The anatomical and micromorphological structure of Myrtus communis L. leaves

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Myrtus communis L. is a promising medicinal plant that has garnered the attention of scientists to introduce it into industrial cultivation as a raw material source for obtaining new phytopreparations. In its chemical composition, *Myrtus communis* L. contains many biologically active substances, which confer upon it a range of medicinal properties including astringent, anti-inflammatory, antiviral, antibacterial, antifungal, antioxidant, antidiabetic, antiulcer, antimutagenic, gastro- and hepatoprotective properties. Published information primarily focuses on the chemical composition and pharmacological activity of common myrtle, with limited data available on its micromorphology.

The aim of the work is to study the morphological and anatomical structure and to determine the general diagnostic microscopic features of common myrtle leaves.

Materials and methods. The object of the research was fresh and dried plant material of common myrtle grown under room conditions. Transverse sections were fixed with Carnois fixative, dehydrated, and paraffinized. The sections were then cut using a Microm HM 325 microtome, followed by deparaffinization. Microscopic analysis of the leaf was performed using temporary preparations on a Carl Zeiss microscope, including the “AxioStar Plus” and “Primo Star”, equipped with a photo attachment for direct and reflected light microscopy.

Results. The external features of common myrtle leaves include their shape, color, size, smell, and type of veining. Anatomically, the leaves exhibit a cuticle, dorsiventral mesophyll, and anomocytic stomata located on the abaxial surface. Additionally, they contain calcium oxalate crystals and druses, as well as simple hairs on the midvein and schizolisogenic secretory receptacles. Histochemical studies of these cavities indicate the presence of lipophilic substances.

Conclusions. The morphological and anatomical analysis of *Myrtus communis* L., a representative of the myrtle genus, allowed for the identification of key diagnostic microscopic features of myrtle leaves. Further research prospects involve conducting a comprehensive set of phytochemical studies to establish standardization parameters for medicinal plant raw materials.

Keywords: Myrtus communis L., plant leaf, leaf anatomy, macro- and microscopic analysis.

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Анатомічна та мікроморфологічна будова листя Myrtus communis L.

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Myrtus communis L. – перспективна лікарська рослина, яка привертає увагу науковців для введення в промислову культуру, оскільки є сировинним джерелом для отримання нових фітопрепаратів. У хімічному складі *Myrtus communis* L. – чимало біологічно активних речовин, що зумовлюють в’яжучі, протизапальні, противірусні, антибактеріальні, протигрибкові, антиоксидантні, протидіабетичні, противиразкові, антимутагенні, гастро- та гепатопротекторні властивості. Опубліковані дані стосуються передусім хімічного складу та фармакологічної активності мирту звичайного, а відомостей щодо мікроморфології майже немає.

Мета роботи – дослідження морфолого-анатомічної будови та визначення загальних діагностичних мікроскопічних ознак листя мирту звичайного.

Матеріали і методи. Об’єкт досліджень – свіжа та висушена рослинна сировина мирту звичайного, вирощеного в кімнатних умовах. Для отримання поперечних зрізів препарати фіксували фіксатором Карнуа, зневоднювали й парафінізували. Поперечні зрізи зробили за допомогою мікротому Microm HM 325 з наступною депарафінізацією. Мікроскопічний аналіз виконали з тимчасовими препаратами листка на мікроскопі Carl ZEISS «AxioStar Plus» та «Primo Star» із фотонасадкою для роботи у прямому й відбитому світлі.

Результати. Наведено зовнішні ознаки листя мирту звичайного: форму, колір, розмір, запах, тип жилкування. Анатомічні особливості листка включають наявність кутикули, дорзивентральний мезофіл, аномоцитні продихи, які розташовані на абаксіальній поверхні

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Keywords: Myrtus communis L., plant leaf, leaf anatomy, macro- and microscopic analysis.

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листка, кристали та друзи оксалату кальцію, прості волоски на середній жилці, схізолізогенні секреторні вмістища. Гістохімічні дослідження цих порожнин дають підстави припустити наявність ліпофільних речовин.

Висновки. Морфолого-анатомічний аналіз представника роду миртових *Myrtus communis* L. дав змогу визначити загальні діагностичні мікроскопічні ознаки листя мирту. Перспективи подальших досліджень полягають у продовженні комплексу фітохімічних досліджень для встановлення параметрів стандартизації лікарської рослинної сировини.

Ключові слова: Myrtus communis L., листя рослин, анатомічна будова листя, макро- та мікроскопічний аналіз.

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Plant extracts and products derived from plants are valuable resources extensively utilized in the treatment of various ailments. Myrtle (*Myrtus communis* L.) is a medicinal plant commonly found in coastal areas of Mediterranean regions, including North Africa and Southern Europe. It also grows in South America, Australia, and certain areas of the Himalayas [1]. Belonging to the family *Myrtaceae (Myrtaceae)*, which encompasses around 3000 species, common myrtle typically grows as an evergreen shrub or a small tree, reaching heights of up to 2.5 meters.

Myrtle has gained widespread use in the pharmaceutical and food industries due to its rich content of biologically active compounds. The chemical composition of *Myrtus communis* L. includes essential oils, such as terpenoids (particularly α-pinene, 1,8-cineole, geranyl acetate, and linalool) [2,3], flavonoids (quercetin, catechin, myricetin derivatives, and anthocyanins), coumarins, oligomeric non-prenylated acylphloroglucin compounds (including myrtucommulone A and B and semimyrtucummulone), haloyl glucosides, ellagitannins, haloylquinic acids, caffeic acid, gallic acid, ellagic acid, and fatty acids (such as linoleic, palmitic, oleic, and stearic acids) [4,5,6].

Myrtle preparations offer a wide range of therapeutic benefits, including astringent, anti-inflammatory, antiviral, antibacterial, antifungal, antidiabetic, antioxidant, gastro- and hepatoprotective properties. As a result, myrtle is effectively used in the treatment of various conditions, including wounds, gastrointestinal diseases, respiratory ailments, urinary tract disorders, rheumatism, diabetes, hypertension, periodontal diseases, and cancer [1,7,8,9,10,11,12,13,14,15].

Myrtle essential oil demonstrated exceptional activity against chronic toxoplasmosis induced by the Tehran *Toxoplasma gondii* strain in mice [16].

Microencapsulated essential oil of the leaves of *Myrtus communis* L., consisting mainly of myrtenyl acetate (30.6 %), linalool (14.9 %), α-pinene (11.10 %), and 1,8-cineole or eucalyptol (9.9 %), showed a powerful anti-inflammatory and antioxidant effect on the gastric mucosa, potentially enabling its use for the treatment of acute gastric ulcers [17].

The results of the research conducted by M. A. Mir et al. demonstrated that *M. communis* leaf extract had a strong inhibitory effect on Gram-positive and acid-fast bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Enterococcus faecalis* (ATCC 29212), *Mycobacterium smegmatis*) with a significant size of the inhibition zone, suggesting its potential as a source of compounds for treating gram-positive bacterial infections in the future [18].

The published information on the chemical composition of *Myrtus communis* L. mainly focuses on studies of specific classes of natural compounds, particularly essential oils and flavonoids. However, microscopic analysis plays a crucial role in pharmacognostic research. When anatomical differences are identified, it becomes essential to develop analytical methods for medicinal plant raw materials. Therefore, it is relevant to determine the diagnostic features of common myrtle to establish parameters for the standardization of medicinal plant raw materials.

Aim

The purpose of the work is to study the morphological and anatomical structure and to determine the general diagnostic microscopic features of common myrtle leaves.

Materials and methods

For microscopic studies, fresh and dried plant material of common myrtle, cultivated under room conditions at the Department of Pharmacognosy, Pharmacology and Botany of Zaporizhzhia State Medical and Pharmaceutical University, was utilized. Mature leaves were collected randomly. Subsequent microscopic research was conducted at the Phytochemical Laboratory of the Educational and Scientific Medical and Laboratory Center with a vivarium at Zaporizhzhia State Medical and Pharmaceutical University.

Illumination of microscopic preparations was achieved by heating the samples in an aqueous solution of 3 % sodium hydroxide (Ukrchemgroup, Spain, series number CAS 1310-73-2) [19]. To obtain transverse sections, fixation was carried out using freshly prepared cool Carnois fixative (consisting of alcohol 96 % (PR JSC BIOLIK, Ukraine), chloroform (Chloroform Pharm, France, series number TAP 1705155) and acetic acid (LLC "VVV" PCC, Ukraine) in a ratio of 6:3:1) [20,21], which exceeded the volume of the studied objects by 15 times (for 20 objects, 80 ml fixative), and left for approximately two hours, followed by washing with three portions of 70 % ethanol. The fixed material underwent dehydration using graded ethanol (10–100 %) and chloroform, then it was infiltrated and embedded in paraffin (Thermo Scientific Inc., UK) in a thermostat at a constant temperature of 56 °C. Transverse sections were cut using a mechanical rotary microtome Microm HM 325 (Germany) at 5 μm and placed on microscope slides. Subsequently, deparaffinization was performed in two portions of xylene (Shanghai Synnad, China), each for 5 minutes, followed by washing in distilled water and soaking for 10 minutes in 96 %, 80 %, and 70 % ethyl alcohol.

Fig. 1. External view of the leaves of *Myrtus communis* L.

Fig. 2. Macroscopic analysis of the length of the common myrtle leaf plate.

Fig. 3. Macroscopic analysis of the width of the common myrtle leaf plate.

Carl ZEISS "AxioStar Plus" and "Primo Star" microscopes equipped with objectives of ×4, ×10, ×20, ×40, and ×100 magnification were utilized for examination under both direct and reflected light. A digital camera AxioCam ERc 5s was employed to record the research findings. Anatomical studies were conducted in statistically significant quantities, with a minimum of 10 observations made for each object.

To identify the localization of the essential oil, a histochemical reaction was conducted using Sudan III (LLC "Trubosnab", Ukraine) solution, which stains the oil-containing structures in an orange-red color [22].

During the microscopic examination of medicinal plant raw materials, attention was focused on the type of leaf blade, the structure of epidermal cells, the presence, number, and type of stomata, as well as the characteristics of hairs and glands.

Results

During the macroscopic analysis, it was determined that the leaves of common myrtle are simple, oblong, lanceolate, or ovoid-elliptic with entire or slightly rounded margins and acute tips, with a rounded or slightly uneven base *(Fig. 1)*. They are arranged oppositely, sessile, or semi-sessile. The upper surface of the leaves is dark green with a glossy texture, while the lower surface is green. The length ranges from 0.5 to 3.5 cm *(Fig. 2)*, and the width ranges from 0.2 to 2.0 cm *(Fig. 3)*. The thickness of the leaf blade is 293.93 ± 2.59 μm. It was observed that the leaves exhibit numerous secondary and tertiary reticulate veins. The venation pattern is brochidodromous (pinnate-loop-like), where the veins extend to the leaf margin, joining with the anterior vein, forming a loop that is barely visible

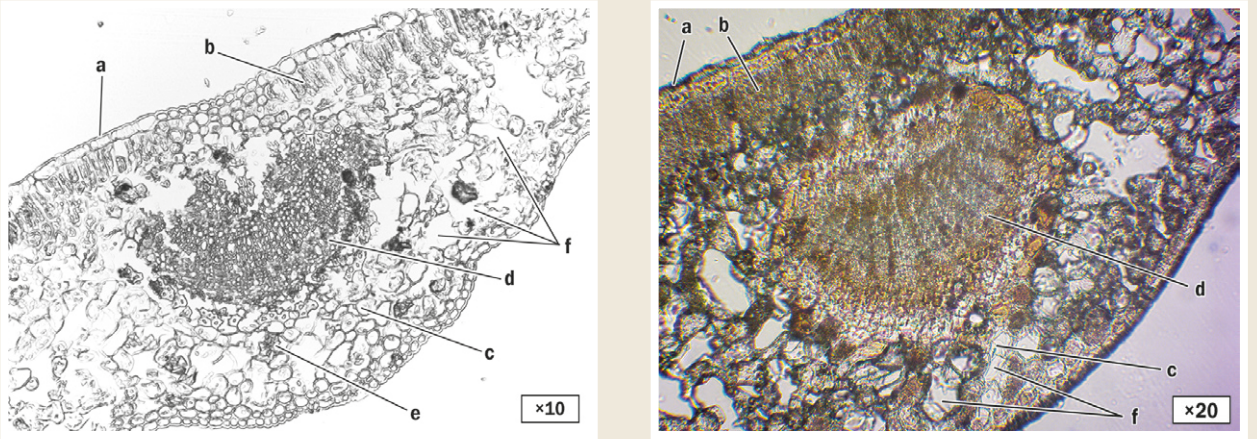


Fig. 4. Transverse section of a leaf plate of *Myrtus communis* L. **a:** cuticle; **b:** columnar mesophyll of the epidermis; **c:** spongy parenchyma; **d:** closed collateral conducting bundle; **e:** calcium oxalate drusen.

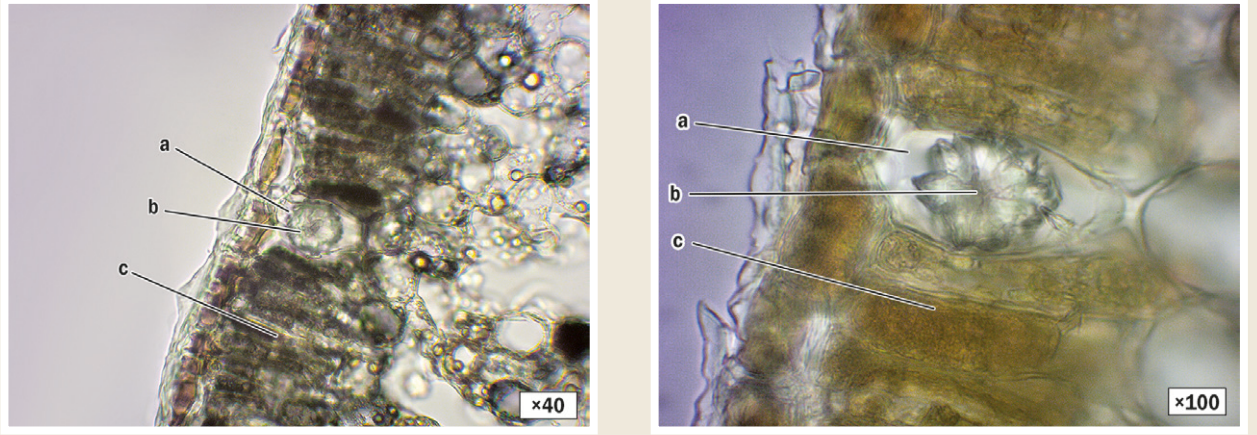


Fig. 5. Cross-section of common myrtle leaves. Subepidermal idioblast containing calcium oxalate drusen. **a:** idioblast; **b:** calcium oxalate drusen; **c:** columnar mesophyll.

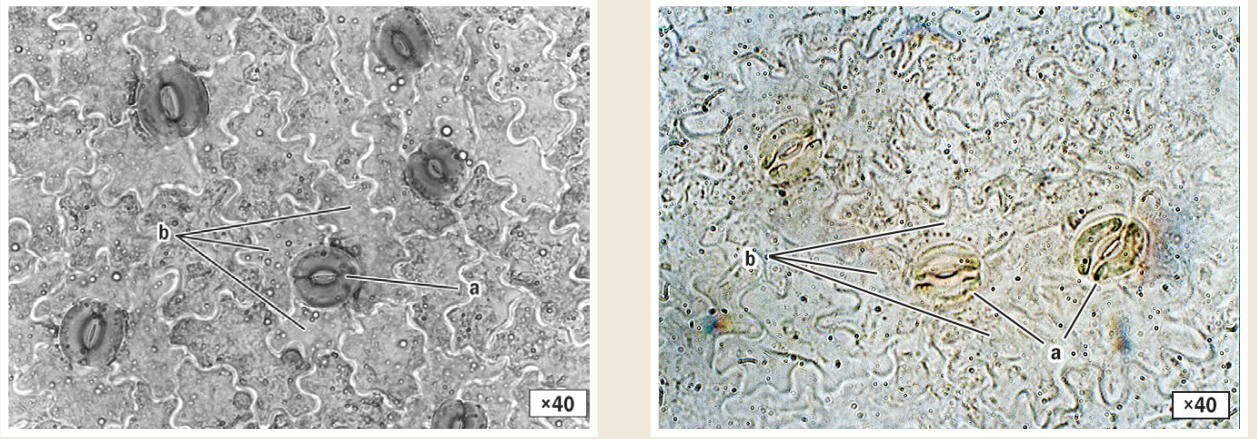


Fig. 6. A fragment of the lower epidermis of a leaf. **a:** stomata; **b:** near stomatal cells.

externally. The leaves emit a characteristic balsamic aroma upon crushing, attributed to the presence of numerous secretory receptacles.

When examining cross-sections of fresh leaves microscopically, a distinct cuticle is noticeable on the surface *(Fig. 4, a)*. The adaxial and abaxial epidermal cells appear compressed, flat convex, and mostly isodiametric. The leaf was determined to be dorsoventral bifacial based on the location of the columnar mesophyll, with the palisade parenchyma positioned beneath the upper side of the leaf blade *(Fig. 4, b)*. The mesophyll exhibits differentiation into palisade tissue and spongy parenchyma *(Fig. 4, c)*. The

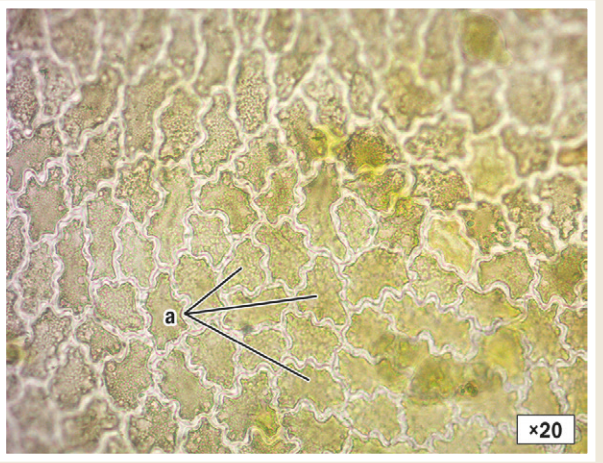
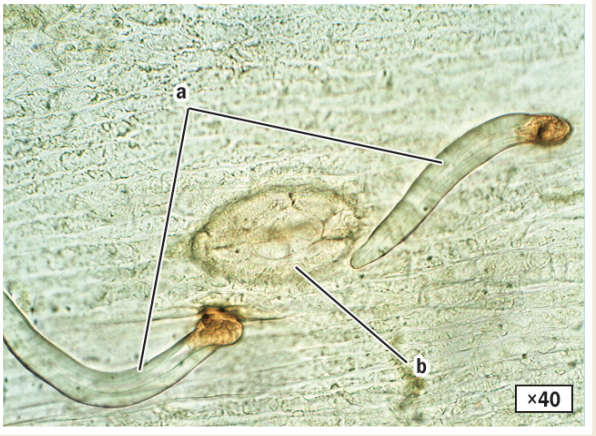
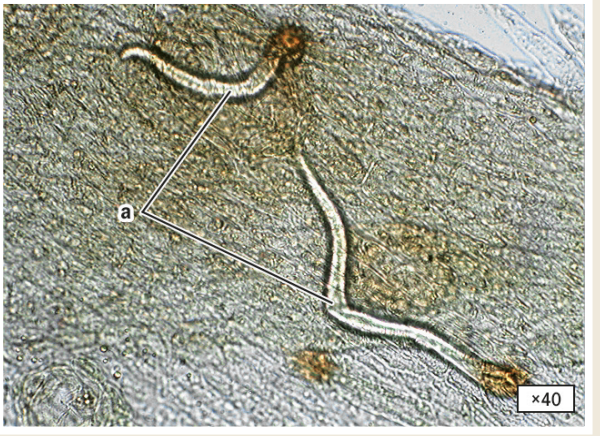


Fig. 7. A fragment of the upper epidermis of a leaf. **a:** epidermal cells.





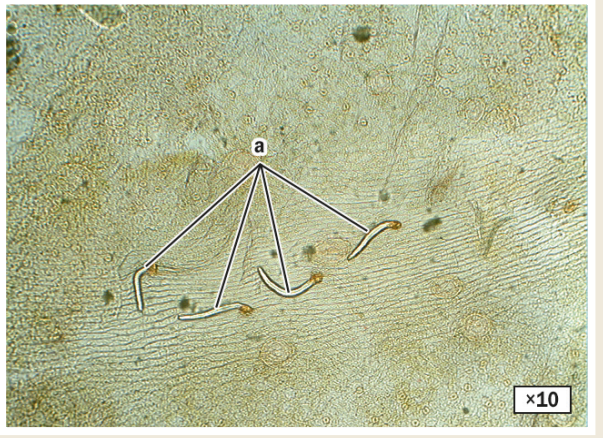


Fig. 8. A fragment of the epidermis of the middle vein of a leaf. **a:** trichomes; **b:** secretory container.

palisade tissue comprises somewhat dense, highly elongated cells densely packed with chloroplasts, characterized by thin primary cell walls. A distinct layer of collecting cells is observed between the palisade and spongy tissues, facilitating the transport of carbohydrates synthesized in the palisade cells to the spongy parenchyma. The cells of the spongy tissue are isodiametric in shape, elongated, and adjacent to the lower epidermis. Large intercellular spaces are evident between them *(Fig. 4, f)*, which are comparable in size to the cavities' airiness. The arrangement of tissues within the leaves serves as a diagnostic feature. Closed, rounded conductive bundles *(Fig. 4, d)* are present in the leaf, with a coating formed by cells with significantly thickened walls on the phloem side. Subepidermal idioblasts containing calcium oxalate crystals (druses) are distributed throughout the columnar parenchyma *(Fig. 5)*.

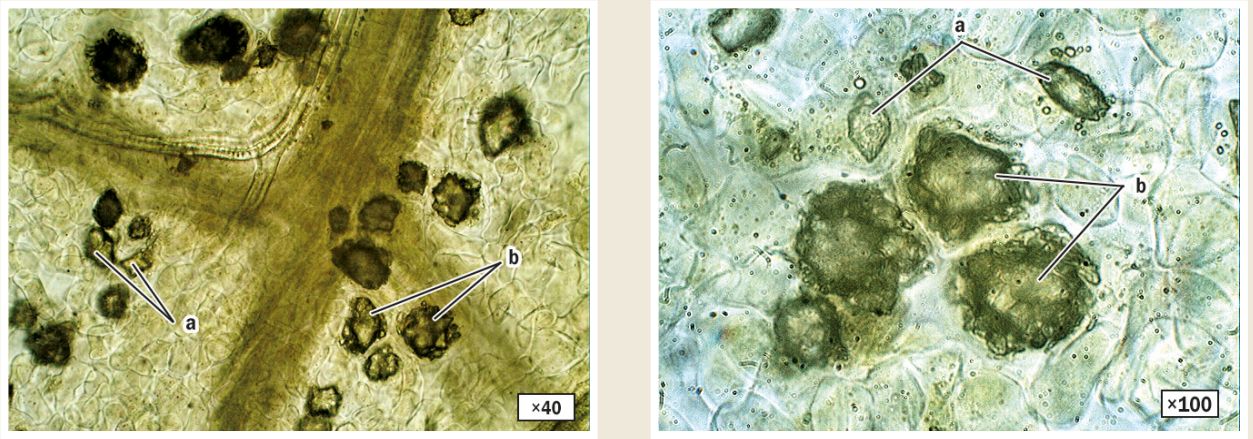


Fig. 9. Drusen and calcium oxalate crystals. **a:** calcium oxalate crystals; **b:** calcium oxalate drusen.

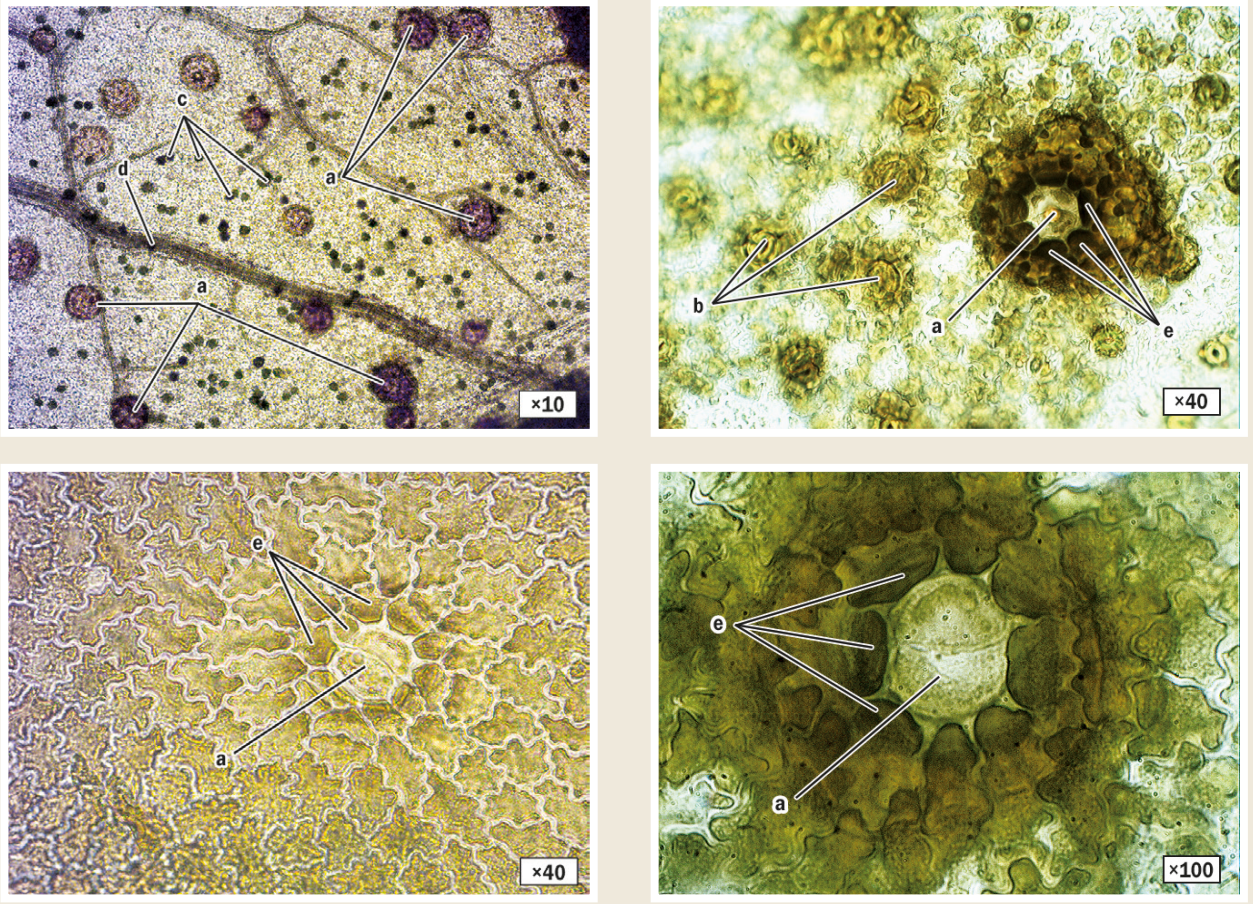


Fig. 10. Schizolisogenic secretory receptacles. **a:** schizolisogenic secretory receptacle; **b:** stomata; **c:** calcium oxalate drusen; **d:** central vein; **e:** epithelial cells located above the receptacles.

Stomata are predominantly located on the lower side of the leaf, indicating a hypostomatic type. The abaxial surface is characterized by deeply convoluted epidermal cells housing anomocytic stomata *(Fig. 6, a)* of oval-round shape. These stomata are surrounded by 3–5 epidermal cells *(Fig. 6, b)* and are positioned at the same level as the surrounding epidermal cells. The guard cells exhibit a kidney shape, and the lumen between them is constricted at the equatorial part. Stomata are uniformly distributed across the leaf surface.

The cells of the upper epidermis appear larger than those of the lower epidermis *(Fig. 7, a)*. The upper epidermis consists of isodiametric or slightly elongated cells with thickened, sinuous walls. Notably, the adaxial surface of the leaf is devoid of trichomes, whereas the abaxial surface exhibits a sparse distribution of trichomes along the midvein *(Fig. 8, a)*.

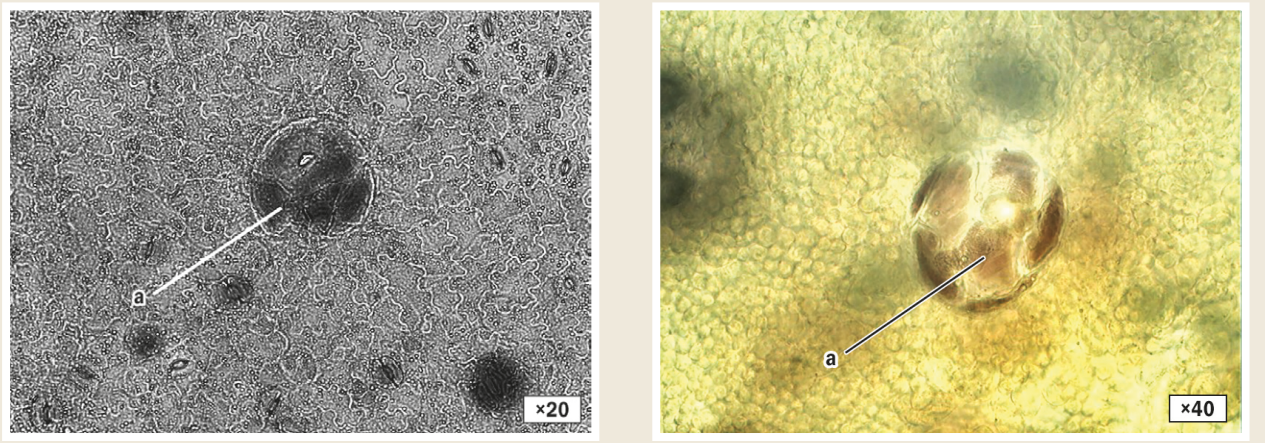


Fig. 11. Idioblast with resin-oil content. **a:** idioblast.

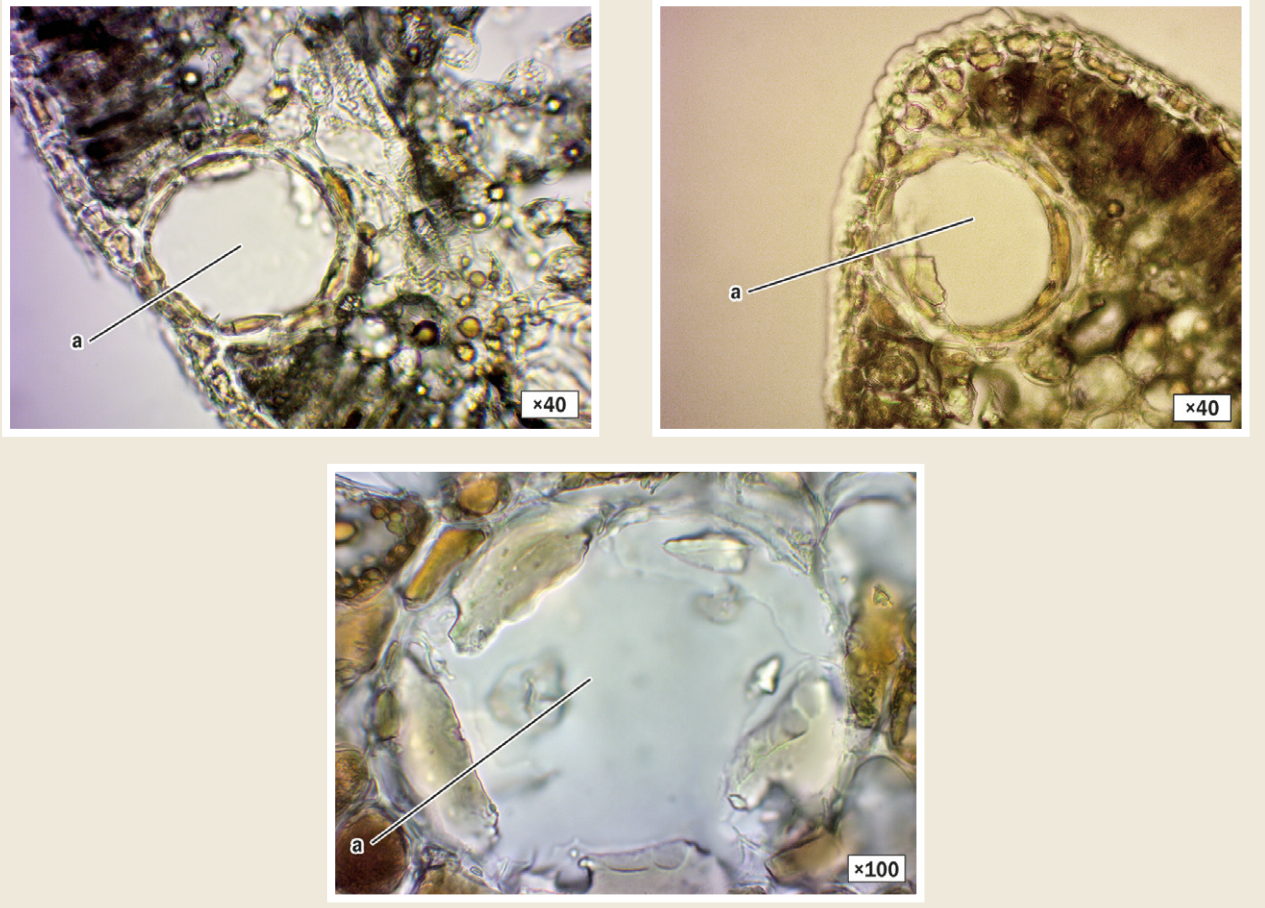


Fig. 12. Transverse section of a leaf showing the schizolisogenic receptacle and epithelial cells on the abaxial surface near the leaf margin. **a:** schizolisogenic receptacle.

These trichomes are simple hairs characterized by smooth, thick walls, and are unicellular, solitary, conical, and slightly undulated.

Common myrtle leaves additionally contain significant quantities of druses *(Fig. 9, b)* and prismatic crystals of calcium oxalate *(Fig. 9, a)*.

In the leaf mesophyll, there are also spherical secretory receptacles *(Fig. 10, a)*, which exhibit the typical schizolisogenic structure. Histochemical tests with Sudan III indicate that the secretory receptacles of *Myrtus communis* mainly produce lipophilic compounds. The epithelial cells situated above the receptacles *(Fig. 10, e)* are larger in size, rounded, with less tortuous walls compared to other cells of the epidermis.

In addition to the containers of essential oil, dark brown idioblasts containing resinous-oil content are also present *(Fig. 11, a)*. These structures are distributed differently

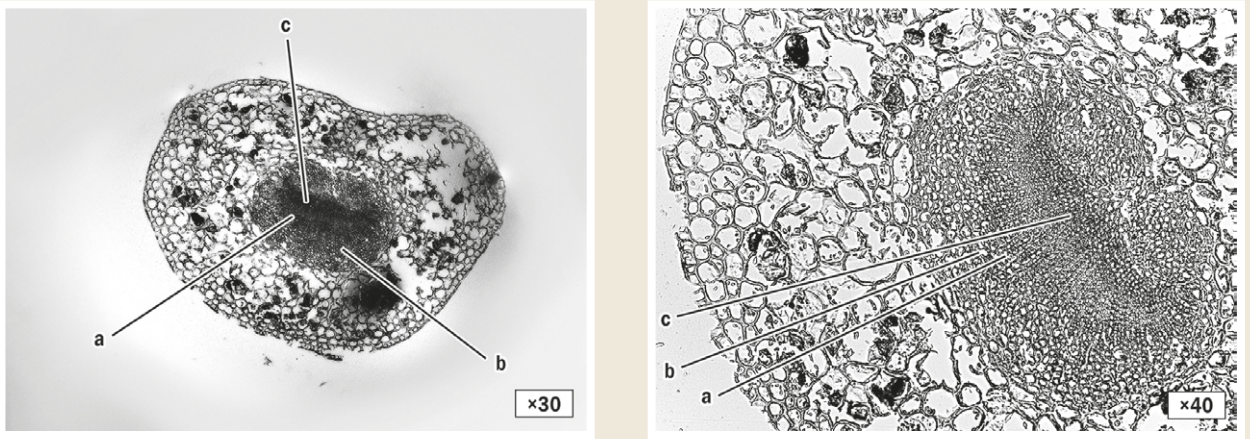


Fig. 13. Transverse section of the petiole of a common myrtle leaf. **a:** vascular bundle; **b:** phloem; **c:** xylem.

throughout the leaf: in the columnar parenchyma, where they are situated directly beneath the adaxial epidermis *(Fig. 12, a)*; and in the spongy parenchyma, particularly in the transitional region with the columnar mesophyll or adjacent to the abaxial epidermis. The cavities typically appeared empty, although at times there was an accumulation of colorless or pale yellow material. This material consisted of small densely packed droplets or large clusters that filled the entire volume of the cavity.

The petiole of the leaf exhibits a heart-shaped cross-section *(Fig. 13)*. Its surface is adorned with trichomes, and the conducting collateral bundle is enclosed *(Fig. 13, a)*. The phloem *(Fig. 13, b)* lies adjacent to the xylem *(Fig. 13, c)* on the lower side, with distinct separation between the adaxial and abaxial phloem. Well-developed curved edges characterize the phloem, while a pronounced sclerenchymal lining of the bundle consists of small cells with lignified cell walls. Collenchyma areas are present in both the upper and lower parts of the bundle, with cells possessing non-lignified, irregularly thickened cell walls.

Discussion

For further research and the introduction of promising phytopreparations based on *Myrtus communis* L. into medical practice, reliable identification signs are necessary for medicinal plant raw materials. To fulfill this purpose, a microscopic study of *Myrtus communis* L. leaves was conducted, establishing characteristic anatomical and diagnostic features of the studied samples. The results of this study reveal that *Myrtus communis* L. shares several anatomical features with other species of the family, including the presence of druses and calcium oxalate crystals, internal phloem, and secretory cavities.

Calcium oxalate crystals are abundant in the leaves of *Myrtus communis* L., particularly in the palisade parenchyma located just below the adaxial epidermis. The precise function of these structures is not fully understood but is believed to be associated with the regulation of calcium and other minerals, as well as serving a defensive role against herbivores and pathogens.

The presence of internal phloem was observed in the midvein as a continuous tissue. Typical features for identifying representatives of the *Myrtaceae* family include the development of adaxial phloem, fusion between adaxial and abaxial phloem, and the continuity of fibers around the midvein [22].

The ontogeny of secretory cavities in *M. communis* L. follows the typical schizogenous pattern commonly observed in *Myrtaceae*. This development combines lysogenic (due to cell breakdown) and schizogenic processes [23,24].

Conclusions

1. During the research, the characteristic features of the appearance of whole common myrtle leaves were determined, including their shape, size, color, and smell. Microscopic studies of plant material identified the diagnostic features of the anatomical structure of the leaves of *Myrtus communis* L. These features include the dorsoventral structure of the leaf plate, the presence of the anomocytic type of stomatal apparatus with a hypostomatic location, simple hairs, the presence of druses and prismatic calcium oxalate crystals, and schizogenous secretory receptacles that produce lipophilic substances.

2. The identified diagnostic signs can be recommended for the identification of *Myrtus communis* L. plants and for establishing parameters for the standardization of medicinal plant raw materials.

Prospects for further research include studying the dynamics of the accumulation of biologically active substances in common myrtle leaves, as they serve as a source of medicinal plant raw materials for the development of new phytopreparations.

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