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DEVELOPMENT OF METHODS FOR STANDARDIZATION OF PRICKLY THISTLE (*CIRSIUM ARVENSE* L.) HERB ACCORDING TO THE CONTENT OF FLAVONOIDS

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The article presents the results of the development of the method of standardization of the prickly thistle (Cirsium arvense L.) herb according to the content of flavonoids. The conditions for the quantitative determination of plant flavonoids were studied and the optimal extraction parameters were selected, such as the concentration of the extraction solvent, the ratio of raw materials to extraction solvent, fineness factor, the time and frequency of extraction, the concentration and amount of the complexing additive added. It is established that the best extraction solvent for the prickly thistle (Cirsium arvense L.) herb is ethyl alcohol at a concentration of 40%, the ratio of raw materials and extraction solvent is 1: 30, the fineness factor is 2 mm, the optimal extraction time is 30 minutes with triple extraction. It is established that the dominant substance is apigenin, which is proposed to be recalculated, and the analytical wavelength for the quantitative determination of flavonoids is 388±2 nm.

Keywords: Prickly thistle (*Cirsium arvense* L.), herb, flavonoids, apigenin, quantitative determination

Medicinal herbs are increasingly attracting the attention of researchers and practical medicine, as they have a wide range of pharmacological activity and can be effectively used for the prevention and treatment of various diseases. The possibility of a rational combination of medicinal plants both among themselves and with chemical medicinal products to enhance the therapeutic effect, their softness of action, the rare manifestation of side effects are the main advantages of medicinal plants. In this regard, a promising medicinal plant for studying is the Prickly thistle (*Cirsium arvense* L.) of the sunflower family *(Asteraceae),* which is widely used in folk medicine as an anti-inflammatory, antioxidant and antimicrobial agent, it is used as a remedy for gout and rheumatism, externally for skin diseases, used for various nervous diseases, epilepsy, diseases of the digestive system [2,3]

Purpose of study is development of methods for standardization of Prickly thistle (*Cirsium arvense* L.) herb according to the content of flavonoids.

MATERIALS AND METHODS

As the objects of the study, we used samples of Prickly thistle (*Cirsium arvense* L.) herb, harvested on the territory of the Republic of Bashkortostan.

The quantitative determination of flavonoids was carried out by the method of differential spectrophotometry in the ultraviolet region using a Shumadzu UV-1800 spectrophotometer with the selection of optimal extraction parameters: extraction solvent, the ratio of raw materials to extraction solvent, fineness factor, the time and frequency of extraction, the conditions for the complex formation reaction. Statistical data processing was carried out in accordance with the requirements of the State Pharmacopoeia of the Russian Federation [1].

RESULTS AND DISCUSSION

The method of differential spectrophotometry with the selection of optimal conditions was used to develop a technique for the quantitative determination of flavonoids in the Prickly thistle herb [4,5]. In the course of the experiment, the absorption spectra of alcohol solutions of the Prickly thistle herb, as well as solutions with adding a complexing additive – a solution of aluminum chloride (III), were studied to exclude the influence of excipients. With a solution of aluminum chloride, flavonoids form complexes that

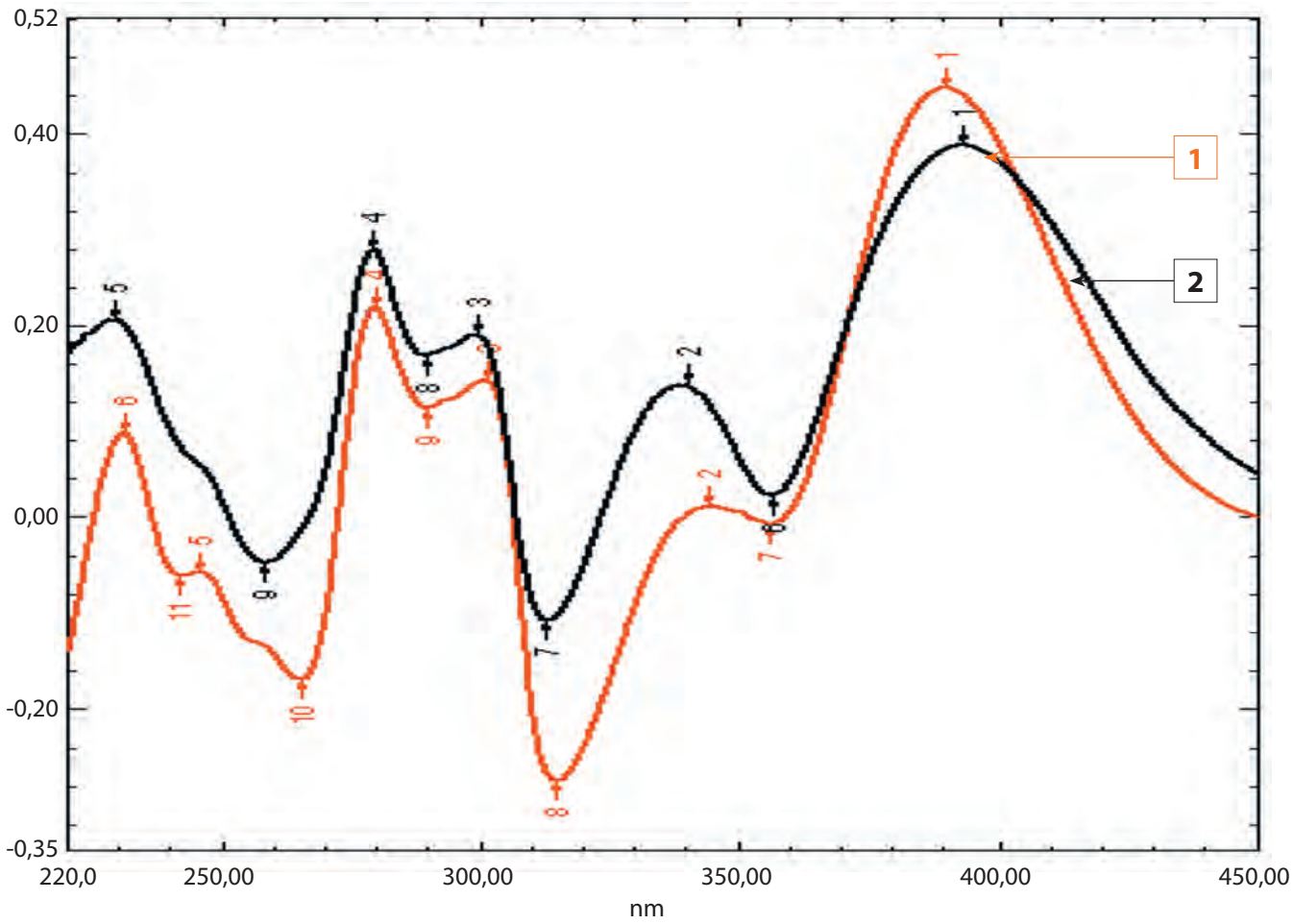

FIG. 1. *Differential spectra:*
1 – SUBSTANCES *of the standard – apigenin; 2 – extracts from Prickly thistle herb with the addition of an aluminum chloride solution (λ_{max}=388±2 nm)*

are stable in acidic environment, and a bathochromic shift is observed with clearly defined absorption maxima (Fig. 1).

It was found that the absorption spectrum of the Prickly thistle herb has a close maximum to the spectrum of apigenin (λ_{max}=388±2 nm), so this flavonoid was chosen as the main one in the amount for which it is proposed to recalculate in the future. The resulted data were consistent with the results of the previously conducted chromatographic analysis of alcohol extracts from the Prickly thistle raw materials.

At the next stage, it was necessary to select the conditions for the extraction of flavonoids that would ensure their maximum yield from the Prickly thistle raw material, namely: concentration of the extraction solvent, the raw material fineness (since the particle size of the raw material affects the completeness and speed of transition to the solution of the substances under study), the ratio of the raw material and the extraction solvent, the time and frequency of extraction.

In a comparative assessment of the effect of the extraction solvent on the yield of flavonoids, ethyl alcohol of various concentrations was used. In the resulted extracts, the optical density was determined in the range of wavelengths which are characteristic of flavonoids, and the content of flavonoids was calculated (Table 1). As a result, it was found that the optimal extraction solvent, when using which the higher values of the content of flavonoids were observed, is 40%

Table 1

EFFECT OF THE EXTRACTION SOLVENTS ON THE YIELD OF FLAVONOIDS

Concentration of ethyl alcohol	Optical density (D average)	Content of flavonoids, %
40%	0.528±0.025	2.89±0.14
50%	0.427±0.022	2.73±0.12
70%	0.425±0.019	2.68±0.11
80%	0.376±0.017	2.51±0.09
90%	0.364±0.008	2.46±0.07

Table 2

EFFECT OF EXTRACTION PARAMETERS ON FLAVONOID YIELD

Parameter	Content of flavonoids, %	
Fineness of raw materials, mm		
1	2.77±0.13	
2	2.84±0.18	
3	2.69±0.10	
Extraction time, min		
15	2.64±0.07	
30	2.88±0.12	
45	2.75±0.09	
60	2.59±0.07	
90	2.39±0.04	
Ratio of raw material and extraction solvent		
1:30	2.84±0.12	
1:50	2.77±0.10	
1:100	2.68±0.08	
Frequency of extraction		
1:30	one-time	2.72±0.03
	two-fold	2.88±0.05
	three-fold	2.92±0.06
1:50	one-time	2.64±0.04
	two-fold	2.75±0.07
	three-fold	2.79±0.11
1:100	one-time	2.59±0.04
	two-fold	2.62±0.05
	three-fold	2.68±0.02

ethyl alcohol, which was selected for further studies.

The results of studies for selection of the fineness factor of raw materials, the extraction time, the ratio of raw materials and extraction solvent, the frequency of extraction are presented in Table 2.

According to the resulting data, the optimum way is the fineness of raw materials to the size of particles passing through a sieve with a mesh diameter of 2 mm, the extraction time at which the maximum extraction of flavonoids occurs is 30 minutes, the ratio of raw materials to extraction solvent is 1: 30 and three-time extraction provides the most complete extraction of flavonoids from the Prickly thistle raw materials.

To determine the optimal parameters of the effect of complexing agent on the concentration of flavonoids, the concentration of an alcoholic solution of aluminum chloride and its amount added to the extraction were studied (Table 3).

Analyzing the data obtained, it can be noted that the most optimal way is the use of a 2% solution of aluminum chloride in amount of 1 ml. When assessing the stability of the resulting complex, it was found that the complex formation reaction develops within 45 minutes and the complex remains stable for an hour.

On the basis of the conducted studies, a method for the quantitative determination of flavonoids in the Prickly thistle herb is proposed: an analytical sample of raw materials is crushed to the size of particles passing through a sieve with a mesh diameter of 2 mm. About 1 g (exact weight) of the raw material is placed into a flask made of heat-resistant glass with a slice with a capacity of 250 ml, 30 ml of 40% ethyl alcohol is added, then it is attached to the backflow condenser and heated in a boiling water bath for 30 minutes. Then the resulting extraction is carefully filtered through cotton wool into a 100 ml volumetric flask, avoiding the ingress of raw material particles into the funnel. To the remaining raw materials in the flask, 30 ml of 40% ethyl alcohol is added and cotton wool, through which the solution was filtered, is attached to the backflow condenser and heated in a boiling water bath for another 30 minutes. The content of the flask is filtered into a measuring flask with the first portion of extraction through cotton wool. The process is repeated one more time according to the above procedure. The resulting extraction is brought to 100 ml in a measuring flask with the same solvent (solution A)

Into a measuring flask with a capacity of 25 ml, 1 ml of solution A is placed, 1 ml of a 2% alcohol solution of aluminum chloride is added

Table 3

EFFECT OF THE COMPLEXING AGENT ON THE YIELD OF FLAVONOIDS

Study sample	Content of flavonoids, %				
	Aluminum chloride concentration				
	1%	2%	3%	5%	10%
Thistle herb	2.63±0.09	2.78±0.12	2.66±0.10	2.54±0.08	2.48±0.09
	Amount of aluminum chloride added				
	1 ml	2 ml	3 ml	5 ml	10 ml
	2.80±0.11	2.68±0.09	2.54±0.08	2.51±0.06	2.44±0.05

Table 4

THE METROLOGICAL CHARACTERISTICS OF THE METHOD OF QUANTITATIVE DETERMINATION OF FLAVONOIDS IN THE PRICKLY THISTLE HERB

Object under study	f	$\bar{x}$	s^2	S_x	P, %	t(P, f)	E_α	ε,%
Prickly thistle herb	5	2.78	0.000038	0.0019	95	2.57	0.09	3.23

and the solution is brought to the mark with 95% ethyl alcohol (solution B). After 45 minutes, the optical density of the solution is measured using a spectrophotometer at a wavelength of 389 nm in a cuvette with a layer thickness of 1 cm. As a reference solution, a solution consisting of 1 ml of A solution and 1 drop of a solution of diluted acetic acid, brought to the mark with 95% ethyl alcohol is used in a measuring flask with a capacity of 25 ml.

In parallel, the optical density of the complex of a solution of an apigenin standard sample with a solution of aluminum chloride is measured: 1 ml of a 2% alcohol solution of aluminum chloride is added to 1 ml of a 0.05% solution of apigenin and the solution is brought to 25 ml with 95% ethyl alcohol in a measuring flask. The measurements are carried out similarly to the test solution

The content of the sum of flavonoids (X) in terms of apigenin and absolutely dry raw materials (in %) is calculated by the formula:

$$X = \frac{A \times a_0 \times 100 \times 1 \times 25 \times 100 \times 100}{A_0 \times a \times 25 \times 1 \times (100 - W)},$$

where A is the optical density of the analyzed solution (solution B); A0 is the optical density of the complex of the standard sample of apigenin with aluminum chloride; a is the weight of the raw material in grams; a0 is the weight of the standard sample of apigenin in grams; W is the weight loss of the raw material during drying, %.

The metrological characteristics of the method of quantitative determination of flavonoids in the Prickly thistle herb are presented in Table 4, the error of the experiment was not higher than the maximum permissible values.

CONCLUSIONS

1. A method has been developed for the quantitative determination of the amount of flavonoids in the prickly thistle herb by differential spectrophotometry in terms of apigenin (λ_{max}=388±2 nm)

2. The optimal parameters of extraction of flavonoids in the prickly thistle raw materials were established: the extraction solvent – ethyl alcohol at concentration of 40%, the ratio of raw materials and extraction solvent – 1:30, the fineness factor – 2 mm, the extraction time – 30 minutes with three-fold extraction, the concentration of the complexing agent – 2%, the amount of the added complexing agent – 1 ml

3. The content of the sum of flavonoids in terms of apigenin is 2,78±0,09%.

REFERENCES

1. *State Pharmacopoeia of the Russian Federation, Edition XIV [Electronic resource] // Federal Electronic Medical Library, 2018. – Available at: http://www.femb.ru.*
2. *Illustrated key to plants of Central Russia. Vol. 3. Angiosperms (dicotyledonous: eleutheropetalous) / I.A. Gubanov, K.V. Kiseleva, B.S. Novikov,*

V.N. Tikhomirov. – M.: Publishing house KMK, Technological Study Institute. – 2004. – 520 p.

3. *Krasnoborov I.M. Genus of Thistle (Cirsium Mill) – Thistle, Cotton thistle // Key to plants of the Republic of Tyva. – Novosibirsk: Publishing House of SB RAS, 2007. – pp. 507–509.*
4. *Krasyuk E.V. Characterization of phenolic compounds of Monarda species introduced in the Republic of Bashkortostan / E.V. Krasyuk, K.A. Pupykina, I.E. Anishchenko // Bashkir Chemical Journal. – 2015. – Vol. 22. – No. 3. – pp. 79–83*
5. *Modern approaches to the issue of standardization of medicinal plant raw materials / A.N. Mironov, I.V. Sakaeva, E.I. Sakanyan, et al. // Gazette of the Scientific Center for the Examination of Medical Products. – 2013, No. 2. – pp. 52–55.*