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Research Article

The Standardization of *Crataegus sanguinea* Fruits Growing on the Territory of Orenburg Region

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ABSTRACT

For standardization of *Crataegus sanguinea* fruits the method of differential spectrophotometry at analytical wavelength 412 nm was used[1]. The optimal extraction conditions of flavonoids from fruits of *Crataegus sanguinea* Pall. - extractant is 70% ethyl alcohol; the ratio of "raw-extractant" - 1:30; extraction time -60 min[1]. The relative degree of the determination of the total flavonoids in fruits of *Crataegus sanguinea* Pall. in used method with confidence probability 0,95 is no more than ±3,5%. The content of total flavonoids in fruits of *Crataegus sanguinea* Pall. varied from 0,18±0,001% to 0,22±0,002% (calculated on hyperoside).

Keyworlds: *Crataegus sanguinea* Pall., fruits, flavonoids, hyperoside, spectrophotometry, standardization.

INTRODUCTION

Crataegus sanguinea is widely used in official and traditional medicine since XIX century[2,3]. The pharmacopoeial raw material of *Crataegus sanguinea* are fruits containing significant quantities of flavonoids [1] (0,06-0,3%), among which the main flavonoid is hyperoside[4]. In Russian literature, the quantitative determination of *Crataegus sanguinea* fruits is carried by spectrophotometry method at wavelength 412 nm, with using standard sample of hyperoside[1]. Flavonoids are responsible for the main pharmacological action[5]. The fruits of *Crataegus sanguinea* are used as a cardiotonic agent in functional disorders of cardiac activity, cardiac weakness after severe diseases and initial forms of hypertension[5]. Research on standardization of medicinal raw materials of *Crataegus sanguinea* are relevant, due to the fact that on the territory of Orenburg region it grows everywhere and widely used by the local population as a cardiotonic agent, however the content of flavonoids in *Crataegus sanguinea* in different parts of Orenburg is vary.

The purpose of the present research - to compare the content of flavonoids of *Crataegus sanguinea* fruits, growing in different parts of Orenburg region.

RESULTS AND DISCUSSION

Objective Materials: industrial designs of *Crataegus sanguinea* fruits (OAO "Krasnogorleksredstva"), fruits of *Crataegus sanguinea*, made in September 2016, in the Orenburg region. Electronic spectra were measured on the UV-spectrophotometers "UNICO".

During the research of flavonoids amount in fruits of *Crataegus sanguinea* studied the UV spectra of solutions of water-alcohol extraction from this raw material, as well as solutions of selected substances.

To quantify flavonoids samples is used the fruits of *Crataegus sanguinea* procedure developed earlier (extractant - 70% ethyl alcohol, the ratio "raw material - extractant" - 1:30, extraction time - 60 min)[1].

Consequently, as analytical wavelength may be used a value of 412 nm, and as the standard sample can be serve the dominant flavonoid - hyperoside. In the case of the absence of this standard in the calculation formula can be used the theoretical value of the specific absorption index (= 330)[1].

It is also important to note that in a direct spectrophotometry (Fig. 1) and the differential spectrophotometry (Fig. 2) were obtained with comparable absorbance values, indicating the possibility of the use of both variants in the methods of quantitative determination of the total flavonoids. In the case of *Crataegus sanguinea* fruits is used the method of differential spectrophotometry.

A technique of quantitative definition of the total flavonoids in fruits of Crataegus sanguinea Pall

Analytical sample species is crushed to the size of the particles passing through a sieve with apertures in diameter of about 1 mm. 1 g chopped species (precise linkage) is placed in a flask with a grinding capacity of 50 ml and 30 ml of 70% ethyl alcohol is added. The flask is closed and weighed on calibrated scale accurate to ±0,01 g. The flask is attached to reverse refrigerator and heated on a boiling water bath (moderate boiling) within 60 minutes. Then the flask is closed with the same tube, weighed again and filled in the missing extragent to the original mass. The solution is filtered through paper filter («red» band) and cooled for 30 minutes. Tested solution is prepared in a following

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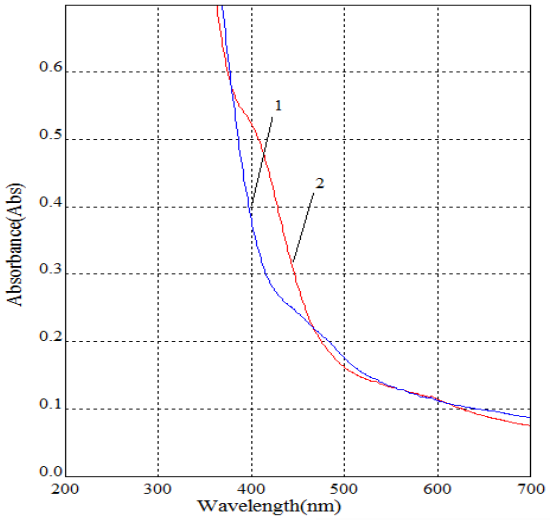

Figure 1: Electronic spectra of aqueous-alcoholic extract from the fruits of *Crataegus sanguinea* (1) and aqueous-alcoholic extract from the fruits of *Crataegus sanguinea* with the addition of aluminum chloride (2).

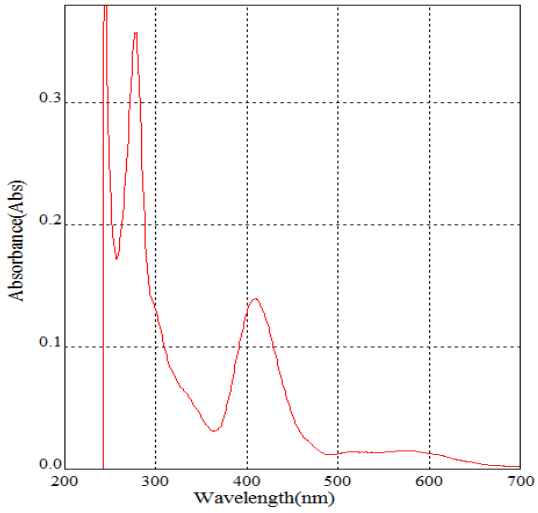

Figure 2: Electronic spectrum of aqueous-alcoholic extract from the fruits of *Crataegus sanguinea* with the addition of aluminum chloride (differential version).

Table 1: Metrological characteristics of the methods of quantitative determination of the amount of flavonoids in fruits of *Crataegus sanguinea* Pall.:

f	$\overline{X}$	*S*	*P, %*	*t (P,f)*	*ΔX*	*E, %*
10	0,19	0,003	95	2,23	±0,066	±3,5

Table 2: The total content of flavonoids in various samples in fruits of *Crataegus sanguinea* Pall.:

№	Characteristics of the sample materials	Contents of total flavonoids calculated on hyperoside and absolutely dry raw material (in%)
1.	Industrial designs of *Crataegus sanguinea* raw materials (OAO "Krasnogorleksredstva")	0,19±0,002
2.	Raw materials of *Crataegus sanguinea* Pall., (September 2016, Orenburg region, botanical garden)	0,18 ±0,001
3.	Raw materials of *Crataegus sanguinea* Pall., (September 2016, Orenburg region)	0,22 ±0,002

way: 5 ml of obtained extract is placed in a volumetric flask with a capacity of 25 ml, 2 ml of a 3% alcohol solution of aluminum chloride is added. The volume of the solution is brought to the mark with 95% ethanol (test solution). As a solution of comparison is using the solution prepared in the same conditions, but without addition of aluminum chloride (reference solution A). Measurement of optical density of the solution is carried out on the spectrophotometer at a wavelength of 412 nm[1].

Note: preparation of the hyperoside solution - standard sample. About 0.02 g (precise linkage) hyperoside is placed in a volumetric flask with a capacity of 50 ml, dissolved in 30 ml of 70% ethyl alcohol during a heating in a water bath. After cooling the contents of the flask to room temperature the volume of the 70% solution of ethyl alcohol is brought to the mark (solution A hyperoside). 1 ml solution A of hyperoside is placed in a volumetric flask 25 ml, 1 ml of a 3% alcohol solution of aluminum chloride is added and the volume of the solution is brought to the mark 95% ethanol (test solution B). As a reference solution is used the solution, which is prepared in the following manner: 1 ml of solution A hyperoside is placed in a volumetric flask and adjusted to 25 ml volume of the solution to the mark with 95% ethanol (hyperoside reference solution B)[1].

Content amount of flavonoids in fruits of *Crataegus sanguinea* in terms on hyperoside and absolutely dry raw materials in percent (X) is calculated by the formula[1]:

$$X = \frac{D * m_0 * 30 * 1 * 25 * 100 * 100}{D_0 * m * 50 * 1 * 25 * (100 - W)}$$

where D is optical density of the test solution;
Do - optical density of the working standard sample hyperoside solution;
m - the mass of raw material, g;
mo - the mass of the working standard sample hyperoside, g;
W - loss of mass on drying in percent.
A simplified calculation formula as an alternative:

$$X = \frac{D * 30 * 25 * 100}{m * 330 * 5 * (100 - W)}$$

where D - optical density of the test solution;

W - loss of mass on drying in percentage; 330 - specific absorption of the working standard sample hyperoside.

Metrological characteristics of the methodology of quantitative measurement of the amount of flavonoids in fruits of *Crataegus sanguinea* Pall. presented in table 1. The results of statistical processing of experiments show that the error of a single determine the amount of flavonoids in practical with confidence probability of 95% is ±3,5% (Table 1).

Using the developed methods we analyzed a number of sample practical (Table 2) and determined that the content of the amount of flavonoids varies from 0,18% to 0,22%, which can be recommended as a lower limit for raw materials this plant the content of the amount of flavonoids not less than 0,18 per cent.

CONCLUSIONS

Based on the literature information of chemical researches used the standardization of *Crataegus sanguinea* Pall. fruits, consisting in the determination of total flavonoids and by using the standard sample of hyperoside. The method of quantitative determination of the content of total flavonoids in *Crataegus sanguinea* Pall. fruits was carried out by using UV-spectrophotometer at the analytical wavelength 412 nm. The research results allow to recommend a lower limit on the content of the total flavonoids in practical not less than 0,18 per cent.

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