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Anatomical study of two Hydrophytes – *Pistia stratiotes* L. and *Centella asiatica* (L.) Urban

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ABSTRACT

Anatomical and structural features of two different hydrophytic plant species i.e., *Pistia stratiotes* L. (Araceae) and *C. asiatica* L. Urban Umbelliferae were studied in the present investigation. Samples for this study were collected from Botanical Garden, GCU, Lahore. The research was based on the investigation of the anatomical adaptions in plants living in extreme waterlogged conditions. For that purpose Vegetative parts of plants were studied under scanning electron microscope and light microscope. Single layered epidermal cells were observed on both sides of the leave's epidermis with greater number of stomata on the upper layer. Collenchyma and sclerenchyma, with large intercellular spaces interrupted in the cortical region. Vessels were observed poorly developed and consisted of protoxylem cells only. In the petiole of *C. asiatica* (L.) Urban presence of calcium oxalate crystals was also noted.

Key Words: Hydrophytes, Angiosperms, Anatomy, Tissue types.

INTRODUCTION

Aquatic macrophytes are the plants adapted to live and grow in permanent or periodic water bodies. These plants (growing only in water e.g., ponds, lakes rivers and coasts) are also referred to as Hydrophytes. These plants cannot live without water for any significant period of time (Tinner, 1991). The selected hydrophytes for the present study i.e., *Pistia stratiotes* L. belongs to family Araceae and *Centella asiatica* (L.) Urban to Umbelliferae. (Seevaratnam *et al.,* 2012).

Ecologically, the members of family Araceae are mostly free-floating type of hydrophytes. This family contains 3790 species and 117 genera (Tripathi *et al.,* 2010). This plant is also known as "Water lettuce" (Cook *et al.,* 1974; Holm *et al.,* 1977). *Pistia stratiotes* L. floats on lakes, ponds, stagnant water and in streams. This plant consists of velvety, light-green leaves, which are covered by short hairs that trap the air and produces buoyancy. The leaves are hairy and ridged, without leaf stalks. Long feathery roots hang *Pistia* freely in the water. Inflorescences are inconspicuous (Buzgo, 2006). Although *Pistia stratiotes* L. degrade water quality by blocking the air-water interface, reduce oxygen levels in the water, and thus threatening aquatic life, it has been tested for metal remediation (Odjegba & Fasidi, 2004), metal detoxification (Tewari *et al.,* 2008), and treatment of urban sewage (Zimmels *et al.,* 2006).

Family umbelliferae is commonly known as celery, carrot or parsley family, the family of aromatic plants with hollow stems. This family consists of 3000 species belonging to 300-400 genera. *Centella asiatica* (L.) Urban is a creeping and perennial herb with up to 2m long slender and horizontal prostrate stolon, characterized by long rooting internodes. Leaves arise from each node of the stems, are green in color and fan-shaped (Jamil *et al.,* 2007; Koh *et al.,* 2009). *Centella asiatica* (L.) Urban is used in wound healing, in digestive disorders (Chevallier, 2001) and is effective in treatment of stomach ulcers, diarrhea, syphilis and asthma (Goldstein & Goldstein, 2012). Triterpenoid and saponins, the primary constituents of *Centella asiatica* (L.) Urban are believed to be responsible for its wide therapeutic actions.

The aim of the study is to compare the anatomical structure of the vegetative parts of two hydrophytes viz. *P. stratiotes* L. and *C. asciatica* (L.) Urban, to identify the adaptive variations of these plants in GCU Botanical garden Lahore. The study was also aimed to observe the anatomical characters of the commonly found hydrophytes with a view to inculcate such characters to enhance their identification.

MATERIALS AND METHODS

The types of plant samples were collected from Botanical Garden, GCU, Lahore situated near Mall road,Lahore. These plants were collected from

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two different ponds. *Pistia stratiotes* L. and *Centella asiatica* (L.) Urban were identified in Dr. Sultan Ahmad Herbarium with the help of flora of Pakistan (Stewart, 1972; Nasir, 1975; Qaiser, 1993). To study anatomical features of the samples, sections (T.S.) of stem and petiole were obtained through free hand section cutting. Sections were preserved in the glass vial, half filled with Glycerin. Best sections were selected and dehydrated by passing through the grades of Alcohol 30%, 50%, 70%, 70% and stained with safranin and fast green for the identification of cells. Lower epidermis of leaves were peeled and studied through Light and Scanning Electron Microscope.

RESULTS AND DISCUSSION

Structures of vegetative parts of the both hydrophytes are illustrated in Plate 1&2. Structure of the leaf epidermis (outermost covering) of *Pistia stratiotes* is illustrated in Fig.,1, through LM. The leaf epidermis is generally uniseriate with thin walled cells. It contains numerous minute opening called stomata, which are mainly involved in transpiration. Stomata are surrounded by two guard cells. Guard cells of stomata are of bean shaped and slightly thick walled. Guard sells are surrounded by subsidiary cells. Epidermis shows the presence of numerous multicellular projections called trichomes. Trichomes are the epidermal appendages that may be multicellular or unicellular. In the Fig., 2 Plate 1 transverse section of stem of *Pistia stratiotes* L. is shown. In these sections single layred epidermis was observed. Below epidermis collenchymatous tissue was present. Parenchyma tissue was also thick layered. These parenchyma cells formed the cortex of the stem. Parenchyma is the predominant and well developed tissue in hydrophytes ranging from oval to polygonal shaped cells. In Fig.3, Plate 1 vascular bundles are shown. 10-12 collateral vascular bundles are scattered irregularly amongst the central aerenchymatous tissues. Sieve tubes present in large number. Vessel is the main mechanical tissue observed in *P. stratoites* L. Intercellular spaces are present in the stem of *Pistia*. In Fig.4, Plate 1 stem structure studied under LM showed the presence of sclerieds towards inner side. Some trichomes were also observed in this section that protrude from the surface.

Leaf epidermis of *Centella asciatica* (L.) Urban is illustrated in Fig.1. It is single layered and is protective in function. The leaf epidermis consisted of thin walled cells without appendages. Epidermis bears numerous minute opening called stomata. Stomata are surrounded by two guard cells. Guard cells of stomata are of kidney shape. They help in exchange of respiratory gases in leaves and are responsible for opening and closing of stomata. Sides of walls of guard cells bordering the aperture of stomata are thickened. *C. asiatica* (L.) Urban is a dicot plant, and in dicot plants stomata are mostly found in lower epidermis. Stomata are Anisocytic type. Epidermal cells surrounding the guard cells are called subsidiary cells or accessory cells. They support the movement of guard cells. The shape of cells is polygonal but slightly elongated axially. Anticlinal walls of cells are wavy. Petiole of *Centella asiatica* (L.) Urban shows a characteristic outline microscopically in Fig.2 and 3, under SEM. In this plant the microscopic structure shows that the petiole contains an epidermis which is single layered. The epidermal cells were arranged in compact form. The cells of epidermis were rounded to cubical covered by a thin cuticle. The cortex was much thickened. Collenchyma cells were present. These cells were 2 or 3 layered. A broad zone of more or less rounded parenchyma cells present within intercellular spaces. In Fig. 6, the section that was studied through SEM revealed the presence of calcium oxalate crystals in parenchyma cells. Vascular tissue is a complex tissue and characteristic of vascular plants. The two main components of vascular tissue are the xylem and phloem. The vascular tissues were arranged in long, discrete strands called vascular bundles. These bundles include xylem and phloem, as well as supporting and protective cells. In Fig.7, the petiole of *C.asiatica* (L.) Urban shows the different structures that are studied under SEM and LM. In Fig.8, the micrograph of petiole under LM shows clear vascular bundles. The transverse section of *Centella asiatica* (L.) Urban stem is described in Fig., 5 and 6, Plate 2. In this structure the boundary of wall is more or less concave to convex. Epidermal cells were found. Epidermis was single layered. Epidermis composed of rounded to cubical cells covered by a thin cuticle. Collenchyma cells were found below the epidermal cells. Two or three layered collenchymatous tissue was also observed. These collenchyma cells were elongated and have unevenly thickened walls. Like parenchyma cells, were still alive when mature. A collenchyma cell provides structural support, particularly in growing shoots and leaves. These cells were followed by 6 to 8 layers of thin-walled, isodiametric, parenchymatous cells. Aerenchyma was also studied. Vascular tissues were observed among these lacunea. Mucilaginous cells were also present. Vascular bundles were collateral, open and

arranged in a ring. These bundles were surrounded by patches of sclerenchymatous cells.

The aim of present investigation was to study the anatomical features through SEM at high magnification power and enhance the identification pattern. These hydrophytes belonging to two different families showed some structural similarities (William & Barbers, 1961) pointed out that the structural similarities correlated to their perspective habitat. The common features in both hydrophytes were thick parenchymatous tissue, air chambers or lacunae, procession of chloroplast, thin cuticle and reduction in mechanical tissues. They are found commonly among the aquatic angiosperms. The presence of strengthening tissue in hydrophytes is responsible for mechanical support rather than water conservation. Stomata occur on the adaxial side of floating leaves of angiosperms. Results showed that most floating leaves have prominently chambered mesophyll (Kaul, 1976). In *Pistia stratiotes* epidermis was dense. There is preponderance of parenchyma tissue while other tissues were less developed. Vascular bundles were found in scattered form. In hydrophytes scattered vascular bundles reported by Pan *et al.,* (2011). Pith is sparse. *Centella asiatica* (L.) Urban studied through SEM showed calcium oxalate crystals (Table 1). Large air spaces were observed. Cortex was dense. Epidermis was organized and densely packed. Aerenchyma were present in large number. *Centella asiatica* (L.) Urban had thinner epidermal layers and high calcium increased the thickness of hypodermal layers as stated by Sarma & Mazumder (2011). Well-developed aerenchyma is a major characteristic of aquatic plants. Pattern of well-developed aerenchyma has been reported by Jung *et al*.,(2008). Pattern of distribution of different tissue type is described in Table below.

Table I: Pattern and distribution of tissues of two hydrophytes

Species name and family	Location and distribution pattern		Distribution of chloroplast in the stem		Type of mechanical tissue
	Location	Distribution pattern	Location	Pattern	
Pistia stratiotes L. Araceae	Pith	Sparse and scattered	Epidermis	Dense	Thick parenchyma and sparse vessels
	Vascular bundle	Scattered	-	-	-
	Small air spaces	Scattered	-	-	Sclerenchyma cells
Centella asiatica (L.) Urban Apiaceae	Epidermis	Organized and densely packed	Cortex	Dense	Collenchyma and parenchyma
	Aerenchyma cells	In greater number	-	-	-

CONCLUSION

From the results of the present investigation it can be concluded that hydrophytes have reduction of protection, support and conducting tissues and the presence of air chambers can be clearly observed in *Pistia stratiotes* L. and *Centella asiatica* L. Difference lies in the type and features of the trichomes, sclerieds, stomata and the number of parenchyma cells dividing the air chambers. In *Centella asiatica* (L.) Urban parenchymatous calcium oxalate crystals were present. The vascular system is represented by a number of collateral vascular bundles in Araceae species. Well-developed phloem consisting of phloem parenchyma and sieve tube were present. Lacunae were extremely reduced being represented by a single large central xylem cavity, and in the phloem at different angles mucilaginous cells were embedded.

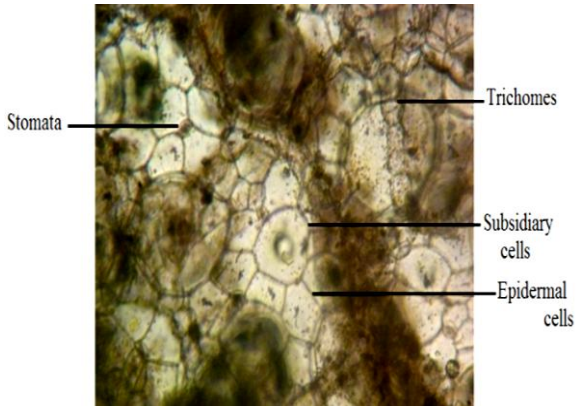

Fig., 1: Micrographs of leaf Epidermis of *C. asiatica*(L.) Urban under SEM

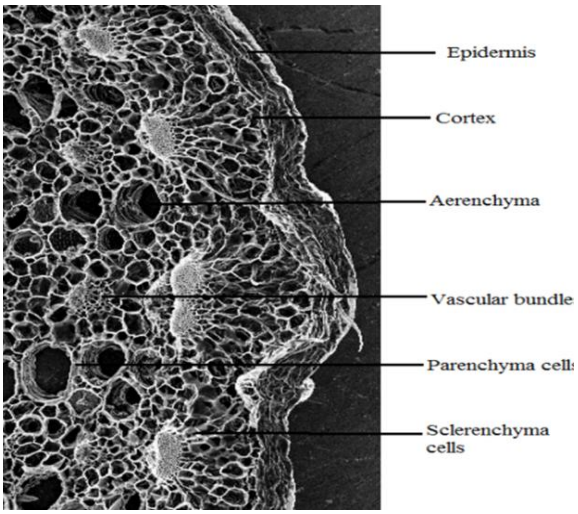

Fig., 2: Micrograph of petiole of *C.asiatica* (L.) Urban under SEM

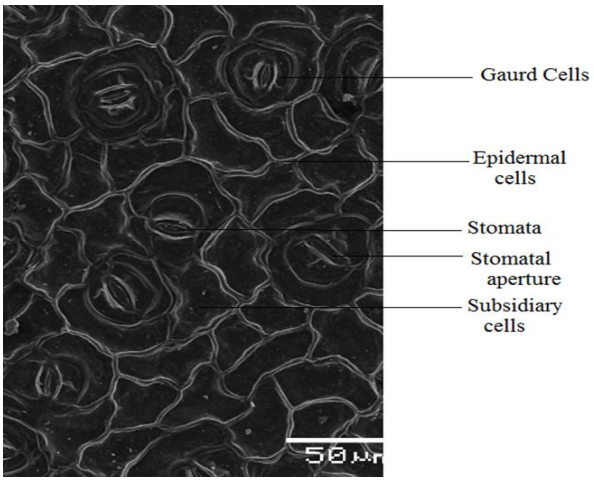

Fig., 5: Micrographs of leaf Epidermis of *C. asiatica*(L.) Urban under SEM

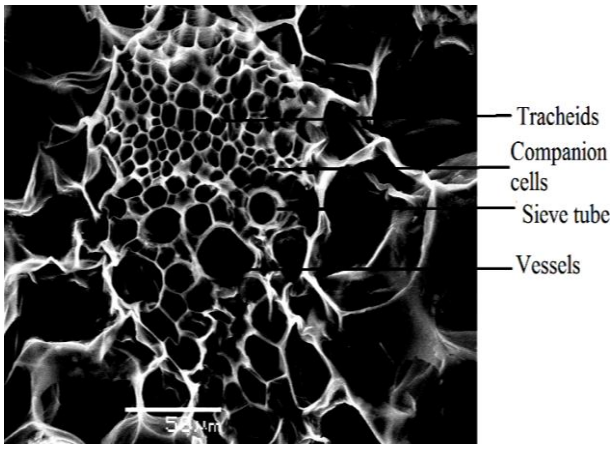

Fig., 3: Micrograph of stem of *P. stratiotes* L. under SEM

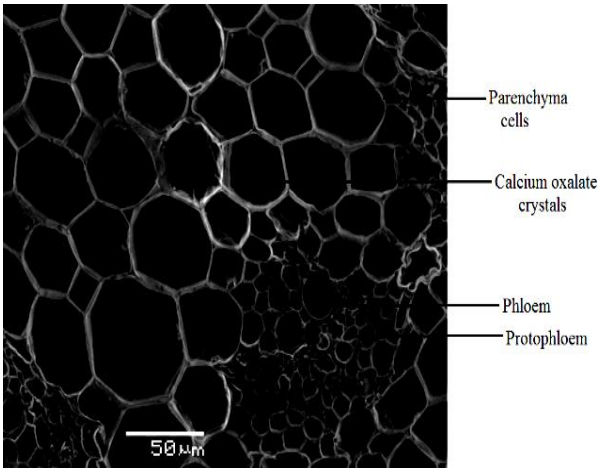

Fig., 6: Micrograph of petiole of *C.asiatica* (L.) Urban under SEM

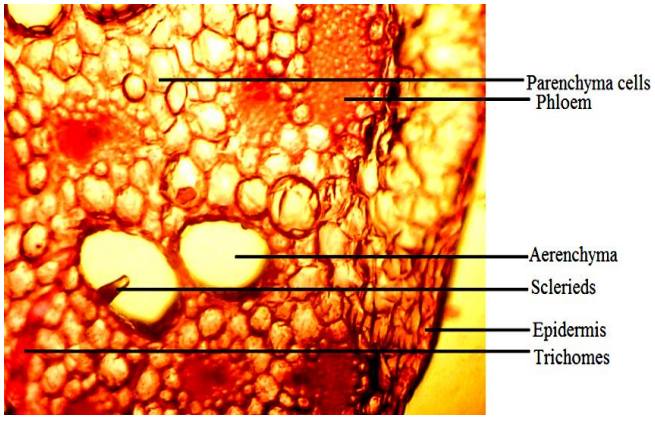

Fig., 4: Micrograph of stem of *P. stratiotes* L. under LM

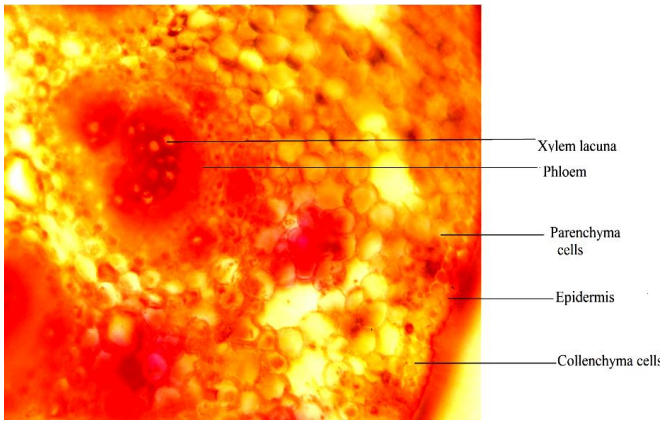

Fig., 7: Micrograph of petiole of *C.asiatica* (L.) Urban under LM

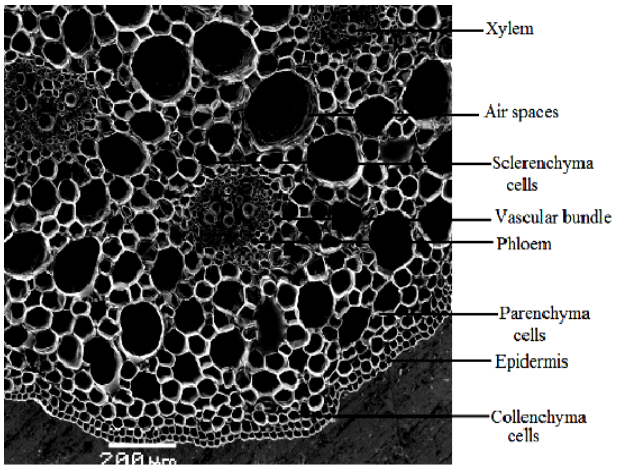

Fig., 8: Micrograph of stem of *C.asiatica*(L.) Urban under SEM

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