DETERMINATION OF THE OPTIMAL EXTRACTANT FOR THE EXTRACTION OF BIOLOGICALLY ACTIVE SUBSTANCES OF SOPHORA FLOWER–BUDS

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Introduction

Herbal medical products (HMP) based on Sophora japonica L., which have anti–inflammatory and immunomodulatory effects, are presented on the pharmaceutical market of Ukraine [1]. Pharmacologic effect of Sophora japonica due to the presence of a complex of flavonoids: rutin, 3–sophoroside kempherol (sophoraflavonoside), 4– genistein glucoside (soforobioside), the main of them is rutin [2, 3]. The content of rutin differs in various types of herbal materials of Sophora and depends on the period of budding. Especially a lot of rutin accumulates in young, rapidly developing organs of the plant, its maximum amount is noted in buds [4].

According to the market products based on Sophora japonica L., extraction of biologically active substances (BAS), flavonoids in particular, is carried out with ethanol in a concentration 50–70% [1]. However, in recent years, in literature data have appeared the information that the use of water–ethanol solutions is not effective for the extraction of biologically active substances and contributes to the release of a large amount of ballast substances. An alternative is to use surfactant–based extractants (SBE) [5,6]. In relation to Sophora Flower–buds, these questions have not been investigated, which determines the relevance of research on the selection of the optimal extractant.

Purpose of the study. The aim of this work was research of determination of the optimal extractant to extract the maximum amount of Sophora Flower–buds flavonoids. To assess the quality of the obtained extracts a quantitative determination of the sum of flavonoids in terms of rutin and the study of antiradical activity were carried out.

Materials and Methods

The object of the research is Sophora Flower–buds, harvested in 2018 during the budding period, supplier – Phytomarket "Mir trav". Herbal material Sophora Flower–buds met the requirements of monographs State Pharmacopoeia of Ukraine 2.1 «Sophora Flower–buds» [7].

At the first stage, determination of Sophora Flower–buds technological parameters was conducted: the degree of grinding of herbal materials, specific mass, bulk density, bulk weight, porosity, permeability and free volume of the layer. Research methods are given in the literature [8].

Sophora Flower–buds extracts were obtained according to the scheme: dry herbal materials, crushed to a particle size passing through a sieve with a hole diameter of 3–5 mm and placed in an extractor. In the extraction process was used *ethanol P* in concentrations 30 %, 40 %, 50 % and an aqueous solution of sodium lauryl sulfate in concentrations 0,5 %, 1,5 %, 2,5 % in the ratio of herbal material –extractant 1:1. Extracts were obtained using a paddle mixer, the extraction time was 1 hour, 2 hours and 3 hours until the biologically active substances were completely extracted from the herbal material.

Determination of dry residue of extracts. The definition of this quality indicator was carried out in accordance with the requirements of the State Pharmacopoeia of Ukraine monograph 2.0 «Determination of dry residue of extracts» *(2.8.16)* [9].

Determination of the amount of flavonoids in terms of rutin. Conducted in accordance with the requirements of the SPhU monograph 2.0 «Absorption spectrophotometry in the ultraviolet and visible regions» *(2.2.25)* and SPhU 2.1 «Sophora Flower–buds» quantification procedures [8].

Determination of antiradical activity. Conducted using spectrophotometric method in accordance with the requirements of the SPhU monograph 2.0 «Absorption spectrophotometry in the ultraviolet and visible regions» *(2.2.25)* [9].

Extract preparation. 1.00 g (accurate weight) of plant extract was placed into a 25.0 mL volumetric flask and filled up to the mark by solvent (30, 40, 50% EtOH or water).

Method. Free radical scavenging activity of samples was estimated by ABTS radical cation assay [10, 11]. The stock solution included 7mM ABTS solution and 2.4 mM potassium persulfate solution were mixed in equal quantities, stored in the dark at the room temperature for 16 h before use. The obtained solution was diluted with water to obtain absorbance of 0.900 at 734 nm. 100 μL of test solution was added to 4.9 mL of ABTS solution, the absorbance was measured in 30 minutes after the initial mixing. An appropriate solvent blank was run in each assay. All determinations were repeated three times. Percent inhibition of absorbance at 734 nm has been calculated as ABTS scavenging activity (%):

$$(\%) = \frac{A_o - A_T}{A_0} \times 100, \text{ where}$$

A_o – absorbtion of ABTS solution;

$A_т$ – absorbtion of ABTS solution mixed with the sample / standard.

The ABTS scavenging capacity of samples was evaluated with the Trolox standard.

Results and Discussion

The results of the determination of technological parameters for herbal material of Sophora Flower–buds presented in table 1. The determination of each parameter was carried out on 3 samples.

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Table 1. The results of the determination of technological parameters of Sophora Flower–buds (n =3)

№	Indicator	Results
1	Disintegration, mm	3–5±0.03
2	Bulk density, g/cm^3	0.65±0.02
3	Bulk weight, g/cm^3	0.50±0.02
4	Specific mass, g/cm^3	0.22±0.03
5	Porosity, g/cm^3	0.72±0.02
6	Permeability, g/cm^3	0.69±0.02
7	Free volume of the layer, g/cm^3	2.01±0.04

The obtained data on the technological properties of the herbal material (table 1) will be used to develop an optimal method for extracting, predicting and standardization of the quality of extracts.

The next step was to prepare the Sophora Flower–buds extracts and a study of their quality indicators. One of the quality indicators that indicates a rationally selected extractant is "Determination of dry residue of extracts". The results are presented in table 2.

Table 2. The results of the determination of the dry residue of Sophora Flower–buds extracts (n = 3)

Extraction time	Dry residue values					
	Ethanol			Sodium lauryl sulfate solution		
	30 %	40 %	50 %	0.5 %	1.5 %	2.5 %
1 hour	5.96±0.24	7.02±0.41	8.24±0.36	5.85±0.32	7.14±0,25	8.07±0.38
2 hours	6.41±0.29	7.59±0.27	8.72±0.28	6.29±0.26	7.64±0.33	8.48±0.32
3 hours	6.49±0.31	7.64±0.36	8.81±0.37	6.35±0.22	7.73±0.34	8.54±0.45

The data obtained in table 2 indicate that the highest values of dry residue were obtained during the extraction for two to three hours using ethanol 50 % and sodium lauryl sulfate solution 2.5 % as the extractant.

The next step was to research a comparative evaluation of the flavonoids content in the extracts obtained. Quantitative determination of the sum of flavonoids was performed by spectrophotometric method according to the SPhU 2.1 method «Sophora Flower–buds». The typical UV spectrum of the tested Sophora Flower–buds extraction solutions is shown in Fig. 1. The results of quantitative determination are given in table 3.

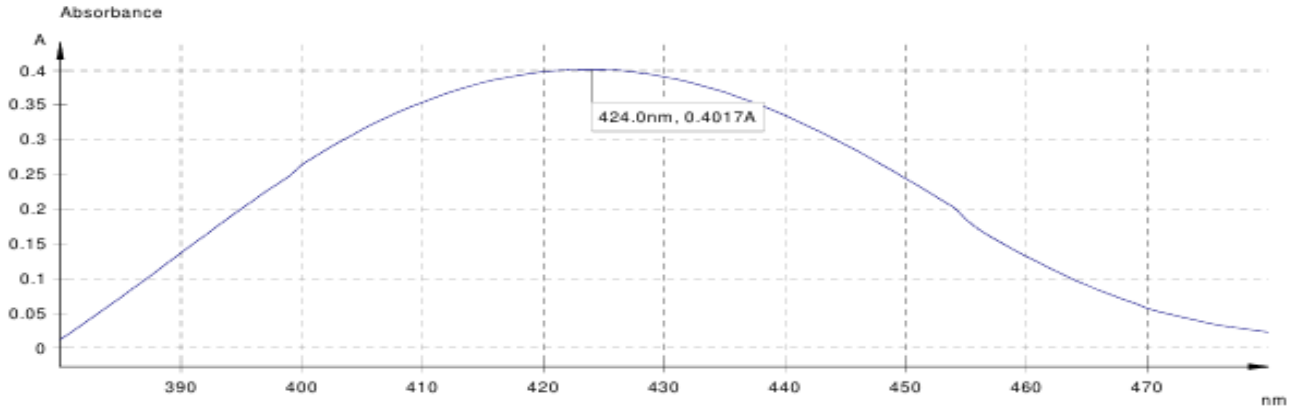

Fig. 1 Typical UV spectrum of the tested extraction solution Sophora Flower–buds

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Table 3. Results of the quantitative content of the sum of flavonoids in extracts Sophora Flower–buds (n = 3)

Extraction time	The content of flavonoids in terms of rutin, %					
	Ethanol			Sodium lauryl sulfate solution		
	30 %	40 %	50 %	0.5 %	1.5 %	2.5 %
1 hour	5.76±0.03	6.89±0.01	8.04±0.03	5.71±0.02	7.09±0.01	7.98±0.03
2 hours	6.12±0.04	7.29±0.02	8.61±0.02	6.12±0.01	7.58±0.03	8.28±0.02
3 hours	6.19±0.01	7.34±0.03	8.75±0.01	6.17±0.02	7.64±0.02	8.43±0.03

In the table. 3, the quantitative results indicate that the maximum amount of flavonoids in the raw material under study is achieved by extraction within three hours using ethanol 50 % and sodium lauryl sulfate solution 2.5 % as the extractant. According to the literature data, the biological action of phenolic compounds is based on their antioxidant properties, which are the ability to react with free radical compounds formed under conditions of oxidative stress [12]. Sophora Flower–buds contains flavonoid compounds of different chemical composition, and their qualitative and quantitative composition in extraction can affect biological activity [13]. Therefore, for a more objective evaluation of the extracting ability of the proposed extractants were conducted studies of antiradical activity. The results are presented in table 4.

Table 4. Results of evaluation of antiradical activity (n = 3)

Extraction time	TEAC (μmol/g)*, mean±SD					
	Ethanol			Sodium lauryl sulfate solution		
	30 %	40 %	30 %	0.5 %	1.5 %	2.5 %
1 hour	4093.71 ±0.02	4633.12 ±0.03	5162.92 ±0.01	4076.47 ±0.03	4687.35 ±0.03	5154.43 ±0.02
2 hours	4207.21 ±0.02	4878.40 ±0.01	5122.92 ±0.02	4242.55 ±0.02	4904.74 ±0.01	5179.14 ±0.01
3 hours	4493.71 ±0.03	5068.40 ±0.03	5162.92 ±0.02	4524.36 ±0.03	5074,74 ±0.03	5207.04 ±0.02

*TEAC, Trolox equivalent of antiradical capacity (μmol) in the samples (g) of dry weight.

Research of the antiradical activity of Sophora Flower–buds (table 4) showed that the maximum value of TEAC is exhibited by such agents as ethanol 50 % and sodium lauryl sulfate solution 2.5 %. The data obtained confirm that the antiradical activity depends on the quantitative content of flavonoids in the studied samples of Sophora Flower–buds.

Conclusions

1. The research of possibility of using surfactants for the extraction of biologically active substances from Sophora Flower–buds was conducted. It is found that the best extractive ability to Sophora Flower–buds flavonoids have extractants: ethanol 50 % and sodium lauryl sulfate solution 2.5 %, which have virtually the same quality indicators: dry residue 8.81±0.37 and 8.54±0.45; the content of the sum of flavonoids 8.75±0.01 и 8.43±0.03.
2. The study of antiradical activity in Sophora Flower–buds extracts was conducted for the first time. Maximum values are obtained in extracts by extraction over two hours using ethanol 50 % sodium lauryl sulfate solution 2.5 %.
3. The data presented can be used in research on the development of Sophora Flower–buds herbal medicinal products in the various dosage form.

Determination of the optimal extractant for the extraction of biologically active substances of Sophora Flower–buds

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Introduction. According to the market products based on Sophora japonica L., extraction of biologically active substances, flavonoids in particular, is carried out with ethanol in a concentration 50–70%. However, in recent years, in literature data have appeared the information that

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the use of water–ethanol solutions is not effective for the extraction of biologically active substances and contributes to the release of a large amount of ballast substances. An alternative is to use surfactant–based extractants. In relation to Sophora Flower–buds, these questions have not been investigated, which determines the relevance of research on the selection of the optimal extractant. **Material & methods.** The object of the research is Sophora Flower–buds, harvested in 2018 during the budding period. The definition of quality indicators was carried out in accordance with the requirements of the State Pharmacopoeia of Ukraine. **Results & discussion.** Determination of Sophora Flower–buds technological parameters was conducted: the degree of grinding of herbal materials, specific mass, bulk density, bulk weight, porosity, permeability and free volume of the layer. The obtained data on the technological properties of the herbal material used to develop an optimal method for extracting, predicting and standardization of the quality of extracts. Studied one of quality indicators, that indicates a rationally selected extractant is "Determination of dry residue of extracts". Also was carried out research a comparative evaluation of the flavonoids content in the extracts obtained. Quantitative determination of the sum of flavonoids was performed by spectrophotometric method according to the SPhU 2.1 method «Sophora Flower–buds». The data obtained indicate that the highest values of dry residue and maximum amount of flavonoids were obtained during the extraction for two to three hours using ethanol 50 % and sodium lauryl sulfate solution 2.5 % as the extractant. Content flavonoid compounds of different chemical composition Sophora Flower–buds can affect biological activity. Therefore, for a more objective evaluation of the extracting ability of the proposed extractants were conducted studies of antiradical activity. Research of the antiradical activity of Sophora Flower–buds showed that the maximum value of TEAC is exhibited by such agents as ethanol 50 % and sodium lauryl sulfate solution 2.5 %. The data obtained confirm that the antiradical activity depends on the quantitative content of flavonoids in the studied samples of Sophora Flower–buds. **Conclusion.** The research of possibility of using surfactants for the extraction of biologically active substances from Sophora Flower–buds was conducted. It is found that the best extractive ability to Sophora Flower–buds flavonoids have extractants: ethanol 50 % and sodium lauryl sulfate solution 2.5 %, which have virtually the same quality indicators: dry residue 8.81±0.37 and 8.54±0.45; the content of the sum of flavonoids 8.75±0.01 и 8.43±0.03. The study of antiradical activity in Sophora Flower–buds extracts was conducted for the first time. Maximum values are obtained in extracts by extraction over two hours using ethanol 50 % sodium lauryl sulfate solution 2.5 %. The data presented can be used in research on the development of Sophora Flower–buds herbal medicinal products in the various dosage form.

Keywords: Sophora Flower–buds, extractant, biologically active substances

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