

Morphological and anatomical study of freshly harvested raw materials from individual representatives of the genus *Hypericum*. Publication 1: *Hypericum perforatum* L. - St. John's wort perforated (common, pierced-leaved)

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SUMMARY

A morphological and anatomical study of the freshly harvested herb St. John's wort was carried out. Diagnostic signs of the aboveground part (grass) were found, which can be used as characteristics of the authenticity of raw materials. The results of the study were used to develop a draft Pharmacopoeia Monograph on freshly harvested raw material of St. John's wort, used in homeopathy.

Key words: St. John's wort, morphological signs, anatomical signs, diagnostic signs.

I. Introduction

At the Institute of Homeopathy and Naturotherapy of the FNECC TMDL, within the framework of the scientific theme "Scientific substantiation, development and introduction into practical health care of means and methods of traditional medicine for the diagnosis and treatment of pathological conditions and medical rehabilitation of patients", a pharmacognostic study of fresh raw materials of closely related species used in homeopathy is carried out. In allopathic and homeopathic practice, it is possible to use medicinal plant materials procured from several representatives of the same genus. In homeopathic pharmacy, as a rule, raw materials of one type are an unacceptable admixture to raw materials of another type of the same kind, since the spectra of clinical homeopathic action (pathogenesis) of monopreparations from raw materials of even closely related species are very different [12, 22].

This publication is devoted to the results of studying the morphological and anatomical diagnostic signs of freshly harvested St. John's wort, a representative of the genus *Hypericum* L. St. John's wort is approved for use in homeopathy in the Russian Federation [2, 8, 15]. However, there is no ND for freshly harvested grass.

Application in allopathy. St. John's wort is widely used in modern and traditional medicine in many countries. It is an integral part of more than 20 complex Russian drugs and elixirs with tonic, anti-inflammatory, hypoglycemic, diuretic, hepatoprotective, antioxidant, antihelminthic, wound healing and other medicinal properties [3, 8, 9, 17]. For example, the herb infusion is a part of the drug "Doppelherz Energotonik", the tincture - in "Capsitrin", the extract - in "Novo-Passit", balsam "Pantaforte", "Faringal", "Novobrassit", "Prostanorm", "Formula longevity", "Yarsin 300",

"Vitaon", "Deprim", "Deprim-forte", "Gelarium Hypericum", "Negrustin", "Hyperforat", "Psychotonin", "Neuropas", etc. A technology for the preparation of "Optimistin" has been developed, which has the properties of a mild antidepressant [1]. The oil fraction of water-alcohol-oil extract from the aerial part of St. John's wort is a part of the drug for the treatment of diseases of the female genital area [16, 20]. In Czechoslovakia, the drug Floristen was obtained from St. John's wort for the treatment of gynecological diseases. Foaming vaginal tablets with dry extract of St. John's wort have been created for the treatment of genital papillomavirus infection [13]. Medicines containing St. John's wort extract are patented in the United States for the treatment of acute and chronic hepatitis C. Experimental studies have shown the possibility of using St. John's wort preparations in the treatment of chronic fatigue syndrome [1]. St. John's wort is included in the British Herbal Pharmacopoeia [23]. Application in homeopathy. In domestic and foreign homeopathic practice, it is used freshly collected aerial part of St. John's wort [2, 10, 15, 25, 26, 28, 29]. St. John's wort is included in the nomenclature of homeopathic medicines in Germany [25], Britain [24], India [28] and France [27].

Fresh raw materials are used to obtain a matrix homeopathic tincture of Hypericum, which is used in various dilutions for the production of monopreparations. So, in dilution D1 is used for the manufacture of monopreparations "Hypericum opodeldoc homeopathic" and "Hypericum homeopathic ointment" (JSC "Moscow Pharmaceutical Factory", Russia), which are prescribed for neuralgic shooting pain spreading from the fingers to the shoulder, as well as from the place where the sciatic a nerve spreading along its course. In D1 dilution, it is part of the complex homeopathic preparations "Memoria" (Richard Bittner, Austria), "Valerianachel" (Biologische Heilmittel Heel GmbH, Germany); in breeding D2 - "Brionia-plus" (LLC "Doctor - N", Russia), "Traumeel S" (Biologische Heilmittel Heel GmbH, Germany), "Urtika-plus" (LLC "Doctor - N", Russia), Hamomilla-plus (Doctor-N LLC, Russia); in breeding D6 - "Repisan" (Richard Bittner, Austria); in breeding C3 - "Aneuro" (Gomeofarma, Russia). Freshly harvested herb St. John's wort is used to obtain the homeopathic ointment Cicaderm (Laboratories Boiron, France) [10, 18]. The main indications for use in homeopathic practice are: craniocerebral trauma, neuralgia, inflammatory skin diseases [2].

II. Research objects

Fresh grass St. John's wort - *Hypericum perforatum* L. (family Hypericaceae), harvested at the beginning of flowering (July 2005) in the Krasnogorsk district of the Moscow region.

III. Research methods.

Devices and equipment

1. The study of the external signs of raw materials was carried out in accordance with the SP XI ed. no. 1 "Methods for the analysis of medicinal plants" [6]. The study of the external signs of raw materials and their description was carried out immediately after the procurement.

2. Microscopic examination was carried out in accordance with GF XI ed., No. 1 "Technique of microscopic and microchemical research of medicinal plant raw materials" in accordance with the State Fund of the XI ed., No. 1 "Technique of microscopic and microchemical research of medicinal plant raw materials" [6]. All plant parts intended for microscopic studies were preserved in an alcohol-glycerol-water mixture (1: 1: 1) immediately after collection.

3. Microscopic examination was performed on an Olympus CX41 microscope (Japan) with 19x eyepieces, 4x, 10x, 20x, 40x and 100x objectives. Photographing was carried out with an Olympus Digital Camera C - 3000 Zoom (Japan).

4. The studies were carried out in three independent replicates in accordance with requirements of the GF XI ed., no. 1, p. 277 "Technique of microscopic and microchemical research of medicinal plant raw materials."

IV. Study of morphological features of freshly harvested St. John's wort perforated

In order to identify the macroscopic diagnostic signs of fresh herb St. John's wort, we analyzed the data from literary sources, and also conducted our own research. To determine external signs, at least 5 samples were used, collected at a distance of at least 1 meter from each other. The results of the studies were summarized by us in Table 1.

On the basis of the research carried out, we formulated a criterion for assessing the authenticity of freshly harvested St. John's wort "External signs". Upper parts of stems with leaves, flowers, buds and unripe fruits. Stems are greenish-yellow or grayish-green, non-pubescent, cylindrical with two prominent longitudinal ribs, oppositely branched at the top, up to 30 cm long.

Leaves are simple, opposite, sessile, dark green or grayish-green, 0.7–3.0 cm long, 0.2–0.8 (1.5) wide; oblong, oblong-oval, oval, ovate, oblong-ovate, elliptical, broadly elliptical, almost heart-shaped or more or less narrow, oblong-linear, lanceolate; entire, glabrous, with a blunt apex, flat or more or less curled along the edge, with rare black dots - containers. When examining the sheet in the light, numerous translucent points are visible - containers.

The flowers are golden yellow or yellow in color, collected in a wide paniculate and / or almost corymbose, or corymbose, inflorescence. Bracts are lanceolate, 0.5 cm long, acute. Perianth double, regular, 5-petal calyx and corolla. The calyx is multi-leaved, five-part or deep five-part, 5 mm long, two or almost two to three times shorter than the corolla. Sepals lanceolate or narrowly lanceolate, linear or linear-lanceolate, 4 (5) mm long, 1 mm wide, equal or longer ovaries, acute or finely pointed, with rare glandular black, mostly oval punctures, even or slightly serrated along the edge. Petals are oblong or oblong-elliptic, obliquely cut at the apex (unequal), dentate, 1.2–1.5 cm long, 0.5–0.6 cm wide, along the edges and upper part with numerous black glands in the form of black dots and dashes and on the surface with many yellow light punctuations and in the form of thin dashes and stripes glands or without black dots. There are many stamens (50-60), accrete at the base in 3 bundles. One pistil, with a three-celled upper ovate ovary 3–5 mm long and free, bent columns twice as long as the ovary. The fruit is a greenish or greenish brown capsule. The smell is weak, peculiar. The taste is bitter, slightly astringent. The fruit is a greenish or greenish brown capsule. The smell is weak, peculiar. The taste is bitter, slightly astringent. The fruit is a greenish or greenish brown capsule. The smell is weak, peculiar. The taste is bitter, slightly astringent.

External signs of fresh herb St. John's wort

Table 1

Морфологический признак		Характеристика	Методика определения
1		2	3
Стебель	Форма	Цилиндрический или округлый с двумя выдающимися диаметрально расположенными ребрышками	Визуально
	Характер ветвления	Вверху супротивно-ветвистый	Визуально
	Опушение	Отсутствует	Визуально, с помощью лупы
	Цвет	Зеленовато-желтый, серовато-зеленый	Визуально, при дневном освещении
Лист	Расположение	Супротивное	Визуально
	Сложность листовой пластинки	Простые	Визуально
	Форма	Продолговатые, продолговато-овальные, овальные, яйцевидные, продолговато-яйцевидные, эллиптические, широко эллиптические, почти сердцевидные или более менее узкие, продолговато-линейные, ланцетные	Визуально
	Вид листа на просвет	При рассматривании листа на просвет видны многочисленные просвечивающие светлые точки – вместилища	Визуально
	Размер: – длина – ширина	0,7–3,0 см 0,2–0,8 (1,5) см	С помощью линейки
	Характер жилкования	Сетчатое	Визуально
	Характер края	Цельный	Визуально
	Опушение	Отсутствует	Визуально, с помощью лупы
	Цвет	Темно-зеленый; серовато-зеленый	Визуально, при дневном освещении
Цветки	Соцветие	Цветки собраны в широко метельчатое и/или почти щитковидное, или щитковидное соцветие	Визуально
	Диаметр соцветия	5–11 см	С помощью линейки
	Околоцветник	Двойной, правильный, состоящий из 5-лепестных чашечки и венчика	Визуально, с помощью лупы
	Чашечка	Пятираздельная или глубокопятираздельная в 2 или почти в 2–3 раза короче венчика	Визуально, с помощью лупы
	Чашелистики	Ланцетные или узко ланцетные, линейные или линейно-ланцетные, равные (или длиннее) завязи, острые или тонко заостренные, с редкими железистыми черными большей частью овальными точками, по краю ровные или немного зубчатые	Визуально, с помощью лупы
	Венчик	Крупный, золотисто-желтый или желтый, состоящий из 5 свободных лепестков	Визуально, с помощью лупы
	Лепестки	Продолговатые или продолговато-эллиптические, заостренные, на верхушке косо срезанные (неравнобокие), зубчатые, по краям и верхней части с многочисленными черными железками в виде черных точек и черточек и на поверхности со многими желтыми светлыми точечными и в виде тонких черточек и полосок железками или без черных точек	Визуально, с помощью лупы
	Тычинки	Тычинок много (50–60), сросшихся у основания в 3 пучка	Визуально, с помощью лупы
	Пестик	Один, с трехгнездной верхней яйцевидной завязью и свободными, отогнутыми столбиками в 2 раза длиннее завязи	Визуально, с помощью лупы
	Размеры цветка	Чашелистики: длина – 4(5) мм, ширина – 1 мм. Лепестки: длина – 1,2–1,5 см, ширина – 0,5–0,6 см	С помощью линейки
	Опушение	Отсутствует	Визуально, с помощью лупы
	Цвет	Золотисто-желтый или желтый	Визуально, при дневном освещении
Запах		Слабый, своеобразный	При растирании
Вкус		Горьковатый, слегка вяжущий	При разжевывании

V. Study of the anatomical features of freshly harvested St. John's wort perforated

We have not found data on the anatomical structure of the fresh herb St. John's wort in the available literature.

In FS No. 52 "St. John's wort" GF XI, no. 2, p. 323 shows microscopy of dry leaves of St. John's wort and spotted [7]. V.A. Kurkin et al. studied the microscopic signs of dried leaves of St. John's wort, spotted, coarse-haired and graceful [14].

Liu Wen-Zhe and Hu Zheng-Hai [26] described the structure and arrangement of secretory structures in the herb St. John's wort. He described three types of intracellular secretory structures: secretory spherical cells (black dots), secretory cavities (translucent points) and secretory channels (translucent stripes). The former are found in flowers, leaves and stems. Black dots - the receptacles are composed of large secretory cells surrounded by two layers of flat, membrane-covered cells. Secretory cavities are presented along the entire length of the leaf blade; secretory canals all over the flower. Both of them consist of 1 or 2 layers of flat cells, divided by a wall into oil chambers or channels. Using histochemical and fluorescence microscopy, it was found that hypericin accumulates in secretory spherical cells.

Rapisarda A., Tauk L. and Ragusa S. proposed the use of scanning electron microscopy, which provides the identification of phytognostic markers for the detection of impurities in the herb St. John's wort (hybrids and other species of St. John's wort). It was found that the key features are the size and shape of leaf surface cells [30].

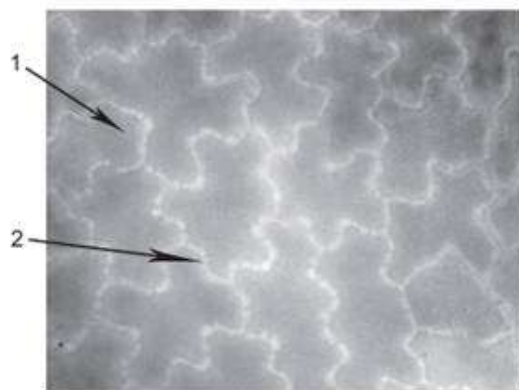
In all studied literary sources, there is no information on the anatomical structure of the stems and flowers of St. John's wort.

In accordance with the FS "Technique of microscopic and microchemical examination of medicinal plant raw materials" GF XI issue. 1, p. 277 [6] when examining a grass, a leaf is taken for analysis, sometimes a piece of a stem and a flower.

According to the results of our own research, microscopic signs of leaves, stems and flowers of St. John's wort were revealed.

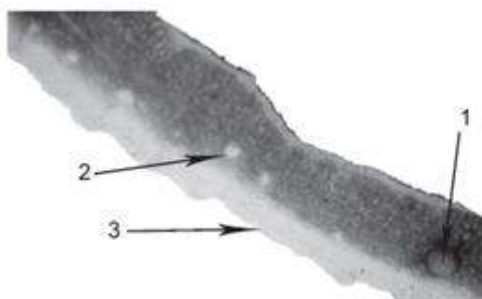
Microscopic signs of leaves

When examining the leaf from the surface, cells of the upper epidermis are visible - slightly sinuous (Fig. 1), the lower ones are strongly sinuous. Their walls have clearly visible thickenings. The stomata are found only on the underside of the leaf. They are surrounded by 3-4 cells of the epidermis (anomocytic type of stomatal complex). There are two types of containers: with pigmented and colorless contents (Fig. 2). Reservoirs with pigmented content (PV) are located mainly along the edge of the leaf blade, and also occur on its surface and apex. By their size, they can be divided into large, medium and small.



Rice. 1. Epidermis of the upper side of St. John's wort leaf (40x): 1 - epidermis cell with winding walls; 2 - clearly visible thickening of the cell wall.

Large receptacles are mainly found at the top and along the entire surface of the leaf, but can also be found along the edge of the leaf blade. Medium and small PV are located along the edge of the leaf blade. Sometimes medium-sized containers are found on the surface of the upper and middle leaves, as well as on the top of the lower leaves, and small containers are found on the surface of the leaf blade near the edge. The receptacles located along the edge of the leaf alternate with each other: through each medium or large PV there is a chain of small ones. In the lower leaves on the surface, there is a greater number of PVs than in the upper and middle leaves, and their number along the edge slightly increases from the upper to the lower leaves. All pigmented containers are round or oval in shape. In the middle and lower leaves, PVs are sometimes found elongated along the length of the leaf. At the apex of the leaf blade there are 2 PVs, one on each side of the main vein. Containers with colorless content (BC) are scattered over the entire surface of the leaf. They are round or oval in shape. There are also BVs elongated along the length of the sheet. The edge of the leaf is uneven, wavy. The leaf blade ends at the top with a narrow or wide apex with a blunt end.



Rice. 2. Containers along the edge of the St. John's wort leaf (10x): 1 - a container with pigmented contents; 2 - shallow container with light content; 3 - edge sheet.

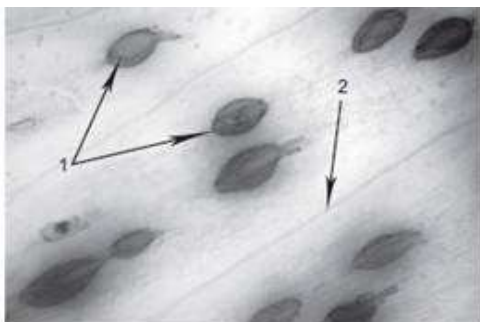
Microscopic signs of the stem

When examining the stem preparation, epidermal cells with straight walls, elongated along its length, are visible from the surface. There are areas of the epidermis where cells

have a shorter length and resemble a "mesh" in appearance. On the entire surface are the stomata of a round or oval shape, surrounded by 3-4, rarely 5 cells of the epidermis (anomocytic type of stomatal complex). There are also stomata surrounded by 3 cells of the epidermis, 1 of which is much smaller than the other two (anisocytic type of stomatal complex). There are containers with pigmented and colorless contents, elongated along the length of the stem. Oval containers are also sometimes found. The cells of the epidermis are filled with small greenish inclusions.

Microscopic signs of flowers

When examining the petal from the surface, cells of the epidermis with wavy are visible, and at the base and sometimes along the edge with straight walls. They are elongated along its length, have beaded thickening of the cell walls and are filled with greenish inclusions of a round and irregular shape. At the base of the petal (approximately in the lower 1/4 part), inclusions are few, and they are very small. In the lower 1/5 of the petal, cells are often colored orange. There are two types of containers: with pigmented and colorless contents. Reservoirs with pigmented content (PW) (Fig. 3) are located at the apex, along the edge and surface of the petal. The PVs located on the surface are generally larger, and sometimes smaller or equal in size to the receptacles along the edge of the petal.



Rice. 3. Reservoirs with pigmented contents in the upper part of the petal (10x): 1 - container with pigmented contents; 2 - vein.

The receptacles located along the edge have a round or oval shape, and on the surface they are elongated along the length of the petal, much less often they have an oval or almost oval shape. Containers with colorless content (BC) are found only on the surface of the petal. Basically, they are elongated along its length, less often they have an oval shape. The contents of light containers have a color from light yellow to yellow-orange, and sometimes reaches reddish. BV are located approximately in the upper half of the petal. Channels with pigmented and colorless contents are visible on the surface. They lie mainly between the veins of the petal, and can also pass next to and along them. Channels with pigmented content (CP) are short or medium in length. Long CPs are less common. Channels with light content (CC) can be short, medium and long. There are much more of them, than KP. The color of the content of the CW varies from light yellow to yellow-orange. There are areas where channels with pigmented and colorless content merge into each other.

When examining the sepal from the surface, cells of the epidermis are visible with sinuous, and closer to the base and sometimes on the sides, straight walls with clearly visible thickenings.

The stomata are found on both sides. They are surrounded by 3-4 cells of the epidermis (anomocytic type of stomatal complex). Often there are stomata surrounded by 3 cells of the epidermis, one of which is much smaller than the other two (anisocytic type of stomatal complex).

There are two types of containers: pigmented and colorless. Vessels with pigmented contents (PV) are located along the edge and surface of the sepal. Sometimes they are absent on the surface. PVs located on the surface are larger than the containers along the edge. They have an oval shape and a rounded or oval shape along the edge. Vessels with light content (BV) are located on the sepal surface. They are mainly elongated along its length, less often oval or round in shape.

There are two types of channels: with pigmented and light content. Channels with pigmented content (CP) run along the veins (Fig. 4) and are short or medium in length. Channels with light content are located between the veins and are of medium length. The content of the KP is dark red, and the KS is light yellow or yellow-orange. Sepals end with a narrow elongated apex. Sometimes small outgrowths are found near it at the top.



Rice. 4. Channels with greenish content along the sepal vein (40x): 1 - channel with greenish content along the vein; 2 - vein.

conclusions

1. For the first time, a morphological and anatomical study of freshly harvested grass was carried out *Hypericum perforatum*.
2. Studied the external signs of fresh grass *St. John's wort*: leaf, stem, flowers. Its morphological diagnostic features were revealed.
3. Studied the anatomical structure of fresh grass *St. John's wort*: leaves, stem, flowers. Revealed its anatomical diagnostic signs.
4. On the basis of the research carried out, a criterion of authenticity was proposed. "External signs" and "Microscopy" for inclusion in the ND project "Fresh *St. John's wort* grass".

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