

## THE ANATOMICAL CHARACTERISTICS OF THE VEGETATIVE AND GENERATIVE ORGANS OF THE MEDICINAL *SILYBUM MARIANUM* L. SPREAD IN THE MOUNTAINOUS REGION OF THE LESSER CAUCASUS

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**Abstract.** In this study, the anatomical characteristics of the vegetative and generative organs of *Silybum marianum* L. were thoroughly investigated. Plant samples were carefully collected from a mountainous area and processed with the necessary chemical reagents to preserve their structure for analysis. Thin sections from different organs were obtained and examined under a microscope to observe detailed tissue arrangements. High-magnification objective lenses were employed to closely study individual tissues, which allowed a more precise observation of their composition. In the leaf, the outer epidermis layer, composed of a single layer of cells, was observed. This layer contained stomata and trichomes, which were also found on the epidermal tissue of other aerial organs. Beneath the epidermis, chlorenchyma cells were found to constitute the mesophyll in the leaf lamina, playing a crucial role in photosynthesis. In the flower stem, a generative organ of the plant, both main parenchyma cells and aerenchyma were identified. Additionally, pith parenchyma was noted in the stem and flower axis, while cortex parenchyma was present in the root. The vascular system in the aerial parts of the plant comprised complex fibrous collateral-type bundles. Furthermore, in the root, secretory cavities were detected along the central cylinder. The xylem of the root displayed a diarch structure, distinguished by parenchymal rays in the central cylinder. This comprehensive anatomical study provides insight into the structural organization of *Silybum marianum* L. across its various organs, contributing to a better understanding of its physiological functions.

**Keywords:** *Epidermis, collateral, main parenchyma, stomata, folium.*

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### 1. Introduction

In this study, cross anatomical sections of the parts of *Silybum marianum* L. were made and the obtained preparations were analyzed under a microscope. The work is presented under the following headings.

**Leaf.** Perpendicular cross-sections of the central and lateral veins of the dorsiventral leaf structure were obtained. Although the central vein is primarily formed by three large vascular bundles, smaller intermediary bundles can occasionally be observed between them. The large bundles are structurally similar and are bordered by a ring of somewhat irregular, thick-walled surrounding cells. The bundles are situated within an expanded area filled with parenchyma cells. These parenchyma cells are larger

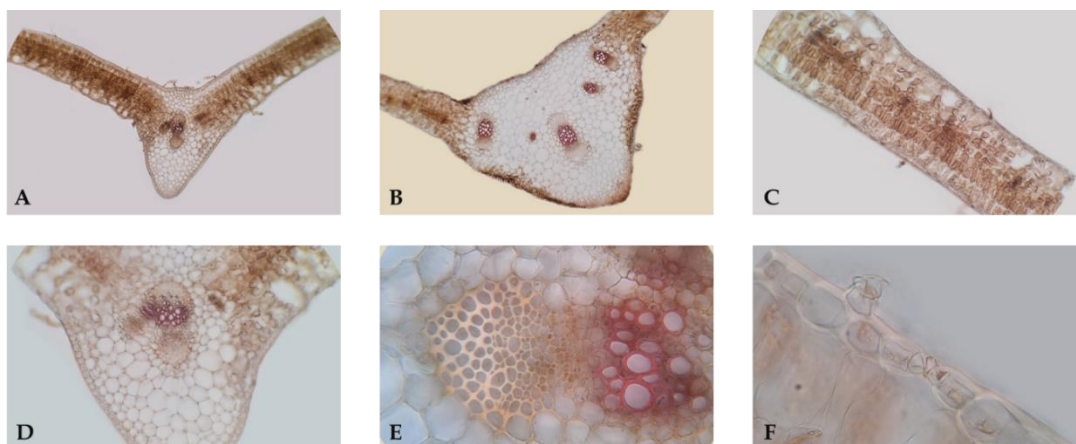
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towards the center and become smaller towards the edges, where they terminate in mechanical tissue elements in the subepidermal regions, specifically under the upper and lower epidermis and in chlorenchyma at the lateral areas (Lotova *et al.*, 2007).

The chlorenchyma tissue, which constitutes the main internal structure of the leaf blade, can be divided into two groups of cells. On the adaxial (upper) side of the leaf blade, the columnar parenchyma consists of elongated cells arranged in three parallel rows. In contrast, the abaxial (lower) side features spongy parenchyma composed of more rounded cells, loosely and irregularly arranged. In the subepidermal region of the upper epidermis, the uppermost layer of the columnar parenchyma shows more sparsely arranged cells. In the subepidermal region of the lower epidermis, the loose arrangement of cells results in the formation of air spaces, which facilitate the exchange of water vapor and other gases.



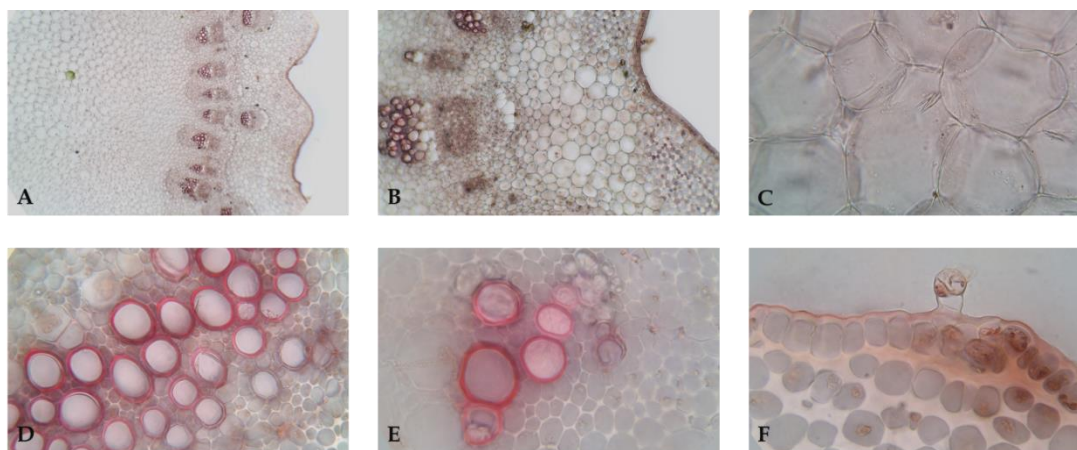
**Figure 1.** The general anatomical structure of the leaf A – cross-section of the lateral vein (4x); B – cross-section of the central vein (4x); C - cross-section of the leaf blade (10x); D, E – conductive bundle of the lateral vein (10x, 4x); F – epidermis with trichomes and stomata (60x)

Along the central axis of the leaf blade, small bundles are regularly spaced among the chlorenchyma cells. These contain a limited number of vascular tissue elements and are primarily involved in the uniform distribution of water and minerals across the entire leaf surface. In the larger bundles, there are more conducting elements (Tutayuk, 1967). The xylem and phloem are positioned adjacent to each other, with the xylem on the upper side and the phloem on the lower side, both encased by mechanical tissue.

A perpendicular section of the lateral vein reveals a single vascular bundle. Under the microscope, a branch that has recently diverged from this vein can also be observed. The structure of the bundle in the lateral vein is similar to that of the bundles in the central vein. The epidermis, which encases the leaf, shows the presence of trichomes and stomata. The lateral margins of the leaves bear spines and in cross-sections of these spines, the thick-walled cells of the mechanical tissue are seen to have developed extensively towards the edges, nearly filling the entire spine cavity. Small vascular bundles are visible in the center and at some distance from the spine.

**Stem.** In the cross-section of a primary structure stem, sparse trichomes are observed on the epidermis. Upon increasing the magnification, the presence of stomata in the epidermis becomes noticeable. The edges of the stem are irregular and it is observed that the mechanical tissue in the convex areas is more pronounced, extending almost

entirely through the subepidermal region. Beneath the subepidermal region lies the cortical parenchyma, mainly composed of spherical cells. Some of these cells contain chloroplasts. This layer is bordered by bicollateral vascular bundles of varying sizes, separated by medullary rays. Additionally, there are vascular bundles within the cortical parenchyma itself.

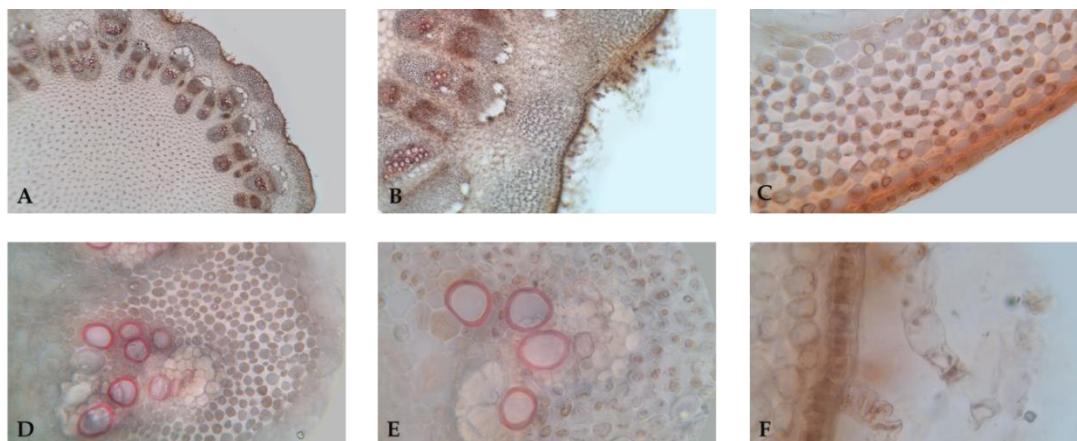


**Figure 2.** The general anatomical structure of the stem A – cross-section of the stem (4x); B – epidermis, cortex and vascular bundles (10x); C – pith parenchyma (40x); D, E – xylem of the vascular bundles (40x, 60x); F – collenchyma, epidermis and trichomes (60x)

In the bundles, the activity of procambium has resulted in the formation of metaxylem and metaphloem, which are more clearly visible in the larger bundles. The xylem is surrounded by phloem on both the external and internal sides. The cells between the externally located phloem and the xylem contain druses and mechanical tissue is located in the outer region (Korovkin, 2007). The cells of the pith parenchyma are smaller within the medullary rays and become larger as they transition towards the pith. However, as they approach the center of the pith, this enlargement gradually decreases.

**Flower Stem.** As observed in the transverse section of the flower stem, the epidermis is covered by a dense layer of trichomes, some of which are simple while others are glandular. The epidermis, where these trichomes originate, also contains stomata in certain areas, allowing for gas exchange, which is essential for the internal tissues of the organ to maintain their living structure. The epidermis is single-layered and beneath it, in the convex regions of the stem, mechanical tissue is clearly visible, providing strength and helping the stem maintain its vertical position (Humbatov, 2017). This function is also supported by the vascular bundles, some of which are located within the cortical parenchyma, while the majority are arranged in a ring near the pith. Within the bundles, the phloem tissue, separated from the metaxylem by a procambial layer, contains druses (complex crystals) in its inward-facing part. The portion of the phloem oriented towards the cortical parenchyma is bordered by a single layer of medium-sized cells, where a significant amount of ergastic substance is accumulated.

As the metaxylem extends inward, it is followed by the protoxylem elements and then the phloem tissue. Medullary rays, composed of cells that increase in size towards the pith, are situated between the bicollateral bundles. The size of the pith parenchyma cells continues to increase relatively as they approach the center of the pith.



**Figure 3.** The general anatomical structure of the flower stem A – cross-section of the flower stem (4x); B – vascular bundles, cortex and epidermis covered with trichomes (10x); C – collenchyma (40x); D – vascular bundle along with mechanical tissue elements (40x); E – xylem of the vascular bundle (60x); F – epidermis along with trichomes (60x)

**Phyllary.** In the microscopic view of a cross-section taken perpendicular to the main vein of the phyllary, one of the first notable features is the presence of aerenchyma tissue with large air spaces. This tissue, which occupies almost half of the internal cavity of the phyllary, consists of cells arranged in a chain-like manner, forming a net-like structure with internal air channels. Additionally, in the upper subepidermal region, these cells are seen to form a continuous cell layer. The lower part of the phyllary primarily consists of chlorenchyma tissue, which gradually transitions into the main parenchyma as it moves laterally toward the edges (Sardarova, 2022a). The main parenchyma is also present around the central bundle but gradually transitions into aerenchyma towards the upper epidermis. In the central bundle area, a widening is observed along the main vein. The bundles are aligned in a row, located at approximately equal distances from both the upper and lower epidermis and are surrounded by protective cells. When examining the structure of the central bundle, xylem, phloem and a small amount of mechanical tissue are visible from top to bottom.

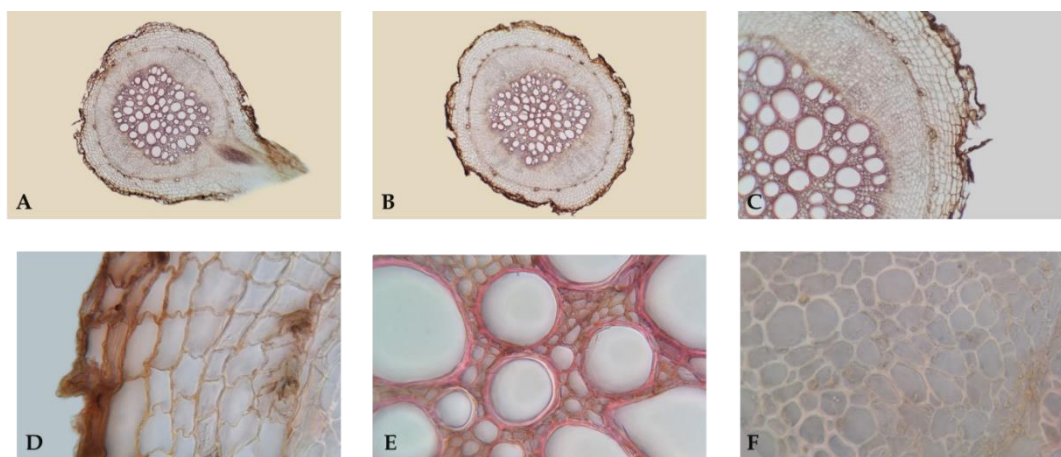


**Figure 4.** The general anatomical structure of the phyllary A, B – central and peripheral regions in the cross-section of the phyllary (4x); C – aerenchyma of the central region of the phyllary (10x); D – aerenchyma cells and epidermis (40x); E – mesophyll and epidermis with stomata (40x); F – vascular bundles (60x)

Both the upper and lower epidermis have a cuticle layer, which contains trichomes and stomata. In the transverse section of the tip of the phyllary, which ends in a spine, small central bundles, as well as lateral bundles extending along a line from the center outward and upward, are observed. Additionally, there are small, scattered bundles that are weakly developed. Mechanical tissue, starting from the subepidermal region, is also noticeable in this area (Gasimov *et al.*, 2010).

**Root.** In microscopic images of transverse sections of medium-sized roots, the structure of the root can be examined. The root is encased by periderm, which is formed by the tissues of the phellogen, including phellem and phelloderm. The center of the root contains xylem, which is interrupted by parenchyma rays extending towards the center as a result of the activity of the vascular cambium. The xylem connects to the phloem through the cambium. Notable are the small cavities along the external boundary of the phloem, which extend the entire length of the root and are characterized by thickened surrounding walls; these are secretory tissues. The transverse section also shows a part of the already-formed lateral root protrusion. The lateral root, in terms of structure and development, is a branch that does not differ from the primary root.

The formation of lateral roots begins in the branching zone of the primary root. In this region, some cells in the pericycle, which divide periclinally (parallel to the surface), form cell clusters (Sardarova, 2022b). The apical meristem of the newly forming lateral root begins to develop in the parts of these clusters located towards the exterior of the primary root.



**Figure 5.** The general anatomical structure of the root  
A, B – cross-section of the root with lateral and simple (4x); C – xylem, phloem and cortex (10x);  
D – cortex (40x); E – xylem: tracheae and libriform fibers (40x); F – phloem (60x)

During this process, the endodermal cells surrounding the pericycle in the periclinal direction divide to form new cell walls perpendicular to the root surface, resulting in a layer known as the root cap. This structure, which surrounds the origin of the lateral root and plays a role in its development, penetrates through the external wall of the primary root and emerges externally once the lateral root has sufficiently elongated.

## 2. Material and methods

The material of the study is *Silybum marianum* plants taken from the mountainous area of the Lesser Caucasus (the area around Maral Gol) and comparatively, from the garden area of the city of Ganja (in situ in the backyard). The plant was studied anatomically (Gurbanov & Mammadova, 2010; Asgarof, 2006). Classical and modern botanical-floristic, systematic, ecological, etc. methods, “Flora Azerbaijan”, “Flora Kavkaza” identifiers and websites were used. Phenological observations were made on the plants taken to study the anatomical features of *Silybum marianum* (Ibadullayeva & Alakbarov, 2010). After the plant growing in natural conditions in the area reaches full morphological maturity, its stem, leaf, root and rhizome are taken (Albrechtova, 2003). A large number of cuttings from the vegetative organs of the species were prepared and analyzed in depth. Both temporary and permanent preparations were prepared by cutting the incisions by hand and with a microtome according to the method adopted in anatomical practice (Ibadullayeva & Gahramanova, 2016). Preparation of preparations was carried out according to generally accepted methods (Barykina *et al.*, 2007). Cuttings were made from fresh or fixed parts of plants. Alcohol or formalin was used to fix the plant. Cuts are made with the help of a sharp razor. At this time, small objects are placed in the core of the kandalash body. Transverse sections were made from the leaf and cylindrical organs (root, stem) of the plant.

In order to achieve a more effective result, thin cuts were made by squeezing the object between two fingers of the left hand professionally. In the sections made with a microtome, the object is placed in paraffin blocks. Sections are added to the dyed water solution in petri dishes, thinner sections are transferred to a clean watch glass and an aqueous solution of the dye is added to it. After a few minutes (depending on the concentration of the dye), the sections were washed several times with water or 50% ethyl alcohol solution to remove the dye.

This process was monitored under a microscope. Afterwards, the washed sections were placed one by one on a glass slide in pre-prepared melted glycerine-gelatin drops. Canada balsam was also used to study cell contours. Anatomical preparations prepared using modern special modern digital and camera NLCD-307 B model, ZEES microscopes, Motic brand made in Germany, XSP 91-06-DN digital), as well as “MBU-6”, MBU-1, MBU-3 microscopes has been studied (Barykina *et al.*, 2007).

## 3. Results and discussion

**1. Stem Anatomy:** Microscopic examination of stem cross-sections revealed that the epidermis is covered with dense trichomes and stomata. The presence of mechanical tissue in the subepidermal region contributes to the stem's mechanical strength, allowing it to maintain its vertical position. This structural adaptation plays a crucial role in enhancing the plant's resistance to environmental stresses. Additionally, the observation of druses and ergastic substances within the phloem and xylem tissues is significant, as these structures are involved in regulating metabolic processes and supporting growth. The organization of xylem and phloem cells optimizes the transport of water and nutrients within the plant.

**2. Root Structure:** Microscopic images of root cross-sections show that the root is encased by the periderm, formed by the periclinal divisions of the pericycle, resulting in the development of the phellogen, phellem and phelloderm tissues. The xylem is centrally

located, surrounded by parenchymatous rays resulting from cambial activity. The xylem connects with the phloem through the cambium and the presence of secretory tissues, characterized by thickened cell walls, is notable. The development of lateral roots begins in the branching zone of the primary root, where pericycle cells divide periclinally to form new lateral roots. This process involves the division of endodermal cells in an anticlinal direction, creating root cap structures that eventually penetrate the outer walls of the primary root as the lateral roots elongate.

**3. Flower Stem Anatomy:** Cross-sections of the flower stem reveal an epidermis covered with dense trichomes and stomata. These structures facilitate gas exchange and transpiration processes. The presence of bicollateral vascular bundles in the flower stem indicates a well-organized system for water and nutrient transport. Druses found in the phloem tissues play a role in various metabolic functions and the presence of mechanical tissue supports the structural integrity of the stem. Both the upper and lower epidermises contain a cuticular layer with trichomes and stomata, contributing to the plant's adaptability to its environment.

**4. Phyllary:** The phyllary exhibits aerenchyma tissue with large air cavities, which are crucial for gas exchange and water regulation. The chain-like arrangement of aerenchyma cells forms a network of internal air channels, supporting the plant's survival in low-oxygen environments. The xylem and phloem organization within the phyllary optimizes nutrient and water transport, while the mechanical tissue provides structural support. The observation of small lateral bundles and dispersed, less-developed bundles in the cross-sections highlights the phyllary's role in maintaining gas exchange and supporting the plant's overall physiological processes.

## 5. Conclusion

This study provides a detailed analysis of the anatomical structures of the plant stem, root, flower stem and phyllary. Microscopic examination revealed that the presence of mechanical tissues, xylem-phloem organization and the formation of druses are crucial for enhancing the plant's resistance to environmental factors and optimizing metabolic processes. The aerenchyma tissue in the phyllary, with its large air cavities, supports gas exchange and water regulation, contributing to the plant's adaptability. These findings offer valuable insights into the anatomical features underlying plant resilience and physiological functions. The results contribute to fundamental scientific knowledge and have potential applications in biology and plant breeding.

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