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Pharmacognostical analysis of leaf and stem of *Ipomoea carnea* (L.) Jacq (Convolvulaceae) grown in India

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Abstract

The medicinal activity of *Ipomoea carnea* exists in the leaf and stem of the plant. Lack of substantial information on pharmacognostical aspects both macro and microscopical features of *Ipomoea carnea*, this work aimed to investigate the pharmacognostic features of stem and leaf. Various morphological characters were studied the leaf was observed to 12-23 c.m. long heart shaped, green, odourless, entire margin, symmetric base, reticulate pinnate venation and hairy surface on both sides. The stem of the plant was observed to be cylindrical in shape, 0.5-0.9 c.m. in diameter, green, odourless and hairy surface. The leaf has paracytic stomata, multicellular glandular trichomes along the leaf margins and vascular bundles on the dorsal side. The stem revealed wide cortex, pith, medullary rays etc. The main features for identification are stomata, trichomes, medullary rays and vascular bundles. The histochemical analysis revealed the presence of lignin, calcium oxalate, resin cell in leaves and starch grains, lignin, calcium oxalate resin cells in stem. The powder microscopy of leaf and stem exhibited the presence of fragments of epidermal cells, trichomes (leaf) resin cells, schizogenous cells, palisade cells (leaf), wood parenchyma and medullary rays (stem), calcium oxalate crystals, xylem vessels (stem), stomata (leaf) and starch grains (stem).

Keywords: *Ipomoea carnea*, stomata, trichomes, histochemical analysis, morphology

Introduction

Plants are the natural laboratories where the simple chemical structure is transformed to a complex drug moiety. They are the rich source of pharmaceuticals new bioactive and cosmetics. Thus wide scope and utilisation of plants for the betterment of human health has call for the development of bonafide guidelines for the herbal standarization. Among the quality control parameters macroscopic evaluation and microscopic evaluation are indispancable. The macroscopic evaluation acts as an important tool in the identification and authentication of a plant or a plant part. However the macroscopic evaluation of the plant is affected by adulterants or its substitutes. So to overcome this issue, if any, the microscopy is performed, which acts as an important diagnostic tool and also assists in the proper identification of the plant.

These parameters are crucial for the preparation of effective, safe and quality herbal drug formulation. Considering the importance of evaluation, the undertaken work was performed to reveal the macroscopic and microscopic features of *Ipomoea carnea*, commonly known Beshram (H) and Morning Glory (E) belonging to the family Convolvulaceae. It is widely distributed throughout the American tropics, Argentina, Brazil and Bolivia^{1, 2}. This plant species has also been reported from India, West-Pakistan and Srilanka. It is a wild herb distributed throughout India. It spite of various reported work on different ipomoea species, till date a comprehensive study of pharmacognostical characters of *Ipomoea carnea* is inaccessible. Therefore the present investigation was undertaken to identify and standardise the features of leaf and stem of *Ipomoea carnea* L. Jacq. species, which retains the medicinal activity of the plant.

Materials and Methods

Selection and collection

Fresh leaves and stem of the selected plant was collected in the month of July from Jabalpur Madhya Pradesh, India. The leaves and stem was dried under shade and stored in air tight container for further studies.

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Authentication of Plant

The plant was authenticated by the Taxonomist, Dr. A. B. Tiwari, Associate Professor, Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India.

Pharmacognostic study of plant

Macroscopic Evaluation

The macroscopic characters for fresh leaves and stem were observed for size and shape, height, diameter, flexibility, colour, odour, surface, venation, apex, margin, base, lamina and texture. (Mousa A.Y. *et al*)

Microscopic Evaluation

The specimens of the proposed study were collected with care. Then selected part of the plant was washed thoroughly with running water to remove adherent soil, dirt etc. and fixed in FAA (Formalin 9: acetic acid 7: alcohol 3) for microscopic investigation. Free hand sections both transverse and longitudinal were taken from the preserved material of the leaf and stem. The section was cleared with chloral hydrate and then stained with hydrochloric acid and saffrine dye to observe the lignifications of cell wall. (Majumdar *et al* 2013 irjp) [6]. Sections were mounted on slide in glycerine and studied under the microscope. Photomicrographs of section were taken by camera (Nikon 14 mp).

Stomatal number

It is the average number of stomata per square mm of the epidermis of the leaf. Stomatal index is the percentage which the number of stomata forms to the total number of epidermal cells, each stomata being counted as one cell.

The middle part of leaf was clear by boiling with chloral hydrate solution. Upper and lower epidermis was peeled out separately. Then mounted with glycerine and observed under the slide. A square of 1 mm was drawn by micrometre. The epidermis cell and stomata was observed with the help of camera. The number of stomata presents in the area of 1 sq. mm was counted. Recorded the result for each of the ten fields and calculated the average number of stomata per sq.mm. (Folorunso A. E. 2013, Gautam *et al* 2010) [4, 5].

Stomatal index can be calculated by using following equation:

$$I = \frac{S}{E+S}$$

I = Stomatal index,

S = No. of stomata per unit area,

E = No. of epidermal cells in the same unit area

Histochemical test

Histochemical test were conducted for starch grain (lugol), lignin (hydrochloric phloroglucin), calcium oxalate crystals (dilute sulphuric acid, Sudan III for lipophilic substances). (Duarte *et al* 2012) [1].

Powder microscopy

The dried stem and leaf were powdered separately. The fine powder was placed on a glass slide mounted with glycerine

and observed under the microscope; photographs were taken. (Kohli *et al* 2011) [7].

Result and Discussion

Ipomoea carnea grows to a height of 5-6 m, but acquires a shorter height in the aquatic habitats. The stem is thick and develops into a solid trunk over several years with many branches from base. The stem is erect, woody, hairy, and more or less cylindrical in shape and greenish in colour. It has alternate leaves. The internodes were present. The leaf is simple and petiolate, petiole is cylindrical. The upper surface of leaf is dull green and the lower surface is paler. The leaves which receive lesser sunlight may grow larger than the leaves which receive full sunlight.

Pharmacognostic study of plant

A. Macroscopic evaluation of leaf

The leaf of *Ipomoea carnea* revealed features as shown in Table 1 and Figure 1

Table 1: Macroscopic Features of *Ipomoea carnea* leaf

S. No.	Parameters	Description
1.	Size	12- 23cm Length and 5.0-9.0 cm in Width
2.	Shape	Heart Shaped
3.	Color	Rich green
4.	Odor	Odourless
5.	Apex	Cordate
6.	Margin	Entire
7.	Base	Symmetric
8.	Venation	Reticulate pinnate
9.	Surface	Hairy on both side
10.	Midrib	Prominent on lower surface

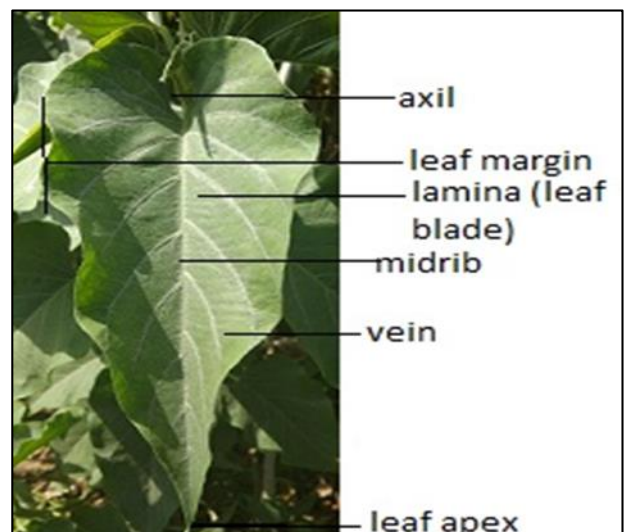


Fig 1: *Ipomoea carnea* Leaf Revealing Various Features

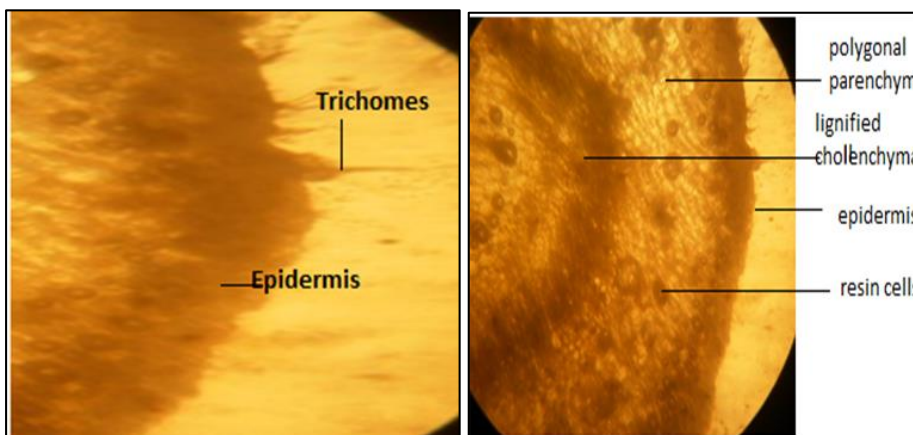
B. Microscopic evaluation of leaf

Microscopic study revealed the presence of epidermis, palisade, parenchyma and collenchyma, trichomes, stomata in *Ipomoea carnea*. The features are discussed as under (Table 2 and Figure 2).

Table 2: Microscopic Features of T.S. *Ipomoea carnea* leaf

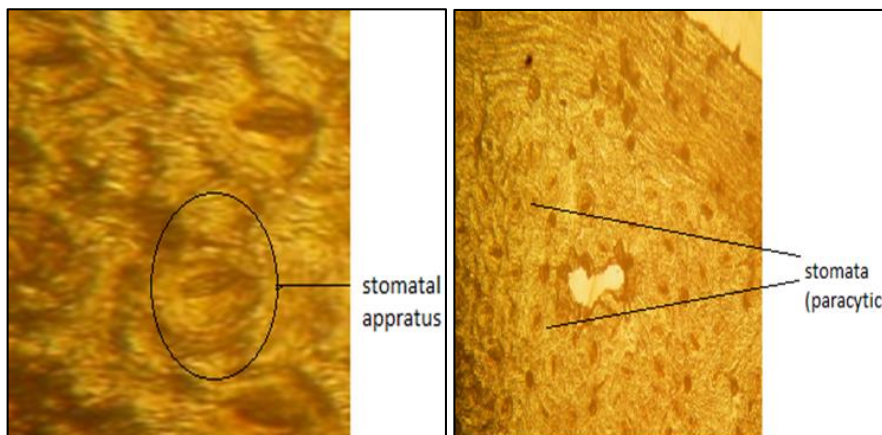
S. No.	Parameters	Description
1.	Upper Epidermis	Single layer Polygonal parenchymatous cells with trichomes
2.	Upper Palisade	2-3 layers of elongated parenchymatous
3.	Spongy parenchyma	Presence of parenchymatous cells between upper and lower palisade

4.	Lower palisade	Same to upper palisade 1-2 layer cells
5.	Lower Epidermis	Similar to upper epidermis with more trichomes
6.	Collenchyma	Presence multiple layers of both upper and lower end of midrib
7.	Vascular Bundle	Dorsal side of leaf contain lignified xylem and non lignified phloem fibre
8.	Cortical parenchyma	Multilayered compactly arranged parenchymatous cells are present approximately $\frac{3}{4}$ of midrib
9.	Trichomes	Glandular epidermal multicellular, branched, thin walled trichomes
10.	Stomata	Presence of paracytic type stomata
11.	Stomatal Index	13.5



a). T.S. of Leaf *Ipomoea carnea*
Show Trichomes

b). T.S. of leaf *Ipomoea carnea*



c) L.S. of leaf *Ipomoea carnea* show paracytic stomata

Fig 2: Microscopic Features of *Ipomea carnea* L. Jacq leaf

C. Macroscopic Evaluation of stem

The stem of *Ipomoea carnea* revealed features as shown in Table 1 and Figure 1

Table 3: Macroscopic Features of *Ipomoea carnea* stem

S. No.	Parameters	Description
1.	Shape	Cylindrical
2.	Color	Green
3.	Odor	No Odor
4.	Diameter	0.5- 0.9 c.m.
5.	Internodes	Hallow 3.2-5.9 cm in length
6.	Surface	Hairy

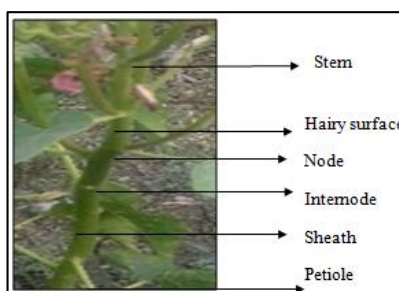


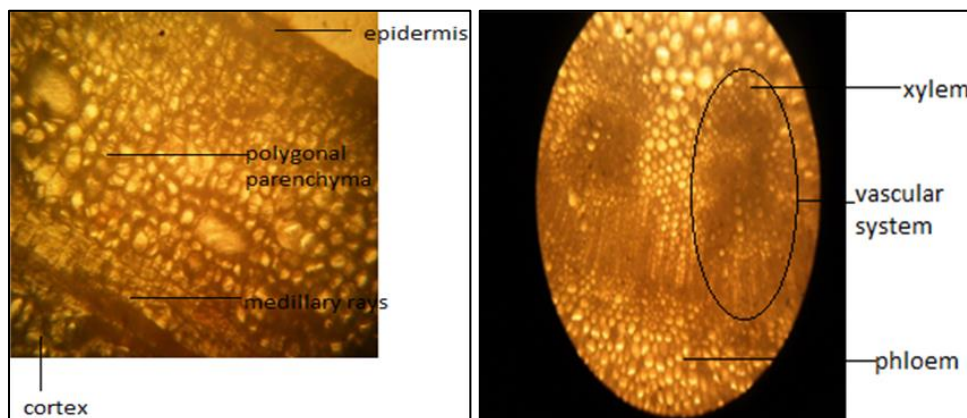
Fig 3: *Ipomoea carnea* stem revealing morphological features

D) Microscopic evaluation of stem

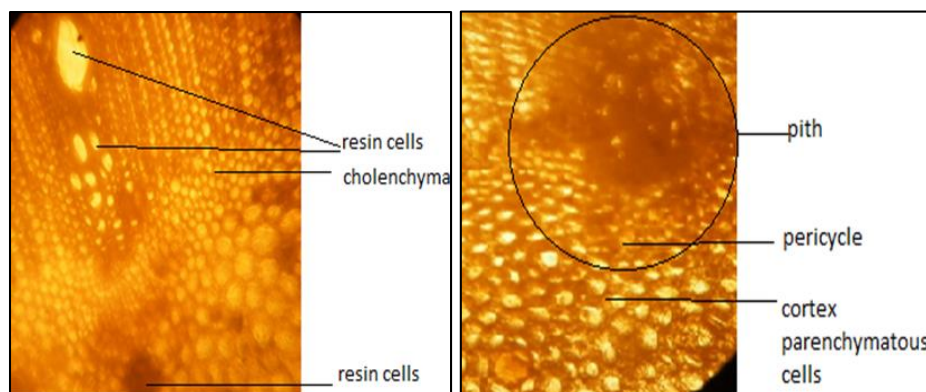
Histological observation of T.S and L.S. of stem have been detailed in Table 4 and Figure 4.

Table 4: Microscopic Features of T.S. and L.S. *Ipomoea carnea* Stem

S. No.	Parameters	Description
1.	Epidermal cells	Oval, thick cuticle, angular and compact parenchymatous cells
2.	Pith	Circular, thin walled parenchymatous cells with large size central pith contain few crystals of calcium oxalate, resin cells and shizogenous glands.
3.	Medullary rays	Three layers of elongated parenchymatous cells with close arrangement, uniseriate three layers of thin walled parenchymatous cells in phloem region and elongated cells with lignified walls in the xylem region.
4.	Vascular bundle	Closely aggregated phloem both outer and inner sides and primary lignified xylem vessels present
5.	Cambium	5-7 layers of tangentially elongated radially arranged cambiform cells



a). L.S. Of Stem *Ipomoea carnea* b). T.S. of stem *Ipomoea carnea* Show vascular bundles



c) T.S. of stem *Ipomoea carnea* d) T.S. of stem *Ipomoea carnea* show pith

Fig 4: Microscopic Features of *Ipomea carnea* L. Jacq Stem

E) Histochemical analysis

Table 5: Histochemical analysis revealed the presence of following constituents

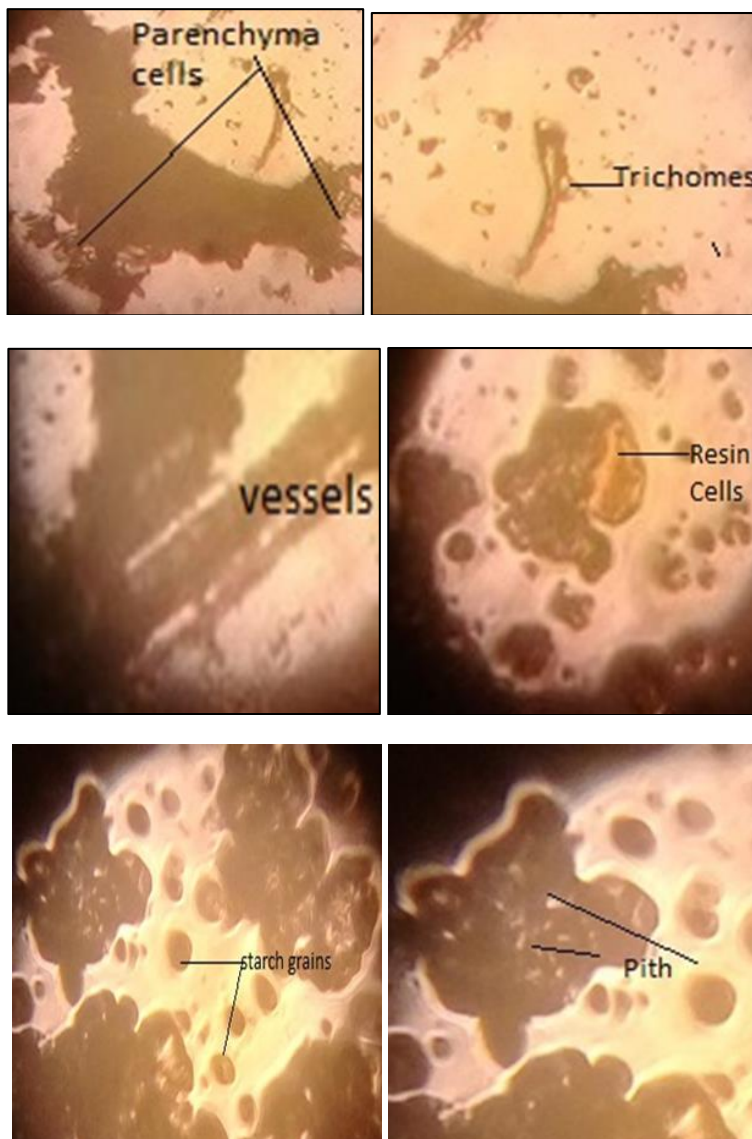
S. No.	Constituents	Observation	
		Leaf	Stem
1.	Starch Grains	- - -	+++
2.	Lignin	+++	++
3.	Calcium oxalate	+++	+++
4.	Resin cells	++	+++

F) Powder Microscopy

Powder microscopy of green color with no odor and taste dried leaves and stem of *Ipomoea carnea* characterized by followings in Table 6

Table 6: Powder Microscopy of *Ipomeea carnea* Leaf And Stem

Powder Microscopy		
S. No	Leaf	Stem
1.	Fragments of upper and lower epidermis with polygonal cells	Fragments of epidermal cells with polygonal cells
2.	Glandular trichomes are present	Fragments of thick lignified wood fibers
3.	Resin cells with yellowish brown contents	Show Resin cells
4.	Less schizogenous glands	Schizogenous glands
5.	Fragments of palisade cells	Fragments of wood parenchyma and medullary rays
6.	Cluster of calcium oxalate crystals	Cluster of calcium oxalate crystals
7.	Fragments of lignified vessels	Fragments of lignified xylem vessels
8.	paracytic stomata	Few starch granules

**Fig 5:** Microscopic features of *Ipomeea carnea* L. jacq stem and leaf powder microscopy

Discussions

The morphological as well as microscopy characteristics of the leaf and stem of *Ipomeea carnea* L. Jacq are in accordance with multiple aspects of convulvaceae family. The morphological analysis of leaf and stem implicates various characters of convulvaceae. The microscopy examination of leaf exhibited upper epidermis, lower epidermis, vascular bundle, midrib and trichomes, stomata. These parameters, were also reported by Folorunso A.E. in a taxonomic evaluation of fifteen species of *Ipomeea carnea* L. Jacq. In general, the leaf midrib leads a significant role in distinguishing the multiple species of plants. *Ipomeea carnea* depicts the prominent midrib on the lower surface. The microscopy of leaf depicts the existence of multiple layers of collenchyma cells

on the upper end lower end of midrib. So far, the multi layer arranged parenchymatous cells occupy approximately three fourth of midrib.

In considering the stem features, the results of work shows epidermis constituted of oval, thick cuticle, angular and compact parenchymatous cells. Distinguishing large central pith, uniseriate medullary rays consisting of thin wall parenchymatous cells, collateral vascular bundle and cambium form of 5-7 layers tangentially elongated radially arranged cambiform cells. These descriptions are in similarity with that reported by Mousa A.Y. *et al.*

Histochemical study demonstrates the presence of lignin, calcium oxalate crystals and resin cells in leaf, while in stem all these constituents along with starch grains were present.

The aim of the conducted research work inclines to powder microscopy of leaf demonstrates the presence of upper and lower epidermis, resin cells, schizogenous glands, fragment of palisade cells, crystals of calcium oxalate and paracytic stomata. The stem exhibits the presence of epidermal cells, thick lignified wood fibres, a few resin cells, schizogenous glands, medullary rays, the crystals of calcium oxalate, fragments of xylem vessels and few starch grains.

The powder microscopy is evaluated as a significant diagnostic tool. Here, in the presence of yellowish resin cells, paracytic stomata and glandular trichomes are the demarcating features of *Ipomoea* leaf.

The powder stem shows epidermal cells, thick lignified wood fibres, a few resin cells, schizogenous glands, medullary rays, the crystals of calcium oxalate, fragments of xylem vessels and few starch grains, fragments of wood fibres are the distinguishing characters of *Ipomoea carnea* stem.

For diagnostic and evaluation purposes, the various features viz, macroscopic, microscopic histochemistry of *Ipomoea carnea* discussed in the work can be utilised as a noticeable tool. These features can also be employed to detect the presence of adulterant and to check the purity of the selected plant. Hence these outcomes would definitely play an important role in the identification, evaluation and standardisation of the selected plant.

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