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Evaluation of diagnostic morphological and anatomical features of *Ocimum americanum* (Lamiaceae) raw material

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Abstract

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Standardisation is an essential factor in determining the quality of raw material, as medicinal plants have a great natural heterogeneity due to their different geographical distribution, ecological growth conditions, and presence of varieties and chemotypes. Morphological and anatomical characteristics are very important in the pharmacognostic identification of plant raw material with valuable therapeutic potential. This study aimed to conduct the macro- and microscopic analysis of *Ocimum americanum* L., a non-traditional medicinal plant. The aerial part of plants cultivated in West Podillya (Ukraine) was harvested during the flowering period. The shape, size, colour, and features of the surface of aboveground plant organs were studied. The revealed specific morphological and anatomical characteristics of the *Ocimum americanum* stems, leaves, and flowers can allow us to identify its raw material and prevent the ingress of contaminants with aerial parts of plants belonging to other taxa of the genus *Ocimum*.

Keywords: flower, leaf, macroscopic features, microscopic features, pharmacognostic characteristics, plant raw material, stem.

INTRODUCTION

Herbal medicines are essential sources of natural medications for treating many diseases and are widely used in healthcare systems globally. Quality control of plant raw materials remains a challenge (Klein-Junior et al., 2021), especially given that new herbal drugs are continuously being introduced to the market (Raclariu-Manolică et al., 2023). Appropriate standardisation is a noticeable factor in determining the quality of plant raw materials, as there is a great natural heterogeneity due to different geographical distribution, ecological growth conditions, and the presence of varieties and chemotypes. The most justified is complex approaches using pharmacognostic analysis of plant raw material (Hordiei et al., 2023; Rustemkulov et al., 2023). Pharmacognostic standardisation and correct identification of plant material in the context of quality assessment are crucial to providing the efficacy and safety of herbal medicines due to inter- and intra-species diversity (Maurya et al., 2019; Rashid et al., 2019). Besides the phytochemical characterisation, it is essential to provide a comprehensive morphological and anatomical authentication of plant raw material, which is often adulterated due to improper identification (Simmler et al., 2018; Rashid et al., 2019; Zhang et al., 2021;

Mykhailenko et al., 2022). For instance, microscopy is a traditional pharmacopoeial method used to identify plants (Ichim et al., 2020; Rustemkulov et al., 2023). Generally, morphological and anatomical characteristics are essential in recognising plant raw material possessing therapeutic potential.

Representatives of the subfamily Nepetoideae Burnett. (Lamiaceae Martinov) are of great interest to researchers as valuable sources of bioactive compounds (Raudone et al., 2016; Karpiński, 2020; Wang & Peters, 2023). This subfamily includes such well-known genera of traditional medicinal plants as *Mentha* L., *Origanum* L., *Salvia* L., *Thymus* L. (Sharifi-Rad et al., 2021; Gerçek et al., 2022; Hafez Ghoran et al., 2022). In recent years, the analysis of the composition and pharmacological properties of bioactive compounds in the aboveground parts of non-traditional species from the genera *Ocimum* L., *Dracocephalum* L., *Satureja* L., etc. has attracted considerable attention of researchers (Ebadollahi et al., 2021; Shanaida et al., 2021 a, b; Moghaddam et al., 2022; Sneha et al., 2022; Rai et al., 2023).

The genus *Ocimum* represents more than 70 species of aromatic plants. Some are economically important because of their medicinal and culinary properties (Gurav et al., 2021). Traditionally, remedies of *Ocimum* are used to heal the common cold, bronchitis, fever, digestive ailments, nerve disorders and arthritis. Such species as *Ocimum basilicum*, *Ocimum gratissimum* and *Ocimum sanctum* are commonly known throughout the world as sources of valuable phytochemicals (WHO, 2004; Gupta et al., 2011; Gurav et al., 2021). The species mentioned above are quite well studied from a pharmacognostic point of view (Jürges et al., 2009; Rastogi et al., 2020). *Ocimum basilicum* plays a significant role in the culinary traditions of some cultures (Rai et al., 2023). Generally, *Ocimum basilicum* could be regarded as the most important species in the *Ocimum* genus (Bernhardt et al. 2014). The flowering tops, leaves, and essential oils of a lot of its cultivars are widely used in the food industry for flavouring foods and beverages as well as in perfumery (e.g. soaps, dental creams, mouthwashes) and traditional medicine as an antimicrobial, insecticidal, hepatoprotective and antiulcerogenic remedy (Labra et al., 2004; Verma et al., 2013).

Ocimum americanum L. is an essential oil-bearing plant native to tropical and subtropical Africa and Asia (Ali et al., 2022). It is introduced mainly into South America and occasionally is cultivated in Europe. *Ocimum americanum* is locally used as a spice, digestive, sedative, and anti-inflammatory remedy (Shanaida et al., 2021). The aerial part of *Ocimum americanum* accumulates such valuable phytochemicals as essential oil, hydroxycinnamic acids, and flavonoids (Jasicka-Misiak et al., 2021; Shanaida et al., 2021a; Ali et al., 2022). Recently, Ali et al. (2022) have conducted the phytochemical screening of *Ocimum americanum* and found a lot of bioactive compounds (tannins, flavonoids, terpenoids, etc.) possessing antimicrobial properties and cytotoxic effects for crustacean *Artemia salina.*

Chowdhury et al. (2017) have concluded that the comprehensive scientific investigations of diagnostic macroscopic and microscopic features of the *Ocimum americanum* herb could be used in standardisation of this plant's raw material. Therefore, this study aimed to establish the specific anatomical and morphological features of the *Ocimum americanum* stems, leaves and flowers, providing the basis for the quality control of this plant's raw material.

MATERIALS AND METHODS

Plant raw material: characteristics and features of its gathering

The aerial parts of *Ocimum americanum* plants were collected during the flowering period in July (Fig. 1) from the experimental plots under cultivation in West Podillya, Ukraine (49°38′03″ N; 25°28′32″ E). Specimens were identified by the first author.

The aerial part of the plant was cut at the level of the lower leaves. Then the leaves from the lower part of the stem were separated, and the stems thicker than 3 mm were removed. Then, the herb was put in a thin layer on the shelves and dried according to requirements (Rashid et al., 2019), provided for essential oil-bearing plants (at a temperature of 30–35 °C in a space protected from sunlight).

Macroscopic and microscopic analysis

Morphological and organoleptic characteristics, including the raw material's shape, size, colour, fracture and texture, were investigated and noted ac-

Fig. 1. Flowers of *Ocimum americanum* under its cultivation in West Podillya (Ukraine). Photograph by M Shanaida

Fig. 2. The dried raw material of *Ocimum americanum*

cording to standard methods (Vlasova et al., 2022). The morphological analysis was conducted with the naked eye and using a magnifying glass. It included analysis of stems, leaves, inflorescences and flowers.

The microscopic features of the structure of the epidermal cells, the type and location of the stomatal apparatus, and the specific features of the indumentum and glandular trichomes of the epidermis were evaluated (Gontova & Zatylnikova, 2013; Vlasova et al., 2022). Features of the anatomical structure of leaves and stems were also studied in cross-section, and parts of the flower were analysed using the microscope without preparation.

Before analysis, the dried plant materials were soaked in water for 24 hours, then in a mixture of 96% ethanol, glycerin and purified water (1 : 1 : 1) for 7–10 days. The structure of the stems and leaves was studied in transverse sections. The epidermis was prepared from the surface of organs. Parts of flowers were examined under a microscope without prior preparation. The microscope *Micromed XS-4130* (China) magnified 400 times (oculars *WF15X*, lenses ×40/0.65, ×10/0.25). *Samsung PL50* camera was applied for photography. Sudan III (1-{[4-(phenyldiazenyl)phenyl]diazenyl}naphthalen-2-ol) and aniline sulphate were used as reagents for colouring essential oil trichomes and lignified tissues, respectively.

RESULTS

The macroscopic analysis of the raw material of *Ocimum americanum* (Fig. 2) revealed that the quadrangular stems were pubescent and covered with short, numerous trichomes. Leaves were arranged cross-oppositely. The leaf blade of *Ocimum americanum* was simple, entire, elliptic-lanceolate, with pinnate venation, up to 5 cm long, 4 cm wide and moderately pubescent with short hairs. The petiole of the leaf was 1.0–2.2 cm long. The veins protruded from the underside of the leaf blade. The upper surface of the leaf was pale green, and the lower surface was greenish white. The leaf edge was serrated and slightly ciliated.

It was found that the lateral shoots ended in thyrsoid inflorescences up to 20 cm long. The flowers were up to 1 cm long and arranged in false whorls surrounded by small oval bracts. The calyx was slightly two-lipped and bell-shaped, up to 0.5 cm long. The white two-lipped corolla was twice as long as a calyx. The lower lip of the corolla was elongated and bent down, while the upper lip was four-lobed. The stamens were protruding significantly from the throat, and the upper two were slightly shorter than the lower two; others were brownish-yellow. The naked pistil protruded from the corolla tube and had a two-part stigma.

Stems and leaves were light green, and corolla was creamy white. The smell of raw material was specific and fragrant; its taste was slightly bitter-burning.

The microscopic investigations revealed that the stem in the middle zone (Fig. 3–5) had four ribs,

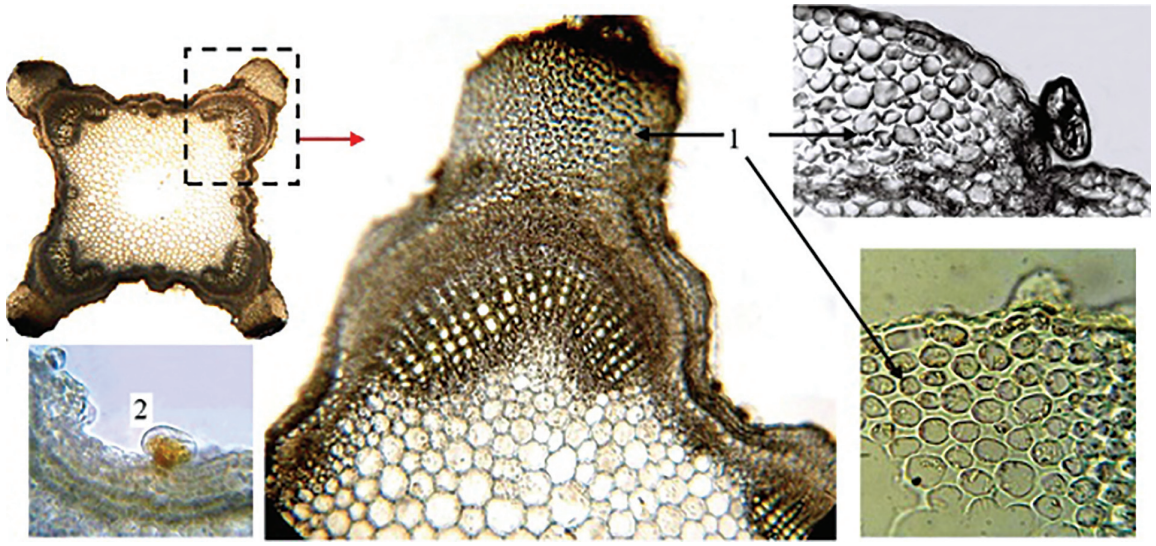

Fig. 3. General scheme of the *Ocimum americanum* stem (fragments of the rib): 1 – angular collenchyma, 2 – secretory gland of the epidermis

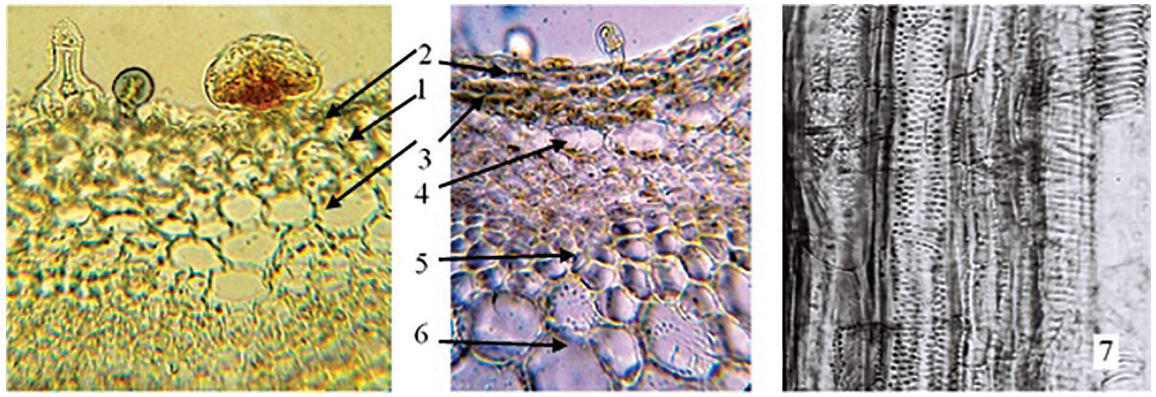

Fig. 4. Fragments of the cross-sections in the middle zone of the *Ocimum americanum* stem (the part of stem between ribs): 1 – angular collenchyma, 2 – epidermis with trichomes, 3 – chlorenchyma, 4 – endoderm, 5 – pericyclic sclerenchyma, 6 – pith parenchyma, 7 – vessels of the xylem (longitudinal section)

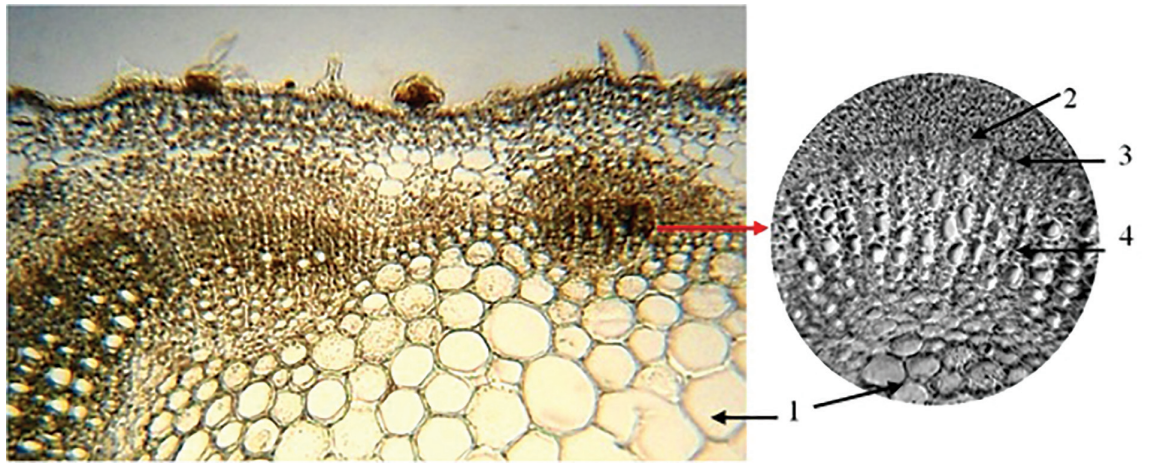

Fig. 5. Fragment of the cross-sections in the middle zone of the *Ocimum americanum* stem and structure of the additional collateral vascular bundle: 1 – pith parenchyma, 2 – phloem, 3 – cambium, 4 – vessels of the xylem (transverse section)

which were more sharp in the upper part of the plant than in the middle and lower ones. The epidermis of the stem had a noticeable layer of cuticle and trichomes. The basic cells of the epidermis were almost rectangular, slightly elongated, with thin side walls.

The stem was covered by trichomes of several types. Trichomes were more or less evenly spaced, but most simple covering hairs were usually concentrated on the ribs. Hairs were unicellular and dead, with warty cuticles, more or less curved. They were surrounded by the slightly elevated rosette of thick-walled cells. There were also frequent living sharp hairs consisting of 3–4 cells with thin walls. Secreting trichomes were represented by essential oil peltate glands (typical structure for Lamiaceae) and glandular hairs (Fig. 4). The heads of glandular (capitate) hairs were roundish-oval, 1–2-celled and colourless; their 1–2-celled stalks were well visible. The peltate glands contained a monocellular stalk with a round multicellular secretory head. The essential oil peltate glands were on a single-cell stalk, slightly embedded in the epidermis.

Generally, the anatomical structure of the stem was non-bundle in the lower zone and transitional in the middle part. Strongly protruding ribs were filled with angular collenchyma (Fig. 3), which is underlain by 1–2 rows of small-celled chlorenchyma. Under the ribs were the main large semilunar vascular bundles. The primary cortex of young stems of the apical zone between the ribs is represented by 1–3 layers of angular collenchyma, 2–3 layers of chlorenchyma and uniseriate endoderm.

Leaves were pubescent with simple and glandular trichomes (Fig. 6–8).

The simple trichomes were more abundant along the edge of the leaf blade and the veins (Fig. 8). The simple sharp unicellular conical hairs and 2–4-cell elongated hairs with warty cuticle and multicellular elevated rosette dominated. The glandular hairs and glands with yellow-brown content were more or less evenly distributed in the lower epidermis of the leaf blade. The glands were at the same level as the epidermal cells or slightly submerged and had a large, dark, spherical 8-cell head and a short stalk. The capitate cells were arranged in a circle. The glandular hairs were much smaller than the glands, and their structure was similar to the structure of the stem. The basal cells of the epidermis were small, with thin, angularly tortuous walls. A large number of diacytic-type stomata were observed (stomatal index was 4.5).

The upper epidermis of the leaf consisted of larger tortuous cells (Fig. 7). There were much fewer stomata found in the lower epidermis. Trichomes were represented mainly by simple unicellular pointed hairs with 4–6-cell rosette and glandular hairs.

It was revealed that the anatomical structure of the leaf blade was amphistomatic dorsoventral or vaguely differentiated (Figs 9–10) with a narrow mesophyll (on average 3–4-layered). Chlorenchyma cells under the upper epidermis were slightly elongated and densely packed (palisade mesophyll). Under the lower epidermis, were large round cells with small intercellular spaces between them (spongy mesophyll). The central vein protruded significantly from the dorsal side and slightly deepened on the ventral. The densest accumulation of trichomes was on the border of a plate and a ledge.

The central vein had one arcuate bundle with dominated xylem and many vascular rays (Fig. 10). The angular collenchyma strengthened the lower and upper parts of the vein. The petiole was densely pubescent, typical for all parts of the leaf trichomes; its cross section was semicircular and slightly notched on the ventral side.

The flower's calyx (Fig. 11) was pubescent, similar to the leaves. Its covering hairs with warty surface consisted of sharp 1–3-cells directed upwards. The capitate hairs were small, with a dark head. The cells of the outer epidermis (Fig. 11, 2) were small and elongated along the axis, with thickened loop-like tortuous walls. The stomata were very rare. The cells of the inner epidermis (Fig. 11, 3) were more elongated than the upper one. The veins were accompanied by fibres.

The corolla of flowers (Fig. 12) was abundantly covered with trichomes. There were many simple covering hairs along the veins, but between the veins, there were more glands and glandular hairs. The cells of the inner (ventral) side of the corolla's four-bladed upper and one-bladed lower part were parenchymal and tortuous. The corolla tube was covered with an epidermis composed of narrow prosenchymal cells. The epidermis of the limb was covered with simple and glandular trichomes. It was quite remarkable that the corolla had no stomata.

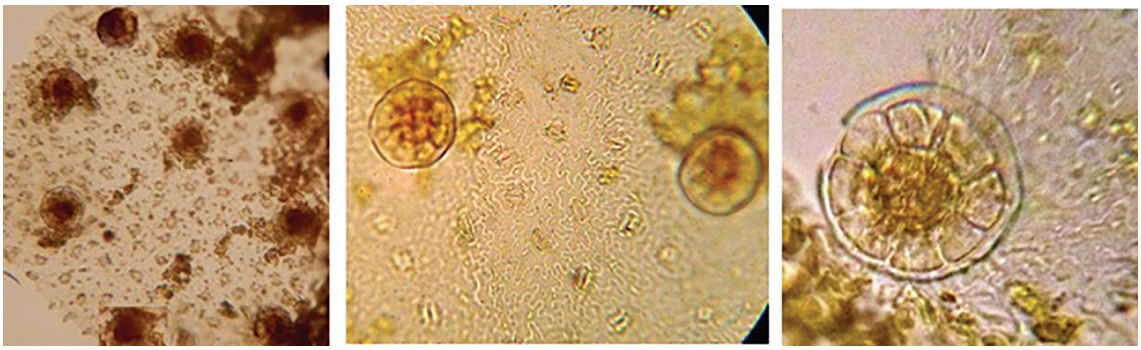

Fig. 6. Fragments of the lower epidermis of leaves with secretory glands

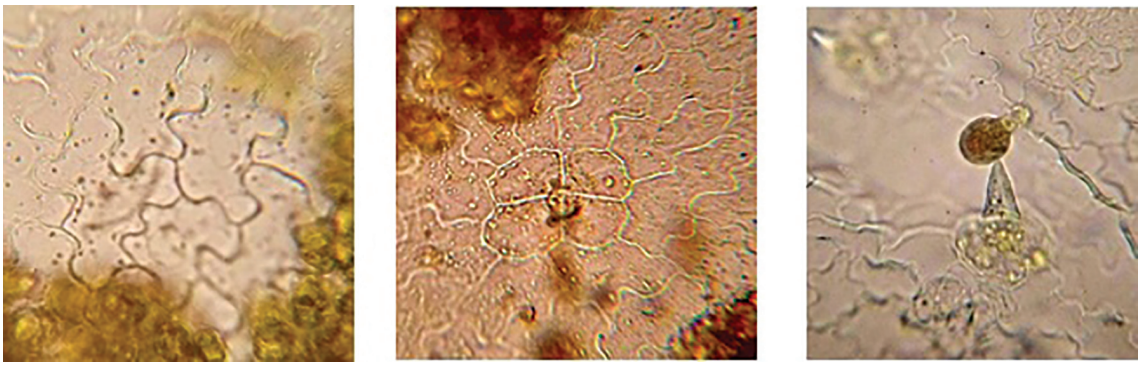

Fig. 7. Fragments of the upper epidermis of leaves

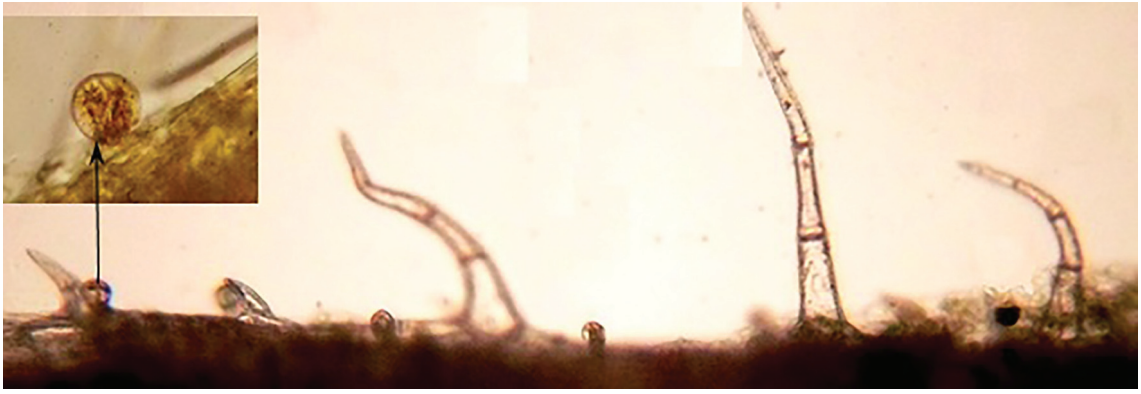

Fig. 8. Simple 2–4-cell elongated and glandular hairs of the *Ocimum americanum* epidermis above the vein

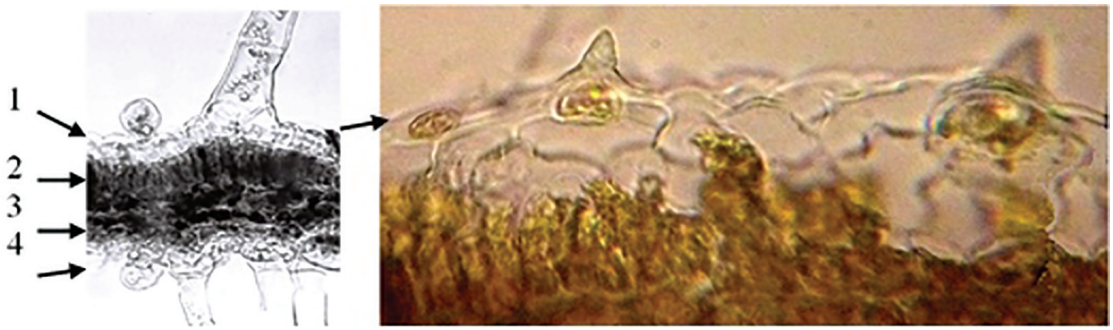

Fig. 9. Fragments of the cross-sections of *Ocimum americanum* leaf blade: 1 – upper epidermis with trichomes, 2 – poorly differentiated columnar chlorenchyma, 3 – spongy chlorenchyma, 4 – lower epidermis with trichomes

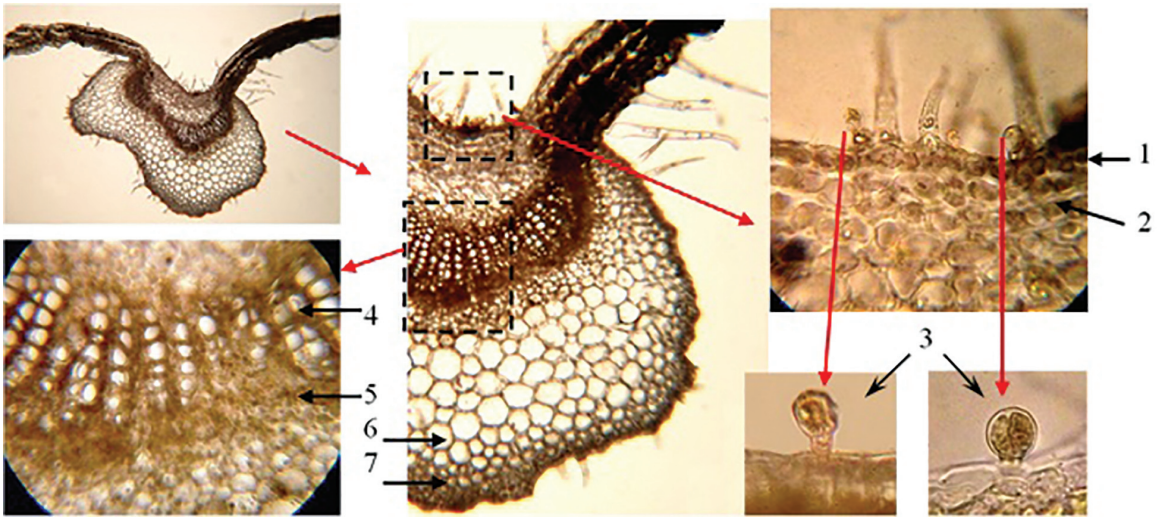

Fig. 10. Fragments of the cross-sections of *Ocimum americanum* leaf blade: 1 – upper epidermis with trichomes, 2 – poorly differentiated columnar chlorenchyma, 3 – glandular hairs, 4 – xylem vascular rays and vessels, 5 – phloem, 6 – parenchyma, 7 – collenchyma

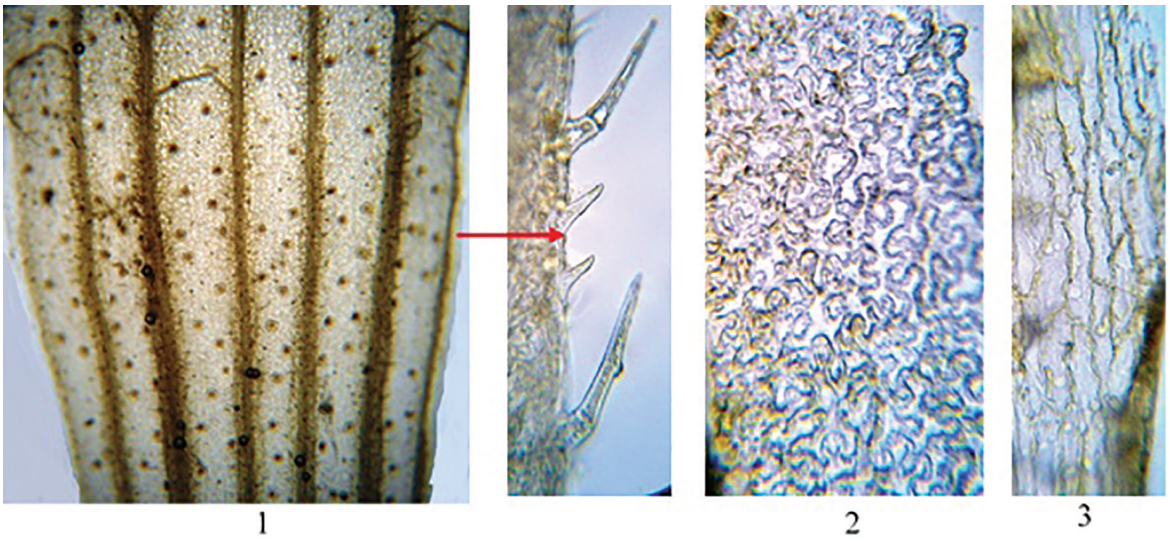

Fig. 11. Fragments of the calyx of the *Ocimum americanum* flower: 1 – general view of the surface and edge of the teeth with simple sharp 1–3-cells hairs, 2 – outer epidermis of the calyx, 3 – inner epidermis of the calyx

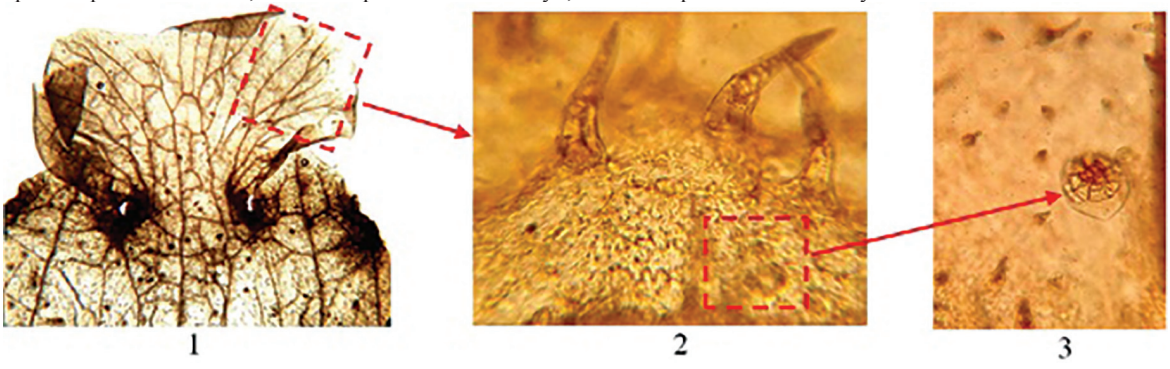

Fig. 12. Fragments of the corolla of the *Ocimum americanum* flower: 1 – epidermis of the limb with simple (2) and glandular (3) trichomes

DISCUSSION

Recently, Chowdhury et al. (2017) have built the dissimilarity dendrogram and showed significant clusters among several *Ocimum* genotypes according to their morphological traits. They have combined four species into the *Ocimum basilicum* group: *Ocimum basilicum*, *Ocimum americanum*, *Ocimum africanum* and *Ocimum kilimandscharicum*. The researchers have noted that unlike the studied by us *Ocimum americanum*, possessing elliptic-lanceolate leaves and white corolla, the *Ocimum basilicum* leaves are ovate-lanceolate, and flowers are whitish pink or pinkish-white (depending on varieties). According to Chowdhury et al. (2017), the plant heights of *Ocimum americanum* and *Ocimum basilicum* are different (ranging from 20 cm to 60 cm and from 45 cm to 100 cm, respectively). Similar results have also been reported by Verma et al. (2013). Moreover, *Ocimum basilicum* is characterised by a significant number of varieties and chemotypes; therefore, it is much more challenging to describe it with evident characteristics than *Ocimum americanum* (Verma et al. 2013).

As it is known, essential oils play an important role in the pharmaceutical and perfume industries. Therefore, it is worth studying the diagnostic microscopic features of the excretory structures of essential oil-bearing Lamiaceae plants that produce them (Maleci & Giuliani, 2006). The peltate and capitate glandular trichomes dominate among thirteen kinds of outgrowths of the epidermis of *Salvia nemorosa* (Talebi et al., 2018). Da Silva et al. (2023) have recently conducted comprehensive morphoanatomical and histochemical investigations of the *Plectranthus ornatus* Codd. They have established a relationship between the density of placement of glandular structures and the level of essential oil accumulation. It has been concluded that environmental factors significantly influence the yield and composition of essential oil of this species because its synthesis is related to the protection against external disturbances such as herbivores (da Silva et al., 2023).

Over the past decades, significant progress has been achieved in characterising the genes and enzymes responsible for the unique metabolic capabilities of glandular trichomes in different taxonomical groups of plants (Zager & Lange, 2018).

In addition to the presence of specific morphoanatomical features, such as glandular structures (peltate and capitate trichoms) and dead simple trichomes, representatives of the *Lamiaceae* family are characterised by the presence of diacytic type of stomata and tetrahedral stems with angular collenchyma on the ribs (Zhang et al., 2021). It should be noted that diacytic stomata are located on the upper and lower epidermis of *Ocimum sanctum* (WHO, 2004), like in the studied *Ocimum americanum* species.

Besides the pattern of features expected for the *Lamiaceae* plants (Sahu et al., 2022; da Silva et al., 2023), some peculiarities of the studied *Ocimum americanum* were found. For instance, *Ocimum americanum* has the specific structure and localisation of simple and glandular trichomes of the epidermis. Thus, the characteristic feature of the studied species' epidermis was that essential oil glands were localised at the same level as the epidermal cells or slightly submerged, with a brownish-yellow spherical 8-cell head and a short stalk. According to monographs on selected medicinal plants (WHO, 2004), capitate trichomes of *Ocimum sanctum* are short and consist of 2–4 cells with rounded heads on a one-cell stalk.

The studied *Ocimum americanum* also differed from the other *Ocimum* species (WHO, 2004; Gupta et al., 2011; Mangla et al., 2020) in the structure and localisation of simple and glandular hairs of the epidermis. It was established that the distinctive features of the raw materials of *Ocimum americanum* were peculiarities of the shape of the stems in cross-section and the localisation of vascular and mechanical tissues. The stems of *Ocimum americanum* had the most pronounced narrow club-shaped protruding ribs. The specific structure and placement of epidermal trichomes and mesophyll structures were also quite distinct.

Recently, Mirgal et al. (2023) have reported that purple varieties of *Ocimum basilicum* accumulate anthocyanins in the epidermal cells of different aboveground organs, while *Ocimum americanum* is not characterised by the presence of anthocyanin-rich forms. Furthermore, *Ocimum americanum* has another epidermal cell wall architecture compared to *Ocimum basilicum* (Mirgal et al., 2023). Generally, Chowdhury et al. (2017) have estimated that *Ocimum americanum* is much less studied from the mor-

phological and chemical points of view than *Ocimum basilicum* or *Ocimum sanctum*.

Thus, the comprehensive macro- and microscopic analysis of stems, leaves and flowers of the non-traditional medicinal plant *Ocimum americanum* revealed its main diagnostic morphoanatomical features. It is recommended to account for them while developing the *Ocimum americanum* standardisation parameters.

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