, Kimya, Taksonomi



Introduction

The advent of industrialization, which is responsible for the production of highly processed food items, has spurred individuals to seek alterations in their dietary choices, specifically gravitating toward nutritious and healthful products derived from organic agriculture, with the goal of enhancing overall well-being (Santos et al., 2022).

Remarkably, there are approximately 390.000 plant species globally, and in Brazil alone, approximately 46.000 species are consumed to elevate dietary standards (RGB Kew, 2017). It is noteworthy that Brazil boasts an immense diversity of plant species, as elucidated by Kinupp and Barros (2007). However, despite this inherent potential, Brazil continues to rely on resources from other continents, thereby contributing to the devaluation of native species. Furthermore, a multitude of plant species remains either underexplored or entirely unknown to the botanical community (Brasil, 2020).

Within this context, it is pertinent to highlight the category of unconventional food plants (UFP). The categorization of a plant as conventional or unconventional varies depending on the geographical location, region, and dietary customs (Ranieri, 2017). It is worth emphasizing that certain UFPs form an integral part of various families' diets, while others remain relatively obscure but possess significant nutritional and pharmacological potential. These plants may harbor bioactive constituents that can either positively or negatively impact human health, potentially leading to toxicological effects. Hence, there is a pressing need to expand our understanding of the utilization of UFPs.

Of relevance in this study is the plant species *Plantago major* L., commonly known as "tanchagem." The circumscription of the genus *Plantago* L. has been broadened by the inclusion of the monotypic genus *Bouqueria* Decne (Rahn, 1996; Ronsted et al., 2002). This genus, belonging to the family Plantaginaceae Juss., has a cosmopolitan distribution, encompassing approximately 200 genera and 2500 species. In Brazil, there exist 18 native genera and approximately 120 species (Flora do Brasil, 2020), some of which serve as sources of food and medicine, among other applications.

In addition to its potential as a UFP, *P.*

major is widely employed in traditional medicine (Barreto and Spanholi, 2019) to address various ailments associated with the genitourinary system (Paiva et al., 2017). Its roots are employed to alleviate dental pain (Duke, 1989), while the leaves are used for their depurative, hemostatic, suppurative, diuretic (Brasil, 2014), and throat-soothing properties (Valeriano et al., 2020). Both the leaves and seeds are harnessed to combat infections (Xavier and Lima, 2020). Furthermore, this plant exhibits antibacterial, antipyretic, antiseptic (Brasil, 2014), anti-inflammatory, and antihemorrhagic properties (Valeriano et al., 2020).

In light of the above, it becomes evident that research aimed at elucidating the morphoanatomical and histochemical attributes of *P. major* is indispensable, given its significance as a potential food source and medicinal resource. Therefore, the primary objective of this study is to undertake a comprehensive characterization of the morphoanatomy and histochemistry of the vegetative organs, accompanied by a phytochemical analysis of leaf extracts from *P. major*. This endeavor contributes to the foundation for future investigations pertaining to the nutritional properties and culinary utilization of this remarkable species.

Material and Methods

Plant Material

Adult specimens of **Plantago major** L. were collected in Muribeca, in the municipality of Jaboatão dos Guararapes, Pernambuco, Brazil (geographic coordinates 8°6'43" S, 35°0'55" W). A herbarium specimen (No. 94321) was prepared and deposited at the Dárdano de Andrade Lima Herbarium, belonging to the Agronomic Institute of Pernambuco (IPA), for botanical identification and authentication.

Macroscopic Characterization

In the investigation of stem and leaf morphology, we scrutinized several characteristics, including phyllotaxy, blade morphology, venation patterns, indumentum, and petiole attributes. The nomenclature employed for describing leaf shapes and venation patterns adhered to that delineated by Hickey (1973). To perform macroscopic assessments, we availed ourselves of

standard measuring instruments such as a ruler, a caliper, and a Binocular Biotika® stereomicroscope, equipped with an integrated camera (Magalhães et al., 2024).

Anatomical Characterization

For anatomical characterization, the specimens were preserved in FAA50 [acetic acid, formaldehyde, and 50% ethyl alcohol (1:1:18 v/v)] (Johansen, 1940). Transverse sections of the root, leaf (petiole and leaf blade), and floral scape were manually obtained using steel blades, with the pith of *Cecropia* sp. petioles used as support. Paradermal sections of the leaf blade were prepared, encompassing both the adaxial and abaxial surfaces. Subsequently, the sections were cleared using a 50% sodium hypochlorite solution (Kraus and Arduin, 1997) and then rinsed with distilled water. The transverse sections were stained according to the technique described by Bukatsch (1972), using safranin and Astra blue. The paradermal sections were stained with methylene blue (Krauter, 1985). Finally, all sections were mounted on semipermanent slides following standard procedures for plant anatomy (Johansen, 1940; Sass, 1951).

Histochemical Characterization

Histochemical analyses were conducted using fresh cross-sections of the leaf blades, following the same sectioning procedures described for the anatomical characterization. A range of histochemical reagents was applied to identify specific classes of metabolites. Potassium dichromate (10%) was used to detect phenolic compounds (Johansen, 1940), while Dragendorff's reagent was employed for alkaloid detection (Yoder and Mahlberg, 1976). Tannins were identified using hydrochloric vanillin (Mace and Howell, 1974), and Sudan III was used to detect lipophilic substances (Sass, 1951). Antimony trichloride was applied for the detection of triterpenes and steroids (Mace et al., 1974). Lugol's solution was used to identify starch, and phloroglucinol was employed to detect lignin (Johansen, 1940).

Mucilage and acidic mucopolysaccharides were evaluated using a combination of 5% tannic acid and 3% ferric chloride (Pizzolato and Lillie, 1973). Ruthenium red (0.1%) was used for pectin detection (Johansen, 1940), whereas Xylidine Ponceau (0.1%) was applied to identify proteins (Vidal, 1970). Hydrochloric acid (10%) was used to investigate the nature of the crystals observed in the plant tissues (Johansen, 1940). For each histochemical assay, control sections without the respective reagent were processed in parallel. Following the reactions, semipermanent slides containing the cross-sections were prepared according to standard procedures (Johansen, 1940; Sass, 1951).

The analyses were performed from digital images obtained using a Leica DM 750M optical microscope coupled with a Leica ICC 50W digital camera. Image acquisition and subsequent processing were carried out using LAS EZ software.

Preliminary Phytochemical Screening

For the phytochemical characterization, the leaf material was analyzed by thin-layer chromatography (TLC) using methanolic extracts obtained by decoction. The analyses were carried out on commercially prepared silica gel plates (Merck), using appropriate mobile phases according to a protocol developed at the Pharmacognosy Laboratory of the Department of Pharmaceutical Sciences, Federal University of Pernambuco (UFPE). The methodology was based on the procedures described by Harborne (1998) and Wagner and Bladt (1996), with modifications proposed by Randau et al. (2004).

For each chromatographic analysis, 5 µL of the sample was applied to the silica gel plates. The chromatographic systems were established according to the metabolite class investigated, including the corresponding mobile phase, reference standard, and visualization reagent. The specific conditions used for the analysis are presented in Table 1.

Table 1. Chromatographic systems and developers used in preliminary phytochemical screening

Metabolites	Eluent system	Ratio	Developer	Evaluation criterion	Standard	Reference
Alkaloids	EtOAc ¹ : Formic acid: Acetic acid ³ :H ₂ O	100:11:11:27	Dragendorff	Visible	Atropine	Wagner & Bladt, 1996
Mono- and sesquiterpenes	Toluene ⁴ :EtOAc ¹	97:3	Sulfuric vanillin + Δ	Visible	Carvacrol	Wagner & Bladt, 1996
Triterpenes and steroids	Toluene ⁴ :EtOAc ¹	90:10	Liebermann–Burchard + Δ	Visible	β-sitosterol	Harborne, 1998
Anthraquinones	EtOAc ¹ :Methanol:H ₂ O	100:13.5:10	10% KOH	Visible and ultraviolet (365 nm)	Sennoside	Brazil, 2010
Coumarins	Toluene ⁴ : Ether ¹ saturated with 10% acetic acid ³	1:1	5% KOH	Ultraviolet (365 nm)	Coumarin	Neu, 1956; Wagner & Bladt, 1996
Phenylpropanoid glycosides	EtOAc ¹ : Formic acid: Acetic acid ³ :H ₂ O	100:11:11:27	NEU	Ultraviolet (365 nm)	Verbascoside	Neu, 1956; Wagner & Bladt, 1996
Cinnamic derivatives	EtOAc ¹ : Formic acid: Acetic acid ³ :H ₂ O	100:3:3:3	NEU	Ultraviolet (365 nm)	Chlorogenic acid	Neu, 1956; Wagner & Bladt, 1996
Flavonoids	EtOAc ¹ : Formic acid: Acetic acid ³ :H ₂ O	100:3:3:3	NEU	Ultraviolet (365 nm)	Rutin	Neu, 1956; Markham, 1982
Condensed tannins	EtOAc ¹ : Formic acid: Acetic acid ³ :H ₂ O	100:3:3:3	Hydrochloric vanillin	Visible	Catechin	Roberts et al., 1957
Hydrolyzable tannins	EtOAc ¹ : Formic acid: Acetic acid ³ :H ₂ O	100:3:3:3	NEU	Ultraviolet (365 nm)	Ellagic acid	Stiasny, 1912
Reducing sugars	EtOAc ¹ : Formic acid: Acetic acid ³ :H ₂ O	100:11:11:27	Thymol 0.5% in EtOH ⁵ + Δ	Visible	Glucose	Metz, 1961

A.Ac³: Acetic acid; Ac6: Acetone; AcOEt¹: Ethyl acetate; AF²: Formic acid; EtOH⁵: Ethanol; NEU: Ethylborylaminoester 1% in methyl alcohol; Tol⁴: Toluene

Results

Macroscopic Characterization

Plantago major L. is a herb characterized by an absence of a stem (Figure 1A). Its leaves exhibit an ovate or elliptical shape with an apex that can vary from acute to obtuse and a base that is obtuse or truncated (Figures 1B, 1C). The leaves possess a membranous to papyraceous texture and are supported by a thickened membranous petiole (Figures 1B, 1C). Notably, rounded glands are present on the leaf blade (Figure

1D). The venation pattern is anastomosed, with a basal acrodrome from which the main vein branches into secondary, tertiary, and quaternary veins (Figure 1E). The floral scape assumes a cylindrical form and is nearly equivalent in length to the spike (Figure 1F). The inflorescence is characterized by a dense axillary spike (Figure 1G). Additionally, the axial roots do not display any significant thickening (Figure 1H).

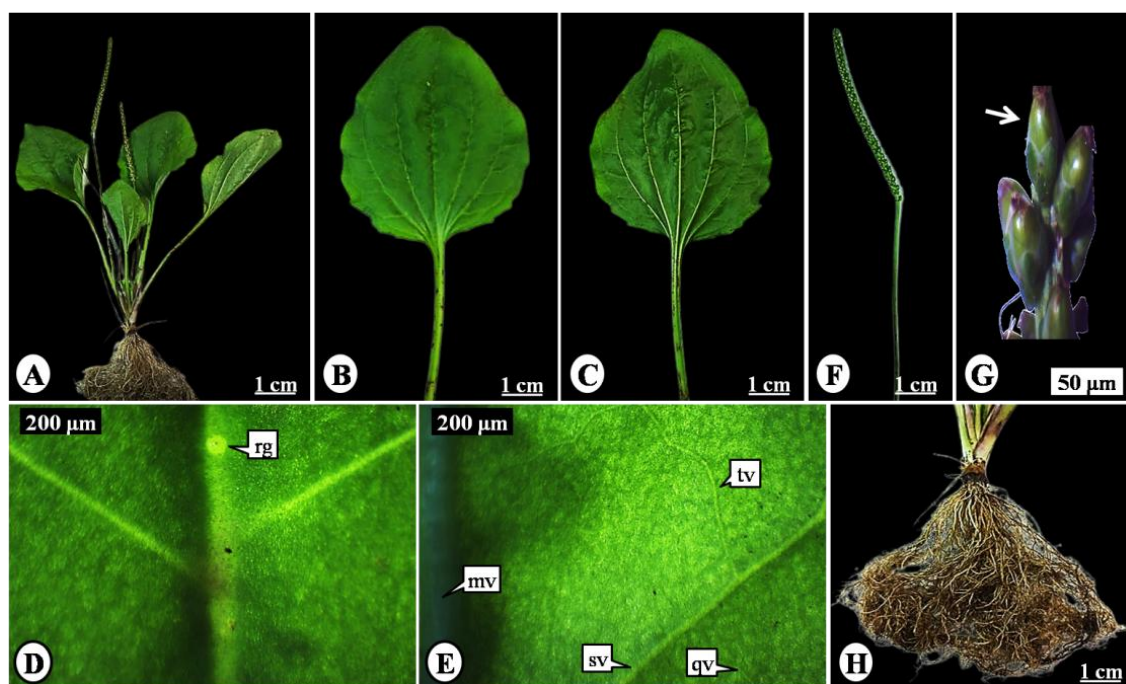


Figure 1. External morphology of *Plantago major* L., A: External general morphology of *P. major*; B-C: Detail of the leaf, adaxial and abaxial views; D: Detail of the gland on the adaxial face; rg: rounded glands; E: Details of ribs; mv: main vein; qv: quaternary vein; sv: secondary vein; tv: tertiary vein; F: Scape detail; G: Detail of the inflorescence; white arrow: inflorescence spikes; H: Root detail

Anatomical Characterization

Root

In the cross-section, the root of *P. major* in its primary growth stage displays a circular outline (Figure 2A). It is characterized by a thin cuticle covering the uniseriate epidermis,

followed by the hypodermis (Figure 2B). The cortical parenchyma fills the entire root (Figure 2B). The endodermis is followed by the pericycle (Figure 2C). The vascular cylinder is triarchic and presents a collateral-type arrangement. (Figure 2C).

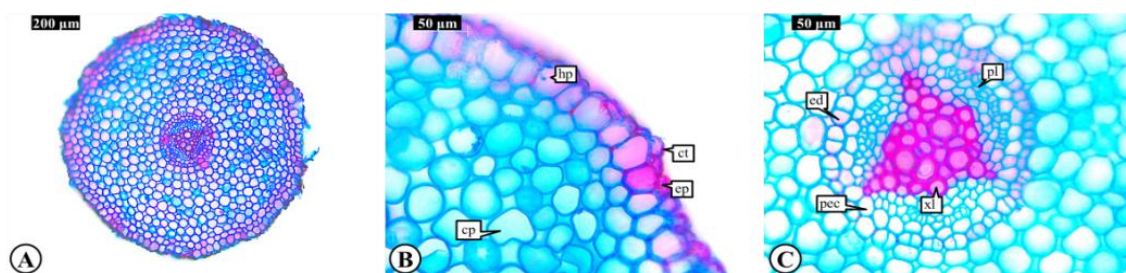


Figure 2. Root cross section of *Plantago major*. A: General appearance of the root; B-C: root details; cp: cortical parenchyma; ct: cuticle; ed: endoderm; ep: epidermis; hp: hypodermis; pec: pericycle; pl: phloem; xl: xylem

Petiole

The cross-section of the petiole in *P. major* exhibits a concave-convex shape with a pronounced elevation on the upper side (Figure 3A). A substantial cuticle covers the uniseriate epidermis (Figure 3B). Adjacent to the epidermis of the abaxial face, one to two layers of angular collenchyma are observed (Figure 3C), with three to five layers present in the prominence of the adaxial face (Figure 3D) and similar tissue located near the

vascular bundle (Figure 3E). The fundamental parenchyma occupies the entirety of the petiole (Figure 3B). Three collateral vascular bundles are present, with one being larger at the center and two smaller in the prominences (Figure 3E). Smaller vascular bundles are interspersed among the larger ones (Figure 3E). Stomata (Figure 3F) and non-glandular trichomes (Figure 3G) are also present.

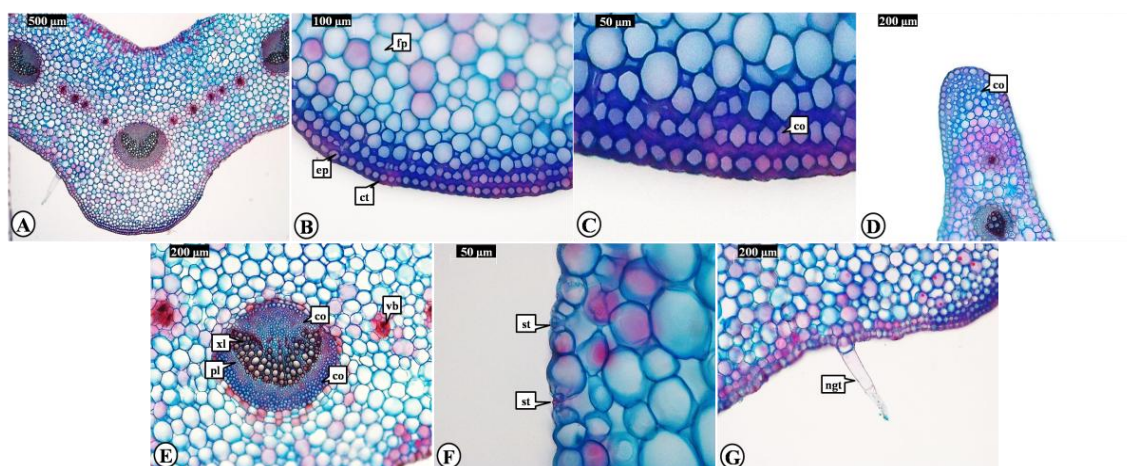


Figure 3. Cross-sections of the petiole of *Plantago major* L. A: General aspect of the petiole; B-G: Petiole details; co: collenchyma; ct: cuticle; ep: epidermis; fp: fundamental parenchyma; ngt: non-glandular trichome; pl: phloem; st: stoma; vb: vascular bundle; xl: xylem

Leaf

In paradermal sections, the leaf blade demonstrates epidermal cells with a slightly sinuous contour on both the adaxial and abaxial surfaces (Figures 4A, 4B). The leaf blade is classified as amphistomatic, featuring anisocytic stomata on both sides (Figures 4A, 4B).

In the cross-section, the midrib displays a concave-convex shape (Figure 4C). A thick cuticle covers the uniseriate epidermis (Figure 4D). Then, 1 layer of angular

collenchyma is observed (Figure 3D), as well as collenchyma close to the vascular bundle (Figure 4E). The fundamental parenchyma pervades the entire vein (Figure 4D). The vascular bundle follows a collateral arrangement (Figure 4E). Captured glandular trichomes (Figure 4F) and non-glandular trichomes (Figure 4G) are present in the epidermis. The mesophyll is dorsiventral with a layer of palisade parenchyma and layers of spongy parenchyma composed of about seven to eight cell layers (Figure 3H).

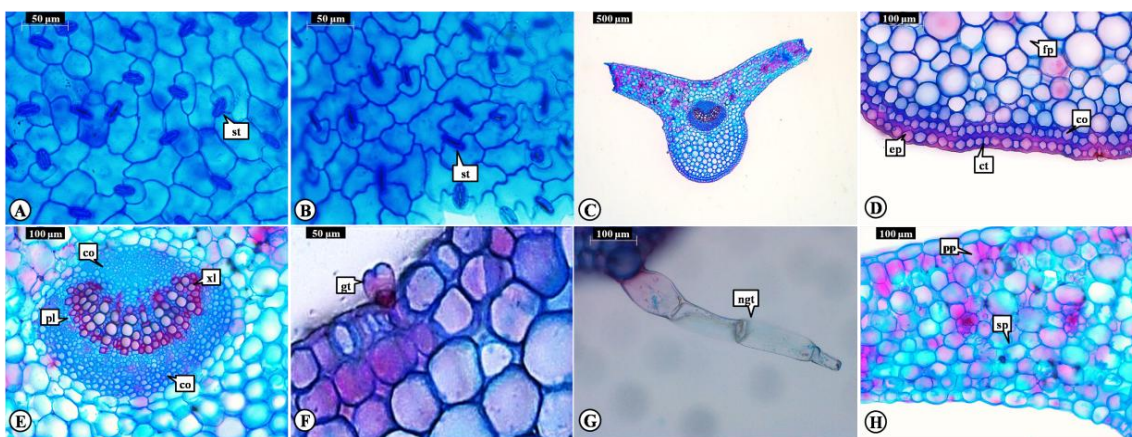


Figure 4. Paradermal and cross sections of the leaf blade of *Plantago major* L. A: adaxial face; B: abaxial face; C: general appearance of the central vein; D-G: details of the midrib; H: detail of the mesophyll; co: collenchyma; ct: cuticle; ep: epidermis; fp: fundamental parenchyma; gt: glandular trichome; ngt: non-glandular trichome; pl: phloem; pp: palisade parenchyma; sp: spongy parenchyma; st: stomata; xl: xylem

Floral Scape

In the cross-section, the floral scape assumes a circular shape (Figure 5A), with a thick cuticle enveloping the uniseriate epidermis (Figure 5B). Stomata are observed at the level of the epidermis (Figure 5B). Adjacent to the epidermis, a single layer of angular collenchyma is found (Figure 5B, 5C), followed by fundamental parenchyma

that fills the floral scape (Figure 5B). The vascular bundle follows a collateral arrangement, distributed in segments throughout the floral scape (Figure 5D). Sclerenchyma tissue forms a closed cylinder adjacent to the bundle in the central region of the scape (Figure 5D, 5E). Non-glandular trichomes are observed on the scape (Figure 5F).

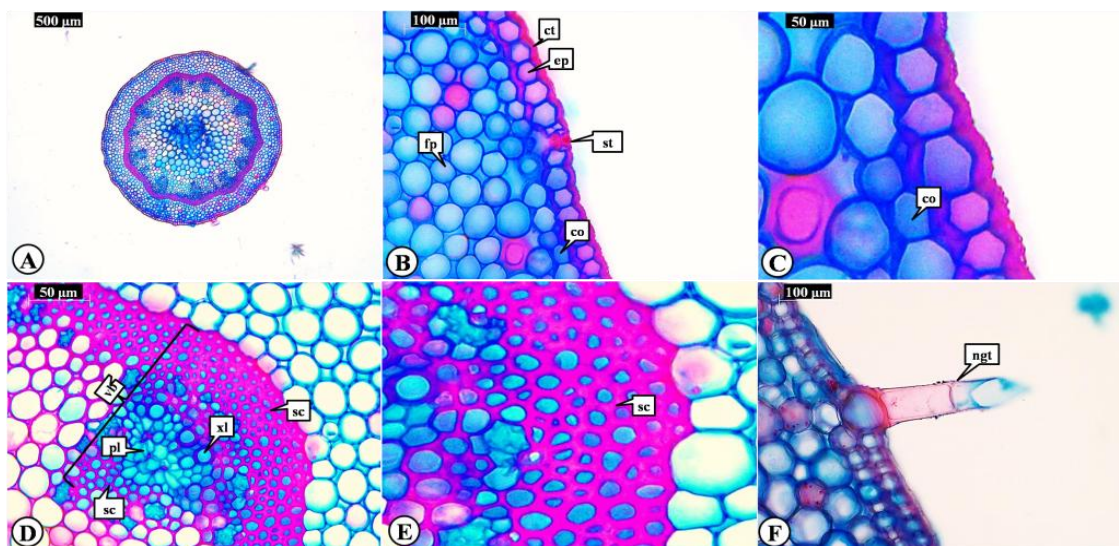


Figure 5. Cross sections of the floral scape of *Plantago major* L. A: general appearance of the scape; B-D: details of the floral scape; co: collenchyma; ct: cuticle; ep: epidermis; fp: fundamental parenchyma; ngt: non-glandular trichome; pl: phloem; sc: sclerenchyma; st: stoma; vb: vascular bundle; xl: xylem

Histochemical Characterization

Figures 6A to 6S illustrate the results of the histochemical characterization of the root and leaf blades of *P. major* in cross-sections. Figures 6A and 6I represent the root and leaf blade, respectively, without the addition of any reagent.

In the root, alkaloids were detected in the parenchyma (Figure 6B), phenolic compounds in the hypodermis (Figure 6C), lipophilic compounds in the cuticle and hypodermis (Figure 6D), and lignin in the xylem (Figure 6E). Mucilages were identified in the parenchyma (Figure 6F), pectins in both the parenchyma and cell wall (Figure 6G), and proteins in the hypodermis and parenchyma (Figure 6H). Tests for

starch, tannins, triterpenes, and steroids yielded negative results for the roots.

In the leaf blade, alkaloids were found in the epidermis (Figure 6J), starch in the parenchyma (Figure 6K), phenolic compounds in the epidermis (Figure 6L) and collenchyma (Figure 6M), lipophilic compounds in the cuticle (Figure 6N), and lignin in the xylem (Figure 6O). Mucilages were observed in the epidermis and parenchyma (Figure 6P), pectins in the epidermis, parenchyma, and cell wall (Figures 6Q, 6R), and proteins in the epidermis, collenchyma, and parenchyma (Figure 6S). Tests for starch, tannins, triterpenes, and steroids were negative for leaf blades.

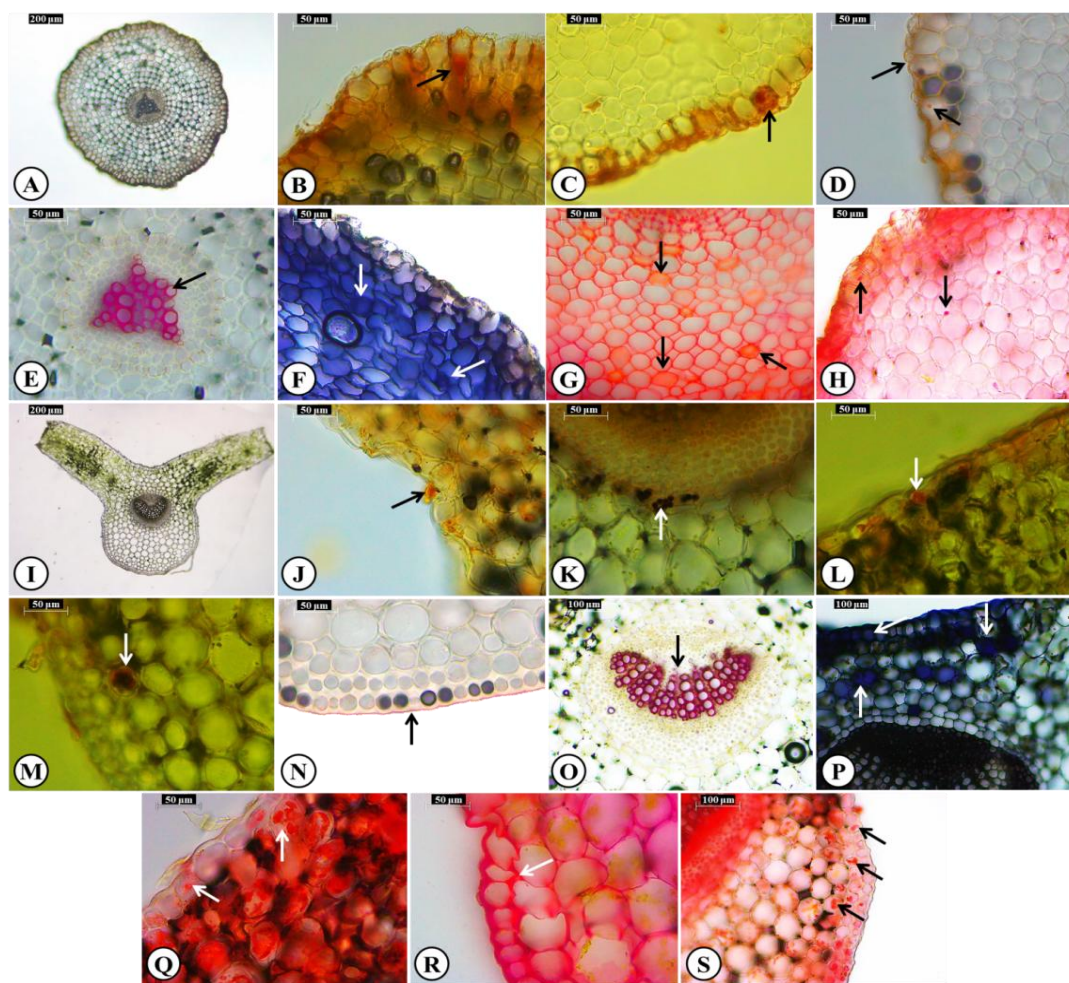


Figure 6. Histochemistry of the root and leaf blade of *Plantago major*. A-H: Root; I-S: Leaf blade; A, I: Control; B, J: Alkaloids; C, L-M: Phenolic compounds; D, N: Lipophilic compounds; E, O: Lignin; F, P: Mucilages; G, Q-R: Pectins; H, S: Proteins; K: Starch. Arrows: Indicate the location of primary and secondary metabolites in tissues

Preliminary Phytochemical Screening

Phytochemical screening revealed metabolites in the methanolic extract of *P. major*, as shown in Table 2, which may include bioactive compounds with potential pharmacological activities.

Table 2. Phytochemical prospection of *P. major*

Metabolites	<i>P. major</i>
Mono and sesquiterpenes	++
Triterpenes and steroids	+++
Alkaloids	-
Flavonoids	+
Cinnamic derivatives	-
Phenylpropanoglycosides	-
Hydrolyzed tannins	-
Anthraquinone	-
Saponins	+
Condensed tannin	+
Hydrolyzable tannin	-

-: absence of bands; +: up to two highlighted bands; ++: two to five bands highlighted; +++: above five bands highlighted.

Discussion

Data pertaining to the anatomical characteristics of species within the genus *Plantago* L., as well as the broader Plantaginaceae family, are notably scarce in the existing literature. Remarkably, there exists only one study concerning *Plantago tomentosa* Lam., a species of relevance to the current investigation. Consequently, there is a conspicuous dearth of anatomical information regarding *Littorella* P.J. Bergius (Hoggard et al., 2003) and *Aragoa* Kunth (Bello et al., 2002), which are closely related genera that constitute the tribe Plantagineae (Albach et al., 2005). Regrettably, similar research endeavors are conspicuously absent. Additionally, our study combined histochemical data with phytochemical analyses, thereby highlighting the need for further investigations into *Plantago* species, as well as related genera and other

constituents of the Plantaginaceae family.

Procópio et al. (2003), in their description of leaf anatomy in *P. tomentosa*, observed amphistomatic leaves characterized by anomocytic stomata present on both the adaxial and abaxial surfaces. The authors also noted the presence of a simple epidermis in this species. The adaxial surface exhibited greater thickness compared to the abaxial surface, with a cuticle adorned by epicuticular wax plaques. Furthermore, the mesophyll was described as homogeneous.

Based on the anatomical results described by Mesquita et al. (2017), it is observed that *P. major*, *Plantago australis* L., *Plantago lanceolata* L., and *Plantago catharinea* L. share a substantial set of leaf characteristics, such as amphistomatic leaves with anomocytic stomata, the presence of non-glandular and glandular trichomes, and a well-defined midrib with a distinct vascular bundle, which explains the high anatomical similarity among these species. However, relevant diagnostic differences were identified. *P. lanceolata* stood out as the most distinct species, being the only one to exhibit an isolateral mesophyll, a collateral vascular bundle, and exclusive glandular trichomes with spindle-shaped glandular cells, in addition to greater height and diameter of the midrib and vascular bundle. *P. major* presented an exclusive glandular trichome characterized by an enlarged basal cell and a collar cell, whereas *P. catharinea* showed a higher density of glandular trichomes on the abaxial surface and increased stomatal density, particularly associated with its adaptation to coastal environments. *P. australis*, in turn, displayed an intermediate anatomical pattern, without marked exclusive structures.

These findings indicate that, although *Plantago* species are often confused due to external morphological similarity, the anatomy of the root, petiole, and leaf blade provides consistent characters for taxonomic and diagnostic differentiation. Variations in mesophyll type, vascular bundle organization, and trichome morphology reflect ecological adaptations and genetic differences among the species, even when cultivated under the same environmental conditions. These data highlight the importance of detailed anatomical analysis as a complementary tool for botanical

identification, especially in the medicinal use of the genus *Plantago*, where improper species substitution may compromise therapeutic efficacy and phytotherapeutic safety.

It is pertinent to acknowledge that anatomical investigations pertaining to floral scapes are considerably fewer in number when compared to those focusing on leaf blades. Nevertheless, it is imperative to underscore that the morphoanatomical attributes of floral scapes hold substantial importance in taxonomic studies across various hierarchical levels (Scatena et al., 1998).

Regarding histochemical data, Silva Neto et al. (2023), during a preliminary phytochemical survey, reported the presence of saponins, triterpenoids, and steroids in the hexane leaf extract, while the ethanolic leaf extract exhibited the presence of flavonoids, steroids, and saponins. In phytochemical tests conducted by Trindade et al. (2019), alkaloids, flavonoids, saponins, and tannins were identified in leaf extracts of *P. major*. Additionally, Nogueira et al. (2021) conducted a chemical screening of ethanolic and hydroalcoholic extracts of *P. major* shoots, revealing the presence of triterpenes, coumarins, phenols, tannins, and flavonoids among the phytoconstituents.

Conclusion

This study provides valuable insights into the morphoanatomical and histochemical parameters essential for the diagnosis of *Plantago major*. It is important to note that similar data are conspicuously scarce for other members of the *Plantago* genus and the broader Plantaginaceae family. This scarcity underscores the pressing need for further anatomical, histochemical, and comprehensive investigations, given the potential inherent in this plant species.

In sum, this study not only contributes significantly to the pharmacobotanical standardization of *P. major* but also furnishes crucial data that are pertinent to both medicinal and culinary applications, as well as taxonomic investigations.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: Auygna P.G.S., Cledson S.M., and Karina P.R.; Investigation: Auygna P.G.S. and Cledson S.M.; Materials and Methodology: Auygna P.G.S. and Cledson S.M.; Visualization: Auygna P.G.S., Cledson S.M., and Marina M.B.O.; Writing – Original Draft: Auygna P.G.S. and Cledson S.M.; Writing – Review and Editing: Cledson S.M., Marina M.B.O., and Karina P.R. The author has read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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