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Original Article

Histo-Anatomical Study of *Campanula saxifraga* subsp. *meyeriana* (Rupr.) Ogan

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ABSTRACT

Background: Representatives of the Campanulaceae family are widespread in Azerbaijan. These plants are rich in biologically active compounds such as flavonoids, essential oils, saponins, coumarins, etc. **Objectives:** This work presents histo-anatomical research on cross-sections of the root, stem, leaf and petal of *Campanula saxifraga* subsp. *meyeriana* (Rupr.) Ogan. **Materials and Methods:** Endemic in the Caucasus, these sub-species inhabit the subalpine zone. The plant grows on horizontal rock crevices in the village of Gryz, the Guba region of Azerbaijan. Blocks were prepared according to the generally accepted methods in electron microscopy. **Results:** The microscopic examination of the cross-section of the stem revealed that the cortex is composed of 8-10 layers of collenchyma and laticifers are seen between the endoderm layer and the phloem. The conductive tissue is in two coextensive rings on the cross-section of the root, and laticifers are detected in the cortex. The leaf has a bifacial structure. On the cross-section of the petal, the mesophilic layer is poorly developed. **Conclusion:** The microscopic examination provides a basis to determine the identity of *C. saxifraga* subsp. *meyeriana* (Rupr.) Ogan.

Keywords: *Campanula saxifraga* subsp. *meyeriana* (Rupr.) Ogan., Histo-anatomical study, Root, Stem, Leaf, Petal.

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INTRODUCTION

The Campanulaceae family includes about 84 genera and more than 2400 species worldwide.[1] Six genera of this family are widespread in Azerbaijan.[2]

Campanula L. is one of the most interesting genus due to the large number of its species, its morphological features and distribution areas. The flora of Azerbaijan includes 32 species (including 10 subsp.) of the genus *Campanula* L.[2] and most of these, also growing in other parts of the globe, have been involved in pharmacognostic research by scientists worldwide. Some biologically active compounds (flavonoids, essential oils, saponins, coumarins, etc.)[3-7] have been obtained from these species as well. Numerous studies have proved that some of these have antioxidant, enzyme inhibitory, antimicrobial activities, anti-inflammatory and wound-healing potential.[8-11]

The topicality of this research is based on the fact that, as a reading of relevant literature informs, limited research has been done into this and adjacent species of bellflower growing in the Azerbaijani flora. Among unstudied species belonging to the Medium D.C. section of this genus, *C. saxifraga* subsp. *meyeriana* (Rupr.) Ogan is more preferable in terms of its distribution area and high raw material reserves. Preliminary phytochemical studies demonstrated that the plant comprises a lot of biologically active substances which makes it a valuable object for investigation. Taking all this into consideration, the purpose of our study was to investigate the histo-anatomical structure of *C.saxifraga* subsp. *meyeriana* (Rupr.) Ogan for the identification of the plant.

MATERIALS AND METHODS

For microscopic research work, *C.saxifraga* subsp *meyeriana* (Rupr.) Ogan was collected on June 12, 2021, during its flowering period, in subalpine meadows. The collection area was cracks in rocks around the Gryz village of the Guba region, 1900 metres above sea level: 41°21'93.2"N; 48°24'55.8"E. The stem, root, leaf and petal of the plant were chosen as an investigation object. Blocks were prepared according to the generally accepted methods in electron microscopy.[12-13]

Fifteen fresh samples from several parts of the plant were fixed in phosphate buffer solution (pH 7.4). After being removed from the solution, the samples were washed three times for 15 min each (45 min in total) in 0.1 M phosphate buffer (pH=7.4). Then the samples were postfixed in a mixture of 1% OsO4, 0.1 M phosphate buffer, and 1.5% red prussiate for 1.5 hr. After the post-fixation stage, the samples were washed three times each for 15 min in 0.1 M phosphate buffer (pH=7.4) and then dehydrated. Next, the samples passed through 50%, 70% and 96% ethanol for 15 min three times each. Afterwards, the

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samples were washed, firstly, in a solution of 96% ethanol and acetone (1:1 ratio) and, secondly, in pure acetone three times. Furthermore, an Araldide-Epon solution was prepared using a mixture of 5 substances (Epon-812, DDSA, Araldide M, Dibutalphthalate and DMP-30). The weight ratio of the above-mentioned substances varies depending on the amount of samples taken. Finally, the block preparation was performed in four stages using pure acetone and Araldide-Epon solution of different ratios.

The pre-selected and numbered forms were stored in a thermostat at 37°C, 45°C and 60°C for one day at each heat accordingly (three days in total). Total of eight blocks was cut using a Leica EM UC7 ultramicrotome and, overall, 84 semithin sections (1–2 µm) were obtained from the blocks. For further investigation under the light microscope (Primo Star, Zeiss), the sections were stained using the polychromatic staining method for epoxy embedded tissue.[12] The investigated samples were photographed with a digital camera (Canon D650, Japan). Overall, 35 photos were taken for the study.

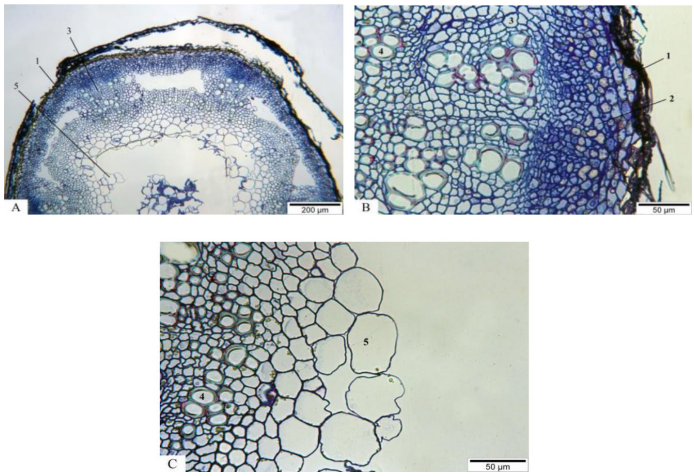

Figure 1: Cross-section of the root: A scale – 200µm, B and C scales – 50 µm. 1 – epidermis, 2 – phelloderm, 3 – phloem, 4 – xylem, 5 – pith.

RESULTS

Root

Microscopic examination shows that the root of the plant has a rounded shape (Figure 1). The root cross-section is composed of periderm, phellogen and phelloderm from outside to inside.

The periderm tissue is comprised of 3-4 layers of squeezed cells. Phellodermic cambium is made up of flat, slightly curved, and thin-walled cellulosic cell layers. The number of layers is up to 4-5. The laticifer cells are visible in the cortex. The conductive tissue is arranged in two coextensive rings. The phloem tissue is formed of sieve tubes, phloem parenchyma, and extra cells. A single-layered cambium is situated between the xylem and phloem. The central part of the root has xylem tissue, which consists of small metaxylem vessels of various sizes. Medullary rays are multicellular and cellulosic in the phloem tissue, whereas, in the xylem tissue, they are multicellular and slightly stiff. The medullary parenchyma is poorly developed.

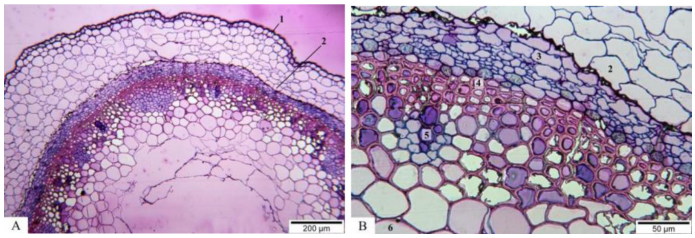

Figure 2: The cross-section of the stem: A scale – 200µm, B scale – 50 µm. 1 – epidermis, 2 – endoderm, 3 – phloem, 4 – cambium, 5 – xylem, 6 – pith.

Stem

On the cross-section, the stem is covered with an epidermis made of a layer of semi-isodiametric structured cells outside (Figure 2). The thick external wall of epidermis is encircled with a cuticle.

The cortex is composed of 8-10 layers of collenchyma. The internal layer of the cortex is parenchymal. Endoderm is visible as a single layer of large cells. Laticifers are visible between the endoderm layer and the phloem. The conducting tissue contains a large number of open collateral tubes of various sizes. Sieve tubes, phloem parenchyma and extra cells constitute the phloem layer. In this area, the medullary rays are cellulosic and made up of more than one cell. The cambium layer is circularly curved. Secondary xylem tissue is surrounded by a cambium layer. Libriform tissue is next to metaxylem vessels. The xylem vessels show reticular and spiral thickening in the longitudinal-radial sections. At the level of xylem, the medullary rays appear multilinear and involve a lot of cells. The medullary parenchyma is developed sufficiently. There is a pith cavity in the central part of the stem.

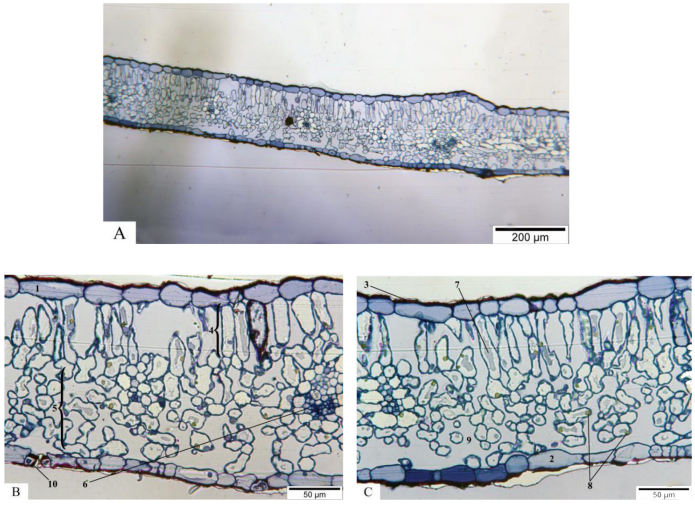

Figure 3: The cross-section of the leaf: A scale – 200µm, B and C scales – 50 µm. 1 – upper epidermis, 2 – lower epidermis, 3 – cuticle, 4 – palisade mesophyll, 5 – spongy mesophyll, 6 – vascular bundle, 7 – vacuole, 8 – chloroplast, 9 – air channel, 10 – stoma.

Leaf

Microscopic investigation of the leaves demonstrated that the upper epidermis consists of large, flat cells with thin radial walls (Figure 3). Epidermal cells vary in size and wall thickness in different parts of the same leaf. The external walls are convex and covered with a thick cuticle. The mesophyll is divided into distinct regions (termed palisade and spongy tissues respectively). Palisade cells are of an elongated form, and intercellular air spaces are easily visible in-between. The elongated cell parenchyma is rich in chloroplasts. The spongy mesophyll is made of variously-shaped cells with many air spaces between them. Centrally located ligneous vascular bundles are surrounded by the assimilation cover. The mesophyll has a bifacial dorsiventral structure. The lower epidermis forms a layer of small cells with thin radial external and

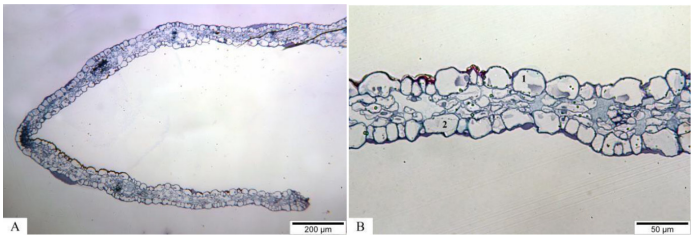

Figure 4: The cross-section of the petal: A scale – 200µm, B scale – 50 µm. 1 – upper epidermis, 2 – lower epidermis.

internal walls. A large number of stomata is detected at the level of the cuticle.

Petal

The investigation established that the petal is made of abaxial and adaxial epidermises and 3-4 layers of undifferentiated isodiametric or elongated cells separated by many air spaces (Figure 4).

The mesophilic layer is poorly developed and interspersed with a few vascular bundles.

DISCUSSION

The structural aspects of *C. saxifraga* subsp. *meyeriana* (Rupr.) Ogan. is identified in this paper through the histo-anatomical analysis. The detected features separate the plant from similar species of this genus.

In our microscopic study, laticifers were found on a cross-section of both the root and stem. In contrast to this, *C. lyrate Lam.* subsp. *Lyrata* has laticifers only in its root.[14] Moreover, similar to the study of *C. persicifolia* L.,[15] we observed densely located ligneous vascular bundles covered by the assimilation layer in the central part of the leaf as well. The leaf has a bifacial dorsiventral structure in both plants. However, unlike the case of *C. persicifolia* L., angular collenchyma was not discovered above the lower epidermis of our plant.

Furthermore, the microscopic examination of *C.carpatica Jacq.* disclosed a circular-shaped stem with five prominent ribs in its cross-section. The collenchyma of the plant is reported to be of 1-2 layers and presented only in the ribs.[16] In our study, no ribs were found on the cross-section of the stem and the cortex is composed of 8-10 layers of collenchyma.

According to the study on *C. davisii Turrill*, the leaf of this plant is covered with a thin layer of cuticle, and the epidermal cells are covered with glandular trichomes.[17] However, in our study trichomes are simple, i.e., not glandular. The cuticle layer of our plant is thicker in comparison with that of *C. davisii Turrill*. But common is the fact that the stomata of both plants occur on adaxial and abaxial surfaces of the leaves (amphistomatic leaves).

CONCLUSION

The performed pharmacognostic study revealed some diagnostic attributes of *C.saxifraga* subsp. *meyeriana* (Rupr.) Ogan. which serve the identification of the plant. The factors allowing to distinguish the plant from other species of the genus are a large number of open collateral tubes of various sizes in the conductive tissue of the stem, laticifers visible between the endoderm and phloem of the stem and in the root, two-faced dorsiventral structure of the leaf, the presence of ligneous vascular bundles in the central area of the leaf, and presence of simple trichomes in the leaf.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DDSA: Dodecenylsuccinic anhydride; **DMP-30:** 2, 4, 6-tris Dimethylamino Methyl Phenol.

SUMMARY

This research studies the histological anatomical structure of the root, stem, leaf and petal of the plant called *Campanula saxifraga* subsp. *meyeriana* (Rupr.) Ogan. The analysis carries importance in terms of the identification of the plant and the quality of its raw materials. To list the findings, the xylem tissue consisting of small metaxylem vessels of various sizes covers the central part of the root; and laticifers are observed in the cross-section. The conducting tissue of the stem consists of a large number of open collateral tubes of various sizes. The leaf has a bifacial dorsiventral structure, and simple trichomes are found at the level of the epidermis. The paper serves the bigger context of the comprehensive study of the mentioned plant.

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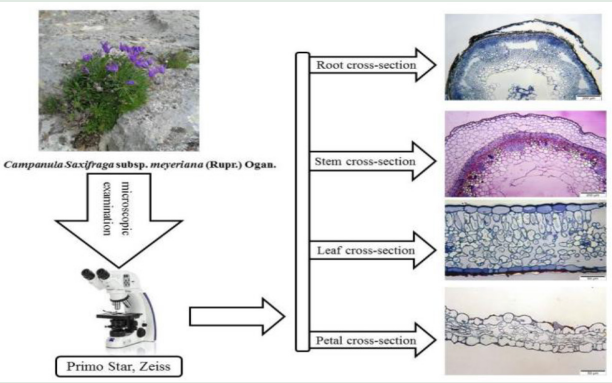
GRAPHICAL ABSTRACT



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