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CHARACTERISTICS OF THE ANATOMICAL AND MORPHOLOGICAL STRUCTURE OF *HIEROCHLOË ODORATA* (L.) P.BEAUV LEAVES AND THE IDENTIFICATION OF THEIR SECONDARY METABOLITES

Fornoliak Daryna Mykolaivna,
Bogomolets National Medical University
ORCID: 0009-0006-3139-1894

Makhynia Larysa Mykolaivna,
Candidate of Biological Sciences,
Associate Professor at the Department of Pharmacognosy and Botany
Bogomolets National Medical University
ORCID: 0000-0002-8095-4255

Karpiuk Uliana Volodymyrivna,
Doctor of Pharmaceutical Sciences,
Professor at the Department of Pharmacognosy and Botany
Bogomolets National Medical University
ORCID: 0000-0002-8316-4910

Minarchenko Valentyna Mykolaivna,
Doctor of Biological Sciences,
Professor at the Department of Pharmacognosy and Botany
Bogomolets National Medical University
ORCID: 0000-0002-5049-7620

Hierochloë odorata (L.) P. Beauv – a plant of the genus Hierochloe, belonging to the family Poaceae, whose roots and above-ground parts have a sweet scent. This rhizomatous perennial plant is native to the cool regions of North America and Europe. It is found in wet meadows, marshes, shaded banks of streams and in mountain canyons, as well as along rivers and lakes. In folk medicine, an infusion of sweet grass is used to stimulate the appetite and improve digestion in chronic gastrointestinal disorders such as gastritis, peptic ulcer disease and colitis, to increase lactation in nursing mothers, and in the treatment of pulmonary tuberculosis. It possesses antiseptic, bactericidal and anti-inflammatory properties; however, its pharmacognostic potential remains underutilized. The study of the macro- and microscopic structure of the leaves, as well as their phytochemical composition, allows for the development of scientifically sound approaches to the standardization and quality control of medicinal plant raw materials. This article analyzes current literature data on the chemical composition, pharmacological properties, and applications of H. odorata. The main microscopic and macroscopic characteristics of the leaves were identified, and groups of biologically active compounds were determined using chemical reactions, which are important for species identification. The morphological and anatomical diagnostic features of H. odorata leaves were investigated, and it was established that the leaves possess paracytic-type stomatal apparatus with gantheloid guard cells, silicate cells, beak-like trichomes along the margin of the leaf blade, and prismatic crystals along the leaf veins. Beneath the upper epidermis are five large motor cells, which are the primary regulators of the leaf's water balance and cause it to curl under adverse conditions. It was found that the leaves of H. odorata contain biologically active substances such as coumarins, tannins, flavonoids, saponins, and polysaccharides. The obtained results can be used to develop methods for quality control of plant raw materials, as well as to compile a monograph on the raw materials of this species.

Key words: *essential oil, grasses, coumarins, saponins, light microscopy, trichomes.*

Дарина Форноляк, Лариса Махиня, Уляна Карпюк, Валентина Мінарченко. Анатомо-морфологічна характеристика листя *Hierochloë odorata* (L.) P. Beauv та ідентифікація її вторинних метаболітів

Hierochloë odorata (L.) P. Beauv – рослина роду Hierochloe, що належить до родини Poaceae, коріння та надземні частини якої мають солодкий аромат. Ця кореневищна багаторічна рослина походить із прохолодних регіонів Північної Америки та Європи. Зустрічається на вологих луках, болотах, затінених берегах струмків і в гірських каньйонах, а також уздовж річок і озер. У народній медицині настій солодкої трави застосовують для стимулювання апетиту та поліпшення травлення при хронічних захворюваннях шлунково-кишкового тракту, таких як гастрит, виразкова хвороба та коліт, для підвищення лактації у годуючих матерів, а також у лікуванні туберкульозу легенів. Вона має антисептичні, бактерицидні та протизапальні властивості; проте її фармаког-

ностичний потенціал залишається недостатньо використаним. Дослідження макро- та мікроскопічної будови листя, а також їхнього фітохімічного складу дає змогу розробити науково обґрунтовані підходи до стандартизації та контролю якості сировини лікарських рослин. У цій статті проаналізовано сучасні літературні дані щодо хімічного складу, фармакологічних властивостей та застосування H. odorata. Визначено основні мікроскопічні та макроскопічні характеристики листя, а також за допомогою хімічних реакцій встановлено групи біологічно активних сполук, що мають важливе значення для ідентифікації виду. Досліджено морфологічні та анатомічні діагностичні ознаки листя H. odorata, встановлено, що листя має продиховий апарат парацитного типу з гантелоїдними охоронними клітинами, силікатними клітинами, дзьобоподібними трихомами вздовж краю листової пластинки та призматичними кристалами вздовж жилок листя. Під верхнім епідермісом розташовано п'ять великих моторних клітин, які є основними регуляторами водного балансу листка і зумовлюють його скручування за несприятливих умов. Встановлено, що листя H. odorata містять біологічно активні речовини, такі як кумарини, дубильні речовини, флавоноїди, сапоніни та полісахариди. Отримані результати можуть бути використані для розробки методів контролю якості рослинної сировини.

Ключові слова: *ефірна олія, трави, кумарини, сапоніни, світлова мікроскопія, трихоми.*

Introduction. *H. odorata*, or sweet grass, is well known for its characteristic sweet aroma, which is due to its high content of coumarin compounds. According to the Global Biodiversity Information Facility, the plant is distributed primarily in the forest zone of Ukraine [1]. It is most often found in pine and mixed forests, on sandy soils, in forest plantations, clearings, and glades. Harvesting is possible in regions such as Volyn, Rivne, Chernihiv, Zhytomyr, and Kyiv [1, 2]. Due to the presence of various groups of biologically active substances, *H. odorata* is used to increase lactation, as a wound-healing and antiseptic agent, for lung inflammation, as well as to stimulate appetite and improve digestion [2]. Sweet grass (*H. odorata*) is a useful plant for the restoration of wetlands and coastal zones and therefore has potential for conservation use in combating soil erosion [3].

There is insufficient information in the literature regarding the pharmacological effects and chemical composition of the bioactive compounds found in sweet grass. However, preliminary screening results of sweet grass have shown that extracts of this plant slow down lipid oxidation [4]. The aim of the study by Lithuanian scientists was to identify the compounds responsible for the radical-scavenging ability of extracts from the aerial parts of sweet grass (*H. odorata*). As a result of multi-stage fractionation of the extract, two new natural compounds with antioxidant activity were isolated. Their structures were determined using nuclear magnetic resonance and mass spectrometry and identified as 5,8-dihydroxybenzopyranone and 5-hydroxy-8-O-β-D-glucopyranosyl-benzopyranone [4].

The activity of the isolated compounds was tested using DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) free radical scavenging assays. For comparison, known natural antioxidants – rosmarinic acid and trolox – were used. The fraction obtained using tert-butyl methyl ether demonstrated the highest radical scavenging capacity. Its activity was comparable to that of rosmarinic acid, which showed a scavenging rate of 90%. Other fractions isolated from the methanol-water extract were characterized by significantly lower activity [4].

Polish scientists investigated the effect of sweet grass on the physicochemical properties of liver cell membranes in rats intoxicated with ethanol. It was found that changes in liver cell membranes under the influence of ethanol are largely associated with reactive oxygen species, which cause oxidative stress. The negative effects of free radicals can be reduced by administering antioxidants. Administration of sweet grass to ethanol-intoxicated rats significantly protects lipids and proteins from oxidative modifications. Thus, the use of *H. odorata* proved effective in minimizing harmful membrane changes caused by ethanol intoxication [5].

Japanese scientists investigated the volatile components of ethanol extracts from the root and aerial parts of *H. odorata* using gas chromatography–mass spectrometry and high-performance liquid chromatography (HPLC). A total of 169 volatile compounds were identified. Coumarins predominated among the volatile components, accounting for 10.3% in the root and 24.9% in the aerial parts of the plant. The content of other compounds was also determined, including isovaleraldehyde – 38.47%, propyl alcohol – 0.51%, isobutyl alcohol – 1.57%, 3-methylbutanol – 3.28%, furfural – 4.97%, and dodecanoic acid – 1.00% in the ethanol extract of the roots; ethylhexadecanoate – 15.70%, furanone – 4.83%, and benzaldehyde – 0.59% in the volatile fraction of the ethanol extract of the aerial parts. The results of quantitative analysis using HPLC showed that the ethanol extract of the roots contains 3.57% coumarins, and the aerial parts contain 3.72%, indicating the plant's value as a source of volatile components [6].

Researchers from Lithuania studied the antioxidant activity of sweet grass and sage (*Salvia officinalis* L.) extracts in emulsions made from lard and rapeseed oil. Soy lecithin was used as an emulsifier, and copper acetate was added to catalyze the oxidation process. The results showed that the antioxidant potential of the extracts was approximately the same in both substances. The use of sweet grass extracts,

sage extracts, or a combination of both significantly increased the resistance of fats to oxidation [7].

According to available literature data, *H. odorata* is characterized by a rich and diverse chemical composition. The following compounds have been identified in this plant: coumarins: coumarin, umbelliferone, β-glucoside of coumarin; hydroxycinnamic acids: *p*-coumaric, *o*-coumaric, ferulic, melilotic, cinnamic, and shikimic acids; phytol, 2-methoxy-4-vinylphenol, certain alkaloids (dynaxin is found in the root and stem of the plant), ascorbic acid, bitter principles, glycosides, fructans, essential oil (the flowers contain hexahydrofarnesol), lauric and palmitic acids (also in the flowers), as well as L-phenylalanine [4, 5, 6, 8, 9]. At the same time, most studies focus on the above-ground parts of the plant in general, while the leaves – a promising part of the plant – remain understudied.

Thus, *H. odorata* is a valuable aromatic and medicinal plant with a wide range of pharmacological properties. Its antiseptic, anti-inflammatory, antioxidant, and hepatoprotective effects have been confirmed by both traditional use and modern experimental studies. Since *H. odorata* is not included in the State Pharmacopoeia of Ukraine, the study of its morphological and anatomical diagnostic features and the phytochemical analysis of its leaves can serve as a basis for developing quality control methods for the plant material of this species.

Aim and objectives. To conduct a phytochemical analysis and investigate the main macroscopic and microscopic characteristics of the leaves of *Hierochloë odorata* (L.) P. Beauv growing in the Kyiv region.

Research methods. Macroscopic and microscopic studies were conducted in accordance with generally accepted classical methods of pharmacognostic analysis described in the literature [10–13].

The plants were collected in the Kyiv region at 50°27'41.6“N 30°02'27.8” E on May 27, 2025, during the flowering phase. The material was collected from 10 survey plots within a single population associated with a mixed herb-grass community covering a total area of approximately 1.5 hectares. The samples were identified at the National Herbarium of Ukraine at the M. G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine: № 129813, KW (Poltava region, Poltava district, between the villages of Umantsivka and Buhaivka); № 114739, KW (Poltava region, Poltava district, village of Kopily); № 041726 (Luhansk region, “Striletsky Steppe” Nature Reserve); № 05668 (outside Kyiv).

Both fresh and dried raw material samples were used to prepare temporary micro-preparations, which were examined in aqueous and glycerin media. The raw material was dried using the air-shade method and stored under standard conditions in a dry, dark, well-ventilated room. This approach allowed for a comparative assessment of the anatomical and morphological characteristics of freshly harvested and dried medicinal plant material.

Microscopic examinations were performed using a ULAB light microscope equipped with ×40, ×100, ×400, and ×1000 magnification systems (the article uses the two most informative images at ×400 and ×1000 magnification), as well as a Canon EOS 550 digital camera, which allowed for detailed visualization of diagnostically significant structures. To increase the reliability and statistical significance of the results, each study was conducted in ten replicates from ten different plants.

During microscopic examinations, histochemical reactions were performed for various groups of biologically active substances: with methylene blue solution (1:5000) for pectin substances; with diazotized sulfanilic acid solution for coumarins; and iron (III) chloride solution for phenolic compounds. To extract biologically active substances for their subsequent identification via chemical reactions, ethanol of various concentrations and purified water were used as solvents. Well-known color and precipitation reactions were performed for the preliminary identification of various groups of metabolites [12, 13].

Research results. The leaves of *H. odorata* are simple, glabrous, alternate, and clasping; they are broadly linear in shape on vegetative shoots and linear-lanceolate on generative stems. The length of the leaf blade ranges from 15 to 35 cm, with an average of 28,3±3,1 cm, while the width ranges from 5 to 7 mm, with an average of 5,8 ± 0,8 mm.

The leaf blade is entire, green with a pointed apex and a sheathing base. The sheath, as the lower part of the leaf, has a tubular shape and tightly encloses the stem, protecting the tender growth zones, particularly the intercalary meristem. The leaf has clearly defined parallel venation, which is clearly visible from the underside and features a prominent central vein that rises above the surface of the leaf blade and is one of the species' characteristic diagnostic features. This venation system ensures the efficient transport of water and minerals along the entire length of the organ. The species-specific combination of morphometric parameters and leaf shape is of great importance for the macroscopic identification of the plant material under study.

For a detailed analysis of medicinal plant material, attention should also be paid to the microscopic characteristics of *H. odorata* leaves, which will subsequently make it possible to distinguish this species from other similar ones. The leaf of *H. odorata* is isolateral and amphistomatic (Fig. 1. A–B).

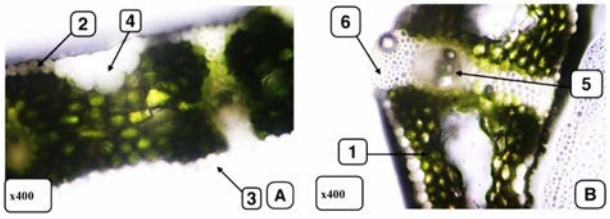


Fig. 1. Cross-section of an *H. odorata* leaf: A, B – ×400: 1 – mesophyll, 2 – upper epidermis, 3 – lower epidermis, 4 – large motor cells, 5 – closed collateral bundle, 6 – sclerenchyma

In a transverse section of the leaf blade, it is clearly visible that between the single-layered upper (Fig. 1. A 2) and lower epidermis (Fig. 1. A 3), there are eight to twelve evenly spaced layers of mesophyll (Fig. 1. B 1). The mesophyll cells are living, parenchymatous, oval or rectangular, and densely filled with chloroplasts. Five large motor cells are located beneath the upper epidermis (Fig. 1. A 4). In a cross-section, they appear fan-shaped, with the central cell being the largest and the lateral ones gradually decreasing in size.

Due to their location and large size, motor cells (Fig. 1. A 4) are easily identifiable in a cross-section, which is an important morphological feature in the study of water and mineral transport in vegetative organs, as well as for the diagnosis of raw materials. Due to their ability to rapidly change volume, they are the primary regulator of the leaf's water regime, ensuring its rolling up under adverse conditions. In a cross-section through a leaf vein, we observe the presence of closed collateral vascular-fibrous bundles (Fig. 1. B 5), which are held in place at the top and bottom by multilayered sclerenchyma (Fig. 1. B 6).

Upon examining the upper and lower epidermis, it should be noted that they consist of rows of cells of various shapes. The majority of epidermal cells are large, rhomboid, elongated cells (Fig. 2. A 1), interspersed with rows of rectangular, thin, rather long cells that cover the veins. Along the veins on the underside of the leaf are prismatic crystals of calcium oxalate (Fig. 2. C 6). On both sides of the cells covering the veins, there are two rows of large, rectangular cells with rounded edges (Fig. 2. A 7, C 7, D 7). There are two rows of rhombic cells with paracytic stomatal apparatus (Fig. 2. A–D 2), which extend in ordered rows along the entire length of the leaf blade. The stomata have a distinctive feature: their guard cells are dumbbell-shaped. At the base of individual lateral cells are round silicate cells (Fig. 2. B 3, D 3). They provide mechanical strength to the leaf and protection against being eaten by animals, which is important for maintaining the structure of the vegetative organs during the transport of water and minerals.

The alternation of rhombic rows of cells with rows of cells containing stomata (Fig. 2. A 5, C 5) and bands of thin cells covering the veins creates a distinct, ordered, systematic structure typical of members of the class Monocotyledons.

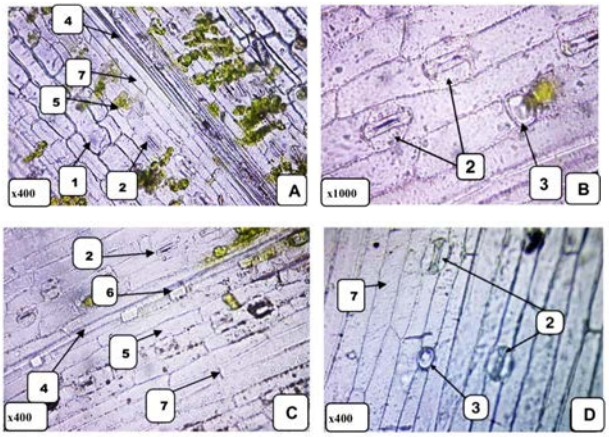


Fig. 2. Epidermis of the leaf surface of *H. odorata*: A, B – adaxial epidermis; C, D – abaxial epidermis. A, C, D – x400, B – x1000: 1 – large rhombic cells, 2 – paracytic stomatal apparatus, 3 – silicate cells, 4 – very narrow elongated cells covering the veins, 5 – rectangular cells containing stomata at their base, 6 – prismatic crystals, 7 – rectangular cells with rounded edges

Along the edge of the leaf blade are retort-shaped, or beak-like, unicellular hairs with a pointed tip (Fig. 3. A, B).

The trichomes point their pointed tips toward the apex of the leaf blade, which requires some caution when harvesting the raw material. In addition to the leaf margin, trichomes are located along the main large veins.

To identify biologically active substances, histochemical reactions were performed on fresh raw material. The presence of phenolic compounds in the raw material was detected using iron (III) chloride reagent, which was applied to the epidermis of the *H. odorata* leaf. The cells along the vein turned blue-violet, indicating a localized accumulation of phenolic compounds (Fig. 4. A 1).

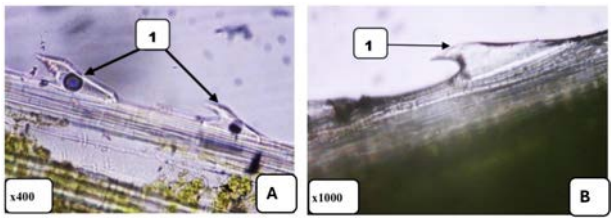


Fig 3. Detail of the margin of a *H. odorata* leaf blade: A – ×400, B – ×1000: 1 – unicellular beak-like trichomes

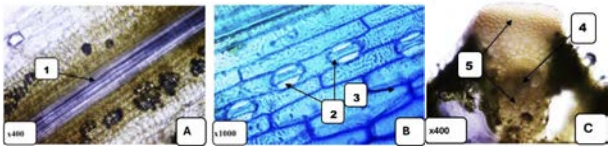
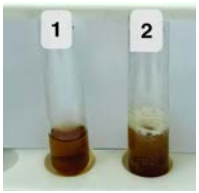
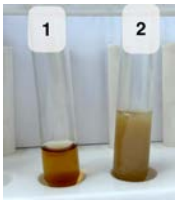
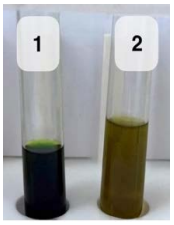
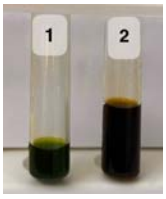
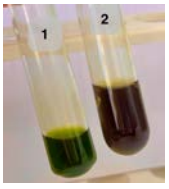
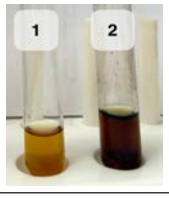


Fig. 4. Results of the histochemical reaction in *H. odorata* leaves: A – with iron (III) chloride, B – using methylene blue solution (1:5000), C – with diazotized sulfanilic acid solution: 1 – cells containing phenolic compounds, 2 – paracytic stomatal apparatus, 3 – the result of pectin substances coloration, 4 – closed collateral bundle, 5 – sclerenchyma stained with diazotized sulfanilic acid solution

Table 1

Results of qualitative reactions

Reaction name	Reagent	Observations	Reaction result
Foaming reaction	Vigorous shaking for one minute	When the aqueous extract was shaken, a stable foam formed that did not dissipate for 1 min, indicating the presence of saponins (2)	
Determination of the chemical nature of saponins	1–5 ml of 0.1 M sodium hydroxide solution 2 – 5 ml 0.1 M hydrochloric acid solution	In the test tube containing an acidic medium (2), the foam column is taller than in the test tube containing an alkaline medium (1), indicating a predominance of triterpene saponins	
Reaction with a 10% solution of lead acetate	4 drops of 10% lead acetate solution	The formation of a precipitate (2) indicates the presence of saponins, flavonoids, and tannins	
Identification of polysaccharides	5 ml of water extract, 25 ml of ethanol	A precipitate was observed, indicating the presence of polysaccharides	
Lactone test	2–5 drops of a 10% alcohol solution of potassium hydroxide; 3–5 drops of a 20% sulfuric acid solution	The initial color change and subsequent formation of a precipitate (2) indicate the presence of coumarins	
Diazotized sulfanilic acid solution reaction	2–5 drops of a 10% potassium hydroxide solution; 3–5 drops of diazotized sulfanilic acid	The solution turned brownish-red (2), indicating the presence of coumarins	
Cyanidin reaction	3 drops of hydrochloric acid and a few grains of magnesium shavings	The change in the extract's color to a deep brownish-red shade (2) indicates the presence of flavonoids in the test material.	
Reaction with iron (III) chloride	5 drops of a 3% iron (III) chloride solution	The solution took on a greenish-brown shade (2), indicating the presence of condensed tannins.	

Note:

1 – control; 2 – reaction result.

To detect pectin substances in the cells of *H. odorata* leaves, a reaction was performed with methylene blue solution. Cell walls containing pectin substances (Fig. 4. B 3) turned a deep blue color, clearly delineating the shape and thickness of the cells.

Since coumarins are among the main biologically active compounds in the *H. odorata* herb, a test with diazotized sulfanilic acid solution was conducted to determine the location of these compounds. The change in the color of the walls to light brown indicates the localization of coumarins in the sclerenchyma surrounding the vascular fiber bundles (Fig. 4. C 5).

The results of the chemical reactions are presented in Table 1.

Conclusions

The morphological and anatomical diagnostic features of *H. odorata* leaves were studied, and it was found that they possess paracytic-type stomatal apparatus with dumbbell-shaped guard cells, silicate cells, beak-like trichomes along the leaf margin, and prismatic crystals along the leaf veins. The main biologically active compounds in *H. odorata* leaves were identified by chemical reactions: coumarins, tannins, flavonoids, saponins, and polysaccharides.

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