

Morphological Investigations on the Diagnostic Features of Six *Hypericum* Species of the Ukrainian Flora

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Ukrayna Florasının Altı Hypericum Türüne ait Tansal Özellikleri Üzerine Morfolojik Araştırmalar

SUMMARY

The results of comparative analysis of the main diagnostic features of the medicinal raw material of six species of the genus *Hypericum* of Ukraine are presented. The most important diagnostic features of *H. alpigenum* Kit, *H. elegans* Steph. ex Willd., *H. hirsutum* L., *H. maculatum* Crantz, *H. montanum* L., and *H. perforatum* L. are the localization, form, and color of secretory structures. Characteristics of the basic morphological features of leaves, sepals, petals, and stems of the studied species are provided. It is emphasized that a comprehensive analysis of the species-specific peculiarities of the main raw organs of species of the genus *Hypericum* allows us to determine their species affiliation clearly. The use of these features makes it impossible to intentionally falsify or incorrectly identify the raw material of a particular species of *St. John's worth* during merchandising analysis.

Key Words: diagnostic features, *Hypericum*, medicinal raw materials, leaves, sepals, petals, stems, secretory structures, Ukraine

ÖZ

Ukrayna'daki *Hypericum* cinsine ait altı türün ilaç hammaddesi olarak kullanılan kısımlarının temel teşhis özelliklerinin karşılaştırmalı analiz sonuçları sunulmaktadır. *H. alpigenum* Kit, *H. elegans* Steph. ex Willd., *H. hirsutum* L., *H. maculatum* Crantz, *H. montanum* L. ve *H. perforatum* L türlerinin en önemli teşhis özellikleri salgı yapılarının yerleşimi, şekli ve rengidir. İncelenen türlerin yaprak, sepal, petal ve gövdelerinin temel morfolojik özellikleri verilmiştir. *Hypericum* droglarının türe özgü özelliklerinin kapsamlı bir analizi, türler arasındaki ilişkiyi açıkça belirlemeye imkan vermektedir. Ticari analiz sırasında bu özelliklerin kullanılması, belirli bir kantaron droğunun kasıtlı olarak değiştirilmesini veya yanlış teşhisini önleyebilecektir.

Anahtar Kelimeler: Teşhis özellikleri, *Hypericum*, ilaç hammaddesi, yaprak, sepal, petal, gövde, salgı yapıları, Ukrayna

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INTRODUCTION

Species of the genus *Hypericum* are extensively studied in the world due to their great phytochemical potential, including petrodiantrons (hypericin, pseudohypericin and their derivatives), phloroglucinols (hyperforin and its derivatives), and flavonoids, which contribute to a wide range of therapeutic effects as an antidepressant, anticancer or antiviral, etc. (Cirak et al., 2016; Gitea et al., 2011; Lotocka, Osinska, 2010; Maggi et al., 2004; Nurk, Crockett, 2011). The concentration of these compounds in plant tissues of *Hypericum* is species-specific and differs between parts of plants (Minarchenko et al., 2020; Onelli et al., 2002; Perrone et al., 2013; Vieira da Silva et al., 2013; Zobayed et al., 2006; Kuchariková et al., 2016).

Special attention is paid to the study of diagnostic morphological and anatomical features of raw parts of species of the genus *Hypericum*, including secretory structures in which various biologically active substances are localized (Nurk, Blattner, 2010; Sagratini et al., 2008; Perrone et al., 2013; Lotocka, Osinska, 2010; Košuth et al., 2011; Zobayed et al., 2006; Soelberg et al., 2007; Piovan et al., 2004).

H. perforatum, which is included in the pharmacopeias of many countries, is the most studied and widely used. In Ukraine, according to the State Pharmacopeia for raw materials «Hyperici herba,» the use of raw materials of two types of *Hypericum* is officially allowed: *H. perforatum* and *H. maculatum* (the synonym is *H. quadrangulum* L.) (SPhU 1, 2). According to recent nomenclature data, *H. quadrangulum* is synonymous with *H. tetrapterum* Fries (Hassler, 2020), and in this context, it is considered to be synonymous with *H. maculatum*, however these are two distinct species that have apparent morphological differences.

According to the latest nomenclature (Mosyakin and Fedoronchuk, 1999), 12 species of the genus *Hypericum* are growing in Ukraine. *H. elegans*, *H. hirsutum*, *H. montanum*, and *H. alpigenum* are the most common and occur as admixtures to the raw materials of the two species of *Hypericum* mentioned above.

In the State Pharmacopeia of Ukraine, it is specified that the use of the whole or cut dried flowering tops of *Hypericum* shoots collected during flowering is allowed (SPhU 1, 2).

Most often, to illustrate the microscopic features of the *Hypericum* herb, a picture is used that shows only the microscopy of the leaf of some pieces of stems and petals, or fragments of tissue. Microphotographs give a more realistic picture of the diagnostic features at the species level and are widely represented in the scientific literature for certain species of *Hypericum* (Lotocka, Osinska, 2010).

In our work, we focus on the morphological and anatomical analysis of the main diagnostic characteristics of raw materials (petals, sepals, leaves, and stems) of six species of *Hypericum*, which may be useful for the authentication of medicinal plant raw materials and their differentiation by species.

MATERIAL AND METHODS

The raw materials (the upper part of flowering shoots – «Herba») of six species of the genus *Hypericum*, which are widespread in Ukraine: *H. alpigenum*, *H. elegans*, *H. hirsutum*, *H. maculatum*, *H. montanum*, and *H. perforatum* were selected for research. Considering the fact, highlighted in the literature (see above) that the main diagnostic morphological characteristics of the raw materials of the studied species are the placement features of secretory structures in different organs of the plant, the main focus of this study is focused on the analysis of these characteristics in sepals, petals, and leaves. Important morpho-anatomical species-specific features of the raw organs of the studied species were also analyzed as an essential component for the identification of their species affiliation.

The research is based on own materials collected during many years of field research by authors in different Ukraine regions, with the additional use of herbarium specimens deposited in the National Herbarium of Ukraine (KW). For the morphological and anatomical analysis of the raw materials, at least ten samples of each species were examined from which fragments of flowers, leaves, and stems

of the upper part of the shoot of each specimen were selected; subsequently boiled for 2–5 min and prepared preparations for light microscopy. Microscopic examination was performed ten times for each organ from different parts of the sample. Observations and microphotographs were made using an Olympus CX23 light microscope, a Philip Harris stereomicroscope, and Levenhuk M1000 PLUS camera software.

RESULTS AND DISCUSSION

The studied species are characterized by common macromorphological features, such as the flower and stem structure, the structure of the leaf blade, leaf placement, etc. At the same time, each of them has different specific characteristics of the mentioned above organs.

Also, the most important diagnostic feature of raw materials of *Hypericum* species is the localization, shape, and color of secretory structures that concentrate valuable secondary metabolites.

Secondary metabolites of *Hypericum* species (so-called biomarkers) mainly belong to xanthenes, petrodianthrones (hypericin and pseudohypericin), derivatives of phloroglucinol (hyperforin), biflavones (I3, II8-biapigenin, amentoflavone, amentoflavonoid), and flavonoid glycosides (rutin, hyperoxide, isoquercitrin, quercitrin, quercetin), which are localized and are often synthesized in a variety of secretory structures mainly of leaves, sepals and petals (Ciccarelli et al., 2007; Lotocka, Osinska, 2010; Vieira da Silva et al., 2013). The localization of secretory structures, their shape, and color of content have species-specific character and differ significantly in the tissues of different organs. Therefore, the level of biologically active substances in a particular tissue of *Hypericum* depends on the relative amount of secretory structures in the raw materials. Thus, the organ dependence of secondary metabolites is a characteristic feature of species of the genus *Hypericum* (Cirak et al., 2016). Most secretory structures in *Hypericum* species plants are concentrated in the perianth (sepals and petals) and leaves, so the quality of the raw material significantly depends on the proportion of these organs in the total

mass of raw materials.

The most studied in this aspect is *H. perforatum* (Ciccarelli et al., 2007; Ciccarelli et al., 2001b; Vieira da Silva et al., 2013 with references); however, there are fragmentary studies of other species, such as: *H. tetrapterum* (Gitea et al., 2018), *H. elodes* (Vieira da Silva et al., 2013), *H. elegans* (Lotocka, Osinska, 2010), etc. There is no uniform terminology for the names of secretory structures of *Hypericum* species. In most studies, they are divided into so-called «dark» (black nodules) and «yellow» (yellow glands, paleglands, translucent glands or dots) and secretory canals (Gitea et al., 2018; Maggi et al., 2004; Nurk, Crockett, 2011), etc. These terms are mainly used to describe the internal secretory structures of leaves and other organs. As for the names of multicellular club-shaped (claviform) protuberances with products of metabolism on the edge of sepals, bracts, and petals, there are even more significant differences. In most works, they are referred to as «black nodules» sometimes – «peduncular black nodules» or «glandular emergences» (Perrone et al., 2013 with references). Considering that these growths are morphologically different from the internal secretory structures, cells of various tissues are involved in their formation, and they differ from the basic structures of the organ in structure and function; we consider the most appropriate name “glandular emergents.”

The comparative description of the diagnostic features of the raw materials (leaves, petals, sepals, and stems) of the investigated species of *Hypericum* is given in the text below.

Leaves

Leaves are entire, mostly sessile, differ in shape (from oblong-lanceolate in *H. elegans* and *H. perforatum* to ovate-lanceolate in *H. alpigenum*) and in size (Table 1, Figure 1 a-f). The surface of the leaf blade in most species is weakly cutinized and smooth, and only *H. hirsutum* is densely covered over the entire body with simple single-tricellic trichomes “huk” and papillae (Figure 1 g-i). *H. montanum* is noticeably cutinized and rough from numerous papillae (Table 1, Figure 1 j), especially on the veins (Figure 1 k).

On the leaf blade of all the studied species, the central vein and 2-3 pairs of lateral veins are clearly distinguished. The tertiary grid is well expressed only in *H. maculatum* (Figure 1 l).

Secretory structures of different types are found in the leaves of all studied species of *Hypericum* (Figure 2). Their localization, color, and shape in the complex are species-specific. Thus, in the leaves of *H. elegans*, *H. hirsutum*, and *H. perforatum*, there is a large number of small translucent rounded essential oil reservoirs on the entire surface (Figure 2 a-c). In contrast, in the leaves of *H. maculatum* and *H. montanum* they are rare in edges of a leaf blade (Figure 2 d-e), and are

almost absent in *H. alpigenum* (Figure 2 g-h). Small elongated secretory channels with yellow content along the veins were found in *H. maculatum* (Figure 2 f).

The leaves of almost all analyzed species, except *H. hirsutum*, are characterized by the presence of rounded secretory structures (nodules) with dark-colored metabolites (black nodules). They can be apparent (*H. alpigenum*, *H. maculatum*, and *H. montanum*) or indistinctly rounded (*H. elegans* and *H. perforatum*); small or large; placed densely (*H. alpigenum* and *H. montanum*) or sparsely; only on the edge of the plate, or in different parts of it (Table 1).

Table 1. Key diagnostic features leaves of species of *Hypericum*

Species name	Leaf shape	Length – width of a plate, cm	Surface structure	Venation	Localization of dark nodules (glands)	Localization and shape of light nodules (glands)
<i>H. alpigenum</i>	Ovate or ovate-lanceolate, with obtuse apex and greatest width in the middle	1,2–3 – 0,8–1,6	Clearly cutinized smooth	2-3 pairs of well-defined lateral veins, which branch out from the central at an acute angle	Clear, small, convex-rounded, densely located on the edge of the leaf blade and single on the surface in the upper half of the leaf	Almost without translucent glands on the surface
<i>H. elegans</i>	Oblong-lanceolate or ovate-lanceolate to triangular-lanceolate with the greatest width at the base	1,5-2,5 (3,3) – 0,3-1,2)	Slightly cutinized smooth	Three pairs of main lateral veins from the lower quarter of the middle vein, weakly expressed tertiary grid	Vaguely rounded, large, systematically along the edge of the leaf blade	Numerous small round translucent secretory structures over the entire surface
<i>H. hirsutum</i>	From ovate-oblong to elliptical with an obtuse apex and the greatest width in the lower third of the leaf	1,7-5 – 1-2	Densely pubescent over the entire surface with simple single-tricellic trichomes “huk” and papillae	Three pairs of main lateral veins, which curve away from the lower half of the middle vein	Absent	Numerous small round translucent secretory structures over the entire surface
<i>H. maculatum</i>	Wide-elliptical with the greatest width in the lower half of the leaf	1,5-4 – 1-2	Slightly cutinized smooth	Three pairs of main lateral veins from the base, well-defined tertiary grid	Rounded, rarely on the edge of the leaf blade	Small round translucent along the edge of the leaf blade, elongated channels along the veins
<i>H. montanum</i>	Egg-shaped or ovate-oblong with the greatest width in the middle or slightly below the middle of the plate	2-6 – 0,5-3,5	Clearly cutinized, rough from numerous papillae on both sides	2-6 pairs of main lateral veins more pronounced in the lower half of the leaf blade	Clear, medium, distinctly rounded, distinctly located on the edge of the leaf blade	Small, rounded, rarely on the edge of the leaf blade
<i>H. perforatum</i>	Oval, oblong-ovate or oblong, obtuse, with a narrowed base and the greatest width in the lower third of the leaf	1-3 – 1,2-1,8	Slightly cutinized smooth	2 (3) pairs of well-defined lateral veins, which branch out from the central at an acute angle	Vaguely rounded, large on the edge of the leaf blade and single on the surface	Small, densely distributed over the entire surface of the leaf blade

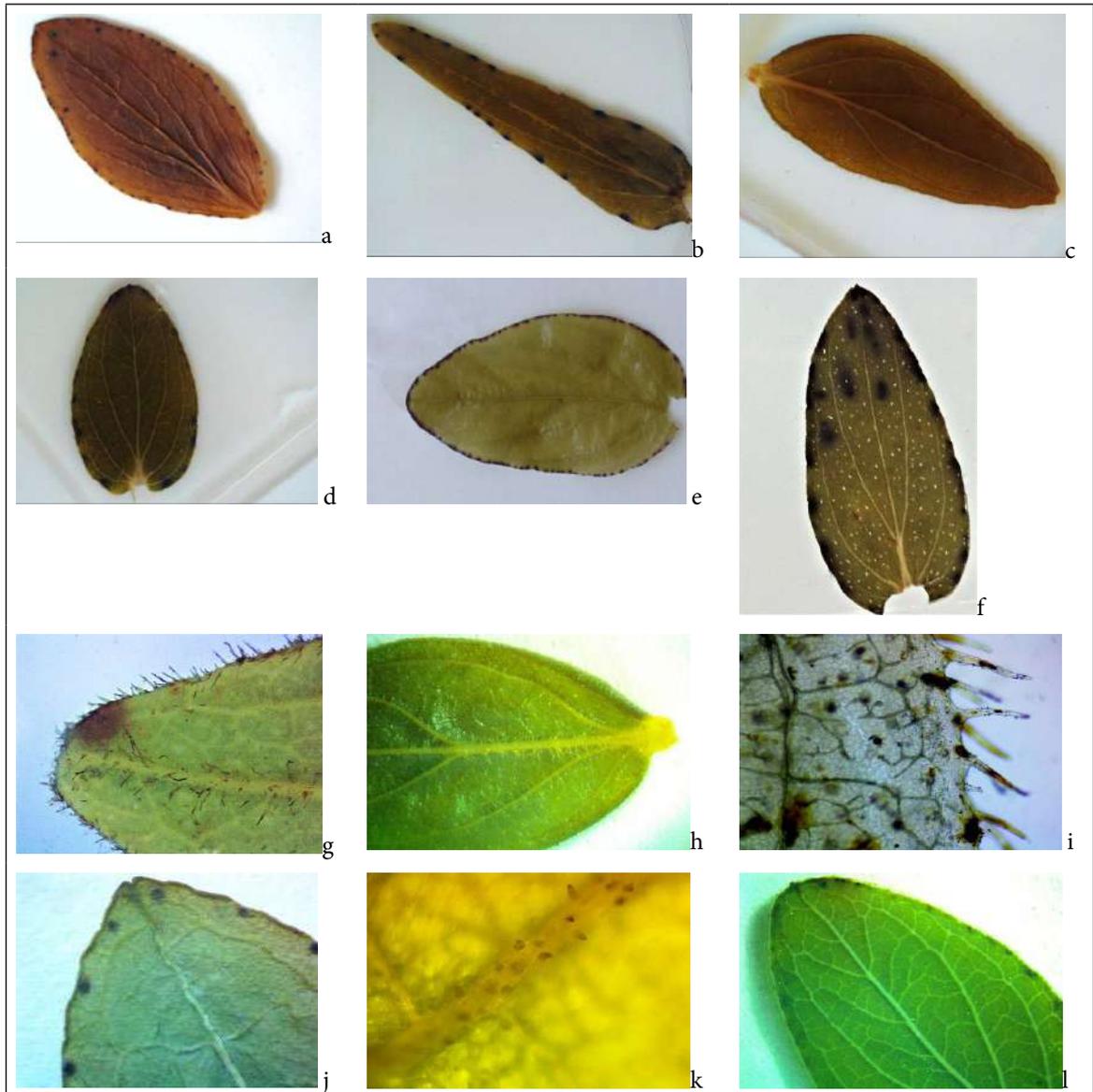


Figure 1. Morphological characteristics of leaves of *Hypericum* species: a) *H. alpigenum*, $\times 10$; b) *H. elegans*, $\times 10$; c) *H. hirsutum*, $\times 10$; d) *H. maculatum*, $\times 10$; e) *H. montanum*, $\times 5$; f) *H. perforatum*, $\times 20$; g) pubescence of leaf *H. montanum* in apical part, $\times 20$; h) pubescence of the leaf *H. hirsutum* in the central and basal part, $\times 10$; i) trichomes and papillae on the surface of the leaf *H. hirsutum*, $\times 100$; j) *H. montanum*, $\times 20$; k) papillae on the central vein of *H. montanum*; l) venation of *H. maculatum* leaf, $\times 10$

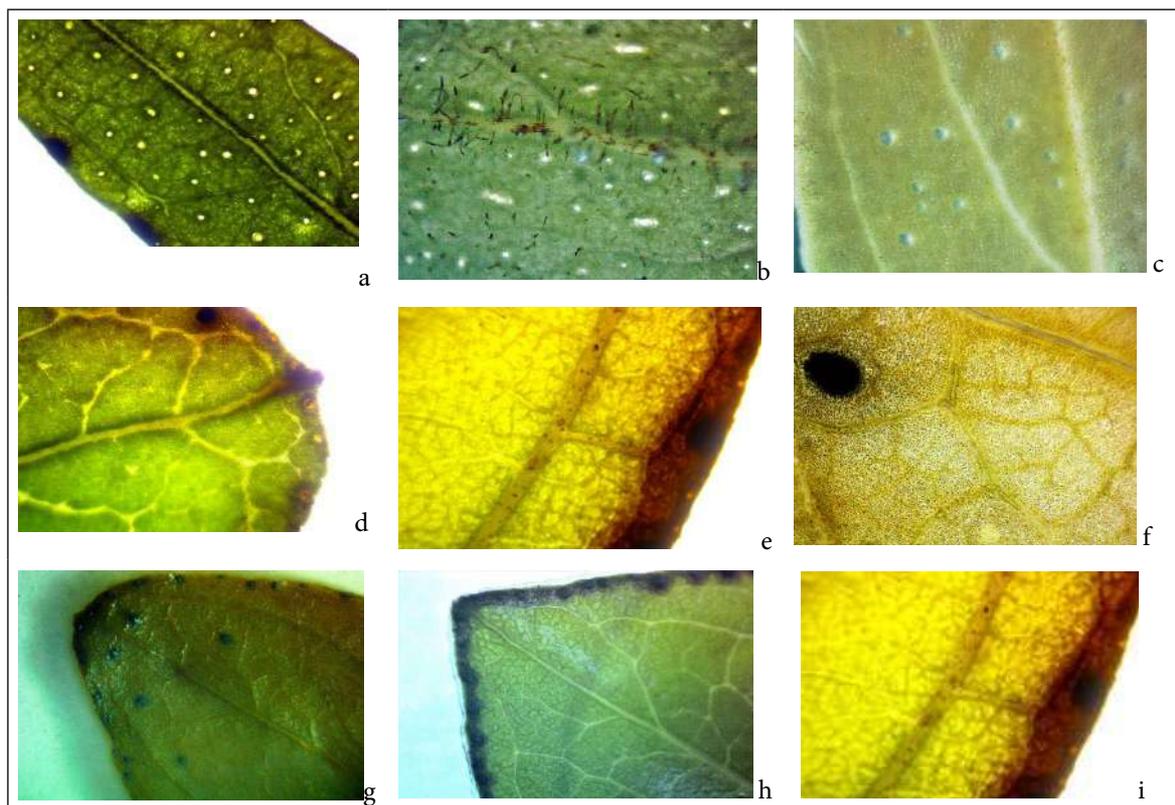


Figure 2. Secretory structures of leaves of *Hypericum* species: a) *H. elegans*, × 40; b) *H. hirsutum*, × 40; c) *H. perforatum*, × 10; d) *H. maculatum*, × 40; e) *H. montanum*, × 40; f); *H. maculatum*, × 100; g) *H. alpigenum*, × 10; h) *H. alpigenum*, × 20; i) *H. montanum*, × 40

Sepals

Due to their small size, the sepals of *Hypericum* species in dry raw materials are usually preserved intact. Hence, their species-specific morphological features make it possible to identify the species of the raw material. The localization and character of secretory structures of sepals also play an important diagnostic role. Sepals of *H. alpigenum* are oval-lanceolate narrowly pointed to the apex. On edge, they have long, slightly club-shaped thickened at the top, secretory growths (emergences) (Figure 3 a, b), present in sepals in most of the studied species. In *H. alpigenum* they are the most numerous and most prolonged, differing in length and width at the base; branches of lateral veins approach them (Figure 3 b). Numerous small convex-oval or elongated, pointed at both ends, secretory structures of almost black color are clearly distinguished on the sepals' surface.

Sepals of *H. maculatum* differ from others by an oval-egg-shaped plate with a blunt apex (Figure 3 c,

d). Between the veins (often along the central vein), the large round dark glands with blurred edges and numerous small oval or elongated yellow color containers are visible (Figure 3 d).

H. hirsutum and *H. montanum* have the same sepals of linear-lanceolate or oblong-lanceolate shape with numerous glandular emergences along the edge (Figure 3 e-h). Despite the general peculiarity of the formation of these structures on the outside of the petal plate or sepals and the dark color of the secretion, they have some species-specific features, such as shape, size, and location. In particular, in *H. hirsutum*, the extended part of outgrowths has a rounded shape (Figure 3 e, f), while in *H. montanum*-cup-shaped (Figure 3 g, h). Also, emergences in *H. hirsutum* are evenly distributed along the edge of the sepals (Figure 3 e), including its apex, and in *H. montanum*, the apex is free (Figure 3 g). Both species have secretory channels along the veins with yellow products of metabolism (Figure 3 e-g). Only

in *H. hirsutum*, they are more pronounced than in *H. montanum*. Also, the surface of the sepals of *H. hirsutum* is densely pubescent with simple protruding trichomes with a broad base (Figure 3 e).

The sepals of *H. elegans* are characterized by the presence of 3 types of secretory structures: glandular emergences with a dark secretion in an oval thickening at the apex along the edge of the sepals, including the apex; numerous yellow oval containers between veins and single dark roundish-convex glands (Figure 3 i-j).

The sepals of *H. perforatum* have an oblong-lanceolate shape of the plate with an elongated narrow apex, which ends with a glandular growth with a dark content (Figure 3 i-l). A few large oval or elongated translucent secretory structures with yellow content are visible on the plate; single large dark glands may be present or absent. In *H. perforatum*, on the edge of the sepals, there are occasional single long, slightly club-shaped thickenings on the top, similar to *H. alpigenum* (Figure 3 k), and light glands at the base merge into continuous channels (Figure 3 l).

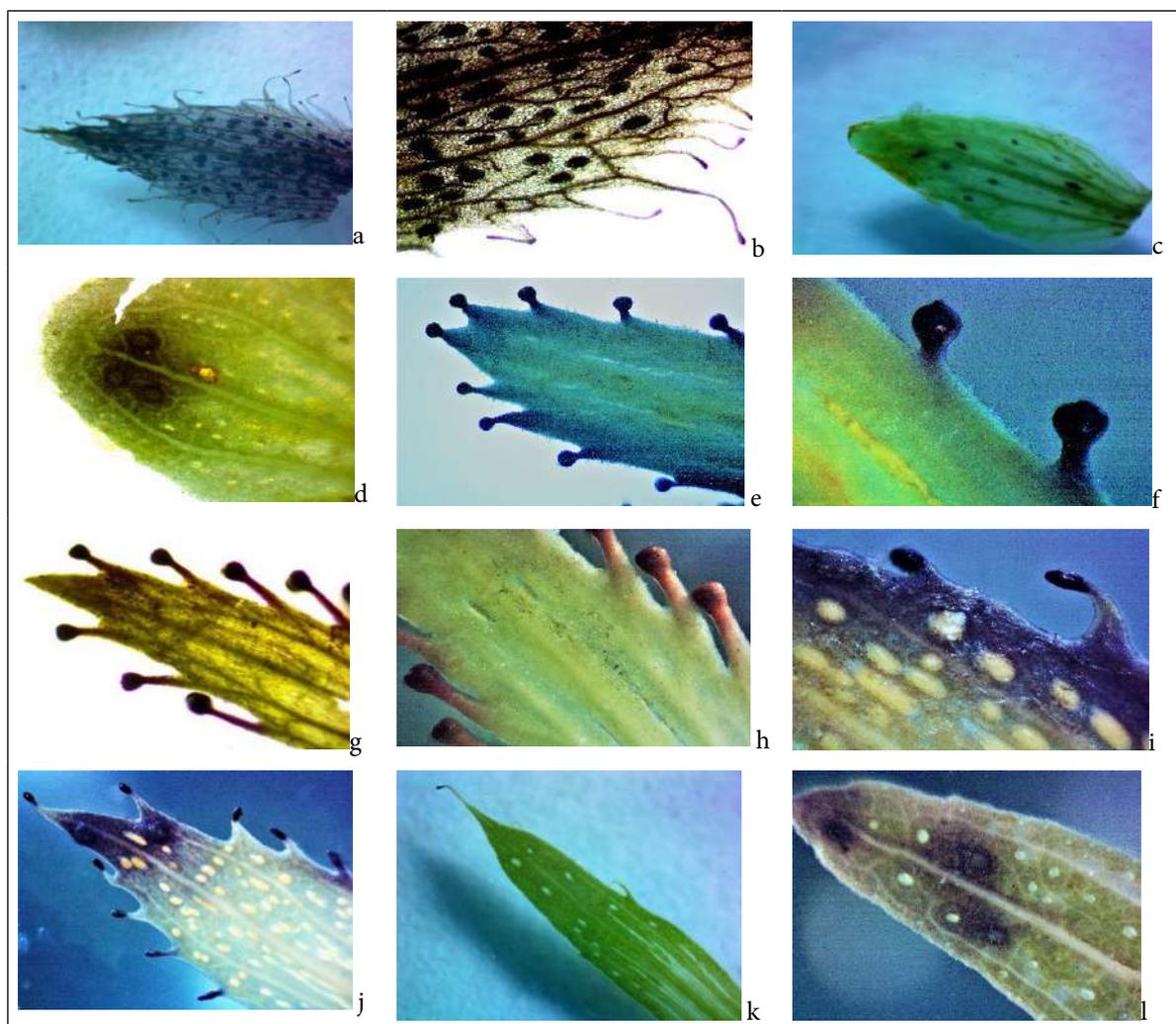


Figure 3. Diagnostic characteristics of sepals of *Hypericum* species: a) general view of *H. alpigenum*, $\times 20$; b) dark glands and emergences on the margin of the sepals *H. alpigenum*, $\times 40$; c) sepals of *H. maculatum*, $\times 20$; d) dark and light secretory structures of *H. maculatum* $\times 40$; e) glandular emergences and secretory canals of the sepals *H. hirsutum*, $\times 40$; f) pubescence and secretory structures of *H. hirsutum*, $\times 100$; g) sepals of *H. montanum*, $\times 40$; h) glandular emergences and secretory canals of *H. montanum*, $\times 40$; i) emergences and yellow glands of the sepals *H. elegans*, $\times 100$; j) secretory structures of the sepals *H. elegans*, $\times 40$; k) sepals *H. perforatum*, $\times 20$; l) dark and translucent glands of *H. perforatum*, $\times 40$

Petals

In all studied species, the petals are free, golden-yellow, unequal, oblong-elliptical, obliquely cut at the top (Figure 4). The main species-specific feature of the petals of the studied species is the location, shape, and color of secretory structures. The latter are least represented in the petals of *H. montanum*, in which individual oval or elongated yellow glands may be singly placed between the veins or absent, as well as dark glands (Figure 4 a, b). Spheroidal dark glands along the beveled edge of the petals are typical for *H. elegans* and *H. perforatum* (Figure 4 c, d). In *H. elegans* they may also be present along the edge of the

petals up to the bottom (Figure 4 e) and occasionally between the veins closer to the edge (Figure 4 f). In both species, oval or elongated secretory structures of yellow color are distinguished between the veins, which merge into channels in the center and base (Figure 4 e-g). Numerous dark oval-convex glands are present on the petals of *H. alpigenum*. Also, in this species, at the edges of the petals in the upper part, there are thin elongated head emergences with dark content, as in the sepals (Figure 4 h). Small headed emergences (one or more) with a dark secretion on the top of the petal are characteristic of *H. hirsutum* (Figure 4 i, k).

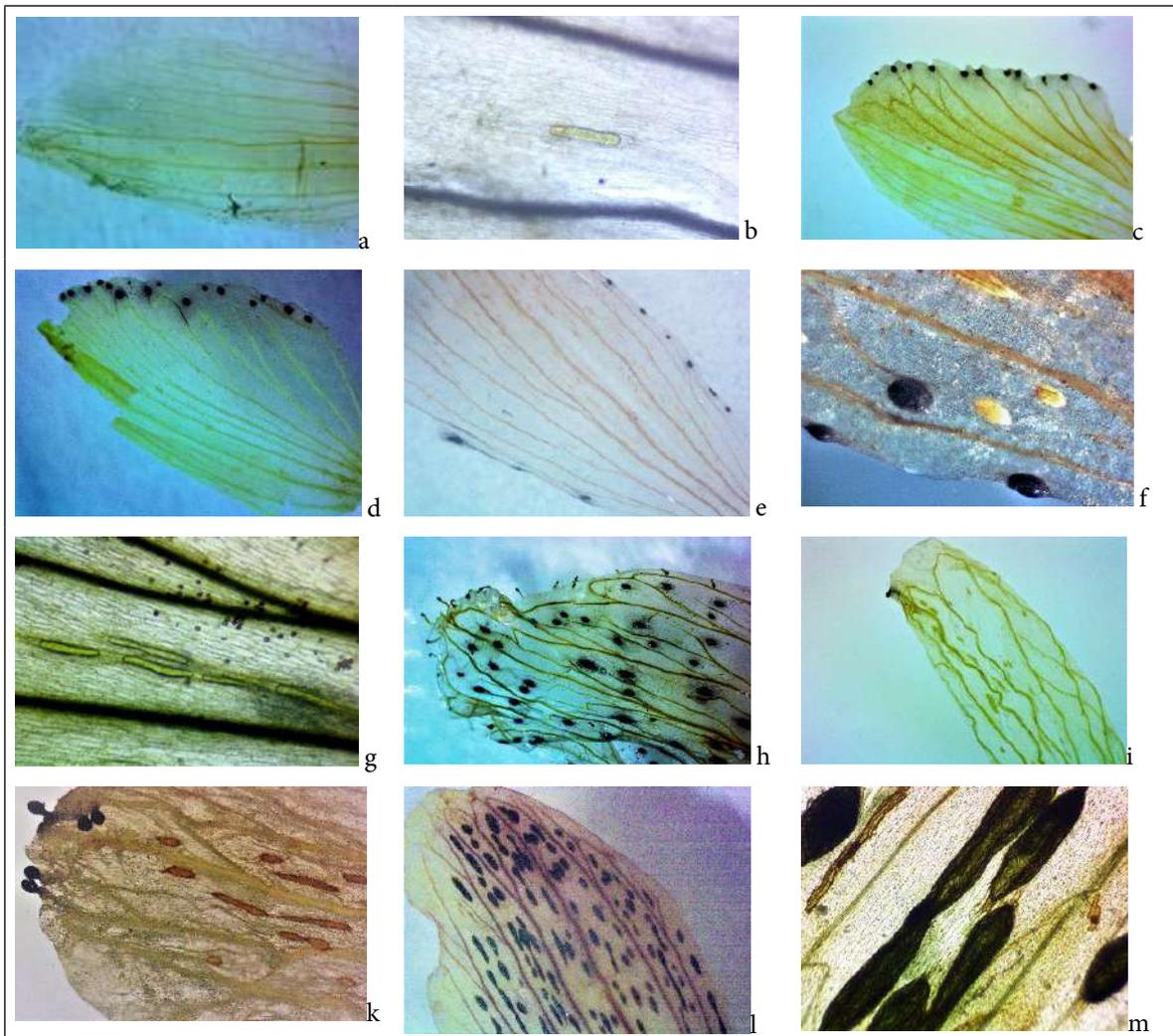


Figure 4. Diagnostic characteristics of petals of *Hypericum* species: a) *H. montanum*, × 20; b) *H. montanum*, × 100; c) *H. elegans*, × 20; d) *H. perforatum*, × 20; e) dark and yellow glands of *H. elegans*, × 40; f) dark and yellow glands of *H. elegans*, × 100; g) *H. perforatum* secretory channels, × 100; h) *H. alpigenum*, × 20; i) *H. hirsutum*, × 20; k) *H. hirsutum*, × 40; l) *H. maculatum*, × 20; m) *H. maculatum*, × 100

Between the veins, there are yellow glands of oval or elongated shape. Most dark glands are present on the petals of *H. maculatum*. They are much larger than in *H. alpigenum*; they have an oval or elongated-oval shape with pointed tips at the top of the petals; in the lower part, they often merge into continuous channels (Figure 4 l, m). Occasionally there are small elongated channels filled with yellow products of metabolism (Figure 4 m). The petals of the studied species of *Hypericum* also differ in size, but even petals of one type of inflorescence vary in size depending on the degree of flowers. Therefore, this feature is less critical in the identification of raw materials.

Stems

Among the macromorphological characteristics of the stem of the studied species of *Hypericum*, the main difference is the severity and number of ribs at

the internodes. The stem of all studied species in the stage of secondary growth of round shape in cross-section with two small wings (ribs) in *H. alpigenum*, *H. elegans*, and *H. perforatum* (Figure 5 a-c); four pairs of different sizes in *H. maculatum* (Figure 5 d) or without them in *H. hirsutum* and *H. montanum* (Figure 5 e, f). Secretory structures are present mainly in the cortex at different depths and in the outer phloem (Figure 5 g). Differences in the anatomical structure of the stem of the analyzed species are less noticeable than morphological. Outside the stems, dark containers and channels are well defined along the ribs of *H. maculatum* and *H. perforatum* (Figure 5 h, i). It has been studied that in the stems of *Hypericum* species, there are no secretory structures in which essential oil accumulates, but tannins, alkaloids, lipids, and resins can accumulate here (Ciccarelli et al., 2001a; Lotocka, Osinska, 10).



Figure 5. Diagnostic characteristics of the stem of *Hypericum* species: a) *H. alpigenum*, $\times 40$; b) *H. elegans*, $\times 40$; c) *H. perforatum*, $\times 20$; d) *H. maculatum*, $\times 40$; e) *H. hirsutum*, $\times 40$; f) *H. montanum*, $\times 40$; g) secretory structures of the stem of *H. maculatum*, $\times 400$; h) dark glands along the ribs of *H. maculatum*, $\times 10$; i) dark glands along the ribs of *H. perforatum*, $\times 10$

CONCLUSIONS

It was found that the raw materials of *H. alpigenum*, *H. elegans*, *H. hirsutum*, *H. maculatum*, *H. montanum*, and *H. perforatum* have some common and specific features. Comprehensive analysis of species-specific characteristics of the main raw material organs (leaves, petals, sepals, and stems) of species of the genus *Hypericum* allows us to determine their species affiliation clearly. Targeted falsification or incorrect identification of a particular species of St. John's wort during the microscopic analysis of raw materials is unlikely. Among the analyzed organs of *Hypericum* species, the species specificity is more pronounced in the secretory structures of the sepals.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

AUTHOR CONTRIBUTION STATEMENT

Developing hypothesis, experimenting, preparing the study text, reviewing the text, analysis and interpretation of the data, literature research (Minarchenko V.), experimenting, analysis and interpretation of the data, literature research (Futorna O.), preparing the study text, reviewing the text, analysis and interpretation of the data, literature research (Pidchenko V.), experimenting, analysis and interpretation of the data, literature research (Tymchenko I., Dvirna T.), preparing the study text, reviewing the text, literature research (Makhynia L.)

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