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ELECTRONIC ABSORPTION SPECTRA OF ST. JOHN'S WORT EXTRACTS

In this study electronic absorption spectra of John's Wort extracts of different types from the collection of Central Botanical Garden of the Belarusian NAS have been investigated. It has been shown that the character of electronic absorption spectra significantly depends on the nature of herbal material, temperature, polarity of solvents and pH value of medium. The *Hypericum perforatum* was found to be the most prospective home herbal material. The optimal conditions of hypericin extraction from John's Wort, including temperature mode and pH value have been established. On the basis of spectral researches it is shown that the greatest extent of hypericin extraction is reached at ethanol and acetone use.

Introduction. Photodynamic therapy is a perspective and actively developing method of treatment of malignant cells. Successful realization of photodynamic therapy of oncological diseases requires a combination of laser radiation and effective photosensitizers. The main requirements to photosensitizers include high selectivity to cancer cells, low toxicity, the ability to be brought out of the organism easily, high quantum exit of triplet state with the energy of not less than 94 kJ/mol. The existence of intensive absorption in red and near infrared spectrum area is also of great importance while chosing a photosensitizer.

In modern medical practice the tendency to expansion of the use of phytopreparations for treatment and prevention of various diseases is observed as medicines on herbal basis combine the wide range and softness of therapeutic action and the absence of a great number of side effects. According to literary data, the main sources of receiving herbal preparations of photosensitizing action are medical plants containing coumarin and anthraquinone derivatives. Among the plants growing on the territory of the Republic of Belarus, the most perspective for extraction of highly effective photosensitizers are the different types of a St. John's Wort containing hypericin and its derivatives. It is known [1] that hypericin possesses rather sharp selectivity to cancer cells, has no impact on the normal dividing (poliferating) cells and it is quickly brought out of an organism. Therefore the optimization of conditions of hypericin extraction from domestic herbal raw materials of some representatives of Hypericum sort (Hypericaceae) is an important task.

Various methods of the analysis [1–4] are applied to identification and definition of hypericin in herbal extraction, however the most available and informative one is a spectrophotometric method. In this regard the study of spectral characteristics of the St. John's Wort extracts, differing in conditions of carrying out the extraction, is of a great scientific and practical interest.

Main part. Objects of research were the ethanol extracts of the touch-and-heal St. John's Wort *Hypericum perforatum L.*, the olympic St. John's Wort *Hypericum olympicum L.*, the bushy St. John's Wort *Hypericum densiflorum L.* from a collection of the Central botanical garden NAS of Belarus. Plants were botanized in a blooming stage in the period from June to August, 2012 according to the general rules of botanizing for these medicinal raw materials [5]. Air-dried herbal raw materials were crushed to the particles of 1 mm size. For hypericin extraction from biomass we used water ethanol solutions with a mass fraction of ethanol 50 wt %. The process of extraction was carried out at the room temperature at continuous mixing during 1 h and without the access of natural light.

Electronic spectra of absorption were written down in the range of 400-1100 nanometers (wavelength pitch 0.1 nanometers) by the Specord-200 device in cuvettes with 10 mm layer thickness. The temperature interval was 5–65 °C with a pitch of 10 °C.

In the experiments ethanol extracts were subjected to vacuum pumping up to the complete removal of ethanol, and the solid residue was dissolved in the solvents of different polarity. Dielectric permeability of solvents varied from 2.20 to 78.3.

For studying of the influence of the acidity of the medium the recording of electronic spectra of absorption of extracts in the range of values pH = 2.0–11.0 with 0.1 interval was carried out. For finishing pH extracts to a target value we used water NaOH and HCl solutions. Control of pH was done by means of pH-meter Hanna HI 8314.

In Fig. 1 the electronic spectrum of a standard sample of hypericin in methanol is presented. The absorption spectrum of hypericin in UF area contains strips with maxima about $\lambda \approx 280$ (proteinaceous strip) and 330 nanometers. In visible spectrum area the wide plateau in the range of wavelengths of 400-500 nanometers is observed and there are strips with maxima at nanometer λ ≈ 547 and 590, and the latter strip is the most intensive. On the basis of literature [3] and obtained by us spectral data this strip of absorption with a maximum at λ = (590 ± 1) nanometer is accepted as the analytical wavelength of hypericin in St. John's Wort extracts.

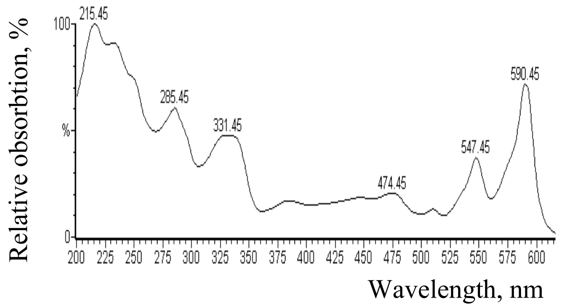


Fig. 1. Electronic spectrum of a standard sample of hyperin in methanol

For screening of the herbal raw materials of the representatives of *Hypericum* growing on the territory of the Republic of Belarus etanol extracts from different types of St. John's Wort were received and their electronic spectrum of absorption were taken. Fragments of electronic spectra of absorption of the received extracts in a visible interval of wavelengths are given in Fig. 2.

Comparative intensity of absorption strips of the studied samples at λ ≈ 590 nanometers shows that the touch-and-heal John's Wort extract contains the greatest number of hypericin (Fig. 2, curve 1). Hypericin concentration in the extract of John's Wort Olympic is practically 10 times less (fig. 2, curve 2), than in *Hypericum perforatum*. In extract of bushy John's Wort hypericin is present in trace quantities (Fig. 2, curve 3).

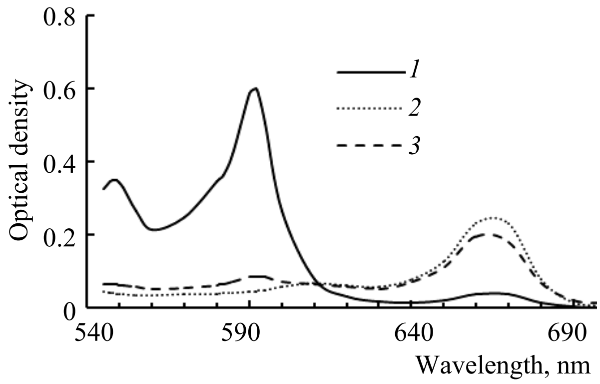


Fig. 2. Fragment of absorption spectra of extracts from different types of St. John's Wort

Thus, the most perspective plant material for obtaining hypericin is the toutch-and-hole St. John's Wort. Therefore the subsequent spectral researches were conducted only with *Hypericum perforatum L* extract.

The typical electronic absorption spectrum of etanol extract of the toutch-and-heal John's Wort is given in Fig. 3.

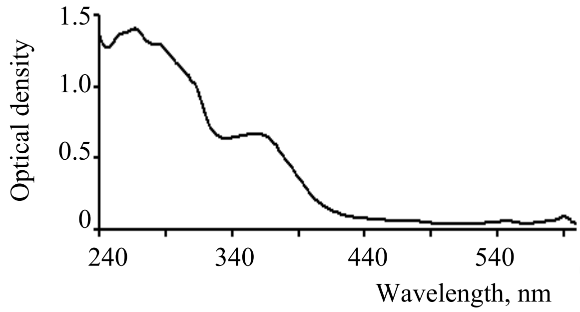


Fig. 3. Electronic absorption spectrum of John's Wort extract

Comparison of the electronic spectra of a standard sample of hypericin and the extract testifies to existence of the general strips of absorption. Hypericin in extract is manifested in the form of absorption strips with maxima of λ ≈ 550 and 590 nm, however their intensity is much lower in comparison with a standard sample. It should be noted that the optical density of the extract in UF area significantly exceeds that one in the spectrum of a standard sample of hypericin Apparently, the absorption of the spectrum of extract samples in UF area is connected with imposing of strips of absorption of hypericin, and of the accompanying compounds existing in the ethanol extracts *Hypericum perforatum* as well.

It is known [1] that the position of a maximum and the intensity of absorption strips of natural pigments depend significantly on the factors the most important of which are the solvent and pH medium nature, and the temperature.

Temperature dependence of absorption spectrum of John's Wort extract in the visible area is given in Fig. 4.

It is seen that heating of the extract leads to decrease of absorption intensity in whole studied interval of wavelengths. The most noticeable decrease of absorption intensity is observed in the field of 590 nm strip because of the increase in extent of the hypericin decomposition.

To establish optimal conditions of hypericin extraction from St. John's Wort the influence of solvent polarity on spectral characteristics of extracts was studied. Values of dielectric permeability of solvents was from 4.22 to 78.30. The analysis of the obtained spectral data showed that the change of polarity of solvent leads not only to the change of intensity of an analytical strip of hypericin absorption (λ ≈ (590 ± 1) nm), but also to the shift of its maximum into the long-wave or short-wave areas.

Data on the influence of dielectric permeability of solvents on the value of optical density of St.

John's Wort extracts with the wavelength corresponding to the maximum of light absorption with a length of wave of λ ≈ of 590 nm are provided in the table.

Dependence of size of the optical density is given in Fig. 5 with a wavelength of a maximum of absorption (λ ≈ of 590 nm) of St. John's Wort extract on the solvent polarity.

Influence of the solvent nature on spectral characteristics of St. John's Wort extracts

Solvent	ε	λ_{max}, nm	A
Diethyl ether	4.22	591.7	0.051
Ethyl acetate	6.00	595.7	0.047
Isoamyl alcohol	14.70	593.4	0.238
Acetone	20.74	597.2	0.265
Ethyl alcohol	25.80	592.0	0.660
Formic acid	57.90	578.9	0.432
Water	78.30	600.2	0.141

From the presented data it is clear that the dependence has an extreme character. As the content of hypericin in the initial etanol extracts was the same, the observed spectral differences at solvent variation are connected both with the electronic state of hypericin itself, and with its diferent solubility. Hypericin is well dissolved in ethanol or acetone having close values of dielectric permeability and it is in a monomeric salt form. Sharp decrease of the optical density of hypericin in the spectrum of water solution is caused by low hypericin solubility in water [6].

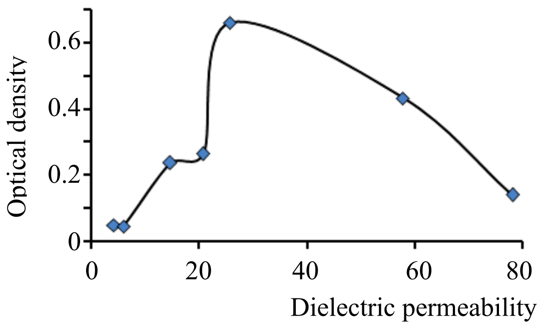
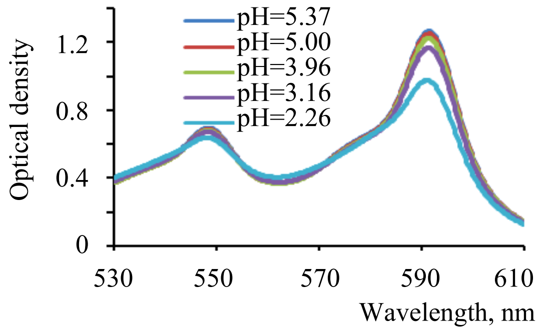


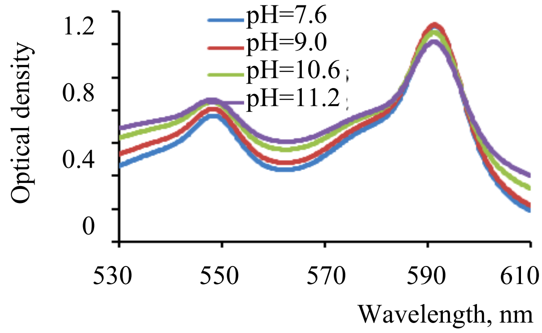
Fig. 5. Influence of solvent polarity on the optical density of St. John's Wort extract at λ ≈ of 590 nm

Using the formic acid as a solvent leads to similar spectral changes. The decrease in optical density with the analytical wavelength of hypericin (λ ≈ of 590 nm) due to the reduction of hypericin solubility in the sour medium is observed in a spectrum. The observed shift of a maximum of the absorption strip at λ ≈ 590 nm into the area of shorter lengths of the waves is connected with formation of the protonated forms of hypericin [6].

The data on the influence of the medium acidity on spectral characteristics of St. John's Wort extracts is the confirmation of the received results. The spectra of absorption of extracts are given in Fig. 6 (a and b) at a variation pH from 2.3 to 11.2.



a



b

Fig. 6. Influence of the medium eacidity on the electronic absorption spectrum of John's Wort extract

From the presented data it is seen that in sour solutions (pH = 2.26) the intensity of the absorption strip at λ ≈ of 590 nm is small and it becomes larger at the increase of pH up to 5.37 units (Fig. 6, *a*). The observed spectral changes are connected with formation of mainly protonated forms of hypericin in sour solutions and also with the fall of its solubility. At the pH increase of the extracts from 5.4 to 9.0 the optical density practically doesn't change (Fig. 6, *b*). According to the literary data [6], monomeric salt forms of hypericin well soluble in water are formed in alkalescent solutions. Further pH increase of the medium is accompanied by some decrease in the intensity of the analytical absorption strip of hypericin.

Conclusion. Thus, the most perspective of the herbal raw materials of the domestic origin investigated by us and suitable for hypericin extraction are *Hypericum perforatum L* plants. The conducted spectral researches allowed to establishthe the preferable conditions of hypericin extraction from St. John's Wort herb. Hypericin extraction needs to be carried out at the room temperature, with the use of the extractive systems including acetone or ethanol. The interval of pH values has to be 5.4–9.0.

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