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Phytochemical screening of twelve species of phytoplankton isolated from Arabian Sea coast

Sushanth Vishwanath Rai*, Madaiah Rajashekhar

Department of Biosciences, Mangalore University, Mangalagangothri-574 199, Karnataka, India

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ABSTRACT

Objective: To analyze the phytochemicals in twelve species of marine phytoplankton.**Methods:** Total phenolic content of methanol extract was estimated by the Folin-Ciocalteu method. Total flavonoid content of the methanol extract was determined by aluminium chloride method. Chlorophylls, β -carotene and astaxanthin were estimated by acetone extraction method. Vitamin C was determined by dinitrophenyl-hydrazine method. Phycobiliproteins such as allophycocyanin, phycocyanin and phycoerythrin in the aqueous extracts were determined.**Results:** Total phenolics varied from 5.41 mg gallic acid equivalents/g dry weight (DW) in *Phormidium corium* (*P. corium*) to 17.37 mg gallic acid equivalents/g DW in *Oscillatoria freyia* (*O. freyia*). Total flavonoids ranged between 0.74 mg quercetin equivalent/g DW in *P. corium* and 9.87 mg quercetin equivalent/g DW in *Nannochloropsis oceanica*. Chlorophyll-a pigment was high in *Chaetoceros calcitrans* (*C. calcitrans*) (15.51 mg/g DW) and low in *P. corium* (1.08 mg/g DW). Chlorophyll-c ranged between 0.07 mg/g DW in *Nannochloropsis oceanica* and 4.62 mg/g DW in *C. calcitrans*. High contents of β -carotene and astaxanthin were found in *C. calcitrans* and low in *P. corium* which ranged from 0.33 to 10.03 mg/g DW and 0.18 to 3.85 mg/g DW, respectively. Vitamin C content varied from 0.50 mg/g DW in *C. calcitrans* to 1.51 mg/g DW in *Phormidium tenue*. *O. freyia* showed highest total phycobiliproteins of 317.05 mg/g DW. High contents of allophycocyanin and phycocyanin were found in *O. freyia*, whereas high contents of phycoerythrin were found in *Oscillatoria sancta*. All the three phycobiliproteins were low in *Chroococcus turgidus*.**Conclusions:** Marine phytoplankton are one of the natural sources providing novel biologically active compounds with potential for pharmaceutical applications.

1. Introduction

Marine phytoplankton represent a major untapped resource of natural bioactive metabolites like pigments, phycobiliproteins (PBP), phenolics, amino acids, polyunsaturated fatty acids and sulphated polysaccharides[1-5]. These metabolites exhibit diverse biological activities, including antioxidant, antimicrobial, anti-inflammatory, anticoagulant, anti-mutagenic, antiproliferative and anti-cancer activities[6-9]. The screening studies reveal the existence of new biomolecules potentially interesting for their biological activities. The studies on biological activities of phytoplankton extracts and biochemical characterization of their metabolites seem to be promising for biotechnology applications[10]. In the present study, twelve species of phytoplankton isolated from Arabian Sea

coast were evaluated for their phytochemical constituents such as phenolics, flavonoids, chlorophylls, β -carotene, astaxanthin, vitamin C and PBPs.

2. Materials and methods

2.1. Isolation and maintenance of phytoplankton cultures

Twelve phytoplankton species including nine cyanobacteria [*Chroococcus turgidus* (*C. turgidus*), *Lyngbya confervoides* (*L. confervoides*), *Nostoc commune* (*N. commune*), *Oscillatoria freyia* (*O. freyia*), *Oscillatoria geminata* (*O. geminata*), *Oscillatoria sancta* (*O. sancta*), *Phormidium corium* (*P. corium*), *Phormidium tenue* (*P. tenue*) and *Spirulina major* (*S. major*)], two diatoms [*Chaetoceros calcitrans* (*C. calcitrans*) and *Skeletonema costatum* (*S. costatum*)] and the planktonic green alga [*Nannochloropsis oceanica* (*N. oceanica*)] were isolated from rocks, puddles and sea water of Arabian Sea coast of Karnataka (west coast of India).

*Corresponding author: Sushanth Vishwanath Rai, Department of Biosciences, Mangalore University, Mangalagangothri-574 199, Karnataka, India.
E-mail: bioraitech@gmail.com

The filamentous cyanobacteria were isolated by micropipette method, whereas unicellular cyanobacterium (*C. turgidus*), diatoms and planktonic green alga were isolated by agar plate method[11]. The cultures were microscopically examined for the assessment of growth and contamination. The successful axenic cultures were diluted and subcultured to 100 mL of culture media in 250 mL conical flasks. The cultures of diatoms and planktonic green alga were maintained in Walne's medium at $(20 \pm 2) ^\circ\text{C}$, whereas cyanobacteria were cultured in f/2 medium at $(28 \pm 2) ^\circ\text{C}$ and incubated under illumination of 1000 lux with 8:16 h light and dark regime.

2.2. Harvesting and storage

The growth phases of the isolated cultures were determined in terms of their chlorophyll-*a* content. The cultures were harvested at exponential growth phase. The phytoplankton biomass was separated from the media by batch centrifugation at 3500 r/min for 10 min, whereas filamentous cyanobacteria biomass was recovered by filtration. The cultures were repeatedly washed in 0.85% saline solution and later by 0.5 mol/L ammonium formate to remove salts without damaging the cells. The biomass was then transferred to the pre-weighed filter paper and oven-dried to obtain constant weight. The dried cultures were stored in air-tight vials at $4 ^\circ\text{C}$ for further use.

2.3. Total phenolic content

The total phenolic content of the methanol extract was estimated by the Folin-Ciocalteu method[12]. About 100 μL of diluted sample was added to 1 mL of 1:10 diluted Folin-Ciocalteu reagent. After 4 min, 800 μL of saturated sodium carbonate (75 g/L) was added. After 2 h of incubation at room temperature, the absorbance at 765 nm was measured. The result was expressed as mg gallic acid equivalent (GAE)/g dry weight (DW) of extract.

2.4. Total flavonoid content

Total flavonoid content of the cultures was determined by aluminium chloride method[13]. About 100 μL of diluted sample was mixed with 0.3 mL of 5% sodium nitrite. After 5 min, 0.3 mL of 10% aluminium chloride was added. After 6 min, 2 mL of 1 mol/L sodium hydroxide was added and the total volume was made up to 10 mL with distilled water. Then solution was mixed well and absorbance was measured at 510 nm. The result was expressed as mg quercetin equivalent (QE)/g DW of extract.

2.5. Chlorophylls

For the extraction of chlorophyll pigments, the cultures were separated from the medium by centrifugation and 10 mL of 90% acetone was added. The tubes were vigorously shaken and homogenized so as to dissolve completely in the solvent. For complete extraction of the pigments, the tubes were kept in refrigerator for 24 h. After the extraction period, the samples were centrifuged and the supernatant was collected. The supernatant was made up to 10 mL with 90% acetone and absorbance was

measured at 630 nm, 647 nm, 664 nm and 750 nm against 90% acetone as blank. The amount of chlorophyll content was calculated using equation given by Jeffrey and Humphrey[14].

$$\text{Chlorophyll-}a \text{ (}\mu\text{g/mL)} = (11.85 \times \text{OD}_{664}) - (1.54 \times \text{OD}_{647}) - (0.08 \times \text{OD}_{630})$$

$$\text{Chlorophyll-}c \text{ (}\mu\text{g/mL)} = - (1.67 \times \text{OD}_{664}) - (7.60 \times \text{OD}_{647}) + (24.52 \times \text{OD}_{630})$$

where, OD expressed optical density.

2.6. β -carotene and astaxanthin

The cultures were harvested by centrifugation at 3000 r/min for 10 min. The pellet was homogenized with 10 mL of 5% potassium hydroxide and 30% methanol. The mixture was kept in water bath maintained at $70 ^\circ\text{C}$ for 5 min and centrifuged for 10 min. About 5 mL of 90% acetone was added to the colorless pellet, homogenized and kept at $70 ^\circ\text{C}$ for 5 min. The supernatant was separated by centrifugation and cooled to room temperature. The absorbance of supernatant was measured at 429 nm, 452 nm and 478 nm for the determination of β -carotene and at 480 nm for astaxanthin against 90% acetone as blank[15].

2.7. Vitamin C

Vitamin C was determined by dinitrophenyl hydrazine method[16]. About 50 mg of cultures were homogenized with 5 mL of extractant solution (3% metaphosphoric acid and 8% acetic acid) and centrifuged for 10 min at 3000 r/min. The supernatant (0.5 mL) was mixed with 1 mL of chromogen solution (2.2% dinitrophenyl hydrazine + 5% thiourea + 0.6% copper sulphate) and incubated for 10 min in boiling water bath under dark condition. The mixture was cooled to room temperature and 4 mL of 65% sulphuric acid was added. The contents were mixed well and incubated for 30 min at room temperature in dark condition. The absorbance was measured at 540 nm using ascorbic acid as standard (5–50 $\mu\text{g/mL}$) and expressed in mg/g DW.

2.8. PBPs

The cultures were separated from the medium by centrifugation at 3000 r/min for 10 min and homogenized with 10 mL of phosphate buffer (0.05 mol/L, pH 7.5). The homogenate was kept at $4 ^\circ\text{C}$ for 1 h and then immediately brought to room temperature. The mixture was centrifuged and the supernatant obtained was used to measure the PBP contents. The absorbance of supernatant solution was recorded at 562 nm, 615 nm and 652 nm with respect to phosphate buffer as blank. The concentrations of PBPs (mg/mL) in the extracts were calculated using the following formulae[17,18]:

$$\text{Allophycocyanin (APC)} = \frac{A_{652} - 0.208 (A_{615})}{5.09}$$

$$\text{Phycocyanin (PC)} = \frac{A_{615} - 0.474 (A_{615})}{5.34}$$

$$\text{Phycocerythrin (PE)} = \frac{A_{562} - 2.41 (\text{PC}) - 0.849 (\text{APC})}{9.62}$$

The final results were expressed in mg of the individual PBPs/g DW of cultures.

2.9. Statistical analysis

All the results were calculated as mean \pm SD ($n = 3$). One way ANOVA was applied to test for significant differences at $P < 0.05$.

3. Results

The phytochemical constituents such as total phenolics, flavonoids, chlorophylls, β -carotene, astaxanthin and vitamin C contents of phytoplankton isolates are shown in Table 1. The total phenolic contents varied from 5.41 mg GAE/g DW in *P. corium* to 17.37 mg GAE/g DW in *O. fremyii*. The species, namely, *N. oceanica* and *O. geminata* also showed high phenolic content with the values of 17.01 mg GAE/g DW and 16.33 mg GAE/g DW, respectively. The total flavonoid contents ranged between 0.74 mg QE/g DW in *P. corium* and 9.87 mg QE/g DW in *N. oceanica*.

The chlorophyll-*a* pigment was found to be high in *C. calcitrans*

(15.51 mg/g DW) and *O. fremyii* (15.42 mg/g DW). The low content was found in *P. corium* (1.08 mg/g DW). The chlorophyll-*c* contents ranged between 0.07 mg/g DW and 4.62 mg/g DW in *N. oceanica* and *C. calcitrans*, respectively. The diatom *C. calcitrans* showed significantly high β -carotene content (10.03 mg/g DW) and low value (0.33 mg/g DW) was found in *P. corium*. The high content of astaxanthin was found in *C. calcitrans* (3.85 mg/g DW) followed by *N. oceanica* (3.83 mg/g DW) and *O. fremyii* (3.36 mg/g DW). *P. corium* had low astaxanthin content (0.18 mg/g DW). The vitamin C content varied from 0.50 mg/g DW in *C. calcitrans* to 1.51 mg/g DW in *P. tenue*.

The PBPs, such as APC, PC and PE contents of isolates were expressed in mg/g DW of cultures (Table 2). *O. fremyii* showed the highest total PBPs of 317.05 mg/g DW. The high content of PBPs was also found in *S. major* (207.56 mg/g DW) and *O. sancta* (106.34 mg/g DW). *C. turgidus* showed low content of total PBPs (4.00 mg/g DW). The APC contents varied between 1.43 mg/g DW

Table 1

Phytochemical constituents in different species of phytoplankton.

Phytoplankton	Phytochemical constituents						
	Total phenolics (mg GAE/g DW)	Total flavonoids (mg QE/g DW)	Chlorophyll- <i>a</i> (mg/g DW)	Chlorophyll- <i>c</i> (mg/g DW)	β -carotene (mg/g DW)	Astaxanthin (mg/g DW)	Vitamin C (mg/g DW)
<i>C. turgidus</i>	7.94 \pm 0.38 ^c	2.96 \pm 0.38 ^c	2.41 \pm 0.03 ^b	0.30 \pm 0.01 ^c	2.26 \pm 0.03 ^d	1.85 \pm 0.03 ^b	1.18 \pm 0.02 ^b
<i>L. confervoides</i>	13.80 \pm 0.26 ^b	3.98 \pm 0.17 ^d	6.03 \pm 0.05 ^f	0.32 \pm 0.02 ^e	0.62 \pm 0.04 ^f	0.42 \pm 0.02 ^e	0.68 \pm 0.02 ^{ef}
<i>N. commune</i>	8.19 \pm 0.18 ^c	2.48 \pm 0.15 ^f	6.41 \pm 0.02 ^e	0.36 \pm 0.03 ^c	1.18 \pm 0.02 ^e	0.91 \pm 0.02 ^d	0.98 \pm 0.03 ^c
<i>O. fremyii</i>	17.37 \pm 0.23 ^a	4.50 \pm 0.06 ^e	15.42 \pm 0.04 ^a	0.95 \pm 0.03 ^b	4.73 \pm 0.03 ^c	3.36 \pm 0.03 ^a	1.02 \pm 0.04 ^c
<i>O. geminata</i>	16.33 \pm 0.10 ^a	4.41 \pm 0.06 ^c	11.54 \pm 0.04 ^b	0.71 \pm 0.02 ^c	1.24 \pm 0.03 ^c	0.89 \pm 0.02 ^d	0.69 \pm 0.01 ^{ef}
<i>O. sancta</i>	7.81 \pm 0.30 ^c	2.39 \pm 0.06 ^{fg}	7.44 \pm 0.06 ^d	0.56 \pm 0.03 ^d	0.44 \pm 0.03 ^g	0.28 \pm 0.01 ^f	0.79 \pm 0.03 ^c
<i>P. corium</i>	5.41 \pm 0.10 ^g	0.74 \pm 0.17 ^j	1.08 \pm 0.03 ^j	0.10 \pm 0.01 ^g	0.33 \pm 0.02 ^h	0.18 \pm 0.02 ^g	0.91 \pm 0.02 ^d
<i>P. tenue</i>	9.22 \pm 0.11 ^d	1.44 \pm 0.38 ⁱ	3.95 \pm 0.04 ^g	0.25 \pm 0.01 ^f	0.40 \pm 0.02 ^g	0.32 \pm 0.02 ^{ef}	1.51 \pm 0.02 ^a
<i>S. major</i>	7.15 \pm 0.07 ^f	2.21 \pm 0.17 ^g	9.90 \pm 0.04 ^c	0.63 \pm 0.03 ^{cd}	2.06 \pm 0.05 ^d	1.45 \pm 0.03 ^e	0.59 \pm 0.03 ^f
<i>C. calcitrans</i>	11.23 \pm 0.19 ^c	5.59 \pm 0.12 ^b	15.51 \pm 0.03 ^a	4.62 \pm 0.03 ^a	10.03 \pm 0.03 ^a	3.85 \pm 0.04 ^a	0.50 \pm 0.03 ^g
<i>S. costatum</i>	10.11 \pm 0.07 ^{cd}	1.79 \pm 0.12 ^h	5.81 \pm 0.04 ^g	0.24 \pm 0.03 ^f	5.81 \pm 0.03 ^b	1.71 \pm 0.03 ^b	0.51 \pm 0.04 ^g
<i>N. oceanica</i>	17.01 \pm 0.09 ^a	9.87 \pm 0.25 ^a	1.65 \pm 0.04 ⁱ	0.07 \pm 0.00 ^g	4.95 \pm 0.03 ^c	3.83 \pm 0.03 ^a	0.90 \pm 0.03 ^d

Values were mean \pm SD of triplicates and values within the columns with different superscripts were significantly different ($P < 0.05$).

and 124.48 mg/g DW. PC contents ranged from 1.54 to 174.05 mg/g DW and PE contents varied from 1.04 to 40.22 mg/g DW. The high contents of APC and PC were found in *O. fremyii*, whereas PE was found to be maximum in *O. sancta*. All the three PBPs were low in *C. turgidus*. The species, namely, *C. turgidus*, *L. confervoides*, *O. fremyii*, *O. geminata*, *P. tenue* and *S. major* showed high content of PC followed by APC and PE, respectively; *N. commune*, *C. calcitrans*, *S. costatum* and *N. oceanica* showed high content of APC followed by PC and PE, respectively; *O. sancta* and *P. corium* showed high content of PE followed by PC and APC, respectively.

Table 2

PBP contents of phytoplankton.

Phytoplankton	APC (mg/g DW)	PC (mg/g DW)	PE (mg/g DW)	Total PBPs (mg/g DW)
<i>C. turgidus</i>	1.43 \pm 0.02 ⁱ	1.54 \pm 0.01 ⁱ	1.04 \pm 0.04 ⁱ	4.00 \pm 0.05 ^k
<i>L. confervoides</i>	12.55 \pm 0.09 ^e	17.65 \pm 0.04 ^d	4.21 \pm 0.02 ^f	34.41 \pm 0.14 ^e
<i>N. commune</i>	14.28 \pm 0.08 ^e	9.07 \pm 0.05 ^f	7.38 \pm 0.03 ^d	30.72 \pm 0.05 ^f
<i>O. fremyii</i>	124.48 \pm 0.23 ^a	174.05 \pm 0.05 ^a	18.51 \pm 0.02 ^b	317.05 \pm 0.29 ^a
<i>O. geminata</i>	10.78 \pm 0.03 ^f	17.27 \pm 0.04 ^d	5.40 \pm 0.01 ^e	33.45 \pm 0.06 ^c
<i>O. sancta</i>	28.52 \pm 0.03 ^c	37.60 \pm 0.03 ^e	40.22 \pm 0.02 ^e	106.34 \pm 0.07 ^c
<i>P. corium</i>	1.48 \pm 0.15 ⁱ	2.24 \pm 0.03 ⁱ	3.24 \pm 0.04 ^g	6.96 \pm 0.17 ^j
<i>P. tenue</i>	4.33 \pm 0.12 ^h	7.41 \pm 0.02 ^g	1.95 \pm 0.03 ^h	13.69 \pm 0.16 ^g
<i>S. major</i>	59.40 \pm 0.03 ^b	140.82 \pm 0.04 ^b	7.33 \pm 0.03 ^d	207.56 \pm 0.08 ^b
<i>C. calcitrans</i>	21.94 \pm 0.04 ^d	14.93 \pm 0.04 ^e	13.16 \pm 0.03 ^e	50.02 \pm 0.03 ^d
<i>S. costatum</i>	3.61 \pm 0.03 ^h	2.89 \pm 0.02 ^h	2.15 \pm 0.04 ^h	8.65 \pm 0.08 ⁱ
<i>N. oceanica</i>	4.63 \pm 0.03 ^g	3.71 \pm 0.03 ^h	3.10 \pm 0.02 ^f	11.44 \pm 0.06 ^h

Values were mean \pm SD of triplicates and values within the columns with different superscripts were significantly different ($P < 0.05$).

4. Discussion

The phenolic compounds, particularly the complex flavonoids, are the important class of antioxidants[5]. In the present study, total phenolic contents varied from 5.41 to 17.37 mg GAE/g DW. These values are supported by the values reported for 12 species of phytoplankton by Hajimahmoodi *et al.*, which ranged from 0.43 to 19.82 mg GAE/g[19]. Li *et al.* evaluated the total phenolic content of 23 species of phytoplankton[20]. The values obtained for all the species (3.59 to 19.03 mg GAE/g) except *Nostoc ellipsosporum* CCAP 1453/17 which showed high value (60.35 mg GAE/g) support the phenolic contents of isolated species. The phenolic contents of 32 phytoplankton species reported by Goiris *et al.* were lower than the values obtained in the present study which was ranged from 0.5 to 4.6 mg GAE/g[8].

Pumas *et al.* reported the phenolic contents of four cyanobacteria species, namely, *Phormidium*, *Leptolyngbya*, *Scytonema* and *Cyanosarcina*, and values were 6.16, 7.44, 3.20 and 2.36 mg GAE/g, respectively[4]. The phenolic contents reported for *Phormidium* and *Leptolyngbya* support the values obtained in the present study for cyanobacteria species, *P. corium* (5.41 mg GAE/g), *C. turgidus* (7.94 mg GAE/g), *N. commune* (8.19 mg GAE/g), *O. sancta* (7.81 mg GAE/g) and *S. major* (7.15 mg GAE/g), but are lower than

the values obtained from *L. confervoides* (13.80 mg GAE/g), *O. fremyii* (17.37 mg GAE/g), *O. geminata* (16.33 mg GAE/g) and *P. tenue* (9.22 mg GAE/g). The phenolic contents of diatoms and planktonic green alga were higher than the values reported by other investigators[3,8].

Shanab *et al.* reported the total phenolic contents of aqueous extracts of nine phytoplankton species, namely, *Anabaena flos-aquae* (0.32%), *Aspergillus oryzae* (0.40%), *Nostoc humifusum* (0.34%), *Nostoc muscorum* (*N. muscorum*) (0.61%), *Oscillatoria* sp. (0.55%), *Spirulina platensis* (*S. platensis*) (0.71%), *Phormidium fragile* (*P. fragile*) (0.36%), *Wolleea saccata* (0.10%) and *Chlorella vulgaris* (*C. vulgaris*) (0.20%)[21]. Phenolic content of *S. platensis* reported by them supported the value obtained from *S. major* in the present study, while the phenolic contents of isolates, namely, *N. commune*, *Oscillatoria* and *Phormidium* species were higher than the values reported for *Nostoc* species, *Oscillatoria* sp. and *P. fragile*. The total phenolics of *S. major* were also supported by the value obtained from *S. platensis* (7.90 mg GAE/g)[22]. It was found that antioxidant activity of phenol compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radical, quenching singlet and triplet oxygen or decomposing peroxides[23].

The study carried out by Klejduš *et al.* indicated the presence of variety of phenolic classes in phytoplankton[24]. According to Abd El-Baky *et al.*[25], the phenolic acids found in *Spirulina maxima* were gallic, *p*-hydroxy benzoic, chlorogenic, vanillin, caffeic, syringic, salicylic, coumaric, ferulic and cinnamic acids.

Several studies have compared antioxidant activity and phenolic content in fractionated extracts of biomass from different species of phytoplankton. The significant relation between antioxidant activities (2,2-diphenyl-1-picrylhydrazyl-radical scavenging and ferric reducing antioxidant potential) and phenolic content of phytoplankton was noticed by Hajimahmoodi *et al.*[19]. The high correlation of total phenolic content in crude extracts of four thermotolerant cyanobacteria with the antioxidative activity was found by Pumas *et al.*[14]. The significant correlation from total phenolic content and ferrous ion chelating activity from a diatom (*Chaetoceros* sp.) and a green microalga (*Nannochloropsis* sp.) was reported by Goh *et al.*[3]. Many studies found that phytoplankton fractions that were rich in phenolic compounds had a high antioxidant capacity[26,27].

Flavonoids have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation, and also as potential therapeutic agents against a wide variety of diseases[28]. The total flavonoid content of isolates ranged from 0.74 to 9.87 mg QE/g DW. These values were supported by the values for *S. platensis* (1.4 mg catechin equivalent/g) reported by Shanmugapriya and Ramanathan[22] and for green microalga *Trentepohlia umbrina* (13.72 mg rutin equivalent/g) reported by Simic *et al.*[29].

Phytoplankton are recognized as an excellent source of natural pigments[30]. The chlorophyll is the primary photosynthetic pigment in all phytoplankton species and they also produce various accessory or secondary pigments, such as PBPs and a wide range of carotenoids[1].

The total chlorophyll contents of the isolates ranged from 1.18 to

20.13 mg/g DW. The chlorophyll contents of phytoplankton reported by Becker ranged from 5 to 15 mg/g DW[31]. The chlorophyll content of all isolates falls within the range given by Becker except *P. corium* and *N. oceanica* which showed low values, 1.18 mg/g DW and 1.72 mg/g DW respectively, whereas *C. calcitrans* showed high value (20.13 mg/g DW)[31].

Apart from the use as food and pharmaceutical colorants, chlorophyll derivatives can exhibit health-promoting activities. These compounds have been traditionally used in medicine due to its wound healing and anti-inflammatory properties[32]. The study conducted by Balder *et al.* has provided evidence linking chlorophyll consumption to a decreased risk of colorectal cancer[33].

The carotenoids seem to function primarily as photoprotective agents and as accessory light harvesting pigment, thereby protecting the photosynthetic apparatus against photodamage[34]. Carotenoids are well known for their ability to deactivate singlet oxygen by physical quenching[8]. The main carotenoids produced by phytoplankton are β -carotene from *Dunaliella salina* (*D. salina*) and astaxanthin from *Haematococcus pluvialis* (*H. pluvialis*)[35,36].

The β -carotene content of cyanobacteria isolates such as *C. turgidus*, *L. confervoides*, *N. commune*, *O. geminata*, *O. sancta*, *P. corium*, *P. tenue* and *S. major* falls within the range reported by Tarko *et al.* in different strains of *Arthrospira* cultured in Zarrouk medium which ranged from 0.074 to 2.26 mg of Trolox/g DW[37]. Compare to this range, high content was recorded in cyanobacterium, *O. fremyii*, diatoms and planktonic green alga. Ranga Rao *et al.* reported 69.5%, 1.7% and 1.5% of total carotenoid contents in *Arthrospira platensis*, *H. pluvialis* and *Botryococcus braunii*, respectively[38]. *D. salina* contains up to 14% β -carotene on DW basis when grown under stress conditions including high salt concentration, high light intensity and nitrogen limitation[39].

In the present study, astaxanthin content varied between 0.18 mg/g DW and 3.85 mg/g DW. Several researchers reported the astaxanthin content in green microalga *H. pluvialis* grown under outdoor conditions which included 1.1%, 3.8% and 2.8%[40-42]. The effect of initial biomass density on astaxanthin production of *H. pluvialis* in a glass column photobioreactor was studied by Wang *et al.*[43]. Of seven initial biomass densities, *i.e.* 0.1, 0.5, 0.8, 1.5, 2.7, 3.5 and 5.0 g/L, 0.8 g/L was optimal and resulted in the highest astaxanthin productivity of 17.1 mg/L/day. Abd El-Baky *et al.* analyzed the carotenoid profile of *D. salina* grown under stress condition and reported that β -carotene content ranged from 3.2 to 62.31 mg/g DW and astaxanthin content varied from 2.13 to 21.31 mg/g DW[44].

Götz *et al.* reported that β -carotene and astaxanthin in cyanobacterium exposed to UV-B radiation exerted their protective function as antioxidants to inactive UV-B induced radicals in photosynthetic membrane[45]. Several studies have demonstrated that carotenoids contribute significantly to the total antioxidant capacity of phytoplankton[46,47]. Phytoplankton are already commercially produced as a source of carotenoid antioxidants for use as additives in food and feed applications, as well as for use in cosmetics and as food supplements[48].

Astaxanthin is a strong coloring agent and has many functions in animals such as growth, vision, reproduction, immune function and

regeneration[49]. It has a great potential to prevent cancer, diabetes and cardiovascular diseases[50,51]. It has also been suggested that astaxanthin has a free radical fighting capacity[30].

Vitamin C is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical[52]. In the present study, average values of vitamin C ranged from 0.50 to 1.51 mg/g DW. This range is comparable with the the range given by Bayanova and Trubachev for seven species of phytoplankton including *C. vulgaris*, *Platymonas viridis*, *Synechococcus elongatus*, *Coccolpidia* sp., *S. platensis*, *Cyanidium caldarium* and *Porphyridium cruentum* (0.54 mg/g in *Platymonas viridis* to 2.4 mg/g in *C. vulgaris*)[53].

Brown and Miller analyzed the ascorbic acid content of eleven species of phytoplankton used in mariculture during both logarithmic and stationary phases of growth and reported the average values of ascorbic acid ranged from 1.1 (*Thalassiosira pseudonana*, stationary phase) to 16.20 mg/g DW (*Chaetoceros gracilis*, logarithmic phase) [54]. This range is higher than the values obtained in the present study except for three species of cyanobacteria (*C. turgidus*, *O. fremyii* and *P. tenue*), whose values fall within this range.

De Roeck-Holtzhauer *et al.* reported ascorbic acid values for *C. calcitrans* and *S. costatum* which were significantly lower than those reported for the same species in the present study[55]. But values obtained from isolates were similar with the values reported by De Roeck-Holtzhauer *et al.* for *Tetraselmis suecica* and *Pavlova lutheri*[55]. They found 0.06 mg/g, 0.12 mg/g, 0.50 mg/g and 0.84 mg/g ascorbic acid in *S. costatum*, *C. calcitrans*, *Tetraselmis suecica* and *Pavlova lutheri*, respectively.

The vitamin contents of phytoplankton fluctuate with environmental factors, harvesting treatment and biomass drying methods[56]. Abd El-Baky *et al.* observed that content of ascorbic acid in *D. salina* significantly increased as results of exposure to UV-B radiation, being grown under nitrogen limitation and high NaCl concentration in growth media[44].

PBPs are water soluble pigments that can be divided into three main groups according to their structures: APC, PC and PE[57]. The contents of APC and PC were high in *O. fremyii*, whereas PE was high in *O. sancta*. Low content of APC, PC and PE was recorded in *C. turgidus*. The total PBPs ranged from 4.00 to 317.05 mg/g, APC contents varied from 1.43 to 124.48 mg/g, PC contents ranged from 1.54 to 174.05 mg/g and PE contents varied from 1.04 to 40.22 mg/g. These values were supported by the values reported for four cyanobacteria species by Pumas *et al.*[14]. Among the four cyanobacteria species, *Leptolyngbya* sp. KC45 showed the highest total PBPs up to 181.63 mg/g DW, followed by 165.47, 72.55 and 37.50 mg/g DW of *Phormidium* sp. PD40-1, *Scytonema* sp. TP40 and *Cyanosarcina* sp. SK40, respectively. The major PBP in *Scytonema* sp. TP40 was found to be PC, while the main PBP in *Leptolyngbya* sp. KC45, *Phormidium* sp. PD40-1 and *Cyanosarcina* sp. SK40 was PE.

Pandey and Pandey evaluated the PBP contents of 13 filamentous cyanobacteria[58]. Their results revealed the presence of all the three types of PBPs (PC, APC and PE) in varying proportions in cyanobacteria species examined. Totally, the PBP content ranged from 48.5 mg/g in *Rivularia aquatica* to 93.3 mg/g in *N. muscorum*

on DW basis. The total PBPs of all isolated cyanobacteria species showed lower values than this range except *O. fremyii*, *O. sancta* and *S. major* which showed high PBPs.

The PBP contents of six species of *Arthrospira* species reported by Tarko *et al.* supported the values obtained from *C. turgidus*, *P. corium*, *P. tenue*, *S. costatum* and *N. oceanica*, but were significantly lower than the values obtained from other isolates[37].

The study conducted by Patel *et al.* on three species of cyanobacteria, namely, *Spirulina* sp., *Phormidium* sp. and *Lyngbya* sp. cultivated in open systems on standard growth medium showed that the contents of three PBPs decreased in each of these species in the order of PC, APC and PE[59]. Pandey and Pandey reported the high content of PC followed by APC and PE in *Oscillatoria proboscidea*, *Oscillatoria limosa*, *Oscillatoria irrigua*, *Phormidium foveolarum*, *P. tenue*, *Anabaena oryzae*, *Rivularia aquatica* and *Hapalosiphon* sp.[58]. The similar variation was observed in the present study for *C. turgidus*, *L. confervoides*, *O. fremyii*, *O. geminata*, *P. tenue* and *S. major*. Similarly other researchers observed high content of PC in *Oscillatoria* sp., *P. fragile*, *Anabaena oryzae* and *Lyngbya* sp.[21,58].

The high content of APC in *N. commune*, *C. calcitrans*, *S. costatum* and *N. oceanica* was supported by the observations made by Shanab *et al.*[21] in *N. muscorum*, *Anabaena flos-aquae*, *S. platensis*, *Wollea saccata* and *Nostoc humifusum*. They found no detectable PE, while PC and APC showed variable contents in the test cyanobacteria species. The PBP contents of *N. muscorum* and *Nostoc linckia* evaluated by Pandey and Pandey showed high content of PC, but in the present study, APC was the predominant PBPs in *N. commune*[58]. The species, namely, *O. sancta* and *P. corium* showed high content of PE followed by PC and APC, respectively. This kind of variation in the individual PBPs was also observed in *Phormidium* sp. PD40-1 and *Leptolyngbya* sp. KC45[4].

The PBPs have been described as the strong antioxidant[60,61]. The antiviral and antifungal activities of APC and PC were reported by Sekar and Chandramohan[62]. The cytotoxic activity of C-PC isolated from *Oscillatoria amphibia* was reported by Demirel *et al.*[63]. In the study of Gardeva *et al.*[64], C-PC from *Arthronema africanum* (cyanobacterium) showed *in vivo* and *in vitro* antitumor activities. Several investigators showed anti-inflammatory activities of PBPs of phytoplankton[57,65].

Marine phytoplankton are one of the natural sources providing novel biologically active compounds with potential for pharmaceutical applications. They have a significant attraction as natural source of bioactive molecules, because they have the potential to produce bioactive compounds in culture, which are difficult to be produced by chemical synthesis. Marine phytoplankton are potential source of new antioxidant, antimicrobial, anti-inflammatory, anticoagulant and cytotoxic compounds because their genetic diversity represents a yet untapped resource of novel natural bioactive compounds that can be harnessed for commercial use.

Conflict of interest statement

We declare that we have no conflict of interest.

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