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MORPHOLOGICAL AND ANATOMICAL CHARACTERISTICS OF THE SPECIES *PALIURUS SPINA-CHRISTI*

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Abstract

In plants and all living beings, the fundamental influence comes from ecological factors that operate simultaneously and in a complex manner. The influence of these factors is expressed in the morphological and anatomical features of the vegetative organs of plants, especially the leaves, which are among the most delicate organs where a series of adaptations occur. Although biological science today recognizes approximately 500,000 plant species, they differ from each other morphologically, anatomically, and genetically because they live in different life conditions and circumstances. Understanding the morphological–anatomical characteristics of plants is highly important for determining their taxonomic and ecological adaptability. This connection between the morphological-anatomical structure of vegetative organs, especially leaves, and external factors has been addressed by many authors: Lakusiç (1962), Greb (1957), Pavlov (1965), Hoxha (1995), Mustafa (1995), and others. To obtain a clearer picture of the impact of ecological factors on the morphological and anatomical characteristics of vegetative organs of plants, we conducted research on the anatomical structure of leaves from different insertions: upper (E) and middle (M) of the plant species *Pailurus spina Christi*.

SYSTEMATICS AND CHARACTERISTICS OF THE FAMILY RHAMNACEAE

Species *Paliurus spina-christi*; Subdivision: *Magnoliophytina (Angiospermae)*; Subclass: *Rosidae*; Class: *Magnoliopsida*; Superorder: *Celastranae*; Order: *Ramnales*; Family: *Rhamnaceae*.

Morphological and Anatomical Characteristics of the Species *Paliurus spina-christi*

The Rhamnaceae is a large family of flowering plants, primarily trees or shrubs with twisted stems or vines. It is characterized by epithelial wreath. The family contains 58 genera and approximately 900 species. Rhamnaceae are distributed worldwide but are most common in subtropical and tropical regions. Fossils date back to the Eocene epoch. The leaves are simple, alternate, and spiral, often with spines. They are distinguished by a deep floral axis and a central or inferior ovary. The flowers are radially symmetrical, with 5 sepals (sometimes 4 or none) and separate petals. The petals can be white, yellow, green, pink, or blue and are small, mainly ornamental plants. Some species of Rhamnus are used for dyeing, whereas R. Chlorophora is used to produce green indigo.

Fig 1. Paliurus spina-christi.

MATERIALS AND METHODS

For research on the anatomical and morphological structure, the leaves of the plant species Paliurus spina Christi were used. Initially, we analyzed the anatomical structure of two insertions: upper and middle. The leaves were cut into pieces (0.5 cm) from each insertion; 8 leaves were taken and placed in a container filled with hydrogen peroxide (H2O2, 30%), to which a small amount of potassium hydroxide (KOH) was added to destroy pigments, specifically chloroplasts.

The leaf pieces remained in this mixture (for a few minutes or hours) depending on the plant species used for research. In this case, the leaf pieces remained for approximately 4 hours, during which time decolorization was continuously monitored until the leaves were whitened by a shade compared with their normal color. After bleaching, the leaf pieces were placed in a container in which chloral hydrate had been added and remained until complete transparency (i.e., full translucency) was reached. A drop of glycerin was then placed on the glass, and the leaf pieces, which were transparent, were carefully placed to avoid air bubbles, which were covered with cover glasses.

The prepared samples were used as objects for observing and counting the stomata and for observing venation. For anatomical structure observation, the prepared leaf pieces were placed on a path and then sliced very thinly with a hand microtome, which were then placed on glass objects in a drop of glycerin and observed under a microscope.

During the observation, we focused on examining several parameters, such as the following:

- Length and width of epidermal cells with cuticles on the upper and lower surfaces of the leaf;
- Thickness of palisade tissue on the upper and lower surfaces;
- Thickness of spongy tissue.

The values obtained during these measurements, such as those for the length and width of epidermal cells with cuticles, the thickness of palisade and spongy tissue, the dimensions of the stomata (length and width), and the thickness of the leaf, were expressed in micrometers (μm), whereas the number of stomata was expressed per mm^2. The venation was measured via a curvimeter and expressed in cm/cm^2. Observations were made with a Spencer microscope at a magnification of 10x43x.

RESULTS AND DISCUSSION

Anatomical structure of leaves
Length of epidermal cells with cuticles

The epidermis is a protective tissue located on the surface of the plant body that is in direct contact with all external environmental influences where the plant lives. Its function is to protect internal tissues from harmful external factors such as excessive water evaporation, mechanical damage, rapid temperature changes, and parasite penetration. This protection particularly applies to the above-ground organs of plants, which is why this paper focuses heavily on the anatomical structure of the leaf epidermis. During the microscopic observation of the leaves of the plant species we used as research subjects, we observed that their epidermis is single-layered and double-layered, consisting of cells that are well adjoined to each other and have a quadrangular shape with somewhat rounded edges. The protective role of the epidermis is enhanced by the presence of cuticles on the surface, which are located on the outer wall in the form of a thin layer.

The analysis revealed that the length of the epidermal cells in the cuticle differed between the epidermal cells on the upper and lower surfaces, as shown in Table 1.

Table 1. Anatomical characteristics of the leaves of the species *Paliurus spina-christi*

Length of epidermal cells with	Width of epidermal cells	Thickness of palisade tissue	Thickness of palisade tissue	Thickness of spongy tissue	Length of epidermal cells with	Length of epidermal cells with	Thickness of the leaf
8.57µmm	5.72µmm	26.55µmm	39.27µmm	28.54µmm	8.73 µmm	5.71 µmm	116.7µmm

On the basis of this table, we can conclude that the epidermal cells with cuticles on the upper surface are slightly smaller (8.57 µm) than those on the lower surface (8.73 µm).

Width of Epidermal Cells

In terms of the width of epidermal cells, on the basis of the results of the measurements presented in the aforementioned table, we can conclude that this parameter does not change in the epidermal cells of either the upper or lower sides of the leaf. The width of these cells on both sides of the leaf is 5.72 µm (Figs. 2-3 and Table 1).

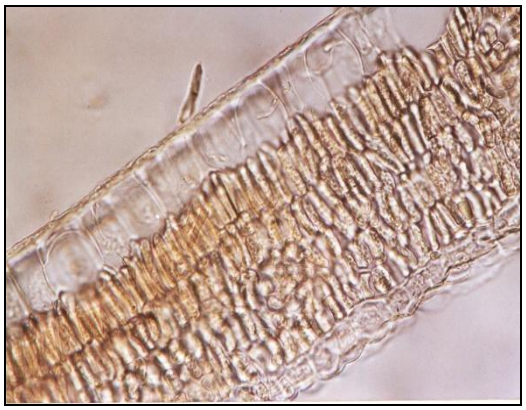

Figure 2. Anatomical structure of the leaf of the species *Paliurus spina-christi*.

Thickness of the Palisade Tissue

The parenchymatic tissue of the assimilation type, called the mesophyll, lies between the upper and lower epidermis of the leaf lobe. This tissue is directly related to the function of the leaf as an organ for CO2 assimilation and for carrying out the photosynthesis process. The mesophyll is differentiated into palisade tissue and spongy tissue.

Palisade tissue usually consists of elongated cylindrical cells arranged perpendicular to the leaf surface. These cells are rich in chloroplasts, making this part of the mesophyll the site where photosynthesis occurs with greater intensity. Hence, it is called true photosynthetic tissue. In the presence of water (H2O), carbon dioxide (CO2), and solar energy, organic matter is synthesized in these cells, while oxygen (O2), which is essential for all living cells, is simultaneously released. The palisade tissue in the leaves of the species Paliurus spina Christi is well developed on both the upper and lower surfaces. On the basis of our observations, we found that the palisade tissue in this plant is one- and two-layered on both the upper and lower sides (Figs. 2-3, Table 1). In terms of the thickness of the palisade tissue, on the basis of the analyses we conducted, the palisade tissue on the lower side of the leaf is more developed (28.54 µm) than the palisade tissue on the upper side (26.56 µm).

Thickness of Spongy Tissue

As mentioned earlier, the mesophyll is differentiated into palisade tissue and spongy tissue. The spongy tissue consists of several layers, depending on the plant species. The cells of this tissue are round or hexagonal. Many intercellular spaces between these tissues are connected into a system that communicates with the external environment through the opening of stomata. The spongy tissue performs the function of gas exchange, and for this reason, it is often called the transpiration parenchyma.

On the basis of our observations of the plant species Paliurus spina-christi, the spongy tissue of this plant is poorly developed and consists of round or hexagonal cells. The measurement results revealed that the thickness of this tissue in this species of plant is, on average, 39.27 µm (Table 1, Figs. 2-3).

Thickness of the Leaf

On the basis of the results obtained during the measurement of the length and height of epidermal cells on both the upper and lower surfaces of the leaf, as well as the height of the palisade and spongy tissues, we determined that the thickness of the leaf of the plant species in question averaged 116.7 µm.

Stomata - Number, Length, and Width

There is a constant exchange of gases between plants and the external environment. Plants absorb oxygen (O2) from the air for respiration and carbon dioxide (CO2) for photosynthesis. On the other hand, through transpiration, they return water vapor and gases to the atmosphere. These processes are carried out by special epidermal structures known as stomata and lenticels. Therefore, we conducted research on the stomata of the plant species Paliurus spina-christi.

Observations of this plant revealed that the stomata are located on both the upper and lower surfaces of the leaf, indicating that the plant is amphistomatic (from the Greek "AMFI" meaning "on both sides"). The results obtained during the measurements revealed that the number of stomata on the upper surface was greater than that on the lower surface. The number on the upper surface is 95.7 per mm² on average, whereas that on the lower surface is 74.5.

Table 2. Number of stomata, length, width, and venation

Surface	Number of stomata per mm²	Length of stomata (µm)	Width of stomata (µm)	Venation (cm/cm²)
Upper surface	97.7	39.4	30.7	
Lower surface	85.5	39	34.1	55.27

In terms of the length and width of the stomata, the stomata on the upper surface are longer than those on the lower surface (39.4µm and 39.0µm, respectively). For width, the opposite is true, meaning that the stomata on the lower surface are wider than those on the upper surface are (34.1µm and 30.7µm, respectively) (Table 2, Figures 5-6). The stomata are positioned at the level of the epidermis.

Figure 3. Stomata on the leaves of the plant species *Paliurus spina-christi* were observed at low magnification.

Figure 4. Stomata on the leaves of the plant species *Paliurus spina-christi* were observed at medium magnification.

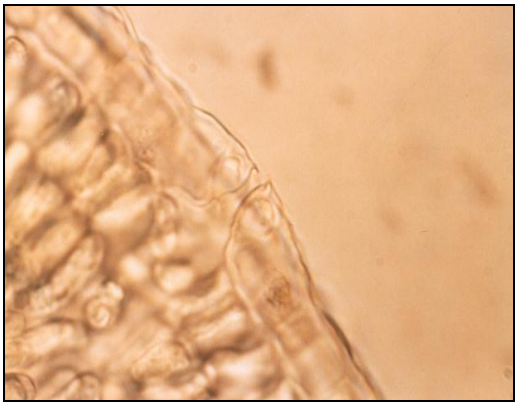

Figure 5. Observation of stomata on the leaves of the plant species *Paliurus spina-christi* at high magnification.

Venation density

In each leaf, especially on the lower surface, we observe veins—vascular bundles of varying sizes that form an entire branching system. Depending on the branching pattern, we distinguish several types of leaves, e.g., leaves with parallel, reticulate, and dichotomous venation.

In the plant species *Paliurus spina-christi*, which we used as the object of study, on the basis of the branching of the veins (venation), it can be concluded that the leaves belong to the type of leaves with parallel venation (Figure 5).

When discussing the venation of leaves, it should be noted that it represents a variable parameter depending on the environment, which actually indicates the adaptation of plants to the action of microclimatic factors derived from the external environment.

The venation density of the studied plant species, which is based on measurements made with a curvimeter, is well developed and averages approximately 55.27cm² (Table 2).

Figure 6. Microscopic observation of venation in the plant species *Paliurus spina-christi*.

CONCLUSIONS

On the basis of the results obtained from the study of the anatomical features of the leaves of the species *Paliurus spina-christi*, we reached the following conclusions:

- The epidermis of the leaves of this species is single-layered and consists of closely packed cells. Above the cells, there is a moderately developed cuticle.
- The palisade tissue is well developed on both surfaces of the leaves. In almost all cases, the tissue is two-layered on the surface and rarely three-layered.
- The spongy tissue is poorly developed with somewhat rounded cells and very few intercellular spaces.

- The stomata are located on both surfaces of the leaf, indicating that the leaves are amphistomatic. The number of stomata is quite high on both surfaces, but it is greater on the lower surface.
- The venation is of the reticulate type and is well developed.

On the basis of the results of this study, we conclude that the species *Paliurus spina-christi* is xerophytic. These types of plants grow in open areas with high temperatures and can withstand drought.

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