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Study Of The Chemical Composition Of The Liquid Extract "Extradent"

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ABSTRACT

The quantitative content of flavonoids of the aerial part of the plant was determined by high performance liquid chromatography (HPLC). Using the inductively coupled plasma mass spectrometry (ICP-MS) method, the quantitative content of vital macro-and microelements in the various vegetative organs of the plant was determined.

KEYWORDS

Flavonoids, microelements, macroelements, HPLC, ICP/MS.

INTRODUCTION

The rich plant resources of Uzbekistan, the centuries-old traditions of using medicinal plant raw materials (MPR) and preparations based on it in medicine cause interest in phytopreparations.

Medicines, including medicinal products, are included in the list of products subject to

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mandatory certification, which implies testing (quality control). Ensuring the proper quality of medicinal products largely depends on the correct organization of control, its effectiveness and efficiency, as well as on the level of requirements laid down in regulatory documents (ND) and the analysis methods used.

At the Department of Pharmaceutical Chemistry, we have developed a new liquid extract for periodontitis from four plants: herbs of peppermint (Polygonum hidropiper L.), shepherd's purse (Bursa pastoris), calendula flowers (Calendulae officinalis) and nettle leaves (Urtica dioica L.).

The raw materials of these plants havebeenused in scientific and folk medicine for many hundreds of years. According to the literature and our own data, the raw materials of these plants are rich in biologically active substances such as flavonoids (quercetin, isorhamnetin, rutin, lutiolin, hyperoside) vitamins B, C, K, polysaccharides, terpenoids and microelements [1-3].

In dental practice, gum diseases, including paradental disease, are more common, given the steady increase in the incidence of this pathology in the local population, it is important to create domestic medicines for both treatment and prevention.

Studies carried out by us earlier showed that preparations from the herb of Knotweed and nettle leaves are characterized by the presence of a hemostatic effect and antimicrobial action [4-8].

The literature data of the above plants were studied, in which rich biological active substances were revealed [9-12]. The creation of effective drugs always begins with the study of the chemical composition of medicinal plant raw materials, as well as with the development of methods for the standardization of raw materials and preparations [13-16].

MATERIALS AND METHODS

Study of proteins

To obtain a liquid extract, the raw materials collected in May 2020 were used. The liquid extract was obtained in the ratio of "raw material-extractant" 1:1 on the basis of 70% ethyl alcohol by the percolation method.

First, the composition of the protein in the preparation was studied. Apparatus, materials and reagents. Determination of protein content was determined by a standard method. We used an analytical balance (0.0001), filter paper, conical funnel, FEK, sodium hydroxide, signet salt, Nessler's reagent, distilled water, concentrated sulfuric acid, concentrated hydrogen peroxide.

The study of protein substances is carried out by various methods. The results will vary depending on the method used. However, all methods of studying proteins are reduced to the following. To isolate proteins, biological material is crushed until the cell walls are destroyed, receiving a homogenate. Then proceed to the extraction of proteins.

To determine the protein content in the isolated fractions, an aliquot of them was taken into a heat-resistant flask (from 5 to 10 ml). Concentrated sulfuric acid H_2SO_4 (1.84 g / cm3) was poured into heat-resistant flasks, to a sampled sample or to an aliquot of the fraction. The flasks were placed in a sand bath, setting the temperature equal to 400 °C. At the same time, it is necessary to avoid violent boiling. Distilled water was carefully poured

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into cooled flasks along the walls and quantitatively transferred into a volumetric flask with a capacity of 50 ml. After cooling, the volume in the flasks was brought to the mark and mixed thoroughly. From a volumetric flask, after mineralization, to determine the protein content by nitrogen, an aliquot was taken, depending on the expected protein content. At a high nitrogen content in the samples, dilution was carried out. To the selected aliquot, up to half the volume of distilled water was added. Then the solution was neutralized. And added 1 ml of Nessler's reagent. The solutions in the flasks were brought to the mark with water and mixed thoroughly. In this case, the solutions should be completely transparent. 15 minutes after painting, the solutions were measured on an electro-photocolorimeter EFK-3 [Ermakov].

Study of flavonoids.

Sample preparation for HPLC.

Flavonoids were studied by thin layer chromatography. The study was carried out on Sigma-Aldrich plates using solvent systems: methanol-chloroform-benzene (1: 19: 1) and n-butanol-ethanol ammonia (7: 2: 5).

The results of TLC studies of the liquid extract indicate that the dominant flavonoid in the liquid extract is robinin and rutin. Also, the qualitative and quantitative determination of flavonoids was studied by HPLC. As a result, the largest amount was made up of flavonoids like robinin and rutin.

In the course of the experiment, we used solutions of the State Standard Samples (GSO) of quercetin and rutin. Quantitative determination of the amount of flavonoids in the liquid extract was carried out using the HPLC method.The detection of substances was carried out by viewing the chromatograms in UV light at a wavelength of 254 and 366 nm, as well as by development with an ammonia solution.

Conditions for chromatographic analysis:

Chromatograph - Agilent Technologies 1200 with autosampler.

Mobile phase (gradient mode) - acetonitrile: acetate buffer pH = 2.92 (4%: 96%) 0-6 min., (10%: 90%) 6-9 min., (20%: 80%) 9-15 ., (4%: 96%) 15-20 min;

Column - Eclipse XDB - C18, 4.6x150mm, 3.0 µm

Injection volume - 20 µl;

Mobile phase speed - 1.0 ml / min,

Detector - diode-matrix, wavelength 370 nm.

Study of macro and microelements.

The elemental composition of the extract was studied on a NEXION-2000 ICP MS instrument, inductively coupled plasma mass spectrometry. Sample preparation was carried out by the method of wet acid microwave ashing. 0.0500-0.5000 g, an exact weighed portion of the test sample is weighed on an analytical balance and transferred to Teflon autoclaves. Then the autoclaves are filled with a corresponding amount of purified concentrated mineral acids (nitric acid (chemically pure) and hydrogen peroxide (chemically pure)). The autoclaves are closed and placed on a Berghof microwave digester with MWS-3 + software or a similar type of microwave digester. Determine the decomposition program based on the type of test substance, indicate the degree of

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decomposition and the number of autoclaves (up to 12 pcs).

After decomposition, the contents in the autoclaves are quantitatively transferred into 50 or 100 ml volumetric flasks and the volume is adjusted to the mark with 0.2% nitric acid. The determination is carried out on an ICP MS device or a similar device (ICP OES) optics of an emission spectrometer with an inductively coupled argon plasma.

In the construction of the sequence of analyzes, the amount in mg and the degree of its dilution in ml are indicated. After receiving the data, the true quantitative content of the substance in the test sample is automatically calculated and entered by the device in the form of mg / kg or μg / g with error limits - RSD in %. Used instruments and utensils: ICP MS NEXION-2000 or a similar mass spectrometer, a microwave decomposition device (Germany) or similar Teflon autoclaves, volumetric flasks

Reagents used: multi-element standard No. 3 (for 29 elements for MS), standard for –Hg (mercury), nitric acid (c / h), hydrogen peroxide (c / h), bidistilled water, argon (gas purity 99.995%) ...

RESULTS AND THEIR DISCUSSION

The results of studying the amount of protein in the liquid extract are shown in Table 1.

Table 1.

Protein analysis results

Experiment	Weight, g	Aliquots, ml	Wavelength 400 nm	Protein,%	Average value,%
Alcohol extract					
1	0,8968	0,2	0,167	5,61	**5,56**
2	0,8854	0,2	0,161	5,52	

According to this table, the amount of protein in the liquid extract was found to be 5.56%, and the nitrogen content was 0.89%.

Analysis of the chromatograms presented in Figures 1 and 2 shows that the peaks with a retention time of 3.12; 3.49 and 17.91 minutes correspond to robinin, rutin and quercetin.

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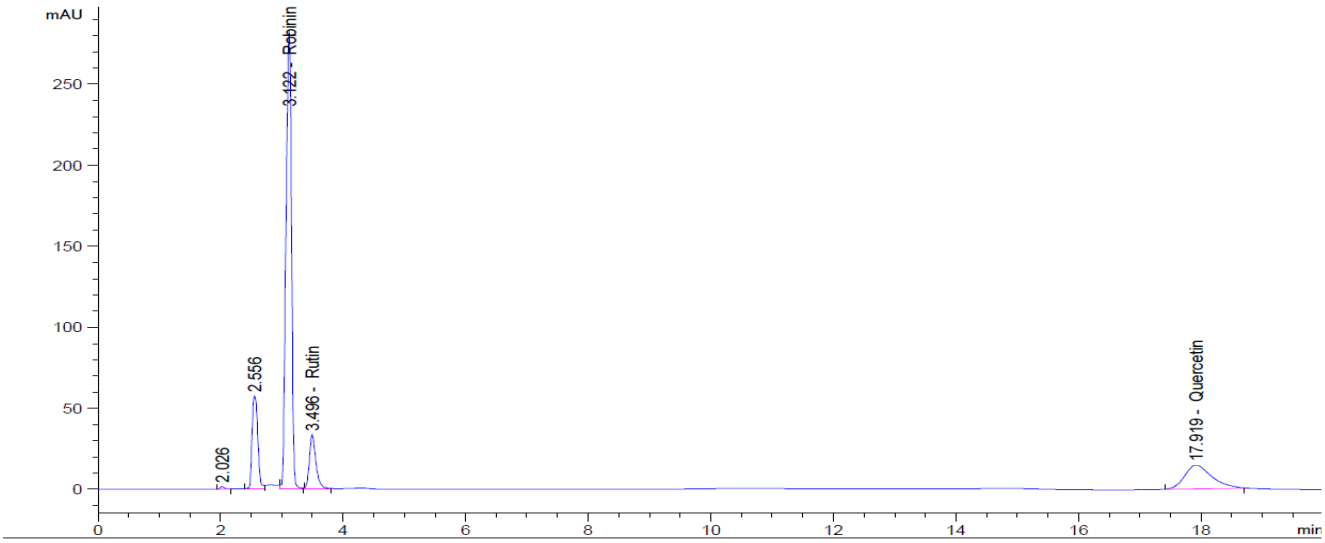

Fig. 1. Chomatogram of mixtures of standard samples.

The identification of flavonoids was carried out by comparing the retention time and peak area of the RCO of rutin, robinin, and quercetin, which was simultaneously subjected to chromatography under the developed conditions (Fig. 1), and the peak obtained on the studied chromatogram (Fig. 2).

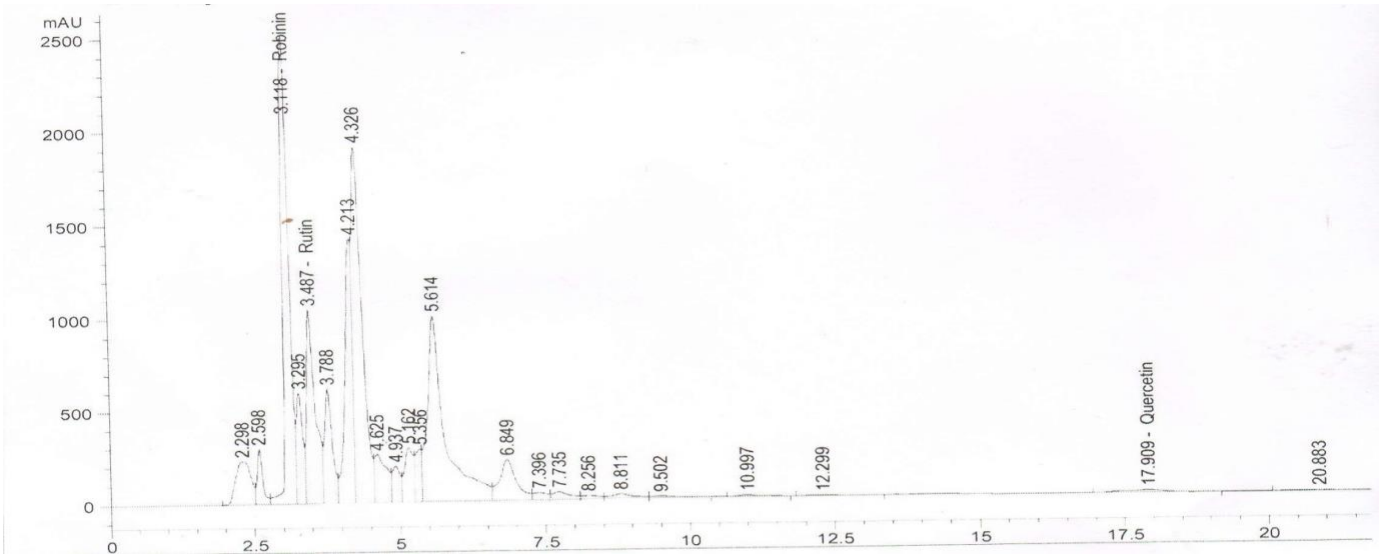

Figure: 2. Chromatogram of the liquid extract "Extradent"

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The rutin content in the liquid extract was calculated using the formula:

$$X = \frac{S \cdot V_1 \cdot a_{ст.} \cdot 100}{S_{ст.} \cdot a \cdot V_2}$$

where, S, Sst - areas of peaks of flavonoids of the test sample and a solution of mixtures of working standard sample, mAu * sec;

V1 is the volume of the solution of mixtures of working standard sample, ml;

V2 — dilution volume of the test sample, ml;

a, ast — weighed portions of the test sample and working standard sample, gr;

Table 3.

Metrological characteristics of the HPLC method for the quantitative determination of flavonoids in the liquid extract "Extradent" (at f=4; t=2,78; P=95%)

№	Content,%	Metrological characteristics of the method					
		$X_{ср.}$	S^2	S	ΔX	$\Delta X_{ср}$	±ε,%
Robinine							
1	0,3776 0,3776 0,3772 0,3770 0,3770	0,3771	0,000000092	0,00030	0,0116	0,0052	0,22
Rutine							
2	0,0146 0,0146 0,0147 0,0145 0,0145	0,0076	0,000000071	0,00008 3	0,0034	0,0015	1,59
Quercetin							

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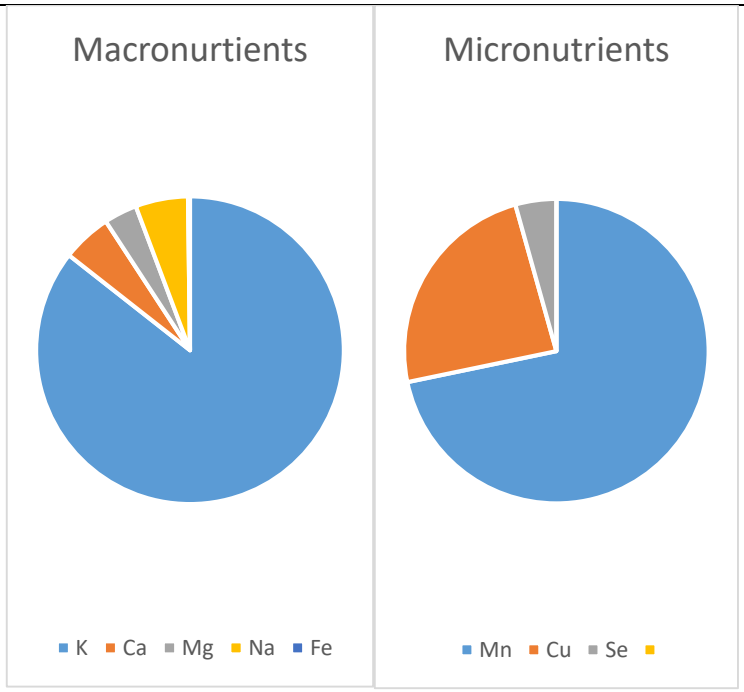
3	0,00045 0,00049 0,00046 0,00041 0,00039	0,00045	0,000000012	0,00001 6	0,0008	0,00071	1,94

As can be seen from the results presented in Table 3, the content of rutin in the herb and motherwort tincture averages 0.296 and 0.032%, respectively, the average relative error of the HPLC method under the selected conditions was 0.22%.

Table 4.

Content of macro- and microelements of liquid extract "Extradent"

№	The elements	Quantitative content mg/kg
1	K	6510,605
2	Ca	395,331
3	Mg	264,437
4	Na	420,249
5	Fe	15,716
6	Mn	0,759
7	Cu	0,253
8	Se	0,046



From the data given in Table 4. It can be seen that the largest amount is observed in macro elements such as potassium (6510.0 mg/l), calcium (395.3 mg/l), magnesium (264.4 mg/kg), sodium (420.2). Which are essential minerals and are stored in large quantities in the body. Of these, the most important element is calcium, which ensures the strength of bones and teeth, activates enzymes, is involved in the regulation of blood pressure, helps muscles to contract, and to transmit a signal to nerves.

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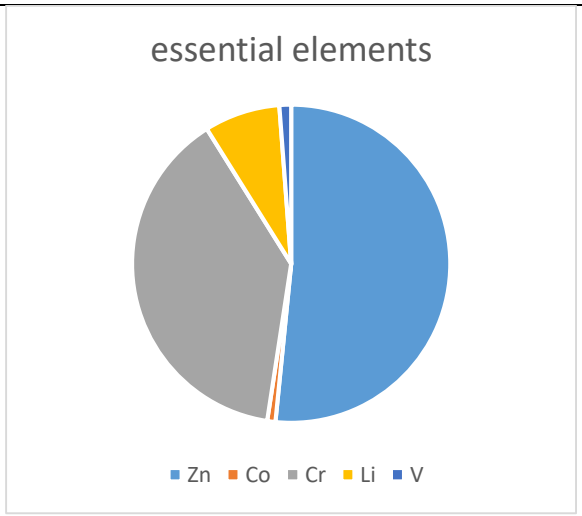
Of the trace elements, copper has the greatest amount of manganese and selenium. Trace elements act in the body by entering in one form or another and in small amounts into the structure of biologically active substances, mainly enzymes (enzymes).

Table 5.

The content of the essential elements of the liquid extract "Extradent"

№	The elements	Quantitative content mkg/kg
1	Zn	0,864
2	Co	0,014
3	Cr	0,648
4	Li	0,129
5	V	0,020



There are also such essential elements as zinc, lithium, vanadium, chromium, which perform a number of functions: they are part of enzymes, provide stabilization of complex protein structures, nucleic acids and membranes and are absolutely necessary at any stage of human development.

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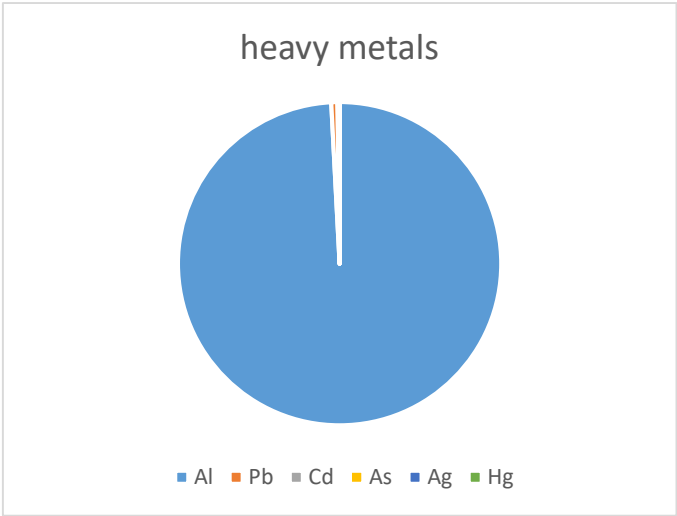
Table 6.

The content of salts of heavy metals of the liquid extract "Extradent"

№	The elements	Quantitative content mkg/kg
1	Al	4,923
2	Pb	0,031
3	Cd	0,002
4	As	0,005
5	Ag	0,003
6	Hg	0,0001



As part of the "Extradent" tincture you can observe salts of heavy elements such as mercury, arsenic, lead, cadmium and aluminum in small amounts. Elemental analysis data showed that the content of heavy metal salts in the liquid extract complies with the norms of Sanitary Nutritional StandardsNo. 0366-19. Hygienic food safety standards.

Thus, based on the data obtained, the following conclusions can be drawn.

CONCLUSIONS

A liquid extract was obtained from the above mentioned plants. Biologically active substances such as flavonoids, elemental composition and quantitative content of total protein have been studied. An HPLC method has been developed for the identification of flavonoids, which will be proposed as an alternative method for standardizing the liquid extract "Extradent", and can also be used for stepwise control of the production of this DF.

REFERENCES

1. Kurkina A.V. New approaches to standardization of water pepper (Polygonum hydropiper L.) raw materials // Fundamental research. - 2013. - No.10. – P.606-609;
2. Seo Sh, Lee Sh, Cha Ph, Kim MY, Min do S, Choi KY. Polygonum aviculare L. and its active compounds, quercitrin hydrate, caffeic acid, and rutin, activate the Wnt/β-catenin pathway and induce cutaneous wound

healing. //Phytother Res. 2016 May;30 (5):848-54. doi: 10.1002/ptr.5593. Epub 2016 Mar 1.

3. Yunuskhodzhaeva N.A., Abdullabekova V.N. Investigation of the chemical composition of the grass POLYGONI AVICULARIS L. // "Chemistry of vegetable raw materials" 2012 y., No. 4., pp. 187-191.
4. Yunuskhodzhaeva N.A., Saidalieva F.A., Kazantseva D.S. Hemostatic properties of the collection from medicinal plants of Knotweed, Knotweed and nettle "" Infection, immunity and pharmacology "2012.- No. 4.- P.76-79.
5. Yunuskhodzhaeva N.A., Abdullabekova V.N., Ibragimova K.S., Mezhlumyan L.G. «Amino-acid composition of Urtica dioica leaves and Polygonum hydropiper and P. aviculare» // Chemistry of Natural Compounds, 2014. -Vol. 50 (5), -P. 970-971. DOI 10.1007/s10600-014-1137-z.
6. Suman Halder and Amit Sharma / A review on Urtica Dioica L // World journal of pharmacy and pharmaceutical sciences - 2017.Volume 6, Issue 10, P.404-421
7. Trineeva OV, Slivkin AI, Safonova EF Determination of hydroxycinnamic acids, carotenoids and chlorophyll in the leaves of stinging nettle (URTICA DIOICA L.) // Chemistry of vegetable raw materials. -2015, -№3.-P.105-110.
8. Balogazyan A., Kurkin V.A., Pravdivtseva O.E., Orekhova A.D. Comparative phytochemical analysis of raw materials of stinging nettle and its main impurities // Questions of biological, medical and pharmaceutical chemistry No. 12, v.19, -2016. Pp. 15-18
9. Амінокислотныйскладтрави Polygonum hydropiper L., Polygonum persicaria L. флориУкраіни/ І. А. Лукіна [идр.]. //Актуальніпитанняфармацевтичної медичноїнаукитапрактики. - 2015. - №1 (17). – С. 56-59.
10. Yunuskhodjaeva N.A., Abdullabekova V.N., Eshbakova K.A. Licviritine and cynnarozide from Polygonum aviculare L //7-th International Symposium on the Chemistry of Natural Compounds: Abstracts. – Tashkent, Uzbekistan, 2007.-P. 353.
11. Kopytko J.F., Lapinskaya E.S., Sokolskaya T.A. Application, chemical composition and standardization of raw materials and preparations of Urtica // Chemical and pharmaceutical journal.-2011.-T.45, -№10. –S.32-40
12. Kiseleva T.L. and others. Development of a draft pharmacopoeial monograph on fresh grass of Knotweed (Polygonum aviculare L.). Man and his health, 2014.-№ 4.-P.71–77.
13. Braslavsky V.B. Standardization of raw materials and preparations of poplar and propolis / V.B. Braslavsky, V.A. Kurkin // Pharmacy. - 2009. - T. 57, No. 4. - P. 53 - 56.
14. Kovaleva T.Yu. Improvement of quality control methods for bearberry leaves and medicinal products based on it. T.Yu. Kovaleva,

I.A. Samylina, V.A. Ermakova // Traditional Medicine. - 2012. - No. 5. - P. 241 - 243.

15. Kovaleva, T.Yu. Requirements of domestic and foreign pharmacopoeias for the standardization of bearberry leaves / T.Yu. Kovaleva // Pharmacy. - 2011. - No. 5.- P.53-56.
16. Kurkina A.V. Actual aspects of standardization of medicinal plant materials and phytopreparations based on flavonoids / A.V. Kurkina // Traditional Medicine. - 2011. - No. 5 (28). - p. 232-239.