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ferns of district Kotli, Azad Jammu and  
Kashmir**

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## Taxonomic and anatomic studies on some ferns of district Kotli, Azad Jammu and Kashmir

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The present study was carried out for taxonomical and anatomical studies of ferns collected from District Kotli, AJK. Total nine ferns were collected and identified as *Adiantum pedatum* L., *Adiantum capillus-veneris* L., *Adiantum caudatum* L., *Pteris multifida* L., *Pteris vittata* L., *Asplenium platyneuron* L., *Christella dentata* Forssk., *Dryopteris carthusiana* Vill., *Aleuritopteris argentea* Fee. *Adiantum pedatum*, *Adiantum capillus-veneris* and *Asplenium platyneuron* had spores on the lower side of pinnae while *Adiantum caudatum* had spores on apex of pinnae. Anatomical characters were observed under a light microscope. The transverse sections of both petiole and stem had cuticle, epidermis, cortex, endodermis, pericycle, parenchyma, sclerenchyma, collenchyma, metaxylem, protoxylem and phloem. Trichomes were found on the epidermis of a few species. Dictyostele was found in all species except *Adiantum pedatum* and *Adiantum caudatum* which had amphiphloic siphonostele. Mesarch xylem was found in *Adiantum pedatum*, *Adiantum capillus-veneris*, *Aleuritopteris argentea*, *Adiantum caudatum* and *Christella dentata*. Diarch xylem was present in rest of the species. Length of cortical cells was highest in *Dryopteris carthusiana* in both petiole and stem i.e. 2.04 $\mu$ m and 2.01 $\mu$ m respectively. Highest width of cortical cells was observed in *Dryopteris carthusiana* i.e. 1.82 $\mu$ m and 1.88 $\mu$ m respectively. In petiole, length of metaxylem was greater in *Pteris vittata* while width was greater in *Adiantum caudatum*. The length of protoxylem was greater in *Pteris multifida* while width was greater in *Pteris vittata*. In stem, length of metaxylem was greater in *Adiantum caudatum* but width was greater in *Dryopteris carthusiana*. The length and width of protoxylem was greater in *Pteris vittata*.

**Keywords:** Morphology, Ferns, petiole, stem, *Adiantum*

## INTRODUCTION

Azad Jammu and Kashmir have a variety of diverse plant life. A wide range of environments, including roadways, farmed fields, wastelands, rivers, lakes, streams, springs, and steep mountain slopes, are residence to a diverse range of plant life. District Kotli is bordered on the east by Occupied Kashmir and is situated between 700 and 1400 meters above mean sea level. It measures 1862 square kilometers in total. In addition, the annual precipitation totals 1227.91 mm, with the highest amounts occurring in July and August (306.93 mm and 256.53 mm, respectively) (Ajaib et al., 2010).

The vascular plants without seeds, or pteridophytes, are classified into 19 orders, 58 families, and 12838 species. They rank as the third largest class of plant life. There are a few species of pteridophytic plants in temperate regions, but the majority is found in tropical and subtropical regions. Even though vascular plants with seeds are currently replacing many plants, they nevertheless account for a significant share of the global vegetation (Gul et al., 2017). The lowest group of plants is ferns. They can be distinguished by their vascularity and spore-bearing ability. Worldwide distribution is exhibited by their species (Sofiyanti et al., 2019). Most commonly, ferns are utilized as food, medicine, textiles, crafts, building materials, or decorative plants. Ferns contain a variety of secondary metabolites that have been

linked to improved human health, including polyphenols, flavonoids, and isoprenoids (Ishaque et al., 2021).

Pteridaceae is a rich family of leptosporangiate ferns, comprising 53 genera, 5 subfamilies, and over 1211 species, according to reports. Pteridaceae are distinguished by long to short creeping rhizomes, scales, simple pinnate leaves, dimorphic or monomorphic blades, vein-free leaves, marginal sori, and sporangia along the veins. Pakistan's six genera and twenty-four species of Pteridaceae are distributed from the north to the southwest. Of these six genera, most species are also found in the temperate coniferous forest of the Malakand division. Five of the genera; *Adiantum*, *Chielanthus*, *Pteris*, *Onychium*, and *Gymnopteris* are found in the humid temperate forest of northern Pakistan (Shah et al., 2019). With over 250 species, the genus *Pteris* L. is widely spread across the world's tropical regions (Gaafar et al., 2018). *Pteris multifida* is an evergreen herbaceous fern that belongs to the Pteridaceae family. East Asian temperate and tropical regions are home to a large population of this species. Additionally, it is utilized in Chinese medicine to cure a few additional illnesses as well as snake bites, diarrhea, jaundice, and the mumps (Hou et al., 2021).

It is said that the fern genus *Adiantum* is big and diverse. It is a member of the Adiantaceae family, which has about 150 species that are found all over

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the world. A perennial herb, *Adiantum capillus-veneris* is a member of the *Adiantum* genus. The salient features of this species are its creeping rhizomes. It reproduces asexually through rhizomatous growth, but sexually through spores (Parthipan and Rajendran, 2015). Because of its vital therapeutic qualities, *A. capillus-veneris* is regarded as an important endangered species of fern (Singh et al., 2020).

In Pakistan and Kashmir's hilly regions, such as Mangora, Murree, Galliyat, and Rawalpindi, *Adiantum caudatum* is found. According to Ahmed et al. (2015), this fern can be used as a natural antibiotic and to treat diabetes, fever, cough, jaundice, and diarrhea. With 720 species, *Asplenium* L. is considered the largest genus within the Aspleniaceae family. The world's temperate and humid tropical regions are also home to the species of this genus (Woch et al., 2021). Pteridaceae is a family of plants that includes the genus *Aleuritopteris* Fee, which is home to tiny cheilanthoid ferns. This genus's members are frequently referred to as silver ferns. Beneath the leaf lamina, they have stunning white or yellow flavonoid farina (Jenkins, 2013).

The family Dryopteridaceae contains approximately 1700 species spread between 40–45 genera. Most of them are located on land. Their rhizome has scales at the tip and can creep, rise, or stand erect. The petiole has ring-shaped vascular bundles (Mir et al., 2014). With over 225 species worldwide, the genus *Dryopteris* is regarded as the largest genera in the family Dryopteridaceae (Sessa et al., 2012). The perennial fern *Dryopteris carthusiana* has its overwintering buds at the soil's surface (Runk et al., 2012). There are 30 genera and 1034 species in the Lypteridaceae family, which is found all over the world. They have stems that are both branching and unbranched, with scales at the tips, and a dictyostele that is radially symmetrical is discovered (Shah et al., 2019). *Christella dentata* is a member of the lypteridaceae family and is widely distributed in high-altitude areas such as Jammu and Kashmir. High humidity levels and shady areas are ideal for its growth. Additionally, Manhas et al. (2018) claim that this kind of fern has antibacterial and antifungal properties.

## MATERIALS AND METHODS

### Collection of samples

In present study, 9 samples (10 replicas / sample) of different species of ferns belonging to 5 families of ferns were collected from different areas in district

Kotli including Khuiratta, Darkoti and Dahmaal during the months of October 2021 to July 2022 (Table 1, Figure 1). They were preserved in Formalin Acetic Acid (FAA). The collected ferns were later described and identified according to Flora of North America (Vol. 2) by Smith (1993) (Figure 2).

### Procedure of section cutting and staining

The sample was handled against the side first finger of the left thumb. Blade was handled in such a position that blade and sample were perpendicular to each other. Several sections were cut at a time, with the help of blade and then placed these sections on a glass watch filled with water. Very fine sections were selected. Sections were placed on glass slides with the help of camel hairbrush. For staining, sections were placed in 30% alcohol and 50% alcohol for 2 minutes each. Then sections were transferred to toluidine blue stain for 5 minutes and in the last step, sections were placed in 70% alcohol for 1 minute. Permanent slides were observed under a microscope and micrometry was performed to measure the size and dimensions of cells.

### Scoring of data and phenogram assessment

All morphological and anatomical traits of the studied species were scored to set up a phenetic analysis. Similarity matrix and cluster analysis and similarity matrix were established by Pclass method (El-Gazzar and Rabei, 2008). The Gower coefficient determined the distances according to the Gower coefficient (Gower, 1982). The Nei genetic similarity index (SI) was used for calculating the pairwise similarities and dissimilarities between the operational taxonomic units (OTUs) based on the equation,  $SI = 2N_{ij} / (N_i + N_j)$ , where  $N_i$  and  $N_j$  are the total number of comparative characters for each species  $i$  and  $j$ , respectively besides  $N_{ij}$  is the number of common ones shared between them. Phenogram was performed based on a sequential agglomerative hierarchical nested clustering where series of successive mergers were used to aggregate studied fern species with similar characters in a method which is called unweight pair group mathematical averages (UPGMA) (Yao et al., 2007; Deya & El-Shabasy, 2020).

### Statistical analysis

Pearson correlation coefficient for each studied fern species against another one was accomplished according to (Shaban, 2005; Tamhane, 2009; Areshi et al., 2023). P values used as significant parameters were based on the degree of freedom according to

**Table 1.** Collection of fern species from different sites of district Kotli, AJK.

Sr. No.	Herbarium Voucher	Fern Species	Collection Site	Collecting Coordinates (Lat. N, long. E)	Collection Date
1	GC Herb Bot 3086	<i>Adiantum pedatum</i> L.	Khuiratta	33°50'N 74°15'E	11 October 2021
2	GC Herb Bot 3087	<i>Adiantum capillus veneris</i> L.	Khuiratta	33°50'N 74°15'E	11 October 2021
3	GC Herb Bot 3088	<i>Pteris multifida</i> L.	Dahmaal	32°44'N 73°04'E	15 March 2022
4	GC Herb Bot 3089	<i>Pteris vittata</i> L.	Darkoti	32°40'N 74°13'E	1 November 2021
5	GC Herb Bot 3090	<i>Adiantum caudatum</i> L.	Darkoti	32°40'N 74°13'E	1 November 2021
6	GC Herb Bot 3091	<i>Aleuritopteris argentea</i>	Darkoti	32°40'N 74°13'E	1 November 2021
7	GC Herb Bot 3092	<i>Dryopteris carthusiana</i> (Vill.)	Darkoti	32°40'N 74°13'E	1 November 2021
8	GC Herb Bot 3093	<i>Christella dentata</i> Forssk.	Kotli	33°31'N 73°54'E	3 July 2022
9	GC Herb Bot 3094	<i>Asplenium platyneuron</i> L.	Kotli	33°31'N 73°54'E	4 December 2021



**Figure 1.** Map of the studied sites with coordinates.

Dutilleul (1993) approaches. All anatomical measurements were subjected to statistical analysis to carry out ANOVA test and standard errors (Al faifi and El-Shabasy, 2021). SPSS software (ver. 22) was utilized to conduct statistical analysis (Atta et al., 2022). The representations of (SLR) represented the significant relationships among studied species (Maindonald, 1992; Miller and Franklin, 2002). They were explored by the linear regression approaches to accomplish the extent effect of valuable parameter.

**RESULTS**

**Key to the species based on petiole and stem anatomy**

- 1. 1-2 vascular strands received by stem 2
- 2a. Stem receives 2 vascular strands at the base which later fused together to form a single strand towards the apex 3
- 2b. Stem receives single vascular strand 4
- 3a. Xylem is mesarch *Christella dentata*
- 3b. Xylem is diarch or triarch *Pteris multifida*
- 4a. Ground tissue is differentiated into outer sclerenchymatous and inner parenchymatous cells *Aleuritopteris argentea*

4b. Ground tissue is differentiated into multiple layers of sclerenchyma and parenchyma	5
5a. Xylem is V-shaped	6
5b. Xylem is U-shaped	<i>Dryopteris carthusiana</i>
6a. Arrangement of xylem with curved ends (petiole)	<i>Pteris vittata</i>
6b. Arrangement of xylem with straight ends	7
7a. Xylem elements are not arranged in triarch pattern	<i>Pteris multifida</i>
7b. Xylem elements are arranged in diarch pattern	8
7c. Xylem elements are arranged in mesarch manner	9
8. Dictyostele is present	<i>Asplenium platyneuron</i>
9a. Vascular strand with semi-lunar shaped xylem	<i>Adiantum pedatum</i>
9b. Amphiphloic siphonostele is present	10
10a. Semi-lunar shaped vascular strand	<i>Adiantum caudatum</i>
10b. Mesarch xylem	11
11a. Xylem is heart-shaped (petiole)	<i>Christella dentata</i>
11b. Xylem is hippocampus-shaped (stem)	<i>Christella dentata</i>

## PETIOLE AND STEM ANATOMY DESCRIPTIONS FOR STUDIED FERN SPECIES

### *Adiantum pedatum* L.

#### Transverse section of Petiole

In the transverse section of petiole (Figure 3A) of *Adiantum pedatum* L., it was observed that a thick cuticle was covering the epidermis. Epidermal cells were found right next to the cuticle. The ground tissue was present inner to the epidermis and was made up of multiple layers of sclerenchyma and parenchyma cells. Tannins were mostly found in cortex which provided dark brown color to the petioles. Vascular bundles including xylem and phloem were surrounded by a single-layered endodermis. The pericycle was present beneath the endodermis and it was made up of 2-3 cell layers. Xylem was a mesarch and consisted of protoxylem and metaxylem and appeared as a semi-lunar shaped structure. Phloem was found outer to protoxylem and metaxylem. Amphiphloic siphonostele was present in the center.

#### Transverse section of Stem

The transverse section (Figure 4A) of stem of *Adiantum pedatum* L. showed that cuticle was present outside single-layered dark brown epidermis. Long hairs were also found on the epidermis. Cortex was present inside the epidermis. Cortex was composed of 3-4 layered sclerenchymatous cells and thin-walled, multilayered parenchymatous cells. Tannin content was found within parenchymatous cells of cortex. Endodermis surrounded the vascular strand. But pericycle was also present inner to the

single-layered endodermis. Vascular bundles consisted of two major xylem groups and phloem. Xylem groups included large, mesarch metaxylem and small patch of protoxylem. Phloem was present outer to the xylem. Amphiphloic siphonostele was present in the stem.

### *Adiantum capillus-veneris* L.

#### Transverse section of Petiole

In the transverse section of petiole (Figure 3B) of *Adiantum capillus-veneris* L., it was observed that a thick, dark brown cuticle was covering the epidermis. Epidermal cells were found inner to the cuticle. The ground tissue was present inner to the epidermis and was made up of multiple layers of sclerenchyma and parenchyma cells. Tannins were mostly found in cortex which provided dark brown color to the petioles. Vascular bundles including xylem and phloem were surrounded by a single-layered endodermis. The pericycle was present beneath the endodermis and it was made up of 2-3 cell layers. Xylem was a mesarch and consisted of protoxylem and metaxylem. Phloem was found outer to protoxylem and metaxylem. Dictyostele was found in the petiole.

#### Transverse section of Stem

The transverse section of stem (Figure 4B) of *Adiantum capillus-veneris* L. showed that single-layered dark brown epidermis was outermost layer but covered with cuticle. The ground tissue i.e., cortex was present inner to the epidermis. It was differentiated into sclerenchymatous cells and thin-walled, parenchymatous cells. Parenchymatous cells were multilayered. Endodermis surrounded the vascular bundles. The pericycle was present inside the single-layered endodermis. Vascular bundles consisted of xylem and phloem. Large, mesarch metaxylem and small patch of protoxylem were included in the xylem groups. Phloem was present surrounding the xylem. Dictyostele was found in the stem.

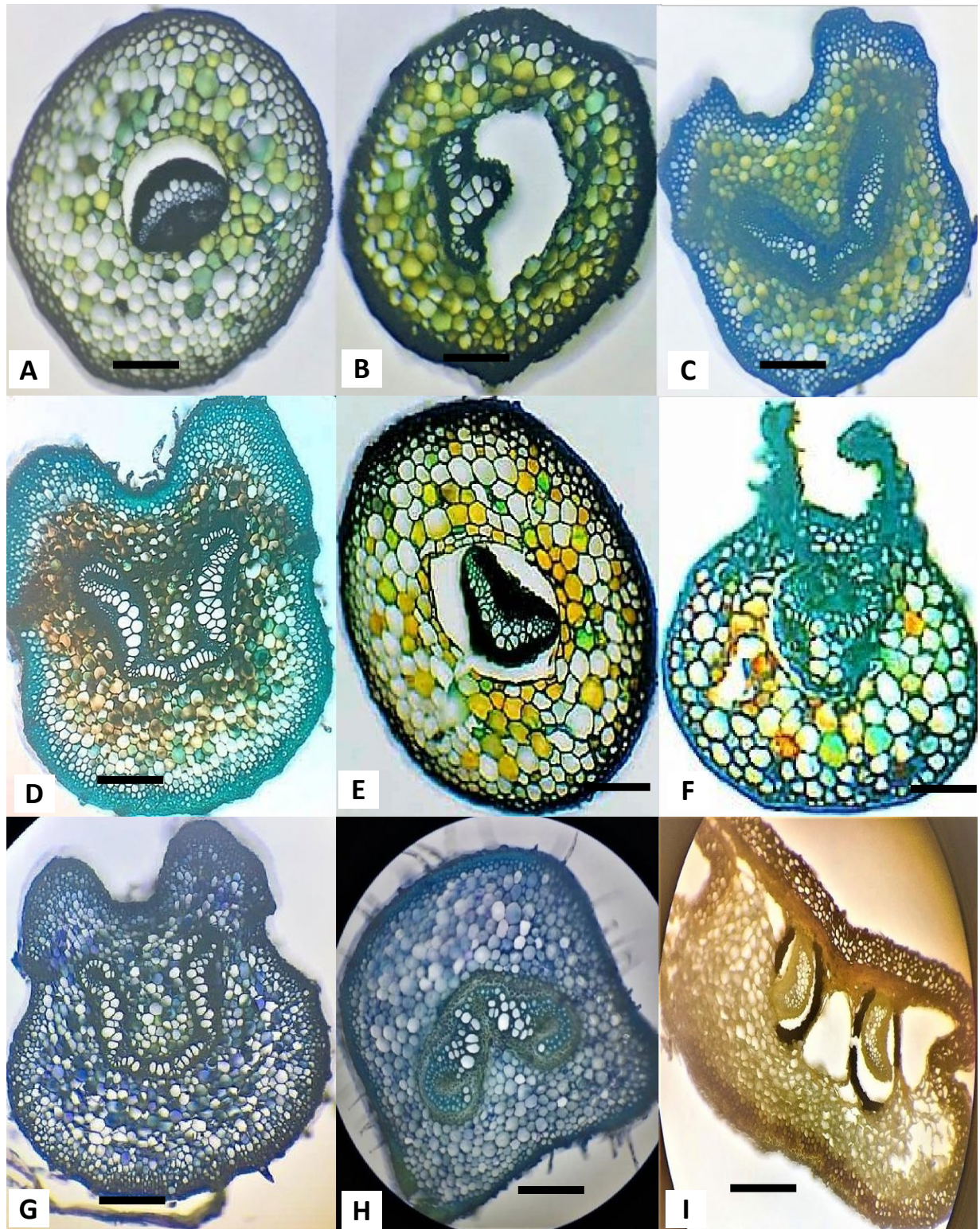
### *Pteris multifida* L.

#### Transverse section of Petiole

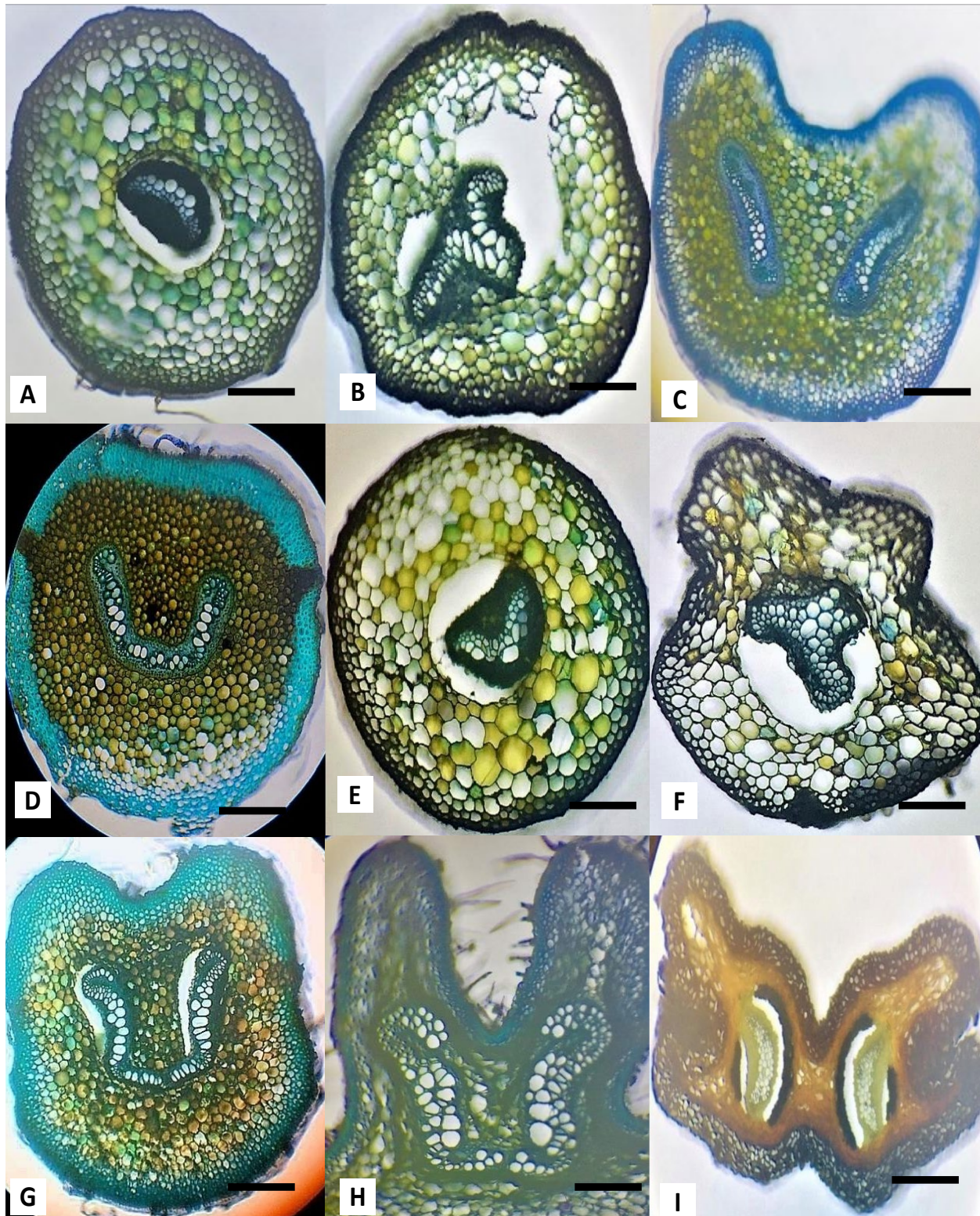
In the transverse section of petiole (Figure 3C) of *Pteris multifida* L., it was observed that scales and trichomes were found on the indumentums of petiole. Trichomes were simple and composed of 5-9 cells. A thick cuticle covered the outermost epidermal layer of petiole. Cortex was present inner to the epidermis and was made up of 2-18 layers of sclerenchyma and parenchyma cells. Tannins were mostly found in cortex which gave dark brown color to the petioles.



**Figure 2.** Fern species A. *Adiantum pedatum* L. B. *Adiantum capillus-veneris* L. C. *Pteris multifida* L. D. *Pteris vittata* L. E. *Adiantum caudatum* L. F. *Aleuropteris argentea* G. D. *Dryopteris carthusiana* Vill. H. *Christella dentata* Forssk. I. *Asplenium platyneuron* L.



**Figure 3.** Transverse section of petioles of nine species A. *Adiantum pedatum* L. B. *Adiantum capillus-veneris* L. C. *Pteris multifida* L. D. *Pteris vittata* L. E. *Adiantum caudatum* L. F. *Aleuritopteris argentea* G. *Dryopteris carthusiana* Vill. H. *Christella dentata* Forssk. I. *Asplenium platyneuron* L. (Scale bar = 1cm).



**Figure 4.** Transverse section of stems of nine species A. *Adiantum pedatum* L. B. *Adiantum capillus-veneris* L. C. *Pteris multifida* L. D. *Pteris vittata* L. E. *Adiantum caudatum* L. F. *Aleuritopteris argentea* G. *Dryopteris carthusiana* Vill. H. *Christella dentata* Forssk. I. *Asplenium platyneuron* L. (Scale bar = 1cm).



V-shaped dictyostele was found in the center. Two vascular bundles were present at the base, and they fused together in the lower third of petiole by abaxial ends. Vascular bundles were surrounded by a monostratified endodermis and pericycle beneath endodermis was made up of 2-3 cell layers. Xylem was diarch with curved ends. Phloem was found outer to protoxylem and metaxylem.

#### Transverse section of Stem

It was observed in the transverse section of stem (Figure 4C) that epidermis was composed of single layered quadrangular cells. Thick cuticle was present around the epidermis. Cortex was differentiated into a few layers of sclerenchymatous cells and a broad area of parenchymatous cells. Leaf and root traces could be found in these areas. Endodermis surrounded the vascular bundles and comprised of casparian strips. Thin walled, 1-2 layered pericycle was also present underneath the endodermis. Phloem consisted of phloem parenchyma and sieve cells. Moreover, phloem surrounded the xylem completely. On the other hand, xylem comprised of xylem parenchyma and tracheids. Protoxylem was smaller in size as compared to metaxylem. Dictyostele was observed in the stem.

#### *Pteris vittata* L.

##### Transverse section of Petiole

The transverse section of petiole (Figure 3D) showed that scales and trichomes were present on the indumentums of petiole. Trichomes were simple and composed of 2-4 cells. The epidermis was covered by a thick cuticle. Cortex was present inner to the epidermis and was composed of 2-18 layers of sclerenchyma and parenchyma. Tannins were mostly found in cortex, and they give dark brown color to the petioles. V-shaped dictyostele was found in the center. Vascular bundles were surrounded by a monostratified endodermis. The pericycle was made up of 2-3 cell layers and was present beneath the endodermis. Xylem was triarch and had curved ends. Phloem was found outer to protoxylem and metaxylem.

##### Transverse section of Stem

The transverse section of stem (Figure 4D) showed that the outline of stem was cordate. Cuticle and ramentum scales covered the epidermis. Epidermis was composed of single-layered parenchymatous cells. Sclerenchymatous and parenchymatous cells made the cortex inner to the epidermis. Tannin cells were found in parenchymatous cells. The vascular

strand was V-shaped and surrounded by endodermis and pericycle. Dictyostele was present. Vascular bundles consisted of xylem and phloem. Xylem was diarch, V-shaped and surrounded by phloem. Protoxylem was present towards the adaxial side.

#### *Adiantum caudatum* L.

##### Transverse section of Petiole

In the transverse section of petiole (Figure 3E) of *Adiantum caudatum* L., it was observed that a thick, dark brown cuticle was covering the epidermis. Epidermal cells were found inner to the cuticle. The ground tissue was present inner to the epidermis and was made up of multiple layers of sclerenchyma and parenchyma cells. Tannins were mostly found in cortex which provided dark brown color to the petioles. Vascular bundles including xylem and phloem were surrounded by a single-layered endodermis. The pericycle was present beneath the endodermis and it was made up of 2-3 cell layers. Xylem was a mesarch and consisted of protoxylem and metaxylem. It was semi-lunar shaped. Phloem was found outer to protoxylem and metaxylem. Amphiphloic siphonostele was found in this species.

##### Transverse section of Stem

The transverse section of the stem (Figure 4E) of *Adiantum caudatum* L. showed single layered and thick-walled outer epidermis. The epidermis was dark brown in color and surrounded by cuticles. A cell layer was present beneath epidermis, towards the inner side, that was termed as cortex. Cortex was differentiated into outer 3 or 4 layered sclerenchymatous cells and thin-walled, multi-layered parenchymatous cells. Parenchymatous cells used to be rich in tannin content. Vascular bundles were surrounded by thick-walled and one-layered endodermis. Multilayered pericycle cells were found inner to the endodermis. A vascular strand was observed in the center in which xylem was surrounded by phloem. Xylem had two groups including metaxylem and protoxylem, which varied in size and were semi-lunar shaped. Metaxylem was large, semi-lunar, mesarch while protoxylem was smaller in size. Amphiphloic siphonostele was present in the stem.

#### *Aleuritopteris argentea* Fee

##### Transverse section of Petiole

In the transverse section of petiole (Figure 3F), it was observed that epidermis was covered by a dark brown colored thick cuticle. Two hook-like trichomes originated from the cuticle. Cortex was present inner

to the epidermis and was made up of 2-3 layers of sclerenchyma and multiple layers of parenchyma cells. Tannins were mostly found in cortex which provided dark brown color to the petioles. Vascular bundles including xylem and phloem were surrounded by a single-layered endodermis. The pericycle was present beneath the endodermis, and it was made up of 2-3 cell layers. Dictyostele was found in the center of vascular bundles. Phloem was found outer to protoxylem and metaxylem. Xylem was a mesarch and consisted of protoxylem and metaxylem. The stele was actinostele.

#### **Transverse section of Stem**

The transverse section of the stem (Figure 4F) of *Aleuritopteris argentea* showed single layered outer epidermis. The epidermis was dark brown and surrounded by cuticles. A cell layer was present beneath epidermis that was termed as cortex. Cortex was differentiated into outer 3 or 4 layered sclerenchymatous cells and thin-walled, multi-layered parenchymatous cells. Vascular bundles were surrounded by thick-walled and one-layered endodermis. Multilayered pericycle cells were found inner to the endodermis. Vascular strand was observed in the center in which xylem was surrounded by phloem. Xylem had two groups, metaxylem and protoxylem. Metaxylem was large, semi-lunar, mesarch while protoxylem was smaller. Stele was simple actinostele.

#### ***Dryopteris carthusiana* (Vill.)**

##### **Transverse section of Petiole**

In the transverse section of petiole (Figure 3G) of *Dryopteris carthusiana*, it was observed that a thick, dark brown cuticle was covering the epidermis. The cortex was present inside the epidermis. Cortex was differentiated into multiple layers of sclerenchyma and thin-walled parenchyma cells. Tannins were mostly found in cortex which provided dark brown color to the petioles. Vascular strand was observed as U-shaped structure. Vascular bundles including xylem and phloem were surrounded by a single-layered endodermis. The pericycle was present beneath the endodermis, and it was made up of 2-3 cell layers. Phloem surrounded the xylem. Xylem was triarch and consisted of protoxylem and metaxylem. Dictyostele was present in this species.

##### **Transverse section of Stem**

It was observed in the transverse section of stem (Figure 4G) of *Dryopteris carthusiana* (Vill.) that epidermis was composed of single layered cells. Thick

cuticle was present around the epidermis. Cortex was differentiated into a few layers of sclerenchymatous cells and a broad area of parenchymatous cells. Vascular strand appeared as a U-shaped structure. Endodermis surrounded the vascular bundles and comprised of casparian strips. Thin walled, 1-2 layered pericycle was also present underneath the endodermis. Phloem consisted of phloem parenchyma and sieve cells. Moreover, phloem surrounded the xylem completely. On the other hand, xylem was made up of xylem parenchyma and tracheids. Protoxylem was smaller in size as compared to metaxylem. The stem of this species had dictyostele.

#### ***Christella dentata* Forssk**

##### **Transverse section of Petiole**

In the transverse section of petiole (Figure 3H), it was observed that small, thick-walled and dark brown epidermal cells were present throughout the length. Smooth, delicate hairs were found on the epidermis. Three or four layered, thick-walled sclerenchymatous cells and thin-walled multilayered parenchymatous layers of ground tissue were found inner to the epidermis. Two widely separated vascular strands arose from rhizome and later got fused together to form a single strand. Vascular bundles were enclosed by endodermis. The pericycle was composed of 1-3 layered thin-walled cells. Xylem was heart-shaped and mesarch. Xylem was also surrounded by phloem. This species had dictyostele.

##### **Transverse section of Stem**

The epidermis was the outermost layer covered with delicate hairs (Figure 4H). It was composed of small, dark brown and thick-walled cells. Cortex was multi-layered and found inner to epidermis. Sclerenchymatous cells consisted of 2-3 layers but thin-walled parenchymatous cells consisted of several layers. Starch grains were found in parenchymatous cells. Endodermis was found outer to the pericycle and surrounded by vascular bundles. Vascular bundles arose from rhizome as two separated vascular strands and later fused together forming a single strand. The pericycle was composed of 1-3 layered thin-walled cells. Dictyostele was present. Phloem was found outer to the xylem. Xylem was mesarch and hippocampus shaped.

#### ***Asplenium platyneuron* L.**

##### **Transverse section of Petiole**

The transverse section of petiole (Figure 3I) showed that the outermost layer was epidermis. Epidermis

was followed by a hypodermis on the inner side. Hypodermis was composed of sclerified cells. Cortex was composed of both sclerenchymatous and parenchymatous cells. Parenchyma consisted of a large portion of cortex. Intercellular spaces were observed in the parenchymatous cells. Two vascular strands containing xylem groups and phloem were found in the petiolar structure. These vascular bundles later fused together towards the rachis, and they were surrounded by endodermis and pericycle. Metaxylem and protoxylem were completely enclosed by phloem. Metaxylem was larger in size as compared to protoxylem. Dictyostele was found in the petiole.

### Transverse section of Stem

The transverse section of stem (Figure 4I) showed that outer layer was epidermis. Hypodermis was composed of sclerified cells. Ground tissue was differentiated into a few layers of sclerenchyma and parenchyma. Parenchymatous cells make up a large portion of cortex. Two vascular strands were surrounded by single-layered endodermis and pericycle. Vascular bundles were fused together afterwards. Xylem consisted of metaxylem and protoxylem while phloem was surrounded with xylem completely.

### Data analysis

The comparative traits were scored to be 69 main characters. They were divided into three categories: morphological (25 characters) (Figure 2), anatomical micro-investigation (20 characters) (Figures 5-8) and anatomical macro-investigation (24 characters) (Figures 3-4). Qualitative characters are represented in both morphological and anatomical macro-investigation. They were characters that were denoted whether present or not. On the other hand, quantitative characters were restricted only in anatomical micro-investigation which expressed on all measurement parameters (Tables 2-4). Quantitative characteristics are not considered as key classification characteristics because they are unstable characteristics that change with changes in the conditions surrounding the plants.

The similarity parameter is the common character within all studied species while the dissimilarity parameter is the character which is absent in even one plant species. So, there were 5 and 4 similarity parameters in morphological and anatomical macro-investigation respectively. The unique parameters are defined as parameters which specifically identify a

species from the others by their presence or absence. The parameters that are present in one species but not found in the others are termed positive unique parameters (PUP) in contrast to the negative unique parameters (NUP) which are absent in one species but present in others. These parameters could be used for taxa identification. (Emad et al., 2023). There were 11 unique parameters among the 69<sup>th</sup> main characters: 6 positive unique parameters and 5 negative unique parameters. They distributed as 6 PUPs (3 morphological traits) and (1 anatomical micro-investigation) besides 5 NUPs (3 morphological ones), (1 anatomical macro-investigation) and (1 anatomical micro-investigation) (Figure 9).

The resulting phenogram revealed that the studied fern species had an average taxonomic distance of 0.80. At this level, two main groups were divided; the first and the second groups were presented at 0.95 and 0.899 respectively. At 0.95, *Adiantum capillus-veneris* L. was delimited as a single sub-clad while *Adiantum pedatum* L. and *Adiantum caudatum* L. were separated as a sub-group at 1.109. The second group was differentiated into two sub-groups; the first and second subgroups were separated at 0.96 and 1.021 respectively. *Pteris multifida* L. and *Pteris vittata* L. were delimited as sub-clads while *Aleuritopteris argentea* Fee. and *Dryopteris carthusiana* (Vill.) presented into a clad at 1.12. The second sub-group was observed at 1.065 including *Christella dentata* Forssk. and *Asplenium platyneuron* L. (Figure 10). As shown in Table 5, The highest similarity value was recorded between *Pteris multifida* L. and *Pteris vittata* L. besides *Aleuritopteris argentea* Fee. and *Asplenium platyneuron* L. (0.84) On the other hand, the lowest similarity value was recorded between *Adiantum pedatum* L. and *Pteris multifida* L. (0.51).

The analysis of variance for studied fern species accomplished by ANOVA tests showed significant differences with high significant in most of them. *F* test values were near to each other (Table 6). Pearson correlation coefficients for each plant species against another species was the highest value (0.848) between *Pteris* and *Asplenium* while the lowest one was between *Dryopteris* and *Christella* (Table 7). In addition, regression was able to describe the co-variation among studied fern plant species variables. SLR curves indicate the significant relationships among them. There was such an extremely high regression between *Adiantum* and *Pteris* whereas *Pteris* and *Aleuritopteris* showed the lowest one (Figure 11).

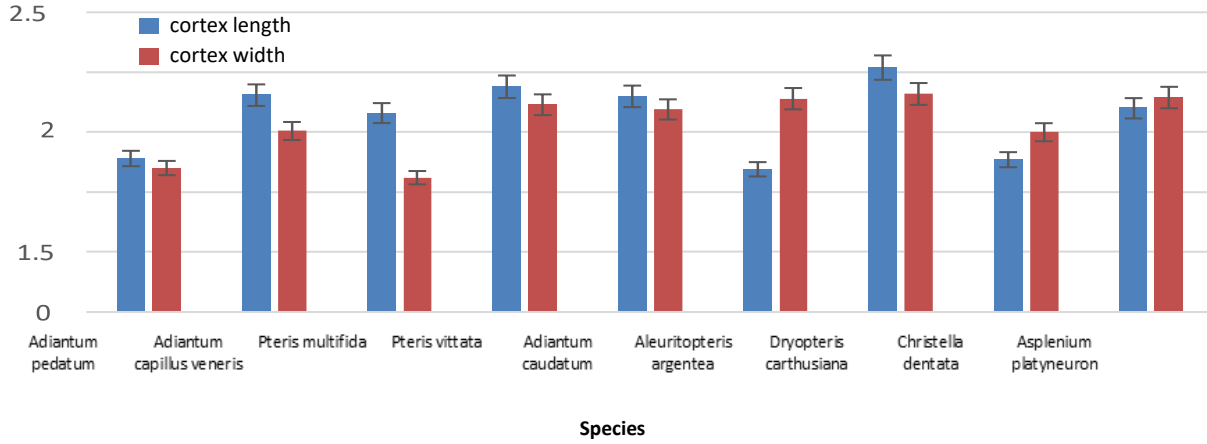


Figure 5. Comparative graph between length and width of Ground tissue of petiole for nine fern species.

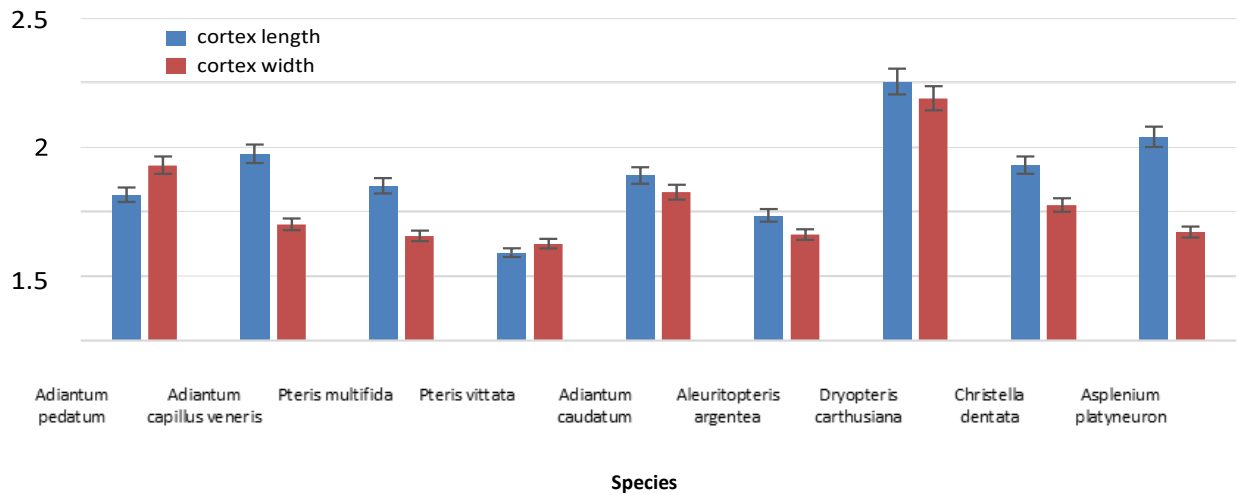


Figure 6. Comparative graph between length and width of Ground tissue of stem for nine fern species.

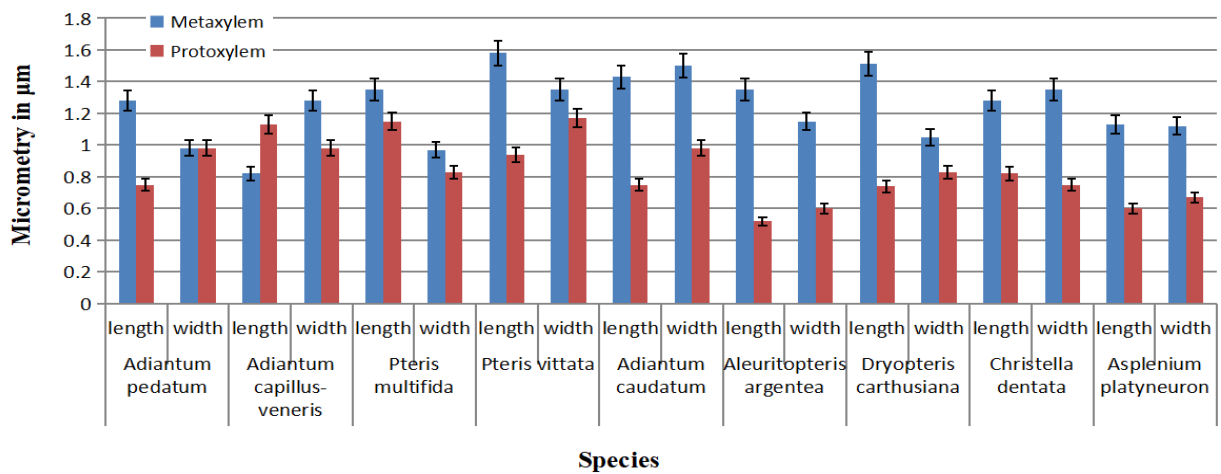


Figure 7. Comparative graph between length and width of Vascular bundles of petiole for nine species.

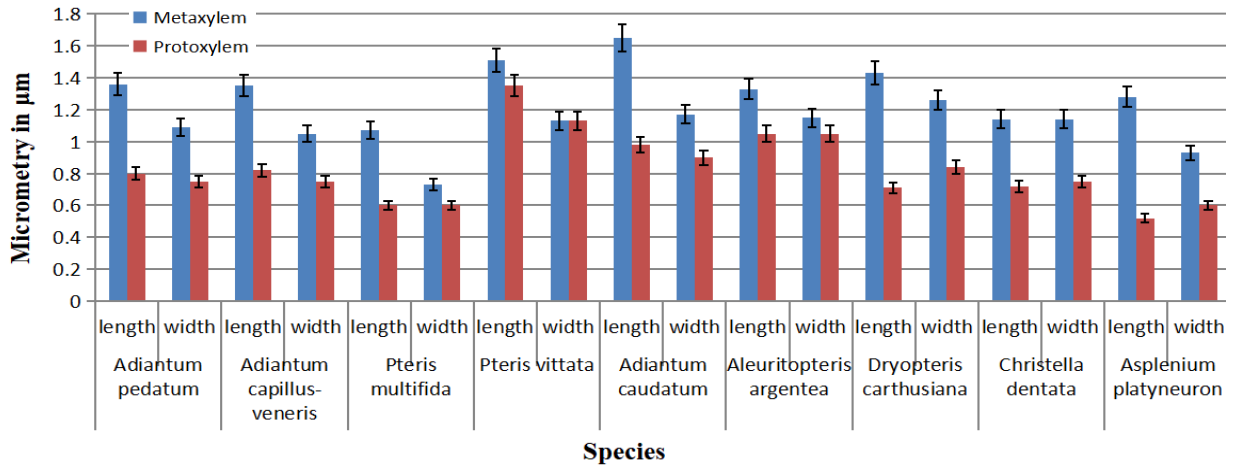


Figure 8. Comparative graph between length and width of Vascular bundles of stem for nine species.

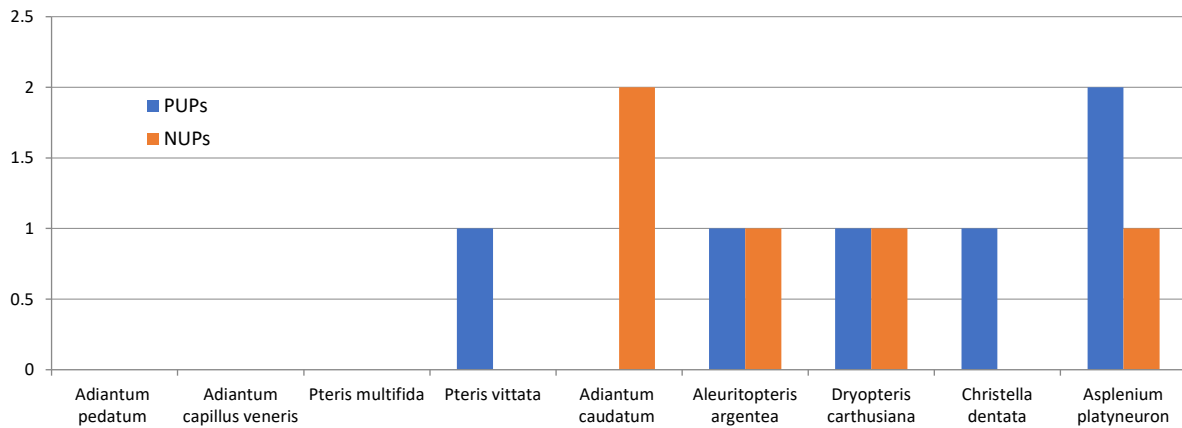


Figure 9. PUPs and NUPs among studied fern species.

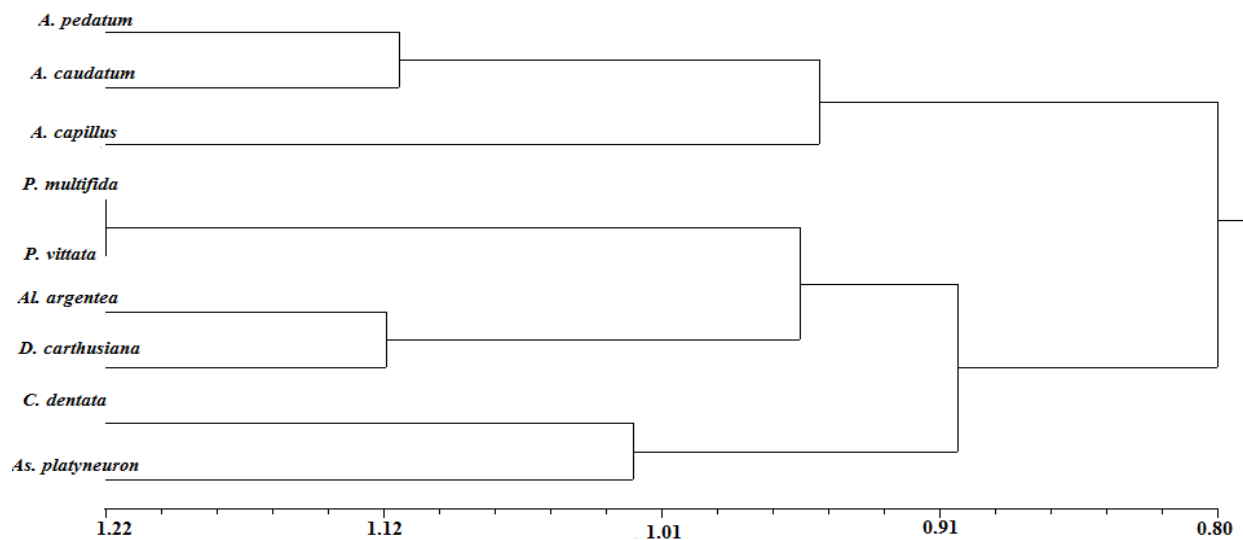


Figure 10. Phenogram among studied fern species.

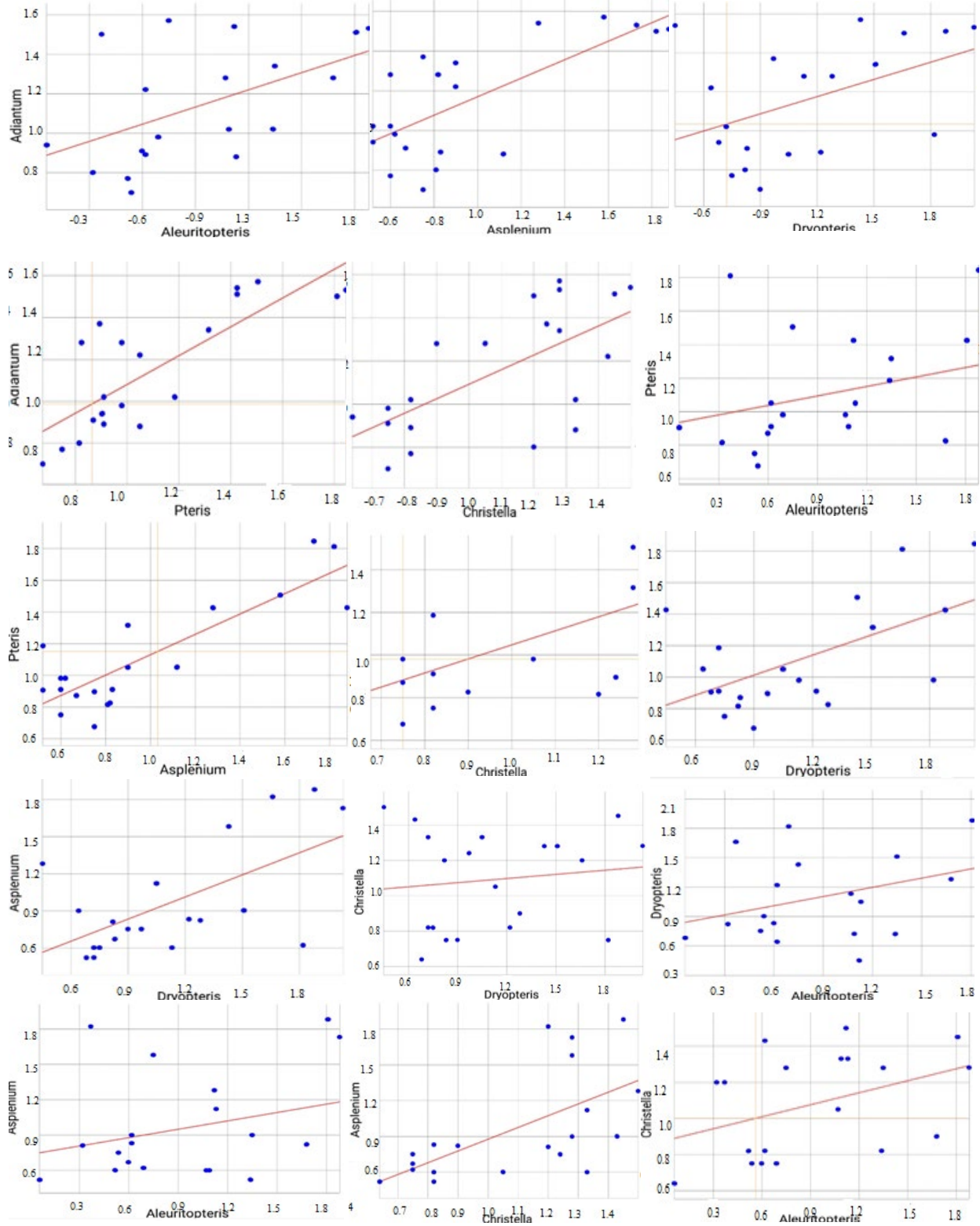


Figure 11. SLR curves among studied fern species

**Table 2.** Binary matrix of morphological traits of studied species (No. 1-9).

Morphological trait	1	2	3	4	5	6	7	8	9
deciduous	1	0	0	0	0	0	1	0	1
herbaceous	1	1	1	1	1	1	1	1	1
Perennial	1	1	1	1	1	1	1	1	1
Rhizome short & erect	1	1	1	1	1	1	1	1	1
Dark scales on rhizome	1	1	0	0	1	1	1	1	1
Clustered fronds	1	1	1	1	1	1	1	1	1
Pinnule	1	1	0	0	1	1	1	1	1
Dimorphic fern	1	1	1	1	1	1	1	1	1
Fan shaped pinnule	1	1	0	0	1	0	0	0	0
Glabrous stipe	1	1	1	1	0	1	1	1	1
Brown stipe	1	0	1	0	1	1	0	1	1
Green pinnae	1	0	1	0	1	1	0	1	1
Bipinnate leaf	1	1	1	1	0	1	1	1	1
Hanging fronds	0	1	0	0	1	0	0	0	1
Opposite pinna	0	0	1	1	1	1	1	1	0
Black stem	1	1	0	0	0	0	0	0	0
Oblanceolate lamina	0	0	1	1	0	1	1	1	1
30cm<Frond length	1	1	1	1	1	1	0	1	1
20cm<Lamina length	1	1	0	1	1	0	0	1	1
10cm<Pinnule length	0	0	0	0	0	0	0	1	0
Winged rachis	0	1	1	1	0	1	1	1	0
Pinna entire margin	1	1	0	1	1	1	1	1	1
Sori at pinna tips	1	1	0	0	0	1	1	0	1
Pinna farinose	0	0	0	0	0	1	0	0	0
Spinny pinna	0	0	0	0	0	0	1	0	0

**Table 3.** The binary matrix of anatomical micro-investigation of studied species (No. 1-9).

Anatomical micro-investigations			1	2	3	4	5	6	7	8	9
Petiole	epidermis	length 0.82cm	1	1	0	0	0	0	1	0	1
		width~1cm	0	1	0	1	0	1	0	0	0
	cortex	length 1.8cm	0	0	1	1	1	1	0	0	0
		width~1.8cm	0	0	0	1	1	1	1	0	1
	proxylem	length~0.8cm	0	1	0	1	0	0	0	1	0
		width ~1cm	1	0	0	1	1	0	0	0	0
	metaxylem	length~1.3cm	0	1	0	0	0	1	0	1	0
		width>1cm	0	1	0	1	1	1	1	1	1
	phloem	length>1cm	0	0	0	0	1	0	1	0	0
		width~0.9cm	0	1	0	0	1	0	1	0	0
stem	epidermis	length~1cm	0	1	1	1	1	0	1	0	0
		width~1cm	0	0	0	0	0	1	0	1	0
	cortex	length~1.85cm	0	1	1	1	1	0	1	1	1
		width>1cm	1	1	1	1	1	0	1	1	1
	proxylem	length~1.2cm	0	1	1	1	0	1	0	0	0
		width>1cm	0	0	0	1	0	0	1	0	0
	metaxylem	length~1.6cm	1	0	0	1	1	0	0	1	0
		width>1cm	1	1	0	1	1	0	0	1	0
	phloem	length>1cm	1	0	0	0	1	1	1	0	0
		width>1cm	1	0	0	0	1	0	0	0	0

## DISCUSSION

Pteridophytes belong to a group of vascular plants which produce spores, and they constitute a considerable percentage of the present-day vegetation across the world. They are mostly found in tropical, sub-tropical and moist temperate areas. The favorable environment for ferns to grow includes cool, moist and shady habitats. It was observed that the richest Pteridophyte diversity was present in Azad Jammu and Kashmir because of thick forest cover,

favourable climate and suitable habitat. Moreover, they are also found in abundance in Khyber Pakhtunkhwa and Punjab province (Irfan et al., 2021).

In this research work, a total of nine species of leptosporangiate ferns belonging to 6 genera and 5 families were reported during the field survey of district Kotli, AJK. Adiantaceae having 3 species (*Adiantum capillus-veneris* L., *Adiantum caudatum* L. and *Adiantum pedatum* L.) and Pteridaceae with 3 species (*Pteris vittata* L., *Pteris multifida* L. and

**Table 4.** The binary matrix of anatomical macro-investigation of studied species (No. 1-9).

Anatomical macro-investigations		1	2	3	4	5	6	7	8	9
Petiole	Thick cuticle	1	1	1	1	1	1	1	1	1
	scales	0	0	1	1	0	0	0	0	0
	trichomes	0	0	1	1	0	1	0	1	0
	epidermis inner to cuticle	0	1	0	0	1	0	0	0	0
	hypodermis	0	0	0	0	0	0	0	0	1
	sclerenchyma & parenchyma in ground tissues	1	1	1	1	1	1	1	1	1
	Tannin	1	1	1	1	1	1	1	0	0
	endodermis	1	1	0	0	1	1	1	1	1
	1-2 layers of pericycle	1	1	1	1	1	1	1	0	0
	mesarch	1	1	1	1	1	1	1	1	1
	diarch	0	0	1	0	0	0	0	1	1
	amphiphloic siphonostele	1	0	0	0	1	0	0	0	0
	dictyostele	0	1	1	1	0	0	1	1	1
	stem	thick cuticle	1	1	1	1	1	1	1	1
scales		0	0	0	1	0	0	0	0	0
hair		1	0	0	0	0	0	0	1	0
hypodermis		0	0	0	0	0	0	0	0	1
multilayered sclerenchyma		1	0	0	0	1	1	1	1	0
tannin		1	0	0	1	1	0	0	0	0
two layered pericycle		0	0	1	0	1	1	1	0	0
amphiphloic siphonostele		1	0	0	0	1	0	0	0	0
mesarch		1	1	1	1	1	1	1	1	1
diarch		0	0	1	1	0	0	1	1	1
dictyostele		0	1	1	1	0	0	1	1	1

**Table 5.** Similarity matrix of studied fern species.

	A. pedatum	A. capillus	P. multifida	P. vittata	A. caudatum	Al. argentea	D. carthusiana	C. dentata	As. platyneuron
A. pedatum	1.00								
A. capillus	0.73	1.00							
P. multifida	0.51	0.61	1.00						
P. vittata	0.55	0.69	0.84	1.00					
A. caudatum	0.80	0.69	0.55	0.55	1.00				
Al. argentea	0.71	0.69	0.76	0.67	0.71	1.00			
D. carthusiana	0.63	0.73	0.71	0.71	0.59	0.78	1.00		
C. dentata	0.65	0.65	0.76	0.71	0.59	0.76	0.69	1.00	
As. platyneuron	0.63	0.69	0.63	0.80	0.55	0.84	0.67	0.76	1.00

**Table 6.** ANOVA test among studied fern species.

	A. pedatum	A. capillus	P. multifida	P. vittata	A. caudatum	Al. argentea	D. carthusiana	C. dentata	As. platyneuron
Ftest	1.106	1.985	1.091	1.591	1.663	1.055	1.064	1.110	1.019
P value	0.88	0.322	0.899	0.500	0.460	0.938	0.928	0.880	0.978
Significant	***	*	***	**	**	***	***	***	***

**Table 7.** Pearson correlation coefficients among studied fern species.

	Adiantum	Pteris	Aleuritopteris	Dryopteris	Christella	Asplenium
Adiantum	-	0.798	0.545	0.465	0.645	0.715
Pteris	0.798	-	0.305	0.576	0.544	0.848
Aleuritopteris	0.545	0.305	-	0.372	0.434	0.290
Dryopteris	0.465	0.576	0.372	-	0.129	0.613
Christella	0.645	0.544	0.434	0.129	-	0.616
Asplenium	0.715	0.848	0.290	0.613	0.616	-



*Aleuritopteris argentea*) were the dominant families whereas Thelypteridaceae (*Christella dentata* Forssk.), Dryopteridaceae (*Dryopteris carthusiana* Vill.) and Aspleniaceae (*Asplenium platyneuron* L.) were having one species each. *Adiantum pedatum* L., *Adiantum capillus-veneris* L. and *Asplenium platyneuron* L. had spores on the lower side of pinnae while *Adiantum caudatum* L. had spores on the apex of pinnae (Iltaf et al., 2012).

The anatomical study of stem can be of great importance in the identification of fern species. Each species possesses distinct anatomical features of stem so this can be helpful to differentiate species from one another. Cuticle was present outside the epidermis in almost all species under observation. It was secreted by epidermal cells and covered the entire outer surface of ferns. In the transverse section of stem, it was observed that a multilayered ground tissue was present. It had outer sclerenchymatous and inner parenchymatous regions in *Adiantum caudatum* L., *Christella dentata* Forssk. and *Pteris vittata* L. Trichomes or scales were also found on the outer surface of epidermis in *Pteris vittata* L., *Dryopteris carthusiana* Vill., *Christella dentata* Forssk. and *Aleuritopteris argentea*. Xylem was hippocampus-shaped in *Christella dentata* Forssk. (Figure 3H), V-shaped in *Pteris vittata* L. (Figure 4D) and semi-lunar shaped in *Adiantum caudatum* L. (Figure 4E). Mesarch xylem was found in *Adiantum pedatum* L. (Figure 4A), *Adiantum capillus-veneris* L. (Figure 4B), *Adiantum caudatum* L. (Figure 4E), *Aleuritopteris argentea* (Figure 4F) and *Christella dentata* Forssk. (Figure 4H). While diarch xylem was present in *Pteris vittata* L. (Figure 4D), *Pteris multifida* L. (Figure 4C), *Asplenium platyneuron* L. (Figure 4I) and *Dryopteris carthusiana* Vill. (Figure 4G). Metaxylem was larger in size but protoxylem made a small patch (Resmi et al., 2016).

The anatomical features of leaf petiole were also reported to be very helpful in fern systematics. The xylem elements and number of vascular strands provide significant information for identification and description of ferns (Hernandez et al., 2012).

The number and type of steles in petiolar structure are also important characteristics for the rapid identification and classification of species. The petiole of some species was covered by trichomes or scales. Tannins used to be found in the cortex, which provided brown to dark brown color to the petiole of ferns. The transverse section of petiole showed a thick cuticle that was found outer to the epidermis. The cortex or ground tissue was composed of several

layers of sclerenchyma and parenchyma cells. Stele was found in the central core of petiole. Vascular bundles were surrounded by endodermis and 2-3 cell layered pericycle. Xylem was mostly surrounded by phloem. Mesarch and diarch xylem were found in most of the species. Xylem had curved ends in *Pteris vittata* L. while it was heart-shaped in *Christella dentata* Forssk. *Asplenium platyneuron* L. was distelic.

Dictyostele having meristeles was found in most of the species including *Adiantum capillus-veneris* L., *Dryopteris carthusiana* Vill., *Asplenium platyneuron* L. and *Christella dentata* Forssk. V-shaped dictyostele was present in *Pteris vittata* L. and *Pteris multifida* L. While amphiphloic siphonostele was found in *Adiantum pedatum* L. and *Adiantum caudatum* L. (Martinez and Vilte, 2012).

The length and width of different cells of stem and petiole were also measured. The epidermal cells, cortical cells, protoxylem, metaxylem and phloem measurements were taken by using ocular and stage micrometer and were represented in  $\mu\text{m}$ . The length and width of cortical cells were greater in *Dryopteris carthusiana* Vill. in petiole i.e.  $2.04\mu\text{m}$  and  $1.82\mu\text{m}$  respectively. Similarly in stem, the highest length and width of cortical cells were shown by *Dryopteris carthusiana* Vill. i.e.  $2.01\mu\text{m}$  and  $1.88\mu\text{m}$  respectively as compared to other species. In petiole, length of metaxylem was greater in *Pteris vittata* L. i.e.  $1.58\mu\text{m}$  while the width of metaxylem was greater in *Adiantum caudatum* L. i.e.  $1.5\mu\text{m}$ , as compared to the rest of species. The length of protoxylem was greater in *Pteris multifida* L. i.e.  $1.15\mu\text{m}$  while the width of protoxylem was greater in *Pteris vittata* L. i.e.  $1.17\mu\text{m}$ . In stem, length of metaxylem was more in *Adiantum caudatum* L. i.e.  $1.65\mu\text{m}$  but width was more in *Dryopteris carthusiana* Vill. i.e.  $1.26\mu\text{m}$ . The length and width of protoxylem was highest in *Pteris vittata* L. i.e.  $1.35\mu\text{m}$  and  $1.13\mu\text{m}$  respectively (Shah et al., 2019).

P- values reported all fern plant species data were more effective in this investigation even though there were different degrees of significance. F tests reflected the degree of accuracy which was carried out in this investigation. Furthermore, Pearson correlation coefficients emphasized that there were more convenient species with related ancestors. Moreover, (SLR) equations indicated the data analysis in the form of exponential curves showing all plant species were compatible to each other with different extents.

Cluster analysis comprises the total visions about plant status and confirms the interrelationships among studied fern species to evaluate the taxonomical position and assess the species role. It relies on parameters like PUP and NUP to crystallize the importance of the species. Unique parameters give the priority to plant fern species to be the most common established like *Asplenium platyneuron* L. having 2 PUPs and 1 NUP otherwise *Adiantum pedatum* L., *Adiantum capillus veneris* L. and *Pteris multifida* L. never had one.

The output phenogram illustrated the taxonomic positions of the studied species. It revealed the transition species between two main groups; *Adiantum pedatum* L. and *Adiantum caudatum* L. that reflected the importance of *Adiantum* sp. as a connecting link among pteridophytes. Despite phenogram analysis, the similarity matrix recorded that *Pteris multifida* L. and *Pteris vittata* L. besides *Aleuritopteris argentea* Fee. and *Asplenium platyneuron* L. were closely related to each other while *Adiantum pedatum* L. and *Pteris multifida* L. were distantly related species.

## CONCLUSION

Finally, the present investigation illustrated the taxonomic value of the most distributed pteridophytes in Pakistan which will help other researchers to draw the whole interrelationships among the same or different genera in other regions or countries.

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