



# FEATURES OF STANDARDIZATION OF C- PHYCOCYANIN,

## EUREKA 2021

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# *Cyanobacteria*

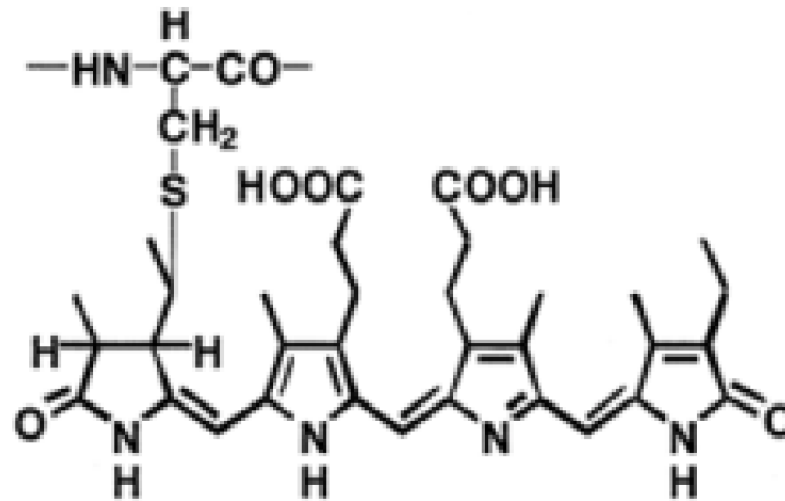
- ❑ Microalgae, including cyanobacteria are the primary producers of aquatic ecosystems.
- ❑ They can flourish almost everywhere like terrestrial, aerial, glaciers, brackish, marine, and fresh water regions
- ❑ Cyanobacteria and microalgae are exploited in recent decades for their products as health supplements, pharmaceuticals, cosmeceuticals and nutraceuticals.
- ❑ Cyanobacteria are known for their structurally distinct compounds that are used as food, feed, fuel, nutraceutical compounds and pharmaceutical drugs.
- ❑ The characteristic blue color of cyanobacteria and other cryptophytes, red algae is due to the presence of **phycocyanin pigment**.

## *Phycocyanin application*

- ❑ Phycocyanin is a blue natural food colorant that can be used as a functional food ingredient for several health benefits and as a natural colorant.
- ❑ For dairy products, it can be used in additives such as dyes with stabilizers and emulsifiers during the manufacturing process.
- ❑ In the food industry, C-PC has been used mainly as a natural dye, replacing artificial dyes that are harmful to health.
- ❑ C-PC is unstable due to sensitivity to temperature, light, pH and oxygen.
- ❑ Therefore, applications of C-PC in food and other products are limited.

# Chemical structure of Phycocyanin

C-PC is classified as a phycobiliprotein, as are other pigments such as allophycocyanin and phycoerythrin. C-PC is a highly fluorescent protein with linear prosthetic groups (bilins) that are linked to specific cysteine residues



**Figure 1** Structure of phycocyanin. Each of phycocyanin subunits contains a polypeptide chain and one ( $\alpha$ -subunit) or two ( $\beta$ -subunit) chromophores-phyco bilin. The connection between the polypeptide chain and the chromophore is a thioester bond.

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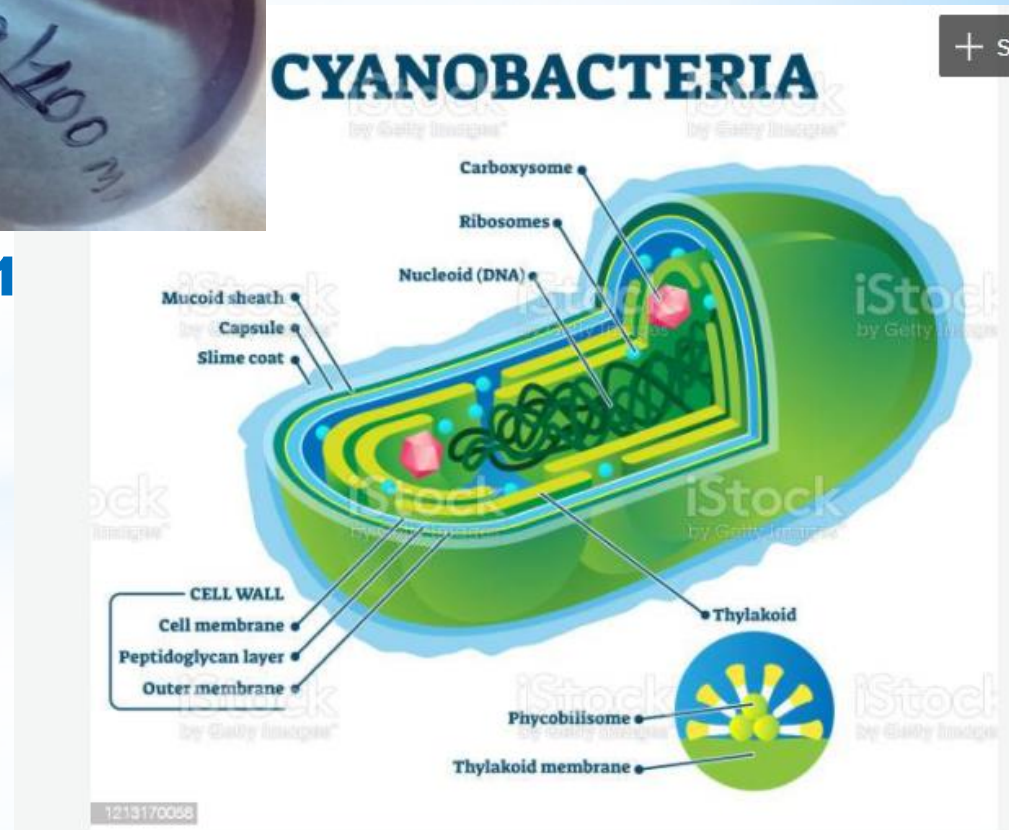
## *Purification of Phycocyanin*

- ❑ Currently, the purification step is a major challenge for industry due to the complexity of the structures and biological properties of different species of microalgae, besides the cost involved in obtaining purified pigment with high yield (which can reach 80% of total costs).
- ❑ Methods for C-PC purification include precipitation with ammonium sulfate, biphasic aqueous system, membrane separation, and chromatographic methods in a fixed-bed or expanded-bed column, etc.

- ❑ **Phycocyanin is blue** colored phycobiliprotein found in phycobilisomes, which are attached to the outer surfaces of thylakoid membrane.
- ❑ Phycobiliprotein are used as natural blue dye additives in food and cosmetics.
- ❑ Our C- Phycocyanin was extracted from cyanobacteria collected in the Šventoji River (Lithuania).



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# DPPH TEST

- ❑ The hydrogen atom or electrons donating ability of the pigment was measured from the bleaching of purple colored ethanol solution of DPPH.
- ❑ The free radical scavenging activity of the solution of an appropriate concentration, based on the scavenging activity of the stable 1,1-diphenyl- 2-picrylhydrazyl (DPPH) free radical, was measured.
- ❑ The sample was added to ethanolic solution DPPH.
- ❑ Absorbance was read at 515 nm after 10, 20, 30 and 40 min, and the percentage scavenging activity was calculated using the formula given below.
- ❑ Solution of DPPH without the test sample was used as a control.

$$\% \text{ Scavenging activity} = [(A_0 - A_1) / A_0] * 100 \%$$

where,

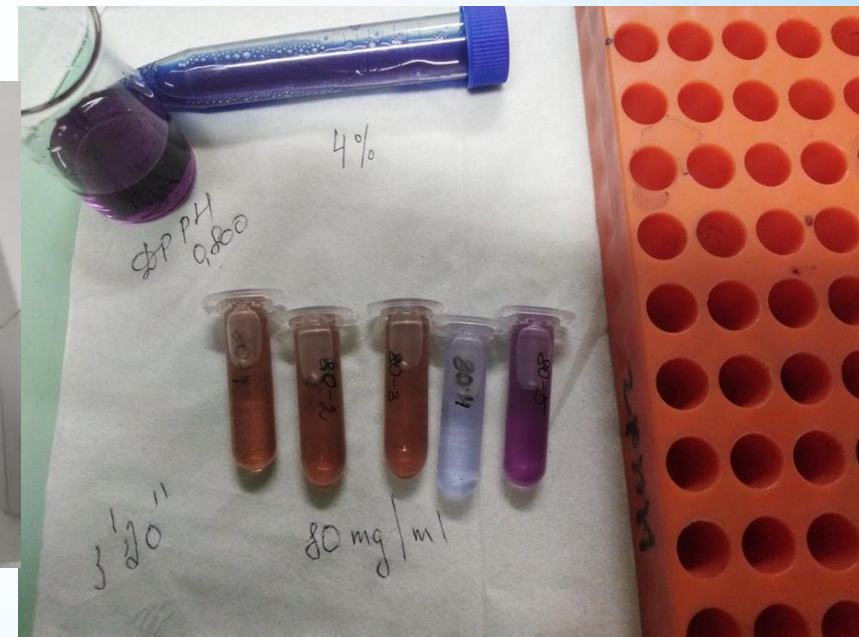
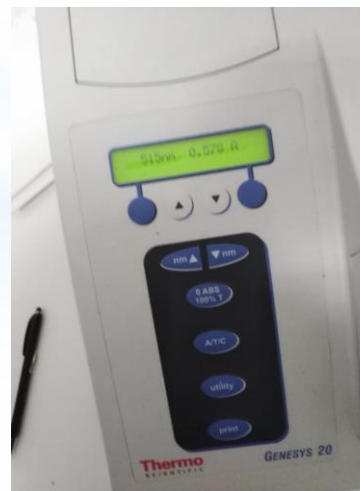
$A_0$  - absorbance of the control,

$A_1$  - absorbance of the test sample

What was sorted out by us:

1. Concentration of ethanol in the solution of DPPH was selected.
2. The ratio of the pigment solution to the DPPH solution was found

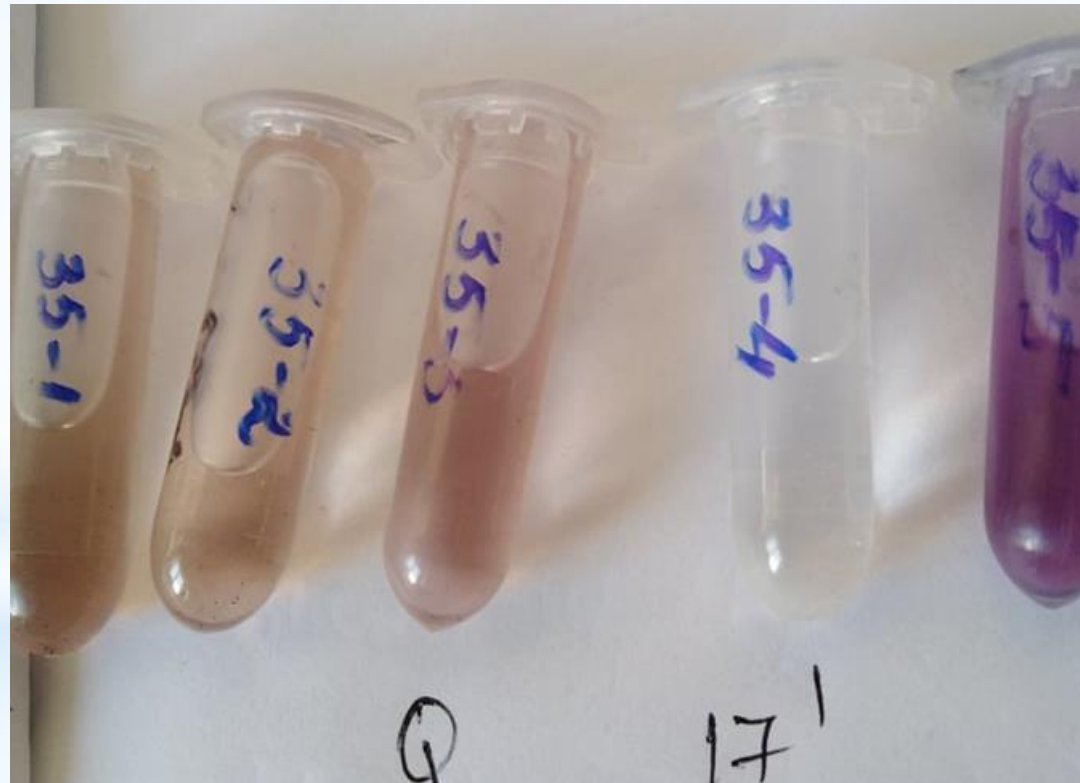
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## WHAT WAS SORTED OUT:

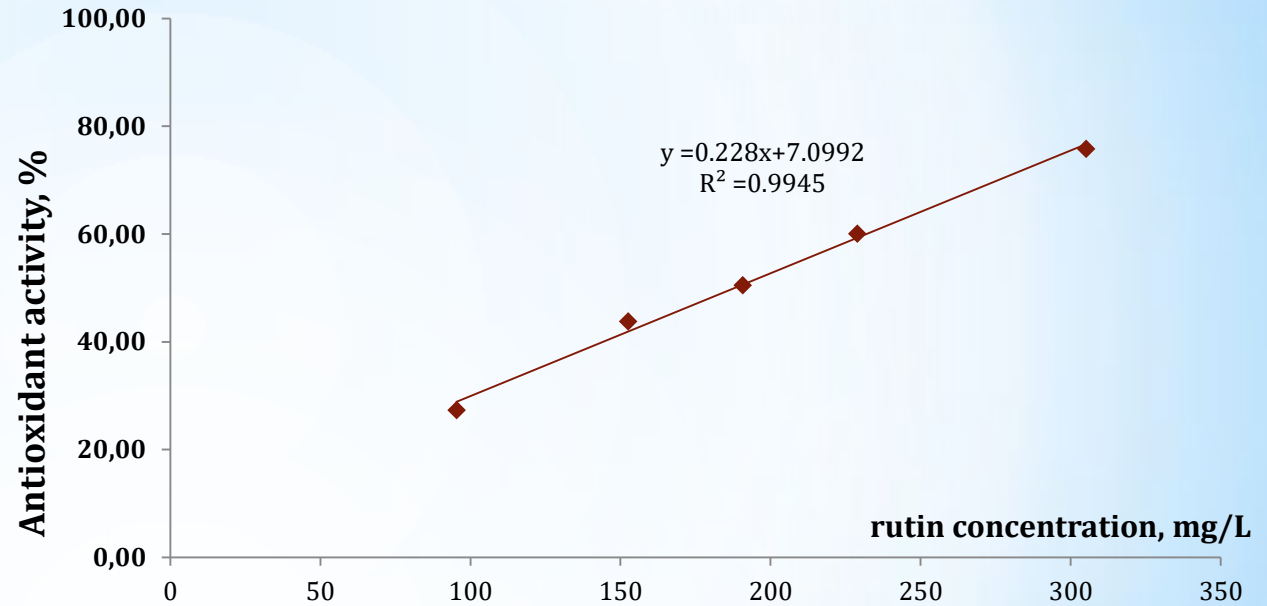
1. Concentration of ethanol in the solution of DPPH
2. The ratio of the pigment solution to the DPPH solution
3. The calibration curves of quercetin and rutin were constructed



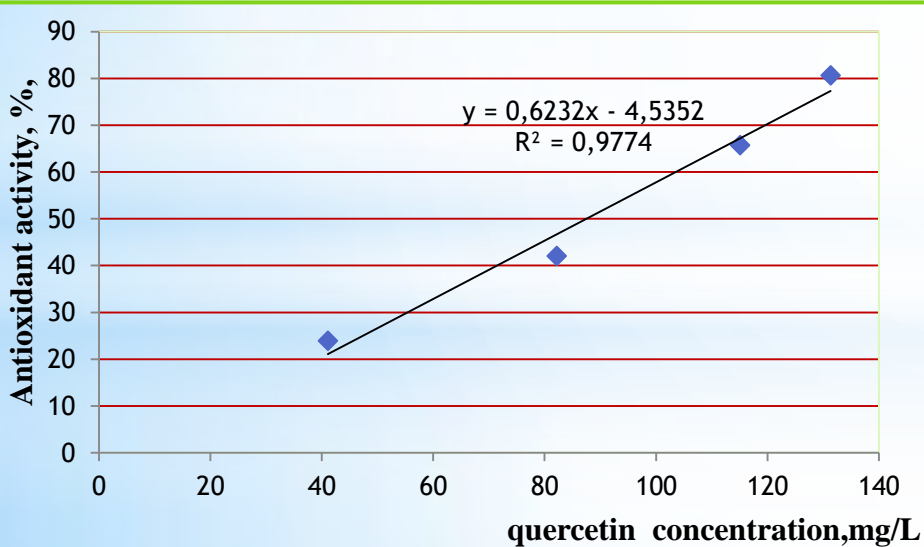


# Elaboration of the DPPH free radical scavenging activity assay

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Calibration curve of rutin

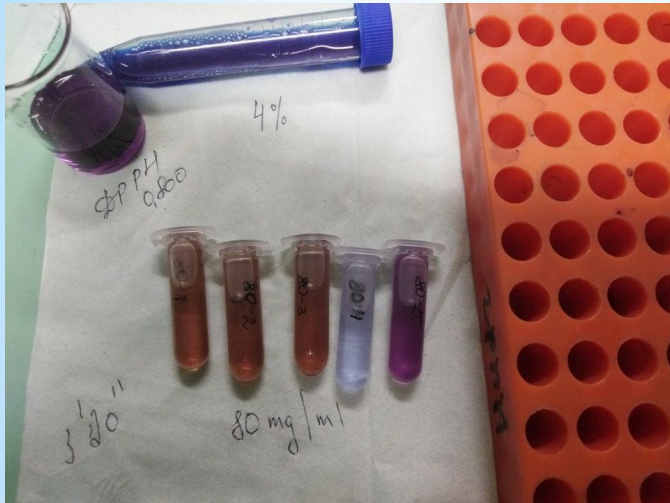


Calibration curve of quercetin

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# Elaboration of the DPPH free radical scavenging activity assay

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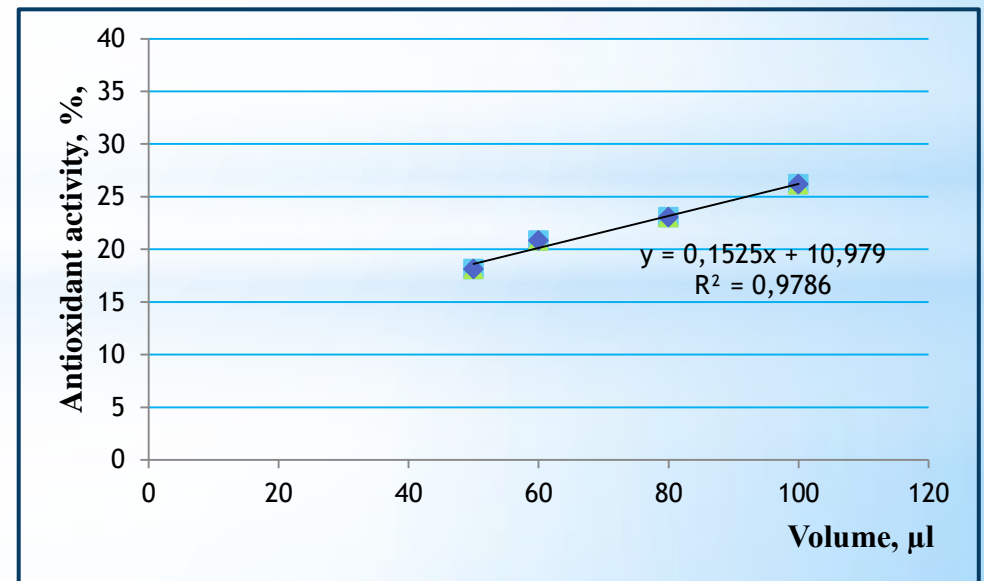
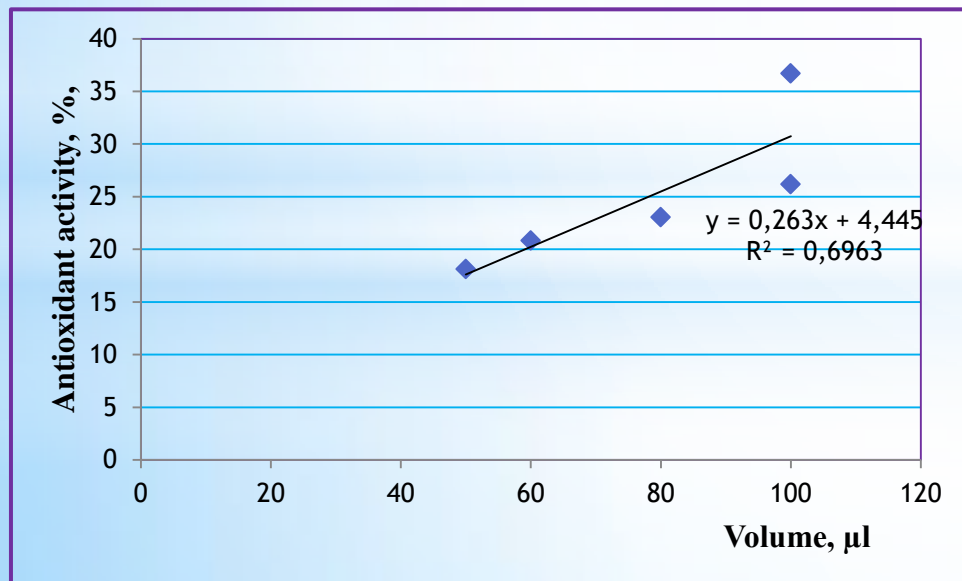
## Results

0.831 mg quercetin equivalents/g

1.627 mg rutin equivalents/g

at 36.68%

The ratio of the pigment solution to the DPPH solution and intraday precision



## CONCENTRATION OF PHYCOCYANIN IN THE POWDER BIOMASS- QUESTION FOR THE DISCUSSION!!!!!!!

- Phycocyanin concentration were determined by spectrophotometry.
- Concentration was calculated with the formula below using absorbance at 620 nm and 652 nm on the spectrophotometer.
- Phycocyanin absorbs light at about 620 nm and emits fluorescence at about 640 nm
- Absorbance at 620 nm indicates the maximum C-PC absorption

$$C - PC \text{ mg/mL} = (\text{Abs}_{620} - 0.474(\text{Abs}_{652}))/5.34$$

$$\text{Yield mg/g} = (C - PC \times \text{Volume})/\text{Biomass}$$

$$C - PC \text{ mg/mL} = (0.504 - 0.474(0.139))/5.34=0.08204 \text{ mg/ml}$$

$$\text{Yield mg/g} = (C - PC \times \text{Volume})/\text{Biomass}= 82 \text{ mg/g of the powder blue mass}$$

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The above study reveals that the phycocyanin pigment isolated from the mixture of cyanobacteria can be a good promising antioxidant compound and with the further study phycocyanin pigment can be used as a potential antioxidant agent and colorant for pharmaceutical technology.

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**Дякую за увагу!!!**

**Thank you for your attention!!!**

**Ďakujem za pozornosť!!!!**

**Dziękuję serdecznie za uwagę!!!!**



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Міжнародною європейською інноваційною  
науково-технічною програмою  
13 «**EUREKA у 2021 році**»