

Study of Morphological and Anatomical Signs of the New Herbal Medicine *Phytourol*

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Abstract

Introduction: Taking into account the great prevalence of kidney stone disease in the structure of the urological diseases, an increase in disease incidence in the modern world, and the high rate of recurrence, the development and introduction of new efficient medicinal herbal preparations intended for treatment and prevention of urolithiasis remain an actual challenge in the modern world.

The scope of the research: Study of external and microscopic signs of perspective Phytourol preparation.

Materials and methods: The object of the study was the preparation containing cowberry powdered leaves, horsetail grass, burdock roots, felon herb and whole fruits of the sulfur root, mixed in the laboratory. The preparation components have been analyzed by pharmacopoeial methods as per the requirements of the State Pharmacopoeia of the Russian Federation, the 13th edition.

Results: The morphological and anatomical signs have been found for the powdered Phytourol preparation developed for the prevention and treatment of kidney stone disease. The description of appearance has been presented, and the microdiagnostic signs of the preparation components have been visualized.

Conclusion: The results have been used to develop the relevant sections of the legal requirements regulating the preparation quality.

Keywords: kidney stone disease, microscopy, medicinal herbal preparation.

INTRODUCTION

The kidney stone disease (KSD, urolithiasis) holds one of the leading places within the structure of urological diseases. Patients with KSD in urological hospitals account for 30 to 50% of the total number of patients [1; 2; 3].

The incidence of KSD in the world is at least 3% and continues to progressively increase [4; 5; 6]. Thus, over the past decade, the absolute number of registered patients with nephrolithiasis has increased by 34.5% in the Russian Federation [7].

The age structure of urolithiasis incidence is characterized by high incidence among working-age persons, especially the age group of 25-50 years. Most often the disease is met among the men: from 70% to 81% of cases, and in women: 47-60% [8; 9; 10]. One of the characteristic features of the disease is the high frequency of recurrence - up to 38.4% [4, 11].

For the prevention of the urinary tract diseases, and also as an effective component of the conservative treatment, the multicomponent drugs based on herbal preparations - the repertoires possessing comprehensive effect on the diseases' etiopathogenesis - are of particular interest. The medicinal herbal preparations are characterized by mild action, the absence of side effects, which makes possible their long-term use to provide multidirectional action in the KSD chronicity [8; 12; 13; 14].

Based on experimental data, as well as information about the etiology and pathogenesis of urolithiasis, chemical composition of biologically active substances of plants, and the experience of their use in official and alternative medicine, the preparation prescription has been developed for the prevention and treatment of urolithiasis, including drug plants (DP): cowberry leaves, horsetail grass, burdock roots, fruits of a sulfur root and felon herb.

Further, the conventional name - Phytourol - has been given to the preparation.

The purpose of the research is to study the external and microscopic signs of the studied Phytourol preparation.

MATERIALS AND METHODS

The object of the research was the preparation consisting of drug plants: cowberry leaves (*Folia Vaccinii vitis-idaeae*), horsetail grass (*Equiseti herba arvensis*), burdock roots (*Arctii radices*), fruits of the sulfur root (*Anethi graveolentis fructus*) and felon herb (*Herba Artemisiae vulgaris*). Industrial samples of the medicinal herbal preparation that had been

purchased in pharmacy organizations of Moscow were used to compile the Phytourol preparation. The preparation under study was prepared in laboratory conditions in accordance with the methodology of the general pharmacopoeia article "Preparations" of the State pharmacopoeia of the Russian Federation, the 13th edition (GF RF, 13th edition.), OFS.1.4.1.0020.15. The material for morphological and anatomical studies and analysis of micropreparations was prepared in accordance with generally accepted pharmacopoeial techniques [15].

External signs of the preparation were determined as follows: "with the naked eye", using a magnifying glass (10×) and a stereomicroscope (15×) in accordance with the general pharmacopoeia article "Preparations" of the State pharmacopoeia of the Russian Federation, the 13th edition, OFS.1.4.1.0020.15 [15]. For a microscopic analysis, series of microscopic preparations in glycerol were made. For long-term storage of drug plants intended for microscopic examination, some of the samples were placed in an alcohol-glycerine mixture. The anatomic and diagnostic signs of the preparation were studied using the MBI-3 microscope (with the magnification of 7×8; 7×20; 7×40; 10×10). The results were documented using the Canon PowerShot G16 camera and the MFN-12 photomicrographic attachment.

RESULTS AND DISCUSSION

According to the results, the Phytourol preparation represents a mixture of disparate pieces of plant material: powdered leaves, stems, and inflorescences, roots and whole fruits, passing through a sieve with the 7 mm holes. The color was yellowish-brownish-green with light green and dark brown inclusions (Figure 1). The smell was soft and unique. The taste of aqueous extract was bitterish, slightly spicy.



Figure 1. Appearance of the preparation

When viewing the preparation under the magnifier (10×) and the stereomicroscope (16×), the following can be seen:

- pieces of leaves of various forms, coriaceous, bare, green on the one side, and light green on the other side with dark brown dots (glands) (cowberry leaves);

- pieces of stems and branches elongated, rigid, segmented, and partially edged ones, with grey-green nodes and axils, sometimes split with a light yellowish core (horsetail grass);

- pieces of roots in transverse and longitudinal sections with the remains of brown and greyish-brown cork, greyish white, sometimes yellowish in color at the fracture (burdock roots);

- pieces of leaves of various shapes: thin, bare and green on the one side, and strongly pubescent with thin white (silvery) hairs on the other side; pieces of green stems in transverse and longitudinal sections with a white spongy core; as well as integral and partially powdered inflorescences - light green capitula with pubescent phyllaries (felon herb);

- integral and partially powdered half-fruits up to 5 mm long, up to 3 mm wide, wide elliptical, slightly convex with three filiform and with two lateral flat pterygoid edges on the one side (back), and flat on the other one; greenish-brown with yellowish edges. The undecomposed cremocarps (Figure 2) are less common (*sulfur root fruits*).



Figure 2. Appearance of whole fruits of the sulfur root (40×). Half-fruits: on the inner and outer sides and undecomposed cremocarp

For microscopic analysis, micropreparations of leaf were prepared from the surface, the transverse and longitudinal section preparations, and "pressed" root preparations, and also raw materials fixed in the alcohol-glycerine mixture were used.

When examining the pieces of a cowberry leaf from the surface, the epidermal cells with more or less sinuous walls can be seen, which correspond to the lower and the upper epidermis of a cowberry leaf. The paracitic type stomata are small, with two periosteal cells located parallel to the stomatal pore. On the epidermis there are glands with brown contents, consisting of plurilocular stem, gradually turning into an oval plurilocular head. In the mesophyll, rare single prismatic crystals of calcium oxalate are encountered (Figure 3).

When looking from the surface of epidermis of the stem pieces, branches and reduced leaves of the *horsetail*, it can be seen that the cells on the edges are very elongated (elongated along the growth axis) with thickened straight or slightly sinuous, porous walls. The cells in the grooves are shorter, their long walls are sinuous (angular and roundly crenate), porous. The stomata are located at the base of the edges, usually in two to three rows (less often in four and one row) and accompany the groove. They are slightly submerged, with characteristic radiant rugosity of the cuticle. The outgrowths of the edge combs are in the form of inclined, sharp, fenced teeth (Figure 4).

When examining the pieces of leaf of the *felon herb* from the surface, it can be seen that epidermal cells with thin sinuous walls are often poorly discernible. The anomocytic type stomata are seen on the underside - they are oval with a well-marked stomatal pore. The hairs are of three types: T-shaped - on a 2-3-cell stem and with a 2-terminal long thin cell attached to the stem in the middle and often twisted with other hairs; the hairs are multicellular (flagelliform) - on a short 2-4-cell stem with a long thin cell, and the short hairs of 2-3 short cells with a small head. On the upper side, T-shaped and short hairs are occasionally found. There are a lot of hairs of a different structure on the underside. The rare glands - oval, divided by a partition into two parts - can also be met (Figure 5).

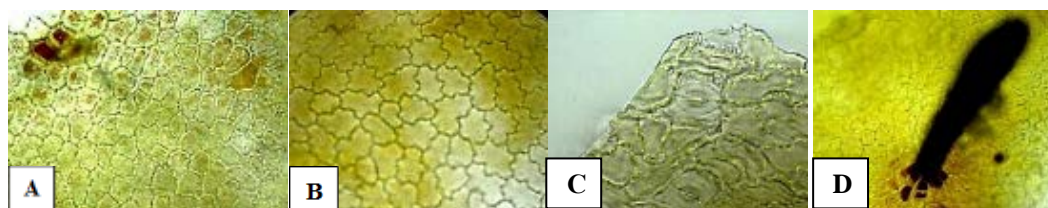


Figure 3. Cowberry leaves: A –upper epidermis (280×); B –lower epidermis (280×); C –lower epidermis with paracitic stomata (400×); D –lower epidermis with a gland (63×)

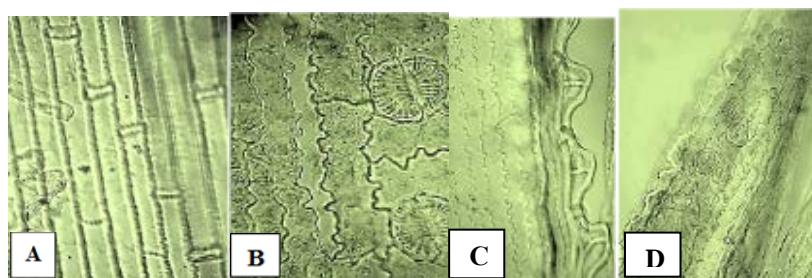


Figure 4. Horsetail grass: A –upper epidermis (280×); B –lower epidermis (280×); C –lower epidermis with paracitic stomata (100×); D –lower epidermis with a gland (100×)

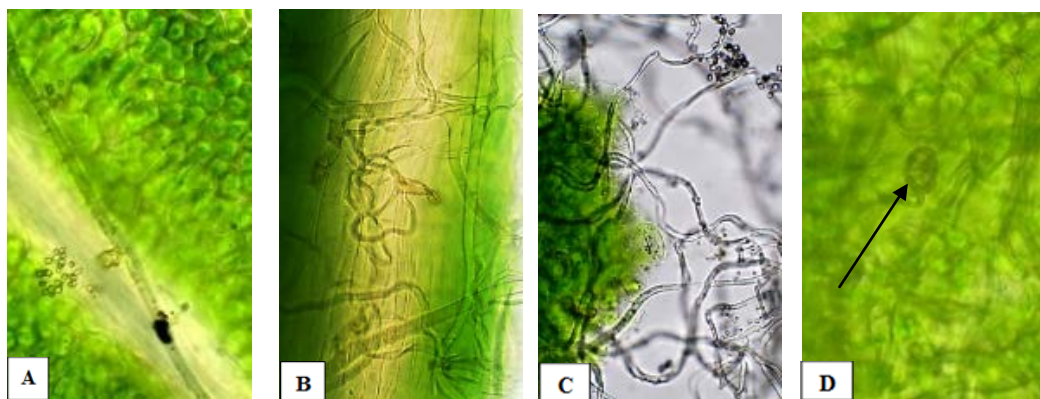


Figure 5. Felon herb (280×):
upper epidermis of the leaf: A –rare hairs along the vein; the lower epidermis of the leaf: B - cephalate and T-shaped hairs along the vein of the leaf; C - stomata at the point of breakage of the leaf limb; D – gland

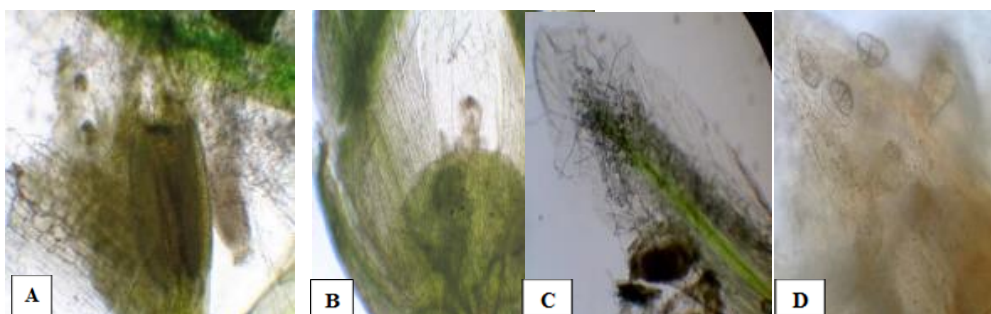


Figure 6. Felon herb: A –flower with anthers (56×); B - floral disk with phyllaries (56×); C –phyllary (56×); D –glands on the bud cover (280×)

When examining the inflorescence (anthodium), separate flowers and a thalamus with phyllaries are visible. The phyllaries are oblong, with filmy edges and a green central part (Figure 6).

Transverse, longitudinal sections were prepared of the pieces of burdock root, and pressed preparations were prepared from heated and not heated pieces. We also collected fresh burdock roots (not subjected to drying and maceration with heating) and fixed them in the alcohol-water-glycerin mixture.

When examining the transverse sections of the roots, the uneven cork line is visible, consisting of several layers of cells with brown walls. Sometimes, the cells (secretory moves) with brown contents were visible in the lower part of the cork. The bark is wide. Many cavities were present in the soaked pieces of roots, and in the freshly-fixed roots there were few such cavities. The primary rays were broad, multirow, widening to the periphery, going almost from the cork through the bark into the wood to the root core. The cambium was seen as a meandering narrow strip of thin-walled slightly brownish cells, separating the core part from the wood one. To the outside of the cambium the phloem (functioning phloem) was visible - the cells were small with strongly sinuous walls, and inside the root, by rays, the vessels and tracheids of xylem were located singly or in small groups in the "cover" from the cells of the parenchyma with slightly thickened walls (Figure 7).

When examining the longitudinal sections, long and short vessels with a porous and reticulate wall and the shorter, narrower tracheids with a retracted sometimes staircase wall were seen (Figure 8).

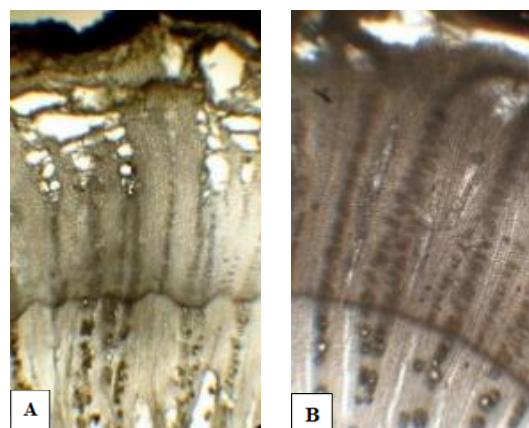


Figure 7. Burdock roots Cross-section (40×)
A - the roots are soaked without heating; B - transverse section of the fresh root



Figure 8. Burdock roots (280×): A –vessels and tracheids in a cross-section; B –vessels and tracheids in a longitudinal section

Inulin forms chords of groups of spheres and hemispheres that are clearly visible in freshly harvested burdock roots fixed in an alcohol-water-glycerin mixture. Small inulin inclusions are found in roots soaked without heating and are invisible in pieces of roots (powdered raw materials) heated for the production of squash preparations (Figure 9).

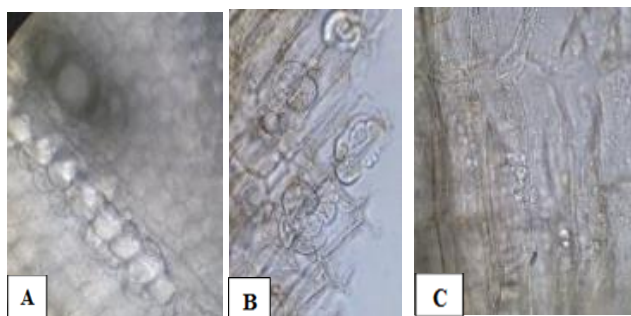


Figure 9. Burdock roots. Inulin aggregates (spheres and hemispheres) (280×):

A – transverse section of the fresh root; B - longitudinal section of the fresh root; C - longitudinal section of the root soaked without heating

When examining the cortex parenchyma, it is also seen that the cells contain droplets (stained with Sudan III) and other inclusions.

When examining a squash preparation, a mesh cork is seen of thin-walled cells with brown walls, parenchyma cells with and without contents, vessels and tracheids (Figure 10).

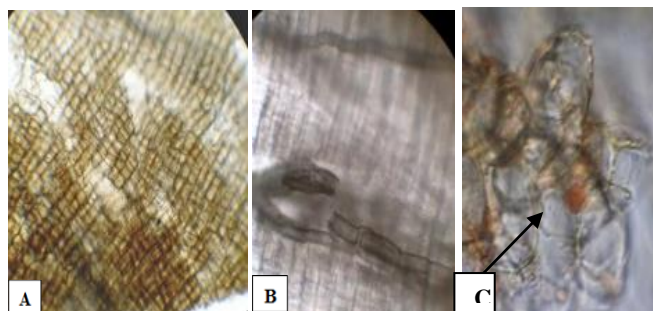


Figure 10. Burdock roots. Squash preparation

A – cork (56×); B – vessel chords (63×); C – fatty oil droplets (Sudan III)

Thus, it was established that all the morphological and anatomical signs of the preparation components corresponded to the regulatory documents and information published in the literature. The photos obtained can be used as an illustrative material of sections of the standard documentation regulating the quality of the Phytourol preparation.

CONCLUSION

1. The morphological and anatomical signs of the components of the preparation developed for the KSD prevention and treatment have been studied and established.
2. The characteristics of the external features of the preparation under study have been given and its microdiagnostic features have been visualized.
3. The obtained results have been used in the preparation of normative documentation regulating the quality of the Phytourol medicinal herbal preparation.

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