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ANATOMICAL STUDIES OF SOME HYDROPHYTES COLLECTED FROM DISTRICT BHIMBER, AZAD JAMMU KASHMIR, PAKISTAN

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Abstract

Hydrophytes play a crucial role in maintaining the ecological balance in aquatic ecosystems. They are bioindicators for water quality, pollution and eutrophication, ultimately assisting habitat restoration. The unique anatomical features of hydrophytes are essential for their survival in waterlogged or submerged environments. Studying these traits reveal how plants evolve to overcome challenges like, low oxygen availability, nutrient absorption and reduced mechanical support**.** The present study was carried out for the anatomical study of hydrophytes collected from district Bhimber, Azad Jammu and Kashmir, Pakistan. Total three species*, Hydrilla verticillata* (L.f.) Royle, *Eichhornia crassipes (*Mart.) Solms and *Nymphaea alba* L. were collected and preserved in the fixative solutions and subjected to anatomical characterization using a light microscope. In the T.S. of the stem of *Nymphaea alb*a L., trichomes were observed (L=4.50±0.7, W=2.11±0.48) profusely surrounding the layer of epidermis. Larger aerenchyma was observed (L=121.50±47.34, W=92.76±32.26). In the T.S of *Hydrilla verticillata* (L.f.) Royle, larger aerenchyma cells were also observed (L=41.84±8.86, W=29.49±2.1). In the T.S of *Eichhornia crassipes* (Mart.) *Solms* aerenchyma was larger among other cells (L=40.50±1.00, W=28.25±1.48). Trichomes were also examined (L=3.46±1.06, W=1.73±0.20). Larger aerenchyma and multicellular trichomes were observed, indicating that these species are better adapted to the influx of excessive water in their aquatic habitats. Overall, the research is correlating the anatomical characterization of the hydrophytes with their environmental and climatic factors helping to understand the mechanism of ecological adaptations to their aquatic environment.

Keywords: Hydrophytes, anatomical study, climatic factors, Trichomes.

INTRODUCTION:

Plant species are commonly classified at the ecological level according to their evolutionary adaptations, phenotypic variations, and the interplay between the evolution of their morphological, physiological, and anatomical traits in response to their environmental conditions. Hydrophytes establish specialized ecological roles in many aquatic environments, including freshwater lakes, marshy areas along the peripheries of the coastal regions, wetlands, ponds and inland marshes (Rodrigo, 2021). Hydrophytes demonstrate a diverse range of phenomenal adaptations that facilitate them to flourish and thrive within aquatic environments (Tiner, 2006). These adaptations encompass a range of anatomical, physiological, and morphological changes that are directed at enhancing their efficiency of nutrition absorption, regulation of buoyancy, photosynthesis, and exchange of gases in order to effectively respond to the demands posed by aquatic environments (Ronzhina *et al*., 2001). Hydrophytes are magnificently essential and are widely used in restorative practices wetland ecosystems (Zhao *et al*., 2016). The examination of the anatomical characteristics of various morphological components of hydrophytes facilitates a comprehensive understanding of their adaptive strategies and enables a more thorough and insightful analysis of the mechanisms they employ to thrive in aquatic environments (Jayeola and Folorunso, 2009). Anatomical analysis is a key factor for the identification of different plant species. In order to study plant systematics, anatomical

analysis is pragmatically necessary. Most of the time, with the change of plant habitat, a change in the epidermal size, type of the stomata, trichomes, and subsidiary cells can be observed (Werker, 2000).

The present research aimed to investigate anatomical features of morphological components of some hydrophytes collected from the district Bhimber, Azad Jammu and Kashmir, Pakistan, as previously no published data is available for the anatomical features of hydrophytes of Azad Jammu and Kashmir. The biodiversity found in Azad Jammu and Kashmir is unrivalled as compared to other regions of Pakistan (Ajaib *et al*., 2012). Bhimber is a well-recognized district that shares a border with Punjab, Pakistan to the south Bhimber is situated in the southern region of Azad Jammu and Kashmir, while its eastern border is adjacent to the Indian-administered Kashmir. The study area (District: Bhimber, Azad Jammu and Kashmir) is located between latitude 32-48° to 33-34° and longitude 73-55° to 74-45°. The total area of district Bhimber is 1516 square kilometers with an altitude of 1118 feet above sea level **(**Maqbool *et al*., 2019**).** District Bhimber exhibits a diverse geography, hydrophytes (I.e. *Nymphaea alba* L., *Hydrilla verticillata* (L.f.) Royle, and *Eichhornia crassipes* (Mart.) Solms) in different aquatic habitats (ponds, ditches, freshwater lakes, and marshes).

Nymphaea is a genus of aquatic plants commonly referred as water lilies. There are many species of *Nymphaea* that are distributed worldwide, with variations in their morpho-anatomical characteristics, range of distribution, and morphological characteristics of flower (i.e. size, colour, shape, arrangement, and differences in the number of sepals, petals, and stamens), Morpho-anatomical characteristics of their leaves (i.e. variation in size, shape, and color of the leaves; the shape of the leaves blade, venation pattern, and texture of the surface of their leaves), and morphological features of their roots or rhizome (Harrison, 1955).

The flowers are the most striking feature of *Nymphaea alba* L. They are large, fragrant, and typically have pure white petals that radiate outward from a yellow centre. Each flower can measure up to 12-15 cm in diameter, making it highly visible in the aquatic environment (Wheeler, 1997) *Nymphaea alba* L. plays a crucial role in improving water quality. Its extensive root system absorbs excess nutrients like nitrogen and phosphorus, which helps prevent water eutrophication and the growth of harmful algae (Nierbauer *et al*., 2014). The stem of *Nymphaea alba* L. has abundant aerenchyma observed along with the presence of multicellular trichomes (Bavaru and Rodica, 2002).

Hydrilla verticillata (L.f.) Royle, commonly known as Water thyme. It is a highly adaptable and invasive aquatic plant that belongs to the Hydrocharitaceae family (Pieterse, 1981**)**. It is native to some warmer areas of Asia, Europe, Africa, the USA, and Australia but has become a notorious invasive species in many regions across the world (Langeland, 1996).

Hydrilla verticillata (L.f.) Royle is characterized by long, slender stems that can grow up to 25 feet in length (Yeo *et al*., 1984). The leaves are arranged in whorls, along the stem. They have serrated and narrow leaves, resembling those of thyme (Verkleij *et al*., 1983; Sumithran *et al*., 2013). *Hydrilla verticillata* (L.f.) Royle is highly adaptable to a wide range of aquatic habitats. It can thrive in both stagnant and flowing water, and it tolerates various water temperatures and depths (Michel *et al*., 2004). It is immensely tolerant to a wide range of pH. It is adapted to carry out the process of photosynthesis even in lower-intensity sunlight (Steward, 1993). They can reproduce vegetatively so they tremendously are fast acting in competing with

other aquatic plants and aggressively colonize wetland ecosystems (Langeland, 1996). It can reduce water clarity, block sunlight penetration, and deplete oxygen levels, which can harm native aquatic species, including fish and invertebrates (Jones *et al*., 2003).

Eichhornia crassipes (Mart.) Solms, commonly known as Water hyacinth, is a free-floating aquatic plant native to south America but has become a notorious aquatic weed due to its invasive nature and widely spread across different regions of the world **(Milne *et al*., 2006).** Water hyacinth is recognized by its attractive appearance, featuring bright green, glossy, oval-shaped leaves and beautiful lavender or violet flowers with a yellow spot. This plant thrives in slow-moving or stagnant freshwater bodies such as ponds, lakes, rivers, and canals. Water hyacinths may widely spread due to vegetative means of reproduction and have a rapid growth rate **(Coetzee *et al*., 2009).** The rapid growth of water hyacinth can lead to the formation of thick mats that cover the surface of the water. This blocks sunlight, which can harm submerged aquatic plants and disrupt the food web (Ayanda *et al*., 2020).

Eichhornia crassipes (Mart.) Solms are widely employed for phytoremediation (Xia and Ma, 2006) and have enormous tendencies to absorb and accumulate heavy metals and toxic pollutants from the water bodies (Jafari, 2010).

This anatomical analysis of hydrophytes is pragmatically essential to understanding the complex morphological, anatomical, and physiological adaptations to survive in aquatic habitats.

Moreover, the present study aims to systematically document and classify hydrophyte species based on their anatomical attributes present in district Bhimber, Azad Kashmir, while also providing valuable perspectives on their evolutionary connections and ecological functions within the indigenous environment.

MATERIALS AND METHODS

Collection of Samples:

The specimens were hydrophytes collected from the marshy areas, ditches, ponds, moist regions, and wet grasslands of the district Bhimber AJK and got authenticated by Dr. Uzma Hanif, Department of Botany, GC University Lahore, Pakistan (Table 1). The specimens were collected and preserved in the fixative solutions as described by Jain and Rao (1977). The species were identified morphologically by using standard methods of flora, dried up and mounted on herbarium sheets. These plants were deposited and got voucher number from Department of Botany, Government College University, Lahore.

Preparation of Reagents:

Fixative Solution:

Leaf tissue was fixed according to the methodology described by Bomblies *et al*. (2008). The Fixative solution was prepared by mixing 500ml 95% ethanol, 100 mL 14% formalin, 50 mL Acetic acid, and the final volume rose up to 1L.

Table 1. Collection of Species from Bhimber AJK

Serial Number	Name of Species	Collection Site	Date of Collection
01	*Nymphaea alba* L.	Bhimber AJK	November 27, 2022
02	*Hydrilla verticillata* (L.f.) Royle	Bhimber AJK	November 27, 2022
03	*Eichhornia crassipes* (Mart.) Solms	Bhimber AJK	November 27, 2022

Preparation of Reagents:

Fixative Solution:

Leaf tissue was fixed according to the methodology described by Bomblies *et al*. (2008). The Fixative solution was prepared by mixing 500 ml 95% ethanol, 100 mL 14% formalin, 50 mL Acetic acid, and the final volume rose up to 1L.

Preparation of Toluidine Blue Stain:

Toluidine blue stain was prepared according to method described by Bergholt *et al*. (2019). 0.5g toluidine blue was dissolved in a small amount of distilled water and then rose the final volume up to 100ml by adding distilled water.

Anatomical Study of Stem:

The following steps were performed to study the anatomical characteristics:

Section cutting procedure:

The section-cutting procedure was performed as described by (Zelko *et al*., 2012).

Toluidine Blue Staining Procedure:

For toluidine blue staining, the following steps were performed according to the method described by Lux *et al*. (2005). The sections were placed in 70% alcohol for approximately 1 minute prior to stain. Then these sections were placed on a glass slide by using a camel hairbrush. The fine sections placed on the glass slide were covered with the coverslips and then observed under the light microscope.

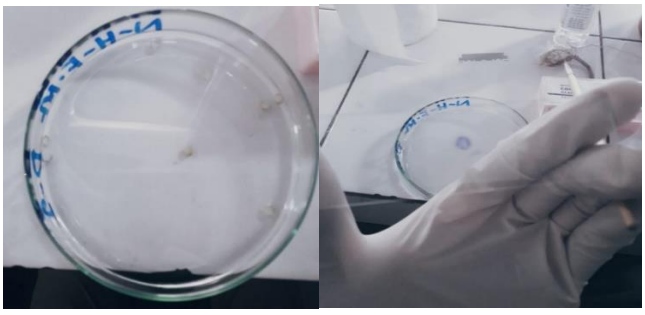

Plate 1: Section cutting and toluidine blue staining

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Micrometry and Microphotography:

Digital photographs were taken by and the measurements of different cells were carried out by using standard methods of micrometry as described by Quesnel (1971).

RESULTS

Anatomical Analysis of the Transverse Sections of Stem of *Nymphaea alba* L.

Stem sections of *Nymphaea alba* L. were examined under the light microscope; a layer of epidermis was clearly observed surrounded by trichomes. The glandular trichomes were profusely surrounding the layer of the epidermis (Plate 2B). Length of the trichomes was observed as 4.50±0.77 (µm) ranging from 3.85 µm to 5.36 µm. However, the width of the trichomes was observed as 2.11±0.48 (µm) ranging from 1.56µm to 2.48µm in different trichomes (Table 2). While studying the *Nymphaea alba* L., aerenchyma was clearly observed. The presence of aerenchyma signified the anatomical adaptation to absorb the excess amount of water in their aquatic habitat (Plate 2B). The length of the aerenchyma was observed as 121.50±47.34 (µm) ranging from 71.03 µm to 164.94µm in different aerenchyma. However, the width of the aerenchyma was observed as 92.76±32.26 (µm) ranging from 70.43µm to 129.76µm in different aerenchyma in the stem of *Nymphaea alba* L. (Table 2). Small vascular bundles were observed in a layer beneath the epidermis (Plate 2B). Length of the small vascular bundles was observed as 7.45±0.31 (µm) ranging from 7.26µm to 7.81µm. However, the width of the small vascular bundles was observed as 92.76±32.26 (µm) ranging from 70.43µm to 129.76µm (Table 4.1). The larger vascular bundle was also observed. The length of the larger vascular bundle was observed as 54.24±5.06 (µm) ranging from 51.16µm to 60.08µm. However, the width of the larger vascular bundle was measured as 59.48±1.29 (µm) ranging from 58.00µm to 60.42µm (Plate 2C, Table 2).

Xylem and Phloem were clearly observed in the larger vascular bundles (Plate 2C). Length of the xylem was measured as 17.91±1.62 (µm) ranging from 16.32µm to 19.56µm. However, the width of the xylem was measured as 6.55±0.79 (µm) ranging from 6.04µm to 7.41µm in different samples of the stem of *Nymphaea alba* L. (Table 2). The phloem was comparatively larger in length and broader in the width than xylem (Figure 2). The length of the phloem was measured as 29.57±1.97 (µm) ranging from 27.62µm to 31.56µm. However, the width of the phloem was observed as 31.47±2.54 (µm) ranging from 29.07µm to 34.14µm in the phloem of different sections of the stem of *Nymphaea alba* L. (Table 2). The length of the collenchyma cells was measured as 40.36±6.67 (µm) ranging from 33.62µm to 46.96µm. The width of the collenchyma cells was measured as 28.85±9.89 (µm) ranging from 17.80µm to 36.88µm (Plate 2A, Table 2).

Anatomical Analysis of the Transverse Sections of the Stem of *Hydrilla verticillata* (L.f.) Royle

In the transverse section of the stem of *Hydrilla verticillata* (L.f.) Royle, a compact arrangement of the cells was observed and larger aerenchyma cells were also observed (Plate 3). However, different cells and tissues such as parenchyma, ground tissues, xylem, and phloem were present and observed using the light microscope at 40X. Collenchyma cells were observed (Plate 3D). Length of the collenchyma cells was measured as 36.93±2.39 (µm) ranging from 34.31µm to 39.00µm. However, the width of the collenchyma cells was measured as 12.94±0.54 (µm) ranging from 12.39µm to 13.48µm (Table 3). Length of the aerenchyma cells was measured as 41.84±8.86 (µm) ranging from 34.29µm to 51.61µm. However, the width of the aerenchyma cells was observed as 29.49±2.1 (µm) ranging from 27.35 µm to 31.67 µm (Plate 3 A, Table 3). Vascular bundles were present in which the xylem and phloem were clearly observed (Plate 3C). Length of the xylem was observed as 21.78±2.16 (µm) ranging from 19.34 µm to 23.45 µm. The width of the xylem was measured as 14.37±0.94 (µm) ranging from 13.45µm to 15.39µm (Table 3). Length of the

phloem was measured as 34.78±2.08 (µm) ranging from 33.14 µm to 37.13 µm. However, the width of the phloem was observed as 13.67±1.00 (µm) ranging from 12.60 µm to 14.58 µm (Plate 3C, Table 3).

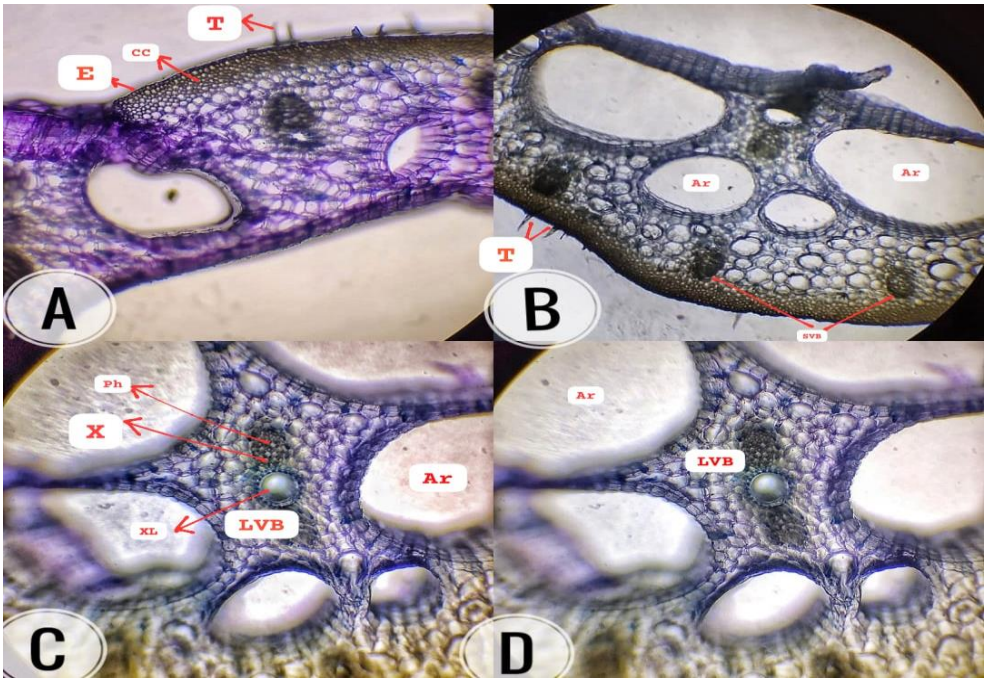

Plate 2: T.S. of the stem of *Nymphaea alba* L. **A.** (**E** epidermis **CC** collenchyma cells **T** trichomes) **B. Ar** aerenchyma **SVB** smaller vascular bundles **T** trichomes) **C. LVB** larger vascular bundles **XL** xylem lacuna **X** xylem **Ph** phloem) **D.** (**Ar** aerenchyma) (40X)

Table 2: Length and width of Different Cells in the T.S. of the stem of *Nymphaea alba* L.

Sr. No.	Parameters	Length (µm)	Range of the length of cells (µm)	Width (µm)	Range of the width of cells (µm)
01	Aerenchyma	121.50±47.34	71.03-164.94	92.76±32.26	70.43-129.76
02	Small vascular bundles	7.45±0.31	7.26-7.81	7.97±1.25	6.71-9.22
03	Collenchyma cells	40.36±6.67	33.62-46.96	28.85±9.89	17.80-36.88
04	Large Vascular Bundle	54.24±5,06	51.16-60.08	59.48±1.29	58.00-60.42
05	Xylem lacuna	21.84±6.59	15.00-28.16	17.77±3.60	14.99-21.84
06	Xylem	17.91±1.62	16.32-19.56	6.55±0.79	6.04-7.41
07	Phloem	29.57±1.97	27.62-31.56	31.47±2.54	29.07-34.14
08	Trichomes	4.50±0.77	3.85-5.36	2.11±0.48	1.56-2.48

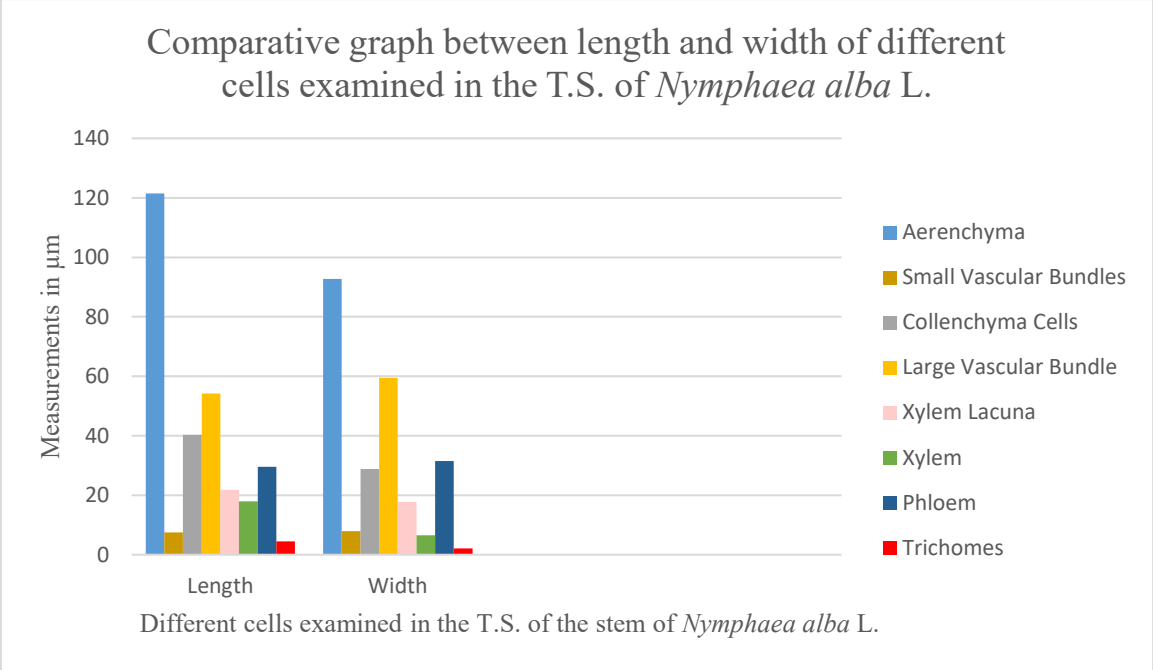

Figure 1: Comparative graph between length and width of different cells examined in the T.S. of the stem of *Nymphaea alba* L.

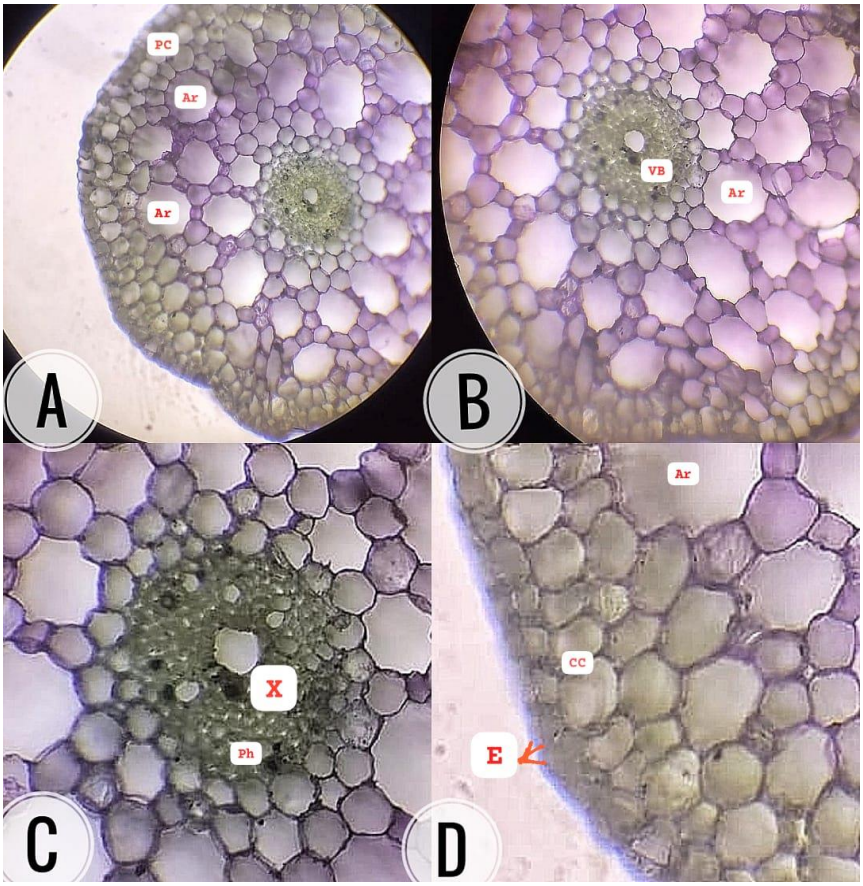

Plate 3: T.S. of the stem of *Hydrilla verticillata* (L.f.) Royle **A.** (**PC** parenchyma cells **Ar** aerenchyma cells) **B.** (**VB** vascular bundle) **C.** (**X** xylem **Ph** phloem) **D.** (**CC** collenchyma cells **E** epidermis cells) (40X)

Table 3: Length and width of different cells examined in the T.S. of *Hydrilla verticillata* (L.f.) Royle

Sr. No.	Parameters	Length (µm)	Range of the length of cells (µm)	Width (µm)	Range of the width of cells (µm)
01	Aerenchyma Cells	41.84±8.86	34.29-51.61	29.49±2.1	27.35-31.67
02	Collenchyma Cells	36.93±2.39	34.31-39.00	12.94±0.54	12.39-13.48
03	Xylem	21.78±2.16	19.34-23.45	14.37±0.94	13.45-15.34
04	Phloem	34.78±2.08	33.14-37.13	13.67±1.00	12.60-14.58

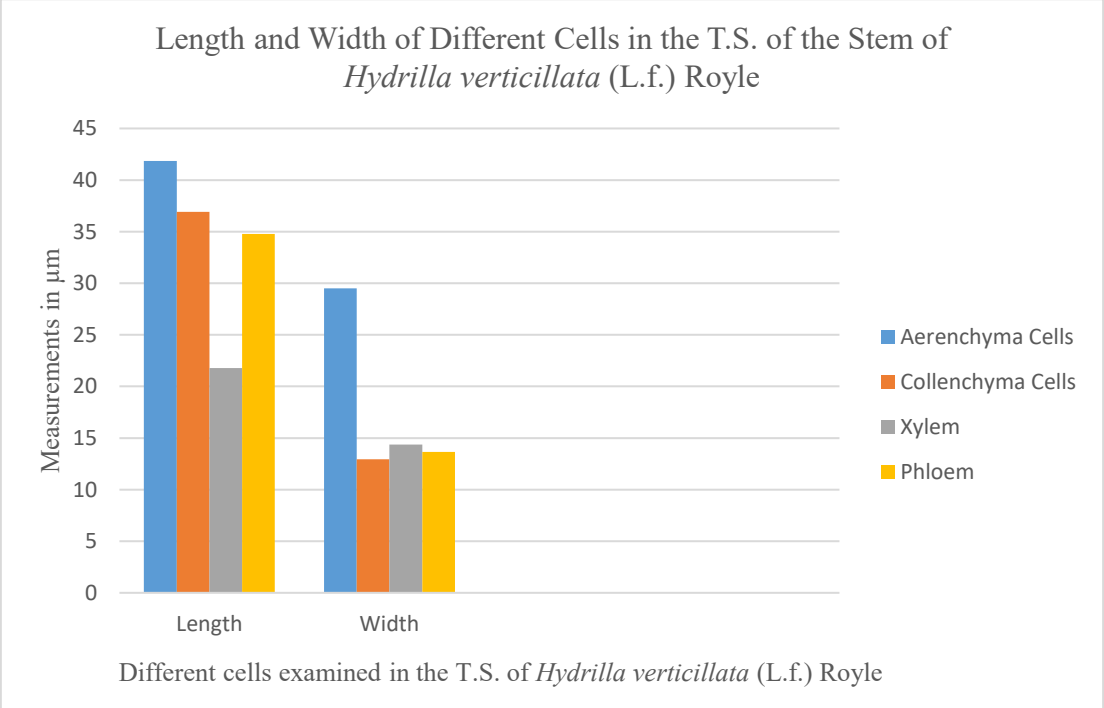

Figure 2: Comparative graph between length and width of different cells examined in the T.S. of the stem of *Hydrilla verticillata* (L.f.) Royle

Anatomical Analysis of the Transverse Sections of the Stem of *Eichhornia crassipes* (Mart.) Solms

The transverse section of the stem of *Eichhornia crassipes* (Mart.) Solms was observed under the light microscope at 40X. Aerenchyma was abundantly present. Vascular bundles were present in which xylem and phloem were observed (Plate 4). The length of the aerenchyma cells was measured as 40.50±1.00 (µm) ranging from 39.58µm to 41.57µm. However, the width of the aerenchyma cells was observed as 28.25±1.48 (µm) ranging from 26.56µm to 29.35µm (Plate 4A, Table 4). Vascular

bundles were present in which the xylem and phloem were clearly observed (Plate 4B). Length of the xylem was observed as 22.98±6.48 (µm) ranging from 16.36µm to 29.32µm. The width of the xylem was measured as 17.36±3.57 (µm) ranging from 13.83µm to 20.98µm. The length of the phloem was measured as 26.74±2.64 (µm) ranging from 23.92µm to 29.17µm. However, the width of the phloem was observed as 7.86±0.71 (µm) ranging from 7.37µm to 8.69µm (Table 4).

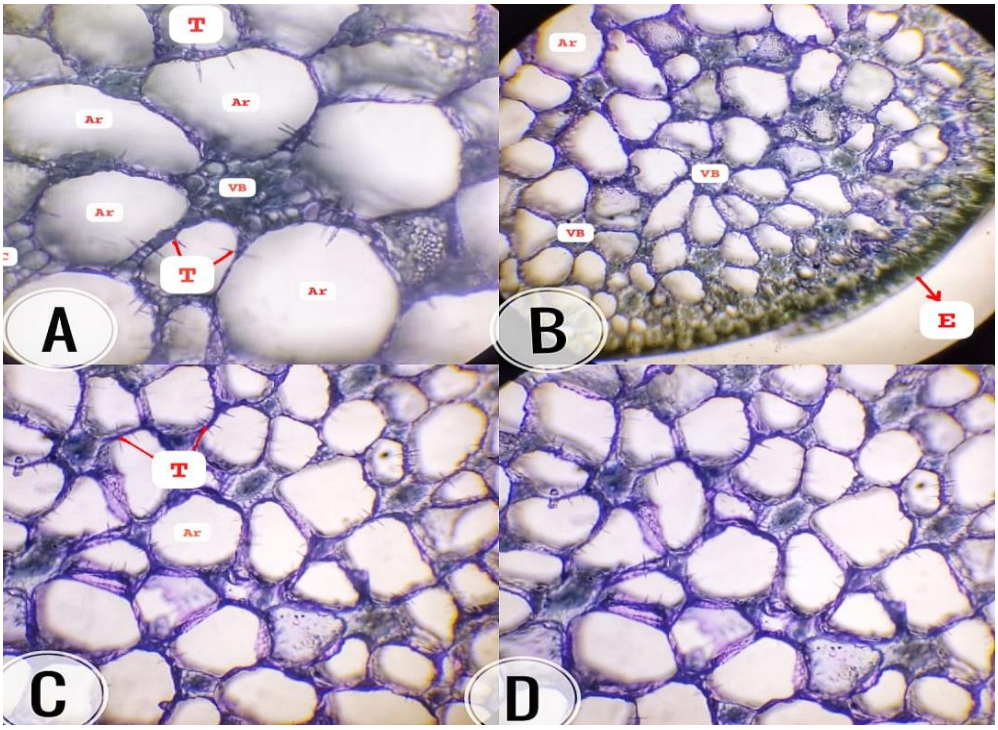

Plate 4: T.S. of the stem of *Eichhornia crassipes* (Mart.) Solms **A.** (**Ar** aerenchyma cells **VB** vascular bundle **T** trichomes) **B.** (**E** epidermis) **C.** (**T** trichomes) (40X)

Table 4: Length and width of different cells examined in the T.S. of *Eichhornia crassipes* (Mart.) Solms

Sr. No.	Parameters	Length (µm)	Range of the length of cells (µm)	Width (µm)	Range of the width of cells (µm)
01	Aerenchyma Cells	40.50±1.00	39.58-41.57	28.25±1.48	26.56-29.35
02	Vascular Bundle	38.27±10.17	32.20-50.02	26.79±1.61	24.93-27.80
03	Xylem	22.98±6.48	16.36-29.32	17.36±3.57	13.83-20.98
04	Phloem	26.74±2.64	23.92-29.17	7.86±0.71	7.37-8.69
05	Trichomes	3.46±1.06	2.35-4.48	1.73±0.20	1.54-1.94

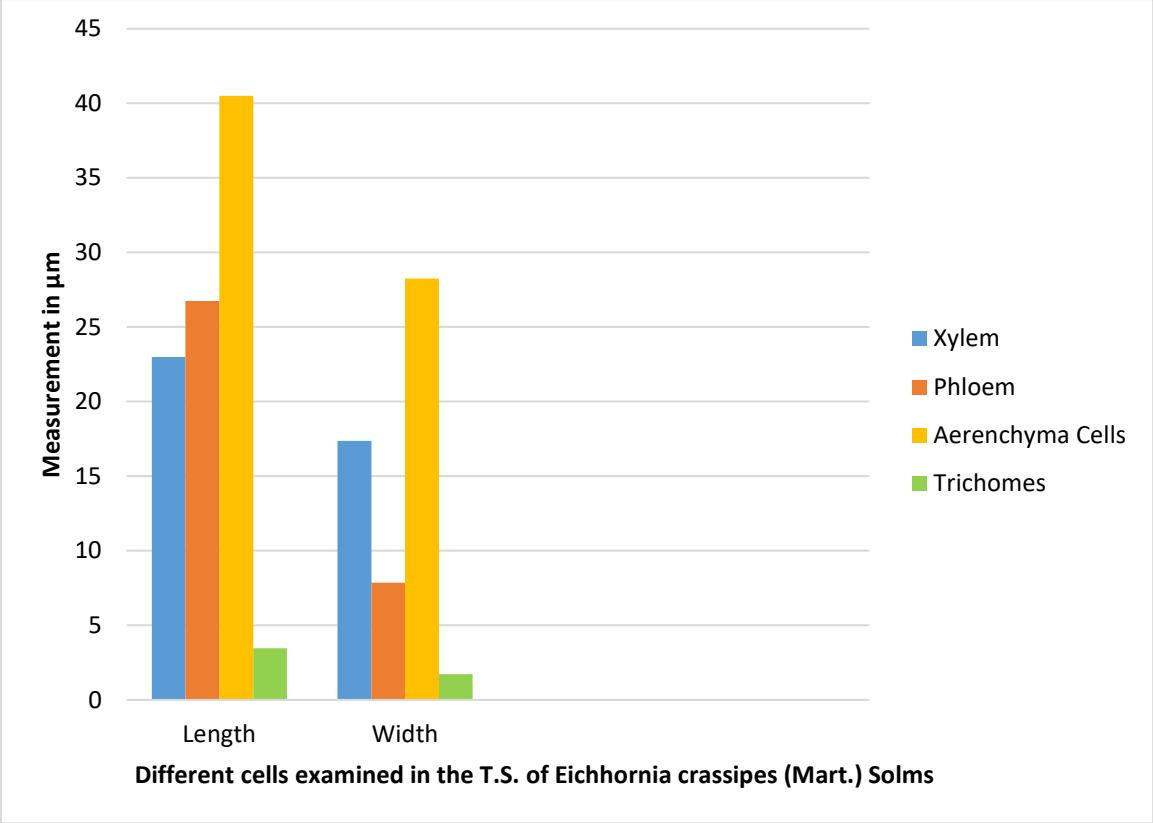

Figure 3: Comparative graph between length and width of different cells examined in the T.S. of the stem of *Eichhornia crassipes* (Mart.) Solms

Table 5: Comparative anatomical analysis of xylem and phloem in *Hydrilla verticillata* (L.f.) Royle, *Eichhornia crassipes* (Mart.) Solms, and *Nymphaea alba* L.

Sr. No.	Name of the Species	Parameters	Length (µm)	Width (µm)
01	*Hydrilla verticillata* (L.f.) Royle	Xylem	21.78±2.16	14.37±0.94
02	*Eichhornia crassipes* (Mart.) Solms	Xylem	22.98±6.48	17.36±3.57
03	*Nymphaea alba* L.	Xylem	17.91±1.62	6.55±0.79
04	*Hydrilla verticillata* (L.f.) Royle	Phloem	34.78±2.08	13.67±1.00
05	*Eichhornia crassipes* (Mart.) Solms	Phloem	26.74±2.64	7.86±0.1
06	*Nymphaea alba* L.	Phloem	29.57±1.97	31.47±2.54

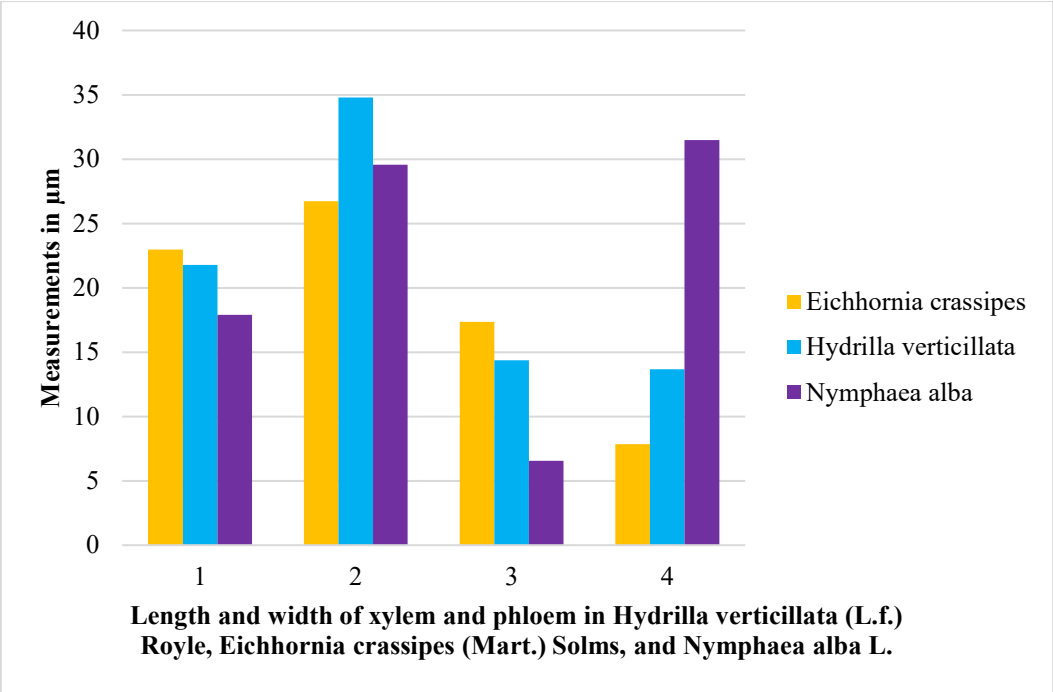

Figure 4: Comparative Anatomical Analysis of Xylem and Phloem in *Hydrilla verticillata* (L.f.) Royle, *Eichhornia crassipes* (Mart.) Solms, and *Nymphaea alba* L.

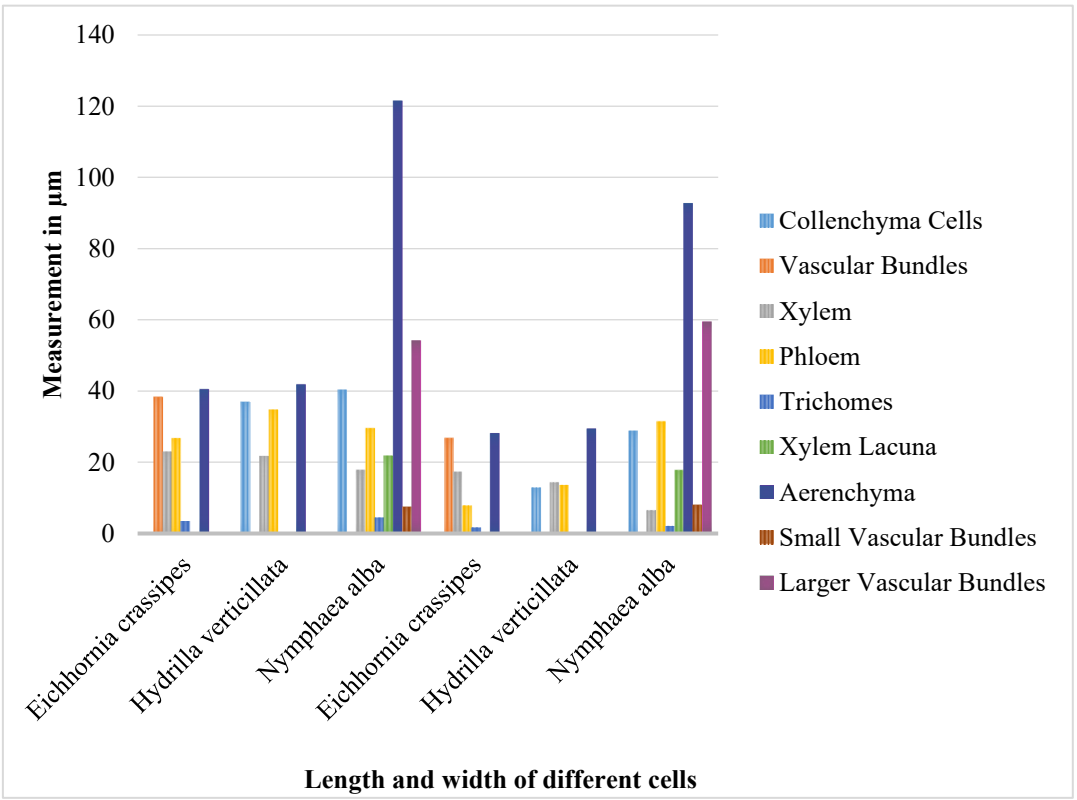

Figure 5: Comparative anatomical analysis (length and width) of different cells examined in the T.S. of *Eichhornia crassipes* (Mart.) Solms, *Hydrilla verticillata* (L.f.) Royle, and *Nymphaea alba* L.

DISCUSSION

Hydrophytes exhibit a wide range of adaptations that facilitate them to grow and thrive within aquatic environments (Tiner, 2006; Rodrigo. 2021). These adaptations encompass a range of anatomical, physiological, and morphological changes that are directed at enhancing their efficiency of nutrition absorption, regulation of buoyancy, photosynthesis, and exchange of gases to effectively respond to the demands posed by aquatic environments (Ronzhina *et al*., 2001). The examination of the anatomical characteristics of various morphological components of hydrophytes facilitates a comprehensive understanding of their adaptive strategies and enables a more thorough and insightful analysis of the mechanisms they employ to thrive in aquatic environments (Jayeola and Folorunso, 2009).

This research work was conducted to collect different hydrophytes of the district Bhimber, Azad Jammu and Kashmir, Pakistan. The focus of the research was to identify and authenticating the taxonomic status of the collected species, based on the anatomical analysis of their morphological parts. In this research, anatomical features of the transverse sections of the stem of *Eichhornia crassipes* (Mart.) Solms, *Nymphaea alba* L., and *Hydrilla verticillata* (L.f.) Royle have been studied by using light microscope (40X). Three hydrophytes, *Eichhornia crassipes* (Mart.) Solms, *Nymphaea alba* L., and *Hydrilla verticillata* (L.f.) Royle, were collected from various aquatic habitats of the district Bhimber, Azad Jammu and Kashmir. The plants were preserved in the fixative solution. Then, they were subjected to the anatomical analyses.

In the stem sections of *Nymphaea alba* L., when examined under the light microscope (40X), a layer of epidermis was clearly observed surrounded by Trichomes. The Trichomes were profusely surrounding the layer of the epidermis. The length of the Trichomes was observed as 4.50±0.77 (µm) ranging from 3.85µm to 5.36µm. However, the width of the Trichomes was observed as 2.11±0.48 (µm) ranging from 1.56µm to 2.48µm. Hanif *et al*. 2017 reported the similar anatomical features in *Eichhornia crassipes* (Mart.) Solms and *Nymphaea rubra*. While studying the *Nymphaea alba* L., aerenchyma were clearly observed, significantly indicating the anatomical adaptation in their aquatic habitats in response of excessive water supply. The length of the aerenchyma was observed as 121.50±47.34 (µm) ranging from 71.03µm to 164.94µm in different aerenchyma. However, the width of the aerenchyma was observed as 92.76±32.26 (µm) ranging from 70.43µm to 129.76µm in different aerenchyma in of the stem of *Nymphaea alba* L. Hanif *et al*. 2017 reported the similar results and suggested the presence of aerenchyma as a main anatomical feature of the hydrophytes to adapt to their ecological habitat. They suggested the presence of six larger aerenchyma surrounding the inner vascular bundle. Jung *et al*. (2008) also identified the anatomical pattern of aerenchyma in hydrophytes.

Xylem and Phloem were clearly observed in the inner vascular bundle. The length of the xylem was measured as 17.91±1.62 (µm) ranging from 16.32µm to 19.56µm. However, the width of the xylem was measured as 6.55±0.79 (µm) ranging from 6.04µm to 7.41µm in different samples of the stem of *Nymphaea alba* L. The phloem was larger in length and broader in the width than xylem. The length of the phloem was measured as 29.57±1.97 (µm) ranging from 27.62µm to 31.56µm. However, the width of the phloem was observed as 31.47±2.54 (µm) ranging from 29.07µm to 34.14µm in the phloem of different sections of the stem of *Nymphaea alba* L. Hanif *et al.* (2017) observed the poorly developed vascular bundles and mainly larger

aerenchyma were observed to adapt to the influx of excessive water in the ponds and aquatic habitats.

In the transverse sections of the stem of *Hydrilla verticillata* (L.f.) Royle, a compact arrangement of the cells was observed. Different cells and tissues such as collenchyma cells, epidermal cells, ground tissues, xylem, and phloem were present and observed using the light microscope (40X). The existence of different sized lacuna, collenchyma cells, parenchyma cells, multicellular trichomes, and distribution of chloroplast in various regions of the stem of hydrophytes were also reported by Jayeola and Folorunso (2009).

The transverse section of the stem of *Eichhornia crassipes* (Mart.) Solms was observed under the light microscope (40X). Aerenchyma cells were prominently observed. Vascular bundles were present in which xylem and phloem were observed. The length of the aerenchyma cells was measured as 40.50±1.00 (µm) ranging from 39.58 µm to 41.57 µm. However, the width of the aerenchyma cells was observed as 28.25±1.48 (µm) ranging from 26.56 µm to 29.35 µm. Similar findings were also observed by Hanif *et al*. (2017) who studied the adaptations of hydrophytes. Larger aerenchyma were reported with the abundant trichome density. Multicellular trichomes were observed. The selected hydrophytes were belonging to different families. However, it was pragmatically observed that, all these hydrophytes were significantly similar in their anatomical features and characteristics to adapt to their ecological environment (i.e. aquatic habitat). These anatomical characteristics are commonly found among aquatic angiosperms and represent and represent adaptations in the aquatic habitats. It was also investigated that similarity in the anatomical features of selected plants of a geographical locality, although belong to different families, is related to the similarity of their habitat (i.e. aquatic habitat) also described by Hanif *et al*. (2017).

This study involves the anatomical characterization of various structures in the transverse sections of the stem of some selected hydrophytes, collected from district Bhimber, Azad Jammu and Kashmir. The anatomical characteristics of these hydrophytes were not investigated before by using microscopic techniques. This thesis provides the evident micrographs of the selected plants along with the measurement of different cells examined in the stem of these plants which will be a significant addition to be studied and utilized as the foundational data base in the regional scientific literature and worldwide.

CONCLUSION

In the present research, anatomical characteristics of the transverse sections of the stem is investigated to comprehend their adaptations to their ecological environment. Larger aerenchyma and multicellular trichomes were observed, indicating that these species (i.e. *Eichhornia crassipes* (Mart.) Solms, *Hydrilla verticillata* (L.f.) Royle, and *Nymphaea alba* L.) were better adapted to their aquatic environment.

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AUTHOR'S CONTRIBUTION

UH and MUF contributed to the study conception and design. Material preparation, sample and data collection and analyses were performed by RA. The first draft of manuscript was written by RA, and all authors commented on previous versions of manuscript. The final draft was written by WA. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

All data presented is primary and included in the manuscript

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CONFLICT OF INTEREST

Authors have no relevant financial or non-financial conflicts of interest to disclose.

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