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Ability Antioxidant and Anti-inflammatory Phycocyanin from Extract *Spirulina platensis*

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ABSTRACT

Increased exposure to free radicals and chronic inflammatory processes are major factors in the development of various degenerative diseases, including cancer, diabetes, and cardiovascular disease. Therefore, the search for natural bioactive compounds with antioxidant and anti-inflammatory properties has become a major focus in pharmaceutical and biomedical research. Phycocyanin is a complex protein pigment from the microalgae Spirulina platensis which is known to have biological activities, especially as an antioxidant and anti-inflammatory. This study aims to evaluate the antioxidant and anti-inflammatory properties of phycocyanin extract obtained through maceration and freeze-thawing methods using PBS solution pH 7.4 and distilled water. Antioxidant activity was evaluated using the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate)) method, while the anti-inflammatory potential was tested using the protein denaturation inhibition method. The test results showed that the phycocyanin extract had quite strong antioxidant activity with an IC_{50} value of 82.86 mg/L. In addition, anti-inflammatory activity was also shown with an IC_{50} value of 73.92 mg/L in the protein denaturation inhibition test. These results indicate that phycocyanin extract from Spirulina platensis has the potential as a natural bioactive agent in the development of nutraceutical, cosmetic, and pharmaceutical products. Further studies are needed to understand the molecular mechanisms and in vivo testing to support its clinical application.

Introduction

Increased exposure to free radicals and chronic inflammatory processes has been identified as a major contributing factor in the development of various degenerative diseases, including cancer, diabetes mellitus, and cardiovascular disorders. Free radicals can induce oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and the biological system's ability to detoxify them, leading to cellular and tissue damage [1]. On the other hand, uncontrolled chronic inflammation exacerbates these conditions by persistently activating pro-inflammatory signaling pathways such as NF-κB and increasing the production of inflammatory cytokines [2].

The search for natural bioactive compounds with antioxidant and anti-inflammatory properties has become a primary focus in the development of nature-based therapeutic agents. *Spirulina platensis*, a species of blue-green microalgae (cyanobacteria), is known to be rich in bioactive compounds, particularly phycocyanin—a complex protein pigment belonging to the phycobiliprotein group [3]. Phycocyanin has been widely reported to exhibit various biological activities, including antioxidant, anti-inflammatory, immunomodulatory, and anticancer properties [4].

The antioxidant activity of phycocyanin is primarily associated with its ability to scavenge free radicals and stabilize ROS, thereby preventing oxidative damage to

biomolecules such as lipids, proteins, and DNA [5]. Meanwhile, its anti-inflammatory activity is linked to the suppression of inflammatory mediators, including prostaglandin E2, TNF-α, and IL-6, as well as inhibition of the COX-2 enzyme [6][7].

This study aims to evaluate the antioxidant and anti-inflammatory potential of phycocyanin extract obtained through maceration and freeze-thawing methods using PBS buffer (pH 7.4) and distilled water. Antioxidant activity was assessed using the ABTS assay, while anti-inflammatory potential was evaluated via the protein denaturation inhibition method. The findings of this study are expected to support the utilization of phycocyanin as a natural active ingredient in the development of nutraceutical, cosmetic, and pharmaceutical products.

Materials and Methods

This study utilized dried *Spirulina platensis* with the commercial brand **Tiens** as the primary raw material. The chemicals used included phosphate-buffered saline (PBS) at pH 7.4, distilled water (aqua destillata), ABTS reagent (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and bovine serum albumin (BSA). Laboratory equipment employed in the study included a centrifuge, micropipettes, UV-Vis spectrophotometer, test tubes, water bath, and freeze dryer.

Phycocyanin Extraction Procedure

Phycocyanin extraction was carried out using a combination of maceration and freeze-thawing methods [4]. A total of 25 grams of *Spirulina platensis* powder was dissolved in phosphate-buffered saline (PBS) pH 7.4 at a ratio of 1:10 (w/v), then homogenized and left to stand for 24 hours at 4°C. The mixture was subsequently subjected to freezing at –20°C for 24 hours followed by thawing at room temperature, repeated for four cycles. The extract was then centrifuged at 10,000 rpm for 15 minutes to obtain a blue-colored supernatant, indicating the presence of phycocyanin. The supernatant was then freeze-dried to obtain phycocyanin in powder form. The purity of the phycocyanin was determined using the formula: OD_{620} / OD_{280}.

Antioxidant Activity Assay Using ABTS Method

Antioxidant activity was evaluated using the ABTS radical scavenging assay and spectrophotometric analysis. Briefly, the ABTS stock solution was prepared by mixing ABTS (7 mM) and potassium persulfate (2.45 mM) in equal volumes. The mixture was then incubated in the dark for 18 hours to allow the formation of the $ABTS^{\bullet+}$ radical cation. The stock solution was subsequently diluted with phosphate-buffered saline (PBS) (0.02 M, pH 7.4) to obtain a working solution with an absorbance of 0.70 ± 0.05 at 734 nm. To assess the antioxidant capacity, 20 µL of phycocyanin in deionized water at various concentrations was mixed with 180 µL of the ABTS working solution. The absorbance of the mixture was measured at 734 nm after 6 minutes of incubation in the dark at room temperature. The radical scavenging activity was calculated using the formula: **% inhibition = [(A_control – A_sample) / A_control] × 100.**

Trolox was used as the reference antioxidant standard. The IC_{50} value, defined as the concentration of the sample required to inhibit 50% of ABTS radicals, was determined from the plot of % inhibition versus sample concentration [8] .

Anti-inflammatory Activity Assay

A total of 0.2 mL of 5% bovine serum albumin (BSA) solution was mixed with 0.2 mL of phycocyanin extract at varying concentrations. The mixture was incubated at 37°C for 15 minutes, followed by heating at 70°C for 5 minutes. After cooling to room temperature, the absorbance was measured at 660 nm [9]. The anti-inflammatory activity was expressed as the percentage inhibition of protein denaturation. The IC_{50} value was calculated to determine the effective concentration required to inhibit 50% of protein denaturation.

Data analysis

Absorbance data obtained from the ABTS and protein denaturation assays were analyzed using Microsoft Excel. IC_{50} values were determined through linear regression analysis. All experiments were performed in triplicate, and results are presented as mean ± standard

deviation.

Results and Discussion

Phycocyanin extracted from *Spirulina platensis* using two different solvents resulted in crude extracts with varying yields and purity levels. Phycocyanin extracted with distilled water yielded a crude extract with a recovery of 48% and a purity value of 0.79.

Table 1. Activity Values Antioxidant and Anti-inflammatory PBS Phycocyanin Extract

Concentration Phycocyanin (mg/L)	Activity antioxidant(% Inhibition)	Activity Anti-inflammatory (%inhibition)
20	13,014	27,425
40	26,123	35,493
60	34,363	42,416
80	49,953	54,806
100	59,644	60,273

Meanwhile, extraction with PBS buffer resulted in a yield of 69.7% and a purity value of 0.96, which falls into the food-grade category, indicating superior extraction quality.

PBS provides a more stable pH and ionic environment, enhancing the solubility and extraction efficiency of phycocyanin from *Spirulina platensis* cells. PBS maintains a stable pH around 7.4, which is optimal for phycocyanin stability and solubility, while also preventing protein denaturation during the extraction process [10]. Its ionic composition facilitates more effective cell membrane disruption, allowing efficient release of phycocyanin into the solution[11]. Additionally, the ionic conditions in PBS improve phycocyanin solubility [12]. Further studies have shown that PBS buffer produces higher phycocyanin concentrations compared to water, as it more effectively disrupts cell membranes and facilitates pigment release [13], resulting in a greater extraction yield than water alone.

Studies also report that phycocyanin extracted using PBS buffer yields a higher concentration, with values reported around 35.31 mg/g, compared to the lower yields from water extraction [11]. The higher yield obtained from PBS extraction correlates with the observed higher purity. PBS's buffering capacity and pH stability improve the solubility and stability of phycocyanin throughout the extraction process. Phycocyanin, as a chromoprotein, is highly sensitive to changes in temperature and pH; thus, a stable pH environment helps maintain its structural integrity and prevent denaturation. Unlike water, PBS minimizes phycocyanin degradation during extraction, which can otherwise lead to protein denaturation [10].

The use of PBS has proven to produce a higher purity ratio (A620/A280), indicating a lower level of contamination from other mobile phase components [11]. This trend aligns with observations in previous studies where a purity ratio of 3.42 was reported using PBS, in contrast to the lower ratio obtained with water [13]. Furthermore, phycocyanin concentration increased by 12% when extracted with phosphate buffer compared to extraction using water and Tris-HCl buffer [14]. This is also consistent with the research by Pang (2014), who concluded that PBS exhibits better extraction efficiency than Tris-HCl buffer [10].

Activity antioxidant

The antioxidant activity assay was conducted on PBS-extracted phycocyanin, which had the highest purity value. Based on the ABTS assay, the IC_{50} value of phycocyanin in scavenging ABTS radicals was found to be **82.86 mg/L**, which is categorized as moderate antioxidant activity. The percentage of inhibition for each concentration of phycocyanin against ABTS radicals is presented in **Table 1**. A previous study (14) using the freeze thawing extraction method reported an IC_{50} value of 0,56 mg/L which is classified as strong antioxidant activity. The linear regression of the antioxidant assay is shown in **Figure 1.**

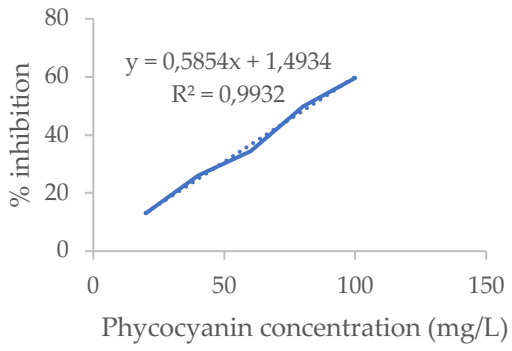


Figure 1. Linear Regression Curve of the

Antioxidant Activity Assay

This assay was selected because the ABTS test can detect a broader range of antioxidants, including those that do not react well with DPPH, such as dihydrochalcones and flavanones [15]. Previous studies have also shown that phycocyanin exhibits strong reactivity in the ABTS assay, indicating its potent antioxidant properties [16]. In general, antioxidant compounds with high pigment content and hydrophilic characteristics are more effectively evaluated using ABTS than the DPPH method [17].

Phycocyanin, a pigment - protein complex, exhibits significant antioxidant properties due to its unique molecular structure, allowing it to donate both electrons and hydrogen atoms to neutralize free radicals. This activity is associated with its ability to donate electrons through chromophore groups [8].Additionally, phycocyanin can donate hydrogen atoms, similar to other polyphenolic compounds, which helps stabilize free radicals and prevent oxidative damage [18]. ABTS radicals can be neutralized by both HAT (hydrogen atom transfer) and ET (electron transfer) mechanisms, enhancing the radical-scavenging activity of phycocyanin through dual mechanisms [19].

Activity anti-inflammatory

The anti-inflammatory activity of phycocyanin was evaluated using the protein denaturation method, which showed an IC_{50} value of **73.92 mg/L**, classified as strong activity. The percentage inhibition at various sample concentrations is presented in **Table 1**, and the linear regression curve is shown in **Figure 2**.

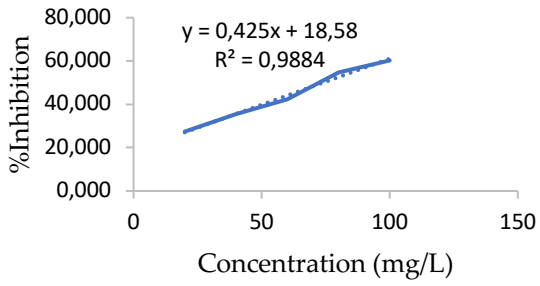


Figure 2. Linear Regression Curve of the Anti-inflammatory Activity Assay

Researchers classify the anti-inflammatory activity of a sample into several categories based on its IC_{50} value: *very active* if IC_{50} is less than 50 ppm, *active* if IC_{50} is between 50–100 ppm, *moderate* if IC_{50} is between 101–250 ppm, *weak* if IC_{50} is between 250–500 ppm, and *inactive* if IC_{50} is greater than 500 ppm [20]. Based on this classification, the purified phycocyanin obtained in this study falls under the *active* category as an anti-inflammatory agent. [3] reported an IC_{50} value of 98.76 ppm for the anti-inflammatory activity of C-phycocyanin extracted from *Spirulina platensis* using the freeze-thawing and ultrasonication method, while [21] compared the anti-inflammatory activity of phycocyanin extracted using Microwave-Assisted Extraction (MAE) and Ultrasound-Assisted Extraction (UAE), resulting in IC_{50} values of 8.0164 mg/mL and 8.5745 mg/mL, respectively.

The antioxidant properties of phycocyanin contribute to its anti-inflammatory effects by reducing oxidative stress, which is known to trigger inflammation. Phycocyanin enhances the synthesis of glutathione, a powerful antioxidant, thereby strengthening the cell's natural defense against oxidative stress. This antioxidant activity is further supported by its ability to inhibit oxidative stress-induced damage in various cellular models [22]. Phycocyanin modulates cytokine production—key mediators in the inflammatory response—and has been observed to suppress pro-inflammatory cytokines such as IL-6 and IL-8, while enhancing anti-inflammatory cytokines like TGF-β1 in intestinal models [23]. This modulation aids in maintaining a balanced immune response and prevents excessive inflammation. Additionally, phycocyanin acts as an inhibitor of inflammatory mediators by suppressing the production of prostaglandin E2 (PGE2) and phospholipase A2 (PLA2), which are crucial mediators in the inflammatory process [24].

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Conclusion

Phycocyanin extract from *Spirulina platensis*, obtained through maceration and freeze-thawing methods, exhibited significant antioxidant and anti-inflammatory activities, with IC_{50} values of 82.86 mg/L (ABTS assay) and 73.92 mg/L (protein denaturation assay), respectively. These findings confirm the potential of phycocyanin as a promising natural bioactive agent for development in the fields of nutraceuticals, cosmetics, and pharmaceuticals. Further studies are required to explore its molecular mechanisms of action and validate its effectiveness through in vivo testing.

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