

MORPHO-ANATOMICAL STUDIES OF INDIGOFERA LINNAEI

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Abstract

Indigoferalinnæi known as Birdsville indigo and nine-leaved indigo. It is a low ground herb and is an important species of herbaceous cover. It forms extensive mats of populations carpeting the soil. The plant provides food for certain local insects and protects the soil cover with its clustered root system and spreading form of multi-stemmed branching pattern. Therefore Indigofera linnaei has the potential for use in the restoration of destroyed degraded and damaged habitats. The present investigation has been carried out to study the morphological and anatomical features of whole plant of Indigofera linnaei. Anatomical studies of plant parts show various significant characters. Epidermal cells of stem are radially elongated, collenchymatous cortex contain starch sheath and resin duct. Pericycle is homogenous formed of several layer of parenchymatous region. Vessels are in radial multiplies some are solitary performing various shape. Two different types of stomata are present on adaxial and abaxial surface. Paracytic type of stomata is present in adaxial surface and anisocytic stomata present in abaxial surface, also variation in both epidermal cells. Cortical region of root contain crystals of calcium oxalate, xylem vessels are radial, predominantly paired. Rachis shows 5 vascular bundles, one is large, lateral two are equal in size and remaining two are smaller.

Keyword: Morphology and anatomy of Indigofera linnaei, Root, stem, leaf and petiole

1. INTRODUCTION

Indigofera linnaei is a species of leguminous shrub in the genus *Indigofera* of the subfamily Papilionaceae. The

genus name *Indigofera* is derived from Latin and means containing Indigo-a purple dye originally obtained from some *Indigofera* species while linnaei derived from Linnaeus. Wilson (1987) and Wilson and Ross (2004) provided a general account on *Indigofera* species.

Indigofera is a large genus of over 750 species of flowering plants of the subfamily papilionaceae belonging to the pea family Fabaceae. It is widely distributed in tropical and subtropical regions. The genus is one of the nine genera which are members of the tribe Galegeae. (Nwachukwu and Mbagwu, 2007).

Indigofera in Greek means Indigo dye which is famous for the natural blue colour obtained from the leaflets and branches of this herb. The fruits are oval shaped and elongated, 4-angled or flattened and often curved with many seeds. The dye which is among the most widely used natural dye in the world is obtained mainly from the leaves through a process of fermentation. *Indigofera* genus possess wide range of uses ranging from several economical and ecological purposes, feed for livestock, ornamental, medicinal, plant recipes as well as dye for commercial purposes. *Indigofera* species are used as food plants by the larvae of some Lepidoptera species including Turnip Moth.

The leafy twigs are the main source of indigo dye used since very ancient times for dyeing textile blue. The leaves and twigs do not contain indigo but colourless precursors that extracted and processed to produce the indigo dye.

The herb layer has structural and functional significance in both forest and non-forest ecosystems. Certain low ground herbs have the ability in retarding soil, water and nutrient erosion and these abilities vary with different weed species. (Kumar, et al. 1997).

Indigofera linnaei is a perennial plant with prostrate sometime, ascending, much-branched stem that can become more or less woody, especially near the base and persist. The plant is harvested from the wild for local use as a medicine, in times of need the plant is harvested from the wild for its seed which are an emergency source of food. It causes however a disease of horses but is obviously suitable for feeding sheep and cattle. The juice of the plant is used as an antiscorbutic and diuretic. It is considered to be alternative in the treatment of old venereal affections.

From the available literature no anatomical studies were carried out on this species. The present investigation has been carried out the details morphological and anatomical study of the complete plant of *Indigofera linnaei*. The study was aimed to provide valuable and reliable illustrated anatomical descriptions of the species.

2. MATERIAL AND METHOD

Mature and fresh samples of root, stem and leaves were collected from grassland area in Amravati region. Fresh materials were fixed in Formalin Acetic Acid (FAA) for 48 hrs., washed in several changes of distilled water, dehydrated through alcohol series (30%, 50%, 70%, 90% and 100%), 2hrs. in each solution and embedded in wax. Sections in each case were cut on a Leica 2125 rotary microtome at thickness 5 μ . The sections were de-waxed with pure xylene and rehydrated in alcohol series following Cutler (1978) with modifications. Staining was achieved by dipping the slides in 1% alcian blue (light green) for about 5 min. washed with distilled water and counter stained with 1% safranin for 2 min. The stained sections were dehydrated through alcohol series and mounted permanently in DPX. Photomicrographs of anatomical sections were taken with a Coslab camera fitted with 4X, 10X, 40X microscopic objective lens.

To study leaf architecture, the mature leaves from fresh materials were cleared by treating them with 5% aqueous sodium hydroxide which was repeatedly replaced by fresh solution until leaf material got cleared, followed by treatment with 2% acetic acid for 1-2 hours to neutralize residual sodium hydroxide. The cleared leaves after washing with distilled water stained with aqueous safranin and mounted in glycerin or dehydrated. Major and minor venation patterns and

details of leaf architecture, were studied under compound microscope. Terminology of Hickey (1971, 1973) is followed for describing leaf architecture. Whole lamina photographs were taken directly using coslab camera fitted with 4X, 10X, 40X microscopic objective lens.

3. RESULT & DISCUSSION

3.1. Morphological features

Diffuse, Prostrate, annual or perennial herb with woody root stock, branches trailing 30-40cm long, grey-pubescent. Stem is branched, herbaceous, cylindrical, and velvet-hairy. Leaves are pinnately compound 1-2cm long cuspidate, imparipinnate with 5-9 alternately arranged leaflets, 7-12mm long, 2-5mm broad, sessile, oblanceolate or obovate. Inflorescence is a spicataracemes, peduncle 0.2cm long. Flowers red, in many flowered axillary crowded, pedicel absent, bract scarious, 2mm long, ovate, acuminate, persistent, sepals-5, polysepalous, 3-4mm long, hairy outside, teeth long. Petals-5, gamopetalous, papilionaceous corolla, bright red, slightly exerted 4mm long. Fruit 3-6mm long, 2-3mm broad, pods very small, cylindric, 3-4mm long, sparsely clothed with white appressed hairs, apiculate, pubescent, 1-2 seeded, globose. Flowering June to December.



Fig. No. 1: *Indigofera linnaei* Habit (Axillary crowded head)

Located along grassland area in Amravati region, common on rocky soil or old walls etc. very common throughout in open grasslands, fields etc.

3.2. Anatomical features

3.2.1. Primary structure (Young Stem)

Young stem roughly triangular in shape, Epidermis is single layered, cells are angular small in size covered with thick cuticle. Following epidermis there is single layer of hypodermal stone cells interrupted by

chollenchymatous cells, cells are loosely placed, Cortex narrow, parenchymatous, cells thin walled, enclosing small intercellular spaces, endodermis distinct, 16 vascular bundles are present, cambium in the form of complete ring from the beginning. Pith large, cells parenchymatous, rounded or oval in shape, loosely arranged with small intercellular space with styloids scattered present in small amount.

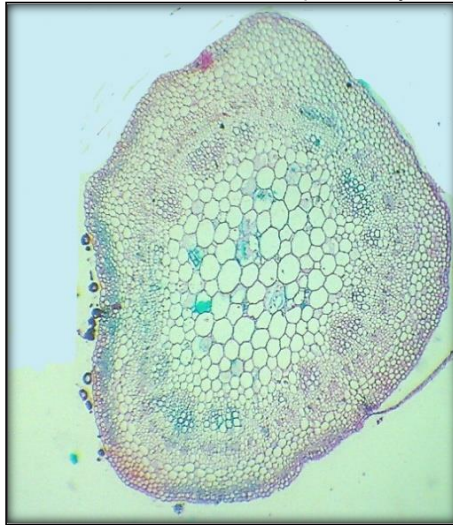


Fig.No.02: Primary structure (Entire View)

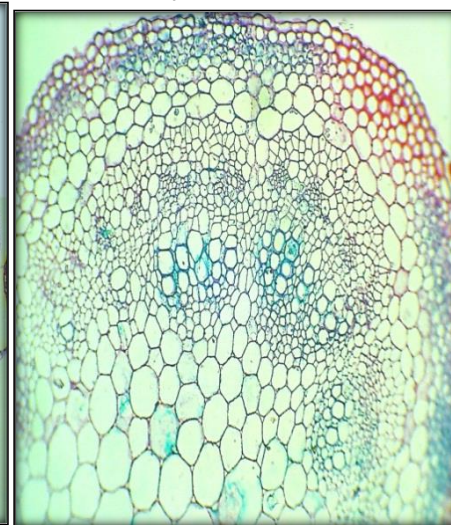


Fig.No.03: Primary structure (Cross View)

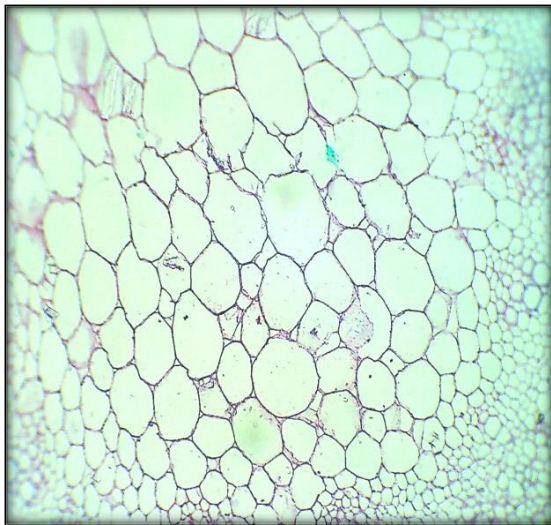


Fig.No.04: Primary structure (Pith)

3.2.2. Secondary structure (Old Stem)

The transverse section is represented in fig.no.5. A thin transverse section of a stem when examined under the microscope showed circular in outline the following regions observed from outside to inside.

- Epidermis: It is the outermost region of the stem, single layered of rectangular cells, radially elongated. The outer surface of the epidermis is covered by a layer of cuticle.
- Cortex: Just below the epidermis cortical region is present, consist of 4-5 layers of collenchymatous cells containing starch sheath. Resin duct is present at the cortical region.
- Endodermis: Distinct endodermis is present next to cortex. It is the parenchymatous barrel shaped cells and is the innermost layer of the cortex.
- Pericycle: Pericycle is homogenous. It lies inside the endodermis and is formed of several layer of parenchymatous region.
- Vascular cylinder:

- a) Secondary phloem: Secondary phloem is found next to pericycle region, 3-4 layered parenchymatous cells.
 - b) Cambium: The cambium has been found to initiate in the vascular bundle between xylem and phloem. Gradually it extends towards the upper part.
 - c) Secondary xylem: Xylem inner to cambium in close cylinder transversed by narrow rays. Vessels in radial multiples of 6-7, some are solitary.
 - d) circular, angular, triangle in outline, secondary xylem with vessels predominantly in radial multiples.
- Pith: Wide, homogenous, cells parenchymatous thin walled, clear, circular or oval, enclosing very small intercellular spaces sheath at meeting corners of walls containing fine starch cells adjoining xylem small, compact.



Fig.No.05: Secondary structure (Entire View)

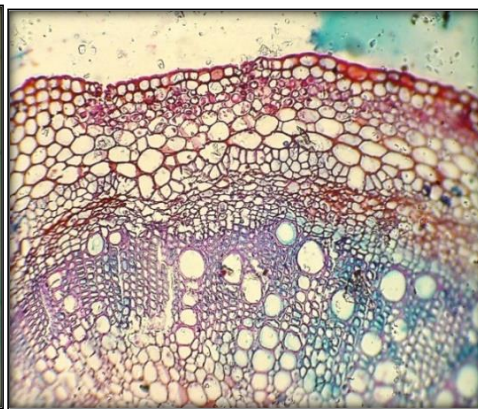


Fig.No.06: Secondary structure (Cross View)

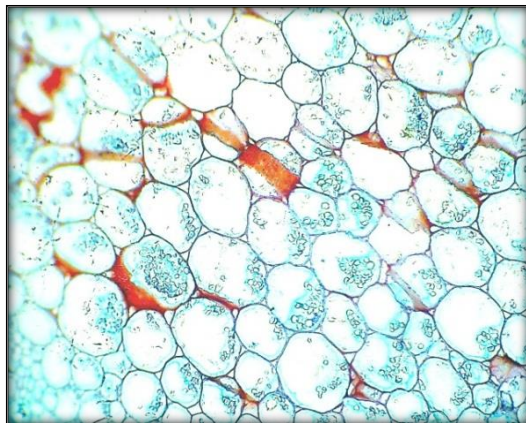


Fig.No.07: Secondary structure (Pith)

T.S. of Root: Outline circular

- Periderm: Multilayered, parenchymatous, rectangular, compactly arranged without intercellular spaces. Cortex: Multilayered, cells parenchymatous, polygonal, thin walled, cortical cells contain crystals of calcium oxalate.

Endodermis: Indistinct. Pericycle: Multilayered, parenchymatous.

- Vascular Cylinder: Secondary phloem in the form of an outer layer, phloem fibres are present in patches. Secondary xylem form inner wide cylinder transversed by medullary rays medullary rays multiseriate xylem vessels are radial predominantly paired.

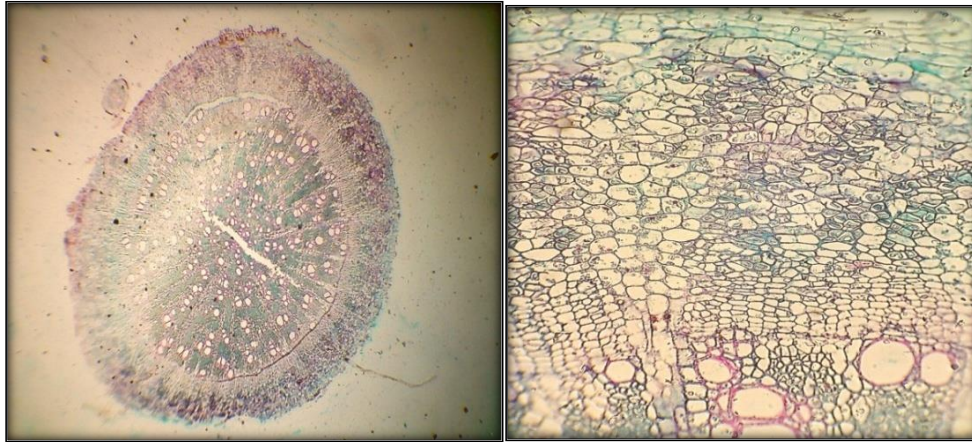


Fig.No.08: Root (Entire View)

Fig.No.09: Root (Cross View)

T. S.of Leaf:

- Lamina: Both upper and lower epidermis is single layered, covered with cuticle, amphistomatic, and compactly arranged parenchymatous cells. Upper epidermal cells are radially elongated; lower epidermal cells are angular and small in size as compared to upper. Stomata present in lower epidermis are anisocytic and upper epidermis is paracytic type. Mesophyll differentiated into palisade and spongy parenchyma. Palisade single

layered, compactly placed, spongy parenchyma dominant, 5-6 layered, barrel shaped, columnar, squaride vein-bundle embedded in spongy parenchyma, bundle sheath sclerenchymatous.

- Margin: Palisade continuous upto margin, single layered, cells elongated, finger like tapering towards lower end.
- Midrib: Vasculature in the form of roughly 'C' shaped, sclerenchymatous, perivascular, xylem surrounded by phloem.

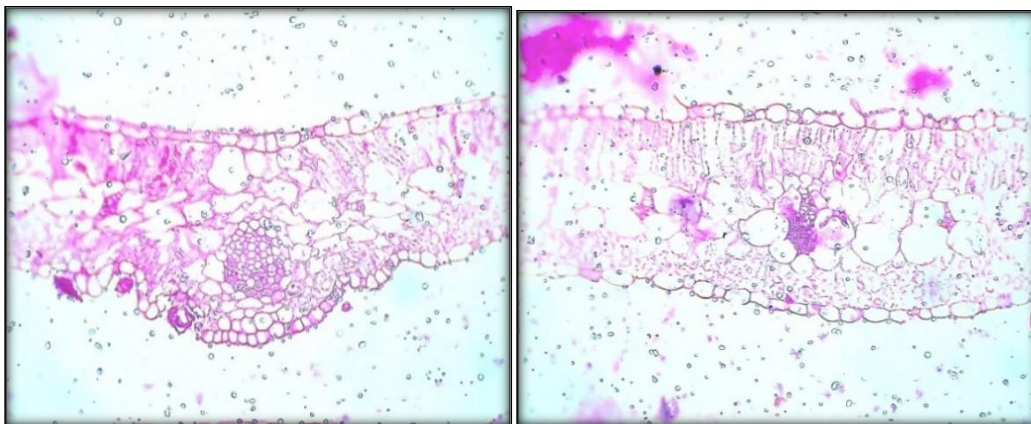


Fig.No.10: Leaf (Showing variation in Epidermal cell) Fig.No.11: Leaf lamina(Showing vein bundle)

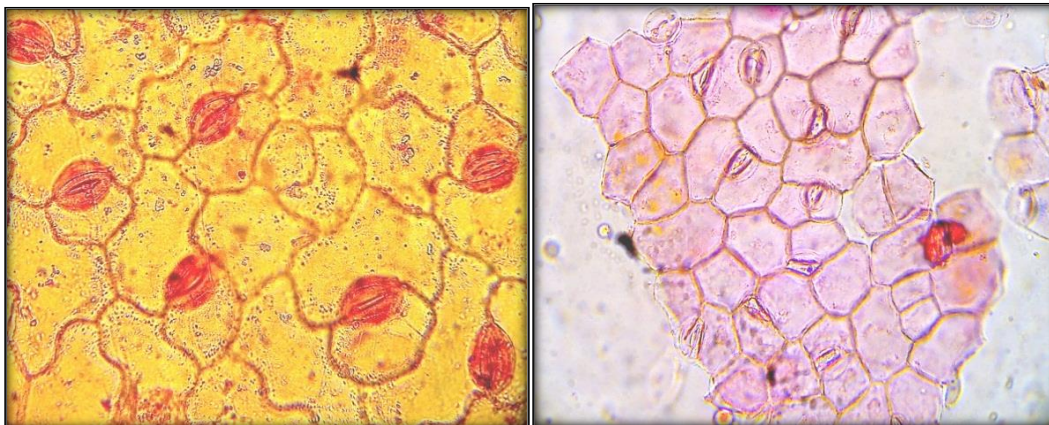


Fig.No.12: Upper epidermis (Paracytic) Fig.No.13: Lower epidermis (Anisocytic)

3.2.3. Trichome (Unicellular macroform two armed hair):

- Foot: Simple with the base circular or oval in outline, cells broader than long, contents scanty on upper and absent in lower. Lateral walls moderately thickened, smooth.
- Body: Unicellular, two armed; arms equal or nearly so, T-shaped, gradually tapering pointed

at both ends; wall moderately thickened, surface smooth or punctuate.

Length of trichome on adaxial surface is 75µm and width in middle is 5µm.

Length of trichome on abaxial surface is 125µm and width in middle is 10µm.

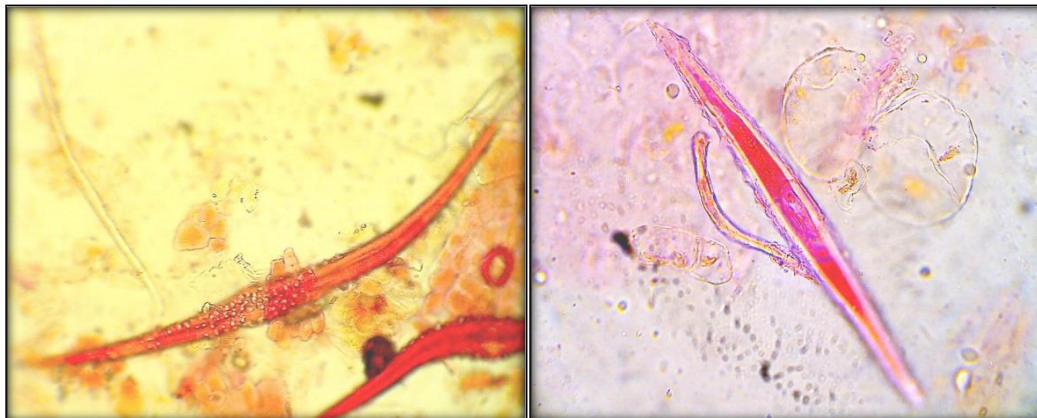


Fig.No.14: Trichome filled with scanty contents (adaxial) Fig.No.15: Trichomewithout contents (abaxial)

3.2.4. Leaf architecture

The basic axis of orientation in the leaflet is apical. The leaf organization is compound with respect to leaf shape and size, the length of leaflet is 7-12mm long and width is 2-5mm broad. The lamina is asymmetrical, base is slight asymmetrical, form is narrow obovate, apex is obtuse and base is acute. The margin is entire, leaf texture is membranaceous. The glands are absent

on the lamina and the petiole is normal. Type of venation is pinnate. Semicraspedodromous, primary vein (1°) is moderate, its course is straight and unbranched secondary vein (2°) is present. The angle of divergen is acute upper secondary veins more obtuse than lower and nearly uniform. Secondary veins are moderate, the course is curved, abruptly; loop forming branches are joining supra adjacent secondary at an acute angle. Intersecondary veins are simple, intermarginal vein is absent. Tertiary veins (3°) are

present. Angle of origin on exmedial and admedial side is RR/RA. The pattern is random reticulate, the precurrents are present, their course is sinous. The relationship to midvein is oblique, tertiary angle with midvein remaining approximately constant. The arrangement is predominantly alternate. Higher order venation forming reticulum in which vein orders are distinct. Quaternary veins (4°) are thin, their course is

random. Quinary veins (5°) are thin, their course is random. Highest vein order of the leaf is 6°. The marginal ultimate venation is complete. Veinlets linear, simple or branched once. Aéroles are perfectly developed arrangement is random and shapes are pentagonal and irregular.

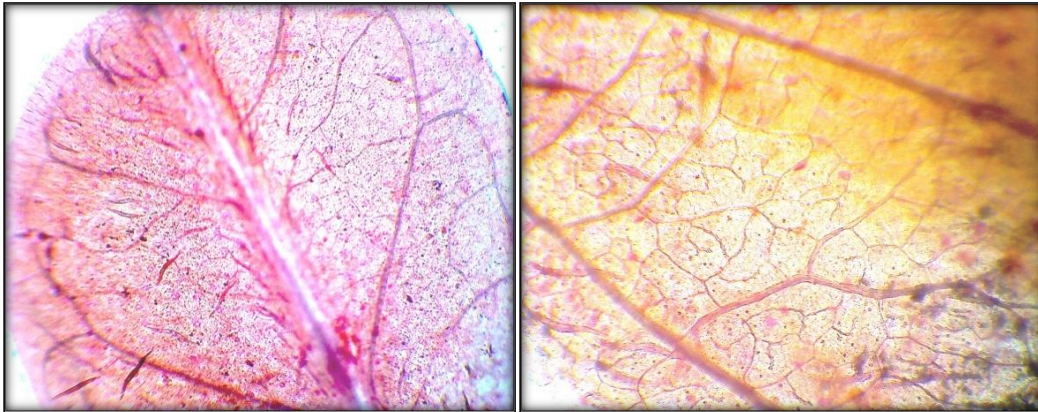


Fig.No.16: Cleared leaf (Apex) Fig.No.17: Leaf Venation showing veinlet

3.2.5. T-S Rachis:

Kidney shaped structure in outline. Rachis shows outer most layer of epidermis covered with thick cuticle. Epidermal cells are angular and small in size followed by single layer of hypodermis; hypodermal cells are collenchymatous, 3-4 layers of chlorenchyma present

below the collenchymatous layer on upper side, cells loosely placed with larger intercellular space. 5 vascular bundles roughly arranged in a ring; one is large, lateral two are equal in size departing to the wings and remaining two are very small in size. Ground tissue parenchymatous, thin walled with small intercellular spaces.

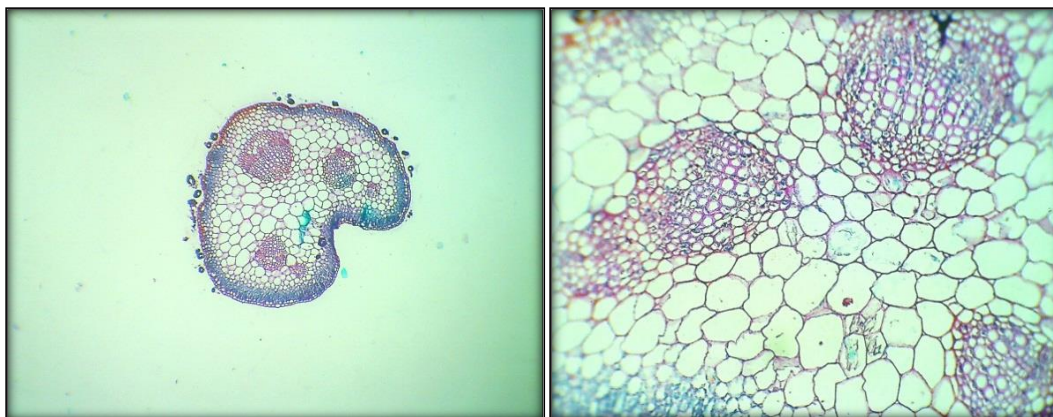


Fig.No.18: T-S Rachis (Entire View) Fig.No.19: T-S Rachis (Middle View)

4.CONCLUSION

Anatomical studies of plant parts show various significant characters. Epidermal cells of stem are radially elongated, collenchymatous cortex contain starch sheath and resin duct. Pericycle is homogenous formed of several layer of parenchymatous region. Vessels are in radial multiplies some are solitary performing various shape. Two different types of stomata are present on adaxial and abaxial surface. Paracytic type of stomata is present in adaxial surface and aniscytic stomata present in abaxial surface, also variation in both epidermal cells. Upper epidermal cells are radially elongated; lower epidermal cells are angular and small in size. Spongy parenchyma is dominant, 5-6 layered, vein-bundle is embedded in spongy parenchyma, and vasculature is roughly C-shaped. Unicellular macroform two armed trichome is present on both the leaf surface, scanty content is present on upper surface trichome and absent on lower surface trichome. Cortical region of root contain crystals of calcium oxalate, xylem vessels are radial, predominantly paired. Rachis shows 5 vascular bundles, one is large, lateral two are equal in size and remaining two are smaller. In general, anatomical characteristics of *Indigoferalinnæi* are very important and could be used in diagnostic key of taxa in all taxonomic level.

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