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Research Article

The Development of New Approaches of Standardization of *Rubia tinctorum* Rhizomes et Radix

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ABSTRACT
A quantitative analysis method for total anthracenderivatives in rhizomes et radices of *Rubia tinctorum* with the using of direct spectrophotometry at analytical wavelength 520 nm there was developed. The developed optimal conditions of extraction of anthracenderivatives in rhizomes et radices of *Rubia tinctorum* L. - extractant is 80% ethyl alcohol; the ratio of "raw-extractant" - 1:30; extraction time -90 min. The relative degree of the determination of the total anthracenderivatives in rhizomes et radices of *Rubia tinctorum* L. in developed method with confidence probability 0,95 is no more than ±3,18%. The content of total anthracenderivatvies in rhizomata et radices of *Rubia tinctorum* L. varied from 4,50±0,02% to 4,85±0,03% (calculated on ruberythrinic acid).

Keywords: *Rubia tinctorum* L., rhizomes et radices, anthracenderivatives, ruberythrinic acid, spectrophotometry, standardization.

INTRODUCTION

Anthracene derivatives - a group of natural compounds, which are based on the anthracen nucleus[1]. Anthracenderivatives belong to the group of laxatives, which cause irritation of receptors of the intestinal mucosa. They act moderately on the motility of the colon. Anthraquinonderivatives and products of its recovery (Antron and Anthranol) are widespread in nature and found in many higher plants[2]. Anthracenderivatives most often found in plants of the families Rubiaceae, Rhamnaceae, Polygonaceae, Fabaceae, Liliaceae[3]. Anthracenderivatives contained dissolved in the cell sap, and can be easily installed microchemicaly[4]. Rhizomes et radices of *Rubia tinctorum* L. containing anthracenderivatives from alizarin group, among which is the main glycoside ruberythrinic acid[5]. *Rubia tinctorum* L. is perennial herb having diuretic, antispasmodic and kidney stones destroyer properties[6]. The pharmacopoeial raw material are rhizomes et radices containing significant quantities of anthracenderivatives[7] (2.5-3,0%), which are responsible for the pharmacological action of the preparations. Research on standardization of medicinal raw materials of *Rubia tinctorum* L. are relevant, due to the fact that in Russian literature[7], the quantitative determination is carried by photoelectrocolorimetry method at wavelength 530 nm, while in the European Pharmacopoeia using spectrophotometry at the same wave length[8]. The purpose of the present research - to develop methods of quantitative analysis of *Rubia tinctorum* L. rhizomes et radices.

RESULTS AND DISCUSSION

Objective. Materials: raw materials of *Rubia tinctorum* L., made in March 2015, in the Krasnodar region. Electronic spectra were measured on the UV-spectrophotometers "UNICO". Previously with the purpose of substantiation of methodical approaches to standardization of *Rubia tinctorum* L. rhizomes et radices conducted a study on the release of substances from these medical plant. It is established that the dominant components are ruberythrinic acid. During the development of a methodology to quantify the amount of anthracenderivatives in rhizomes et radices of *Rubia tinctorum* L. studied the UV spectra of solutions of water-alcohol extraction from this raw material, as well as solutions of selected substances. Research UV spectra showed that the maximum absorption of the ammonia solution alkaline aqueous-alcoholic extract of *Rubia tinctorum* L. rhizomes et radices in the wavelength region of the spectrum is at 520 +2 nm (Fig. 1). In the long-wavelength region of the electron spectrum alkaline ammonia solution of ruberythrinic acid also observed distinct absorption maximum at 520 + 2 nm (Fig. 2). It should be noted that evaluation indicative authentication solutions are differential version of spectra (spectra of the test solutions on a background of reference solution) (Fig. 3). Consequently, as the analytical wavelength may be used value 520 nm, and the standard model can serve as dominant anthracenderivatives - ruberythrinic acid, and in the absence of a standard in the calculation formula can be used in the theoretical value of the specific absorption

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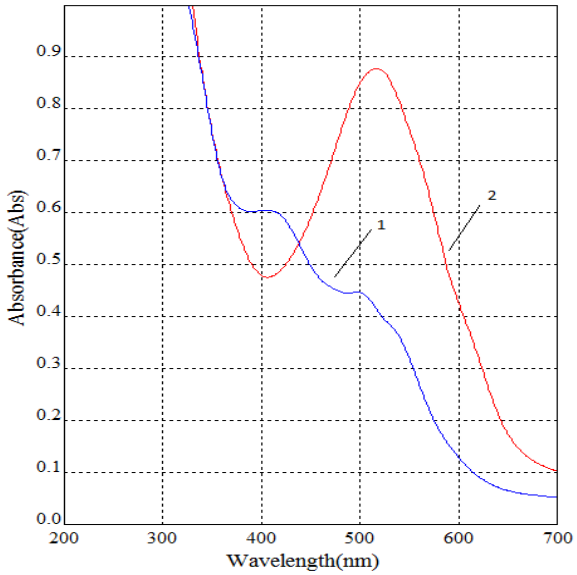

Figure 1: Electronic spectra of the initial solution (1) and alkali-ammoniac solution (2) aqueous-alcoholic extract of *Rubia tinctorum* L. rhizomes et radices.

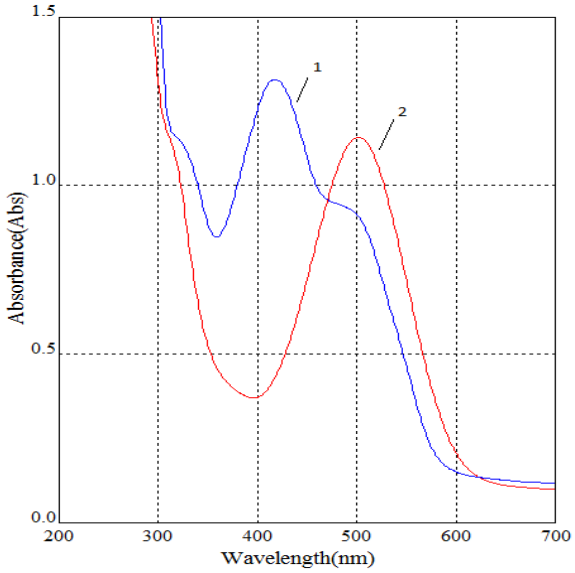

Figure 2: Electronic spectra of the initial solution (1) and alkali-ammoniac solution (2) of ruberythrinic acid.

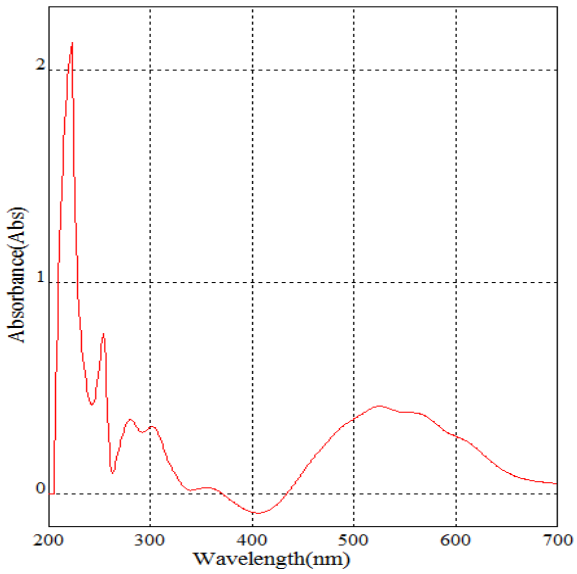

Figure 3: Electronic spectrum of alkaline ammonia solution of *Rubia tinctorum* L. rhizomes et radices (differential version).

coefficient (E) - 520. To develop methods of quantitative determination of the amount of anthracenderivatives us the optimal conditions for the extraction of anthracenderivatives from the rhizomes et radices of *Rubia tinctorum* L.: extragent 80% ethyl alcohol; the ratio of "raw materials-extracting" - 1:30; the extraction time - extraction on a boiling water bath (moderate boiling) within 90 min (Table. 1).

A technique of quantitative definition of the total anthracenderivatives in rhizomes et radices of Rubia tinctorum L. 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 100 ml, add 50 ml of 80% ethyl alcohol. Closed the flask and weigh on calibrated scale accurate to ±0,01 g. Flask attached to reverse refrigerator and heated on a boiling water bath (moderate boiling) within 90 minutes. Then the flask close with the same tube, weighed again and fill in the missing extragent to the original mass. Removing filtered through paper filter («red» band) and cool for 30 minutes. Tested solution is prepared as follows: 1 ml of obtained extract placed in a volumetric flask with a capacity of 50 ml and bring the volume of the solution to the mark alkali-ammoniac solution (test solution). The test solution A placed in a flask with a capacity of 50 ml and heated for 15 min in a boiling water bath with reverse refrigerator. After cooling measure the optical density of the solution on the spectrophotometer at a wavelength of 520 nm. The reference solution is purified water.

Note: preparation of the ruberythrinic acid solution - standard sample. About 0.02 g (precise linkage) ruberythrinic acid placed in a volumetric flask with a capacity of 50 ml, dissolved in 30 ml of 70% ethyl alcohol when heated in a water bath. After cooling the contents of the flask to room temperature bring the volume of the 70% solution of ethyl alcohol up to the mark (solution A fragulin A). 1 ml solution A of ruberythrinic acid placed in a volumetric flask 25 ml and bring the volume of the solution to the mark alkali-ammoniac solution (test solution B). Solution B is placed in a flask with a capacity of 50 ml and heated for 15 min in a boiling water bath with reverse refrigerator. After cooling measure the optical density of a solution on the spectrophotometer at a wavelength of 520 nm. The reference solution is purified water. Content amount of anthracenderivatives in rhizomes et radices of *Rubia tinctorum* L. in terms on ruberythrinic acid and absolutely dry raw materials in percent (X) is calculated by the formula:

Table 1: Dependence of the completeness of extraction amount of anthracenderivatives in rhizomes et radices of *Rubia tinctorum* L..

S. No	Extractant	The ratio of raw material: extractant	Extraction time, min	Contents of total anthracenderivatives calculted on ruberythrinic acid and absolutely dry raw material (in%)
1.	40% ethanol	1:50	90	2,28 ± 0,06
2.	50% ethanol	1:50	90	2,85 ± 0,05
3.	60% ethanol	1:50	90	3,09 ± 0,04
4.	70% ethanol	1:50	90	2,72 ± 0,05
5.	80% ethanol	1:50	90	3,23 ± 0,07
6.	95% ethanol	1:50	90	1,52 ± 0,02
7.	80% ethanol	1:50	30	2,71 ± 0,06
8.	80% ethanol	1:50	45	2,41± 0,05
9.	80% ethanol	1:50	60	2,76 ± 0,06
10.	80% ethanol	1:50	90	3,20 ± 0,08
11.	80% ethanol	1:50	120	2,80 ± 0,05
12.	80% ethanol	1:30	90	4,85 ± 0,06
13.	80% ethanol	1:100	90	1,97 ± 0,03
14.	80% ethanol	1:200	90	1,29 ± 0,02

with confidence probability of 95% is ±3,18% (Table 2).

Table 2: Metrological characteristics of the methods of quantitative determination of the amount of anthracenderivatives in rhizomes et radices of *Rubia tinctorum* L.:

f	$\overline{X}$	*S*	*P, %*	*t (P,f)*	*ΔX*	*E, %*
10	4,85	0,0692	95	2,23	±0,154	±3,18

Table 3: The total content of anthracenderivatives in various samples of *Rubia tinctorum* L. rhizomes et radices:

S. No	Characteristics of the sample materials	Contents of total anthracenderivatives calculated on ruberythrinic acid and absolutely dry raw material (in%)
1.	Raw materials of *Rubia tinctorum* L., (March 2015, Krasnodar region)	4,85 ±0,10
2.	Raw materials of *Rubia tinctorum* L., (March 2014, Krasnodar region)	4,5 ±0,12

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

where D is optical density of the test solution;

Do - optical density of the solution nor ruberythrinic acid;

m - the mass of raw material, g;

mo - the mass of the working standard sample ruberythrinic acid, g;

W - loss of mass on drying in percent.

A simplified calculation formula as an alternative:

$$X = \frac{D * 50 * 50 * 100}{m * 520 * (100 - W)}$$

where D - optical density of the test solution;

W - loss of mass on drying in percentage;

520 - specific absorption of the working standard sample ruberythrinic acid.

Metrological characteristics of the methodology of quantitative measurement of the amount of anthracenderivatives in rhizomes et radices of *Rubia tinctorum* L. presented in table 2. The results of statistical processing of experiments show that the error of a single determine the amount of anthracenderivatives in practical

Using the developed methods we analyzed a number of sample practical (Table 3) and determined that the content of the amount of anthracenderivatives varies from 4,50% to 4,85%, which can be recommended as a lower limit for raw materials this plant the content of the amount of anthracenderivatives not less than 4,5 per cent.

CONCLUSIONS

Developed methodological approaches to the standardization of *Rubia tinctorum* L. rhizomes et radices, consisting in the determination of anthracenderivatives and the using of techniques of the analysis of standard sample of ruberythrinic acid. The method of quantitative determination of the total anthracenderivatives in terms ruberythrinic acid practical using 80% ethyl alcohol as a solvent and UV-spectroscopy at the analytical wavelength 520 nm. The research results allow to recommend a lower limit on the content of the total anthracenderivatives in practical not less than 4,5 per cent.

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