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EXTRACTION ISOLATION OF HYPERICINS FROM PARTICULAR KINDS OF ST. JOHN'S WORT (HYPERICUM PERFORATUM)

The paper deals with development of the effective mode of isolation of hypericins from St. John's Wort including Soxhlet extraction with ethanol as a solvent. In Central Botanical Garden of the NAS of the RB were found out the kinds of St. John's Wort, which contain the largest amounts of hypericins. They may be used as material for producing of phytopreparations and drugs for treating oncological diseases by the method of photodynamic therapy.

Introduction. Hypericins are red pigments in St. John's Wort and represent condensed derivatives of anthraquinone. Due to chromophore atomic group contained in given compounds they could be widely applied as photosensitizes when treating oncological diseases by the method of photodynamic therapy [1]. In St. John's Wort there are two red pigments – hypericin and pseudohypericin. The structures of these compounds are presented in the figure.

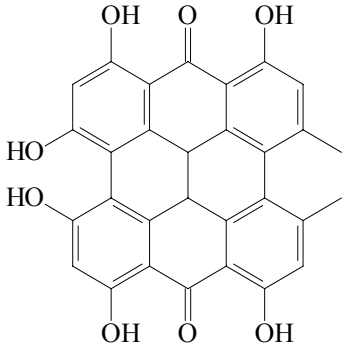
Hypericin and pseudohypericin are located in glandules of plant flowers, leaves and stalks. But to some authors they are present in enchylema as well. The content of hypericin in St. John's Wort is from 0,03 to 0,34%, therefore it may be a potential source for isolation of these compounds [2].

Main part. This work aimed at selecting of optimal conditions for extraction of hypericins in particular kinds of St. John's Wort. Four kinds of St. John's Wort cultivated in Central Botanical Garden of the NAS of the RB were analyzed for reaching this goal.

The most effective method of natural compound isolation from vegetable raw material, among them hypericins, is extraction. It means that substances from vegetable raw materials are relocated into solution by appropriate solvent.

There are a lot of extraction methods, which are employed not only for isolation of biologically active compounds from vegetable raw materials but also for substance mixture splitting and for purifying of particular organic substances from admixtures. The easiest and most effective methods are: maceration, digestion, percolation, perforation [3, 4].

When determining the most effective solvent for hypericin isolation from St. John's Wort the extraction was carried out by the method of maceration within ten days in darkness at room temperature. As solvents were used ethanol, acetone, butanone-2, cyclohexanone-2, ethyl acetate, chloroform. Screening of the most suitable solvents was carried out according to their capability to extract substances and hypericins.



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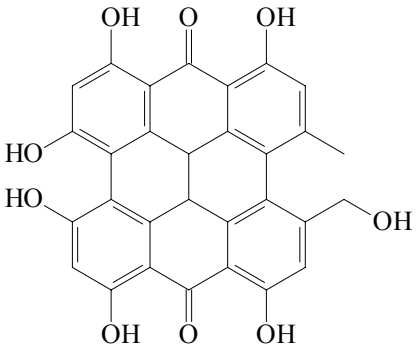

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Structural formulae of hypericin (1) and pseudohypericin (2)

Quantitative characteristics of extraction with different solvents by the method of maceration are presented in Table 1.

Table 1

The solvent influence on extraction output of St. John's Wort

Solvent	Extract output towards to St. John's Wort, wt %
1 ethanol	12.53
2. acetone	3.90
3. butanone-2,	9.66
4. cyclohexanone-2	10.00
5. ethyl acetate	2.23
6 chloroform	3.56

Hypericin qualitation in obtained extracts was carried out by the method of thin-layer chromatography (TLC) according to bright red fluorescence and proper rate R_f [2, 5].

There were no hypericins in extracts obtained with help of butanone-2, cyclohexanone-2, ethyl acetate, chloroform as extractants, whereas in extracts with acetone and ethanol there were observed hypericin as well as its structural analog pseudohypericin. As a result extraction by the method of maceration allowed to find out that acetone and ethanol are the most effective solvents for hypericin isolation.

As the method of maceration is a statistic approach and not always results in maximum output of extraction substances we have tested extraction by method of percolation in Soxhlet device in presence of ethanol and acetone. Screening of the most effective method of extraction was realized not only according to extraction outputs but also to quantitative content of hypericins in them.

The quantitative content of hypericins was determined by method of HPLC (high-performance liquid chromatography) with help of chromato-mass-spectrometer "Waters Micromass ZQ 2000" and column BDS HYPERSIL C18 250×4.6 мм. Detection was carried out by diode matrix detector with wave length of 590 nm and by mass-detector with electrosprayionization. Elution was realized in linear gradient assisted by the system consisting of acetonitrile and ammonium acetate water solution 0,01 M with flow rate 1 ml/min [6].

The results of comparative hypericin extraction from St. John's Wort "K-8" carried out by the method of maceration assisted by Soxhlet device in presence of ethanol are presented in Table 2.

Table 2

Influence of extraction conditions on effectiveness of hypericin eduction from St. John's Wort

Method of extraction	Extract output, wt %	Hypericin content in extract wt %
In Soxhlet device	32.4	0.129
The method of maceration	12.5	0.070

The Table shows that extraction assisted by Soxhlet device is more effective in comparison with extraction by the method of maceration.

In order to detect the most effective solvent for obtaining of hypericin containing extract in Soxhlet device extraction was made from St. John's Wort "Amber" by ethanol and acetone. Quantitative characteristics of extraction processes are presented in Table 3.

As follows from Table 3 the hypericin content is equal in both extracts but application of ethanol as extractant provides a higher output of extraction substances in comparison with application of acetone as solvent. As a result the most acceptable solvent for hypericin extraction is ethanol.

Table 3

Influence of solvent on effectiveness of hypericin extraction from St. John's Wort in Soxhlet device

Extractant	Extract output, wt %	Hypericin content in extract wt %
Ethanol	22.0	0.23
Acetone	7.2	0.23

Thus, application of ethanol and Soxhlet device for extraction is most effective for hypericin eduction from vegetable raw materials in comparison with extraction by the method of maceration and using of other solvents as extractants. The developed successful mode of hypericin extraction was employed for their eduction from 4 kinds of St. John's Wort cultivated in Central Botanical Garden of the NAS of the RB for identifying and carrying out quantitative analysis. Quantitative hypericin content in different samples of St. John's Wort was detected also by method of HPLC. The results are presented in Table 4.

Table 4

Detection of hypericin content in different kinds of St. John's Wort

Kind	Hypericin content in extract wt %	Hypericin content in St. John's Wort wt %
1. Amber	0.230	0,051
2. Wild from Hungary	0.081	0.02
3. K-8	0.129	0.042
4. Wild from woodland	0.084	0.016

From Table 4 is obvious that the samples 1 and 3 contain the largest amount of hypericin therefore they can be used as feed stock for hypericin eduction and producing on their basis medicines for photodynamic therapy.

Conclusion. In this way the effective method of hypericin eduction from St. John's Wort has been developed. It includes extraction by ethanol in Soxhlet device and provides high output of substance extraction with high hypericin content.

It was revealed that the sorts of St. John's Wort «Amber» and «K-8», cultivated in Central Botanical Garden of the NAS of the RB contain the largest amount of hypericin (0,051 и 0,042% respectively) and are the most beneficial materials for producing medicines for photodynamic therapy.

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