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Anatomical and phytochemical study of *Ocimum basilicum* L. leaves of two popular varieties in Ukraine

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Abstract: one of the important sources of flavonoid phytonutrients is basil (*Ocimum basilicum* L.). Vegetative and generative organs of which have essential oils, in particular 1,8-cineole, estragole and eugenol, ascorbic acid, a rich pigment complex, as well as polyphenolic compounds with antioxidant effects. Basil realizes its antioxidant potential through anthocyanins in varieties with purple leaves and flavonoids in varieties with green leaves. The level of their accumulation, as well as other biologically active substances, depends on the variety to which the plant belongs, the degree and spectrum of illumination, soil moisture, and mineral nutrition (Priss OP, et al., 2019; Joshi RK, 2014; Trichopoulou, et al., 2000). That is why our attention was drawn to two of the most popular basil varieties in Ukraine: «Dolly» with green leaves and «Rosie» with purple leaves. Our research was focused on morphological, anatomical and phytochemical studies of *O. basilicum* leaves of the above-mentioned raw materials. To realize this goal, microscopy was performed using State Pharmacopoeia of Ukraine methods. Groups of biologically active substances were determined by standard qualitative reactions and the procedure was carried out using extracts or by applying directly to the sections. It was found that *O. basilicum* chateaux of «Dolly» and «Rosie» varieties have a number of common and distinctive features. Both varieties of *O. basilicum* are characterized by the presence of dorsoventral, amphistomatic leaves, diacytic stomatal apparatus and biserial glands. The leaves differ in color, shape of the leaf blade margin, outlines of the upper epidermal cells, presence of idioblast cells with anthocyanins in the «Rosie» variety, and values of stomatal indices. The stomatal index is $27.5 \pm 2.3\%$ for the «Dolly» variety and $25.4 \pm 2.5\%$ for the «Rosie» variety. It was found that the leaves of *O. basilicum* varieties «Dolly» and «Rosie» contain such biologically active substances as anthocyanins, flavonoids, essential oil, triterpene saponins, especially expressed in the variety «Rosie» and tannins. The research results can be used in the development of a monograph on the raw materials of this species.

Keywords: [Anthocyanins](#); [Essential Oil](#); [Flavonoid](#); [Ocimum basilicum](#); [Phytonutrients](#).

Introduction

According to the 2019 Global Burden of Disease (GBD), an independent health research organization, cardiovascular diseases, mainly coronary heart disease and stroke, are the leading causes of death and one of the main factors of disability worldwide (Institute for Health Metrics and Evaluation).

Compounds of flavonoid nature and anthocyanins in particular play an important role in the prevention of cardiovascular diseases. Their action is closely related to protection against oxidative stress (Lila M. A., 2004; Youdim K. A. et al., 2000).

One of the important sources of flavonoid phytonutrients is a representative of the Lamiaceae family – true Sweet Basil or basil (*Ocimum basilicum* L.). Its vegetative and generative organs contain essential oils, in particular 1,8-cineole, estragole and eugenol, ascorbic acid, a rich pigment complex, which is represented by carotenoids (more in green varieties), as well as polyphenolic compounds with antioxidant effects. The manifestation of antioxidant properties in basil with purple leaves is due to anthocyanins, and in plants with green leaves – to flavonoids (Priss OP, et al., 2019; Joshi RK, 2014; Trichopoulou, etc., 2000). That is why our attention was attracted by two of the most popular varieties of basil in Ukraine «Dolly» with green leaves and «Rosie» with purple leaves (State Register of Plant Varieties Suitable for Distribution in Ukraine).

The stable, available and inexpensive sources of anthocyanins obtained from *O. basilicum* are attractive for use in the pharmaceutical, medical and food industries. The abundance of acylated and glycosylated anthocyanins in basil extracts can also serve as a unique source of stable red pigments for the needs of pharmaceuticals and food industry. Identification of high-performance sources of anthocyanins will allow us to continue our work on the study of antioxidant properties and quantitative content in different varieties of basil.

Aim

To conduct anatomical, morphological and phytochemical analysis of *O. basilicum* leaves in the raw materials of the two most popular varieties of Ukraine – «Dolly» and «Rosie».

Materials and methods

To realize this aim, microscopy was performed according to the method of V. S. Dolya (Dolya V.S., et al., 2003).

In order to prepare the micro preparations, we took raw materials, both fresh and dried, after soaking them in water. First, we boiled the leaf epidermis in a 5% NaOH solution for 5 min, as a result of which it was easier to detach from the mesophyll. The clarification of microsections was performed by boiling them in an aqueous solution of chlorohydrate (4:1). The prepared micro preparations were examined in aqueous medium and water-alcohol medium of different concentrations under a ULAB microscope (×40, ×100, ×1000) equipped with a Canon EOS 550 digital microphotographic camera. To provide more objective research, 10 micro preparations were examined for each sample of medicinal plant material.

Determination of the stomatal index. The stomatal index was calculated according to the method specified in the State Pharmacopoeia of Ukraine (State Pharmacopoeia of Ukraine, 2015).

Formula for determining the stomatal index:

$$\text{Stomatal index} = \frac{100 \times S}{E + S}$$

S – the number of stomata per certain surface area of the leaf blade;

E – a certain number of epidermal cells (including hairs and glands) per the same leaf surface area.

For each *O. basilicum* variety, at least ten samples of leaf blades were collected and the average value was calculated.

Groups of biologically active substances (BAS) were determined using standard chemical reactions and the procedure was carried out using extracts or by applying directly to the sections (State Pharmacopoeia of Ukraine, 2016; State Pharmacopoeia of Ukraine, 2018; Kovalev V. M. et al., 2014).

Review and discussion

The leaves of *O. basilicum* make up a significant part of the phytomass used for pharmaceutical and medical purposes. Taking into account the presence of fifteen varieties of basil according to the State Register of Plant Varieties suitable for distribution in Ukraine, it is important to establish the main, diagnostic, species-specific features for the primary diagnosis of raw materials of the most popular varieties, in particular «Dolly» and «Rosie».

O. basilicum leaves of «Dolly» and «Rosie» varieties have a number of common features: type and shape of the leaf blade; sinuous-walled shape of the cells of the lower epidermis, presence of diacytic stomatal apparatus and eight-celled essential oil glands, and distinctive features: color of the leaf blade, shape of the leaf margin, shape of the cells of the upper epidermis, different values of stomatal indices, presence of idioblast cells with anthocynes in «Rosie» variety (Fig. 1.).

The leaf petioles of both varieties have a rather similar structure (Fig. 2. A, B). Under the epidermis layer there are several rows of elongated, rect-

angular, thick-walled palisade parenchyma cells filled with brownish-olive substance (Fig. 2.D).

The basis of petiole mesophyll consists of 10-12 rows of thick-walled cells of spongy parenchyma with irregular shape. In the upper part of the spongy parenchyma there are 5 to 7 closed collateral vascular fiber bundles, which are lined up in one continuous line (Fig. 2. A, C, 1).

Both varieties of *O. basilicum* are characterized by the presence of dorsoventral, amphistomatic leaves. The upper epidermis of the leaf variety «Dolly» is represented by rather large, clearly zigzag-shaped cells, among which diacytic sto-



Figure 1. Morphometric parameters of *O. basilicum* leaves of varieties: A – «Dolly», B – «Rosie»

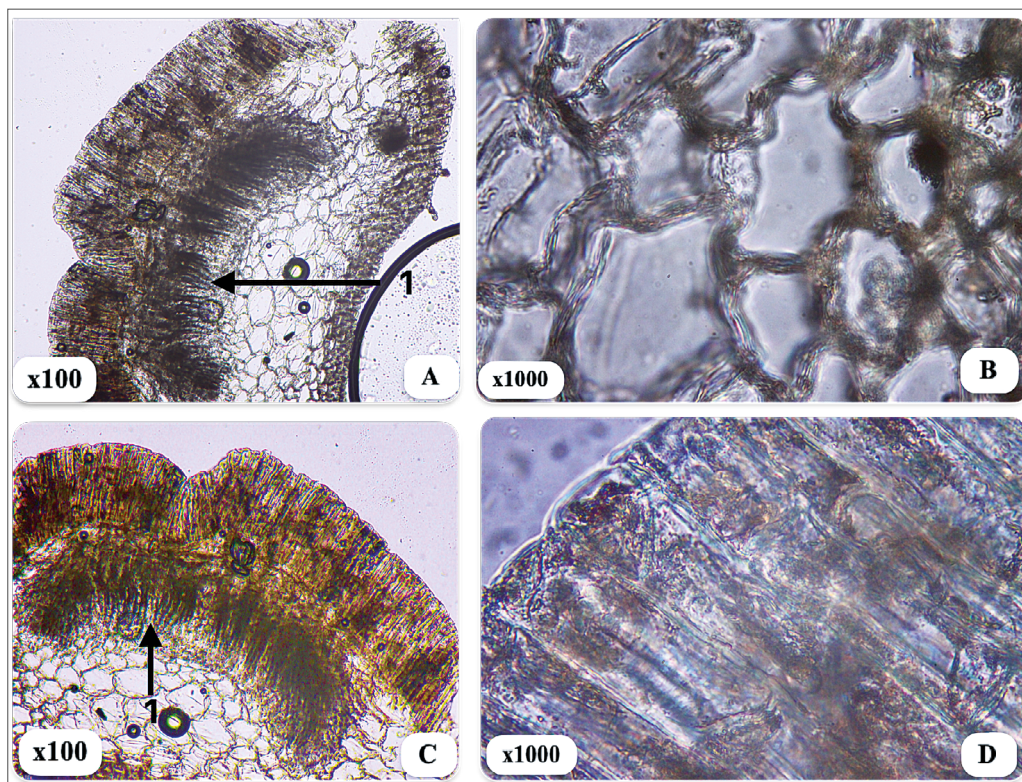


Figure 2. Cross section of *O. basilicum* petiole: A, B – «Dolly» variety, C, D – «Rosie» variety. 1 – vascular fiber bundle

matal apparatus are quite common (Fig. 3.A2). In some places, essential oil glands are located over the entire surface (Fig. 3.A, B1).

The upper epidermis of the leaf of *O. basilicum* variety «Rosie» is formed by thin-walled, tortuous cells with a slightly smaller number of diacytic stomata per leaf blade surface area (Fig. 3.C, E2). The entire surface of the leaf is covered with a large number of point glands – idioblast cells with anthocyanin content (Fig. 3. D3). Occasionally there are also large eight-celled essential oil glands, characteristic of Lamiaceae (Fig. 3. C, E1). In addition, along the veins, characterized by elongated, more rectangular epidermal cells with geometrically smooth edges, there are simple 3-4-celled simple hairs with a several-celled base (Fig. 3.D4). Often, when the

epidermis is removed, a layer of mesophyll with rather large chloroplasts located along the perimeter of the cell's tortuous wall is attached to its surface (Fig. 3.E5).

The lower epidermis of both varieties is characterized by the presence of tortuous wall cells and a large number of diacytic stomata (Fig. 4.A-C2). The stomatal index for the lower epidermis of «Dolly» is $27.5 \pm 2.3\%$, and for «Rosie» – $25.4 \pm 2.5\%$. In both representatives, essential oil bead glands are not often found in the lower epidermis, but in «Rosie» there are also idioblasts with anthocyanin content and the epidermal cells themselves have anthocyanins in the cell sap, which gives them a purple color (Fig. 4.-D1,3).

The macroscopic and microscopic studies made it possible to identify a number of features

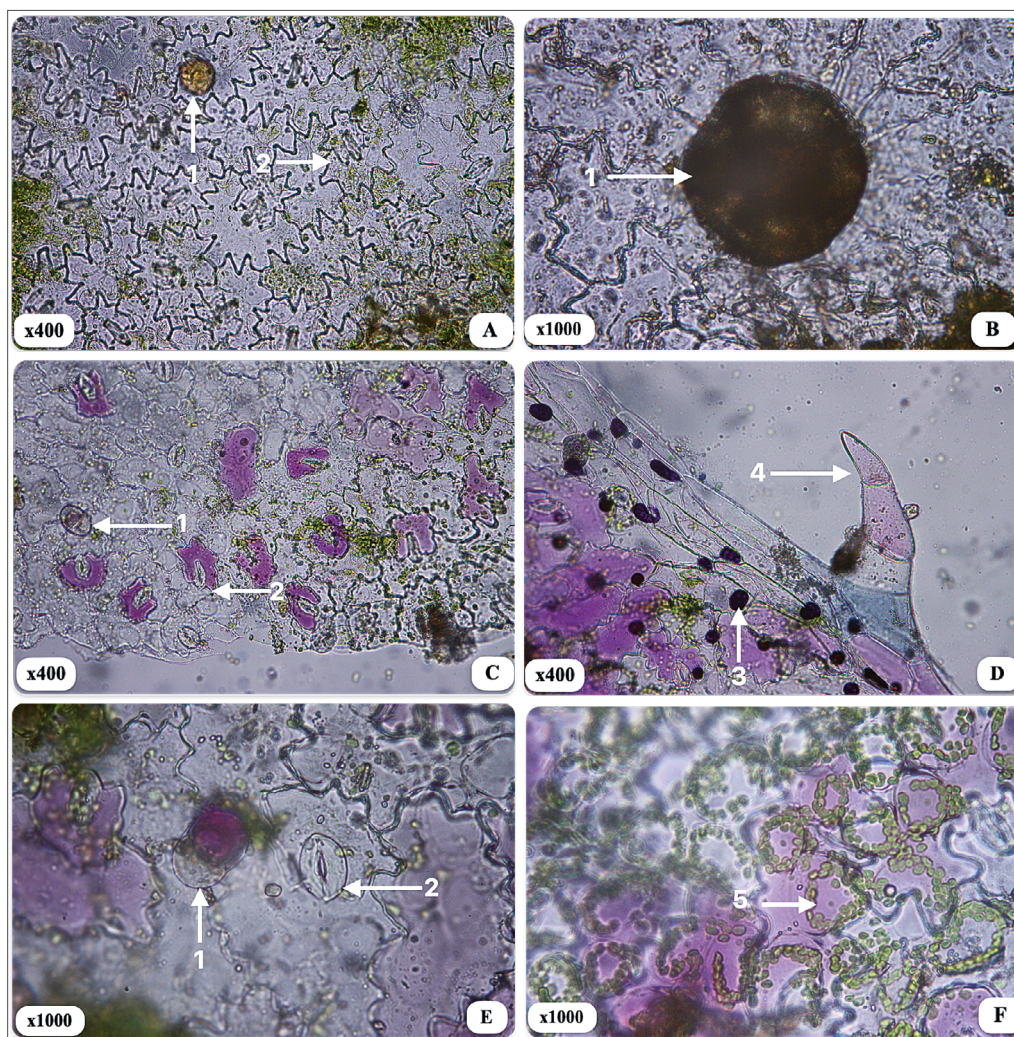


Figure 3. Upper leaf epidermis of *O. basilicum*: A, B – variety «Dolly», C, D, E, F – variety «Rosie».

1 – essential oil gland, 2 – diacytic stomatal apparatus, 3 – idioblasts, 4 – simple multicellular hair, 5 – chloroplasts.

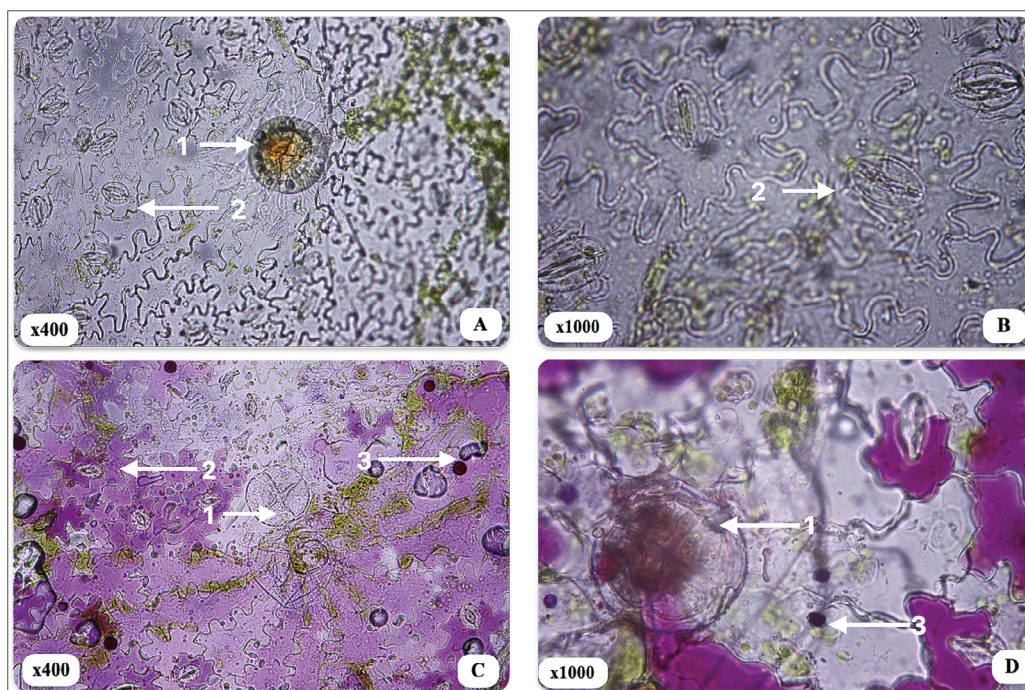


Figure 4. Lower leaf epidermis of *O. basilicum*: **A, B** – variety «Dolly», **C, D** – variety «Rosie». **1** – essential oil gland, **2** – diacytic stomatal apparatus, **3** – idioblasts

inherent in a particular variety, which can be further used to identify raw materials and detect and separate impurities, in particular *Origanum vulgare* L. for the «Dolly» variety, *Lagonosticta rubricata* Lichtenstein for the «Rosie» variety.

Since our raw materials contain essential oil glands and anthocyanin-containing compounds, histochemical reactions to identify their localization and frequency of occurrence will allow us to further develop storage standards and the degree

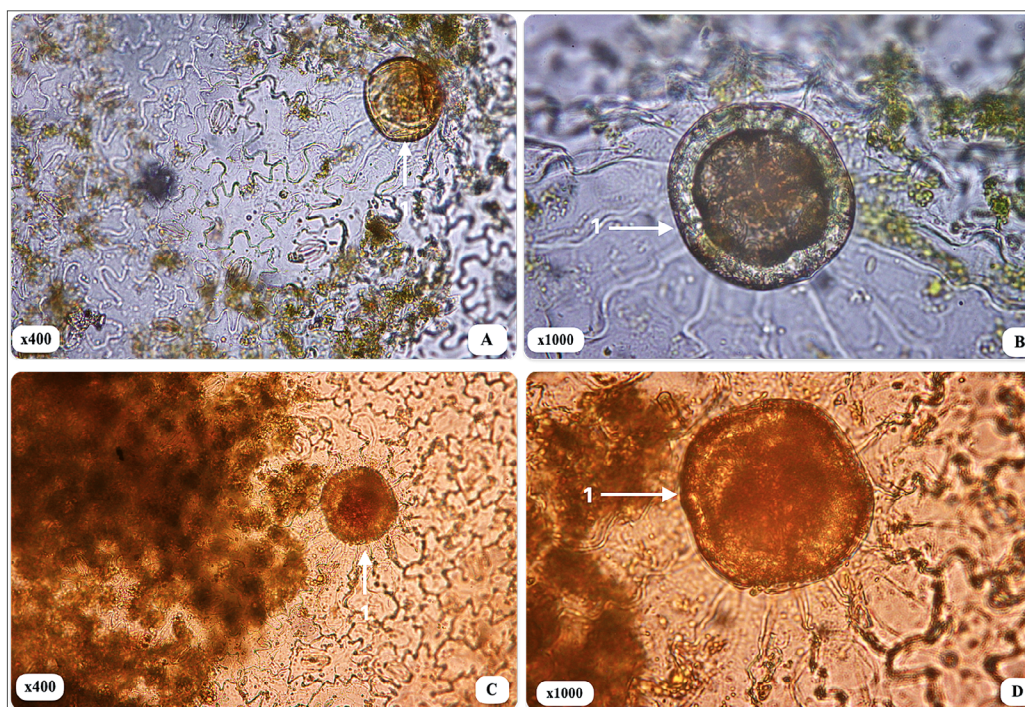


Figure 5. Lower leaf epidermis of *O. basilicum* with Sudan III: **A, B** – variety «Dolly», **C, D** – variety «Rosie». **1** – essential oil gland

of grinding of the raw materials under study to minimize the loss of valuable BAS at the initial stages of work with raw materials.

Reaction to the detection of essential oil. Microscopic studies revealed a large number of essential oil glands, the lower surface of *O. basilicum* leaves was immersed in a solution of Sudan III, and after a couple of minutes the preparation was washed from the remnants of Sudan.

The essential oil glands changed their color to orange-brown, which proves the presence of essential oils in the leaves of both varieties of basil.

Reaction to the detection of anthocyanins. To confirm the presence of anthocyanins in the leaves of *O. basilicum*, a few drops of hydrochloric acid 10% were applied to the lower epidermis of the leaf blade (Fig. 6.A-D).

We observed the appearance of crimson-red color in the places of localization of anthocyanins, namely: idioblast cells of the «Rosie» variety (Fig. 6), in the cells of the «Dolly» variety leaves the color became brick red, indicating a mediocre accumulation of anthocyanins in individual samples.

To confirm the histochemical reactions and identify other groups of BAS in the studied raw materials, qualitative reactions were performed. The results of the reactions are shown in Table 1.

The qualitative reactions indicate the presence of saponins, flavonoids, and tannins, in addition to specific BAS for this raw material. These basil varieties are quite promising for further phytochemical studies.

Conclusions

It was found that the leaves of *O. basilicum* varieties «Dolly» and «Rosie» have a number of common features: type and shape of the leaf blade; sinuous-walled shape of the cells of the lower epidermis, the presence of diacytic stomatal apparatus and eight-celled essential oil glands, and distinctive features: color of the leaf blade, shape of the leaf margin, shape of the cells of the upper epidermis, different values of stomatal indices, presence of idioblast cells with anthocyanins in the variety «Rosie». It was found that the leaves of *O. basilicum* varieties «Dolly» and «Rosie» contain such biologically active substances as anthocyanins, flavonoids, essential oil,

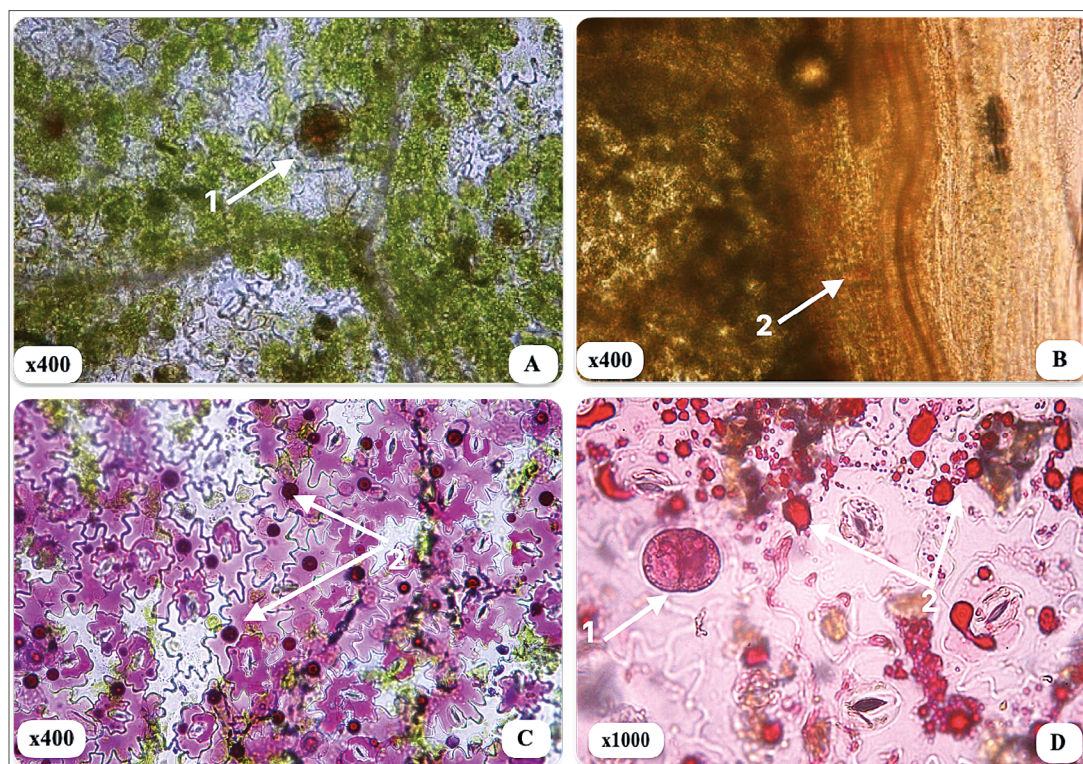


Figure 6. Lower leaf epidermis of *O. basilicum* with HCl (10%): **A, B** – «Dolly» variety, **C, D** – «Rosie» variety. **A, C** – before the reaction, **B, D** – the result of the reaction.

1 – essential oil gland, 2 – idioblast cells with anthocyanins

Table 1. Results of the qualitative reactions

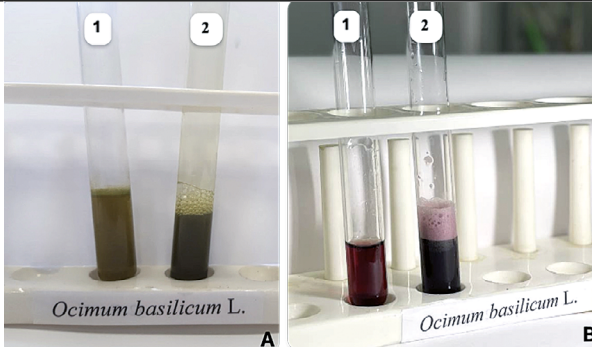
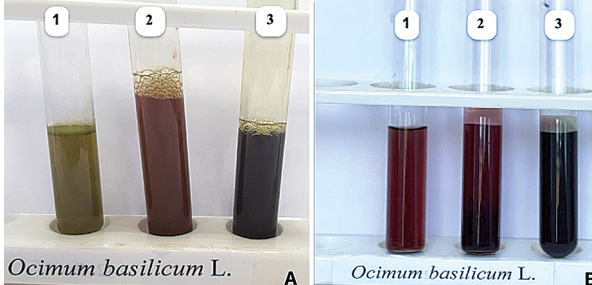
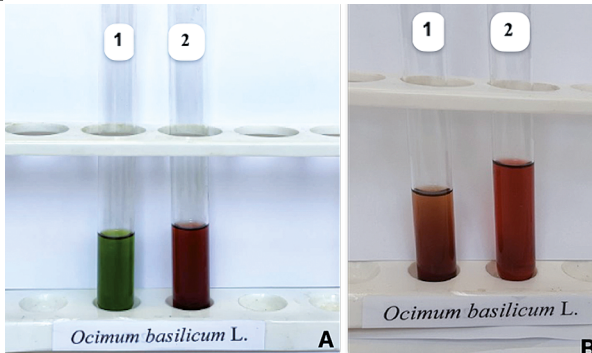
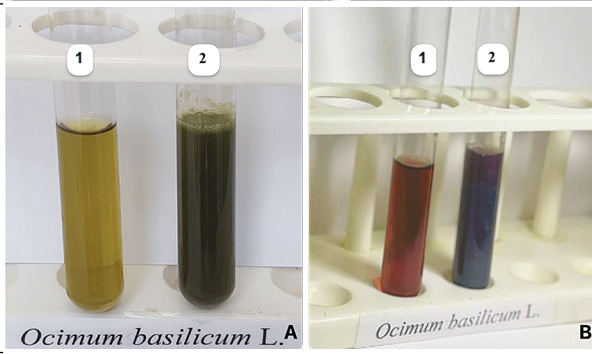
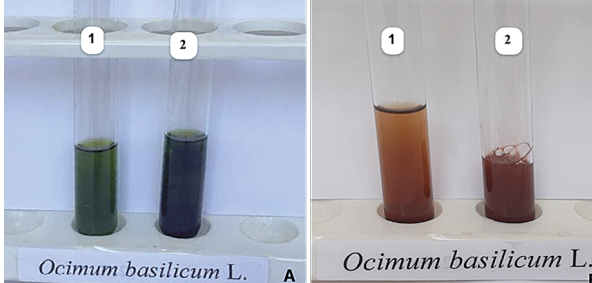
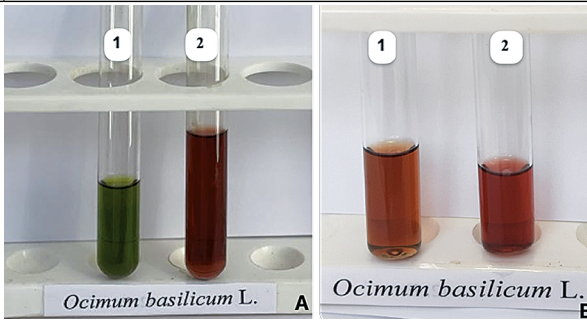
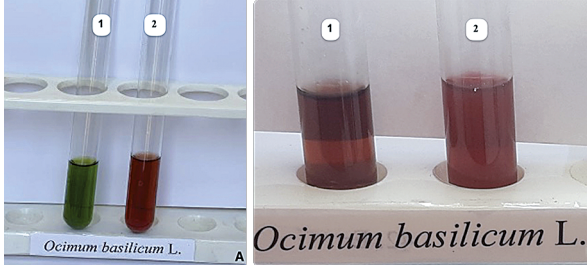
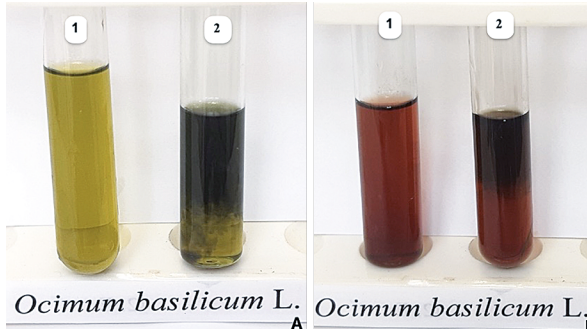
Name of the reaction	Reactive	The result of the reaction	Image *
Test for foam formation (for saponins)	Shake intensively for a minute	A stable foam has formed	
** Determination of chemical nature of saponins	5 ml of 0.1 N HCl – test tube 2. 5 ml of 0.1 N NaOH – test tube 3. Shake intensively for a minute.	Stable foam in extracts from both varieties indicates the presence of triterpene saponins	
Salkovsky test (for saponins)	1 ml of chloroform and 5-6 drops of concentrated sulfuric acid	The extracts have turned red	
Reaction with lead acetate (for saponins, flavonoids, tannins)	4 drops $Pb(C_2H_3O_2)_2$	Muddy sediment has fallen out	
Cyanidin reaction (for flavonoids)	5 drops HCl (con.), 10-15 mg metalline Zn	The extracts became rich emerald and burgundy in color	

Table 1 (continued)

Name of the reaction	Reactive	The result of the reaction	Image *
Reaction with 1% vanillin solution (for catechins)	3 drops 1% vanillin solution in HCl (con.)	Solutions of both varieties turned pink in color	
Detection of anthocyanins	To 2 ml of the alcohol extract, 2 drops of 10% H ₂ SO ₄ were added	The solutions of both varieties acquired a bright red color	
Reaction with iron-ammonium alum (for tannins)	4 drops 1% iron-ammonium alum solution	The color of the solutions became dark green-black	

*A – «Dolly» variety, B – «Rosie» variety:

1 – analyzed leaf extract of *O. basilicum*; 2 – the result of the reaction.

** A – variety «Dolly», B – variety «Rosie»:

1 – analyzed leaf extract of *O. basilicum*; 2 – the result of the reaction with the addition of HCl;

3 – the result of the reaction with the addition of NaOH.

triterpene saponins, especially expressed in the variety «Rosie», tannins. The obtained research results can be used in the development of a monograph on the raw materials of this representative or as methods of quality control of *O. basilicum* raw materials.

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This study did not receive external funding.

Conflict of interest

There is no conflict of interest in this article. No rewards received.

Consent to publication

All authors of the article are acquainted with the final version of the manuscript and have no

objections to its publication. The article does not use personal data and information about patients.

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of article.

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Анатомічне та фітохімічне дослідження листків *Ocimum basilicum* (L.)

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Актуальність: одним з важливих джерел фітонутрієнтів флавоноїдного спрямування є базилик (*Ocimum basilicum* L.). Вегетативні та генеративні органи якого мають ефірні олії, зокрема 1,8-цинеол, естрагол і евгенол, аскорбінову кислоту, багатий пігментний комплекс, а також поліфенольними сполуками з антиоксидантною дією. Базилик, свій антиоксидантний потенціал, реалізовує через антоціани у сортів з фіолетовим листям і флавоноїди – у сортів з зеленим. Рівень їх накопичення, як і інших біологічно активних речовин, залежить від сорту до якого належить рослина, ступеня і спектру освітленості, вологості ґрунту та мінерального живлення (Прісс О. П., і т.д., 2019; Joshi RK, 2014; Trichopoulou, etc., 2000). Саме тому нашу увагу привернули два найпопулярніші в Україні сорти базилюку з зеленим листям «Доллі» та з фіолетовий – «Розі» (Державний реєстр сортів рослин, придатних для поширення в

Україні). Наші дослідження було зосереджено на морфолого-анатомічних та фітохімічних дослідженнях листя *O. basilicum* вищезгаданої сировини. Для реалізації даної мети мікроскопію було виконано за допомогою методик ДФУ. Групи біологічно активних речовин визначалися за допомогою стандартних якісних реакцій та здійснювали процедуру із використанням витягів, або шляхом нанесення безпосередньо на зрізи. Встановлено, що лиски *O. basilicum* сортів «Доллі» та «Розі» мають ряд спільних та відмінних ознак. Для обох сортів *O. basilicum* характерна наявність дорзовентральних, амфістоматичних листків, діацитних продихових апаратів та бісеріатних залозок. Листки відрізняються за кольором, формою краю листкової пластинки, обрисами клітин верхньої епідерми, наявністю клітин-ідіобластів з антоціанами у сорту «Розі», значеннями продихових індексів. Продиховий індекс становить $27,5 \pm 2,3\%$ для сорту «Доллі» та $25,4 \pm 2,5\%$ для сорту «Розі». Виявлено, що листя *O. basilicum* сортів «Доллі» та «Розі» містить такі біологічно активні, речовини, як антоціани, флавоноїди, ефірна олія, тритерпенові сапоніни, особливо виражені в сорту «Розі», таніни та полісахариди. Отримані результати досліджень можуть бути використані при розробці монографії на сировину даного представника.

Ключові слова: антоціани, ефірна олія, *Ocimum basilicum*, фітонутрієнти, флавоноїди.



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