Characterization and bioactivity of phycocyanin isolated from *Spirulina maxima* grown under salt stress

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In this study, *Spirulina maxima* (SM) has been selected following preliminary investigations, for cultivation in either normal (0.02 M) or stress (0.1 M) NaCl medium (Zarrouk) under room conditions to evaluate the possibility of increasing the total phycobiliprotein content (TPC) and their chemical constituents: C-phycocyanin (C-PC), allophycocyanin (APC) and phycoerythrin (PE). TPC material was separated, purified and characterized by various spectroscopic techniques (UV-Vis and IR spectra). The antioxidant activity against free radicals of DPPH, ABTS, superoxide (\( \cdot O_2^- \)), hydroxy (OH) and reducing power potential were determined. Results indicated a highly significant correlation between increased TPC content in SM cells and the increasing concentration of NaCl in medium, and its chemical constituents were significantly different (\( P > 0.05 \)). TPC of SM (grown in stress NaCl containing high amounts of C-PC groups, showed strong antioxidant activity compared with ascorbic acid (standard antioxidant). Although, it activity against different free radicals were found to be variable and dose-dependent. Moreover, the TPC showed lower antimicrobial activity (MIC values in the range of 250–300 \( \mu \)g mL\(^{-1} \)) than that of chloramphenicol (30 \( \mu \)g mL\(^{-1} \), reference antimicrobial). Therefore, *Spirulina maxima* could be cultivated in a salinated open pond, and considered as highly healthy foods and source of natural pigments.

Introduction

Recently, cultivation of blue green microalgae such as *Spirulina* species has become an attractive process for obtaining the value-added biochemical compounds, such as sulphate polysaccharides, glycolipids, \( \omega \)-3-fatty acids, chlorophyll and other pigments.\(^1\)\(^\text{-}\)\(^2\) These compounds possess a wide range of biological properties such as antimicrobial, antiviral, antioxidant, anti-inflammatory, antiallergic and antiin thrombotic activities.\(^3\)\(^-\)\(^4\) Additionally, *Spirulina* sp. have been widely used for 100 years as excellent nutrient supplements for human beings and animals due to their excellent nutritional profile and high-protein content (55–65%) with all the essential amino acids in perfect balance.\(^5\) In 1996, the United Nations World Health Organization declared that *S. platensis* can be considered as the best for tomorrow and it has since gained popularity as a food supplement.

Nowadays, *Spirulina* sp. among cyanobacteria are attracting commercial conglomerates, as a source of natural pigments due to their high-end application and easy separation procedures. Currently many of the commercially produced pigments are from either synthetic source or from plant materials. Nevertheless, there is a rapid increase in alternate source of pigments from cyanobacteria especially from *S. platensis* and *S. fusciformis*. Phycobiliproteins (PCs) are the major photosynthetic pigments in cyanobacteria (may account for 20% based on dry weight of the cell proteins).\(^6\) These pigments, a type of large supra-molecular aggregate that attach to the thylakoid membrane of blue-green and red algae, have a function in light catching and energy migration, particularly energy transfer in the following direction CPC \( \rightarrow \) APC \( \rightarrow \) PSII.\(^6\)\(^-\)\(^7\) PCs are water-soluble proteins, having covalently attached to tetrapyrroles, and they have three-dimensional structures and exhibit a strong red fluorescence when present in native and concentrated form.\(^8\) However, PCs are not only used as natural dye, nutrient ingredient and natural coloring for food and cosmetics\(^9\) but also used as a potential therapeutic agent in the treatment of oxidative stress-induced diseases\(^10\) and as fluorescent markers in biomedical research.\(^9\) Moreover, PCs have pharmaceutical potential characteristics such as antioxidant, anti-tumoural and anti-inflammatory activities.\(^10\)\(^,\)\(^11\) Also, PCs have been used for the treatment of Alzheimer’s and Parkinson’s diseases\(^12\)\(^-\)\(^13\) and the prevention of many cancer types.\(^14\)\(^-\)\(^16\)

Free radical reactions, especially those with participation of oxidative elements, have been shown to be associated with many biological processes that damage lipids, proteins, cell membranes and nucleic acids, ultimately resulting in various diseases.\(^17\) More recently, there has been an explosive interest in the use of antioxidant nutritional supplements.\(^18\)^{19} Several reports demonstrated that intake of some vitamins, minerals, and other food...
constituents may help to protect the body against many diseases, and antioxidants may have a protective effect, either in preventing these diseases or decreasing their severity.\textsuperscript{20-23} Several activities of the antioxidants are mediated by inhibition of reactive oxygen species (ROS), which are generated during the oxidative burst. Thus, the usefulness of antioxidants in protecting cellular components against oxidative stress is well established.\textsuperscript{24} *Spirulina* contains a whole spectrum of natural mixed carotene and xanthophyll phytopigments which, together with phycocyanin, seem to be related to its antioxidant activity.\textsuperscript{25,26}

In this work, *Spirulina maxima* (SM) microalgae was cultivated either in normal or NaCl-stress defined medium (Zarrouk) under room conditions and the TPC and its chemical constituent (C-PC, APC and PE) content were evaluated for purity ratios and chemical characterization. The antioxidant and antimicrobial activities of TPC were also measured by different methods.

**Materials and methods**

**Algal source**

Blue-green algae, *Spirulina maxima*, was obtained from the culture collection of Botany Department, Texas University (Austin, Texas, U.S.A).

**Reagents**

All reagents and chemicals used in the experiments were of analytical grade.

**Cultivation of algae**

The *S. maxima* was cultivated (during spring season 2009, in National Research Centre, Egypt) in Zarrouk’s medium\textsuperscript{27} containing normal concentrations of NaCl (0.02 M) and sodium nitrate as a nitrogen source (2.50 g L\textsuperscript{-1}). For salt stress, the salinity of fresh medium was adjusted to 0.1 M NaCl by supplementation with NaCl. Aeration was accomplished using air pumps to achieve an air flow rate of 20 L h\textsuperscript{-1}. The cultures were gassed with air/CO\textsubscript{2} (0.03\%) mixture. The temperature of the culture was maintained at 25 \textdegree C \pm 3 \textdegree C. The pH of all media was adjusted to 9.5. The cultures were illuminated with continuous 10 cool white fluorescent lamps (Philips 40 W) provided an illumination of 2500 lux. In all cultivated flasks, conductivity, salinity, pH and temperature were measured daily with Hanna (HI 99812-5) conductivity meter. The purity of cultures was periodically checked by microscopic observation following taxonomy guidelines. All solutions and glassware were autoclaved at 121 \textdegree C for 15 min prior to use.

**Growth measurements and harvesting**

The growth rate of *Spirulina maxima* was monitored every three days through the entire cultivation period by determining the dry weight (d. w.) and optical density at 670 nm.\textsuperscript{28} The cells were harvested at the stationary phase, by centrifugation at 10 000 g (4 \textdegree C) for 15 min and the cell masses were stored at –20 \textdegree C until analysis.

**Separation of phycobiliproteins**

Fresh algae (10 g) cells were added to 100 ml of 0.05 M phosphate buffer (PB, pH 6.7) and kept in the dark at 4 \textdegree C for 12 h, then clarified by centrifugation at 10 000 g (4 \textdegree C) for 15 min. The blue supernatant was decanted, then ammonium sulfate solution (100 ml, 25\% M) was added and the mixture was left in the dark for a further 12 h at 4 \textdegree C. The blue pigment proteins (C-PC crude extract) were precipitated by (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} (50 ml, 60\%). After 6 h at 4 \textdegree C, C-PC was collected by centrifugation at 10 000 g for 15 min and the above steps were repeated until a colorless supernatant was obtained. Then, the protein pellets containing blue pigments were suspended in phosphate buffer and the final volume was recorded.

**Determination of phycocyanin**

Various absorbance of phycocyanin in the supernatant were spectrophotometrically determined at different wavelengths (620, 652 and 562 nm). The concentrations of phycocyanin (C-PC), allophycocyanin (APC) and phycoerthrin (PE) were deduced using the following formula:\textsuperscript{29}

\[
\text{C-PC (mg mL}^{-1}\text{)} = \frac{[A_{620} \text{ nm} - 0.474 (A_{652} \text{ nm})]}{5.34},
\]

\[
\text{APC (mg mL}^{-1}\text{)} = \frac{[A_{652} \text{ nm} - 0.208 (A_{620} \text{ nm})]}{5.09},
\]

\[
\text{PE (mg mL}^{-1}\text{)} = [A_{562} \text{ nm} - 2.41(\text{PC}) - 0.849 (\text{APC})]/9.62.
\]

**Determination of total proteins**

The total proteins’ containing mainly phycocynin in the supernatant was spectrophotometrically determined at 280 nm. Bovine serum albumin (BSA) was used as a protein standard.\textsuperscript{29}

**Spectroscopic measurements**

**UV-Vis absorption spectra.** The absorption spectra of phyco-cyanin extracts were recorded on a UV-Vis spectrophotometer (Thermo, USA). The maximum absorption wavelength for the extract was compared with those reported for C-PC (620 nm) and APC (652 nm) in the literature.\textsuperscript{30} The purity was evaluated according to the absorbance ratio (A\textsubscript{620} for TCP/A\textsubscript{280} for total proteins).

**Infrared spectroscopic measurements.** IR spectra of freeze-dried phycocyanin extract as KBr pellets were recorded on a Perkin-Elmer spectrophotometer.

**Antioxidant activity**

The antioxidant activity was measured by the scavenging ability of hydroxyl, ABTS and DPPH radical and reducing power methods. All tests were run in triplicates and averaged.

**DPPH scavenging radical assay.** The ability of the PC-SM samples to scavenge DPPH radical was estimated according to...
the method of Tagashira and Ohtake.\textsuperscript{31} Ascorbic acid (5–50 \(\mu\)g mL\(^{-1}\)) was used as a reference antioxidant standard. The radical-scavenging activity of CP-extract was calculated from a calibration curve. The concentration providing 50\% inhibition (IC\(_{50}\)) was calculated from a graph representing the inhibition percentage against PC concentration.

**ABTS scavenging radical assay.** TBAS radical scavenging activity of the CP-SM extract was determined according to the method of Re, \textit{et al.}\textsuperscript{32} with some modifications. The reduction percentage of absorbance at 15 min compared to the initial value was determined.

**Hydroxyl radical assay.** Hydroxyl radical scavenging activity was carried out according to the method of Muller.\textsuperscript{33} BHT and ascorbic acid were used as positive controls.

**Reducing power assay.** Total reducing power was spectrophotometrically determined at 700 nm according to the method described by Zhu, \textit{et al.}\textsuperscript{34}

**Superoxide anion radical scavenging assay.** The superoxide anion scavenging activity was spectrophotometrically determined at 560 nm according to Nishikimi, \textit{et al.}\textsuperscript{35} Ascorbic acid was used as a control. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity.

The percent inhibition (%) of superoxide anion generation was calculated as follows:

\[
\% \text{ Inhibition} = \left[\left( A_0, \text{control} - A, \text{sample}\right)/A_0, \text{control}\right] \times 100
\]

where, \(A_0, \text{control}\) and \(A, \text{sample}\) were absorbances of control and sample, respectively.

**Antimicrobial assay**

**Preparation of bacterial cultures.** Four species of Gram-positive bacteria (\textit{Bacillus subtilis}, ATCC 6633; \textit{Bacillus cereus}, ATCC 14579, \textit{Staphylococcus aureus}, ATCC 27840 and \textit{Micrococcus luteus}, ATCC 4698) and two Gram-negative bacteria (\textit{Klebsiella pneumoniae}, ATCC 13883 and \textit{Serratia marcescens}, ATCC 13880) were used for measuring the antimicrobial activity of CP-SM extracts. These specific strains were recommended for antibacterial screening purposes. The bacteria were sub-cultured on nutrient agar at 37 °C prior to nutrient broth overnight. These cultures were standardized using sterile saline solution to produce approximately 1.5 \(\times\) \(10^7\) colony forming units (cfu) per ml.

**Disc agar diffusion method.** The antibacterial activity of the CP-SM was examined by the disc agar diffusion method as mentioned by Abd El Baky, \textit{et al.}\textsuperscript{36} The antimicrobial activity was evaluated by measuring the inhibition zones expressed as mm of inhibition against the tested organisms.

**Minimum inhibitory concentration.** The minimum inhibitory concentration (MIC) values were determined for the bacterial strains, as described by Daw, \textit{et al.}\textsuperscript{37} The MIC is defined as the lowest concentration of tested samples showing no visible bacterial growth after a 24 h incubation period at 37 °C.

**Statistical analysis**

All results are expressed as mean values ± S. D. The statistical differences between experimental groups were assessed by analysis of variance (ANOVA) using the COSTAT software package (Cohort Software, CA, USA). The mean values were compared with LSD test (\(P < 0.05\)).

**Results and discussion**

**Growth properties**

\textit{S. maxima} (SM) has been selected as the one of blue green algae responsible for enhancing the production of natural pigments and its biological action in response to NaCl stress at concentration of 0.1 M NaCl. This concentration was chosen after preliminary studies that cells can grow without any significant change in growth rates and SM biomass. As shows in Table 1, the values of growth parameters (GPs) include biomass productivity and \(\mu_{\text{max}}\) of \textit{S. maxima} grown in normal (0.02 M NaCl, NC) and NaCl-stress (0.1 M NaCl, SC) media were ranged from 139.50 to 221.33 mg L\(^{-1}\) day\(^{-1}\), and 0.089 to 0.097 day\(^{-1}\), respectively. Since, these values in cells grown at 0.1 M NaCl were lower than that of cells grown in normal NaCl concentration, all GPs were significantly enhanced (\(P > 0.05\)) as response to exposure to NaCl stress, these changes may reflect the change in metabolic pathways in SM.

**Phycobiliprotein contents**

Table 2 indicates that the increase of NaCl concentration in the Zarrouk nutrient media led to a high changes in TPC contents (% of dry weight) of SM cells and its constituents including phycocyanin (CPC), allophycocyanin (APC) and phycoerythrin (RPC). The values of these components were increased in algal cells grown at 0.1 M NaCl by 2.51\%, 4.11\% and 9.01\% as great as that found in SM cultured in normal NaCl concentration 2.2\%, 3.57\% and 6.3\%, respectively. Thus, increase of NaCl concentration in nutrient medium elicits a significant increase of TPC content and its constituents. In addition, the total soluble protein content was significantly higher (47.75 mg g\(^{-1}\)) in SM grown in 0.10 M NaCl culture may increase the protein synthesis required for increase the intracellular osmotic compounds in order to balance the high osmotic of the medium.\textsuperscript{26} However, it is well known that the nitrogen concentration in medium has a great influence on phycocyanin content of several species of \textit{Spirulina}.\textsuperscript{36,38} Piorreck, \textit{et al.}\textsuperscript{39} reported that \textit{Spirulina} and other algae species grown at different nitrogen levels showed great variations in pigments and total protein contents. In addition, Becker\textsuperscript{38} and Ciferri and Tiboni\textsuperscript{40} reported that \textit{Spirulina} spp grown in a nitrogen-rich medium had the ability to accumulate high considerable quantity of proteins (up to 60\%) and up to 20\% of this protein fraction was TPC blue pigment.
SM grown in a normal medium (2.50 g L\(^{-1}\) NaNO\(_3\) + 0.02 M NaCl)
SM grown in a salt stress medium (0.1 M NaCl)

- The data refer to mean value ± standard deviation.
- \(\mu_{\text{max}}\) = maximum specific growth rate.
- \(\rho_{\text{R432}}\) = productivity at 432 h = 18 days.

### Spectroscopic analysis

The UV-Vis spectra of purified TPC obtained after purification steps showed maximum absorption at \(\lambda = 280\) and 620 nm, which had a typical UV spectrum of pure phycocyanin (Fig. 1). Infrared spectra (Fig. 2) (KBr, cm\(^{-1}\)) of purified TPC of *S. maxima* showed the main characteristic bands of PC: 1670 (amide I) and 1543 (amide II) (C–N stretching), 1411 (C–N), and 3400 (N–H bond). The absence of intense bands at ranges of 1000–1100 cm\(^{-1}\) is an indication of the presence of high amounts of phycocyanin (C–N stretching) and successive improvements in purity of TPC produced by precipitation was achieved by Huang, et al.\(^{43}\) Moreover, this value can be regarded as purity of TPC in range of food grade (0.7 < \(x < 3.9\)). In this respect, Rito-Palomares, et al.\(^{44}\) stated that the TPC purity values of 0.7, 3.9 and >4 are considered as food grade, reactive grade and analytical grade, respectively.

### Purification of phycobiliproteins

In order to investigate the biological function of phycobiliproteins separated from SM, the total protein extract was partially purified by precipitation with saturated ammonium sulfate solution. The conventional protein purification techniques were employed in two stages. Firstly, precipitation by ammonium sulfate (30%) was performed to remove the majority of the undesirable protein. Secondly, saturated ammonium sulfate (50%) was used to precipitate approximately all TPC proteins.\(^{43,44}\) Based on the \(A_{280}:A_{238}\) ratio, the quantity of the purified phycocyanin was found to be 2.25 for partially purified TPC of SM proteins grown at 0.10 M NaCl (SC). This value was significantly higher than that found in crude protein extract, which is an indication of the presence of high amounts of phycocyanin and successive improvements in purity of TPC produced by precipitation was achieved by Huang, et al.\(^{43}\)

Moreover, this value can be regarded as purity of TPC in range of food grade (0.7 < \(x < 3.9\)). In this respect, Rito-Palomares, et al.\(^{44}\) stated that the TPC purity values of 0.7, 3.9 and >4 are considered as food grade, reactive grade and analytical grade, respectively.

### Table 1  Growth parameters of *Spirulina maxima* as affected by salt stress\(^a,b,c\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(\mu_{\text{max}}) (day(^{-1}))</th>
<th>Biomass productivity (\rho_{\text{R432}}) (mg L(^{-1}) day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM grown in a normal medium (2.50 g L(^{-1}) NaNO(_3) + 0.02 M NaCl)</td>
<td>0.089 ± 0.005</td>
<td>139.5 ± 0.12</td>
</tr>
<tr>
<td>SM grown in a salt stress medium (0.1 M NaCl)</td>
<td>0.097 ± 0.003</td>
<td>221.33 ± 0.21</td>
</tr>
</tbody>
</table>

\(^{a}\) The data refer to mean value ± standard deviation. \(^{b}\) \(\mu_{\text{max}}\) = maximum specific growth rate. \(^{c}\) \(\rho_{\text{R432}}\) = productivity at 432 h = 18 days.

### Antioxidant activity

In the present study, scavenging of DPPH, ABTS, superoxide \((^\cdot O_2^–)\) and OH radicals were used to investigate the antioxidant activity of TPC obtained from SM cultured in NaCl stress (0.10 M) medium.

### Scavenging activity of \(^\cdot O_2^–\) and OH radicals PCT of SM

Antioxidant activity of TPC obtained from SM cultured in stress NaCl (0.10 M) medium against \(^\cdot O_2^–\) and OH radicals generated from non-enzymatic systems and commercial antioxidant are shown in Table 3. In both assays, the TPC extract of SM showed a relatively higher scavenging activity than that of ascorbic acid, and their activity was in a concentration dependent manner. The IC\(_{50}\) values for scavenging \(^\cdot O_2^–\) and OH radicals were 25 and 20 mg mL\(^{-1}\), compared with 30 and 41 mg mL\(^{-1}\) for ascorbic acid.

### ABTS and DPPH scavenging radical assay

Table 3 and Fig. 3 show that TPCs extracts of SM cultured NaCl stress medium possessed an apparent scavenging abilities on both DPPH and APTS radicals in concentration dependent manner, with IC\(_{50}\) values of 20 and 16 mg mL\(^{-1}\), respectively. TPC extracted from SM grown in stress medium possessed good scavenging effect towards DPPH and ABTS radicals than commercial antioxidant (ascorbic acid, IC\(_{50}\) 19 \(\mu\)g mL\(^{-1}\)). On other hand, the radical scavenging activity of the SM extract was higher in the ABTS assay (IC\(_{50}\) = 16 \(\mu\)g mL\(^{-1}\)) than the DPPH (IC\(_{50}\) = 20) assay of the same concentration. Therefore, the discrepancies in the scavenging activity of MS extract against both radicals are due to different mechanisms. In the DPPH assay, the antioxidant effect was likely to be due to the hydrogen donating ability of the extract, whereas in the ABTS assay, the antioxidant effect is due to scavenging proton radicals induced through donation of electrons.\(^{46,47}\)

### Table 2  Phycocyanin content of *Spirulina maxima* (SM) as affected by salt stress

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phycocyanin pigment (%)</th>
<th>Total phycocyanin (%)</th>
<th>Ratio of treatment : control</th>
<th>Soluble proteins (mg g(^{-1}))</th>
<th>Ratio of treatment : control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM grown in a normal medium (2.50 g L(^{-1}) NaNO(_3) + 0.02 M NaCl)</td>
<td>2.205</td>
<td>3.577</td>
<td>6.3</td>
<td>11.08</td>
<td>0.0</td>
</tr>
<tr>
<td>SM grown in a salt stress medium (0.1 M NaCl)</td>
<td>2.51</td>
<td>4.11</td>
<td>9.01</td>
<td>15.63</td>
<td>1.41</td>
</tr>
</tbody>
</table>

**Table 2**  Phycocyanin content of *Spirulina maxima* (SM) as affected by salt stress

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Reducing power

Table 3 and Fig. 3 show the reducing power (RP) of TPC of SM cultured in stress NaCl medium. The values of RP increased with increasing concentrations of TPC-SM, their values were 0.1, 0.2 and 0.3 at concentration of 5, 20 and 50 \( \text{mg} \cdot \text{L}^{-1} \), respectively. In addition, the RP of SM extracts was far better than that of ascorbic acid. However, Shahidi and Wanasundara\(^4\) reported that RP of antioxidant compounds may be attributed to its hydrogen-donating ability, and ascorbic acid had a high reduction that could readily donate a hydrogen atom to a free radical, that terminating free radical reaction. Also, Revananappa and Salimath\(^4\) stated that these reductions have efficient RP due to their hydrogen donating ability.

In general, a relationship was found to exist between the concentration of PC-SM and both its scavenging activity and RP. In addition, the increased PC level was positively correlated with its scavenging activity against DPPH, ABTS, OH and \( \cdot \text{O}_2 \) radicals. Moreover, this effect was dose-dependent (Table 3) and was higher than of ascorbic acid. Previous studies demonstrated that there was a positive correlation between antioxidant property of \textit{Spirulina} in various oxidative systems and phycocyanin content.\(^25,45,50–53\) However, the covalently linked tetapyrrole chromatophore phycocyanobilin is suggested to be involved in the scavenging activity of phycocyanin.\(^7\) Similar results were found in many reports that the phycocyanin as the major water-soluble in \textit{Spirulina} sp. had greater antioxidant activity with about 20 fold than that of ascorbic acid. The extract of \textit{Spirulina} has been shown to be effective in free radical-induced lipid peroxidation and possessed protective activity in the major organs including the heart. Moreover, both \textit{Spirulina} and C-phycocyanin induced antioxidant activity as evidenced from the scavenging of superoxide radicals and has the ability to chelate free iron. Additionally, \textit{Spirulina} has been shown to scavenge peroxyl, hydroxyl \(^54,55\) and superoxide,\(^56\) act as a potent antioxidant and inhibit lipid peroxidation mediated by ROS.\(^54\)

### Superoxide anion radical scavenging

The antioxidant activity of \textit{Spirulina} phycocyanin was evaluated based on scavenging superoxide anion radicals in order to characterize the antioxidant propensities of phycocyanin. The mechanism of reaction is known to be complex and depends on the physico-chemical parameters of the test reagents and substrates (NADH).\(^57\) These superoxide radicals are scavenged by the donating electrons of CP-SM. As shown in Table 3 and Fig. 3, ascorbic acid showed lower superoxide anion radical scavenging potential of 86.11% (at 50 \( \text{mg} \cdot \text{L}^{-1} \)) compared with that of 96.14% for the \textit{Spirulina} phycocyanin at the same concentration. Thus, the efficiency of CP-SM was excellent and acted in a dose-dependent manner.

### Antimicrobial activity

The antibacterial activity of TPCs obtained from SM cultured in NaCl stress (0.1 M) medium and chloramphenicol (CAP, standard reference of antibiotic) were evaluated by the inhibition zone (IZ) method against a panel six bacteria selected on basis of their relevance to public health (Table 4). The present results revealed that the PCs had lower levels IZ ranged from 10–20 mm.

### Table 3  In vitro antioxidant assays of ascorbic acid and \textit{Spirulina maxima} phycocyanin\(^a\)\(^b\)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Ascorbic acid (( \mu \text{g} \cdot \text{mL}^{-1} ))</th>
<th>SM phycocyanin (( \mu \text{g} \cdot \text{mL}^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>ABTS (% scavenging ABTS radical)</td>
<td>25.5</td>
<td>36.1</td>
</tr>
<tr>
<td>Superoxide (% scavenging ( \cdot \text{O}_2 ) radical)</td>
<td>14.5</td>
<td>26.3</td>
</tr>
<tr>
<td>DPPH (% scavenging DPPH radical)</td>
<td>21.3</td>
<td>31.1</td>
</tr>
<tr>
<td>OH (% scavenging OH radical)</td>
<td>19.5</td>
<td>25.1</td>
</tr>
<tr>
<td>Reducing power (absorbance at 700 nm)</td>
<td>0.07</td>
<td>0.09</td>
</tr>
</tbody>
</table>

\(^a\) The target oxidative substances values represent the % of scavenging activity.\(^b\) Each value represents the mean of three replicates (\(n = 3\)).

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Fig. 1  UV–Vis absorption overlay spectra of phycocyanin (a) phycocyanin standard and (b) \textit{Spirulina maxima} phycocyanin.

Fig. 2  The IR spectra of phycocyanin (a) phycocyanin standard and (b) \textit{Spirulina maxima} phycocyanin.
against the all tested bacteria, compared with CAP. The anti-
bacterial activity of PC-SM was found to be dose-dependent.
Also, no differences in sensitivity were observed even between
bacteria belonging to the same species. By comparison, PSs
derived from many blue-green algae, such as *S. platensis* and *S.
fussiformis* have been reported to possess moderate levels of
antibacterial activity against several panel of bacteria. In
general, PCs showed potential antimicrobial activity against all
tested bacterial strains, with MIC value ranged from 0.3 mg
mL\(^{-1}\). SM exhibited moderate antibacterial activity against
Gram +ve and Gram -ve bacteria. The comparison between
chloramphenicol (MIC was 0.020 mg mL\(^{-1}\)) and the antibacterial
activity of PCs of SM lead to conclude that the later compounds
were not effective as commercial drugs.

**Table 4** Antibacterial activities (inhibition zone in diameter (mm)\( ^{a} \) around the disks and MIC\(^{b} \)) of *Spirulina maxima* phycocyanin

<table>
<thead>
<tr>
<th>Bacterial species (Gram + or -)</th>
<th>Reference antibiotic (chloramphenicol)</th>
<th>SM phycocyanin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 (mg per disk)</td>
<td>1 (mg per disk)</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> (+)</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> (+)</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (+)</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em> (+)</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td><em>Serratia marcescens</em> (-)</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> (-)</td>
<td>16</td>
<td>13</td>
</tr>
</tbody>
</table>

\( ^{a} \) Values represent the mean of three replicates. \( ^{b} \) MIC: Minimum inhibition concentration, values given as mg mL\(^{-1}\) for samples and for chloramphenicol.
Acknowledgements

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References