Investigation of phenolic compounds of the Canadian small petal (Erigeron canadensis L.)

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The study of Erigeron canadensis L. phenolic compounds

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SUMMARY

The HPLC method was used to analyze the qualitative composition and quantitative ratio of phenolic compounds of the canadensis grass (Erigeron canadensis L.). In the alcohol-water extract obtained by the method of extraction with heating, 22 substances were found, 17 of them were identified: among them phenolcarboxylic acids, coumarins, flavonoids.

A method for the spectrophotometric determination of the quantitative content of the sum of flavonoids in the herb of canadian small petals has been developed and its validation has been carried out. The following validation parameters of the method were established: precision (repeatability, intra-laboratory reproducibility), correctness (accuracy) and linearity.

In the course of determining the linearity of the method, it was found that the graphs of dependence are linear. The correlation coefficient is close to one and is equal to 0.9992 for the GSO Rutin and 0.9991 for the alcohol-water extract from the canadian small petal grass. The relative standard deviation in determining the convergence was 2.97%. The acceptance criterion, expressed as a relative standard deviation, did not exceed 3.84%.

In the course of the studies carried out, it was found that this express method is reproducible and affordable.

Key words: canadian small petals, HPLC, phenol carboxylic acids,flavonoids, validation.

RESUME

Analysis of the qualitative and quantitative composition of herb Erigeron canadensis L. phenolic compounds has been made by us using HPLC method. In water-spirit fraction obtained by extraction with heating, we have found 22 substances, 17 of them have been identified: phenolic acids, coumarins, flavonoids.

The technique of the spectrophotometric determination of quantitative content of the amount of flavonoids in the herb of Eri-geron canadensis L. has been developed and its validation has been carried out. We have established the following validation parameters of technique: precision (repeatability, reproducibility), accuracy and linearity.

In determining linearity of the techniques we have established that the plots are linear. The correlation coefficient is close to one and it is equal to 0.9992 and 0.9991 for rutin and water-spirit extract of Erigeron canadensis L. herb respectively. In determining repeatability value of the relative standard deviation (RSD) was equal to 2.97%. Acceptance criteria, expressed by value of RSD don't exceed 3.84%.

As a result of the spent researches we have concluded that the technique is reproducible, affordable and express.

Keywords: Erigeron canadensis L., HPLC, phenolic acids, flavonoids, validation.

Introduction

At the current stage of development of medical science, much attention is paid to the problem of using pharmaceutical preparations of herbal origin. This trend is associated, first of all, with a fairly wide range of pharmacological activity and low

toxicity of products of natural origin.

Phenolic compounds are one of the most widespread and numerous groups of natural compounds that are of interest to researchers due to the wide spectrum of their biological activity.

The genus small-petal (Erigeron) is one of the largest in the family of Compositae, has about 200 species, common on all continents, but mainly in North America. In Russia, small petals are represented by about 70 species [4]. The object of this study was the Canadian small petal (Erigeron canadensis L.), which is one of the most widespread species of this genus in our country and has a large resource base, in particular, on the territory of the Central Black Earth Region. The canadian small petal is not used in official medicine, which is explained, in our opinion, by its insufficient chemical and pharmacological knowledge.

The aim of this work was to study the phenolic composition of the aerial part of the canadian small petals.

Materials and research methods

For phytochemical research, we used aboveground plant organs collected during the growing season: “end of flowering - beginning of fruiting” in the Kursk region in 2010–2011.

The study of the phenolic compounds of canadian small petal grass was carried out by high performance liquid chromatography (HPLC) on a Gilston chromatograph (manual injector, model Rheodyne 7125 USA) with subsequent computer processing of the research results using the Multichrome program for Windows. The stationary phase was a 4.6 x 250 mm Kromasil C 18 metal column with a particle size of 5 microns. A system of solvents methanol-water-concentrated phosphoric acid in a ratio of 400: 600: 5 was used as a mobile phase. The analysis was carried out at room temperature. The flow rate of the eluent is 0.8 ml / min. Analysis duration 70 min. Detection was carried out using a Gilston UV / VIS UV detector at a wavelength of 254 nm.

The raw material was crushed to a particle size passing through a sieve with a hole diameter of 2 mm (GOST 214-83). 7.0 g of raw material was placed in a flask with a capacity of 200.0 ml, 60 ml of 70% ethyl alcohol was added, connected to a reflux condenser and heated in a boiling water bath for 60 minutes from the moment the alcohol-water mixture boiled in the flask. After cooling, the mixture was filtered through a paper filter into a 100.0 ml volumetric flask and brought to the mark with 70% ethyl alcohol (test solution).

At the same time, a series of 0.05% solutions of standard samples (GSO) in 70% ethyl alcohol was prepared: rutin, quercetin, luteolin, luteolin-7-glycoside, hesperidin, apigenin, hyperoside, dihydroquercetin, kaempferol, vitexin, isovitexin, naringenin, bainetoram , gallic, coffee, chlorogenic, chicory, cinnamic, ferulic, ellagic, o-coumaric acids, umbelliferone, esculetin, coumarin, methoxycoumarin, epigallocatechin gallate, epicatechin. 50 μL of the test solution and GSO were injected into the chromatograph and chromatographed as described above.

The quantitative determination of flavonoid compounds in the herb of canadian small petals was carried out using the spectrophotometric method of analysis [2, 6]. An analytical sample of the air-dry raw material was crushed to a particle size passing through a sieve with a diameter of 1 mm. 1.0 g of crushed raw material (accurately weighed) was placed in a flask with a thin section with a capacity of 100.0 ml, 50.0 ml of 70% ethyl alcohol was added. The flask was closed, weighed to the nearest 0.01 g and heated under reflux in a water bath for 30 minutes. Then the flask with the contents was cooled, weighed, the weight of the flask was brought to the initial 70% with ethyl alcohol and filtered (solution A). In parallel, a 0.05% alcohol solution of GSO rutin was prepared: about 0.05 g (accurately weighed) of GSO rutin, previously dried at a temperature of 130-135 ° C for 3 hours, was dissolved in 85,

with a capacity of 100.0 ml when heated in a water bath, cooled, brought the volume to the mark with alcohol of the same concentration (solution B).

Test solution. 2.5 ml of solution A and 2.0 ml of a 2% solution of aluminum chloride (in 70% ethanol) were placed in a 50.0 ml volumetric flask and made up to the mark with 70% ethyl alcohol.

Reference solution. 2.0 ml of solution B and 2.0 ml of a 2% solution of aluminum chloride (in 70% ethanol) were placed in a 50.0 ml volumetric flask and made up to the mark with 70% ethyl alcohol.

Compensation solution (a). 2.5 ml of solution A was placed in a 50.0 ml volumetric flask and the volume of the solution was brought to the mark with 70% ethanol.

Compensation solution (b). 2.0 ml of solution B was placed in a 50.0 ml volumetric flask and the volume of the solution was brought to the mark with 70% ethanol.

The optical density of the test solution and the reference solution was measured at 414 nm after 30 minutes, using compensation solution (a) and compensation solution (b) as zero solutions, respectively.

The content of the sum of flavonoid compounds in the herb of canadian small petals in terms of rutin and absolutely dry raw materials in percent (X) was calculated by the formula:

$$X = \frac{D \times m_0 \times V_{k1} \times V_{k2} \times V_{n0} \times 100 \times 100}{D_0 \times m \times V_{k3} \times V_{k4} \times V_{n} \times (100 - W)}$$

where D is the optical density of the test solution; D_0 - optical density of GSO routine; m is the weight of the sample of raw materials in grams; m_0 - weight of sample of GSO routine in grams; W is the percentage loss on drying; V_{k1} - the volume of the extractant for the extraction of flavonoids from the herb; V_{k2} - the volume of the flask for diluting the extract of the herb of small-petal canadian; V_{k3} - the volume of the flask for the 1st dilution of the GSO rutin; V_{k4} –Volume of the flask for the 2nd dilution of the GSO rutin; V_{NS} - the volume of the pipette (the volume of the canadian small petal herb extract taken for dilution); V_{n0} - the volume of the pipette (the volume of the GSO rutin solution taken for dilution).

To experimentally prove the suitability of the developed technique, the above-described technique for the quantitative determination of the amount of flavonoids in the herb of canadian small petals was validated. The validation of the developed methodology was carried out according to the following indicators: precision (repeatability, intra-laboratory reproducibility), correctness (accuracy) and linearity [3].

Convergence, as a characteristic of the accuracy of the method, was determined on one sample in 6 replicates under the same conditions. The acceptance criterion was expressed by the value of the relative standard deviation (RSD), which should not exceed 10%.

The linearity of the method was assessed at 8 concentration levels for GSO rutin and at 5 for extraction. The criterion for the acceptability of linearity is the correlation coefficient, the value of which must be at least 0.99.

The determination of the intralaboratory reproducibility of the technique was carried out in parallel by two researchers. The tests were carried out on three different samples in triplicate. Three replicates were performed at different times and on different devices. An SF-2000 spectrophotometer and an SF-46 spectrophotometer were used. The acceptance criterion was expressed as the value of the relative standard deviation, which should not exceed 10%.

To check the correctness of the method, a model preparation was prepared with the maximum possible content of the analyte. Then the model was diluted with 70% ethyl alcohol, thus obtaining 3 levels of concentration, and the original preparation (model) is not included in the number of experimental dilutions. In this case, the content of the determined component was taken as the reference value. Acceptance criterion - openness, a value that should be within 100 ± 5% and a relative standard deviation [1].

Research results and their discussion

The data obtained using HPLC indicate the content of various phenolic compounds in the canadian small petals grass: phenylbenzo-γ-pyrone derivatives (flavonoids), benzo-α-pyrone (coumarins), as well as phenol carboxylic acids (Table 1).

The quantitative ratio of substances of polyphenolic nature in the studied extract

established by the method of normalization by the areas of chromatographic peaks [5]. Based on the data presented in table. 1, of the identified flavonoids, dihydroquercetin and hesperidin (to a lesser extent luteolin-7-glycoside, quercetin and rutin) prevail, of phenol carboxylic acids - chlorogenic, gallic, chicoric, and ferulic. Coumarins are represented by umbelliferone and dihydrocoumarin.

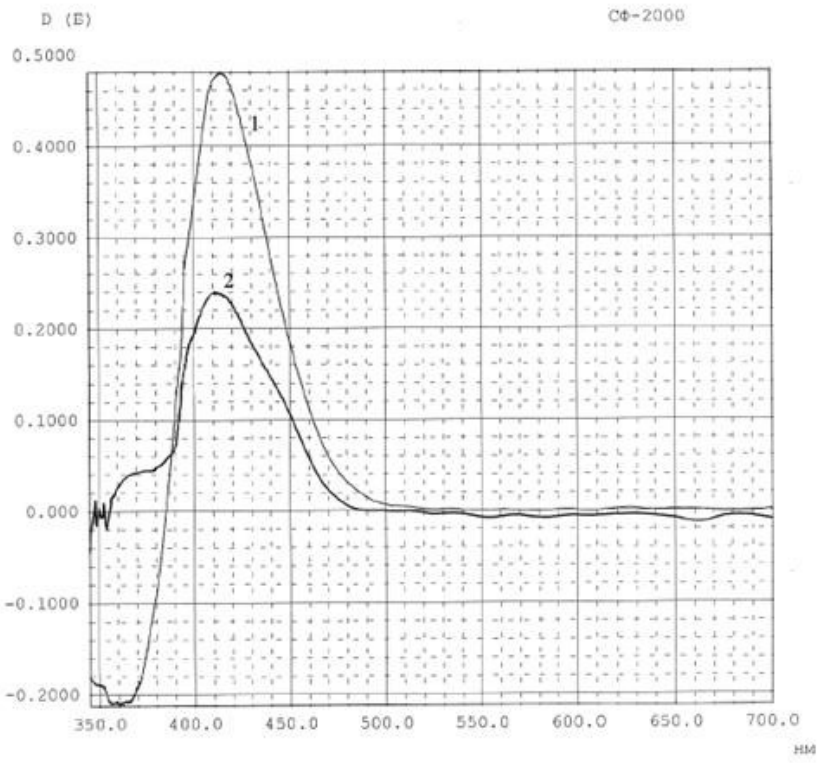
An analysis of various batches of raw materials showed that the content of the sum of flavonoids in terms of rutin and absolutely dry raw materials in the herb of canadian small petals ranges from 0.89 to 1.24%, which makes it possible to recommend that the content of the total of flavonoids is not less than 0 as a numerical indicator of the raw materials of canadian small petals, eight %.

When developing a spectrophotometric technique for the quantitative determination of the sum of flavonoids in order to select an analytical wavelength, the absorption spectra of flavonoid complexes of alcohol-water extract from the herb of small petals of Canada and the standard sample of GSO rutin with aluminum chloride were studied (Fig. 1). Since the interval between the maximum of the differential absorption spectrum of the extraction and the long-wavelength absorption band of the standard sample does not exceed half the half-width of the absorption band of the standard sample, the error will be insignificant. This makes it possible to use a wavelength of 414 ± 2 nm as a working one, and GSO routine as a standard sample in this experiment.

Table 1

Results of the study of phenolic compounds by HPLC in small petal grass Canadian

№	Время удерживания, с	Количественное соотношение, %	Наименование вещества
1	179,7	4,17	Умбеллиферон
2	189,7	5,82	Галловая кислота
3	224,1	2,23	Неидентифиц.
4	237,0	4,94	Эпигаллокатехин галлат
5	299,1	5,07	Цикориевая кислота
6	314,4	5,89	Хлорогеновая кислота
7	385,0	2,63	Кофейная кислота
8	450,1	6,53	Феруловая кислота
9	548,9	3,72	Дигидрокумарин
10	694,7	4,88	Эпикатехин
11	803,5	4,24	Лютеолин-7-гликозид
12	872,2	9,70	Гесперидин
13	968,4	1,99	Гиперозид
14	1084,2	2,61	Рутин
15	1100,0	16,03	Дигидрокверцетин
16	1413,0	0,71	Коричная кислота
17	1636,0	5,80	Неидентифиц.
18	1874,0	3,37	Неидентифиц.
19	2133,0	1,95	Неидентифиц.
20	2459,0	1,67	Катехин
21	2776,0	2,74	Неидентифиц.
22	3516,0	3,30	Кверцетин



Rice. 1. Differential absorption spectra of alcohol-water extract from grass canadian small petals (2) and GSO rutin (1), filmed under the same conditions.

In the course of determining the linearity (the linear dependence was estimated for the GSO rutin and alcohol-water extraction separately) for the method for the quantitative determination of flavonoids in the herb of canadian small petals, it was found that the dependence graphs are linear and are described by the equations y = 246.46x - 0.0186 and y = 2 , 41x - 0.0046 for GSO rutin and canadian petal grass extract, respectively. The correlation coefficients for GSO rutin and alcohol-water extraction are 0.9992 and 0.9991, respectively. This indicates a linear dependence of the values of optical density on the concentration of active substances in the range of certain concentrations (diagrams 1, 2; Table 2).

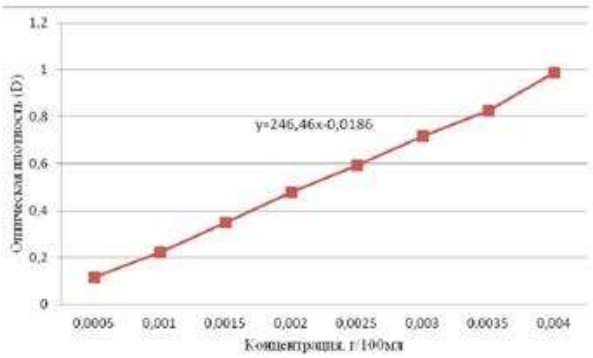


Diagram 1. Dependences of optical density on the concentration of GSO rutin.

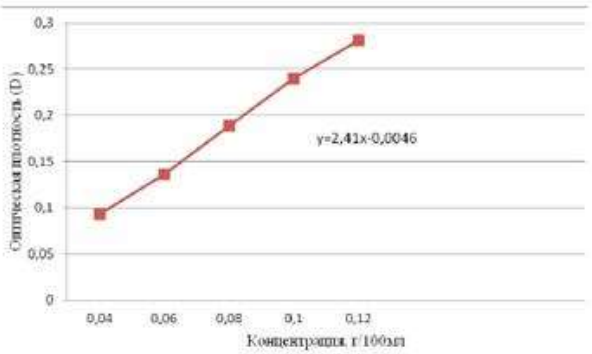


Diagram 2. Dependence of optical density on the dilution of alcohol-water extract from canadian small petal herbs.

table 2

Determination of the linearity of the method for quantifying flavonoids in grass canadian small petal

№	ГСО Рутин		Спиртово-водное извлечение	
	Аналитический отклик (оптическая плотность)	Концентрация, г/100 мл	Аналитический отклик (оптическая плотность)	Концентрация, г/100 мл
1	0,1157	0,0005	0,0931	0,04
2	0,2216	0,0010	0,1358	0,06
3	0,3476	0,0015	0,1889	0,08
4	0,4781	0,0020	0,2401	0,10
5	0,5944	0,0025	0,2816	0,12
6	0,7164	0,0030	–	–
7	0,8260	0,0035	–	–
8	0,9881	0,0040	–	–

The relative standard deviation in determining the convergence of the developed method was 2.97%, which indicates the precision of the method (Table 3).

When determining the within-laboratory reproducibility by two chemists, the value of the relative standard deviation did not exceed 3.05%, which indicates the precision of the method under conditions of replication (Table 4).

Determination of the correctness showed that the open rate is within acceptable limits, and the RSD value is 3.84%, which corresponds to the optimal value for this method of analysis (Table 5).

In the course of research, it was found that the described express method is reproducible and available.

The data obtained on the composition of the polyphenolic fraction and the quantitative content of the sum of flavonoids in the herb of canadian small petals determine the prospects of its further research.

Table 3

Determination of the convergence of the developed method of quantitative determination flavonoids in small petal grass

Количество повторностей	Содержание суммы флавоноидов в пересчете на рутин и абсолютно сухое сырье, %
1	1,1282
2	1,1056
3	1,1568
4	1,1243
5	1,1345
6	1,0593
Среднее значение (Xср)	1,1181
Стандартное отклонение (SD)	0,0332
Относительное стандартное отклонение (RSD), %	2,9719

Table 4

Determination of the within-laboratory accuracy of a quantitation procedure flavonoids in small petal grass

Повторяемость	Аналитик	Содержание суммы флавоноидов в пересчете на рутин и абсолютно сухое сырье, %					
		Образец 1	Дата	Образец 2	Дата	Образец 3	Дата
1	1	1,1845	13.12.11	1,1349	14.12.11	0,9512	15.12.11
2	1	1,2103	13.12.11	1,1487	14.12.11	0,8893	15.12.11
3	1	1,1754	13.12.11	1,1211	14.12.11	0,8959	15.12.11
4	2	1,1732	13.12.11	1,1054	14.12.11	0,9321	15.12.11
5	2	1,1643	13.12.11	1,1566	14.12.11	0,9456	15.12.11
6	2	1,2368	13.12.11	1,0942	14.12.11	0,8945	15.12.11
Среднее значение (Xср)		1,1908		1,1268		0,9181	
Стандартное отклонение (SD)		0,0275		0,0244		0,0280	
Относительное стандартное отклонение (RSD), %		2,3106		2,1689		3,0525	

Table 5

Evaluation of the correctness of the method for determining the amount of flavonoids in the herb canadian small petals (initial content 1.24%)

№	Разведение препарата	Найденное содержание суммы флавоноидов, % (с учетом показателя разведения)	Расчетное содержание суммы флавоноидов, % (с учетом показателя разведения)	Открываемость (R), %	Метрологические характеристики
1	1:2	0,39	0,41	95,12	Rср = 100,14 SD = 3,84 RSD = 3,84 %
2	1:2	0,43	0,41	104,88	
3	1:2	0,42	0,41	102,44	
4	1:1	0,65	0,62	104,84	
5	1:1	0,60	0,62	96,77	
6	1:1	0,61	0,62	98,39	
7	1:0,5	0,85	0,82	103,66	
8	1:0,5	0,79	0,82	96,34	
9	1:0,5	0,81	0,82	98,78	

Literature

1. Gavrilin M.V., Senchenko S.P. Analytical Method Validation Guidelines for graduate students and students [Text]. - Pyatigorsk: GOU VPO Pyatigorskaya GFA Roszdrav, 2009. - pp. 8–25.

2. Gubanova E.A. Quantification of flavonoids in clary sage herb // Pharmacy from century to century: collection of articles. tr. SPHFA. - SPb, 2008. - Part III. - S. 20-24.

3. Evdokimova O.V. Validation of the method for quantitative determination of the amount of flavonoids in columns with corn stigmas // Pharmacy. - 2008. - No. 7. - S. 14-17.

4. Plant life: Flowering plants [Text]. - M, 1981. T.5., Part II. - S. 470–471.

5. Styskin E.L., Itsikson LB, Braude E.V. Practical high performance liquid chromatography [Text]. - M., 1986 .-- 204 p.

6. Shimko OM, Khishova OM Quantification of flavonoids in Potentilla herb white // Advances in fundamental, clinical medicine and pharmacy. Materials of the 64th jubilee scientific session of the university dedicated to the 75th anniversary of its foundation. - Vitebsk, 2009. - pp. 110–112.

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Investigation of phenolic compounds of the Canadian small petal (Erigeron canadensis L.) / A.Yu. Botov, V. Ya. Yatsyuk, G.V. Siplivy, L.E. Husky // Traditional medicine. - 2012. - No. 3 (30). - S.48-53.