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Standardization of the Complex Urological Herbal Medicine *Phytourol*

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Abstract

Much attention is paid to the development and standardization of multicomponent preparation based on pharmaceutical substances of vegetable origin. Currently, there is a limited choice of vegetable compositions having comprehensive influence on the etiology and pathogenesis of kidney stone disease as well as of those, which can be used as effective drugs for the preventive therapy of urolithiasis, taking into account the chronic nature of the disease and the high frequency of relapses. Based on a review of the literature data, and pharmacological research, the complex Phytourol urological preparation was developed. This article presents the results of the research on the development of methods for qualitative and quantitative determination of biologically active substances of the initial preparation, which were included in the draft regulatory documentation for the Phytourol urological preparation.

Keywords: medicinal herb preparations, medicinal plants, urolithiasis, standardization.

INTRODUCTION

According to the medical statistics in recent decades, there has been a steady growth of urologic disease of the population due to one of the major reasons - the lack of efficiency of the disease prevention system [1].

The kidney stone disease (KSD, urolithiasis) holds one of the leading places within the structure of urological diseases [2-4]. Patients with KSD in urological hospitals account for 30 to 50% of the total number of patients [5, 6]. KSD leads to long-term disability and invalidization of the population. In addition, the high recurrence rate is a characteristic feature of the disease [7-10]. The effective therapy and prevention of KSD include interventions aimed at addressing the etiological factors of the disease and pathogenetic conditions of lithiasis [11, 12]. 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 repertories possessing a 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].

Today, 4 plant preparations recommended in the comprehensive treatment of urological diseases are registered in the State Registry of Medicinal Remedies [15]. No plant compositions intended for KSD conservative treatment and prevention were registered and officially approved. Thus, there is a need to expand the line of Russian-made complex herbal preparations that will ensure an individual approach to the choice of the pharmacotherapy of the urinary tract diseases and extend the potential of the KSD conservative treatment.

Therefore, the development and implementation of the original preparation designed for KSD conservative treatment and prevention are of priority.

We have developed the complex Phytourol urological preparation consisting of drug plants (DP) for conservative urolithiasis treatment and prevention, as follows: cowberry leaves, horsetail grass, burdock roots, sulfur root fruits and felon herb. The drug plants of the preparation under study have long been used in traditional and official medicine as diuretic, antispasmodic, antiseptic, anti-inflammatory, choleretic agents and have a well-fixed raw material base. Medicinal plants included in the proposed preparation contain flavonoids, phenolic acids, phenolglycoside arbutin, tannins, coumarins, polysaccharides (inulin) and essential oils.

The purpose of this work is to develop techniques for qualitative and quantitative determination of biologically active substances of the complex urological Phytourol preparation.

MATERIALS AND METHODS

The object of the study was the complex urological Phytourol preparation containing the following drug plants: cowberry leaves, horsetail grass, burdock roots, sulfur root fruits, and felon herb.

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 (OFS. 1.4.1.0020.15).

Determination of the content of extractive substances extracted by water in the preparation under study was carried out according to the procedure of the general pharmacopoeia article "Determination of the content of extractive substances in drug plants and herbal preparations" of the State Pharmacopoeia of the Russian Federation, the 13th edition (OFS. 1.5.3.0006.15).

The quantitative determination of arbutin was carried out by iodometric titration according to the State Pharmacopoeia, 11th ed., Vol. 2, Art. 27.

Physicochemical methods of analysis were used for qualitative and quantitative determination of BAS (biologically active substances) in the preparation: thin-layer chromatography (TLC), gas-liquid chromatography (GLC), high-performance liquid chromatography (HPLC), spectrophotometry (SPM).

The BAS analysis in the preparation was carried out by TLC on Kieselgel 60 F254 plates, TLC Silicagel 60 F254 (Merck, Germany) measuring 20×20 cm, 15×20 cm. The R_f

values of the substances identified are the average of five measurements.

The component composition of the essential oils obtained from the preparation was carried out by GLC method on the Crystallux-4000M (Meta-chrome, Russia) chromatograph, followed by computer processing of the study results using the NetChrom, V2.1 program for Windows. The HP-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm) filled with 5%-phenyl-95%-methylpolysiloxane was used as a stationary phase. Nitrogen was used as the mobile phase. The flow rate was 1 ml/min, the temperature: evaporator - 200 °C, detector - 250 °C. The initial thermostat temperature at the time of the analysis beginning was 100 °C. During the experiment, it changed at a rate of 5 deg/min and reached 150 °C. The detector was of the flame-ionization type. The analysis duration was 10 min. The sample volume was 1 μl of the solution with a 1:27 flow separation.

The HPLC analysis of phenolic compounds, organic acids, sugars and arbutin was carried out on a high-performance Gilson liquid chromatograph (Gilson, France) with a Rheodyne manual injector, model 7125 (Rheodyne, USA), followed by computer processing of the results using the Multichrom program for Windows. The Kromasil C18 metal column measuring 4.6×250 mm, 5 microns was used as a stationary phase for phenolic compounds and arbutin; and the Altech OA-1000 OrganicAcids metal column measuring 6.5×300 mm was used for sugars and organic acids. The methanol-water-phosphoric acid (concentrated) (400:600:5) was used as the mobile phase for phenolic compounds and arbutine; and 0.005 M sulfuric acid solution was used for sugars and organic acids. The study was carried out at room temperature. The eluent flow rate and the analysis duration for phenolic compounds and arbutin were 0.8 ml/min and 70 min; for sugars and organic acids - 1 ml/min and 20.38 min, respectively. The sample volume was 50 μl. Detection was carried out with the Gilson UV/VIS UV detector model 151, at a wave length for phenolic compounds and arbutin - 254 nm; for sugars and organic acids - 190 nm.

The spectrophotometric analysis of phenolic compounds, fructosans and fructosides was carried out on a HeliosAlfa self-recording spectrophotometer (SpectronicUnicam, Great Britain). A cuvette with a layer thickness of 10 mm was also used. The validation evaluation of spectrophotometric techniques was additionally carried out on the Cary100 Scan (Varian, USA) and SchimadzuUV-1800 (Schimadzu, Japan) spectrophotometers.

The standard samples (SS) from Sigma-Aldrich were used in the methods of qualitative and quantitative determination of BAS: luteolin-7-glycoside (CAS No. 5373-11-5), arbutin (CAS No. 84380-01-8), rutin (CAS No. 207671-50-9), quercetin (CAS No. 6151-25-3), caffeic acid (CAS No. 331-39-5), gallic acid (CAS No. 5995-86-8), chlorogenic acid (CAS No. 327-97 -9), luteolin (CAS No. 491-70-3), kaempferol (CAS No. 520-18-3), apigenin (CAS No. 520-36-5), hyperoside (CAS No. 482-36-0) and fructose (CAS No. 57-48-7).

The validation of the methodologies in accordance with the general pharmacopoeia article of the State pharmacopoeia of the Russian Federation, 13th edition, according to GOST and methodical instructions was carried out according to the following criteria: accuracy, precision (convergence and reproducibility), specificity, linearity.

Results And Discussion

Using UV spectrophotometry techniques, TLC, the presence of the major biologically active substances being the part of the preparation drug plants was set and confirmed, namely: flavonoids (rutin, hyperoside, luteolin, quercetin, luteolin-7-glucoside, naringenin), phenolic carboxylic acid (gallic), oxycinnamic acids (chlorogenic, ferulic), free sugars (sucrose, glucose, fructose), phenolic glucoside (arbutin), tannins and polysaccharides (inulin).

The HPLC analysis of phenolic compounds in the preparation allowed confirming the presence of those previously identified phenolic compounds and also determining the following: phenolic carboxylic acid (ellagic); oxycinnamic acids (neochlorogenic, isoferulic, coffee, chicory, cinnamic acids); derivatives of tannins (epicatechin, catechin, epigallocatechin gallate); coumarins (dicoumarin, methoxycoumarin) (Table 1).

Table 1. Component composition of the phenolic compounds of the Phytourol preparation, determined by the HPLC method

Item No.	Component	Retention time, min	Peak height,mV	Peak area,mV*s
1.	Arbutin	3.543	750.23	13,571.88
2.	Unidentif.	3.878	353.71	3,021.63
3.	Gallic acid	4.072	485.42	8,342.63
4.	Catechin	4.541	219.65	3,576.67
5.	Unidentif.	4.718	200.92	1,642.30
6.	Epigalocatechin gallate	4.986	278.92	3,753.88
7.	Unidentif.	5.146	293.81	3772.91
8.	Epicatechin	5.337	216.18	2,920.52
9.	Chlorogenic acid	5.759	136.84	2,667.54
10.	Chichoric acid	6.021	131.34	1,595.12
11.	Unidentif.	6.248	161.40	4,090.54
12.	Caffeic acid	6.829	120.17	3,652.42
13.	Unidentif.	7.414	61.75	1,132.44
14.	Neochlorogenic acid	7.75	65.08	1,941.11
15.	Unidentif.	8.405	76.25	3,578.12
16.	Dicoumarin	9.432	37.33	1,569.26
17.	Ferulic acid	10.33	43.19	1,706.49
18.	Isoferulic acid	11.47	44.86	2,302.84
19.	Unidentif.	12.14	40.47	1,452.85
20.	Unidentif.	13.16	84.92	4,860.71
21.	Luteolin-7-glycoside	16.06	160.85	16,032.53
22.	Hyperoside	18.66	83.55	8130.76
23.	Rutin	19.39	76.66	5996.24
24.	Unidentif.	22.25	88.47	9720.98
25.	Unidentif.	24.28	75.52	8,531.89
26.	Unidentif.	25.81	64.77	3,518.88
27.	Ellagic acid	26.62	64.91	5893.48
28.	Unidentif.	27.98	61.67	9,245.05
29.	Cinnamic acid	31.29	50.78	8,452.20
30.	o-methoxycoumarin	34.44	38.71	6,261.28
31.	Naringenin	39.51	41.81	12,778.97
32.	Quercetin	45.69	58.69	18,180.92
33.	Luteolin	51.61	21.70	5,202.74

The presence of organic acids (succinic acid, oxalic acid) and free sugars (fructose, glucose) was also determined in the water extraction of the Phytourol preparation by HPLC. Using the GLC method in the essential oil obtained from the preparation, limonene, camphor, geraniol, anethole, bornilacetate were established.

Development of methodology for the quantitative determination of the sum of flavonoids in the preparation

To quantify the amount of flavonoids in the preparation under study, a spectrophotometric technique based on the flavonids' complex formation reaction with aluminum chloride was used. As a standard sample, luteolin-7-glycoside was used, which, with aluminum chloride, had a similar curve and a near maximum absorption in one region of 402±5 nm with the maximum absorption of the flavonoids' preparation with the use of the same complex formation agent (Fig. 1).

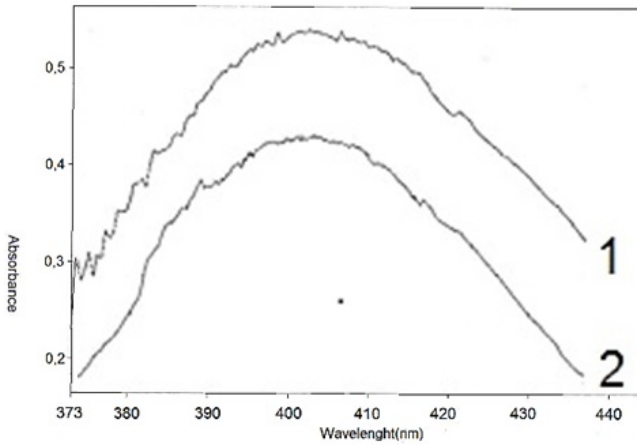

Fig. 1. Absorption spectra of flavonoid complexes of the preparation under study (1) and SS of luteolin-7-glycoside with aluminum chloride (2)

To select the optimal conditions for the methodology, the influence of several parameters was studied, namely: the nature of the solvent, the crushing of the raw material, the ratio of the raw material and the extractant, and the extraction time and frequency. It has been found during the experiment that optimal analysis conditions are the following: extractant - 70% alcohol, the ratio of raw material and extractant - 1:10, crushing of the preparation - 2 mm, and double extraction for 60 minutes.

The content of the amount of flavonoids in the preparation under analysis in terms of luteolin-7-glycoside and absolutely dry raw materials in percent (X) shall be calculated by the following formula:

$$X = \frac{D^* \times 100 \times 25 \times M \times 1 \times 100 \times 100}{D \times M^* \times 100 \times 1 \times 25 \times (100 - W)}$$

where

D* is theoptical density of the solution tested;

D isthe optical density of SS of luteolin-7-glycoside;

M* is thesample weight of the preparation analyzed, g;

M is themass of SS of luteolin-7-glycoside, g; and

W is theweight loss during drying of the preparation under study, %.

The methodology developed has been validated according to the following parameters: accuracy, precision (convergence and reproducibility), specificity and linearity. Based on the results obtained, the methodology developed can be considered as validated.

The content of extractives extracted by water and the main BAS groups were defined in the preparation (Table 2):

Table 2. Quantitative content of the extractives and BAS in the preparation under study (n=5, p=0.95)

No.	Content, %						
	Extractants extracted by water	Sum of flavonoids	Arbutin	Sum of polysaccharides (inulin)	Essential oil	Sum of tannins	Sum of organic acids
1	21.2	0.46	1.39	1.77	0.36	3.38	1.02
2	23.7	0.47	1.49	1.72	0.34	3.43	1.07
3	21.1	0.45	1.48	1.83	0.33	3.39	1.10
4	22.7	0.47	1.35	1.84	0.35	3.34	1.07
5	23.1	0.48	1.41	1.74	0.33	3.33	1.03
aver age	22.36± 1.44	0.466± 0.01	1.42± 0.07	1.78± 0.06	0.34± 0.02	3.37± 0.05	1.06± 0.04

The studies have identified that the quantitative content of extractives in the Phytourol preparation samples varies from 21.1 to 23.7%, flavonoids - from 0.45 to 0.48%, arbutin - from 1.35 to 1.49%, polysaccharides (inulin) - from 1.72 to 1.84%, essential oil - from 0.33 to 0.36%, tannins - from 3.33 to 3.43%, and organic acids - from 1.02 to 1.10%.

Taking into account the pharmacological focus of the preparation, the standardization based on quantitative contents of flavonoids and amount of arbutin is advisable. Assuming that the Phytourol preparation is intended for the preparation of aqueous extracts, it is rational to make standardization based on the content of extractives extracted by water. In the light of the data and the statistical analysis results, it was suggested to establish that the content of extractive substances extracted by water should be at least 21%, the content of the amount of flavonoids in terms of luteolin-7-glycoside - not less than 0.4%, and the content of arbutin - not less than 1.3%.

CONCLUSION

1. The composition and content of BAS was determined in the preparation under study as follows: flavonoids 0.47±0.01%, arbutin 1.4±0.07%, polysaccharides (inulin) 1.78±0.07%, tannins 3.37±0.05%, organic acids 1.06±0.04%, essential oils 0.34±0.02%, coumarins and free sugars.
2. In our opinion, the Phytourol preparation should be standardized based on quantitative contents of flavonoids, arbutin and extractive substances extracted by water.
3. The results obtained were used to develop the sections of the draft regulatory documentation for the complex Phytourol urological preparation.

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