

Effect of the application of phycocyanin from *Arthrospira platensis* (*Spirulina platensis*) as fertiliser on bioactive compounds in microgreens

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Abstract: Bio-fertilisation has gained popularity due to its sustainability in agricultural practice. One of the cyanobacterial-based bio-fertilisers is *Arthrospira platensis* (*Spirulina platensis*), which contains phycocyanin, a blue protein pigment that is abundant in *Spirulina* sp., that boosts the growth yield and bioactive content of plants. The aim of the current study is to determine the alteration in antioxidant and phenolic content in red cabbage microgreens (*Brassica oleracea* var. *capitata* f. *rubra*) by treating different organs of the plant with phycocyanin from *Arthrospira platensis*. The phycocyanin application did not significantly affect the phenolic content of the cotyledon leaves. However, soaking with phycocyanin solution significantly affected the phenolic content of seeds. Regarding the antioxidant capacity of the microgreens, no significant effect was detected on the antioxidant activity of the seeds among the phycocyanin concentrations. The phycocyanin application significantly increased antioxidant activity compared to control soaked in deionised water. The results clearly demonstrate that phycocyanin application to seed and cotyledon leaves affected the antioxidant capacity and phenolic content of the microgreens. Although there are several studies about the effects of phycocyanin on growth parameters, this study has different perspectives in terms of the method of phycocyanin application and evaluation of the bioactive compounds in microgreens.

Keywords: microalgae; bio-fertiliser; microgreen; phenolic content; antioxidant activity

In traditional agriculture, chemical fertilisers overused with the aim of increasing yield per unit area reduce the soil fertility and biological diversity and cause the emergence of new pests and diseases. The use of agricultural chemicals at amounts that the ecosystem cannot sustain negatively affects soil and plant health. For sustainable agriculture, in addition to providing the

organic content of soil with natural fertilisers, it is necessary to enrich the microorganism diversity and amount to ensure physical and chemical balance in soil. If this is not done, yield and product quality fall. As a result, there is a need for practices that increase soil quality to obtain quality products (Aguilar-Paredes et al. 2020; Yadav 2020; Shelar et al. 2023). In agricultural product activity,

the ability to ensure healthy development with matter obtained by the plant from the soil is correlated with the physical and chemical features of the production environment in which the plant is grown. A method to improve soil properties and ensure continuity may be ensured when items added to soil are materials with an organic source (Bender et al. 1998). Organic agricultural activities sensitive to humans and health keep agricultural production at the fore. The target should be to use sustainable sources specific to human health and agricultural ecology as agricultural inputs in production.

Microalgae are organisms that have been a focus in recent years in terms of their high nutrient content. Microalgae are known to be the most productive biological systems that use solar energy for the production of organic components through photosynthetic processing. Microalgae are microorganisms without complex reproductive organs. This situation ensures the opportunity to harvest and use the whole biomass. Several species of microalgae may be used for the production of proteins, carbohydrates, lipids and pigments with commercial value and with high concentrations of the desired components. Microalgae such as *Porphyridium cruentum*/Rhodophyta and green algae of *Chlorella* strains are used to produce polysaccharides (Plaza et al. 2009; Mišurcová et al. 2015). *Chlorella* is also an alternate source of vitamin B₁₂ due to its rich content. In addition to this, *S. platensis* is a natural source of docosahexaenoic acid (DHA) (ω -3). Therefore, microalgae are described as a good alternative to chemical synthesis for several phytonutrients (Plaza et al. 2009; Mobin et al. 2019). In several regions with low yield due to a lack of freshwater or poor soils, a way to increase yield and ensure simple protein production is the production of microalgae cultivated using seawater or brackish water in poor soils. Microalgae biomass production system may easily adjust to a variety of functional or technological ability levels, from production units requiring intense labour to fully automatic systems requiring high investment (Genç 2019).

To date, one of the most important algal species that has been cultivated is *Arthrospira platensis* (*Spirulina platensis*). *Arthrospira platensis* (*A. platensis*) was first isolated by Turpin in 1827 (Vonshak 1997) and has the highest production among microalgae production systems. It is

a green-blue prokaryotic cyanobacteria that can photosynthesise (Fernandez, Alvitez 2009). It has high chlorophyll and phycocyanin content. The composition includes several nutrients like B vitamins, β -carotene, linoleic acid, bioflavonoids, protein and Fe, Ca Mg, Mn, K, Se, and Zn (Turner 2017). Effects like increasing yield and product quality may also assist in increasing nutrient intake (Morsy 2019). *A. platensis* appears to have the ability to correct the atmospheric environment. It contributes to the development of plant vegetative parts and especially plant root systems, and is reported to contribute to macro-micro nutrient matter content of the cultivation environment (Faiz 2019; Shedeed et al. 2022). Phycocyanin obtained from *A. platensis* culture can be used in plant production and use is becoming popular around the world (Refaay et al. 2021; Chen et al. 2022). Phycocyanin is a blue accessory protein pigment that is abundant in *Spirulina* sp. (Athiyappan et al. 2024).

Microgreens are an exotic species of renewable greens, in addition to being called vegetable confetti. They are renewable cotyledon leaves of grasses, cereals and vegetables or young seedlings with 1.5-2 first true leaves (Xiao et al. 2012; Lenzi et al. 2019). They are different from sprouts because they require light and a specific cultivation environment and have longer growth cycles, like 7–28 days (Di Gioia et al. 2017). Microgreens are plants which have gained increased interest from producers in recent years due to their short cultivation cycle, ease of cultivation, ability to produce all year round, high potential profitability for the producer due to popularity and high sustainability in production (Kyriacou et al. 2020). Microgreens may contain higher amounts of phytochemicals, minerals and vitamins compared to mature counterparts. Microgreens are considered to be a substitute for sprouts due to rich nutrient content and more intense flavour and taste (Zhang et al. 2021; Barut Gök et al. 2024).

In recent years, applying fertiliser with irrigation water by dissolving in water appears to be a more practical method. Just as this system lowers costs due to the lack of any disinfection process, it removes infection risk and produces up to 40% production increases (Çetin et al. 2016).

In this study, the aim was to research the effects of phycocyanin obtained from *Arthrospira platensis*, included among micro and macro al-

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Table 1. Phycocyanin application to different microgreen parts

Abbreviation	Treatment	Phycocyanin amount (mL/100 mL)
SC	seed control	–
ST_1	phycocyanin application to the seed (soaking)	1/100
ST_2	phycocyanin application to the seed (soaking)	2/100
CC	cotyledon control	–
CT_1	phycocyanin application to cotyledon leaves (spraying)	1/100
CT_2	phycocyanin application to cotyledon leaves (spraying)	2/100

gae with high nutrient values and content used for plant nutrition, animal feed and human food supplements, added to different organs of the plant during cultivation of *Brassica oleracea* var. *capitata* f. *rubra* microgreens on antioxidant and phenolic compounds content.

MATERIAL AND METHOD

Plant material and production of microgreens. Red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) was used as plant material. The research was carried out in a climate-controlled laboratory. The research was designed with two replications according to a randomised block experimental design. As a microgreen growing medium, a plastic container of 125 × 195 × 70 mm dimensions (1 000 g) made of cellulose fabric was used. Seed sowing was carried out very dense by the spread out method on May 15, 2023, to create confetti (approximately 2 000 plants in a plastic container). Temperature (°C) and humidity (%) were measured during the experiment, and an average temperature of 22 °C and an average humidity of 65% were noted. Phycocyanin solutions prepared with

different concentrations were applied by soaking seeds (10 grams of seeds for each application) or by spraying the solution onto the cotyledon leaves of microgreens (approximately 2 000 plants in a plastic container). The seeds were soaked in 1 mL/100 mL (ST_1) and 2 mL/100 mL (ST_2) phycocyanin solution for 60 s, and excess water was removed by using filter paper. Following this process, the seeds were placed on cellulosic paper in the plastic container for germination. Alternatively, 1 mL/100 mL (CT_1) and 2 mL/100 mL (CT_2) phycocyanin solutions were sprayed onto the cotyledon leaves (Table 1). Samples without applied phycocyanin were labelled seed control (SC) and cotyledon control (CC), and prepared by using only distilled water. Sowing and spraying applications were performed in triplicate (Figure 1). Preservatives and pesticides were not used on the seeds.

Preparation of microgreens. Plants with cotyledon leaves were harvested 14 days after sowing. Harvesting was carried out by cutting microgreens from the root collar area (Figure 2A) with a sharp and sterile knife. Following the harvest, the material was passed through distilled water and quickly



Figure 1. Seeds soaked into the solution

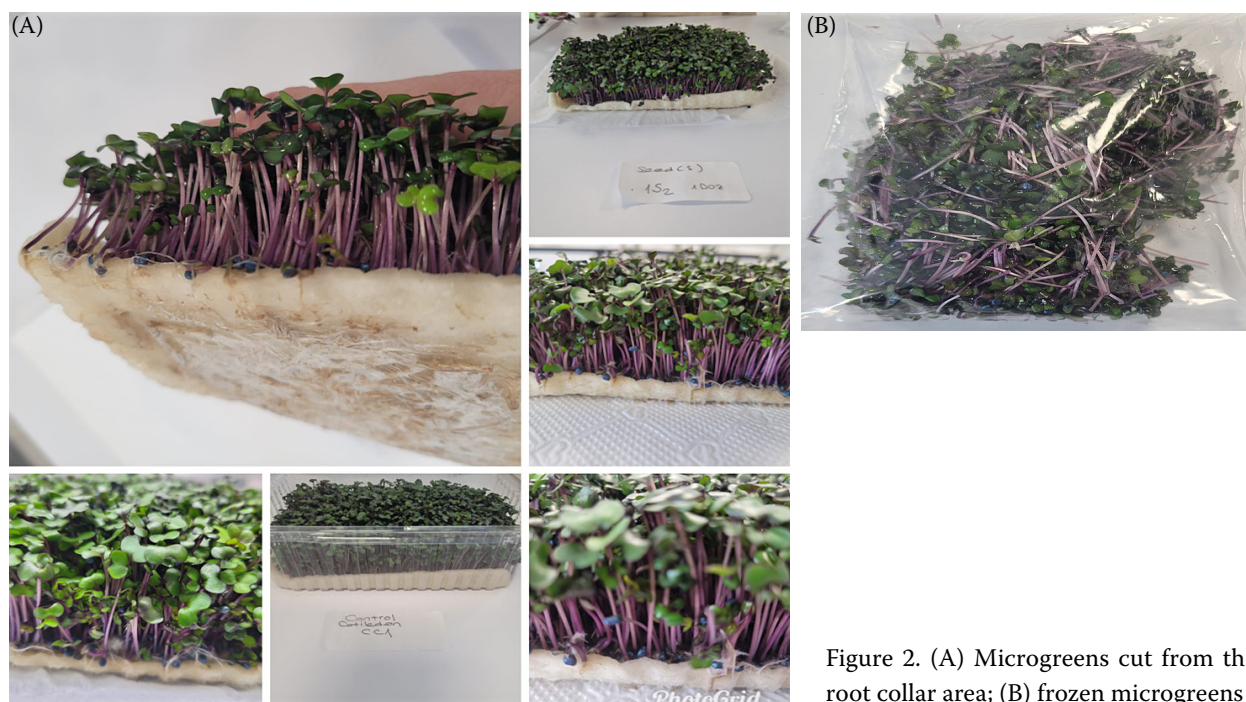


Figure 2. (A) Microgreens cut from the root collar area; (B) frozen microgreens

frozen at -20 ± 1 °C (Figure 2B) and left in these conditions until analysis.

Cultivation of *Arthrospira platensis*. The strain *A. platensis* (*S. platensis*) was obtained from a local producer in Türkiye and was cultivated in the microalgae department of the laboratory for aquatic laboratory animals at Tekirdağ Namık Kemal University. For this study, a standard Zarrouk medium with a salinity of 21.0 practical

salinity units (PSU) was used to grow the culture (Table 2). The cultivation of the culture was initially carried out by inoculation into Zarrouk agar medium (Figure 3). After incubation for a certain period, the cultures grown on solid medium were transferred to a closed system. In the closed system, round plastic drums with a volume of 5 litres were used for the culture. The purity of the culture was determined by examination under a fluorescence

Table 2. Standard Zarrouk medium for the production of *Arthrospira platensis*

Component	Stock solution (g/L dH ₂ O)	Quantity used for medium (g/L dH ₂ O)
NaNO ₃		2.5
K ₂ HPO ₄		0.5
K ₂ SO ₄		1
NaCl		1
MgSO ₄ ·7H ₂ O		0.2
CaCl ₂ ·2H ₂ O		0.04
FeSO ₄ ·7H ₂ O		0.01
EDTA		0.08
NaHCO ₃		16.8
Micronutrient solution (mL)		1
H ₃ BO ₃	2.86	
MnCl ₂ ·4H ₂ O	1.81	
ZnSO ₄ ·4H ₂ O	0.222	
Na ₂ MoO ₄	0.0177	
CuSO ₄ ·5H ₂ O	0.079	

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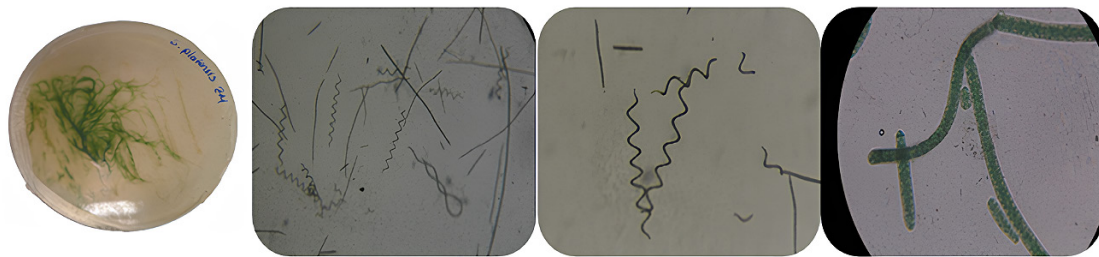


Figure 3. Picture of a planting in a petri dish, images of *Arthrospira platensis*, taken with a fluorescence microscope (40×, 100×, 1 000×)

microscope (Soif Smart-2 (Plan Achromat) Binocular Microscope, Shanghai, China), as shown in Figure 3. A white LED lamp (1 600 lumens) was used to illuminate the culture, and a 16-h light-8-h dark light cycle was applied. Continuous aeration was provided during 24 h using a motor with a flow rate of 80 m³/h. During the period from sowing to harvesting, the ambient temperature was maintained at an average of 32 ± 0.615 °C. When cultivating the culture, 5% of the culture was placed in 5-litre containers, and harvesting was performed after 14 days. The growth parameters were measured using a spectrophotometer (UV-VIS Shimadzu, UVmini-1240 Spectropho-

tometer, Japan). The biomass concentration of the culture was determined at a wavelength of 565 nm, the amount of chlorophyll at a wavelength of 680 nm and the visible turbidity at a wavelength of 750 nm (Figure 4).

Harvesting of *Arthrospira platensis*. The harvesting time of *A. platensis* was determined based on the pH of the culture medium. Once a specific pH range was reached, the harvesting process was carried out, ensuring optimal biomass yield and cellular integrity. Harvesting was performed when the pH of the culture reached 10 (Figure 5). The cultures were filtered using a silk sieve with

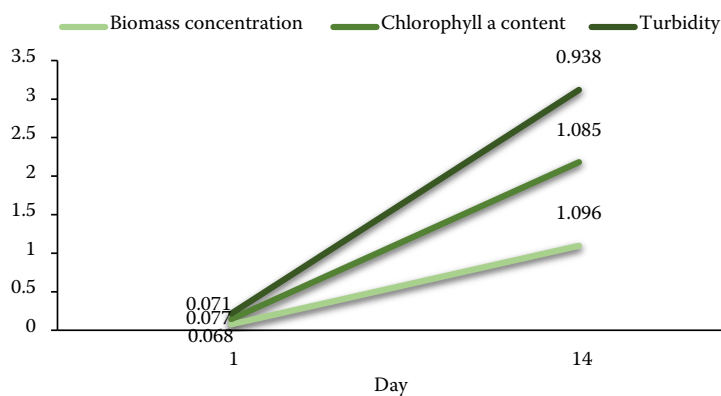


Figure 4. Evaluation of the growth parameters of *Arthrospira platensis*

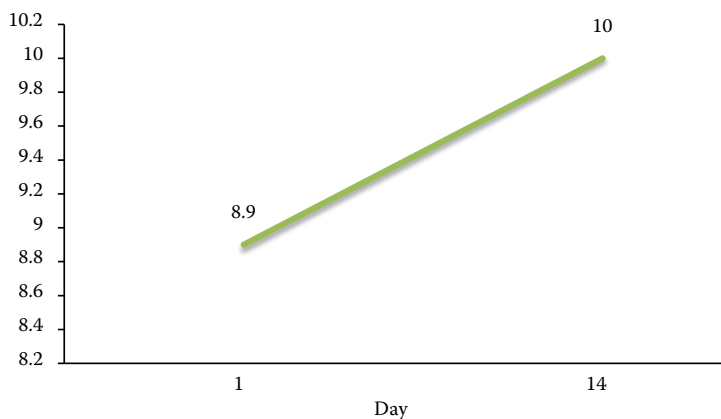


Figure 5. pH change of *Arthrospira platensis* from sowing to harvest

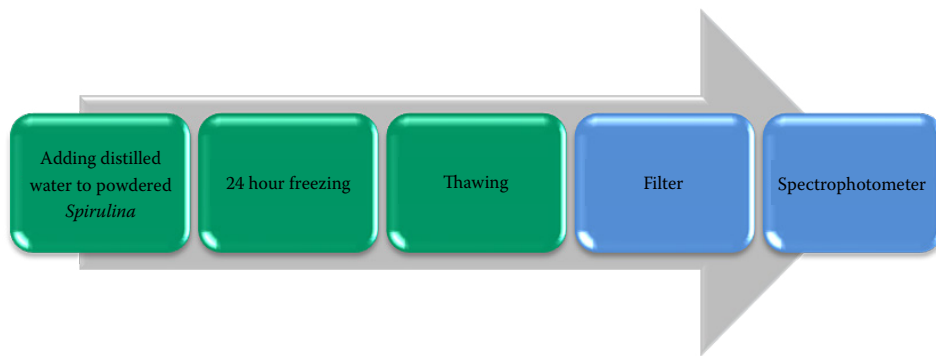


Figure 6. Method of phycocyanin extraction from spirulina by freezing and thawing

a mesh size of 30–50 micrometres. The filtered cultures were dried for 12 h with an infrared lamp at an average temperature below 40 ± 1 °C, and the dry weights were measured.

Phycocyanin extraction. For this, 1 g of *Spirulina* powder was mixed with 3 mL of pure water to obtain phycocyanin from the *Spirulina* culture. This was thawed at room temperature and filtered with coarse filter paper after being frozen (-18 °C) for 24 hours. Measurements of optical density (OD) were performed with a spectrophotometer (UV-VIS Shimadzu UVmini-1240 Spectrophotometer, Japan) at wavelengths of 615 nm (OD_{615}) and 652 nm (OD_{652}) to determine the amount of liquid phycocyanin (Figure 6). The amount of phycocyanin was calculated using the following formula (Equations 1, 2, 3) (Katari et al. 2023; Sulistiawati et al. 2023). The phycocyanin content determined in the study using the standard Zarrouk medium was 159.6 ± 2.494 mg/L.

$$\text{Allophycocyanin (CPC) (g/L)} = \frac{(OD_{615} - 0.474(OD_{652}))}{5.34} \quad (1)$$

$$\text{Chloro-phycocyanin (APC) (g/L)} = \frac{(OD_{652} - 0.208(OD_{615}))}{5.09} \quad (2)$$

$$\text{Total phycocyanin (Pct) (g/L)} = \text{CPC} + \text{APC} \quad (3)$$

Extraction of phenolic content from microgreens. The extraction procedure was performed according to the method of Khattak et al. (2007) with some modifications (Barut Gök, Erdoğan 2024). A 2 g portion of the fresh plant was weighed into dark glass bottles and extracted at room temperature for 15 min in an ultrasonic bath. The suspension was centrifuged at 4 500 rpm for 10 min

at room temperature with 5 mL of 80% (v/v) aqueous MeOH, and the supernatant was collected. The extraction was carried out in triplicate. The supernatant was filtered and the filtrate was used to detect total phenolic content and antioxidant capacity of microgreens.

Total phenolic content assay. Phenolic compounds were determined using the Folin–Ciocalteu reagent method by reading the absorbance at 760 nm with a UV–Vis spectrophotometer (UV-2500, Shimadzu, Kyoto, Japan) according to the method of Ainsworth and Gillespie (2007). Gallic acid was used as a standard, and the results are expressed as milligrams of gallic acid equivalent (GAE)/kg of fresh weight (FW).

DPPH radical scavenging activity assay. Antioxidant activities of seed and microgreen extracts (ME) were identified according to Brand-Williams et al. (1995). Different concentrations (between 100 to 1 000 μ L) of MEs were placed in tubes, and 600 μ L of 1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH*) radical solutions were added to each tube; the total volume was completed to 6 mL with methanol. After mixing and incubating the tubes for 30 min at room temperature in a dark environment, absorbance was read at 517 nm wavelength against the control. By using the absorbance value, the % inhibition of DPPH radicals (I %) for each of the samples was calculated by using Equation (4). In Equation (4), the absorption of control (methanol instead of SE) is expressed as A_{control} and the absorption of the analysed sample is expressed as A_{sample} .

$$\text{Inhibition \%} = ((A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}})) \times 100 \quad (4)$$

Inhibition values were graphed against different concentrations for each extract and linear regres-

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sion analysis was applied in order to obtain the equation defining the curve. By using the equation, the EC₅₀ value was calculated. The EC₅₀ value is the amount of antioxidant necessary to decrease the initial DPPH* concentration by 50%.

Statistical analysis. The results of the experiments were evaluated using SPSS (version 16.0) statistics software. ANOVA variance analysis and Duncan multiple comparison tests were conducted on the research results.

RESULTS AND DISCUSSION

Total phenolic content (TPC). The analysis of variance results for the TPC of microgreens are presented in Table 3. Soaking in phycocyanin solution significantly affected the phenolic content of seeds. The concentration of the solution increased and the phenolic content increased. However, there was no significant difference among the concentrations in terms of seed application. Regarding the treatment of cotyledon leaves, CT₂ was sprayed with the highest amount of phycocyanin of 2 mL/100 mL and increased the phenolic content of cotyledon leaves. However, the phycocyanin application did not significantly affect the phenolic content of the cotyledon leaves. *A. platensis* (*S. platensis*) was reported to improve the biological activity of soil as a bio-fertiliser (Nosheen et al. 2021; Ammar et al. 2022). In addition, *A. platensis* boosts plant development (Alobwede et al. 2019). The phenolic content of the cotyledon leaves was detected at higher values than the content in the seeds. However, the phycocyanin application influences the seeds and the cotyledon

leaves in different ways. Different growth stages of plants could affect the nutritional metabolites of plants (Zou et al. 2021). Significant changes in metabolic profile, primary metabolites and glucosinolate, a secondary metabolite in Brassicaceae vegetables, were detected during different development stages in terms of microgreens, seedlings and adult choy sum plants (Zou et al. 2021). Gluconapoleiferin (Zou et al. 2021) and total aliphatic glucosinolates significantly decreased from microgreens to seedlings and in adult plants such as cabbage, broccoli, etc. (Bellostas et al. 2007; Björkman et al. 2011; Zou et al. 2021). In addition to this, total phenolic content dropped significantly from cotyledon to true leaf in garden cress, red beetroot, dill and radish (Barut Gök et al. 2024). One of the crucial factors that affects bioactive compounds and the quality of vegetables was reported to be the plant growth stage. The phenolic content is closely related to the growth stages of the plant (Delgado et al. 2004; Navarro et al. 2008). Besides factors such as cultivation, environmental conditions, and agronomic practices, plant maturity has a crucial role in determining the phenolic profile of the plant (Björkman et al. 2011; Neugart et al. 2018; Zou et al. 2021). Recent studies focused on nutritional and bioactive characteristics of microgreens and showed that microgreens had higher levels of secondary and primary metabolites than adult vegetables (Xiao et al. 2012; Waterland et al. 2017; Tan et al. 2020; Zou et al. 2021; Barut Gök et al. 2024). A unique blend of phytonutrients is present in the young plant compared to the mature stage (Yadav et al. 2019). This could be attributed to the biosynthetic

Table 3. Total phenolic content and antioxidant activity of different microgreen parts

Treatment	<i>Brassica oleracea</i> var. <i>capitata</i> f. <i>rubra</i>	
	total phenolic content (mg GAE/kg FW)	antioxidant activity (EC ₅₀ µg/mL)
SC	165.60 ± 13.8 ^a	0.82 ± 0.03 ^c
ST ₁	182.92 ± 2.4 ^{bc}	0.52 ± 0.07 ^a
ST ₂	186.07 ± 7.2 ^c	0.53 ± 0.03 ^a
CC	182.34 ± 6.3 ^{bc}	0.56 ± 0.06 ^{ab}
CT ₁	168.52 ± 9.0 ^{ab}	0.62 ± 0.03 ^b
CT ₂	186.37 ± 3.6 ^c	0.50 ± 0.02 ^a

SC – seed control; ST₁ – phycocyanin application to seeds as 1 mL/100 mL; ST₂ – phycocyanin application to seeds as 2 mL/100 mL; CC – cotyledon control; CT₁ – phycocyanin application to cotyledon leaves as 1 mL/100 mL; CT₂ – phycocyanin application to cotyledon leaves as 2 mL/100 mL; values are the mean of three replicates ± standard deviation; different letters in the same column represent statistically different results; ^athe lowest value; ^cthe highest value

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pathways of phenolic content (Yu et al. 2018); however, phenylalanine ammonia-lyase (PAL) activity could also be substantial in plants based on growth stage and variety (Medda et al. 2020; Zou et al. 2021; Barut Gök et al. 2024).

Total antioxidant activity (TAC). The analysis of variance results for the antioxidant activity of microgreens are presented in Table 3. The results are in accordance with the phenolic content of the samples. No significant effect was detected on the antioxidant activity of the seeds among the phycocyanin concentrations. However, phycocyanin application significantly increased the antioxidant activity compared to the control soaked in deionised water. Spraying phycocyanin on the cotyledon leaves did not affect the antioxidant capacity of the plant compared to controls. Similar to phenolic content, different concentrations of phycocyanin had a significant effect on the antioxidant activity of cotyledon leaves. In accordance with the results, *Spirulina* extracts increased the antioxidant capacity of whole grains (El-Baky et al. 2010). Several studies reported that oxidative stress is reduced with *Spirulina* treatment. However, the antioxidant effect of *Spirulina* results from components such as phycocyanin (Li et al. 2009; Hwang et al. 2013; Xia et al. 2016) and β -carotene, which have antioxidant potential (Upasani, Balaraman 2003; Abdel-Daim et al. 2013; Wu et al. 2016). Chentir et al. (2019) reported an increase in bioactive characteristics, such as antioxidant activity, of a potential food packaging film by incorporating phycocyanin into gelatine. In conclusion, besides its role as a bio-fertiliser, *A. platensis* might be used as an additional antioxidant agent due to enhancing the phenolic and antioxidant capacity of microgreens, which naturally contain antioxidant chemicals including polyphenols and vitamins (Ebert 2022; Partap et al. 2023).

In conclusion, phycocyanin, which is a protein pigment present in *Spirulina sp.*, has natural properties such as antioxidant, antimicrobial, anti-anaemic, etc. effects. There are several studies about the effects of *A. platensis* (*S. platensis*) and phycocyanin on root weight, size, growth rate and yield. However, there is limited knowledge about the effects on bioactive components of plants. There is no study about the effect of phycocyanin on the bioactive components in microgreens. The current study demonstrated that the phycocyanin application to seed and cotyledon leaves affected

the antioxidant capacity and phenolic content of microgreens.

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