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# Study of Chemical Composition of the New Urolithic Herbal Tea

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**Abstract**

A phytochemical study of multicomponent herbal tea for the urolithiasis treatment and prevention was conducted. The initial composition of phytocomposition was selected based on the literature data on pharmacological effects of each component on the body, taking into account the preliminary pharmacological tests data. As a result of this study, the qualitative and quantitative contents of main groups of biologically active compounds included in the herbal tea were determined. The qualitative and quantitative methods of active substances determination were developed to evaluate the herbal urolithic product quality.

## Keywords

Chemical study; Urolithiasis; Medicinal herbs; Medicinal herbal teas

## Introduction

Despite the progress achieved in recent years in the delivery of care to patients with urolithiasis, the prevention and treatment issues continue to be relevant to date. The incidence rate of urolithiasis is not less than 3% in the world and continues to progressively increase. For example, for the period 2002-2009 the absolute number of registered patients with nephrolithiasis has increased by 17.3% in the Russian Federation.

Having said that in the absence of preventive measures a recurrence of stone formation is observed nearly in 40% patients over 3 yrs [1].

Urolithiasis is considered pluricausal disease, since the plurality of etiological factors may be involved in its contraction. This is not one disease, but a large group of disorders and syndromes, not homogeneous on the causes and mechanisms of development, but united by one common factor – stone formation in the urinary organs [2].

In this regard, a pressing task is the development of complex herbal remedies, containing an overall combination of biologically active compounds in their natural form and providing the comprehensive pharmacotherapeutic effect on various links in the disease pathogenesis.

We have proposed a multicomponent herbal tea for the urolithiasis treatment and prevention based on leaves of *Vaccinium vitis-idaea* (*L.*), herb of *Equisetum arvense* (*L.*), roots of *Arctium lappa* (*L.*), fruits of *Anethum graveolens* (*L.*), and herb of *Artemisia vulgaris* (*L.*). The initial composition of our herbal tea was selected based on the literature data on pharmacological effects of each component on the body, taking into account the preliminary pharmacological tests data. The main groups of biologically active substances (BAS), having urolithic effect, are presented by flavonoids, arbutin, tannins, polysaccharides, organic acids, essential oils, and silicon [3-6].

## Materials and Methods

The object of this study is a new multicomponent herbal tea that has the urolithic effect. As objects under study, the water and alcohol extraction of herbal tea were used.

The identification of main groups of BAS was carried out using the commonly accepted qualitative contents [7-9]. Flavonoids were determined by the cyanidin reaction: to 1 ml of water extraction we added 1 ml of 95% alcohol, 0.1 g of magnesium powder, and 1 ml of concentrated hydrochloric acid; red staining was appeared gradually [8].

Arbutin determination was carried out by the qualitative reaction using 2% solution of dichloroquinone-chloroimide in ethyl alcohol, 2% solution of sodium carbonate by the appearance of blue staining [9].

When we added a few drops of ammonium iron alum into the water extraction, the black-and-blue staining was observed (tannins) [8]. When we added double amount of 95% ethyl alcohol into the water extraction of herbal tea, the flocculent clots precipitated upon standing were appeared (polysaccharides) [8]. When we applied some particles of crushed roots, a few drops of thymol 20% alcohol solution, and drops of concentrated sulfuric acid to the scrape, the orange-red staining was observed (inulin) [7]. When you add the Felling reagent to the water extraction and heat it to boiling point, the tile-red sediment settles out (sugars) [8]. During the histochemical reaction with sudan III solution, the drops of essential oil are stained orange [7].

The phytochemical analysis was performed using the qualitative chemical reactions and sorbent thin-layer chromatogram (TLC).

To separate and identify the phenolic compounds, the TLC [10] was carried out on the "Kiesel gel 60 F254" plates by Merk Company in the solvent system of chloroform–ethyl acetate–methanol–5% solution of acetic acid in the water (20:20:20:5). As a reference, solution used 0.05% working standard sample (WSS) solution of apigenin, hyperoside, luteolin-7-glycoside, luteolin, rutin, naringenin, quercetin, gallic acid, chlorogenic acid, and ferulic acid. The detection of adsorption zones was carried out by 5% solution of phosphomolybdic acid in 95% ethanol (after heating at 100-105°C).

To identify the fraction of flavonoids in the object under study by the TLC [11], the analysis was carried out on the "Kieselgel 60 F254"

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plates by Merk Company in the solvent system of isopropanol–formic acid–water in a ratio of (2:5:5). As a reference solution was used as 0.05% WSS solutions of apigenin, hyperoside, luteolin-7-glycoside, luteolin, rutin, naringenin, and quercetin. The visualization of adsorption zones was carried out by 2% solution of aluminum chloride in 95% ethanol (after heating at 100-105°C).

The identification of arbutin by TLC method [9] was carried out on the "Kieselgel 60 F254" plates by Merk Company in the solvent system of formic acid–water–ethyl acetate (6:6:88). As a reference solution was used as the WSS solutions of arbutin, hydroquinone, and gallic acid in methanol. The detection of adsorption zones was carried out, at first, by 1% solution of dichloroquinonechlorimide in methanol and then by 2% solution of anhydrous sodium carbonate.

To determine the free sugars by TLC method [12], the analysis was carried out on "TLC Silicongel 60, E. Merк" plates in the solvent system of pyridine–ethylacetate–water (5:12:4). As a reference solution was used as the WSS solutions of sugars in the ethyl alcohol of glucose, fructose, sucrose, mannose, arabinose, galactose, and rhamnose. The visualization of adsorption zones after treatment with a mixture: thymol-concentrated sulfuric acid-95% ethyl alcohol (0.5:5:95); heating for 10 min in a dry air oven at 100-105°C.

The TLC of free amino acids [13] was carried out on the "Kieselgel 60 F254" plates by Merk Company in the solvent system of *n*-butanol–glacial acetic acid–water (50:25:25). As a reference solution was used 0.05% WSS solutions of amino acids of arginine, glutamic acid, proline, serine, histidine, glycine, methionine, and tyrosine. The visualization of adsorption zones was carried out after treatment by 0.2% solution of ninhydrin in 95% ethanol (after heating at 100-105°C for 5 min).

BAS quantification in the herbal tea of flavonoids was carried out by the spectrophotometric method [14]; of polysaccharides by the spectrophotometric method [15]; of free organic acids by the titrimetric method calculated with reference to malic acid [8]; of tannins by the permanganometric method [7]; of amino acids by the spectrophotometric method [13,16]; of arbutin by the high-performance liquid chromatography [17]; of silicon by the atomic absorption spectrophotometry [18]; fructosans and fructosides calculated with reference to inulin by the spectrophotometric method [19]; and of essential oil by the steam distillation [7].

## Results and Discussion

At the first stage of the study, the qualitative reaction with water and alcohol extraction was carried out. In this composition the presence of following groups of BAS was expected: flavonoids, arbutin, tannins, polysaccharides, and essential oils.

In order to establish the qualitative composition of BAS in the urolithic herbal tea TLC method was used.

The results of chromatographic analysis of water and alcohol extractions from the urolithic herbal tea are shown in Figures 1-5.

As a result of TLC study of phenolic compounds, we could detect seven zones colored in blue and purple with Rf ~ 0.12 (chlorogenic acid); Rf ~ 0.38 (gallic acid), Rf ~ 0.44 (hyperoside); Rf ~ 0.47 (rutin); Rf ~ 0.73 (luteolin); Rf ~ 0.87 (quercetin); and Rf ~ 0.85 (ferulic acid) in the chromatogram. The identified phenolic compounds belong to flavonoids (rutin, hyperoside, luteolin, quercetin) and phenol carbonic acids derivatives (chlorogenic, gallic, ferulic) in their structure (Figure 1).

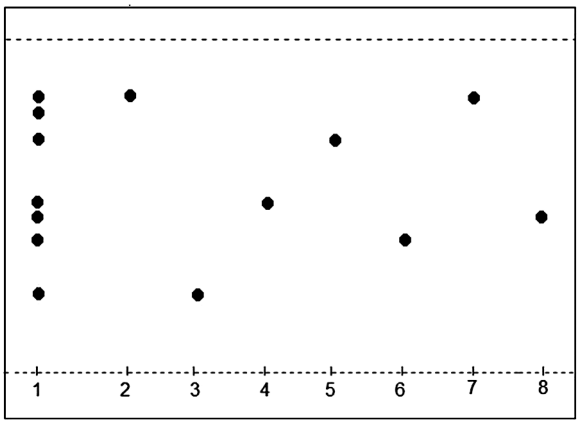


**Figure 1:** Chromatogram scheme of phenolic compounds: 1 – alcohol extraction from the urolithic herbal tea; WSS of: 2 – quercetin; 3 – chlorogenic acid; 4 – rutin; 5 – luteolin; 6 – gallic acid; 7 – ferulic acid; 8 – hyperoside

The identification of flavonoids in the chromatographic analysis after the chromatogram development with an alcoholic solution of aluminum chloride showed us five zones colored in yellow with Rf ~ 0.14 (quercetin); Rf ~ 0.42 (luteolin); Rf ~ 0.70 (hyperoside); Rf ~ 0.78 (rutin); and Rf ~ 0.88 (naringenin) (Figure 2).

The identification process of arbutin using the chromatographic analysis of methyl extraction from the herbal medicinal product is shown in Figure 3. After the chromatogram detection, we observed the occurrence of two blue zones with Rf ~ 0.2 (arbutin), Rf ~ 0.85 (hydroquinone) and a brownish zone with Rf ~ 0.67 (gallic acid).

The results of qualitative composition study of free sugars contained the urolithic herbal tea are shown in Figure 4. In the chromatogram of the herbal tea test sample, three red-violet zones with Rf ~ 0.39 (sucrose); Rf ~ 0.50 (glucose); and Rf ~ 0.55 (fructose) are marked.

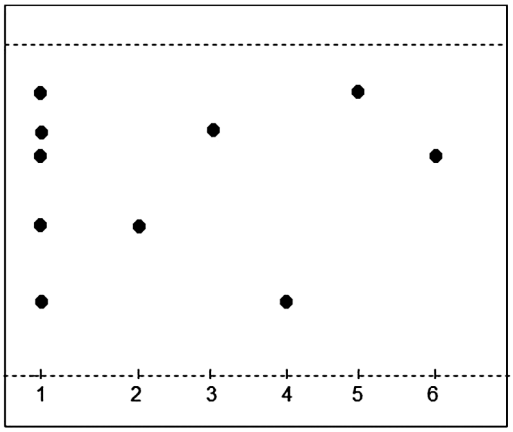


**Figure 2:** Chromatogram scheme of flavonoids: 1 – alcohol extraction from the urolithic herbal tea; WSS of: 2 – luteolin; 3 – rutin; 4 – quercetin; 5 – naringenin; 6 – hyperoside

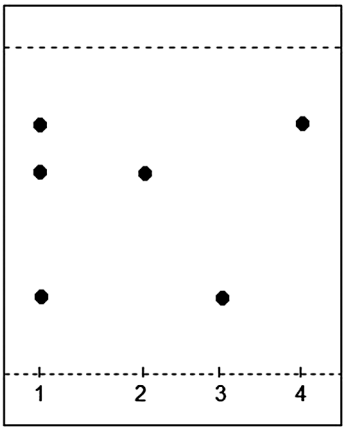


**Figure 3:** Chromatogram scheme of arbutin: 1 – alcohol extraction from the urolithic herbal tea; WSS of: 2 – gallic acid; 3 – arbutin; 4 – hydroquinone

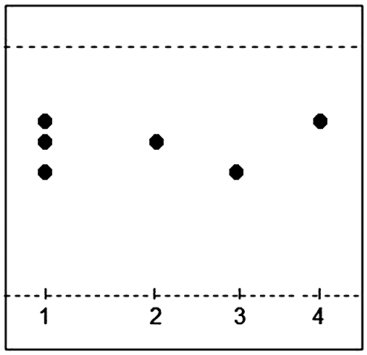


**Figure 4:** Chromatogram scheme of sugar: 1 – alcohol extraction from the urolithic herbal tea; WSS of: 2 – glucose; 3 – sucrose; 4 – fructose

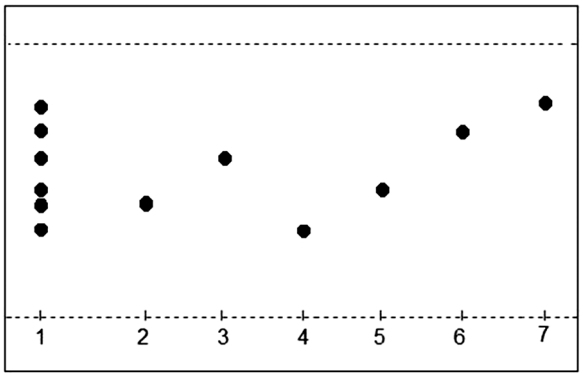


**Figure 5:** Chromatogram scheme of amino acids: 1 – water extraction from the urolithic herbal tea; WSS of: 2 – glycine; 3 – glutamic acid; 4 – proline; 5 – serine; 6 – methionine; 7 – tyrosine

| Name of the BAS | Content (%) |
|---|---|
| Flavonoids | 0.46 ± 0.01 |
| Arbutin | 0.79 ± 0.02 |
| Polysaccharides | 10.28 ± 0.19 |
| Tannins | 3.38 ± 0.08 |
| Organic acids | 1.09 ± 0.13 |
| Amino acids | 0.94 ± 0.01 |
| Essential oils | 0.33 ± 0.03 |
| Fructosans and fructosides | 1.78 ± 0.02 |
| Silicon | 0.16 ± 0.02 |

**Table 1:** BAS content in the urolithic herbal tea

The results of chromatographic analysis of free amino acids due to the water extraction from the phytocomposition are shown in Figure 5. In the chromatogram of the test sample, six colored zones with Rf ~ 0.39 (proline); Rf ~ 0.46 (glycine); Rf ~ 0.48 (serine); Rf ~ 0.56 (glutamic acid); Rf ~ 0.65 (methionine); and Rf ~ 0.72 (tyrosine) are identified.

Also, we found out the presence of phenolic compounds, flavonoids, arbutin, free sugars, and free amino acids in the herbal tea composition.

Based on the data obtained by the qualitative composition of the object under study, we conducted the BAS quantification under the proposed methods. The results are shown in Table 1.

## Conclusions

Based on phytochemical study of urolithic herbal tea, the following can be concluded:

1. Using the qualitative reactions and TLC methods, we revealed the presence of flavonoids (rutin, hyperoside, luteolin, quercetin, naringenin), phenol carbonic acids derivatives (chlorogenic, gallic, ferulic), free sugars (sucrose, glucose, fructose), arbutin, free amino acids (proline, glycine, serine, glutamic acid, meteonin, tyrosine), tannins, polysaccharides, inulin, and essential oil.
2. The quantitative methods of the herbal tea standardization on the content of polysaccharides, amount of flavonoids, fructosans and fructosides, amino acids by the spectrophotometric method; of free organic acids by the titrimetric method; of tannins by the permanganometric method; of arbutin by the high-performance liquid chromatography; of silicon by the atomic absorption spectrophotometry; and of essential oil by the steam distillation were proposed.
3. The quantitative analysis detected the content of basic groups of BAS: flavonoids – 0.46 ± 0.01%, arbutin – 0.79 ± 0.02%, polysaccharides – 10.28 ± 0.19%, tannins – 3.38 ± 0.08%, organic acids – 1.09 ± 0.13%, amino acids – 0.94 ± 0.01%, essential oils – 0.33 ± 0.03%, fructosans and fructosides – 1.78 ± 0.02%, silicon – 0.16 ± 0.02%.
4. The developed methods of qualitative detection and quantitative determination of BAS can be used to evaluate the quality of our proposed urolithic herbal tea and included in the appropriate regulatory documentation.

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