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Studies on anatomy and phytochemical analysis of *Ipomoea pes-tigridis* L.

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Abstract
The present study has been carried out to determine the requisite anatomical features of root, stem, leaf, petiole and phytochemical analysis for evaluating the *Ipomoea pes-tigridis* an important medicinal plant used in the traditional systems of medicine. This investigation provides referential anatomical and phytochemical information for correct identification of this plant.

Keywords: *Ipomoea pes-tigridis*, convolvulaceae, pharmacognosy, phytochemical analysis, anatomy

Introduction
Plant drugs are extensively utilized in many formulations in Ayurveda, Siddha and other traditional systems of medicine. To ensure the originality of the herbal raw drugs and to identify the adulterations, a valid pharmacognostic study is required for each raw drug. Usually the herbal drugs are collected by traditional practitioners who have inherited Ayurvedic, Siddha or other herbal practices. Their identification is mostly based on external features or other traditionally known characters. In such cases, there is a possibility of selecting incorrect raw drugs/adulterants. Therefore, an extensive histological and phytochemical screening is needed for each raw drug to avoid any ambiguity and such a study will serve also as a reference for further studies [1]. Anatomical studies are useful in describing a particular drug with a special emphasize on quantitative microscopy, such as sclereids, starch grains, crystals, stomata, and trichomes, and qualitative microscopy, such as xylem, phloem, and other tissues [2].
Ipomoea pes-tigridis L. is an annual twining hispid herb, belongs to the family Convolvulaceae, well known for its wide range of medicinal properties such as purgative, laxative, diuretic and used in sores and pimples treatment, haemorrhoids, arthritis, rheumatism, dropsy, swellings, oedema, gout, venereal diseases, boils, carbuncles and dog bites, pain killer, antidotes for venomous stings, snake bites etc [3-8]. The present study has been carried out to standardize the anatomical features of leaf, stem, petiole, roots and phytochemical analysis to serve as a possible tool for proper identification of *Ipomoea pes-tigridis* L.

Materials and Methods
Anatomical studies
For the present study, fresh whole plant (1.5 m length) (Figure – 1:1) was collected from Redhills, near Chennai and authenticated using regional flora [9]. Root, stem, petiole and leaf samples were cut in to small pieces and fixed immediately in Formalin-Acetic-Alcohol for 24h. After fixation they were washed thoroughly in distilled water, dehydrated, embedded in paraffin wax after infiltration and sectioned using rotary microtome to the thickness of 8-12 µm [10]. Sections were stained with Toluidine blue and photographed.

Fig 1: 1. Habit, 2. Dried plant

Phytochemical analysis
For the phytochemical analysis, whole plant was shade dried (Figure – 1:2) for a week and powdered. Powdered samples were subjected to physico-chemical analysis, such as the

percentage of water and alcohol soluble extractive, total ash, acid insoluble ash [11] and preliminary phytochemical screening was carried out using standard procedures [12-14].

Thin Layer Chromatographic (TLC) Analysis

Two grams of powdered sample was refluxed with 10 mL of methanol in a water-bath at 60 °C for 30 min, consecutively 3 times, and then concentrated and dried. The final extract was re-dissolved in methanol and used for the TLC analysis. Precoated Silica Gel F^{254} (Merck) plate was used for stationary phase and Toluene: Ethylacetate (3:1). After development, the plate was observed under UV-254nm and spots recorded. Then dipped in 1% vanillin sulphuric acid and heated at 105 °C in hot air oven for 5 min to develop the colour and the spots recorded.

Results

Anatomy of root (Figure – 2:1-4)

In transverse section of root measuring about 4-5 mm in diameter is roughly circular in outline with small fissures (Figure – 2:1. Outermost zone consist of radial bands of rectangular, tangentially elongated, thin-walled periderm about 8-12 layers (Figure – 2:3). Secondary phloem composed of phloem fibres in patches with thin walled parenchyma in between (Figure – 2:3). Starch grains and druse crystals of calcium oxalate found scattered in phloem parenchyma and cortex region (Figure – 2:4). The secondary xylem is splitted by medullary rays and formed fan wing shaped. Growth rings are fairly distinct and demarcated by small zone. Vessel elements are oval or circular in shape, wide, arranged in solitary (Figure – 2:1-2).

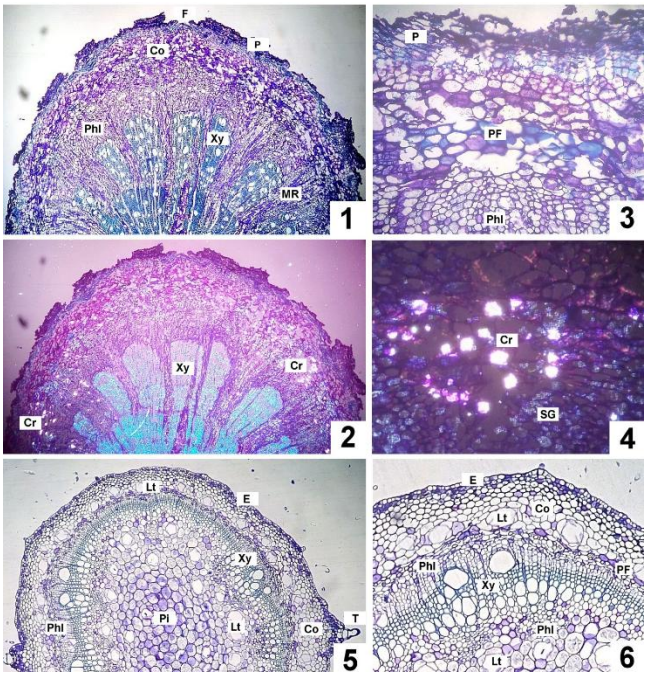

Fig 2: 1. Cross section of root, 2. Cross section of root under polarized light, 3.Periderm and cortex region, 4. Periderm and cortex region under polarized light, 5. Cross section of stem, 6. Cross section of stem – Portion enlarged

Anatomy of stem (Figure – 2:5-6)

In transverse section of stem measuring about 2.5-3 mm in diameter is roughly circular in outline with single layer epidermis covered with long unicellular filiform trichomes. Followed by epidermis 4-5 layers of collenchyma cells and then parenchyma cells 2-3 layers present. Well developed, broad laticifers cells are arranged circularly in the cortex and scattered in the pith region. A thin layer of phloem fibres are arranged continuously or discontinuously just above the phloem. Vascular bundles are well developed, collateral, open type, phloem region slightly compressed. Vessel elements are oval or angular in shape. Druse crystals of calcium oxalate found scattered in cortex region. Starch grains abundantly present in parenchyma cells of pith region (Figure – 2:6).

Anatomy of petiole (Figure – 3:4-5)

Petiole about 1-1.2 mm in diameter shows circular in outline and clear furrow in the adaxial side, consist single layer of epidermis with compactly arranged cubical cells and covered by filiform unicellular trichomes. 3-4 layers of collenchymatous cells present just below the epidermis. A few druses type of calcium oxalate crystals found scattered in the collenchymatous tissue (Figure – 3:5). Five vascular bundles arranged as three dorsal, two ventral and open type. Dorsal three bundles are fused and middle one is small comparatively and form girdle shape. The arrangement of vascular bundles expressed as 3 + 2.

Anatomy of leaf (Figure – 3:1-3; 6-7)

The epidermis consists of cubical or somewhat conical shaped cells, covered with trichomes (Figure – 3:7). Midrib and lamina regions are very distinct. Midrib is triangular in shape consist vascular bundle in center region. In the vascular region phloem surrounds the central xylem bundle (Figure – 3:1). In the lamina mesophyll cells are differentiated in to palisade and spongy parenchyma. Palisade parenchyma present below the upper epidermis consists of continuous single layered cells and vertically elongated. Spongy parenchyma lies below the palisade parenchyma and loosely arranged. Spongy parenchyma consist druses type of calcium oxalate crystals (Figure – 3:2-3). Stomata are amphistomatic and paracytic type (Figure – 3:6).

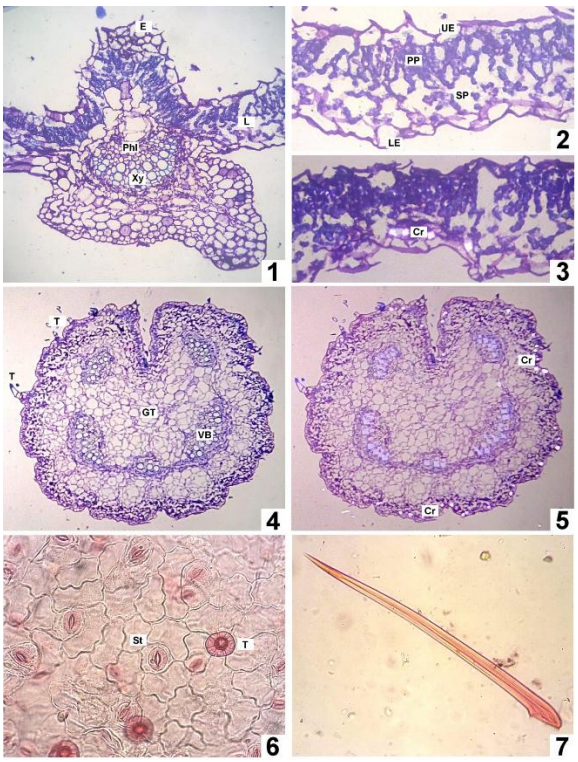

Fig 3: 1. Cross section of leaf midrib, 2. Lamina, 3. Lamina under polarized light, 4. Petiole, 5. Petiole under polarized light, 6. Stomata, 7. Filiform trichome

Phytochemical Analysis

The results of physico-chemical analysis, presence and absence of different phytoconstituents and TLC fingerprint profiles are presented below.

Physico-chemical analysis

Parameters	Values (%)
Water soluble extractives	17.44 ± 0.50
Alcohol soluble extractives	7.28 ± 0.24
Total Ash	10.43 ± 0.22
Acid insoluble ash	0.85 ± 0.04

Value from triplicate (Mean ± SD)

Preliminary phytochemical evaluation

Chemical constituents	Results
Flavonoid	+
Alkaloid	+
Tannin	+
Terpenoid	+
Steroid	+
Saponin	+
Cardiac glycosides	+
Anthraquinone glycosides	+

+ - Positive

Thin Layer Chromatographic (TLC) analysis (Figure – 4: A-B)

Rf – values (UV – 254 nm)	Rf – values (Visible Light)
--	0.24 (Grey)
0.30 (Black)	0.30 (Light grey)
0.42 (Black)	0.42 (Blue)
--	0.48 (Violet)
--	0.54 (Purple)
0.62 (Black)	0.62 (Violet)
--	0.66 (Dark blue)

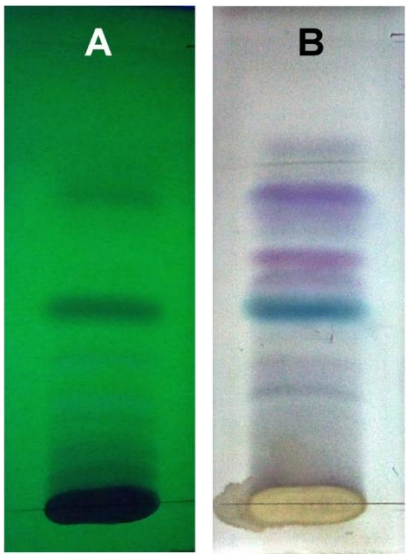

Fig 4: A. Under UV-254 nm, B. Under visible light

(Co – Cortex; Cr – Crystal; E – Epidermis; F – Fissure; GT – Ground tissue; L – Lamina; LE – Lower epidermis; Lt – Laticifer; MR – Medullary rays; P – Periderm; PF – Phloem fibre; Phl – Phloem; Pi – Pith; PP – Palisade parenchyma; SG – Starch grains; SP – Spongy parenchyma; St – Stomata; T – Trichome; UE – Upper epidermis ; VB – Vascular bundle; VE – Vessel element; Xy – Xylem)

Discussion and Conclusion

Traditional systems of medicine such as Ayurveda and Siddha uses majority of the crude drugs from plant origin. It is necessary that standards have to be laid down to control and ensure the identity of the plant and ascertain its quality before use. A comprehensive pharmacognostic evaluation therefore is highly essential prerequisite [15]. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [16].

Ipomoea pes-tigridis L. widely used in traditional medicines has tremendous medicinal potential owing to its multifaceted biological functions [17]. However, there are no detailed anatomical and phytochemical studies on whole plant to help in the proper identification. Hence, the present study was undertaken with the aim to provide key diagnostic tools of identification. The following anatomical and phytochemical features of the above drug are the key features that can be used to diagnose this plant.

Root:

Well developed periderm with small fissure. Presence of starch grains and druse crystals in phloem parenchyma and cortex region. Wing shaped secondary xylem with large medullary rays. Distinct growth ring and oval or circular shape vessel elements, arrangement in solitary.

Stem

Single layer epidermis covered with unicellular filiform trichomes. Presence of laticifers in the cortex and pith region. Phloem fibres are arranged continuously or discontinuously. Collateral and open type of vascular bundles. Vessel elements are oval or angular in shape. Presence of druse crystals in cortex region. Presence of starch grains in pith region.

Petiole

Epidermis covered by filiform unicellular trichomes. Presence of druse crystals in collenchyma. Five vascular bundles arranged as three dorsal, two ventral and open type. Dorsal vascular bundles girdle shaped.

Leaf

Epidermis covered with trichomes. Midrib triangular in shape consist vascular bundle in center region. Presence of druse crystals in spongy parenchyma and paracytic type of stomata.

Phytoconstituents

Presence of flavonoid, alkaloid, tannin, terpenoid, steroid, saponin, cardiac glycosides, anthraquinone glycosides.

TLC profile

Two major spots at 0.42 & 0.62 under UV-254nm and three major spots at 0.42 (Blue), 0.54 (Purple) & 0.62 (Violet) under visible light.

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