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### EVALUATION OF THE SPECTRAL CHARACTERISTICS, PURITY AND ANTIOXIDANT ACTIVITY OF C-PHYCOCYANIN FROM THE CYANOBACTERIA COLLECTED IN KAUNAS LAGOON (LITHUANIA)

N. HUDZ<sup>1,2✉</sup>, V. TURKINA<sup>3</sup>, O. YEZERSKA<sup>1</sup>, L. KOBYLINSKA<sup>4</sup>, A. FILIPSKA<sup>1</sup>,  
J. KAROSIENĖ<sup>5</sup>, D. GALINYTĖ<sup>6</sup>, G. BALČIŪNAITĖ–MURZIENĖ<sup>7</sup>,  
S. KHOMYAK<sup>8</sup>, N. SAVICKIENĖ<sup>9</sup>

<sup>1</sup>Department of Drug Technology and Biopharmacy,  
Danylo Halytsky Lviv National Medical University, Lviv, Ukraine;

<sup>2</sup>Department of Pharmacy and Ecological Chemistry, University of Opole, Poland;

<sup>3</sup>Research Institute of Epidemiology and Hygiene,  
Danylo Halytsky Lviv National Medical University, Lviv, Ukraine;

<sup>4</sup>Department of Biochemistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine;

<sup>5</sup>Laboratory of Algology and Microbial Ecology, Nature Research Centre, Vilnius, Lithuania;

<sup>6</sup>Department of Pharmacognosy, Lithuanian University of Health Sciences, Kaunas, Lithuania;

<sup>7</sup>Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Kaunas, Lithuania;

<sup>8</sup>Department of Technology of Biologically Active Substances, Pharmacy and  
Biotechnology, Lviv Polytechnic National University, Lviv, Ukraine;

<sup>9</sup>Department of Pharmacognosy, Lithuanian University of Health Sciences, Kaunas, Lithuania;

✉ e-mail: natali\_gudz@ukr.net

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The physicochemical characteristics of phycocyanin extracted from cyanobacteria collected in Kaunas Lagoon were studied (spectrum characteristics, C-PC content in the dry mass and chemical purity). It was determined that the tested concentrations of C-PC in purified water should be in the range of 0.02–0.16% for measuring C-PC content in the dry mass and its spectrum characteristics. The two clear absorption maxima were detected in the spectrum of C-PC at the wavelengths of 277 and 619 nm. The content of C-PC in the dry powder form was in the range of 7.25% to 9.30% depending on its concentration in the solution and type of spectrophotometer. Furthermore, a purity factor of 1.5 was calculated, which indicated the food qualification of the obtained biomass of C-PC. Finally, the analytical procedure for studying the pro- and anti-oxidant activity of C-PC was developed and the antioxidant activity of C-PC was measured for the available markers. It was revealed that C-PC has dual properties (pro- and anti-oxidant ones) depending on its concentration, more exactly, its content in reaction mixtures with 2,2-diphenyl-1-picrylhydrazyl (DPPH). The following issues were resolved during the research: the concentration of ethanol in the DPPH solution was chosen in order to avoid precipitation of proteins in the reaction mixtures (50%); the ratio of the solution of C-PC to the DPPH solution was selected; the selected concentrations of the markers for the construction of their calibration curves were chosen for quercetin and for rutin. The antioxidant activity of the obtained C-PC sample was determined.

**Key words:** C-phycocyanin, cyanobacteria, antioxidant activity, DPPH, rutin, quercetin.

**C**-Phycocyanin belongs to the family of phycobiliproteins present in cyanobacteria [1]. Cyanobacteria contain a high amount of phy-

cobiliproteins (PBPs), which are principal metabolic products associated with the light-harvesting complex in photosystems, called phycobilisomes (PBS).

PBS contain the core and rods assembly that contain allophycocyanin (APC) ( $\lambda_{\max} = 650\text{--}655\text{ nm}$ ) as a core surrounded by C-PC ( $\lambda_{\max} = 610\text{--}620\text{ nm}$ ) and occasionally C-phycoerythrin (C-PE) ( $\lambda_{\max} = 540\text{--}570\text{ nm}$ ) in rods [2].

The biological activities of C-PC include antioxidant, antibacterial, antitumor properties, etc. [3-9]. C-PC stimulates the immune system and exhibits hepatoprotective, antiplatelet and neuroprotective activities as well [10, 11]. Many authors state about the significant antioxidant activity of C-PC [9, 12, 13]. C-PC contains high levels of glutamic acid, aspartic acid, alanine, leucine, arginine, isoleucine, serine, glycine and threonine. These amino acids are reported to have antioxidant activity [14]. Antioxidants are important as substances that can inhibit the formation of free radicals by scavenging them or reducing them with hydrogen ions [9, 15]. An antioxidant can be defined as any molecule capable of preventing or delaying oxidation of other molecules, usually such as lipids, proteins or nucleic acids [9, 15-17].

DPPH test is widely used for measuring the antioxidant activity of C-PC [18]. Different authors use different markers for the calculation of the antioxidant activity of C-PC. Among them mainly are Trolox and ascorbic acid [1, 14, 19]. Sometimes authors use butylated hydroxyanisole [3]. However, there are a few publications related to the study of prooxidant and antioxidant properties of C-PC simultaneously with reference to rutin and quercetin.

To the best of our knowledge, there are only a few publications giving the determination of C-PC content in its samples. Therefore, our primary aim was to test the analytical procedure for measuring the content and chemical purity of our sample of C-PC, which was obtained from Kaunas Lagoon (Lithuania), in order to possess the information of the C-PC quality. The secondary aim of the study was to elaborate the analytical technique of measuring the prooxidant/antioxidant activity of C-PC. Finally, our purpose was to determine the antioxidant activity for such commercially available markers as rutin and quercetin and calculate the antioxidant activity of C-PC with reference to these markers.

## Materials and Methods

C-PC was obtained from cyanobacteria collected in Kaunas Lagoon (Lithuania).

*Extraction and purification of C-PC.* The blue-green algal biomass for the C-PC extraction was

collected in Kaunas Lagoon in June 2021. This biomass contained the different species of cyanobacteria. The harvested biomass was frozen and stored at  $-20^{\circ}\text{C}$  until analysis. To obtain the maximum amount of the target pigment and to reduce the production cost in the initial stage, the selection of a suitable cell wall disruption method and a buffer solution for the C-PC extraction was crucial. The extraction was performed according to the method described by Khazi et al. [20]. Five ml of culture centrifuged for 5 min at 10,000 rpm. The pellet was suspended in 5 ml of 100 mM sodium phosphate buffer (pH 7). To extract C-PC, the cell suspension was sonicated at a frequency of 20 kHz for 2 min. The suspension was then centrifuged at 4,500 rpm for 5 min. The purification of C-PC was performed using gel chromatography and ion-exchange chromatography. Sephadex G-25 and ion exchange Q-Sepharose XL were used as the sorbents for gel chromatography. C-PC in the powder form was obtained after lyophilisation. This form of C-PC was used for the analytical studies described in this paper.

*Spectral characteristics.* In order to evaluate the spectral characteristics of the tested sample of C-PC, we recorded the spectrum of the C-PC solutions in the range of 200 to 800 nm and determined their absorption maxima. The structure of the spectrum was compared to the published data.

*Calculation of the C-PC content.* The C-PC concentration and purity in the solutions of an appropriate concentration were determined by the spectrophotometric method.

The C-PC concentration of the solution in mg/ml was calculated by measuring the absorbance at 620 and 652 nm using the following equation [1, 9, 21-24]:

$$\text{C-PC (mg/ml)} = (A_{620} - 0.474 \times A_{652}) / 5.34,$$

where  $A_{620}$  is the absorbance of the solution at a wavelength of 620 nm,  $A_{652}$  is the absorbance at a wavelength of 652 nm and 5.34 is the constant factor.

*C-PC purity.* The chemical purity of C-PC was monitored according to the  $A_{620}/A_{280}$  ratio. The absorbance at a wavelength of 620 nm indicates maximum absorption of C-PC, while the absorbance at a wavelength of 280 nm is related to proteins and nucleic acids in the solution [9, 21, 24-28].

Among the main tasks of the determination of the C-PC spectrum, content and chemical purity were the choice of the solution concentration for the studies and repeatability of the results of the studies

performed on different days and in different laboratories.

**Determination of pro- and anti-oxidant activity (DPPH radical-scavenging activity).** The DPPH radical scavenging test is widely used with the purpose of evaluating the free radical scavenging activity of antioxidants, C-PC, herbal preparations, food products and beekeeping products [2, 9, 17, 24, 29-31]. The antioxidant activity of the obtained sample was determined according to the elaborated procedure of the DPPH test. DPPH radical-scavenging activity of C-PC was determined as described by Hudz et al. [29] and Shanaida et al. [30] with the slight modifications. Briefly, about 3.0 mg of DPPH (1,1-diphenyl-2-picrylhydrazyl, Sigma–Aldrich, Germany) were dissolved in 100 ml of 50% ethanol. This solution of DPPH was used on the day of its preparation after the previous determination of its absorbance at a wavelength of 515 nm. The absorbance of the DPPH solution should be in the range of 0.760 to 0.840, namely,  $0.800 \pm 5\%$ . If the absorbance was not in this range, there was a correction of the solution by adding DPPH or 50% ethanol depending on the absorbance. The different volumes of the C-PC solution were added to 1.95 ml of the DPPH solution in 2 ml tubes. Due to the blue coloration of the C-PC solutions, it was necessary to prepare a blank for the mixtures of the C-PC solution with DPPH. This blank consisted of the same volume of the C-PC solution and 1.95 ml of 50% ethanol, while 50% ethanol was the blank for 0.003% solution of DPPH. The mixtures were then mixed vigorously and allowed to stand at room temperature in the dark for 40 min. The absorbance of the resulting mixtures was read at a wavelength of 515 nm each 10 min for 40 min, using the spectrophotometers: Genesys 20 or ULAB 108U. The DPPH radical-scavenging activity was calculated according to the following equation:

$$\begin{aligned} \text{DPPH radical-scavenging activity (\%)} = \\ = (A_{\text{control}} - A_{\text{sample}} \times 100\%) / A_{\text{control}}, \end{aligned} \quad (1)$$

where  $A_{\text{control}}$  is the absorbance of the solution of DPPH against 50% ethanol,  $A_{\text{sample}}$  is the absorbance of the reaction mixtures of the C-PC solutions with DPPH at a wavelength of 515 nm against the same volume of C-PC solution and 1.95 ml of 50% ethanol. The reaction mixture for measuring  $A_{\text{control}}$  consisted of 1.95 ml of 0.003% solution of DPPH and the same volume of 50% ethanol that was equal to the volume of C-PC taken for the analysis. If we obtained the values  $A_{\text{sample}}$  more than  $A_{\text{control}}$  and respec-

tively negative values DPPH radical-scavenging activity (%), we considered them as prooxidant values of C-PC.

0.05 ml of the different volumes of the solutions of quercetin and rutin was added to 1.95 ml of the DPPH solution in 2 ml tubes. The mixtures were then mixed vigorously and allowed to stand at room temperature in the dark for 40 min. The absorbance of the resulting mixtures was read at a wavelength of 515 nm at each 10 min for 40 min, using the spectrophotometers: Genesys 20 or ULAB 108U. The DPPH radical-scavenging activity was calculated according to equation 1.

**Statistical analysis.** The statistical analysis was employed for the comparison of the C-PC contents determined on two spectrophotometers. The decision rule in all the cases was: with  $\alpha = 0.025$ , critical values of  $t^*$  should be in the range of  $-3.18$  to  $+3.18$  or  $-4.30$  to  $+4.30$ . The null hypothesis ( $H_0$ ) was rejected if the value of  $t^*$  was out of these ranges [32, 33].

Excel was used for the calculations of coefficients of correlation.

## Results and Discussion

C-PC is a blue powder. Its solutions are blue of different tincts depending on concentration. Our main accent was made on the spectrophotometric and antioxidant properties of C-PC. We found the suitable concentrations of C-PC in order to determine the spectral characteristics, the concentration of C-PC in the solution after its dissolution and C-PC purity. The spectrum of 0.10% solution of C-PC is provided in Fig. 1.

As can be seen in Fig. 1, the spectrum contained two main absorption maxima at the wavelengths of 277 and 619 nm that is in line with the published data [3, 6]. Gantar et al. revealed that the absorbance spectrum of C-PC in the dimer form extracted from *Limnothrix* sp. strain 37-2-1 showed the absorption maximum at a wavelength of 620 nm [6]. The same absorption maximum was for the C-PC from the Saharian *Arthrospira* sp. strain [3]. Moreover, in all the tested concentrations there were these two absorption maxima.

The C-PC concentration (mg/ml) in the solution and purity were computed by measuring the absorbances at the wavelengths of 280, 620 and 652 nm, using the following known equations [9, 26]:

$$\begin{aligned} \text{C-PC (mg/ml)} = (A_{620} - 0.474 \times A_{652}) / 5.34 \text{ and} \\ A_{620} / A_{280}. \end{aligned}$$

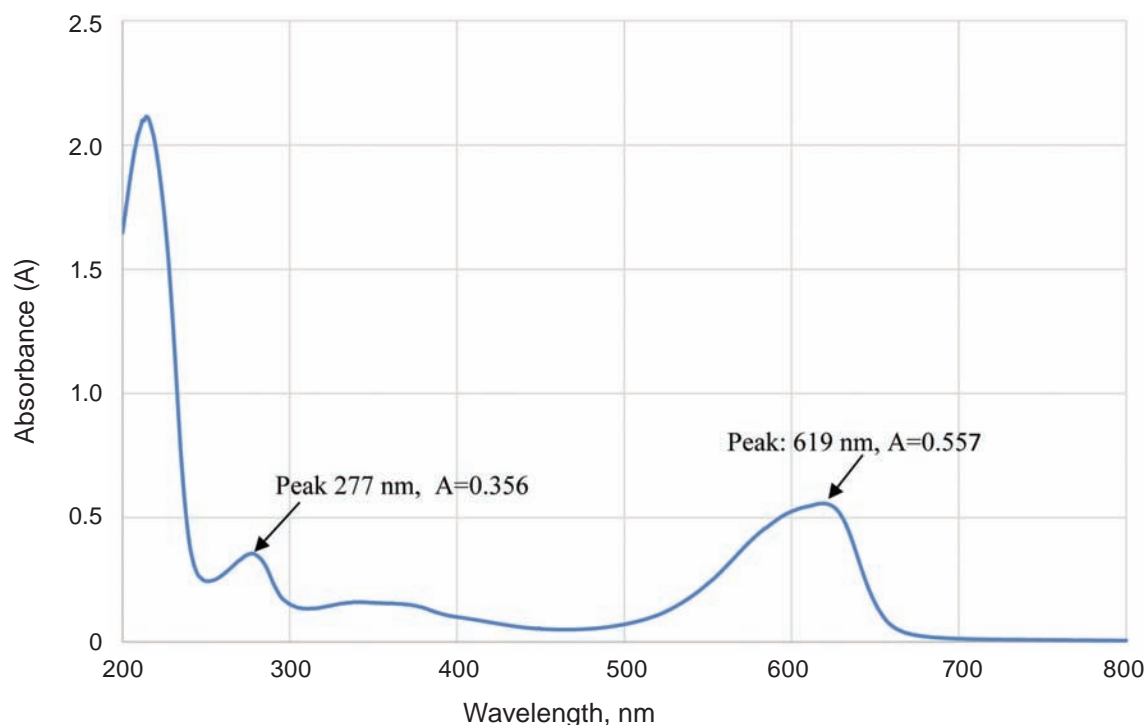


Fig. 1. The spectrum of 0.10% aqueous solution of C-PC

As can be seen in Table 1, we dealt with the food qualification of C-PC as the chemical purity was about 1.3–1.5. The chemical purity of the food qualification should be more than 0.7 and not more than 3.9 [5, 9, 25, 26, 34]. This qualification can also be acceptable for feeding animals, including aquaculture.

Comparing the results of the determination of the C-PC content obtained on the two spectrophotometers, it was revealed that these results slightly differed. The values obtained on the spectrophotometer “Genesys 20” ( $8.22 \pm 0.02\%$ ) slightly differed from ones obtained on the second spectrophotometer, namely, the first results ( $8.22 \pm 0.02\%$ ) were in the range of the second results ( $8.78 \pm 0.59\%$ ) (line 1 of Table 2). Secondly, using the null hypothesis it was confirmed that there was no influence of the spectrophotometers on the content of C-PC. Moreover, it was revealed that there is no effect of different days of the analysis on the content of C-PC performed on the same spectrophotometer (line 2 of Table 2). However, there was an influence of the spectrophotometers on the content of C-PC if we compared the values provided in line 3 (Table 2) that could be explained by using different C-PC concentrations (0.02%, 0.1% and 0.1%, 0.16 %).

The hydrogen atom or electrons donating ability of C-PC was measured from the change of the purple color of the ethanolic solution of DPPH (0.003%). The free radical scavenging activity of the C-PC solutions of an appropriate concentration was measured, based on the scavenging activity of the stable DPPH free radical. Fifty percent ethanol was used for dissolving DPPH because 96% ethanol precipitated the proteins that were present in the tested sample of C-PC, especially in the case of a concentration of 4% that made it impossible to perform spectrophotometric analytical studies. The C-PC solution was added to the ethanolic solution of DPPH in an appropriate ratio. The absorbance was read at a wavelength of 515 nm after 10, 20, 30 and 40 min in order to study kinetics parameters of the reaction, establish an optimum time of the reaction of C-PC with DPPH and calculate the percentage of scavenging activity.

Studying the antioxidant activity of individual compounds or their mixtures, scientists often use  $IC_{50}$  value (inhibitory concentration), which is defined as the concentration of test compounds that can decrease the content of free radicals by 50%. The smaller  $IC_{50}$  value, the higher is the activity of reduction of free radicals [35].

Table 1. The content and purity of the tested sample of C-PC\*

Date	Weight and volume of water/C-PC concentration	A <sub>620</sub>	A <sub>652</sub>	A <sub>280</sub>	C, mg/ml	Content, %	A <sub>620</sub> /A <sub>280</sub>	Content ±SD, %
<i>Spectrophotometer "Genesys 20"</i>								
3.11.2021	20 mg in 100 ml/0.02%	0.105	0.036	–	0.017	8.23	–	8.22 ± 0.02%
	Stability of the solution in 2 h	0.092	0.031	–	0.015	7.25	–	
3.11.2021	100 mg in 100 ml /0.10%	0.505	0.136	–	0.082	8.20	–	
	Stability of the solution in 2 h	0.497	0.134	–	0.081	8.10	–	
<i>Spectrophotometer "ULAB 108U"</i>								
1.12.2021	43 mg in 25 ml/0.172%	0.855	0.225	0.657	0.140	8.14	1.30	8.78 ± 0.59%
	50 mg in 50 ml/0.10%	0.540	0.137	0.417	0.089	8.90	1.29	
	80 mg in 50 ml/0.160%	0.898	0.230	0.652	0.148	9.30	1.38	
6.12.2021	100 mg in 100 ml/0.10%	0.557	0.132	0.350	0.093	9.30	1.56	9.20 ± 0.14%
	Stability of the solution in 1 h	0.561	0.135	0.356	0.093	9.30	1.58	
	80 mg in 50 ml/0.16%	0.879	0.211	0.564	0.146	9.10	1.56	
	Stability of the solution in 1 h	0.880	0.216	0.573	0.146	9.10	1.54	

\*The absorbance was measured 3 times for each solution



23 *Table 2. Statistical analysis for comparison of the content of C-PC determined in different days and on different spectrophotometers*

No	Comparable mean values of the content, mg/g		Standard deviations (SD) of mean values		$\bar{X}_1 - \bar{X}_2$	$S_p^2$	$t$	$t^*$	Conclusion 1	Conclusion 2
	$\bar{X}_1$	$\bar{X}_2$	SD <sub>1</sub>	SD <sub>2</sub>						
1	8.22	8.78	0.02	0.59	0.56	0.116	-1.81	-3.18 to +3.18	H <sub>0</sub> is accepted	The two means are equal. There is no influence of the spectrophotometers on the content of C-PC
2	9.20	8.78	0.14	0.59	0.42	0.239	0.94	-3.18 to +3.18	H <sub>0</sub> is accepted	The two means are equal. There is no effect of different days of the analysis on the content of C-PC determined on the same spectrophotometer
3	8.22	9.20	0.02	0.14	0.98	0.10	-9.80	-4.30 to +4.30	H <sub>0</sub> is rejected	The two means are statistically significantly different. There is a clear influence of the spectrophotometers on the content of C-PC

*Table 3. Antioxidant and prooxidant characteristics of C-PC*

Date	Concentration, volume, weight	Mean absorbance/AA, %					AA <sub>(40min)</sub> ± SD, % (n = 3)
		10 min	20 min	30 min	40 min	A <sub>blank</sub>	
5.11.2021	0.02%, 50 µl, 0.01 mg	0.795	0.799	–	0.782	0.767	–(2.0±1.3)%
	0.10%, 50 µl, 0.05 mg	0.859	–	–	0.846	0.777	–(8.9±0.4)%
8.11.2021	4%, 50 µl, 2.0 mg	0.659/13.3%	0.645/15.6%	0.629/17.5%	0.624/18.2%	0.762	18.2%±1.2%
	4%, 60 µl, 2.4 mg	0.667/14.5%	0.647/17.1%	0.626/19.7%	0.616/20.8	0.778	20.8%±0.9%
	4%, 80 µl, 3.2 mg	0.630/17.3	0.610/20.0	0.590/22.6	0.582/23.0	0.756	23.0%±1.3%
	4%, 100 µl, 4.0 mg	0.601/21.7	0.582/24.2	0.567/25.5	0.563/26.3	0.764	26.3%±1.6%
9.11.2021	4%, 100 µl, 4.0 mg	0.489/35.3	0.485/35.8	0.450/36.5	0.478/36.8	0.756	36.8%±0.8%

In our studies C-PC showed the dual abilities, namely, generating and scavenging DPPH radicals depending on its concentration – in other words, C-PC had prooxidant and antioxidant properties depending on its concentration (Table 2, Fig. 2). These studies are in line with the published studies [36]. In these studies, C-PC has the opposite abilities of generating and scavenging hydroxyl radicals generated by the iron/H<sub>2</sub>O<sub>2</sub> system. The generation of radicals was facilitated by illumination and low concentrations of C-PC. However, when the hydroxyl radical generating ability reached an optimum, it started to reduce if the concentration of C-PC increased [36].

As can be seen in Table 1, reducing by 11.9% means that 2 hours are not acceptable for the storage of C-PC solutions in the concentration of 0.02%. Using the spectrophotometer “Genesys 20,” the solutions in the concentrations of 0.02% and 0.10% give the same content of C-PC. The content of C-PC was 8.22% ( $X_{\text{mean}} \pm \text{SD} = 8.22 \pm 0.02\%$ ). Reducing by 1.6% means that 2 hours are acceptable for the storage of C-PC solutions in the concentration of 0.10%. Using the spectrophotometer “ULAB 108U,” the content of C-PC ranged from 8.14% to 9.30% ( $X_{\text{mean}} \pm \text{SD} = 8.78 \pm 0.59\%$ ). The chemical purity was characterized as 1.29–1.38. Measuring the content of C-PC another day, it was established that the content of C-PC ranged from 9.10% to 9.30% and this interval was in the previous range ( $X_{\text{mean}} \pm \text{SD} = 9.20 \pm 0.14\%$ ). The chemical purity was 1.56. The absence of changes in the content meant that 1 hour was acceptable for the storage of the C-PC solutions in the concentration of 0.10 and 0.16%.

As can be seen in Table 3, the C-PC solution in the concentration of 0.02% did not have antioxidant activity. The C-PC solution in the concentration of 0.10% did not have antioxidant activity as well. On the contrary, we can say about prooxidant activity. It was revealed that DPPH should be dissolved in 50% ethanol in order to avoid the precipitation of proteins present in the sample. It was found that increasing in the amount of C-PC increased antioxidant activity, namely, there is a strong correlation between the antioxidant activity and the C-PC mass in the range of 2 to 4 mg ( $R^2 = 0.9818$ ). There is a deviation of  $36.8 - 26.3 = 10.5\%$  that could be explained by illumination in the different days, special features of the analytical procedure itself and other unknown factors.

The phycobilin moiety is the principal part of C-PC involved in scavenging hydroxyl radicals [36]. Other authors showed that the antioxidant activities of phycocyanobilin and C-PC in equal concentrations with reference to the phycocyanobilin basis in the AAPH-containing reaction mixture were almost the same. C-PC was taken from spray-dried *Spirulina* [27].

Our studies showed that C-PC concentration directly affects the prooxidant/antioxidant activity of C-PC, namely, C-PC had the opposite abilities to generate and scavenge DPPH radicals depending on its concentration. The low concentrations of C-PC had the properties of the generation of DPPH radicals – in other words, C-PC had prooxidant properties. We suppose that if the radical generating ability reached an optimum, it started to decrease at the increase in the concentration of C-PC. The antioxidant activity finally enhances if the content of C-PC significantly increases in the reaction mixtures with DPPH (Fig. 2).

Moreover, we observed a real increase in the antioxidant activity if the content of C-PC increased in the reaction mixtures (Fig. 2). Finally, the coefficient of correlation was very high in the range of the content of 2.0 to 4.0 mg of C-PC in the reaction mixtures ( $R^2 = 0.9818$ ) (Fig. 2).

One more task of our study was to establish an appropriate time for the reaction of C-PC with DPPH. Different authors use various times in the DPPH test. For example, Gabr et al. used 30 min as the time of the reaction and Venugopal et al. used 20 min [19, 24].

As can be seen in Table 4, 40 min is enough for the reaction between DPPH and C-PC. It can be explained by that the coefficients of correlation are significantly decreased at 40 min compared to 30 min. Moreover, such a time was also established for the extracts of *Schizandra chinensis* according to the same analytical technique [37].

One more task was to study the interday precision in the determination of antioxidant activity. It was revealed that the antioxidant activity was 36.77%. Such a difference (approximately 10%) could be mainly explained by the influence of illumination, which did not undergo the control of an analyst and by other unknown reasons. Zhou et al. stated that illumination facilitates the generation of free radicals [36]. According to the developed technique, the half-maximal effective concentration ( $EC_{50}$ ) value of rutin and quercetin is about

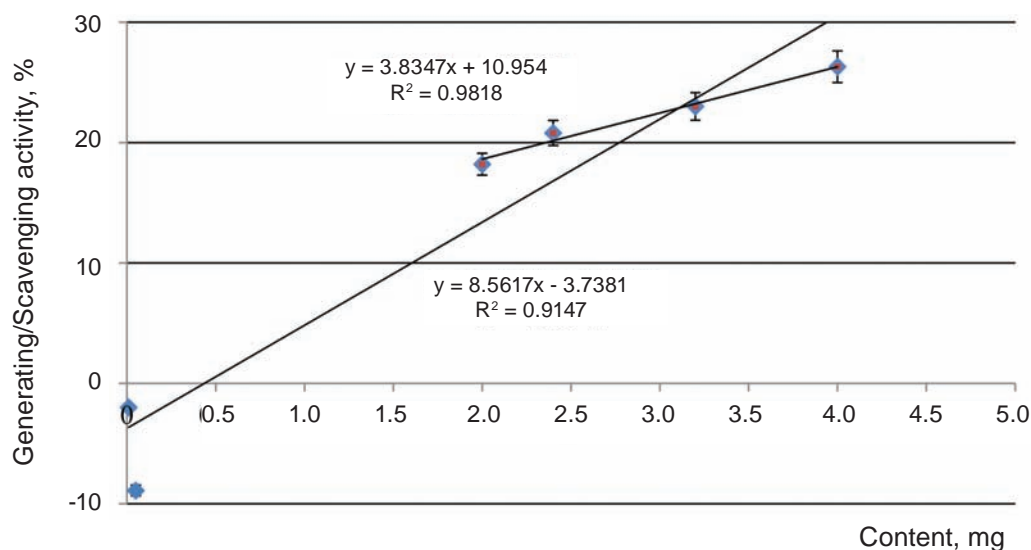


Fig. 2. Prooxidant and antioxidant properties of C-PC depending on its content in the reaction mixture with DPPH

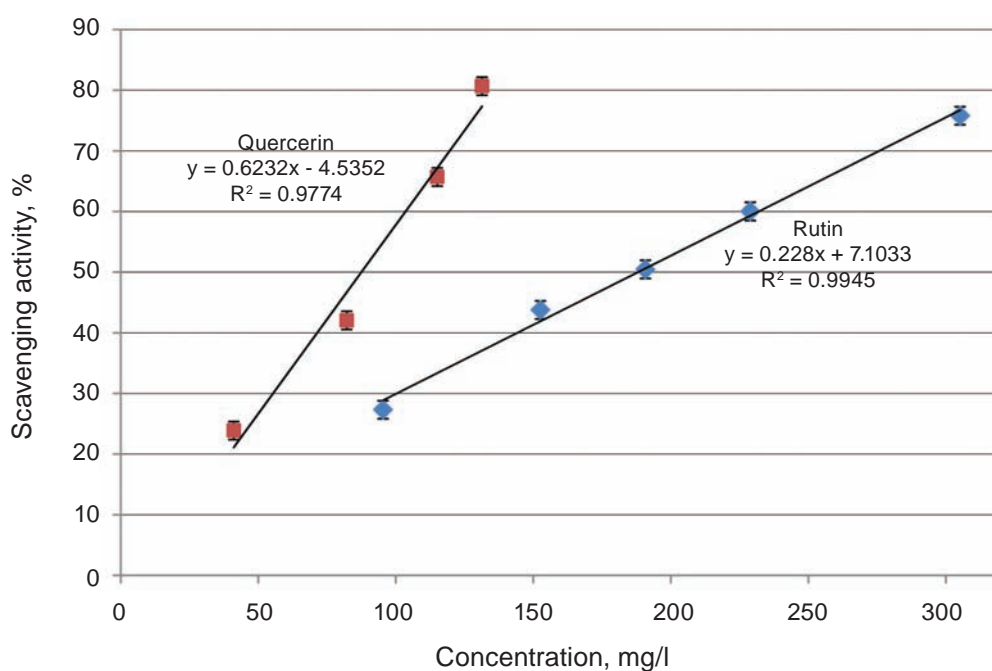


Fig. 3. The calibration curves of rutin and quercetin for the elaborated procedure of the DPPH test

188 and 88 mg/l (Fig. 3). Such a difference could be explained by the chemical structure of these flavonoids. One drawback of our study was not reaching a reduction of the absorbance of the DPPH solution by 50% in order to establish the  $EC_{50}$  value of C-PC. This drawback could be explained by the following factors. Firstly, our analytical technique was developed for 2.0 ml for herbal preparations. At 50  $\mu$ l of 4% solution of the sample and 1.95 ml of solution of

DPPH, AA was 18.2%. Secondly, we did increase the volume of 4% solution of the sample to 60, 80 and 100  $\mu$ l violating insignificantly the total volume of the reaction mixture to 2.05 ml (2.5%). The next increase would have induced a higher deviation. Therefore, we did not increase the volume of 4% solution of the sample in order to reach 50% of the absorbance of the DPPH solution.



Table 4. Kinetics characteristics of the reaction between DPPH and C-PC depending on the mass of C-PC

Mass of C-PC, mg	40 min		30 min	
	Equation and R <sup>2</sup>	Coefficient of the correlation (r)	Equation and R <sup>2</sup>	Coefficient of the correlation (r)
2.0	y = 0.166x + 12 R <sup>2</sup> = 0.9535	0.976	y = 0.21x + 11.267 R <sup>2</sup> = 0.997	0.998
2.4	y = 0.215x + 12.65 R <sup>2</sup> = 0.9716	0.986	y = 0.26x + 11.9 R <sup>2</sup> = 1	1.000
3.2	y = 0.197x + 15.8 R <sup>2</sup> = 0.9263	0.962	y = 0.265x + 14.67 R <sup>2</sup> = 0.9999	1.000
4.0	y = 0.151x + 20.65 R <sup>2</sup> = 0.9385	0.969	y = 0.19x + 20 R <sup>2</sup> = 0.9678	0.984

Patel et al. indicated the dose-dependent antioxidant activity of C-PC with a value of  $72.85 \pm 2.1\%$  at the dose of 200  $\mu\text{g/ml}$ , whereas DPPH-scavenging activity of ascorbic acid was  $99.82 \pm 1.9\%$  at 200  $\mu\text{g/ml}$  [2]. However, these authors did not provide the correlation coefficients between the antioxidant activity and concentration. This actually complicates the evaluation of antioxidant activity as, from our experience with the antioxidant activity of ascorbic acid in the DPPH test, it was revealed that antioxidant activity did not increase if the ascorbic acid concentration increased in the defined range. For example, at concentrations of 0.4 and 0.6 mg/ml, the antioxidant activity was equal to 92% [29].

In the process of our studies measuring antioxidant activity, the following issues were sorted out: the concentration of ethanol in the solution of DPPH was selected with the purpose of avoiding the precipitation of proteins in the reaction mixtures; the ratio of the C-PC solutions to the DPPH solution; the calibration curves of quercetin and rutin were constructed ( $y = 0.6232x - 4.5352$ ,  $R^2 = 0.9774$  and  $y = 0.228x + 7.0992$ ,  $R^2 = 0.9945$ , respectively); the antioxidant activity was determined in the range of 2 to 4 mg of C-PC in the reaction mixture with DPPH.

**Conclusions.** We identified C-PC by the spectrophotometric method. The spectrum had two main

absorption maxima: 277 and 619 nm. The purity of C-PC was about 1.5, which indicates the possible usage of the obtained C-PC in the food industry and as colorant in the pharmaceutical industry. As a result of our studies, we determined the antioxidant activity of the obtained sample of C-PC. It was equal to 0.831 mg quercetin equivalents/g and 1.627 mg rutin equivalents/g at a value of the antioxidant activity of the C-PC solution of 36.68%. It was revealed that C-PC had dual properties depending on its concentration: prooxidant and antioxidant. Prooxidant properties were expressed at low concentrations (0.02 and 0.1%).

**Conflict of interest.** Authors have completed the Unified Conflicts of Interest form at [http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi\\_disclosure.pdf](http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf) and declare no conflict of interest.

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## ОЦІНКА СПЕКТРАЛЬНИХ ХАРАКТЕРИСТИК, ЧИСТОТИ Й АНТИОКСИДАНТНОЇ АКТИВНОСТІ С-ФІКОЦІАНІНУ З ЦІАНОБАКТЕРІЙ, ЗІБРАНИХ У КАУНАСЬКІЙ ЛАГУНІ (ЛИТВА)

Н. Гудзь<sup>1,2✉</sup>, В. Туркіна<sup>3</sup>, О. Єзерська<sup>1</sup>,  
Л. Кобилінська<sup>4</sup>, А. Філіпська<sup>1</sup>, Я. Каросене<sup>5</sup>,  
Д. Галініте<sup>6</sup>, Г. Бальчюнайте-Мурзісене<sup>7</sup>,  
С. Хом'як<sup>8</sup>, Н. Савіцкене<sup>9</sup>

<sup>1</sup>Кафедра технології ліків та біофармації  
Львівського національного медичного університету  
імені Данила Галицького, Львів, Україна;

<sup>2</sup>Кафедра фармації та екологічної хімії  
Опольського університету, Польща;

<sup>3</sup>Науково-дослідний інститут епідеміології та гігієни  
Львівського національного медичного університету  
імені Данила Галицького, Львів, Україна;

<sup>4</sup>Кафедра біологічної хімії Львівського  
національного медичного університету імені  
Данила Галицького, Львів, Україна;

<sup>5</sup>Лабораторія альгології та мікробної екології,  
Центр природничих досліджень, Вільнюс, Литва;

<sup>6</sup>Кафедра фармакогнозії, Литовський університет  
наук про здоров'я, Каунас, Литва;

<sup>7</sup>Інститут фармацевтичних технологій Литовського  
університету наук про здоров'я, Каунас, Литва;

<sup>8</sup>Кафедра технології біологічно активних  
речовин, фармації та біотехнології  
Національного університету «Львівська  
політехніка», Львів, Україна;

<sup>9</sup>Кафедра фармакогнозії, Литовський  
університет наук про здоров'я, Каунас, Литва

✉e-mail: natali\_gudz@ukr.net

Вивчено фізико-хімічні характеристики С-фікоціаніну (С-ФЦ), екстрагованого з ціанобактерій, зібраних у Каунаській лагуні (спектральні характеристики, вміст С-ФЦ в сухій масі й хімічна чистота). З'ясовано, що досліджувані концентрації С-ФЦ в очищеній воді повинні бути в межах 0,02–0,16% для вимірювання вмісту С-ФЦ у сухій масі і його спектральних характеристик. У спектрі С-ФЦ виявлено два чітких максимуми поглинання за  $\lambda$  277 і 619 нм. Вміст С-ФЦ у сухому порошку знаходився в діапазоні від 7,25% до 9,30% залежно від його концентрації в розчині і типу спектрофотометра. Розраховано коефіцієнт чистоти 1,5, який вказує на харчову кваліфікацію отриманої біомаси С-ФЦ. Запропонована аналітична методика дозволила виявити, що С-ФЦ має

про-/антиоксидантні властивості залежно від концентрації, а саме його вмісту в реакційних сумішах із 2,2-дифеніл-1-пікрилгідразилом (DPPH). У ході досліджень вирішено наступні питання: підібрано концентрацію етанолу в розчині DPPH для уникнення осадження протеїнів у реакційних сумішах (50%); підібрано співвідношення розчину С-ФЦ до розчину DPPH; побудовано калібрувальні криві для кверцетину й рутину; визначено антиоксидантну активність отриманого зразка С-ФЦ.

**Ключові слова:** С-фікоціанін, ціанобактерії, антиоксидантна активність, DPPH, рутин, кверцетин.

### References

1. Izadi M, Fazilati M. Extraction and purification of phycocyanin from *Spirulina platensis* and evaluating its antioxidant and anti-inflammatory activity. *Asian J Green Chem.* 2018; 2(4): 364-379.
2. Patel HM, Rastogi RP, Trivedi U, Madamwar D. Structural characterization and antioxidant potential of phycocyanin from the cyanobacterium *Geitlerinema* sp. H8DM. *Algal Res.* 2018; 32: 372-383.
3. Chentir I, Hamdi M, Li S, Doumandji A, Markou G, Nasri M. Stability, bio-functionality and bio-activity of crude phycocyanin from a two-phase cultured Saharian *Arthrospira* sp. strain. *Algal Res.* 2018; 35: 395-406.
4. Chiu HF, Yang SP, Kuo YL, Lai YS, Chou TC. Mechanisms involved in the antiplatelet effect of C-phycocyanin. *Br J Nutr.* 2006; 95(2): 435-440.
5. de Amarante MCA, Braga ARC, Sala L, Kalil SJ. Colour stability and antioxidant activity of C-phycocyanin-added ice creams after *in vitro* digestion. *Food Res Int.* 2020; 137: 109602.
6. Gantar M, Simović D, Djilas S, Gonzalez WW, Miksovská J. Isolation, characterization and antioxidative activity of C-phycocyanin from *Limnospira* sp. strain 37-2-1. *J Biotechnol.* 2012; 159(1-2): 21-26.
7. Hirata T, Tanaka M, Ooike M, Tsunomura T, Sakaguchi M. Antioxidant activities of phycocyanobilin prepared from *Spirulina platensis*. *J Appl Phycol.* 2000; 12(3): 435-439.
8. Jiang L, Wang Y, Yin Q, Liu G, Liu H, Huang Y, Li B. Phycocyanin: A Potential Drug for Cancer Treatment. *J Cancer.* 2017; 8(17): 3416-3429.

9. Safari R, Raftani Amiri Z, Esmacilzadeh Kenari R. Antioxidant and antibacterial activities of C-phycoerythrin from common name *Spirulina platensis*. *Iran J Fish Sci*. 2013; 19(4): 1911-1927.
10. Hudz NI, Turkina VA, Filipaska AM, Kuzminov OB, Korytniuk RS, Lubenets VI, Wiczorek PP, Savickiene N. Protein c-phycoerythrin, structure, physicochemical and biological properties, methods of extraction. *Biopolym Cell*. 2021; 37(6): 407-418.
11. Park WS, Kim HJ, Li M, Lim DH, Kim J, Kwak SS, Kang CM, Ferruzzi MG, Ahn MJ. Two Classes of Pigments, Carotenoids and C-Phycocyanin, in *Spirulina* Powder and Their Antioxidant Activities. *Molecules*. 2018; 23(8): 2065.
12. Aoki J, Sasaki D, Asayama M. Development of a method for phycocyanin recovery from filamentous cyanobacteria and evaluation of its stability and antioxidant capacity. *BMC Biotechnol*. 2021; 21(1): 40.
13. Finamore A, Palmery M, Bensehaila S, Peluso I. Antioxidant, Immunomodulating, and Microbial-Modulating Activities of the Sustainable and Ecofriendly *Spirulina*. *Oxid Med Cell Longev*. 2017; 2017: 3247528.
14. Wu HL, Wang GH, Xiang WZ, Li T, He H. Stability and antioxidant activity of food-grade phycocyanin isolated from *Spirulina platensis*. *Int J Food Prop*. 2016; 19(10): 2349-2362.
15. Prez J, Alejandro T. Chemistry of natural antioxidants and studies performed with different plants collected in Mexico. In book: *Oxidative Stress and Chronic Degenerative Diseases – A Role for Antioxidants*. Ed. Morales-González JA. InTech: Rijeka, Croatia, 2013; P. 59-85.
16. Agrawal M, Bansal S, Chopra K. Evaluation of the in vitro and in vivo antioxidant potentials of food grade Phycocyanin. *J Food Sci Technol*. 2021; 58(11): 4382-4390.
17. Liang N, Kitts DD. Antioxidant property of coffee components: assessment of methods that define mechanisms of action. *Molecules*. 2014; 19(11): 19180-19208.
18. Asatiani MD, Elisashvili VI, Wasser SP, Reznick AZ, Nevo E. Free-radical scavenging activity of submerged mycelium extracts from higher basidiomycetes mushrooms. *Biosci Biotechnol Biochem*. 2007; 71(12): 3090-3092.
19. Gabr GA, El-Sayed SM, Hikal MS. Antioxidant activities of phycocyanin: A bioactive compound from *Spirulina platensis*. *J Pharm Res Int*. 2020; 32(2): 73-85.
20. Khazi MI, Demirel Z, Conk Dalay M. Enhancement of biomass and phycocyanin content of *Spirulina platensis*. *Front Biosci*. 2018; 10(2): 276-286.
21. Garcia AB, Longo E, Bermejo R. The application of a phycocyanin extract obtained from *Arthrospira platensis* as a blue natural colorant in beverages. *J Appl Phycol*. 2021; 33(5): 3059-3070.
22. Minato T, Teramoto T, Adachi N, Hung NK, Yamada K, Kawasaki M, Akutsu M, Moriya T, Senda T, Ogo S, Kakuta Y, Yoon KS. Non-conventional octameric structure of C-phycoerythrin. *Commun Biol*. 2021; 4(1): 1238.
23. Safaei M, Maleki H, Soleimanpour H, Norouzy A, Zahiri HS, Vali H, Noghabi KA. Development of a novel method for the purification of C-phycoerythrin pigment from a local cyanobacterial strain *Limnospira* sp. NS01 and evaluation of its anticancer properties. *Sci Rep*. 2019; 9(1): 9474.
24. Venugopal VC, Thakur A, Chennabasappa LK, Mishra G, Singh K, Rathee P, Ranjan A. Phycocyanin Extracted from *Oscillatoria minima* Shows Antimicrobial, Algicidal, and Antiradical Activities: *In silico* and *In vitro* Analysis. *Antiinflamm Antiallergy Agents Med Chem*. 2020; 19(3): 240-253.
25. Kamble SP, Gaikar RB, Padalia RB, Shinde KD. Extraction and purification of C-phycoerythrin from dry *Spirulina* powder and evaluating its antioxidant, anticoagulation and prevention of DNA damage activity. *J Appl Pharm Sci*. 2013; 3(8): 149-153.
26. Khandual S, Sanchez EOL, Andrews HE, de la Rosa JDP. Phycocyanin content and nutritional profile of *Arthrospira platensis* from Mexico: efficient extraction process and stability evaluation of phycocyanin. *BMC Chem*. 2021; 15(1): 24.
27. Panchal J, Shrivastav A. Extraction and purification of phycocyanin from *spirulina platensis* by using different methods: A Review. *Int J Biol Pharm Allied Sci*. 2020; 9(7): 1653-1669.
28. Soni A, Dubey M, Verma M, Dhankhar R, Kaushal V, Atri R, Sabharwal R. Revisiting the

- role of phycocyanin in current clinical practice. *Int J Pharm Sci Res.* 2015; 6(11): 4950-4600.
29. Hudz N, Ivanova R, Brindza J, Grygorieva O, Schubertová Z, Ivanišová E. Approaches to the determination of antioxidant activity of extracts from bee bread and safflower leaves and flowers. *Potr S J F Sci.* 2017; 11(1): 480-488.
  30. Shanaida M, Hudz N, Jasicka-Misiak I, Wieczorek PP. Polyphenols and Pharmacological Screening of a *Monarda fistulosa* L. dry Extract Based on a Hydrodistilled Residue By-Product. *Front Pharmacol.* 2021; 12: 563436.
  31. Kobylinska L, Klyuchivska O, Lesyk R, Stoika R. Targeting of the pro-oxidant-antioxidant balance *in vitro* and *in vivo* by 4-thiazolidinone-based chemotherapeutics with anticancer potential. *Ukr Biochem J.* 2019; 91(2): 7-17.
  32. Hudz N, Yezerska O, Grygorieva O, Brindza J, Felšöciová S, Kačániová M, Wieczorek PP. Analytical procedure elaboration of total flavonoid content determination and antimicrobial activity of bee bread extracts. *Acta Pol Pharm.* 2019; 76(3): 439-452.
  33. Daniel WW. Biostatistics: A Foundation for Analysis in the Health Sciences, 9th ed.; Wiley: Hoboken, NJ, USA. 2009. P. 215–244.
  34. Song W, Zhao C, Wang S. A large-scale preparation method of high purity C-phycocyanin. *Int J Biosci Biochem Bioinforma.* 2013; 3(4): 293-297.
  35. Hadiyanto, Suzery M, Setyawan D, Majid D, Sutanto H. Encapsulation of phycocyanin-alginate for high stability and antioxidant activity. IOP Conf Ser: *Earth Environ Sci.* 2017; 55: 012030.
  36. Zhou ZP, Liu LN, Chen XL, Wang JX, Chen MIN, Zhang YZ, Zhou BC. Factors that effect antioxidant activity of C-phycocyanins from *Spirulina platensis*. *J Food Biochem.* 2005; 29(3): 313-322.
  37. Hudz N, Sedláčková VH, Yezerska O, Zhurba M, Brindza J. Methodological approach to the elaboration of the analytical procedure of the antioxidant activity determination of the *Schisandra chinensis* (Turcz) Baill. extracts. *Agrobiodivers Improv Nutr Health Life Qual.* 2021; 5(2): 250-257.