

## METHOD FOR THE QUANTITATIVE DETERMINATION OF BLACK-EYED SUSAN FLOWERS ANTHOCYANINS

### МЕТОД КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ АНТОЦИАНИНОВ В ЦВЕТКАХ РУДБЕКЦИИ ШЕРШАВОЙ

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**Резюме.** В ходе работы разработана методика количественного определения антоцианов рудбекии шершавой цветков при использовании в качестве основного экстрагента водно-ацетоновой смеси. Исходя из полученных результатов, наиболее эффективное извлечение антоцианов наблюдается при температуре экстракции – 60°C, соотношении сырья и экстрагента – 1:100, продолжительности экстракции – 1,5 ч при однократном извлечении, предпочтительный размер частиц сырья – менее 180 мкм, концентрация кислоты хлористоводородной в растворе – 25 г/л, продолжительность проведения реакции – 20 мин.

**Ключевые слова:** Антоцианы, количественное определение, рудбекия шершавая.

**Abstract.** In the course of the work, a method was developed for the quantitative determination of Black-eyed Susan flowers anthocyanins using a water-acetone mixture as the main extractant. Based on the results obtained, the most effective extraction of anthocyanins is observed at an extraction temperature of 60 °C, plant raw material and extractant the ratio of 1: 100, an extraction time of 1.5 h with a single extraction, the preferred feed particle size is less than 180 µm, and the concentration of hydrochloric acid in solution – 25 g/l, reaction time – 20 min.

**Keywords:** Anthocyanins, quantitative determination, Black-eyed Susan.

### Introduction

The results of previous studies have demonstrated that Black-eyed Susan flowers are a rich source of anthocyanins [1], which are one of the most promising subgroups of flavonoids for research and have a wide range of pronounced pharmacological effects.

For the rational use of medicinal plant raw materials, its standardization is provided, which includes method of quantitative determination of active substances. It was previously found that the method for the quantitative determination of anthocyanins in begonia leaves, set out in the State Pharmacopoeia of the Republic of Belarus [2], showed the highest content of anthocyanins in Black-eyed Susan flowers, therefore, in the study of the optimal conditions for the quantitative determination of anthocyanins, we initially used the main extraction parameters from this method. However, it is necessary to clarify them, since a new research object is used.

Also, earlier studies of the extraction ability of various solvents were carried out, according to the results of which it was revealed that 70% of acetone has the highest extraction ability, which determines its further use for the development of a method for the quantitative determination of anthocyanins in Black-eyed Susan flowers [3].

Based on the above, the goal of the study was formulated: develop a method for the quantitative determination of Black-eyed Susan anthocyanins.

### Materials and methods

The object of the study was Black-eyed Susan flowers harvested during the period of mass flowering from cultivated forms in mid-July 2019 in the vicinity of Vitebsk.

For the study, the following parameters were selected: extraction temperature, ratio of plant raw material and extractant, duration of extraction, frequency of extraction, particle size of plant raw materials, concentration of hydrochloric acid, reaction time.

During the study, the following reagents were used: hydrochloric acid solution, 70% acetone.

The content of the sum of anthocyanins was determined in terms of cyanidin chloride by the method of a calibration graph. Statistical processing was carried out using the Data Analysis package of the Microsoft Office Excel 2010 computer program. The results were presented as the mean and the half-width of its confidence interval ( $n = 3$ ;  $P = 95\%$ ).

### Results and discussions

During the study of the influence of temperature on the extraction of anthocyanins, the following temperatures were studied: 20 (room temperature), 40, 60, 80 and 100 °C (boiling water bath). The experimental data obtained in this case are presented in figure 1.

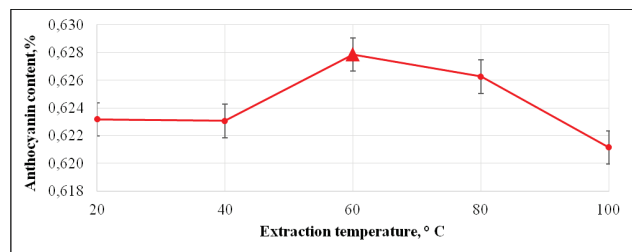


Fig. 1. Dependence of the content of anthocyanins on the extraction temperature

Based on the results presented in figure 1, it can be seen that the most complete extraction of anthocyanins occurs at a temperature of 60°C (0.628 ± 0.0314%).

The next step was to study the influence of the ratio of plant raw materials and extractant (g/ml). The studied ratios are 1 to 5, 1 to 10, 1 to 25, 1 to 50, 1 to 100. The data is shown in figure 2.

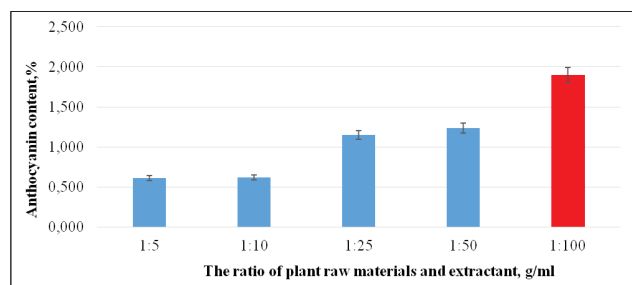


Fig. 2. Dependence of the content of anthocyanins on the ratio of plant raw materials and extractants

The largest extraction of anthocyanins fell on the ratio of raw materials and extractants – 1: 100 (1.90 ± 0.095%).

The next investigated parameter was the duration of the extraction. Extractions were obtained by thermal extraction for 30 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours and 6 hours. The results are shown in figure 3.

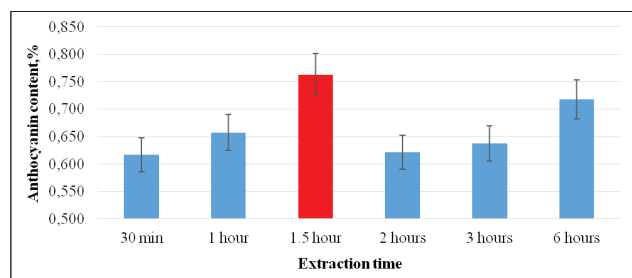


Fig.3. Dependence of anthocyanin content on extraction time

The largest amount of anthocyanins was released at an extraction time of 1.5 hours (0.763 ± 0.0382%).

The next step was to study the effect of the extraction rate on the anthocyanin content in the extraction. Studied one-, two- and threefold extraction. The results are shown in figure 4.

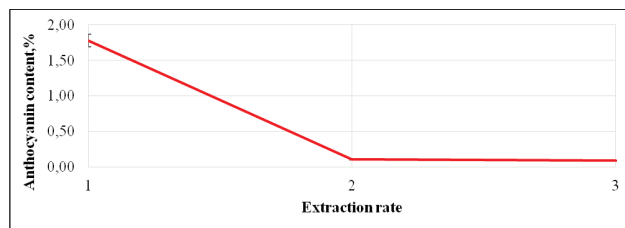


Fig. 4. Dependence of the content of anthocyanins on the extraction rate

During the work, it was found that two- and threefold extraction (0.106 ± 0.00530% and 0.0891 ± 0.00446%, respectively) did not carry a significant amount of anthocyanins, in contrast to the extraction obtained with a single extraction (1.78 ± 0.0890%), which indicates the unambiguous effectiveness of the latter.

The next step was to study the effect of the particle size of Black-eyed Susan flowers on the content of anthocyanins in the extract. Particles larger than 2 mm (> 2 mm), from 2 to 1.4 mm (<2 mm), from 1.4 mm to 355 μm (<1.4 mm), from 355 μm to 180 μm (<355 μm), less than 180 μm (<180 μm). The results are shown in figure 5.

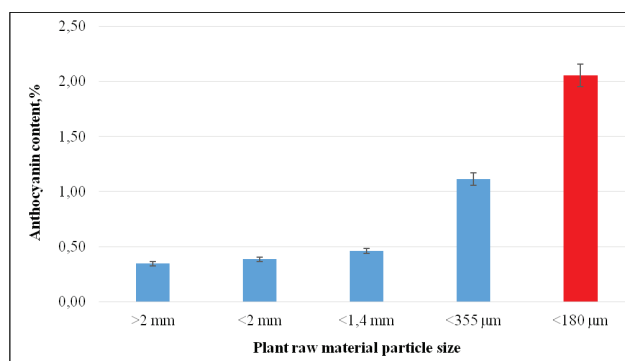


Fig.5. Dependence of the content of anthocyanins on the particle size of plant raw materials

It has been established that the content of anthocyanins in the extraction is in direct proportion to the degree of extraction of plant raw material particles. The best results were obtained for particles less than 180 μm (2.05 ± 0.103%).

Further, the course of work was transferred to the study of a solution of hydrochloric acid, which ensures the conversion of anthocyanins into the state of pyrylium salt with a characteristic red color, which ensures the detection of compounds spectrophotometrically in the visible region of the spectrum.

The concentration of hydrochloric acid in the solution and the reaction time were investigated.

In the course of studying the effect of concentration on the quantitative determination of anthocyanins, the following concentrations were investigated: 5, 10, 15, 20, 25 and 30 g/l. The obtained experimental data are shown in figure 6.

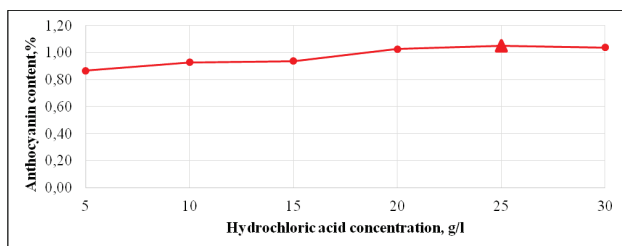


Fig.6. Dependence of the content of anthocyanins on the concentration of hydrochloric acid

The most effective concentration for the quantitative determination of anthocyanins of Black-eyed Susan flowers is 25 g/l ( $1.05 \pm 0.0525\%$ ).

The last studied parameter for the quantitative determination of anthocyanins was the reaction time of the obtained extract with hydrochloric acid. The reaction was carried out for 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 minutes. The data are presented in figure 7.

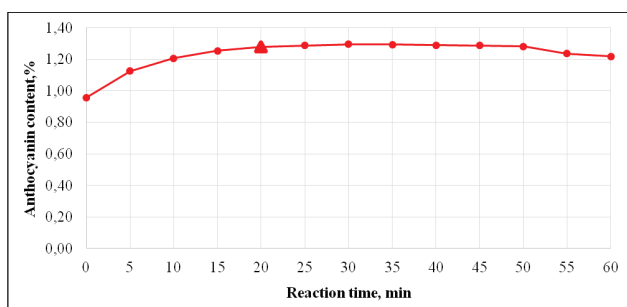


Fig.7. The dependence of the content of anthocyanins on the reaction time

It was revealed that the most optimal reaction time is 20 minutes ( $1.28 \pm 0.064\%$ ), after which the anthocyanin content reaches a plateau for 25-50 minutes, then by 55 minutes ( $1.24 \pm 0.062\%$ ) it begins to decrease.

When performing the method according to all the selected parameters, the average content of the sum of anthocyanins in terms of cyanidin chloride was  $2.79 \pm 0.194\%$ . the standard error of determination was 8.19%.

## Conclusions

In the course of the work, the optimal conditions for the quantitative determination of anthocyanins were established: a single extraction at a temperature of 60 °C for a duration of 1.5 hours with the plant raw material to extractant ratio of 1: 100, plant raw material particle size of less than 180  $\mu\text{m}$ , a hydrochloric acid concentration of 25 g/l with a reaction duration 20 minutes. Determined that the method has good reproducibility (RSD = 6.94%).

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## References

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