

## COMPARATIVE MORPHOLOGICAL AND ANATOMICAL STUDY OF LEAVES AND STEMS OF *IRIS PSEUDACORUS* AND *IRIS SIBIRICA*

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### ABSTRACT

**Objective:** A comparative morphological and anatomical study of leaves and stems of two species of *Iris* from Limniris section of Iridaceae family – *Iris pseudacorus* L. and *Iris sibirica* L. has been carried out.

**Methods:** The morphological and anatomical analysis of medicinal plants.

**Result:** Their distinguishing and common features have been determined.

**Conclusion:** Study results will be used in standardization of raw materials and development of an article for the State Pharmacopoeia of Ukraine, as well as for determining systematic position of the species.

**Keywords:** *Iris pseudacorus* L., *Iris sibirica* L., Anatomical studies, Morphological studies, Leaves, Flower stalks.

### INTRODUCTION

Nowadays, there is an increasing interest to creation of vegetable preparations, which is conditioned by their mild action, less habit-forming, and practical absence of side effects. Therefore, issues of standardization and quality control of medicinal plants remain topical. At present, in Ukraine the quality of raw material is controlled by the State Pharmacopoeia of Ukraine (SPhU) [1-2]. Based on the concept of harmonization of national legislation base on quality drug control with the European Pharmacopoeia (EurPh), corresponding articles of EurPh are used as the base document for the development of SPhU articles. Articles of the United States Pharmacopoeia are slightly different. Article "Vegetable drugs" lists a series of pharmacognosy methods; there are articles on specific drugs and raw materials. However, all the pharmacopoeias in addition to quality evaluation criteria of medicinal plant materials,

pay attention to authenticity of raw materials – establishment of their macro- and microscopic features.

This work is devoted to the study of diagnostic signs of the *Iris pseudacorus* and *Iris sibirica* for further development of articles for SPhU and use in standardization of herbal drugs, as well as for increase of systematic position issue of *Iris* family species [3].

*Iris pseudacorus* L. (Fig. 1) and *Iris sibirica* L. (Fig. 2) are representatives of the *Iris* genus (*Iris* L.), Limniris subgenus (Limniris), *Iris* family (Iridaceae L.). The genus is represented by 200 species [4]. According to the Mosyakin and Fedorchuk (1999), 16 *Iris* species grow in Ukraine, two of which are cultivated and one species (*I. musulmanica* Fomin) is an ergasiophyte. The "Red Book of Ukraine" includes *I. pineticola* Klok., *I. pseudacorus* Schur and *I. pontica* Zapal.[5].



Fig. 1: *Iris pseudacorus* L., Iridaceae



Fig. 2: *Iris sibirica* L., Iridaceae

Irises are widely used in folk medicine as anti-inflammatory, diuretic, analgesic, and wound-healing agents [6-8]. Rhizomes are used for treating inflammation of the upper respiratory tract, gastrointestinal

tract, in gynecology and oncology practice [9-12]. In order to expand raw material base, it was decided to investigate not only the underground organs of irises, but also the aboveground ones: leaves and flower stalks.

To identify common and distinctive diagnostic features of the two species belonging to one section, comparative morphological and anatomical studies of leaves and flower stalks of *Iris pseudacorus* and *Iris sibirica* was conducted.

#### MATERIAL AND METHODS

Objects of the study were leaves and flower stalks of *Iris pseudacorus*, which had been collected in spring of 2011 in the village of Borschova, Kharkiv region, and *Iris sibirica* collected in spring of 2012 in Botanical Garden's territory of V.M. Karazin Kharkiv National University. For macro- and microscopic studies, fresh and fixed in a mixture of alcohol-glycerin-water (1:1:1) plant material was used. The structure of leaves and flower stalks were studied on the cross and longitudinal sections, whereas epidermis – from the surface [13-19]. The following devices were used in work: magnifying glass, binocular microscope MBS-9, MBI-6 LOMO with 80, 120, 300 and 600-time magnification capacity. Diagnostic features were fixed with digital camera OLYMPUS FE-140. Photos were processed in program "Adobe Photoshop 9.0 SS2"

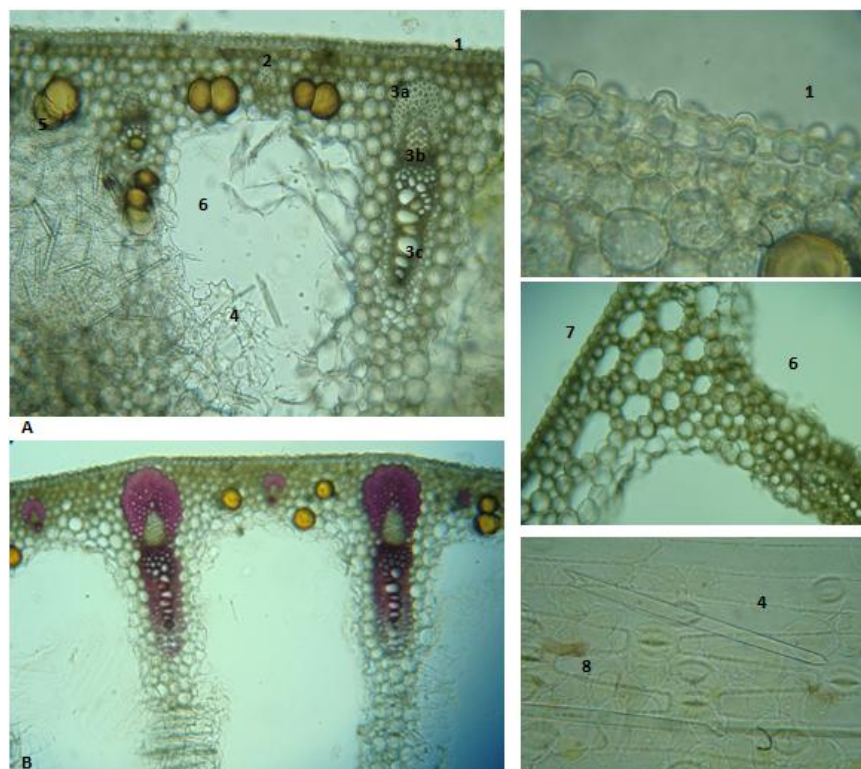
#### RESULTS AND DISCUSSION

Comparative study of leaves and flower stalks of both iris species revealed common and distinctive morphological features listed in the table below.

As it is seen in the table, the species are different as for leaf size, their shape, length of flower stalks and their fullness (see the Table). The length of *Iris pseudacorus* leaves can coincide with that of peduncle, but in *Iris sibirica* the leaves are 2-3 times smaller than the stem. Leaves of both species are long, vaginal with parallel venation. In *Iris pseudacorus*, the leaf blade is up to 150 cm long, it is wider and has a pronounced central vein, other veins protrude slightly above the surface; lower leaves have a well-developed vagina. In *Iris sibirica*, on the contrary, leaves are linear, up to 70 cm long, veins are feebly shown; lower leaves are also vaginal, but stem ones are clasping. The flower stalk of *Iris pseudacorus* is long, thick, and dense, whereas in *Iris sibirica* it is hollow and thin. In both species the top of the shoot is branching.

**Table 1: Morphological characteristics of leaves and flower stalks of *Iris pseudacorus* L. and *Iris sibirica* L**

Characteristic	Species	
	<i>Iris pseudacorus</i> L.	<i>Iris sibirica</i> L.
Size of leaf lamina	100-150 cm long, 2-3 cm wide	15-70 cm long, 0.5-1 cm wide
Form of leaf	widely ensiform, pointed	linear, pointed
Venation	parallel, with a long central vein, second-order veins – thin, numerous, significantly protruding	parallel, veins – thin, numerous, poorly protruding
Edge	thin, almost filmy, sometimes with thin fibers that exfoliate	thin, filmy
Color	yellow-green at the top, brown-green at the bottom near the base	light green at the top, light yellow at the base of the leaf
Taste	bitter	bitter
Odor	specific	specific
Flower stalk	60-150 cm tall, filled	30-80 cm tall, hollow



**Fig. 3: Common mikrodiagnostic evidence of leaves of *Iris pseudacorus*. The upper epidermis (A, without coloring): 1 – papillary growths, 2 – spongy mesophyll, 3 – fibrovascular collateral bundles (a – sclerenchyma, b – phloem, c – xylem), 4 – styloid, 5 – cells-idioblast, 6 – air cavity, 7 – aerenhima; 8 – tetracytic type of stomata apparatus; B – upper leaf of epidermis of *Iris pseudacorus* colored phloroglucinol (sclerenchyma is colored purple, cell idioblast in yellow).**

### Anatomical research

During anatomical studies of *Iris pseudacorus* and *Iris sibirica* their common features have been revealed. Leaf blade is of isolateral type, they covered with a layer cutin on both sides. Cells of upper and lower epidermis are prosenchimatous, with slightly thickened cell walls and distinct evident straight pores. In the epidermis of leaves there are sparse papillary growths with rounded tips (Fig. 3a). Stomata are large, oval and ordered. The type of stoma apparatus is tetracytic (Fig. 3). Mesophyll is homogeneous; cells are round or oval, thick-walled. The cells of chlorenchyma are situated more closely near of upper epidermis, but the lower ones – loosely. There is chlorenchyma along the leaf edge. In subepidermal layers of the mesophyll near the vascular bundles, often there are elongated cells-idioblasts with yellow contents (Fig. 3c). In leaf mesophyll there are raphyds or styloids (Fig. 3). The system of fibrovascular collateral bundles is well developed. The bundles are elongated (Fig. 3). Above phloem, there is a significantly evident area of sclerenchyma; phloem is represented by sieve tubes with well seen companion cells. Xylem vessels have wide lumen; they are situated one above the other forming a chain.

Distinguishing features of leaf anatomical structure in *Iris pseudacorus* include presence of well developed aerenchyma, formation of large cavities filled with air between conductive bundles, which is typical for hygrophytes. In *Iris sibirica*, air cavities between conductive bundles are small, and cells-idioblasts occur really seldom.

The type of anatomical structure of flower shoots in both species is primary, fascicular (Fig. 4, 5). Epidermis is single layered, covered by cuticle; cells are large, shells are thickened, stomas are dipped into epidermis. Primary cortex is weakly expressed, cells are small, ordered. Percyclic sclerenchyma forms a whole ring, which separates primary cortex from central cylinder. Closed collateral conductive bundles are located disorderly in cortex part and central axial cylinder (Fig. 4, 5). Conductive bundles are small, rounded; sclerenchyma is located above phloem in small regions. Xylem vessels are few, located in half-moon form. The number and density of conductive bundle location increases closer to periphery. Parenchyma cells of flower stalk are rounded, large, filled with granule-like content. Among cells of cortex parenchyma and axial cylinder of flower stalks, in leaf mesophyll, cells-idioblasts with yellow and red secretion are often found. Histochemical reactions prove that these cells accumulate substances of phlobaphene type – oxidation products of tanning agents [4].

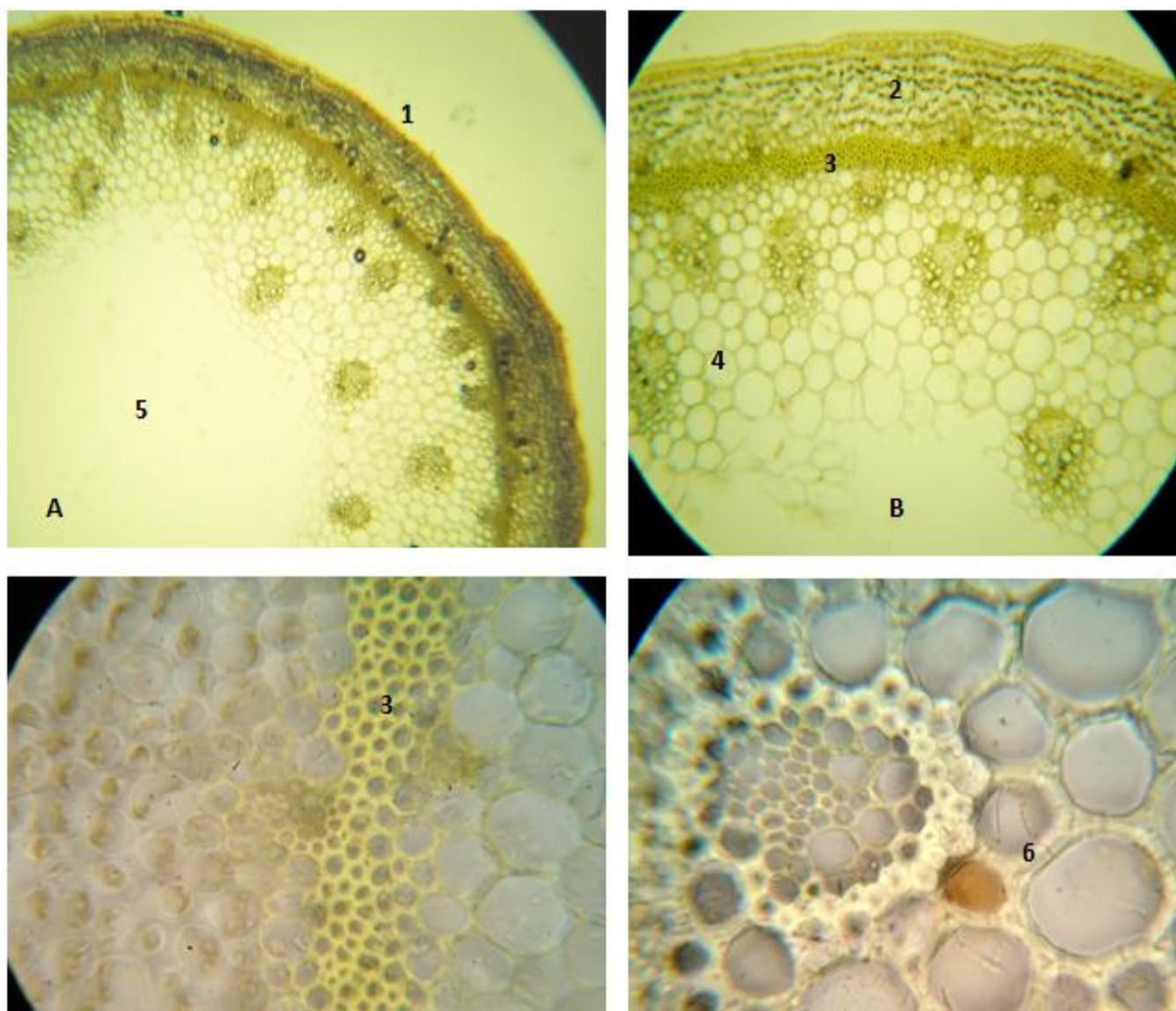
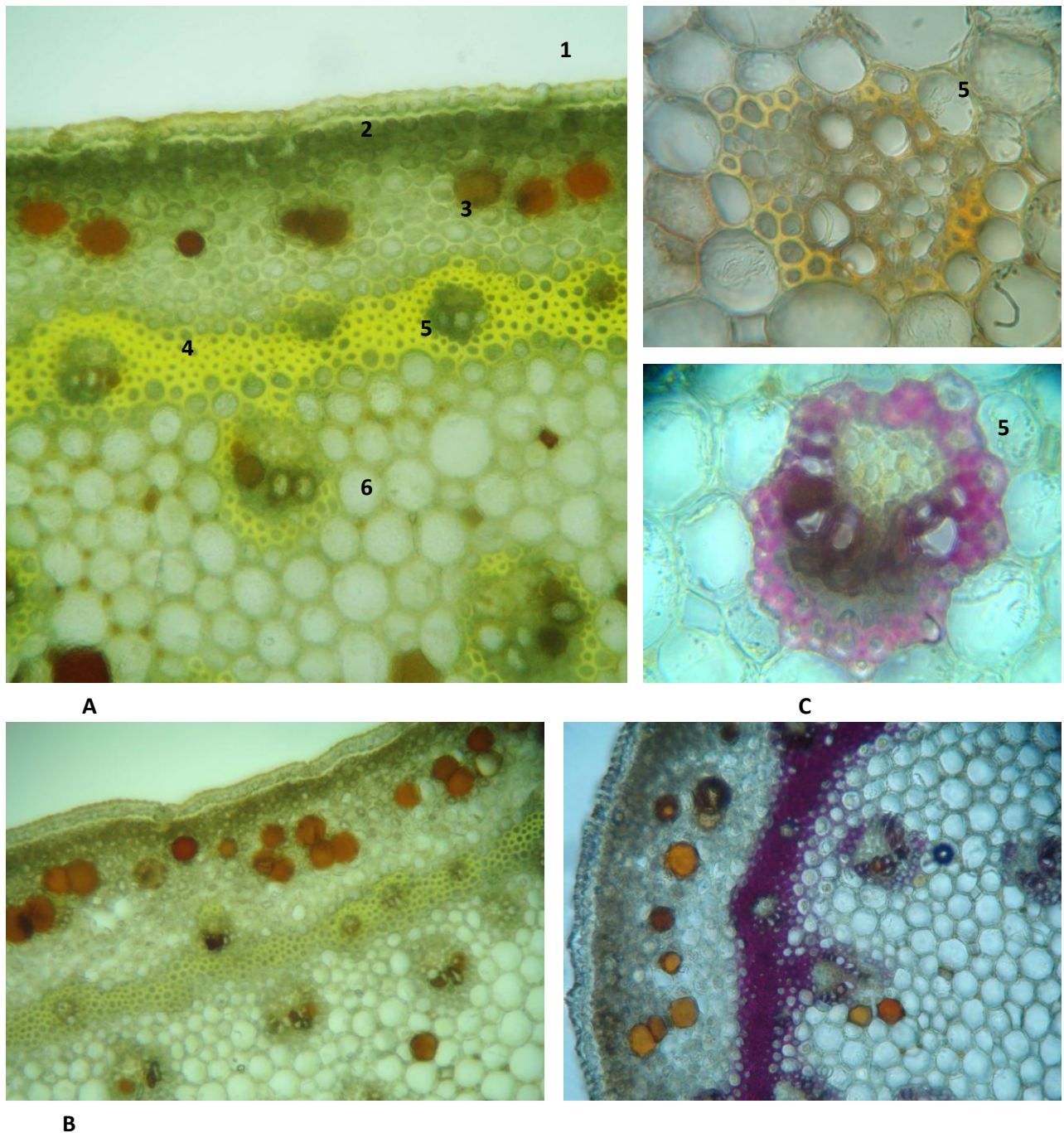


Fig. 4: Mikrodiagnostic evidence of flowering shoots *Iris sibirica* (cross-section) A (without coloring): 1 – stomas into epidermis, 2 – chlorenchyma, 3 – perecyclic sclerenchyma, 4 – closed vascular bundles 5 – cavity, 6 – rare cells-idioblast. B – colored aniline sulfate.



**Fig. 5: Mikrodiagnostic evidence of flowering shoots of *Iris pseudacorus* (cross-section), A (colored aniline sulfate): 1 – epidermis with stomata and papillae outgrowths, 2 – chlorenchyma, 3 – cells-idioblast, 4 – pericyclic sclerenchyma, 5 – closed vascular bundles, 6 – cell parenchyma. B – colored alkali, C – colored phloroglucinol.**

The distinctive features reveal include the one that the core of flower stalk in *Iris pseudacorus* is filled, represented by parenchyma cells (Fig. 5), and the one in *Iris sibirica* is hollow with distinct edges (Fig. 4a). In *Iris sibirica*, conductive bundles are rounded; xylem vessels surround well expressed phloem in a V-form, whereas in *Iris pseudacorus*, sclerenchyma is located in a ring around the conductive bundle; in *Iris sibirica* sclerenchyma is more evident than in *Iris pseudacorus*.

#### CONCLUSIONS

1. Comparative morphological and anatomical studies of leaves and flower stalk of *Iris pseudacorus* and *Iris sibirica* have been carried out.

2. There are common diagnostic features in both species: cutin layer on leaf epidermis; immersed stomata, tetracitic type of stomata apparatus; papillary growths on epidermis; presence of raphids; single styloids in leaf mesophyll; cells-idioblasts with secretion; close collateral conductive bundles.
3. *Iris pseudacorus* differs from *Iris sibirica* due to a number of morphological and anatomical features: size of leaf blade, degree venation development, fullness and length of a flower stalk; presence of aerenchyma in leaf mesophyll, disposition of sclerenchyma around conductive bundle.
4. Morphological and anatomical characteristics of leaves and flower stalks of *Iris sibirica* and *Iris pseudacorus* will be used in

standardization of raw materials, development of articles for SPhU and determination of species systematic position.

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