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Histochemical analysis of plant metabolites in *Pithecellobium dulce*: Unveiling plant chemical diversity

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Abstract
This study investigates the tissue-specific localization of bioactive compounds in *Pithecellobium dulce*, a tropical plant widely used in traditional medicine. Known for its therapeutic potential, the plant contains diverse phytochemicals such as quercetin, kaempferol, dulcitol, tannins, phenols, flavonoids, and saponins. Fresh sections of leaf, stem, fruit, and bark were subjected to histochemical staining using specific reagents to detect major metabolites including saponins, phenols, tannins, flavonoids, alkaloids, and glycosides. The analysis revealed distinct patterns of metabolite distribution across different tissues, highlighting the plant's chemical diversity. These findings provide insight into the localization of pharmacologically important compounds and support the medicinal relevance of *Pithecellobium dulce*. The study lays a foundation for further exploration of its therapeutic applications and potential in drug development.

Keywords: *Pithecellobium dulce*, phytochemicals, bioactive compounds, tissue-specific localization, metabolite distribution

Introduction
Pithecellobium dulce (Roxb.) Benth popularly called as manila tamarind, is an ever green tree belongs to the family Leguminosae, subfamily Mimosoideae, broadly distributed throughout India [1]. *P. dulce* is evergreen tree compose of pairs of leaflets with greyish and rough bark, it produces green and red variety of rolled pods covering the period from February to August. The pods enclose 5 to 12 arils which are white and pink each containing black seeds [2, 3]. Pithecellobium species are rich in diverse phytochemicals [4]. These phytochemicals are organic compounds, typically classified into primary and into secondary metabolite groups [5]. Primary metabolites are vital compounds involved in key biological processes such as photosynthesis, respiration, overall growth and development [6]. Secondary metabolites are naturally occurring organic molecules which do not contribute directly to the typical growth and development of an organism [7, 8]. They are often produced in highest concentrations during the transition from active growth to the stationary phase [7, 9]. These metabolites can be broadly classified into major three groups: terpenes (such as carotenoids, sterols, cardiac glycosides, and plant volatiles), phenols (including phenolic acids, lignans, coumarins, flavonoids, tannins, and lignin), and nitrogen-containing compounds (like Alkaloids, glucosinolates) [7, 10, 11]. Plants are the primary source of secondary metabolites [12], accounting for approximately 80%, together with fungi, bacteria and marine organisms also playing a role [13]. These plant compounds are crucial in pharmaceuticals, flavors, fragrances, insecticides, and dyes [13, 14]. The seeds of *P. dulce* are rich in fixed oil, primarily olein, and also contain lipids, phospholipids, steroids, saponins, glycosides, glycolipids, and polysaccharides [15, 16, 17]. Quercetin, a glycoside, has been derived from the plant. The bark yields catechol-type tannins [18], while the leaves contain quercetin, kaempferol, dulcitol, and afezilin [19, 20]. Additionally, *P. dulce* fruit contains alkaloids, tannins, phenols, saponins, flavonoids, glycosides, and anthraquinones [21, 22, 23]. This comprehensive profile highlights the significant potential of *P. dulce* for health benefits and pharmacological uses [24, 25]. *P. dulce* shows diverse medicinal properties in various parts [26]. The leaves and bark are known to have anti-diabetic properties, while the fruit peel exhibits significant antibacterial, antioxidant, wound-healing, and diabetes-controlling properties [27, 28].

Additionally, plant phytochemicals exhibit strong insecticidal activity, particularly against adult mosquitoes [29]. The medicinal attributes of plants are primarily due to secondary metabolites namely flavonoids, alkaloids, tannins, and saponins [30], which exhibit significant biological activities. To confirm and identify these compounds, histochemical techniques are used to study the distribution and presence of these metabolites within plant tissues [30, 31]. Histochemistry involves the detection, identification, and localization of bioactive substances within biological cells utilizing indicators, stains, and light microscopy techniques. This technique is vital for investigating the structures responsible for secretion in plants, as their classification is depends on the composition of the substances they secrete [32]. Histochemistry offers an in-depth chemical analysis of cells and tissues, highlighting their structural organization. This is particularly significant because a single gland or even an individual glandular cell can simultaneously produce multiple metabolic products. Thus, a thorough histochemical analysis is crucial for accurately profiling the variety of metabolites generated by plant secretory structures [31].

In this study, we aim to use histochemical techniques to detect and map the distribution profile of secondary metabolites within *P. dulce*. By applying these methods, we aim to investigate the presence of key bioactive compounds present in the plant's tissues that contribute to its diverse medicinal properties. Histochemistry facilitates accurate detection and characterization of metabolites, providing valuable information pertaining the secretory structures of the plant species and their functions in producing compounds that are biologically active. This thorough profiling is crucial for exploring the pharmacological potential of *P. dulce* as a guidance for future research.

Material and Methods

Plant Collection

Different plant parts of *P. dulce* was collected from Ghatkopar, Mumbai and was identified & authenticated taxonomically from Blatter herbarium from St. Xaviers College, Mumbai, Maharashtra, India. The specimen is matches with Blatter herbarium specimen no JP-3229 OF J. Pallithanam [33].

Macroscopical observations

Macroscopical observations were conducted through visual, physical, and organoleptic evaluation techniques. Colour and shape were assessed under natural daylight. Surface characteristics, such as texture and appearance, were examined using a hand lens [32, 34].

Histochemical localisation of the plant metabolites

To localize plant metabolites by histochemistry technique, samples were prepared by free-hand sectioning and staining methods, using standard protocols. Transverse section of plant tissues were treated with specific reagents to localized tissue area. The treated sections were observed by microscope (Models: Stereomicroscope WF 10X/20, Optical microscope WF-10X/20MM and Motic microscope-WF-10X/20MM) [35, 36].

The fresh plant samples were sectioned (leaf, stem, bark, and fruit) to perform histochemical localization. These sections were treated with the appropriate reagents [32]. Following reagents were used for histochemical localization were viz concentrated H_2SO_4, 1% barium hydroxide, calcium chloride, potassium dichromate to detect saponins, dilute acidic $Fecl_3$ solution (0.5% to 1% of ferric chloride in 0.1 N HCl) to detect tannins [37], Dragendorff's reagent for detection of alkaloids, 10% Lead acetate for flavonoids, 10% Alcoholic ferric chloride for detecting phenols [33], Concentrated H_2SO_4 was used to detect terpenes, 1% NaOH for detection of glycosides [38]. The metabolites were confirmed by the corresponding colour development within the specific region of the plant tissue.

Results and Discussion

Macroscopical observations

P. dulce (Roxb.) Benth is an evergreen tree from the Leguminosae family, typically reaching heights of 10-15 meters, though it can grow between 15-18 meters [19]. It features a broad, spreading crown with irregular branches extending up to 30 meters [39]. Its leaves are bipinnate with two pairs of kidney-shaped leaflets, and thin spines ranging from 2 to 15 mm are found in pairs at the base of the leaves [39]. Small white flower heads, approximately 1 cm in diameter, are formed by the tree, each having a hairy corolla and calyx that surround around 50 thin stamens united at the base. The bark starts off grey and smooth, gradually becoming rough and peeling with maturity [1]. The inner bark is thick, smooth, and lightly brown. The pods, measuring 10-15 cm by 1.5 cm, turn reddish-brown and contain 5-10 glossy black seeds. A distinguishing feature of *P. dulce* is its tightly coiled seed pods, which make identification straightforward [1, 3].

A) B) C)

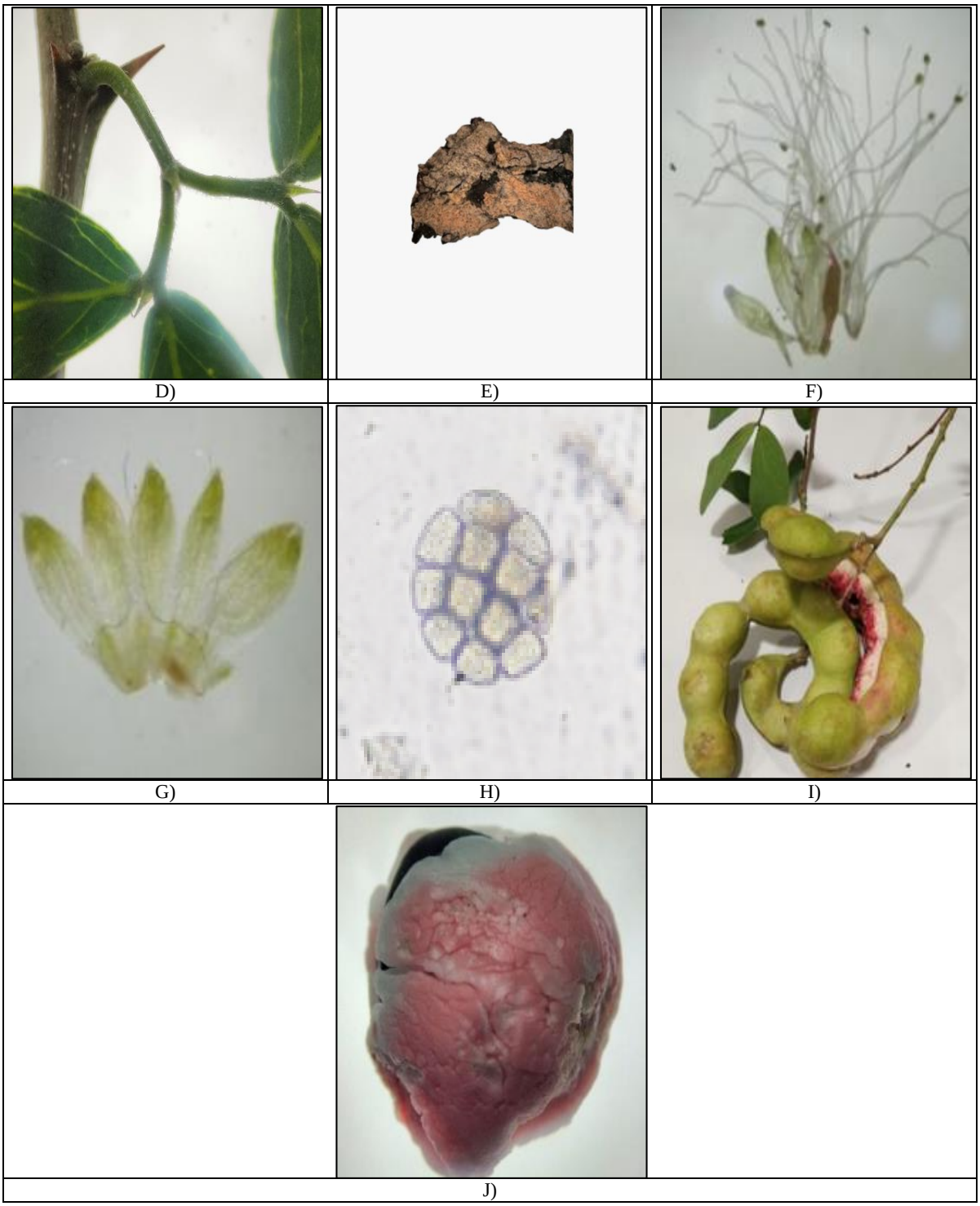


Fig 1: A) *P. dulce* entire plant, B) Inflorescence, C) bipinnate leaf, D) pair of sharp spine, E) hard bark, F) L.S. of flower with ovary, style & stigma, G) corolla, H) single pollen grain, I) fresh fruit, J) seed with pulp.

Histochemical localisation of plant metabolites

Histochemical localization tests identify the presence and distribution of active metabolites within tissues, thus providing critical insights into their medicinal potential and acting as a foundational method for isolating natural products [31]. The analysis of stem metabolites given in (Figure 2) and (Table 1) revealed distinct patterns of localization and colouration. Saponins, which appeared yellow, were concentrated in the cortical region. Tannins, exhibiting a blue-green colour, were distributed across the cortical and parenchymatous regions. Alkaloids, marked by a reddish-orange colour, were located in hypodermis and the xylem bundles. Glycosides, showing yellow, were present in the sclerenchyma cells of the hypodermis and vascular region, while flavonoids, also yellow, were localized primarily in vascular region. These findings highlight the specific distribution and potential functional roles of these metabolites within the stem.

Histochemical localization of the stem in *P. dulce*

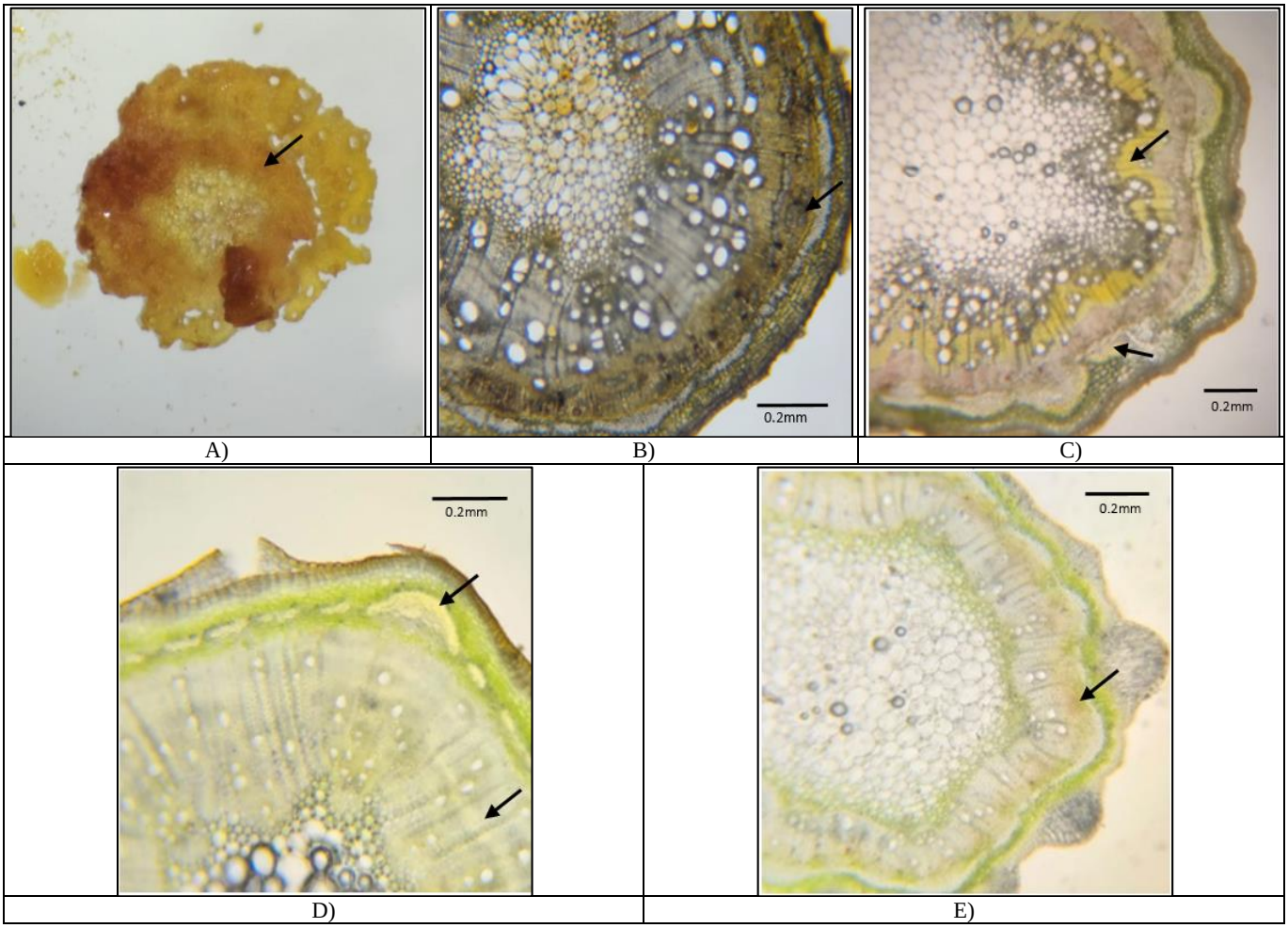


Fig 2: Histochemical tests performed on transverse sections of the stem in *P. dulce* A) Saponins, B) Tannins, C) Alkaloids, D) Glycosides, E) Flavonoids.

Table 1: Histochemical localization of the stem in *P. dulce*

Sr. No.	Plant Metabolites	Reagents used & treatment	Localization of Stem	
			Colour observed	Region
1	Saponins	Section treated with Conc. H_2SO_4 for 30 min. followed by overnight treatment with 1% barium hydroxide then section is washed with calcium chloride and then placed in potassium dichromate.	Yellow	Cortical region
2	Tannins	Dilute acidic $Fecl_3$ solution	Blue green	Cortical & parenchymatous region
3	Alkaloids	Dragendorff's reagent	Reddish Orange	Hypodermis & xylem bundles
4	Glycosides	1% Sodium Hydroxide	Yellow	Sclerenchyma cells in the hypodermis & vascular region
5	Flavonoids	10% Lead acetate	Yellow	Vascular region

The analysis of leaf metabolites given in the (Figure 3) and (Table 2) the Saponins were detected in mesophyll tissue and bundle sheath, exhibiting a yellow colouration. Tannins, which appeared blue-green, were concentrated in the vascular region and the palisade layer of the mesophyll. Alkaloids, characterized by a reddish-orange colour, were located in the bundle sheath cells. Glycosides, observed as yellow, were found in epidermal cells beneath the vascular bundle of the mid vein. These findings highlight the diverse localization of metabolites within leaf tissues, suggesting their specialized roles in leaf structure and function.

Histochemical localization of the leaf in *P. dulce*

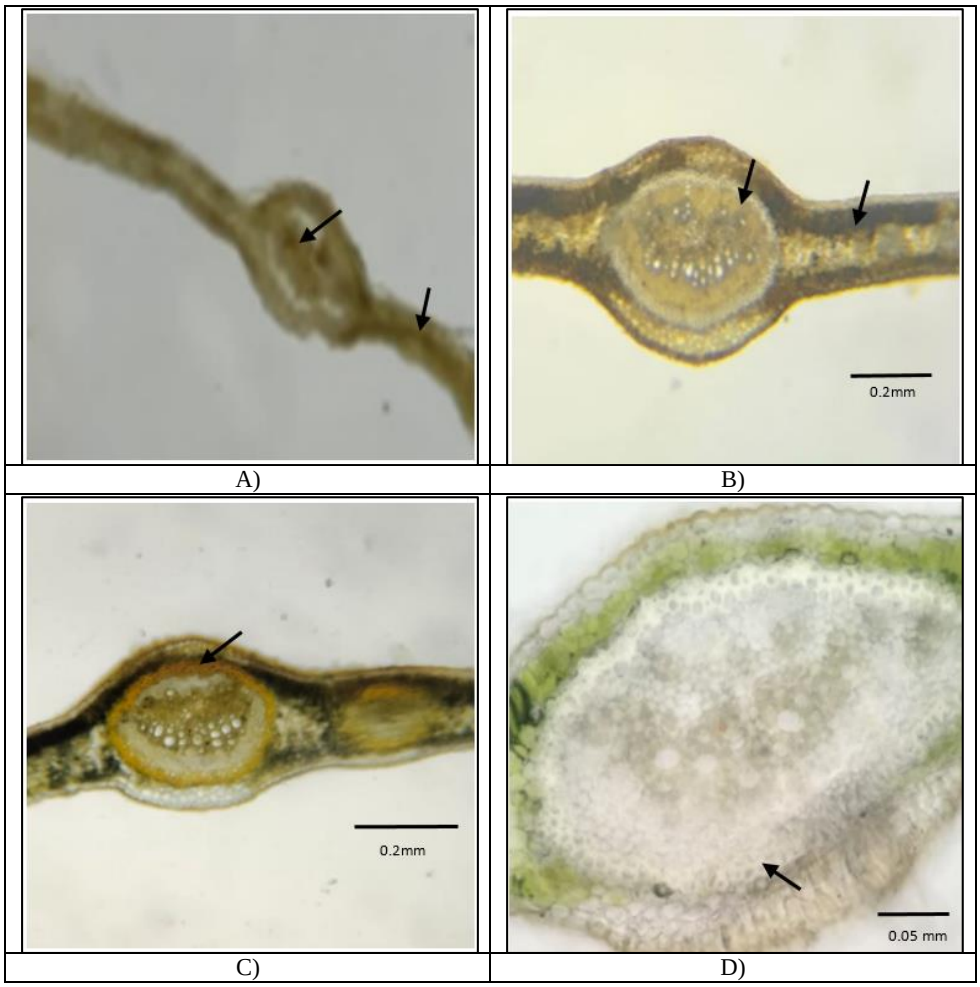


Fig 3: Histochemical tests performed on transverse sections of the leaf in *P. dulce* A) Saponins, B) Tannins, C) Alkaloids, D) Glycosides.

Table 2: Histochemical localization of the leaf in *P. dulce*

Sr. No.	Plant Metabolites	Reagents used & treatment	Localization of Leaf	
			Colour observed	Region
1	Saponins	Section treated with Conc. H_2SO_4 for 30 min. followed by overnight treatment with 1% barium hydroxide then section is washed with calcium chloride and then placed in potassium dichromate	Yellow	Mesophyll tissue and bundle of sheet
2	Tannins	Dilute acidic $Fecl_3$ solution	Blue green	Vascular region and palisade layer of mesophyll region
3	Alkaloids	Dragendorff's reagent	Reddish Orange	Bundle of sheath cells
4	Glycosides	1% Sodium Hydroxide	Yellow	Epidermal cells below the vascular bundle of mid vein

The analysis of bark metabolites given in the (Figure 4) and (Table 3) the Saponins were predominantly localized in the phloem fibres, where they appeared yellow. Tannins, displaying a blue-green colour, were present in both some phloem fibres and medullary rays. Alkaloids were concentrated in medullary rays, showing a reddish-orange colouration. Phenols were identified in phloem fibres and medullary rays with a green colour. Flavonoids, marked by yellow, were found in the parenchyma just beneath the cork cells and in some of the phloem fibres. Glycosides also appeared yellow and were distributed in the parenchyma and medullary parenchyma beneath the sclerenchymatous layer, along with a few phloem fibres. Lastly, terpenoids were observed as a light purple colouration in phloem fibres. These observations underscore the diverse localization of these metabolites in bark, pointing to their varied roles in structural support and defence mechanisms within the tissue. Histochemical localization of the Bark in *P. dulce*

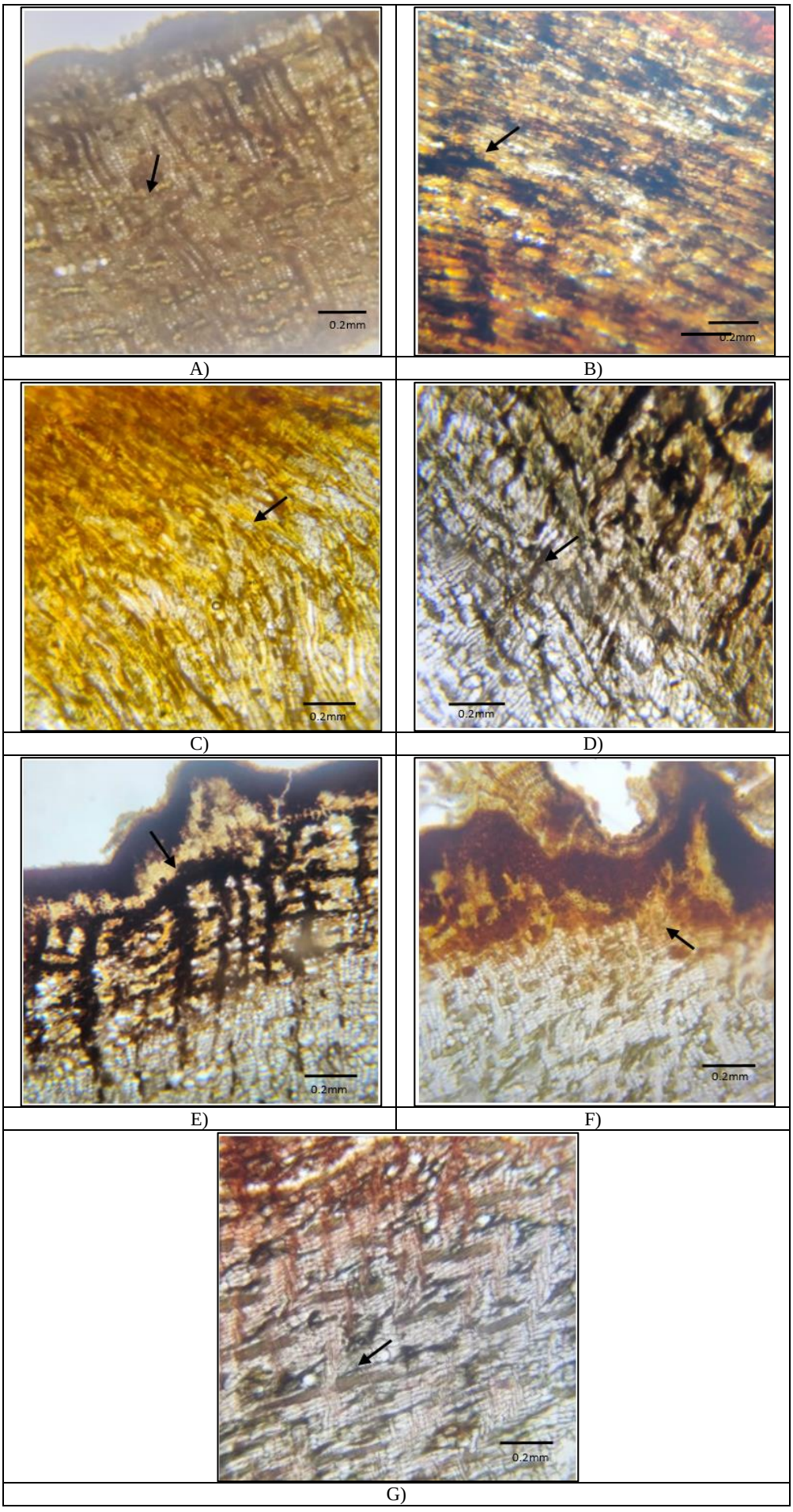


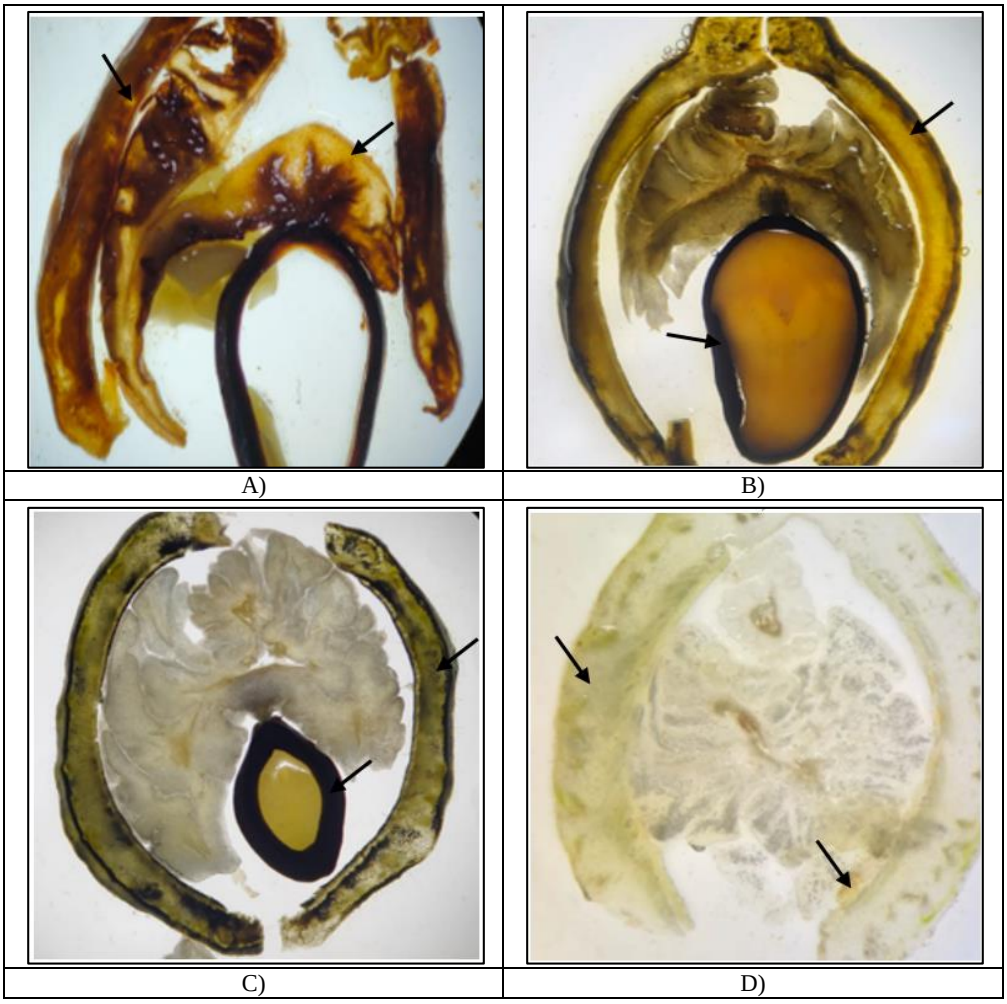
Fig 4: Histochemical tests performed on transverse sections of the Bark in *P. dulce* A) Saponins, B) Tannins, C) Alkaloids, D) Phenols, E) Flavonoids, F) Glycosides, G) Terpenoids.

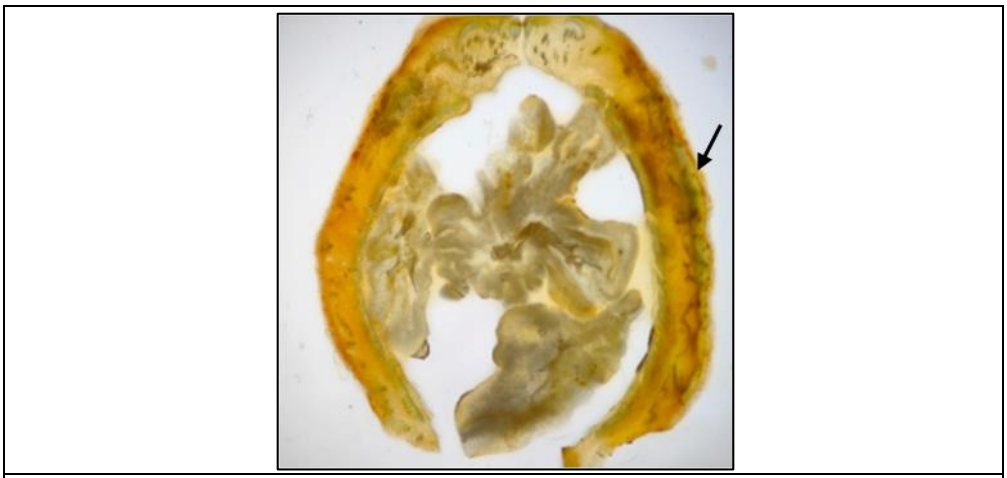
Table 3: *Histochemical localization of the Bark in P. dulce*

Sr. No.	Plant Metabolites	Reagents used & treatment	Localization of Bark	
			Colour observed	Region
1	Saponins	Section treated with Conc. H_2SO_4 for 30 min. followed by overnight treatment with 1% barium hydroxide then section is washed with calcium chloride and then placed in potassium dichromate	Yellow	Phloem fibres
2	Tannins	Dilute acidic $Fecl_3$ solution	Blue green	Some phloem fibres & medullary rays
3	Alkaloids	Dragendorff's reagent	Reddish Orange	Medullary rays
4	Phenols	10% Alcoholic ferric chloride	Green	phloem fibres & medullary rays
5	Flavonoids	10% Lead acetate	Yellow	Parenchyma inner to the cork cells and some phloem fibres
6	Glycosides	1% Sodium Hydroxide	Yellow	Parenchyma & medullary parenchyma below the sclerenchymatous layer & few phloem fibres
7	Terpenoids	Concentrated H_2SO_4	Light purple colour	Phloem fibres

The analysis of fruit metabolites given in the (Figure 5) and (Table 4) the Saponins were detected in the epicarp and mesocarp regions, displaying a yellow colour. Tannins were concentrated in the epicarp and the seed coat, appearing blue-green. Phenols were found in both the inner and outer layers of the epicarp and the seed coat, with a green colouration. Flavonoids, also yellow, were localized in certain areas of the epicarp and the outer layer of the mesocarp. Glycosides were detected throughout the entire epicarp and in certain areas of the mesocarp, exhibiting a yellow hue. These results indicate a targeted distribution of these compounds in the fruit, likely contributing to its structural integrity and protective functions.

Histochemical localization of the Fruit in *P. dulce*





E)

Fig 5: Histochemical tests performed on transverse sections of the Fruit in *P. dulce* A) Saponins, B) Tannins, C) Phenols, D) Flavonoids, E) Glycosides.

Table 4: Histochemical localization of the Fruit in *P. dulce*

Sr. No.	Plant Metabolites	Reagents used & treatment	Localization of Fruit	
			Colour observed	Region
1	Saponins	Section treated with Conc. H_2SO_4 for 30 min. followed by overnight treatment with 1% barium hydroxide then section is washed with calcium chloride and then placed in potassium dichromate	Yellow	Epicarp & mesocarp region
2	Tannins	Dilute acidic $Fecl_3$ solution	Blue green	Epicarp & seed coat
3	Phenols	10% Alcoholic ferric chloride	Green	Inner and outer layer of epicarp & seed coat
4	Flavonoids	10% Lead acetate	Yellow	Some region of epicarp & outer layer of mesocarp
5	Glycosides	1% Sodium Hydroxide	Yellow	Entire epicarp & some region of mesocarp

Conclusion

The study of metabolite localization in the leaf, fruit, bark, and stem through histochemical analysis revealed distinct patterns that underscore the specialized functions of each compound within the plant. Saponins, tannins, alkaloids, phenols, flavonoids, glycosides, and terpenoids were each identified in specific tissues, reflecting their roles in structural reinforcement, defence, and other physiological processes. Histochemistry proved invaluable in this research, allowing for precise visualization of these metabolites within their native tissue contexts, thereby providing insights into their functional roles. The ability to localize these compounds at the cellular level enhances our understanding of plant biology, offering critical information for improving plant resilience, developing targeted agricultural strategies, and advancing the study of plant-environment interactions. This approach not only contributes to the fundamental knowledge of plant physiology but also has practical implications for the optimization of plant resources in various applications.

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Abbreviation

NaOH-(Sodium Hydroxide), H_2SO_4-(Sulphuric Acid), $Fecl_3$-(Ferric Chloride), mm-(mili meter), cm-(centimeter)

References

1. Sneha D, Prashanth S, Kaveti VS, Boggula N. Systematic review of *Pithecellobium dulce* (Roxb.) Benth.: a traditional medicinal herb. Journal for Innovative Development in Pharmaceutical and Technical Science. 2020 May;3(5):1-9.
2. Vargas-Madriz ÁF, Kuri-García A, Vargas-Madriz H, Chávez-Servín JL, Ferriz-Martínez RA, Hernández-Sandoval LG, *et al.* Phenolic profile and antioxidant capacity of *Pithecellobium dulce* (Roxb) Benth: a review. Journal of Food Science and Technology. 2020 Dec;57(12):4316-4336.
3. Murugesan S, Lakshmanan DK, Arumugam V, Alexander RA. Nutritional and therapeutic benefits of medicinal plant *Pithecellobium dulce* (Fabaceae): a review. Journal of Applied Pharmaceutical Science. 2019 Jul 1;9(7):130-139.
4. Shukla M, Singh A, Ghosh P, Chatterjee S, Singh P. Systematic review on evidence-based therapeutic potential of *Pithecellobium dulce* for health benefits. Toxicology International. 2024 Apr 15;31(2):249-256.
5. Monari S, Ferri M, Salinitro M, Tassoni A. New insights on primary and secondary metabolite contents of seven Italian wild food plants with medicinal applications: a comparative study. Plants. 2023 Sep 5;12(18):3180.
6. Crozier A, Clifford MN, Ashihara H. Plant secondary metabolites: occurrence, structure and role in the human diet. Oxford: Blackwell Publishers; 2006. p. 1-384.

7. Bizzo HR, Silveira D, Gimenes MA, Agostini-Costa TS, Vieira RF, Bizzo HR, *et al.* Chromatography and its applications. 2012 Mar 16. p. 1-131.
8. Zandavar H, Babazad MA. Secondary metabolites: alkaloids and flavonoids in medicinal plants. In: Herbs and Spices-New Advances. IntechOpen; 2023.
9. Čihák M, Kameník Z, Šmídová K, Bergman N, Benada O, Kofroňová O, *et al.* Secondary metabolites produced during the germination of *Streptomyces coelicolor*. Frontiers in Microbiology. 2017 Dec 13;8:2495.
10. Kaushik B, Sharma J, Kumar P, Shourie A. Phytochemical properties and pharmacological role of plants: secondary metabolites. Biosciences Biotechnology Research Asia. 2021;18(1):23-31.
11. Sharma I, Sharma S, Kumar A, Kumar V, Sharma AK. Plant secondary metabolites. In: Sharma AK, editor. Singapore: Springer; 2022. p. 329-353.
12. Twaij BM, Hasan MN. Bioactive secondary metabolites from plant sources: types, synthesis, and their therapeutic uses. International Journal of Plant Biology. 2022 Feb 21;13(1):4-14.
13. Thirumurugan D, Cholarajan A, Raja SS, Vijayakumar R. An introductory chapter: secondary metabolites. In: Secondary Metabolites-Sources and Applications. 2018 Sep 5. p. 1-13.
14. Pagare S, Bhatia M, Tripathi N, Pagare S, Bansal YK. Secondary metabolites of plants and their role: overview. Current Trends in Biotechnology and Pharmacy. 2015;9(3):293-304.
15. Kulkarni KV, Jamakhandi VR. Medicinal uses of *Pithecellobium dulce* and its health benefits. Journal of Pharmacognosy and Phytochemistry. 2018;7(2):700-704.
16. Rangarajan H, Elumalai A, Chidanand DV. Traditional fruits of South India: bioactive components and their potential health implications in chronic diseases. Journal of Food Biochemistry. 2021 Mar;45(3):e13266.
17. Alhamed AS, Alqinyah M, Alghaith AF, Algahtani MM, Alqahtani F, Nasr FA, *et al.* Phytochemical analysis and anticancer activity of the *Pithecellobium dulce* seed extract in colorectal cancer cells. Open Chemistry. 2023 Jul 20;21(1):20230362.
18. Al-Obaidi FJ, Almehemdi AF. Nutritional and medicinal properties of *Pithecellobium dulce*: a review article. Iraqi Journal of Desert Studies. 2024 Jul 1;14(1):1-10.
19. Hameed A, Srivastav Y, Afaq H, Hashmi A, Ahmad M. Outline of the pharmacological and phytochemical profile of the jungle jalebi (*Pithecellobium dulce*). Asian Plant Research Journal. 2024 Mar 9;12(2):22-27.
20. Dhanisha SS, Drishya S, Guruvayoorappan C. Traditional knowledge to clinical trials: a review on nutritional and therapeutic potential of *Pithecellobium dulce*. Journal of Basic and Clinical Physiology and Pharmacology. 2022 Mar 28;33(2):133-142.
21. Kondabolu UL, Babitha B, Kalagatur NK, Nagaraj A, Velumani S. Phytochemical analysis in *Pithecellobium dulce* fruit peel extract. Current Trends in Biotechnology and Pharmacy. 2023;17(3):1052-1059.
22. Suprada Rao MS, Punarvasu TP, Pramod Kumar P, Angaswamy N, Sharada AC. Phytochemical analysis of *Pithecellobium dulce* (Roxb.) Benth unripe aril via LC-MS/MS and evaluation of its antiproliferative activity against human colon carcinoma cells. Annals of Phytomedicine. 2023 Dec 30;12(2):479-489.
23. Aleman-Ramirez JL, Okoye PU, Torres-Arellano S, Sebastian PJ. Challenges and prospects in energetic application of *Pithecellobium dulce* (Roxb.) Benth as a bioenergy tree. Biofuels, Bioproducts and Biorefining. 2024 Sep;18(5):1658-1675.
24. Saha P, Reddy MK, Ramya CH, Pavithra Y, Manasa V, Vamsi G. Evaluation of *Pithecellobium dulce* (Camachile) fruit and its aril powder. International Journal of Chemical Studies. 2021;9(2):671-676.
25. Kavita K. Development of jungle jalebi (*Pithecellobium dulce*) fruit powder (JJFP) and effect of its intervention on blood glucose level of NIDDM patients [doctoral dissertation]. Udaipur: MPUAT; 2023.
26. Devhare LD, Gokhale N. A brief review on: phytochemical and antiulcer properties of plants (Fabaceae family) used by tribal people of Gadchiroli Maharashtra. International Journal of Pharmaceutical Sciences and Research. 2023;14(4):1572-1593.
27. Sukantha TA, Sripathi SK. Isolation and characterization of secondary metabolites from *Pithecellobium dulce* Benth fruit peel: a review. International Journal of Pharmacy and Pharmaceutical Sciences. 2015 Sep 22;7(11):199-203.
28. Katekhaye SD, Nagmoti DM. α-Glucosidase and α-amylase inhibitory activities of *Pithecellobium dulce* bark and leaves. Phytopharmacology. 2013;4(1):123-130.
29. Murugesan S, Lakshmanan DK, Arumugam V, Alexander RA. Nutritional and therapeutic benefits of medicinal plant *Pithecellobium dulce* (Fabaceae): a review. Journal of Applied Pharmaceutical Science. 2019 Jul 1;9(7):130-139.
30. Mahendra C, Sudarshana MS, Jagannath S. Histochemical localization of secondary metabolites *in vivo*, *in vitro* leaf and leaf-derived callus of *Salacia macrosperma* Wight. No journal details provided—please confirm.
31. Badria FA, Aboelmaaty WS. Plant histochemistry: a versatile and indispensable tool in localization of gene expression, enzymes, cytokines, secondary metabolites and detection of plants infection and pollution. Acta Scientific Pharmaceutical Sciences. 2019 Jul;3(7):88-100.
32. Bhavadharaniparkavi K, Abirami P. Histochemical investigation and localization of secondary metabolites of *Acalypha paniculata* Miq. International Journal of Pharmaceutical Sciences Review and Research. 2022 Jan 15;72(1):80-89.
33. Yogeshwari C, Kumudha P. Histochemical localization of primary and secondary metabolites in *Tiliacora racemosa* Colebr. Research Journal of Pharmacognosy and Phytochemistry. 2018;10(2):152-156.
34. Ondora OM, Gikonyo NK, Nyambaka HN, Thoithi GN. Macroscopic and microscopic features of diagnostic value for *Warburgia ugandensis* Sprague leaf and stem-bark herbal materials. Journal of Pharmacognosy and Phytotherapy. 2020 Apr 30;12(2):36-43.
35. Lux A, Morita S, Abe JU, Ito K. An improved method for clearing and staining free-hand sections and whole-mount samples. Annals of Botany. 2005 Nov 1;96(6):989-996.

36. Zholdasbayev ME, Ishmuratova MY, Atazhanova GA, Musozoda SM, Poleszak E. Histochemical study of above-ground parts of *Prunella vulgaris* L. growing on the territory of the Republic of Kazakhstan. Research Journal of Pharmacy and Technology. 2023;16(12):5944-5947.
37. Dhale DA. Histochemical investigation of some medicinal plants. Advanced Research in Pharmacy and Biological Sciences. 2011 Oct;1:147-154.
38. Deshmukh MA, Theng MA. Phytochemical screening, quantitative analysis of primary and secondary metabolites of *Acacia arabica* bark. International Journal of Current Pharmaceutical Research. 2018 Mar 15;10(2):35-37.
39. Rao BG, Samyuktha PSP, Ramadevi DRD, Battu HBH. Review of literature: phyto-pharmacological studies on *Pithecellobium dulce*.