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Concentration-dependent photostability of phycocyanin under UV-A and UV-B irradiation

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ABSTRACT

Background: Indonesia's tropical location results in intense UV exposure, necessitating effective photoprotective agents. Phycocyanin from *Spirulina platensis* shows promise as a natural sunscreen ingredient, yet systematic evaluation of its photostability across concentrations remains limited.

Objectives: To evaluate phycocyanin stability and antioxidant activity under UV-A and UV-B irradiation across different concentrations.

Methods: Phycocyanin (200, 250, 300, and 350 ppm) was exposed to UV-A (365 nm, 2.8 mW/cm^2) and UV-B (312 nm, 3.2 mW/cm^2) irradiation for up to 30 minutes. Pigment concentration and DPPH radical scavenging activity were measured using UV-Vis spectrophotometry.

Results: Phycocyanin exhibited concentration-dependent stability, with 300–350 ppm demonstrating optimal performance. UV-B caused greater degradation than UV-A, with concentration losses of 14.19–43.43 ppm (UV-B) versus 6.85–16.63 ppm (UV-A) after 30 minutes. Antioxidant activity decreased minimally under UV-A (≤1.85%) but more substantially under UV-B (≤1.97%). The 350 ppm concentration showed highest stability and antioxidant retention (98.9% and 98.0%, respectively).

Conclusion: The 300–350 ppm range represents the optimal concentration for photoprotective applications, supporting phycocyanin's potential as a natural sunscreen ingredient.

Keywords: antioxidant, phycocyanin, photostability, unscreen, UV radiation

Introduction

Ultraviolet (UV) radiation from sunlight is classified by wavelength into UV-C (270-290 nm), UV-B (290-320 nm), and UV-A (320–400 nm). While the Earth's ozone layer largely absorbs UV-C radiation, both UV-A and UV-B penetrate the atmosphere and pose significant health risks, particularly to human skin [1,2]. Both wavelengths contribute to sunburn, actinic keratosis, premature aging, and carcinogenesis [3]. UV-B is primarily responsible for erythema and exhibits 1,000-10,000 times greater carcinogenic potential than UV-A, whereas UV-A penetrates deeper into the dermis, contributing to photoaging and photocarcinogenesis [4].

Although the Montreal Protocol has partially mitigated ozone depletion since 1987, UV exposure remains a critical public health concern, particularly in tropical regions experiencing high solar intensity. While melanin provides natural photoprotection, excessive UV exposure can induce hyperpigmentation and cellular damage [5] Sunscreens offer additional defense through UV filters that absorb, reflect, or scatter radiation, with effectiveness measured by Sun Protection Factor (SPF), which quantifies protection against UV-B-induced erythema [6]. Modern photoprotective formulations increasingly incorporate antioxidants to neutralize reactive oxygen species (ROS) generated by UV exposure, thereby reducing oxidative stress, protecting cellular macromolecules, and promoting skin regeneration and collagen synthesis [7–9].

Natural antioxidant sources, particularly microalgae, have attracted increasing attention for cosmetic and pharmaceutical applications.

Indonesia harbors over 2,060 microalgae species, representing a biodiversity hotspot for bioactive metabolites [10–13]. Among these, *Spirulina platensis* is widely distributed and thrives in alkaline waters (pH 8–11) at 35–40°C [14]. This cyanobacterium is rich in proteins, essential fatty acids, vitamins, and minerals, exhibiting antiviral, antioxidant, anti-inflammatory, and immunomodulatory activities [15].

As a photosynthetic organism, *S. platensis* produces chlorophylls, carotenoids, and phycobiliproteins, with phycocyanin being the most abundant pigment. Phycocyanin is a blue phycobiliprotein widely used as a natural food and cosmetic colorant, exhibiting multiple biological activities including antioxidant, anti-inflammatory, anticancer, and hepatoprotective effects [16–18]. Structurally, phycocyanin consists of an apoprotein bound to phycocyanobilin, an open-chain tetrapyrrole chromophore responsible for its characteristic blue color and antioxidant properties [19]. In cyanobacteria, phycocyanin functions in light-harvesting complexes alongside chlorophyll and carotenoids [20].

Previous study has demonstrated that UV radiation affects cyanobacterial pigment biosynthesis, with UV-A reducing pigment production and UV-B inducing protein degradation and significant reductions in phycocyanin and phycoerythrin levels [21]. Although research has demonstrated photoprotective effects of *Spirulina* extracts in cellular models [22] and assessed phycocyanin stability using spectrophotometric methods [23], systematic evaluation of purified phycocyanin across multiple concentrations remains limited. Most studies examining UV stability have focused on single concentrations or absorbance changes without quantifying actual concentration loss or functional antioxidant activity [24,25]. Moreover, the influence of phycocyanin concentration on photostability has not been systematically addressed, despite concentration being a critical factor in sunscreen formulation efficacy [26].

The growing consumer demand for natural and sustainable cosmetic ingredients, combined with increasing awareness of synthetic chemical risks, has created market opportunities for plant- and algae-derived photoprotective compounds. Phycocyanin's dual functionality—both as a UV-absorbing pigment and as an antioxidant—positions it as a promising candidate for next-generation sunscreen formulations. However, translating laboratory findings into commercial applications requires establishing optimal concentration ranges that balance photoprotection, stability, and formulation compatibility.

This study investigates the photostability of purified phycocyanin extracted from *S. platensis* under controlled UV-A and UV-B irradiation by monitoring both pigment concentration and antioxidant activity (DPPH radical scavenging capacity) across different concentrations (200, 250, 300, and 350 ppm). By identifying the concentration range that optimally maintains phycocyanin integrity and biological activity under UV exposure, this work provides fundamental insights into its suitability as a natural photoprotective agent and establishes evidence-based guidelines for sunscreen formulation development.

Methods

Phycocyanin extraction and purification

Phycocyanin was extracted using a modified maceration method [23]. *Spirulina platensis* biomass (5 g), obtained from the Department of Biology, Padjadjaran University, Indonesia, was mixed with phosphate buffer (100 mL, pH 7.0, 1:20 w/v) prepared using monosodium phosphate and disodium hydrogen phosphate (Merck, Darmstadt, Germany). The mixture was stirred at 4°C for 20 minutes, incubated at −4°C for 20 minutes, then centrifuged at 3,500 rpm (1,200 × g) for 20 minutes. The supernatant was stored at −4°C in amber glass containers.

Purification employed ammonium sulfate precipitation with modifications [27]. Sequential saturation at 25% and 50% (w/v) using ammonium sulfate (analytical grade, Merck) separated the pigments, with the blue precipitate from 50% saturation collected, re-dissolved in phosphate buffer (pH 7.0), and dialyzed through cellulose membrane (12–14 kDa MWCO) for six days at 4°C with daily buffer replacement. Complete ammonium sulfate

removal was confirmed using 1% $BaCl_2$ solution (Merck). The dialyzed solution was freeze-dried at −50°C and 0.1 mbar for 48 hours and stored at −20°C until use.

Phycocyanin stability under UV irradiation

Phycocyanin stability was evaluated following established methods with modifications [21]. Purified phycocyanin was dissolved in phosphate buffer (pH 7.0) to obtain concentrations of 200, 250, 300, and 350 ppm (0.2–0.35 mg/mL). Samples (3 mL) in quartz cuvettes were placed 15 cm beneath UV lamps in a darkened chamber with temperature actively monitored using a digital thermometer and maintained at 25 ± 2°C throughout the exposure period. Samples were exposed to either UV-A irradiation (365 nm, 2.8 mW/cm^2) provided by T5 8W UV lamps (EVACO, Bandung, Indonesia) or UV-B irradiation (312 nm, 3.2 mW/cm^2) using TL 18W UV lamps (Exoterra Reptile UVB 150, Rolf C. Hagen Corp., USA) for 15, 20, 25, and 30 minutes. Irradiance was verified using a UV340A radiometer (Lutron Electronic Enterprise Co., Ltd., Taiwan). Non-irradiated controls were wrapped in aluminum foil and maintained under identical conditions.

Absorbance was measured at 620 nm and 652 nm against phosphate buffer blank using a Shimadzu UV-2700i spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Phycocyanin concentration was calculated using the equation:

$$\text{Phycocyanin (mg/mL)} = \frac{A_{620} - 0{,}474\ (A_{652})}{5{,}34} \quad \text{.....(1)}$$

where A_{620} and A_{652} represent absorbance values at 620 nm and 652 nm, respectively. Identical procedures were followed for both UV-A and UV-B exposure experiments. Each concentration was tested in triplicate across three independent experimental runs using separate phycocyanin extractions.

Antioxidant stability assessment

DPPH radical scavenging activity was measured following established methods with modifications [28]. UV-irradiated phycocyanin samples (4 mL) were mixed with freshly prepared DPPH solution (2 mL, 40 ppm in methanol ≥99.8%, Merck) using 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, St. Louis, MO, USA) in amber vials, vortexed for 10 seconds, and incubated in darkness at 25 ± 2°C for 30 minutes. Absorbance was measured at 517 nm using the Shimadzu UV-2700i spectrophotometer with methanol as blank. DPPH radical scavenging activity (inhibition percentage) was calculated using the equation:

$$Q = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \quad \text{....(2)}$$

where A_control represents the absorbance of DPPH solution mixed with phosphate buffer only (without phycocyanin), and A_sample represents the absorbance of DPPH solution mixed with phycocyanin sample. Each sample was analyzed in triplicate across three independent experimental runs.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) from three independent experiments (n = 3). Prior to analysis, normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. One-way analysis of variance (ANOVA) with Tukey's post hoc test was performed using SPSS 26.0 (IBM Corp., Armonk, NY, USA) with statistical significance set at $p < 0.05$. Graphs were prepared using GraphPad Prism 9.0 (GraphPad Software, USA).

Results

Phycocyanin pigment stability under UV-A irradiation

Phycocyanin exhibited concentration-dependent stability under UV-A exposure, with higher concentrations demonstrating superior pigment retention (Figure 1). All concentrations showed gradual decline over the 30-minute exposure period, with concentration losses ranging from 4.58–14.12 ppm after 15 minutes and 6.85–16.63 ppm after 30 minutes (Table 1). The 350

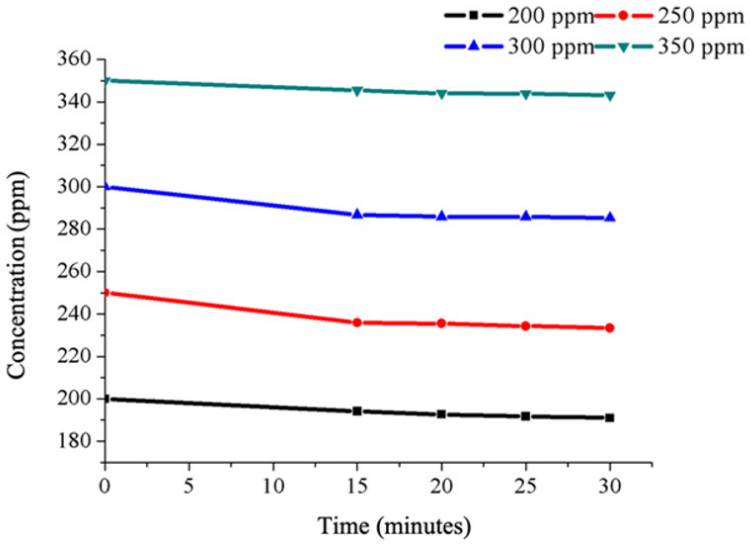

Figure 1. Effect of UV-A irradiation on phycocyanin concentration at 200–350 ppm over 30 minutes. Data represent mean ± SD (n = 3). Different letters indicate significant differences ($p < 0.05$) by Tukey's test.

Table 1. Phycocyanin concentration decrease after UV-A exposure

Concentration (ppm)	Decrease after 15 min (ppm)	Decrease after 30 min (ppm)	% Loss (30 min)
200	5.88 ± 0.42	8.98 ± 0.65	4.5
250	14.12 ± 1.08*	16.63 ± 1.21*	6.7
300	13.23 ± 0.95*	14.66 ± 1.03*	4.9
350	4.58 ± 0.38	6.85 ± 0.51	2.0

Note: Data represent mean ± SD (n = 3). Asterisks indicate significant difference from 350 ppm ($p < 0.05$).

ppm sample demonstrated the highest stability with only 6.85 ppm reduction after 30 minutes, representing 2.0% total loss. In contrast, the 250 ppm concentration showed the greatest decrease (16.63 ppm, 6.7% loss), followed by 300 ppm (14.66 ppm, 4.9% loss). The 200 ppm sample exhibited intermediate stability with 8.98 ppm loss (4.5% loss). Statistical analysis revealed significant differences between the 350 ppm and 250 ppm groups ($p < 0.01$), while no significant difference was observed between the 200 ppm and 350 ppm groups ($p > 0.05$).

Phycocyanin pigment stability under UV-B irradiation

UV-B irradiation induced substantially greater degradation than UV-A, with a clear concentration-dependent protective effect evident across all exposure times (Figure 2). After 30 minutes, concentration losses ranged from 14.19 ppm (350 ppm) to 43.43 ppm (200 ppm), representing 4.1% and 21.7% reductions, respectively (Table 2). The 200 ppm sample showed approximately 3-fold greater degradation than 350 ppm ($p < 0.001$), with rapid decline observable within the first 15 minutes (13.14 ppm loss). Intermediate concentrations (250 and 300 ppm) exhibited comparable decreases after 15 minutes (6.54 and 6.41 ppm, respectively), but diverged after 30 minutes, with 300 ppm experiencing 10% greater loss than 250 ppm (33.78 vs. 30.71 ppm, $p < 0.05$). The 350 ppm concentration maintained the highest integrity throughout the exposure period, with only 14.19 ppm decrease after 30 minutes.

Antioxidant stability under UV-A irradiation

DPPH radical scavenging activity decreased gradually under UV-A exposure, with reductions ranging from 0.20–0.88% after 15 minutes and 0.80–1.85% after 30 minutes (Figure 3, Table 3).

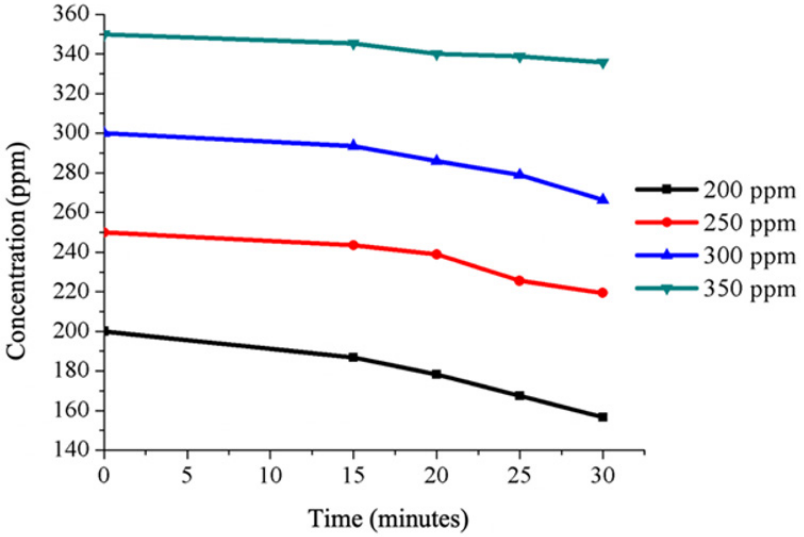

Figure 2. Effect of UV-B irradiation on phycocyanin concentration at 200–350 ppm over 30 minutes. Data represent mean ± SD (n = 3). Different letters indicate significant differences ($p < 0.05$) by Tukey's test.

Table 2. Phycocyanin concentration decrease after UV-B exposure

Concentration (ppm)	Decrease after 15 min (ppm)	Decrease after 30 min (ppm)	% Loss (30 min)
200	13.14 ± 1.15***	43.43 ± 2.87***	21.7
250	6.54 ± 0.58*	30.71 ± 1.92**	12.3
300	6.41 ± 0.52*	33.78 ± 2.15**	11.3
350	4.63 ± 0.41	14.19 ± 1.08	4.1

Note: Data represent mean ± SD (n = 3). Asterisks indicate significance vs 350 ppm: *p < 0.05, **p < 0.01, ***p < 0.001

Initial antioxidant activities before UV exposure were concentration-dependent: 63.2% (200 ppm), 66.8% (250 ppm), 70.3% (300 ppm), and 72.1% (350 ppm). After 30 minutes UV-A irradiation, the 350 ppm sample retained highest activity (71.3%, 1.1% decrease), while 250 ppm showed greatest reduction to 64.9% (2.8% decrease). Interestingly, 300 ppm exhibited larger decline (1.39%) than 250 ppm after 30 minutes despite higher initial activity, suggesting non-linear response patterns. Statistical analysis indicated no significant differences between groups after 15 minutes ($p > 0.05$), but significant variation emerged at 30 minutes between 350 ppm and other concentrations ($p < 0.05$).

Antioxidant stability under UV-B irradiation

UV-B exposure caused more pronounced antioxidant depletion than UV-A, though decreases remained relatively modest (Figure 4). After 30 minutes, DPPH inhibition decreased by 1.41–1.97% across concentrations (Table 4). The 200 ppm sample experienced greatest decline from 63.2% to 61.3% (1.93% reduction, $p < 0.01$ vs 350 ppm), while 350 ppm maintained 70.7% activity (1.41% reduction, 98.0% retention). Contrary to expectations, 300 ppm showed slightly greater activity loss (1.97%) than 250 ppm (1.55%) after 30 minutes, despite initially higher antioxidant capacity (70.3% vs 66.8%). This pattern mirrors observations under UV-A and suggests concentration-specific photochemical responses. All groups showed significant differences from 350 ppm at 30 minutes ($p < 0.05$), confirming superior antioxidant stability at highest concentration tested.

Comparative analysis of UV-A and UV-B effects

Table 5 summarizes the comparative effects of UV-A and UV-B irradiation on phycocyanin stability and antioxidant activity across all tested concentrations. UV-B consistently induced 2–3

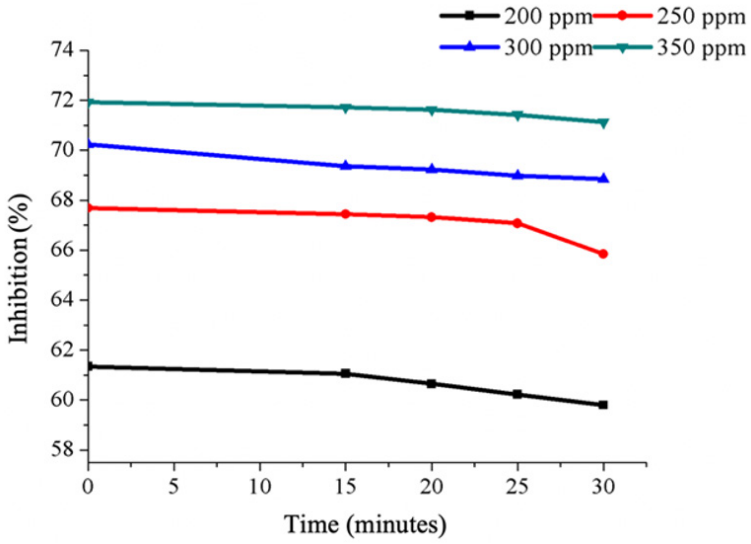

Figure 3. DPPH radical scavenging activity of phycocyanin under UV-A irradiation at 200–350 ppm. Data represent mean ± SD (n = 3). Different letters indicate significant differences ($p < 0.05$) by Tukey's test.

Table 3. Decrease in DPPH inhibition percentage after UV-A exposure

Concentration (ppm)	Decrease after 15 min (%)	Decrease after 30 min (%)	Retention (30 min, %)
200	0.28 ± 0.03	1.54 ± 0.12	97.6
250	0.25 ± 0.02	1.85 ± 0.15*	97.2
300	0.88 ± 0.07	1.39 ± 0.11	98.0
350	0.20 ± 0.02	0.80 ± 0.06	98.9

Note: Data represent mean ± SD (n = 3). Asterisk indicates significant difference from 350 ppm ($p < 0.05$)

fold greater pigment degradation than UV-A at all concentration levels. The protective effect of higher concentrations was more pronounced under UV-B exposure, with 350 ppm showing 5.3-fold better stability than 200 ppm under UV-B compared to only 1.9-fold better stability under UV-A. Antioxidant activity retention patterns were similar between UV-A and UV-B conditions, though UV-B caused slightly greater functional decline. The concentration-dependent protective effect was evident for both pigment stability and antioxidant retention, with optimal performance consistently observed at 300–350 ppm under both UV conditions.

Discussion

This study provides a systematic evaluation of phycocyanin photostability under UV-A and UV-B irradiation by quantifying both concentration loss and antioxidant activity across multiple pigment concentrations. The key findings demonstrate that: (i) phycocyanin exhibits concentration-dependent photostability, with 300–350 ppm showing optimal performance; (ii) UV-B causes 2–3 fold greater degradation than UV-A across all concentrations; (iii) antioxidant activity is relatively preserved under both UV conditions, with retention rates exceeding 96%; and (iv) concentrations below 200 ppm and above 350 ppm show suboptimal stability and efficacy. These findings establish critical concentration guidelines for developing phycocyanin-based photoprotective formulations.

While previous research has assessed phycocyanin stability primarily through absorbance measurements [23], the present work advances understanding by directly quantifying concentration changes and functional antioxidant capacity. Earlier studies have generally examined single phycocyanin concentrations, leaving the influence of varying

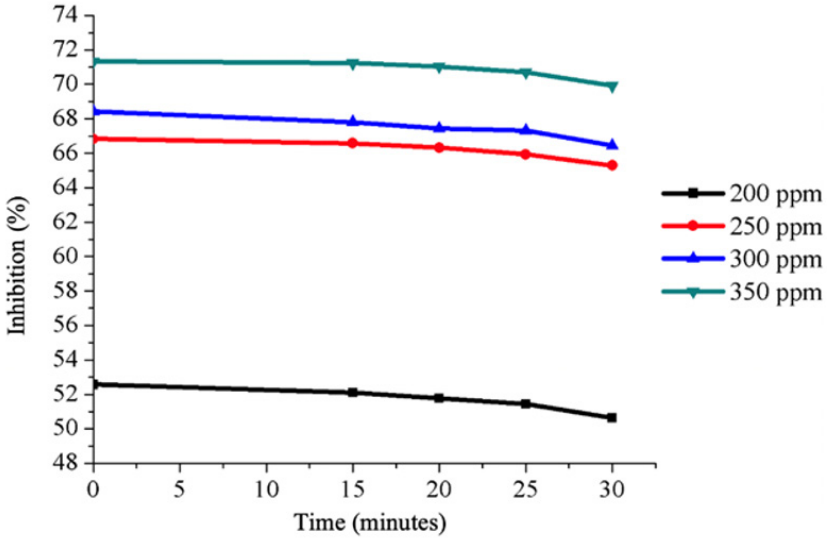

Figure 4. DPPH radical scavenging activity of phycocyanin under UV-B irradiation at 200–350 ppm. Data represent mean ± SD (n = 3). Different letters indicate significant differences ($p < 0.05$) by Tukey's test.

Table 4. Decrease in DPPH inhibition percentage after UV-B exposure.

Concentration (ppm)	Decrease after 15 min (%)	Decrease after 30 min (%)	Retention (30 min, %)
200	0.48 ± 0.04**	1.93 ± 0.16**	96.9
250	0.26 ± 0.02	1.55 ± 0.13*	97.7
300	0.62 ± 0.05*	1.97 ± 0.17**	97.2
350	0.10 ± 0.01	1.41 ± 0.11	98.0

Note: Data represent mean ± SD (n = 3). Asterisks indicate significance vs 350 ppm: *p < 0.05, **p < 0.01

Table 5. Comparative effects of UV-A and UV-B on phycocyanin after 30 minutes exposure

Concentration (ppm)	UV-A Loss (ppm)	UV-B Loss (ppm)	UV-B/UV-A Ratio	UV-A Retention (%)	UV-B Retention (%)
200	8.98	43.43	4.8	97.6	96.9
250	16.63	30.71	1.8	97.2	97.7
300	14.66	33.78	2.3	98.0	97.2
350	6.85	14.19	2.1	98.9	98.0

Note: Loss values represent pigment concentration decrease; Retention values represent antioxidant activity preservation

pigment levels on photoprotective performance unexplored [24,25]. By evaluating a concentration range from 200 to 350 ppm, this research identifies optimal levels for photoprotective applications and reveals non-linear stability patterns that have important formulation implications.

UV-A irradiation caused minimal concentration reduction (6.85–16.63 ppm over 30 minutes), consistent with reports that UV-A exerts limited direct effects on phycobiliproteins in cyanobacteria such as *Lyngbya* sp., which showed no significant degradation after five hours of exposure [21]. Rather than directly degrading pigments, UV-A primarily suppresses biosynthesis by inhibiting amino acid synthesis in photosynthetic organisms [29]. This mechanism explains why UV-A-induced degradation in purified phycocyanin solutions remains relatively modest—the purified pigment lacks the biosynthetic machinery that UV-A typically affects. The concentration-dependent protection

observed suggests that higher phycocyanin concentrations may provide enhanced shielding through increased UV absorption cross-sections, reducing photon penetration and subsequent photochemical damage to individual molecules.

In contrast, UV-B irradiation significantly compromised phycocyanin stability, with concentration losses reaching 14.19–43.43 ppm after 30 minutes, representing 2–3 fold greater degradation than UV-A. This heightened susceptibility stems from UV-B's higher photon energy (approximately 4.0–4.3 eV compared to UV-A's 3.1–3.9 eV), which induces both direct photochemical breakdown of the tetrapyrrole chromophore and indirect oxidative damage mediated by reactive oxygen species (ROS) [21]. Phycocyanin's chromophore groups can act as photosensitizers, enhancing ROS generation under UV-B exposure and leading to structural alterations including protein denaturation, chromophore bleaching, and peptide bond cleavage that collectively impair function [30]. The 350 ppm concentration demonstrated 5.3-fold better stability than 200 ppm under UV-B (Table 5), suggesting that higher pigment concentrations provide mutual photoprotection through enhanced light screening and potentially through radical scavenging by neighboring molecules.

The differential UV sensitivity observed has direct relevance to skin photobiology and sunscreen development. UV-A (320–400 nm) penetrates deeply into the dermis and promotes melanin oxidation, causing immediate pigment darkening as a short-term photoprotective response [31,32]. Additionally, UV-A generates ROS that damage collagen and elastin fibers, leading to photoaging characterized by wrinkles, loss of elasticity, and solar elastosis. Conversely, UV-B (290–320 nm) penetrates primarily the epidermis but exerts more damaging biological effects due to higher photon energy, causing direct DNA damage through cyclobutane pyrimidine dimer formation, sunburn, erythema, and mutagenic events that can lead to skin cancer [33]. Since UV-B-induced erythema forms the basis for Sun Protection Factor (SPF) determination, phycocyanin's concentration-dependent resistance to UV-B degradation directly supports its potential as a natural photoprotective ingredient. The superior stability at 300–350 ppm suggests these concentrations would maintain photoprotective efficacy throughout typical sun exposure periods (2–4 hours), a critical requirement for practical sunscreen applications.

Antioxidant activity decreased modestly under both UV conditions, with reductions of 0.80–1.85% (UV-A) and 1.41–1.97% (UV-B) after 30 minutes, corresponding to retention rates of 97.2–98.9% and 96.9–98.0%, respectively. This functional preservation reflects phycocyanin's chromophore phycocyanobilin, an open-chain tetrapyrrole that efficiently donates hydrogen atoms to neutralize free radicals such as DPPH through its conjugated double-bond system and hydroxyl groups [19,34]. The mechanism involves hydrogen atom transfer from phycocyanobilin to the DPPH radical, forming a stable reduced DPPH molecule and a phycocyanobilin radical that is stabilized through resonance delocalization. However, prolonged UV-B exposure generates cumulative ROS including superoxide anions ($O_2\bullet^-$), singlet oxygen ($^1O_2$), and hydroxyl radicals (•OH) that eventually overwhelm antioxidant defenses [35]. Beyond pigment degradation, this oxidative imbalance induces protein modifications including carbonylation, oxidation of aromatic amino acids (tryptophan, tyrosine, histidine), and disulfide bond disruption that impair cellular function and contribute to photodamage [36,37].

The concentration-dependent stability pattern reveals a critical functional window for formulation development. At 200 ppm, rapid degradation occurred under both UV conditions, indicating insufficient molecular resilience and inadequate light-screening capacity. The 250 ppm concentration provided borderline performance with inconsistent stability between UV-A and UV-B exposure. At 300 ppm, stability improved substantially but showed slight irregularities in antioxidant retention patterns. Optimal performance emerged at 300–350 ppm, where phycocyanin maintained both pigment integrity and strong antioxidant activity under UV stress. Importantly, preliminary observations at concentrations exceeding 350 ppm (400 ppm, data not shown) suggested potential pigment aggregation and reduced DPPH scavenging efficiency. Such

aggregation can cause spectroscopic interference in DPPH assays, diminish bioavailability due to reduced surface area for radical interaction, and create formulation challenges including precipitation, color inconsistency, and reduced skin penetration [38]. Therefore, the effective concentration range of 300–350 ppm provides optimal balance between photostability, antioxidant performance, formulation stability, and practical application feasibility.

Comparing these findings to commercial sunscreen standards provides perspective on phycocyanin's potential. Typical organic UV filters such as avobenzone and octinoxate are formulated at concentrations of 2–3% (20,000–30,000 ppm) to achieve SPF 15–30 protection. While phycocyanin at 300–350 ppm (0.03–0.035%) represents significantly lower concentrations, it should be considered as a complementary antioxidant ingredient rather than a primary UV filter replacement. Modern broad-spectrum sunscreens increasingly incorporate antioxidants (vitamin C, vitamin E, niacinamide) at concentrations of 0.01–0.1% to neutralize ROS and provide secondary photoprotection [7–9]. Within this context, phycocyanin at 300–350 ppm falls within the optimal range for antioxidant photoprotection while offering the additional benefit of natural blue pigmentation that could enhance product aesthetics and consumer appeal in the growing natural cosmetics market.

Several strategies could further enhance phycocyanin stability in sunscreen formulations. High-pressure processing (400–600 MPa) has been shown to improve storage stability by inducing conformational changes that protect the chromophore from oxidation [39]. Polysaccharides (chitosan, alginate, carrageenan) and sugar alcohols (sorbitol, mannitol, glycerol) provide thermal and oxidative protection through hydrogen bonding interactions that stabilize protein structure and scavenge free radicals [25,40]. Encapsulation techniques including nanoemulsions, liposomes, and biopolymer-based carriers (whey protein, zein, alginate beads) offer promising protection against UV-induced degradation by creating physical barriers that limit light penetration and oxygen diffusion while improving skin adhesion and controlled release [41–44]. Integration of such stabilization approaches with the optimal 300–350 ppm concentration identified here could substantially improve phycocyanin's commercial viability as a natural photoprotective agent.

Figure 5 provides a schematic representation of the concentration-dependent photostability and antioxidant activity patterns observed in this study. The visualization illustrates how stability and antioxidant retention increase with concentration from 200 to 350 ppm, with UV-B consistently causing greater degradation than UV-A across all levels. The optimal performance zone at 300–350 ppm represents the balance point where photoprotection is maximized without encountering aggregation-related efficacy loss that may occur at higher concentrations. This conceptual framework can guide formulation scientists in selecting appropriate phycocyanin concentrations for specific product applications, considering factors such as desired SPF enhancement, antioxidant capacity, cost constraints, and regulatory requirements.

Several limitations should be acknowledged. First, the purification method employed (ammonium sulfate precipitation) may not achieve the purity levels obtainable through column chromatography, potentially leaving residual proteins or metabolites that influence photostability measurements. However, this method better represents industrial-scale production conditions, enhancing the practical relevance of findings. Second, this study examined only 30-minute UV exposure periods, whereas typical outdoor sun exposure extends 2–4 hours or longer. Extended exposure studies are needed to assess long-term stability and establish degradation kinetics for realistic use conditions. Third, all experiments were conducted in phosphate buffer solution rather than actual cosmetic formulations, where pH, emulsifiers, preservatives, and other ingredients may significantly affect phycocyanin stability and activity. Fourth, the DPPH assay, while widely used, represents only one measure of antioxidant capacity; additional assays (ABTS, ORAC, FRAP) would provide more comprehensive antioxidant profiles. Finally, in vitro photostability does not predict in vivo photoprotection—human clinical

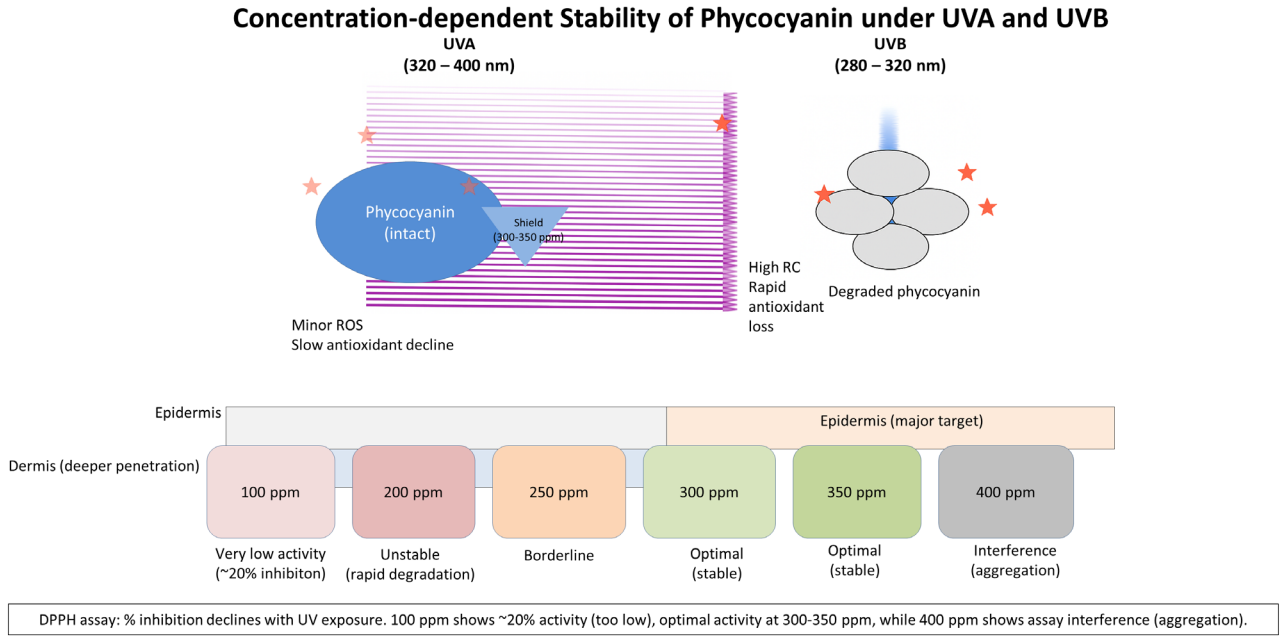

Figure 5. Schematic representation of phycocyanin stability and antioxidant activity under UV-A and UV-B irradiation across different concentrations (200–350 ppm). The shaded zone (300–350 ppm) represents the optimal concentration range for photoprotective applications. Higher concentrations (>350 ppm, indicated by dashed lines) show diminished performance due to aggregation effects.

trials measuring SPF, immediate pigment darkening (IPD), and persistent pigment darkening (PPD) are essential to validate phycocyanin's practical efficacy as a sunscreen ingredient.

This study establishes that phycocyanin photostability and antioxidant capacity follow concentration-dependent rather than strictly linear patterns, with the 300–350 ppm range offering optimal photoprotection under both UV-A and UV-B exposure. Lower concentrations (≤200 ppm) exhibited rapid degradation and weak antioxidant performance, while excessively high levels (≥400 ppm, preliminary observations) showed diminished efficacy likely due to aggregation and assay interference. UV-B induced significantly greater damage than UV-A, consistent with its higher photon energy and stronger oxidative effects. These findings emphasize the importance of concentration optimization alongside molecular stabilization strategies to preserve phycocyanin functionality under UV exposure. The work advances understanding of phycocyanin as a natural bioactive pigment and supports its development as a safe, effective antioxidant and photoprotective compound for sunscreen applications, particularly as a complementary ingredient to enhance the antioxidant capacity of broad-spectrum formulations.

The concentration-dependent stability pattern reveals a critical functional window. At 200 ppm, rapid degradation occurred even before UV exposure, indicating insufficient molecular resilience. The 250 ppm concentration provided borderline performance, while 300 ppm showed improved but inconsistent stability. Optimal performance emerged at 300–350 ppm, where phycocyanin maintained both pigment integrity and strong antioxidant activity under UV stress. Importantly, concentrations exceeding 350 ppm may prove counterproductive, as preliminary observations at 400 ppm suggested pigment aggregation and reduced DPPH scavenging efficiency. Such aggregation can cause assay interference and diminish bioavailability, as reported in studies of phycocyanin extraction and purification [38]. Therefore, selecting an effective concentration range between 300 and 350 ppm provides optimal balance between stability, antioxidant performance, and formulation reliability.

Several strategies could further enhance phycocyanin stability in sunscreen formulations. High-pressure processing improves storage stability [39], while polysaccharides and sugar alcohols provide thermal and oxidative protection [25,40]. Encapsulation techniques including nanoemulsions, liposomes, and biopolymer-based carriers offer promising protection against UV-induced degradation [41–44]. Integration of such stabilization approaches with the optimal 300–350 ppm concentration identified here could substantially improve phycocyanin's commercial viability as a natural photoprotective agent.

This study establishes that phycocyanin photostability and antioxidant capacity follow concentration-dependent rather than linear patterns, with the 300–350 ppm range offering optimal photoprotection. Lower concentrations (≤200 ppm) exhibited rapid degradation and weak antioxidant performance, while excessively high levels (≥400 ppm) showed diminished efficacy likely due to aggregation and assay interference. These findings emphasize the importance of concentration optimization alongside molecular stabilization strategies to preserve phycocyanin functionality under UV exposure. The work advances understanding of phycocyanin as a natural bioactive pigment and supports its development as a safe, effective antioxidant and photoprotective compound for sunscreen applications.

Conclusion

This study establishes that phycocyanin from *Spirulina platensis* exhibits concentration-dependent photostability, with 300–350 ppm providing optimal performance under both UV-A and UV-B irradiation. UV-B induced 2–3 fold greater pigment degradation than UV-A, while antioxidant activity remained well-preserved (>96% retention) across all tested concentrations. These findings provide evidence-based concentration guidelines for formulating phycocyanin as a natural photoprotective and antioxidant agent in sunscreen products. Future research should focus on stabilization strategies including encapsulation technologies, comprehensive formulation optimization in actual cosmetic matrices, and in vivo efficacy and safety evaluation to advance commercial development.

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Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

NU: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. HSHM: Conceptualization, Methodology, Supervision, Writing – review & editing, Project administration. GGG: Conceptualization, Methodology, Formal analysis, Writing – review & editing, Validation All authors have read and approved the final version of the manuscript.

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