Research Article

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ANATOMICAL INVESTIGATION ON THE LEAVES AND STEM OF *PASSIFLORA INCARNATA* (PASSIFLORACEAE)

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ABSTRACT

The present study was designed to explore the anatomical features of leaf and stem of *Passiflora incarnata*. Macroscopical, microscopical analysis for the standardization of *Passiflora incarnata* (Passifloraceae) which has been widely used to treat various disorders like cancer, insomnia, ulcer and convulsion etc. Leaves-green colour, palmate shape with 3 lobes, obtuse apex, serrate margin, pinnate venation. Stem- green colour, cylindrical shape. Microscopic evaluation revealed the presence of anamocytic or anisocytic stomata, unicellular or uniseriate covering trichomes, calcium oxalate crystals, simple unbranched termination, biforked termination and repeatedly branched dendroid type of termination. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. These characters are especially important for identification of drugs. These studies will contribute further investigation on this plant.

Keywords: *Passiflora incarnata*, leaf, stem, insomnia, calcium oxalate crystals

INTRODUCTION

Passiflora incarnata Linn is dicotyledonous plant belonging to the family Passifloraceae. A perennial, creeping herb, climbing by means of axillary tendrils. Leaves alternate, palmate three to five serrate lobes. Flowers large, solitary, with long peduncles, whitish, with a triple purple and pink crown. Fruits are ovate berries containing numerous ovoid, flattened seeds covered with a yellowish or brownish aril¹⁻³.

It has been reported to contain flavonoids as the major phytoconstituents. These include apigenin, luteolin, quercetin, kaempferol etc. It has also been reported to contain alkaloids like harman, harmol. Carbohydrates such as raffinose, sucrose, d-glucose and d-fructose, amino acids, cyanogenic glycoside gynocardin and Cylcopentenoid cyanogenic glycosides- Passibiflorin, eoipassibiflorin, passitrifasciatin^{4,5}.

Based on the ethano-medicinal information it has been revealed that *Passiflora incarnata* has been used as an antispasmodic, sedative, narcotic, neuralgia, insomnia, epilepsy, ulcer, haemorrhoids⁶.

MATERIALS AND METHODS

Collection of plant material

The leaves and stem of *Passiflora incarnata* were collected from Salem, Tamilnadu, India. The plant was identified and authenticated (PARC/2015/3037) by senior plant Taxonomist at Plant Anatomy Research Centre (PARC).

Specimen preparation

Petiole, leaf, stem and tendrils were fixed in FAA solution (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary –Butyl alcohol as per the schedule given by Sass, (1940). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60° C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was about 10-12 µm. Dewaxing of the sections was done by customary procedure⁷. The sections were stained with Toluidine blue as per the method given by O'Brien⁸.

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared⁹. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining⁷.

Photomicrographs

Photomicrographs were taken with the help of Nikon lab photo 2 microscopic Unit. For normal observations bright

field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringence property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars.

RESULTS & DISCUSSION

Macroscopical studies (Table 1 and 2, Figure 1-2.4)

Table: 1 Organoleptic Evaluation of leaf

Colour	Green colour
Shape	Palmate with 3 lobes
Odour	No distinct odour
Texture	Soft and coriaceous (Leathery)
Apex	Obtuse
Base	Symmetrical
Petiole	Petiolate
Leaf arrangement	Alternate
Margin	Serrate
Venation	Pinnate

Leaf also consists of extra floral nectaries and stipules

Table 2: Organoleptic evaluation of stem

Colour	Green
Shape	Cylindrical
Odour	No distinct odour

Stem bears tendrils in leaf axils

Microscopic studies of the leaves¹⁰⁻¹³

Structure of the leaf

In cross sectional view the leaf exhibits prominent midrib and thin, smooth and glabrous lamina (Figure 3.1). The midrib consists of thick and wide adaxial cone and prominent semi-circular abaxial part. The midrib is 900 µm thick. The adaxial cone is 300 µm wide and the abaxial part is 900 µm wide.

The midrib consists of well-defined epidermal layer of cells which are squarish with short, cuticular echinate outgrowths. The ground tissue in the adaxial cone is collenchymatous and in the remaining part the ground tissue is parenchymatous. The cells are angular, thin walled and compact. The vascular system is multistranded. There are 3 vascular bundles forming an abaxial arc and a single adaxial bundle. All the bundles are collateral and are more or less wedge shaped. In all the bundles, the xylem elements are directed towards the centre of the midrib. The bundles have compact masses of wide, circular-thick walled xylem elements. Phloem occurs in large compact mass on the outer part of the xylem strands. The phloem elements are wide and thick walled.

Lateral Vein

The lateral vein consists of a prominent adaxial cone with collenchymatous inner cells. The abaxial part is semicircular and consists of thick walled epidermal cells followed by 2 or 3 layer of similar type of thick walled

cells. The ground tissue is parenchymatous, angular and compact. The vascular strand is single, fairly large and collateral. The xylem elements are in diffuse masses having thick walled cells with wide lumen. Phloem elements occur in a few discrete masses Figure (3.2).

Lamina

The lamina is distinctly dorsiventral and amphistomatic. The adaxial epidermis consists of thick rectangular cells with thick cuticle. The abaxial epidermal cells are wide and rectangular with thin cuticle. The mesophyll tissue consists of adaxial layer of single vertically elongated thin palisade cells and abaxial zone of spherical and lobed spongy mesophyll cells. The abaxial layer is undulate with shallow depressions (Figure 3.3). The lamina is 120 µm thick.

Leaf margin

The leaf margin is broadly conical measuring 100 µm in thickness. It includes thick walled, squarish epidermal layer with highly prominent cuticle. The inner tissues of the marginal part include angular and circular thick walled compact mass of cells. Palisade and spongy mesophyll tissue are not evident in the marginal part. (Figure 3.4).

Epidermal cells and stomata

Paradermal sections were prepared for studying the epidermal tissues of the lamina. The epidermal cells are smaller with thin highly wavy anticlinal walls. The stomata are abundant and diffuse in distribution. Stomata are either anamocytic (Figure 4.1) or anisocytic. In the anamocytic type of stomata no distinct subsidiary cells are evident. In the anisocytic type the stomata have one smaller and 2 larger subsidiary cells. The adaxial epidermis have fairly wider epidermal cells with wavy anticlinal walls. The stomata are less in frequency. The stomata are elliptical measuring 45x60 µm in size. The stomatal pore is narrow slit like in appearance.

Trichomes

The lower epidermis has numerous unicellular or uniseriate covering trichomes (one to three cells) approximately 80–250 µm long, are located predominantly along veins (Figure 4.3).

Crystals

Calcium oxalate druses are densely distributed along the veins of the lamina. The druses are in uniseriate strand (Figure 4.2). They are about 15 µm in diameter.

Venation pattern

The venation is densely reticulate and the veins are gradually reduced in thickness. The ultimate veins forms the well-defined wide polygonal vein islets. Vein terminations are well developed in all islets. They vary

from simple unbranched termination, biforked termination and repeatedly branched dendroid type of termination. The terminations are uniformly thin, straight or curved (Figure 5).

Petiole

The petiole is horizontally flat with adaxial shallow groove (Figure 6). It is multistranded having about 8 vascular bundles organized into distinct pattern, amidst the 8 vascular bundles, 7 bundles are arranged in a shallow arc with wide gaps in between them. All the eight bundles are located in the adaxial median part.

The petiole consists of thin layer of epidermis and 3 or 4 layers of thick walled cells resembling the epidermal cells. The remaining ground tissue consists of large, angular or circular thin walled parenchyma cells. The vascular bundles are collateral and the xylem elements are facing towards the centre. The xylem elements are short, parallel, compact and they are circular and thick walled. Phloem occurs in thick mass on the outer part of each vascular bundle.

Stem

The stem is circular in sectional view measuring 2.4 mm in diameter. It consists of a continuous epidermal layer, narrow cortex with a circle of several discrete fibre strands and a wide hollow cylinder of vascular elements. There is a wide pith with central empty cavity (Figure 7). The epidermal layer consists of small, thin walled elliptical cells. The cortical zone includes 6 or 7 layers of rectangular parenchyma cells. Along the inner border of the cortex occur thick segments of thick walled lignified fibres.¹⁴⁻¹⁵

The vascular cylinder consists of outer thin continuous layer of secondary phloem and inner cylinder of secondary xylem and primary xylem.

The secondary phloem includes wide, angular and thick walled sieve elements with well-preserved companion cells. The phloem parenchyma has small, thick walled cells. The secondary xylem consists of compact radial files of thick walled and lignified xylem fibres and a few wide, circular thick walled cells.

The inner boundary of the xylem cylinder exhibits the primary xylem strands. The primary xylem consists of 1 or 2 wide meta xylem elements and a group of small proto xylem elements. The secondary xylem vessels are up to 80 µm in diameter.

Tendrils

The tendril is circular in sectional view with slightly undulate outline (Figure 8). It is 1.25 mm thick. The tendril consists of fairly large radially oblong thin walled epidermal cells with thick cuticle.

The cortical zone includes a small rectangular outer layer of thick walled cells and 2 or 3 inner layer of wide thin walled parenchyma cells. The vascular cylinder consists of an outer circle of thick curved segments of fibres and a thin layer of phloem encircling a thick cylinder of xylem. The xylem includes mostly thick walled wide lignified fibres and a few vessels which are not well distinct from xylem fibres.

The central part of the tendril consists of circular, fairly thick walled and lignified compact cells

Powder microscopy

The powder microscopical study showed the presence of the following microscopical features (Figure 9)

Druses

Calcium oxalate druses were located in the vertical strands along the veins. The druses are quite wide and thick measuring 20 µm in diameter.

Trichomes

Uniseriate trichomes with one to three thin-walled cells, straight or slightly curved, ending in a point or sometimes as a hook.

Vein termination

Fragments of lamina showed the presence of branched dendroid type of vein terminations.

Stomata

Abaxial epidermal peelings showed the presence of epidermal cells and stomata. The epidermal cells are amoeboid in outline with wavy anticlinal walls. Stomata were densely distributed and they are anamocytic type. The guard cells are elliptical and thin walled.

Fibres

Xylem fibres are predominantly seen in the powder. They are of 2 types, some are wide and others are narrow. The wide fibres are thin walled, short with wide lumen. They are 400 µm long and 20 µm wide. The narrow fibres are comparatively thick walled with reduced lumen. They are also 400 µm wide but 10 µm thick.

Parenchyma cells

Parenchyma cells are abundant and scattered in the powder. They vary in shape and size. They may be narrow and rectangular or wide and elongated. Some of the parenchyma cells are squarish and thin walled. The parenchyma cells are up to 140 µm long and 20 µm thick. Some of these cells have granular inclusions. These cells are also attached laterally with the vessel elements.

Tracheids

Occasionally tracheids are seen in the powder. The tracheids are long, narrow with dense pits on the lateral wall. The tracheids do not have end wall perforation.

Vessel elements

Vessel elements are more common in the powder. They are mostly long narrow cylinders. Some vessel elements are comparatively shorter and wider. The vessel elements have wide, circular, horizontal end wall perforations on the lateral walls are seen dense multiseriate and circular bordered pits. The narrow vessel elements are 320 μm long and 50 μm wide. The short wide vessel elements are 270 μm long and 80 μm wide.

Figure 1: Aerial parts of *P. incarnata*

Figure 2.1: Serrate margin

Figure 2.2: Extra floral nectaries

Figure 2.3: Tendrils

Figure 2.4: Stipules

Macroscopical (Features of *P. incarnata*)

Figure 3.1: T.S of midrib enlarged

Figure 3.2: T.S of lateral vein

Figure 3.3: T.S of lamina

Figure 3.4: T.S through margin

Figure 4.1: Stomata enlarged

Figure 4.2: Vertical row of druses

Figure 4.3: Trichomes

Figure 5: Venation pattern

Figure 6: T.S of petiole

Figure 7: T.S of stem

Figure 8: T.S of tendril

Druses

Trichomes

Venation

Stomata

Fibers

Parenchyma Cells

Tracheids

Vessel Elements

Figure 9: Powder Microscopy

CONCLUSION

The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. These characters are especially important for identification of powdered drugs, because in these cases most of the morphological diagnostic features are lost. Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials. In our present work we selected the plant *P. incarnata* L. (Passifloraceae). The macroscopic and organoleptic characters of the plant can serve as diagnostic parameters. The microscopic study revealed the presence of anamocytic or anisocytic stomata, unicellular or uniseriate covering trichomes, calcium oxalate crystals, simple unbranched termination, biforked termination and repeatedly branched dendroid type of termination. In conclusion, the present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant.

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