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COMPARATIVE ECOLOGICAL ANATOMICAL STUDY OF THE STRUCTURAL ADAPTATION OF *Althaea officinalis* L. (Malvaceae Juss.) IN IN SITU AND EX SITU CONDITIONS

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Abstract. The ecological-anatomical characteristics of vegetative and generative organs of *Althaea officinalis* L. ecotypes were examined. Microscopic analyses involved comparative assessment of transverse sections from samples collected under in situ and ex situ conditions, where statistically significant structural differences were identified. In situ specimens showed denser tissue organization, intensive accumulation of ergastic substances, well-developed sclerenchyma and palisade parenchyma, as well as a higher density of secretory structures and trichomes. In contrast, ex situ specimens exhibited hypertrophy and hyperplasia in vegetative organs, resulting in enlarged parenchymatous tissues and xylem vessels with greater diameters. Functional activity of stomatal apertures was also observed. The results indicated enhanced development of anatomo-functional structures and formation of intracellular inclusions under in situ conditions. Differences in the stomatal apparatus - small guard cells with closed stomata in situ (xerophytic trait) versus large guard cells with open stomata ex situ (mesophytic trait) - demonstrate the species' phenotypic plasticity and adaptive strategies. This ecological-anatomical comparison, conducted for the first time within the flora of Azerbaijan, provides a valuable scientific basis for understanding the anatomical foundations of ecological adaptation in medicinal plants.

Keywords: *Stellate trichome, ecological anatomy, aerenchyma, protoxylem and metaxylem, druse, bicatenary palisade.*

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1. Introduction

Althaea officinalis L. (Malvaceae) is a perennial medicinal plant that occurs naturally in the flora of Azerbaijan and is also cultivated (Gurbanov, 2024). The species demonstrates high phenotypic plasticity, developing distinct morpho-anatomical traits under varying ecological conditions. Ex situ individuals are characterized by taller growth, thicker stems and shallow, highly branched lateral roots, whereas in situ specimens display drought-adapted slender stems, deep root systems and a greater degree of pubescence. These morphological differences reflect the adaptive strategies of the species in contrasting environments.

A. officinalis has long been used in folk medicine and phytotherapy. Its flowers are primarily applied in pulmonology, while the roots and leaves are incorporated into diverse anti-inflammatory preparations (Uzunhisarcıklı & Vural, 2012; Bonaterra *et al*., 2020; Xue *et al*., 2023; Bahari *et al*., 2025). The roots are particularly valued for their high content of mucilage, polysaccharides, flavonoids, phenolic acids and tannins, which provide considerable potential for pharmaceutical and cosmetic applications (Ibadullayeva, 2024).

In the modern era, climate change, soil salinity and anthropogenic pressures

strongly influence the structural-functional properties of medicinal plants (Lata *et al*., 2021; Kolupaev & Blume, 2022; Joshi & Trivedi, 2023). Consequently, investigating anatomical and functional modifications of medicinal plants under different ecological conditions has become highly relevant. Recent studies show that eco-anatomical approaches yield fundamental insights into the dynamics of endogenous and exogenous secretory tissues, the development of sclerenchyma and parenchyma and the localization of biologically active compounds (Sun *et al*., 2022). Although similar investigations have been conducted on several plant species worldwide (Pereira *et al*., 2009; Amoroso *et al*., 2023; Khamraeva *et al*., 2021, 2024; Apostol *et al*., 2024), comprehensive eco-anatomical studies within the flora of Azerbaijan remain scarce and are largely confined to recent work by the present author (Sardarova, 2025a, 2025b; Sardarova & Ibadullayeva, 2025).

To date, a systematic comparative eco-anatomical analysis of medicinal plants under in situ and ex situ conditions in Azerbaijan has not been undertaken. In particular, limited information is available regarding the visual-anatomical characterization of tissues and organs responsible for the accumulation of bioactive metabolites in pharmacologically valuable species such as *A. officinalis*. The absence of such analyses has created knowledge gaps concerning metabolite localization at the tissue level, the functional mechanisms of secretory structures and anatomo-functional adaptations. Addressing these gaps is essential for understanding the biosynthesis of bioactive compounds, functional anatomy and ecological adaptation mechanisms of medicinal plants, thereby holding both scientific and applied significance. Accordingly, visual-anatomical studies of *A. officinalis* will not only advance knowledge of the flora of Azerbaijan but also provide valuable insights into the species' bioecological and pharmacological potential.

This study is guided by the following hypotheses:

1. Contrasting ecological conditions (in situ vs. ex situ) induce significant anatomical variations in *A. officinalis*.

2. These variations influence the species' adaptive potential, the localization of intracellular inclusions and its ecotypic structural variability.

Thus, for the first time within the flora of Azerbaijan, the present research provides a comparative eco-anatomical analysis of *A. officinalis*, bridging existing knowledge gaps and establishing a scientific foundation for pharmacological, biotechnological and conservation-oriented applications.

2. Materials and Methods

Plant Material Collection. Specimens of *Althaea officinalis* were collected in May 2021 from Khoshbulag village, Dashkasan region (40°26′52″N, 46°02′09″E) and initially cultivated ex situ in container culture under the environmental conditions of Bala Baghman, Ganja (40°39′57″N, 46°20′36″E). After one year, the plants were transplanted into open soil, where they developed under natural field conditions without artificial intervention. The elevation and climatic characteristics of both collection and cultivation sites were recorded and analyzed to evaluate their ecological influence on the species (Figures 1-3).

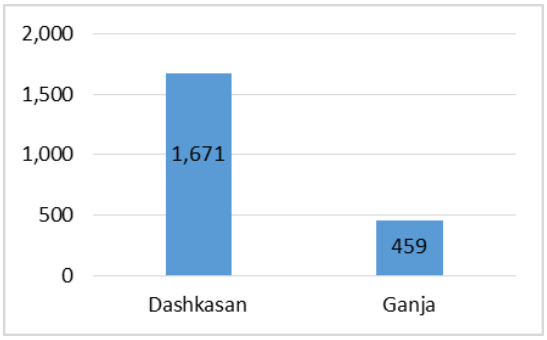


Figure 1. The elevation of the research areas (in meters)

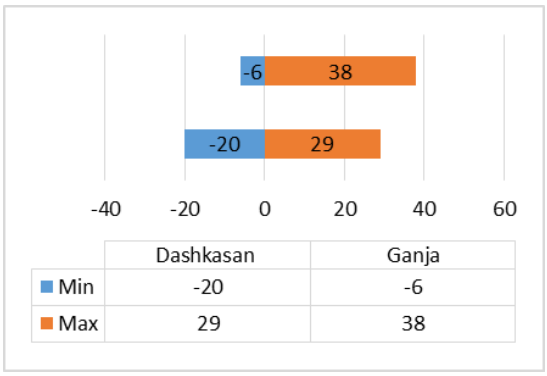


Figure 2. Temperature variation (Min-Max) across sites (°C)

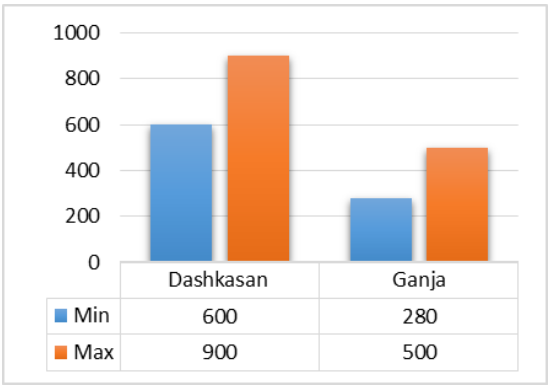


Figure 3. Annual minimum and maximum precipitation (mm)

Sample Preparation. In 2023, plant samples were collected from both in situ and ex situ populations and fixed in appropriate solutions. Anatomical sections were obtained using a rotary microtome (RADICAL, RMT-5, India). Paraffin wax (BW Blended Waxes, Inc., USA) was used during infiltration and sectioning (Erişen *et al*., 2024). Section thickness was controlled with a micrometric adjustment screw, ensuring precise calibration within the 6-8 µm range. Following microtomy, histochemical methods were applied to stain tissues with specific dyes: Safranin O (KimyaLab, Turkey), Fast Green, Sudan III, Toluidine Blue, Fluoroglucinol-HCl and Methylene Blue (INOVATING SCIENCE, USA). Additional reagents including xylene, carboxylol and ethylene (Mir Nauki, Russia) were used. Selective staining of tissue components was achieved via stepwise decolorization protocols (Pradhan Mitra & Loqué, 2014; Moyo *et al*., 2015; Bozdağ *et al*., 2016; Da Silva *et al*., 2020; Engin *et al*., 2024). The application of multiple

sequential staining techniques enabled high-resolution comparative analysis of eco-anatomical structures in *A. officinalis*. Permanent slides were prepared using Canada balsam (INOVATING SCIENCE, USA). A drop of balsam was placed on each section mounted on a glass slide (AmScope, USA), sealed with a coverslip and cured in a temperature-controlled incubator (20-25°C) to ensure complete hardening. Fully dried slides were then analyzed microscopically.

Microscopy. Observations were conducted in the Department of Biology Laboratory at the Azerbaijan State Agricultural University using a Carl Zeiss Axio Imager A2 microscope (ZEISS, Germany) equipped with LED illumination and high-precision objective lenses (5×, 10×, 20×, 40×, 100×). An LCD Digital Microscope NLCD-307B (Wincom Company Ltd., China) was employed during sample preparation to assess section quality and staining efficiency. Final photomicrographs and biometric measurements were obtained using the Carl Zeiss Axio Imager A2 system. For high-magnification observations, immersion oil (RMY, USA) was applied with the 100× objective to enhance resolution and contrast. Macroscopic observations were also performed using stereoscopic microscopes: Zeiss Stemi508 (ZEISS, Germany) and YK-SM067B2 (Wincom Company Ltd., China).

Biometric Measurements. To verify the accuracy of automatically acquired micrometric data, both ocular and stage micrometers (MUHVA, China) were used. Calibration of the ocular micrometer was performed with the stage micrometer prior to measuring plant structures. A digital caliper (Jiavarry, China) was used to determine the macroscopic dimensions of plant organs selected for sectioning. All measurements were documented on corresponding photomicrographs (Jambor *et al*., 2021).

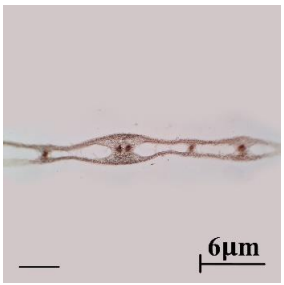
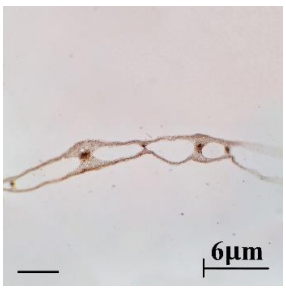
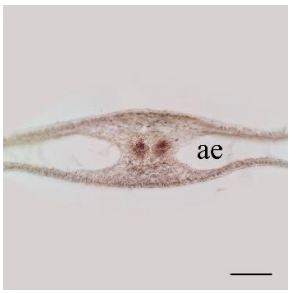
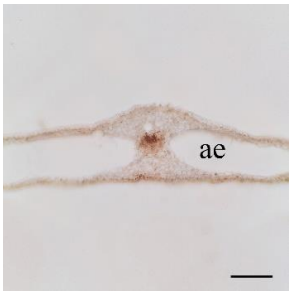
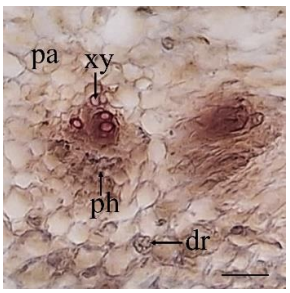
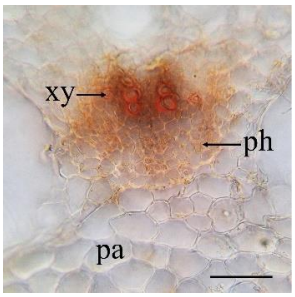
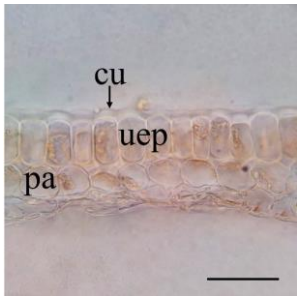
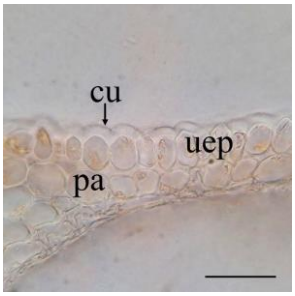
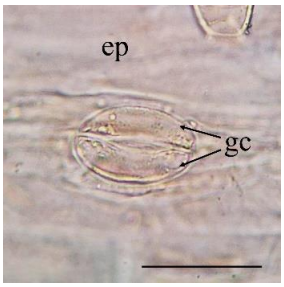
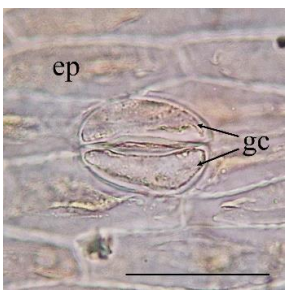
Herbarium Specimen Preparation. Systematically prepared herbarium specimens of *A. officinalis* serve as reference material for ecological, anatomical, pharmacognostic and phytotherapeutic evaluation (Uma & Düzenli, 2012). Comparative herbarium samples from in situ and ex situ populations were deposited in the herbarium collection of the Department of Biology, named after Academician Valida Tutayuq, at the Azerbaijan State Agricultural University. These specimens are available for research, teaching and reference purposes.

Statistical Analyses. *A. officinalis* samples from two ecologically distinct regions of Azerbaijan were analyzed using biometric and anatomical parameters. From each population, 8-12 individuals were randomly selected. Vegetative and generative organs were sampled from each plant and 10-15 transverse sections were prepared per organ. Quantitative measurements were statistically analyzed using Jamovi software (version 2.6.26, University of Sydney, Australia). Results were expressed as mean ± standard deviation (SD). The Shapiro-Wilk test was applied to assess data normality ($W > 0.05$, $p > 0.05$). An Independent Samples T-Test was then conducted to determine whether statistically significant differences existed between the two ecotypes. Results ($p < 0.05$) confirmed notable structural variations between plants grown under in situ and ex situ conditions.

3. Results

Petal. In transverse section, petal thickness was markedly greater in ex situ specimens. Internally, the petal exhibited prominently developed aerenchyma cavities. In situ specimens displayed curvature of the vascular bundle region in both inward and outward directions, whereas ex situ specimens showed bulging exclusively outward (Table 1).

Table 1. Anatomical differences in petals of *Althaea officinalis* from different conditions

Description of anatomical features	In situ	Ex situ
Transverse section of the petal (scale bar: 500 μm).		
Central part of the petal, ae-aerenchyma (scale bar: 200 μm).		
Central vascular bndles, pa-parenchyma, dr-druse, xy-xylem, ph – phloem (scale bar: 50 μm).		
Upper epidermal region, cu-cuticle, uep-upper epidermis, pa-parenchyma (scale bar: 50 μm).		
Structure of the stomata, ep – epidermis, gc-guard cells (scale bar: 50 μm)		

Note: "6 μm" shown on the firs images indicates the section thickness

Anatomical investigations revealed that in situ petals contained a centrally located dual-bundle vascular system, while ex situ samples possessed a single enlarged vascular bundle (Table 2).

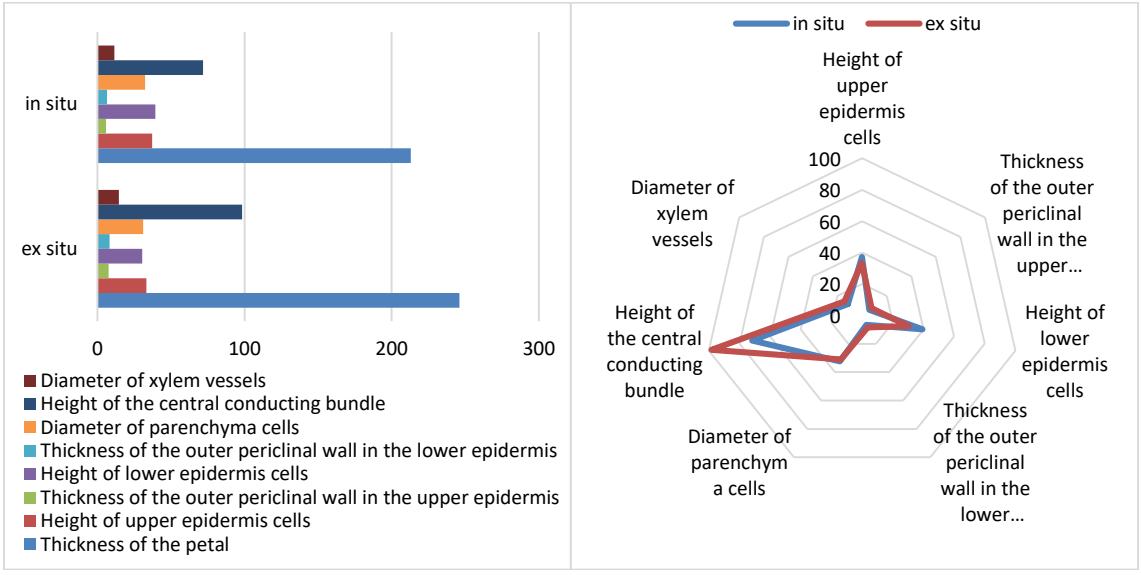
Table 2. Summary of *Althaea officinalis* petals measurements and t-test analysis

Indicators	Mean ± SD (µm)		Independent Samples T-Test
	In situ	Ex situ	p
Thickness of the petal	213.01 ± 23.47	246.06 ± 25.43	0.0073
Height of upper epidermis cells	37.22 ± 3.61	33.32 ± 3.81	0.0304
Thickness of the outer periclinal wall in the upper epidermis	5.94 ± 1.06	7.63 ± 1.59	0.0119
Height of lower epidermis cells	39.42 ± 7.98	30.40 ± 6.87	0.0144
Thickness of the outer periclinal wall in the lower epidermis	6.56 ± 1.33	8.35 ± 1.83	0.0226
Diameter of parenchyma cells	32.38 ± 1.10	31.02 ± 0.94	0.0081
Height of the central conducting bundle	71.74 ± 14.52	98.27 ± 20.51	0.0037
Diameter of xylem vessels	11.59 ± 1.89	14.47 ± 2.60	0.0109

Note: SD - standart deviation. Shapiro-Wilk test confirmed normal distribution (W>0.05, p > 0.05); t - test indicated a significant difference between groups (p < 0.05).

These structural differences were interpreted as adaptations to distinct ecosystem conditions. The xylem vessel diameters were wider in ex situ samples, whereas in situ vessels were more compactly organized (Figure 4).

Constitutional materials such as calcium oxalate druses were primarily localized in the parenchyma surrounding the vascular bundles, with more pronounced development in in situ specimens. In both ecotypes, the petal surface was covered with epidermal cells and an overlying cuticle layer. Ex situ specimens exhibited crescent-shaped guard cells with larger dimensions and open stomatal apertures, indicating a turgid state. By contrast, in situ specimens possessed smaller guard cells with closed apertures, a condition attributed to the onset of plasmolysis caused by reduced turgor pressure, as determined through microscopic analysis.

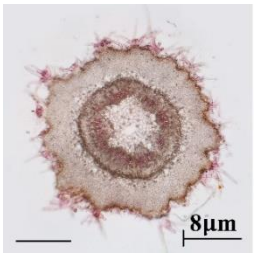
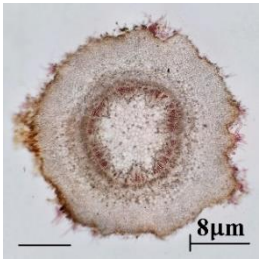
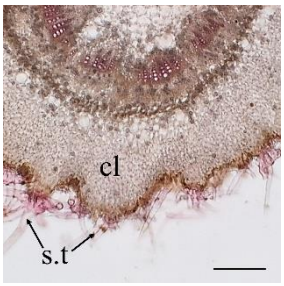
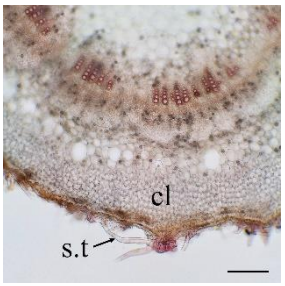
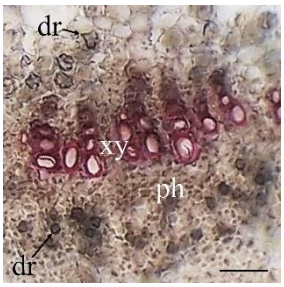
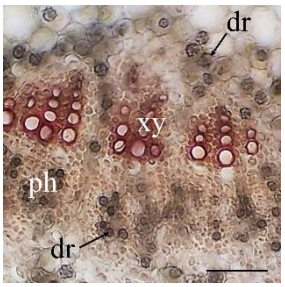
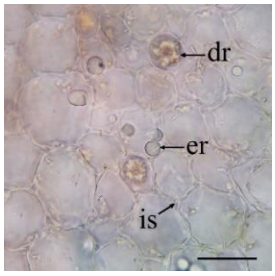
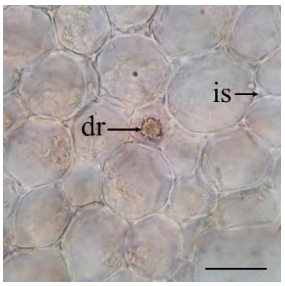


(A) (B)

Figure 4. Bar (A) and Web Chart (B) comparison of species *Althaea officinalis* petal measurements from two sites

Pedicel. The central region of the pedicel was occupied by the pith, clearly encircled by vascular elements. Pith parenchyma cells were noticeably larger in ex situ specimens. Microscopic analysis identified aerenchyma formation exclusively in the pith of in situ specimens. Ergastic substances, including druse crystals, were detected in the pith and cortical parenchyma, vascular tissue and collenchyma in both ecotypes, but were more abundant and intensively accumulated in in situ plants. Photomicrographs revealed a significantly higher density and frequency of druse crystals around vascular bundles in in situ specimens, with larger crystal sizes also observed (Table 3).

Table 3. Anatomical differences in pedicels of *Althaea officinalis* from different conditions

Description of anatomical features	In situ	Ex situ
Transverse section of the pedicels (scale bar: 500 μm).		
Vascular system and cortex, cl-collenchyma, s.t-stellate (scale bar: 200 μm).		
Structure of the vascular tissue, dr-druse, xy-xylem, ph – phloem (scale bar: 50 μm).		
Pith parenchyma, dr-druse, er-ergast substances, is-intercellular spaces (scale bar: 50 μm).		

Note: "8 μm" shown on the firs images indicates the section thickness

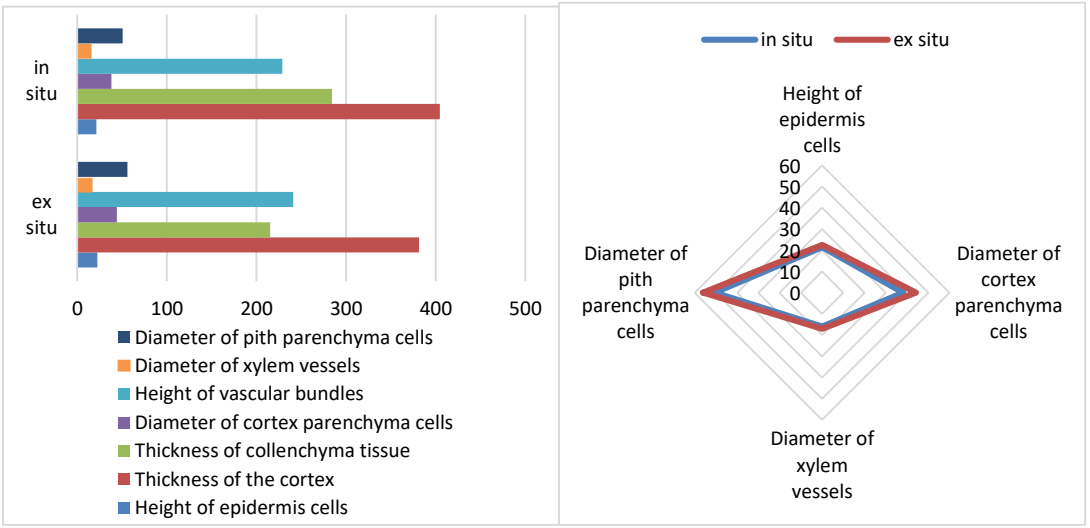
The vascular system exhibited a more compact organization in in situ specimens, whereas ex situ specimens possessed bulkier vascular bundles with xylem vessels of larger diameter (Table 4). The phloem region appeared more developed and protoxylem elements were identifiable toward the inner direction of the radially arranged xylem

strands. A distinct and actively functioning vascular cambium was observed along the conductive zone, with vascular bundles sharply demarcated from the cortex. Angular collenchyma tissue formation in the subepidermal region was more pronounced in in situ specimens. Cortical parenchyma cells, however, were considerably larger in ex situ samples (Figure 5).

Table 4. Summary of *Althaea officinalis* pedicels measurements and t-test analysis

Indicators	Mean ± SD (µm)		Independent Samples T-Test
	In situ	Ex situ	p
Height of epidermis cells	21.43 ± 1.03	22.42 ± 1.07	0.0486
Thickness of the cortex	404.73 ± 15.90	381.41 ± 13.47	0.0023
Thickness of collenchyma tissue	284.21 ± 35.99	215.23 ± 28.29	0.0002
Diameter of cortex parenchyma cells	38.19 ± 3.28	44.23 ± 4.80	0.0041
Height of vascular bundles	228.95 ± 8.50	240.96 ± 11.40	0.0155
Diameter of xylem vessels	15.91 ± 0.88	16.97 ± 0.81	0.0113
Diameter of pith parenchyma cells	50.66 ± 4.40	56.07 ± 5.03	0.0197

Note: SD - standart deviation. Shapiro-Wilk test confirmed normal distribution (W>0.05, p > 0.05); t - test indicated a significant difference between groups (p < 0.05).



(A) (B)

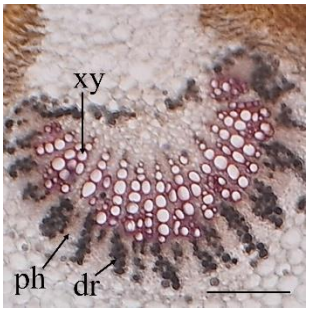
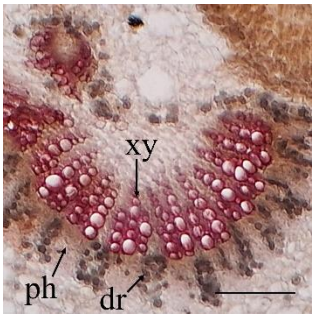
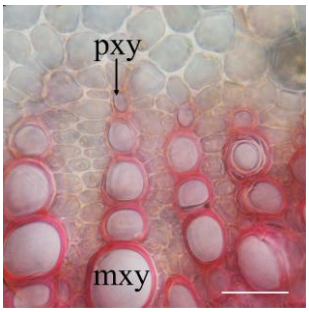
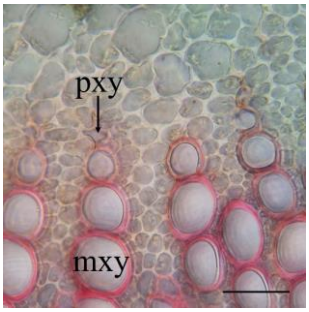
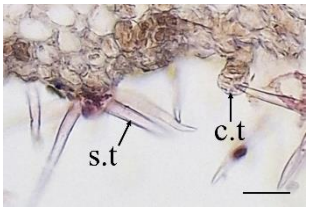
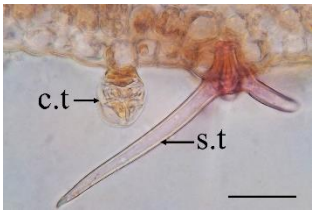
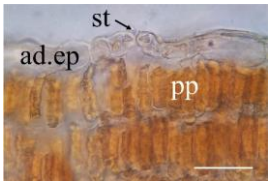
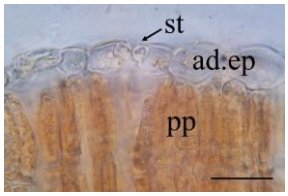
Figure 5. Bar (A) and Web Chart (B) comparison of species *Althaea officinalis* pedicel measurements from two sites

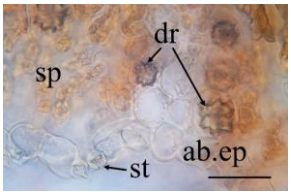
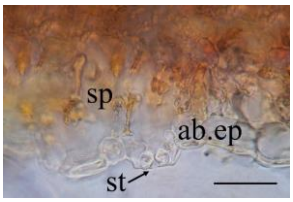
The pedicel surface of ex situ plants appeared smoother than that of in situ specimens, likely due to the presence of larger parenchymal cells in both cortical and pith regions. Characteristic stellate trichomes, typical of *A. officinalis*, were also observed, with higher functional activity in in situ specimens.

Leaf. In transverse section, the leaf exhibits a dorsoventral structure. A prominent ventral protrusion and a smaller dorsal bulge are observed in the central vascular region, where collenchyma tissue is formed - more extensively in in situ specimens. Parenchyma cells located in the adaxial and abaxial bulges are larger in the in situ sample. The main arc-shaped portion of the vascular system lies on the adaxial side of the leaf, with the phloem oriented ventrally. The vascular bundle comprises 18-22 xylem rays and 2-6

vessel elements, predominantly metaxylem. These quantitative traits are similar in both specimens; however, the vascular bundle is larger in in situ plants due to the greater volume of phloem tissue and the presence of thick-walled xylem vessels (Table 5). Druze crystals occur within the parenchyma rays of the phloem, with more pronounced development in in situ specimens.

Table 5. Anatomical differences in leaves of *Althaea officinalis* from different conditions

Description of anatomical features	In situ	Ex situ
Transverse section of the leaf (scale bar: 500 μm).		
Central vascular bundle, xy – xylem, ph – phloem, dr-druse (scale bar: 200 μm).		
Elements of xylem tissue, pxy-protoxylem, mxy-metaxylem (scale bar: 50 μm).		
Trichomes on the epidermis s.t-stellate trichomes, c.t-capitate trichomes (scale bar: 50 μm).		
Adaxial side of the leaf lamina, ad.ep–adaxial epidermis, st-stoma, pp-palisade parenchyma (scale bar: 50 μm)		

Description of anatomical features	In situ	Ex situ
Abaxial side of the leaf lamina, ab.ep–abaxial epidermis, st-stoma, sp-spongy parenchyma, dr-druse (scale bar: 50 μm)		

Note: "6 μm" shown on the firs images indicates the section thickness

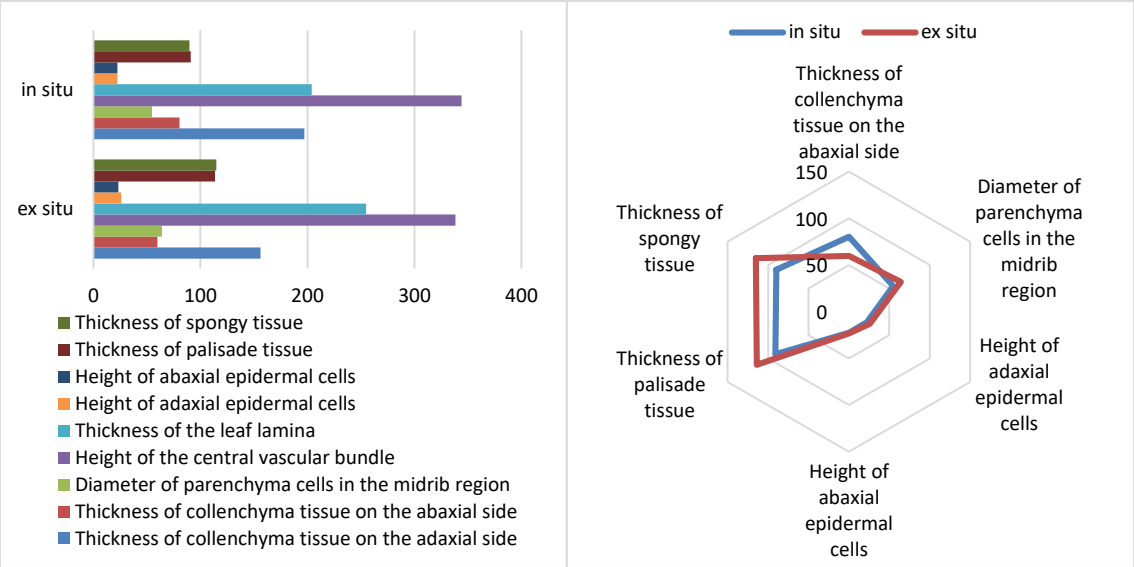
A distinct structural feature of *A. officinalis* is the presence of bicatenary palisade parenchyma in both specimens, although the parenchyma cells are larger in ex situ leaves (Table 6). In situ leaves display more compact palisade and spongy mesophyll layers. Ergastic and constitutive substances are accumulated in mesophyll cells of both samples, but this accumulation is notably weaker in ex situ leaves. Anatomical analysis revealed comparatively larger adaxial and abaxial epidermal cells in ex situ specimens (Figure 6). Stellate and capitate trichomes were observed in both ecotypes but were more actively developed in in situ plants. Stomatal distribution indicates that the leaf is amphistomatic.

Table 6. Summary of *Althaea officinalis* leaves measurements and t-test analysis

Indicators	**Mean ± SD (μm)**		**Independent Samples T-Test**
	In situ	**Ex situ**	**p**
Thickness of collenchyma tissue on the adaxial side	197.21 ± 28.78	156.17 ± 27.34	0.0043
Thickness of collenchyma tissue on the abaxial side	80.36 ± 15.85	59.85 ± 13.79	0.0064
Diameter of parenchyma cells in the midrib region	54.84 ± 5.40	64.15 ± 6.53	0.0027
Height of the central vascular bundle	344.15 ± 5.26	338.44 ± 3.53	0.0106
Thickness of the leaf lamina	204.15 ± 32.45	254.94 ± 31.06	0.0022
Height of adaxial epidermal cells	22.48 ± 2.47	26.00 ± 3.02	0.0107
Height of abaxial epidermal cells	22.54 ± 0.74	23.39 ± 0.95	0.0395
Thickness of palisade tissue	90.83 ± 13.72	113.80 ± 19.49	0.0069
Thickness of spongy tissue	89.67 ± 14.25	114.88 ± 20.24	0.0047

Note: SD - standart deviation. Shapiro-Wilk test confirmed normal distribution ($W>0.05$, $p > 0.05$);
t - test indicated a significant difference between groups ($p < 0.05$).

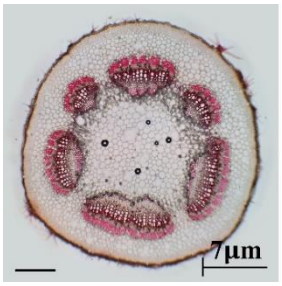
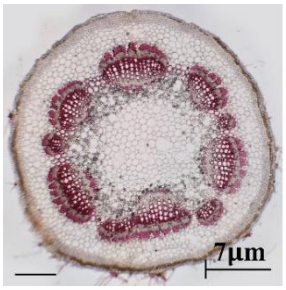
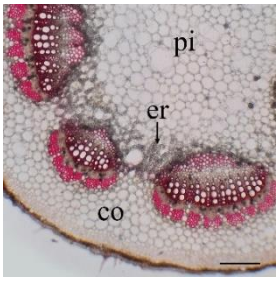
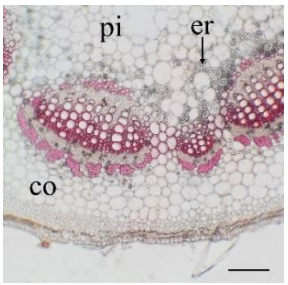
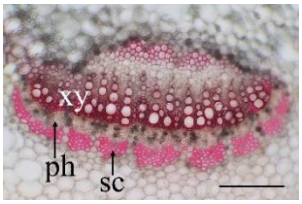
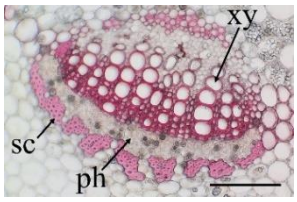
Petiole. In transverse section, the petiole displays a circular outline. Its outermost layer consists of epidermis, beneath which chlorenchymatous parenchyma cells are arranged in 2-3 layers in the in situ sample and 1-2 layers in the ex situ sample. Below this, collenchyma tissue occurs in both specimens, but is more extensively developed in the in situ petiole (Table 7). Cortex parenchyma is noticeably thicker in the ex situ specimen, with larger cells. Ergastic substances, including druses, are localized in the parenchyma surrounding the vascular bundles, more prominently in in situ plants.

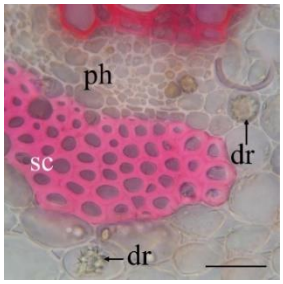
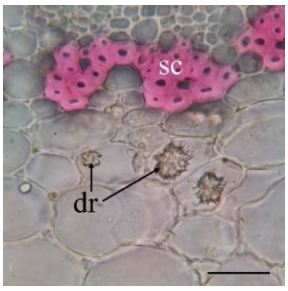
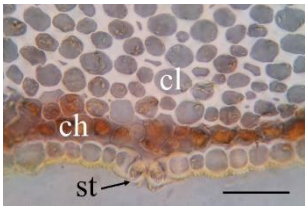
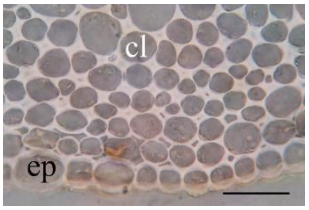
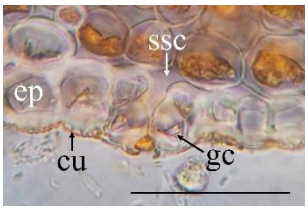
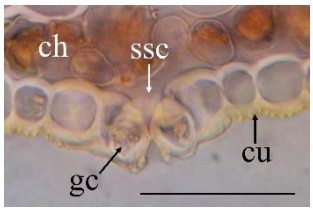


(A) (B)

Figure 6. Bar (A) and Web Chart (B) comparison of species *Althaea officinalis* leaf measurements from two sites

Table 7. Anatomical differences in petioles of *Althaea officinalis* from different conditions

Description of anatomical features	In situ	Ex situ
Transverse section of the petiole (scale bar: 500 µm).		
A part of the petiole, pi-pith, er-ergastic substrances, co-cortex (scale bar: 200 µm).		
Structure of the vascular bundles, xy-xylem, ph-phloem, sc-sclerenchyma (scale bar: 200 µm).		

Description of anatomical features	In situ	Ex situ
Sclerenchyma tissue and calcium oxalate crystals in the surrounding parenchyma cells, sc-sclerenchyma, ph-phloem, dr-druse (scale bar: 50 μm).		
Peripheral part of the cortex, ep-epidermis, cl-colenchyma, ch-chlorenchyma, st-stoma (scale bar: 50 μm)		
Structure of the stomata, ep-epidermis, ch-chlorenchyma, cu-cuticle, gc-guard cells, ssc-sub-stomatal cavity (scale bar: 50 μm)		

Note: "7 μm" shown on the firs images indicates the section thickness

Microscopic analysis further showed that pith parenchyma cells are larger in the in situ sample. Sclerenchyma tissue surrounds and penetrates the vascular bundle, occurring as isolated groups near the cortex and as a single group adjacent to the pith. The process of sclerenchymatization is more intensive in in situ specimens (Table 8).

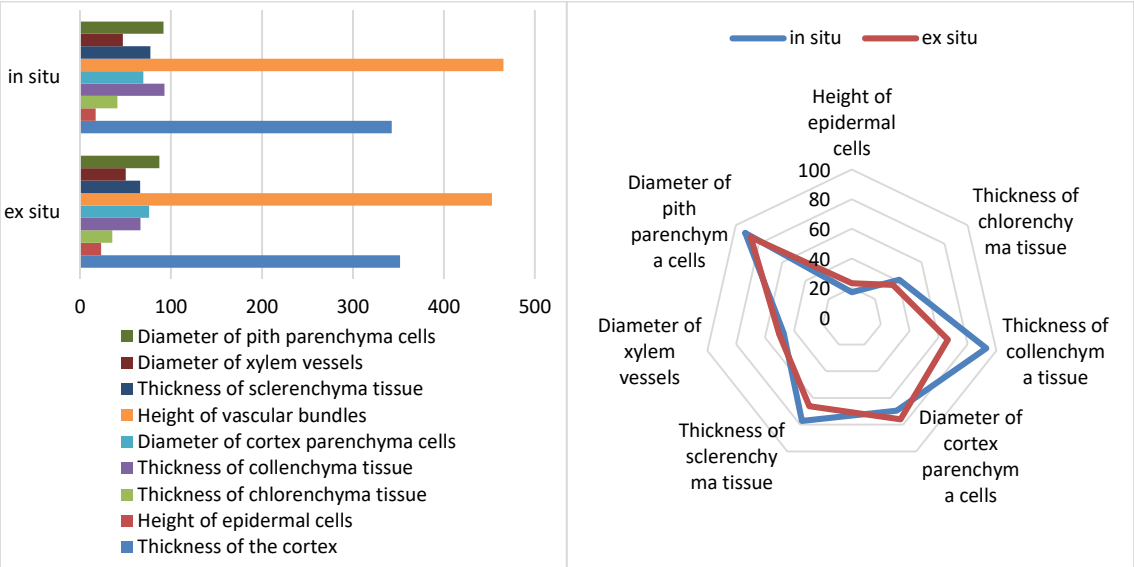
Table 8. Summary of *Althaea officinalis* petioles measurements and t-test analysis

Indicators	**Mean ± SD (μm)**		**Independent Samples T-Test**
	In situ	**Ex situ**	**p**
Thickness of the cortex	342.65 ± 5.21	351.94 ± 7.23	0.0040
Height of epidermal cells	17.17 ± 4.09	23.17 ± 5.57	0.0132
Thickness of chlorenchyma tissue	41.07 ± 4.85	35.37 ± 3.99	0.0102
Thickness of collenchyma tissue	92.97 ± 15.98	66.47 ± 16.46	0.0018
Diameter of cortex parenchyma cells	69.57 ± 4.31	76.00 ± 5.00	0.0065
Height of vascular bundles	465.77 ± 11.06	453.03 ± 10.84	0.0181
Thickness of sclerenchyma tissue	77.27 ± 9.34	66.17 ± 8.76	0.0134
Diameter of xylem vessels	47.17 ± 2.57	50.17 ± 3.26	0.0347
Diameter of pith parenchyma cells	91.75 ± 4.21	87.28 ± 4.20	0.0286

Note: SD - standart deviation. Shapiro-Wilk test confirmed normal distribution ($W>0.05$, $p > 0.05$); t - test indicated a significant difference between groups ($p < 0.05$).

Within the vascular system, protoxylem elements are directed toward the pith, while metaxylem and libriform fibers are oriented toward the cortex. Notably, ex situ specimens exhibited a higher number of xylem elements with larger metaxylem vessels (Figure 7). The phloem elements are arranged toward the cortical side of the vascular bundle. Stellate and capitate trichomes are better developed in in situ petioles. In both specimens, the

epidermis is covered with a cuticle layer, but thickened epidermal walls and a more distinct cuticular layer are evident in in situ specimens.

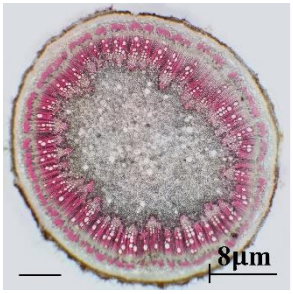
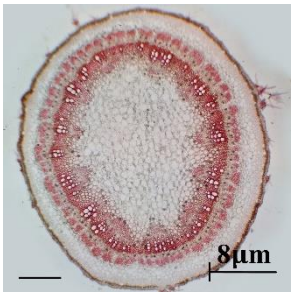
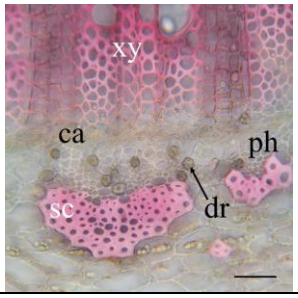
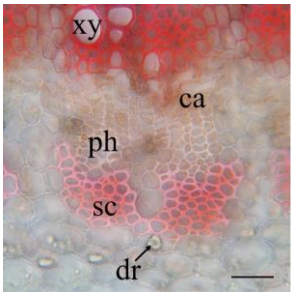


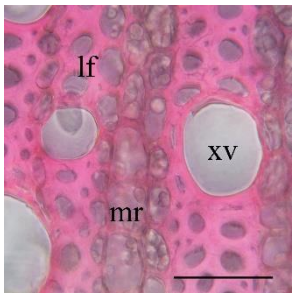
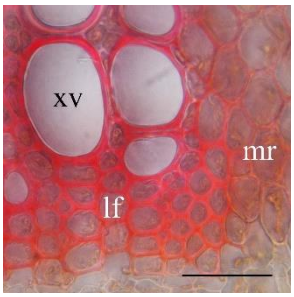
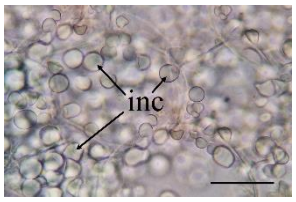
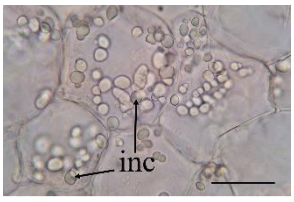
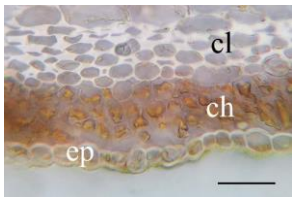
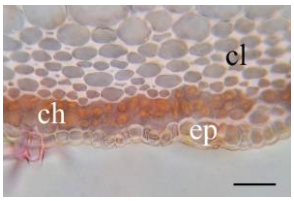
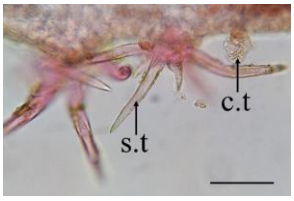
(A) (B)

Figure 7. Bar (A) and Web Chart (B) comparison of species *Althaea officinalis* petiole measurements from two sites

Stem. In transverse section, numerous bifurcated and stellate trichomes are clearly visible on the epidermis, often arranged in groups with fused bases. Small capitate trichomes are also present. Eco-anatomical analysis indicates that these trichomes are more prominently developed in in situ samples. Stomatal structures are also detected on the stem epidermis. Beneath the epidermis lies a subepidermal layer composed of two to three rows of chloroplast-containing cells, followed by a layer of collenchyma. Toward the pith, large parenchymatous cells are located centrally (Table 9).

Table 9. Anatomical differences in stems of *Althaea officinalis* from different conditions

Description of anatomical features	In situ	Ex situ
Transverse section of the stem (scale bar: 500 µm).		
Elements of the vascular system, xy-xylem, ph-phloem, ca-cambium, dr-druse, sc-sclerenchyma (scale bar: 50 µm).		

Elements of the xylem tissue, lf-libriform fibers, xv-xylem vessels, mr-medullary rays (scale bar: 50 µm).		
Pith parenxyma cells, inc-intracellular inclusions (scale bar: 50 µm).		
Peripheral part of the cortex, ep-epidermis, ch-chlorenchyma, cl-collenchyma (scale bar: 50 µm)		
Trichomes on the epidermis, s.t-stellate trichomes, c.t-capitate trichome (scale bar: 50 µm)		

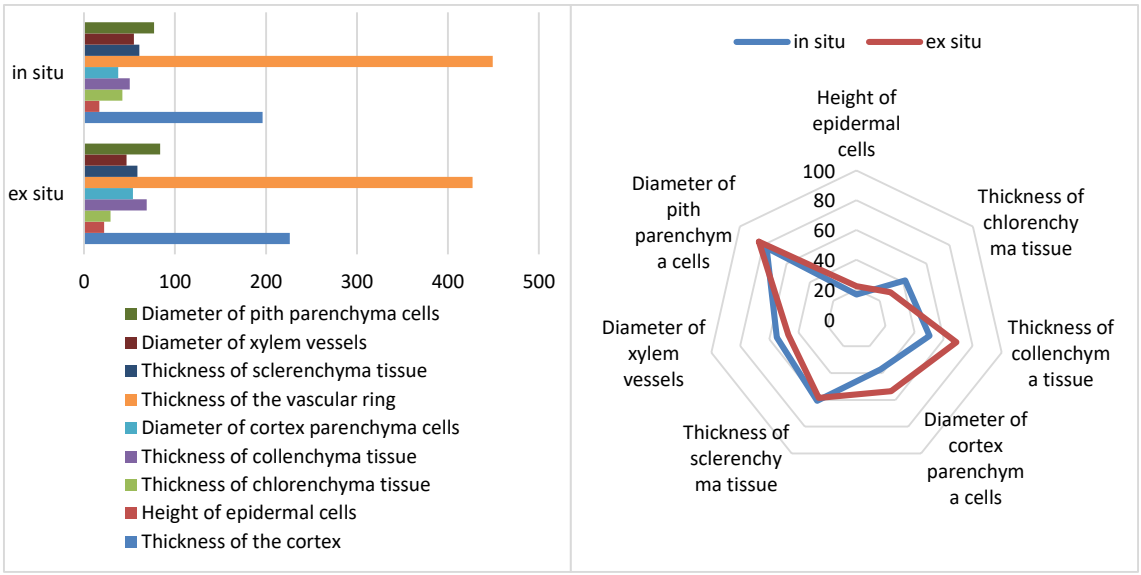
"8 µm" shown on the firs images indicates the section thickness

These parenchyma cells are bounded internally by a sclerenchyma layer. Under ex situ conditions, the stem shows more actively developed cortical and medullary parenchyma, with a comparatively thicker collenchyma layer (Table 10; Figure 8).

Table 10. Summary of *Althaea officinalis* stems measurements and t-test analysis

Indicators	**Mean ± SD (µm)**		**Independent Samples T-Test**
	In situ	**Ex situ**	**p**
Thickness of the cortex	196.27 ± 21.72	226.27 ± 25.45	0.0110
Height of epidermal cells	16.67 ± 3.70	22.21 ± 5.58	0.0175
Thickness of chlorenchyma tissue	41.94 ± 8.46	29.05 ± 8.21	0.0028
Thickness of collenchyma tissue	50.17 ± 13.01	68.87 ± 13.70	0.0058
Diameter of cortex parenchyma cells	37.43 ± 12.20	53.63 ± 15.78	0.0194
Thickness of the vascular ring	449.23 ± 23.48	427.05 ± 22.34	0.0442
Thickness of sclerenchyma tissue	60.75 ± 2.23	58.55 ± 1.29	0.0147
Diameter of xylem vessels	54.89 ± 5.78	46.83 ± 6.28	0.0079
Diameter of pith parenchyma cells	77.07 ± 5.92	83.73 ± 6.08	0.0232

Note: SD - standart deviation. Shapiro-Wilk test confirmed normal distribution ($W>0.05$, $p > 0.05$); t - test indicated a significant difference between groups ($p < 0.05$).



(A) (B)

Figure 8. Bar (A) and Web Chart (B) comparison of species *Althaea officinalis* stem measurements from two sites

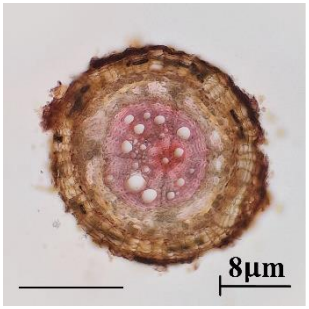
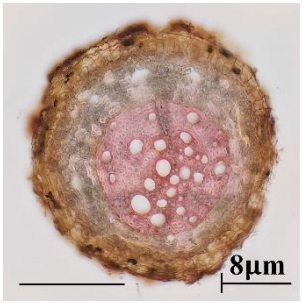
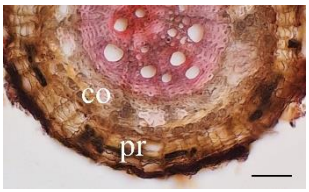
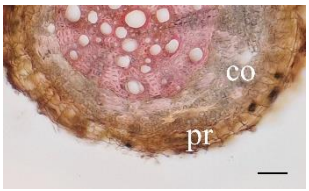
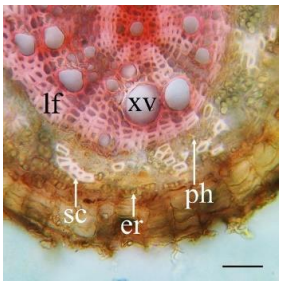
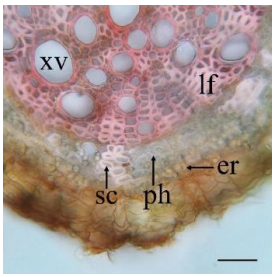
In situ specimens exhibit pith cells filled with intracellular inclusions, whereas their abundance is markedly lower in ex situ pith parenchyma. Lignification of sclerenchymal fiber walls is also more pronounced in situ. Phloem groups are distinctly visible, comprising sieve tubes and small companion cells, with numerous druse crystals observed in the phloem of in situ specimens. The vascular system consists of protoxylem elements positioned near the pith, followed by radially arranged metaxylem elements that increase in size toward the cortex. Libriform fibers occur between metaxylem vessels. Xylem development is more advanced in situ, both in number and structural thickness. Secondary wall thickening is also more pronounced in libriform fibers of the in situ sample, while those in ex situ samples appear thinner. The inner xylem boundary (adjacent to the pith) contains sclerenchymal fibers, which are more abundant in situ. Between xylem and phloem lies the cambium, composed of elongated, narrow meristematic cells, reflecting active periclinal divisions in this zone.

Root. In both conditions, transverse sections reveal that in situ roots display smaller overall diameters (Table 11). These differences are statistically supported at the micrometric level through micrographic analysis.

The outermost layer is covered by a periderm, which is more strongly developed in situ (Table 12). Greater accumulation of ergastic and constitutive substances is observed in the periderm and cortical parenchyma of in situ roots.

In contrast, the vascular system of ex situ specimens contains more numerous xylem elements with larger vessel diameters (Figure 9). Cortical parenchyma is also more actively developed in ex situ roots. However, sclerenchyma tissue within the cortex is more pronounced in situ, reflecting enhanced mechanical adaptation to natural environmental stress.

Table 11. Anatomical differences in roots of *Althaea officinalis* from different conditions

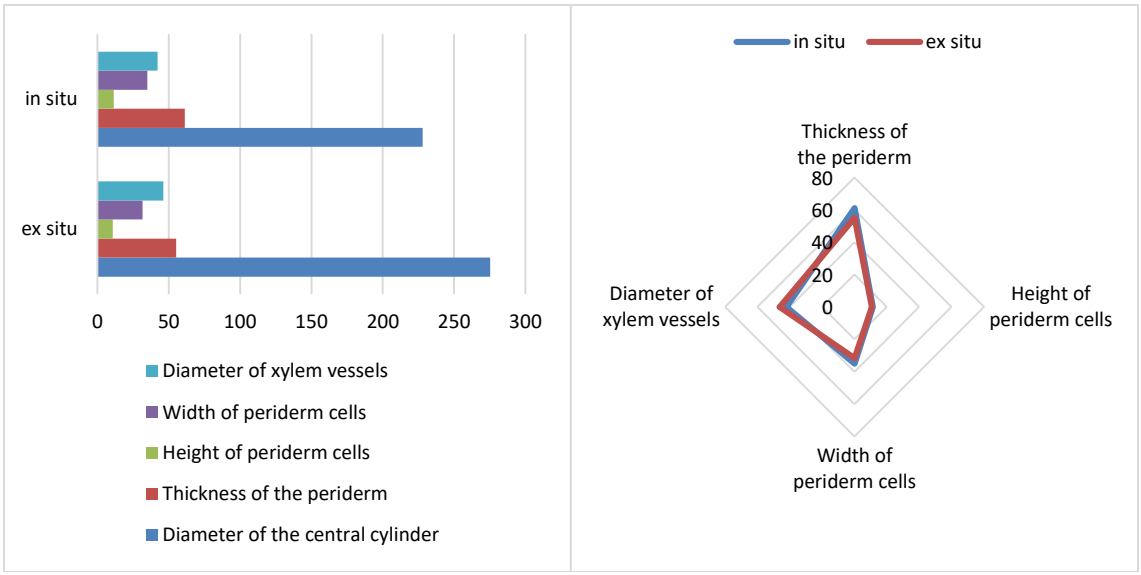
Description of anatomical features	In situ	Ex situ
Transverse section of the root (scale bar: 200 μm).		
A part of the root, co-cortex, pr-periderm (scale bar: 50 μm).		
Vascular system and cortex elements, lf-libriform fibers, xv-xylem vessels, sc-sclerenchyma, ph – phloem, er-ergastic substrances (scale bar: 50 μm).		

"8 μm" shown on the firs images indicates the section thickness

Table 12. Summary of *Althaea officinalis* roots measurements and t-test analysis

Indicators	**Mean ± SD (μm)**		**Independent Samples T-Test**
	In situ	**Ex situ**	**p**
Diameter of the central cylinder	228.05 ± 32.92	275.24 ± 34.79	0.0060
Thickness of the periderm	61.14 ± 6.57	55.11 ± 5.31	0.0366
Height of periderm cells	11.34 ± 0.71	10.78 ± 0.42	0.0441
Width of periderm cells	34.97 ± 2.88	31.68 ± 2.39	0.0124
Diameter of xylem vessels	42.08 ± 3.12	46.12 ± 3.76	0.0177

Note: SD – standart deviation. Shapiro-Wilk test confirmed normal distribution ($W>0.05$, $p > 0.05$); t-test indicated a significant difference between groups ($p < 0.05$).



(A) (B)

Figure 9. Bar (A) and Web Chart (B) comparison of species *Althaea officinalis* root measurements from two sites

4. Discussion

Significant anatomical differences were observed in the petals of *Althaea officinalis* between in situ and ex situ environments. The central petal region exhibited distinct vascular bundle counts, with in situ samples possessing two bundles and ex situ samples only one, as confirmed microscopically. In situ specimens displayed more intensive parenchyma development and greater cellular differentiation. Ergastic substances, including druse-type calcium oxalate crystals and constitutive compounds, were localized within parenchymal cells, suggesting enhanced biosynthetic and defensive functions under natural conditions (Bertoft, 2017; Cuéllar-Cruz *et al*., 2020; Apriyanto *et al*., 2022). Although similar structures were present in ex situ specimens, their abundance and functional activity were considerably reduced. These findings highlight the adaptive plasticity of petal secretory and protective mechanisms in response to environmental conditions.

Anatomical changes in the stomatal apparatus also reflected environmental influences. In in situ specimens, guard cells were smaller, with predominantly closed stomatal apertures, indicating reduced turgor pressure, minimized water loss and the development of xeromorphic traits typical of arid and mountainous regions (Potkay & Feng, 2023; Brodersen *et al*., 2025). Conversely, ex situ specimens exhibited larger guard cells with open apertures, suggesting optimal water balance, higher turgor pressure and reduced abiotic stress typical of mesophytic environments. Open stomata in these plants facilitated more active photosynthesis and transpiration. These structural-functional variations underscore stomatal plasticity and phenotypic flexibility, demonstrating the capacity of the same genotype to express distinct traits under variable conditions. In situ plants exhibited well-developed cuticles, mechanical tissues and storage parenchyma, whereas ex situ samples showed expanded palisade and spongy parenchyma, indicating enhanced gas exchange and hydraulic efficiency.

The pedicel of in situ plants displayed higher density and diversity of epidermal trichomes, presumed to function in secretion and protection against abiotic stressors

(Wang *et al*., 2021). The more developed vascular system, particularly phloem and xylem tissues, suggested optimized nutrient and water transport under natural conditions. Ex situ specimens exhibited less developed vascular elements but more abundant central parenchyma, likely reflecting lower demands for water and minerals under cultivation. The observed anatomical variability of the pedicel reflects key traits associated with ecological adaptation.

In the leaves, numerous capitate trichomes and druse-shaped calcium oxalate crystals in the central vascular phloem were present under both conditions, but development was more pronounced in in situ specimens. Palisade parenchyma was denser and more compact in situ, while vascular bundles in the stem and petiole were surrounded by well-developed sclerenchyma tissues, particularly in naturally grown plants. Although bioactive compounds accumulated in stem parenchymal cells under both conditions, their quantity and distribution were greater in in situ specimens.

Roots also exhibited clear eco-anatomical differences. In situ roots displayed more intense sclerification, with sclerenchyma complexes in the cortical region and a multi-layered periderm, indicating structural adaptation to environmental stress. Accumulation of ergastic substances and localization of bioactive compounds in secretory parenchyma reflected higher metabolic activity and secondary metabolite synthesis in natural settings (Zhang *et al*., 2023; Pramanik *et al*., 2024). Ex situ roots, by contrast, contained more numerous xylem vessels, likely associated with greater water availability and irrigation. Overall, root anatomy demonstrated high plasticity, emphasizing the importance of in situ conservation for preserving natural adaptive traits.

Previous studies support these findings. Arabameri et al. (2020) classified trichomes in *Alcea* species and our observations in *A. officinalis* confirm both non-glandular stellate and glandular capitate types. Sagyndykova et al. (2023) reported thick leaf mesophyll with druse crystals in palisade and spongy parenchyma. Our study observed thicker leaf lamina in ex situ samples, with more intense calcium oxalate accumulation in in situ specimens. The presence of stellate trichomes and sclerenchyma in the stem aligns with previous reports, particularly in naturally grown plants. Osipova and Pogotskaya (2021) described dense trichomes formed by fused single cells on the leaf surface; our study confirms a higher density of such stellate trichomes in in situ leaves. The distribution of druse crystals around vascular bundles, as reported earlier, was verified in both environmental conditions in our study.

5. Conclusion

Ecological-anatomical investigations demonstrated that the vegetative and generative organs of *Althaea officinalis* exhibit notable morpho-anatomical variations under different ecological conditions. In situ specimens displayed denser tissue organization, thickened sclerenchyma, enhanced differentiation of palisade parenchyma and a more developed vascular system. These plants also showed higher densities of stellate and capitate trichomes, accompanied by intensive accumulation of druse crystals and other ergastic substances within parenchymal cells. Under ex situ conditions, hyperplasia and hypertrophy led to enlarged parenchymatous tissues, as confirmed by statistical analyses, along with the formation of xylem vessels of larger diameter. Distinct ecological differences were also observed in the stomatal apparatus: in situ specimens possessed smaller guard cells and predominantly closed stomata, whereas ex situ specimens exhibited larger guard cells with mostly open stomata. Collectively, these

findings provide compelling evidence of ecotypic structural variability and morpho-anatomical adaptation strategies in the species.

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