



Microscopic analysis of *Valeriana stolonifera* and *Valeriana collina* 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

Plants of the *Valeriana* species are distributed in various parts of the world, especially in Europe and Asia. A high content of polyphenolic compounds, including flavonoids and hydroxycinnamic acids with expressed biological activity, was previously identified within the herb of the studied *Valeriana* species. Morphological and anatomical data can be used in phylogenetics of species and genera to find out diagnostic and age-related characteristics of plants. This led us to microscopic studies of aerial organs of the above-mentioned *Valeriana* species.

The aim of the work is to conduct a comparative study of the diagnostic features within the morphological and anatomical structure of *Valeriana stolonifera* and *Valeriana collina* leaves.

Materials and methods. Both raw and dried plant material of *V. stolonifera* and *V. collina* was used for microscopic studies. Temporary micropreparations were made using generally accepted methods. The anatomical features of the raw material were examined using Carl ZEISS “AxioStar Plus” and “Primo Star”.

Results. The study identified key morphological and anatomical features of the species, which should be considered for the identification and standardization of promising medicinal plant materials and within the development of methods of analytical and regulatory documentation.

Conclusions. The key microscopic differences in the leaves of the studied species lie in the structure of the adaxial and abaxial surfaces of the leaf blade. The upper epidermis consists of large elongate cells with wavy walls. The cells of the lower epidermis are smaller and have more sinuous walls compared to the upper epidermis cells. The degree of wall sinuosity varies between species – *V. collina* is characterized by more sinuous cells in both the upper and lower epidermis compared to *V. stolonifera*. The absence of stomata on the upper epidermis is a common feature for both species.

Keywords: *Valeriana officinalis*, *Valeriana stolonifera*, *Valeriana collina*, leaf anatomy, microscopic analysis.

Current issues in pharmacy and medicine: science and practice. 2024;17(3):207-214

Мікроскопічний аналіз листя *Valeriana stolonifera* and *Valeriana collina*

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Рослини виду *Valeriana* поширені в різних частинах світу, особливо в Європі та Азії. В траві досліджених видів *Valeriana* попередньо встановлено високий вміст поліфенольних сполук, зокрема флавоноїдів і гідроксикоричних кислот із вираженою біологічною активністю. Морфолого-анатомічні дані можуть бути використані у філогенетиці видів і роду для з'ясування діагностичних і вікових ознак рослин. Це спонукало нас до мікроскопічних досліджень надземних органів двох цих видів валеріани.

Мета роботи – порівняльне дослідження діагностичних ознак у морфолого-анатомічній будові листків *Valeriana stolonifera* та *Valeriana collina*.

Матеріали і методи. Для мікроскопічних досліджень використали свіжу та висушену рослинну сировину *V. stolonifera* та *V. collina*. Тимчасові мікропрепарати виготовляли за загальноприйнятими методиками. Ознаки анатомічної будови сировини вивчили за допомогою мікроскопів Carl ZEISS «AxioStar Plus» та «Primo Star».

Результати. У результаті дослідження встановлено основні морфолого-анатомічні ознаки видів, які слід враховувати під час ідентифікації та стандартизації перспективної лікарської рослинної сировини та розроблення аналітично-нормативної документації.

Висновки. Основні відмінні мікроскопічні ознаки листя досліджених видів – структура клітин адаксіальної та абаксіальної поверхні листової пластинки. Верхній епідерміс складається з великих видовжених клітин із хвилястими стінками. Клітини нижнього епідермісу є меншими за розміром та мають більш звивисті стінки порівняно з клітинами верхнього епідермісу. Ступінь звивистості

ARTICLE INFO



UDC 615.322:582.971.3-145.086.2/3
DOI: [10.14739/2409-2932.2024.3.311562](https://doi.org/10.14739/2409-2932.2024.3.311562)

Current issues in pharmacy and medicine: science and practice. 2024;17(3):207-214

Keywords: *Valeriana officinalis*, *Valeriana stolonifera*, *Valeriana collina*, leaf anatomy, microscopic analysis.

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Received: 03.09.2024 // Revised: 16.09.2024 // Accepted: 24.09.2024

стінок варіює залежно від виду: для *V. collina* притаманні більш звивисті клітини верхнього та нижнього епідермісу порівняно з *V. stolonifera*. Відсутність продихів на верхньому епідермісі є спільною ознакою для видів, що дослідили.

Ключові слова: *Valeriana officinalis*, *Valeriana stolonifera*, *Valeriana collina*, анатомічна будова листя, мікроскопічний аналіз.

Актуальні питання фармацевтичної і медичної науки та практики. 2024. Т. 17, № 3(46). С. 207-214

Species of the *Valerianaceae* family are widely spread worldwide, including Ukraine. In Ukraine, the collective species *V. officinalis* L. encompasses 13 species: *V. stolonifera* Czern, *V. collina* Wallr., *V. dioica* L., *V. rossica* P. Smirn., *V. tuberosa* L., *V. exaltata* Mikan, *V. tripteris* L., *V. transsilvanika* Schur, *V. sambucifolia* Mikan, *V. tanaitica* Worosch, *V. nitida* Kreyer, *V. simplicifolia* (Reichenb.) Kabath, *V. grossheimii* Worosch [1].

Most studies on *V. officinalis* focus on the official medicinal raw material – roots and rhizomes. However, in recent years, the number of studies on aerial parts, such as leaves, inflorescence and seeds, has increased [2,3,4,5].

The phytochemical composition of the underground organs of *Valerian* species has been extensively studied, with more than 800 compounds being isolated, or identified, including essential oils [6], flavonoids [7,8], iridoids [9], lignanoids [6], alkaloids [7] and other classes of biologically active compounds. Due to these compounds, *V. officinalis* exhibits sedative [10], antidepressant [9], anxiolytic [11], anticonvulsant [12], antiepileptic [12], antiarrhythmic [9], antioxidant [13], insecticidal [13], antibacterial [13], anti-inflammatory [14] and antiviral [15] effects.

Traditionally, *V. officinalis* phytomedicines were used to treat insomnia, epilepsy, neurasthenia, manic and depressive states, tachycardia, arrhythmia, high blood pressure, anaemia, various types of pain, digestive disorders (diarrhoea, vomiting) [16,17,18].

Two species of *V. officinalis*, namely *V. stolonifera* and *V. collina*, are widespread in the Zaporizhzhia region (Ukraine). *V. stolonifera* predominantly grows in oak and hornbeam forests, pine forests, forest glades, shrublands and meadow steppes. *V. collina* grows on dry hillsides, in oak forests and shrubs.

Creeping *Valerian* (*V. stolonifera*) is a perennial herbaceous plant with a short, cylindrical rhizome with long underground shoots, or stolons. Its roots are of medium thickness (1.5–2.0 mm). The stems are 40–130 cm tall, ribbed, smooth, and slightly rough at the base. The leaves are pinnate with 6–15 pairs of leaflets, measuring 2–8 cm in length and 0.5–2.0 cm in width. The leaflets are sessile, lanceolate or linear and lanceolate, entire, or sometimes toothed and smooth. The inflorescence is 11–35 cm long, 4–22 cm wide with white, pale pink, and pink flowers, blooming from May to June.

Hill *Valerian* (*V. collina*) has a short rhizome with short underground shoots and roots of medium thickness. The stems are 30–120 cm tall, ribbed, smooth. The leaves are pinnate with 5–14 pairs of leaflets, measuring 2–8 cm in length and 0.4–1.5 cm in width. The leaflets are sessile, lanceolate, or linear and lanceolate, entire, or sometimes toothed and smooth. The inflorescence is 10–30 cm long, 5–18 cm

wide with white, pale pink, and pink flowers, blooming from May to June.

The main morphological difference between these species is that *V. stolonifera* is characterized by a creeping growth form with stolons, i. e., horizontal connections between plants that enable vegetative reproduction. Its leaves are generally narrow with fewer teeth along the edges; the stems are often smooth and may have a reddish tint; *V. collina*, on the other hand, does not produce stolons. It has a more vertical growth form with several stems emerging from a single rhizome. The leaves have more distinct teeth and are wider compared to *V. stolonifera*.

Microscopic analysis of the leaves of the studied species provides critical insights into the taxonomy, morphology and potential pharmacological properties of medicinal plants. This type of pharmacognostic research aids in the precise identification and classification of different species, contributing to the broader fields of plant taxonomy and botany.

The review of the specialized references shows a lack of systemic data concerning the anatomical structure of aerial parts of *V. stolonifera* and *V. collina*. Meanwhile, morphological and anatomical data can be used in the phylogenetics of species and genera to find out diagnostic and age-related characteristics of plants. This led us to microscopic studies of aerial organs of the above-mentioned *Valeriana* species.

Aim

The aim of the work is to conduct a comparative study of the diagnostic features within the morphological and anatomical structure of *V. stolonifera* and *V. collina* leaves.

Materials and methods

Both raw and dried plant material of *V. stolonifera* and *V. collina* was used for microscopic studies. The raw material was collected in July 2023 (Kantserivska balka in the Zaporizhzhia region and the right bank of the Dnipro River in Zaporizhzhia).

The microscopic study was carried out in 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 % [20,21], PR JSC BIOLIK, Ukraine), chloroform (Chloroform Pharm, France, series number TAP 1705155), and acetic acid (LLC “VVV” PCC,

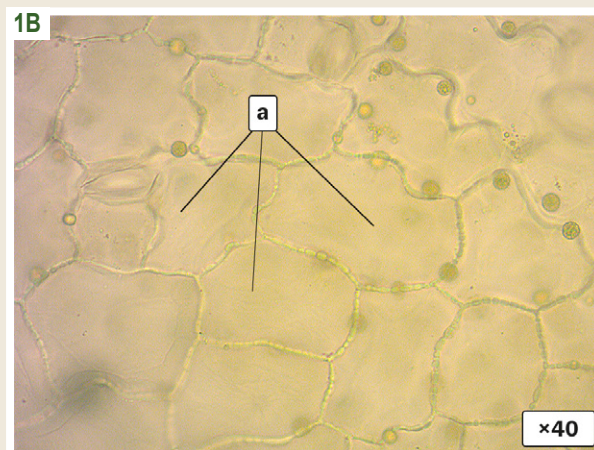
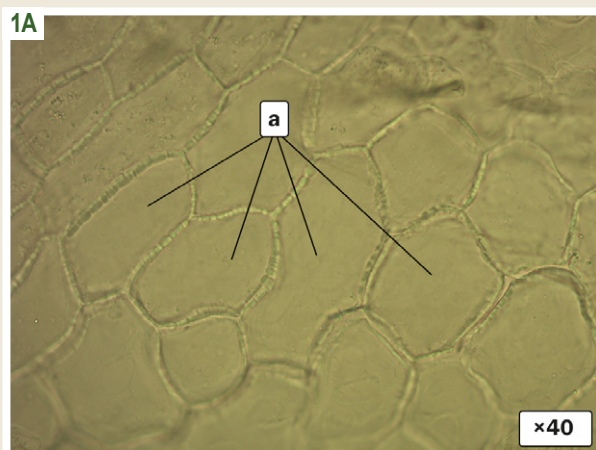


Fig. 1. The adaxial surface of the leaf plate of *V. stolonifera* (A) and *V. collina* (B). a: large wavy cells of the epidermis.

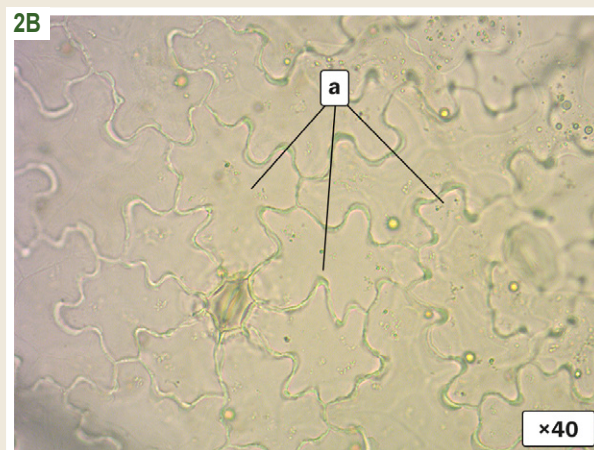
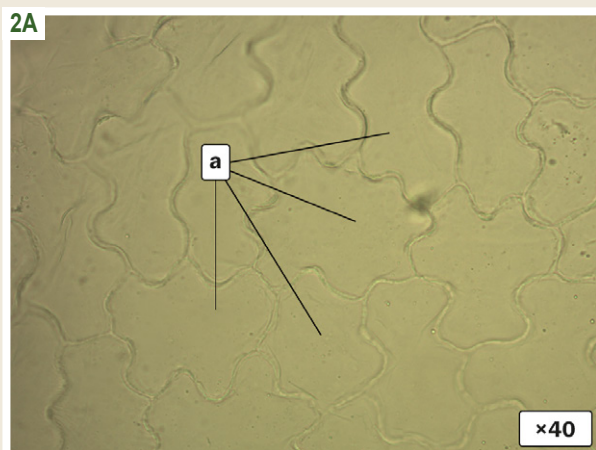


Fig. 2. The abaxial surface of the leaf plate of *V. stolonifera* (A) and *V. collina* (B). a: convoluted cells of the epidermis.

Ukraine) in a ratio of 6:3:1), 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.

The anatomical features of the raw material were examined using Carl ZEISS “AxioStar Plus” and “Primo Star” microscopes both under direct and reflected light (lenses ×4, ×10, ×20, ×40, ×100) with a digital camera AxioCam ERc 5s. The results were recorded using ZEISS ZEN (blue edition) software. Anatomical studies were carried out in statistically reliable quantities (at least 10 for each sample).

Results

During the study of the anatomical structure of the leaves, we analyzed the following features: the structure of the lower and upper epidermis, the type of stomatal apparatus, characteristics of trichomes, presence of exogenous and endogenous structures and inclusions.

The following structure was common to all studied samples: the leaf had a dorsoventral type of structure, and the epidermis of the upper side of the plate consisted of large, elongated cells with sinuous walls, without stomata (Fig. 1, a). The epidermal cells on the lower leaf surface were smaller and had a more sinuous contour (Fig. 2, a).

Stomata of the anomocytic type (Fig. 3, a, b) were located on the lower surface (hypostomatic), with typical four epidermal cells surrounding the stomata (Fig. 3, c).

On both sides of the leaf two types of trichomes were systematically observed: simple unicellular – long (Fig. 4, a), short (Fig. 4, b) and glandular trichomes (Fig. 5). Long (70–100 µm) unicellular trichomes were in a greater number of the veins, while short (35–50 µm) warty trichomes were found across the entire surface and along the edge of the leaf plate. The trichomes had a sharply conical shape and were

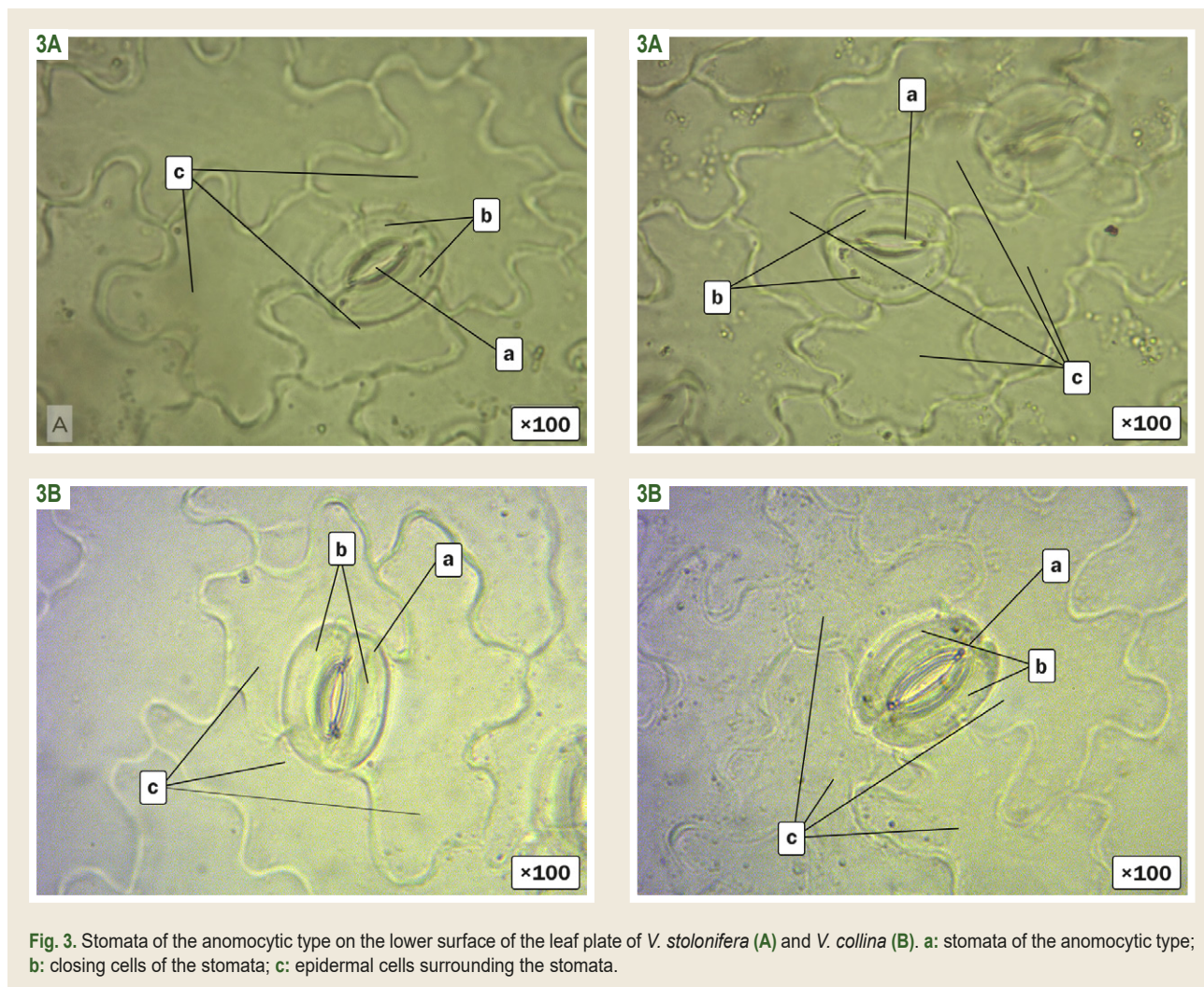


Fig. 3. Stomata of the anomocytic type on the lower surface of the leaf plate of *V. stolonifera* (A) and *V. collina* (B). a: stomata of the anomocytic type; b: closing cells of the stomata; c: epidermal cells surrounding the stomata.

oriented towards the tips of the lobes and segments of the plate. At the base, the trichomes were surrounded by several rectangular cells (Fig. 4, c), forming a rosette-like structure. The cavity of the trichomes was filled with a greyish granular content. The glandular trichomes consisted of a multicellular (4–6 cells) head (Fig. 5, a) with a brownish content and a unicellular stalk (Fig. 5, b).

The petiole of the leaf in the transverse section (Fig. 6) had a structure from U-shaped to V-shaped with collateral open vascular bundles (Fig. 6, a), arranged in a single row. The vascular bundles were surrounded by a 1–3-layered endodermis, whose fibres in the transverse section were angular, with thickened porous partially lignified cell membranes. The endodermis is underlain by a 2–3-layered boundary parenchyma. The cells are elongated along the bundle, rounded in the transverse section, lacking chloroplasts and crystals, with thin cell walls. The phloem (Fig. 6, b) is ring-shaped, composed of small cells, adjacent to narrow spiral vessels of the procambial xylem. The second vessels (Fig. 6, c) have larger openings and are porous. Besides, they form dense core rays, separated from each other by the parenchyma.

Epidermal cells on the lower side of the petiole (Fig. 7, a) are narrow, elongated, homogeneous, and without stomata.

Occasionally, multicellular subepidermal emergences are found on the upper side of the petiole, protruding above the surface in a spherical shape. The outer cells of the sphere are dense, and flattened, with slightly thickened, suberized walls. The cells embedded in the parenchyma are larger, and rounded, with more thickened walls.

Discussion

A comprehensive morphological and anatomical study of various *Valerian* species was carried out. It carefully describes the structural characteristics of the leaves, providing an idea of the epidermis structure, stomatal apparatus, types of trichomes and petiole anatomy.

The study highlights several key findings. The epidermal structure: the leaves show a dorsiventral structure with significant differences between the upper and lower epidermis.

The upper epidermis consists of large, elongated cells with wavy walls and without stomata. In contrast, the lower epidermis consists of smaller cells with more sinuous contours and has anomocytic stomata, usually surrounded by four epidermal cells.

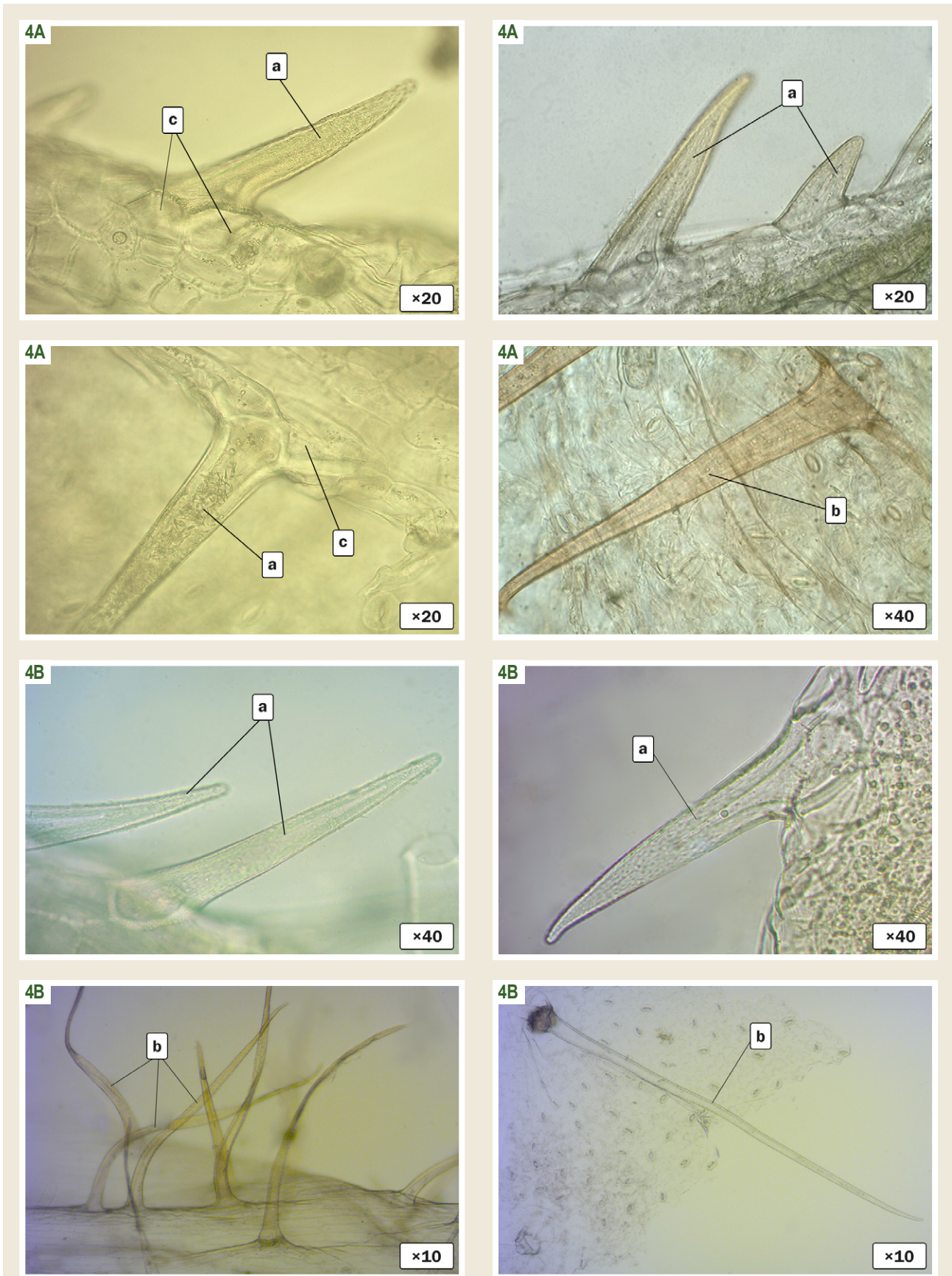


Fig. 4. Simple trichomes of *V. stolonifera* (A) and *V. collina* (B). a: simple unicellular trichomes; b: long simple trichomes; c: rectangular cells; at the base of the simple trichomes.

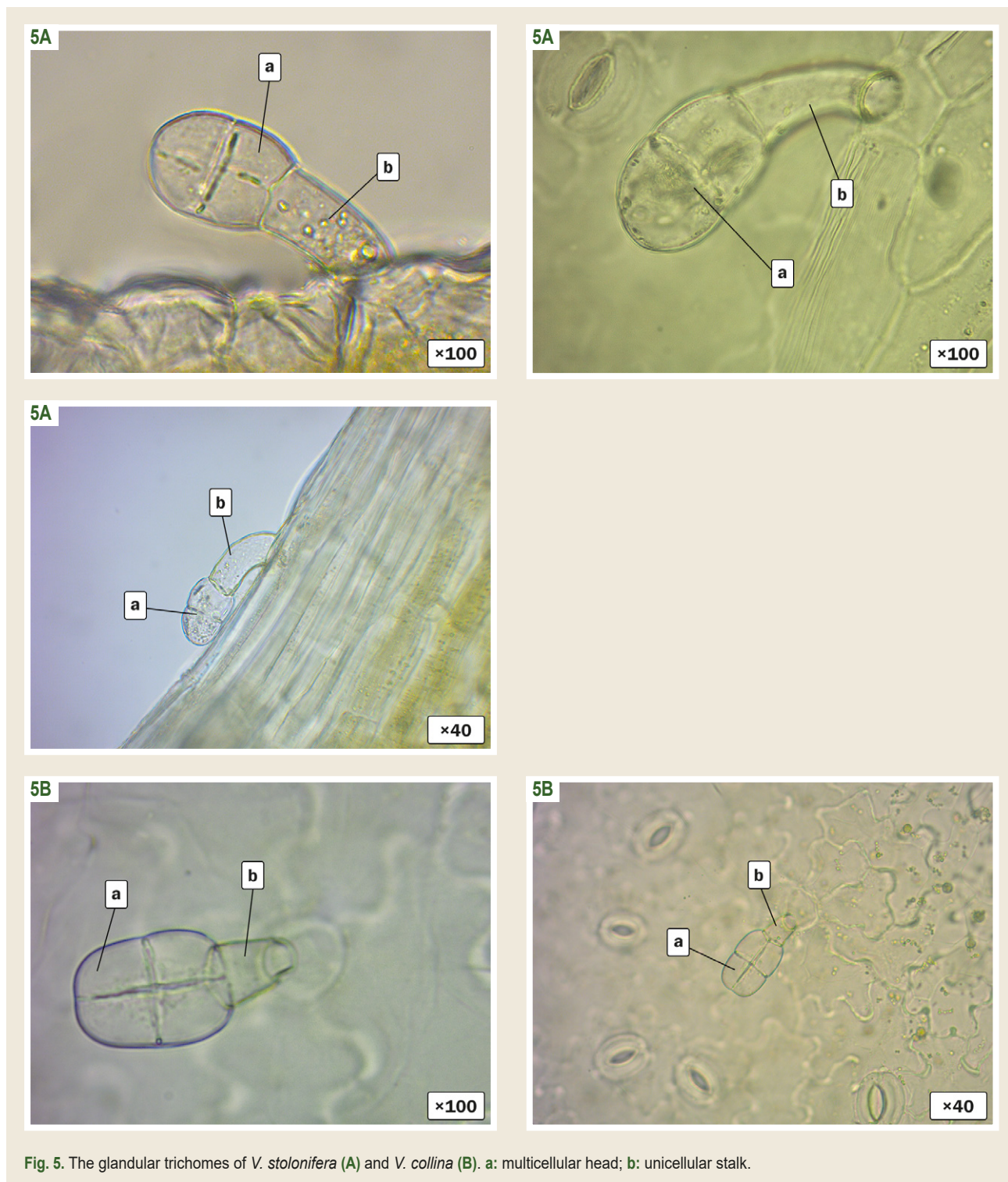


Fig. 5. The glandular trichomes of *V. stolonifera* (A) and *V. collina* (B). a: multicellular head; b: unicellular stalk.

The stomatal apparatus: stomata are located only on the lower surface of the leaves, indicating a hypostomatic leaf type. This arrangement is typical for many plant species adapted to reduce water loss while optimizing gas exchange.

Types of trichomes: the study identifies two types of trichomes on the leaves: simple unicellular trichomes and glandular ones. The unicellular trichomes vary in length, with longer trichomes primarily found on the veins and shorter warty trichomes on the leaf surface and edges. The glandular

trichomes have a multicellular head with a unicellular stalk containing a brown substance.

The petiole structure: the anatomy of the petiole transverse section reveals a structure from a U-shaped to a V-shaped form with collateral open vascular bundles, arranged in a single row. These bundles are covered by a multi-layered endoderm and are surrounded by a layer of parenchyma, highlighting the complex organization for the efficient transport of nutrients and water.

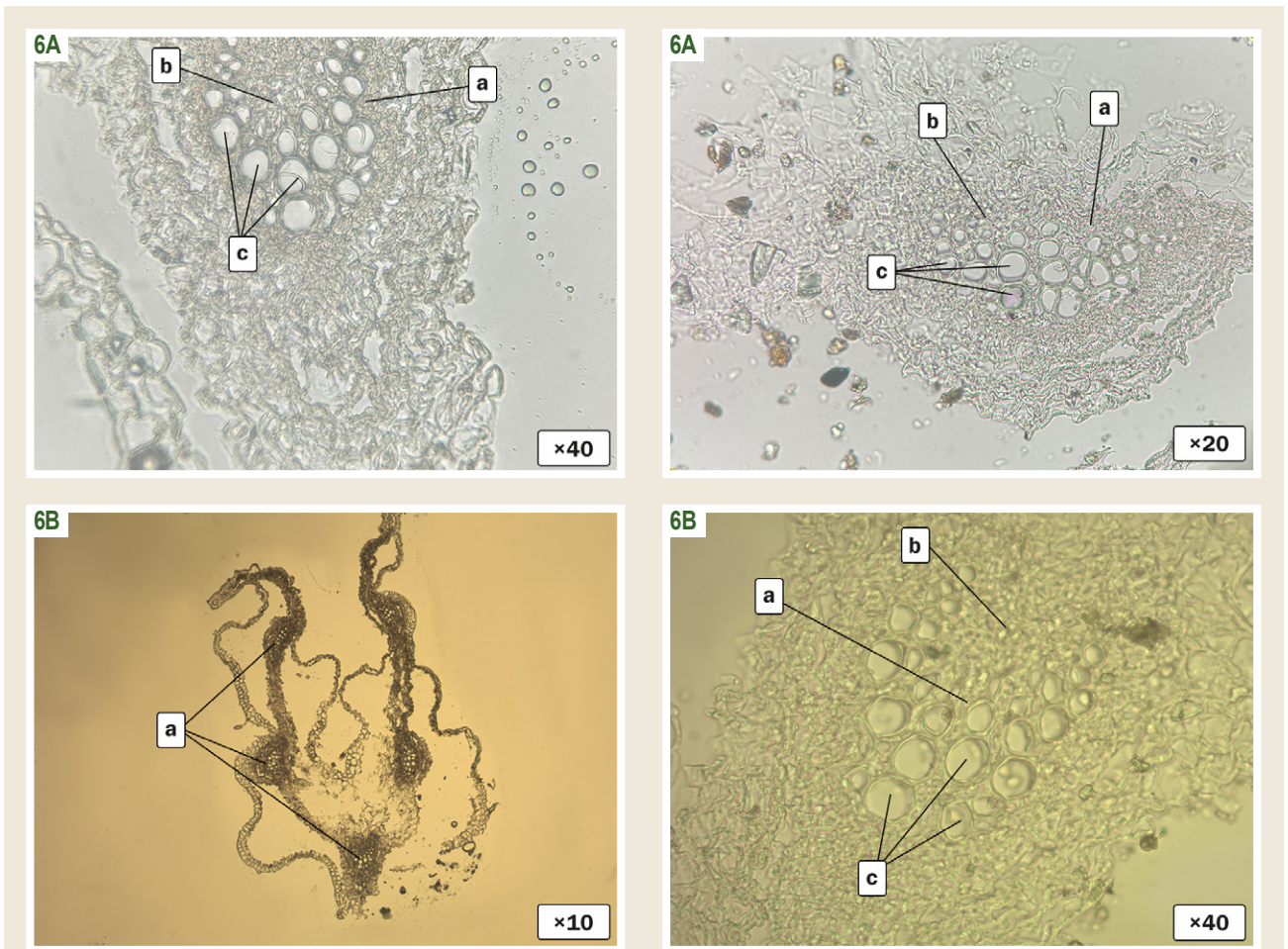


Fig. 6. Fragments of transverse sections of the petiole of *V. stolonifera* (A) and *V. collina* (B). a: collateral open vascular bundles; b: phloem; c: xylem.

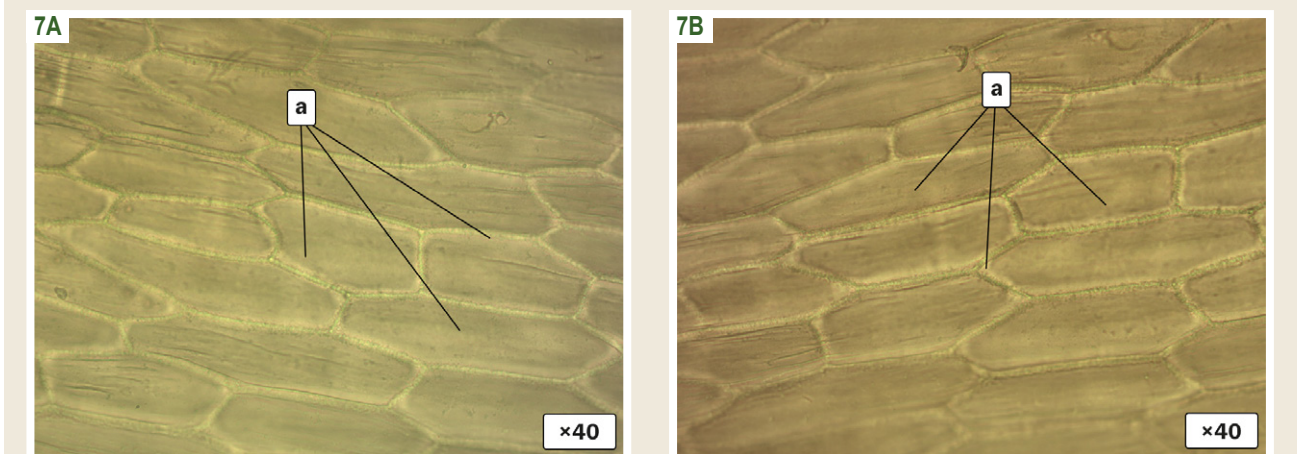


Fig. 7. Epidermis of the petiole of *V. stolonifera* (A) and *V. collina* (B). a: epidermal cells on the lower side of the petiole.

Conclusions

1. A microscopic analysis of *V. stolonifera* and *V. collina* leaves was conducted, revealing their potential as promising sources of various classes of biologically active substances. This indicates the rationale for continued pharmacognostic research on this plant material to develop new phytomedicines.

2. The primary morphological differences between the studied species are stolons in *V. stolonifera*, which are completely absent in *V. collina*. *V. stolonifera* leaves are often narrower with fewer teeth along the edges, whereas *V. collina* has more distinct teeth and broader leaves compared to *V. stolonifera*.

3. The key microscopic differences in the leaves of the studied species lie in the structure of the adaxial and abaxial

surfaces of the leaf blade. The upper epidermis consists of large elongate cells with wavy walls. The cells of the lower epidermis are smaller and have more sinuous walls compared to the upper epidermis cells. The degree of wall sinuosity varies between species, such *V. collina* is characterized by more sinuous cells in both the upper and lower epidermis compared to *V. stolonifera*. The absence of stomata on the upper epidermis is a common feature for both species.

4. Based on the research results, the main morphological and anatomical features of the species were identified. These data are recommended for consideration during the identification and standardization of promising medicinal plant materials and within the development of methods of analytical and regulatory documentation.

Conflicts of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

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