

Comparative Micromorphological Microscopic including Micrometric Evaluation of Important *Tephrosia* species - Leaves

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ABSTRACT

Tephrosia purpurea Pers., *Tephrosia candida* DC & *Tephrosia jamnagarensis* Sant. belongs to the family Fabaceae. All plants were used in Ayurveda. *Tephrosia purpurea* Pers. commonly known as Sharpunkha. It is also called as Plihasatru meaning an enemy of the spleen (splenic diseases)¹. Till date the comparative pharmacognostical work had been not reported regarding leaves. In the present study detail comparative study of *Tephrosia purpurea* Pers., *Tephrosia candida* DC. & *Tephrosia jamnagarensis* Sant. has been under taken. The pharmacognostical evaluation showed that all the three species with prismatic and rhomboidal crystals of ca. oxalate, fibers, oil globules in the sections. Whole powder microscopic showed pollen grains of *T. candida* and *T. jamnagarensis* and border pitted vessel, starch grains are observed.

Keywords

Leaves, pilhasatru, Pharmacognosy, Sharpunkha



Greentree Group

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INTRODUCTION

India is virtually a herbarium of the world, using plants and herbs as the basic source of medicine. Herbs which form a part of our nutrition and provide us an additional therapeutic effect are in demand and *Tephrosia* species is one of such plant. *Tephrosia* species (Fabaceae) are well known medicinal plants. All the three plants commonly found in India and other tropical countries. *Tephrosia purpurea* Pers., *Tephrosia candida* DC. and *Tephrosia jamnagarensis* are herbs naturally available as weed of road side and agricultural fields. They are Laxative, Diuretic, Tonic¹². Till date the comparative pharmacognostical work had not been reported. So in the present study detailed comparative study of *Tephrosia purpurea* Pers., *Tephrosia candida* DC. and *Tephrosia jamnagarensis* Sant. has been under taken.

MATERIALS AND METHODS

Collection of Samples:

Fresh leaves of all the three species of *Tephrosia* were collected by the scholar from out skirts' of Jamnagar during the month of October-November 2015. Then the plant was authenticated in the pharmacognosy lab. IPGT & RA, Jamnagar, herbarium

submitted in the department (Specimen No. PHM 6161/62/63/2015). The leaves were first washed with tap water then few leaves were stored in solution of AAF (70% Ethyl alcohol: Glacial acetic acid: Formalin) in the ratio of (90:5:5) to utilize them for microscopic studies whenever needed. The remaining parts were dried under the shade and then were subjected for 60# powdering.

Morphological Study:

Morphological characters of leaves of *Tephrosia* were carried out as per visual observation, following standard procedure and scientifically studied as per taxonomy and verified with existing floras for authentication^{1,2}.

Microscopic evaluation:

Thin free hand transverse section of leaf i.e. petiole and lamina through midrib for all the three species of *Tephrosia* were taken. Sections were first observed in distilled water, stained with suitable staining reagents (phloroglucinol+HCl, Iodine) and again examined to assess different cellular structure and content. The samples were observed under Carl Zeiss trinocular microscope with camera^{3,4}.

Surface Study:

Leaf surfaces were studied by peeling up of both the surfaces of leaves, then washed with chloral hydrate and observed under microscope with distilled water for stomatal structure and distribution along with stomatal index and palisade ratio^{5,6}.

Micrometric evaluation:

Measurement of the length, breadth of stomata, crystal, trichomes are studied mean value was taken into consideration for micrometric evaluation with the help of Carl Zeiss trinocular microscope attached with camera with preloaded micrometric analysis software^{7,8}.

Organoleptic characters:

The colour, odour, taste and touch of leaves were recorded separately through visual and sensory observation¹¹.

Powder microscopic evaluation:

Cut pieces of leaf of all the three species of *Tephrosia* were dried under shade, powdered with the help of mechanical grinder and sieved through mesh no. 60 #. Then leaf powder were studied under microscope with distil water and also examined after staining with different suitable reagent⁹.

RESULTS AND DISCUSSION

T. purpurea:

Morphological study:

Macroscopic investigation showed that the leaves are 3-8 cm long; leaflets 9-13, 0.5-2.3 X 0.2-1.2 cm, obvate to oblanceolate, appressed-silky-hairy beneath¹⁰.

Microscopical study:

Transverse section of Main rachis:

The T.S of main rachis is monkey face shape in outline. Outer epidermis followed by cortex, pericyclic fiber, vascular Bundle and central pith.

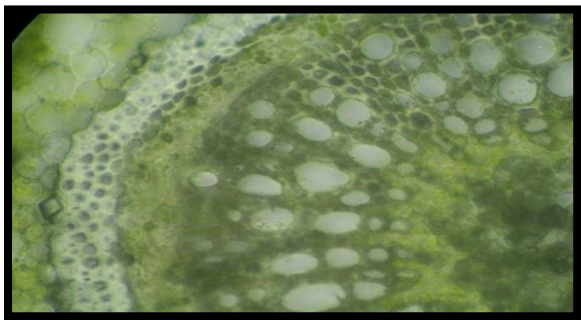
Detailed T.S. shows that epidermis is made up of single layered oval to barrel shaped compactly arranged cells interrupted by simple warty trichomes. Epidermis followed by 3 to 4 layered cholenchyma tissue, compactly arranged without any intercellular spaces forming hypodermis. Cortex is madeup of parenchyma cells loaded with chlorophyll pigments, oil globules, starch grains and rhomboidal crystals of Calcium oxalate. At the corner of ridge two meristemes were observed in the cortical zone. Cortex ends with discontinuous band of circularly arranged 3 to 4 layers of lignified pericyclic fibers beneath the single layered endodermis. Vascular bundle made up of xylem & Phloem. Xylem arranged radially consisting of metaxylem towards

corticle zone, protoxylem towards pith. Xylem consist xylem parenchyma & its fiber. Phloem present above the xylem made up of phloem fibers & sieve elements. Pith occupies larger portion of the section made-up of parenchyma cells loaded by oil globule, starch grain, prismatic crystals (Plate 1A–H).

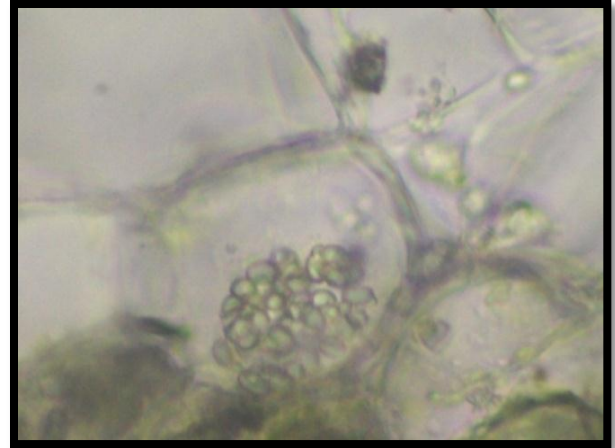
Plate No. 1 Transverse section of Main rachis of *T.purpurea*



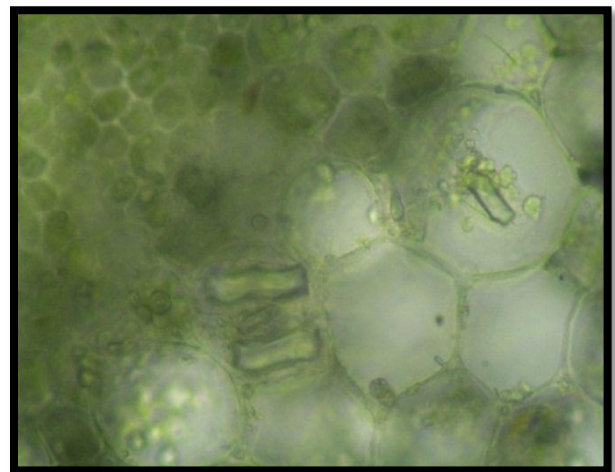
A – Diagrammatic section



B- Cortex, pericyclic fiber & V.B.



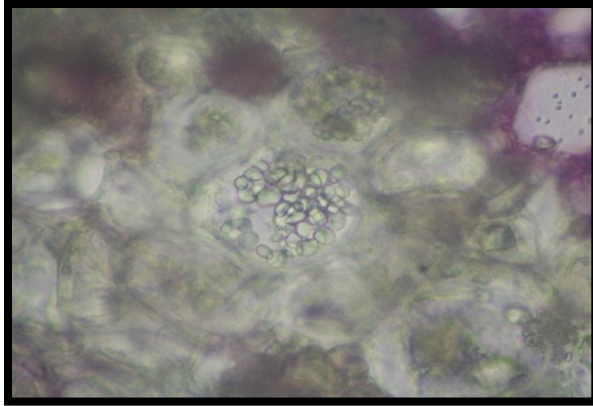
C- Parenchyma cells with Starch Grain



D- Parenchyma cells with Rhomboidal crystals



E- Stained diagrammatic section



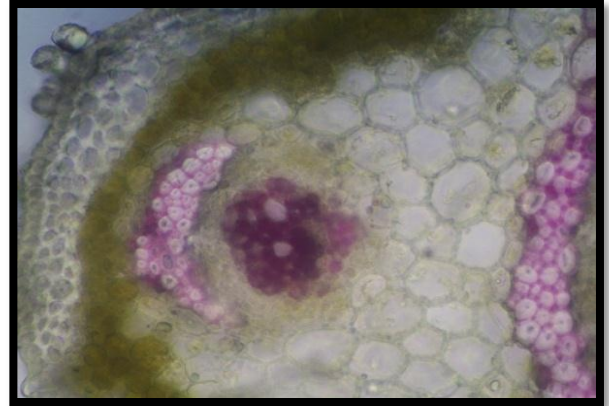
G- Lignified parenchyma cells with Starch Grain

T.S Through midrib:

The T. S of leaf showed upper and lower epidermis with mesophyll tissue having upper palisade and lower spongy parenchyma cells. Section through midrib showed centrally located vascular bundle covered with ground tissue. On the lower side of the transverse section 1–3 layers of collenchymatous cells were present (Plate 2A–F).

Epidermis was Single layered, barrel shaped epidermal cells both on upper and lower epidermis with unicellular trichomes. Epidermis was covered with cuticle. Trichomes were more in lower epidermis than upper epidermis. Stomata found on both the epidermis.

Mesophyll tissue was differentiated into two layers. Upper 2-3 layers of compactly arranged palisade parenchyma with oil globules, and rich in chloroplast pigments,



H- Section with Meristematic

rarely with some rhomboidal crystals of calcium oxalate, lower 3–5 layers of spongy parenchyma cells, loosely arranged with air spaces and loaded with prismatic crystals of calcium oxalate.

Section through mid-rib showed a large vascular bundle located at the centre, 1–3 layers of collenchyma tissue present at the lower epidermis surrounding the ground tissue. Ground tissue was made up of thin walled compactly arranged parenchyma cells heavily loaded by rhomboidal and prismatic crystal of calcium oxalate and some oil globules.

Inner to the ground tissue, single layered, somewhat elongated thin walled cells forming endodermis. Inner to the endodermis 4–5 layers of pericyclic fibers above and below the vascular bundle.

Vascular bundle was open and collateral in type. Phloem presents below the xylem with

some phloem fibers and sieve elements, metaxylem facing towards lower epidermis and protoxylem facing towards upper

epidermis. Xylem bundles were made up of xylem parenchyma and fibers.

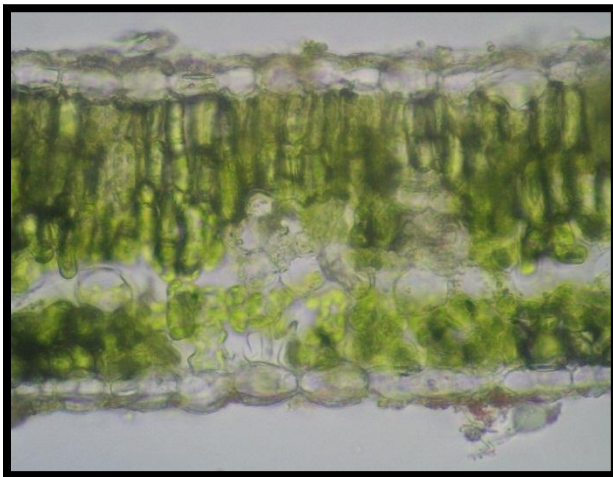
Plate No. 2: Transverse section through midrib of *T. Purpurea*



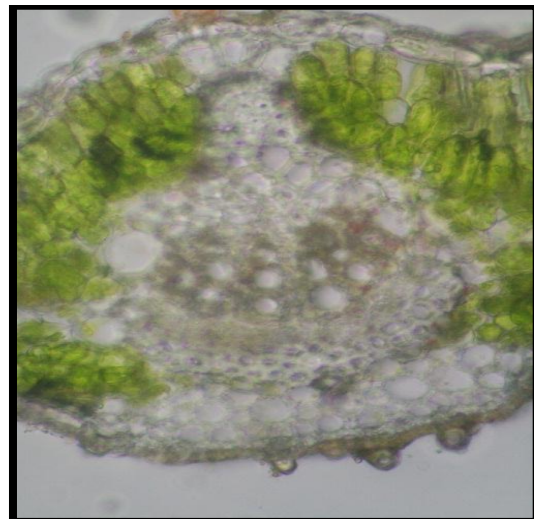
A. T.S. with Lamina and Central V.B.



B. Diagrammatic Section of leaf



C. Upper & Lower epidermis with mesophyll



D. Central V.B.



E. Pericyclic fiber, phloem, Xylem

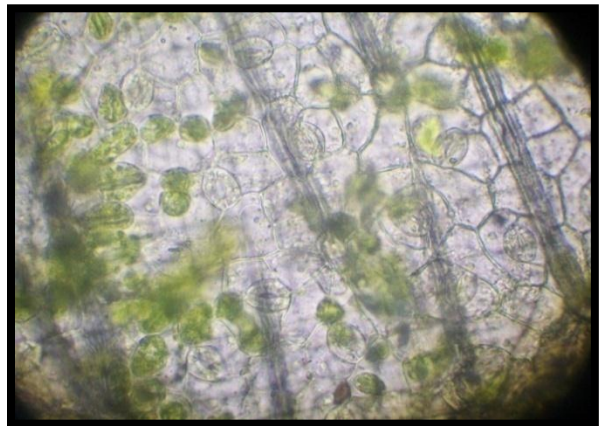
Surface Study:

Surface study of epidermis was carried out to determine type and distribution of stomata, epidermal cell and trichomes. Stomata were present both upper and lower epidermis and consists only wavy epidermal cells. Some of the trichomes and cicatrix were also observed.

Both epidermis consists stomata of paracytic type. Some of the trichomes and cicatrix were also observed. The stomatal number, stomatal index, stomatal size, epidermal cell size were calculated by trial and error method (by taking 3–5 successive readings. Mean value was taken into consideration.) Results are tabulated in Table – 1.



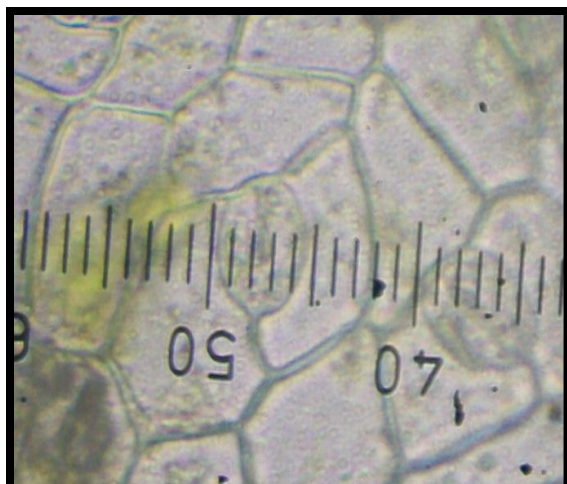
F. Spongy & Palisade Parenchyma



A. Stomatal Index



B. Measurement of Stomata



A- Measurement of Stomata

Table 1 Surface study of *T. purpurea* leaf

Sr. No.	Parameters	Results U.E	Results L.E
1	Type of the stomata	Paracytic	Paracytic
2	Length of the stomata	0.6 μ m	0.6 μ m
3	Width of the stomata	0.5 μ m	0.6 μ m
4	Number of the stomata	10, 12, 14, 13, 12	15,16,14,13,16
5	Number of epidermal cells	40, 48, 56, 52, 48	60,64,58,54,64
6	Stomatal index	20	20

Quantitative microscopy:

Quantitative microscopy of leaves was carried out to determine size of epidermal cell,, size of the stomata, trichomes etc. are scientifically studied and depicted in the Table No. 4.

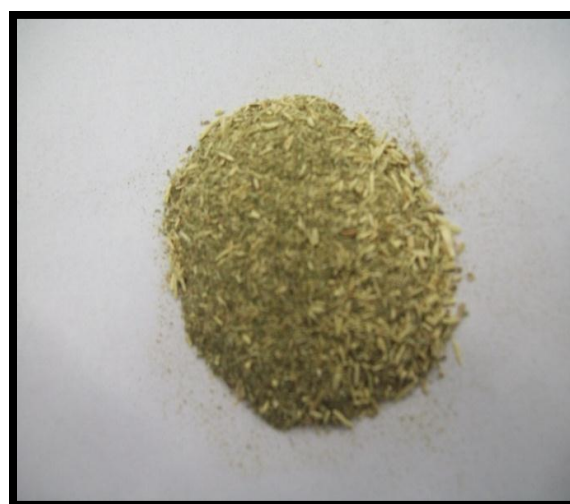
Whole plant powder of *T. purpurea*:

Powder microscopy of the dried leaf powder was carried out following standard guidelines. Organoleptic characters showed the presence of greenish color with leafy odour and bitter taste with slight mucilaginous and fine coarse in touch.

Microscopic characters

Diagnostic characters of powder microscopy showed the paracytic stomata, epidermal cells of upper epidermis, unicellular, simple horn shaped trichomes from epidermis, rhomboidal crystals of calcium oxalate from mesophyll and ground tissue. Border pitted and annular vessels from vascular bundles, fragment of pericyclic fibre, paracytic stomata, lignified fibers, brown content are observed. (Plate 3A-N)

Plate No. 3 Whole plant powder of *T. purpurea*:



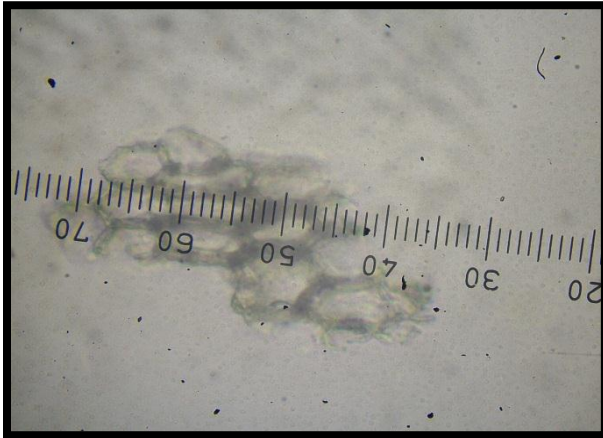
Powder of *T. Purpurea*



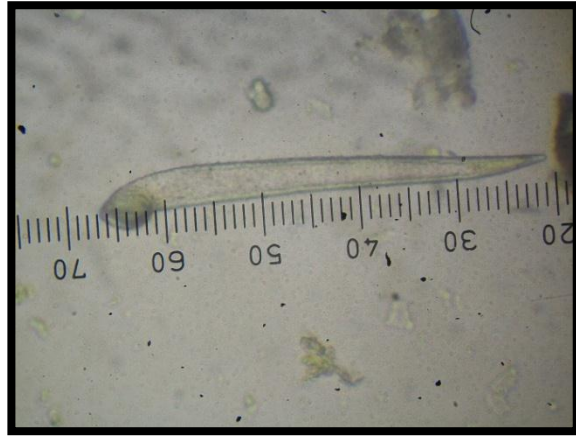
A- Epidermal Cell



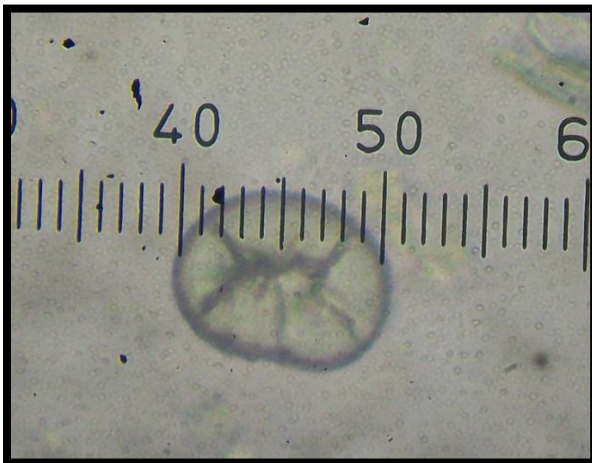
B- Measurement of Epidermal Cell



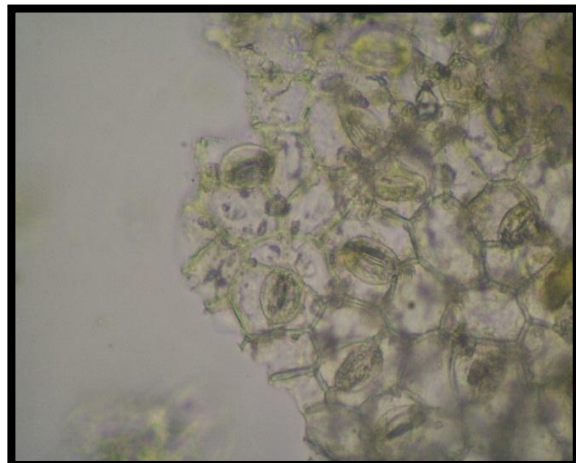
C- Cork in Surface view



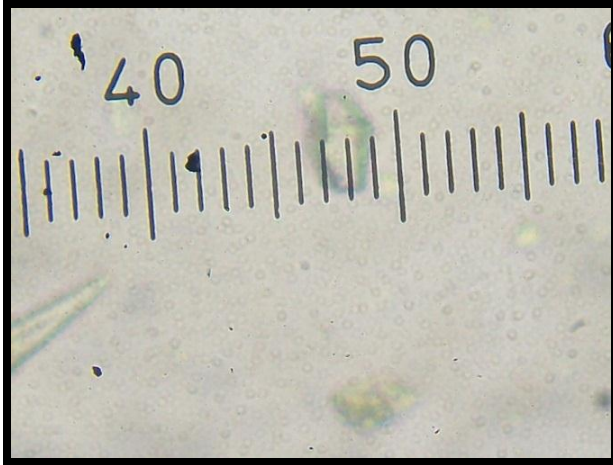
D - Simple warty Trichome



E- Simple Starch Grain



F- Epidermal cells with Stomata



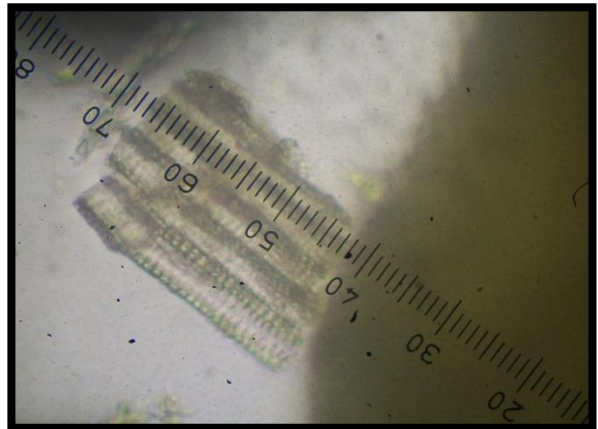
G - Rhomboidal Crystal



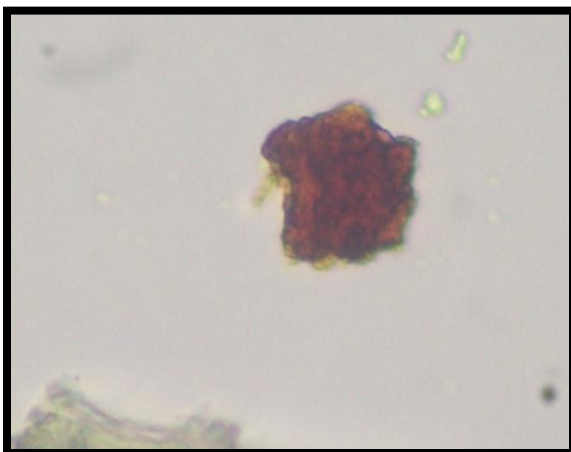
H - Epidermal Cells With Stomata



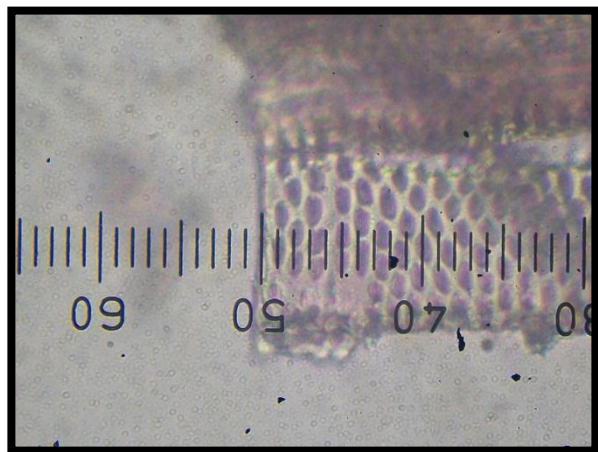
I - Rhomboidal Crystal



J- Annular Vessel



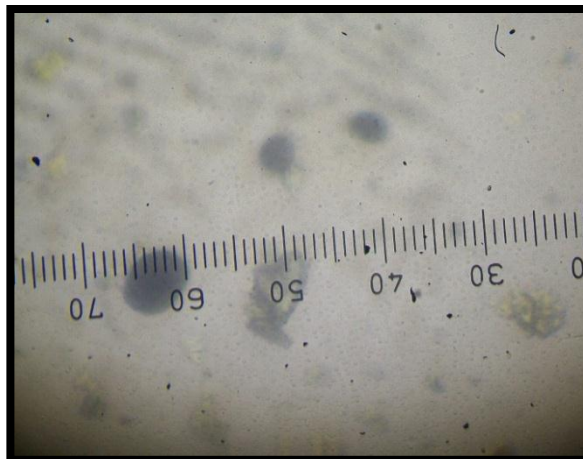
K- Brown Content



L- Lignified Border Pitted vessel



M- Lignified fibers



N- Iodine stained Starch grain

T. candida*:*Morphological study:**

Macroscopic investigation showed that the leaves are 5-7 cm long; leaflets 11-13, opposite, 1-3.8 X 0.2-1 cm, elliptic-oblong or oblong-lanceolate, glabrous above, appressed-pubescent beneath¹⁰.

Microscopical study:**Transverse section of Main rachis of *T. candida*:**

The T.S of Main rachis is oval to monkey face shaped in outline. Outer epidermis followed by cortex, pericyclic fiber, Vascular Bundle and central large pith.

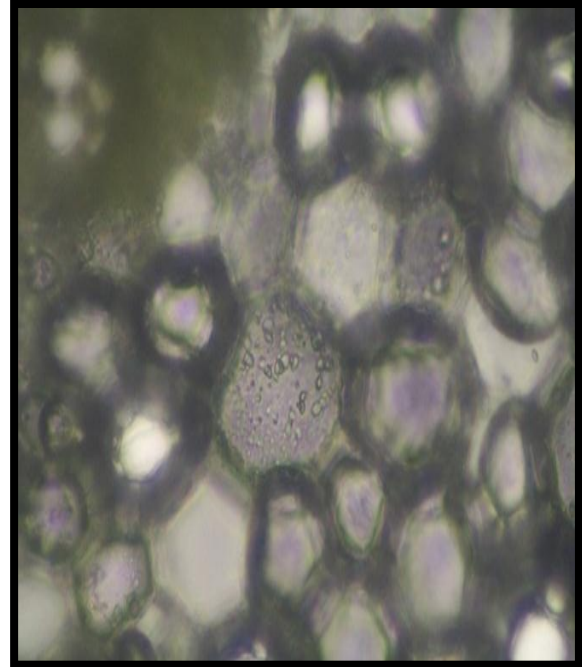
Detail T.S. shows that epidermis made up of single layered oval to barrel shaped compactly arranged cells interrupted by large number of simple warty trichomes. Epidermis followed by 2 to 3 layers of cholenchyma tissue which are compactly

arranged without any intercellular spaces. Cortex made up of parenchyma cells loaded by chlorophyll pigments, oil globules, starch grains and rhomboidal crystals of Ca. oxalate. At the corner of ridge two meristeles were observed in the cortical zone. Cortex ends with discontinuous band of circularly arranged 3 to 5 layers of lignified pericyclic fibers beneath the single layered endodermis. Vascular bundle made up of xylem & Phloem. Xylem radially & circularly arranged & made up of metaxylem towards corticle zone, protoxylem towards pith. Xylem consist xylem parenchyma & its fiber. Phloem present above the xylem made-up of phloem fibers & sieve elements. Pith occupies larger portion of the section as compared to *T.purpuria* made up of parenchyma cells loaded by oil globule, starch grain, prismatic crystals. (Plate 4 A-F)

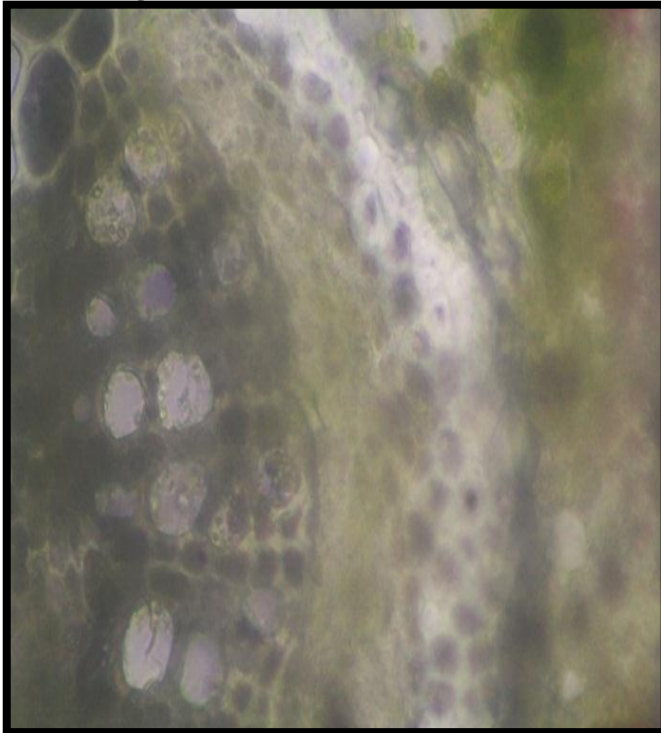
Plate No. 4 Transverse section of Main rachis of *T. candida*:



A- Diagrammatic section



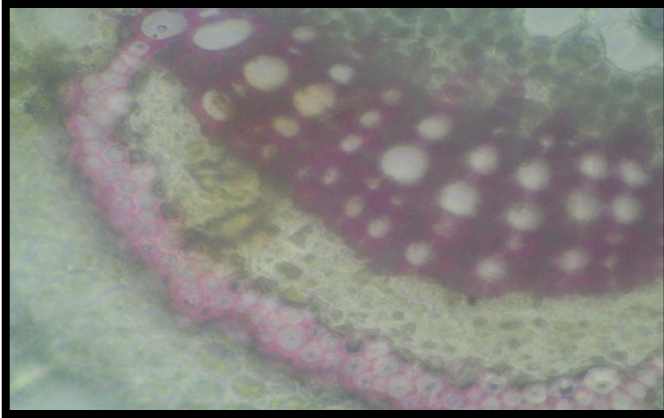
B- Parenchyma Cells with starch grain



C- Cortex, pericyclic fiber, Phloem & Xylem



D- Stained diagrammatic section



E- Cortex, Parenchyma & V.B.

Transverse section through midrib of *T. candida*:

The T. S of leaf showed upper and lower epidermis with mesophyll tissue having upper palisade and lower spongy parenchyma cells. Section through midrib showed centrally located vascular bundle covered with ground tissue. On the lower side of the transverse section 3–4 layers of collenchymatous cells were present (Plate 5A–E).

Epidermis was Single layered, barrel shaped epidermal cells both on upper and lower epidermis with unicellular simple and warty trichomes. Epidermis was covered with cuticle. Trichomes were more in both epidermis stomata found on both epidermis. Mesophyll tissue was differentiated into two layers. Upper 1–2 layers of compactly arranged palisade parenchyma rich in brown content and chloroplast pigments,



F- Epidermis, Hypodermis, Cortex, Parenchyma & phloem

rarely with some rosette crystals of calcium oxalate. Lower 3–5 layers of spongy parenchyma cells.

Section through mid-rib showed a large vascular bundle located at the centre, 1–3 layers of collenchyma tissue present at the lower epidermis surrounding the ground tissue. Ground tissue was made up of thin walled compactly arranged parenchyma cells rarely loaded by rosette crystals of calcium oxalate and some oil globules.

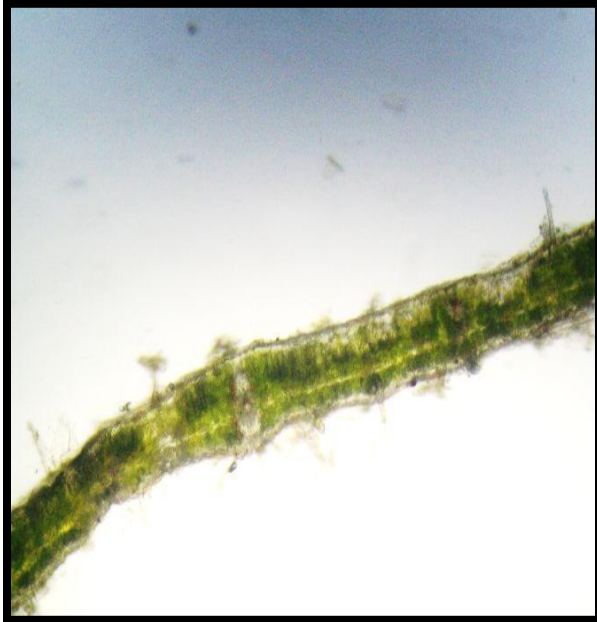
Inner to the ground tissue, single layered, somewhat elongated thin walled cells forming endodermis. Inner to the endodermis 4–5 layers of pericyclic fibers forming a ring like structure covering the vascular bundle.

Vascular bundle was open and bi-collateral in type. Phloem's present around the xylem with some phloem fibers and sieve elements formed a ring like structure, metaxylem

facing towards lower epidermis and protoxylem facing towards upper epidermis. The xylem bundles were separated by

uniseriate medullary rays along with xylem parenchyma and fibers.

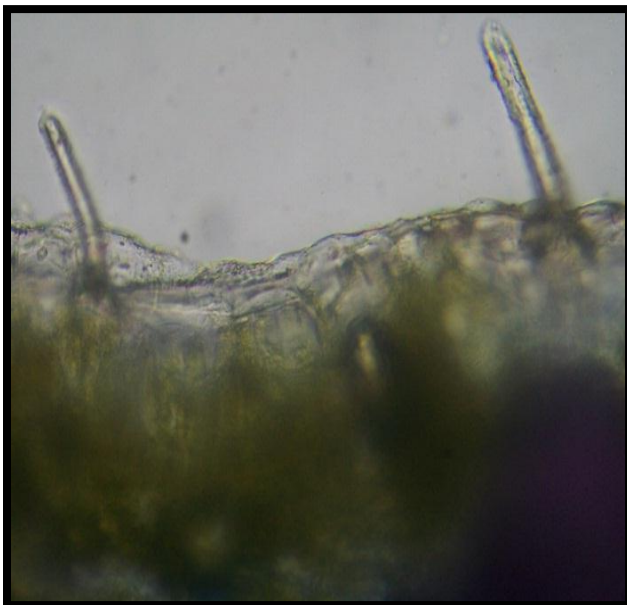
Plate No. 5: Transverse section through midrib of *T. candida*:



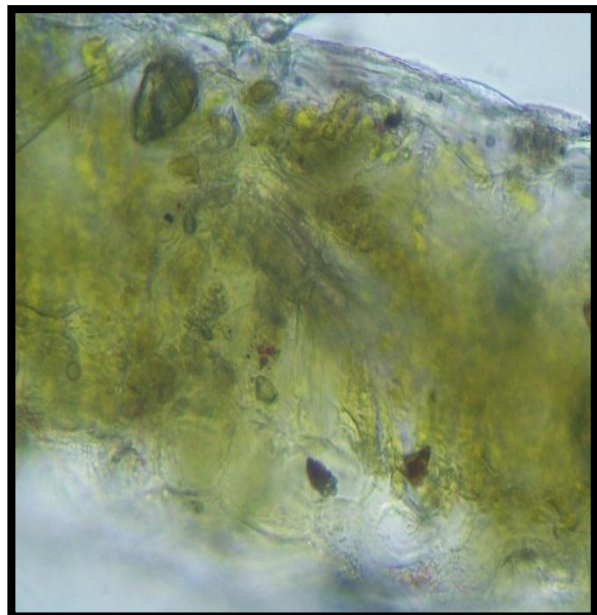
A- Diagrammatic sketch of leaf



B- Lamina & central V.B.



C- Epidermal cells with trichome



D- Lower Epidermis with stomata & crystals



E- Xylem & Phloem

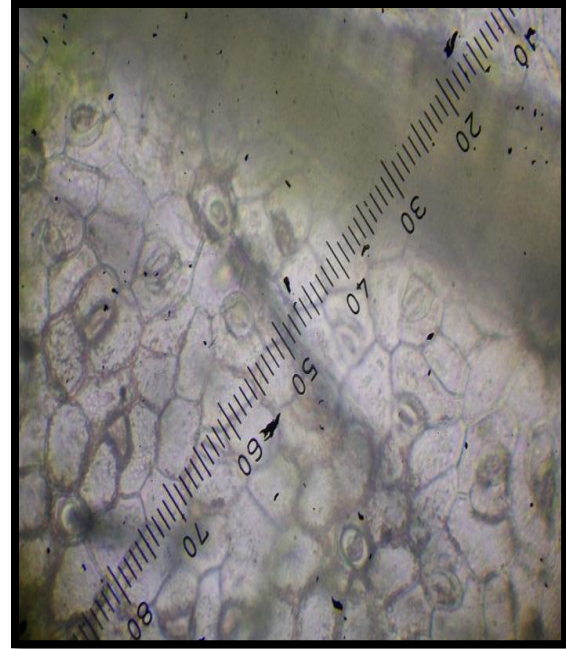
Surface Study:

Surface study of epidermis was carried out to determine type and distribution of stomata, epidermal cell and trichomes. Stomata were present both upper and lower epidermis and consists only wavy epidermal cells. Some of the trichomes and cicatrix were also observed.

Both epidermis consists stomata of paracytic type. The stomatal number, stomatal index, stomatal size, epidermal cell size were calculated by trial and error method (by taking 3–5 successive readings. Mean value was taken into consideration.) Results are tabulated in Table – 2.

Table 2 Surface study of *T. candida* leaf

Sr. No.	Parameters	Results U.E	Results L.E
1	Type of the stomata	Paracytic	Paracytic
2	Length of the stomata	0.5 μm	0.5 μm
3	Width of the stomata	0.4 μm	0.3 μm
4	Number of the stomata	18,20,18,20,20	25, 27, 26,27,27
5	Number of epidermal cells	54,60,54,60,54	45,50,60,50,50
6	Stomatal index	25	34

**A-Stomatal Index****B-Measurement of stomata****C-Measurement of Stomata****Quantitative microscopy:**

Quantitative microscopy of leaves was carried out to determine size of epidermal cell, size of the stomata, trichomes etc. are scientifically studied and depicted in the Table No. 4.

Whole plant powder of *T. candida*:

Powder microscopy of the dried leaf powder was carried out following standard guidelines. Organoleptic characters showed the presence of greenish color with leafy odour and bitter taste with mucilaginous and fine coarse in touch.

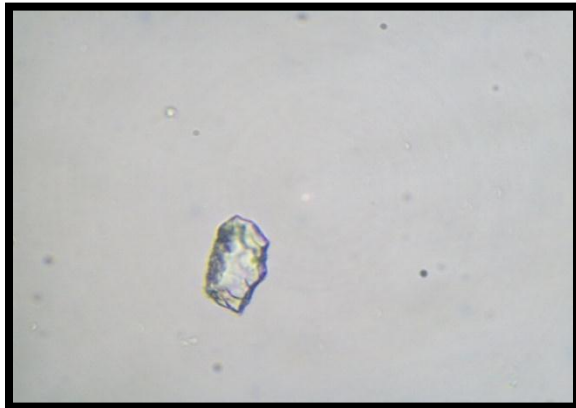
Plate No. 6 Whole plant powder of *T. candida*:

Microscopic characters

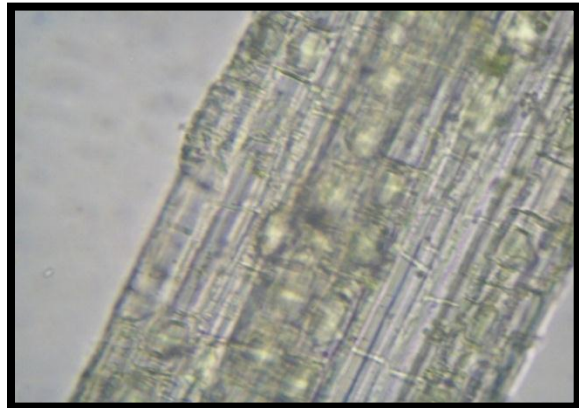
Diagnostic characters of powder microscopy showed the paracytic stomata, epidermal cells of upper epidermis, unicellular, simple horn shaped trichomes from epidermis, rhomboidal crystals of calcium oxalate from mesophyll and ground tissue. Border pitted and annular vessels from vascular bundles, pollen grains from flower, fragment of pericyclic fibre, paracytic stomata, lignified fibres, brown content, and crystalline fibers are observed (Plate 6A–H).



Powder of T. candida



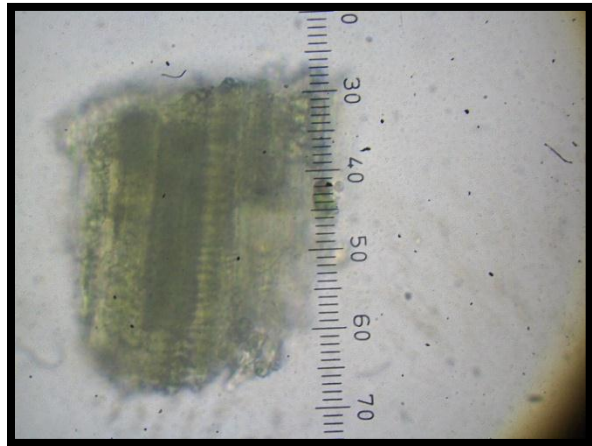
A- Rhomboidal Crystal



B- Crystal Fibers



C- Simple warty trichome



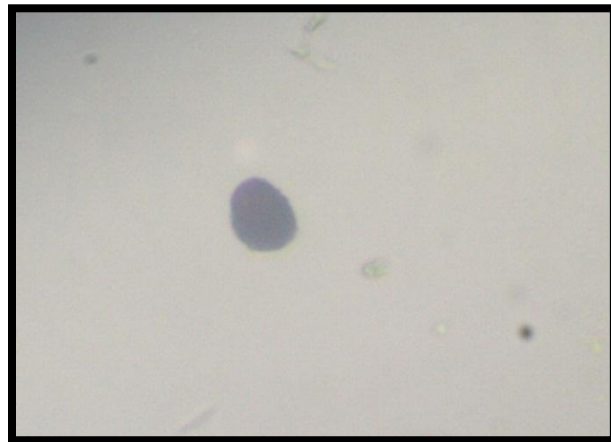
D- Annular vessels



E- Oil Globule



F- Stomata



G- Iodine stained Starch Grain



H- Lignified crystal fibers

T. jamnagarensis*:*Morphological study:**

Macroscopic investigation showed that the leaves are 1.4-6.2 X 0.3-0.8 cm, linear, linear-lanceolate, densely appressed-hairy beneath^[10].

Microscopical study:**Transverse section of Main rachis of *T. jamnagarensis*:**

The T.S of Main rachis is monkey face shaped in outline. Outer epidermis followed

by cortex, pericyclic fiber, Vascular Bundle and central large pith.

Detail T.S. shows that epidermis made-up of single layered oval to barrel shaped compactly arranged cells interrupted by large number of simple warty trichomes. Epidermis followed by 2 to 3 layers of cholenchyma tissue which are compactly arranged without any intercellular spaces, cortex madeup of parenchyma cells, loaded by chlorophyll pigments, oil globules, starch grains and rhomboidal crystals of Ca. oxalet.

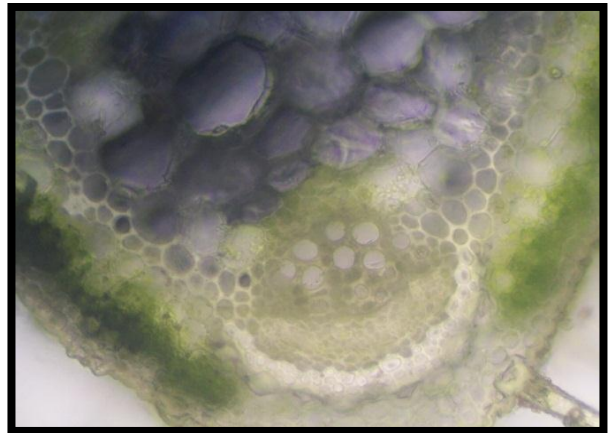
At the corner of ridge two meristeles were observed in the cortical zone. Cortex ends with discontinuous band of circularly arranged 3 to 5 layers of lignified pericyclic fibers beneath the single layered endodermis, vascular bundle made-up of xylem & Phloem. Xylem arranged radially consisting of metaxylem towards corticle zone, protoxylem towards pith. Xylem

consist xylem parenchyma & its fiber. Phloem present above the xylem made up of phloem fibers & sieve elements. Pith occupies larger portion of the section made-up of parenchyma cells loaded by oil globule, starch grain, prismatic crystals (Plate 7A–D).

Plate No.7 Transverse section of Main rachis of *T. jamnagarensis*



A- Diagrammatic section



B- Section with Meristal



C- Stained diagrammatic section



D- Parenchyma cells, Phloem, Xylem with Meristal

Transverse section of leaflet through midrib of *T. jamnagarensis*:

The T. S of leaf showed upper and lower epidermis with mesophyll tissue having

upper palisade and lower spongy parenchyma cells. Section through midrib showed centrally located vascular bundle covered with ground tissue. On the lower side of the transverse section 1–3 layers of collenchymatous cells were present.

Epidermis was single layered, barrel shaped epidermal cells both on upper and lower epidermis with unicellular trichomes. Epidermis was covered with cuticle. Trichomes are more in lower portion than upper portion. Stomata found on both the epidermis.

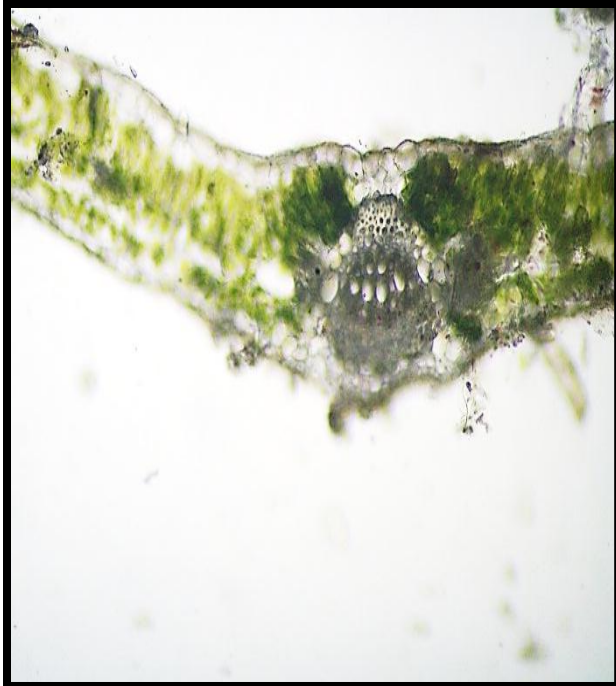
Mesophyll tissue was differentiated into two layers. Upper 2–3 layers of compactly arranged palisade parenchyma with oil globules, brown content and rich in chloroplast pigments, rarely with some rhomboidal crystals of calcium oxalate, lower 3–5 layers of spongy parenchyma cells, loosely arranged with large intercellular spaces and loaded with prismatic calcium oxalate.

Section through mid-rib showed a large vascular bundle located at the centre, 1–3 layers of collenchyma tissue present at the lower epidermis surrounding the ground tissue. Ground tissue was made up of thin walled compactly arranged parenchyma cells heavily loaded by rhomboidal and prismatic crystal of calcium oxalate and some oil globules.

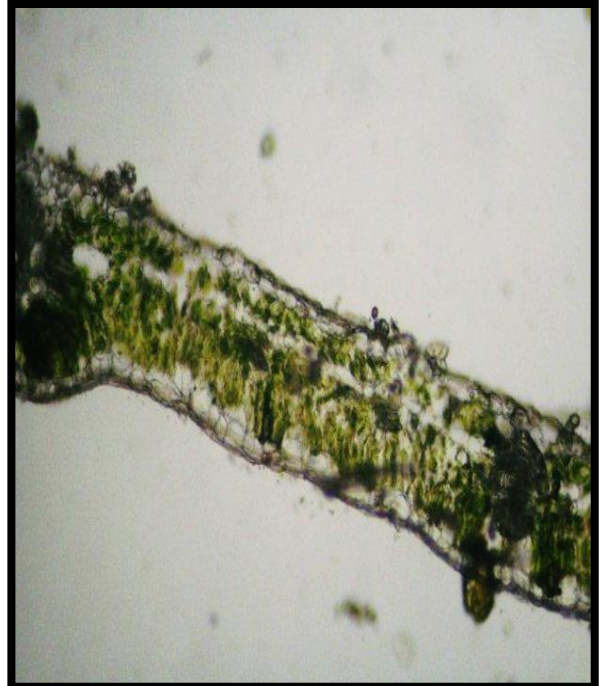
Inner to the ground tissue, single layered, somewhat elongated thin walled cells forming endodermis. Inner to the endodermis 4–5 layers of pericyclic fibers above and below the vascular bundle.

Vascular bundle was open and collateral in type. Phloem presents below the xylem with some phloem fibers and sieve elements, metaxylem facing towards lower epidermis and protoxylem facing towards upper epidermis. Xylem bundles were covered with xylem parenchyma and fibers (Plate 8A–F).

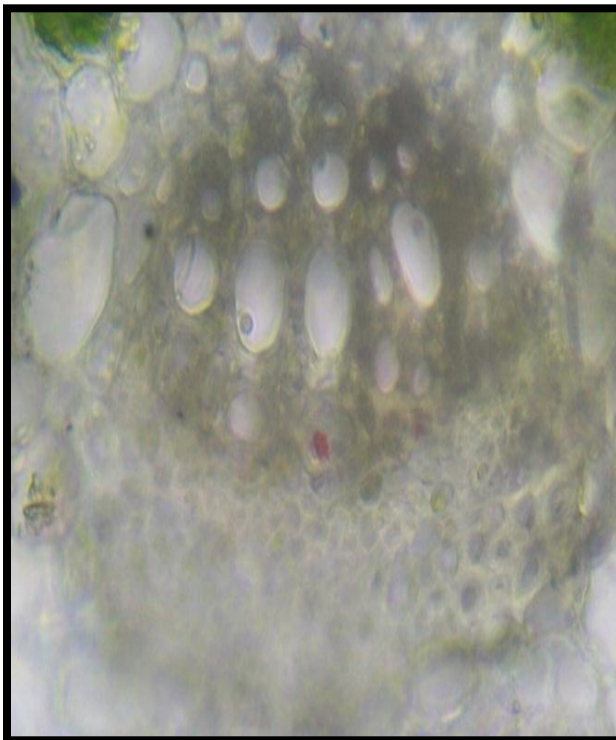
Plate No. 8 Transverse section through midrib of *T. jamnagarensis*



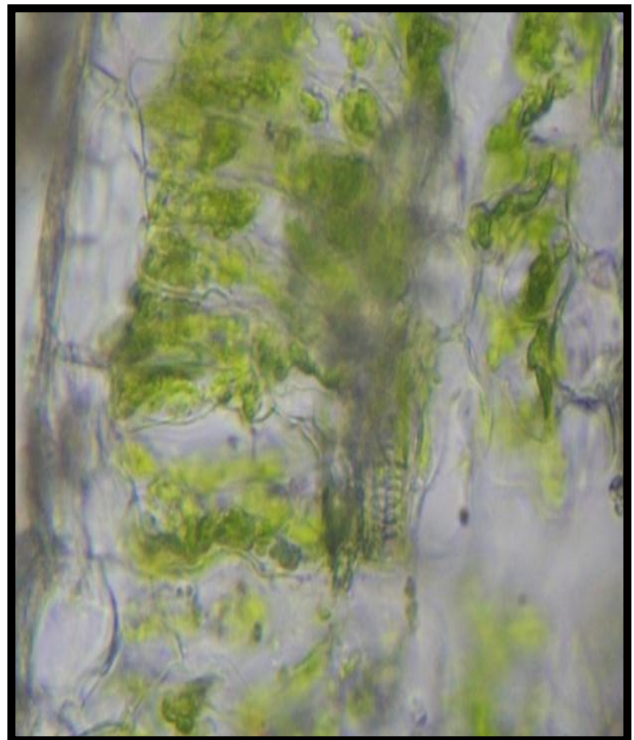
A- Diagrammatic section



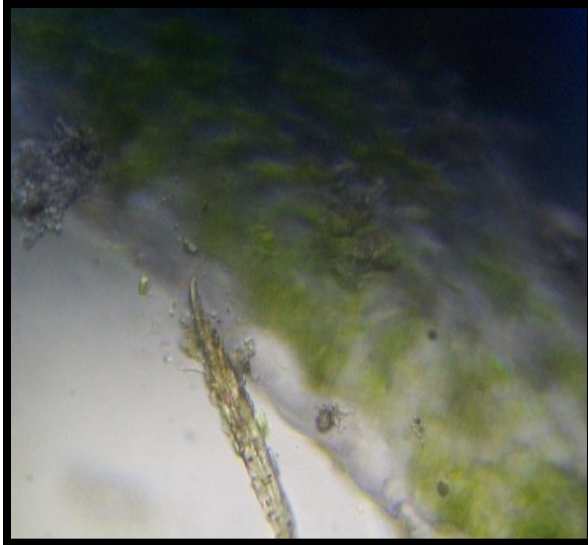
B- Upper & Lower Epidermis with mesophyll



C- Paricyclic fiber, Phloem, Xylem



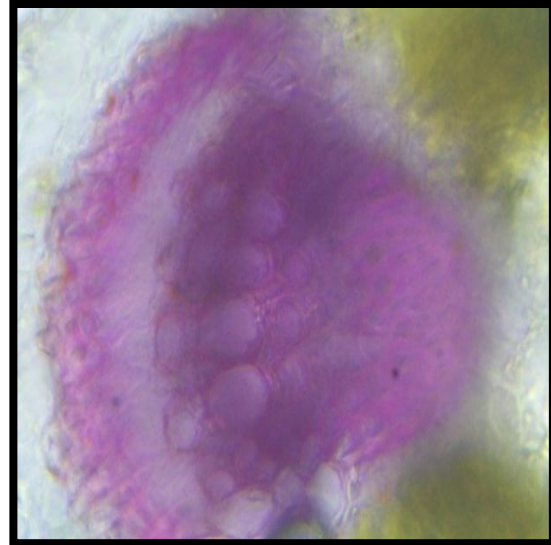
D- Paliside & Spongy Parenchyma



E- Epidermis with warty Trichome

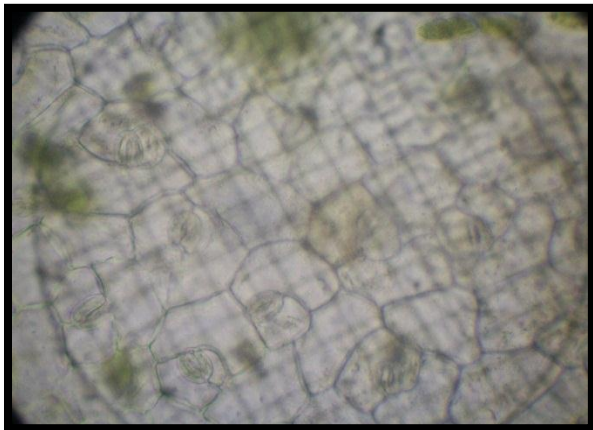
Surface Study:

Surface study of epidermis was carried out to determine type and distribution of stomata, epidermal cell and trichomes. Stomata were present both upper and lower epidermis and consists only wavy epidermal cells. Some of the trichomes and cicatrix were also observed.

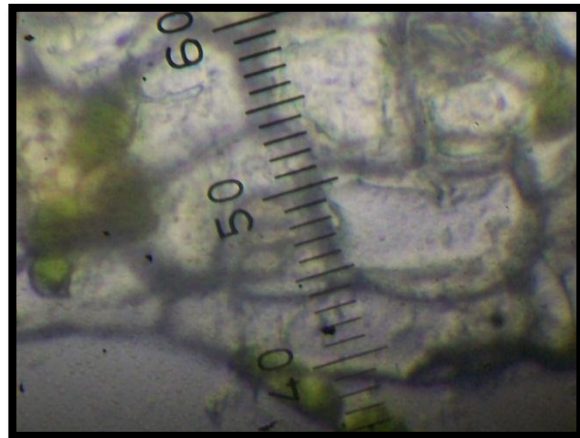


F- V.B. with Xylem, Phloem

Both epidermis consists stomata of paracytic type. Some of the trichomes and cicatrix were also observed. The stomatal number, stomatal index, stomatal size, epidermal cell size were calculated by trial and error method (by taking 3–5 successive readings. Mean value was taken into consideration.) Results are tabulated in Table – 3.



A- Stomatal Index



B- Micro measurement of stomata

C-

Table 3 Quantitative microscopy of *T. jamnagarensis* leaf:

Sr No	Parameters	Results U.E	Results L.E
1	Type of the stomata	Paracytic	Paracytic
2	Length of the stomata	0.5 μ m	0.6 μ m
3	Width of the stomata	0.4 μ m	0.4 μ m
4	Number of the stomata	6,4,7,8,13,12	18,23,24,23,24
5	Number of epidermal cells	43,42,42,40,42	50,53,58,53,58
6	Stomatal index	12	31

Quantitative microscopy:

Quantitative microscopy of leaves was carried out to determine size of epidermal cell, size of the stomata, trichomes etc. these

are scientifically studied and depicted in the Table no. 4.

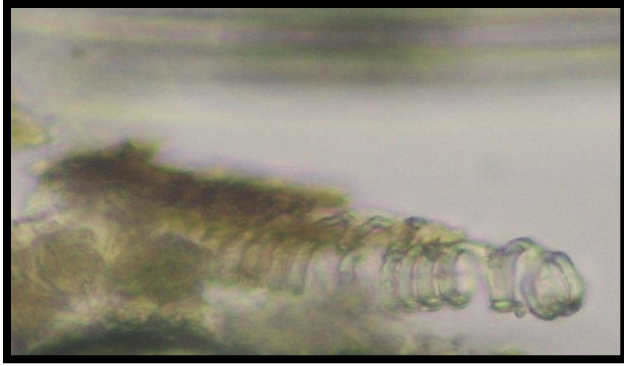
Whole plant powder of *T. jamnagarensis*:

Powder microscopy of the dried leaf powder was carried out following standard guidelines. Organoleptic characters showed the presence of greenish color with leafy odour and bitter taste with highly mucilaginous and fine coarse in touch.

Microscopic characters

Diagnostic characters of powder microscopy showed the paracytic stomata, epidermal cells of upper epidermis, unicellular, simple horn shaped trichomes from epidermis, prismatic crystals of calcium oxalate from mesophyll and ground tissue. Spiral vessels from vascular bundles, pollen grains from flower, fragment of pericyclic fibre, paracytic stomata, lignified fibres, brown content, fibers with stomata are observed (Plate 9A–H).

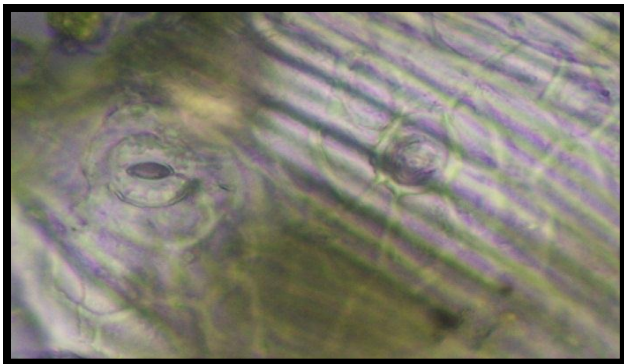
Plate No.9 Whole plant powder of *T. jamnagarensis*Powder of *T. jamnagarensis*



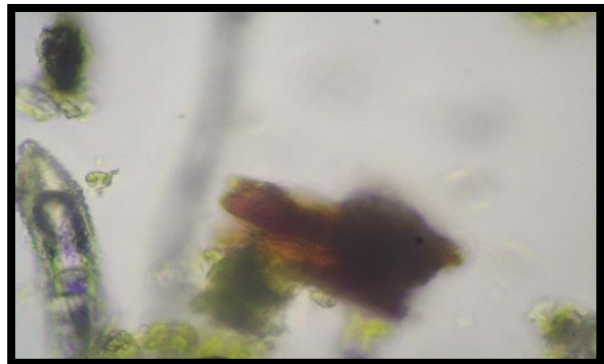
Spiral Vessel



Prismatic Crystals



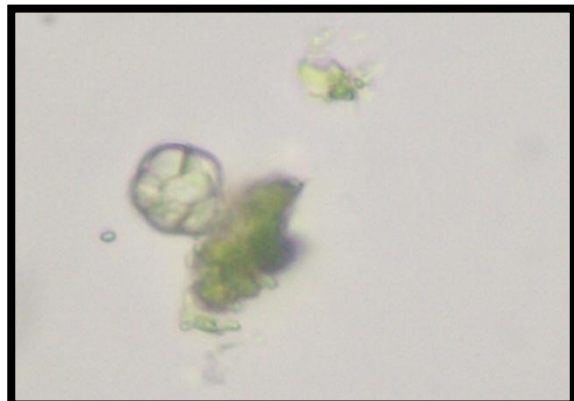
Epidermal cells with Stomata



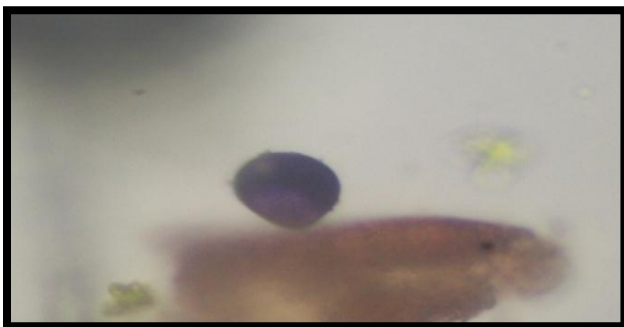
Brown Content



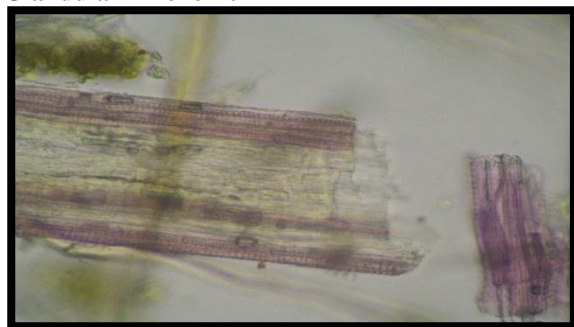
Pleuricellular Trichome



Glandular Trichome



Iodine stained Starch Grain



Annular Vessel along with fibers

CONCLUSION

Leaf of *Tephrosia* (Fabaceae) can be identified on the basis of key microscopical characters like paracytic stomata, unicellular, simple horn shaped trichomes, rhomboidal and prismatic crystals of calcium oxalate, bi-collateral vascular bundles, annular and spiral vessels, tannin, crystal fibers and lignified fibers. Stomatal index in U.E. of *T. purpurea* is 20, *T.*

candida is 25 & *T. jamnagarensis* is 12 in no. and stomatal index in L.E. of *T. purpurea* is 20, *T. candida* is 34 & *T. jamnagarensis* is 31 in no. Stomatal number and index of the species always constant and helps for the identification up to species level These observed parameters could be useful to establish certain botanical standards for identification and standardization of all the three species of *Tephrosia*.

Table 4 Caparative quantitative microscopy of all the three species of *Tephrosia*

Sr. No.	Characters	<i>T. purpurea</i> mm	<i>T. candida</i> mm	<i>T.jamnagarensis</i> mm
01	Trichome	4.5	2.5-5.5	4.8
02	Epidermal cell	1.7 X 1.4	1.5 X 1.3	1.7 X 1.5
03	Starch grain	1	0.3	0.7
04	Oil globule	0.5	0.5	0.5
05	Rhomboidal Crystal	0.5 X 0.3	0.4 X 0.5	0.5 X 0.3
06	Prismatic Crystal	0.8 X 0.4	0.8 X 0.6	0.7 X 0.4
07	Xylem vessel	3	3.8	3.3

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