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PREPARATION OF HYPERICIN ENRICHED ST. JOHN'S WORT EXTRACTS

Results of solid phase extraction (SPE) of St. John's Wort extracts were described. In order to obtain the extract the air-dried plant material of *Hypericum perforatum* L. (cultivar Yantar) grown in the Central Botanical Garden of NAS of Belarus was used. Analysis of the St. John's wort extract solutions was carried out by electronic absorption spectroscopy. It was shown the influence of the nature of the solvent on the spectral characteristics of the extract. The sorbent Waters Sep-Pak S18®Vac RC was used for solid-phase extraction. The methanol solutions of different concentrations were used as an eluent system. For SPE metanol fractions absorption and fluorescence spectra were registered. It is shown that the main part of hypericin was eluted with 80% methanol. The values of quantum yields were calculated. These results suggest that solid phase extraction on the non-polar sorbent is a promising way to increase the content of the hypericin in the St. John's Wort extracts.

Key words: hypericin, solid phase extraction, *Hypericum perforatum*.

Introduction. One of the promising methods of cancer treatment is a method of photodynamic therapy. Effective photo sensitizers are required to implement this method. They can accumulate in tumor cells without affecting normal tissue, enabling to prevent or reduce damage to healthy cells during tumor destruction. Hypericin, a component of the herb St. John's wort extract exhibits these properties [1].

Previous studies [2] have shown that the two-phase processing of the herb hypericin with the extracts system followed by purification of the obtained extract from impure compounds using aqueous solutions of gelatin, ammonia and formic acid produces compounds containing hypericin at least 5.5–5.9 wt %.

The purpose of this work is to get hypericin enriched extracts of the herb St. John's wort using solid phase extraction (SPE).

The main part. The air-dried samples of the herb *Hypericum perforatum* L. (cultivar Yantar) were used in the work. The plant material was obtained in the areas of introduction in the Central Botanical Garden of NAS of Belarus in 2012. The harvesting of the herb St. John's wort was carried out in the flowering phase in the period from June to August according to the general rules for herborization of medicinal plants. The air-dried plant material was ground to a particle size of 1 mm.

Herb extracts were prepared using a two-phase solvent system at room temperature and continued stirring for 90 minutes. A mixture system of chloroform : ethanol : water (8 : 10 : 10) was used as extracting solvent [3]. Extracts were prepared according to the procedures described in the reference literature [2].

To evaluate the effect of solvent composition on the absorption maximum and the molar extinction coefficient, the electronic absorption spectra of the herb extract of *Hypericum* (Fig. 1) in various solvents like ethanol (*1*), methanol (*2*), acetonitrile (*3*) and 80% methanol (*4*) were registered.

Comparison of the spectral characteristics shows that the nature of the solvent influences the position of the maximum absorption intensity of hypericin, but the error of hypericin quantitative determination caused by replacing 100% methanol to 80% methanol does not exceed 10%.

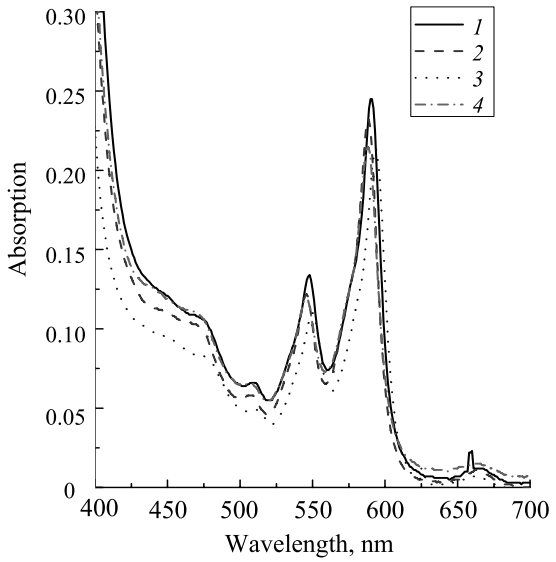

Fig. 1. Absorption spectrum of the crude extract in various solvents in the 400–700 nm area (extract concentration 287 mkg/ml)

A non-polar sorbent WatersSep-Pak S18®Vac RC was used for the solid phase extraction. This sorbent is a silica gel grafted with octadecyl groups. The methanol solutions of different concentrations were used as an eluting system. The characteristics of the obtained fractions are shown in the Table 1.

Table 1

Fractions obtained by solid phase extraction

Fraction	Eluent	Eluent volume, ml
1	25% methanol	3.0
2	50% methanol	3.0
3	75% methanol	3.0
4	80% methanol	3.0
5	100% methanol	3.0
6	100% methanol	9.0
7	Chloroform	9.0

Analysis of the fractions was carried out by electron absorption spectroscopy. To minimize the errors of hypericin content quantitative determination which may occur when changing the polarity of the medium, before recording the spectra, all the samples were diluted with pure absolute methanol of 50-fold ratio.

Fig. 2 shows the absorption spectra of the fractions *1–7* in the visible area.

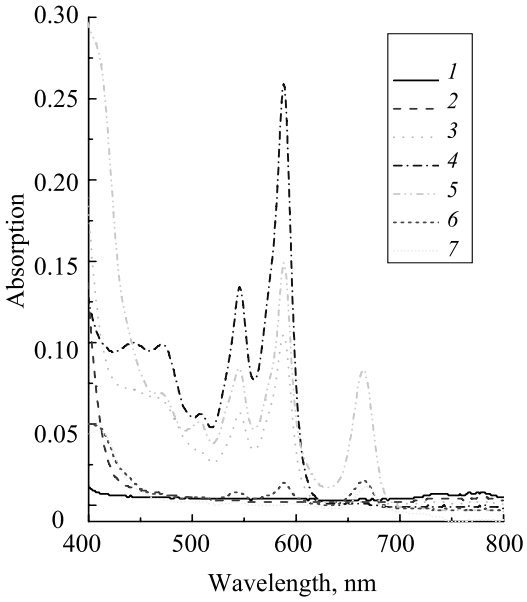

Fig. 2. Absorption spectra of fractions *1–7* in 400–800 nm area

The data in Fig. 2 show that the bulk of hypericin is eluted in fraction 4.

To assess the purity of the obtained fractions, the intensities of the absorption bands were measured at 280 and 590 nm and their relationship were calculated. The results are shown in Table 2. From the data it is evident that the chromatographic separation of the crude extract on a sorbent WatersSep-Pak S18®VacRC made possible to obtain a drug with a high content of hypericin; it is sufficiently greater than the initial one, about 1.7 times.

Table 2

Spectral characteristics of fractions

Fractions	Optical density of solutions at λ = 280 nm and λ = 590 nm		A_{590} / A_{280}
	A_{280}	A_{590}	
1	0.13	0.002	0.015
2	0.565	0.014	0.025
3	1.177	0.105	0.089
4	0.758	0.259	0.342
5	1.202	0.143	0.119
6	0.034	0.014	0.412
7	0.0183	0.002	0.109
Standard sample of hypericin in methanol	–	–	0.581

Fractions 2 and 3 produced by the elution of 50% methanol and 75% contain primarily accompanying compounds which absorb in UV area. The data for the standard sample of hypericin in methanol are shown in Table 2 for comparison [4].

To characterize the fluorescent properties of the SPE-fractions, their emission spectra were taken at the optimum length of the fluorescence excitation wavelength (λ_{exc} = 470 nm), which are shown in Fig. 3.

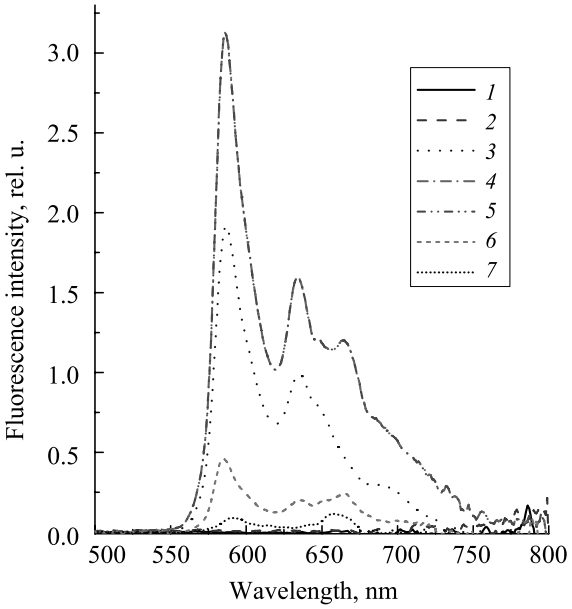

Fig. 3. Fluorescence emission spectra (λ_{exc} = 470 nm) of fractions *1–7*

The values of the quantum yield of fractions 3 and 4 are based on the calculated spectral characteristics, which are shown in Table 3. The apparent low quantum yield of hypericin extracts is due to the presence of impurities that can cause fluorescence quenching.

Table 3

The quantum yield of fluorescence of fractions 3 and 4

Parametre	Fraction		Extract with impurities
	3	*4*	
Fluorescent integral intensity of the sample	107.2	195.9	324.1
Fluorescent integral intensity of the standard (flourescein in 0,1 n. NaOH, quantum yield 0,93)	768.1	901.5	3,658.2
Fluorescence intensity relation	0.14	0.22	0.09
Relative quantum yield	0.13	0.20	0.08

According to the reference literature [4], the quantum yield of hypericin standard solution in methanol is 0.27. Comparing the data from the literature and the experimental data we have obtained by the values of the quantum yield of the crude extract of St. John's wort and fraction 4, it can be noted that the SPE-separation of purified extract on the sorbent WatersSep-Pak S18®VacRC produces the drug of the herb St. John's wort with a high content of hypericin.

Conclusion. Studies indicate that the solid-phase extraction on octadecylsilyl sorbent can be used to enrich the herb St. John's wort extracts with hypericin.

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