Anatomical and histochemical characterization of three endangered species in Fabaceae

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Abstract

Back ground: The study of plant tissues and cells is to to learn more about the way these plants are constructed and how they work. The anatomical and histochemical studies are helpful for taxonomic plant identification and phytochemical analysis. *Methods.* The present study deals with the Anatomical and histochemical characterization of three endangered species in the Westernghats of family Fabaceae– *Prioria pinnatum (*Roxb. Ex DC.) Breteler, *Humboltia bourdilloni* Prain *and Humboltia vahiliana Wight*'. These plants are critically endangered plant species of Western ghats in Kerala. Stem, leaves, rachis & pulvinus leaf base were characterized using anatomical and histochemical analysis. *Findings*: Macroscopic characterization of the selected species showed almost similar morphological charecters and some differences also. All species have compound leaves. Microcopic study revealed the presence of similar characters like uniseriate epidermis, amphivasal vascular bundle etc. Two species of Humboltia possess unicellular non-glandular trichomes in the stem. Sclerenchymatous bundle cap is present in all. Secretory glands are observed in the leaf bases of *Prioria pinnatum* and *Humboltia bourdilloni*. Transverse section of petiole of *P. pinnatum* and *H. vahiliana* showed the presence of a vascular bundle in the centre region of pith. All species have paracytic stomata. Presence of starch grains are observed in cortex medullary rays and pith in all species. Both Humboltia species have winged petiole. In histochemical view, these three species show similarities. Presence of starch, proteins, lipids, essential oils, phenols and Alkaloids are present. Starch granules are commonly found in all species.

Key words: Anatomy, Fabaceae, Endangered, Alkaloids, Phenols , Starch, Lipids

1. Introduction

Plants are an incredibly important kingdom of organisms. Plant anatomy is the study of plant tissues and cells in order to learn more about the way these organisms are constructed and how they

work. Fabaceae**,** also called Leguminosae**,** pea family of flowering plants (angiosperms), within the order Fabales. Fabaceae, which is the third largest family among the angiosperms after Orchidaceae (orchid family) and Asteraceae (aster family), consists of more than 700 genera and about 20,000 species of trees, shrubs, vines, and herbs and is worldwide in distribution. Fabaceae has traditionally been divided into three subfamilies: Caesalpinioideae, Mimosoideae, and Faboideae (or Papilionoideae), each of which have been considered a separate plant family in the past. The subfamily Caesalpinioideae is a heterogeneous group of plants with about 160 genera and some 2,000 species. The selected three endangered plants are comes under the sub family–Caesalpinioideae[2].

Plant anatomy is also known as Phytotomy. Anatomical characters of vegetative and floral parts of flowering plants have been successfully employed to solve taxonomic problems and for the explanation of phylogenetic relationship. Anatomical evidence can be useful in systematic in several ways. When morphological characters prove to be of no help in the preliminary identification of herbarium material, anatomical study may prove to be helpful. Anatomical data has proved to be very useful in understanding evolutionary trends and interrelationship of taxa at and above the species level and at higher taxonomic categories. They are most useful in determining relationship between different genera, families, orders and other taxonomic categories. Plant anatomy provides characters such as trichomes, stomata, cuticular pattern, leaf venation, wood anatomy, growth rings etc. to aid in species identification and in performing physical matches of evidence. Plant anatomy can be important as a forensic tool in criminal investigations. The Knowledge of the preparation of plant fragments, the analysis of these fragments and the interpretation of the data obtained all must be part of forensic botany[9].

Histochemical analysis is essential for the study of plant secretory structures whose classification is based, at least partially, on the composition of their secretion. As each gland, may produce one or more types of substances, a correct analysis of its secretion should be done using various histochemical tests to detect metabolites of different chemical classes. Histochemical studies are used to confirm identification of cellular and tissue chemical components (secondary metabolites). Histochemical methods are employed in the identification, density of accumulation and distribution of chemical compounds within biological cells and tissues in different organs under microscopes using the colour stain reaction technique and photographic recording. These include the preparation of fixed variably stained specimens and then the examination under the microscopic devices. It is successfully applied in detection and localization of cellular components of active cell constituents such as proteins,

carbohydrates, lipids, nucleic acids, and a range of ionic elements occurring in the cell solutions, in addition to identifying the characterization of secretory structures and the chemical nature of the secreted compounds. Histochemical analysis reveals the presence of alkaloids, phenols and proteins in epidermis, cortex and vascular bundles of root, stem and leaf [6]. This work intended to full the 'Anatomical and histochemical characterization of three endangered species in the Westernghats of family Fabaceae– *Prioria pinnatum (*Roxb. Ex DC.) Breteler, *Humboltia bourdilloni* Prain *and Humboltia vahiliana Wight*'.

2. Materials and methods

Present study aims to compare anatomical and histochemical features of three plants belongs to the family fabaceae, *Prioria pinnatum (*Roxb. Ex DC.) Breteler, *Humboltia bourdilloni* Prain *and Humboltia vahiliana Wight*' collected respectively from Nadugani & Kulamavu- Idukki district and Vazhachal from its natural habitat. These plants are critically endangered plant species of Western ghats in Kerala. Stem, leaves, rachis & pulvinus leaf base were characterized using anatomical and histochemical analysis. The materials and methods used for the study are mentioned below;

2.1 Plant materials

1. ***Prioria pinnatum* (Roxb. Ex DC.) Breteler**

It is an endemic and endangered tree of the Southern Western ghats, belonging to the family Leguminosae and subfamily Caesalpinioideae. It carried out in the area of the Western Ghats. Seventeen populatons of *P. pinnatum* were Identified in 13 forest locatons of the Kerala area of Western Ghats. *P. pinnatum* is listed as an endangered plant species under IUCN red listed plants.Flowers very small, numerous, in panicles of racemes. *P. pinnatum* known as the 'Malabar mahogany', a vulnerable and endangered medicinal plant is used in curing sores of elephants. The oleo-gum-resin of this plant species is used in gonorrhoea and catarrhal conditions of genito-urinary and respiratory tracts. Mainly overexploitation leads to the extinction of the species.

2. ***Humboldtia bourdillonii* Prain**

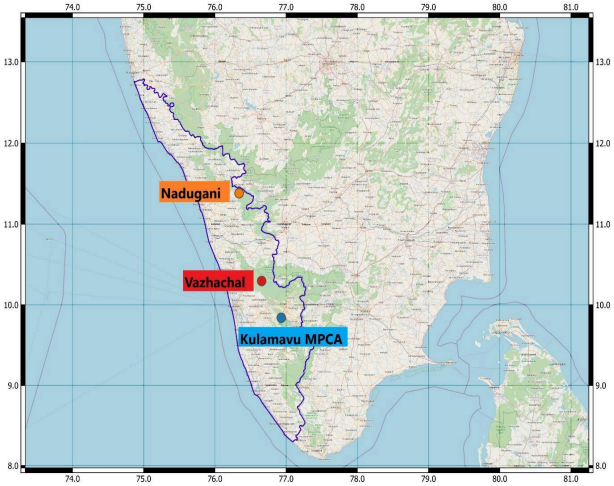
Humboldtia bourdillonii is reported from the Southern Western Ghats, India. But further information on its population and distribution is lacking and thus the species gained the 'endangered' status of IUCN. The study revealed that *H. bourdillonii* occurs in tropical wet evergreen forest from 450 – 800 m altitude as growing along and away from water courses in the

Arjunan Kotta and Poonkavanam forest of southern Western ghats. The species has a discrete distribution, found in seven patches, with an area of occupancy of 0.06 Km^2 and area of occurrence of approximately 2 Km^2.

***3. <u>Humboldtia</u> <u>vahliana</u>* Wight**

It is an endemic plant of Southern Western Ghats, Family: Fabaceae/leguminosae (subfamily: Caesalpinioideae) was carried out in wild conditions.

2.2 Study area



Nadukani is located on the Calicut- Nilambur-gudalur road 20 km north east of Nilambur town. *Prioria pinnatum* were collected from this location. Kulamavu is best known for being one of the three dams of the Idukki reservoir project. It constructed in 1961 to restrict the flow of water into Kallivally rivulet. Study material, *Humboldtia bourdillonii* were collected from this location. Vazhachal Forest stretches from Anaimalai to Athirapilli Falls (60 km) in Tamil Nadu, Kerala border. These forests are situated on the western slope of the Western Ghats and therefore receive copious amount of rainfall. The rest of the area is under various *Humboldtia Vahliana* were collected from this place.

<u>2.3 Stains used for histochemical studies</u>

<u>Lugol's reagent</u>

For examining the presence of starch grains. A solution composed of iodine and potassium iodide. Dissolve KI in about 20-30 ml of distilled water. Add iodine and heat gently with constant mixing until iodine is dissolved. Dilute to 100 ml with distilled water.

Coomassie blue

For examining the presence of proteins. To prepare this solution, dissolve 2g Coomassie Blue (Serva Blau) in 250ml water and slowly add 75ml of glacial acetic acid. Add 500ml of ethanol and makeup to 1000 ml with water.

Sudan black

To test the presence of lipids. It is made by preparing a staining solution of 500 mg Sudan Black B in 20 ml of acetone. This is added to 15 ml of acetic acid, and then added to 85 ml of water. Stir the mixture for 30 minutes and centrifuge to remove the precipitate.

NADI solution

For examining the presence of essential oils. This solution is made by dissolving 1% alpha-naphthol in 95% ethanol (solution 1). Solution 2 is made by adding 1% N, N-dimethyl-p-phenylenediamine HCl in water. Solutions 1 and 2 are mixed in equal volumes just prior to use.

Ferric chloride

For examining the presence of phenolic compounds. Dissolve 5 g of ferric chloride in 100 ml of water.

Wagner's reagent

To test the presence of alkaloids. To prepare this reagent 2 g iodine 6 g of potassium iodide (KI) in 100 ml of water to produce solution.

Glycerin gelatin

It is used as the mounting medium.

2.4 Stains used for permanent slide preparation

Safranin, Alcohol, Fast green, Clove oil , Xylene, Dibutylphthalate polystyrene Xylene (DPX)

2.5 Materials used in the maceration

Nitric acid (HNO_3), Test tube, Test tube holder, Spirit lamb

2.6 Equipments used

Blade, Microscope slides, Coverslip, Cavity block, Compound microscope (10-100x)

Steriomicroscope (Steriomicroscope (Olympus) with camera attachment is used. It helps to view sections at lowest magnification and gradually increase magnification to closely examine macroscopic details of the sample).

2.7 Methods of study

Study was conducted during the period of March – June 2022 at the Korambayil Ahamed Haji Memorial Unity Women's College, Manjeri. Plant materials were collected from natural habitats of Idukki, Trissur (Kerala) and Nilgiris (Tamilnadu).

Microscopic or anatomical evaluation

Thin transverse sections of the fresh stems, leaves, petiole and pulvinous leaf bases were taken by hand. The microscopical evaluation of sections was carried out to confirm the structural details of the material. For effective results permanent slide preparation were also done. Chemical constituents of plants were also evaluated by applying the histochemical stains on small quantities.

Histochemical staining procedures.

In order to estimate the presence of various cell inclusions like Starch grains, proteins, lipids, essential oils, phenolic compounds and alkaloids. The following methods were followed.

Test for Starch:

To examine the presence of Starch

- Sections were Submerge in the Lugol's reagent for 10 min.
- Rinse briefly with distilled water.
- Mount the slides using distilled water or Lugol's reagent itself.
- This reaction highlights the starch grains in dark blue to black.

Test for Proteins

To examine the presence of proteins

- Stain the sections in 0.25% Coomassie blue for 15 min.
- Differentiate in 7% acetic acid.
- Rinse briefly in distilled water.

- Mount in glyceringelatin.
- It stains proteins blue.

Test for Lipids

To examine the presence of lipids

- Stain the sections with Sudan black B for 20 min.
- Rinse briefly in 70% ethanol.
- Wash in distilled water.
- Mount in glyceringelatin.
- It stains lipids dark blue to black.

Test for Essential oils

- To examine the presence of essential oils, Apply NADI reagent for 1 h in the dark.
- Wash in sodium phosphate buffer (0.1 M, pH 7.2) for 2 min.
- Mount in the same buffer.
- This reagent produces differential staining, with essential oils staining blue and resins staining red.

Test for Phenolic compounds

For examining the presence of phenolic compounds,

- Apply the sections with10% ferric chloride for 30 min.
- Wash twice in distilled water to remove surplus ferric chloride.
- Mount in glyceringelatin. This method highlights phenolic compounds through iron precipitation, producing a dark color , usually black , sometimes brown.

Test for Alkaloids

For examining the presence of Alkaloids,

- Apply Wagner's reagent for 20 min.
- Rinse briefly in distilled water. Mount in distilled water.
- This method stains alkaloids red or red brown .

Maceration

Small pieces of wood of the plant materials are heated with concentrated nitric acid in a test tube with the help of a test tube holder. The complete volume should not be larger than 1/10 of the reaction container, because a lot of gas develops very quickly at cautious heating. The surfaces of the wooden pieces are strongly attacked. The single cells can be scraped off after washing of the preparation and examined under the microscope.

3.RESULTS

In the present study, it is aimed to study the microscopic features of three members of the family, Fabaceae – *Kingiodendron pinnatum, Humboldtia bourdillonii And Humboldtia Vahliana*, collected from their natural habitat. The stem, leaves, petiole and leaf bases are selected for the anatomical and histochemical studies. This study also includes the study of stomatal index and tracheids by maceration. The results obtained can be summarised as follows.

***1.Prioria pinnatum* (Roxb. Ex DC.) Breteler**

Stem cylindrical 4 to 8 mm diameter, internodes being 4.5 to 5 cm in length, nodes are prominent, bark 5-8 mm thick, surface greyish-brown with green blotches, rough, exuding a reddish sticky resin. The outline of the transverse section is almost circular. Periderm is composed of phellem, phellogen and phelloderm. The cells of the phellem are rectangular, thickly suberized and dead. A few lenticels also occur in the phellem. There is no hairs or trichomes present in the stem. Cortex is undifferentiated and wholly parenchymatous, the cells are filled with starch. Small intercellular spaces are present. Secretory glands are present in the cortex. Vascular bundles are arranged in a ring, secondary growth is present. Vascular bundle is conjoint, collateral, endarch and open. Secondary xylem is grooved at four places. Secondary phloem present above the secondary xylem. A few phloem cells are thick walled. These are called bast or phloem fibers. A well defined parenchymatous pith present in the centre. Prismatic crystals present in the pith region. They are calcium oxalate Crystals**.** Presence of Starch grains observed in the Stem.

The transverse section of the lesf shows central bulged region and lateral wings

Lamina is dorsiventral with palisade and spongy cells.

Epidermis is bounded by thin cuticularised upper and lower epidermis. The upper epidermis is uniseriate thick walled cells with cuticle. The lower epidermis is made up of uniseriate row of rectangular cells and contain paracytic stomata. The outline of the lower epidermis is angular. Epidermis is followed 4-5

layered mesophyll differentiated into palisade and spongy tissue. Palisade cells contain a large number of chloroplasts which is meant for photosynthesis and spongy tissues are arranged below the palisade cells with large intercellular spaces. Vascular bundles are almost parallel series. Vascular bundles are surrounded by a thick layer of sclerenchymatous cells. Vascular bundles are endarch. Vascular bundles are also seen in the wings of leaf.

Petiole round, 5-10 mm, stout, grooved above, glabrous, lateral nerves 8-13, pinnate, slender, prominent.The outline of the transverse section is almost circular**.** Detailed TS shows a single layered epidermis with elongated compact parenchymatous cells. Trichomes absent. Just below the epidermis, there is 6-7 layered chlorenchymatous hypodermis and few layered parenchymatous tissues. Prismatic crystals present in the hypodermis. Vascular bundles are arranged in a concentric ring and they form additional bundles with in the central region. In the outer vascular bundles xylem is endarch,closed .Central vascular bundles is amphivasal vascular bundle . In Pulvinous leaf base Epidermis is uniseriate parenchymatous cells.Cortex is undifferentiated and wholly parenchymatous, the cells are filled with starch. Small intercellular spaces are present. Secretory glands are present in the cortex.Vascular bundles endarch, conjoint and crescent shaped with leaf traces. Vascular bundles are surrounded by a thick layer of Sclerenchymatous cells. Vascular bundles are endarch.

Table 1- Comparison between two species of *Humboldtia– Humboldtia bourdillonii and Humboldtia vahliana*

Characters		***Humboldtia bourdillonii***	***Humboldtia vahliana***
Stem	Epidermis	Diagrammatic view of the T.S. has a wavy outline with four pronged sides.	Diagrammatic view of the T.S. has a wavy outline with four pronged sides
		Trichomes present	Trichomes absent
	Hypodermis	2-3layered sclerenchymatous cells present	2-3 layered sclerenchymatous cells present
		Secretory glands absent	Secretory glands present
		Sclerenchyma fibres present	Sclerenchyma fibers present

	Vascular bundle	Conjoint,collateral and open	Conjoint**,** collateral and open
	Pith	Large pith made of thin walled parenchymatous cells	Large pith made of thin walled parenchymatous cells
Leaf	**Epidermis**	Formed of single layer of square cells	Formed of single layer of square cells
		Paracytic stomata present on the lower epidermis	Paracytic stomata present on the lower epidermis
	Hypodermis	Palisade cells 2 layered	Palisade cells 2 layered
		2-3layered sclerenchymatous cells present	3-4 layered sclerenchymatous cells present
	Vascular bundle	Crescent shaped	Crescent shaped
		Amphivasal	Amphivasal
Petiole	**Epidermis**	Uniseriate epidermis	Uniseriate epidermis
	Hypodermis	Parenchymatous 5-6 layers.	Parenchymatous and sclerenchymatous
		2- 3 layered Sclerenchymatous cells present	2- 3 layered Sclerenchymatous cells present
	Vascular bundle	Crescent shaped	Arranged in a wavy ring
		Amphivasal	Amphivasal
	Pith	Large	Large, also contains an amphivasal vascular bundle smaller than outer bundle
Leaf base	**Epidermis**	Uniseriate epidermis	Uniseriate epidermis

	Cortex	Wide & consists of parenchymatous soft tissues	Wide & consists of parenchymatous soft tissues
		Secretary glands absent	Secretary glands present
	Vascular bundle	Conjoint, collateral amphiphloic	Conjoint, collateral amphiphloic
		Crescent shaped	Crescent shaped

3.2 Histochemical analysis

Histochemical analysis like the presence of starch, proteins, lipids, essential oils, phenols and alkaloids detected in this study. Findings are concluded as following:

Table 2- Histochemical analysis of *Kingiodendron pinnatum*

	Stem	Leaf	Petiole	Leaf base
Starch	✓	✓	✓	✓
Proteins	✓	✓	✓	✓
Lipids	×	✓	✓	✓
Essential oils/resins	✓	✓	✓	✓
Phenols	✓	✓	✓	✓
Alkaloids	✓	✓	×	×

Table 3- Histochemical analysis of *Humboldtia bourdillonii*

	Stem	Leaf	Petiole	Leaf base
Starch	✓	✓	✓	✓
Proteins	✓	✓	✓	✓
Lipids	✓	✓	×	✓
Essential oils/resins	✓	✓	✓	✓
Phenols	✓	✓	✓	✓
Alkaloids	✓	✓	✓	✓

Table 4- Histochemical analysis of *Humboldtia Vahliana*

	Stem	Leaf	Petiole	Leaf base
Starch	✓	×	✓	×
Proteins	✓	✓	✓	✓
Lipids	✓	✓	✓	✓
Essential oil/resins	✓	✓	✓	✓
Phenols	✓	✓	✓	✓
Alkaloids	×	×	✓	×

STOMATAL INDEX

Stomatal index of the leaf is the ratio of the number of stomata to the total number of stomata and epidermal cells.

$$\text{Stomatal index} = \frac{\text{No. of stomata cells per unit area}}{\text{No. of epidermal cells per unit area}}$$

Prioria pinnatum

	No. of stomata	No. of epidermal cells	Stomatal Index (%)
1	7	33	17.5
2	9	36	20
3	8	35	19.04
4	7	25	21.9

$$\text{Stomatal index} = \frac{17.5+20+19.04+21.9}{4} = 19.61$$

Humboldtia bourdillonii

	No. of stomata	No. of epidermal cells	Stomatal Index (%)

1	11	62	15.1
2	12	60	16.7
3	11	65	14.4
4	14	70	16.6

Stomatal index = 15.1+16.7+14.4+16.6 = 15.7

4

Humboldtia vahliana

	No. of stomata	No. of epidermal cells	Stomatal Index (%)
1	13	51	20.32
2	9	64	12.32
3	11	60	15.4
4	13	59	18.05

Stomatal index= 20.32+12.32+15.4+18.05 = 16.5

4

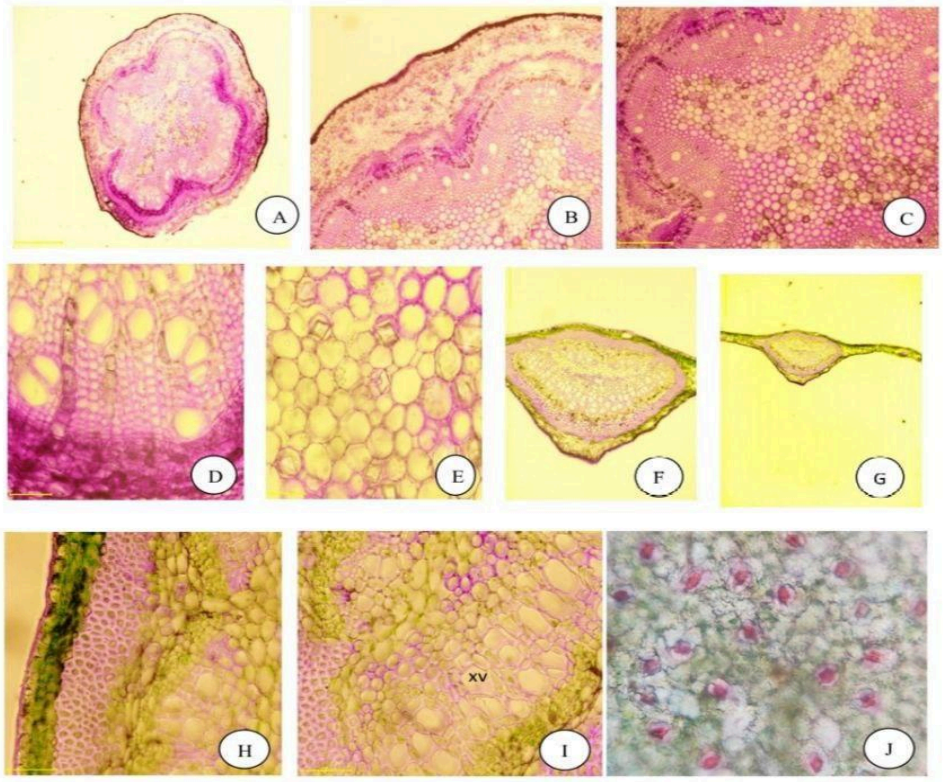


Plate – 4: Fig (A-J) Microscopy of *Prioria pinnatum (*Roxb. Ex DC.) Breteler

(A) T. S. of the entire stem, (B) Portion enlarged, composed of phellum, phellogen and phelloderm, (C) T. S. showing pith region, (D) T. S showing xylem regions. Vascular bundles are arranged in a ring, (E) Detailed T. S. showing calcium oxalate crystals in the pith, (F) T. S. of leaf in the midrib region enlarged, (G) T. S. showing midrib region, (H) T. S. showing outer portion of leaf, (I) Xylem region (xv-xylem vessels), (J) Lower epidermal peeling showing paracytic stomata.

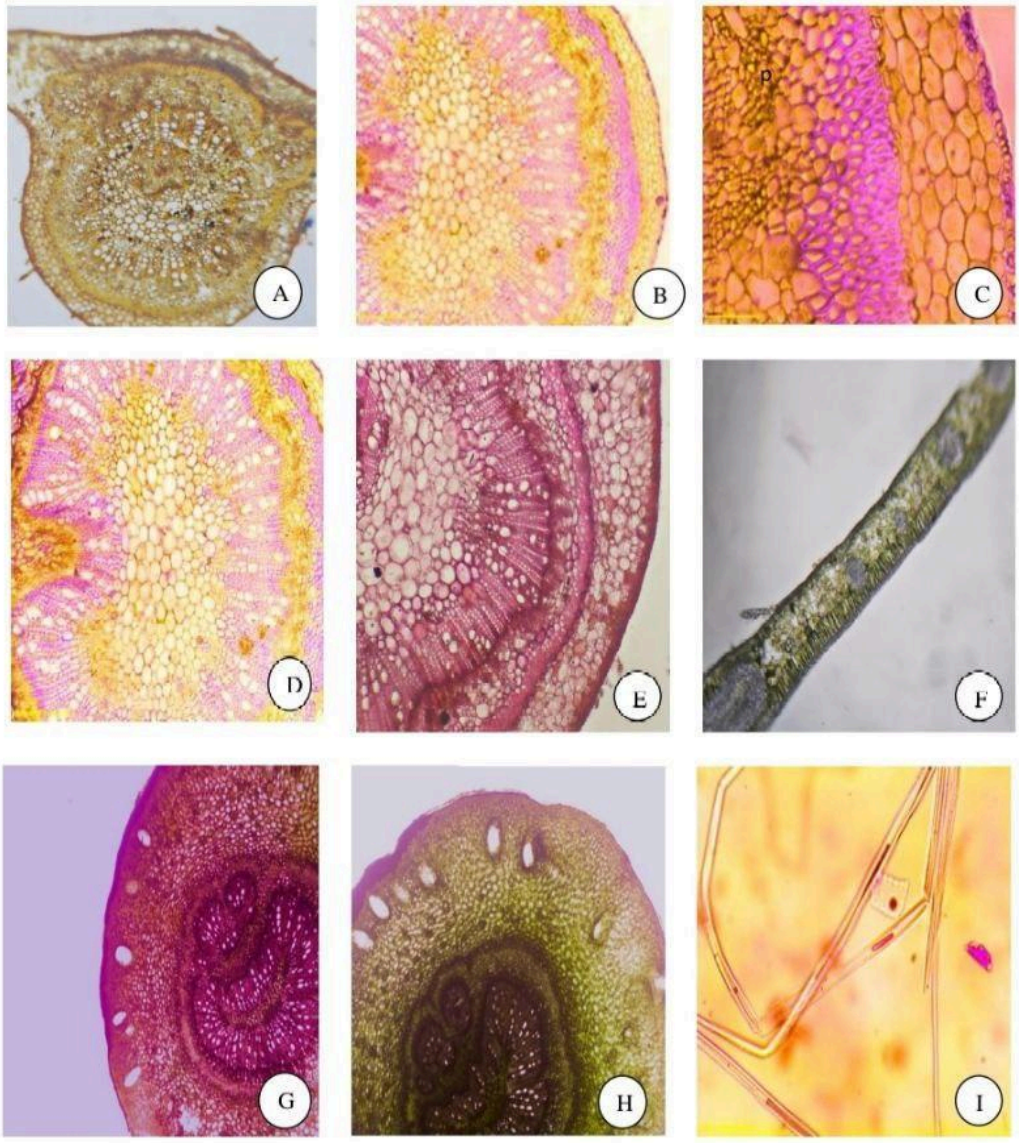


Plate – 7: Fig (A-I) Microscopy of *Humboldtia bourdillonia* prain.

(A) T. S. of entire petiole, (B& C) T. S. showing portion enlarged (P-Phloem), (D) T. S. showing pith region (E) T. S. showing enlarged outer portion, (F) T. S. showing lamina region of the petiole, (G) T. S. Showing leaf base, (H) T. S. showing leaf base outer and inner regions, (I) Tracheids.

5.Discussion

The present study provides macroscopic, microscopic and histochemical characterization of the stem, leaves, petiole and leaf base of *Prioria pinnatum, Humboldtia bourdillonii & Humboldtia Vahliana* belongs to the family fabaceae. There is no evidences for the regarding studies.The macroscopic study of the stem, leaves, people and leaf base showed similarities in their appearances. Since all the three species belong to the same family, fabaceae, are almost same. Stem Morphology is same in the two species of Humboldtia . But it is different in case of P. pinnatum, which is cylindrical in structure.In case of leaves, all species has compound leaves of paripinnate, slightly glabrous, having pulvinus leaf base.The petiole of the both humboldtia species is winged. Anatomical characterization revealed that the basic structure of stem, leaves, petiole and leaf base of the selected plants are almost. There are certain differences also seen. Diagrammatic T. S of the stem of the *P. pinnatum* showed circular outline with distinct epidermis, hypodermis, cortex,Stele and pith in the centre. In *H. bourdillonii* & *H.vahiliana* , the outline have a wavy nature with four- grooved sides. All species show uniseriate epidermis of thick walled cells. Cells are squarish to rectangular in shape. High variation is seen in trichome structure. It is absent in *P.pinnatum* and *H.vahiliana, H. bourdillonii* possess unicellular, non- glandular trichomes on its epidermis. Xylem fibers are present in the stem of Humboldtia species. Oil glands are present in the hypodermis region of stem of the H.vahiliana. Xylem region consists of radially arranged vessels of different sizes,fibres and alternating uniseriate medullary rays in all species. Microscopic structure of leaves of all Species are almost same. Comparison of leaves shows uniseriate upper and lower epidermis present in all species which are made up of thick walled cells. Trichomes are absent in all species. All three species show paracytic stomata in the lower epidermis. Upper epidermis is devoid of stomata. In *P. pinnatum* epidermis is followed by 3-4 layer of chlorenchymatous. sclerenchymatous bundle cap is present in all species. Several radial rows of xylem arranged in a crescent shape. Collenchymatous cells are present in the Midrib region. Vascular bundles are conjoint collateral and Amphivasal. Small vascular bundles are present in the leafy region. Microscopic comparison of petiole shows high variation in diagrammatic level. The petiole of both *humboldtia* species is winged, but that of *P. pinnatum* is not winged. The petiole of three species having uniseriate epidermis. *P.pinnatum* have cuticle covering. Following the epidermis, *P.pinnatum* have 7-8

layer of chlorenchymatous cells, *H. bourdillonii* have 5-6 layer of parenchymatous tissues and *H. Vahliana* have parenchymatous and chlorenchymatous cells. Sclerenchymatous bundle cap is present in all the three species. Vascular bundle of *P. pinnatum* is arranged in a ring. The pith region also contain a vascular bundle of the same pattern but small. Vascular bundle of *H. bourdilloni* is crescent shaped and Amphivasal. Pith large.*H. vahiliana* has Vascular bundle arranged in a wavy ring structure.pith also contain Amphivasal vascular smaller than surrounding bundle.In the leafy region vascular bundles are arranged at regular intervals surrounded by sclerenchymatous tissue. Microscopic comparison of leaf bases shows almost same structure. All species have pulvinus leaf base. Epidermis is uniseriate parenchymatous in all species. Cortex is undifferentiated made up of parenchymatous soft tissues. Cortex of *P.pinnatum* & *H.valiliana* contains secretary glands.it is absent in *H.bourdilloni.* Vascular bundle of *P. Pinnatum* is endarch, conjoint, surrounded by thick layer of parenchymatous cells. Vascular bundle of H. *bourdillonii* is conjoint, collateral and amphiploic and that of *H. vahiliana* is crescent shaped, it is surrounded by sclerenchymatous fibres[7] . Histochemical analysis reveals the presence of starch, proteins, lipids, essential oils, phenols and Alkaloids in the stem, leaves, petiole and leaf base. *P. pinnatum* contains starch grains in stem, Leaf, petiole and leaf base in the hypodermis and cortical regions. Presence of proteins also confirmed lipids present in the vascular region of leaf, petiole and leaf base. It is absent in the stem. Presence of essential oil is examined by NADI test. It is present in the almost all regions of the stem, leaf, petiole and leaf base. Phenols present in all sections of *P. pinnatum* [4]. Alkaloids present in the stem and leaf in cortex and vascular regions. It is absent in petiole and leaf base[4]. In *Humboldtia bourdillonii*, starch and proteins are present in stem, leaf, petiole and leaf base at random regions. Lipid is absent in petiole, but present in the vascular region of the stem, leaf and leaf base. The presence of essential oils, phenols and alkaloids are confirmed in stem, leaf, petiole and leaf base of *H. bourdillonii* [3]. In case of *Humboldtia vahliana*, Starch grains are present in the stem and petiole, absent in the leaf and leaf base. Starch granules mainly seen in the pith region. Protein is present in the all regions of *H. vahiliana* [11]. Lipids, essential oils, phenols are present in all among them in variable regions. Alkaloids are detected only in the petiole.it is absent in the other parts[8]. Stomatal index is the ratio of the number of epidermal cells to the number of stomata in a given area of a leaf. The three species have high stomatal index. High stomatal index correlate with a higher net rate of photosynthesis [10]. *P. pinnatum* shows higher stomatal index as compred to *Humboltia vahiliana* and *Humboltia bourdillonii.* Anatomical characters are conserved and stable and thus can be used as a taxonomic character for plant taxonomy. Anatomical features can be used in taxonomy for the

identification of plants, establishing genetic relationships and solving taxonomic disputes. All the three members possess paracytic stomata in their leaves. Paracytic stomata have one or more subsidiary cells parallel to the opening between the guard cells. All species have uniseriate epidermis, paracytic stomata on the abaxial surface.

5. **Conclusion**

This is the first attempt to compare the microscopy and histochemistry of *Prioria pinnatum, Humboltia bourdilloni & Humboltia vahiliana.* Macroscopic characterization of the selected species showed almost similar morphological characters and some differences also. In histochemical view, these three species show similarities. Starch granules are commonly found in all species. From the study it is clear that the basic structure is same in the stem, leaves, petiole and leafbase of *Prioria pinnatum, Humboltia bourdilloni and Humboltia vahiliana.* The minor differences observed can be taken as diagnostic characters for the identification of the selected species.

6.Acknowledgement

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