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Anatomy of Vegetative Parts of *Bergenia ciliata* (Haw.) Sternb. (Saxifragaceae): A Potential Medicinal Herb

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Abstract: Morphological and anatomical study of many herbaceous Saxifragaceae has still been lacking. A detailed anatomical structure of vegetative organs: leaf, petiole, rhizome and root of a highly medicinal plant *Bergenia ciliata* were described for the first time. Cells in all parts were rich in starch grains and some cells contain calcium oxalate crystals. Some important anatomical features were: a hood like structure found in its lower portion of petiole; petiole supplied with numerous conjoint, collateral and open vascular bundles with poorly differentiated bundle sheath; leaves amphistomatic; ten to fifteen conjoint, collateral and open vascular bundles found in mid vein of leaf. Globular trichomes were present in abaxial side of leaf and petiole; cork of rhizome divided into two zones; cork cambium multilayered; secondary growth evident in rhizome and root; activity of interfascicular cambium in rhizome was lacking. Endodermis and pericycle only found in root was crushed during secondary growth; polyarch xylem and phloem in root; vessels short and narrow with simple perforation plates. There was no comprehensible difference in size of central and lateral vascular bundles of leaf and petiole as in some other Saxifragaceae. Well developed bundle sheath which was the characteristic feature of many Saxifragaceae was feebly distinguished in the leaf and petiole of *B. ciliata*. The globular trichomes found in leaf and petiole of *B. ciliata* is probably a new feature for Saxifragaceae.

Key words: Anatomical features, *Bergenia ciliata*, Saxifragaceae, vegetative organs

INTRODUCTION

Bergenia ciliata (Haw.) Sternb. (Saxifragaceae) is a perennial rhizomatous herb, up to 35 cm tall, usually found in rocky cliffs. Leaves are few in number, spreading, glabrous, suborbicular to orbicular or broadly obovate. Length of petiole 1-2 cm long glabrous or hirsute and a hood like outgrowth occurs either at lower half or upper half of the petiole (Chowdhary and Verma, 2010). Inflorescence a one sided raceme or corymbose, often subtended by an ovate leafy bract; flowers white or pink; fruit capsule. It is one of the most popular medicinal plants used in traditional Ayurvedic medicine for the treatment of several diseases in Nepal, India, Pakistan, Bhutan and some other countries. Rhizomes and roots are bitter in taste, astringent, diuretic, demulcent, aphrodisiac. Extract of rhizome and root is used to cure fever, diarrhea, pulmonary affection and renal and muscular calculus (Rai and Sharma, 1994; IUCN Nepal, 2004). *B. ciliata* rhizome has anti-bacterial and anti-tussive properties (Sinha *et al*., 2001a, b) and reported to be helpful in dissolving kidney stones (Singh and Aswal, 1992). Due to this nature in Sanskrit it is called as Pashanbhed (literally ‘to dissolve the stone’).

Anatomical feature of leaf and petiole have wide systematic value and useful for recognizing or circumscribing generic, tribal or family level (Dickison, 1989; Dickison and Weitzman, 1996; Souza *et al*., 2004). Moreover, some anatomical feature including stem can also reflect particular habits of the plant (Carlquist *et al*., 1995). Anatomical studies of family Saxifragaceae have been started from late 19th century (Thouvenin, 1890; Holle, 1893) and they are mainly focused on woody species. However, the species studied by Thouvenin, (1890) (*Ixerba*, *Bauera*, *Anopterus*, *Abrophyllum* and *Dontia*) and Holle (1893) (*Bauera*, *Anopterus*, *Abrophyllum*, *Argophyllum*, *Carpodetus* and *Anodopetalum*) have already been excluded from Saxifragaceae in present systematics. Later also, the anatomical study on Saxifragaceae *sensu lato* mainly concentrated on the woody species (Watari, 1939; Stern, 1974, 1978; Styer and Stern, 1979a, b; Hils, 1985;

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Hils *et al*., 1988; Dickison *et al*., 1994). Thus, data on anatomical study of herbaceous species of family Saxifragaceae *sensu strict* is very rare in previous literature.

There are many reports on ethno-botanical uses and phytochemical analysis of the different species of *Bergenia* (Sinha *et al*., 2001a, b; Islam *et al*., 2002; IUCN Nepal, 2004; Srivastava and Rawat, 2007, 2008; Singh *et al*., 2007; Rajkumar *et al*., 2010). In spite of the economic importance and huge ethnomedicinal value of *Bergenia* the anatomical aspect of this genus in previous works is very rare. Only fragmented data of internal structure of rhizome were listed by Srivastava and Rawat (2008). Due to the paucity of information found in the available literature on anatomy of vegetative parts of *B. ciliata*, the objective of this work is to explore the detailed morphology and anatomy of all vegetative parts (leaf, petiole, root and rhizome) of the species. This result will certainly help to compare anatomical features within genus *Bergenia* and family Saxifragaceae.

MATERIALS AND METHODS

Well developed and healthy leaves, rhizome and root of *Bergenia ciliata* were collected from glass house grown plant at Kangwon National University Chuncheon, South Korea during June-July 2011. Collected material were fixed in FAA (5 parts formalin: 5 parts glacial acetic acid: 90 parts 50% ethanol) for a week and preserved in 50% ethanol. Preserved plant parts were dehydrated with ethanol series (50, 70, 80, 90, 95% and absolute ethanol). After complete dehydration, plant parts were embedded in Technovit 7100 resin (Heraeus Kulzer GmbH, Wehrhein Germany) and histo-blocks were prepared. Embedded materials in histo-blocks were sectioned with disposable knife by using rotary microtome. Serial sections of 4-5 μm thickness were cut, dried in slide warmer, stained with 0.1% Toluidine blue O (Research Organics Inc, Cleveland United States) and de-stained in running tap water. All stained slides were mounted with Entellan. Prepared slides were observed with BX-50 light microscope (Olympus Co., Japan). Photographs were taken by digital camera system attached to microscope.

RESULTS

Leaf and petiole anatomy: Upper half of the petiole of mature leaf is pure circular in outline and devoid of wings (Fig. 1a); however, lower half of the petiole encompasses a hood like structure which looks like a large wing in cross section (Fig. 1b). Lower half is more or less circular in abaxial side but more flat in adaxial side. Multicellular and globular secretory trichomes attached to the epidermal layer (Fig. 1a, b). A continuous layer of collenchymas observed both on abaxial and adaxial side. The massive ground tissues composed of thin walled parenchyma cells which are filled with starch grains and some of such cells contain rosette calcium oxalate crystals. A number of veins, representing single vascular bundle run along the whole ground region of the petiole and few of them form a circle towards outskirts, while many of them pass through the centre. Although the size of the vascular bundle is diverse, shape of the individual vascular bundle is constantly crescentiform (Fig. 1a, b). Occasionally some veins to the core of ground tissue interconnected and form a cluster of two to four. Vascular bundles are conjoint, collateral and closed type (the xylem is adaxial and the phloem is abaxial) and are always supported by layers of collenchymas.

The dorsiventral leaf blades are enclosed from both surfaces by uniseriate epidermis. Epidermal cells are barrel shaped and mostly abaxial surface has smaller cells than adaxial one. Bulbous trichomes are observed only in abaxial surface (Fig. 1c, d). The outer wall of the epidermis are usually thickened and covered by waxy cuticle which is thicker in adaxial surface than abaxial surface. Stomata are found on both sides (Fig. 1d) although most abundance in abaxial side. Mesophyll tissue divided into two regions, palisade parenchyma and spongy parenchyma (Fig. 1d). Cells of palisade parenchyma are slightly elongated, round or oval in shape and arranged quite compactly in about 3-4 layers beneath the adaxial epidermis. Spongy parenchyma found in abaxial side, cells are loosely arranged and irregular in shape with large intercellular spaces. The general anatomy of mid rib is similar with petiole (Fig. 1c). One or two layers of collenchymas positioned in abaxial side and veins are few in number than in the petiole. Cells in ground tissue are full of starch grains and some also have rosette crystals (Fig. 1e). There are about 10-15 crescentiform vascular bundles, some of them are interconnected to each other and nature of vascular bundle is similar with petiole (Fig. 1c, e).

Rhizome anatomy: In cross-section, rhizome of *B. ciliata* appears round in shape which is covered by scaly bark (Fig. 2a). The bark is thick and the cork is divided into two regions; outer region consists of tangentially elongated and compressed cells, whereas inner region contains irregular parenchymatous cells with intercellular spaces. Inner cork zone is much thicker than outer and multilayered cork cambium lies just below this zone (Fig. 2b). Few cell layers stuck between two cork zones also retain cambial activity. Cortical region is occupied by thin walled isodiametric parenchymatous cells filled with starch grains and tannin (Fig. 2b, c). Some of them contain rosette calcium oxalate crystals (Fig. 2c). Secretory ducts

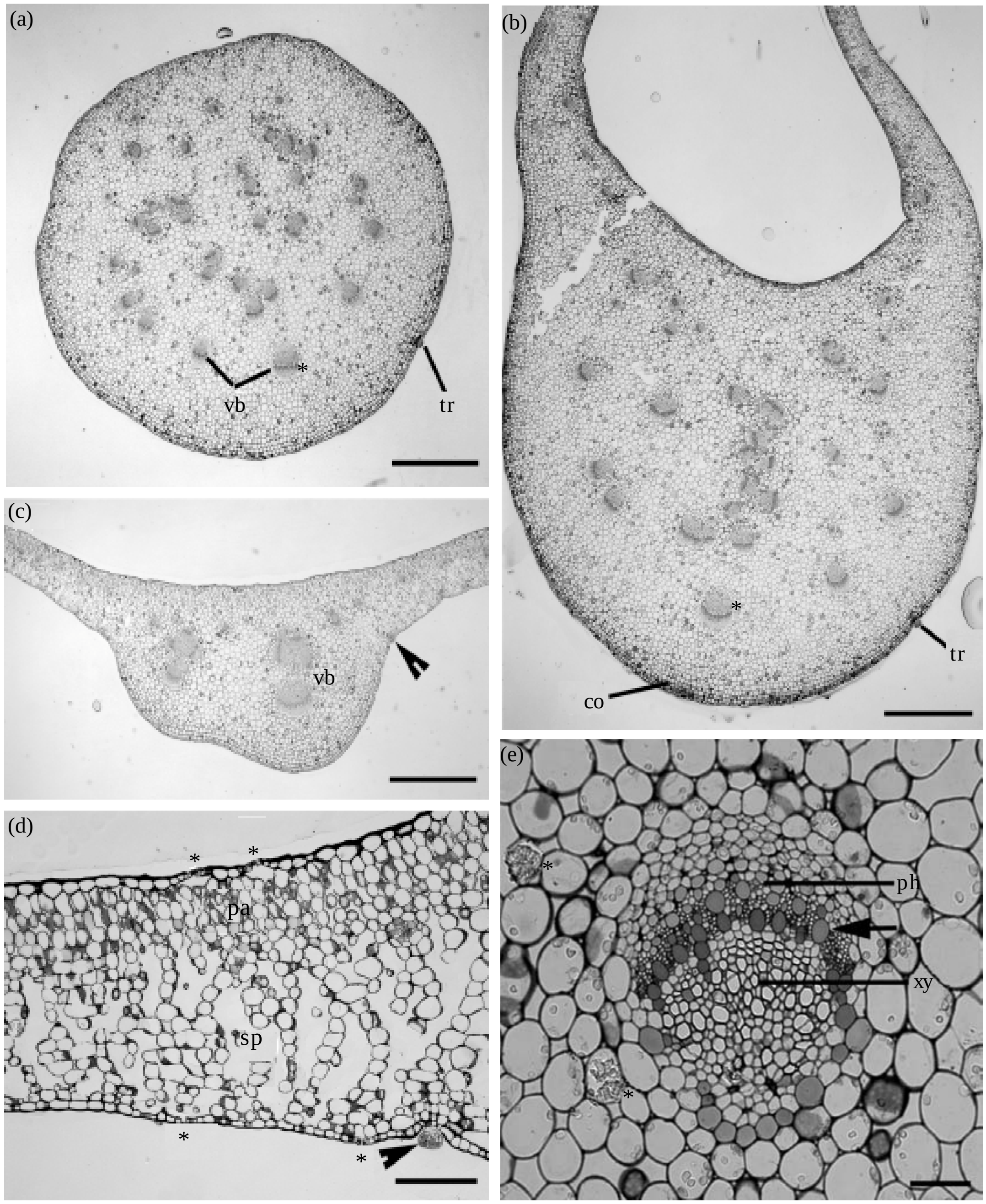

Fig. 1(a-e): Transverse section of *B. ciliata* petiole and leaf, (a) Upper half of petiole (*large vascular bundle), (b) Lower half of petiole showing hood like structure (*large vascular bundle), (c) Leaf lamina through mid vein (arrow head: Trichome), (d) Leaf lamina showing stomata, trichome and mesophyll (*stomata, arrow head: Trichome) and (e) Vascular bundle of the leaf (*rosette calcium oxalate crystals; arrow head: Collenchymas), co: Collenchymas, pa: Palisade parenchyma, ph: Phloem, sp: Spongy parenchyma, tr: Trichome, vb: Vascular bundle, xy: Xylem, Scale bars (a, c) 100 μm (b) 125 μm and (d, e) 20 μm

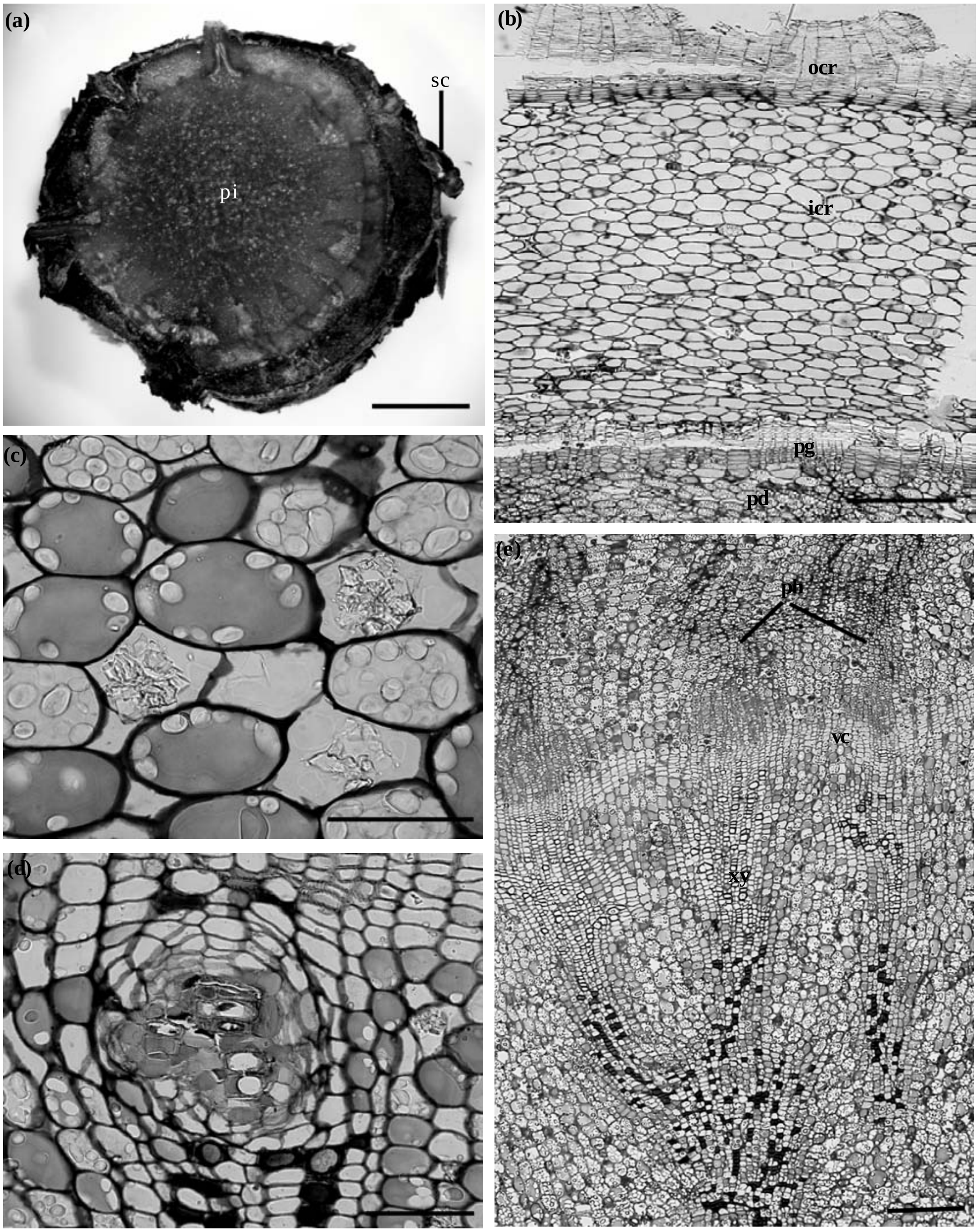

Fig. 2(a-e): Transverse section of *B. ciliata* rhizome, (a) Whole structure, (b) Cork and cork cambium, (c) Starch grain and rosette crystals in ground tissue (magnified), (d) Secretory duct and (e) Vascular bundle, icr: Inner cork zone, ocr: Outer cork zone, pd: Phelloderm, pg: Phellogen, ph: Phloem, pi: Pith, sc: Bark scale, vc: Vascular cambium, xy: Xylem, Scale bars (a) 500 μm and (b, c, d, e) 20 μm found in the rhizome are generally located in vascular region, infrequently these may pass through vascular bundles too (Fig. 2d). Numerous long, narrow vascular bundles are arranged in circle and separated by broad region of parenchyma, surrounding prominent large pith in the core (Fig. 2a, e). Vascular bundles are conjoint, collateral and open type and almost fusiform in outline (Fig. 2e). Xylem consists of tracheid, vessel, fiber and xylem parenchyma, whereas phloem consists of sieve tube elements, companion cells and phloem parenchyma. Pith is very large, composed of thin walled parenchyma cells and rich in starch (Fig. 2a). No visible endodermis and pericycle is found in cross section. Secondary growth is noticeable in vascular region but there are no distinct ray cells in between vascular bundles.

Short and narrow vessel elements are usually surrounded by fibers and elongated parenchyma cells (Fig. 3a). End walls of vessel elements are oblique (Fig. 3b). Wall thickening is helical to reticulate, bars usually forked and interconnected in many places to form network like structure and intervessel pits are mostly scalariform (Fig. 3b), infrequently transitional and are circular to elongated. Perforations are exclusively simple (Fig. 3c). Tracheids are very few in comparison to vessel. They are similar but narrower than vessel. Bordered pits are prominent, arranged in single row and circular in outline (Fig. 3d).

Root anatomy: Root of *B. ciliata* is almost circular in cross section. Histologically, it is divided into epidermis, cortex, vascular region and pith. The root epidermis is uniseriate and cells are small, thin walled and devoid of cuticle (Fig. 4a). Unicellular root hairs are evident and arise from the epidermal cells. Beneath the epidermis there are 1-2 layered suberised exodermis (Fig. 4a). Cortical region is wide and formed by asymmetrical parenchymatous cells with conspicuous intercellular spaces. No starch grains and crystals have observed in primary structure of root but both structures are abundance after secondary growth. Innermost layer of cortex develops into endodermis and well developed endodermis in young root loses its appearance in secondary structure (Fig. 4b, c). Casparian stripe appears in radial and cross wall of endodermal cells. Pericycle is 4-6 layered parenchymatous and disappears during secondary growth along with endodermis (Fig. 4b). There is a large parenchymatous conjunctive region in between pericycle and vascular region (Fig. 4b). Primary vascular system is polyarch:

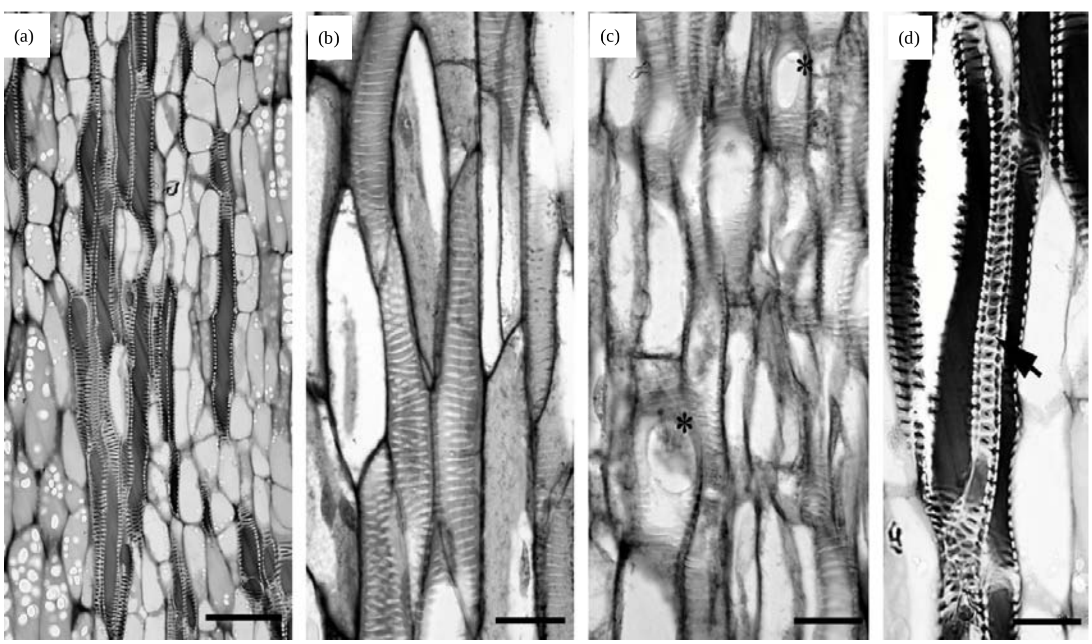

Fig. 3(a-d): Longitudinal section of rhizome, (a) Vessels, tracheids and parenchyma, (b) Scalariform vessel wall, (c) Simple perforation plates in vessel (asterisk) and (d) Tracheid with circular bordered pits (arrow head), Scale bars (a) 20 μm and (b, c, d)10 μm

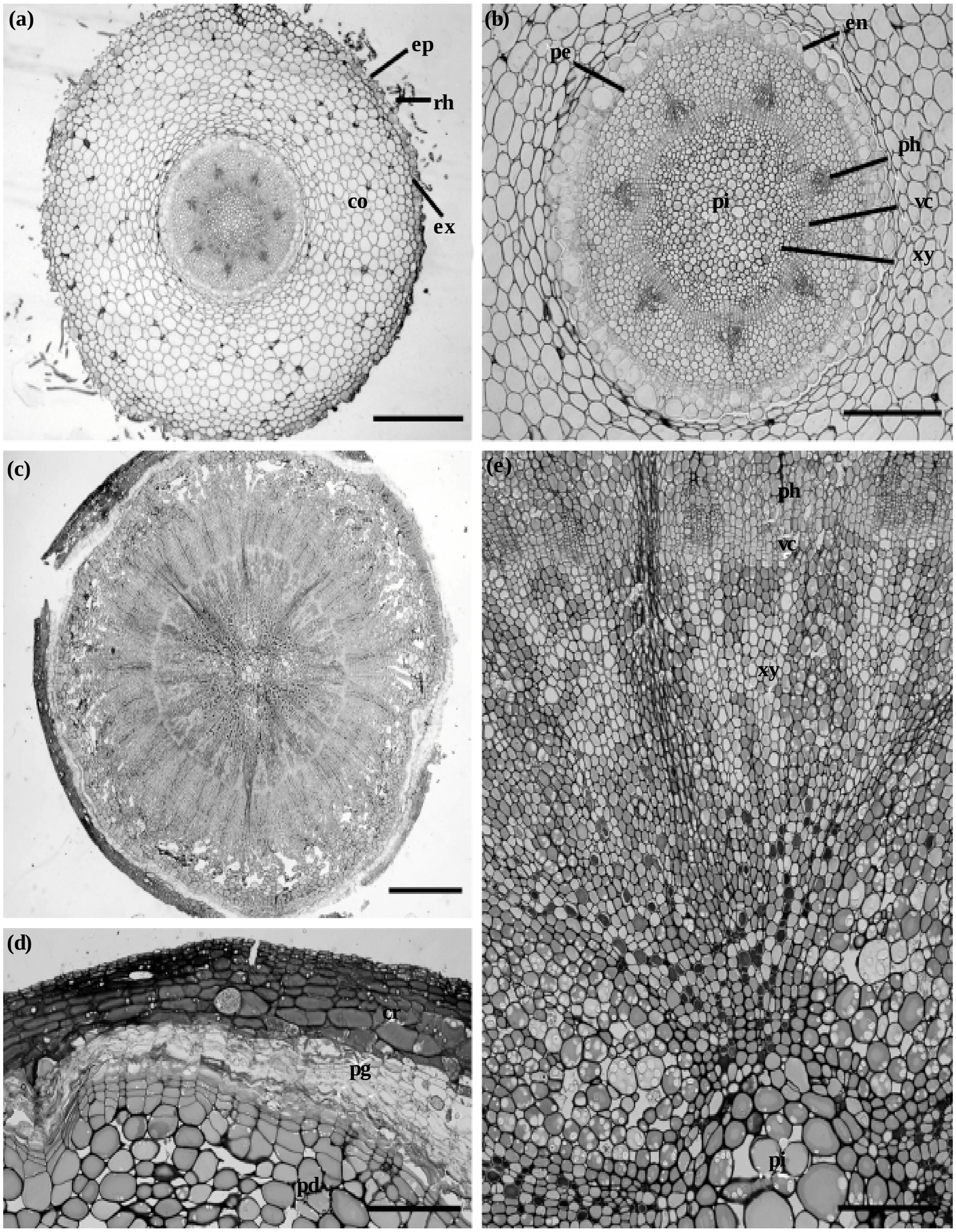

Fig. 4(a-e): Transverse section of *B. ciliata* root, (a) Whole young root (before secondary growth), (b) Vascular region before secondary growth (magnified), (c) Whole mature root (after secondary growth), (d) Secondary structure in cortical region and (e) Secondary growth in vascular region, co: Cortex, cr: Cork, ep: Epidermis, en: Endodermis, ex: Exodermis, pd: Phelloderm, Pe: Pericycle, pg: Phellogen, ph: Phloem, rh: Root hair, vc: Vascular cambium, xy: xylem, Scale bars (a) 50 μm, (b, c, d) 20 μm and (e) 100 μm seven separate phloem and xylem strands appear and are separated by conspicuous multilayered cambium (Fig. 4a, b). The central region of the root is occupied by wide parenchymatous pith (Fig. 4b).

Secondary growth is evident in root. The cambium divides tangentially and produces secondary tissue. The cambial cells produce more xylem elements on its inner side than phloem on the outer side (Fig. 4c, e). The secondary vascular tissue form a continuous cylinder and primary xylem usually embedded in secondary xylem. Central pith region encroached by vascular elements and thus pith is very little after secondary growth (Fig. 4c, e). Simultaneously, the periderm develops in the outer region. Some cells of multilayered pericycle become meristematic and divide, giving rise to cork cambium (Fig. 4d). It produces few layers of cork cells outwardly and phelloderm inwardly (Fig. 4d). The pressure caused by secondary tissue ruptures the cortex with endodermis, which is ultimately crushed.

DISCUSSION

Some peculiar morphological structures in petiolar region of *Bergenia* species has been described by Chowdhary and Verma (2010). They found a hood like outgrowth either at lower half or upper half of the petiole in *B. stracheyi*. Here, we also found similar structure at the lower half of the petiole of *B. ciliata*. Petiole is supplied by numerous veins with various sizes and in some places two or three bundles were interconnected each other. This is the general feature of the petiole in most of the angiosperms that the biggest vascular bundle is found towards the abaxial surface whereas lateral bundles are comparatively smaller in size. However, in *B. ciliata* we couldn't find such remarkable variation in shape instead one abaxial vascular bundle was ineffectually larger than others. Hare (1943) realized that the distinctive features of petiole anatomy should be regarded as mainly adaptive and functional, with little phylogenetic significance. In the petiolar anatomy, vascular system has received most attention. According to Olowokudejo (1987) vascular structure of the petiole in genus *Biscutella* (Cruciferae) is most taxonomically significant anatomical feature and he described three basic shape of vascular bundles; shallow crescentiform, deep crescentiform and U-shaped in 46 species. Similarly in *Aphanopetalum*, a member of Saxifragaceae, the shape of central bundle and laterals are different: large central bundle is flat, undivided arc to circular, whereas lateral bundles are always circular in outline (Dickison *et al*., 1994). However, we did not observe the dual form of vascular bundle in petiole of *B. ciliata*. Both central and lateral bundles were crescentiform.

Amphistomatic and anomocytic stomata found in *Bergenia ciliata* are also common in other Saxifragaceae (Moreau, 1984) except few woody genera: *Tetracarpea* (Hils *et al*., 1988) and *Aphanopetalum* (Dickison *et al*., 1994). The mesophyll is bifacial in structure with a well differentiated triseriate palisade region and a lacunose spongy region. There are more than 10 major and minor bundles in the mid vein region and some of them are interconnected each other. Bundle sheath is poorly developed in *B. ciliata*. Although much about the foliar bundle sheath in herbaceous Saxifragaceae has not been described so far, however, well developed bundle sheath is the characteristic feature of many woody Saxifragaceae (Stern, 1974, 1978; Styer and Stern, 1979a, b; Hils, 1985). On the contrary, bundle sheath of *Aphanopetalum* is less well-developed and distinct than other woody Saxifragaceae (Dickison *et al*., 1994) whereas *Tetracarpea* almost lacks foliar bundle sheath and bundle sheath extensions (Hils *et al*., 1988). In this report, one or two layers of collenchymas have been observed in abaxial part of the mid vein. The collenchyma cells are more or less turgid, generally found on both region of mid vein just below the leaf epidermis in dicotyledonous plants and so gives the strength to the leaf (Fahn, 1990).

The occurrence of starch grains and calcium oxalate crystals in rhizome are common in *Bergenia* (Srivastava and Rawat 2008) and also observed in species of *Micranthes* and *Saxifraga* (Engler, 1930; Gornall, 1987). The endodermis or starch sheath present in root of *B. ciliata* has not found in rhizome. In contrary, uniseriate endodermis with Casparian strip was observed in young stem of *Aphanopetalum* (Dickison *et al*., 1994). In *Rhaponticum* (Asteraceae), a starch sheath was found in the root and hypocotyl but not in the rhizome (Lotocka and Geszprych, 2004). The endodermis with Casparian strip is rarely formed in shoot of herbaceous dicotyledons, however it has been observed in underground rhizome of many plants (Esau, 1953). In *B. ciliata*, vascular bundles occurred in a more or less continuous cylinder, secondary growth well observed in vascular region and cambial activity is lacking in interfascicular areas. Cortical and/or medullary bundles are also reported in family Saxifragaceae (Soltis, 2007). Cells of secondary cortex are parenchymatous, irregular to polygonal with intercellular spaces and cork is divided into two zones. *B. ligulata* and *B. stracheyi* also have two separate cork zone, outer zone is made of compressed cells and inner zone composed of thin walled, tangentially elongated cells followed by a single layered cambium (Srivastava and Rawat, 2008). Contrary to the result of Srivastava and Rawat (2008), we found multilayered cork cambium in *B. ciliata*. Vessel-segments have simple perforation plates and tracheids are very few with circular border pits. In saxifragaceous plants, the vessels may have simple or scalariform perforation plates and tracheids if present are small, imperforated and with bordered pits (Bensel and Palser, 1975). Ray cells are absent in *B. ciliata* but are generally found in woody Saxifragaceae (Hils, 1985). Ray cells of *Aphanopetalum* and *Tetracarpaea* lack crystals but contain abundant dark-staining deposits (Hils *et al*., 1988; Dickison *et al*., 1994).

Roots are raised from lower part of the underground rhizome. It is the common feature of the root that the epidermal layer is usually devoid of cuticle. However, sometimes the outermost cell walls including root hairs are covered by cuticle (Scott *et al*., 1963; Guttenberg, 1968). Specialized trichoblast cells which are only capable for root hair production in many plants (Rudall, 2007) are lacking in *Bergenia ciliata*. Therefore, root hairs develop from some of the normal epidermal cells. Suberised exodermis found below the epidermis in the root of *B. ciliata* was also reported in many angiosperm plants like *Citrus* (Cossmann, 1940), *Oryza* (Clark and Harris, 1981), *Smilax* and *Iris* (Fahn, 1990). There is often variation in the number of vascular strands, sometimes even within the same plant, depending upon the diameter of root (Rudall, 2007). In general, dicotyledonous roots possess relatively few xylem strands usually two, three or four. However, we observed seven primary vascular bundles (polyarch) alternating with wide parenchymatous region. Xylem and phloem in bundles are separated by multilayered vascular cambium. Interestingly, polyarch vascular bundle is the characteristic feature of adventitious roots of monocotyledons (Fahn, 1990; Rudall, 2007).

In conclusion, detailed anatomy of vegetative parts of *B. ciliata* no longer comparable with previous reports of woody Saxifragaceae. Many anatomical features are reported first time for the genus and some are new to family Saxifragaceae. In spite of systematically important characters revealed here we couldn't make comparative and systematic analysis on this basis due to lack of sufficient previous reports on herbaceous Saxifragaceae. Further studies are required on other herbaceous genera of Saxifragaceae for comparative study and systematic treatment of anatomical features within the family.

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