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A comparative analysis of mucilage quantities in different organs of *Malva* species and varieties and *Althaea ludwigii* L. via anatomical and biochemical approaches

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Abstract
Mucilages are hydrocolloids that form complex polysaccharides (consisting of sugars and uronic acids) and glycoproteins, which are found in many higher plants. This study aimed to correlate anatomical features of mucilage-secreting cells with phytochemical mucilage content in three varieties of *Malva sylvestris* var. *mauritiana*, var. *eriocarpa*, and var. *sylvestris*, *Malva parviflora* var. *microcarpa*, and *Althaea ludwigii*, collected from distinct ecological regions in Iran. Leaf, petal, fruit, and root tissues were analyzed using light microscopy and Ruthenium Red staining for anatomical characterization, while mucilage content was quantified via hot water extraction (HEM). Results revealed that mucilage-secreting cells were predominantly located in the epidermis of leaves and petals, with the highest density and cell area observed in var. *mauritiana*. Phytochemical analyses confirmed that leaves contained the highest mucilage content (up to 27.86% DW), followed by petals, roots, and fruits. A significant positive correlation was found between anatomical parameters (cell number and area) and extracted mucilage yield, validating microscopic analysis as a rapid, cost-effective predictive tool. Ecological altitude was identified as a key factor influencing mucilage production, with higher yields observed in populations from higher elevation regions (e.g., Gilavand). No significant diagnostic anatomical characters were found among varieties, except for the presence of prismatic calcium crystals in *Malva sylvestris* var. *sylvestris* roots, indicating the limited taxonomic value of anatomical traits at the infraspecific level. These findings support the use of anatomical screening for preliminary selection of high-yield mucilage sources in pharmaceutical applications.
Keywords: Altitude, Anatomical correlation, ImageJ, *Malva sylvestris* varieties, Mucilage, Phytochemical quantification, Iran.

Introduction

Malva L. is a large genus of Malvaceae, including mainly herbs of Mediterranean to Southwestern Asian distribution, with 65 species in the world and 7 species in Iran (Pakravan, 2005; 2007). It is characterized by small flowers, 2-3 epicalyxes, schizocarp fruit and circular, somewhat expanded mericarps. *Malva sylvestris* L., commonly known as common mallow, is a polymorphic species with several recognized varieties across its range. There are three prominent varieties of *M. sylvestris*: var. *mauritiana* (L.) Boiss. and var. *eriocarpa* Boiss. and var. *sylvestris* in Iran. These varieties are differentiated by mericarp surface sculpturing. *Malva parviflora* L., is distinguished by small flower (10-15 mm in diameter) (large in *M. sylvestris* about 40-55 mm in diameter), linear epicalyx and somewhat smooth mericarp (Pakravan, 2008). It has three varieties in Iran which distinguished by mericarp characteristics. Systematic studies of *Malva* species have been carried out by different botanists. For example, morphological (Pakravan, 2008; Pour et al., 2019), cladistic (Pakravan & Nemati, 2011) and phylogenetic investigations (Escobar et al., 2009). Despite extensive phytochemical investigations into their therapeutic potentials of *M. sylvestris* (Sharifi Rad et al., 2019), anatomical studies focusing on mucilage-secreting structures and their quantitative correlation with mucilage yield in this species, remain scarce (Tabaraki et al., 2012). Mucilages are natural hydrophilic biopolymers widely distributed in higher plants, particularly within the Malvaceae (Tabaraki et al.,

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2012), composed of acidic polysaccharides and glycoproteins. They serve critical roles in water retention, seed dispersal, wound healing, and defense mechanisms (Munir et al., 2021). Acidic or enzymatic hydrolysis of mucilage results in the release of pentose and hexose monosaccharides, including arabinose, xylose, rhamnose, mannose, galactose, and glucose (Kumar & Neeraj, 2019). In the realm of industrial applications, mucilages are extensively utilized across multiple sectors owing to their unique physicochemical properties, including gel-forming capacity, high viscosity, thermal stability, and biocompatibility. In the pharmaceutical industry, they serve as excipients in tablets and syrups, and as thickening agents (Bosabalidis, 2014). Also, it has used in the cosmetic and food industry (Nep et al., 2013, Chandravanshi et al., 2022, Krasteva et al., 2023). Mucilages are deposited in a wide range of plant organs, including stems, roots, leaves, flowers, tubers, bulbs, floral buds, fruits, and seeds. Their physiological functions are organ-specific and context-dependent. In stems and leaves, mucilage accumulation may mitigate water loss (Christodoulakis et al., 2010), thereby contributing to enhanced drought tolerance (Karawaya et al., 1980; Moyna & Difabio, 1978). In seed coats, mucilage serves a dual function: it facilitates seed dispersal, for example, by promoting adhesion to soil particles or animal vectors and critically supports germination by modulating hydration kinetics, gas exchange, and interactions with soil microbiota (Tamada et al., 1989). These biopolymers are distributed in various plant families, including Plantaginaceae, Cactaceae, Malvaceae, Papilionaceae, Crassulaceae, Boraginaceae, Amaryllidaceae and etc. (Sawidis, 1998). Evidence indicates that mucilage content in wild plant species increases in response to environmental stressors such as drought, salinity, and suboptimal temperatures (Ranˇci´c et al., 2018). This adaptive trait enhances the economic and ecological viability of utilizing wild plants over cultivated species grown under controlled conditions. Wild plants are generally more abundant, cost-effective to harvest, and ecologically better adapted to native environments, thereby offering sustainable advantages in biopolymer sourcing (Gupta et al., 2015). In studies concerning the extraction and application of mucilage, accurate quantification of its content within plant tissues is of paramount importance. Various methodologies have been developed for mucilage quantification, including physicochemical approaches (e.g., swelling factor measurements) and microscopic techniques (Ranˇci´c et al., 2018). Among these, one rapid, low-cost, and non-destructive method involves measuring the cross-sectional area occupied by mucilage-containing cells in histological sections, a technique that has been successfully employed by multiple researchers (Bykova &Yakovleva, 1991, 1996; Pakravan et al., 2007; Ranˇci´c et al., 2018). This approach allows for relative quantification of mucilage while preserving tissue integrity, making it particularly suitable for comparative and developmental studies. As an illustrative example, Bykova and Yakovleva (1991), in their study on *Alcea* species, demonstrated a statistically significant correlation between the cross-sectional area of mucilage-containing cells in leaf tissues and the quantity of mucilage subsequently extracted. They concluded that this histomorphometric approach could serve as a reliable proxy for the quantitative estimation of mucilage content. Similarly, Turowska et al. (1966) conducted analogous research on the roots of *Alcea rosea* L., wherein they quantified the number of mucilage-producing cells in transverse root sections. The anatomical data obtained were validated against physicochemical measurements derived from the swelling factor method, confirming the robustness and reproducibility of microscopic quantification as a complementary analytical tool (Turowska et al., 1966). These studies collectively underscore the utility of histological and morphometric techniques in mucilage quantification, particularly when integrated with conventional physicochemical assays, offering a non-invasive, cost-effective, and biologically informative strategy for assessing mucilage accumulation in plant tissues. In a comprehensive study, Ranˇci´c et al. (2018) not only mapped the anatomical distribution of mucilage-secreting cells in both cultivated and wild populations of *Althaea officinalis* L., but also quantified their mucilage content using physicochemical assays. Their results revealed that wild specimens exhibited significantly higher mucilage yields compared to their cultivated counterparts. This finding strongly supports the hypothesis that mucilage biosynthesis is environmentally inducible, particularly under abiotic stress conditions and underscores the role of ecological factors as natural elicitors capable of enhancing the production of this valuable biopolymer. Consequently, wild-harvested plants may represent a more efficient and sustainable source for mucilage extraction in industrial and pharmaceutical applications (Ranˇci´c et al., 2018, Goksen et al., 2023, Pedroza-Sandoval, 2025). Species such as *Malva* L (Munir et al., 2021), *Althaea* (Ranˇci´c et al., 2009, Moazzezi et al., 2022), *Alcea* L. (Sharifi et al., 2024) and *Hibiscus* (Vignesh & Bindu, 2018, Saha et al., 2024) are renowned for their high mucilage content (Dugani et al., 2016) and have been traditionally used in Persian and global herbal medicine for treating diabetes, inflammation, respiratory ailments, and gastrointestinal disorders (Ameri et al., 2014). This study was designed to investigate the occurrence and distribution of mucilaginous cells in different tissues of three varieties of *Malva sylvestris* and *M. parvifolia* var. *microcarpa* collected from diverse habitats in Iran, with a comparative assessment involving *Althaea ludwigii*, to compare anatomical and biochemical mucilage yields, the latter quantified through biochemical analyses conducted in the Pharmaceutical Raw Material Laboratory. Moreover, we compared mucilage content across populations originating from distinct ecological settings to test the effect of altitude, this research aimed to provide new insights into the potential influence of environmental conditions on mucilage production. Furthermore, we aim to evaluate taxonomic utility, determine whether anatomical characteristics are valuable for taxonomic identification of *Malva* species. Understanding the anatomical distribution and quantitative variation of mucilage across different tissues and habitats not only provides valuable insights into the adaptive strategies of these species but also contributes to their taxonomic characterization. Furthermore, documenting mucilage content in previously unstudied species and plant organs, such as the petals of *Malva sylvestris* and *Althaea ludwigii*, offers novel data that can inform future pharmacognostic and industrial applications

Materials and Methods

Plant Material and Sampling

Dried herbarium specimens of *Malva parviflora* var. *microcarpa*, *M. sylvestris* L. var. *mauritiana*, var. *eriocarpa*, var. *sylvestris* and *Althaea ludwigii* were collected from several distinct populations in Iran: Voucher specimens (Table 1) were deposited at the Herbarium of Alzahra University (Tehran, Iran). Fresh leaf (leaves below the inflorescence), petal, and root samples were stored in 70% ethanol for 14 days. Free-hand sectioning from the middle of leaves and epidermal peel sections were prepared and stained with Ruthenium Red (specific for acidic polysaccharides) (Ruzin, 1999), following Pakravan et al. (2007). Sections were observed and photographed using an Olympus BX51 light microscope and a DP12 digital camera. Moreover, Lugol test was carried out to assess the presence of starch in root samples.

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Table 1. The list of studied Iranian taxa along with related data.

Species	Locality, collector & voucher number	Herbarium Name
M. Sylvestris L. var. *sylvestris*	Tehran: Damavand, 2014 m a.s.l., Abedinzade, 3124	ALUH
	Tehran: Evin, 1650 m a.s.l., Abedinzade 3130	ALUH
	Tehran: Evin, 1640 m a.s.l., Abedinzade 3132	ALUH
M. Sylvestris L. var. *mauritiana* (L.) Boiss.	Tehran: Damavand, 1700 m a.s.l., Abedinzade 3125	ALUH
	Tehran: Damavand, Gilavand, 1800 m, Abedinzade 3126	ALUH
	Tehran: Evin, 1650 m a.s.l., Abedinzade 3131	ALUH
M. Sylvestris L. var. *eriocarpa* Boiss.	Tehran: Damavand, 1950 m a.s.l, Abedinzade, 3119	ALUH
	Tehran: Evin, 1640 m a.s.l., Abedinzade 3133	ALUH
	Kerman: Road of Bam to Jiroft, 180 m a.s.l., Pakravan 3047	ALUH
M. parviflora L. var. *microcarpa* (Pers.) Fiori & Paol.	Khuzestan: Mollasani, 23 m a.s.l., Farasat 18440	ALUH
	Khuzestan: Mollasani, 23 m a.s.l., Farasat 18443	ALUH
	Qom: Road from Qom to Kashan, 850 m a.s.l., Abedinzade 3140	ALUH
Althaea ludwigii L.	Hormozgan: Genu Mt., 1200 m a.s.l., Pakravan 2890.	ALUH
	Kerman: Baft, Gijuiyeh village, 2977	ALUH

Anatomical Quantification

For each taxon, three distinct populations were selected. From each population, two individual plants were randomly sampled, and five anatomical replicates were prepared per individual, resulting in a total of 30 replicates per variety (n = 30). Epidermal peels and transverse sections were examined microscopically, and digital images were analyzed using ImageJ v1.53 (Schneider et al., 2012). Following scale calibration (converting pixel dimensions into micrometers), the cross-sectional area (µm²) of mucilage-secreting cells was manually delineated and measured. This procedure was repeated for at least 30 cells from three independent leaf samples (10 cells per sample).

Phytochemical Extraction and Quantification

Dried plant organs (leaf, petal, root, fruit) were ground and subjected to hot extraction (HEM) (Karawaya et al., 1980; Brautigam & Franz, 1985; Pakravan et al., 2007). Briefly, 5 g of powder was extracted in 100 mL of distilled water (pH adjusted to 3.5 with HCl) at 96°C for 12 h . The extract was precipitated with 96% ethanol (8:2 v/v). The resulting extract was filtered, then concentrated under reduced pressure (4°C, 24 h) and subsequently centrifuged (3000 rpm, 15 min). To the obtained solution, four volumes of 96% ethanol were added, and the mixture was allowed to precipitate overnight at 0–4°C. The precipitated polysaccharides were collected by filtration onto filter paper, dried at 40°C for 24 hours, and then weighed. Mucilage content was expressed as % dry weight (g/100g DW). (Clifford et al., 2002). The data were determined in triplicate, and analyzed by analysis of variance (ANOVA) in SPSS software ver. 25 (2017).

Statistical analysis

The relationship between mucilaginous cell area and mucilage content (% dry weight) was evaluated using regression analysis, and the correlation coefficient was also estimated using SPSS software ver. 25 (2017).

Results

Anatomical Observations of Leaf Structure

Epidermal cells ranged from smooth to undulate; mucilage-secreting cells were interspersed among them, fully occupied by mucilage, and exhibited diverse shapes including dumbbell, pyriform, rectangular, and oval forms. Upon Ruthenium Red staining, mucilage exhibited a distinct red coloration. Stomata were predominantly located on the abaxial epidermis, of the anisocytic type, with a stomatal index ranging from 11 to 20 (Table 3; Fig. 1). Glandular, stellate, trichotomous, dichotomous, and simple trichomes were observed on both surfaces (Fig. 2). Notably, 2–4 mucilage cells were consistently located beneath trichome bases (Fig. 2C). The mesophyll was dorsiventral, consisting of two rows of palisade parenchyma (adaxial) and spongy parenchyma (abaxial) (Fig. 2A, B, C, F). Mucilage in the mesophyll was restricted to intercellular spaces adjacent to vascular bundles, particularly in contact with xylem tracheids (Fig. 2C, E, F). In all *Malva* species, the adaxial surface of the midvein was elevated with a curved apex (Fig. 2D), whereas in *A. ludwigii* it was cleft and V-shaped (Fig. 2E). Two to three layers of sclerenchyma occurred on the upper and lower sides of the midrib (Fig. 2D). In *A. ludwigii*, however, only one to two layers of sclerenchyma were present on the underside of the midrib (Fig. 2E). Among the populations of *Malva sylvestris* var. *sylvestris*, the largest leaf mucilage cell area was observed in the Damavand population (elevation 2014 m a.s.l.), ranging from approximately 15.0 to 15.73 µm2, along with the highest number of mucilage cells (296). The smallest leaf mucilage cell area in this variety was recorded in the Evin population (elevation 1640 m a.s.l.), ranging from 14.5 to 14.8 µm2, with the lowest number of mucilage cells (192). In the populations of *M. sylvestris* var. *eriocarpa*, the Damavand population (elevation 1950 m a.s.l.) exhibited the largest leaf mucilage cell area, ranging from about 19.7 to 22.18 µm², and also had the highest number of mucilage cells (784). The smallest leaf mucilage cell area in this variety was recorded in the Kerman population (elevation 180 m a.s.l.), ranging from 19.94 to 21.4 µm², with the lowest number of mucilage cells (237). Among the populations of *M. sylvestris* var. *mauritiana*, the Gilavand population (elevation 1800 m a.s.l.) showed the largest leaf mucilage cell area, ranging from approximately 27.9 to 28.63 µm², as well as the highest number of mucilage cells (932). Conversely, the smallest leaf mucilage cell area in this variety was observed in the Evin population (elevation 1640 m a.s.l.), ranging from 27.72 to 28.20 µm², with the lowest number of mucilage cells (590). In the populations of *M. parviflora* var. *microcarpa*, the Qom population (elevation 850 m a.s.l.) had the largest leaf

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mucilage cell area, ranging from 6.71 to 7.33 µm², and also exhibited the highest number of mucilage cells (444). The smallest leaf mucilage cell area in this variety was found in the Khuzestan population (elevation 23 m a.s.l.), ranging from 6.28 to 6.89 µm², with the lowest number of mucilage cells (218). Among the populations of *Althaea ludwigii*, the Hormozgan population (elevation 1200 m a.s.l.) exhibited the largest leaf mucilage cell area, ranging from 15.7 to 16.87 µm², as well as the highest number of mucilage cells (784). The smallest leaf mucilage cell area in this species was observed in the Kerman population, ranging from 15.2 to 15.4 µm², with the lowest number of mucilage cells (591). The quantitative analysis revealed that the highest density of mucilaginous cells on the leaf surface (934 cells per cm²) occurred in *Malva sylvestris* var. *mauritiana*, whereas the lowest density was observed in *M. parviflora* var. *microcarpa* (230 cells per cm²). Furthermore, the mean area of mucilaginous cells was greatest in *M. sylvestris* var. *mauritiana* (28.63 µm²), while the smallest values were recorded in *M. parviflora* var. *microcarpa* (6.28 µm²). At the epidermal level, the highest number of mucilaginous cells was detected in the Evin population of var. *mauritiana* (No. 3131), whereas the Evin population of var. *sylvestris* (No. 3123) exhibited the lowest count.

Table 2. Number and area of mucilaginous cells in petal and leave of the studied taxa.

Species	No. Msc. petal	Area Msc. Petal µm²	No. Msc. Leaf	Area Msc. Leaf µm²
M. sylvestris L. var. *sylvestris*	192-437	22.8-24.22	192-296	14.5-15.73
M. sylvestris L. var. *mauritiana* (L.) Boiss.	229-637	17.62-22.9	590-932	27.72- 28.63
M. sylvestris L. var. *eriocarpa* Boiss.	250-380	13.24-15.7	237-784	19.94-22.18
M. parviflora L. var. *microcarpa* (Pers.) Fiori & Paol.	230-455	16.6-17.69	218-444	6.28-7.33
Althaea ludwigii L.	326-490	20.85-21.75	591-784	15.2-16.87

No. Msc.: Number of mucilaginous cells per mm²

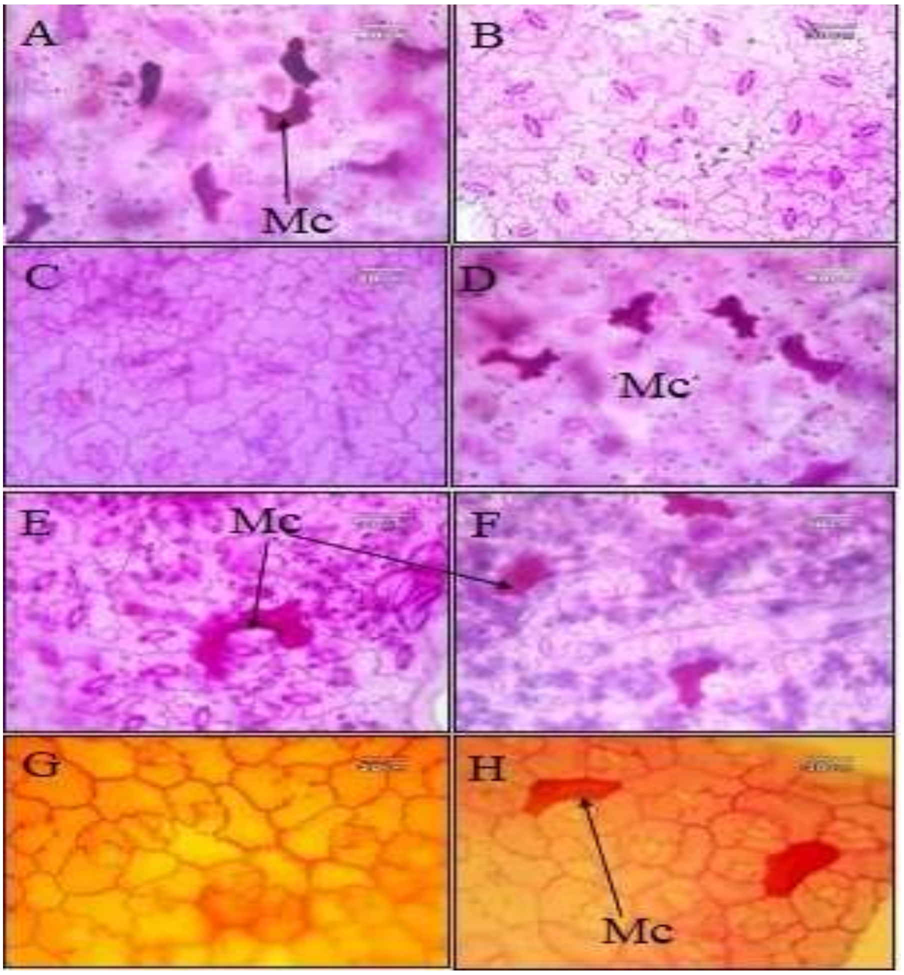

Fig. 1. Leaf epidermis characteristics in the studied taxa. A& B: *Malva sylvestris* var. *mauritiana*, C & F: *M. sylvestris* var. *sylvestris*, D: *M. sylvestris* var. *eriocarpa*, E: *M. parviflora* var. *microcarpa*, G & H: *Althaea ludwigii.* Mc: mucilaginous cell

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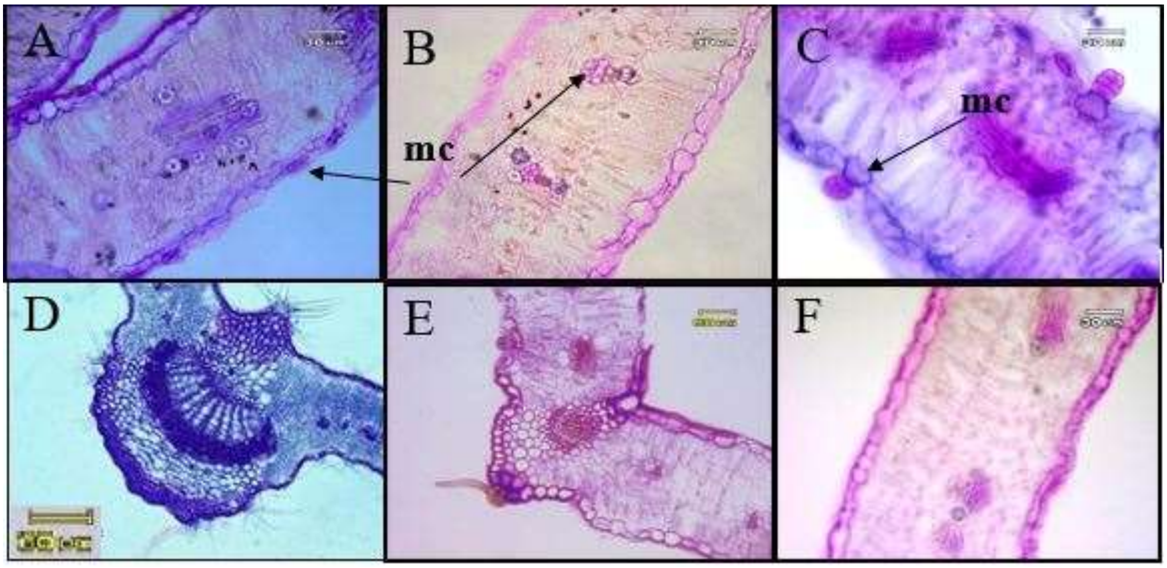

Fig. 2. Leaf cross section characteristics in the studied taxa. A: *M. sylvestris* var. *sylvestris*, B: *Malva sylvestris* var. *mauritiana*, C: *M. parviflora* var. *microcarpa*, D & F: *M. sylvestris* var. *eriocarpa*, E: *Althaea ludwigii.* Mc: mucilaginous cell

Anatomical characteristics of the petal

The anatomical study of the petal demonstrated that it is composed of two cell layers, with 4–5 additional cell rows restricted to the midrib region (Table 3 and Fig. 3C). Mucilage was observed both within the epidermal cells and in the intercellular spaces adjacent to the midrib (Fig. 3). Epidermal mucilaginous cells were elongated and cylindrical in form (Fig. 3). Among the populations of *Malva sylvestris* var. *sylvestris*, the largest petal mucilage cell area was observed in the Damavand population (elevation 2014 m a.s.l.), ranging from approximately 23.8 to 24.22 µm2, along with the highest number of mucilage cells (437). The smallest petal mucilage cell area in this variety was recorded in the Evin population (elevation 1640 m a.s.l.), ranging from 22.8 to 23.4 µm2, with the lowest number of mucilage cells (192). In the populations of *M. sylvestris* var. *eriocarpa*, the Damavand population (elevation 1950 m a.s.l.) exhibited the largest petal mucilage cell area, ranging from about 14.5 to 15.7 µm2, and also had the highest number of mucilage cells (380). The smallest petal mucilage cell area in this variety was recorded in the Kerman population (elevation 180 m a.s.l.), ranging from 13.24 to 14.8 µm2, with the lowest number of mucilage cells (250). Among the populations of *M. sylvestris* var. *mauritiana*, the Gilavand population (elevation 1800 m a.s.l.) showed the largest petal mucilage cell area, ranging from approximately 20.5 to 22.9 µm2, as well as the highest number of mucilage cells (637). Conversely, the smallest petal mucilage cell area in this variety was observed in the Evin population (elevation 1640 m a.s.l.), ranging from 16.6 to 19.76 µm2, with the lowest number of mucilage cells (229). In the populations of *M. parviflora* var. *microcarpa*, the Khuzestan population (elevation 1443 m a.s.l.) exhibited the largest petal mucilage cell area, ranging from 17.21 to 17.69 µm2, and also showed the highest number of mucilage cells (455). The smallest petal mucilage cell area in this variety was found in the Khuzestan lowland population (elevation 23 m a.s.l.), ranging from 16.6 to 16.9 µm2, with the lowest number of mucilage cells (230). Among the populations of *Althaea ludwigii*, the Hormozgan population (elevation 1200 m) exhibited the largest petal mucilage cell area, ranging from 21.16 to 21.75 µm2, along with the highest number of mucilage cells (490). The smallest petal mucilage cell area in this species was observed in the Kerman population, ranging from 20.85 to 21.0 µm2, with the lowest number of mucilage cells (326). Among the examined taxa, the highest mean number of mucilaginous cells was found in *M. sylvestris* var. *mauritiana* (637), whereas the lowest was recorded in var. *sylvestris* (192). Similarly, the largest mucilaginous cell area occurred in *M. sylvestris* var. *mauritiana* (22.9 µm²), while the smallest was measured in *M. sylvestris* var. *eriocarpa* (15.7 µm²). At the population level, the Gilavand population of *M. sylvestris* var. *mauritiana* exhibited the highest number of mucilaginous cells (637), in contrast to the Evin population of *M. sylvestris* var. *sylvestris*, which showed the lowest (192) (Table 2).

Table 3. Anatomical characteristics of leaves in the studied taxa.

Species	Leaf Trichome	adaxial midrib	Shape of cell wall	Stomatal index	Leaf crystal
M. Sylvestris L. var. *sylvestris*	Adaxial	Elevated with curved apex	undulate	20-21.05	Druse, prismatic
M. Sylvestris L. var. *mauritiana* (L.) Boiss.	Adaxial	Elevated with curved apex	Angular to undulate	20-20.45	Druse
M. Sylvestris L. var. *eriocarpa* Boiss.	Both sides	Elevated with curved apex	undulate	19.27-20.83	Druse
M. parviflora L. var. *microcarpa* (Pers.) Fiori & Paol.	Abaxial	Elevated with curved apex	Angular to undulate	17.63-19.03	Druse
Althaea ludwigii L.	Abaxial	Cleft	Angular to undulate	18.6-20	Druse

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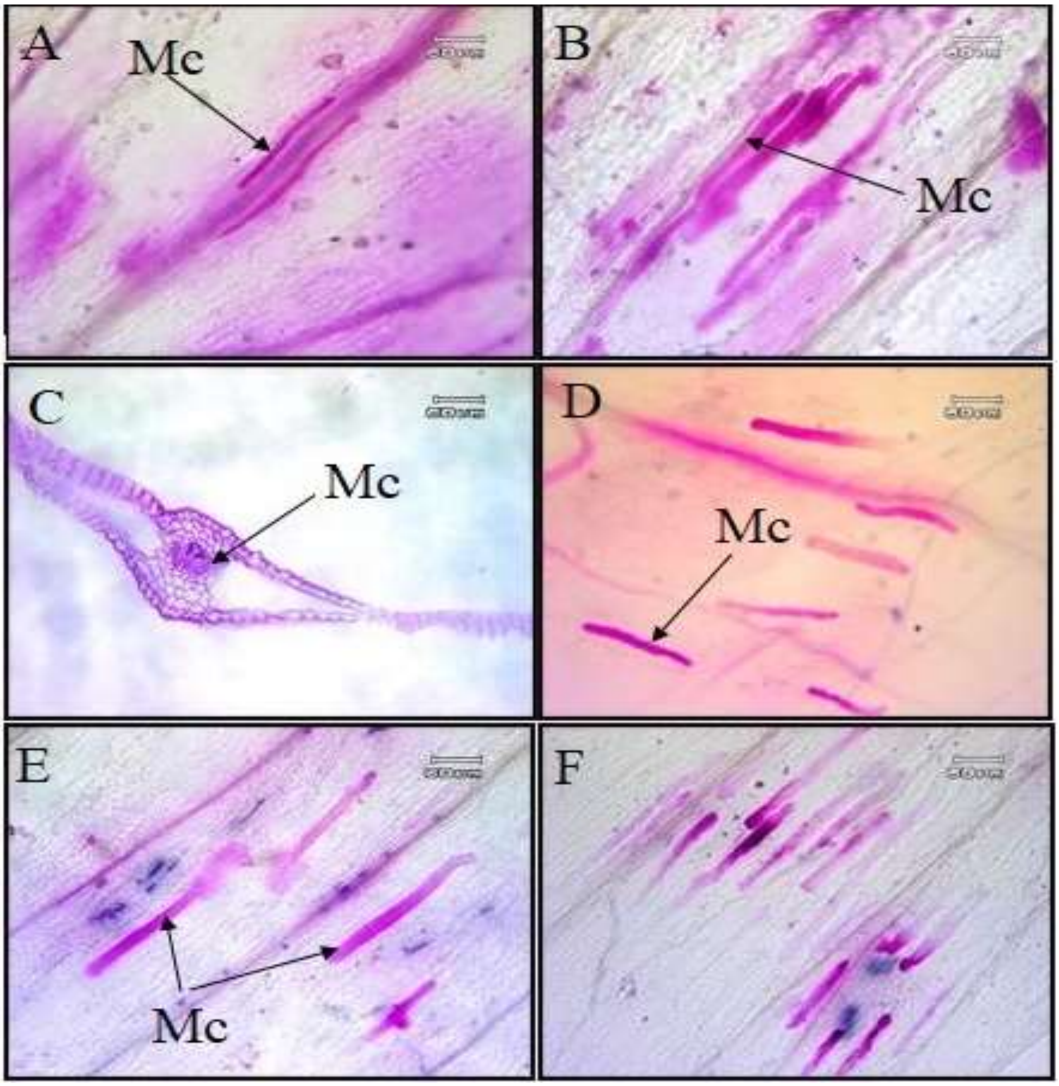

Fig. 3. Petal epidermis characteristics in the studied taxa. A: *Malva sylvestris* var. *mauritiana*, B: *Althaea ludwigii,* C & E: *M. sylvestris* var. *sylvestris*, D: *M. sylvestris* var. *eriocarpa*, F: *M. parviflora* var. *microcarpa*. Mc: mucilaginous cell

Root Structure

Mucilage was stored within idioblasts. The presence of mucilage in the idioblasts was examined using Lugol's iodine staining. While Lugol's reagent typically produces a blue coloration in the presence of starch, the idioblasts observed in the root did not turn blue, suggesting that these cells contain mucilage rather than starch. However, precise area measurement was unfeasible due to indistinct cell boundaries (Fig. 4). Calcium oxalate druse crystals were present in all root samples except in *Althaea ludwigii*. Only in *M. sylvestris* var. *sylvestris* were additional crystal forms (prismatic calcium oxalate crystals) observed (Fig. 4F). The abundance of crystals varied among organs: their density was low in the leaves and relatively high in the roots.

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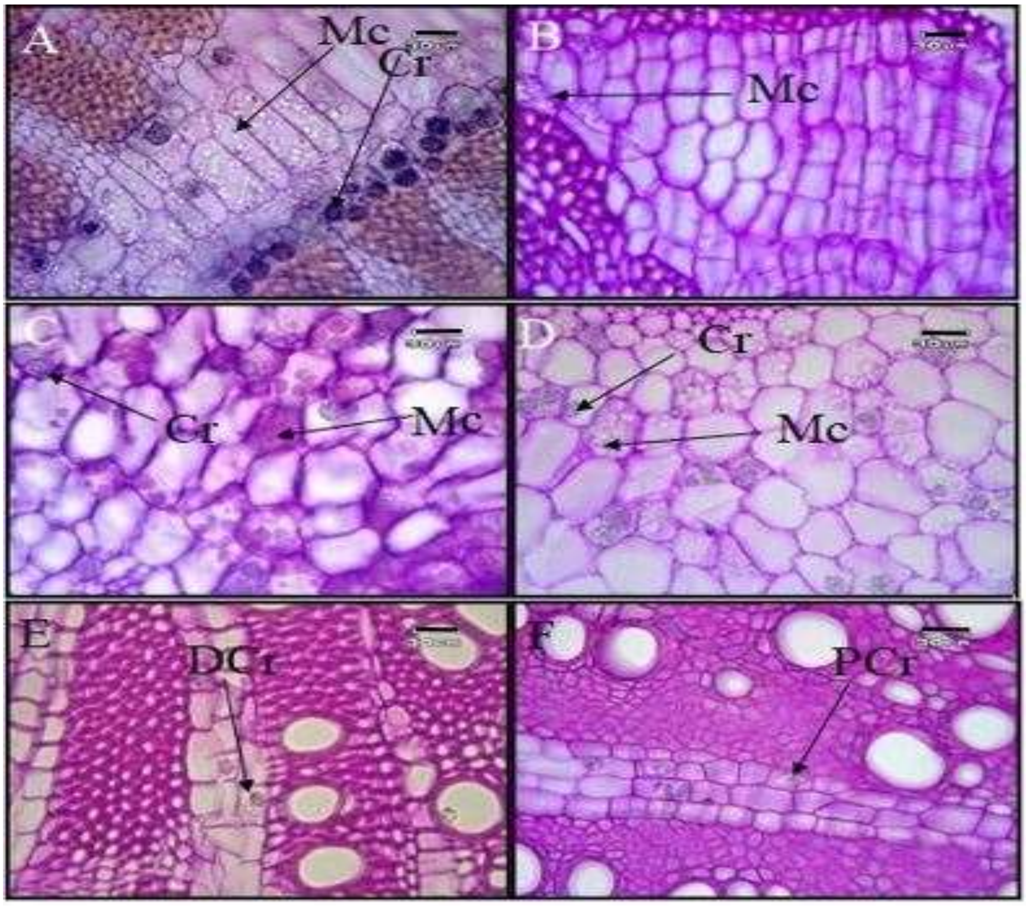

Fig. 4. Root cross section characteristics in the studied taxa. A: *Malva sylvestris* var. *mauritiana*, B: *Althaea ludwigii*, C & D: *M. sylvestris* var. *eriocarpa*, E: *M. parviflora* var. *microcarpa*. F: *M. sylvestris* var. *sylvestris.* Scale bar: 30 µm. Mc: mucilaginous cell, Cr: crystal, DCr: druse crystal, PCr: prismatic crystal.

Phytochemical analysis

The amount of mucilage extracted from the studied taxa was determined using the HEM method and expressed as a percentage of dry weight. In all taxa examined, mucilage content decreased progressively from leaves to flowers, fruits, and roots. Accordingly, leaves contained the highest amounts of mucilage, whereas fruits exhibited the lowest values. In contrast, Pakravan et al. (2007) reported a higher mucilage content in flowers compared to leaves in *Alcea angulata*. Among the examined taxa, leaf mucilage content was greatest in *M. sylvestris* var. *mauritiana* (27.28%) and lowest in *M. parviflora* var. *microcarpa* (25.70%). Petal mucilage content was greatest in var. *mauritiana* (19.57%) and lowest in *M. sylvestris* var. *eriocarpa* (16.62%). Fruit mucilage content was highest in *M. sylvestris* var. *sylvestris* (15.96%) and lowest in *Althaea ludwigii* (15.4%). In roots, *M. sylvestris* var. *eriocarpa* exhibited the highest mucilage content (7%), while the lowest value was observed in *M. parviflora* var. *microcarpa* (5.20%) (Table 4). To the best of our knowledge, the mucilage content of petals in *M. sylvestris* and *A. ludwigii* has not been previously documented. Therefore, the present study represents the first report of petal mucilage content in these species, providing novel insights into their phytochemical characteristics and potential taxonomic value.

Table 4. Mucilage contents in different organs of studied taxa.

Species / **Organ**	**Root (DW%)**	**Fruit (DW%)**	**Petal (DW%)**	**Leaf (DW%)**
M. sylvestris L. var. *sylvestris*	5.97	15.96	17.72	25.15
M. sylvestris L. var. *mauritiana* (L.) Boiss.	5.25	15.72	19.57	27.86
M. sylvestris L. var. *eriocarpa* Boiss.	7.00	15.40	16.62	25.70
M. parviflora L. var. *microcarpa* (Pers.) Fiori & Paol.	5.20	13.54	18.42	12.68
Althaea ludwigii L.	5.86	13.34	18.87	25.60

DW: Dry weight

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Statistical analysis

The linear regression analysis revealed a strong positive relationship between cell area and mucilage content in *Malva* tissues. The Pearson correlation coefficient (r = 0.89, p ≈ 0.000008) indicated a very strong and statistically significant correlation (Fig. 5).

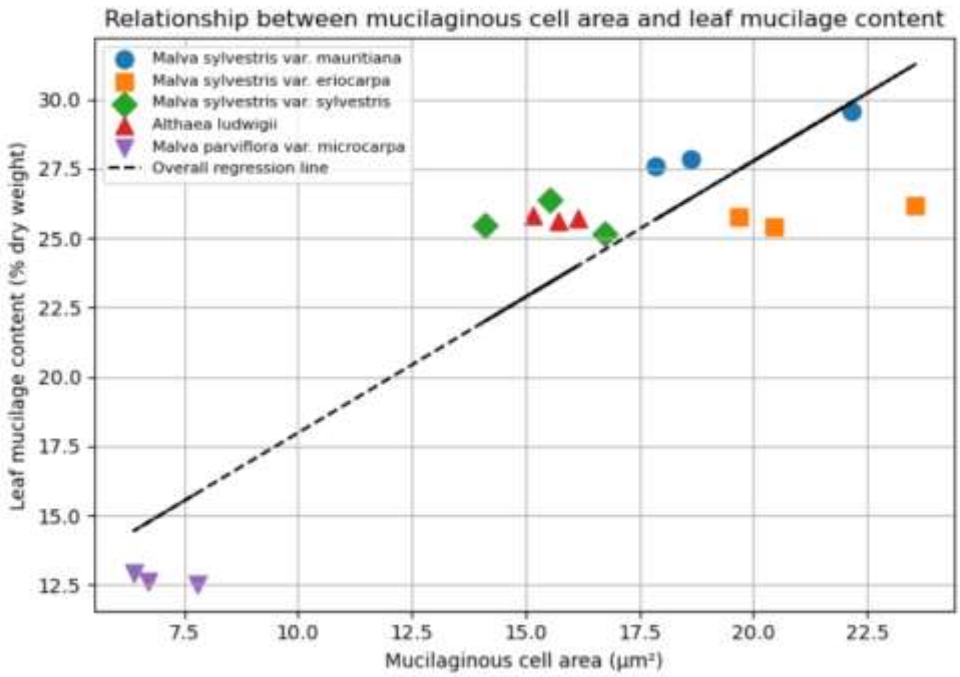

Fig. 5. Scatter plot showing the positive relationship between mucilaginous cell area and leaf mucilage content (% dry weight) across *Malva* species, with each taxon represented by a different symbol and an overall regression line included.

Discussion

In this study, the mucilaginous cell area in cross section of the leaves in *Malva* taxa corresponds with the content of mucilage and relatively high correlation coefficient (0.89), suggesting that larger cell areas are closely associated with higher mucilage content. This result confirms a strong positive correlation between anatomical features and phytochemically quantified mucilage yield, which, validating anatomical screening as a predictive, non-destructive tool, as finding consistent with Pakravan et al. (2007) and Rancić et al. (2018). Except for *M. parviflora* var. *microcarpa* which petals had higher content of mucilage, the mucilage content in leaves was consistent with the findings of Pakravan et al. (2007), who reported that in *Malva neglecta* Walls. and *M. nicaeensis* All., the leaf tissue contained higher mucilage content than other plant organs. But it was in contrast with earlier reports in *Althaea officinalis*, where flowers showed higher yields (Pakravan et al., 2007), highlighting species-specific allocation strategies. The restriction of mesophyll mucilage to vascular-adjacent zones suggests a potential role in hydraulic regulation or pathogen defense. The influence of abiotic factors, particularly altitude, on mucilage production was evident. The Gilavand population (from a higher elevation) consistently exhibited superior yields across all organs. This aligns with the hypothesis that environmental stressors such as increased UV radiation, lower temperatures, and hydraulic challenges at high altitudes trigger enhanced mucilage biosynthesis as an adaptive response (Karawaya et al., 1980; Moyna & Difabio, 1978). In these environments, mucilage likely aids in desiccation tolerance by retaining moisture and may also serve as a physical barrier against damaging UV-B radiation, as suggested by its gel-forming properties (Moyna & Difabio, 1978). This pattern is further supported by Rancić et al. (2018), who reported increased mucilage in *Althaea* roots at higher altitudes. The presence of mucilage in the petals of the studied specimens also plays a protective role for floral organs. In organs whose development is incomplete, as well as in reproductive structures potentially exposed to desiccating conditions, the presence of hydrophilic substances such as mucilage can maintain adequate moisture levels until efficient water-conducting systems are fully developed *(*Pereira et al., 2025*)*. Anatomically, no diagnostic characters reliably distinguished the three varieties, except for the presence of prismatic calcium crystals in addition to druses in var. *sylvestris* roots. This suggests that anatomical traits in *Malva* (studied taxa) possess limited taxonomic value and are more reflective of phenotypic plasticity under environmental influence. This study highlights the anatomical quantification (via microscopy + ImageJ) as a rapid, low-cost, non-destructive method that strongly correlates with biochemical mucilage content, enabling preliminary screening of high-yield plant materials. Among the taxa examined, leaves *of Malva sylvestris* var. *mauritiana* are the optimal source for mucilage extraction, exhibiting the highest cell density, cell area, and phytochemical yield (27. 86% DW). Also, altitude significantly enhances mucilage production, with high-elevation populations (e.g., Gilavand) showing superior yields, a critical consideration for cultivation and wild harvesting. Moreover, taxonomic utility of anatomical traits is limited among *Malva* species, indicating that observed variations are ecophenotypic rather than genotypic. This study provides the first quantitative mucilage data for petals of *M. sylvestris* and *Althaea ludwigii*, filling a significant gap in

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ethnopharmacological literature. These findings offer practical guidelines for the pharmaceutical and nutraceutical industries in selecting optimal plant sources and populations for mucilage extraction, while also contributing to the understanding of plant adaptation mechanisms under environmental stress.

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