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Anatomy and Developmental Changes in Stem of *Leucas aspera* (Willd.) Link. (Lamiaceae)

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ABSTRACT

Present study deals with anatomical characteristics of immense medicinal precious plant *Leucas aspera* (Willd.) Link. A detailed study on anatomy has carried out in fresh hand cross section of stem, leaf and root of field grown plant after staining of safranin. Quadrangular stem consists of a single layered epidermis having a number of non-glandular and glandular trichomes. Hypodermis consists of 3-5 layers of circular, oval or irregular collenchymatous cells at the ridge. The xylem tissue is radially organized and the parenchymatous phloem tissue is very narrow in stems. Dramatic anatomical changes were observed in the developmental stages. At the younger stage, patches at the corners made of collenchymatous tissue observed as mechanical tissues before developing xylem ring. It was disappeared with the development of xylem ring at the matured stages. Leaf lamina was dorsiventral where epidermis was single layered followed by single layered palisade and 4-6 layered spongy cells, bears non-glandular and glandular trichomes as that same found on stem. Amphistomatic leaf consists paracytic stomata. The midrib located conjoint and collateral vascular bundle associated with a parenchymatous pericycle layer on lower side. TS of root was circular with a single layered thick walled epidermis. Xylem vessels, fibers and medullary cells were distinct with peripheral secondary phloem tissue. Xylem reached at the center of pith. This study clears the anatomical features that was lacking previous studies and also notify changes of anatomy at the developmental stages.

Key words: Anatomy, Developmental changes, Leaf, *Leucas aspera*, Root, Stem

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1. INTRODUCTION

Leucas aspera (Willd.) Link. (Family: Lamiaceae) commonly known as Danda-kolosh or Shetodron in Bangladesh and 'Thumbai' in India (Rai et al., 2005). *L. aspera* is commonly found throughout India and the Philippines as well as the plains of Mauritius and Java (Srinivasan, 2011). In India and the Philippines, it is a very common weed (Deb, 1983). *L. aspera* is typically found in dry, open, sandy soil and is abundant in areas with waste. It is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid acutely quadrangular stem and branches. Leaves are sub-sessile or shortly petiolate, linear or linearly lanceolate, obtuse, pubescent, with entire or crenate margin; flowers white, sessile small (Kirtikar & Basu, 1975; Hooker, 1984). In entire plant of *L.*

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aspera examined presence of triterpenoids as preliminary chemical (Kamat & Singh 1994). Three compounds such as oleanolic acid, ursolic acid and 3-sitosterol contain in whole plant is accounted (Chaudhury & Ghosh, 1969). The plant is used traditionally as an antipyretic and insecticide. Flowers are valued as stimulant, expectorant, aperient, diaphoretic, insecticide and emmenagogue. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites (Rai et al., 2005; Shirazi, 1947). *In vitro* study of chloroform and ether extracts of *L. aspera* revealed its antifungal activity against *Trichophyton* and *Microsporum gypseum*. *L. aspera* had both fungistatic and fungicidal actions (Thakur et al., 1987). *L. aspera* was tested for its prostaglandin (PG) inhibitory and antioxidant activities (Sadhu et al, 2003). The smoke of leaves of *Vitex negundo* and *L. aspera* are more toxic to the filarial vector mosquito, Culex quinquefasciatus than the synthetic mosquito mats, which contain 4% d-allethrin (Selvaraj et al., 1994). The essential oils from *L. aspera* possessed bacteriostatic activity (Rao & Narasimha, 1971).

Comparative anatomy is used as a tool in the plant systematic studies. Anatomical characters are very important in perceiving systematic and phylogenetic relationships of particular plant groups. Plant taxonomic studies traditionally use morphological and karyological (Stebbins, 1953), as well as micromorphological characters (pollen and trichomes) (Hayat et al., 2009; Bak & Ozcan, 2018). Indeed, anatomical features can provide useful characters which could help in identification of problematic plant taxa, as well as establishing their taxonomic relationships (Metcalfe & Chalk, 1957; Scatena et al., 2005; Makbul et al., 2011; Sosa et al., 2014; Karanović et al., 2015). According to Dengler (2002), anatomical data can also be useful in determination of the systematic status and evolutionary relation among the genera and species. The limited number of plants of the Lamiaceae family had been undertaken in anatomical study previously. There are a number of studies on anatomy (Metcalfe & Chalk, 1972; Kahraman et al., 2009; Kahraman et al., 2010a,b), trichome morphology (Serrato-Valenti et al., 1997; Corsi & Bottega, 1999; Kaya et al., 2003; Siebert, 2004; Kamatou et al., 2007; Ozkan, 2008) and palynology (Henderson et al., 1968; Cantino et al., 1992) of the genus. The usefulness of the structure of the vascular bundles in petioles for species identification in the family Lamiaceae has been demonstrated (Metcalfe & Chalk, 1972). The taxonomic significance of the structure of trichomes is well known in the Lamiaceae and related families (Metcalfe & Chalk, 1972). Pollen morphology has been pointed out to be useful in systematics of the Lamiaceae (Abu-Asab & Cantino, 1994). The anatomical, palynological and trichome structure of most *Salvia* species in Turkey have been already investigated (Kahraman et al., 2010). However, a few anatomical studies have been carried out in *Leucas aspera* (Yashvanth et al., 2011; Kumar & Devanna, 2016; Prajapati et al. 2010). Since the experimental plant is very important biochemically and has a great impact in socioeconomic, an overall anatomical study is crucial. A detail anatomical study in stem, leaf and root was undertaken in *L. aspera* in the present study.

2. MATERIALS AND METHODS

Naturally field grown *Leucas aspera* (Willd.) Link. plants were collected from the inferior dry land of Barind region (Fig. 2A, B). Healthy whole plants with stems, branches, leaves and roots were brought to Crop Botany laboratory of

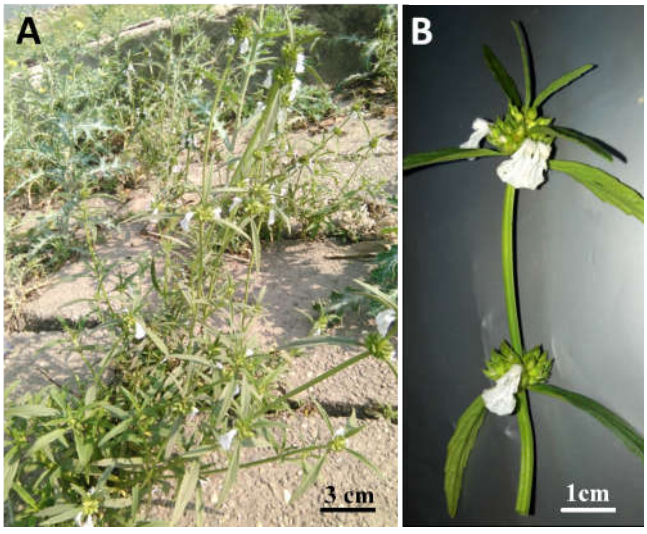

Fig. 1 Naturally grown *Leucas aspera* (Willd.) Link. in field. A) Plants grown in the field. B) A close view of a flowering part of experimental plant.

EXIM Bank Agricultural University Bangladesh, Chapainawabganj. The stem and root slices and whole leaves were separated and washed them through running tap water to remove dirt and soil. The stem slices were taken from three positions at 2nd internodes, 4th internodes and 6th internodes from the tip. Free hand transverse sections were prepared from fresh samples of stems, roots and leaves. For preparation of stem and root sections, slices of them were cut into thin transverse sections with stainless steel razor blade by hand and all sections were immerged into water in a petri dish. The transverse sections were prepared from leaf by cutting thin with stainless steel razor blade by hand with the help of a potato block and all sections were placed into water in a petri dish. Peel of leaf was used to observed stomata at upper and lower surface. Thin and uniform transverse sections of stem, root, leaf and peel of leaf were isolated and kept into 1% (w/v) safranin solution for a period of 4-5 minutes and rinsed with distilled water for 1 minutes in several times to remove stain. The stained sections and peel were placed on fresh glass slide and mounted with a clean cover slip by few drops of glycerin and then observed under light microscope. The photographs were taken with digital camera and different types of cells undertaken to measure cell diameter. Ten cells for each type from ten

sections were used to measure cells or stomata number and diameter. Numbers of stomata counted from a square unit (200 µm^2).

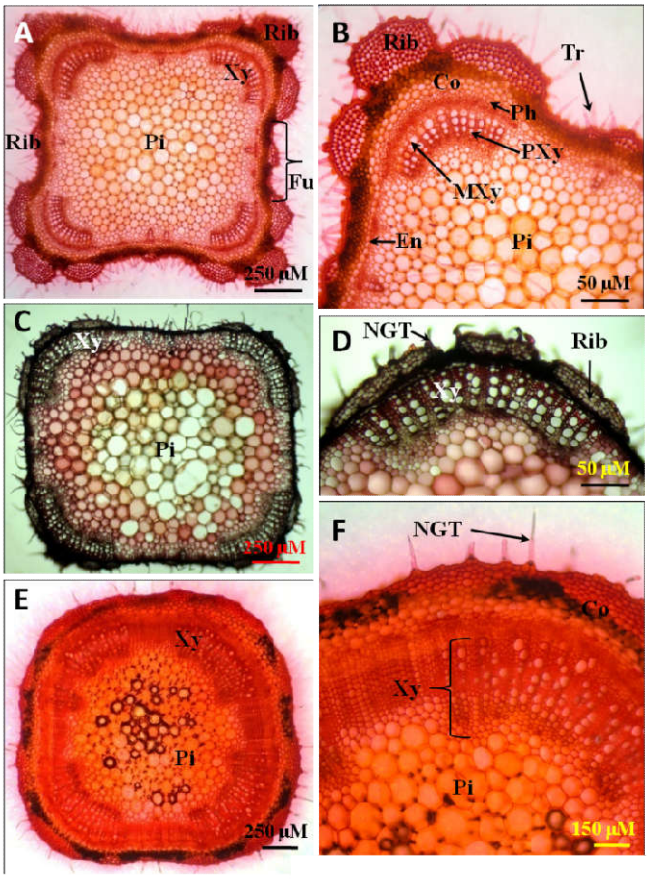

Fig. 2 Stem anatomy and developmental changes at different stages of *Leucas aspera* (Willd.) Link. A) Transverse section of 2nd internode, B) A close view of a part of Fig. A, C) Transverse section of 4th internode, D) A close view of a part of Fig. C, E) Transverse section of 6th internode, F) A close view of a part of Fig. E. Fu-Furrow, Xy-Xylem, Pi-Pith, Co-Cortex, Tr-Trichome, Ph-Phloem, PXy-Protoxylem, MXy-Metaxylem, En-Endodermis, NGT-Non-glandular Trichome.

Table 1 Leaf parameters of *Leucas aspera*. (100 samples were taken for each parameter).

	Parameters	Upper surface (Mean± SE)	Lower surface (Mean± SE)
1	Stomata Number/ 200 square µm	9.6	15.8
2	Stomata Diameter (µm)	42.41±1.26	41.21±0.94
3	Subsidary cell Diameter (µm)	73.10±2.03	73.45±4.33
4	Epidermal cell Diameter (µm)	86.90±4.71	86.21±4.27

SE- Standard Error

3. RESULTS AND DISCUSSION

3.1. Anatomy of Stem

TS of young stem shows quadrangular with four prominent furrows and each angular and middle of furrows contained three and one pronounced patches, respectively composed of collenchymatous tissue (Fig. 2 A, B). These patches serve as diagonally placed I-girders for withstanding flexion. The pronounced patches in stem reported in *Xeranthemum annuum* (Gavrilovic et al., 2019). Section consists of a single layered epidermis that composed of oval to rectangular, thin-walled cells (average diameter 44.48 ± 2.00 µm, Fig. 3). The epidermis of the

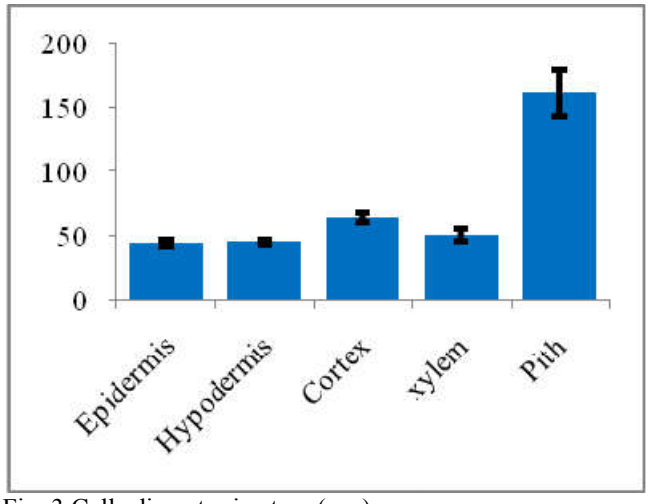

Fig. 3 Cells diameter in stem (µm).

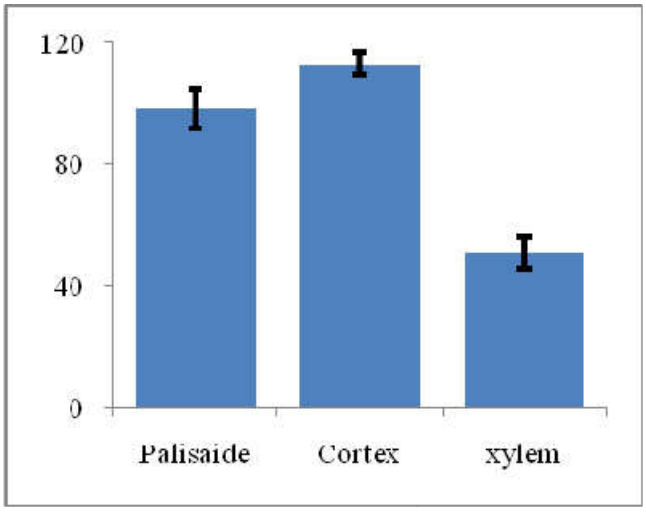

Fig. 4 Cells diameter in leaf (µm).

stem is covered in a thick waxy cuticle. As dermal tissue system, epidermis having a number of multicellular trichomes and contains few traversed stomata. Trichomes are both non-glandular and glandular (capitate sessile glandular) with multicellular head (Fig. 5). The types of capitate glandular trichomes observed in *S. smyrnea* corresponded

respectively to the three types of capitate trichomes described by Werker et al. (1985), Bourett et al. (1994) and Serrato-Valenti et al. (1997) in their study of some Lamiaceae species.

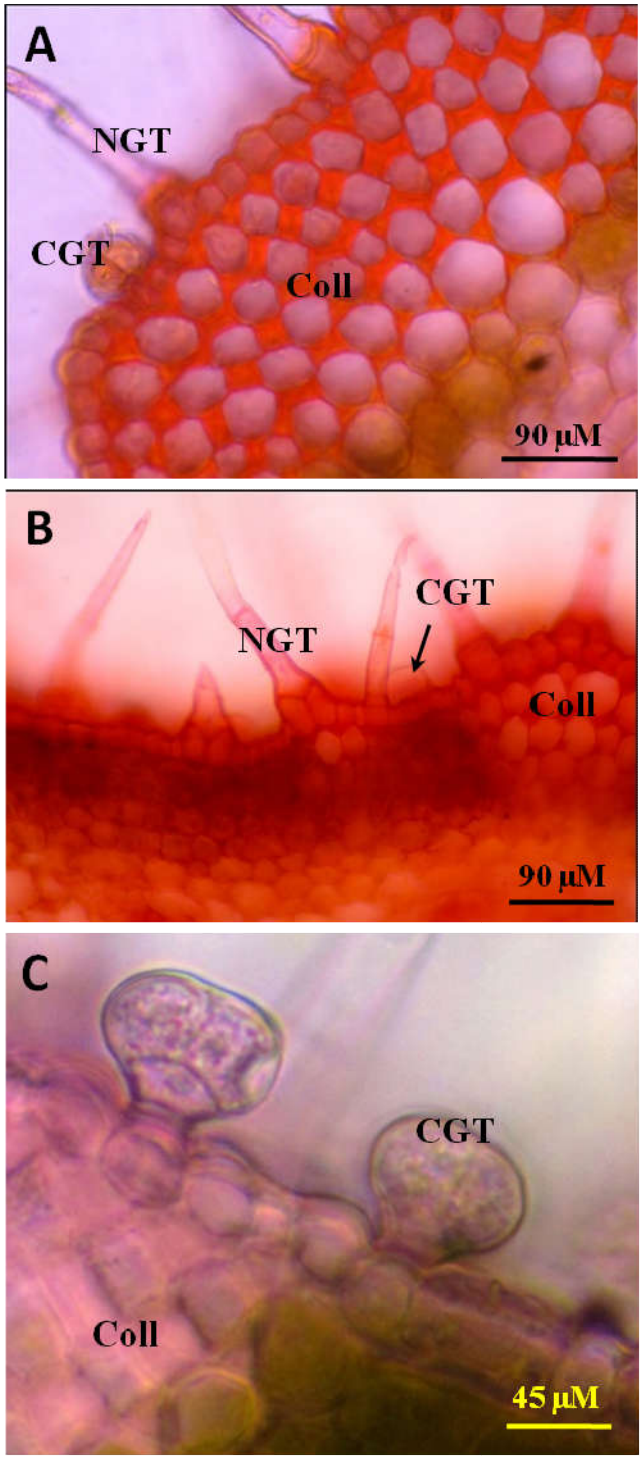

Fig. 5 Close views of stem sections of *Leucas aspera*. A) Collenchyma cells in rib of stem, B) Non-glandular trichomes, C) Glandular trichome. NGT-Non-glandular trichome, CGT-Capitate glandular Trichome.

Hypodermis consists of circular, oval or irregular collenchymatous cells at the patches 5-9 layers of young stems (Fig. 2 A, B) and at the ridge 3-5 layers of mature stem (average diameter 45.17 ± 1.56 µm) (Fig. 2 E, F, Fig. 3). Two-eight-layered collenchymatous tissue is located at the corners of the stem in *Salvia chrysophylla* (Lamiaceae), was reported previously (Kahraman et al., 2010c). The cortex was 3-5 layered composed of thin-layered parenchymatous larger cells (Fig. 2 E, F) and average diameter showed 63.97 ± 3.57 µm (Fig. 3). Endoderm is single layered, consisting of barrel shaped, thin-walled cells; pericycle single layered of thin-walled cells comparatively smaller than the cells of endodermis. A wide stele supposed to be found in the sections. Typically in younger stems the xylem tissue is radially organized and the parenchymatous pholem tissue is very narrow. Xylem consists of metaxylem (average diameter 50.34 ± 5.14 µm, Fig. 3) and protoxylem, fibers, fibers and large amount of xylem parenchyma. Pith wide consisting of circular to oval cells was of thin walled parenchymatous and showed average diameter 50.34 ± 5.14 µm (Fig. 3). The comparable characteristics in cortex, endoderm, pericycle, xylem tissue and pith of *Leucas aspera* have also reported by Yashvanth et al. (2011) and Kumar & Devanna (2016).

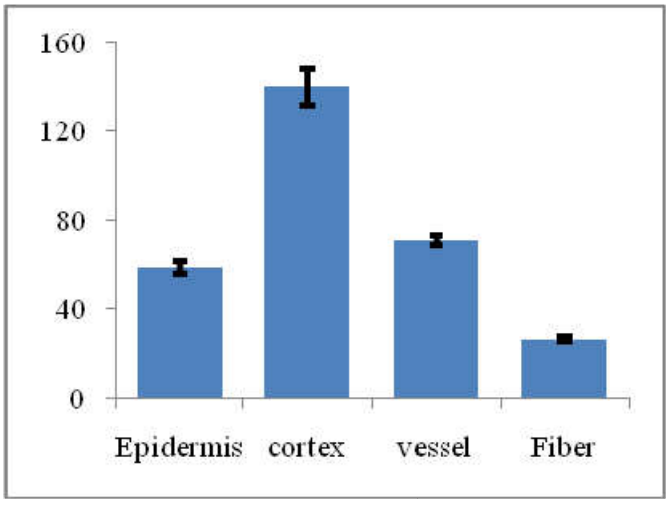

Fig. 6 Cells diameter in root (µm).

3.2. Anatomical Changes in Stem at Developmental Stages

The anatomical changes at the developmental stages in stem section were observed at 2nd internode, 4th internode and 6th internode from the tip of *L. aspera*. The 2nd internode was younger stage, showed quadrangular outline with four furrows and pronounced patches. The patches were made of collenchymatous cells. It was existed not only on four angular (three ribs in each angular) but also within furrows (one rib in each furrows) (Fig. 2 A, B). Among the patches on corner, middle patch consisted of 8-10 layers collenchymatous cell layer and 4-5 layers in side patches. The patches in furrows were contained 2-3 layers of collenchymatous cell. Four angular stem shape also found in

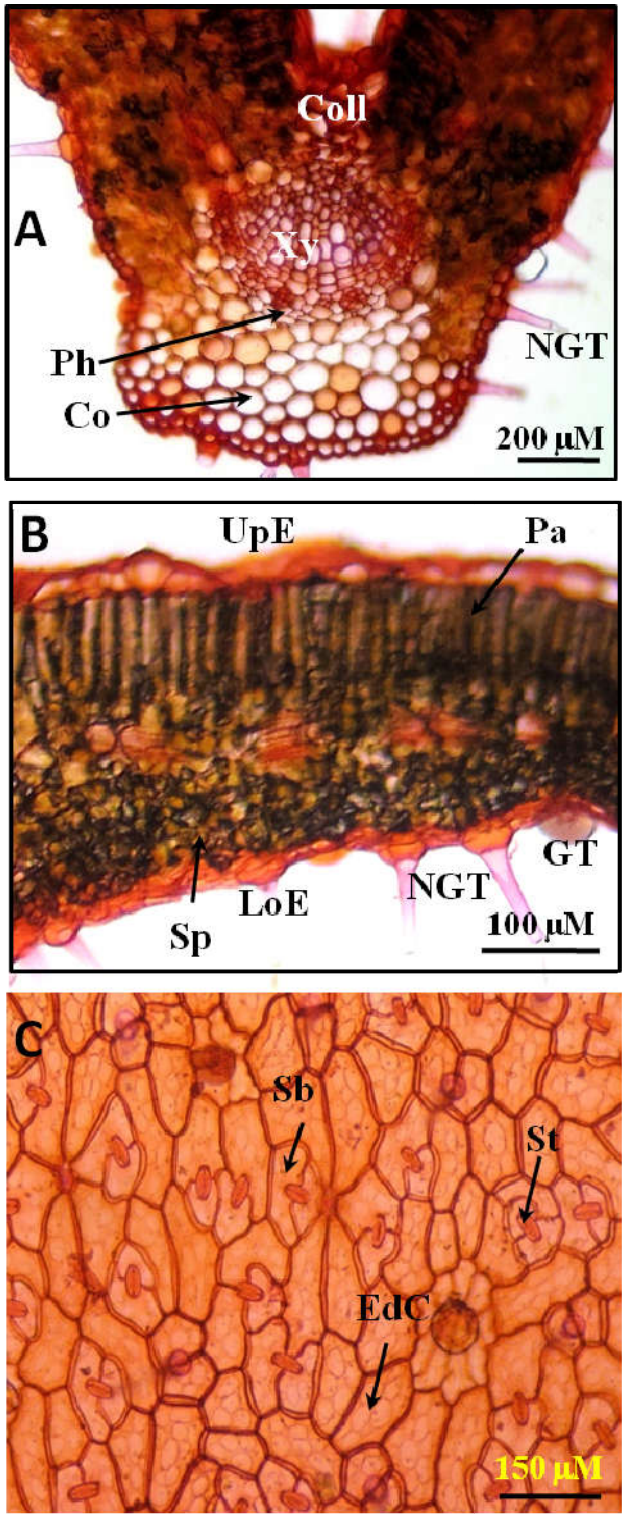

Fig. 7 Anatomical features in leaf sections of *Leucas aspera*. A) Section of a midrib, B) Section of lamina, C) Lower surface of leaf. Coll-Collenchyma, Xy-Xylem, Ph-Phloem, NGT-Non-glandular Trichome, Co-Cortex, UpE-Uper-epidermis, Pa-Palisade cell, Sp-Spongy cell, LoE-Lower-epidermis, GT-Glandular trichome, St-Stomata, Sb-Subsidiary cell, EdC-Epidermal cell.

the TS section of 4th internodes but patches were not so prominent and it was absent in furrow. The middle patch exposed 4-5 layers collenchyma and side patches showed 3-4 layers collenchyma cells (Fig. 2 C, D). In TS of 6th internode, outline showed almost round shape. The patches were approximately absent (Fig. 2 E, F). A single-layered epidermis was present in TS sections of 2nd internode, 4th internode and 6th internode. The trichome number of 2nd internode was higher and it was gradually decreased in 4th internode and 6th internode sections (Fig. 2). Cortex was 5-7 layered in 2nd, 4th and 6th internode section. Xylem tissue constituted with schelerenchyma cells was discontinuously constructed just placed bellow the corner in 2nd internode sections. Twelve-fifteen xylem strands with a 3-5-celled line were present. A few strands (1-2 strands) were found in furrow region. Pronounced collenchymatous patch at 2nd internode sinks by half number of cell layer at 4th internode sections with increasing double number of xylem layer. Though xylem did form ring shape in this growing stage. Collenchymatous patches demolished at 6th internode sections where xylem layer formed a ring shape structure with 15-20-celled line (Fig. 2). It was clarified that demolition of collenchymatous patch with detonation of xylem layer. The collenchyma cell and xylem are given mechanical support to the plant. Therefore, it is suggested that collenchyma as mechanical supporting tissues developed first that support to young stem. With forming of xylem mechanical tissues, collenchymatous tissues will be decreasing.

3.3. Anatomy of Leaf

Leaf of *Leucas aspera* is opposite, subsessile or short petioled, linear or narrowly oblong- lanceolate, entire or distantly crenate, obtuse, narrowed at the base (Deb, 1983). Leaf showed distinct midrib. Lamina showed 2-4 side veins. In dorsiventral lamina, epidermis was single layered followed by single layered palisade cells and 4-6 layered parenchymatous spongy mesophyll cells, covered with thick cuticle (Fig. 7). The results were agreed with the observation of Kumar & Devanna (2016) in *Leucas aspera*. The non-glandular and glandular trichomes (capitate sessile glandular) both were present in leaf as that found on stem (Fig. 7). The both types of trichomes observed in *Salvia chrysophylla* (Lamiaceae) (Kahraman et al., 2010c). Paracytic stomata were observed on both the surfaces (amphitomatic) (Fig. 7C), was also investigated formerly (Yashvanth et al., 2011) in *Leucas aspera*. The stomata were counted on upper and lower surfaces by 9.6 and 15.8 at 200 µm^2 area, respectively (Table 1). Dorsiventral and amphistomatic leaf blade in *Salvia chrysophylla* reported previously (Kahraman et al., 2010c).The diameter of subsidiary cell and epidermal were recorded at 50.34 ± 5.14 µm and 50.34 ± 5.14 µm, respectively (Table 1). Collenchymatous tissue underneath both the epidermis and one layered palisade tissue occupying the major area of the section and spongy parenchyma (Fig. 7B). TS of leaf passing through the midrib is broadly convex on the lower side and

slightly grooved or flat on the upper side, a centrally located conjoint and collateral vascular bundle associated with a parenchymatous pericycle layer surrounded (Fig. 7A). The same characteristics as midrib (convex and flat type) and xylem surrounded by parenchyma reported earlier in *Salvia chrysophylla* (Kahraman et al., 2010c). Region below vascular bundle was filled with collenchymas (Fig. 7A). The position of collenchyma was same in the midrib of *Leucas aspera*, illustrated by Yashvanth et al. (2011) and Kumar & Devanna (2016).

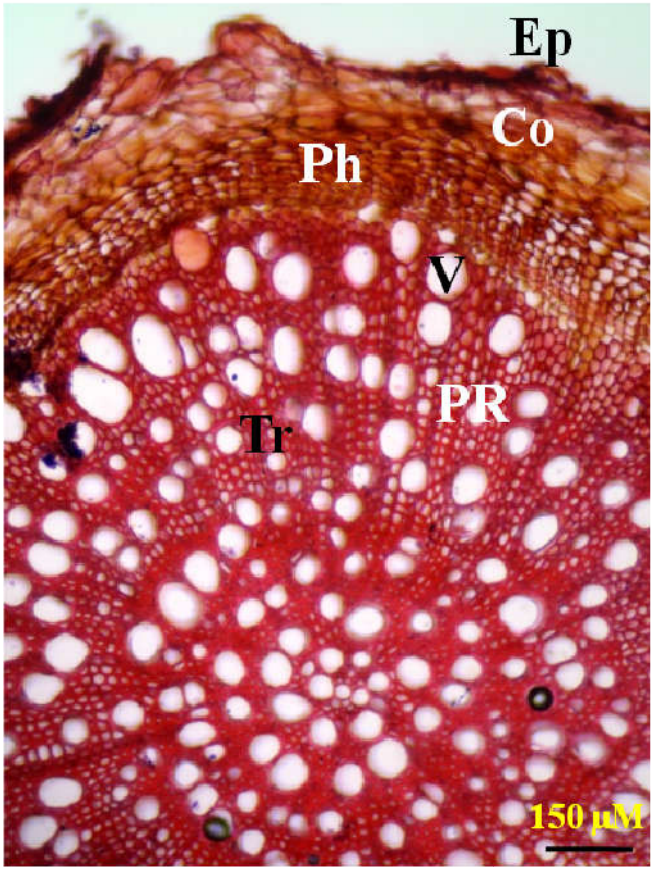

Fig. 8 Anatomical features in root sections of *Leucas aspera*. Ep-Epidermis, V-vessel, Ph-Phloem, Tr-Tracheid, PR-Pith ray.

3.4. Anatomy of Root

The outline in cross section of root was circular in *Leucas aspera*. A single layered endodermis was present on the surface of the root composed of rectangular cells (Fig. 8). Three-five layered cortex was parenchymatous with tangentially elongated and composed of irregular cells, similar to the consequence of Yashvanth et al. (2011) and Kumar & Devanna (2016) in *Leucas aspera*. Since secondary growth was observed in this plant, secondary tissues were noticed in the cross-sections. The dominant part of the root cross sections was the secondary xylem composed of vessels and fibers, similar report to Gavrilovic et al. (2019) in *Xeranthemum annuum* (Compositae). The peripheral secondary phloem tissue was existed beneath the cortex layer (Fig. 8). From the root anatomy point of view, *Leucas aspera* has medullary rays composed of many rows of cells 2-4 seriate like other species of Lamiaceae previously studied (Kahraman et al., 2009; Kahraman et al., 2010a,b,c). Due to dominant part of secondary xylem, parenchymatous pith was not in attendance (Fig. 8).

4. CONCLUSION

The general anatomical features in stem of *Leucas aspera* studied were similar to previous report in this plant. The dramatic changes in anatomical features observed in developmental stages. The collenchyma, mechanical tissues were originated at the younger stages i.e., 2nd internode sections. The collenchyma tissues gradually decreased at the more developmental stages i.e., 4th and 6th internodes sections. On the other hand, xylem, mechanical tissues discontinuously observed in younger stages i.e., 2nd internode sections. It was gradually increased at the more developed stages. The collenchyma tissues were diapeared with increased of xylem tissue. The non-glandular and glandular trichomes were present in stem and leaf sections. The number of trichomes reduced with the development of stems. Leaf was amphistomatic and paracytic type stomata showed. The secondary xylem composed of vessels and fibers was the main part of the root cross sections and it attended to center of root, as a result parenchymatous pith was not there. The present study will be helpful for further anatomical studies of this plant as well as other species of the Lamiaceae family.

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