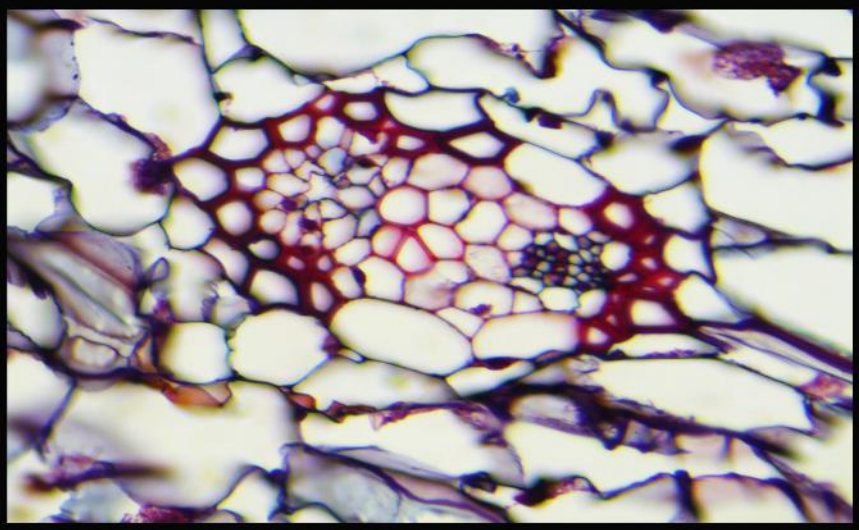
CHAPTER 4
ANATOMY



4.1. INTRODUCTION

Plant anatomy is the branch of botany that deals with anatomical and histological study of plant tissues and cells in order to study more about architecture and working of plants. It is also known as phytotomy. The significance of these studies provides a better comprehension for different phenomenon occurring in plants, their biology, preservation and conservation, as well as give necessary information about their protection from different weather conditions, pathogens and diseases (Cutler et al., 2007).

Plant anatomy plays a vital role in exploring plant biology and taxonomy. In the current scenario plant anatomy along with other branches like systematic, forensics, palaeobotany and pharmacognosy forms an interdisciplinary advanced field which helps to understand many aspects of plant biology and also an essential part of evolutionary biology. The incorporation of anatomical data with gross morphology, pollen, cytology, physiology, chemistry, molecular biology and similar disciplines enables to make revision of plant classification to elucidate phylogenetic relationships in more systematic way (Dosmann and Groover, 2012; Rouhan and Gaudeul, 2014).

An understanding of the biological functions of plants, its growth and development or its evolution, necessitates precise description of their morphology and anatomy at various scales which includes the whole plant, its organs and cells within a tissue, the cell walls, or the organelles within cell. The awareness of the uniqueness, regularities and repetition of characters at different levels of structural patterns leads to compiled data which represent the identity of an individual genus of plants. An anatomical study includes anatomical and histochemical characters of particular parts of plants which were observed by using microscopic and even sub microscopic techniques (Alvin and Boulter, 1974). Conventional microscopy has been usual technique for investigating plant anatomy at the cellular or tissue scale (Dixon et al., 2018). The anatomical characters of the vegetative organs and more specifically of the leaf, are known to undergo for variation based on the habitat of the plant (Fhitsch, 1903).

Anatomical evidence can be useful in systematics in several ways. It can well be exploited taxonomically in the identification of fragmentary material, even a small piece of wood (Sivarajan and Robson, 1991). When morphological characters are not sufficient in the preliminary identification of voucher specimen, anatomical study may prove

significant (Culley, 2013). Anatomical data has proved to be very useful in discriminating evolutionary trends and interrelationships of taxa at and above the species level and at higher taxonomic categories (Taia, 2005). They are useful in determining relationship between various genera, families, orders and other taxonomic categories (Sharma, 2011).

4.2. REVIEW ON ANATOMICAL STUDIES OF ORCHIDS

The identification of plants is very crucial from taxonomical point of view. The anatomical characters are very important tool for identification of various species of plants (Albert et al., 2004; Banerjee et al., 2004). Orchidaceae are one of the most complex family of flowering plant. Most of the times it is very difficult to identify them morphologically during vegetative stage. Hence, anatomical data are very significant for the identification of closely allied genera and species (Williams, 1974). Various anatomists have carried out their work on anatomy of leaf, stem/pseudobulb and root of various species of orchids.

4.2.1. Leaf

Rosso (1966) reported that the undulating walls in leaf are unique feature in most *Cypripedium* species. The comparative study of leaf anatomy within the members of Oncidinae was done by Ayensu and Williams (1972). Atwood and Williams (1978), carried out a work on the species of Genus *Paphiopedilum* and *Phragmipedium* and concluded that epidermal features might be useful for differentiating the species of this genera.

Kaushik (1983) recorded peculiar type of water storage cells with spiral cellulosic thickening in hypodermis of *Coelogyne flaccida* and in ground tissue of *Aerides*, *Bulbophyllum pencillium*, *B. reptans*, *B. triste*, *Cleisostoma*, *Luisia*, *Rhynchostylis* and *Vanda*. Similar type of cells was also observed in *Bulbophyllum andersonii* and *B. dyerianum* by MohanaRao and Khasim (1987c) and in *Dendrobium rotundatum* by Khasim and MohanaRao (1989). The comparative leaf anatomy of four *Dendrobium* species in section Rhizobium were studied by Stern et al. (1994). He also observed water storage cells with banded thickening in the ground tissue. The vegetative anatomical studies in few primitive orchids (three species of *Neuwiedia* two species of *Apostasia* and one unknown species of *Apostasia*) were done by Stern et al. (1993a).

Glandular and non-glandular trichomes are usually accompanied with plicate leaves and bracts (Rosso, 1966). Pridgeon (1981) reported glandular trichomes with absorptive nature in 120 Pleurothallid members. Later, Pridgeon and Stern (1982) observed sunken, glandular trichomes in almost all Pleurothallids members except *Myoxanthus.* Absorbing trichomes have been reported in the members of Coelogyninae except *Pholidota imbricata* by MohanaRao and Khasim (1987a).

The stomatal diversity was studied by Rasmussen (1981) in 26 species of Orchidoideae. Dermal anatomy of few orchids was studied by Mulgaonkar (2005b) from Sahyadri region of Western Ghats. Abraham et al. (2016) studied stomatal types in five epiphytic orchids (*Acampe praemorsa, Aerides ringens, Bulbophyllum sterile, Dendrobium aphyllum* and *Oberonia brachyphylla*) from Central Travancore region and reported anomosytic stomata in all studied species.

Chesselet (1989) had carried out extensive study on the systematic of leaf anatomy and palynology in the subtribe Disinae and Coryciinae. Pridgeon and Chase (1995) investigated 145 species from 37 genera (Diurideae) and two outgroup genera (*Spiranthes* and *Disa*) for morphological, anatomical and systematic significance. Morris et al. (1996) worked on leaf anatomy of 100 species of subtribe Dendrobiinae to check their taxonomical features and phylogenetic importance. The leaf anatomy of Aporum and Rhizobium section of genus *Dendrobium* was studied by Carlsword et al. (1997) followed by their systematics. Vegetative anatomy of different species from subtribes Habenariinae and Orchidinae were examined by Stern (1997a, b). Prete and Miceli (1999) carried out a systematic review on anatomical data of genus *Orchis* which successfully differentiated the species of this genus.

Isolated cuticular membranes of 15 vascular epiphytes including few members of Orchidaceae were studied by Helbsing et al. (2000) which justifies their drought prone habitat. Emphasis on comparative vegetative anatomy and systematics of different species from subtribe Catasetinae and Cymbidieae were done by Stern and Judd (2001, 2002). The study on leaf and stem anatomy of *Nervilia aragona* was carried out by Myint (2002). Yukawa and Stern (2002) examined the comparative vegetative anatomy and systematics of few species of genus *Cymbidium*.

The monophyly among different species of Calypsoeae and Laeliinae were performed by Stern and Carlswood (2008, 2009) using vegetative anatomical studies and systematics. Arevalo et al. (2011) found various xerophytic characters that were well suited in some epiphytic species (*Oncidium abortivum, Epidendrum excisum, Rodriguezia lehmannii, Hirtzia escobarii, Elleanthus oliganthus, Elleanthus purpureus* and *Pleurothallis cordifolia*) for the storage of water. Guan et al. (2011) studied the leaf anatomy and other parameters to check the divergence in leaf trait of *Paphiopedilum* and *Cypripedium*. Noguera-Savelli and Jauregui (2011) emphasized on the comparison of leaf anatomy and phylogenetic relationships of 11 species from Laeliinae.

Vegetative anatomy of 13 species of genera *Neottia, Cephalanthera, Epipactis, Limodorum, Spiranthes, Platanthera, Serapias, Himantoglossum* and *Anacamptis* were studied by Aybeke (2012) to provide additional tool for identification of orchids. Vegetative anatomy of *Acampe papillosa* from north-east India were examined by Sonowal and Baruah (2012). Smidt et al. (2013) reported leaf anatomical and molecular data of *Bulbophyllum* section Micranthae to develop a phylogenetic hypothesis. Andreota et al. (2015) studied the leaf anatomy of few species of orchids from tribe Cranichidinae.

Leaf anatomy of six epiphytic orchid species (*Cleisostoma filiforme, Eria pannea, Oberonia oklongensis, Papilionanthe teres, P. vandarum* and *Schoenorchis gemmata*) were investigated by Angela et al. (2015). Their morphological and anatomical features support the xeromorphic nature of all these species. Yang et al. (2016) found that thick cuticle in leaves and water storage capacity of pseudobulbs is the adaptive water use strategies in *Dendrobium* species. Comparative vegetative anatomy of south Indian *Vanda* (*viz. V. spathulata, V. tessellata, V. testacea* and *V. wightii*) were examined by Kowsalya et al. (2017) to develop dichotomous key for the identification species of *Vanda*. Vegetative anatomical studies of *Epidendrum radicans* by Muthukumar and Shenbagam (2017) reveals that all the characters are helpful to adapt the species in stress condition. The adaptation from drought and relationship with habitat were established by thorough examination of anatomical characters in 2 species of *Dendrobium* (*D. capra* and *D. arcuatum*) by Metusala et al. (2017). Adams et al. (2018) studied the morphological, anatomical and histochemical aspects of *Crepidium acuminatum* for identification and authentication of the species. Muthukumar and Shenbagam (2018)

concluded that vegetative anatomical features of *Bulbophyllum sterile* were helpful in adaption for the species in xeric conditions.

4.2.2. Pseudobulb/Stem

Curtis (1917) had carried out a pioneering work on the pseudobulb of orchids. He observed large, thin-walled cells and spirally marked water storage cells in the pseudobulb of *Bulbophyllum pygmaeum* and *B. tuberculatum*. Olatunji and Nengim (1980) made a critical study on the occurrence of tracheoidal elements in stem and pseudobulb of west African orchids.

The water storage cells with spiral cellulosic thickening were observed in the ground tissue of pseudobulb in *Bulbophyllum pencillium, B. reptans, B. triste*, some *Coelogyne* spp. (Kaushik, 1983) *B. andersonii, B. dyerianum* (MohanaRao and Khasim, 1987b) and *Dendrobium rotundatum* (Khasim and MohanaRao, 1989).

Morris et al. (1996) worked on the stem of 100 species of subtribe Dendrobiinae to check their taxonomical features and phylogenetic importance. Kurzweil et al. (1995) worked on 123 species of orchid belonging to tribe Disease. He observed sclerenchyma cap in the vascular bundle and the degree of dissection of the siphonostele of the tuber (‘polystelic’ or ‘monostelic’ clear differentiation of cortex and ground tissue).

4.2.3. Root

The foremost study on family Orchidaceae was carried out by Link (1824) on the roots of genus *Epidendrum.* The most important findings in his research was specialized multi-layered cells in the epidermis which turns white after drying, but he was unable to explain its origin or function. The presence of secondary thickenings on the walls of velamen tissues in terrestrial and epiphytic orchids was described by Meyen (1837). Later on, Link (1849 – 50) contributed significantly on these secondary thickenings. The term ‘Velamen radicum’ was coined by Schleiden (1849) for the multi-layered epidermis and speculated its application as water and gases absorption. Later few anatomist namely Chatin (1856), Schacht (1856), Fockens (1857), Oudemans (1861), Leitgeb (1864), Zankowski et al. (1987) and Porembski and Barthlott (1988) were carried out their work on root anatomy. Dycus and Knudson (1957) observed absorptive region in velamen root which is impermeable to water and certain solutes. Sanford and Adanlawo (1973) worked

on velamen and exodermis of West African epiphytic species and proposed the term 'epivelamen' for outermost multiseriate velamen. Noel (1974) observed helical thickenings in velamen cells of *Ansellia gigantean.* The root anatomy of nine species of orchids were studied by Oliveira and Sajo (1999). They reported presence of a multiseriated velamen, a parenchymatous cortex and endodermis.

Meinecke (1894), Pfitzer and Kraenzlin (1907) and Sprenger (1904) observed suberized and lignified fibrous bodies along with rod like structures in roots of few orchid genera. Later these types of bodies were also observed in *Dendrochilum* by Pfitzer & Kraenzlin (1907) and *Bulbophyllum*, *Cirrhopetalum* and *Megaclinium* by Sprenger (1904). The SEM studies on fibrous bodies of *Sobralia macrantha* reveals these fibrous bodies may work as protective plugs and barriers in transpiration (Benzing et al., 1982). These bodies were named as 'tilosomes' by Pridgeon et al. (1983).

Solereder and Meyer (1930) observed branched root hairs in the root of *Chysis bractescens* and *Haemaria discolour*.

Cheadle (1942) reported vessel elements from roots and tracheids from stems and leaves of some genera. Later, Olatunji and Nengim (1980) made a critical study on the occurrence of tracheoidal elements in root, stem and pseudobulb of 88 species of west African orchids. The advancement in vessel element from terrestrial to epiphytic orchids was investigated by Carlquist & Schneider (2006).

Stern et al. (1993b) examined the cortical root cells of *Pelexia adnata*: a species from Spiranthoideae in search of specialised amyloplasts. Morpho anatomical characterization of vegetative organs of *Miltonia regnelli* and their usefulness were examined by Dettke et al. (2008) to check their flourishment in epiphytic environment. Moreira and Isaias (2008) performed the comparative root anatomy of epiphytic and terrestrial orchids and concluded that these characters might be helpful to achieve various adaptations suitable for their growth.

Root anatomy of 12 species of Catasetinae was carried out by Pedroso-de-Moraes et al. (2012). Barretta-Dos-Santos et al. (2015) examined the root of *Galeandra leptoceras* and described adaptations related to hydric relations and characters of taxonomic interest. Piazza and Smidt (2015) investigated the comparative vegetative

anatomical studies of *Bulbophyllum* section *Didactyle* and *B.* section *Xiphizusa*. Silva et al. (2015) mentioned the root anatomy of eight species of genus *Catasetum* for vegetative identification between them as well as verified their adaptation related to habit using anatomical features. Description of morphological and anatomical characteristics along with mycotrophy in root and rhizomes of *Eulophia epidendraea* and *Malaxis acuminata* were studied by Uma et al. (2015). The study on morphology, anatomy and mycorrhizal relation in the roots of few orchids namely *Ascochilus emarginatus, Taeniophyllum biocellatum,* and *Thrixspermum subulatum* from Sempu Island, Indonesia were carried out by Nurfadilah et al. (2016).

In the present study comparative leaf anatomy was carried out in both epiphytic and terrestrial species. It includes comparative studies of the structure and arrangement of cell layers like cuticle, epidermis, mesophyll, vascular tissues etc. that could be helpful for identification key aiding to morpho-taxonomical characters.

4.3. MATERIALS AND METHOD

4.3.1. Sample collection

1 – 2 mature and fresh leaf samples were collected from different locations and durations as mentioned in section 2.3.3 for both epiphytic and terrestrial species.

4.3.2. Killing and Fixation

Samples were cleaned and washed properly to remove dirt and impurities. Immediately after cleaning, sample were fixed in FAA (Formaldehye: acetic acid: ethanol 10: 5: 50, v/v/v) solution for preservation (Berlyn and Miksche 1976). After 72 hours of fixation they were transferred to 70% alcohol for further processing and storage.

4.3.3. Microtomy and Staining

The fixed leaf samples were dehydrated with a graded series of TBA and processed for paraffin embedding (Johansen, 1940). The blocks were trimmed and fixed on wooden blocks for microtomy. Transverse section (T.S.) of 15 – 20 µm thickness was taken by using Leica rotary microtome. The staining process was carried out with Safranin-Astra blue stain combination and dehydrated through Ethanol-Xylene series and mounted with DPX for permanent slide. Slides were observed under Leica DM 1200 Microscope.

4.4. RESULTS AND DISCUSSION

All the orchids were successfully processed and studied for their anatomical features except *Zeuxine strateumatica* in which cells were ruptured and sections were not proper due to the delicate tissue. The comparative study of the structure and arrangement of different cell layers like cuticle, epidermis, hypodermis, mesophyll, vascular tissues etc. were described here in detail.

4.4.1. General Leaf Anatomy of Orchidaceae

Cuticle is usually smooth and often ornamented in some of the taxa. The thickness of cuticle varies in every genus which provides impenetrable wall for the prevention of water loss. Cuticle is followed by epidermis, hypodermis, ground tissues and vasculature.

Stomata are hypostomatic (restricted to abaxial surface) in most of the genera, however, they are amphistomatic (present on both the surface) in some genera (*Oberonia* and *Vanda*). Cuticular horn like projection on stomata with sub-stomatal cavity is the most common feature observed in all the species. Some epiphytic genera (e.g. *Aerides* and *Vanda*) have sunken stomata where as in some genera (*Dendrobium*, *Habenaria*, *Nervilia* etc.) they are present superficially on the epidermis.

Epidermis is single layered with either thin or thick-walled cells. The epidermal cells are usually rectangular, barrel or polygonal in shape with straight or undulating anticlinal walls (shown with description of individual genera). It provides support as well as help in maintaining the turgor pressure of the cell.

The epidermal cells are usually larger on adaxial surface than abaxial however, *Habenaria* is an exceptional case in which the adaxial epidermal cells are highly elongated and occupies one-half of the leaf volume (Solereder and Meyer, 1930; Stern, 1997a). These cells are more likely to have the function of water storage. (Möbius, 1887; Metzler, 1924; Chesselet, 1989), although there is no scientific data available till date to support the claim. The size of epidermal cells varies in the midrib region and in some cases the larger cells on the adaxial surface are said to perform osmoregulatory function and serve as lamina expansion cells.

The layer immediately subtending the epidermis is hypodermis. Sometimes, the hypodermis is misinterpreted as multi-layered epidermis. It is absent in the leaves of

Orchidoideae but present in several epiphytic members of Epidendroideae (Stern and Carlsward 2008). These epiphytic species have 1 – 3 layers of hypodermis either restricted to one of the surface or both the surface. However, in most of the species, hypodermis is restricted towards adaxial surface. Hypodermal cells are either parenchymatous and/or sclerenchymatous, continuous or as interrupted layers. Parenchymatous layers certainly function in water storage and sclerenchyma fibres provides the mechanical support.

Mesophyll layer comprises thin walled, irregular size, homogenous (no differentiation in palisade and spongy tissues) also referred as isobilateral parenchyma cells. The main function of mesophyll is in photosynthesis using chloroplast from chlorenchyma cells while spongy cells lacking chloroplast provides the storage of water and starch. The cells storing starch are called as assimilatory cells.

Vascular bundles are conjoint, collateral and closed in all the members of Orchidaceae and usually arranged in a single series; however, in some species they are arranged in two rows (e.g. *Oberonia*). Some of the Epidendroideae members shows well developed phloem sclerenchyma as compared to xylem sclerenchyma, but both are absent in most of the Orchidoideae (*Habenaria* and *Peristylus*) (Möbius, 1887; Solereder and Meyer, 1930; Stern, 1997a, b) and some Epidendroideae members (*Crepidium mackinnonii*, *Dendrobium microbulbon, D. peguanum*) (Solereder and Meyer, 1930). A single or double layered sclerenchyma 'bridge' often separates xylem and phloem.

Cluster of starch granules and bundles of raphides are the most common cellular inclusions. Mucilage is much more common in orchid stems and roots, especially tubers in Orchideae, but Möbius (1887) reported cells with a mucilage matrix in leaves of *Aerides* and *Rhynchostylis* (Aeridinae), in either chlorenchyma, hypodermis, or spirally thickened idioblasts. Accumulation of tannin have been observed in the epidermis and mesophyll of many unrelated taxa (e.g. Malte, 1902; Zörnig, 1903; Solereder and Meyer, 1930; Kurzweil et al., 1995) reported oil droplets (*Elaio sphären*) in several species of Epidendreae, Cymbidieae, Dendrobieae and Vandeae.

4.4.2. Leaf anatomy of Orchids

The orchids collected from various regions of Gujarat are mainly belongs to the subfamily Epidendroideae and Orchidoideae. In this section genera of both the subfamily arranged alphabetically in their respective subfamilies. The description includes detailed anatomical features of all the studied species belonging to particular genera.

4.4.2.1. Epidendroideae

It is the largest subfamily among all the five subfamilies, comprised of approximately 21,160 species. Most of them are epiphytic, some are terrestrial, lithophytic herbs or rarely scrambling climbers. Their growth patterns are either sympodial or monopodial, with short to long rhizomes; plants rarely heteromycotrophic (symbiotic relationship of plant rhizome with that of fungi), achlorophyllous (chlorophyll is absent).

This section includes leaf anatomy of eight genera belonging to four tribe namely Nervilieae (*Nervilia*), Malaxideae (*Crepidium*, *Dendrobium* and *Oberonia*), Cymbidieae (*Eulophia* and *Geodorum*) and Vandeae (*Acampe*, *Aerides*, *Rhynchostylis* and *Vanda*).

4.4.2.1.1. Acampe and Rhynchostylis

In *A. praemorsa* and *R. retusa* leaves are isobilateral where in there is no differentiation between palisade and spongy layer. The epidermis is single layered which encloses the hypodermis and ground tissue. The outermost protective layer i.e. cuticle, is thick and smooth and present on both the abaxial and adaxial surface of leaves (Figure 4.1a). The stomata are restricted to lower epidermis in both the genus with pronounced cuticular projections and elongated sub-stomatic chamber (Figure 4.1b). The epidermal cells are spherical to polygonal in shape having thick deposition of lignin on the epidermal walls of both the surface. Hypodermis is also a single layered, similar to that of epidermis on both adaxial and abaxial side, with relatively thick lignified walled cells. In *Acampe* the mesophyll cells are irregular in size with large and small cells intermixed while *Rhynchostylis* shows slight dorsiventral nature having upper mesophyll cells more or less elongated and lower cells more of isodiametric in nature. The walls of mesophyll cells are undulating (Figure 4.1c) in *Rhynchostylis* while no such undulating cells are observed in *Acampe*. The intercellular spaces are few in number and small in size in both the genera. Large number of water storage and assimilatory cells are present in the

mesophyll layer of both genus. Some cells in the mesophyll layer also contain tannin droplets. The vascular bundles are conjoint, collateral and closed. The median vascular bundle present in the midrib region is larger in size as compared to other subsidiary bundles. The phloem sclerenchyma is thick walled, highly lignified and arranged in 5 – 6 layers in both the species wherein, xylem sclerenchyma is comparatively thin walled and 7 – 8 of layers in *Acampe* and they are thick walled and 3 – 4 of layers in *Rhynchostylis*. (Figure 4.1d). Xylem in both the species is composed of narrow lumened vessels and fibres while phloem consists of parenchyma cells, sieve elements and companion cells.

Abraham et al. (2016) and Sonowal and Baruah (2012) observed hypostomatic condition in *Acampe praemorsa* leaf which is similar to our findings. The observations of Vattakandy et al. (2014) shows amphistomatic condition in *A. rigida* and *R. retusa* which is contradictory to our findings. Other anatomical features observed by Vattakandy et al. (2014) in *R. retusa* and Sonowal and Baruah (2012) in *A. papillosa* (Syn. *A. praemorsa*) are in good agreement with ours results.

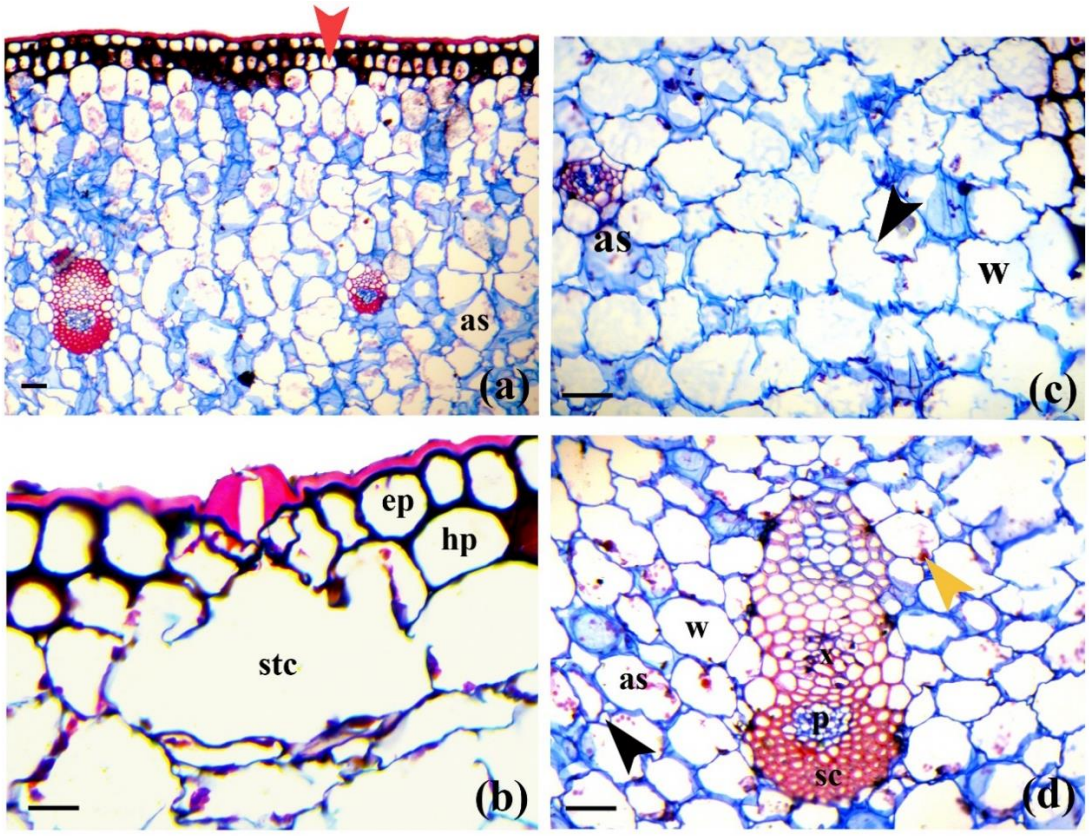


Figure 4.1: *Acampe* and *Rhynchostylis* leaf. Scale bars = 20 µm. **(a)** T.S. of *R. retusa*

showing epidermis (ep), hypodermis (hp) (red arrowhead) and vascular bundles; **(b)** T.S. of *R. retusa* showing thick cuticle, cuticular projection of stomata and stomatal cavity (stc); **(c)** T.S. of *R. retusa* showing undulating walls in mesophyll cells (black arrow head); **(d)** T.S. of *A. praemorsa* showing midvein vascular bundle with phloem sclerenchyma cap (sc), intercellular space (black arrowhead) and tannin droplets (yellow arrowhead). assimilatory cell (as), water storage cell (w), xylem (x), phloem (p).

4.4.2.1.2. Aerides

In both the species of genus *Aerides* that is *A. maculosa* and *A. ringens*, cuticle is thick, smooth and undulating, encircling the epidermal cells of both adaxial and abaxial surfaces. The stomata are sunken with horn like cuticular projection and are present on both the surface (Figure 4.2a). Single layered epidermis comprises of thin walled, oval to polygonal cells on both abaxial and adaxial side of the leaf. Hypodermis is absent. Beneath the epidermis, a multi-layered, oval to elongated shaped, ground mass of parenchymatous cells are present. Intercellular spaces are not prominent. Some of the parenchyma cells showed deposition/accumulation of raphide bundles (Figure 4.2b) and tannin droplets (Figure 4.2c and d) in both the species. *A. maculosa* shows presence of mucilage cell (Figure 4.2c). The vascular bundle in both the species are conjoint, collateral and closed type. The xylem and phloem derivatives are surrounded by thick walled sclerenchyma fibre. In *A. ringens*, sclerenchyma fibres form a cap on both abaxial and adaxial side of the vascular bundle whereas in *A. maculosa* it is only at abaxial side (Figure 4.2c and d). The phloem sclerenchyma is 7 – 8 layered in *A. ringens* while in *A. maculosa* it is 1 – 2 layered. The phloem derivatives are completely encircled by sclerenchyma cells and fibres in *A. ringens*. The distribution of phloem sclerenchyma cells differs on both the sides. These cells form a cap of 7 – 8 layers on the outer side of phloem (towards epidermis) while 1 – 2 layers on the inner side (towards xylary elements). In *A. ringens*, thick walled sclerenchyma cells form a band of 4 – 5 layers below the fibrous cap on the adaxial side. However, in *A. maculosa* they are thin walled, unlignified and irregular shaped with undulating walls above the xylary elements.

Available literature indicates that there are no systematic anatomical studies carried out till date on above described species of *Aerides*. Only few information is available on the dermal anatomy of some species of *Aerides* (Mulgaonkar 2005a; Abraham et al., 2016). The amphistomatic condition reported in *A. ringens* and *A.*

maculosa by Mulgaonkar (2005a) is similar to our observation. However, hypostomatic condition is reported in *A. ringens* by Abraham et al. (2016).

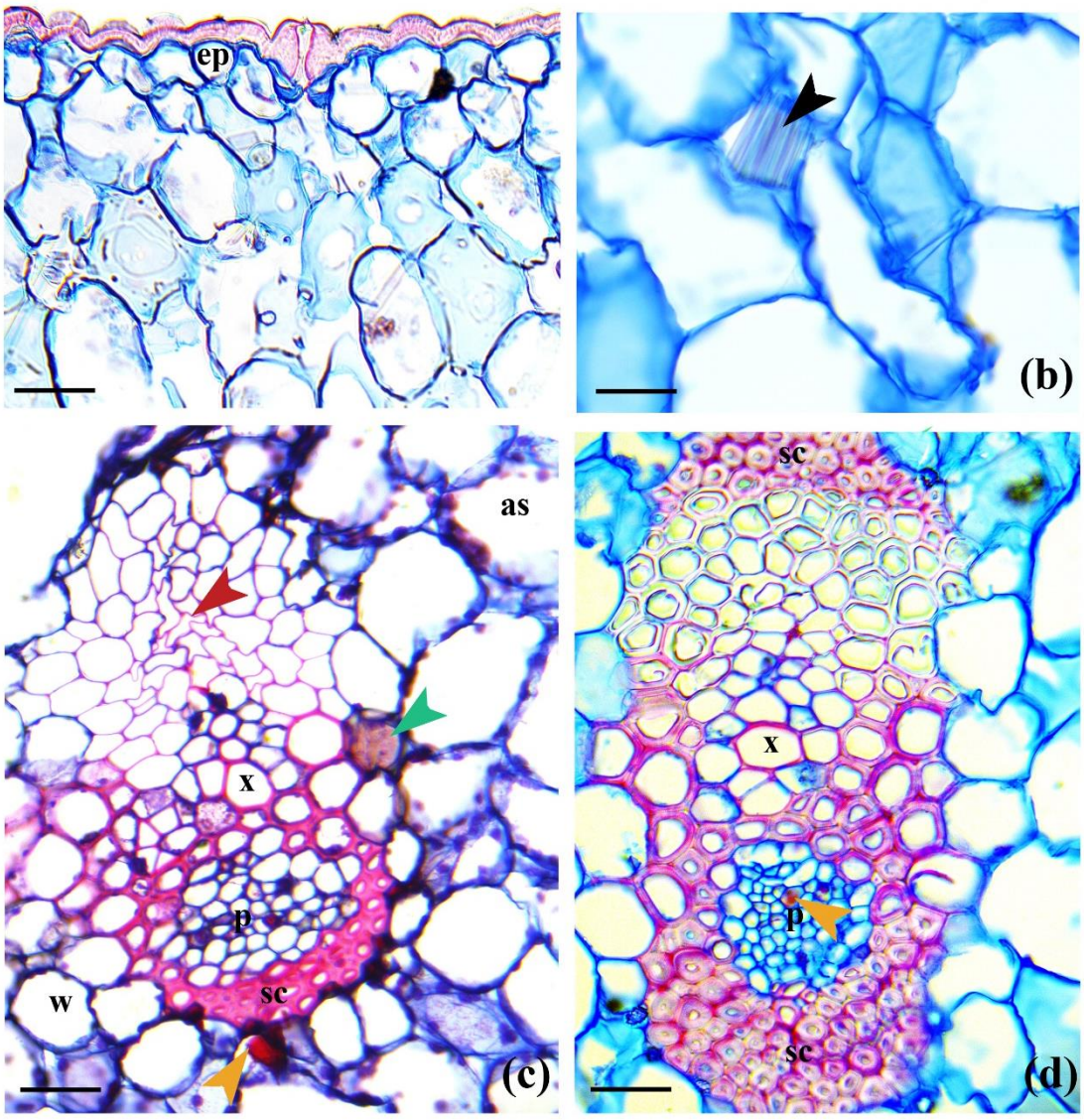


Figure 4.2: *Aerides* leaf. Scale bars = 20 µm. **(a)** T.S. of *A. ringens* showing thick curvilinear cuticle, stomata with cuticular projection and epidermis (ep); **(b)** T.S. of *A. maculosa* showing raphide bundle in mesophyll layer (black arrowhead); **(c)** T.S. of *A. maculosa* showing vascular bundle with phloem sclerenchyma and undulated parenchyma cells above the xylem elements (red arrowhead), tannin droplets (orange arrowhead) and mucilage cell (green arrowhead); **(d)** T.S. of *A. ringens* showing vascular bundle surrounded by sclerenchyma cap (sc) on both the poles and tannin droplet (orange arrowhead). assimilatory cell (as), water storage cell (w), xylem (x), phloem (p).

4.4.2.1.3. Crepidium

The cuticle is smooth and thin in *C. mackinnonii*. Stomata are present superficially on the abaxial side of lamina. Epidermis is single layered comprising thin walled, elongated, rectangular to polygonal shaped cells with curvilinear walls on the abaxial surface (Figure 4.3a). Hypodermis is absent. Mesophyll cells are homogenous, large and irregular in shape, loosely arranged with prominent intercellular space. Some of the parenchyma cells in the mesophyll tissue contains starch grains. Raphides are absent. Vascular bundles are poorly differentiated and shows absence of sclerenchymatous cells in this species. The midvein vascular bundle is slightly larger than the subsidiary bundles. Above the xylary elements narrow, elongated cells with undulating walls are present (Figure 4.3b).

In *C. mackinnonii*, the cuticle is thin and uniform while it is moderately thick and wavy in *C. acuminatum*. The vascular bundles are without fibre cap in *C. mackinnonii* while it is restricted to the abaxial side in *C. acuminatum* (Adams et al., 2018). *C. mackinnonii* is used as a substitute of *C. acuminatum* which is one of the plant in Ashtavarga. Therefore, presence of fibre cap can be used as a crucial character to distinguish both the species. Other anatomical features of *C. mackinnonii* in the current study remains similar to those reported by Adams et al. (2018) in *C. acuminatum*. In genus *Crepidium*, most prominent characters reported are epidermal hairs, sunken stomata and reticulately thickened water storage cells. However, such features were not observed in *C. mackinnonii*. Moreover, Adams et al. (2018) also reported rod shaped bacterial cells in the mesophyll cells which are absent in our sections.

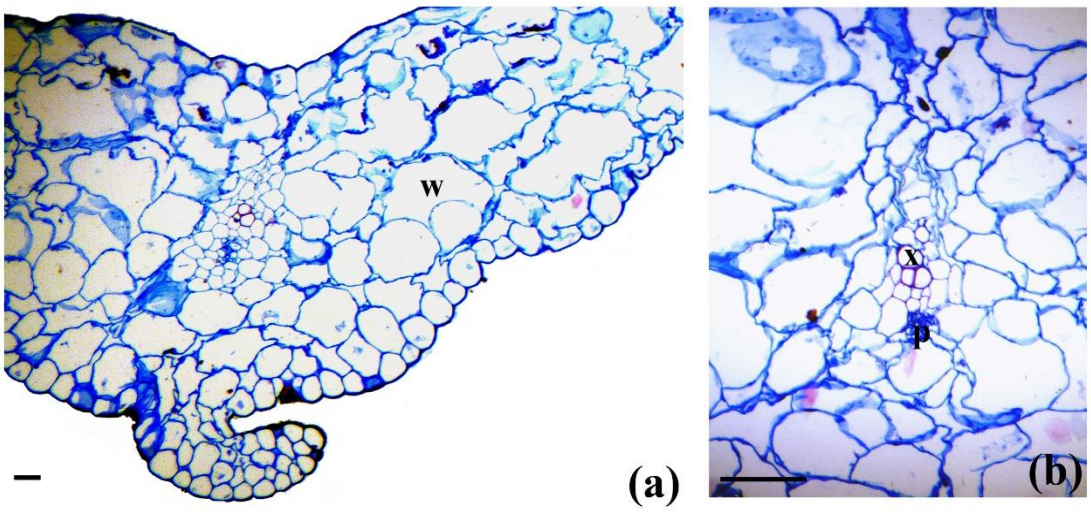


Figure 4.3: *Crepidium* leaf. Scale bars = 20 µm. **(a)** T.S. of *C. mackinnonii* leaf showing abaxial epidermal cells with curvilinear walls; **(b)** T.S. of *C. mackinnonii* showing poorly differentiated vascular bundle. water storage cell (w), xylem (x), phloem (p).

4.4.2.1.4. Dendrobium

Cuticle is smooth and thin to moderately thick in all the species of *Dendrobium* (*D. barbatulum*, *D. microbulbon*, *D. ovatum*) except *D. peguanum.* It is irregularly verrucose on the abaxial surface in *D. peguanum* (Figure 4.4a). The stomata are restricted to abaxial epidermis with small cuticular projection and sub-stomatic chamber (Figure 4.4b). In *D. barbatulum, D. peguanum* and *D. microbulbon* epidermis is of single layered and the epidermal cells on the adaxial side are larger while in *D. ovatum,* there is no variation in the size of epidermal cells. In *D. barbatulum,* the anticlinal walls of adaxial epidermis are undulated, while no such undulating walls are observed in other species. The shape of epidermal cells varies from square, rectangular to hexagonal in all four species. Hypodermis is absent. Beneath the epidermis, multi-layered mesophyll cells are present which are of irregular shape in *D. barbatulum* and *D. microbulbon* while they are isodiametric in *D. ovatum* and *D. peguanum*. Intercellular spaces are small in size and few in number in all the species. Moreover, in *D. peguanum* they are large and more prominent. Raphide bundles, starch grains and tannin droplets are present in few of the mesophyll cells (Figure 4.4c). The median vascular bundle is comparatively larger than the subsidiary bundles in *D. barbatulum* and *D. ovatum* while there is no major difference in their sizes in remaining two species. The vascular bundle is lined/encircled by lignified sclerenchyma cells on both the side in *D. barbatulum* and *D. ovatum* wherein, xylem sclerenchyma forms a band of 2 – 3 layers and phloem sclerenchyma are of 4 – 6 layered (Figure 4.4d). In contrast, sclerenchymatous cap are absent in *D. microbulbon* and *D. peguanum* (Figure 4.4e).

This is the first anatomical comparative data on the four selective species of *Dendrobium.* Out of four species, *D. microbulbon* was described by Ramesh (2014), which is in good agreement with our observations.

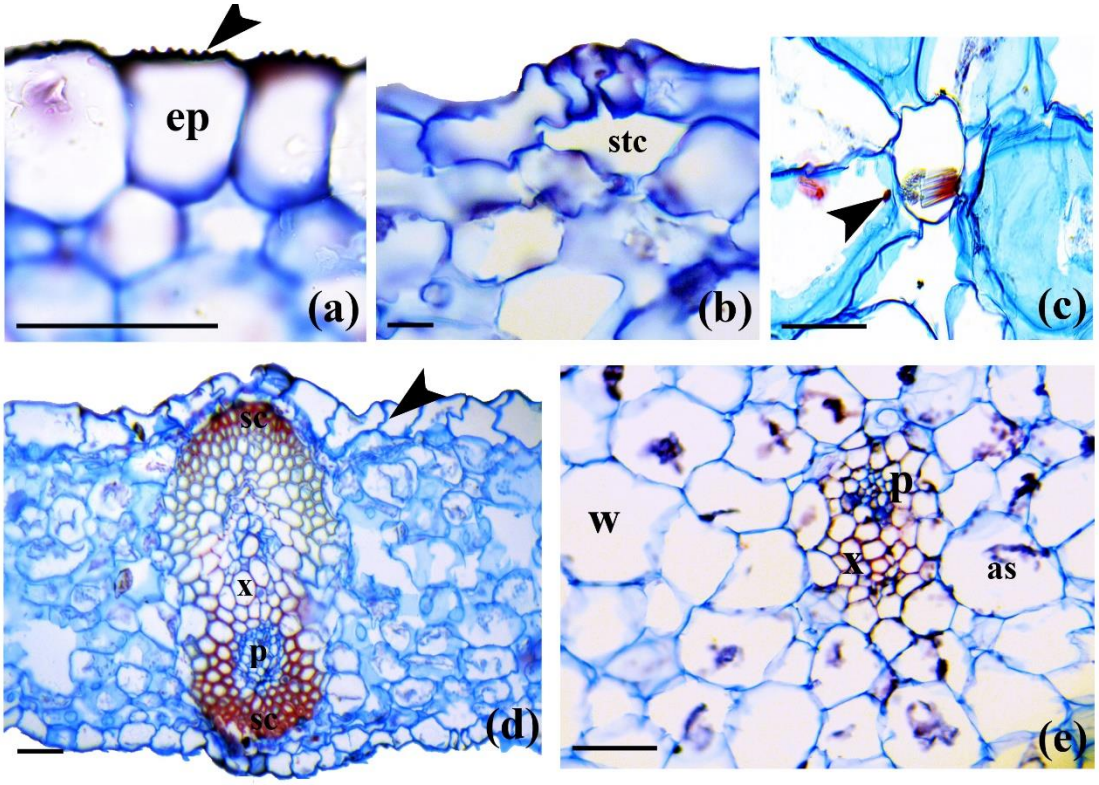


Figure 4.4: *Dendrobium* leaf. Scale bars = 20 µm. **(a)** T.S. of *D. peguanum* showing verrucose cuticle on abaxial surface (black arrowhead); **(b)** T.S. of *D. barbatulum* showing abaxial stomata with cuticular projection and stomatal cavity (stc); **(c)** T.S. of *D. ovatum* showing raphide bundle and tannin droplet (black arrow head) in the mesophyll layer; **(d)** T.S. of *D. barbatulum* showing epidermal cells with undulating walls (black arrowhead) on adaxial surface, vascular bundle with sclerenchymatous cap on both the poles; **(e)** T.S. of *D. peguanum* showing vascular bundle lacking sclerenchymatous cap. epidermis (ep), assimilatory cell (as), water storage cell (w), xylem (x), phloem (p).

4.4.2.1.5. Eulophia and Geodorum

This section comprises the detailed anatomical features of two species of *Eulophia* (*E. herbacea* and *E. ochreata*) and one species of *Geodorum* (*G. laxiflorum*). Cuticle is smooth, moderately thick and curvilinear on both the surface in all the three species. The mid rib region is spherical with a large protrusion on the abaxial side. The stomata are superficial, restricted to abaxial surface with pronounced cuticular projections. The epidermis is single layered with barrel to polygonal shaped cells. The epidermal cells on the abaxial surface are quite smaller in size as compared to adaxial side. Mesophyll cells are irregular in shape and size, with 7 – 10 cells wide in the laminar

region and 1 – 2 in the midvein region. Intercellular spaces are more prominent in the midvein region. Some of the parenchymatous cells in mesophyll layer shows presence of raphide bundle, starch grains and mucilage cells. The mesophyll also contains fibre bundles which are more towards the lower epidermis in all the three species investigated (Figure 4.5a). Vascular bundles are scattered throughout section; however, the midrib vascular bundle is comparatively larger than the subsidiary bundles. In the laminar region, small and large vascular bundles are intercalated. The xylem and phloem cells are surrounded by sclerenchymatous fibre cap at the polar region. In *E. ochreata* and *G. laxiflorum*, these fibre caps are crescent shape, somewhat appear like 'U' shape on abaxial side while inverse 'U' shape on adaxial side. These fibre caps face each other but fail to form a compete ring (Figure 4.5b) while in *E. herbacea* it forms more or less complete circle (Figure 4.5c). These sclerenchymatous cap is 2 – 3 layered and connected laterally by parenchyma cells in *E. ochreata* and *G. laxiflorum* while in *E. herbacea*, these caps are interconnected by a continuous layer of sclerenchyma cells (Figure 4.5c). In these genera, vessels are relatively larger in diameter compared to others. The small vascular bundles are observed on the lateral surface, above the abaxial fibre cap. These vascular bundles are 5 – 6 in *E. herbacea* and 2 – 3 in *E. ochreata* and *G. laxiflorum.*

The results of Devarkar (2001) on anatomical work of *E. pratensis* was matching with our current observation in *E. ochreata*. Devarkar (2001) observed compactly arranged mesophyll cells in *E. pratensis* whereas, in our observation, in *E. ochreata* they are loosely arranged with prominent intercellular spaces. Stern et al. (2001, 2002) carried out anatomical studies on *Geodorum purpureum, Eulophia callichroma, E. gracilis, E. guineensis, E. keithii, E. macrobulbon* and *E. petersii*. The presence of stomata on abaxial epidermis in all the species of *Eulophia* except *E. macrobulbon* and *E. petersii* is in agreement with our observations. Stern et al. (2001, 2002) observed the presence of stegmata and absence of hypodermis in all the investigated species except *E. petersii*. The anatomical feature of *G. laxiflorum* shows similarity with *G. purpureum* except raphides and stegmata which are not observed in our sections. However, these anatomical features are lacking in our plant sample. Absence of this feature is may be due to specific variation or ecological condition.

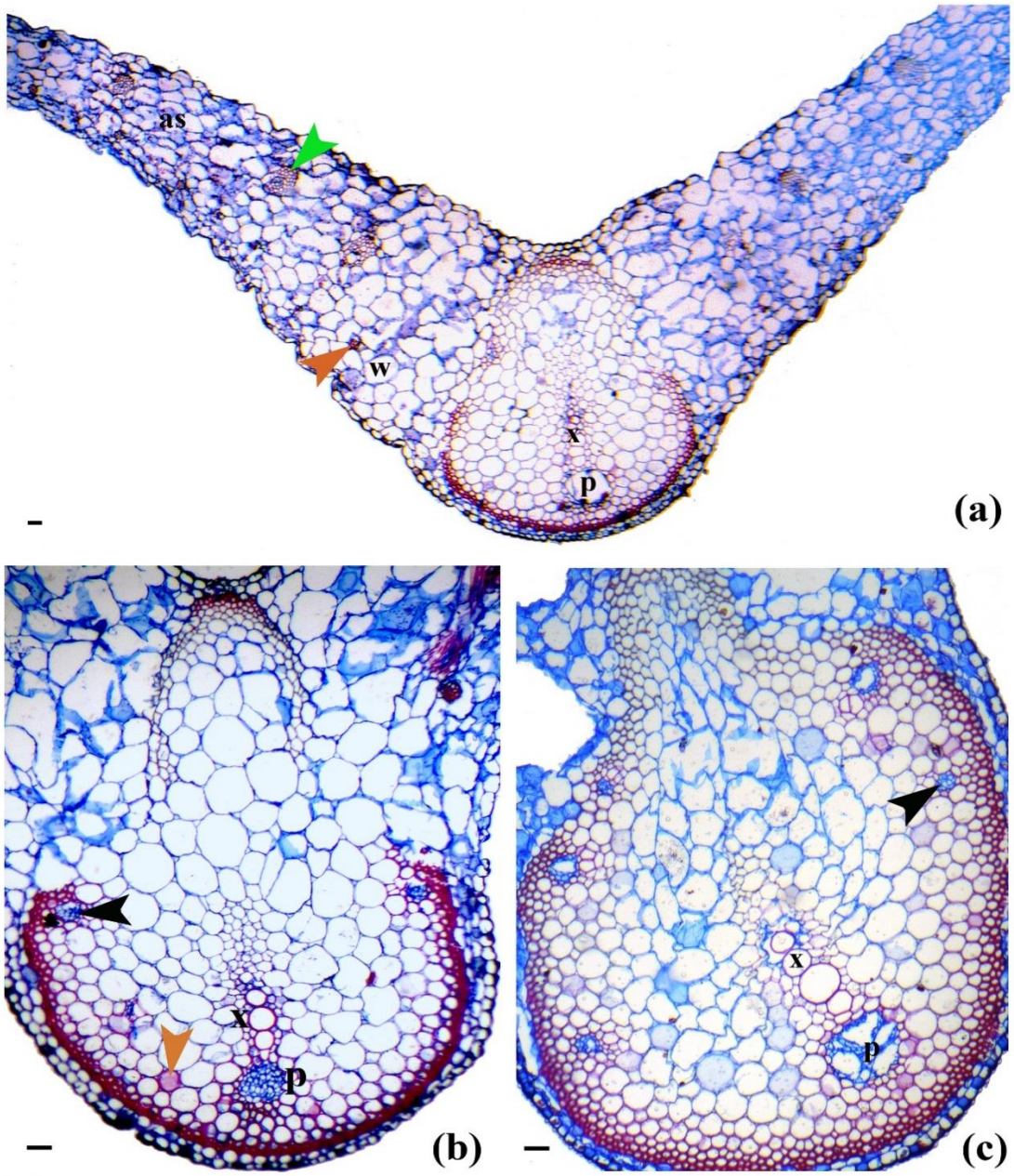


Figure 4.5: *Eulophia* and *Geodorum* leaf. Scale bars = 20 µm. **(a)** T.S. of *G. densiflorum* showing single layered epidermis, fibre bundle (orange arrowhead), subsidiary vascular bundle (green arrowhead) and midvein vascular bundle with crescent shaped fibre cap on both the poles; **(b)** T.S. of *E. ochreata* showing midvein vascular bundle with two crescent shape fibre cap interconnected by parenchyma cells, small vascular bundle on the lateral surface of abaxial fibre cap (black arrowhead), mucilage cell (orange arrowhead); **(c)** T.S. of *E. herbacea* showing midvein vascular bundle with two crescent shape fibre cap (red arrowhead) interconnected by sclerenchyma cells, small vascular bundle on the lateral surface of abaxial fibre cap (black arrowhead). assimilatory cell (as), water storage cell (w), xylem (x), phloem (p).

4.4.2.1.6. Nervilia

In *N. concolor* and *N. plicata*, cuticle is smooth and thin. The side vein region is having V shaped protrusion externally on both the surface. Stomata are more abundant on abaxial side whereas, less on adaxial side with prominent cuticular projections and sub-stomatal cavity. Single layered epidermis comprises square, rectangular to polygonal shaped thin walled cells. Mesophyll layer is polygonal shape. In *N. concolor* the mesophyll cells are with undulated walls whereas it is lacking in *N. plicata* (Figure 4.6a). Intercellular spaces are large and prominent in both the species. Some of the parenchyma cells in mesophyll layer shows presence of water storage cells, mucilage cells, raphide bundle, starch grains and tannin droplets. Vascular bundles are scattered, small in size and surrounded by thin walled parenchymatous cells. There is no distinct variation in the size of midvein and subsidiary vascular bundle. Subsidiary vascular bundles are more towards the protrusion (Figure 4.6b). Phloem cells are interspersed with thin walled, parenchymatic water storage cells (Figure 4.6c).

Similar results were obtained in the studies of Myint (2002) and Stern (2014) in *N. aragona* (Syn. *N. concolor*).

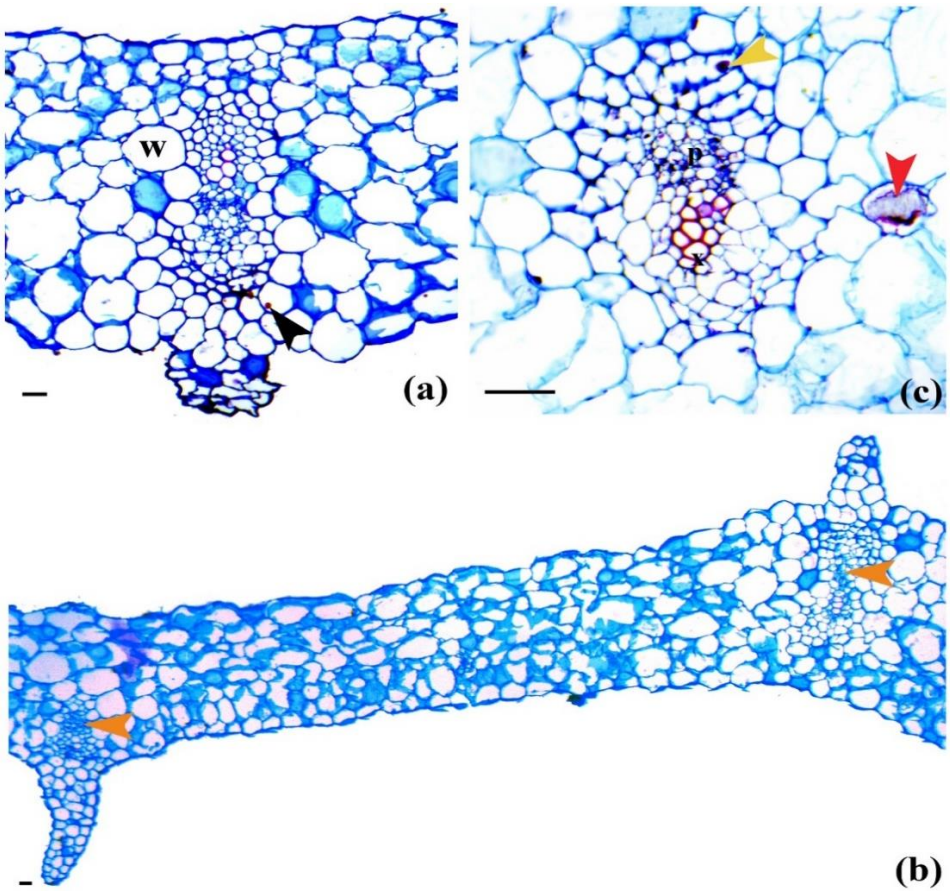


Figure 4.6: *Nervilia* leaf. Scale bars = 20 µm. **(a)** T.S. of *N. concolor* showing undulated walls of mesophylls cells, large intercellular space, midvein vasculature and tannin droplet (black arrowhead); **(b)** T.S. of *N. concolor* showing protrusion on both the surface and subsidiary vascular bundles (orange arrowhead); **(c)** T.S. of *N. plicata* showing vasculature, phloem cells interspersed with large parenchyma cells and tannin droplets (yellow arrowhead) and mucilage cell (red arrowhead). water storage cell (w), xylem (x), phloem (p).

4.4.2.1.7. Oberonia

The T.S of *O. falconeri* and *O. mucronata* is convex in outline. The cuticle is smooth, moderately thick and curvilinear followed by single layered epidermis. The adaxial and abaxial surface of epidermis is interrupted by stomata with small cuticular projections and sub-stomatal cavity (Figure 4.7a). The epidermal cells are thin walled and convexly domed in both the species. Hypodermis is absent. Mesophyll layer comprises ground mass of multi-layered, oval to elongated parenchymatous cells. It shows presence of water storage cells with broad spiral thickenings (Figure 4.7b and c). Raphides are absent. Vascular bundles are arranged in two rows (Figure 4.7d). Moreover, the vascular bundle pair in the midvein is comparatively larger than the pair of subsidiary bundles in the laminar region. The xylem and phloem elements are covered with 1 – 2 layered sclerenchymatous sheath (Figure 4.7e).

Angela et al. (2015) and Stern (2014) reported water storage cells with broad spiral thickening in the genus *Oberonia*, similar cells are also observed in our studies. The other anatomical features of both the species mentioned above are matching with the characters described by Stern (2014) for the genus *Oberonia*. There is no information available on the anatomy of *O. falconeri* and *O. mucronata*.

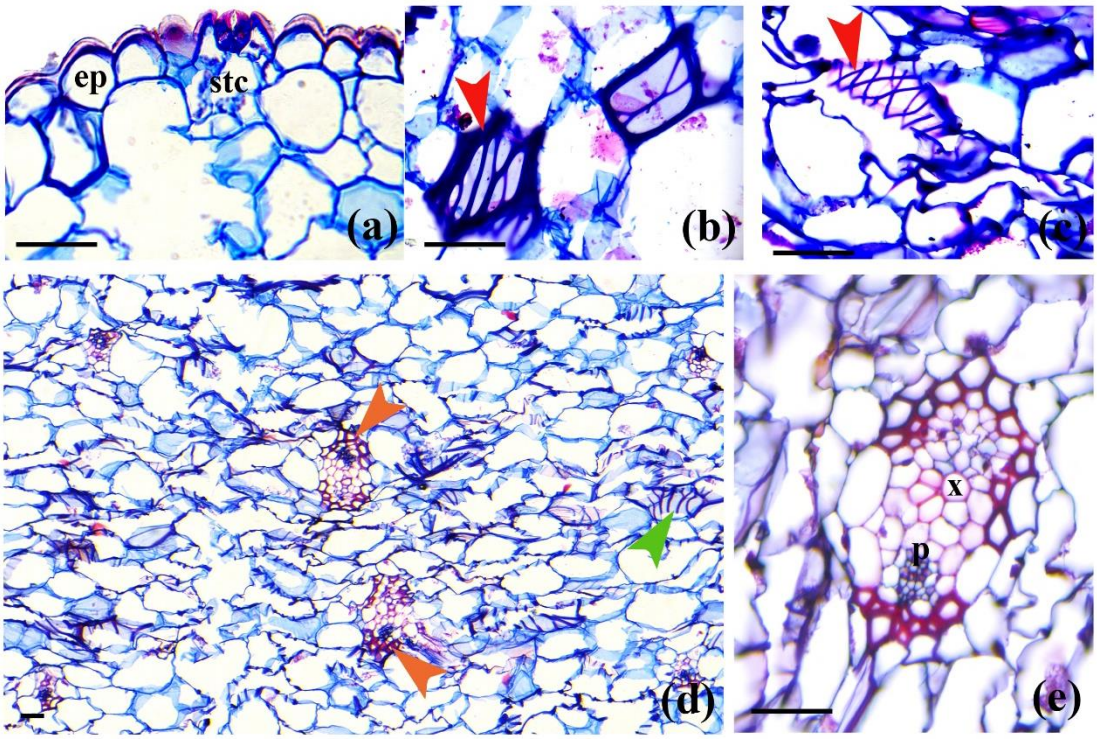


Figure 4.7: *Oberonia* leaf. Scale bars = 20 µm. **(a)** T.S. of *O. falconeri* showing stomata with small cuticular projection, curvilinear cuticle and convexly dome shaped epidermal cells (ep); **(b)** T.S. of *O. mucronata* showing water storage cells with broad spiral thickening (red arrowhead); **(c)** T.S. of *O. mucronata* showing spiral thickening in water storage cells (red arrowhead); **(d)** T.S. of *O. falconeri* showing vascular bundles arranged in two rows (orange arrowhead) and water storage cells (green arrowhead); **(e)** T.S. of *O. mucronata* showing midvein vascular bundle with sclerenchyma cap on both the poles. stc (stomatal cavity), xylem (x), phloem (p).

4.4.2.1.8. Vanda

The outline of T.S is 'V' shaped in both the species of *Vanda* (*V. tessellata* and *V. testacea*). Cuticle is smooth and thick on both the surface adaxial as well as abaxial. The epidermis is single layered, continuous with small, oval to elliptic and thick walled cells. The epidermis is interrupted by stomata situated in the depression of epidermal cells on both the surface with cuticular horns (Figure 4.8a). A 2 – 3 layers of hypodermis lies beneath the epidermis, which is composed of polygonal cells intermixed with sclerenchymatous fibres (Figure 4.8b). These sclerenchymatous fibres may be solitary or form a 2 – 4 cells wide band. The deposition of lignin is very high in these cells so that the size of lumen decreases. Ground mass of leaf lamina in transverse view is composed

of tightly arranged large polygonal cells intermixed with columnar cells. Some parenchyma cells show the presence of starch grains. Raphide bundles are absent. Some cells in the ground tissue contains thick walled water storage cells in both the species. Like other species, median vascular bundle is larger in size compared to subsidiary bundles. Phloem is completely encircled with thick walled, highly lignified sclerenchyma fibre forming cable like covering (Figure 4.8c). In *V. tessellata*, the sclerenchymatous cap was 2 – 3 layers wide while in *V. testacea* it is 4 – 5 layers. The xylem elements are surrounded by xylem parenchyma and thin walled, less lignified sclerenchyma cells.

Similar anatomical characters are also observed by Kowsalya et al. (2017) in both species of *Vanda* using different staining techniques. The amphistomatic condition reported by Vattakandy et al. (2013) in *V. tessellata* and Kowsalya et al. (2017) in *V. tessellata* and *V. testacea* are in good agreement with our observation. This characteristic is an adapted feature of epiphytic orchids growing in dry and humid habitats (Carlsward et al., 1997; Mulgaonkar, 2005b; Abraham et al., 2016). Hypodermis with sclerenchyma fibres investigated in our sections are similar to that of Carlsward et al. (2006) in *V. flabellata.* Our plant material shows lack of raphide bundles in both the species as reported by Kowsalya (2017) and Carlsward et al. (1997).

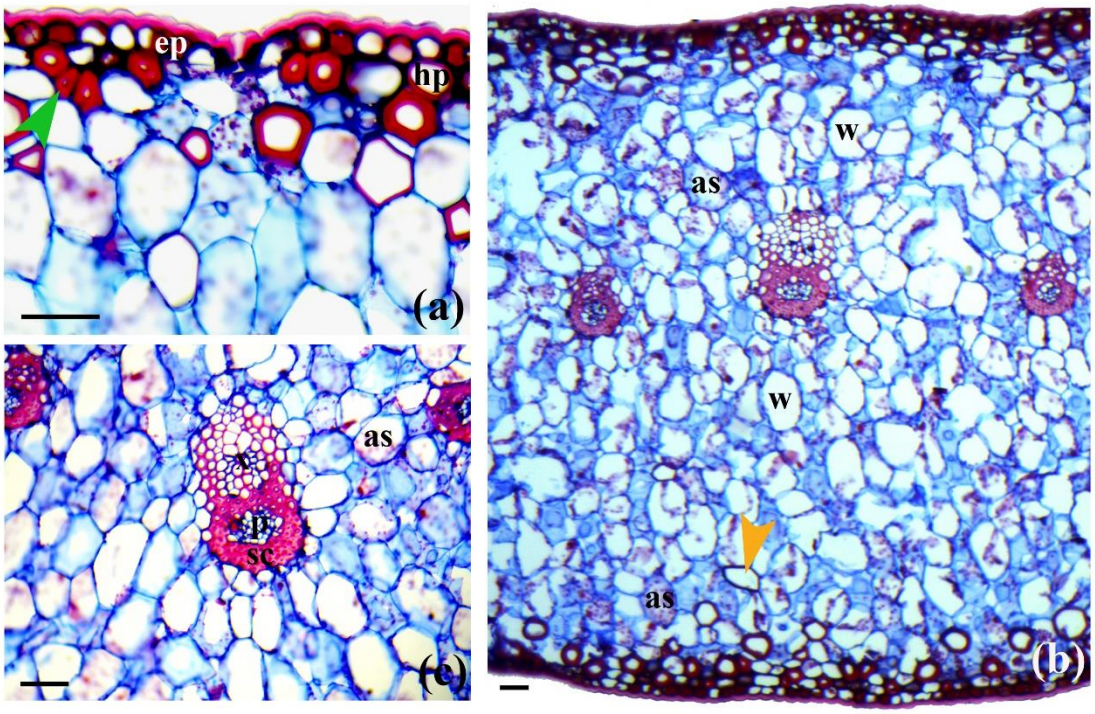


Figure 4.8: *Vanda* leaf. Scale bars = 20 µm. **(a)** T.S. of *V. tessellata* showing thick cuticle, stomata with cuticular projection, epidermis (ep), hypodermis (hp) and

sclerenchymatous band in hypodermis (green arrowhead); **(b)** T.S. of *V. testacea* showing epidermis, hypodermis, thick walled water storage cells (w) (yellow arrowhead), midvein and subsidiary vascular bundle; **(c)** T.S. of *V. testacea* showing midvein vascular bundle with sclerenchyma cap (sc) encircling the phloem. assimilatory cell (as), xylem (x), phloem (p).

4.4.2.2. Sub-family - Orchidoideae

The Orchidoideae consist of approximately 62 genera with 3200 species, mainly includes terrestrial orchids and very rarely epiphytic. This section includes leaf anatomy of two genera viz. *Habenaria* and *Peristylus* belonging to tribe Orchideae.

4.4.2.2.1. Habenaria

The current section includes anatomical studies of seven species of *Habenaria* namely *H. furcifera*, *H. gibsonii*, *H. grandifloriformis*, *H. longicorniculata*, *H. marginata*, *H. plantaginea* and *H. rariflora*. The cuticle is smooth and thin followed by single layered epidermis in all the species. The abaxial epidermis is interrupted by superficial stomata having cuticular extension and sub-stomatal cavity (Figure 4.9a). The adaxial epidermal cells are anticlinal, oval to rectangular and highly elongated in all the species of *Habenaria* except *H. plantaginea* in which these cells are periclinal, not elongated and comparatively smaller in size. The adaxial epidermal cells in *H. grandifloriformis*, *H. marginata* and *H. rariflora* occupy one half of the leaf volume whereas in *H. furcifera*, *H. gibsonii* and *H. longicorniculata*, covers one third of leaf volume (Figure 4.9b and c). The anticlinal walls of adaxial epidermal cells are undulating in *H. grandifloriformis*, *H. marginata* and *H. rariflora* while no such undulating walls are observed in other species. In *H. longicorniculata*, *H. marginata*, *H. furcifera* and *H. rariflora*, 2 – 3 adaxial epidermal cells just above the midvein are smaller in size compared to other epidermal cells. Mesophyll cells are isodiametric in all the species. The intercellular spaces are prominent in midrib region of all *Habenaria* species. Raphides, starch grains and tannin droplets are present in some of the *Habenaria* species (Figure 4.9d). Vascular bundles are conjoint, collateral and closed with larger one in the midvein and smaller in the side vein region. The vasculature of *H. plantaginea* shows some characteristic features which were not found in the other species. These features include development of primary phloem just above the xylary elements in the midrib region and prominent 2 – 3 layered sclerenchymatous fibre cap (Figure 4.9e).

Stern (1997a) studied several species under the sub tribe Habenariinae (*H. comuta*, *H.dirtans*, *H. holothlix*, *H. leonensis*, *H. longicomiculata*, *H. mononhka*, *H. occidentalis*, *H. odontopetala*, *H. plantaginea*, *H. repens*, *H. rhodocheila*, *H. snowdmii*, *H. tridac* and *H. vaginata*). According to Stern (1997a), the vascular fibre cap is absent in all *Habenaria* species studied. However, our observation shows presence of fibre cap on both the poles in *H. plantaginea* however, it is absent in the remaining species. The other anatomical characters found by Stern (1997a) are similar to our observations.

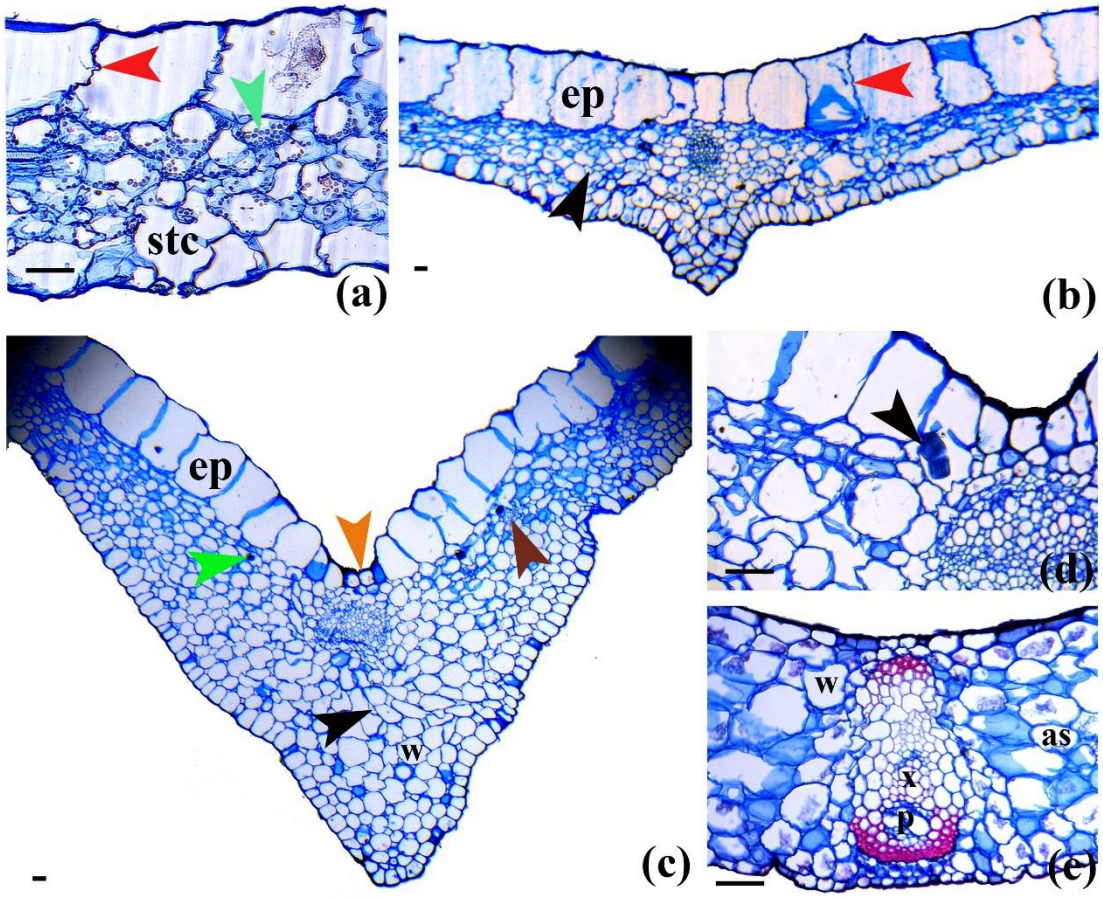


Figure 4.9: *Habenaria* leaf. Scale bars = 20 µm. **(a)** T.S. of *H. furcifera* showing undulating anticlinal walls on adaxial epidermal cells (red arrowhead), starch grains (green arrowhead), stomata with small projection and stomatal cavity; **(b)** T.S. of *H. marginata* showing adaxial epidermis (ep) occupying $^1/_2$ of the leaf volume, undulating anticlinal walls of adaxial epidermal cells (red arrowhead), midvein vasculature and intercellular space (black arrowhead); **(c)** T.S. of *H. longicorniculata* showing adaxial epidermis occupying $^1/_3$ of the leaf volume (ep), two small epidermal cells on the adaxial surface above the midvein (orange arrowhead), intercellular space (black arrowhead),

midvein and subsidiary (blue arrowhead) vascular bundle lacking sclerenchyma cap and tannin droplet (green arrowhead); **(d)** T.S. of *H. longicorniculata* showing raphide bundle (black arrowhead); **(e)** T.S. of *H. plantaginea* showing midvein vascular bundle with sclerenchyma cap (sc) on both the poles (black arrowhead) and primary phloem above the xylary elements. assimilatory cell (as), water storage cell (w), xylem (x), phloem (p).

4.4.2.2.2. Peristylus

This section comprises anatomical studies of four species of genus *Peristylus* namely *P. constrictus*, *P. lawii*, *P. plantagineus* and *P. stocksii*. The cuticle is thin and curvilinear in all the species. Moreover, it is verrucose on abaxial surface in *P. constrictus*, *P. lawii* and *P. plantagineus* (Figure 4.10a) whereas smooth in *P. constrictus* (variant) and *P. stocksii*. The stomata are glandular and restricted to abaxial surface with needle like cuticular projection and sub-stomatal cavity (Figure 4.10b). The epidermis is single layered with elongated, square to barrel shaped cells. The anticlinal walls of epidermal cells are undulating on both the surface in *P. lawii* and *P. stocksii* (Figure 4.10c). Again, in both the species, the adaxial epidermal cells of midvein are smaller in size compared to other cells whereas no such cells are observed in remaining species. Mesophyll cells are multi-layered, spherical to polygonal in shape and irregular in size with water storage cells. The intercellular spaces are large and prominent in all the species. Raphide bundle and starch grains are present in few of the parenchymatous cells (Figure 4.10d). Vascular bundles are collateral in all the species of *Peristylus*. The midrib vascular bundles are comparatively larger than the subsidiary bundles. In all the species, phloem derivatives are interspersed with large thin walled parenchymatous cells in midvein vascular bundle (Figure 4.10e). The vascular sclerenchyma is absent in all the studied species of genus *Peristylus*. This is a first and foremost report on detail description of the anatomical leaf features on *Peristylus* genus.

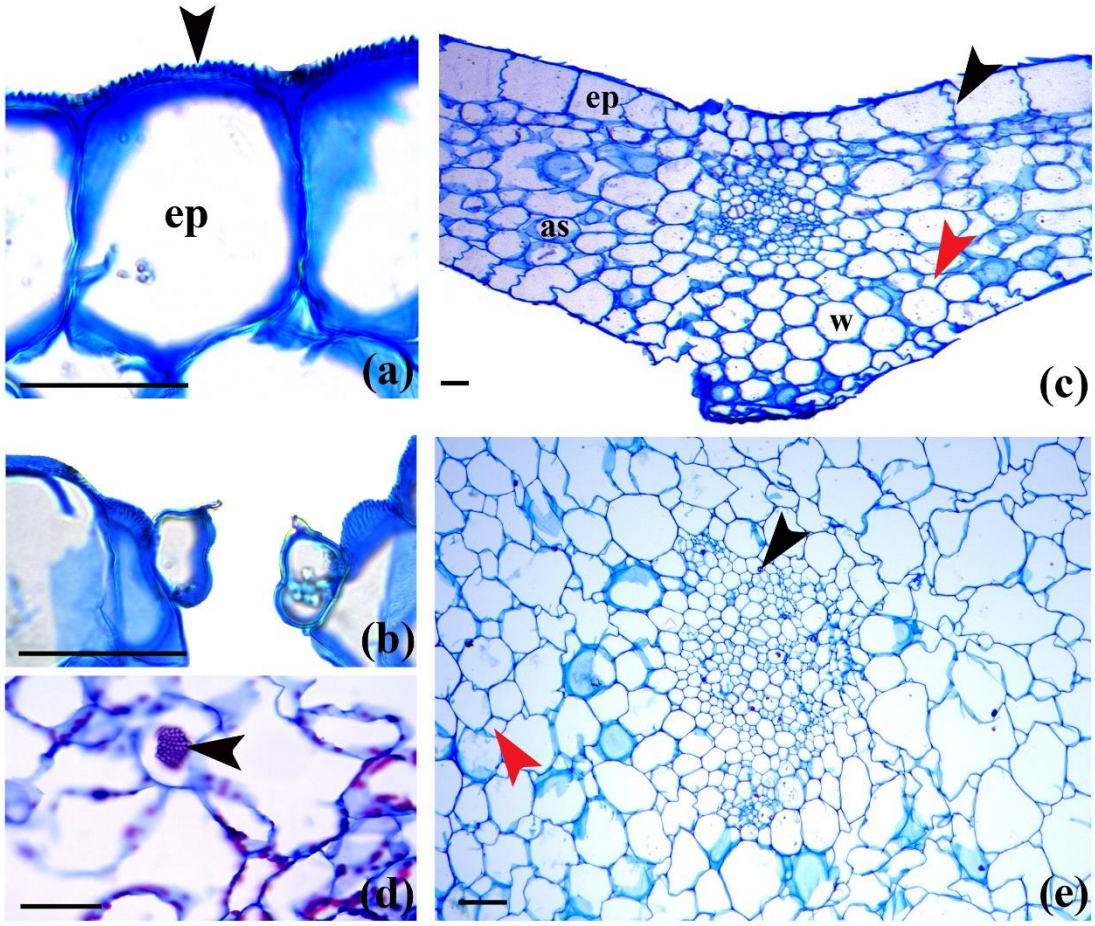


Figure 4.10: *Peristylus* leaf. Scale bars = 20 µm. **(a)** T.S. of *P. constrictus* showing verrucose cuticle in *P. constrictus* (black arrowhead); **(b)** T.S. of *P. plantagineus* showing glandular stomata with cuticular projection; **(c)** T.S. of *P. stocksii* showing epidermal cells with undulating anticlinal walls on both the surface (black arrowhead) and midvein vascular bundle, large intercellular space (red arrowhead); **(d)** T.S. of *P. plantagineus* showing raphide bundle (black arrowhead); **(e)** T.S. of *P. constrictus* showing midvein vascular bundle without sclerenchyma cap , large intercellular spaces (red arrowhead) and tannin droplet (black arrowhead). epidermis (ep), assimilatory cell (as), water storage cell (w), xylem (x), phloem (p).

4.4.3. Identification Keys to the Genera

1a. Plants epiphytic……..…….….**2**

1b. Plants terrestrial……..…….**7**

2a. Cuticle thick…….……**3**

2b. Cuticle thin……..**6**

3a. Hypodermis present……...……..**4**

3b. Hypodermis absent……...**Aerides**

4a. Hypodermis with sclerenchyma band……..…….….**Vanda**

4b. Hypodermis without sclerenchyma band..……..….**5**

5a. Mesophyll cells with undulating walls……..**Rhynchostylis**

5b. Mesophyll cells without undulating walls……**Acampe**

6a. Vascular bundle arranged in single row……...**Dendrobium**

6b. Vascular bundle arranged in two rows……..…**Oberonia**

7a. Vasculature poorly developed……...……..**Crepidium**

7b. Vasculature well developed……..…..**8**

8a. Vascular bundle with crescent shape fibre cap……..…..**9**

8b. Vascular bundle without sclerenchyma cap…….……..**10**

9a. Midrib region with prominent spherical protrusion on abaxial side…….**Eulophia**

9b. Midrib region without protrusion ……...**Geodorum**

10a. V shaped protrusion on both the surface…….……**Nervilia**

10b. Protrusion absent……**11**

11a. Cuticle smooth, upper epidermal cells are larger…….……..**Habenaria**

11b. Cuticle verrucose, upper epidermal cells are comparatively smaller……...**Peristylus**

As per the morphoanatomical observation, *Habenaria* and *Peristylus* are very closely allied with each other and their morphology in vegetative stage is also very similar. There were no distinguishing anatomical feature observed between these two genera. Thus, the detailed anatomical studies using other parts (stem/pseudobulb, root) are required for the confirmation of both the genera in vegetative stage.

Furthermore, the confirmation of all the genera will be based on the combined result of all the techniques *viz.* morphology, anatomy and molecular studies.

4.5. CONCLUSION

From the leaf anatomical studies of the orchids it is concluded that the selective characters like thickness of cuticle, stomata, epidermal cell type, hypodermis, fibre bundles, fibre cap in vascular bundle and arrangement of vascular bundle in mesophyll layer provides significant information for the identification of orchids. The presence of sclerenchyma band in hypodermis of *Vanda*, water storage cells with spiral banded thickening and vascular bundles arranged in two rows in *Oberonia* are the distinguishing feature of these genera. The absence of sclerenchyma cells in the vasculature of *Habenaria* (except *H. plantaginea*) and *Peristylus* are the characteristic of these genera. The species from different genus possess unique anatomical character which could help in identification and authentication of species as well as to prevent adulteration because of much similarities in the morphology.

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