Cellular Dynamics in *Passiflora tarminiana*: Insights from Histological Investigations

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Abstract
The objective of the study was to investigate the visible and microscopic features of Passiflora tarminiana fruit. The plant specimen was collected in May 2023 from Ooty, The Nilgiris District, Tamil Nadu. The evaluation was carried out using macroscopy, microscopy and histochemical tests. The fruit's characteristic components were identified by dissecting it and their structures were visually documented through photographs. Macroscopy revealed the fruit's sensory properties while microscopy showed the presence of oil globules, stone cells, simple pitted, reticulated and spiral vessels and cluster crystals from the seeds.

In histochemical tests, cutin, alkaloid, lignin, mucilage, oil globules and tannins were observed in different regions of the pericarp. Cutin, aleurone grains and oil droplets were observed in various regions of the seed. The analysis of macroscopic, microscopic and histochemical features was found to be the first step in determining the quality, identity and purity of plant material that will be used in formulating a natural medicine. Additionally, the findings will be useful in distinguishing different species of Passiflora.

Keywords: *Passiflora tarminiana*, fruit, macroscopy, microscopy, histology.

Introduction

For centuries, Passiflora species have been traditionally utilized primarily for their anxiolytic and sedative properties. In recent times, these species have gained recognition from the pharmaceutical, food and cosmetic industries. Passiflora species, including *Passiflora tarminiana*, commonly known as banana passion fruit, have been identified for their therapeutic qualities as sedatives and mild tranquilizers, making them recommended for addressing conditions such as hysteria, insomnia, epilepsy and exhibiting antidiabetic activity[7].

Belonging to the Passifloraceae family, *P.tarminiana* is a perennial plant native to South America and widely distributed across many countries. Besides its medicinal uses, it is also valued as an ornamental plant, showcasing attractive pale pink flowers. Notably, it is more frost-tolerant than the common passion fruit and can endure brief exposure to temperatures as low as 0 - 5º C. The mature fruit, categorized as a pepo, is elliptic to oblong, yellow or light orange in color, measuring 10 to 12 cm in length and 3.8 to 4.5 cm in width. The fruit contains numerous seeds enclosed in an edible orange aril, imparting a slightly sour turning to astringent taste with a characteristic odor. *P.tarminiana* is recognized for its disease resistance, particularly when compared to other Passiflora species and is deemed edible.

In addition to its consumption as food, *P.tarminiana* finds applications in various industrial formulations. It is popularly utilized in the production of jellies, jams, gelatin desserts, cocktail drinks and ice creams. Numerous native fruits are globally accessible and esteemed for their contributions to food, nutrition, human health and overall well-being. Additionally, these fruits boost high levels of flavonoids, triterpenes and phenolic acids, endowing them with significant antioxidant properties that make them effective against various diseases.

Moreover, diverse Passiflora species have been recognized for their abundant content of indole alkaloids and anthocyanins. Consequently, the current study focuses on *P.tarminiana*, indigenous to the Nilgiris based on limited available reports detailing the utilization and composition of these fruits.

The categorization of medicinal plant materials relies on their macroscopic, microscopic and sensory attributes, constituting the initial step in ascertaining the identification and purity of these components. The crucial role of implementing these observations before proceeding with additional examinations has been emphasized. Medicinal plant materials are susceptible to common adulteration or substitution practices, driven by factors such as morphological resemblances, confusion in regional nomenclature from classical texts, the presence of similar active principles in substitute materials. These factors can compromise the therapeutic efficacy of the final dosage form[8].

Systematic identification is imperative for producing finished, standardized medicinal plant-based products. Achieving the goal of developing effective, safe and affordable plant-derived pharmaceuticals necessitates a meticulous study of plant compounds[12]. Microscopy stands as a reliable approach for obtaining comprehensive powder microscopic characteristics of crude medicines. In the evaluation of the quality and purity of crude powder, a thorough microscopic examination of *P.tarminiana* fruits

was conducted. The microscopic examination serves multiple purposes including the identification of organized pharmaceuticals, the definition of cellular structures based on histological characteristics and the detection of potential adulterants[9].

Microscopic studies may encompass the examination of whole plants, specific morphological plant components, or powdered versions of unprocessed pharmaceuticals. In cases where crude pharmaceuticals are in powder form, morphological characteristics may not accurately predict the substance's identity, making microscopical inspection the sole viable option[1]. Additionally, the characteristics of medicinal plant cell inclusions including types, numbers, sizes and other attributes have been observed to vary geographically[13].

In this study, *P.tarminiana* fruits were collected in May 2023 in and around Ooty, Nilgiris district, Tamilnadu, India, with the aim of defining criteria for their uniqueness, purity and quality. The anatomical structure of *P.tarminiana* fruits was investigated and their structures were documented through photographs. Future research endeavors will focus on employing chemical methods to identify the chemical composition of *P. tarminiana* fruit samples.

Material and Methods

Collection, Authentication and Processing: Fruits were gathered from the vicinity of Ooty, Tamil Nadu, with meticulous attention given to selecting healthy specimens. The freshly collected fruit sample underwent identification and authentication at the Siddha Central Research Institute, Chennai. The voucher specimen, bearing the number P12052304T, is housed in the Department of Biotechnology, JSS College of Life Sciences, Ooty, Tamil Nadu, along with authentication numbers. To ensure the removal of contaminants adhering to the surface, the essential fruit sample was extracted from the plant and thoroughly cleaned under running water. Subsequently, the cleaned fresh fruits were subjected to shade-drying, followed by grinding into a fine powder using a pulverizer. The resulting powder was then sieved through a 60-mesh screen to obtain a fine powder suitable for use in powder microscopic research[2].

Methodology: The sensory, external, or macroscopic examination of the test sample was documented using a Nikon D-5600 Digital camera. For microscopic investigations, the material was immersed in a fixative solution of formalin acid alcohol (FAA) for approximately 48 hours. Subsequently, the preserved specimen was sectioned into thin transverse fragments using a sharp knife and stained with safranin. Transverse sections were then photographed under bright field light using a Zeiss Axiocam 208 color digital camera attached to an Axiolab 5 trinocular microscope. The scale bar was included to indicate magnifications[17]. In the analysis of the powdered sample, the powder was treated with a saturated chloral hydrate solution and mounted on a microscopic slide with a drop of 50% glycerol. To confirm the presence of starch grains, the sample was treated with iodine solution. Diagnostic characters were observed under bright field light using a Nikon ECLIPSE E200 trinocular microscope, which was equipped with a Zeiss ERc5s digital camera. Photomicrographs of these diagnostic characters were captured and recorded[11].

Histochemical examinations were conducted on plant sections to identify various constituents. To detect crystals, the section was immersed in water and acetic acid was applied to one end of the cover slip. The observation of air bubbles indicated the presence of calcium carbonate crystals. If no air bubbles are formed, the experiment was repeated with concentrated hydrochloric acid (HCl), where the dissolution of crystals and the formation of calcium sulfate needles suggested the existence of calcium oxalate crystals.

For fats, fatty oils, volatile oils and resins, Sudan-IV (1 to 2 drops) was added to the section and allowed to stand. The presence of fatty oil substances was indicated by orange-red/pink/red-colored globules while red-colored irregular contents indicated the presence of resin. 2% iodine water solution was applied and a blue color indicated the presence of starch. Alcoholic ferric chloride (a drop) was added and bluish-black colored contents were observed for starch presence. Ruthenium red (a drop) was used to observe pink to red-colored contents, indicating the presence of mucilage.

For lignified cell walls, a drop of phloroglucinol was added and left to stand for approximately two minutes, or until it was nearly dry. The addition of 50% HCl turned the cell wall color from pink to cherry red, confirming the presence of lignin, observed through a glass cover. Suberized or cuticular cell walls were tested by adding a drop of Sudan red III, allowing it to stand for a few minutes and gently warming it. An orange-red or red stain on the cell wall indicated the deposition of suberin or cutin. To confirm alkaloids, a drop of Wagner's reagent was added and the observation of yellow to reddish-brown colored contents confirmed their presence[3].

Results and Discussion

The identification of a specific species is achieved through organoleptic and morphological observations facilitated by sensory organs. The matured fruit, categorized as a pepo, exhibits a yellow or light orange hue and possesses an elliptic to oblong shape that tapers at both ends, measuring 10 to 12 cm in length and 3.8 to 4.5 cm in width. Numerous seeds are embedded within an edible orange aril. The fruit imparts a slightly sour taste that transforms into an astringent flavor, accompanied by a distinctive odor (Figure 2).

Figure 1 illustrates the comprehensive microscopic structure of the *P.tarminiana* fruit. The transverse section (T.S.) of the fruit reveals an outer layer of thick-walled epicarp (Figure 3), covered by cuticles. Subsequently, a broad mesocarp region is observed (Figure 3a). Within the mesocarp region

(Figure 3b), two to three layers of hypodermal cells are present, with the lower layer displaying sporadic patches of stone cells. Below this layer, isodiametric, pitted parenchyma cells are observed, spanning 20 to 26 layers of thick-walled cells, succeeded by 15 to 18 layers of thin walled radially elongated parenchyma cells. Vascular strands traversing the mesocarp layer are formed of conventional vascular elements (Figure 3c). Additionally, a layer of endocarp cells (Figure 3d) is visible on the inner section of the fruit.

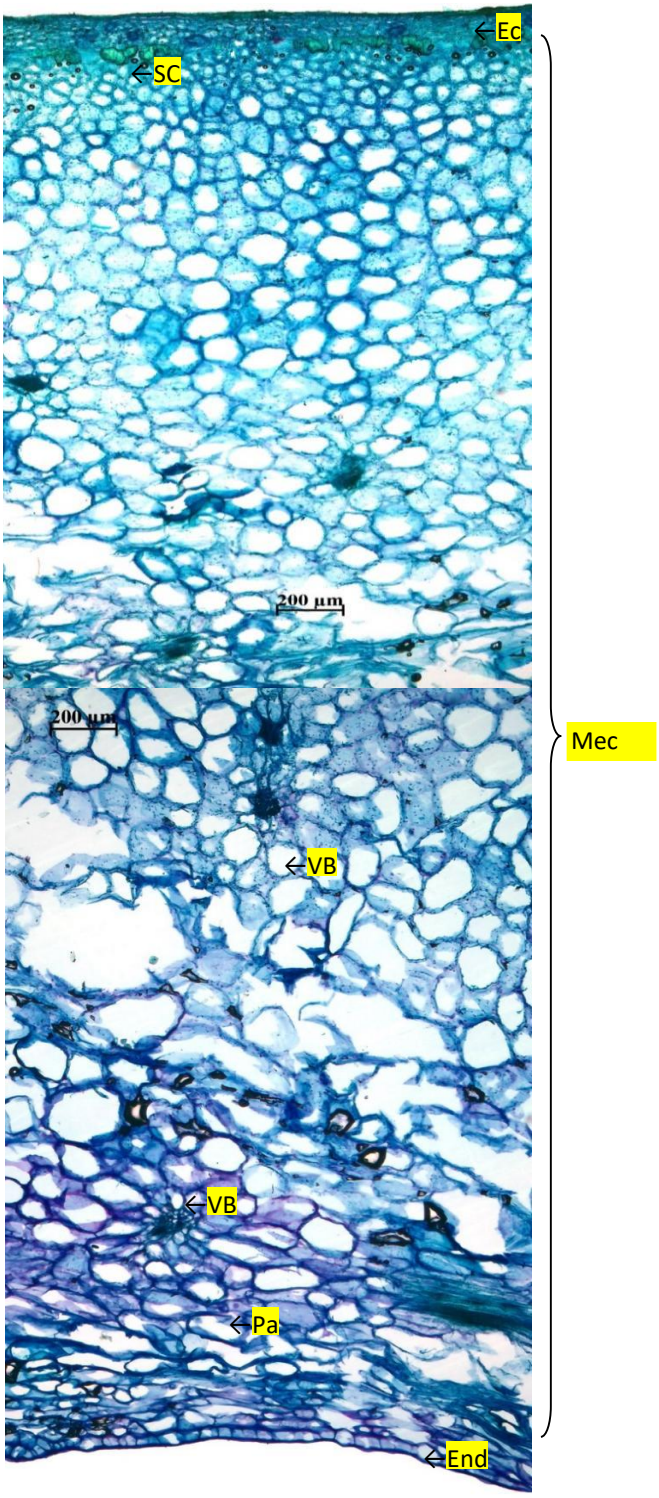

Figure 1: Microscopy of *Passiflora tarminiana* fruit

The powder exhibits a chocolate brown color with a distinctive odor and a taste from sour to astringent. It displays various characteristics including the surface view of epicarp cells (Figure 4a), pitted parenchyma cells from the mesocarp (Figure 4b) and thin-walled parenchyma cells containing oil globules (Figures 4c and 4d). Stone cells from the mesocarp are also evident (Figure 4e) including those with reddish-brown content (Figure 4f). Additionally, the powder showcases simple pitted vessels (Figure 4g), vessels with reticulate patterns (Figure 4h) and spiral thickening (Figure 4i), along with cluster crystals from the seed (Figure 4j).

In the histochemical analysis, the pericarp exhibited the presence of tannin deposition (Figure 5a) in the mesocarp region, along with the presence of mucilage (Figure 5b) and lignin (Figure 5c) in the same region. Alkaloids (Figure 5d) were detected in both the epicarp and mesocarp cells, while cutin (Figure 5e) was observed on the epicarp walls. Oil globules (Figure 5f) were noted in the parenchyma cells of the mesocarp. Cutin was identified in the outer layer of cotyledonary cells (Figure 5g) of the seeds and oil droplets were observed in the endosperm and cotyledon cells (Figure 5h, i). Lignin and tannin were not observed, but aleurone grains (Figure 5j) were present in the endosperm cells.

Plants possess structural support systems, exemplified by the persistent deposition of polymers like cutin and suberin into specific cell walls. Tannins, with their antioxidant qualities, find wide applications in the food and medicinal industries. Numerous studies in recent years have focused on determining the antioxidant activity of tannins and their attention has increased due to their potential in preventing cancer, heart disease and osteoporosis[16].

Figure 2: Macroscopy of *Passiflora tarminiana* fruit

Mucilage, a complex polysaccharide that dissolves in water, primarily consists of uronic acid and monosaccharides connected by glycosidic linkages, glycoproteins and bioactives. Mucilage is considered a favorable option for incorporation into food formulations because of its properties such as thickening, emulsifying and modification

of viscosity/ consistency. An alkaloid is any naturally occurring organic base containing nitrogen. Alkaloids have various physiological effects on humans and other animals, with well-known examples including nicotine, ephedrine, quinine, strychnine and morphine.

These compounds are primarily found in plants, with certain flowering plant families exhibiting higher concentrations. Alkaloids can function as defense mechanisms in plants, effectively deterring pathogens and predators due to their toxic nature.

As the drying and pulverization processes can lead to the loss of morphological identity, a thorough examination through both macroscopic and microscopic means was conducted to establish the identity and quality of the fruits before further analysis. The standardization of *P. tarminiana* fruits in this study was based on their external features, transverse sections and histochemical tests.

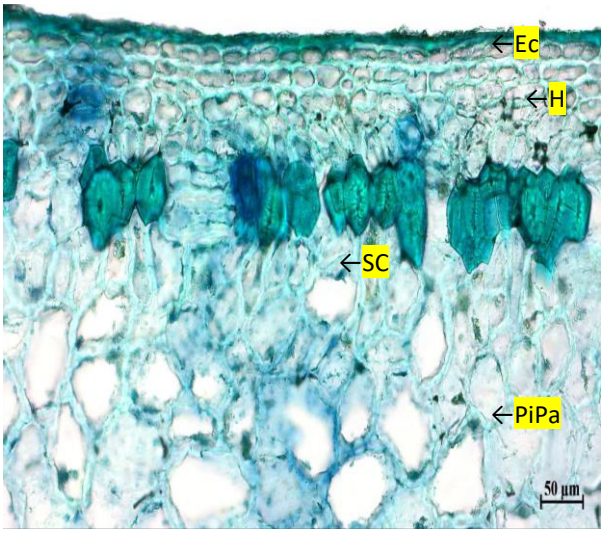

Figure 3: T.S of pericarp – Upper region

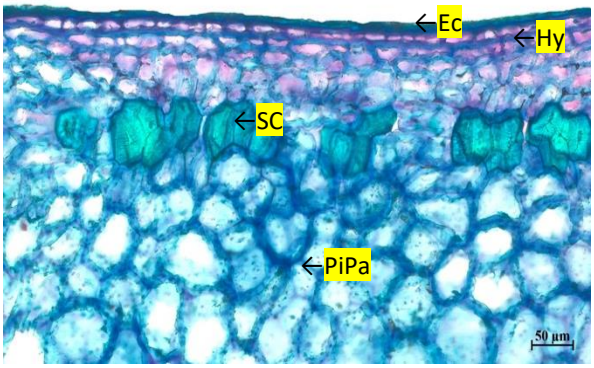

Figure 3a: Epicarp and mesocarp regions enlarged

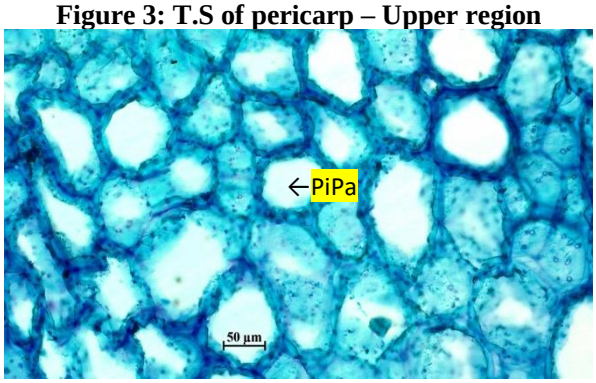

Figure 3b: Mesocarp cell

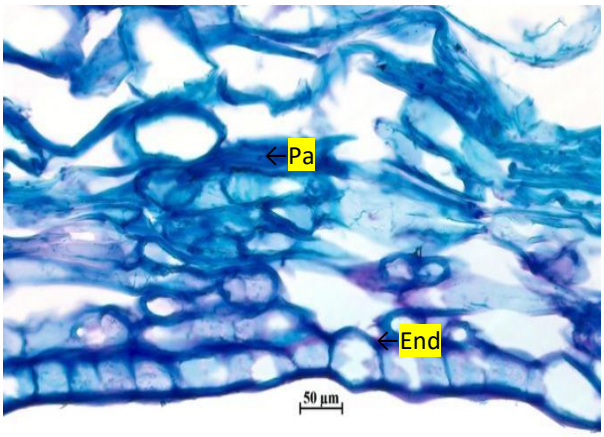

Figure 3c: Vascular strand in mesocarp

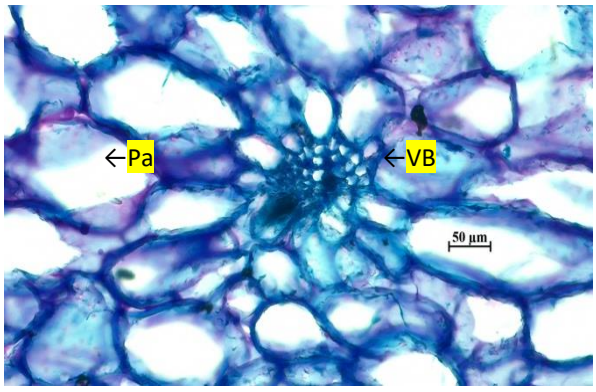

Figure 3d: Endocarp enlarged

Ec - Epicarp; End - Endocarp; Hy - Hypodermis; Mec – Mesocarp; Pa - Parenchyma; Pipa - Pitted Parenchyma; SC - Stone cell; VB - Vascular Bundle

Figure 3: T.S of *Passiflora tarminiana* fruit

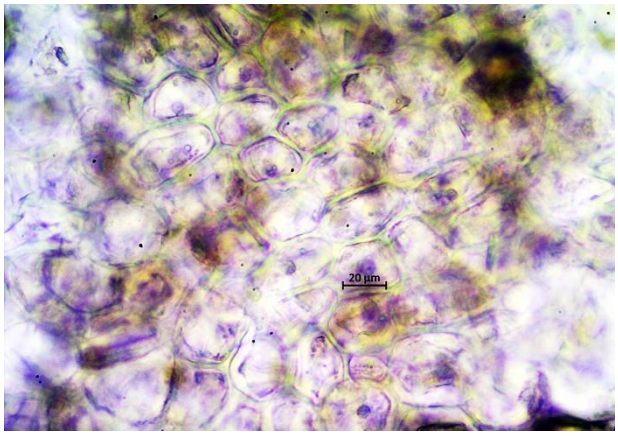

Figure 4a: Epicarp cells in surface view

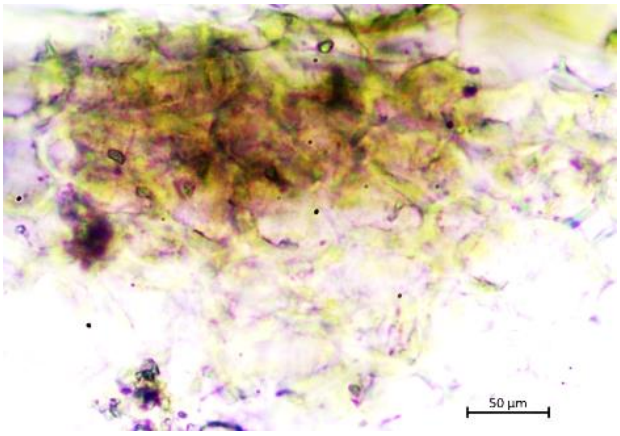

Figure 4b: Pitted parenchyma from the mesocarp

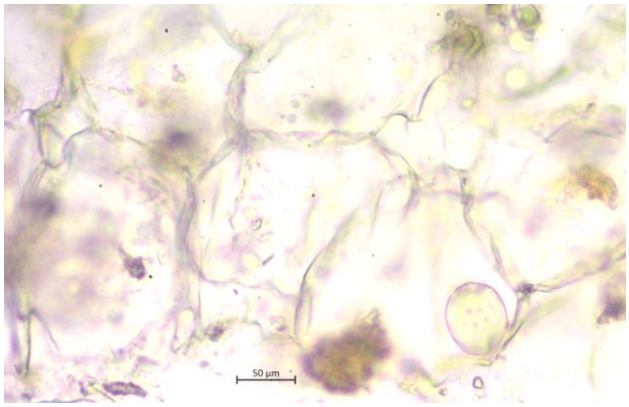

Figure 4c: Parenchyma with oil globules

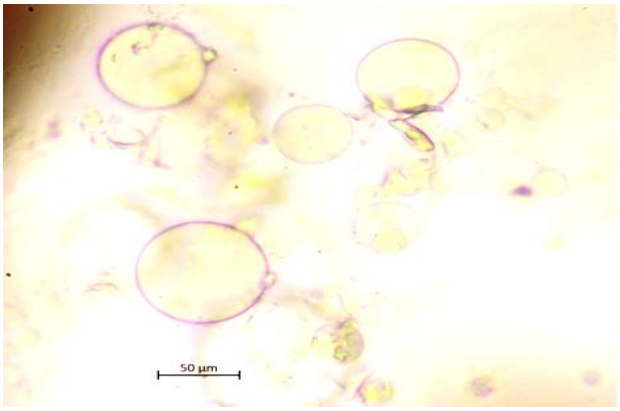

Figure 4d: Oil globules

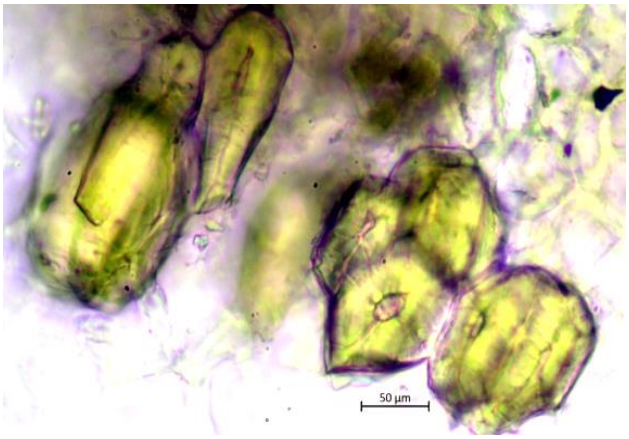

Figure 4e: Stone cells

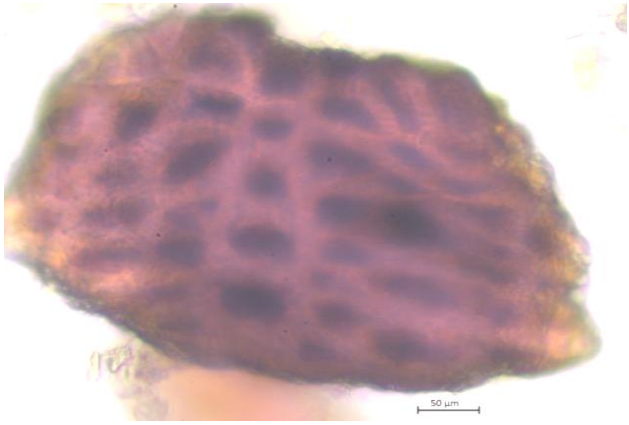

Figure 4f: Stone cells enlarged

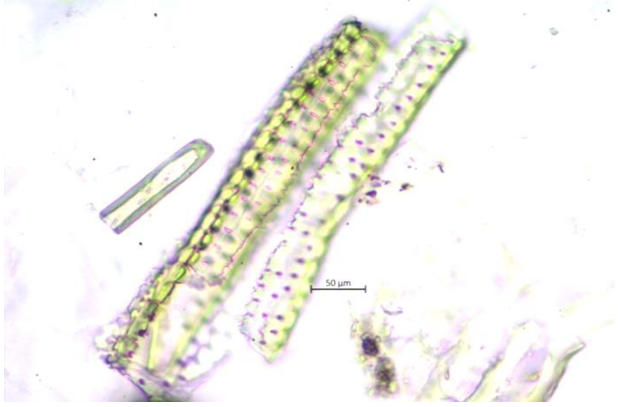

Figure 4g: Small pitted vessels

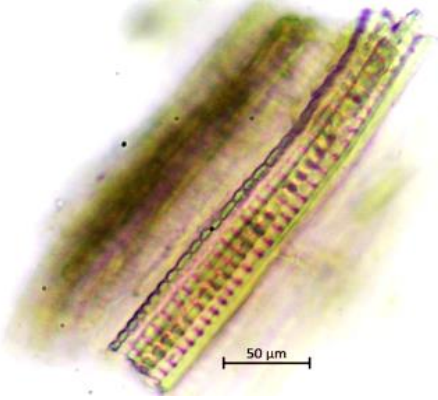

Figure 4h: Reticulate vessels

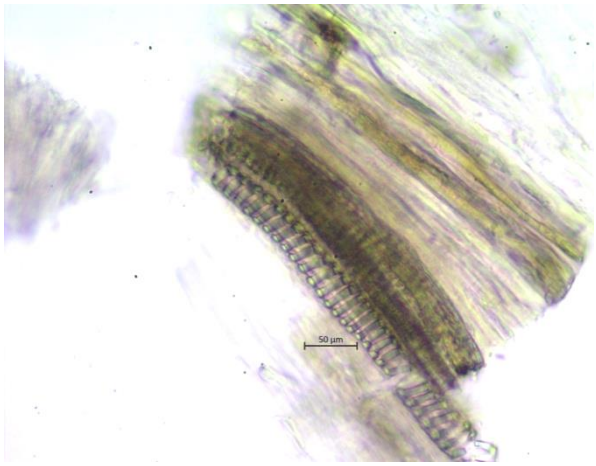

Figure 4i: Spiral vessels

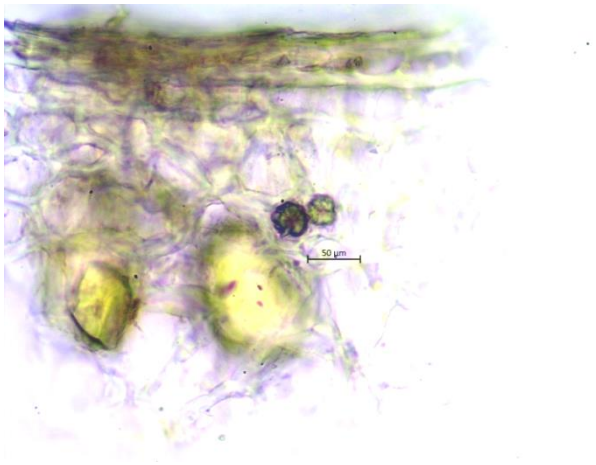

Figure 4j: Cluster crystals from seed

Figure 4: Powder Microscopy of *Passiflora tarminiana* Fruit

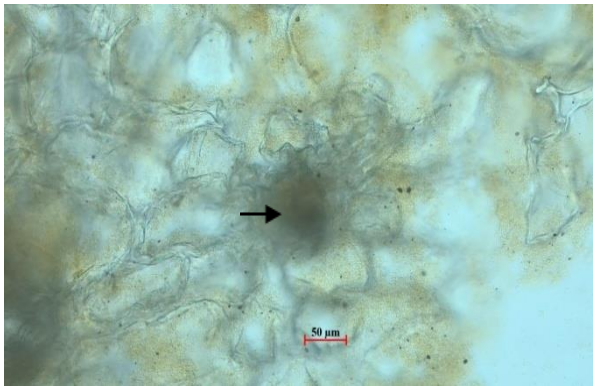

Figure 5a: Tannins

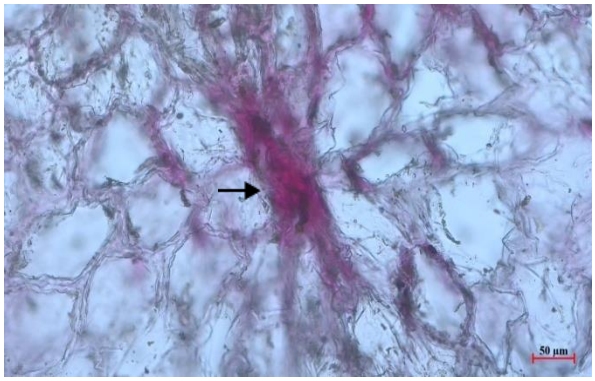

Figure 5b: Mucilage

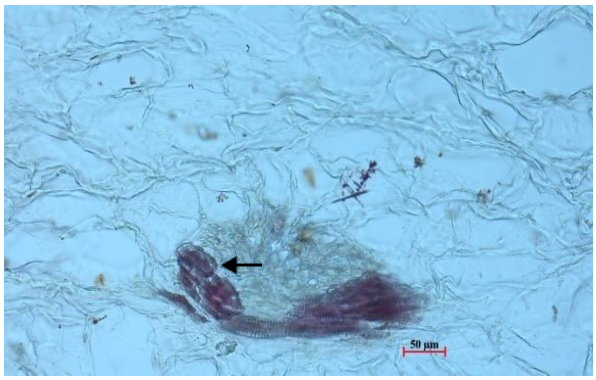

Figure 5c: Lignin

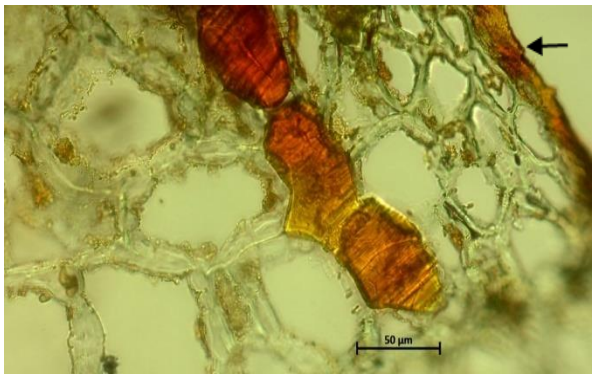

Figure 5d: Alkaloid

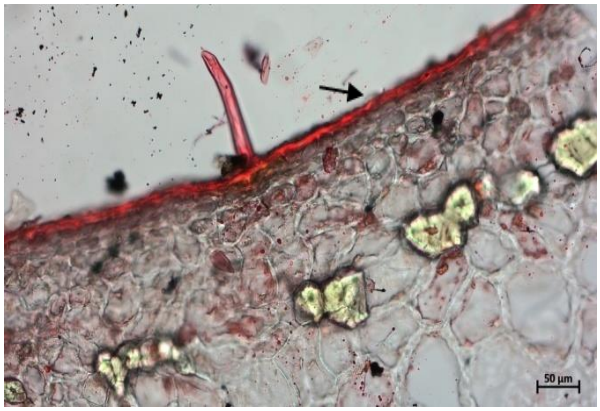

Figure 5e: Cutin

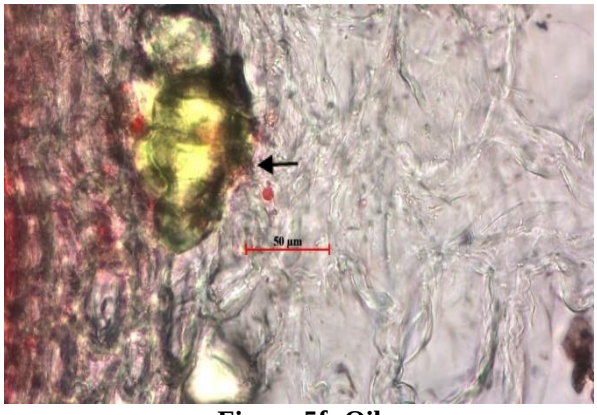

Figure 5f: Oil

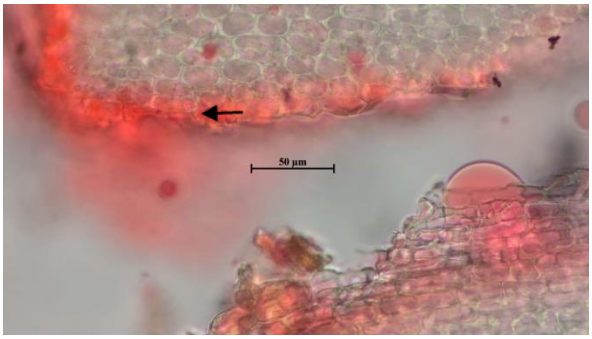

Figure 5g: Cutin (Cotyledon)

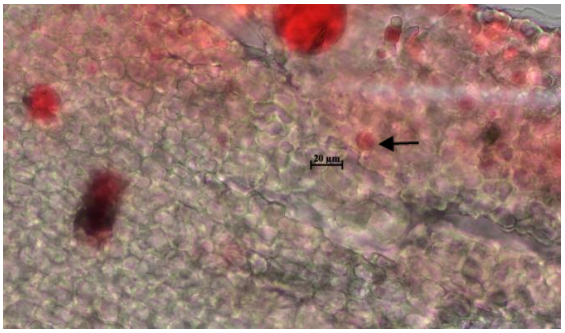

Figure 5h: Oil (Cotyledon)

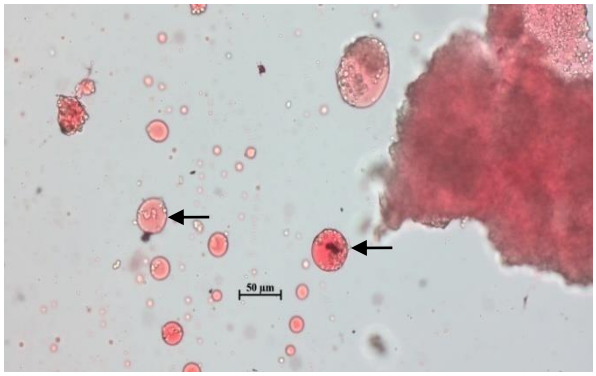

Figure 5i: Oil

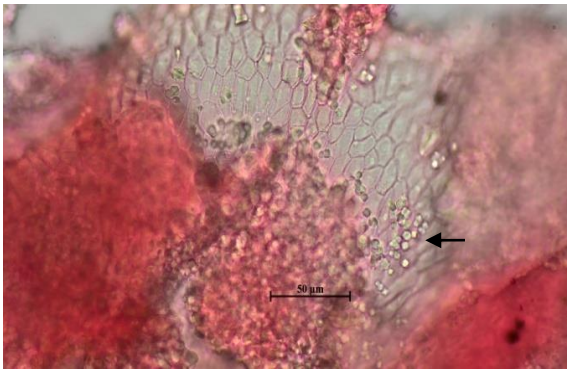

Figure 5j: Aleurone grains (Endosperm)

Figure 5: Histochemistry of *Passiflora tarminiana* pericarp

Conclusion

Ensuring the quality control of raw drugs and herbal formulations is crucial for their acceptance in the modern system of medicine. However, a significant challenge faced by the herbal drug industry is the absence of a consistent quality control profile for both raw herbal material and its formulations. A literature review highlighted the lack of pharmacognostic data on the fruits of the *P. tarminiana* plant. Consequently, this study was undertaken. Microscopical standardization plays a vital role in maintaining the quality control of herbal drugs, encompassing the entire spectrum of research from a plant's inception to its clinical application. The study involved macroscopy and a microscopical examination including transverse sections and powder analysis of *P. tarminiana* fruits.

The present study suggests that microscopical examinations will furnish the necessary standards for identifying and authenticating the plant material. The ongoing assessment of additional parameters including proximate analysis such as moisture content, ash values, extractive values and preliminary phytochemical screening, will contribute to establishing comprehensive quality control parameters.

Using the findings from these investigations, the data generated in this study can be applied for standardizing powdered samples of *P. tarminiana* fruits. This standardization is essential to uphold the quality of the powdered crude drug during the formulation process for *P. tarminiana* fruits.

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References

1. Azhagu R.C. and Ajithadas A., Pharmacognostic study and development of quality parameters of whole plants of *Trichodesma indicum* (Linn.) R., *Asian Journal of Pharmaceutical and Clinical Research*, **6(3)**, 167-169 **(2013)**

2. Chandima W. and Sirimal P., Comparative powder microscopy of *Alpinia calcarata* Roscoe and *Alpinia galanga* (Linn.) Willd., *Ayu*., **33(3)**, 441-443 **(2012)**

3. Fahn A., Plant anatomy, 3rd ed., Pergamon Press, Oxford **(1980)**

4. Geo C.E., Victoria E.B., Peter M.J. and John M.M., Passiflora tarminiana, a new cultivated species of Passiflora subgenus Tacsonia (Passifloraceae), *NovonA Journal for Botanical Nomenclature*, **11(1)**, 8–15 **(2011)**

5. Haseeb A., Shannon R.S., David M.E., Thomas C.T., Farrukh A., Hasan M. and Nihal A., Protective effect of sanguinarine on ultraviolet B-mediated damages in SKH-1 hairless mouse skin: implications for prevention of skin cancer, *Photochemistry and Photobiology*, **83**, 986–993 **(2007)**

6. Hulya C., Merve M. and Seyma M.Y., Determination of the effects of flaxseed (*Linum usitatissimum L.*) sprouts powder on the gluten-free cupcake quality, *Journal of Food Processing and Preservation*, **45(5)**, 15435 **(2021)**

7. Ingale A.G. and Hivrale A.U., Pharmacological studies of *Passiflora* sp. and their bioactive compounds, *African Journal of Plant Sciences*, **4(10)**, 417–426 **(2010)**

8. Jayanthy A., Prakash Kumar U. and Remashree A.B., Seasonal and geographical variations in cellular characters and chemical contents in *Desmodiumgangeticum* (L.) DC. – an ayurvedic medicinal plant, *International Journal of Traditional Herb and Medicine*, **2(3)**, 34-37 **(2013)**

9. Jignesh K. and Bhupesh P., Identification of fruits of *Tribulus terrestris* Linn. and *Pedalium murex* Linn: a pharmacognostic approach, *Ayu*., **32(4)**, 550–553 **(2011)**

10. Joshi A., Pathak A.K. and Mukul T., Comparative pharmacognostic characterization of selected species of *Ocimum*., *Journal of Drug Delivery and Therapeutics*, **4(2)**, 72-80 **(2014)**

11. Khandelwal K.R., Practical Pharmacognosy, 19th ed., Niraliprakashan **(2008)**

12. Kripa K.G., Sangeetha R. and Chamundeeswari D., Pharmacognostical and phytochemical evaluation of the plant *Leucas aspera*, *Asian Journal of Pharmaceutical and Clinical Research*, **9(2)**, 263-268 **(2016)**

13. Mandal B. and Madan S., Comparative standardisation and physicochemical evaluation of the leaves of Stevia rebaudiana Bertoni from different geographical sources, *Pharmacognosy Journal*, **3(25)**, 21-26 **(2011)**

14. Rahul K.L., Deeba N.S., Vaqar M.A., Mohammad I.K. and Hasan M., Dietary Polyphenols in prevention and treatment of prostate cancer, *International Journal of Molecular Sciences*, **16**, 3350-3376 **(2018)**

15. Samira B., Arezou K., Seid M.J., Zahra P. and Amir M.M., Seed mucilages as the functional ingredients for biodegradable films and edible coatings in the food industry, *Advances in Colloid and Interface Science*, **280**, 102164 **(2020)**

16. Squillaro T., Cimini A., Peluso G., Giordano A. and Melone M.A.B., Nanodelivery systems for encapsulation of dietary polyphenols: an experimental approach for neurodegenerative diseases and brain tumors, *Biochemical Pharmacology*, **154**, 303-317 **(2018)**

17. Wallis T.E., Analytical Microscopy - Its aims and methods in relation to foods, water, spices and drugs, 3rd ed., Boston, Little, Brown and Company **(1965)**.

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