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Original Article

Macro-Microscopical and Histochemical Analysis of *Justicia tranquebariensis* L. (*Acanthaceae*): A Woody Undershrub

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ABSTRACT

Background: Medicinal plants are a crucial source of therapeutic agents for treating various human ailments. *Justicia tranquebariensis*, a small woody shrub widely distributed in southern India is traditionally used for managing conditions such as respiratory problems, kidney infections, gastrointestinal diseases, anemia and more. Despite its extensive use in traditional medicine, detailed pharmacognostic studies on this plant are limited. **Materials and Methods:** Sections were prepared using a rotary microtome and histochemical analysis was conducted following standard protocols. **Results:** The macroscopical, microscopical and histochemical studies revealed the occurrence of key phytochemicals such as carbohydrates, proteins, lipids, alkaloids, flavonoids, tannins, phenols, starch and saponins. These findings contribute to the understanding of the plant's medicinal properties and provide a foundation for future pharmacological research.

Keywords: *Justicia tranquebariensis*, Macroscopical study, Microscopical study, Histochemical analysis.

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INTRODUCTION

Medicinal plants are an exclusive gift from nature to mankind as they are the sources of important therapeutic aids for alleviating human ailments.[1] After years of fascination with modern medicine, people have increasingly embraced the "back to nature" concept due to the undesirable side effects associated with pharmaceutical treatments. Therefore, validating the microscopic characteristics of plants will aid in their standardization and accurate identification. Demonstrating the microscopical characteristics of the plant contributes to the standardization and precise identification of plant samples.[2] Despite contemporary methods, pharmacognostical research remains a more dependable method for the correct identification of the plant.[3] Standardization is a vital step in the quality assurance program for producing herbal drugs.[4] Macroscopic and microscopic examinations are among the simplest and most cost-effective techniques for accurately identifying the source material.[5] The family *Acanthaceae* has a pantropical distribution with approximately 240 genera and 3250 species.[6] The genus *Justicia*, which includes around 600 species found in tropical and temperate regions worldwide, has been traditionally utilized in herbal medicine for the treatment of fever,[7] pain,[8] inflammation,[9] diabetes[10] and liver diseases.[11]

Justicia tranquebariensis Linn. (Synonym: *Justicia glauca* Rottl., *Justicia parvifolia* Lam., *Adhatoda tranquebariensis* Nees.) is a small subshrub which is widely distributed in all districts of Peninsular India and Sri Lanka. The Common name: Tarangambadi justicia; Tamil name: Punnaku Poodu, Thavasi murungai, Sivanarvembu; Sanskrit: Pindi; Telugu: Chikerachettu; Kannada: Kaddiyarakina gida (Http://Zipcodezoo.Com/Plants/J/Justicia, 2009).[12] In the traditional system of medicine this plant is claimed to be useful in treating common colds, coughs, as an expectorant and also finds use in the treatment of smallpox in children and contusions.[13] Root paste is applied for toothache,[14] leaf juice serves as a cooling agent and aperients and is also given to children with smallpox,[13] to treat jaundice[15] and bronchial asthma.[16] A leaf paste is applied to the affected area to treat skin diseases,[15] applied externally to reduce swelling and alleviate pain (Sandhya *et al.*, 2006)[14] and as an antidote for cobra bite.[17] This plant is also reported to possess anti-arthritic activity and anti-inflammatory[18] cardioprotection[19] and is also used to treat gastric ulcers.[20] Despite these extensive medicinal benefits, the perusal of published literature revealed that pharmacognostic studies on the aerial parts of *Justicia tranquebariensis* Linn. remain limited. Therefore, our study focused on microscopical and histochemical localization of phytochemicals in *Justicia tranquebariensis* which provides the foundation for pharmacological research and aids

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in understanding the presence of phytochemicals that may contribute to its medicinal properties.

MATERIALS AND METHODS

Collection and authentication of the plant

The stem and leaves of *Justicia tranquebariensis* L. were collected from Thindal, Erode district, Tamil Nadu, India and subsequently authenticated and deposited at the PG and Research Department of Botany, Vellalar College for Women in Erode, where a herbarium specimen has been archived for future reference. The plant materials were dried separately in the shade, then stored in a closed container for later use and ground into a powder utilizing a mechanical grinder (Plate 1).

Macroscopical Studies

Macroscopic characters are simple determination techniques that comprise organoleptic studies like shape, size, color, odor and its additional features. The various plant parts (stem and leaves) of *Justicia tranquebariensis* L. (*Acanthaceae*) were analyzed in studied in their natural habitat, photographed on-site and evaluated botanically.[21]

Microscopical Studies

Preparation of Specimens

Healthy aerial plant sections of *Justicia tranquebariensis* were carefully chosen. Fresh samples of various sections (leaves and stems) were cut into small pieces and preserved in FAA (5 mL of farmalin 5 mL of acetic acid and 90 mL of 70% ethanol). Following a 24 hr fixing period, the samples underwent dehydration using a graded sequence of Tertiary Butyl Alcohol (TBA) in accordance with the timetable provided by Sass (1940).[22] Paraffin wax (melting point (58-60°C) was added slowly to the specimens until the TBA solution reached super saturation. The samples were poured into blocks of paraffin.

Sectioning

A Rotary Microtome was utilized to section the specimens fixed in paraffin, yielding sections with a thickness of 10-12 µm. The sections were de-waxed following the established protocol (Johansen, 1940) and subsequently stained with Toluidine blue according to the method described by O'Brian *et al.* (1964).[24]

Staining

For anatomical studies, a staining protocol was established in which Toluidine blue stain was prepared by dissolving 0.25 g of the dye in a mixture containing 0.25 g of benzoic acid, 0.29 g of sodium benzoate and 200 mL of distilled water, achieving a pH of 4.2-4.4. As a polychromatic stain, Toluidine blue provided exceptional staining results, revealing distinct cytochemical reactions: cellulose walls appeared pink, lignified cells stained blue, suberin took on a dark green hue, mucilage was colored violet and protein bodies appeared blue. When necessary, additional sections were stained with safranin, Fast-green and IKI (Lugol's iodine), which is a brown solution that turns black in the presence of starch. To investigate stomatal morphology, venation patterns and trichome distribution, paradermal sections (taken parallel to the leaf surface) were prepared, alongside leaf clearing with 5% sodium hydroxide or epidermal peeling through partial maceration using Jeffrey's maceration fluid (Sass, 1940). Glycerin-mounted temporary preparations were created for the macerated materials. Following staining, powdered components from various tissues were cleaned with NaOH and mounted in a glycerin medium, with various cell components being measured and examined.

Photomicrographs

Following staining, all permanent slides were dehydrated using a graduated sequence of Xylol+Ethanol and then mounted in DPX. Where applicable, micrographs were included to complement the microscopic descriptions of the tissues. Nikon Lab Photo-2 Microscope was used to capture images at various magnifications on Konica color film (100ASA). Observations were made using bright-field microscopy, while polarized light was employed to examine crystals, starch grains and lignified cells, as these structures exhibit birefringent and appear bright against dark backgrounds. The scale bars show the figures' magnifications. The conventional anatomy manuals (Esau, 1964)[25] have descriptive labels for the anatomical features.

Histochemical Studies

Using conventional sectioning and staining techniques, microscopical characterization was performed in accordance with the usual process outlined by O'Brien *et al.* (1964).[24] Using the appropriate chemicals, the stem and leaf of the research plant were histochemically examined to determine the cell configurations, size, shape, inclusions, synthesis and distribution. The results were captured on a photonic microscope (Model Ax70 TRF, Olympus optical).

Fresh hand sections of *J. tranquebariensis* (stem and leaves) were subjected to histochemical analysis using specific reagents to identify the presence or absence of metabolites, synthesis and their storage area. The presence of these metabolites was confirmed through the color changes observed due to the interaction between the cells and the reagents.[26,27]

RESULTS

Macroscopical Studies

Justicia tranquebariensis L. (*Acanthaceae*) is a woody undershrub which can grow up to 70cm height, stem is whitish grey, glaucous, pubescent with many stiff branches, long petiole, leaves are simple, opposite, decussate, ovate-orbicular, cuneate at base, entire, ciliate at margin, obtuse-rounded at apex, membranous

and pubescent. Inflorescence is a spike, terminal or axillary, rarely solitary; flowers sessile or sub-sessile, white with pinkish-purple striate; bracts ovate-orbicular, minutely pubescent; bracteoles usually narrow. 5 Calyx, narrow; corolla 2-lobed, tube cylindrical, white pubescent upper lip with a purple spot and a lower lip that is 3-lobed and pink-striated. 2 Stamens, filaments often dilated: anthers 2-celled, purple striate. Ovary is ellipsoid, 2-loculed with 4 ovules and style slender, stigma shortly bifid with minutely apiculate at apex. Fruit is a long capsule, solid at base or not, often papery. Seeds are 4, oblong, more or less compressed, tuberculate, echinate. Flowering and fruiting throughout the year[21] Table 1 and Plate 1.

Microscopic Studies

Microscopic evaluation permits a more detailed investigation of a drug, facilitating its identification based on the known histological characteristics associated with its plant origin. The histological studies were made from very thin sections of stem and leaf of *Justicia tranquebariensis* L. The plant parts showed the following anatomical features:

Stem

The stem is circular and 2.2 mm thick, (Plate 2). The outer most part consists of crushed epidermal cells of periderm is 50 µm thick, often bears glandular trichomes and followed by a cortex is differentiated into outer collenchyma cells and inner parenchyma

Table 1: Macroscopic evaluation of aerial plant parts of *Justicia tranquebariensis* L.

Sl. No.	Features	Observations		
		Stem	Petiole	Leaf
1.	Condition	Fresh	Fresh	Fresh
2.	Colour	Brownish Green	Light Green	Green
3.	Odour	Characteristic smell	Characteristic smell	Characteristic smell
4.	Taste	Bitter	Bitter	Bitter
5.	Size	10 cm length and 0.3 cm width.	5 cm length and 0.1 cm width.	2.8 cm length and 2 cm width.
6.	Shape	Cylinder	Cylinder	Obovate
7.	Texture	Rough	Rough	Rough

Plate 1: Habit-*Justicia tranquebariensis* L. Aerial parts of plant powder.

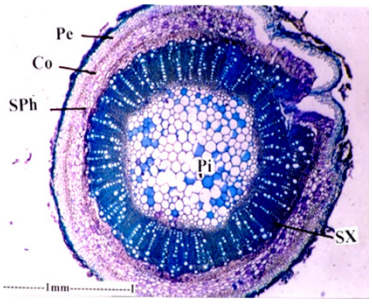

Plate 2: T.S. stem of *Justicia tranquebariensis*.

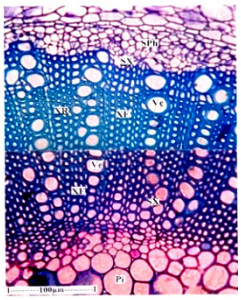

Plate 3: Enlarged portion of secondary xylem and secondary phloem. Tr-Trichome, Pe-Peridermis, Co-Cortex, SPh-Secondary Phloem, SX-Secondary Xylem, Pi- Pith.

cells. The vascular cylinder is composed of an outer thin layer of secondary phloem, which features a continuous arrangement of sieve elements and parenchyma cells. The secondary xylem, measuring 150 µm in thickness, contains vessels, xylem fibers and xylem rays. The vessels are large radial multiples of narrow thick-walled circular cells with 20 µm wide, xylem fibres are narrow cells with very thick lignified walls, xylem rays are less prominent, thin radial lines of narrow radically elongated cells (Plate 3).

Petiole

It is semicircular in outline with flat adaxial surface, 920 µm in vertical plane and 2.1 µm in horizontal plane and thin continuous, a single layer of elongated epidermal cells, followed by a layer of ground tissue composed of thin-walled, circular and closely packed homogeneous parenchyma cells. A singular, wide, arc-shaped vascular bundle is present, which is conjoint, collateral and closed. This bundle features several closely arranged parallel lines of angular, thick-walled and wide xylem elements, with a narrow band of phloem positioned beneath the xylem. Surrounding the entire vascular bundle is a thick sclerenchyma sheath. Additionally, there are two small circular accessory bundles, located on either side of the main vascular bundle. The accessory bundles are also collateral, comprising of a cluster of xylem and thin lateral phloem (Plate 4).

Leaf

The leaf is dorsiventral, thick midrib; conical adaxial prominent slightly curved pointed beak is 100 µm in height. The abaxial part of the midrib is wide and 450 µm in thick, semicircular shape is 270 µm in horizontal plane (Plate 5.1). The lamina is smooth on both surfaces and thick. The epidermal cells exhibit a spindle shape, while the adaxial beaks are small and thick-walled. Inside the beak, there is a small cluster of collenchyma cells. The palisade cells of the lamina extend and become horizontally transcurrent towards the adaxial beak region. The remaining ground tissue consists of small, angular, compact and thin-walled parenchyma cells. In the midrib region, there is a single large vascular bundle that is broadly conical and collateral, with the xylem elements located on the adaxial side and the phloem elements on the abaxial side (Plate 5.2). The xylem comprises vessels and fibers, with the vessels being circular, thick-walled and arranged in vertical compact lines. Beneath the xylem strand occur small discrete units of phloem. The entire vascular strands are surrounded by a thick sclerenchyma sheath.

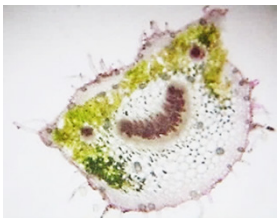

Plate 4: T.S. of petiole of *Justicia tranquebariensis.*

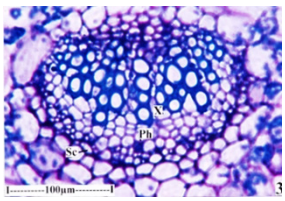

Plate 5.2: Enlarged Vascular bundle-Midrib Region. AdB-Adaxial Beak, GP-Ground parenchyma, MR-Midrib, VB-Vascular Bundle, La-Lamina Sc-Sclerenchyma, X-Xylem, Ph-Pholem.

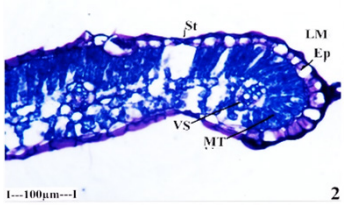

Plate 6.2: T.S of Leaf margin.

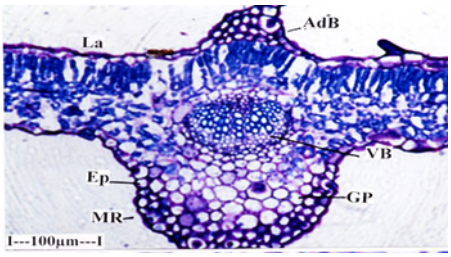

Plate 5.1: T.S. of Leaf-Entire view.

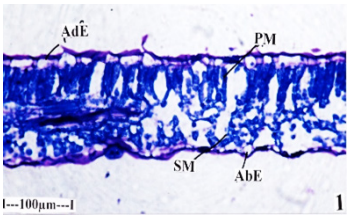

Plate 6.1: T.S. of lamina of the leaf.

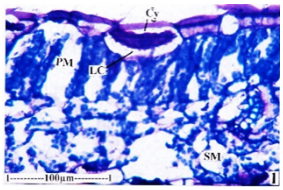

Plate 6.3: The epidermal cell showing cystoliths. AbE-Abaxial Epidermis, AdE-Adaxial epidermis, St-Stomata, LM-Leaf Margin, PM-Palisade Mesophyll, SM-Spongy Mesophyll, VS-Vascular Strand, Cy- Cystolith, Lc –Lithocyst.

Table 2: Histochemical analysis of aerial parts of *Justicia tranquebariensis* L.

Sl. No.	Reagents	Colouration	Phytochemicals	Tissue location		
				Stem	Petiole	Leaf
1.	Fehlings's	Pink	Carbohydrate	Cortex, xylem, pith region.	Epidermis, collenchyma, ground tissue, xylem, pith.	Epidermis, collenchyma, ground tissue, xylem, phloem, clerenchyma sheath.
2.	Biuret	Yellowish brown	Protein	Cortex, xylem, pith region.	Epidermis, collenchyma, ground tissue, phloem, xylem, pith region, sclerenchyma sheath.	Epidermis, collenchyma, few ground tissue, xylem, phloem, sclerenchyma sheath.
3.	Sudan	Yellow	Lipid	Cortex, pith region.	Epidermis, collenchyma, ground tissue, phloem, sclerenchyma sheath.	Absent
4.	Wagner's	Orange brown	Aalkaloids	Epidermis, xylem region.	Epidermis, ground tissue, xylem, sclerenchyma sheath.	Epidermis, collenchyma cells, xylem, sclerenchyma sheath.
5.	10% Lead acetate	Magenta	Flavonoid	Epidermis, cortex, xylem, pith region.	Epidermis, xylem region.	Epidermis, collenchyma, ground tissue, xylem, phloem, sclerenchyma sheath.
6.	10% Ferric chloride	Black	Tannin	Epidermis, xylem, few pith cells.	Xylem, ground tissue.	Xylem, mesophyll tissue.
7.	Anhydrous Ferric chloride	Brown to black	Phenol	Epidermis, cortex, phloem, xylem.	Epidermis, xylem, phloem, sclerenchyma sheath.	Epidermis, xylem, sclerenchyma sheath.
8.	Lugol's iodine solution	Dark brown to pink	Starch	Epidermis, xylem, pith region.	Epidermis, xylem region.	Epidermis, collenchyma, ground tissue, xylem, sclerenchyma sheath.
9.	Conc. H_2SO_4	Bluish black	Saponin	Parenchyma cortex, xylem, pith.	Ground tissue, mesophyll tissue.	Xylem region.

Lamina

The lamina is 160 µm thick. The mesophyll tissues consist of a single layer of fairly thick cylindrical palisade parenchyma cells on adaxial part and spongy parenchyma cells are lobed, loosely arranged and interconnected in abaxial part. (Plate 6.1). The marginal part of the lamina is slightly bulged and bent down. The mesophyll tissues of the marginal part are different from that of the lamina. In the leaf margin the palisade cells are thicker'; the cells are elongated more in vertical plane. The cuticle is more prominent and a small vascular bundle is located at the submarginal part (Plate 6.2). Cystolith is calcium carbonate crystals (60 µm long and 12 µm thick) are widely distributed in all parts of plant organ and more common in the leaf epidermal cells. The cystoliths in the epidermal cells are elongated cylindrical bodies, located horizontally inside wide dilated specialized cells called lithocyst (Plate 6.3).

The surface view reveals the epidermal peelings, which consist of thick-walled, wavy and sinuous epidermal cells. The stomata are of the diacytic type, with each stoma encircled by a pair of subsidiary cells whose common wall is oriented at right angles to the guard cells. Glandular trichomes are frequently seen especially on the adaxial surface of lamina. It consists of a wide circular basal stalk cell which is buried in the epidermal layer. The upper part of the gland has a multicellular globose secretary head and 30 µm in height; the secretary body is 30µm wide. The gland is partly buried in the lamina. Non-Glandular trichomes are elongated and uniseriate structures that consist of multiple cells, typically five and are present on both surfaces of the epidermis (Plate 7).

Histochemical Studies

Histochemical analysis is a crucial standardization method for assessing the quality of crude drugs, particularly for detecting

Plate 7: T.S of Lamina with glandular Trichome. Ep-Epidermis, St-Stomata, NGTr-Non-Glandular trichome, GTr-Glandular Trichome, Sc-Sclerenchyma. PM -Palisade Mesophyll.

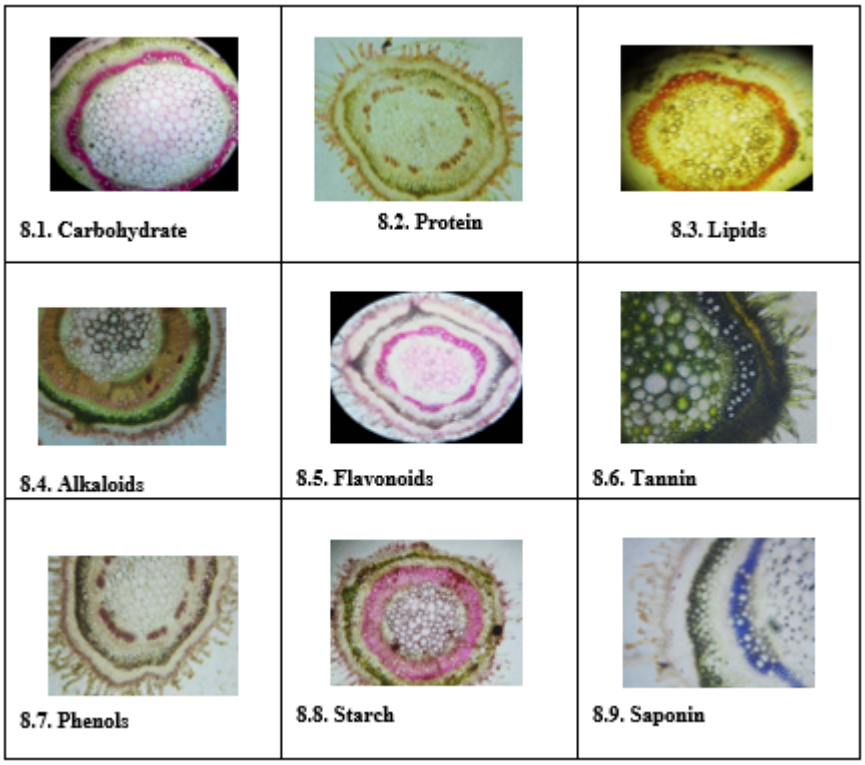

Plate 8: T.S. Stem of *Justicia tranquebariensis*.

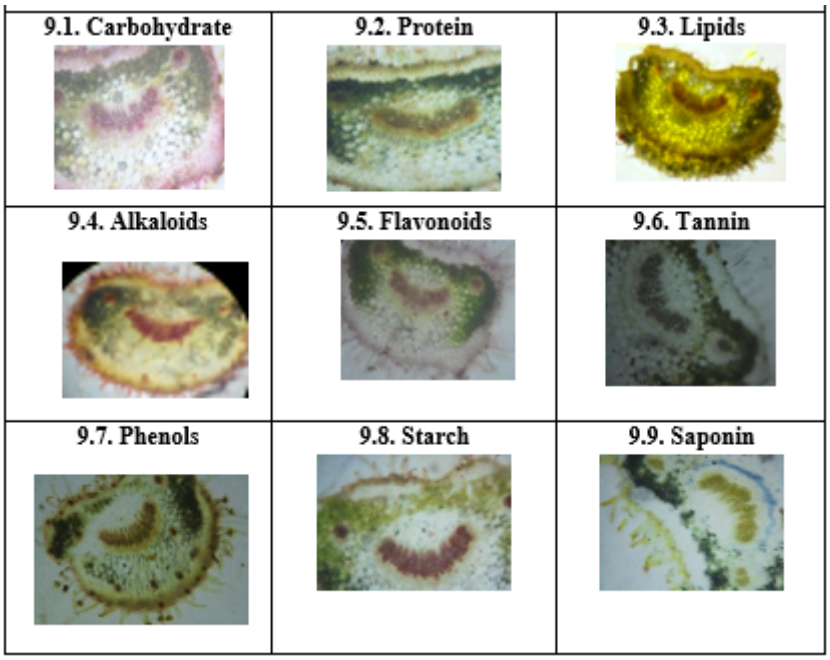

Plate 9: T.S. Petiole of *Justicia tranquebariensis*.

elastic cell contents within the plant's histological zones. Defined as the study of the identification and distribution of chemical compounds within and between the microstructures of biological cells, histochemistry employs histological techniques such as staining, indicators and microscopy. It has been utilized to identify various groups of phytochemicals localized in different tissue zones of plant parts. The findings from this research indicated the presence of carbohydrates, proteins, lipids, alkaloids, flavonoids, tannins, phenols, starch and saponins in various tissue zones of the aerial parts of *Justicia tranquebariensis*, as summarized in Table 2 and illustrated in Plates 8, 9 and 10. This analysis underscores that vascular bundles and the cortical zone serve as primary sites for the synthesis or storage of diverse phytochemical groups.

DISCUSSION

Pharmacognosy is a straightforward and dependable method for obtaining comprehensive information about crude drugs.[28-30] Pharmacognostic investigations, which aid in the identification and verification of plant materials and guarantee their quality, efficacy and safety, can be used to gradually standardize the procedure.[31,32] As a result, there has been a rapid increase in the standardization of several selected medicinal plants that hold potential therapeutic significance in recent years. Plant anatomy has long been thought to provide very reliable recommendations for diagnosing fragmented plant.[33,34] The anatomical features may be useful for specific delimitation in family *Acanthaceae*, once morphological boundaries are still unclear for genera circumscription. In the present study, a comprehensive analysis of the anatomical features of *Justicia tranquebariensis* was conducted.

In the present study, the stem showed a circular outline with well-defined zones of periderm, cortical layer, secondary phloem and secondary xylem. The secondary phloem consists of a continuous layer of parenchyma cells and sieve elements, while the xylem is composed of vessels, fibres and rays. This is in par with Patil *et al.* (2024)[35] in *Barleria* species of *Acanthaceae*. In the present findings the petiole showed two small circular accessory bundles placed on either side of the main vascular bundle. This arrangement is similar in other *Acanthaceae* species as reported by Patil and Patil (2012).[36] Similar vascular arrangements have been reported by Mohd. Tajudin *et al.* (2022)[37] in *Justicia gendarussa, Justicia procumbens* and *Justicia adathoda* and Verdam *et al.* (2012)[38] in *Justicia acuminatissima.* The present findings exhibited sclerenchyma cells encircling the phloem tissue of both the main and accessory vascular bundles in the petiole and midrib. This anatomical feature was similarly noted in three species of *Justicia* by Mohd.Tajudin *et al.* (2022).[37]

Certain plant species and families can be identified using the various forms and types of cystolith cells.[39] Various parts of the plant, especially the leaves, contain cystolith cells in addition to xylem and phloem rays.[40] Studies by Nurul-Aini *et al.* (2018)[41] and Maisarah *et al.* (2020)[39] the variation of cystolith cells can be used to distinguish between *Acanthaceae* species. The presence of cystoliths in the vegetative parts of *Acanthaceae* species is widely documented by numerous researchers.[39-41] In *Justicia*, cystoliths are commonly found in the epidermal layers of leaves and sepals.[42] Variations in their shapes and sizes have been employed to establish taxonomic distinctions either on their own or alongside other internal or external structures of these plants.[43] The results showed the presence of elongated cylindrical cystoliths in the epidermal cells. This finding is reinforced by Pierantoni *et*

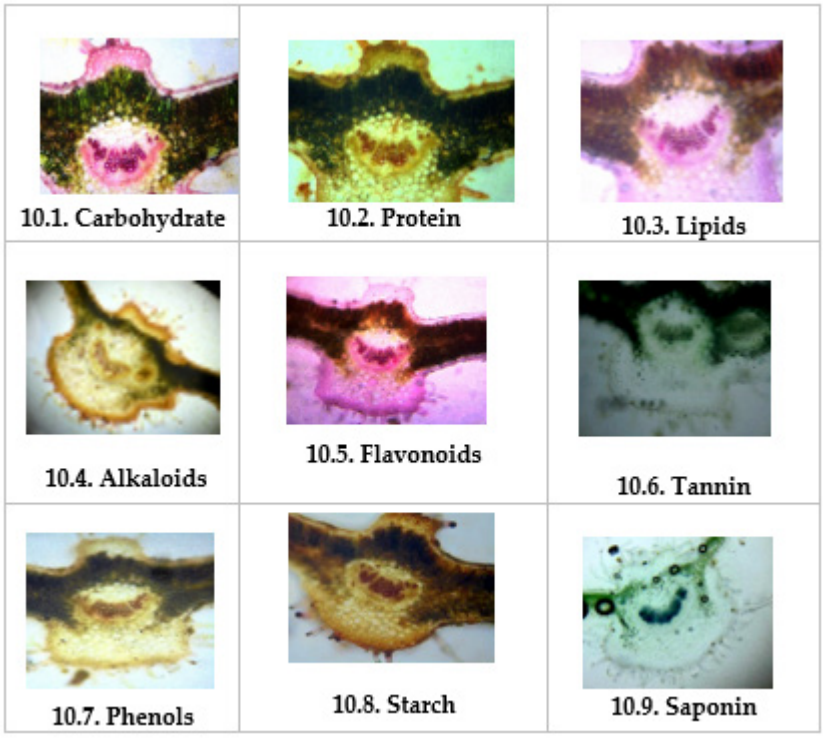

Plate 10: T.S. Leaf of *Justicia tranquebariensis*.

al. (2018), who mentioned that cystolith deposition can occur in either the abaxial or adaxial epidermis.[44] Additionally, Mohd. Tajudin *et al.* (2022)[37] observed cystoliths in the epidermal layer, collenchyma cells and parenchyma cortex of all three examined *Justicia* species. These plants can be identified by differences in the shape and quantity of trichome cells, even when the plants are in powder form.[37] In the present investigation, long multicellular non-glandular trichomes and uniseriate non-glandular trichomes, generally 5-celled, were found on both sides of the epidermis.[45] Singh and Jain (1975)[46] reported 40 different types of trichome comprising 19 glandular and 21 simple non-glandular trichomes across 41 taxa in the *Acanthaceae* family. Nurul Aini *et al.* (2014)[47] reported 12 distinct trichome types in selected *Staurogyne* species of *Acanthaceae*. Dhale and Kalme (2012)[48] reported glandular and non-glandular trichomes in the stem of *Adhatoda zeylanica*. Mohd. Tajudin *et al.* (2022)[37] also reported five distinct trichome types in three *Justicia* species (*J. gendarussa, J. procumbens* and *J. adathoda*). This discrepancy in trichome morphology highlights its potential utility in identifying and differentiating species within the genus.

The present study recorded diacytic type of stomata on the epidermal surfaces. Related studies done by Verdam *et al.* (2012)[38] in the leaves of *Justicia acuminatissima* and Ramasubramanian Raja and Jeevan Reddy (2014)[49] in the leaves of *Andrographis echioides* also showed diacytic stomata on both the adaxial and abaxial surfaces of epidermis. Suman Kumar *et al.* (2014)[50] reported the existence of diacytic stomata on the lower leaf epidermis of *Andrographis paniculata*. Similarly, Mohd. Tajudin *et al.* (2022),[37] Nurul-Aini *et al.* (2014)[47] and Noor-syaheera *et al.* (2015)[51] also observed diacytic stomata in most *Acanthaceae* species.

One important method for standardizing the quality control of crude pharmaceuticals is histochemical analysis, which may be used to find the presence of elastic cell contents in the plant's histological zones.[52] In the present study, histochemical analysis confirmed the existence of carbohydrates, proteins, alkaloids, saponin and phenolic compounds. Specifically, carbohydrates (stained pink) were observed in the cortex, phloem, xylem and pith of the stem; the epidermis, collenchyma, ground tissue, phloem and xylem of the petiole; and the epidermis, collenchyma hypodermis, ground tissue, phloem and xylem of the leaf. These findings align with those of Sudipa and Subrata (2014)[53] who found carbohydrates in the xylem and hypodermis of the stem, a few cortical cells of the petiole and the epidermis, hypodermis, a few cork cells and secondary phloem of the root in *Barleria lupulina*.

Proteins were detected in the cortex, xylem and pith cells of the stem; the epidermis, collenchyma, ground tissue, phloem, xylem and sclerenchyma bundle sheath of the petiole; the epidermis, collenchyma, ground tissue, phloem, xylem and sclerenchyma bundle sheath of the leaf. These results mirror those obtained by Sudipa and Subrata (2014),[53] who noticed proteins in the cork cells, epidermis, hypodermis, secondary xylem and pith cells of the root, stem, leaf of *Barleria lupulina*. Furthermore, the presence of alkaloids, phenolic compounds and saponins was also noted by Verdam *et al.* (2012)[38] in *Justicia* acuminatissima.

Overall, the morphological, anatomical and chemical features observed in *Justicia tranquebariensis* are consistent with those found in many species within the *Justicia* genus. This comprehensive characterization provides valuable insights into the plant's identity and highlights its common traits across the genus. Consequently, this report is instrumental for the accurate identification of *Justicia tranquebariensis*.

CONCLUSION

This study provides a comprehensive anatomical analysis of *Justicia tranquebariensis*, revealing key anatomical features and histochemical localization of various phytochemicals. The findings confirm the presence of significant phytochemicals in the stem and leaves, supporting its traditional use in treating various ailments. In-depth anatomical and histochemical studies contribute to accurate plant identification and standardization, while also providing a foundation for future pharmacological research. The observed phytochemical distribution within the plant tissues highlights its potential therapeutic applications, emphasizing the importance of *Justicia tranquebariensis* in traditional medicine and modern pharmacognosy.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

%: Percentage; **°C:** Degree Centigrade; **µm:** Milli micron; **cm:** Centimeter; **DPX:** Depaffinated Xylene; **dia:** Diameter; **FAA:** Formalin acetic acid; **hr:** Hour; **mL:** Milliliter; **mm:** Millimeter; **TBA:** Tertiary butyl alcohol, **g:** Gram, **NaoH:** Sodium hydroxide.

SUMMARY

Medicinal plants are vital for their therapeutic properties and *Justicia tranquebariensis*, a small woody shrub native to southern India, is traditionally used for treating various ailments. Despite its traditional, detailed pharmacognostical studies on this plant are limited. This study investigates the anatomical and histochemical localization of phytochemicals in the stem, petiole and leaves of *Justicia tranquebariensis* to assist in proper identification and standardization.

The anatomical analysis revealed that the stem exhibits a thick epidermis with an underlying cortex and a prominent vascular bundle system. The petiole shows a similar structure with distinct vascular bundles and the leaves possess a mesophyll structure with palisade and spongy parenchyma. Histochemical studies identified phytochemicals such as carbohydrates, proteins, lipids, alkaloids, flavonoids, tannins, phenols, starch and saponins. These findings increase an understanding of the plant's medicinal properties and provide a foundation for future pharmacological research.

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