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Full Length Research Paper

Microanatomical and high performance thin layer chromatographic (HPTLC) standardization of *Ipomea pes-tigridis* L (Convolvulaceae) aerial parts

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Ipomoea pes-tigridis **is an annual herbaceous twining vine which belongs to the family Convolvulaceae. Each part of the plant is used for multitude biological uses. The current work is oriented towards exploring the microanatomical, physicochemical and phytochemical aspects of the plant. The transverse section (TS) of the leaf and TS and longitudinal section (LS) of the stem were studied. The powdered microscopical studies of leaf, stem and fruits were observed. The chemo microscopical studies using various staining reagents like toluidine blue, safranin, schules reagent and sudan III were performed. The physicochemical parameters performed were determination of ash values, extractive values and fluorescence analysis. The aerial parts of the plant was dried and powdered and then after defatting with petroleum ether was extracted with methanol and then subjected to phytochemical screening. The thin layer chromatographic (TLC) analysis was carried out for the methanolic extract followed by the high performance thin layer chromatographic (HPTLC) standardization. The anatomy of the leaf, stem and fruits showed abundant long, slender, uniseriate covering trichomes and sessile glandular trichomes. The main tissue of diagnostic tissue of the stem was found to be spiral shaped vessels. The vascular bundles were bicollateral which was considered to be a typical character of Convulvulaceae family. The chemomicroscopical studies revealed the presence of suberin, cutin, lignin and cellulose in the cell walls. Stomatal number was determined to be high in the upper epidermis than the lower one. Microscopic measurements revealed the length of trichomes to be high in the stem region. Fluorescence analysis showed wide range of fluorescence colours and all the three parts showed similar colours with slight changes in intensity. Water soluble extractive values were found to be high which shows that most of the phytoconstituents may be polar soluble. The aerial part after defatting with petroleum ether was extracted with methanol and the percentage yield obtained was found to be 12.55% w/w. The preliminary phytochemical chemical tests performed showed the presence of flavonoids, alkaloids, tannins and cardiac glycosides. The TLC studies foe flavonoids showed five prominent spots with yellow and blue colour fluorescence. The HPTLC standardization revealed the presence of scopoletin, flavonoid I and flavonoids glycoside and six unknown compounds. The pharmacognostical studies performed has identification of the significant features and standards for the proper identification of the plant from its related species as well as in the detection of adulteration/substitution. Hence, the matter embodied in this article will be helpful in establishment of suitable monograph for the plant.**

Key words: Trichomes, sessile, chemomicroscopy, Schulzes, fluorescence, toluidine.

INTRODUCTION

Medicinal plants constitute a source of raw materials for both traditional systems of medicine and modern medicine. Phytochemical screening involves botanical identification, extraction with suitable solvents, purification and characterization of the active constituents of pharmaceutical importance (John, 2007; Yi-Zeng, et al, 2004).

Ipomoea genus is one of the largest genera of family

Figure 1. Young leaf-mature leaf of *I. pes-tigridis*.

Figure 2. A typical mature leaf with fruit.

Convolvulaceae with about 600 species of annual, perennial herbaceous and shrubs. They primarily occur in subtropical and tropical regions of the world (Fang and George, 1995). *Ipomoea pes-tigridis* is an annual herbaceous vine, twining, with spreading hispid axial parts. All parts are more or less covered with long, spreading, pale or brownish hairs. Leaves are palmate and deeply divided into segments (3 - 5 - 9), elliptic oroblong, heart-shaped at the base and somewhat hairy on both surfaces (Figures 1 to 3). Flowers are white in colour and fruits are rounded, 6 to 7 mm in diameter (Figure 4). It can be found flowering through out the year when sufficient water is available. It is called “Tiger Foot Morning Glory” in English. Its geographical distribution includes the Sahel zone from Senegal to Niger and North Nigeria, and dispersed across tropical Africa and into Asia and Australasia, Mascarene Island and Malaysia (Flowers of India, 2010). It usually found in bush land, grassland, riverside, waste places, cultivated ground and sandy soil. The plant is determined to be a rich source of alkaloids, flavonoids, fatty acids, mucilage, resins, tannins, astringents, cardiac glycosides, saponins, resins and carbohydrates (Danial, 1975; Plants.jstor.org/flora, 2010; National Plant Data Center, 2010). The traditional claims of different parts of India were found to be as mentioned: leaves are used to treat poulticing sores and pimples, haemorrhoids, arthritis, rheumatism, dropsy, swellings, oedema, gout, venereal diseases, in boils, carbuncles and dog bites (Sudhir, 2010). Petiole is used as diuretics, laxatives and pain killer. Leaf sap is used as antidotes for venomous stings, snake bites, etc. Seeds are used to treat stomach troubles. Stem is used in the treatment of tumours and cancers. Entire creeper is crushed and the juice extracted and taken orally for treatment of or prevention of rabies if bitten by a rabid dog. The plant is used for healing wound and leaf powder is smoked to get relief from bronchial spasm.

MATERIALS AND METHODS

The fresh plant of *I. pes-tigridis* was collected in regions of Bollapally village of Kattamgur Mandal, Nalgonda district in the month of November to December, 2010. A herbarium was prepared and submitted to Department of Botany, Osmania University. It was identified and authenticated under the Voucher no: 0396 (*I. pes-tigridis* L) by Prof. Dr. B. Badraiah, H.O.D of Botany Department, Osmania University, Hyderabad, Andhra Pradesh, India.

Microanatomical study

Transverse section (TS) of leaf and stem

Fresh leaf and stem of *I. pes-tigridis* was used for this purpose. Free hand sectioning was performed to obtain a thin transverse section of leaf and stem, and microphotographs were taken for identification and arrangement of cells and tissues (Iyengar, 2004; Kokate, 2008; Khandelwal, 2009).

Longitudinal section (LS) of stem

Fresh stem of *I. pes-tigridis* was sectioned longitudinally and

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Figure 3. Mature leaf and fruit with clothing hairs.

Figure 4. Flower and fruit of *I. pes-tigridis*.

studied (Iyengar, 2004).

Powder macroscopy

Shade dried areal parts of *I. pes-tigridis* that is, leaf, stem and fruit were powdered with the help of an electric grinder till a fine powder was obtained. The organoleptic characters like colour, odour, taste and fracture were studied for leaf, stem and fruit powder (Evans, 2009). This fine powder was subjected to powder microscopy, as per standard procedures mentioned (Iyengar, 2005; Kokate, 2008; Khandelwal, 2009; Wallis, 2005).

Chemomicroscopy

TS and LS of the leaves and stems were stained with various staining reagents like pholoroglucinol-hydrochloric acid, Schulzes' solution, toluidine blue, safranin and sudan III to identify the different chemical present in the cell wall as well as its distribution throughout the section (Kokate, 2008).

Determination of leaf constant

The parameters studied under this category were stomatal number, stomatal index, vein islet number and vein termination number, and was performed as per standard procedures mentioned (Kokate, 2008).

Microscopical measurements

The microscopic measurements were performed for the important characters like trichomes, vessels, calcium oxalate crystals and starch grains. The experiment was performed as per standard reference books (Kokate, 2008).

Ultraviolet (UV) fluorescence analysis

Two (2 g) of powdered drug sample was taken in a beaker and dissolved in 5 ml of ethanol. The sample was transferred to a watch glass and observed under a UV chamber for colour and fluorescence reported. Similar procedure and observations were reported with different chemicals such as 50% H_2SO_4, 50% HNO_3, 5% NaOH, 1 N methanolic NaOH, 1 N KOH, 5% KOH, 5% $FeCl_3$, methanol, conc. HCl, conc. H_2SO_4, ammonia and conc .HNO_3 (Sandhya, 2010a, b).

Proximate analysis physicochemical analysis

Determination of moisture content (loss on drying), crude fibre content, total ash value, acid-insoluble ash, water soluble ash, alcohol-soluble extractive value and water soluble extractive values were performed as per standard procedures (Kokate, 2008; Sandhya, 2010a, b).

Extraction

200 g of the whole aerial part of the plant was taken and defatted with petroleum ether and then with methanol for 32 h at 40°C. The thick mass obtained was evaporated with help of rotary vacuum evaporator and percentage yield was calculated.

Thin layer chromatographic (TLC) analysis

The TLC was performed for flavonoids by using the adsorbent as precoated Silica gel $60F_{254}$ plates, solvent system as chloroform: Acetone : formic acid (7.5:1.65:0.85). The detection systems used were UV chamber and aluminium chloride solution (Wagner, 2004).

High performance thin layer chromatographic (HPTLC) analysis for flavonoid profile

The dried plant extract was dissolved in 1 ml of methanol and centrifuged at 3000 rpm for 5 min. 2 µl of the solution was loaded as 6 mm band length in the 2 × 10 cm Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase (Chloroform: Acetone : formic acid (7.5:1.65:0.85) and the plate was developed in the respective mobile phase up to 90 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254 nm and UV 366 nm. The developed plate was sprayed with

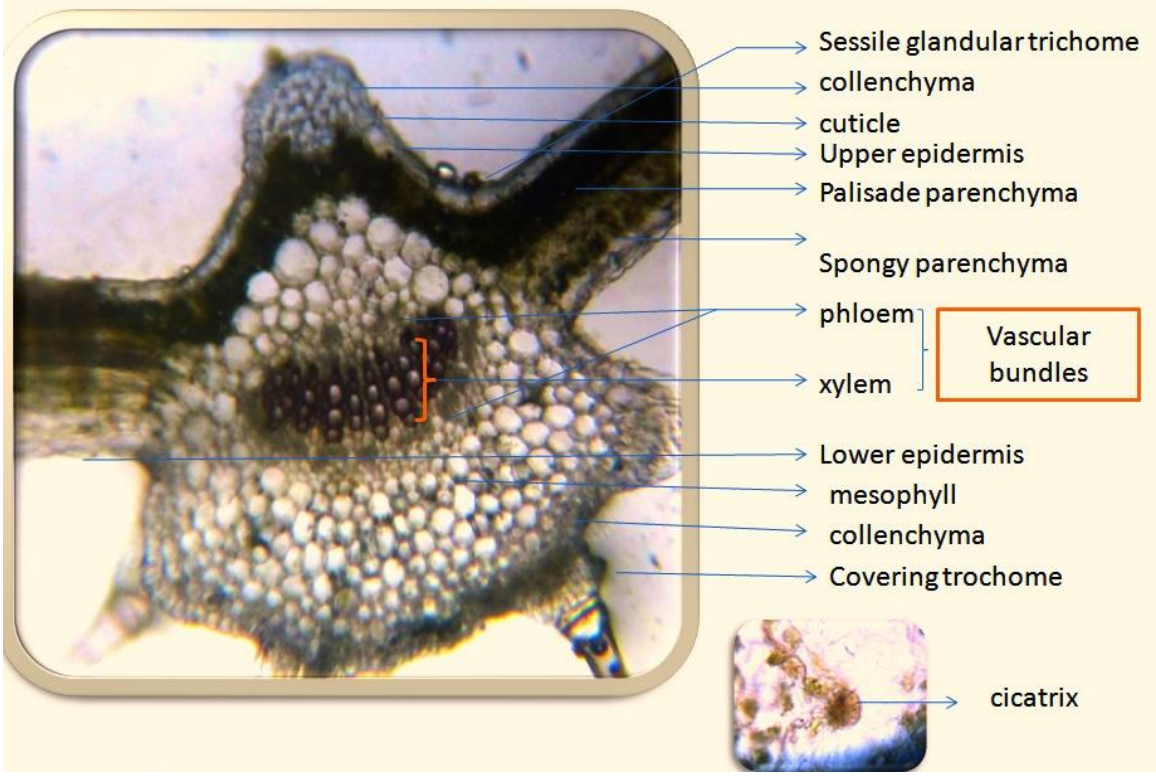

Figure 5. TS of midrib and lamina of *I. pes-tigridis*.

aluminium chloride (flavonoid) and dried at 100°C in hot air oven. The plate was photo-documented at day light and UV 366 nm mode using photo-documentation (CAMAG REPROSTAR 3) chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 365 nm. The peak table, peak display and peak densitogram were noted.

RESULTS

Anatomy of leaf

Transverse section of leaf showed dorsiventral nature. It was divided into lamina and midrib region (Figure 5).

Lamina region

Upper epidermis consists of single layered rectangular cells with cuticulerized outer walls. Abundant covering and glandular trichomes were observed in this region. Covering trichomes were long, slender, unicellular, uniseriate with pointed apex and bulbous base. Glandular trichomes were sessile with unicellular head. Mesophyll region was differentiated into palisade cells and spongy parenchyma. Palisade parenchyma was found to be single layered, compactly and radially arranged. Spongy parenchyma was found in many layers, loosely arranged with intercellular spaces. Lower epidermis was identical to upper epidermis. Chlorophyll was found in these cells. Stomata and numerous trichomes (both covering and glandular) were seen in lower epidermal region too. Epidermal peelings of leaf showed the presence of abundant cicatrix, covering and glandular trichomes.

Midrib region

Epidermal layers of lamina were continuous in the midrib region. Strip of collenchyma in 2 to 3 layers above the upper epidermis and below the lower epidermis were observed. This was followed by cortical parenchyma cells and the centre of these region arch shaped bicollateral vascular bundles was observed. Below the vascular bundles 5 to 6 layers of loosely arranged parenchyma cells with intercellular spaces were observed. Paracytic stomata and cluster crystals of calcium oxalate crystals were observed in this region.

Anatomy of stem

Transverse section of stem showed a circular shape. It was divided into the region as follows:

Epidermis: It was found to be the outermost layer and was made up of single layer of cells. The outer surface of

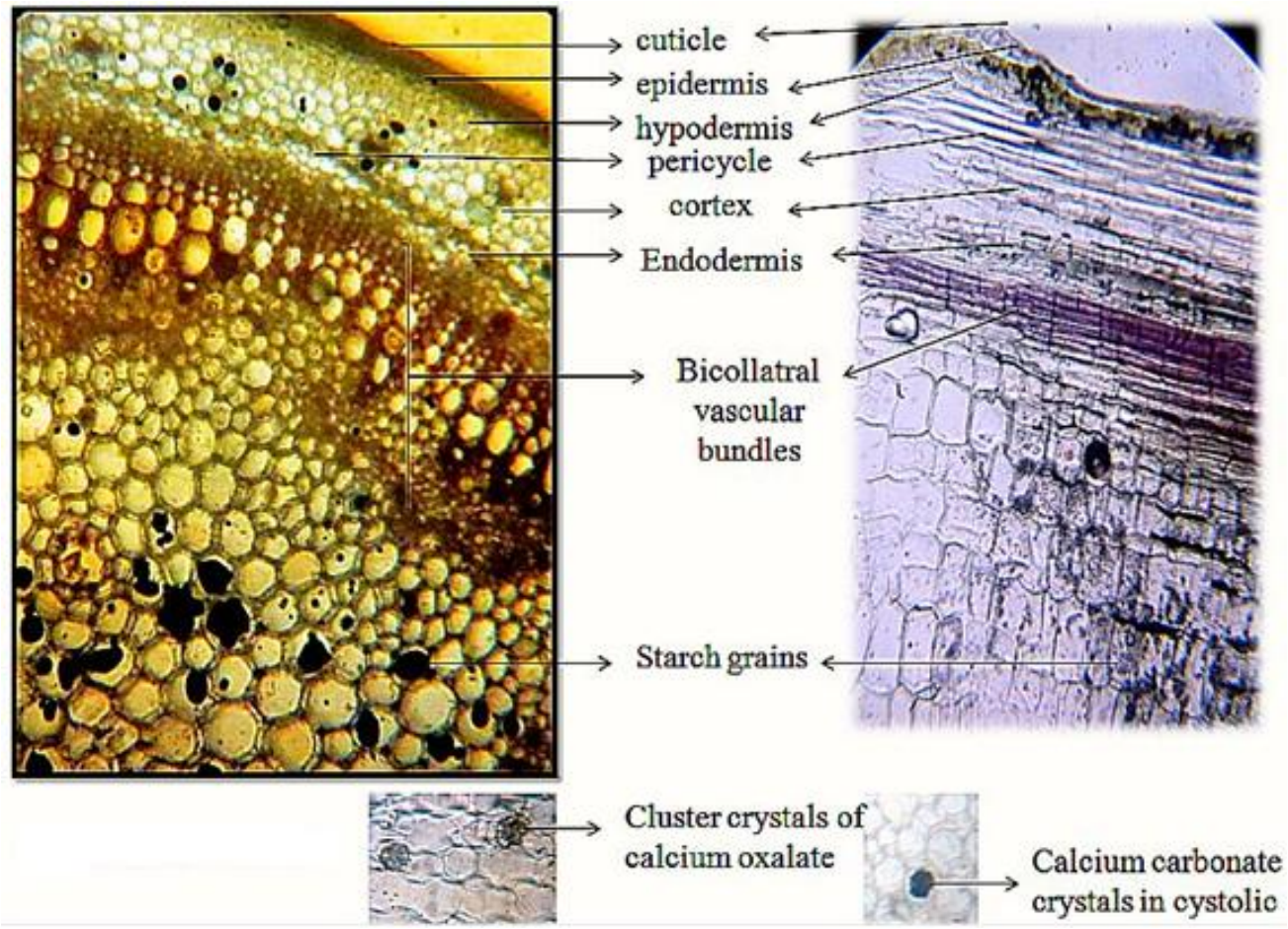

Figure 6. TS and LS of stem of *I. pes-tigridis*.

the epidermis was covered by a thin layer of cuticle. Abundant covering trichomes also were observed. Hypodermis was seen just inner to the epidermis and it consist of collenchymatic cells (Figure 6).

Cortex: Cortex was found next to hypodermis and it was made of thin walled parenchymatic cells arranged in several layers with intercellular spaces. Pericycle was made up of two types of cells that is, parenchymatic cells and schlerenchymatic cells in 2 to 3 layers. The schlerenchymatic cells were lignified. Next to the pericycle a single layer of wavy barrel shaped endodermis was found.

Vascular bundles: Bicollateral vascular bundles were present beneath the pericycle. Endarch metaxylem and exarch protoxylem were seen. The central region of the stem was occupied by the pith. It was made up of thin walled parenchymatic cells with intercellular spaces. Pith contained abundant starch grains, cluster crystals of calcium oxalate and calcium carbonate crystals in the form of cystolith (Figure 6).

Powder macroscopy

I. pes-tigridis powder organoleptic evaluaton:

For leaf:

1) Colour - Green
2) Odour - Characteristic
3) Taste - Bitter, astringent
4) Fracture - Uneven

For stem:

1) Colour - Light yellow
2) Odour - Characteristic
3) Taste - Bitter
4) Fracture - Uneven, flaky

For fruit:

1) Colour - Brown
2) Odour - Characteristic

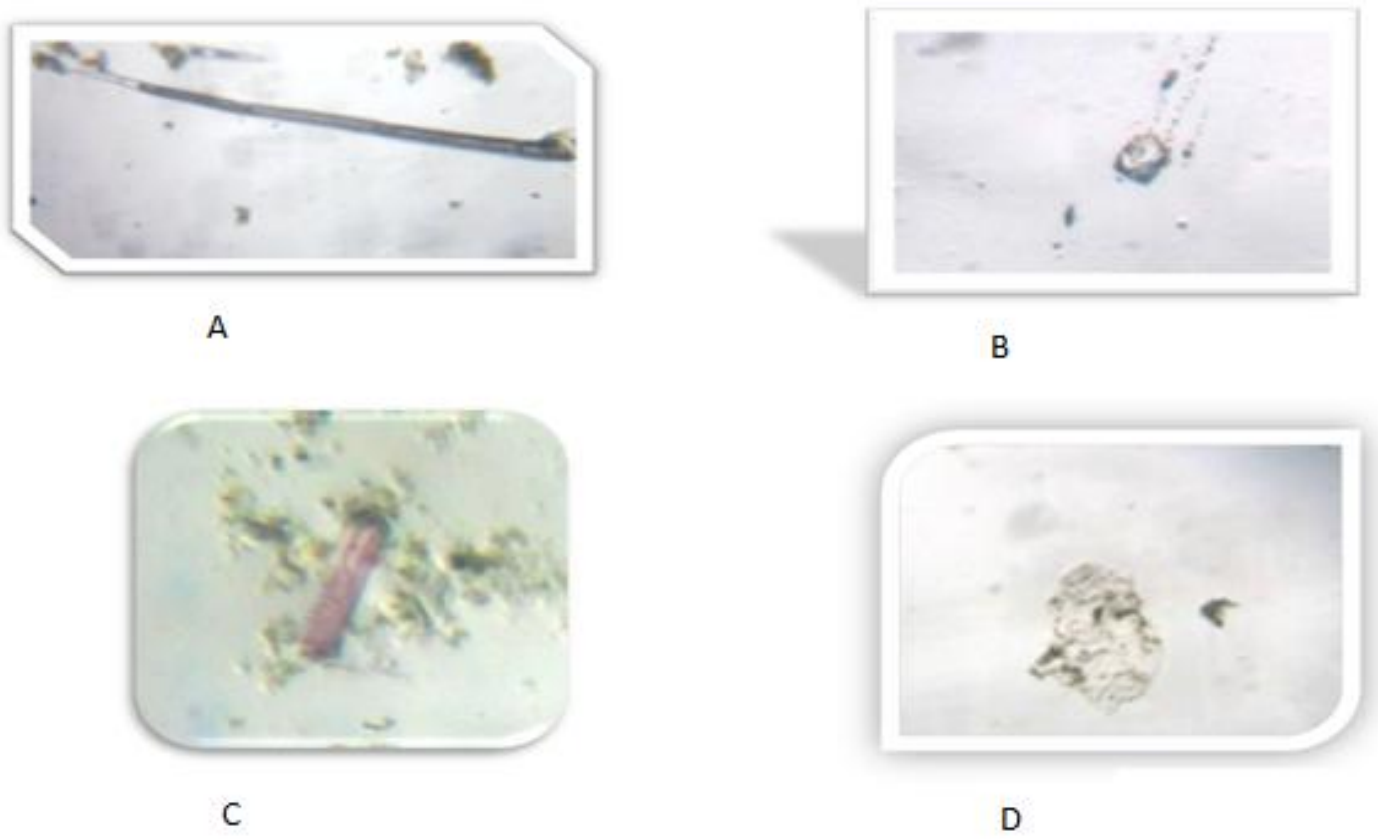

Figure 7. Powder microscopic characters of leaf. A, Covering trichome; B, calcium oxalate crystals; C, xylem vessels; D, epidermal cells.

3) Taste - Bitter, astringent
4) Fracture - uneven, flaky

Powder microscopy for leaf

Long slender, uniseriate, unicellular covering trichome with pointed apex and bulbous base were found in abundance (Figure 7A). Rectangular calcium oxalate crystals (Figure 7B), spiral lignified xylem vessels (Figure 7C) and polygonal shaped epidermal cells (Figure 7D) were observed throughout the powder.

Powder microscopy for stem

Lignified clustered fibres which were long and broken pieces (Figure 8A), polygonal shaped epidermal cells with fibres (Figure 8B), lignified spiral xylem vessels with lateral wall pits (Figures 8C, D and E), long slender covering trichomes (Figure 8F), isolated broken pieces of long non lignified fibres (Figure 8G) and polygonal epidermal cells (Figure 8H) were main characters which were observed in large quantities throughout the stem powder.

Powder microscopy of fruit

Non lignified isolated fibres similar to stem powder were observed even in the fruit powder (Figure 9A). The other characters found were yellowish brown coloured endosperm (Figure 9B), polygonal colourless parenchyma cells (Figure 9C), covering trichomes similar to leaf and stem powder (Figure 9D) and short slender lignified fibres with tapering ends (Figure 9E) were the major characters observed in the powder microscopy of fruit.

Chemomicroscopy

The TS of the leaf and stem when stained with polychromatic stain toluidine blue stained lignin containing cells blue to blue-green (Figure 10). When leaf section was stained with sudan III the cellulose containing cells were stained pink in colour (Figure 11). The LS of the stem when stained with Schulze's solution the lignified xylem was stained pink and the starch grains were stained bluish purple. It was observed that the grains were accumulated in the pith region (Figure 12).

The TS of stem when stained with safranin the lignin

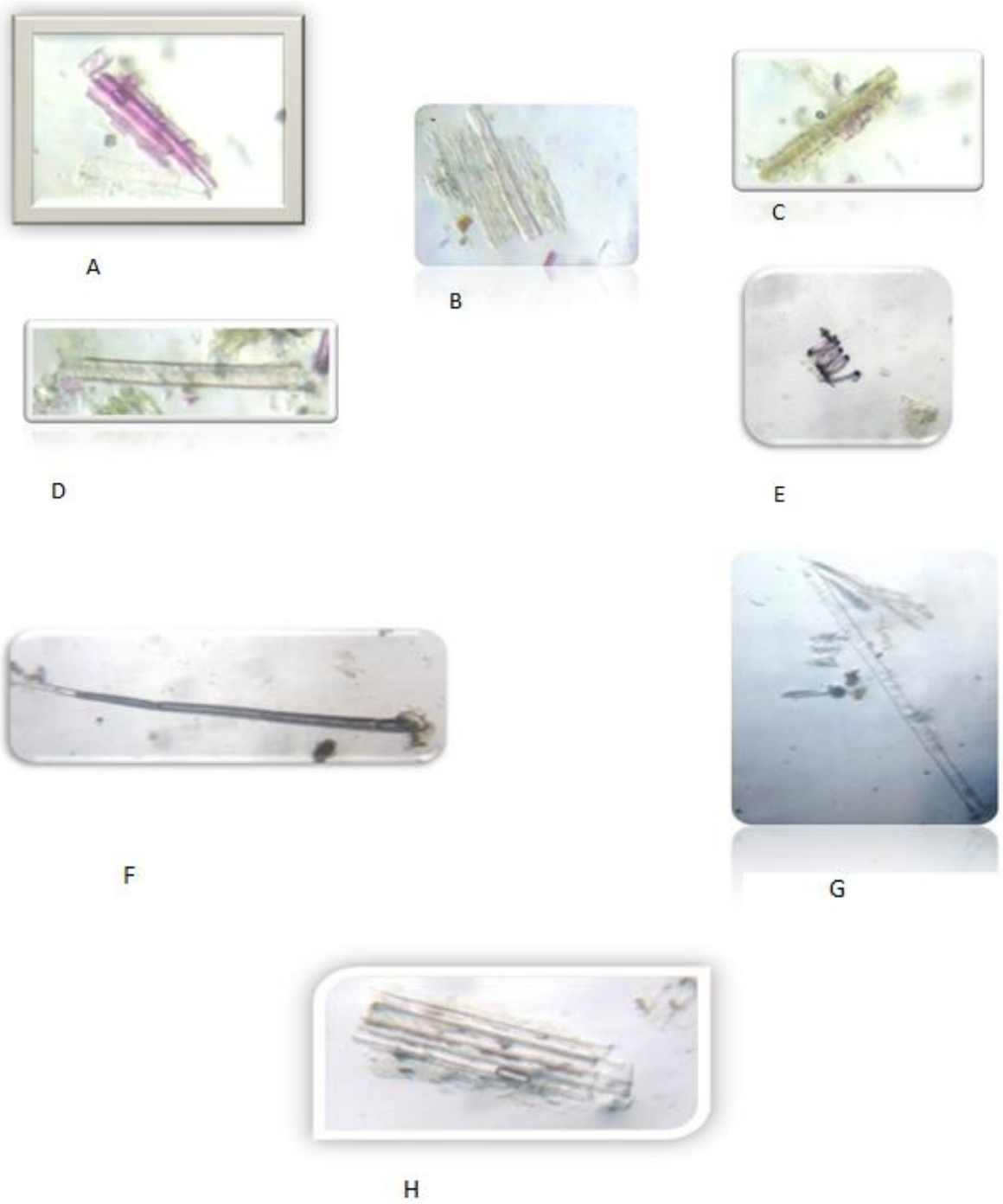

Figure 8. Powder microscopic characters of stem of *I. pes-tigridis.* A, Cluster of lignified fibres; B, epidermal cells with non lignified fibre; C, lignified spiral arrangement of vessels; D, vessels with lateral wall pits; E, spiral xylem vessels; F, covering trichome; G, non-lignified fibres; H, epidermal cells.

present in the phloem region showed faint pink colour and the cellulose were observed as blue colour (Figure 13). The TS of stem when stained with toluidine blue he lignified xylem vessels were stained bluish green colour where as the ploem contents of phloem were stained faint reddish blue colour (Figure 14).

Determination of leaf constants

The different parameters like stomatal number, stomatal index, vein is-let number and vein termination numbers was determined as per standard procedure and are shown in Table 1. It was observed that stomatal number

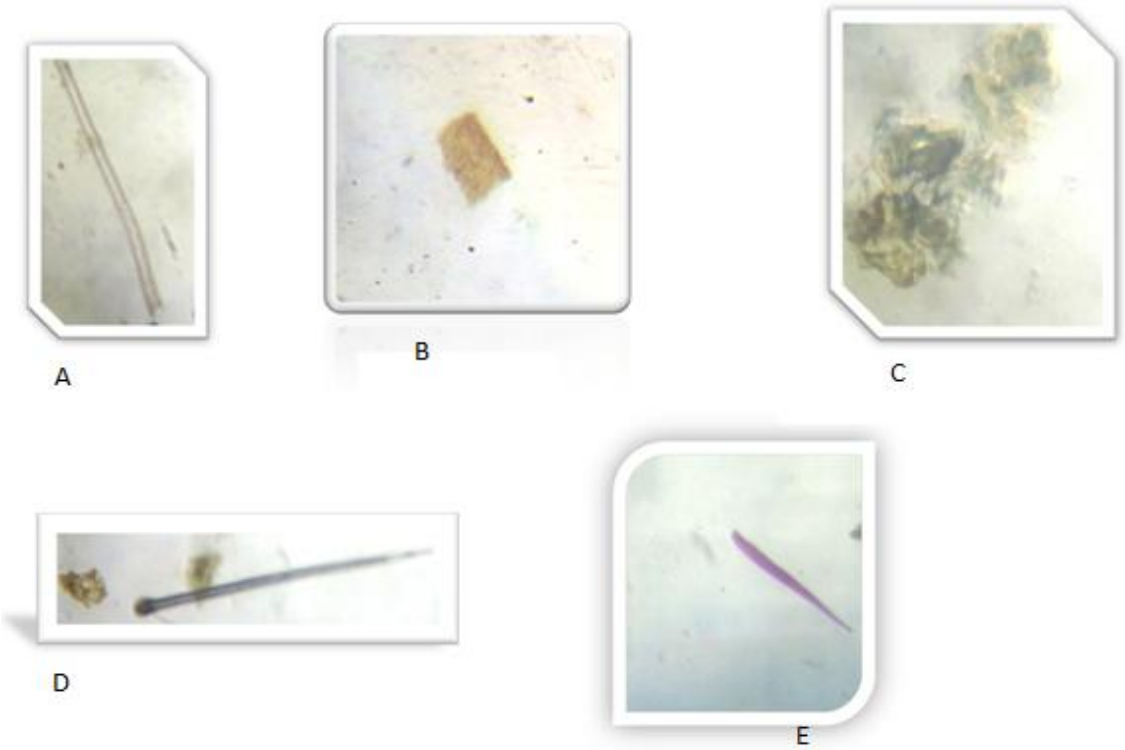

Figure 9. Powder microscopic characters of fruit of *I. pes-tigridis*. A, Nonlignified fibre; B, portion of endosperm; C, parenchymatic cells; D, covering trichomes; E, lignified fibres.

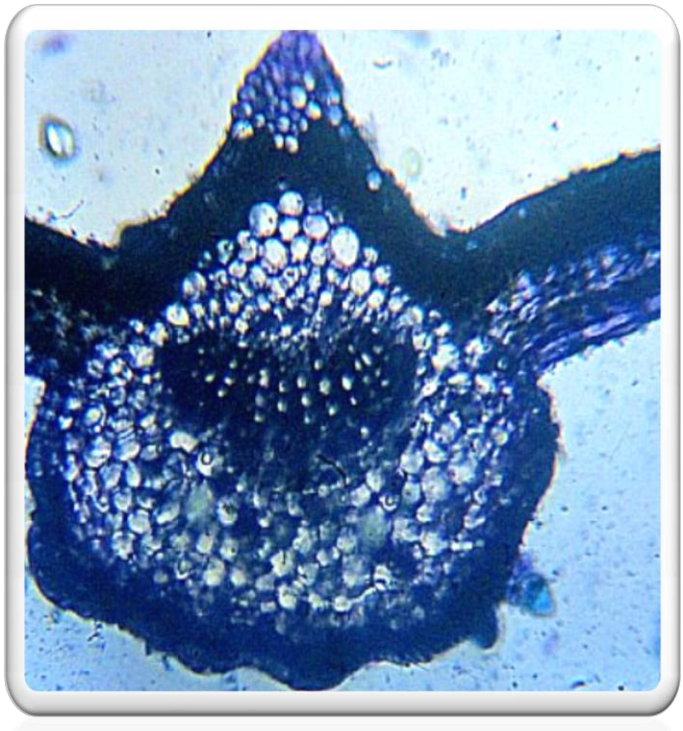

Figure 10. Section of leaf stained with toluidine blue.

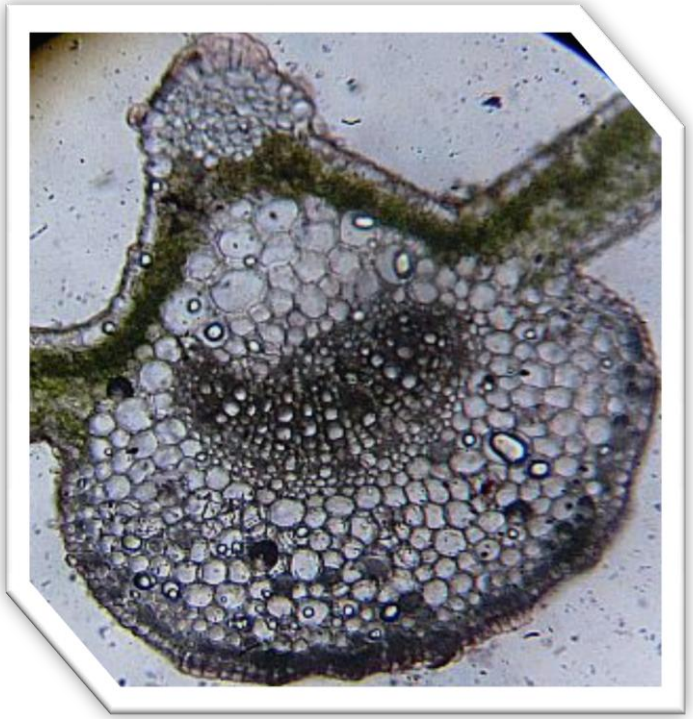

Figure 11. Section of leaf stained with Sudan III.

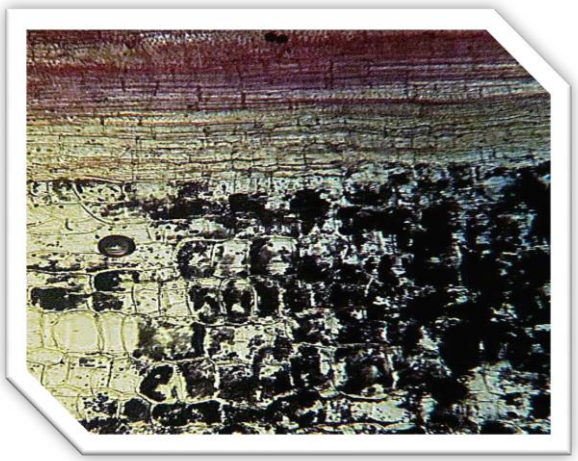

Figure 12. L.S of stem with Schulze's reagent.

Figure 13. Section of stem with safranin.

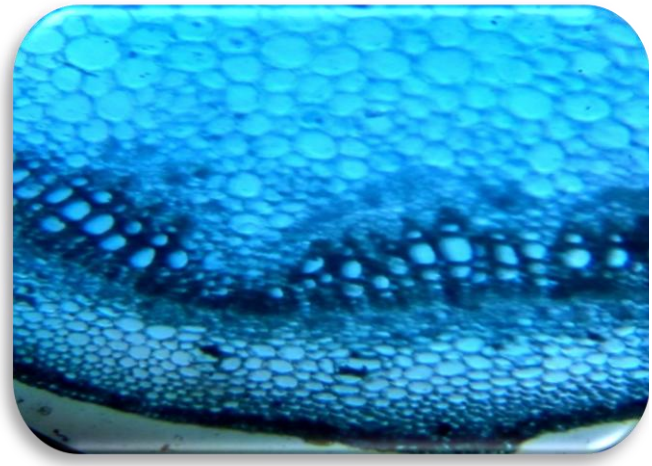

Figure 14. Section of stem with toluidine blue.

Table 1. Leaf constants of *I. pes-tigridis*.

Stomatal number	Upper epidermis	9
	Lower epidermis	6
Stomatal index	Upper epidermis	27.2
	Lower epidermis	22.2
Vein terminations		33
Vein islets		15

was more in the upper epidermal region than lower region.

Microscopic measurement

The length of trichome was highest for the stem; starch grain present in stem and fruit were found to be of same size. The dimensions of calcium oxalate crystals were also found to be in the similar range for leaf and stem (Table 2).

Fluorescence analysis

The fluorescence analysis revealed wide range of fluorescence colours. In comparison, it was observed that all the parts showed similar colour ranges with mild differences (Tables 3, 4 and 5).

This may be due to the presence of similar phytoconstituents.

Proximate analysis

Determination of extractive values

The alcohol soluble and water soluble extractive values were highest for stem followed by fruit and finally by leaf. Among the two extractive values, water soluble extractive values was found to be high for all the three parts (Table 6).

Determination of ash values

Total ash value was found to be highest for leaf and stem, whereas the acid insoluble ash value was highest for the fruit. Water soluble ash value was almost same for stem and fruit powder whereas leaf showed low value. Sulphated ash value was highest for stem. Loss on drying and crude fibre content was highest for fruit (Table 7).

Table 2. Measurements of *I. pes-tigridis* leaf, stem and fruit.

Parameter	Leaf (µm)	Stem (µm)	Fruit (µm)
Length of the trichome	112.5 - 423 - 1125	137.5 - 527.5 - 937.5	112.5 - 1200 - 2287.5
Width of fibre	-	13.04 - 37.7 - 65.2	-
Diameter of starch grain	-	6.25 - 17.5 - 25	6.25 - 17.5 - 25
Diameter of calcium oxalate crystal	12.5 - 42.5 - 72.5	12.5 - 34.06 - 72.5	-

Table 3. Fluorescence analysis of *I. pes-tigridis* leaf.

Reagent	Day light	Short wavelength UV (256 nm)	Long wavelength UV (365 nm)
Powder + 50% H_2SO_4	Brown	Light green	Green
Powder + 50% HNO_3	light brown	green	Dark brown
Powder + 5% NaOH	light brown	Green	Dark green
Powder + 1N methanolic NaOH	Green	Green	Purple
Powder + 1N KOH	Yellow brown	Green	Greenish blue
powder + 5% KOH	yellowish brown	Yellowish green	Dark green
Powder + 5% $FeCl_3$	Brown	Green	Dark green
Powder + Methanol	Brown	Yellow	Purple
Powder + Conc. HCl	Brown	Green	Dark green
Powder + Conc. H_2SO_4	Reddish brown	Light green	Dark green
Powder + Ammonia	Green	Dark green	Brown
Powder + Conc .HNO_3	Light brown	Light green	Dark green

Table 4. Fluorescence analysis of *I. pes-tigridis* stem.

Reagent	Day light	Short wavelength UV (256 nm)	Long wavelength UV (365 nm)
Powder + 50% H_2SO_4	Light brown	Light green	Dark green
Powder + 50% HNO_3	Yellowish brown	Green	Blue
Powder + 5% NaOH	Brown	Green	Yellowish green
Powder + 1N methanolic NaOH	Light brown	Light green	Blue
Powder + 1N KOH	Yellow	Yellowish green	Greenish blue
Powder + 5% KOH	Brown	Green	Dark green
Powder + 5% $FeCl_3$	Orange	Green	Dark green
Powder + Methanol	Pale yellow	Yellow	Blue
Powder + Conc. HCl	Brown	Pale green	Green
Powder + Conc. H_2SO_4	Dark brown	Light green	Greenish black
Powder + Ammonia	Brown	Light green	Greenish blue
Powder + Conc. HNO_3	Reddish brown	Green	Black

Extraction and phytoconstituents

The percentage yield obtained for the methanolic extract of the parts of plant was found to be 12.55% w/w. The preliminary phytochemical studies revealed that the aerial parts of *I. pes*-tigridis contained alkaloids, flavonoids, saponin glycosides, cardiac glycosides, resins, tannins and phenolic components and carbohydrates in methanolic extract.

Thin layer chromatographic (TLC)

The TLC for flavonoids showed five fluorescent spots

Table 5. Fluorescence analysis of *Ipomoea pes-tigridis* fruit.

Reagent	Day light	Short wavelength UV (256 nm)	Long wavelength UV (365 nm)
Powder + 50% H_2SO_4	Brown	Light green	Green
Powder + 50% HNO_3	Yellowish brown	green	Blue
Powder + 5% NaOH	Yellowish brown	Green	Dark green
Powder + 1 N methanolic NaOH	Light yellow	Light Green	Blue
Powder + 1 N KOH	Yellow bown	Green	Greenish blue
powder + 5% KOH	Yellowish brown	Yellowish Green	Dark green
Powder + 5% $FeCl_3$	Greenish orange	Yellowish Green	Black
Powder + Methanol	Brown	Yellow	Blue
Powder + Conc. HCl	Brown	Light Green	Green
Powder + Conc. H_2SO_4	Brown	Light green	Dark green
Powder + Ammonia	Green	Dark Green	Brown
Powder + Conc .HNO_3	Light brown	Light green	Dark green

Table 6. Extractive values of *Ipomoea pes-tigridis* leaf, stem and fruit.

Parameter	Leaf (%w/w)	Stem (%w/w)	Fruit (%w/w)
Ethanol extractive value	1.3	3.28	2.18
Water extractive value	4.71	9.52	7.61

Table 7. Ash values of *Ipomoea pes-tigridis* leaf, stem and fruit.

Parameter	Leaf (%w/w)	Stem (%w/w)	Fruit (%w/w)
Total ash value	10.5	10.65	7.45
Acid insoluble ash	0.55	0.35	0.95
Water soluble ash	6	7.5	7
Sulphated ash	8.5	13	10.45
Loss on drying	4.5	6.4	10.5
Crude fibre content	37	51	75

under UV light with Rf values 0.061 (yellow), 0.102 (yellow), 0.142 (blue), 0.51 (blue) and 0.61 (blue). After derivatization with aluminium chloride the quenching of the fluorescent spots were observed.

High performance thin layer chromatographic (HPTLC)

Yellow coloured fluorescent zone at UV 366 nm mode present in the given plant extract was observed in the chromatogram after derivatization, which may be the presence of flavonoid in the extract.

The HPTLC finger printing for the flavonoids was identified as scopoletin, flavonoid glycoside, an unknown flavonoid and six unknown components (Table 8) and (Figures 15 to 17).

DISCUSSION

The quantitative determination of pharmacognostical parameters is useful for setting standards for crude drugs. Identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. Microscopic studies or a structural detail helps the secondary identification of drugs. Clothing hairs have protective function (Evans, 2009). Glandular trichomes secrete essential oils or oleo-resins. The microscopical studies of the leaf showed the presence of sessile glandular trichomes and long slender uniseriate, unicellular covering trichome with bulbous

Table 8. HPTLC of *Ipomoea pes-tigridis* for flavonoids.

Track	Peak	Rf	Height	Area	Assigned substance
Spot a	1	0.01	319.6	3506.3	Unknown
Spot b	2	0.05	224.2	6059.0	Flavonoid glycoside
Spot c	3	0.12	94.4	2631.1	Flavonoid 1
Spot d	4	0.45	26.6	934.7	Unknown
Spot e	5	0.56	37.5	1264.0	Unknown
Spot f	6	0.71	47.9	1778.8	Scopoletin
Spot g	7	0.82	88.6	3984.6	Unknown
Spot h	8	0.90	39.5	1061.5	Unknown
Spot i	9	0.95	115.2	4190.0	Unknown

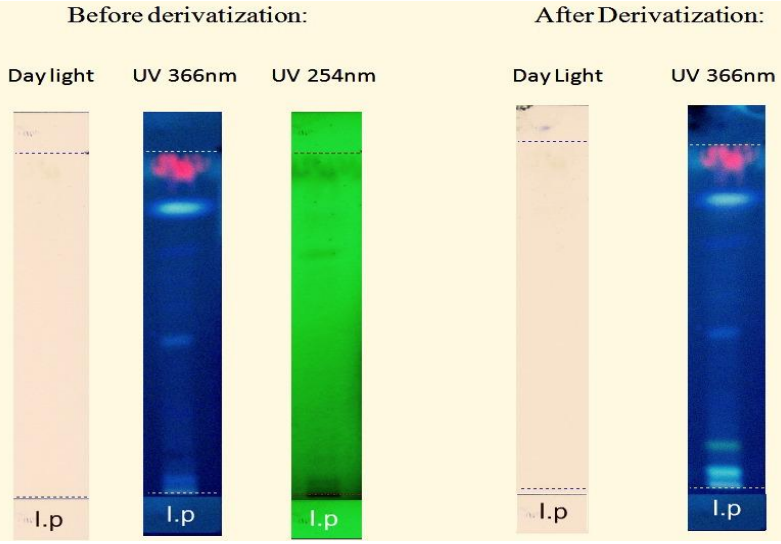

Figure 15. HPTLC figure printing of flavonoids.

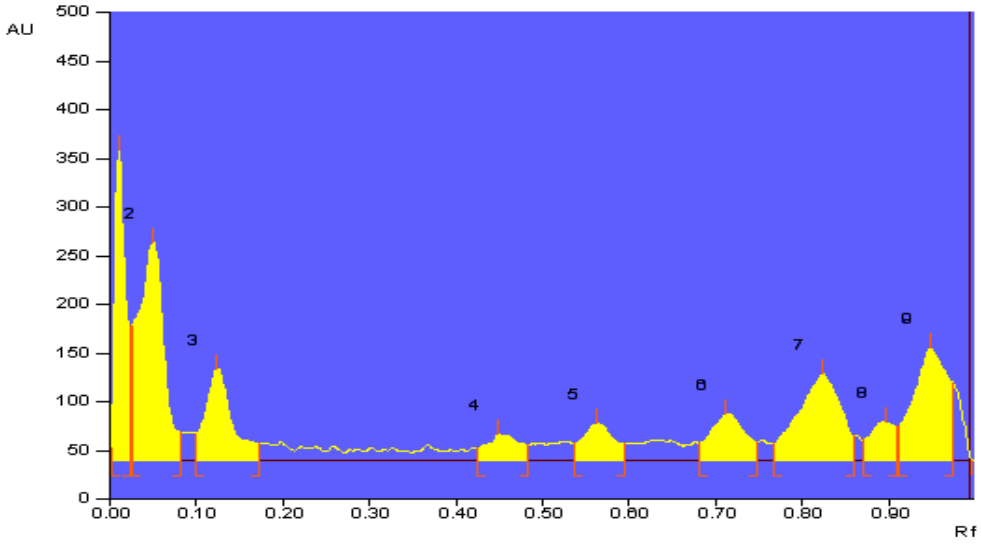

Figure 16. Ethanol extract densitogram display (scanned at 365 nm).

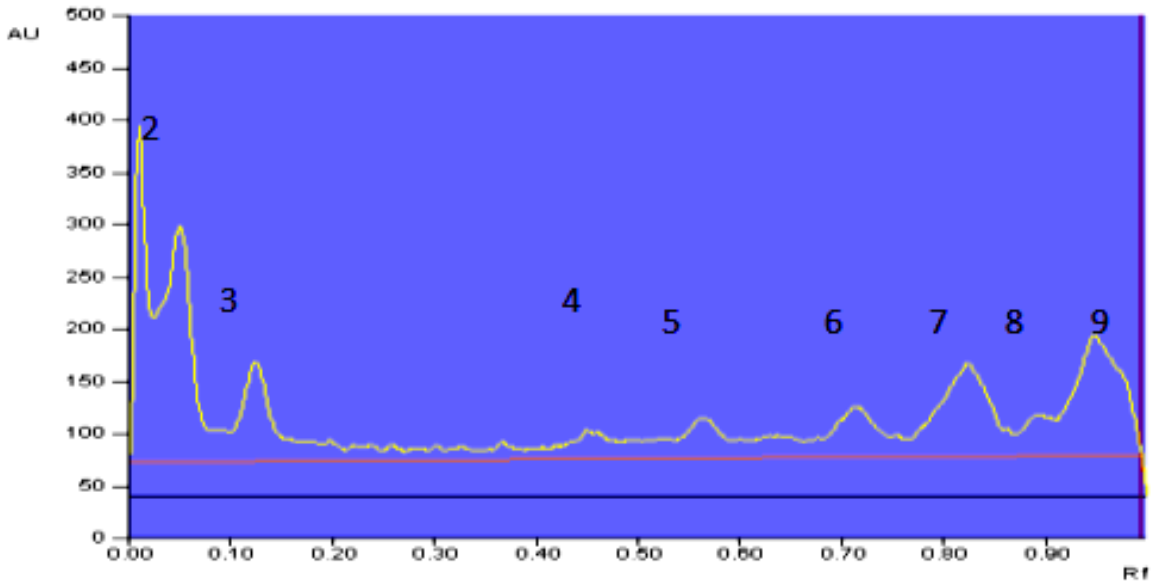

Figure 17. Ethanol extract peak baseline display (scanned at 365 nm).

base and pointed apex which can be considered as the tissue of diagnostic importance in the leaf. These trichomes can be correlated with morphological characteristics since they were seen in abundance. Abundant cicatrix (scar of the fallen trichome) was observed in epidermal peelings which were due to shedding of the trichomes. As the midrib of the leaf was found to be well differentiated, the palisade tissue was found to be interrupted in the midrib region. Bicollateral vascular bundles were observed which are usually found to be very rare in dicotyledonous plants. The cell walls of this tissue were thick due to presence of the cellulose which provides greater mechanical strength to the plant.

The main tissue of diagnostic tissue of the stem was found to be spiral shaped vessels. Presence of non-lignified fibre was also found in stem and fruit. The parenchymatic cells of outer layers of the section of the stem showed chlorophyll which forms the epical character of the aerial stem. The vascular bundles were bicollateral which was typical character of Convulvulaceae family (Evans, 2009). The microscopy of entire aerial part (leaf, stem and fruits) showed the presence of abundant epidermal trichomes which can be correlated with the morphology of the parts as they were covered with clothing hairs.

The chemomicroscopical study is application of chemical tests to small quantities of its histological section which helps for the study of different constituents of drugs. In the microscopy, cell wall may during the differentiation of the cell, undergo various chemical modifications which profoundly change its physical properties; among these are the deposition of further cellulose or hemicellulose and incrustation of the wall by lignin and cutin. This study gives chemical composition of the cell walls of the tissue in the pant. When leaf and stem sections were stained with safranin, sudan III and toluidine blue showed presence of suberin, cutin, lignin and cellulose in the cell walls. Schulze's solution stained the starch grains in the stem section (Kokate, 2008). This study helped to identify the location of starch in the stem part of the plant.

Microscopical measurement which helps for the identification of drugs belongs to the same family and species. It also set a limit and range for identification of authenticity that can be present in the plant specimen. Measurements of the length of the trichomes in the order of friut>stem>leaf. Starch is absent in the leaf. Starch grains of the fruit were found to be more than that of the stem. Determination of leaf constants is a very critical step in standardization of crude drugs as it gives a clear numerical value for plant cell structures and which makes it easy for identification from other adulterants and similar looking sub species. Stomatal number is relatively a constant for particular species of same age and hence taken into consideration as a diagnostic character for identification of leaf drug. Stomatal number and index were more in the upper epidermis. This may be due to the abundant clothing hairs which provide protection for the plant parts. Vein islet number is a constant for a given species of the plant and it does not alter with the age of the plant and is independent of the size of the leaf. The vein termination number was found to be more than the vein islet number.

Test for ash values was performed to determine quality and purity of a crude drug. Ash contains inorganic radicals like phosphates, carbonates, silicates of sodium, potassium, magnesium, calcium etc. Sometimes inorganic variables like calcium oxalate, silica, carbonate

content of crude drug affects ‘total ash value’ (Kokate et al, 2006). The total ash values were more for the fruit and stem than the leaf probably because of absence of calcium oxalate crystals in the leaf. Among the three parts, acid-insoluble and sulphated ash were high in stem when compared with the leaf and fruit. Extractive values useful for the evaluation of a crude drug which gives an idea about the nature of the chemical constituents present in a crude drug and also useful or the estimation of specific constituents, soluble in that particular solvent used for extraction. In this plant, water extractive values were more when compared with the methanolic extractive values. The crude fibre content of the fruit was more than that of the stem and leaf due to presence of the polysaccharides.

Fluorescence studies of the leaf stem and fruit exhibited wide range of colours at day light, UV-chamber (256 nm and 365 nm). These colour changes reflect the nature of the chemical components present in the plant parts when exposed to the respective chemical reagent. Hence, this parameter is a very important technique for the proper identification of the plant species.

The TLC analysis of flavonoids showed fluorescent quenching after spraying with the aluminium chloride which reveals the presence of high concentration of flavonoids. The HPTLC standardization showed the presence of scopoletin and from the literature survey, it was found that the same compound was isolated from the roots of *Ipomoea batatas* (Yong-Qin and Ling-Yi, 2008).

The present research investigation on *I. pes-tigridis* has revealed out various microscopical as well as proximate standards which will help in setting a suitable plant profile for the proper identification of the species from its related species as well as in the detection of adulteration or substitution.

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