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Pharmacognostic Studies on Two Himalayan Species of Traditional Medicinal Value: *Allium wallichii* and *Allium stracheyi*

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Abstract

The present research was aimed as a pharmacognostic study of whole plants of *Allium wallichii* and *Allium stracheyi,* both of which are very important traditional medicinal plants of North-West Himalayas. This study was carried out in terms of macroscopic and microscopic analyses and standard histochemical methods were followed for detecting starch, calcium oxalate, tannins, total lipids and alkaloids. *Allium wallichi* can be distinguished from *A. stracheyi* by possessing polyarch roots, mycorrhizal fungi in the outer cortical cells and triangular leaf midrib. The present study is the first to describe the pharmacognosy in terms of anatomical and histochemical features of these two Himalayan *Allium* species. Although they are listed in Ayurvedic database, the API so far has not given an account on these two species and hence this work is of high importance. Also, the herbal industries, researchers and traditional medicine can now use the distinguishing characters of these species listed in the current paper, while specifically acquiring them from local markets without any confusion.

Keywords: *Allium sp.*, conservation, histology, histochemistry, pharmacognosy

Introduction

In recent times Traditional Knowledge (TK) - based medicine is being increasingly recommended for a healthy life of humans. All traditional systems of medicine mainly focus their attention on the natural products that help the human race to sustain longer and stronger life. Among the world population, 80% of those belonging to developing countries still rely on traditional medicine because of its efficacy, cultural acceptability and lesser side effects (Shrestha and Dhillion, 2003). In India, which is a mega biodiversity country, out of 17500 plant species, 1500 plants are mentioned in ancient literatures for their medicinal properties, but only 800 plant species are actually used in traditional medicine (Kamboj, 2000). The allopathic system of medicine is now increasingly becoming dependent on herbal based treatments. Nearly 25% of its medicine is plant derived (Tripathi, 2002).

Among the Indian traditional medicinal plants, species belonging to the genus *Allium* are particularly important. *Allium* is one of the largest genera of the petaloid monocots, encompassing over 750 species (Friesen *et al*., 2006), which are found primarily throughout the temperate, semi-arid and arid regions of northern hemisphere (Fritsch and Friesen, 2002). Among the 34 species of *Allium* found in India, onion and garlic are very commonly used as vegetable and medicine throughout India and elsewhere. *Allium wallichii* Kunth and *Allium stracheyi* Baker are less known species of *Allium* of high medicinal and culinary value and are commonly used by Himalayan tribal communities like *Bhutia, Bhoxas, Tharus, Koltas, Kinnauries, Junsaris*, etc. (Anonymous, 1961; Atkinson, 1882; Berreman, 1972; Joshi, 1929; Manandhar, 2002; Tiwari, 2010).

The bulbs of these two taxa are boiled, then fried in ghee, and eaten for treatment of cholera and dysentery. The raw bulb is chewed to treat cough and cold and also in case of altitude sickness; they also show good antimicrobial activity against major pathogens. Their juice is used as a moth repellent. Out of these two *A. stracheyi* has become a vulnerable category of species (Ved *et al*., 2003).

A critical survey of literature on the various species of *Allium* (DeManson, 1980; Miryeganeh and Movafeghi, 2009; Uysal, 1999) has revealed that a structural evaluation of these two species has not been done so far.

Hence, this study has been made on morphological taxonomic, anatomical and histochemical features so as to help proper identification and authentication of the two species in samples sold in local markets in the Himalayan region.

Materials and methods

Collection and documentation

The two *Allium* species samples were collected in Uttarakhand, both in dried and fresh forms. Autumn is the best season for the collection of roots, and spring for collection of aerial parts. Accordingly, the samples were collected and stored with the assignment of FRLH ID in the Biocultural Repository, Bangalore. Voucher herbarium sheets for further reference are also deposited in FRLHT's Herbarium.

Histology

Fresh samples of leaves were used for taking leaf and scape epidermal peelings. They were prepared according to the modified method of Clark (1960). The fresh parts were placed in a tube filled with 88% lactic acid, kept hot in boiling water bath for about 50-60 minutes. Lactic acid is used to soften the tissues of leaf so that the peelings could be taken. The samples preserved in formalin-acetic acid-alcohol (FAA) (40% formalin - 5 ml, 50% Ethanol - 90 ml, Glacial acetic acid - 5 ml) (Krishnamurthy, 1988) were used for taking transverse sections of various parts of the plants. These sections were stained with Toluidine Blue O (TBO) 0.05% in benzoate buffer (benzoic acid 0.25 g in 200 ml water pH 4.4) (Krishnamurthy, 1988), washed with water, observed under a microscope (Olympus BX 41, Tokyo, Japan). The photographic images were captured using a digital Olympus camera fixed with the microscope. The images were processed on Image Pro Express 6.0.

Histochemistry

Transverse sections were stained using specific histochemical procedures mentioned by Krishnamurthy (1988) for localizing starch, lignin, calcium oxalate, tannins and total lipids. Dragendorff reagent was used for localizing alkaloids (Joger, 1998; Yoder and Mahlberg, 1976). Photographic images were captured as above.

Results and discussion

Description of plants

Allium wallichii Kunth, from the Alliaceae family (Hooker, 1843; Murti, 1892) is a herbal plant of 40-65 cm height; with cylindrical bulbs, insignificant, stem base covered by old fibrous leaf bases, profusely rooting at base, strongly smelling like leek (Fig. 1). Roots elongate, thick. Bulbs, solitary or clustered, cylindrical, tunic yellowish brown, laciniate or fibrous to subreticulate. Stem 3-8 mm in diam. Glabrous, triangularish. Leaves linear to oblong-lanceolate or lanceolate, shorter than to subequaling scape, (2-)5-20 mm wide, mid vein distinct, base narrowed into a petiole or not. Scape lateral, (10)20-50(110) cm, triangled, sometimes narrowly 3-winged, covered with leaf sheaths only at base or for ca. 1/2 of its length. Spathe 1- or 2-valved, deciduous. Umbel hemispheric, laxly or densely flowered. Pedicels subequal, 2-4 × as long as perianth, ebracteolate. Perianth stellately spreading, recurved after anthesis, pale red, red, or purple to blackish purple, rarely white; segments oblong-elliptic to narrowly so, 5-9 × 1.5-2 mm, apex retuse or obtuse. Filaments subulate, shorter than to subequaling perianth segments, connate at base and adnate to perianth segments. Ovary obovoid-globose, smooth; ovules 2 per locule. Style longer than ovary. Capsule globose to oboided, 4-6x 5-8 mm, light brownish to yellow, trilobed. Seeds oblong, 3.5-4x2 mm, brownish black.

Fig. 1. Habitat of *Allium wallichi* (A) and *Allium strachey* (B)

Local denomination: Gogpa (Bhotia), doona, gobka, laddu, ladu (Hindi), ujnad (Khasi), bpyazi (Nepali) and ksirapalandu (Sanskrit). The last name means safe onion/white onion (Sharma, 1994).

Distribution: This species occurs in India, Nepal, Bhutan, China and Myanmar (Dasgupta, 2006; Hooker, 1894; Rawat et al., 2012) (Fig. 2).

Ecology

It grows above 3200-3625 m, aside running water, hilly slopes or rocky areas, in open conifer forests, in upper sub alpine region and relatively dry and open area.

Flowering phenology: July to November.

Classical usage: It is used as a vegetable and neutraceutical in traditional home gardens (Hanelt, 2001).

Trade aspects: In Nepal dried *Allium wallichii* is sold at the rate of Rs 300-400/kg and dust of Dhupi is sold at the rate of Rs 30-50/kg in the local markets (Chhetri and Gupta, 2007).

Allium stracheyi Bake is a 20-30 cm high slender herb, having, 3.5-5 x 5-1-1.7 cm, oblong-ovoid, fibrous bulbs (Fig. 1). The stem is leafy at the base, terete, glabrous. Leaves 2-4, arising 2-8 cm above the bulb, linear 11-30 cm x 1-2 mm. Umbel subglobose or hemispherical, 1.0-2.5 cm in diam., 8-10 flowered, loose; spathe bifid, ovate, c. 1 x 0.5 cm, acuminate. The plant has oblong-campanulate flowers; with 3-5 mm long pedicels. Tepals rosy purple or yellow, often white with pink veins, broadly-oblong or ovate-oblong, 4-5 x 2-2.5 mm, outer slightly shorter, convex, obtuse, midvein prominent, coherent and pouched at base. Filaments purple, linear, 7-8 x 0.5 mm, free, adherent to perianth at base up to 1 mm, exserted after anthesis; anthers yellow, oblong, 1-1.5 x 0.5-0.7 mm. Ovary oblong, 1.5-2.5 x 1.2 mm, deeply trilobed, lobed winged, covered with large necteral pits by hood like projections; style purple, linear, 3.5-8 mm long, trisulcate; stigma obscure. Capsules globose-oblong, ca. 4-3 mm; pericarp thin papery, yellow, glabrous. 2 seeds in each locule. Seeds elliptic, plano-convex, ca. 3 x 1.7 mm, black, surface uneven.

Local denomination: Jambu (Garhwal and Kumaon).

Distribution: This species occurs in Jammu Kashmir, Himachal Pradesh and Uttarkhand, India, Nepal and Pakistan (Dasgupta, 2006; Hooker, 1894; Rawat *et al*., 2012) (Fig. 2.).

Ecology: It grows above 3200-3625 m, aside running water, on dry open slopes or among rocky boulders.

Flowering phenology**:** July to October.

Classical usage: It is used as a traditional vegetable (Hanelt, 2001).

Trade aspects**:** In Nepal dried *Allium stracheyi* is sold at the rate of Rs. 300-400/kg and dust of Dhupi is sold at the rate of Rs. 30-50/kg in the local markets (Chhetri and Gupta, 2007).

Medicinal uses of the two species: Both species have a common medicinal and nutraceutical value. Young leaves are cooked as vegetable. Dried leaves are used as a condiment in curries and pickles. Raw bulbs are used as flavouring materials for cooking curries. The cloves are used as a substitute for garlic. Flowers are used as a garnish on salads. The juice of the plant is used as a moth repellent. The whole plant is said to repel insects and moles. The bulbs, boiled then fried in ghee, are eaten for the treatment of cholera and dysentery. The raw bulb is chewed to treat cough and cold. It is said that eating the bulbs can ease the symptoms of altitude sickness. It contains sulphur compounds (which give them their onion flavour) and when added to the diet on a regular basis they help to reduce blood cholesterol levels, acts as a tonic to the digestive system and also in vigor the circulatory system.

Histology and histochemistry of *Allium wallichii*

Bulb and scape**:** The bulb consists of a condensed and telescoped underground stem, on whose upper region a large number of leaves arise from a shoot apical meristem and a few stout adventurous roots arise from its middle and lower parts. The scape is located at the centre of the bulb, enclosed by leaves. It consists of a single layer of epidermal cells, covered by a thick cuticle. The epidermis is followed by a parenchymatous region (4-6 layers) and continues sheath of sclerenchyma cells (4-5 layers). The center of the scape is occupied by the ground tissue of parenchyma, in which are embedded variable numbers of collateral vascular bundles (6-8), almost in a ring (Fig. 3).

Root: The roots have single epidermal layer and a wide cortex region consisting of 19-25 layers of parenchymatous cells layers; a few parenchymatous cells of the outermost cortex contain mycorrhizal fungi. The innermost cortical layer is endodermis, whose cells are thickened with lignin. There is a stele at the centre, whose outermost layer is the pericycle. There are 5-9 radiating xylem groups that alternate with an equal number of phloem groups. The protoxylem is exarch (pointing towards periphery). There is no pith. The parenchyma cells of the cortex and stele contain starch grains, lipid droplets and also a few alkaloid deposits (Fig. 4).

Fig. 2. Distribution of *Allium wallichii* and *A. strachey* in the Himalayan region

Leaf: The leaf has a distinct, almost triangular wedge-shaped midrib region as seen in transverse section, with lamina extending on opposite directions from it. The midrib region has two vascular strands, while the lamina on each lateral side may have 6-7 vascular strands. Each vascular strand has a collateral arrangement of xylem and phloem. The xylem region may contain tracheary elements in a group or the elements are a little scattered with intervening parenchyma cells. The mesophyll is homogenous with only spongy parenchyma. Occasional lactiferous canals are found scattered in the mesophyll. The epidermis is very distinct, with large tangential elongated cells. Stomata are arranged in regular longitudinal rows, with regular spatial intervals between them. The whole epidermis is covered by a very prominent and thick cuticle on the outside, which often extends to some distance in the radial walls. Some parenchyma cells contain lipid droplets and / or alkaloids (Fig. 5).

Histology and histochemistry of *Allium strachey*

Bulb and scape**:** The bulbs contain a centrally located underground stem covered by basal sheath of several leaves on the upper side and a number of thin roots that arise from the stem on the lower part. The scape arises at the stem at the center of spem region of the bulb and is either circular or hemispherical in outline, in T.S. depending upon the height at which the sections are taken, with two small depressions in one side; it has a well-defined epidermis, covered by a very thin cuticle and an inner region of ground tissue, fully parenchymatous. A number of collateral vascular bundles are embedded in the ground tissue in two almost concentric rings. The outer rings of bundles are small, while the inner are fairly large. Totally, there are about 7-8 outer bundles and 4-5 inner bundles. A number of lactiferous tubes are seen in the inner ground tissue (Fig. 6).

Root: The transversal section of root shows epidermis, wide cortex and a stele. The stele has etrach-xylem tissue with a large central metaxylem and four small exarch protoxylem groups. The endodermis is the innermost layer of the cortex and is distinguishable very clearly from cortex and stele cells. The cortex is uniformly parenchymatous and no mycorrhizal association is seen in the outer cells of cortex. The epidermis forms a single layer (Fig. 7).

Leaf: Leaves are cylindrical and form almost concentric sheaths around the scape. The midrib, although slightly thicker than lamina, is not wedge shaped as in the previous species and has only one vascular bundle. Several vascular strands are found in lamina. The mesophyll is made up of only spongy tissue in between the adaxial and abaxial epidermises, whose structure is almost similar to that described for the above species. The outer leaves slowly shrink outer and their mesophyll disintegrates slowly only the two epidermises. The outer epidermis becomes almost fully sclerencymatous with only the stomata remaining non-sclerenchymatous.

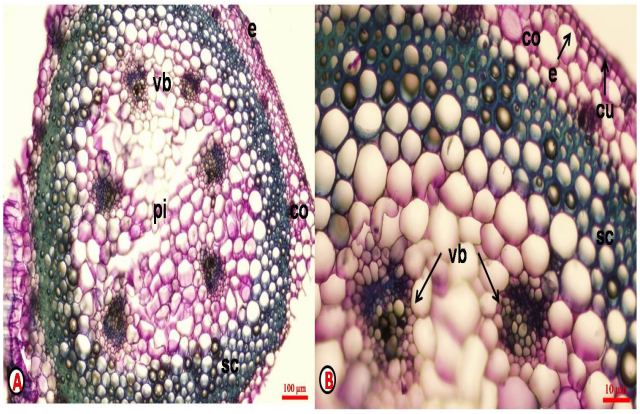

Fig. 3. Transversal section of *Allium wallichii* scape: A) Showing the entire structure of the Scape; B) A portion of the same, showing the two vascular bundles and the peripheral part of the scape; cu - cuticle layer; co - cortex; e - epidermis; sc - sclerenchyma; pi - pith; vb - vascular bundle.

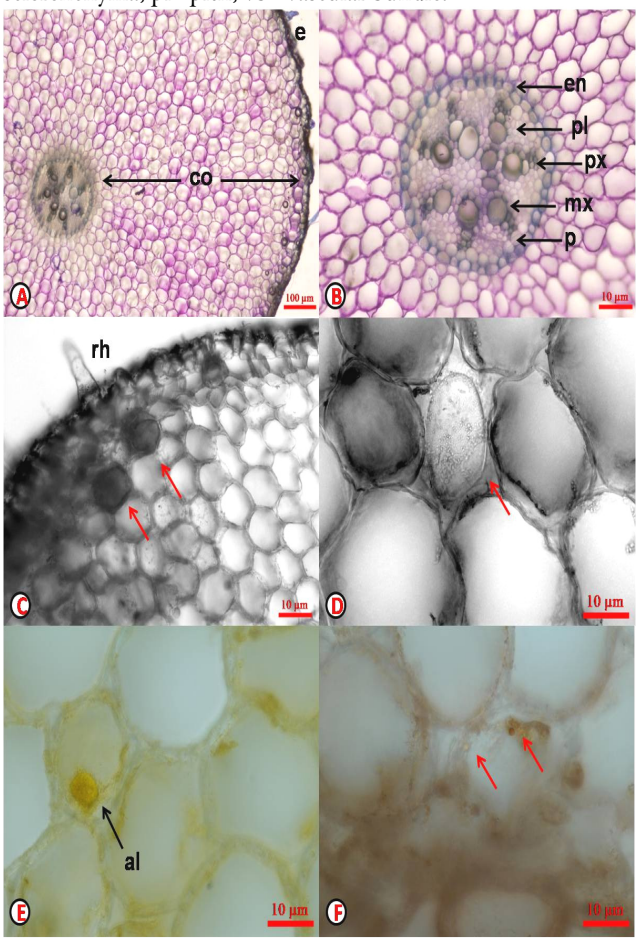

Fig. 4. Transversal section of *Allium wallichii* root: A) T.S. of showing the stele, cortex and epidermis of root; B) Magnified portion of the stele; C) A portion of a T.S. showing the presence of cells with mycorrhizal fungi (see arrows) near the root hair; D) Portion enlarged of C) showing the presence of mycorrhizal fungi; E) Presence of alkaloid (see arrow) treated with Dragondroff's reagent; F) Presence of total lipid (see arrows) stained with Sudan III; al - alkaloid; co - cortex; e- epidermis; en - endodermis; mx - metaxylem; p - pericycle; pl - phloem; px - protoxylem; rh - root hair.

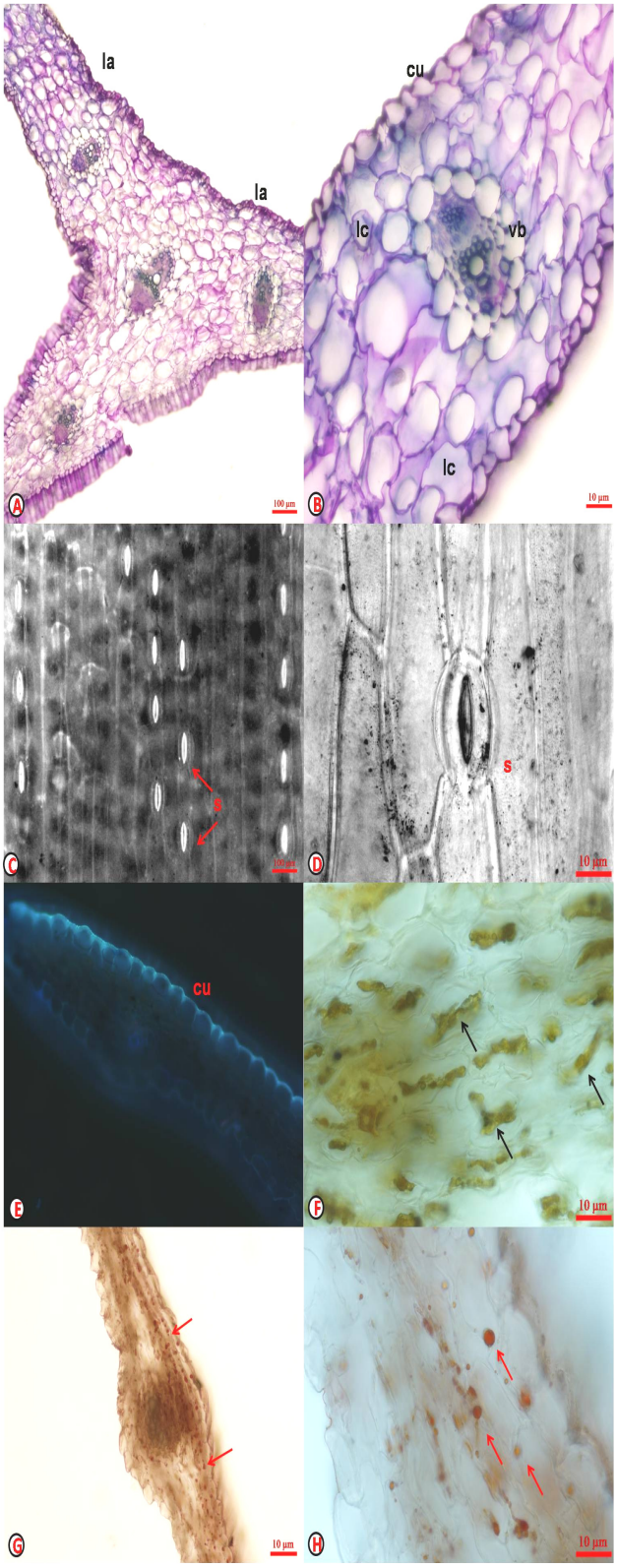

Fig. 5. Transversal section of *Allium wallichi* leaf midrib: B) T.S. of a portion of leaf lamina; C) Leaf peel showing the stomatal arrangement; D) Leaf peel showing magnified image of a stoma. E) Cuticle layer (also with suberin) observed under UV; F) Presence of alkaloid (black arrow); G-H) Presence of lipid droplets (red arrow); cu - cuticle layer; la - lamina; lc - lactiferous cell; vb - vascular bundle, s - stomata.

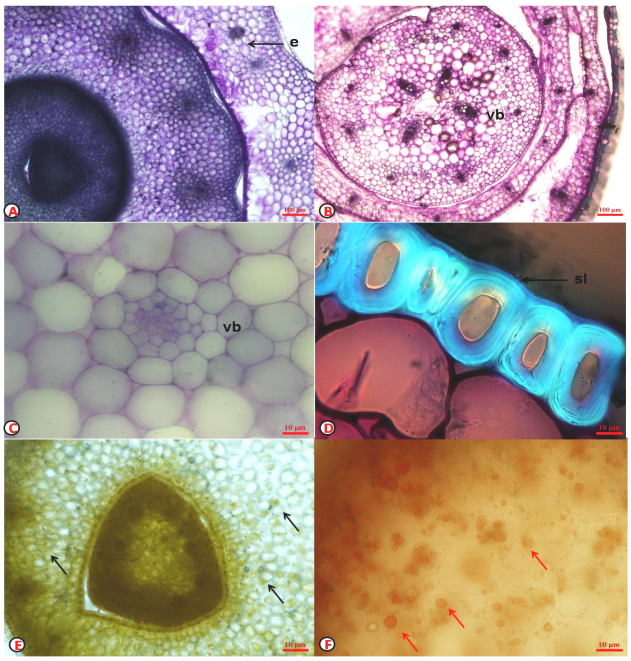

Fig. 6. Transversal section of *Allium stracheyi*. A) T.S. of bulb just above the shoot apex showing concentric rings of successively older leaves; B) T.S. of a bulb with scape enclosed by leaves; C) Vascular bundle leaf magnified; D) T.S. of outermost leaf showing sclereids; E) Presence of alkaloid (black arrows) in leaf cells; F) Presence of total lipids (red arrows); e - epidermis; sl - sclereid; vb - vascular bundle

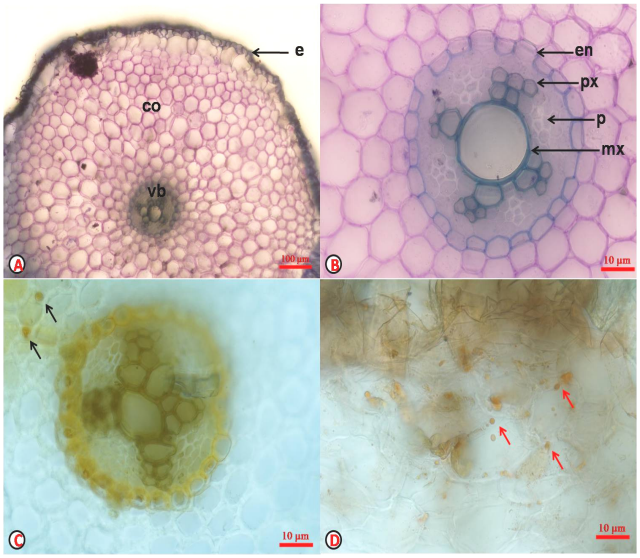

Fig. 7. Transversal sections of *Allium strachey* root: A) T.S. of root showing stele, cortex and epidermis; B. T.S of stele region; C) Presence of alkaloid (black arrow) in cortical cells; D) Presence of lipid droplets (red arrow) in cortical cells; co - cortex; e - epidermis; en - endodermis; mx - metaxylem; p - pith; px - protoxylem

Conclusions

The observations made in this work are important in three respects. Although anatomical studies on vegetative parts of a number of *Allium* species have been done so far by many authors (Miryeganeh and Movafeghi, 2009; Özdemir *et al*., 2011; Yousaf *et al*., 2008), no study has been made so far for the two species studied, which are essentially restricted to Himalayan region. Hence these add information over the anatomy of two more species of *Allium*, along with important additional histochemical data.

The *A. stracheyi* and *A. wallichii* are two important plants of Ayurdevic medicine and have been listed in the database of Ayurveda plants prepared by FRLHT, Bangalore. However, no details on these plants are found anywhere, and particularly in Ayurvedic pharmacopeia of India (API) of both the Editions I & II. Hence, the details obtained from the study can become part of Ayurvedic pharmacopeia of India, which may be revised in future.

Since both plants are found in Himalayan region and both are used in traditional medicine, it is necessary to authenticate the two species as being different from one another, including in market samples sold in Himalayan region. Details given in this paper would help traditional medicine people, Ayurvedic physician and Ayurvedic drug companies to distinguish the two species. *Allium wallichi* can be distinguished from *A. stracheyi* in the following respects: roots are polyarch in the former and tetrarch in the latter; mycorrhizal fungi are present in some of the outermost cortical cells in the former, but not in the latter; leaf midrib is triangular in outline in the former, but not so in the latter.

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