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RESEARCH ARTICLE

Carotenoid composition and investigation of the antioxidant activity of *Phormidium* sp.

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ABSTRACT

Microalgae metabolites are used for health, feed additives, cosmetic industries, food and biodiesel production. *Phormidium* species have an important position in medical studies because they contain essential components. In this study, carotenoid profile and content were analyzed using the HPLC method. Antioxidant activities for *Phormidium* sp. were determined using DPPH and FRAP assays. BHT and ASC were used as control samples in antioxidant assays. The method used to resolve a number of carotenoids from saponified *Phormidium* sp. proved acceptable separation, as evidenced by retention factor (k) values of 0.54 to 3.83 and separation factor (α) values greater than 1. Main carotenoids were dominated by the two main derivatives, all-trans form of lutein 41.35% (1.25 mg/g) and 9- or 9'-cis-β-carotene 36.43% (1.10 mg/g). Auroxanthin and cis neoxanthin were identified as epoxy-containing compounds. It is also understood that considering the DPPH assay, the extract of *Phormidium* sp. (IC50:127.6 mg/L) exhibited clearly low radical scavenging activity compared to the standards ASC (IC50: 0.02 mg/L) and BHT (IC50: 0.19 mg/L). In the FRAP antioxidant experiment, the mean ASC and BHT equivalent amounts were determined as 828.6 and 124.6 mg/L, respectively. Quantitatively, *Phormidium* sp. was predominated by cis-Lutein as a major constituent, being 41.35% (3.02 mg/g) in total carotenoids (Tc). The antioxidant capacity of *Phormidium* sp. that considering the DPPH and FRAP were compared to control standards were showed considerably low effects.

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Introduction

Microalgae are single-celled microscopic organisms capable of producing bioactive compounds and are the primary producers of the aquatic food chain. Microalgae are microorganisms that can preserve complex organic compounds in their bodies and release them out of the cell with the help of sunlight (Chacon-Lee & Gonzalez-Marino, 2010; Batista et al., 2013). Carotenoids of structural and functional roles are indispensable in photosynthetic life. They prevent the formation of photooxidative damage by providing the transfer of excess energy from the photosynthetic apparatus (Stange, 2016). Reactive oxygen species (ROS) are a natural biochemical product involved in the aerobic metabolism processes of the cell (Hancock et al., 2001). Increased exposure to ROS causes disruption of redox signaling and control known as oxidative stress, resulting in cell membrane, enzyme, and DNA fragmentation as a result (Zuluaga et al., 2017). New therapeutic molecules that can block oxidative stress are very important in the prevention and improvement of chronic disease due to oxidative stress. Because of their antioxidant properties, carotenoids have been suggested for the prevention of chronic diseases. Current studies show that carotenoids prevent oxidation of cholesterol, proteins or DNA by scavenging free radicals and reducing stress-induced by ROS (Rao & Rao, 2007; Khansari et al., 2009; Gong & Bassi, 2016).

The antioxidant properties of carotenoids are explained by the scavenging of singlet molecular oxygen and peroxyl radicals in general, due to the mixture of various isomers in their structures and their chemical formation with other bioactive compounds (Niyogi et al., 1997). It is known that natural carotenoid isomers are more active for direct human consumption than their synthetic counterparts dominated by all-trans compounds (Patrick, 2001).

Rodríguez-Meizoso et al. (2008) detected various carotene groups such as β-carotene, lutein, violaxanthin and neoxanthin by extraction with different temperatures and solvents in their study with *Phormidium* species and stated that they showed high antioxidant activity. In a different study, Rodrigues et al. (2015) investigated the carotene profile and antioxidant effect of *Phormidium autumnale,* as a result, 24 carotenoids, 3 phycobiliproteins, and 2 chlorophylls of this species were identified (Rodrigues et al., 2015). The high antioxidant effect of *Phormidium* species has been demonstrated in many studies (Soni et al., 2008; Shanab et al., 2012; Chatterjee & Bhattacharjee, 2014).

The potential of different species, which may differ in the carotenoid compositions of many algae species, is being investigated. Comparisons are used to understand the local environments of different growing conditions over similar or identical species. For this purpose, it focused on elucidating the profile of carotenoids and antioxidant activity performance of the microalgae *Phormidium* sp. isolated from Kapulukaya Reservoir (Kirikkale, Turkey).

Material and Methods

Phormidium sp. were isolated from freshwater samples of the River Kızılırmak in Kırıkkale Province (Turkey). Basal Bold Medium (BBM) (Bischoff & Bold, 1963) was selected for the growth of *Phormidium* sp. using illumination of 16:8 h light-dark cycle of 4000 lux light intensity under temperature conditions kept at 25±1°C. Centrifugated biomass at 3000 rpm for 10 min. was lyophilized for 48 hours at -83°C and 1.33 Pa (Richmond & Hu, 2013) and stored at –80°C until used.

Extraction and Profiling of Carotenoids

The details of both the extraction procedure (Chen et al., 1991) and the analysis of carotenoids were given in a previous study by Aluç et al. (2018). Briefly; 0.1 g of the microalgae sample was stirred for 1 hour using 3 mL of a hexane-ethanol-acetone-toluene mixture (10:6:7:7, v/v). 1 mL of 40% methanolic KOH was added to wait 16 hours to achieve saponification. Then hexane and 10% sodium sulfate were added. The carotenoids were then allowed to separate under dim light and nitrogen gas. Collected extracts dried by evaporation were subjected to HPLC analysis in mobile phase solvent. Two mobile phases (A and B) of methanol–acetonitrile-water (84:14:2, v/v/v) and methylene chloride (100%) with an arranged flow rate of 0.6 mL/min were used in a gradient manner from 100% A and 0%.

Antioxidant Capacity Assays

For the extraction of antioxidants, the microalgae biomass (0.1 g) was mixed with 1 ml methanol-toluene (3:1) in a volumetric flask. Following homogenization, insoluble biomass was separated from the supernatant by centrifugation at 13500 rpm for 10 min. (Chen et al., 1991).

DPPH (2,2-Diphenyl-1-picrylhydrazyl) activity of the *Phormidium* sp. extract solutions was determined based on the method described by Blois (1958). Volumes of 62.5 µL from each extract solution prepared with methanol at concentrations of 2, 10, 25, 50, 100 µg/mL, respectively, were added to a mixture of methanol (125 µL) and DPPH (62.5 µL) and then left

for incubation in the dark at room temperature. The reaction values measured at 515 nm against methanol extract as blank were used to calculate the percentage radical scavenging activity as below.

So that the control included DPPH and methanol while sample consisted of microalgae extract, DPPH and methanol. The EC_{50} values which were calculated from the plot of scavenging activity against the concentration of sample indicated the half-maximal potency of microalgal extract to scavenge DPPH radicals. For the Ferric Reducing Antioxidant Power Assay (FRAP), a 1 ml of *Phormidium* sp. extract solutions prepared at graded concentrations (2, 5, 10, 25, 50, 100 µg/mL) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (200 µL, 1%) and left for incubation at 50°C for 20 min. Trichloroacetic acid (TCA, 10%, 200 µL) was added to the mixture and centrifuged at 3750 rpm for 10 min. (Oyaizu, 1986). A 125 µL of the upper layer was separated and mixed with 25 µL distilled water and 20 µL $FeCl_3$ (0.1%). The absorbance of the mixture was measured at 665 nm by a UV-spectrophotometer.

Statistical Analysis

Means of data were obtained from triplicate analysis during HPLC analysis. Results from antioxidant assays were obtained via triplicate experiments. Relationships between antioxidant activity and concentrations of algal extracts and standards were depicted by regression analysis. Stepwise multiple regression analysis was used to STATISTICA (version 7) statistical software.

Results and Discussion

Identification of Carotenoids

The method proved an acceptable separation as inferred from the retention factor (k) ranging between (0.54-3.83) (Liu et al., 2004; Inbaraj et al., 2006). They were all positively identified based on retention time and Q ratio values of standards designation which was tentative and made by the comparison of retention time and absorption spectra values given in the literature (Tables 1 and 2) (Inbaraj et al., 2006; Aluç et al., 2018). For the identification of carotenoids in *Phormidium* sp. and the photoisomerized standards were subjected to HPLC analysis. Carotenoids spectral characteristics of *Phormidium* sp. samples were designated. The chromatogram of the extract solution from the *Phormidium* sp. cells revealed 16 resolution peaks assigned to carotenoids (Figure 1 and Table 1). All-*trans* forms of lutein (0.023 mg/g), zeaxanthin (0.074 mg/g), β-cryptoxanthin (0.005 mg/g), α-carotene (0.000 mg/g) and β-carotene (0.321 mg/g) were assigned to peaks 6, 8, 11, 13 and 14, respectively (Figure 1 and Tables 1 and 2). The *Phormidium* sp. eliquates contained 3.005 mg/g of total carotenoids (TC), dominated by the two main derivatives, trans and *cis* forms of cis-lutein 41.35% (1.25 mg/g) and 9-or 9'-cis-β-carotene 36.43% (1.10 mg/g), All-trans-zeaxanthin 2.43% (0.074 mg/g), was represented by a small amount in Tc (Table 2).

Wojtasiewicz & Ston-Egiert (2016) identified the *Phormidium* sp. (CCNP 1317) species obtained from the Pomeranian lakes and investigated pigment composition under optimized growing conditions by cultivating. Total carotenoid content was found to be around 15.80 mg/m^3 under optimized conditions. The corresponding values of β-carotene and zeaxanthin were around 14.66 mg/m^3 and 1.44 mg/m^3 (Wojtasiewicz & Ston-Egiert, 2016). In another study, Morone et al. (2020) identified the *Phormidium* sp. (LEGE 05292) compounds consisted of three xanthophylls, lutein (28.65 µg/g), canthaxanthin (23.48 µg/g), echinenone (105.81 µg/g) and one carotene β-carotene (62.94 µg/g) total carotenoid content was found 215.88 µg/g. All of these findings are much less than those obtained in our study.

Various factors play a role in the biosynthesis of carotenoids and the distribution of their derivatives in algae. Although it varies species-specific (Gong & Bassi, 2016), it can also differ in different environments and conditions of the same species (Ho et al., 2014). Additionally, variations may arise due to different culture conditions, as noted in studies for direct determination of carotenoids or those applying stress conditions to potentially increase carotenoid content (Goiris et al., 2012; Rodrigues et al., 2015; González-López et al., 2018).

Antioxidant Capacity

One of the most important features of photosynthetic organisms such as algae for human needs is based on the antioxidant potential of free radical extracts, which are produced by the physiological and biochemical processes of cells. In this study, the methods to measure antioxidant activity represented the DPPH and FRAP the single electron transfer reactions (ET). The antioxidant activities of the algal extract determined by DPPH and FRAP assays were evaluated against those of BHT (BHT or its chemical name 2,6-di-tert-butyl-p-cresol (DBPC) is a synthetic phenolic antioxidant widely used as a food additive) (Leclercq et al., 2000). The advantage of the DPPH method is that DPPH is a stable radical that is often used to measure the antioxidant activity of plant extracts. The DPPH

method can be used as samples or solid solutions and is not specific for certain antioxidant components. In addition, this method is simple, accurate, fast and inexpensive to test the ability of components to capture radical compounds (Sri Mariani, 2018). The advantages of the FRAP method are that it is fast and inexpensive, the reagents used are very simple, and there are no special tools used to calculate total antioxidants. The FRAP method is a plant antioxidant test method. The FRAP method was used to measure the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} (Tahir et al., 2018). However, both methods have disadvantages. The DPPH method can only be used to measure antioxidants that are soluble in organic solvents, especially alcohol, and are very sensitive to light, oxygen, pH and solvent type. On the other hand, the FRAP method cannot measure antioxidants with thiol groups (including -SH) such as glutathione (Putri et al., 2020).

Table 1. Retention time, retention factor (k), separation factor (α), peak purity and resolution of carotenoids in *Phormidium* sp.

Peak no.	Compound	Retention time (min)	k[a]	α[b]	Peak purity (%)	Resolution
1	Auroxanthin	8.89	0.00	0.00	98.1	0.00
2	Auroxanthin	10.47	0.18	0.00	89.2	4.14
3	Cis-neoxanthin	13.73	0.54	3.07	97.5	8.34
4	13-or-13'-cis lutein	20.57	1.31	2.41	99.7	18.53
5	cis-lutein	22.63	1.54	1.18	99.7	4.61
6	All-trans-lutein	24.42	1.75	1.13	99.9	3.74
7	Cis-lutein	25.19	1.83	1.05	95.4	1.31
8	All-trans-zeaxanthin	26.19	1.94	1.06	99.9	1.67
9	9-or-9'-cis-lutein	27.66	2.11	1.09	99.7	3.08
10	Cis-lutein	31.05	2.49	1.18	98.2	6.97
11	All-trans-beta-cryptoxanthin	33.36	2.75	1.10	98.6	4.67
12	13-or-13'-cis-beta-carotene	36.92	3.15	1.15	92.6	6.71
13	All-trans-alfa-carotene	37.31	3.19	1.01	99.3	0.42
14	All-trans-beta-carotene	41.31	3.64	1.14	99.8	4.64
15	9-or 9'-cis-alfa-carotene	41.92	3.71	1.02	99.6	1.33
16	9-or 9'-cis-beta-carotene	42.924	3.83	1.03	99.9	2.15

Note: a: retention factor; b: selectivity (separation factor).

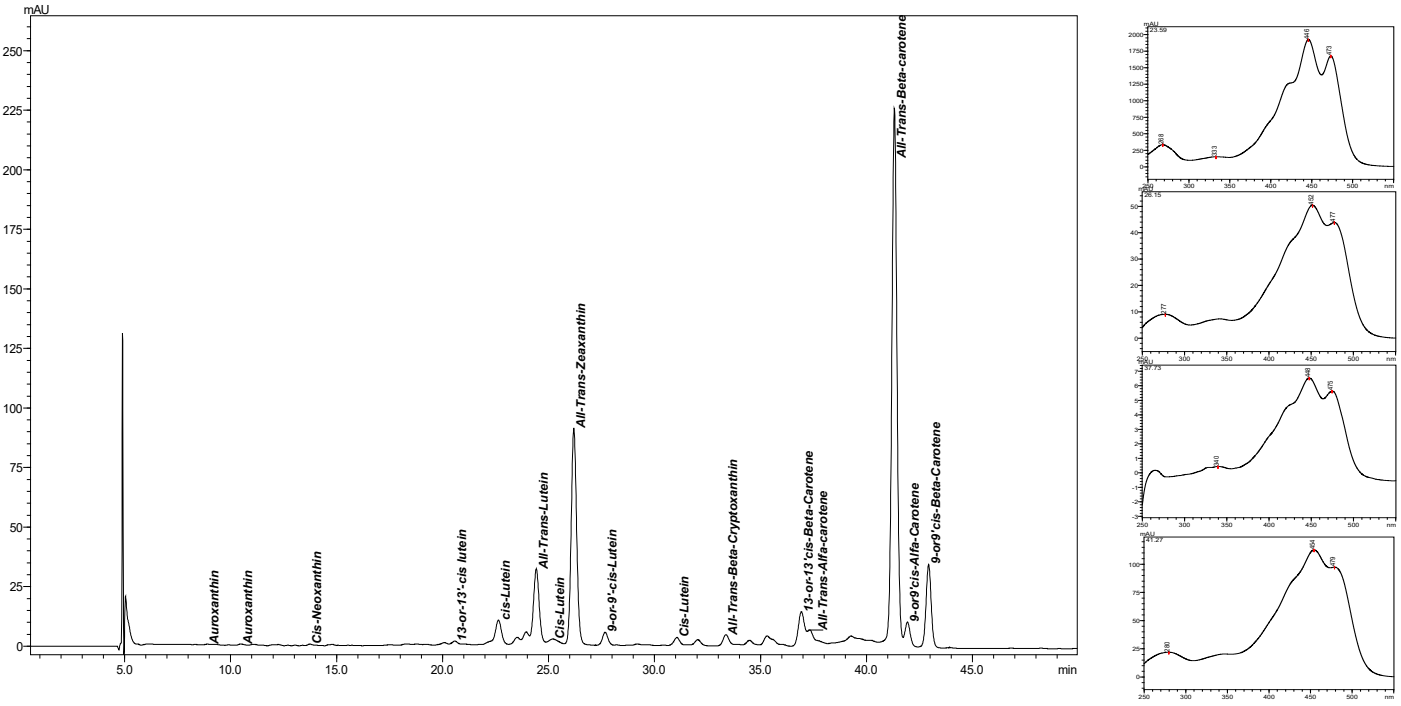

Figure 1. The HPLC chromatogram of the carotenoids obtained from *Phormidium* sp. (left) and the profiles of all-trans carotenoids (right)

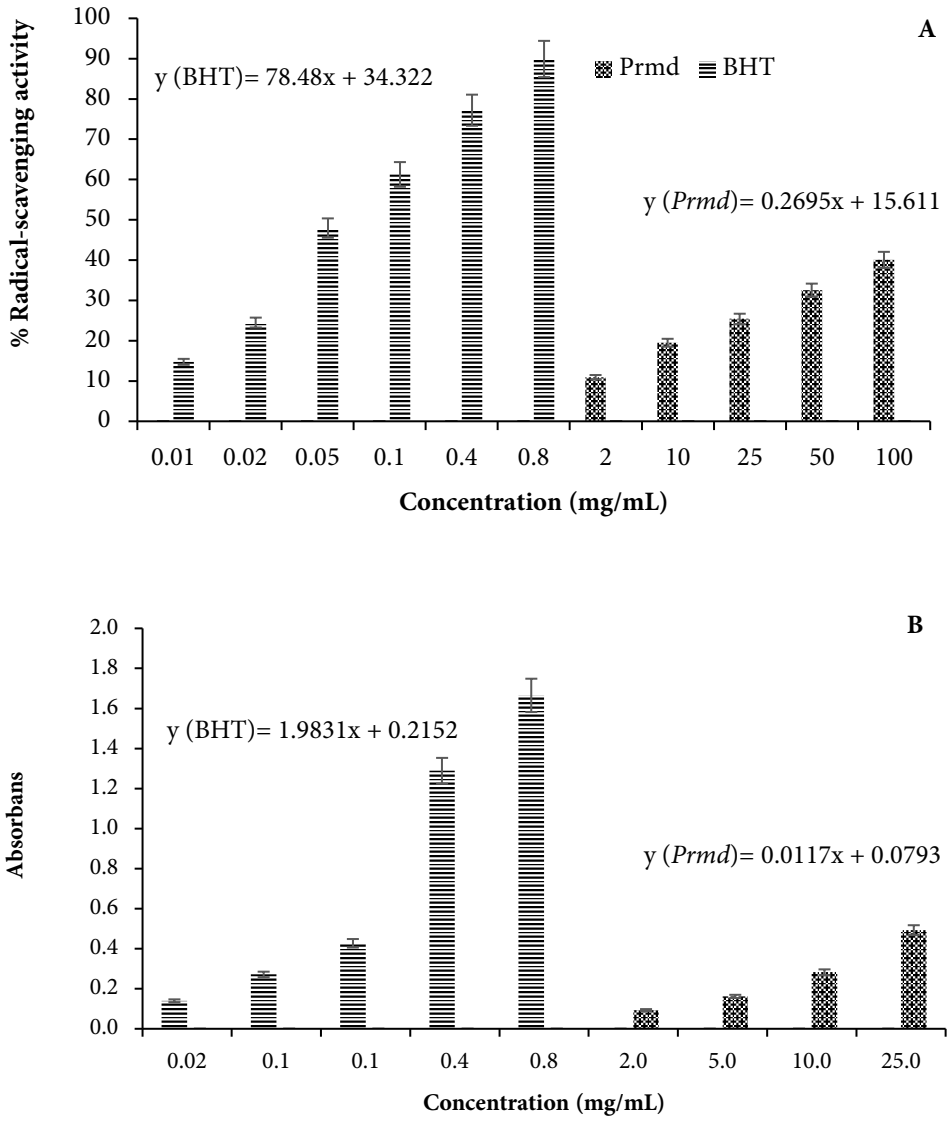

Figure 2. Collective display of DPPH radical sweeping (A) and FRAP inhibition (B) effect of *Phormidium* sp. extract. Column legends and x-axis titles are shown in the graph

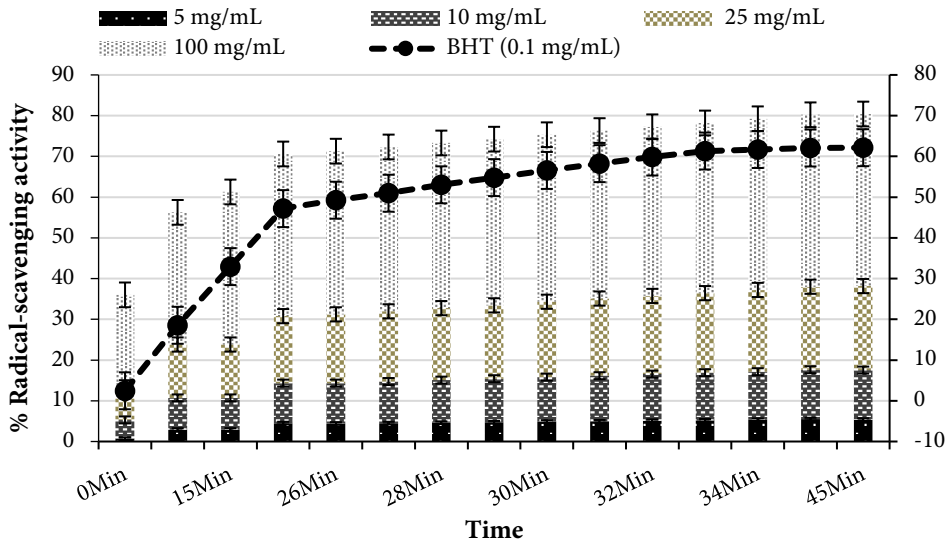

Figure 3. % Radical-scavenging activity of BHT and *Phormidium* sp. in a time-dependent manner

Table 2. Assignment data for all-trans and cis forms of carotenoids and their amounts (mg/g) in *Phormidium* sp.

Peak no.	Compound	Retention time (min.)	λ (nm, inline)				λ (nm, reported)				Q-ratio[e]	Amount (mg/g)
1	Auroxanthin	8.89	-	-	424	-	-	398	422[b]	-	0.35	0.000
2	Auroxanthin	10.47	-	-	424	-	-	398	422[b]	-	0.32	0.000
3	Cis-neoxanthin	13.73	-	396	416	-	-	411	429	459[c]	0.26	0.000
4	13-or-13'-cis lutein	20.57	-	-	443	459	-	415	440	464[b]	0.22	0.006
5	cis-lutein	22.63	339	-	-	458	331	-	440	467[a]	0.31	0.023
6	All-trans-lutein	24.42	-	-	451	477	-	423	446	470[d]	0.18	1.249
7	Cis-lutein	25.19	344	-	451	-	344	427	452	476[b]	0.15	0.000
8	All-trans-zeaxanthin	26.19	-	-	452	478	-	-	452	477[a]	0.19	0.074
9	9-or-9'-cis-lutein	27.66	-	-	460	-	332	416	440	470[b]	0.12	0.049
10	Cis-lutein	31.05	344	-	447	469	332	416	440	470[b]	0.14	0.100
11	All-trans-beta-cryptoxanthin	33.36	345	-	452	480		414	450	476[a]	0.16	0.005
12	13-or-13'-cis-beta-carotene	36.92	342	-	447	469	344	422	452	476[b]	0.12	0.079
13	All-trans-alfa-carotene	37.31	343	-	447	471	344	426	449	476[b]	0.11	0.000
14	All-trans-beta-carotene	41.31	-	-	454	480	350	430	458	482[b]	0.13	0.321
15	9-or 9'-cis-alfa-carotene	41.92	-	-	445	469	344	421	446	470[b]	0.15	0.000
16	9-or 9'-cis-beta-carotene	42.924	-	344	449	474	344	428	452	476[b]	0.17	1.101
Total												**3.031**

Note: a: A gradient mobile phase of methanol-acetonitrile-water (84:14:2, v/v/v) and methylene chloride (from 100:0, v/v to 45:55, v/v) was used by Aluç et al. (2018); b: A gradient mobile phase of methanol-acetonitrile-water (84:14:2, v/v/v) and methylene chloride (from 100:0, v/v to 45:55, v/v) was used by Inbaraj et al.(2006); c: A gradient mobile phase of methanol-2-propanol (99:1, v/v) and methylene chloride (from 100:0, v/v to 70:30, v/v)was used by Chen et al. (2004); d: A gradient mobile phase of methanol-2-propanol (99:1, v/v) and methylene chloride (from 100:0, v/v to 70:30, v/v) was used by Liu et al. (2004); e: Q-ratio is defined as the height ratio of the cis peak to the main absorption peak.

The IC50 values calculated of DPPH activity *Phormidium* sp. extract (IC50 127.60 mg/mL) and BHT equivalent values (14.32 µmol/g DW) as given in Table 3. The results of DPPH scavenging activity (%) of *Phormidium* sp. extract and both standard solutions were presented with positive and significant correlations in the DPPH and FRAP assays (Figures 2A and 2B). In addition, in Figure 3, the % radical scavenging activity of BHT and *Phormidium* sp. were given in a time-dependent manner. DPPH cleaning capacity in the literature; IC50 values of cyanobacterial species such as *Synechocystis*, *Oscillatoria*, and *Phormidium* have reported antioxidant values of 56.79-83.08 g/mL. As for our data, the IC50 value (IC50 127.60 mg/mL) was not as low as those reported in previous studies for other cyanobacterial species. It is also obviously understood that considering the DPPH assay, the extract of *Phormidium* sp. exhibited low activity compared to the BHT. Considerably high amounts of extracts (i.e., 100 mg/mL) reached only a maximum of 40.05% scavenging ability whereas standard of BHT (IC50: 0.19 mg/mL) had strong scavenging effects around (89.2%) despite their small amounts i.e., 0.8 mg/mL. The FRAP assay also generated similar results; the effect of *Phormidium* sp. extract being the lowest (i.e., 0.49 at 25 mg/mL) followed by BHT (0.43 at 0.1 mg/mL). The DPPH and FRAP methods were tested statistically using a paired T-test with a 95% confidence level to determine whether there was a significant difference in IC50 values. Results of the paired test showed an insignificant value ($p < 0.01$); This means that there is a significant difference in the antioxidant activity value of BHT and *Phormidium* sp. using the DPPH and FRAP methods. Based on the research of Maesaroh et al. (2018), the DPPH method has been shown to be more effective and efficient than the FRAP method. This is because the FRAP method is less sensitive to samples than the

DPPH method. In general, these two methods affect each other and may even be interchangeable (Maesaroh et al., 2018).

Table 3. IC50 values of *Phormidium* sp. and standards (BHT) in DPPH assay, and equivalent BHT of *Phormidium* sp. in both assays

Parameters	DPPH	FRAP
Phormidium sp. IC_{50} (mg/mL)	127.60	25.105
BHT Equivalent (µmol/g DW)	14.32	38.59

Babu & Wu (2008) applied a β-carotene-linoleate assay to identify the antioxidation activity of *M. aeruginosa, C. raciborskii, Oscillatoria* sp., *B. braunii* and were shown that the extracts of all four tested species exhibited positive antioxidation activity. They proposed in their studies that algal species produce a natural BHT that demonstrates antioxidant activity similar to that of synthetic BHT. In general, algae display two main types of defense mechanisms against ROS. These are defined as enzymatic and non-enzymatic antioxidant systems. It has been reported that the non-enzymatic antioxidant mechanism studied in *Ulva fenestrata* protects the cell from photooxidation by changes in the lipid composition of the cell under different radiations (Khotimchenko & Yakovleva, 2004). The enzymatic mechanisms studied in *Nodularia*, *Microcystis*, and *Anabaena* showed increases in ascorbate peroxidase and superoxide dismutase activity at high irradiance (Canini et al., 1996). Benedetti et al. (2010) reported the phycobiliprotein found in *Aphanizomenon flos-aquae*, a commercial microalgae species, is 10 times lower than that obtained from *Phormidium autumnale*.

A wide variety of factors play a role in the biosynthesis of carotenoids. Although species-specific varies, it can also differ in different environments and conditions of the same species. In addition, studies for the direct determination of carotenoids can reveal different variations due to stress conditions applied to potentially increase the carotenoid content and different media and culture conditions of the same species. The amount of total carotenoids that the string of a local species, *Phormidium* sp., contained could be considered as satisfactorily, relying upon comparisons to other species of cyanobacteria species. Considering that many other bioactive compounds are present in microalgal cells very high domination of lutein and β-carotene in the composition of total carotenoids ascertained for carotenoids, chlorophylls and phycocyanins that indicating the potential as a renewable source of these pigments.

Conclusion

Phormidium species can be considered an exciting crop for discovering bioactive compounds. Algae biotechnology can be made more proficient by getting over the disadvantages of traditional systems with advanced attempts that will also take into account some other factors such as high sustainable production potential.

Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Aluç, Y., Başaran Kankılıç, G., & Tüzün, İ. (2018). Determination of carotenoids in two algae species from the saline water of Kapulukaya reservoir by HPLC. *Journal of Liquid Chromatography & Related Technologies*, *41*(2), 93-100. https://doi.org/10.1080/10826076.2017.1418376

Babu, B., & Wu, J. T. (2008). Production of natural butylated hydroxytoluene as an antioxidant by freshwater phytoplankton. *Journal of Phycology*, *44*(6), 1447-1454. https://doi.org/10.1111/j.1529-8817.2008.00596.x

Batista, A. P., Gouveia, L., Bandarra, N. M., Franco, J. M., & Raymundo, A. (2013). Comparison of microalgal biomass profiles as novel functional ingredient for food products. *Algal Research*, *2*(2), 164-173. https://doi.org/10.1016/j.algal.2013.01.004

Benedetti, S., Benvenuti, F., Scoglio, S., & Canestrari, F. (2010). Oxygen radical absorbance capacity of phycocyanin and phycocyanobilin from the food supplement Aphanizomenon flos-aquae. *Journal of Medicinal Food*, *13*(1), 223-227. https://doi.org/10.1089/jmf.2008.0257

Bischoff, H. W., & Bold, H. C. (1963). Some soil algae from Enchanted Rock and related algal species. Phycological Studies IV. University of Texas Publication.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199-1200. https://doi.org/10.1038/1811199a0

Canini, A., Albertano, P., Leonardi, D., Di Somma, D., & Grilli Caiola, M. (1996). Superoxide dismutase in cyanobacteria of the Baltic Sea. *Algological Studies/Archiv für Hydrobiologie, Supplement Volumes*, *83*, 129-143. https://doi.org/10.1127/algol_stud/83/1996/129

Chacon-Lee, T. L., & Gonzalez-Marino, G. E. (2010). Microalgae for "healthy" foods-possibilities and challenges. *Comprehensive Reviews in Food Science and Food Safety*, *9*(6), 655-675. https://doi.org/10.1111/j.1541-4337.2010.00132.x

Chatterjee, D., & Bhattacharjee, P. (2014). Supercritical carbon dioxide extraction of antioxidant rich fraction from *Phormidium valderianum*: Optimization of experimental process parameters. *Algal Research*, *3*, 49-54. https://doi.org/10.1016/j.algal.2013.11.014

Chen, B. H., Yang, S. H., & Han, L. H. (1991). Characterization of major carotenoids in water convolvulus (*Ipomoea aquatica*) by open-column, thin-layer and high-performance liquid chromatography. *Journal of Chromatography A*, *543*, 147-155. https://doi.org/10.1016/S0021-9673(01)95763-2

Goiris, K., Muylaert, K., Fraeye, I., Foubert, I., & Cooman, L. D. (2012). Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *Journal of Applied Phycology*, *24*(6), 1477–1486. https://doi.org/10.1007/s10811-012-9804-6

Gong, M., & Bassi, A. (2016). Carotenoids from microalgae: A review of recent developments. *Biotechnology Advances*, *34*(8), 1396-1412. https://doi.org/10.1016/j.biotechadv.2016.10.005

González-López, C., Camacho-Rodríguez, J., López-Rosales, L., García-Camacho, F., & Molina-Grima], E. (2018). Maximizing carotenoid extraction from microalgae used as food additives and determined by liquid chromatography (HPLC). *Food Chemistry*, *15*, 257, 316-324. https://doi.org/10.1016/j.foodchem.2018.02.154

Hancock, J. T., R. Desikan, and S. J. Neill. (2001). Role of reactive oxygen species in cell signalling pathways. *Biochemical Society Transactions*, *29*(2), 345-349. https://doi.org/10.1042/0300-5127:0290345

Ho, S. H., Chan, M. C., Liu, C. C., Chen, C. Y., & Chang, J. S. (2014). Enhancing lutein productivity of an indigenous microalga *Scenedesmus obliquus* FSP-3 using light-related strategies. *Bioresource Technology*, *152*(1), 275-282. https://doi.org/10.1016/j.biortech.2013.11.031

Inbaraj, B. S., Chien, J. T., & Chen, B. H. (2006). Improved high performance liquid chromatographic method for determination of carotenoids in the microalga *Chlorella pyrenoidosa*. *Journal of Chromatography A*, *1102*(1-2), 193-199. https://doi.org/10.1016/j.chroma.2005.10.055

Khansari, N., Shakiba, Y., & Mahmoudi, M. (2009). Chronic inflammation and oxidative stress as a major cause of age- related diseases and cancer. *Recent Patents on Inflammation & Allergy Drug Discovery*, *3*(1), 73–80. https://doi.org/10.2174/187221309787158371

Khotimchenko, S. V., & Yakovleva, I. M. (2004). Effect of solar irradiance on lipids of the green alga *Ulva fenestrata* Postels *et* Ruprecht. *Botanica Marina*, *47*(5), 395-401. https://doi.org/10.1515/bot.2004.050

Leclercq, C., Arcella, D., & Turrini, A. (2000). Estimates of the theoretical maximum daily intake of erythorbic acid, gallates, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in Italy: A stepwise approach. *Food and Chemical Toxicology*, *38*(12), 1075-1084. https://doi.org/10.1016/s0278-6915(00)00106-x

Liu, H. L., Kao, T. H., & Chen, B. H. (2004). Determination of Carotenoids in the Chinese medical herb jiao-gu-lan (*Gynostemma pentaphyllum* MAKINO) by liquid chromatography. *Chromatographia*, *60*(7/8), 411-417. https://doi.org/10.1365/s10337-004-0418-2

Maesaroh, K., Kurnia, D., & Anshori, J. A. (2018). Perbandingan metode uji aktivitas antioksidan DPPH, FRAP dan FIC terhadap asam askorbat, asam galat dan kuersetin. *Chimica et Natura Acta*, *6*(2), 93-100. https://doi.org/https://doi.org/10.24198/cna.v6.n2.19049

Morone, J., Lopes, G., Preto, M., Vasconcelos, V., & Martins, R. (2020). Exploitation of filamentous and picoplanktonic cyanobacteria for cosmetic applications: Potential to improve skin structure and preserve dermal matrix components. *Marine Drugs*, *18*(9), 486. https://doi.org/10.3390/md18090486

Niyogi, K. K., Björkman, O., & Grossman, A. R. (1997). The roles of specific xanthophylls in photoprotection. *Proceedings of the National Academy of Sciences*, *94*(25), 14162-14167. https://doi.org/10.1073/pnas.94.25.14162

Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics*, *44*(6), 307-315. https://doi.org/10.5264/eiyogakuzashi.44.307

Patrick, L. (2001). Beta carotene: The controversy continues. *Alternative Medicine Review: A Journal of Clinical Therapeutic*, *5*(6), 530-545.

Putri, M. D., Arumasi, A., Kurniaty, N. (2020). Review artikel: Uji aktivitas antioksidan ekstrak daging buah semangka dan albedo semangka (*Citrullus lanatus*) dengan metode DPPH dan FRAP. *Prosiding Farmasi*, *6*(2), 992-997. https://doi.org/10.29313/.v6i2.24206

Rao, A. V., & Rao, L. G. (2007). Carotenoids and human health. *Pharmacological Research*, *55*(3), 207–216. https://doi.org/10.1016/j.phrs.2007.01.012

Richmond, A., & Hu, Q. (Eds.) (2013). *Handbook of microalgal culture: Biotechnology and applied phycology*. Wiley-Blackwell Publishing.

Rodrigues, D. B., Menezes, C. R., Mercadante, A. Z., Jacob-Lopes, E., & Zepka, L. Q. (2015). Bioactive pigments from microalgae *Phormidium autumnale*. *Food Research International*, *77*, 273-279. https://doi.org/10.1016/j.foodres.2015.04.027

Rodríguez-Meizoso, I., Jaime, L., Santoyo, S., Cifuentes, A., Reina, G. B., Señoráns, F., & Ibáñez, E. (2008). Pressurized fluid extraction of bioactive compounds from *Phormidium* species. *Journal of Agricultural and Food Chemistry*, *56*(10), 3517-3523. https://doi.org/10.1021/jf703719p

Shanab, S. M. M., Mostafa, S. S. M., Shalaby, E. A., & Mahmoud, G. I. (2012). Aqueous extracts of microalgae exhibit antioxidant and anticancer activities. *Asian Pacific Journal of Tropical Biomedicine*, *2*(8), 608-615. https://doi.org/10.1016/s2221-1691(12)60106-3

Soni, B., Trivedi, U. B., & Madamwar, D. (2008). A novel method of single step hydrophobic interaction chromatography for the purification of phycocyanin from *Phormidium* fragile and its characterization for antioxidant property. *Bioresource Technology*, *99*(1), 188-194. https://doi.org/10.1016/j.biortech.2006.11.010

Sri Mariani, N. R. d. S. (2018). Antioxidant activity test of watermelon (*Citrullus lanatus*) fruit extracts. *Jurnal Akademika Kimia*, *7*(2), 96-101.

Stange, C. (Ed.) (2016). *Carotenoids in Nature: Biosynthesis, Regulation and Function*. Springer.

Tahir, M., Heluth, A. C., & Widiastuti, H. (2018). Uji aktivitas antioksidan ekstrak buah semangka (*Citrullus lanatus*) dengan metode FRAP. *As-Syifaa Jurnal Farmasi*, *8*(1), 31-38. https://doi.org/10.33096/jifa.v8i1.155

Wojtasiewicz, B., & Ston-Egiert, J. (2016). Bio-optical characterization of selected cyanobacteria strains present in marine and freshwater ecosystems. *Journal of Applied Phycology*, *28*, 2299-2314. https://doi.org/10.1007/s10811-015-0774-3

Zuluaga, M., Gueguen, V., Pavon-Djavid, G., & Letourneur, D. (2017). Carotenoids from microalgae to block oxidative stress. *Bioimpacts*, 7(1), 1–3. https://doi.org/10.15171%2Fbi.2017.01