

Internal Structure of Vegetative Part in *Euphorbia helioscopia* L.

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Abstract: A notable progress has occurred in the anatomical study of vascular plants and its applications in taxonomy. The major criteria selected for the study involves internal structures of *Euphorbia helioscopia* L. Free hand transverse sections were prepared from stems, leaves and roots and after stained it was observed under light microscope. Foliar surface was also studied in the present investigation. As major portions in stem section, cortex, vascular and pith zones were occupied by 19.70%, 35.60% and 44.70%, respectively. At the periphery beneath epidermal layer, the hypodermis was formed by an angular collenchymatous parenchyma with small intercellular spaces and contained laticiferous canals. The stem axis contains a continuous ring of vascular tissue. The xylem was radially arranged with uniserrated medullary rays. The phloem was narrow containing laticiferous canals. Pith was made up of parenchymatous cellulosic cells with numerous auriferous cavities. The results of leaf anatomy showed dorsiventral mesophyll where palisade cells (35.80 μ m in diameter) and spongy cells (12.80 μ m in diameter) were arranged. Laticiferous canals were observed in mesophyll cells. The ratio of midrib region composed of 61.54% cortical zone and 38.46% vascular zone. The transverse section of root was circular in outline, encircled by cork cells. A wide xylem layers was present and it was radially arranged vessels, tracheids, uniserrated and multiserrated medullary rays and extended up to phloem. The major portion i.e., cortex, vascular and pith were composed of 25%, 65% and 10%, respectively. Laticifers were readily seen in the cortical parenchyma and secondary phloem in the root. The study conducted here layout a clear anatomical view of *Euphorbia helioscopia* will be helped to solve the taxonomical obscurity.

Keywords: Anatomy, *Euphorbia helioscopia*, Laticifers, Taxonomic Problem, Vegetative Part

1. Introduction

Euphorbia helioscopia (Sun Spurge) belongs to the Euphorbiaceae family, is a smooth annual plant with an erect, stout stem from eight to twelve inches high, often branched from the base. It is an annual medicinal plant commonly grown as weeds during winter season, used in folk medicine of various countries around the world. It has green or reddish stalks in upright or prostrate position (either single numerous, simple or ramified). The branches, as well as the main stem, end in a more or less compound umbel which is subtended by a circle of leaflets. The plant contains white lacteal juice. The extract of this plant have anticancer, activation of tumors, inhibition of HLV-1 multiplication, eye burns, antibacterial, viral and fungal effect [1-3].

During the last decades a remarkable evolution has occurred in the anatomical study of vascular plants and its

applications in taxonomy [4]. Metcalfe and Chalk [5] stated that the anatomical features of *Euphorbia* show a wide range of differentiations in relation to the habitat diversity and that any prominent trait does not occur throughout the numerous tribes into which the family is divided.

The earlier anatomy studying on Euphorbiaceae had been reported about formation of laticifer [6]. Epidermal characteristics of plants were chosen because they are mildly influenced by ecological conditions and are of high diversity in structure [7]. Former studies [8-10] showed that epidermal characteristics are also reliable in plant systematics and classification. Stem and leaf anatomy *E. helioscopia* with other plants of *Euphorbia* species were studied [11]. Talebi *et al.* [12] reported on the leaf anatomy of 18 *Euphorbia* taxa including *E. helioscopia*. Foliar anatomy of some taxa of Euphorbiaceae had been reported [13]. Singh and Isfaq [14] investigated leaf and stem anatomy of *E. helioscopia*. Zahra *et al.* [15] investigated eight leafy species of *Euphorbia* for

differences in the features of foliar epidermal anatomy. Furthermore, leaf epidermal features of 150 species of *Euphorbia* were examined by Kakkar and Paliwal [16]. Their study showed that papillae in different shapes and sizes were found on these cells. In addition, various types of stomata as well as epidermal cell shapes have been recorded.

Comparative internal structure study on *Euphorbia* carried out for first time [6]. Stem and leaf anatomy of 11 species in longitudinal and cross sections were investigated. The present study deals with anatomical characterization of various plant parts along with determination of its phytoconstituents. The stem and leaf anatomy of *E. helioscopia* were studied a limited area but root anatomy was not so far. The root anatomy of *E. helioscopia* will be first report in the present study.

2. Materials and Methods

Euphorbia helioscopia is naturally grown in different places at Rajshahi, Bangladesh. The plants were collected from the Agricultural field of Rajshahi University during December-February at the optimum growing period. Some healthy plants were selected at the research sites and then whole plants. Stems, leaves and roots were the plant materials of the present study (Figure 4A). The stems, leaves and roots of these plants were separated from the whole body and washed them running tap water to remove dirt. The section preparation and staining methods were done following by the procedure of Sultana [17], Rahman and Sultana [18-20], Rahman et al. [21], Sultana and Rahman [22], and Sultana [23, 24]. Free hand transverse sections were prepared from stems, leaves and roots. For preparation of stem and root sections, slices of them were cut into thin and uniformed sections. For preparation of leaf sections, the leaf slice holding midrib were placed in a potato block and then sections were cut thin with stainless steel razor blade by hand. Thin and uniformed sections were separated carefully. For observation of the stomatal and epidermal tissues, the fresh leaves were used. Small sizeable portions of the leaf specimens were obtained from standard median level of matured and well-expanded leaves. Epidermal peels of both abaxial and adaxial surfaces of leaf were made and placed it on a clean glass slab, with the surfaces to be studied facing down. The specimens were irrigated with water holding and it downwards from one end, and then the epidermis above the desired surface was scraped-off carefully with a sharp razor blade. The loose cells were washed away from the epidermal peels with the aid of hair brush and water until the desired epidermis below was reached. The sections of stem, root and epidermal peels were stained in 1% aqueous solution of Safranin for 4 minutes, rinsed carefully in water to remove excess stain and mounted in 10% glycerol. Keen and minute observation was made for each of the 25 slides. Microphotographs were taken with digital camera. The numeric data were determinate at the studied specimens in microphotographs.

3. Results

3.1. Stem Anatomy

The major criteria selected for the study involves internal structures of *E. helioscopia*. The stem anatomy of *E. helioscopia* shows epidermis, cortex region, laticiferous canal, xylem, phloem, medullary ray, and pith. As major portions in stem section, cortex, vascular and pith zones covered by 19.70%, 35.60% and 44.70%, respectively (Figure 1). The transverse section of stem was circular at outline where outer most layer epidermal cells were isodiametric (21.10 μm in diameter) with external thick wall and covered with thin cuticle (Figure 4B, 5A). It was usually smooth or straight around the surface of plant. At the periphery beneath epidermal layer, the hypodermis was formed by an angular collenchymatous parenchyma with small intercellular spaces and contained laticiferous canals (Figure 4B). The cells of hypodermis was 28.8 μm in diameter (Figure 5A). In the internal cortex, big auriferous cavities and small cortical vascular bundles were observed (Figure 4B). At the phloem periphery and in the upper third of the stem, cordons of incipient sclerenchymatous fibers were present. In the rest of the stem, these fibers are complete enlightened, having very thick walls, but slightly lignified in the mass (Figure 4A). Endodermis was well developed and phloem was narrow containing laticiferous canals. The cambium was present between the xylem and phloem, whereas xylem was radially arranged, with uniserrated medullary rays. The stem axis contains a continuous ring of vascular tissue. The metaxylem was distinct that was measurable (35.7 μm in diameter). In the centre, pith was present and made up of parenchymatous cellulosic cells (78.20 μm , Figure 3A). In the pith, numerous auriferous cavities were observed.

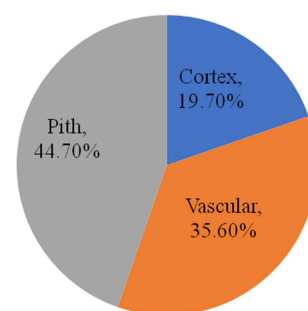


Figure 1. The parentage of major regions covered the cross section of stem.

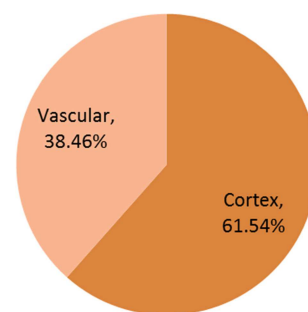


Figure 2. The parentage of major regions covered the cross section of leaf.

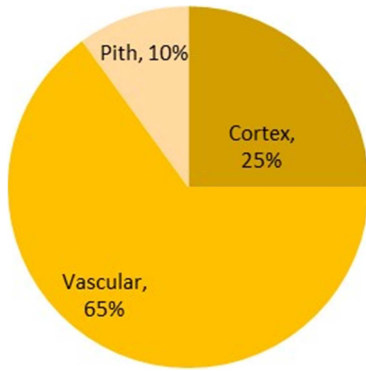


Figure 3. The parentage of major regions covered the cross section of root.

3.2. Leaf Anatomy

Leaves of *E. helioscopia* were alternate, exstipulate and bi- or hetero-facial structure. The transverse section of the studied plant samples, leaf was covered with adaxial (upper epidermis) and abaxial (lower epidermis). The epidermal cells were slightly tangentially elongated. The shapes of leaf epidermal cells were recorded as polygonal in *E. helioscopia*. The shape and size of epidermal cells was similar in both adaxial (15.60µm) and abaxial (14.10µm) surfaces (Figure 4C). The patterns of the anticlinal walls of epidermal cells were straight. No trichomes were present on both abaxial and adaxial surfaces in leaf of *E. helioscopia*.

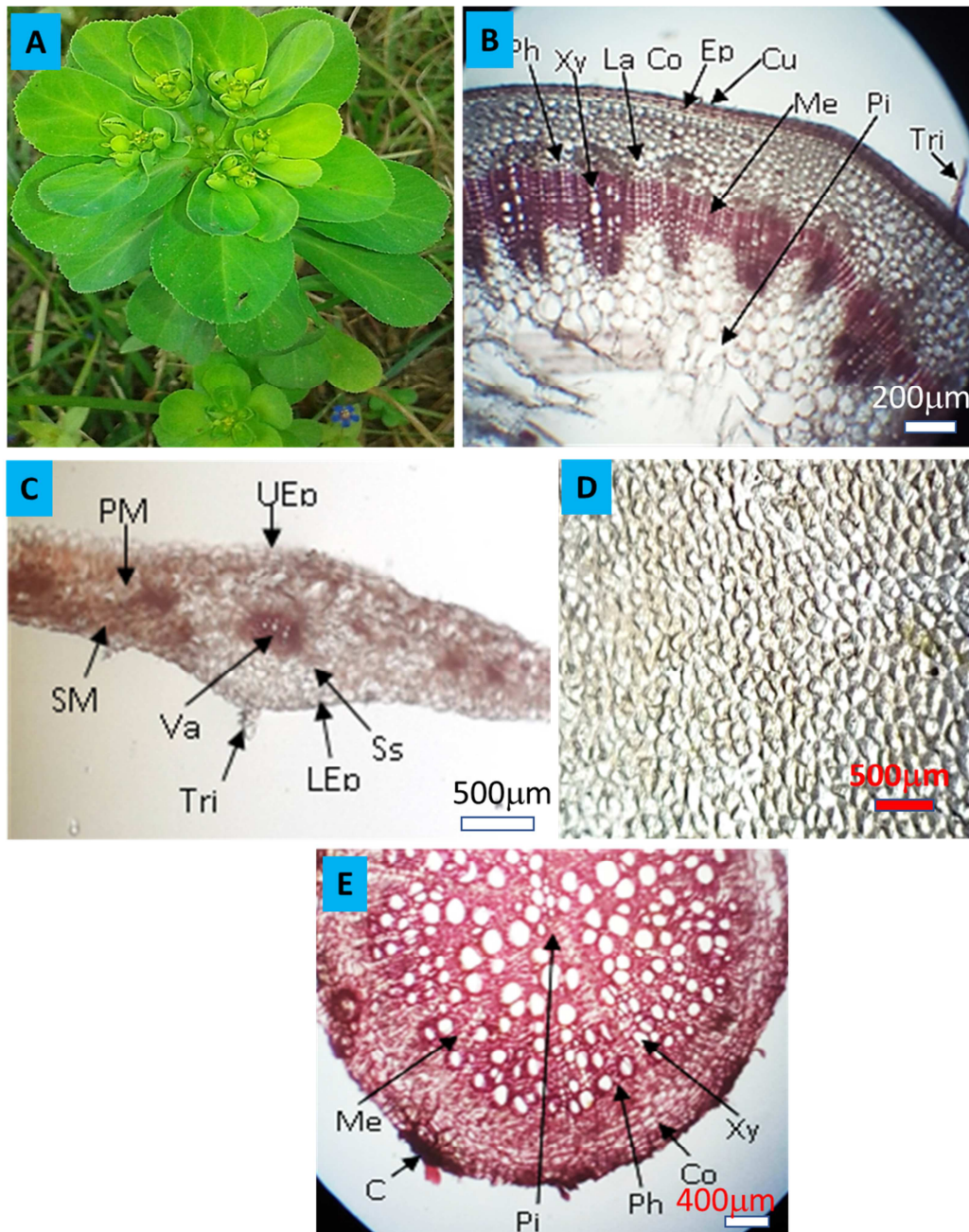


Figure 4. Micrographs of stem, leaf and root of *Euphorbia helioscopia* L. A) Plants grown in the field B) T. S of Stem, C) T. S of Leaf, D) Lower surface of leaf, E) T. S of Root. Ep: Epidermis, La: Laticiferous canal, Co: Cortex, Ph: Phloem, Pi: Pith, LEp: Lower Epidermis, UEp: Upper Epidermis, SM: Spongy Mesophyll, PM: Palisade Mesophyll, Tri: Trichome, C: Cork, Me: Medullary ray, Va: Vascular bundle, Ss: Sunken stomata.

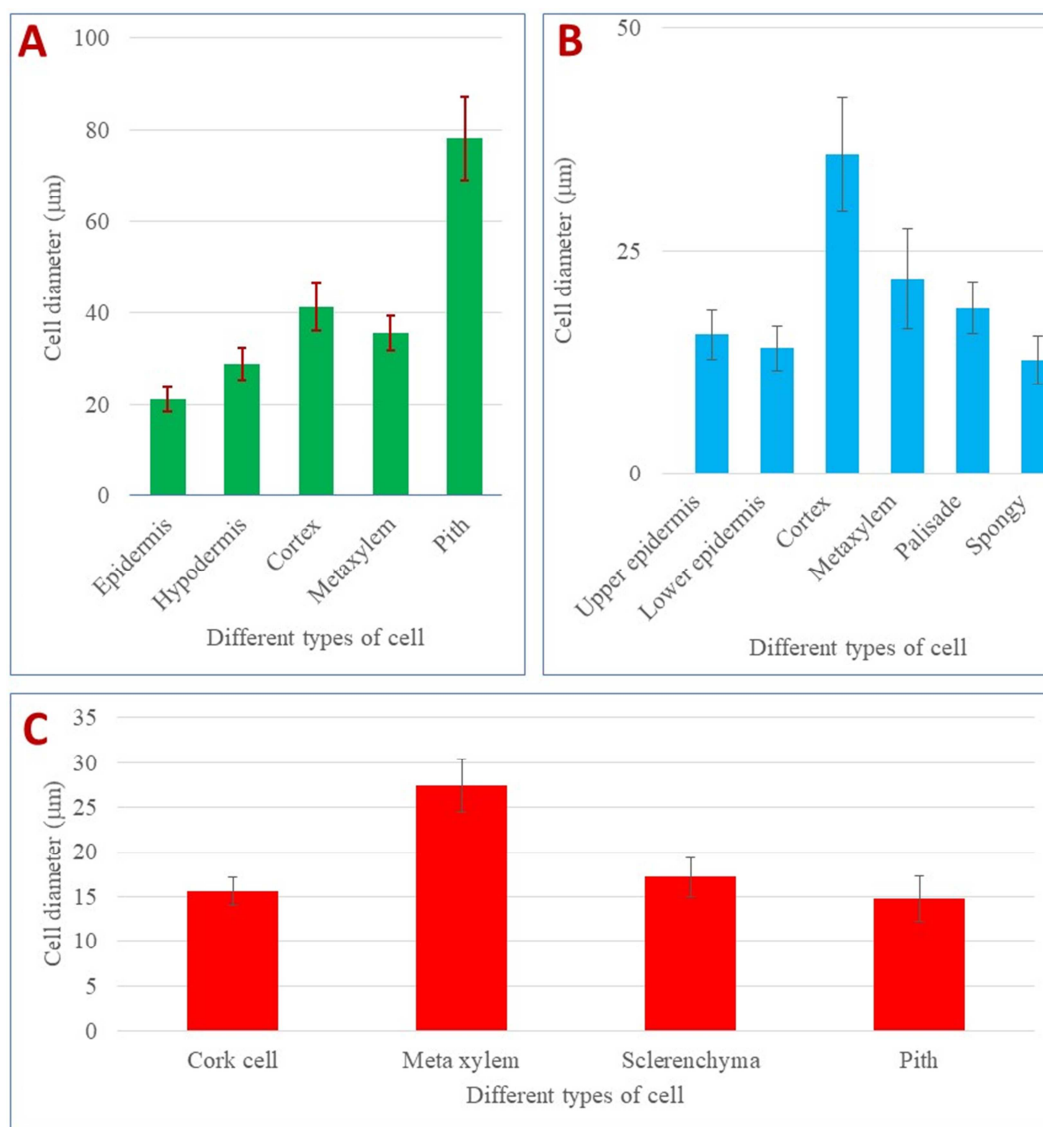


Figure 5. Size of different cells in *Euphorbia helioscopia* L. A) Cell diameters in stem, B) Cell diameters in leaf, C) Cell diameters in root.

The surface of the limb presented cells with right lateral walls. The limb was amphistomatic; the stomata were actinocytic and anomocytic types (Figure 4D). Number of stomata was $0.061/\mu\text{m}^2$ and stomatal index was 8.1. Sunken stomata were also present at the lower epidermis that exhibited the xerophytic adaptation. Between the upper and lower epidermal cells, mesophyll cells were arranged. The results of leaf anatomy showed dorsiventral mesophyll where palisade cells ($35.80\mu\text{m}$ in diameter) and spongy cells ($12.80\mu\text{m}$ in diameter) were arranged. Laticiferous canals were also observed in mesophyll cells. The ratio of midrib region composed of 61.54% cortical zone and 38.46% vascular zone (Figure 2). In the leaf, laticifers were distributed on both sides in the vascular bundle of the median nervure.

3.3. Root Anatomy

In the present study, an attempt was done on the root anatomical details of *E. helioscopia*. The root anatomy of *E. helioscopia* shows cork, epidermis, cortex region, xylem,

phloem, medullary ray and pith, etc. The major portion i.e., cortex, vascular and pith were composed of 25%, 65% and 10%, respectively (Figure 3). In the studied plant samples, the root evidenced a secondary structure resulted from the activity of both lateral meristem i.e., cambium and the phellogen. The phellogen was differentiated from the cortical layer producing a few cork layers. The external ones were being exfoliated. The cork has a typical organization and the phellodermis was mildly collenchymatous (Figure 4E).

The transverse section of root was circular in outline, encircled by cork cells. Phelloderm was 6-8 layered (Figure 4E). Endodermis was well developed. Phloem was narrow. In the center, a wide xylem layers was present, radially arranged vessels (metaxylem was $27.4\mu\text{m}$ in diameter, Figure 5C) and tracheids, uniserrated and multiserrated medullary rays were present and extended up to phloem. The xylem was the most voluminous tissue of the root. The axle of the root comprises a variable number of primary vascular bundles, according to which the stele was of two types: triarch and tetrach. Triarch type in the root of *E. helioscopia* was observed (Figure 4E).

In the root, laticifers were readily seen in the cortical parenchyma and secondary phloem in *E. helioscopia* L. The pith in root was occupied a very small area.

4. Discussion

The investigation showed that, the outline shape was mostly circular outline in examined samples. The results were in harmony with the finding of Luza *et al.* [25] who recorded that the stem transverse section showed a circular outline. The stem anatomy of *E. helioscopia* shows epidermis, cortex region, laticiferous canal, xylem, phloem, medullary ray and pith, etc. Outer most layer epidermis cells were isodiametric with external thick wall and covered with thick cuticle. Epidermal layer is simple (present in one layer) in the examined species but some *Euphorbia* genus (e.g. *E. grandicornis* and *E. heterophylla* L.) have multiple epidermis (hypodermis) [26]. These results were also in agreement with those obtained by Kakkar and Paliwal [16], Gales and Toma [27], who recorded that the epidermis was covered by a cuticle of variable thickness, the stem cork was present. At the periphery, the cortex was formed by an angular collenchymatous parenchyma with small intercellular spaces and contained laticiferous canals. In cortex, laticifer canals were also observed in the stem cortical. These results are in agreement with those obtained by Jafari and Nasseh [11]; Lukovic *et al.* [28]; Sultana [24] who recorded that both the parenchyma and chlorenchyma cells were present in cortex of *Euphorbia* sp. Laticifer canals and storage parenchyma were also present in the cortex layer. The cambium was present in between the xylem and phloem, whereas xylem was radially arranged, with uniserrated medullary rays. In the phloem, laticifer canals are recorded in the examined samples. The results are in harmony with the finding of Bercu [29] who recorded that in the phloem zone, few laticifers were present. In the centre, pith was present and made up of parenchymatous-cellulosic cells. In the pith numerous auriferous cavities observed. The stem pith was usually consisted of parenchyma cells. Aerenchyma cells and air cavities are also observed in the pith of *E. helioscopia* L. The results were in agreement with the finding of Gales and Toma [27] who recorded that the pith was parenchyma cells, often becomes hollow. Air cavities and laticifer canals were also observed in the pith.

Epidermal characteristics of leaves were chosen because they were mildly influenced by ecological conditions and are of high diversity in structure [7]. Former studies [8-10] showed that epidermal characteristics of leaves were also reliable in plant systematics and classification. In transverse section, leaf was covered with both upper epidermis and lower epidermis. The epidermis cells are slightly tangentially elongated. The shapes of epidermal cells varied among the different taxa. All of our observations on epidermal cells were done on the middle parts of lamina, because the size, shape, and orientation of these cells vary considerably in various lamina parts of a single leaf. For instance, there are often distinct variations among epidermal cells overlying the

veins and the cells located above the mesophyll between the veins [5]. The limb was amphistomatic; the stomata were of actinocytic and anomocytic types. Previous studies show that different types of stomata are found in the members of Euphorbiaceae family [5]. The study of 150 *Euphorbia* species showed that the majority of types of dicotyledon stomata present in this genus [30], the most important of which were anomo, aniso, para and cyclocytic [16]. Metcalfe and Chalk [5] reported the presence of paracytic stomata in the tribe Euphorbieae and this is the most common type of stomata [31]. Although stomata types in our studied species were similar in both adaxial and abaxial surfaces, in some species of this genus—*E. gerardiana*, *E. exstipulata*, *E. milii*, *E. arizonica*, and *E. bertheloti* – more than one type of stomata were present on the same surface of the leaf [16].

In the present study, an attempt to study was laid on the root anatomical details of *Euphorbia helioscopia*. This study on root anatomy of *E. helioscopia* is the first report. The root anatomy of *E. helioscopia* shows cork, epidermis, cortex region, xylem, phloem, medullary ray and pith, etc. In the centre, wide xylem was present, radially arranged vessels and tracheids, uniserrated and multiserrated medullary rays were present and extended up to phloem. Similar studies on morphological and anatomical characterization have been reported by Ramona and Constantin [32] on different species of *Euphorbia*. Laticifers were readily seen in the cortical parenchyma and secondary phloem in root of *E. helioscopia*. The investigated taxa of this paper present a number of common characters, already mentioned in the literature on the anatomy of other *Euphorbia* genus [33, 34].

5. Conclusion

The present study was conducted anatomical characterization of three major parts, stem, leaf and root of *E. helioscopia*. Though anatomy of stem and leaf were studied a limited area, the root anatomical study was not so far. In the present study, the root anatomy of *E. helioscopia* will be first report. As an important feature, laticiferous canals were existed in three parts of plants studied here. A rich anatomical view of *E. helioscopia* conducted here that will be helped to explain the taxonomical complications.

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