การเพิ่มการผลิตซี–ไฟโคไซยานินและพอลิไฮดรอกซีบิวทิเรตใน อาร์โทสไปรา พลาเทนซิส ที่เพาะเลี้ยงด้วยไดโอดเปล่งแสง (แอลอีดี)

ฉันท์ชนก ดวงศรี[1] วินัย จรูญไชยพิพัฒน์[1] คมสัน สัจจะสถาพร[1] อุเทน พรมอริยะ[1] นาฏอนงค์ หมุดธรรม[1] ณัฐพงศ์ อัคริมาจิรโชติ[2] สุชนิทธิ์ งามกาละ[1] และวุฒินันท์ รักษาจิตร์[1*]

[1]โปรแกรมเทคโนโลยีสุขภาพสัตว์ คณะเทคนิคการสัตวแพทย์ และ [2]ภาควิชาสรีรวิทยา คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ กรุงเทพฯ 10900

[*]E-mail: cvtwnr@ku.ac.th

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บทคัดย่อ

ไซยาโนแบคทีเรียม อาร์โทสไปรา พลาเทนซิส เป็นทรัพยากรที่มีศักยภาพสูงในการผลิตสารประกอบที่มีมูลค่า เช่น ซี–ไฟโคไซยานิน (ซี-พีซี) และ พอลิไฮดรอกซีบิวทีเรต (พีเอชบี) การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อประเมินผลกระทบของไดโอดเปล่งแสง (แอลอีดี) (แสงสีแดง สีเขียว สีน้ำเงิน และสีขาว) ภายใต้ความเข้มแสงต่ำ ปานกลางและสูง (LL, ML, HL; 15, 30, 60 ไมโครโมลโฟตอนต่อตารางเมตรต่อวินาที ตามลำดับ) ต่อปริมาณชีวมวล คลอโรฟิลล–*เอ* ซี–พีซี และพีเอชบีของอาร์-โทสไปรา พลาเทนซิส อีกทั้งประเมินแอคติวิตีการกำจัดอนุมูลอิสระของซี–พีซีของอาร์โทสไปรา พลา-เทนซิส ผลการศึกษาพบว่า ค่าสูงสุดของปริมาณชีวมวล (2.80 ± 0.11 กรัมต่อลิตร) และปริมาณคลอโรฟิลล์–*เอ* (5.80 ± 0.12 มิลลิกรัมต่อลิตร) พบในเซลล์ที่เพาะเลี้ยงภายใต้แสงสีแดงที่มีความเข้มแสงปานกลาง ในขณะที่ค่าต่ำสุดของปริมาณชีวมวลและและปริมาณคลอโรฟิลล์–*เอ* พบในเซลล์ที่เพาะเลี้ยงภายใต้แสงสีน้ำเงินที่มีความเข้มแสงปานกลาง ในทางกลับกัน ซี–พีซี มีปริมาณเพิ่มขึ้นเท่ากับ 2.78 ± 0.07 มิลลิกรัมต่อกรัม ในเซลล์ที่เพาะเลี้ยงภายใต้แสงสีน้ำเงินที่มีความเข้มแสงสูง เมื่อเทียบกับแสงสีแดง สีเขียว และสีขาวที่ความเข้มแสงเดียวกัน นอกจากนี้พบว่า ซี–พีซี ที่ความเข้มข้น 100 ไมโครกรัมต่อมิลลิลิตรซึ่งสกัดได้จากเซลล์ที่เพาะเลี้ยงภายใต้แสงสีน้ำเงินที่มีความเข้มแสงสูงแสดงค่าแอคติวิตีการกำจัดอนุมูลอิสระเท่ากับร้อยละ 52.1 ± 5.6 นอกจากนี้พีเอชบีมีปริมาณสูงสุด (9.18 ± 0.32%(w/w) DW) ในเซลล์ที่เพาะเลี้ยงภายใต้แสงสีขาวที่มีความเข้มแสงสูงและขาดแหล่งอาหารไนโตรเจนในอาหารเลี้ยง เมื่อเทียบกับแสงสีแดง สีเขียว และสีน้ำเงินที่ความเข้มแสงเดียวกัน การศึกษาครั้งนี้ให้ข้อมูลทางสรีรวิทยาที่เป็นประโยชน์เกี่ยวกับผลกระทบของสเปกตรัมแสงที่แตกต่างกันและความเข้มของแสงต่อการเจริญเติบโต ปริมาณรงควัตถุสังเคราะห์ด้วยแสง และพีเอชบี ซึ่งเป็นแนวทางเบื้องต้นในการปรับการผลิตสารประกอบที่มีมูลค่าสูงจากการเพาะเลี้ยงอาร์โทสไปรา พลา-เทนซิส ให้เหมาะสม

คำสำคัญ: อาร์โทสไปรา พลาเทนซิส ซี–ไฟโคไซยานิน แอลอีดี พอลิไฮดรอกซีบิวทีเรต

Enhanced Production of C–Phycocyanin and Polyhydroxybutyrate in the *Arthrospira platensis* Cultured with Light–Emitting Diodes (LEDs)

Chanchanok Duangsri[1], Winai Charunchaipipat[1], Khomson Satchasataporn[1], Authen Promariya[1], Nat–Anong Mudtham[1], Nattaphong Akrimajirachoote[2], Suchanit Ngamkala[1] and Wuttinun Raksajit[1*]

[1]Program of Animal Health Technology, Faculty of Veterinary Technology, and [2]Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand

[*]E-mail: cvtwnr@ku.ac.th

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Abstract

The cyanobacterium *Arthrospira platensis* is considered a highly potential resource for the production of valuable compounds such as C–phycocyanin (C–PC) and poly–3–hydroxybutyrate (PHB). This study aimed to evaluate the effects of different coloured light–emitting diodes (LEDs) (red, green, blue, and white light) under low, medium, and high light intensity (LL, ML, HL; 15, 30, 60 μmol photons $m^{-2}s^{-1}$, respectively) on the biomass, chlorophyll–*a* (Chl–*a*), C–PC, and PHB contents of *A. platensis*, and further determine the free–radical scavenging activity of *A. platensis* C–PC. The results exhibited that the highest values for biomass (2.80 ± 0.11 g L^{-1}) and chlorophyll–*a* (Chl–*a*) content (5.80 ± 0.12 mg L^{-1}) were obtained in cells cultured under ML red light, while the lowest values for biomass and Chl–*a* content were observed in cells cultured under ML blue light. On the other hand, the C–PC value increased to 2.78 ± 0.07 mg g^{-1} in cells cultured under HL blue light compared to red, green, and white lights with equal intensities. Besides, the purified C–PC obtained from cells cultured under HL blue light showed 52.1 ± 5.6% of free–radical scavenging activity at a C–PC concentration of 100 μg mL^{-1}. In addition, the maximum PHB content (9.18 ± 0.32%(w/w) DW) was obtained in cells cultured under HL white light with nitrogen deprivation compared to red, green, and blue lights with equal intensities. The results of the current study provide useful physiological information regarding the effects of different light spectra and light intensity on growth, photosynthetic pigments, and PHB as a prerequisite to optimize the production of high–value compounds from cultures of *A. platensis*.

Keywords: *Arthrospira platensis*, C-Phycocyanin, LEDs, Polyhydroxybutyrate

Introduction

Arthrospira platensis is a filamentous cyanobacterium that is rich in valuable pigments like chlorophyll–*a*, carotenoids, phycobiliproteins (PBPs), and biopolymers (Lupatini *et al*., 2017; Milia *et al*., 2022; Nematollahi *et al*., 2020). *A. platensis* biomass can be applied in the food sector for animal and human consumption. PBPs are major pigment–protein components of the cyanobacterial cells and can constitute up to 50% of the cell materials. PBPs are divided into four types according to their maxima light absorption wavelength: phycocyanin (λ=615–640 nm), phycoerythrin (λ=565–575 nm), phycoerythrocyanin (λ=560–600 nm), and allophycocyanin (λ=650–660 nm). Among them, phycocyanin (C–PC) is a hydrophilic, blue–coloured fluorescent protein covalently bound to chromophore tetrapyrrole groups (Minic *et al*., 2018). It is a pigment associated with other phycobiliproteins, such as allophycocyanin and phycoerythrin. Furthermore, it is a water–soluble compound with constituents of 17% proteins (Patel *et al*., 2005). Besides, C–PC has been shown to exhibit antioxidant, anti–inflammation, anticancer, and immunity enhancement properties (García *et al*., 2021; Sonani *et al*., 2017). Phycocyanin from *S. platensis* LEB–52 can enrich nutritional value and provide a natural colour to dairy products (Campos Assumpção de Amarante *et al*., 2020). In addition, *A. platensis* have a biological system with the potential to synthesize biopolymers such as poly–3–hydroxybutyrate (PHB) under various culture conditions (Duangsri *et al*., 2020). The highest accumulation of PHB in *Arthrospira platensis* was observed in cells grown photoautotrophically under nitrogen deprivation with acetate supplementation (Duangsri *et al*., 2020). Most PHB is produced and accumulated intracellularly as a carbon storage material and an energy reservoir. PHB is an attractive alternative to common thermoplastics due to its hydrophobicity, biodegradability, and biocompatibility. Furthermore, PHB nanofiber from *Spirulina platensis* has exhibited properties equal to or better than nanofiber made with commercially available PHB (Moradi *et al*., 2021). The accumulation of photosynthetic pigments, PHB, and biomass in *A. platensis* can be achieved by optimizing the availability of growth factors such as light, temperature, pH, and nutrients. Particularly, light quality affects the growth and biochemical properties of phototrophic organisms (Khatoon *et al*., 2018). Recently, studies have focused on investigating the effects of light intensity and light quality on microalgal growth, pigmentation, and photosynthesis (Klepacz–Smółka *et al*., 2020; Wicaksono *et al*., 2019). Various light sources such as sunlight, fluorescent light, and light–emitting diodes (LEDs) have been used to produce biomass and valuable biochemical components (da Fontoura Prates *et al*., 2020). In practice, artificial illumination LEDs tend to be the preferred choice for

acclimatizing a cultivation system into an intensive one in a small area. LEDs emit a single wavelength of light and have low power consumption interpreted to low carbon emissions (Van Hieu *et al*., 2021). Notably, LED lights produce less of an environmental impact than traditional lighting sources. Therefore, LEDs are considered an attractive artificial light for microalgal production. Even though many studies have focused on various lighting conditions during the cultivation of *Arthrospira* sp. to enhance biomass and C–PC production, little or no attention has been given to the co–production of C–PC and PHB using LEDs during cultivation. Hence, this research aimed to investigate the effects of different LEDs (white, red, green and blue light) on *A. platensis* biomass, photosynthetic pigments (chlorophyll–*a*, carotenoid, and C–PC), and PHB content, as well as further determine the *in vitro* free–radical scavenging activity of *A. platensis* C–PC.

Research Methodology

Cell cultivation and biomass determination

The *A. platensis* used in this study was obtained from the Institute of Food Research and Product Development, Kasetsart University (IFRPD). The cells were cultivated in 250–mL Erlenmeyer flasks (Schott Duran, Germany) containing 50 mL Zarrouk medium under photoautotrophic conditions at 32 ± 0.5°C, pH 10.0. Experiments for the two–stage cultivation were conducted as follows. In the first stage, *A. platensis* was cultivated under continuous illumination with white fluorescent at 30 µmol photons m^{-2} s^{-1} for 7 days until reaching the late logarithm phase (optical density, OD~1–1.2). After that, white fluorescent was replaced with blue, green, red and white LEDs with wavelengths of 430–465 nm, 525–550 nm, 630–665 nm, and 420–665 nm, respectively. The *A. platensis* was further cultivated under three light intensities designated as low–intensity (LL, 15 µmol photons $m^{-2}s^{-1}$), medium–intensity (ML, 30 µmol photons $m^{-2}s^{-1}$), and high–intensity (HL, 60 µmol photons m^{-2} s^{-1}) for 5 days. The biomass concentration (g L^{-1}) was determined spectrophotometrically according to Duangsri *et al*., (2020). The irradiance of the LEDs was measured using a quantum meter (Biospherical Instruments, model QSL–100).

C–phycocyanin extraction, partial purification and determination

The *A. platensis* cells were harvested by filtration and then suspended in a 0.1 M sodium phosphate buffer (pH 7.0) at a ratio of 1:15 under darkness on ice, while cells were extracted by sonication at 20 kHz for 10 min with three repeated cycles of freezing and thawing (RFT cycles). The suspension was frozen for 3 hrs at –20°C and thawed for 1 hr at 25°C. After 3×RFT cycles, the mixture containing C–phycocyanin (C–PC) was centri-

fuged for 30 min at 4,000×*g* at 4°C to remove the cell residues. Partial purification of C–PC was done using ammonium sulfate precipitation. Solid ammonium sulfate was gradually added into the beaker containing crude extracts of C–PC to obtain 40% saturation with continuous stirring for 1 hr. The solution was stored overnight at 4°C under dark conditions, and the precipitation was collected by centrifugation at 15,000×*g* for 15 min at 4°C. The clear supernatant was discarded, and the blue precipitate was dissolved in 0.1M PBS pH 7.0 then stored at 4°C in an amber bottle until examination (Kumar *et al*., 2014). After that, the C–PC contents were calculated using Eq. (1) as described by Bennett and Bogorad (1973).

$$\text{C–PC (mg g}^{-1}) = (A_{620} - 0.474 \times A_{652})/5.34) \quad (1)$$

where A_{620} and A_{652} are the optical density at 620 and 652 nm, respectively, for phycocyanin.

Chlorophyll–*a* extraction and determination

The chlorophyll–*a* contents (mg L^{-1}) were determined according to MacKinney (1941). Briefly, one milliliter of cell culture was centrifuged at 8,000×*g* for 10 min. The biomass was resuspended in 1 mL of 99.9% methanol. The samples were incubated under darkness overnight. The upper aqueous layer was kept and determined by a spectrophotometer at 665 nm.

PHB induction and quantification

The microalgal biomass of *A. platensis* (7 days) was harvested and washed twice with fresh nitrogen-free Zarrouk medium (ZN_o), and inoculated into 50 mL of ZN_o followed by cultivation under blue, green, red, and white LEDs with low–intensity (LL, 15 µmol photons m^{-2} s^{-1}), medium–intensity (ML, 30 µmol photons m^{-2} s^{-1}), and high-intensity (HL, 60 µmol photons m^{-2} s^{-1}) for 5 days. PHB was quantified by high–performance liquid chromategraphy (HPLC, Waters Corp., USA) using a C18 reverse–phase column (I.D. 4.6×150 nm) with a UV detector set at 210 nm (Duangsri *et. al*., 2020). Briefly, dry cells were boiled at 100°C for 60 min with H_2SO_4. Boiled samples were filtered using a 0.45 µm polypropylene membrane filter. The acid–hydrolyzed PHB (crotonic acid) monomer was detected using HPLC. The isocratic solvent system was run at 40% (v/v) of acetonitrile and 60%(v/v) of 0.1% acetic acid with a flow rate of 0.6 mL min^{-1}. Authentic commercial PHB from Sigma–Aldrich (USA) was used as a standard which was prepared similarly to that of the cell sample. The unit of PHB content was % PHB weight per biomass dry weight (%(w/w DW)).

Antioxidant activity using DPPH assay

DPPH (2,2–diphenyl picrylhydrazyl)

free radical scavenging activity was studied using the method described by Roy and Pabbi (2022). Briefly, two milliliters of samples were mixed with 1 mL of DPPH solution (200 µM in ethanol), incubated at room temperature in the dark for 30 min followed by measuring the absorbance of the samples at 517 nm. The DPPH reduction percentage was determined using Eq. (2), where A_i is the initial absorbance and A_f is the absorbance after incubation for 30 min.

DPPH free–radical scavenging activity (%)

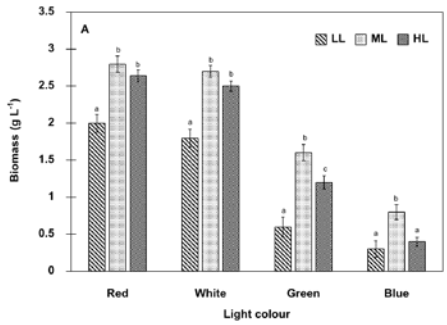
$$= (A_i - A_f)/A_i \times 100 \quad (2)$$

Statistical analysis

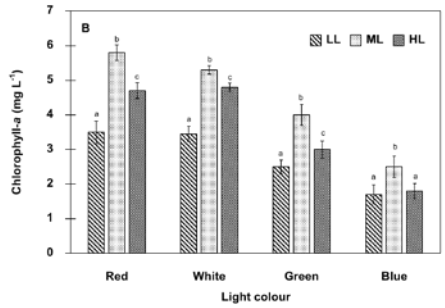
All data obtained in this study represent the means of three independent biological replicates, and the error bars represent the standard deviation (Mean ± SD, n = 3). The statistical analysis was evaluated by one-way analysis of variance (ANOVA) and the significant difference ($p < 0.05$) was compared by Duncan's multiple range tests using SPSS version 22 (IBM, USA).

Results and Discussion

One of the important requirements for cyanobacteria metabolism is light. Light intensity directly affects the rate of photosynthesis. Cyanobacteria grown under various light intensities show changes in biomass production, photosynthetic pigments, and lipid contents (Gim *et al*., 2016; Maltsev *et al*., 2021; McGee *et al*., 2020). The highest biomass content (2.80 ± 0.11 g L^{-1}) was obtained under ML red LED, which was significantly higher than LL red LED ($p < 0.05$), and higher than ML white, ML green and ML blue LEDs (Figure 1A). Previously, it was found that the highest growth rate was obtained with red LED in batch cultures of *A. platensis* (Markou, 2014), while the lowest biomass productivity was obtained with ML blue LED. In addition, the red LED showed the most effective emission for *S. platensis* growth, followed by white, yellow and green LEDs with a similar growth pattern (Chen *et al*., 2010). Furthermore, the levels of chlorophyll–*a* in *A. platensis* under ML intensities of all colour LEDs were significantly higher than that cells under HL and LL intensities about 0.7– to 1.5–fold, respectively ($p < 0.05$). The highest chlorophyll–*a* content (5.80 ± 0.12 mg L^{-1}) was obtained under ML red LED (Figure 1B). In particular, the chlorophyll–*a* levels obtained under red and white LEDs were significantly higher than those reached under green and blue illumination for ML conditions ($p < 0.05$). Additionally, C–phycocyanin (C–PC) content (2.55 ± 0.09 mg g^{-1}) was 1.7–fold higher under LL red LED compared to ML red LED, and significantly higher than those reached



(A)



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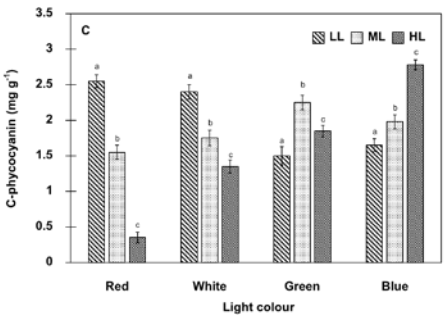

(C)

Figure 1 Effects of light colour and light intensity on biomass (A) chlorophyll–*a* (B) and C–PC (C) of *A. platensis*. Cells were cultivated under three light intensities designated as low–intensity (LL, 15 µmol photons m^{-2} s^{-1}), medium–intensity (ML, 30 µmol photons m^{-2} s^{-1}), and high–intensity (HL, 60 µmol photons m^{-2} s^{-1}) for 5 days. Bar graphs represent mean values (± SD) of three independent experiments. Different letters (a, b, or c) indicate significant differences ($p < 0.05$) between the groups obtained from cells cultured in LL, ML and HL.

under green and blue illumination for LL and ML conditions ($p < 0.05$) (Figure 1C). Remarkably, the highest value for C–PC increase (2.78 ± 0.07 mg g^{-1}) was obtained in cells cultured under HL blue light compared to red and white lights. Chen *et al*. (2010) reported that the chlorophyll–*a* and C–PC contents of *S. platensis* under green and blue LEDs were significantly lower than those produced under the red, white and yellow LEDs. On the other hand, it was reported that the highest pigment production rate for *Spirulina fussiformis* was obtained under blue light (Madhyastha and Vatsala, 2007). A possible explanation is that the authors used other light sources instead of LEDs to obtain the desired colour. All results demonstrated that this cyanobacterium absorbed blue light much less efficiently than white and red light. These findings are consistent with previous investigations conducted with *A. platensis* (Markou, 2014; Wang *et al*., 2007). Our results support the hypothesis that the blue LED creates an imbalance between photosystems I and II, with an excess of energy capture at the PSI side and a lack at the PSII side of the photosynthetic electron transport chain of *A. platensis* (Luimstra *et al*., 2018). Moreover, cyanobacteria β–carotene, which absorbs blue light efficiently, is more abundant in PSI than in PSII, hence further contributing

to the photosynthesis light–harvesting capacity of PSI. Our results showed that *A. platensis* cells acclimate to blue light by enhancing the production of phycocyanin in an attempt to restore the balance between photosystems I and II. These findings corroborate the notion that a culture grown under blue light has a much lower growth rate than red and white lights, but a significantly higher phycocyanin content. Moreover, Chen *et al*. (2010) demonstrated that blue light showed a trend of more intensity accompanied with a larger *m* value which is the empirical constant in kinetic model of product formation. This meant that blue light was capable of inducing phycocyanin production at a higher light intensity as compared to lower light intensity.

PHB accumulation is induced by optimal nutrients and light intensities as well (Ansari and Fatma, 2016; Costa *et al*., 2018; Monshupanee and Incharoensakdi, 2014). Indeed, PHB production in cyanobacteria was induced when the cells were first cultivated in a nutrient–rich medium, followed by a second step, where the medium was deprived of a nitrogen source and/or supplemented with an organic carbon source to stimulate PHB accumulation (Charunchaipipat *et al*., 2020; Duangsri *et al*., 2020; Panda and Mallick, 2007). A similar approach was used to assess the capacity of PHB production by *A. platensis*. No PHB was detectable in cells grown in a nutrient–rich medium, while a substantial level of PHB production was apparent under nitrogen deprivation. The highest PHB content was obtained under HL white LED (9.18 ± 0.32%(w/w) DW) which was significantly higher than HL red, green, and blue LEDs ($p < 0.05$) (Figure 2). A previous study demonstrated that a moderately HL white intensity (200 μmol photons $m^{-2}s^{-1}$) was optimal for the co–production of glycogen, lipid and PHB in *Synechocystis* sp. PCC6803 (Monshupanee and Incharoensakdi, 2014). Besides, Abiusi *et al*., (2014) reported that *Tetraselmis suecica* F&M–M33 grown in red LED attained the highest protein content, while cultures grown under blue and green lights showed the highest lipid and carbohydrate content, respectively. It has been shown that the total lipid content of three microalgal species, namely *Isochrysis galbana* LB987, *Nannochloropsis oculata* CCAP849/1, and *Dunaliella salina*, increases with increasing light intensity (80–100 μmol photons $m^{-2}s^{-1}$). This is because excessive light energy was converted into lipid (Gim *et al*., 2016). However, a shift from low to high irradiance is known to cause an immediate increase in photosynthetic activity and growth rate (Warner and Madden, 2007), as well as induce an initial

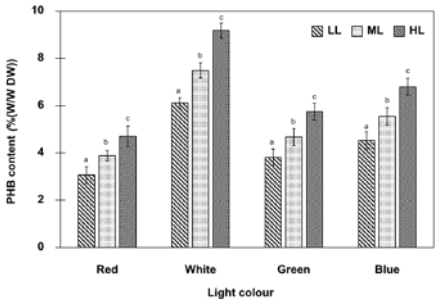

Figure 2 Effects of light colour and light intensity on PHB content of *A. platensis*. Cells were cultivated under three light intensities designated as LL, ML, and HL for 5 days. Bar graphs represent mean values (± SD) of three independent experiments. Different letters (a, b, or c) indicate significant differences ($p < 0.05$) between the groups obtained from cells cultured in LL, ML and HL.

accumulation of carbohydrates. High light irradiance (440 μmol photons $m^{-2}s^{-1}$) activated the rapid accumulation of glycogen by up to 34% of cell dry weight after 9 hrs but did not generate PHB in *Spirulina maxima* (de Philippis *et al*., 1992). C–Phycocyanin (C–PC) is known to expand the light absorption spectra and is thought to have high antioxidant potential that can help sustain *A. platensis* growth under such extreme conditions (Mogany *et al*., 2020; Roy and Pabbi, 2022). The antioxidant potential of C–PC was determined by measuring DPPH (2,2–diphenyl picrylhydrazyl) radical scavenging activity. The reduction in the optical density of DPPH after interaction with the antioxidant was estimated. The C–PC concentrations from 10 to 100 μg mL^{-1} extracted from cells cultured under HL blue light were incubated with DPPH solution for 30 min. Maximum inhibition was found at 100 μg mL^{-1} of C–PC concentration with antioxidant activity of 52.1 ± 5.6% for *A. platensis* (Figure 3). The DPPH radical scavenging activity of ascorbic acid at 100 μg mL^{-1} concentration was 80.2 ± 4.8%. Comparable antioxidant activity of the C–PC concentration was also detected in *Geitlerinema* sp. TRV57 and *Geitlerinema* sp. H8DM (Patel *et al*., 2018; Renugadevi *et al*., 2018). The reaction capacity of antioxidants depends on their structural characteristics more specifically the hydroxyl groups available for reduction. Moreover, Pan–utai and Iamtham (2019) showed similar results to our study in terms of C–PC antioxidant activity having equal crude concentration in the range of 30–60% for DPPH

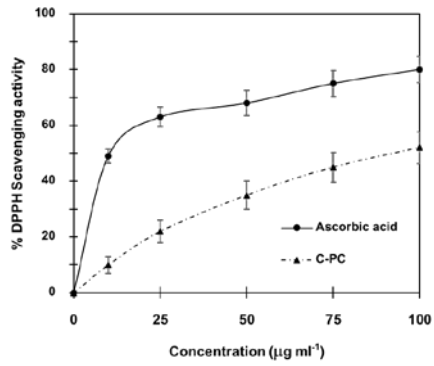

Figure 3 *In vitro* antioxidant activity of partial purified C–PC of *A. platensis*. Ascorbic acid was used as a reference antioxidant. Error bar represent ± SD of three independent experiments.

scavenging under normal cultivation condition.

The commercialization of many natural products requires a large amount of biomass and a high percentage of the desired valuable pigments in the biomass. In this respect, the growth of *A. platensis* under LL and ML red LEDs fulfils the requirement for a higher concentration of chlorophyll–*a* and C–PC in the biomass. Although lower biomass was observed in cells cultured under HL blue LED, a high amount of C–PC was obtained compared to that continuously grown under red LED. Moreover, the use of red LEDs could result in a more economically viable production process if we consider that leftover biomass after the extraction of C–PC still contains valuable products such chlorophyll–*a* and valuable proteins.

Conclusion

This study concluded that light–emitting diode (LEDs) radiation enhanced biomass, chlorophyll–*a*, C–phycocyanin, and PHB production in *Arthrospira platensis*. Red LED supported biomass and chlorophyll–*a*, while blue LED enhanced phycocyanin. Besides, white LED increased PHB content. The results of this study offer a promising option for the utilization of alkaliphilic cyanobacteria in the production of C–PC and PHB.

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