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THE DEVELOPMENT OF NEW APPROACHES OF STANDARDIZATION OF RHEUM PALMATUM ROOTS

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Abstract

In the present work are discussed the actual aspects of the development of methods of standardization of Rheum palmatum roots. During previous studies, we isolated the active ingredients, set dominant components (frangula-emodin, catechin) from roots of Rheum palmatum. In this paper, we describe a qualitative analysis technique developed by us using a Fourier transform infrared spectroscopy and the dominant substance – frangula-emodin. For qualitative analysis, optimal conditions of extracting active substances from the roots of Rheum palmatum, were selected and by using a zinc selenide support. New approaches to the standardization of Rheum palmatum roots by the quantitative content of tannins by ultraviolet spectrophotometry using the standard - dominant tannin - catechin at an analytical wavelength of 282±2 nm are proposed. In order to maximize the extraction of tannins from raw materials of Rheum palmatum, optimal conditions were selected - extractant-water, the ratio of raw materials-extractant 1 to 50, the extraction time in a boiling water bath-15 minutes. Ultraviolet spectra of water-alcohol extracts solutions from raw materials, were studied using the Unico 2800 spectrophotometer. The relative degree of the determination of the total tannins in roots of Rheum palmatum in developed method with confidence probability 0,95 is no more than ±7,40%. The content of the total of tannins in roots of Rheum palmatum varied from 21,17% to 24,68%, (calculated on catechin). The proposed methods of standardization of Rheum palmatum roots are simple, accessible and do not require the use of a large number of reagents.

Keywords: Rheum palmatum L., anthracenderivatives, tannins, standardization, Fourier transform infrared spectroscopy, ultraviolet spectrophotometry.

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INTRODUCTION

The Rheum palmatum (fam. Polygonaceae) is a medicinal plant used in traditional medicine of the European Union, traditional and non-traditional Chinese medicine and homeopathy. Interest in this medicinal plant is due to its unique chemical composition. The leading groups of biologically active compounds of Rheum palmatum roots are substances that have absolutely opposite pharmacological properties – tannins and anthracenderivatives [1, 2, 3, 4]. The nature of the effect of extracts from Rheum palmatum on the gastrointestinal tract depends on the dosage: astringent, anti-inflammatory effect is provided by small amounts of extracts, while large amounts have a laxative effect on the contrary [1, 3, 5]. State Pharmacopoeia of the Russian Federation XIV edition in the FS.2.5.0092.18 contains a method for qualitative analysis of anthracenderivatives by thin-layer chromatography and a method for quantitative determination of tannins by direct permanaganatometric titration by the Leventhal-Kursanov method. In accordance with this method, tannins are determined by oxidizing them with potassium permanganate in slightly diluted solutions in the presence of indigosulfonic acid [6, 7]. This method is quite time-consuming and inaccurate. To determine the tannins in the roots of Rheum palmatum, a method of spectrophotometric determination in terms of catechin was proposed. By experiment there was selected the optimal conditions for extraction of tannins from raw materials. At the present time there are no methods of qualitative analysis of Rheum palmatum roots by Fourier transform infrared spectroscopy (FTIR). FTIR is a fast and nondestructive analytical method and becoming a suitable technique for analysis of herbal medicine. This paper focuses on the recent developments and updates for the qualitative analysis of herbal medicine using FTIR.

MATERIAL AND METHOD

The objects of the study were the roots of Rheum palmatum LLC "Staroslav", Russia, Novosibirsk region, Berdsk, 2018. Electronic spectra were measured using a «Unico 2800» UV spectrophotometer and Fourier transform infrared spectroscopy (FTIR) «Infralum FT-02 (Thermo) ».

Method of infrared spectroscopic analysis of Rheum palmatum roots: 1 g (exact weight) of Rheum palmatum roots, pre-crushed to the size of particles passing through a sieve with holes 1 mm in diameter, was placed in a conical flask with a capacity of 100 ml and added 50 ml of 70% ethyl alcohol. The flask with the contents was attached to the reverse refrigerator and heated in a boiling water bath for 30 minutes. Then 15 minutes were extracted in an ultrasonic bath at a

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temperature of 40^0C, an ultrasound power of 60W and a frequency of 40 kHz. The contents of the flask were defended for 10 minutes, filtered through a paper filter with a red stripe into a 200 ml volumetric flask. A sample was prepared from the resulting extract by watering: 100-250 microl of the extract was placed on a horizontal substrate. The substrate material is zinc selenide. The substrate with the sample was dried at room temperature. After complete removal of the solvent, a thin film of the test sample remained on the substrate. The sample obtained in this way was placed in the infralum FT- 02 infrared Fourier spectrometer. The infrared spectrum was shot in the range of 500-4000 cm^{-1}. A plate made of the same material, the same thickness as the substrate for filling films, was used as a reference sample for removing spectra. In the case when the optical density values of the obtained film sample did not allow making unambiguous conclusions, the fill was performed repeatedly, until reliable values were obtained.

Method of quantitative determination of tannins in Rheum palmatum roots: analytical sample of raw materials is milled to a particle size passing through a sieve with holes of 1 mm diameter. About 1 g of crushed raw material (precise linkage) is placed in a flask for 250 ml, add 50 ml of water. The flask is closed, weighed to an accuracy of ±0.01, attached to the reverse refrigerator and heated in a boiling water bath (moderate boiling) for 15 minutes from the moment of boiling the extractant. After that, the contents of the flask are cooled, weighed and, if necessary, brought to the initial mass with water. The resulting extract is filtered through a paper filter, discarding the first 10 ml of filtrate (solution). 1 ml of solution A is placed in a 50 ml volumetric flask and the volume of the solution is brought to the mark with water. The optical density is measured on a spectrophotometer at an analytical wavelength of 282 nm in a cuvette with a layer thickness of 10 mm. The content of the amount of tannins in terms of catechin and completely dry raw materials as a percentage (X) is calculated using the formula:

$$X = \frac{A \times 50 \times 50 \times 100}{A_{1см}^{1\%} \times 1 \times a \times (100 - W)}$$

where

A – optical density of the test solution;

$A_{1см}^{1\%}$ – specific absorption index of catechin at a wavelength of 282 nm, equal to 144;

50 – volume of extractant, ml;

50 – volume of the test solution, ml;

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1 - volume of aliquot solution A, ml;

a – linkage of raw material, g;

W – raw material humidity,%.

RESULTS AND DISCUSSION

Absorption bands characteristic of phenolic compounds are observed in the region of 1200-1400 cm-1 infrared spectra (Fig. 1), such as anthracene derivatives and flavonoids [8, 9]. The pronounced absorption band at 1600 cm^{-1} indicates the presence of a carbonyl group (C=O) in anthracene derivatives (Fig. 1) [10, 11]. In the region of 2900-3000 cm^{-1} infrared spectra, an absorption band is also observed due to the valence fluctuations of the aromatic CH=CH (Fig. 1).

Fig. 1: Infrared spectra of water-alcohol extraction from the roots of Rheum palmatum;

Notation: 1- infrared spectrum of water-alcohol extraction from the roots of Rheum palmatum; 2-infrared spectrum of frangula-emodin

Previously with the purpose of substantiation of methodical approaches to standardization of Rheum palmatum roots conducted a study on the release of substances from these medical plant. It is established that the dominant components is catechin. During the development of a methodology to quantify the amount of tannins in roots of Rheum palmatum studied the UV spectra of solutions of water extraction from this raw material, as well as solutions of selected substances.

Research UV spectra showed that the maximum absorption of the aqueous extract of Rheum palmatum roots in the wavelength region of the spectrum is at 282 ±2 nm (Fig. 3) [1, 12]. In the

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electron spectrum of alkaline ammonia solution of catechin also observed distinct absorption maximum at 282 ±2 nm (Fig. 2).

Consequently, as the analytical wavelength may be used value 282 nm, and the standard model can serve as dominant tannins - catechin, and in the absence of a standard in the calculation formula can be used in the theoretical value of the specific absorption coefficient ($A_{1см}^{1\%}$) - 144.

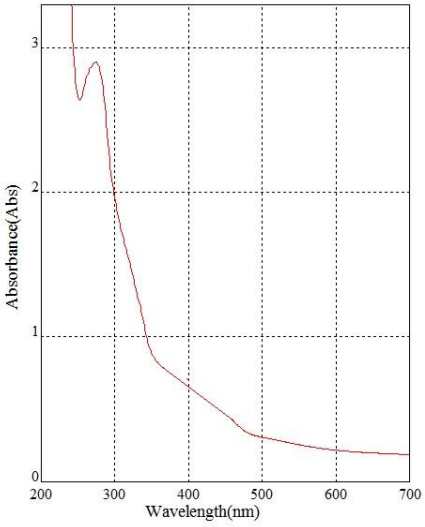

Fig. 2: Electronic spectra of the initial catechin solution

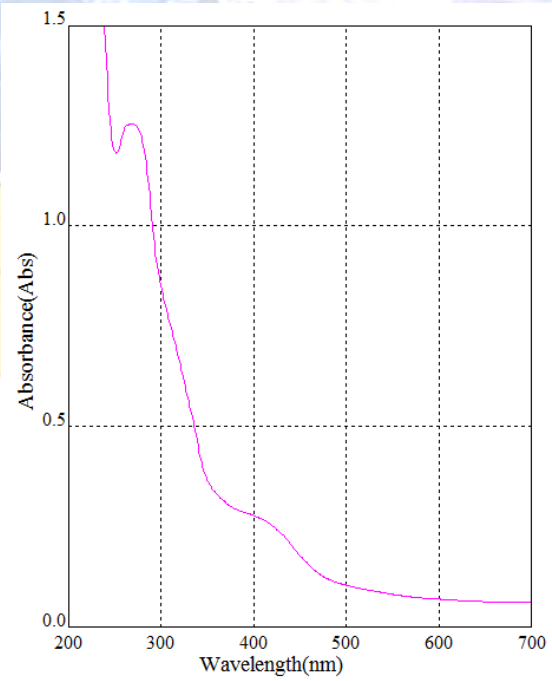

Fig. 3: Electronic spectra of water-alcohol extraction from the roots of Rheum palmatum

Metrological characteristics of the method for quantifying the content of tannins in the roots of Rheum palmatum are presented in **Table 1**. The results of statistical processing of the conducted

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experiments indicate that the error of a single determination of tannins in the roots of Rheum palmatum is ±7,40% [13].

Table 1: Metrological characteristics of methods for quantitative determination of tannins in the roots of Rheum palmatum

F	$\overline{X}$	*S*	*P, %*	*t (P,f)*	*ΔX*	*E, %*
10	23,67	0,7855	95	2,23	±1,752	±7,40

Using the developed methods we analyzed a number of sample practical (**Table 2**) and determined that the content of the amount of tannins varies from 21,17% to 24,68%, which can be recommended as a lower limit for raw materials this plant the content of the amount of tannins not less than 21,17 per cent.

Table 2: The total content of tannins in various samples of Rheum palmatum roots

Characteristics of the sample materials	Contents of total tannins calculated on frangula-emodin and absolutely dry raw material (in %)
Staroslav LLC, Russia, Novosibirsk region, Berdsk, (2016)	24,21±1,79
Staroslav LLC, Russia, Novosibirsk region, Berdsk, (2017)	23,59±1,75
Staroslav LLC, Russia, Novosibirsk region, Berdsk, (2018)	21,17±1,57
Energia Trava LLC, Russia, Krasnodar region, Goryachy Klyuch, (2016)	22,98±1,70
Energia Trava LLC, Russia, Krasnodar region, Goryachy Klyuch, (2017)	24,68±1,83
Energia Trava LLC, Russia, Krasnodar region, Goryachy Klyuch, (2018)	24,55±0,29
Botanical garden of Samara state University, (September 2016)	23,91±1,77
Botanical garden of Samara state University, (September 2017)	24,01±1,55
Botanical garden of Samara state University, (September 2018)	23,17±1,72

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CONCLUSION

A method of qualitative analysis of anthracenderivatives in the roots of Rheum palmatum by using Fourier transform infrared spectroscopy and the standard compound frangula-emodin was developed. The developed method can be recommended for the purposes of qualitative analysis of the roots of Rheum palmatum.

A method of quantitative determination of tannins in the roots of Rheum palmatum by ultraviolet spectrophotometry in terms of catechin at an analytical wavelength of 282±2 nm has been developed. The maximum extraction of tannins from raw materials of Rheum palmatum, was achieved by using optimal conditions - extractant-water, the ratio of raw materials-extractant 1 to 50, the extraction time in a boiling water bath-15 minutes. The research results allow to recommend a lower limit on the content of the total tannins in practical not less than 21,17 per cent. This method is fast, allows you to extract a high content of tannins, is simple and does not require the use of reagents, so it can be recommended to extract tannins from other types of medicinal plant raw materials

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