

Stem, Leaf and Root Anatomy of *Eclipta alba* (L.) Hassk. (Asteraceae)

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ARTICLE INFO	ABSTRACT
<p>Received date: August 25, 2018 Accepted date: Dec. 18, 2018</p>	<p>Anatomical studies on transverse sections of stem, leaf and root were conducted under light microscope to find out identical characteristics of <i>Eclipta alba</i> (L.) Hassk, belongs to the Asteraceae family. Free hand transverse sections stained with safranin were studied here. In stem section, epidermis, hypodermis, endodermis, vascular bundle and pith characters were general without cortex. A number of air cavities setup scatteredly in cortex, where seen secretory structures closed to air cavities. Average diameters determined 34, 62, 20 and 92 μm in cortex, air cavity, metaxylem and pith cells, respectively. Amphistomatic leaf sections presented a relatively thick cuticle layer on the adaxial surface. Anisocytic stomata ($19 \pm 0.1 \mu\text{m}$ in diameter) at a large quantity in the abaxial surface, counted 4 stomata per μm^2. The mesophyll was dorsiventral, composed of one layer palisade and four layers spongy parenchyma. The vascular system in leaf was open, collateral conjoint forming a flattened arch in the triangular shape. Secretory structure as single or group located laterally in the cortical parenchyma of leaf sections. At leaf blade side, upper epidermis, spongy and palisade cells showed 37, 33 and 74 μm diameter, respectively. Upper epidermis, cortex and metaxylem cells diameter at midrib were 12, 38 and 14 μm, respectively. In root section, the cortical zone (cortex cell 49 μm) was wide and a large region occupied with air cavities (72 μm) although other parts like epidermis (32 μm), endodermis and vascular bundle (metaxylem 23 μm) characteristics were standard. The thick cuticle on the adaxial surface of leaf is a special feature for the plant studied. The presence of air cavity in stems and roots suggested to adaptability in aquatic condition. The secretory structure in stems and leaves can be an identical character for the experimental plant as well as Asteraceae family.</p>

Key words: Anatomy, Asteraceae, *Eclipta alba*, Leaf, Root, Secretory structure, Stem

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1. INTRODUCTION

Eclipta alba (L.) Hassk. (also known as *Eclipta prostrata* Roxb.) belongs to the Asteraceae family, is commonly

known as false daisy in English and bhringoraj in Bengali. It is regarded as a weed of ethnomedicinal significance, is a small C_3 annual herb whose stem is usually erect, round, blackish green, profusely branched and pubescent

(Mansoorali et al., 2012; Shaikh et al., 2012; Sivarajan, 1994; Neeraja & Margaret, 2011). *E. alba* being as a broad-based in distribution, is abundantly found in India, China, Brazil, Bangladesh and the United States. Habitat destruction, reclamation of wetlands and changes in climate seem to cause pressure on the survival and distribution of this plant as a result it has become rare and endangered in many parts of the world especially in Bangladesh and India (Soni and Soni, 2017).

Many chemical compounds have been isolated from *E. alba* like resins, ecliptine, nicotine and glucosides. The plant extract accommodates plenty of bio-active steroidal alkaloids that have cytotoxicity property against certain cells. Extract of *E. alba* has been shown to inhibit snake venom phospholipase A2 activity of rattle snakes venom. The inhibitory activity from snake venom has been attributed to the coumestans, wedelolactone and demethylwedelolactone present in the extract of *E. alba* (Diogo et al., 2009). A number of other chemicals that have so far identified to exist in the plant extract are wedelic acid, apigenin, luteolin, b-amyirin, etc. The compounds wedelolactone, apigenin, and luteolin have been phytochemically analyzed a dose dependent Hepatitis C virus inhibition and anti-HCV replication activity in cell culture system (Tabassum & Agrawal, 2004). Wedelolactone has been reported to have the property used for treating hepatitis and cirrhosis (Wagner et al., 1986), as antibacterial, and antihemorrhagic (Kosuge et al., 1985). Petroleum ether and ethanol extract of *E. alba* have reduced time of hair growth (Roy et al., 2008).

The plant anatomy is a general term for the study of the internal structure of plants. It is now frequently investigated at the cellular level, and often involves the sectioning of tissues and microscopy. Anatomical studies have much significance in different sectors of investigation, can explain where, what, when and how level chemical compounds deposited. It can also describe cellular changes; cellular abnormalities and can clarify the qualities of the wood properties. Anatomical studies have shown to be an important tool associated with taxonomic studies, mainly when there is no reproductive organ in the investigated sample (Solereder, 1908; Metcalfe & Chalk, 1950). The limited number of plants of the Asteraceae family had been undertaken in anatomical study previously. Castro et al. (1997) reviewed the types of secretory structures in the leaves of 72 plants including *E. alba* of the Asteraceae family. When analyzed together these secretory structures, eight types were mentioned: ducts, cavities, idioblasts, laticifers, hydathodes, extrafloral nectaries, trichomes and glandular appendixes. Ekeke & Mensah (2015) reported on secretory ducts in leaf midrib of 17 species of Asteraceae and observed that twelve species have secretory duct. According to Metcalfe & Chalk (1950), anatomical diversity is commonly observed in leaves structure of species belonging to the Asteraceae. Among the features that vary are: stomata distribution on leaf surfaces; guard cell positioning in relation to ordinary epidermal cells; hypoderm development on the upper side of the leaf

surface; mesophyll and fibrovascular system differentiation; and wax secretion on leaf surface (Solereder, 1908). The anatomical features that can be observed in Asteraceae are: presence of various types of glandular or covering trichomes; papillae on the abaxial epidermis; anomocytic, anisocytic and rarely heliocytic stomata; presence of hydathodes; presence of hypoderm; homogeneous or heterogeneous mesophyll; and vascular bundles with parenchymatic sheath composed by large cells (Metcalfe & Chalk, 1979; Rahman et al., 2013; Milan et al., 2006). Cellular nature of pericarpic region was studied in a special type of fruit of Asteraceae known as cypsela (Paul, 2017). The study of Gharage & Menon (2017) investigated influence of polluted water with industrial effluents on the morphology and anatomy of *E. alba*. Stem anatomy of *E. alba* examined by Pimentel et al. (2012), observed that greater secretory structures over the phloem and fundamental parenchyma. The vascular bundles are arranged in a circular line inner the central cylinder and colorless medullar parenchyma. Khan et al. (2013) studied on transverse section of root anatomy.

Eclipta alba has great importance in the fields of cosmetics and pharmacy due to the production of essential oils. Regarding the importance of this species as medicinal plants there are few information about their stem, leaf and root (organ used for medicinal purposes) anatomy. From the above description, it was also clarified that leaf anatomy literature are perceptible but stem and root anatomical studies were very rare. Therefore, the objective of this paper was to describe the anatomy of mature stem, leaf and root of *Eclipta alba* with emphasizing to identify typical structures.

2. MATERIALS AND METHODS

Naturally grown *Eclipta alba* (L.) Hassk. was collected from the inferior dry land of Barind region (Fig. 2A). Healthy whole plants with stems, branches, leaves and roots were brought to laboratory of EXIM Bank Agricultural University, Bangladesh. The stem, root and leaves were separated and washed them through running tap water. Free hand transverse sections were prepared from fresh samples. For preparation of stem and root sections, slices of them were cut into thin transverse sections with stainless steel blade by hand and all sections were immersed into water in a petridish. The transverse section prepared from leaf by cutting thin with stainless steel blade by hand with the help of a potato block and all sections were placed into water in a petridish. Thin and uniform transverse sections of stem, root and leaf were isolated and kept into 1% (w/v) safranin solution for a period of 10 minutes and rinsed with distilled water for 5 minutes in several times to remove stain. The stained sections were placed on fresh glass slide and mounted with a clean cover slip by few drops of glycerin and then observed under light microscope and photographs were taken with digital camera. Ten cells of a photograph and photographs of ten sections were used to measure cells or stomata diameter. Number of stomata counted each μm^2 .

3. RESULTS AND DISCUSSION

3.1. Stem Anatomy

The explanation of anatomical characteristics is an effective tool in interpreting phyletic evaluations and systematic descriptions (Metcalfe & Chalk, 1950).

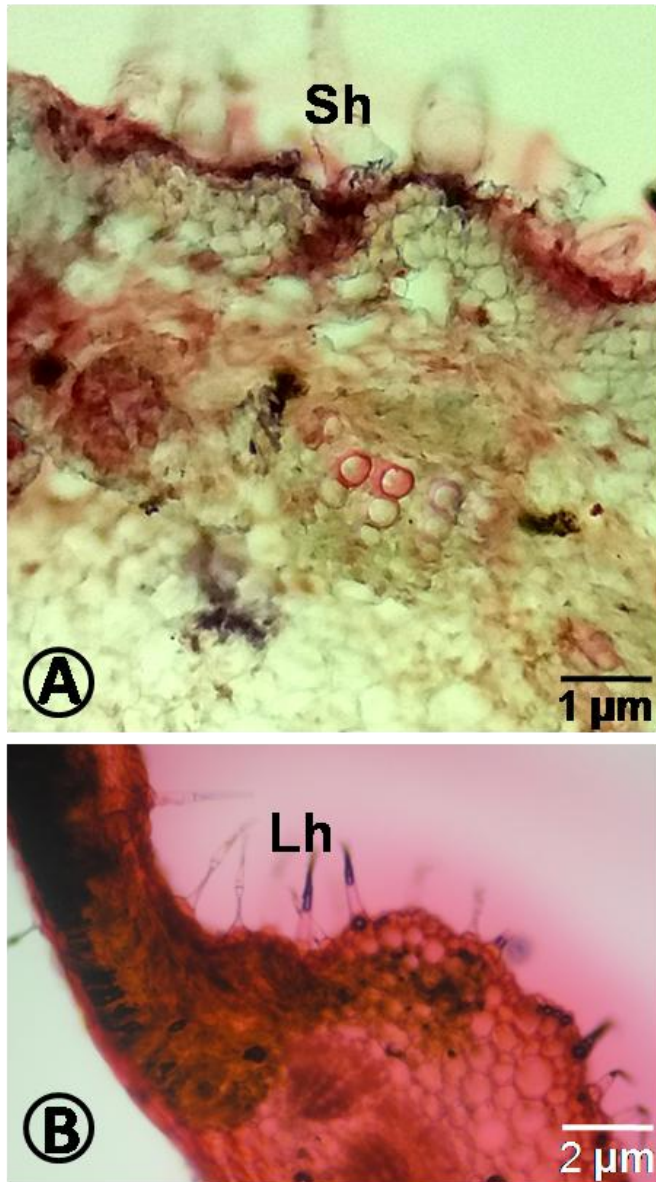


Fig. 1 Transvers sections of stem and root of *Eclipta alba* (L.) Hassk. A) Stem section at juvenile stage, showed stem hair (trichome). B) Leaf section showed leaf hair (trichome). Sh: stem hair, Lh: leaf hair.

This study was undertaken to assessment of anatomical features in stem of *E. alba*. Cross section of the stem was generally circular shape, covered with thick cuticle layer. Epidermis was the outermost covering a layer of the stem, was a single layer of compactly arranged by barrel-shaped parenchyma cells with slightly thick walled where intercellular spaces were absent.

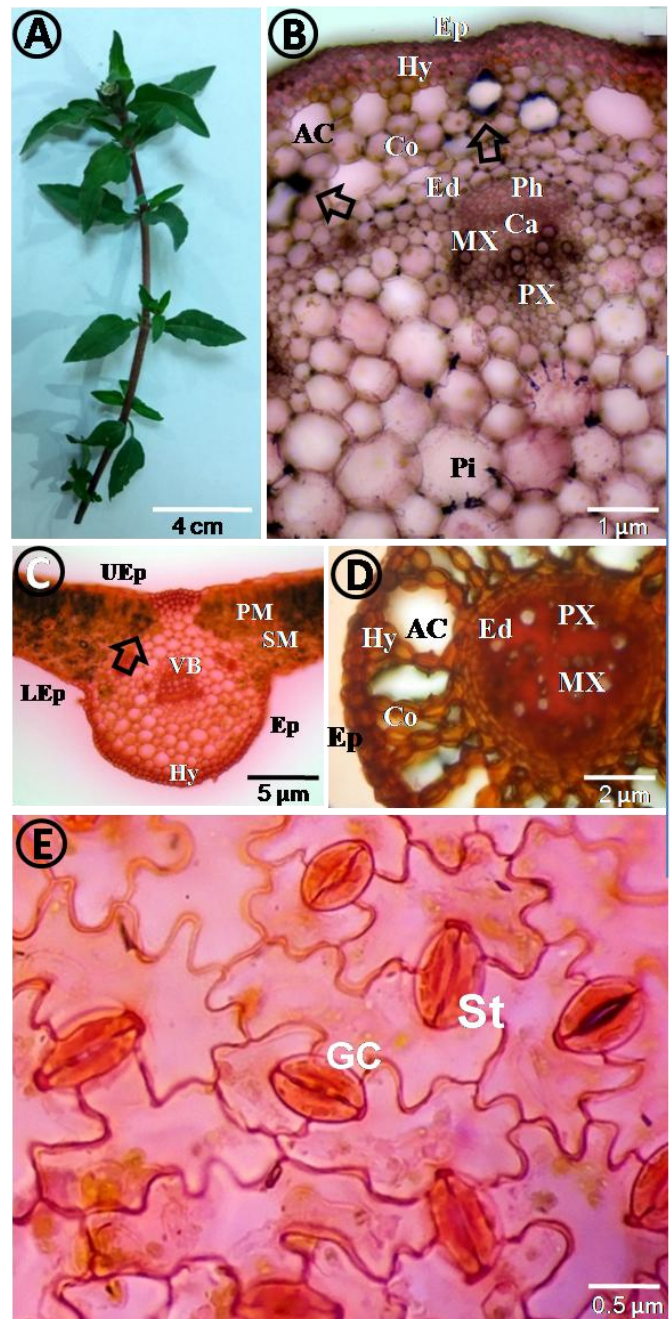


Fig. 2 Anatomical study on *Eclipta alba* (L.) Hassk. A) Plant of *Eclipta alba*. B, C, D) Stem, Leaf, Root section, respectively; E) Stomata on lower surface of leaf. Ep: Epidermis, Hy: Hypodermis, Ac: Air cavity, Co: Cortex, Ed: Endodermis, Ph: Phloem, Ca: Cambium, MX: Metaxylem, PX: Protoxylem, Pi: Pith, UEp: Upper Epidermis, LEp: Lower epidermis, PM: Palisade mesophyll, SM: Spongy mesophyll, VB: Vascular bundle, St: Stomata, GC : Guard cell. Arrows indicate secretory structures.

Epidermis did not show trichomes in mature stages (Fig. 2B) but it was present at the juvenile stages (Fig. 1A). Hypodermis was a region lying immediately below the

epidermis in the stem sections. It was characterized by a few layers (3-5 layers) of collenchyma cells with angular thickenings. The cells were compactly arranged without any intercellular spaces (Fig. 2B).

Cortex is the major part of the stem represented by several layers of loosely arranged parenchyma cells and intercellular spaces were prominent. The average diameter was examined by $34 \pm 0.2 \mu\text{m}$ (Fig. 3). The several number of small and large air cavities (average diameter $62 \pm 0.3 \mu\text{m}$) were found in the cortex area and their distribution was scattered (Fig. 3). The secretory structures were available found near to some air cavities (Figs. 2B). The presence of secretory structure in *E. alba* was contradictory observation of Ekeke and Mensah (2015). A wavy endodermis was the innermost layer of cortex corresponds to compactly arranged barrel-shaped cells without any intercellular spaces (Fig. 2B). The experimental plant is a vascular plant; therefore xylem and phloem were present as conducting tissue. The vascular bundles were conjoint collateral open type and xylem arrangement showed endarch (Fig. 2B). The average diameter of metaxylem was $20 \pm 0.1 \mu\text{m}$ (Fig. 3). The cambium layer was distinguished with several layers (3-4 cells layers). Pith was an innermost part of the stem formed by a group of loosely arranged parenchyma cells (average diameter $92 \pm 0.6 \mu\text{m}$) where intercellular spaces were prominent (Fig. 2B, 3). The anatomical structure in the cross section of the stem was studied with the general characteristics of some species of the Asteraceae (Milan et al., 2006; Rahman et al., 2013; Ekeke & Mensah, 2015). In stem section of *E. alba*, description of the anatomical features by Pimentel et al. (2012) were not detailed. The present study screened detail feature in stem of studied plant within the presence of air cavity and secretory structure in cortical region.

Table 1 Different cell diameters in leaf of *Eclipta alba*.

Parameters	Range of diameter (μm)	Diameter (μm) $\bar{x} \pm \text{SE}$
Leaf blade		
Upper epidermis	20 - 60	37 ± 0.1
Spongy cells	20 - 56	33 ± 0.1
Palisade cells	51 - 100	74 ± 0.1
Midrib		
Upper epidermis	10 - 21	12 ± 0.2
Cortex cell	16 - 61	38 ± 0.2
Meta xylem	10 - 23	14 ± 0.2
Stomata		
Stomata on lower surface	13 - 28	19 ± 0.1
Subsidiary cells on lower surface	22 - 75	38 ± 0.2

3.2. Leaf Anatomy

Leaves of *E. alba* are simple (i.e., lobed or unlobed but not separated into leaflets), opposite: there are two leaves per node along the stem. The edge of the leaf blade has teeth

(Fig. 2A). At transversal view, the leaf is covered with a simple uniseriate epidermis with regular sized greater cells ($37 \pm 0.1 \mu\text{m}$ diameter) (Table 1). As epidermal tissue system, cuticle, stomata and trichome were found on epidermis layer of *E. alba* (Fig. 1A, 2B, 2C). Both surface of epidermis was warped with cuticle layers where a thick cuticle layer obtained on the adaxial surface. The presence of cuticle on aerial leaf surface is a common feature. The thick cuticle in leaf surface can be a special treat for a plant. The thick cuticle on the adaxial surface (Fig. 2C) of the *E. alba* species must be an important characteristic for protection from microorganism or insect. It has been interpreted as an adaptive strategy in many plants (Dickison, 2000; Larcher, 2000). The cuticle recovers the epidermis of the plant and acts as an interface between the inside and outside of the organism (Bukovac, 1990) and must be considered in the interventions aiming at the chemical control of plants (Procopio, 2003).

The stomata occur in both surfaces (i.e., amphistomatic leaf) of *E. alba*. It was located at various levels in different epidermal layers, in the form of anisocytic at a large quantity in the abaxial surface, counted 4 stomata per μm^2 (Fig. 2E). The average diameter of stomata was $19 \pm 0.1 \mu\text{m}$. Subsidiary cells were large and average diameter found $38 \pm 0.2 \mu\text{m}$ (Table 1).

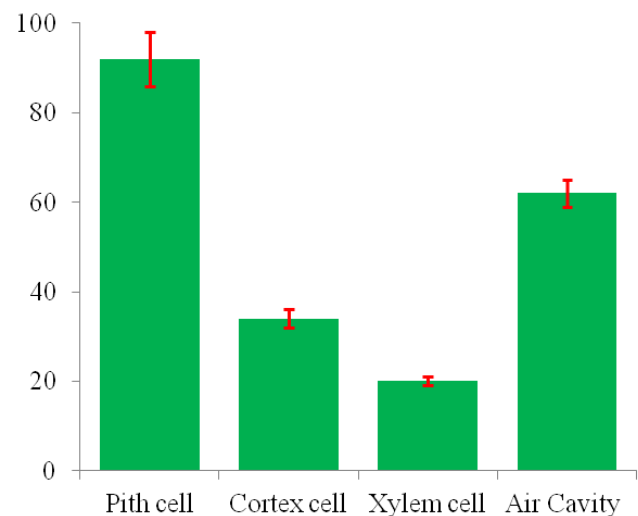


Fig 3 Different cell diameters in stem of *Eclipta alba*.

The amphistomatic leaves generally present larger quantities of stomata at the epidermis of the abaxial surface (Greulach, 1973), to which it seems to be a preventive mechanism against photoinhibition, since the adaxial surface is more exposed to solar radiation, as most of the leaves are in the horizontal position (Smith et al., 1998). Stomata on two leaf surfaces characterized in some species of Asteraceae (Kaur & Nagpal, 2016; Milan et al., 2006) and are ecologically less variable characters (Dilcher, 1974).

Trichomes, particularly the secretory ones, are considered important tools for taxonomy (Solender, 1908; Metcalfe & Chalk, 1950; Fahn, 1979; Theobald et al., 1979).

In the present study, distribution of non-glandular trichomes was scattered through the entire leaf blades (Fig.1B), are of the conical type, presenting a large base and a thin extremity, and ornamentations on the wall. Trichomes were multicellular and presented a set of bulky epidermis cells arranged radially at the base. They were visually larger and more numerous, frequency in the abaxial surface (Fig. 1B). The glandular and nonglandular trichomes are distributed widely in the some species of Asteraceae family (Milan et al., 2006; Aschenbrenner et al., 2013; Saha & Mukherjee, 2012) including many *Eclipta* species (Sharma et al., 2017).

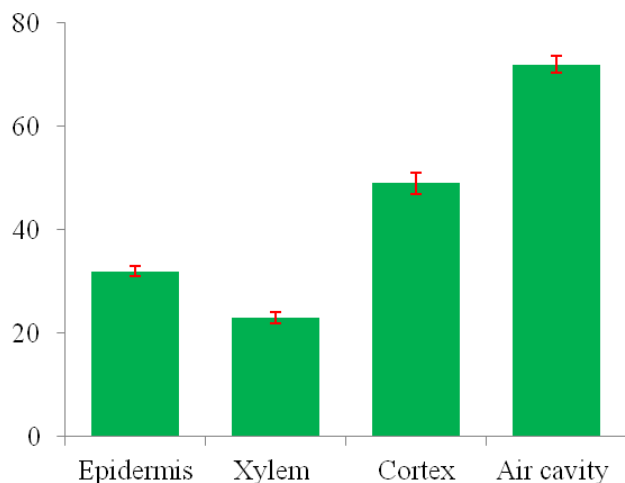


Fig. 4 Different cell diameters in root of *Eclipta alba*.

It is not a unique character for the plants of Asteraceae due to absence in *Porophyllum ruderale* Cass. (Milan et al., 2006). The multicellular non-glandular trichomes observed in *E. alba* in the present study, similar statement reported in this plant earlier (Rahman et al., 2013). The largest frequency of the trichomes in the adaxial surface of *E. alba* can be related to several factors such as protection against excessive radiation and high temperatures, as reported in the literature (Valkama et al., 2003). The penetration of herbicides in the plant tissues is essential for the effective chemical control of weed (Procopio, 2003). The anatomical characteristics practically determine the ease with which these products can be absorbed (Hess & Falk, 1990). Trichomes in the leaf surface can intercept pulverized drops, preventing these from reaching the epidermis. The efficiency of herbicide absorption by the trichomes and their translocation to the epidermic cells are still partially unknown (Hess & Falk, 1990). However, the absorption of these substances can in part take place through the trichomes (Hull, 1970). Some authors consider that trichomes, especially the non-glandular types, are an efficient point for the penetration of herbicides in the plant tissue. Hess & Falk (1990) observed the negative relation between herbicide adherence in trichomes and the efficiency of these products. Thus, the high frequency of non-glandular trichomes in the adaxial surface of *E. alba* would be advantageous for the

species when undergoing chemical control strategies, which are important distinctive characteristics for this species.

The average diameter of epidermis cell measured $37 \pm 0.1 \mu\text{m}$ at leaf blade portion (Table 1). One layer of collenchyma was on upper rib and two layers of collenchyma on lower rib in the median region of the main vein. The mesophyll is dorsiventral, with one layer of palisade ($74 \pm 0.1 \mu\text{m}$ in diameter) and 4 layers spongy ($33 \pm 0.1 \mu\text{m}$ in diameter) with conspicuous intercellular lacunae (Table 1, Fig. 1C). The vascular system was open, forming a flattened arch in the triangular shape (Fig. 1C). The dorsiventral arrangement in mesophyll observed in *E. alba*, is common characteristic in plants of the Asteraceae family (Milan et al., 2006). The vascular bundle sheath was absent in leaf, therefore it was a C_3 plant. The contrast of this result was observed in 13 species of *Isostigma* (Asteraceae) (Peter & Katinas, 2003).

At midrib section, the average diameter of epidermis cell measured $12 \pm 0.2 \mu\text{m}$ (Table 1). Secretory structure is an identical characteristic for plant. In the plant studied here, the secretory structures located as single or group structures in adaxial surface, mesophyll cells and cortical parenchyma cells ($38 \pm 0.2 \mu\text{m}$ in diameter) of leaf vein (Table 1, Fig. 2C). The vascular bundles are arranged in a circular line inner the central cylinder and colorless medullar parenchyma in experimental plant. The size of metaxylem measured $14 \pm 0.2 \mu\text{m}$ in diameter (Table 1). The presence of secretory structure in *E. alba* was reported previously as ducts (Pimentel et al., 2012). Existing secretory structure in *E. alba* would be an identical characteristic. Thus, this characteristic could be a unique for this species as well as plants of Asteraceae family, being important for taxonomic purposes.

3.3. Root Anatomy

Transverse section of the root showed the presence of periderm which consisted phloem containing blackish brown matter. The phellogen (cork cambium) showed two rows of cells encircling the single layer of phelloderm. The root was rough with irregular fissures on the surface (Fig. 2D). The cortical region comprising of randomly arranged parenchymatous cells, was lying below the epidermal. The cortical zone was wide where cells attached together as column at certain regions by a three-celled layer and larger other regions occupied bare, known to as air cavity. The cortical region composed of thick walled barrel shaped cells (Fig. 2D). The average diameter of epidermis cell, cortical cell, air cavity and metaxylem cell were $32 \pm 0.1 \mu\text{m}$, $49 \pm 0.2 \mu\text{m}$, $72 \pm 0.16 \mu\text{m}$ and $23 \pm 0.1 \mu\text{m}$, respectively (Fig. 4). Endodermis was prominent and single layered, was arranged with barrel-shaped parenchymatous cells. Underneath the endodermis, one layer of a pericycle was surrounded as circle. The vascular bundle was collateral in nature with phloem arranged outwards and the xylem situated inwards occupying the whole area of the section. The phloem elements were located in a tiny area. The central cylindrical secondary xylem was lignified. Cambium has separated xylem and phloem bundles. The xylem was exarch in nature

i.e., the arrangement in which the protoxylem is directed towards the periphery and metaxylem towards the centre (Fig. 2D). The large, solitary, circular and thick walled vessels located at the periphery of stele. The central pith was found to be absent (Fig. 2D). The observation in root anatomy was similar to the study of Khan et al. (2013).

4. CONCLUSION

The results presented in this study allowed the identification of stem, leaf and root anatomical characteristics. The thick cuticle on leaf can be a vital character for acting defence mechanism of *Eclipta alba* (L.) Hassk. Trichomes of leaf and stem were exposed in immature stages of plant and it disappears at the mature stage. The presence of air cavity in cortex of stem and root is to be an identical characteristic of *E. alba*. This characteristic is a hydrophytic trait although experimental plant was collected from dry land terrestrial habitat. The secretory structure located in the cortex in stem as well as in periderm, mesophyll and cortical parenchyma in leaf. It can be an identical anatomical characteristics that allowed to distinguish *E. alba* from the other species of Asteraceae, which is being important taxonomically. The present study will be helpful for further anatomical studies of this plant as well as other species of the Asteraceae family.

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