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Production, Large-Scale Extraction, and Purification of Phycocyanin by Different Cyanobacteria Isolated from Various Environments

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Abstract: The current research aims to investigate the isolates with the highest phycocyanin yield among five cyanobacterial isolates obtained from the wastewaters of the Porsuk River, thermal springs, and boron-containing waters of Eskişehir province in Türkiye. The study focused on optimization, purification, and characterization to increase phycocyanin yield. Phycocyanin yield was initially analyzed using four extraction methods: freeze-thaw, sonication, homogenization, and treatment with lysozyme. The sonication method determined the thermophilic *Geitlerinema* sp. as the isolate with the highest phycocyanin yield (116±0.18 mg g-1 C-phycocyanin). The crude extract obtained following optimization studies was first purified by solid ammonium sulfate precipitation/dialysis at 50% and 75% saturation, followed by ion-exchange chromatography using two anionic resins (DEAE-cellulose and Q-sepharose). The purity rate determined in the spectrum was recorded as 4.12 with 48.9% recovery (A_{620}/A_{280}). Characterization using the SDS-PAGE method found molecular weights of 17 kDa and 19 kDa for subunit α and subunit β, respectively. Additionally, we evaluated the stability of the purified thermal phycocyanin at different temperatures, with the pigment displaying resistance to high temperatures up to 60°C, as shown by its CR value.

Keywords: C-phycocyanin; *Geitlerinema* sp; cyanobacteria; extraction; purification; thermal stability.

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1. Introduction

Blue-green cyanobacteria, a group of gram-negative prokaryotes, have undergone a long evolution, play vital ecological roles, and are morphologically diverse and phylogenetically compatible with cyanobacteria. The thylakoid membrane's outer surface is surrounded by phycobiliproteins combining and collecting light energy to photosynthetic reaction centers [1]. These proteins are classified into three pivotal groups based on different chromophores or absorption spectra, including phycocyanin (C-PC blue λ610-625 nm), phycoerythrin (C-PE red λ540-565 nm), and allophycocyanin (C-APC bluish-green λ650-655) [2]. Phycocyanin is a non-toxic, light-sensitive protein pigment known as "Lina blue," with an intense blue color. Phycocyanin constitutes 75% of the colored phycobiliprotein family [3]. Since its introduction in 1982, the application of phycobiliproteins, particularly phycocyanins, as colorants has steadily expanded because of their unique properties [4]. Phycocyanin is a

unique pigment with no side effects compared to synthetic food colorants, which can be toxic and carcinogenic [4]. The potential use of C-PC and its production have increased significantly in recent years [5]. During phycocyanin extraction from cyanobacterial cells, cell walls are disrupted, and water-soluble coloring proteins are removed, followed by concentration and purification if necessary [6].

The commercial interest in C-phycocyanin has mainly increased due to its easy extraction, high purity, and yield. The usage area of phycocyanin is determined according to its purity rate. Phycocyanin, with a purity rate of 0.7 and above, is considered suitable for food, 3.9 is reactive, and 4 and above is analytically pure. Furthermore, phycocyanin can dissolve in water and facilitate purification, separating these molecules from other oil-soluble pigments. Some operations require large facilities, equipment, and long processing times. However, more is needed for C-PC efficiency [7]. Recent studies on the purification of C-PC have used various methods, including a combination of different techniques such as ammonium sulfate precipitation, ultrafiltration/diafiltration, ion-exchange chromatography, gel filtration chromatography, chromatography on hydroxyapatite, and expanded-bed adsorption chromatography [8-9]. Consequently, the extraction and purification protocol may vary according to the desired protein.

Factors such as previous research, pretreatment, solvent selection, extraction techniques, and processing parameters must also be carefully considered [10]. As is known, very few cyanobacterial species can produce C-PC in high amounts, which is necessary to determine more productive strains. Thus, this study aims to determine the most suitable isolate by investigating the C-PC production efficiency of five cyanobacterial isolates, optimizing extraction and purification techniques, and investigating the thermal properties of the obtained phycocyanin. The results of our study will contribute to cheap phycocyanin production that can be used as a pigment in many fields, including the food and textile industries, pharmacology, etc.

2. Materials and Methods

The cyanobacterial isolates examined in this study were obtained from diverse environments and displayed growth potential and adaptability to specific environmental conditions. These are *Geitlerinema* sp., obtained from thermal hot springs; *Nodosilinea* sp., isolated from the Porsuk River; *Phormidium lumbricale*, *Leptolyngbya norvegica*, and *Leptolyngbya* sp., isolated from boron-containing wastewaters.

These pure cyanobacterial cultures were preserved in the microbiology laboratory of Eskişehir Technical University. First, the isolates were incubated for 28 days in BG11 medium at pH 7.0 (pH adjusted to 10 by adding 4 g of boric acid per 1 L for a wastewater sample with boron) in 250 mL Erlenmeyer flasks. Cyanobacterial isolates from the Porsuk River and boron area were grown at room temperature (25°C) in the illuminated area. In contrast, thermal isolates were cultured in a climate control device (ECOCELL, MMM group) for 24 hours, with a light intensity of 150 µmol photon m-2 s-1 and a temperature set at 45°C. After extraction, biomass from the thermophilic cultures, totaling eight liters in volume, was rapidly and efficiently produced using twenty-eight-day air compressors in eight 2L Erlenmeyer flasks under identical conditions.

2.1. Cyanobacterial growth conditions.

The grown isolates were centrifuged at 9000 g for 10 minutes (Hettich universal 320 Centrifuge, Germany). The resulting pellet was washed twice with distilled water and subjected to overnight sterilization in a Thermo Scientific Heraeus Instruments T20 sterilizer at low temperature using the oven-drying technique, with the temperature set at 40°C by assuming that phycocyanin is heat-sensitive at 45°C. After pretreatment, the biomass was weighed and determined to be dry weight. Then, it was dissolved in 0.1 M sodium phosphate buffer (pH 7.0) at a concentration of 100 mg/mL per dry weight and suspended under shaking conditions at 150 rpm for 30 minutes. The entire extraction process was conducted in the dark, with the samples wrapped in aluminum foil [4].

2.2. Extraction analysis of C-phycocyanin.

The following extraction methods were applied to break down the cyanobacterial cell wall.

A: Freezing/thawing: Each suspended sample was subjected to three cycles of freezing at -20 °C in the freezer and thawing at 4°C for one hour under dark conditions.

B: Sonication. To induce cell disruption, each suspended sample underwent sonication using a Sonics & Materials Vibracell for 2 minutes at a constant frequency of 20 kHz, with an amplitude rate between 15% and 20%. All sonication experiments were conducted in an ice bath to mitigate the device's potential heat generation [29].

C: Homogenization: Each suspended sample was homogenized for approximately 10 minutes at 11,000 rpm using a homogenizer (ICA Ultra-Turrax T-25, Germany). All experiments regarding the heat the device might generate for a long time were carried out similarly [11-12].

D: Lysozyme: Dry biomass was suspended in 200 mL of 0.1 M phosphate buffer (pH 7.0) containing 100 mg lysozyme and 10 mM EDTA, followed by incubation at 30°C for 24 hours at 150 rpm under shaking conditions [12-13].

2.3. Analytical evaluation of phycocyanin.

The resulting extracts were centrifuged at 9000 g and 4°C for 15 minutes, following which the blue supernatants were collected and incubated at 4°C overnight.

The concentration of C-phycocyanin was determined using a Shimadzu UV-3150 PC spectrophotometer. It was calculated using the equation developed by Bennett and Bogorad (1976), which is widely used to determine phycocyanin levels in different algae species [14]:

$$C - PC(mg/mL) = \frac{A_{max} - (0.474 \times A652)}{5.34} \tag{1}$$

A_{max} – The absorbance point where the phycocyanin pigment peaks, usually observed at wavelengths $A_{610\text{-}625}$.

A_{652} – The absorbance point of the allophycocyanin pigment where the maximum peak is detected.

The amount of phycocyanin per dry weight was calculated using the formula presented by Silveira et al. (2007) [15]:

$$C - PC(mg/g) = \frac{C - PC(mg/mL)x\ Solvent\ volume\ (mL)}{Dry\ mass\ (g)} \quad (2)$$

The purity of C-PC used in the extraction and all subsequent steps was calculated with the ratio of A_{620}/A_{280} [16].

2.4. The effect of wet and dry biomass on phycocyanin yield.

After determining the best extraction method and the isolate displaying the phycocyanin activity, the same extraction process was applied to the incubated dry and wet biomass. After centrifugation, the resulting biomass was weighed in a 250 mL Erlenmeyer flask. The amount of wet biomass was 1 gram, and the amount of dry biomass was 0.04 grams. The solvent volume was calculated based on the dry weight (0.001 g/mL), and 4 mL of the solvent was added to each sample [11].

2.4.1. Determination of optimum extraction solution.

To determine the buffer solution where the highest amount of phycocyanin was obtained, extraction was performed with 1.5% $CaCl_2$, 0.1 M sodium phosphate buffer, and distilled water solutions, and the amount of phycocyanin was determined [32].

2.4.2. Determination of optimum sonication parameters.

Various sonication parameters were tried to obtain the highest phycocyanin yield. Changes in C-PC efficiency were examined by applying sonication intensity at a constant frequency (20 kHz) at different amplitude rates (5%, 10%, 15%, 20%, and 25%) and a constant amplitude at various times (2, 4, 6, 8, and 10 minutes) [33].

2.5. Purification analysis of phycocyanin.

The recycling efficiency of C-PC in the pre- and post-purification processes at each purification step was calculated using the following formula [15]:

$$Recovery(\%) = \frac{C - PC(mg/mL) \times V_{AP}}{C - PC(mg/mL) \times V_{BP}} \times 100 \quad (3)$$

V_{AP}– volume after purification, V_{BP} – volume before purification

2.5.1. Ammonium sulfate precipitation/dialysis.

Ammonium sulfate precipitation/dialysis was carried out in a cold room at 4°C. In the first step, solid ammonium sulfate was gradually added to the crude extract until a 25% saturation was achieved. After allowing it to stand at 4°C for 4 hours with continuous stirring, the obtained homogenates were centrifuged at 8,000 g for 15 minutes at 4°C. In the second step, solid ammonium sulfate was added until the supernatant reached a 75% saturation and stirred at 4°C overnight. The resulting solution was centrifuged at 8,000 g for 15 minutes. The

supernatant was discarded, after which the precipitated proteins were collected, dissolved in 10 mM sodium phosphate buffer (pH 7.0), and transferred to the dialysis membranes (Sigma-D9277). C-PC samples were mixed continuously for two days in 10 mM sodium phosphate buffer at 4°C, and the buffer was changed twice [23].

2.5.2. Ion exchange chromatography.

To achieve analytical purity, partially purified phycocyanin isolates underwent ion-exchange column chromatography using Q-Sepharose^{TM} fast flow (Cytiva, USA) fillers, one weak DEAE-cellulose (Sigma Aldrich, Germany) and the other strong anionic resin, loaded onto a 1.5x30 cm column with a bed volume of 5 mL. First, the column was equilibrated with 25 mM sodium phosphate buffer (pH 7.0). The flow rate was adjusted with a peristaltic pump at 1 mL per minute with a linear flow rate of 30 mm/hour. Then, the C-PC sample was added carefully to not disturb the filler's surface. Then, 5 column volumes of elution buffer (25 mM sodium phosphate buffer, pH 7.0) and binding buffer were eluted with increasing step gradient from 0.5 to 1 M NaCl concentration, and the C-PC-rich copper blue-colored fractions were collected into glass tubes [13].

2.6. Characterization analysis of C-phycocyanin.

2.6.1. Spectroscopic measurement UV–Vis absorption spectra.

Most characterization phases of C-PC involved spectrophotometric measurements. The solutions used in each step of other purification techniques, especially extraction, were chosen as the blank value. The scanning speed of the crude and purified isolates and the UV-Vis absorption spectra of 1 nm were observed in the wavelength range of 250-700 nm (absorbance) [16].

2.6.2. Molecular weight determination by the SDS-PAGE method.

In each successive purification step, C-phycocyanin was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Phycocyanin pigments were separated on a 4% stacking gel and 12% polyacrylamide solvent adjusted for protein size. A standard protein marker in the 11 to 190 kDa range was utilized to determine its molecular weight (Prestained Protein Marker, Broad Range). To show the subunits more clearly in the dissolved gel, they were stained with Coomassie Brilliant Blue G-250. The proteins' molecular sizes in the gel photographs taken by the gel imaging device (Gel Doc™ EZ imager) were calculated based on the standard protein [17-20].

2.7. Effect of temperature on phycocyanin stability.

To determine the thermal stability of partially purified phycocyanin, samples were transferred to 1.5 mL Eppendorf tubes at mg/mL concentrations and stored in a water bath (Julabo TW20) at different temperatures (30°C, 50°C, 60°C, 70°C, and 80°C). Then, they were incubated for 30 minutes, and spectrum analyses of C-phycocyanin were examined in the range of 250-700 nm (absorbance). After C-PC mg/mL concentrations were determined, the remaining phycocyanin concentration (CR %) relative to the initial concentration was calculated using the equation below [17, 21-23]:

$$C_R = \frac{C}{C_0} \times 100\% \tag{3}$$

Here, C_R is expressed as a percentage (%), and C and C_0 are the final and initial mg/mL concentrations of C-PC, respectively.

2.8. Statistical analysis.

Results were calculated as mean ± standard deviation, with experiments conducted in triplicate (n=3). Statistical evaluations and graph plotting were performed using GraphPad Prism 8.0 and Microsoft Office Excel. Data were analyzed by applying one-way ANOVA and posthoc Tukey's test, with the following significance values: $p>0.05$ (no difference), $p<0.05$* (difference), $p<0.01$** (significant difference), $p<0.001$*** (significant difference), and $p<0.0001$**** (very significant difference) [13].

3. Results and Discussion

3.1. Selection of isolates and optimal extraction methods according to phycocyanin yield.

This study assessed different isolates and various extraction methods to maximize phycocyanin yield (mg/g) with purity rates.

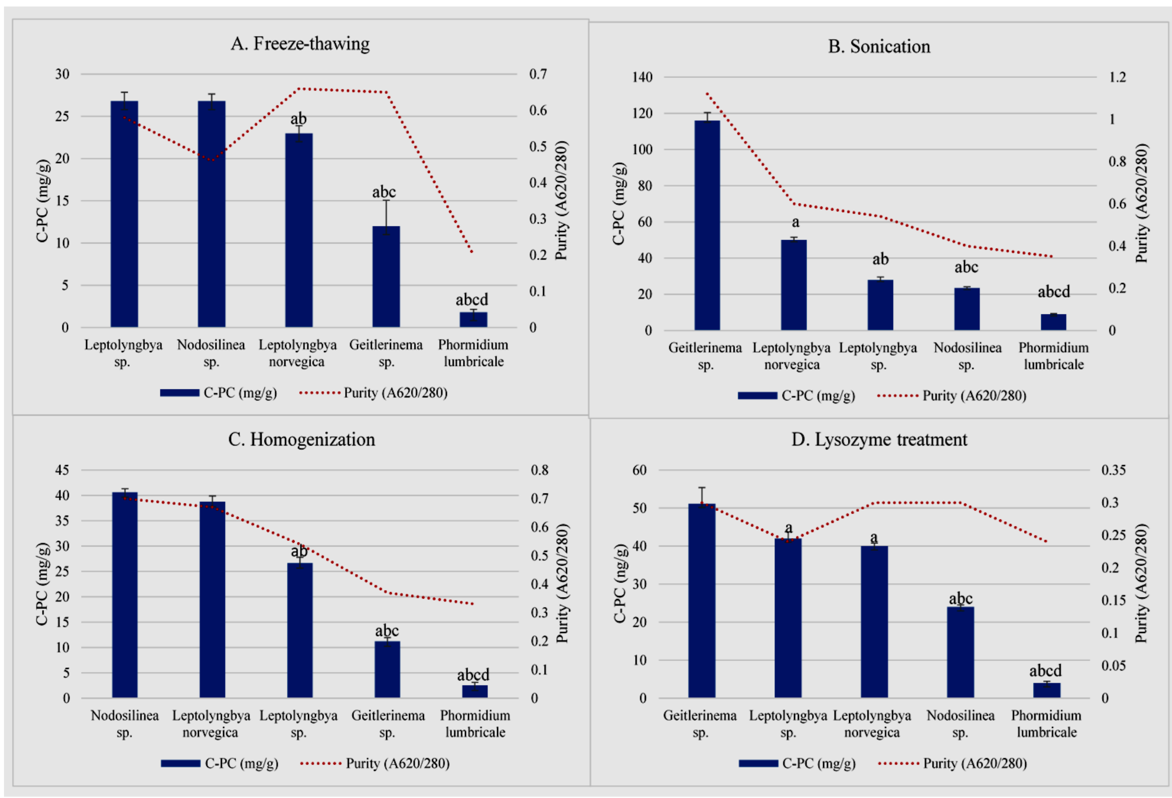

Figure 1. The comparison of different extraction methods and isolates based on C-phycocyanin yield. Four methods were employed: A) freeze-thaw, B) sonication, C) lysozyme, and D) homogenization. Error bars in the graph represent standard deviations calculated based on three replicates (N=3), with coefficients of variation used to measure variability among replicates. Statistical significance between groups was determined using Tukey's post hoc analysis, with different letters denoting significance for the highest phycocyanin yield mean ($p<0.0001$). This rigorous statistical analysis allowed for a robust comparison and identification of superior extraction methods and isolates regarding C-phycocyanin yield.

Figure 1 shows that C-phycocyanin concentrations varied significantly among the isolates and extraction methods. The freeze-thaw method (Method A) yielded the lowest C-PC concentration, indicating the least extraction efficiency across all isolates. On the other hand, the sonication method (Method B) demonstrated superior performance, with significantly higher pigment concentration and purity rates than other methods.

Geitlerinema sp. was the best phycocyanin-producing isolate, yielding 116±4.4 mg/g of C-PC using the sonication method. In contrast, the *Nodosilinea* sp. isolate obtained the highest pigment yield of 40.63±0.7 mg/g using the homogenization method. Remarkably, the lysosome treatment method (Method C) produced 44.10% lower phycocyanin concentrations (51.16±4.2 mg/g) in *Geitlerinema* sp. compared to the sonication method, although with lower impurity rates.

Phormidium lumbricale exhibited insufficient C-phycocyanin yield across all extraction methods. Consequently, upon analyzing the results from all extraction methods, the isolate was ranked as follows in terms of C-PC yield: *Geitlerinema* sp. > *Leptolyngbya norvegica* > *Leptolyngbya* sp. > *Nodosilinea* sp. > *Phormidium lumbricale*, which reflects a decreasing order based on the amount of C-PC obtained.

3.2. Optimization of C-phycocyanin extraction.

The impact of C-PC on yield and purity was examined, considering the potential bacterial degradation of cyanobacterial dry and wet biomass, which could influence pigment production. As a result, the extraction of the biomass soaked with the same solvent revealed no significant difference in yield and purity between both types of biomass (Table 1).

Table 1. Effect of wet and dry biomass on phycocyanin yield.

Biomass	**C-PC yield (mg/g)**	**C-PC purity (A_{620}/A_{280})**
Dry	95.2 ± 0.17	0.9 ± 0.08
Wet	96.8 ± 0.25	1.23 ± 0.21

To determine the effects of solutions on phycocyanin isolated from *Geitlerinema* sp., the highest purity was obtained with $CaCl_2$ (A620/280 = 1.5), while the highest phycocyanin content was achieved with sodium phosphate buffer (79.2 mg/g). A lower yield was obtained with distilled water than other buffers (Figure 2). Statistical differences were found between the solutions used regarding phycocyanin amount and purity ($p<0.0001$).

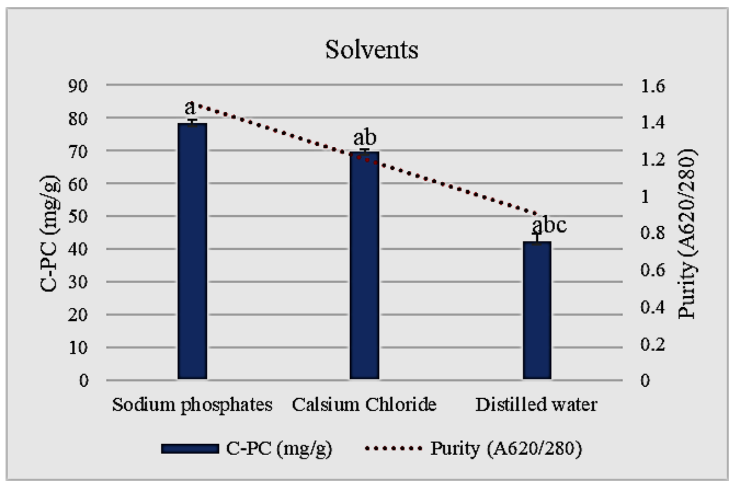

Figure 2. Effects of buffer solutions on phycocyanin yield and purity. Standard deviations are plotted with error bars as coefficients of variation for n = 3 replicates. a. Different letters denote statistically significant differences in mean phycocyanin yield ($^{****}p<0.001$) based on Tukey's test results.

Another optimization study on C-phycocyanin investigated changes in sonication parameters. In the first experiment, amplitude ratios were varied in the sonication device, with vibration frequencies increasing from 5% to 25% over a fixed period of 2 minutes. Optimal C-PC yield (108 mg/g) with a purity of 1.2 was observed at 20% amplitude (see Figure 3 A). In another experiment, the purity factor gradually decreased with increasing sonication time intervals. At the same time, C-PC yield reached 126 mg/g at a sonication time of six minutes (see Figure 3B).

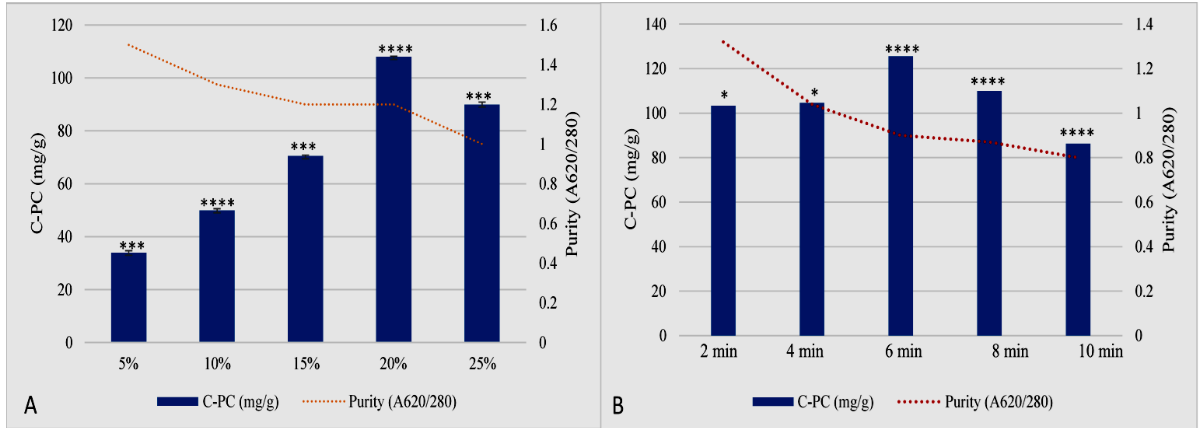

Figure 3. A) Phycocyanin yield at different amplitude ratios; **B)** Phycocyanin yield at different sonication times (there is a significant difference: *p<0.0.5 ***p<0.001, ****p<0.0001).

3.3. Analysis of phycocyanin purification.

The study obtained analytically pure phycocyanin, and Table 2 summarizes the purification profile results.

Table 2. Purity and yield values of all methods used for the purification of phycocyanin.

Method	C-PC yield (mg/g)	Recovery (%)	Purity (A_{620}/A_{280})
Extraction	103.2	100	1.84
Ammonium sulfate precipitation/dialysis	62.11	60.19	2.79
Ion-exchange chromatography (DEAE-cellulose)	22.23	38.8	4.0
Ion-exchange chromatography (Q-sepharose)	28.02	48.9	4.12

The phycocyanin purification procedure began with centrifugation of eight-liter samples and extraction with 1.5% $CaCl_2$ solution. Initial C-PC yield was calculated according to absorbance values, with a purity of 1.84 at 103.2 mg/g per dry weight. Then, purity (A_{620}/A_{280}) and recovery efficiency were calculated after ammonium sulfate precipitation and dialysis, yielding a recovery of 60% with a purity of 2.79 at 62.11 mg/g. Hence the increased purity factor resulted in decreased yield. Ion exchange chromatography was preferred as the last step to achieve high purity in the next step. Here, the purity factor, C-PC yield, and recycling rate of strong (Q-sepharose) and weak anionic resins (DEAE-cellulose) used as column fillers are compared. Q-sepharose was selected as the effective resin for phycocyanin purification, resulting in a C-PC yield of 28.02 mg/g, final recycle of 48.9%, and purity of 4.12 [14].

3.4. Spectrophotometric identification of phycocyanin.

In this study, spectrum scanning of each obtained fraction was performed at each step, including extraction and purification. Figure 4 shows the increase and decrease rates of peaks at wavelengths A_{620} and A_{280}, which are two necessary peaks. Here, A_{280} is the absorbance of

the total proteins, and with the decrease in this ratio at each purification step, the high peak formed by A_{620} proved that pure C-PC was obtained. In the spectrum absorption of purified C-phycocyanin, no allophycocyanin was observed at any peak indicated by the shoulder, particularly at A_{652} nm.

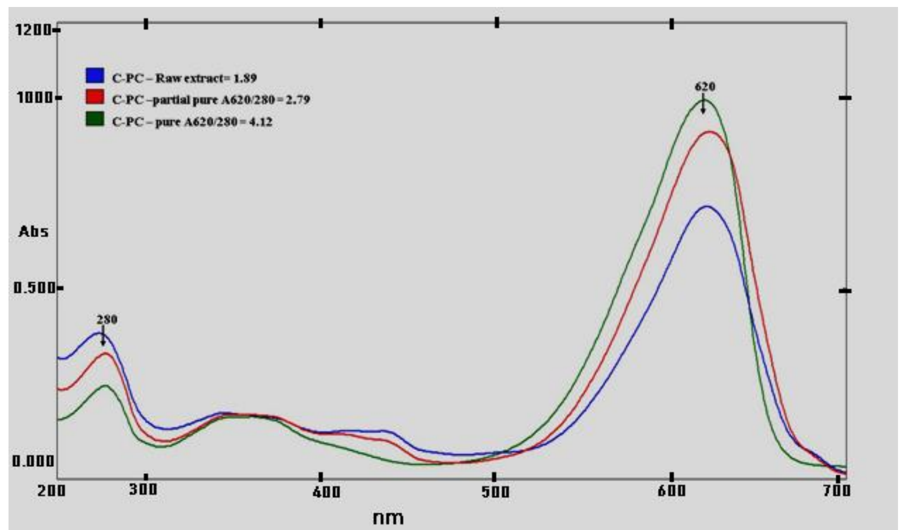

Figure 4. Systematic examination of spectrum scanning at each stage: extraction, partial purification, and final purification.

3.5. Molecular weight determination of phycocyanin.

The α and β subunits of C-PC were observed at approximately 17 kDa and 19 kDa, respectively, based on the bands identified in the gel. The intense bands in the first two strips (A and B) revealed the presence of other proteins in the crude phycocyanin extract. All impurities disappeared in the C and D bands, and clearer bands emerged (Figure 5.a). The same results were obtained with pure and homogeneous bands after loading the pure C-PC isolates obtained from the two resins used as column fillers onto the gel, and the experiment was confirmed for the second time. The molecular weight of a phycocyanin monomer was determined as 36 kDa (17 kDa+19 kDa) (Figure 5. b).

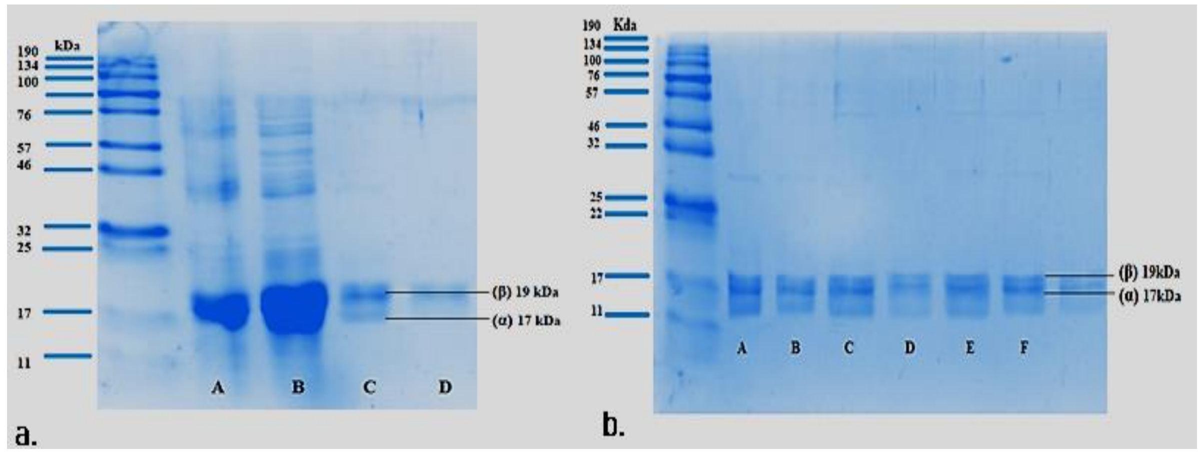

Figure 5. a) SDS-PAGE profile of purified C-PC: (A) – crude extract, (B) ammonium sulfate precipitation/dialysis, (C) ion exchange chromatography; **b)** Pure phycocyanin (A620/280 <4.0) obtained from isolates. Q-sepharose (a,b,c), DEAE-cellulose (d,e,f).

3.6. Effect of temperature on phycocyanin stability.

The stability of C-PC decreased at decreasing rates from 100%, 99.0%, 76.71% to 26.71% and 14.66%, respectively (Figure 6).

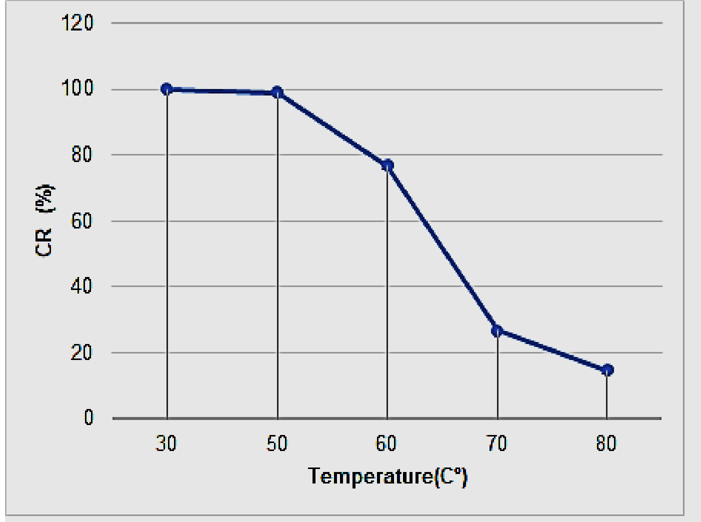

Figure 6. The effect of temperature increase on C-phycocyanin CR (%) values ($p^{****}<0.0001$).

The mg/mL values of C-PC remained at the same level, with temperature increasing from 30°C to 50°C, and there was no significant change in CR (%) values. However, starting from 60°C, the CR values of phycocyanin decreased with the increasing temperature. These results were also reflected in the spectrum properties (Figure 7). Variations in the spectrum of C-phycocyanin at A_{620} nm are shown in different colors, indicating changes in the structural conformation of C-phycocyanin.

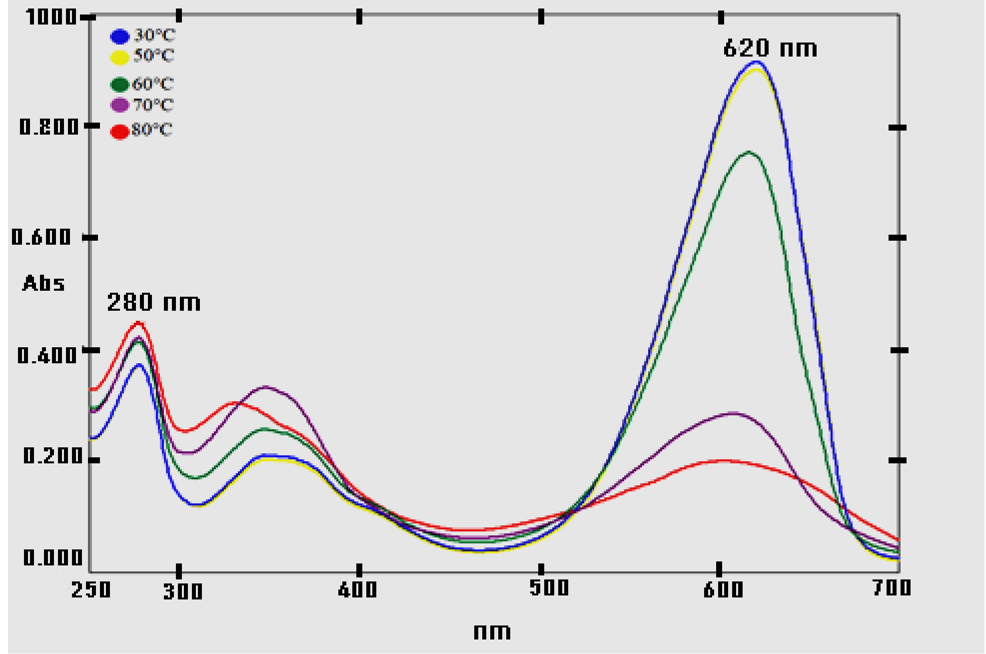

Figure 7. Effect of temperature increase on C-phycocyanin spectrum (Abs 250-700nm).

Additionally, high temperatures causing protein denaturation significantly affected the color stability of phycocyanin, which is clearly seen in Figure 8.

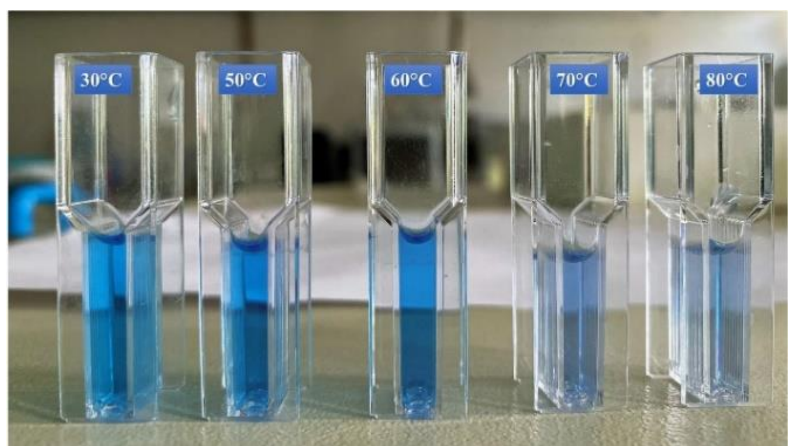

Figure 8. The color change caused by different temperatures in the phycocyanin solution.

As far as we know, the core substances of pigments originate from nature. Cyanobacteria, which can develop spontaneously in nature without chemical or physical effects, can be an excellent biotechnological natural resource for producing a natural blue color (C-PC) instead of being released into the environment as waste [18, 24-26].

Spirulina plantesis stands out as a significant source of high-quality protein for phycocyanin, an important cyanobacterial accessory pigment used in many industries [24]. Recent studies have successfully extracted and purified phycocyanin from *Spirulina fusiformis* [27], *Calothrix* sp. [29], *Synechococcus* sp. [16], *Thermosynechococcus elongatus* [30], and *Leptolyngyba* sp. [31]. The present study employed traditional methods to extract C-PC: freeze-thaw, sonication, homogenization, and enzymatic treatment with lysozyme. According to previous reports, the most common way to break down the cyanobacterial cell wall is to subject the biomass to multiple freeze-thaw cycles [32]. However, the study (Figure 1) found three consecutive freeze-thaw cycles to be insufficient compared to other extraction methods, and *Leptolyngbya* sp. (26.8±1.05 mg/g) and *Nodosilinea* sp. (26.8±0.85 mg/g) were the isolates with the highest phycocyanin yield.

Despite its economic advantages, the aforesaid method is long and requires significant energy and human labor. Limitations on storage and transportation can also cause additional problems, such as bacterial contamination and the onset of spoilage-producing malodorous compounds [13]. Another method is enzymatic treatment, which has been examined by numerous researchers [11, 13]. Although the isolates' pigment concentration efficiency decreased by 51.16±4.4 mg/g, it yielded a moderate yield. However, this method resulted in a very low purity of the isolates. The purity factor calculated for the isolates was less than the mean determined as food grade (<0.7).

Therefore, another extraction step is required to separate enzymes from the environment [33]. Among the sonication and homogenization techniques with similar characteristics according to the cell lysis mechanism, the highest pigment concentration was obtained using the sonication method in the isolate *Geitlerinema* sp. Then, various pivotal parameters were tried, such as buffer solution, dry and wet biomass, and extraction time that can affect extraction from the determined isolate. Numerous studies have discussed the effects of dry and wet biomass on pigment concentration [34-35]. This research found both methods suitable for C-PC production. However, the following studies preferred the wet biomass method due to the time and labor differences. The study investigated the effects of two solvents, except the selected solution buffer (0.1 M sodium phosphate, pH 7.0), and found the $CaCl_2$ solution to be more suitable for this method. The main reason for preferring the aforementioned

solution was that the crude extract obtained with the $CaCl_2$ solution had a bright blue color and high purity compared to other solutions. According to the report, $CaCl_2$ was a better solution because the cell membrane quickly absorbs calcium ions and interacts with pores. Additionally, Mishra et al. stated in their report (2014) that $CaCl_2$ effectively preserved phycocyanin stability at 4°C [36].

In another optimization study, power and time parameters were changed in the sonication method, resulting in a higher phycocyanin yield. The intensity and duration of sonication are essential in breaking different types of cyanobacterial cell walls [37]. The above-mentioned method is more advantageous for C-PC since it minimizes losses and is quick and efficient. According to the results, the increase in the power and time parameters reduced the purity and yield of C-PC by interacting with the solvent and other pollutants. In terms of purity and yield, the optimal time is 2 - 6 minutes, and the optimal power is 20% amplitude in the sonication method.

Following optimization studies, purification was carried out in two steps. A purity of 2.79 was achieved with a total recovery of 60.19% after ammonium sulfate precipitation (50% and 75% saturation) in the first step and after dialysis, reaching an acceptable purity level for industrial use as a colorant in cosmetic products. This process is beneficial for salting unwanted proteins and concentrating the parent phycocyanin. In the next step, ion exchange chromatography was performed to obtain a pure C-PC protein pigment. There was a significant difference due to time and efficiency in the resins used. In the treatment with DEAE-cellulose, a weak anionic resin, pure C-PC isolates were eluted with 38% recycling in an average of one and a half hours, yielding 22.23 mg/g. The isolates were eluted with Q-sepharose with 48.9% recycling in a longer time (average 3 to 4 hours), yielding 28.02 mg/g. This may be attributed to the strong adsorption of ionic charges in the protein onto the resin in the column.

The purity rate we obtained in the final purification step (Table 2) was almost comparable to several previous studies using similar methods [14]. In the UV-vis visible spectrum, crucial in the concentration and purity of C-PC, all the tubes eluted at each purification step were analyzed and released a distinct peak at 620 nm (Figure 4). The SDS-PAGE method characterized the samples, resulting in high purity. The presence of single bands in the α-subunit (17 kDa) and β-subunit (19 kDa) of phycocyanin confirmed that pure phycocyanin was obtained (Fig. 5.a, b). Our findings based on the SDS-PAGE method are similar to those of Hazra and Kesh (2017) and Minkova et al. (2007) for *Geitlerinema* sp. Another study reported that the α-subunit of phycocyanin obtained from the *Geitlerinema* sp. H8DM isolate was approximately 17.5 kDa, and the β-subunit was 18.1 kDa, which can be considered close to our results.

In the study by Samsonoff and MacColl (2001), phycocyanins of thermophilic cyanobacteria can make a difference by resisting thermal denaturation at high temperatures compared to mesophilic organisms. In the study, C-phycocyanin sourced from *S. lividus* was thermally denatured at 66°C, while mesophilic C-phycocyanin was denatured at 49-51°C. This study investigated the thermal stability of the partially purified phycocyanin obtained from the thermophilic *Geitlerinema* sp., which remained stable up to 60°C. Denaturation in the spectrum density in the temperature range of 70-80°C was apparent (Figure 7), indicating that the pigment is thermally unstable at high temperatures. The findings obtained from the study on stability were the same as the report by [38].

4. Conclusions

The cyanobacterial isolates investigated in this study have received limited attention regarding their capacity for phycocyanin production. Therefore, the data obtained from the study can enrich our knowledge of cyanobacteria as a resource for biotechnology and other related industries. In this study, *Geitlerinema* sp., isolated from thermal springs, produced the highest pigment yield. In contrast, decreasing amounts of the phycocyanin pigment were found in other species (*Leptolyngbya norvegica, Nodosilinea* sp., *Phormidium lumbricale*, and *Leptolyngbya* sp.). Varying phycocyanin concentrations may have originated from the need to combine different buffers or methods for each isolate. Suggestions for phycocyanin studies are presented by obtaining simple and remarkably positive results in the extraction, purification, and characterization steps of C-phycocyanin isolated from *Geitlerinema* sp. In conclusion, C-phycocyanin proved that it can preserve the structure up to 60°C. The thermostable C-phycocyanin pigment obtained can be a natural resource that can be used in various industrial and health fields, such as food supplements, the textile industry, reactive analyses, and pharmaceuticals, according to every purity degree.

This study significantly advances our understanding of the biotechnological implications of cyanobacteria. Cyanobacteria have attracted increasing interest due to their capacity to produce bioactive compounds with diverse industrial applications. Natural pigments such as phycocyanin have great potential across various sectors, including pharmaceuticals, cosmetics, and food technology. The considerable production of phycocyanin, particularly by *Geitlerinema* sp., underscores the biotechnological relevance of this species and provides a foundational framework for further research and use.

Investigating varying levels of phycocyanin yield among different cyanobacterial species offers valuable insights into isolate-specific biotechnological potentials. This understanding facilitates the selection of cyanobacterial isolates tailored for specific biotechnological processes, optimizing efficiency and efficacy.

In general, this study sheds light on the biotechnological significance of cyanobacteria and paves the way for future research aimed at harnessing their potential for various industrial and biomedical applications.

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Conflicts of Interest

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