Exploration of using the algal bioactive compounds for cosmeceuticals and pharmaceutical applications

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Purpose
The research is aimed at describing the extraction and determination methods of carotenoids, fatty acids, phenolic, flavonoids, proteins, and carbohydrates as bioactive compounds from different microalgae species grown in different media.
Materials and methods
The microalgae used in this study are *Scenedesmus* spp. and *Spirulina platensis*, which are microalgae species of freshwater; *Dunaliella salina* and *Oscillatoria limnetica*, which are of salt stress media; and microalgae community Bloom of municipal wastewater plant at Zenin, Giza. Carotenoids were extracted using jojoba oil as fatty oil, and their contents were determined using high-performance liquid chromatography analysis. From the cell residue, total lipid was extracted by ultrasonic bath with cosolvent mixture of *n*-hexane and isopropanol (3 : 2 v/v). The fatty acid profile is determined by gas chromatographic analysis. Phenolic contents are determined with Folin–Ciocalteu reagent after preparing the ethanolic extract. Methanolic extraction was done for determination of total flavonoid contents by colorimetric method. Total protein was determined by Kjeldahl method, and finally, total carbohydrate content is evaluated after extraction with barium carbonate by spectrophotometer at 485 nm.
Results and conclusion
The results of each assay showed that *D. salina* and *O. limnetica* accumulated carotenoids more than microalgae cells of Bloom, which have carotenoids content more than *Scenedesmus* spp. and *S. platensis* (151.4, 136.3, 123.4, 11.93, and 7.5 μg/g, respectively). Gas chromatographic analysis of extracted lipid exhibited the high percentage of polyunsaturated fatty acids (ω-3 and ω-6) found in *O. limnetica* and Bloom. Total phenolic compounds were presented in amounts higher than flavonoids in all the studied strains. All of the biomass of microalgae that was subject to analysis had a relatively high protein content, where *Scenedesmus* spp. had the highest value (210 mg/g) and *D. salina* the lowest (21.5 mg/g. The total carbohydrate content, which plays an important role in cosmetics products, was detected at the highest concentration in *Scenedesmus* spp. (6.6 mg/g), followed by *S. platensis* (6.0 mg/g), *O. limnetica* (3.5 mg/g), *D. salina* (2.3 mg/g), and Bloom (1.5 mg/g). The results obtained in this study prove that microalgae are a rich source of bioactive compounds for pharmaceutical and cosmeceutical industries.

Keywords:
bioactive compounds, carotenoids, cosmeceuticals and pharmaceuticals applications, jojoba oil, microalgae

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Introduction

Microalgae are perfect agents for large-scale production of valuable compounds because they grow fast with minimal nutrient requirement. Generally, several species are regarded as safe for human consumption.

In recent years, microalgae have gained attention as an alternative source of fuel, and as a natural source of several interesting valuable compounds relevant to human health such as essential fatty acids (EFAs), carotenoids, phenolic, flavonoids, carbohydrates, and proteins [1].

Carotenoids are a group of natural pigments that are responsible for the color of the different natural matrices. The carotenoid structure consists of eight isoprenoid units that constitute the skeleton of the carotenoid compound with a long chain of conjugated double bonds, and such arrangement is responsible for the antioxidant activities of carotenoids [2].

In microalgae biotechnology, the production of carotenoids has been one of the successful activities [3].

Carotenoids like β-carotene and lycopene act as antioxidant in photo-oxidation by quenching singlet mole oxygen and proxy radical during peroxidation [4].

β-Carotene is the pigment responsible for the orange color of carrots, and it is found in many other fruits and vegetables. It is a precursor of vitamin A and used in the formulation of cosmetics and personal care products as aftershave lotions, makeup, hair conditioners, shampoos, skin care, and suntan products [5].

Astaxanthin is one of the valuable carotenoids produced from microalgae. It has a pinkish-red color and is responsible of salmon, trout, and shrimp color. Astaxanthin is considered as an antioxidant having highly efficient properties of scavenging of free radicals buildup within the human body [6]. Astaxanthin protects the skin against ultraviolet (UV)-induced photo-oxidation, and it is used for the treatment of damage related with age. So, it is considered a natural compound that enhances the athletic performance by increasing the power and decreasing the time of muscle recovery [7].

Lutein and zeaxanthin belong to carotenoids that have a role as photosynthetic accessory pigments in the plant and have extensive applications in nutraceutical and pharmaceutical industries. They are well known for their role in eye health. Several medical studies have proved that lutein and zeaxanthin protect the macula against photo-oxidative damage, which can cause age-relate macular degeneration and blindness, because they act by absorbing the damaging blue and near-UV light in human retina, where these carotenoids are concentrated in the macula luteal or 'yellow spot' [3,8].

Lutein is present in significant quantities in the skin, breast, and brain. Lutein has an important role in skin health owing to its ability to absorb high energy of blue-light and scavenging of free radicals that develop from the exposure to sunlight [3,9].

Phenolic compounds include more than 8000 compounds significantly different in structure. According to their chemical structure, they can be divided into different classes. They are considered as natural antioxidants through single electron transfer and hydrogen atom transfer [10]. Phenolic compound concentration in microalgae is lower than that in terrestrial plants [10]. Some studies showed that in microalgae the complex phenolic compounds may be produced from several classes of flavonoids. So, the identification and characterization of phenolic compounds in microalgae cells are desired, where they may contain new compounds of phenolic group [1].

The flavonoids compounds that are present in the algal cells have an important as natural products; they are the essential components in different products of cosmeceutical, pharmaceutical, and nutraceutical industries owing to their characterizations as antioxidant, anticarcinogenic, and anti-inflammatory [10].

The microalgae cells have highly valuable proteins for human health with low-cost production, such as vaccines, therapeutic proteins, and antibodies [11]. Proteins perform a large fraction of the biomass although they are generally undervalued compared to minor products such as omega fatty acids and carotenoids [12].

Microalgae accumulate carbohydrates in several forms, such as starch, sugars including glucose, and polysaccharides [13]. Algal biomass contains proteins and carbohydrates, which are considered a large fraction of the biomass [14]. The composition of carbohydrates (mainly starch and cellulose) in microalgae may differ significantly from species to species [15] and also according to growth conditions [16].

The microalgae contain minerals, fats, proteins, carbohydrates, and bioactive compounds such as antioxidants (phenols and flavonoids) and pigments (carotenoids, chlorophylls, and phycobilin) which possess several properties such as antiviral, anti-inflammatory, antibacterial, and antioxidative [17].

The extract of microalgae is rich in some health molecules like EFA, amino acids, and vitamins (A, B, C, and E) [18], which are important for improving the cosmeceutical products [19]. The marine algae are considered as not only sea vegetables for consumption but also as medicinal [20] for skin diseases.

Algae species that are found in both marine and freshwater environments transform solar energy through photosynthesis process into chemical energy which is stored in the form of chemical compounds with particular biological activities, termed bioactive compounds. So, they have emerged as promising organisms for many different applications. Microalgae are considered as a natural source of a variety of drugs for pharmaceutical and nutraceutical industries for health treatments [21]. The bioactive

compounds that are derived from microalgae biomass have anti-inflammatory, antioxidant, and antimicrobial properties [22–25].

The research is aimed at describing the extraction method of bioactive compounds from different algal species and determining their percentage. Moreover, the recent cosmetic and pharmaceutical uses of bioactive compounds from algae species extracts would be cleared through the study.

Materials and methods

Dry biomass of microalgae *Scenedesmus* spp. was obtained from the Algal Biotechnology Unit, NRC, Giza, Egypt.

Spirulina platensis dry powder was obtained from the Microbiology Department Soils, Water and Environment Research Institute, ARC, Giza, Egypt

Microalgae community (Bloom) was collected from the high-rate algal pond constructed to treat municipal wastewater at Zinin Wastewater Treatment Plant, Giza, through the project of 'Biodiesel production from microalgae in stabilization pond for municipal wastewater treatment' in Water Pollution Research Department, NRC, and Giza, Egypt.

Dunaliella salina and *Oscillatoria limnetica* microalgae spp. were obtained as slurry from Botany and Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Egypt.

Jojoba oil, citric acid, potassium hydroxide, and solvents used for extraction were of analytical grade and those used for chromatography were of high-performance liquid chromatography (HPLC) grade.

The wet microalgae biomass was dried over night at 60°C. All the aforementioned algal species were subjected to the following experiment work.

Extraction of algal bioactive compounds

Carotenoids

A volume of 10 ml of distilled water was added to 10 g of algae cells for slurry, 5 g of dry granular citric acid added to the slurry, and Jojoba oil was added as 1 : 1 w/w of algal biomass.

The mixture was homogenized at 1000 rpm for ~15 min (Homogenizer Model Wise Tis HG-150, Germany). The slurry reaction mixture is stirred for 45 min at temperature 80°C, the mixture is neutralized using potassium hydroxide, and then the temperature is reduced to 45°C.

Centrifugation of the mixture was done to obtain two layers: the upper layer was the carotenoid with jojoba oil and the lower layer was biomass and water. Decantation of the upper layer was done, and it was stored in a bottle at −20°C until HPLC analysis. The remaining biomass was used for procuring lipid, phenolic, flavonoid, protein, and carbohydrate.

Ultrasound-assisted bath lipid extraction

The remaining algal biomass is mixed with 60-ml mixture of *n*-hexane and isopropanol (3 : 2 v/v). The extraction process is performed in the ultrasonic bath (model WUC-D10H, 60 Hz, 230 v, 665 W, 3 amps) at room temperature (30–35°C) under reflux condenser for 30 min. Cells residues were removed by filtration. The filtrate of solvent mixture was evaporated using a rotary evaporator to enable gravimetric quantification of total lipid extract. The crude lipid was re-dissolved in *n*-hexane (~5 ml) for further analysis.

Phenolic compounds

Overall, 4 g of algae cells was resuspended in 20 ml of 80% ethanol, and the mixture was homogenized for 3 min at 4°C to disrupt the cells. The homogenate was centrifuged at 2000 rpm for 15 min at 4°C.

The resulting supernatant was centrifuged again at 2000 rpm for 10 min, and the residue was repeatedly extracted with the same solvent until it was colorless. Then, the supernatant was filtered through Millipore filters, Sigma Aldrich Company, Germany. The filtrate was evaporated to dryness to give a crude algal ethanolic extract (enrich in phenolic compounds) and immediately analyzed [26,27]. Total phenolic contents were determined with Folin–Ciocalteu reagent [27] using gallic acid as a standard phenol compound. The concentration of total phenol contents was measured as milligram of gallic acid equivalent (in mg/g of the sample). All the determinations were carried out in triplicates.

Flavonoids [28]

One gram of algal biomass was crushed in motor, and 5 ml of distilled water was added to it. The mixture was centrifuged at 1000 rpm for 15 min and incubate at 4°C for 20 min. Then it was transferred to water bath adjusted at 100°C for 20 min. The supernatant was filtrated through filter paper no. 1. The filtrate was adjusted to 25 ml with 95% methanol solution and incubated at room temperature for 48 h for determination of

flavonoid content (FC). Total flavonoid was measured by a colorimetric assay according to Dae-Ok *et al.* [28] with some modifications.

Determination of the content of bioactive compounds

High-performance liquid chromatography

HPLC analysis was carried out using Agilent Technologies (California, USA) 1100 series liquid chromatograph equipped with an autosampler and a diode-array detector (461 nm). The analytical column was Eclipse XDB-C18 (150×4.6 μm; 5 μm) with a C18 guard column (Phenomenex, Torrance, California, USA). Chromatograms were monitored at 450 nm; the mobile phase was acetonitrile–2-propanol–ethyl acetate (40 : 40 : 20, v/v/v); the flow rate was 0.8 ml/min; the pressure was 850–1050 Psi; and the recorder chart speed was 0.5 cm/min. The carotenoid extracts obtained as mentioned before were dissolved in mobile phase (1 ml), filtered through a 0.45-μm membrane disc (Schleicher and Schuell, Dassel, Germany), and injected into chromatograph (injection volume: 10 μl). The column was regenerated by washing with 2-propanol after analysis, and then equilibrated with the mobile phase.

Standard solution

Standard of β-carotene (1 g enclosed in vial) was obtained from Sigma Aldrich company. Stock solution of β-carotene was prepared by weighing 10 mg in 100 ml petroleum ether (40–60°C). The concentration of the standard solution was 100 ppm. The stock solution was diluted to different known concentration, for example, 20, 40, 60 ppm, and dilutions were obtained in 5 ml of each petroleum ether [29]. Standard calibration graphs were prepared for β-carotene by plotting peak area measurements at 450 nm versus concentration. Linearity, reproducibility, and recovery were determined routinely [30].

Gas chromatography analysis

The FAs profiles of algal oil are determined using gas chromatography (GC) provided with a split automatic injector and silica capillary column DB-5 (length: 60 m; ID: 0.32 mm). Helium is used as carrier gas at a flow rate of 1 ml/min. The column was held at 150°C for 1 min and ramped to 240°C, at a rate of 30°C/min, and held at 240°C, for 30 min. Standards are used to give rise to well-individualized peaks that allow identification of the FAs composition.

Spectrophotometric determination of total phenolic content

Total phenols in the algae extracts were determined in triplicate by the Folin–Ciocalteau reagent using gallic acid as a standard [31]. The extract was diluted in ethanol as 1 : 10 g/ml; 0.5 ml was taken of it and mixed with 5 ml Folin–Ciocalteau reagent (1 : 10 diluted with distilled water) and 4 ml of 1-M Na_2CO_3 solution. Distilled water was added until a final volume of 10 ml was reached. The mixture was heated for 5 min in a 50°C thermostatic water bath. After cooling at room temperature, absorbance was measured at 765 nm. Using a UV-1203 spectrophotometer (Shimadzu Corporation, Japan). the standard curve of gallic acid with different known concentrations, and the total phenol values were expressed as milligram of gallic acid equivalent to gram of dry mass. Folin–Ciocalteau reagent consists of 100 ml of sodium tungstate dihydrate, 25 ml of sodium molybdate dihydrate, 50 ml of 85% phosphoric acid solution, and 100 ml of 36% hydrochloric acid solution. The volumes of control solution used ranged from 5 to 400 μl

Colorimetric assay for total flavonoid content

Total FC of algal extract was determined by following colorimetric method with some modifications [32]. A volume of 20 μl of extract was added to a 1-ml volumetric flask and thoroughly mixed with 20 μl of 10% aluminum chloride, 20 μl of 1 mol/l potassium acetate, and 180 μl of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was recorded at 415 nm. The calibration curve was prepared by using Rutin methanolic solutions at concentrations of 12.5–100 μg/ml. FC was expressed as milligram Rutin equivalents per gram of dried extract (mg RE/g) [33].

Determination of total protein content

Total protein content was determined by Kjedahl method and then multiplied with a factor of 6.25 to give the total protein content according to Chapman and Pratt [34]. Overall, 0.5 g of catalyst and 2 ml of H_2SO_4 were added to 0.2 g of algal sample to digest until the color become clear. Two drops of methyl orange indicator and 15 ml of 40% NaOH solution were added to the clear sample and transfer to distillation apparatus. The liberated ammonia is received in 10 ml of 4% boric acid and two drops of Tashiro's indicator. When the liberation of ammonia stopped, the solution titrates against 0.01N HCl.

Catalyst: 10 g; K_2SO_4+1 g $CuSO_4.5H_2O$+0.5 g selenium.

Tashiro's indicator: 0.24 g methylene blue+0.375 g methyl red in 30 ml methyl alcohol.

Total carbohydrates content [35]

A volume of 25 ml of 1-N H_2SO_4 was added to 0.1 g of algae sample, and the mixture was hydrolyzed for 2 h on a boiling water bath, a flocculent precipitate was noticed at the end of hydrolysis. This precipitate was freed of sulfate by precipitation with barium carbonate. The solution was filtered and complete to 100 ml with distilled water. One milliliter of filtrate was mixed with 1 ml 5% phenol solution and 5 ml concentrated H_2SO_4 and measured by spectrophotometer at 485 nm.

Results and discussion

Carotenoid profiles

The carotenoids composition varied between different microalgae strains even if they were of the same class. The main carotenoids in chlorophyta (green) and cyanophyte (blue-green) microalgae classes in our study were β-carotene and lutein (Table 1).

In algal samples of *Scenedesmus* spp., Bloom, and *D. salina*, which belong to chlorophyta class, β-carotene concentrations in *D. salina* (141.3 μg/g) were higher than that of Bloom (72.3 μg/g) and *Scenedesmus* spp. (9.9 μg/g). At the same time, it was found that *D. salina* accumulated lutein less (10.1 μg/g) than Bloom (36.4 μg/g), which also contained an amount of lycopene (14.7 μg/g). In cyanophyte strains, *O. limnetica* cells contained large amounts of β-carotene (102.6 μg/g) and (lutein 33.7 μg/g) than the other strain from the same class (*S. platensis*).

The β-carotene production was highly affected by the growth conditions such as starvation, light intensity, and salt stress [10,36–39].

D. salina was the most attractive which accumulated higher carotenoids under salt stress conditions (70 g/l NaCl) [40,41], and it is famous for its ability to accumulate β-carotene [10,42]. *O. limnetica*, which grow in salty media of 30 g/l NaCl, accounted for high amounts but not as much as in *D. salina*.

The microalgae community (Bloom) synthesize a significant quantity of β-carotene to protect themselves from damage caused by stress from their cultural environment, such as nutrient depletion and high light irradiance [40,43].

S. platensis and *Scenedesmus* spp. were grow in normal culture conditions without stress or starvation, so they accumulated the lowest amounts of carotenoids among the other species under study.

There is no standard extraction procedure for carotenoids because of the different and the existence of *cis-trans* isomers forms of the carotenoid compounds. Carotenoids are polyisoprene compounds with skeleton of C_{40}. The hydrocarbon carotenoids, named carotenes, consisting of C and H, and xanthophyll carotenoids, which have some oxygen groups such as hydroxyl and ketone groups.

Jojoba oil was chosen as an extraction solvent of carotenoids from the aforementioned algal cells. Jojoba oil is used medicinally because of the following [44]: (a) contains several vitamins (E, C, and B complex); (b) has few amounts of minerals such as tin, copper, sulfur, silicon, chromium, and cobalt; (c) is a powerful anti-inflammation agent because the presence of a high percentage of iodine; (d) is a liquid wax similar to person serum and highly penetrating; and (e) has a long shelf life and antioxidant activity.

The conventional extraction method of carotenoids using organic solvents is sufficient, but not acceptable to customers who are claiming natural products through environment friendly conditions [45,46].

HPLC analysis for extracted carotenoids showed some unidentified carotenoid peaks, which represent approximately 14, 28, 10, and 40% of total carotenoids percentage of *Scenedesmus* spp., Bloom, *D. salina*, and *S. platensis*, respectively (Table 1). The detected carotenoids were the most valuable

Table 1 Carotenoids contents detected (μg/g) and the percentages of undetected carotenoids

Algal strains	β-Carotene orange-red (μg/g)	Lutein yellow (μg/g)	Lycopene red (μg/g)	Total carotenoids detected (μg/g)	Undetected carotenoids (%)
Chlorophyta					
Scenedesmus spp.	9.9	2.03	ND	11.93	14
Bloom	72.3	36.4	14.7	123.4	28
Dunaliella salina	141.3	10.1	ND	151.4	10
Cyanophyte					
Oscillatoria limnetica	102.6	33.7	ND	136.3	0
Spirulina platensis	7.5	ND	ND	7.5	40

and required as natural coloring agents produced by microalgae [47].

The natural β-carotene is a mixture of *trans* and *cis* isomers [48,49], has anticancer activity, and is absorbed by the body 10 times more easily than synthetic one [50,51].

Each molecule of carotenoids can produce two molecules of vitamin A, so carotenoids possess higher activity than that of vitamin A, which is essential for vision and correct functioning of the immune system. It is considered as one of the health food colorants because of its strong antioxidant capacity, which reduces the harmful effects of free radicals in various disorders. Furthermore, β-carotene could enhance the immunity against different infectious diseases [51,52].

Lutein is found in microalgae species of the study, except *S. platensis*. Several studies have concluded that lutein, like zeaxanthin, are yellow pigments that are responsible for the maintenance of the natural visual role of eye macula of humans [53,54], where other carotenoids are not present or found in few amounts [55]. The eye macula is protected against any adverse photochemical reactions because of the antioxidant activity of carotenoids. The main source for the vision loss in persons older than 65 years old has been attributed to the reduction of zeaxanthin and lutein levels [56,57].

Lycopene was present in Bloom microalgal biomass, and it has a red color, and according to different studies, this compound significantly decreased the proliferation of prostate cancer in mice [58].

Lycopene reduced cholesterol by lowing the density of lipoprotein level and improved rheumatoid arthritis [58–60].

The carotenoids cannot be synthesized again by human or animal and can only be obtained through the food or metabolic pathway of the precursor compounds [51].

The carotenoids that have been extracted from microalgae are considered ecofriendly colorants, so can be safely used in the food industries instead of the synthesized pigments. Previous studies have proved that carotenoids can treat the oxidation damage caused by free radicals [61,62].

The advantages of natural carotenoids make it suitable for several pharmaceutical products. The biological features of algal carotenoids, such as anti-inflammatory antioxidant, antitumoral, and vitamin A activities, contribute to the quality of the product. Moreover, the natural carotenoids have several applications in cosmetology, as antiaging and sunscreen compounds (Fig. 1).

Many studies have established that carotenoids participate significantly in the total antioxidant ability of microalgae and used a different algae strains to investigate the possibility of using the biomass as a source of bioactive compounds [63].

Total lipid compositions in the algae species: the extraction of total lipid from the microalgae cells after extracting carotenoids content decreased the lipid percentage obtained owing to the extraction a part of lipid with carotenoids.

Figure 2 represents the percentage of total lipid in studied algal strains, where *D. salina* produced the highest lipid percentage (6.5%) followed by *O. limnetica* (6%), *S. platensis* (5.3%), and Bloom (5%), with *Scenedesmus* spp. having the lowest one (4.3%).

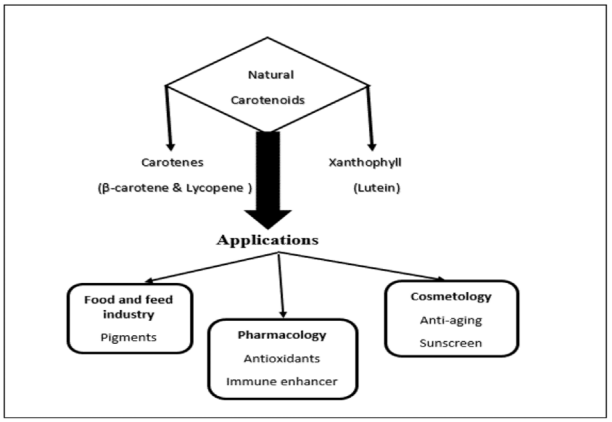
Separately, lipids of each strain provided by GC are tabulated to demonstrate FA profiles (Table 2).

As shown in Table 2, all advised microalgae strains contain valuable FAs for human health with variable quantities.

They have omega-9 FAs, which are a family of monounsaturated FAs, and are not similar to EFAs, because they can be created by the human body from unsaturated fat.

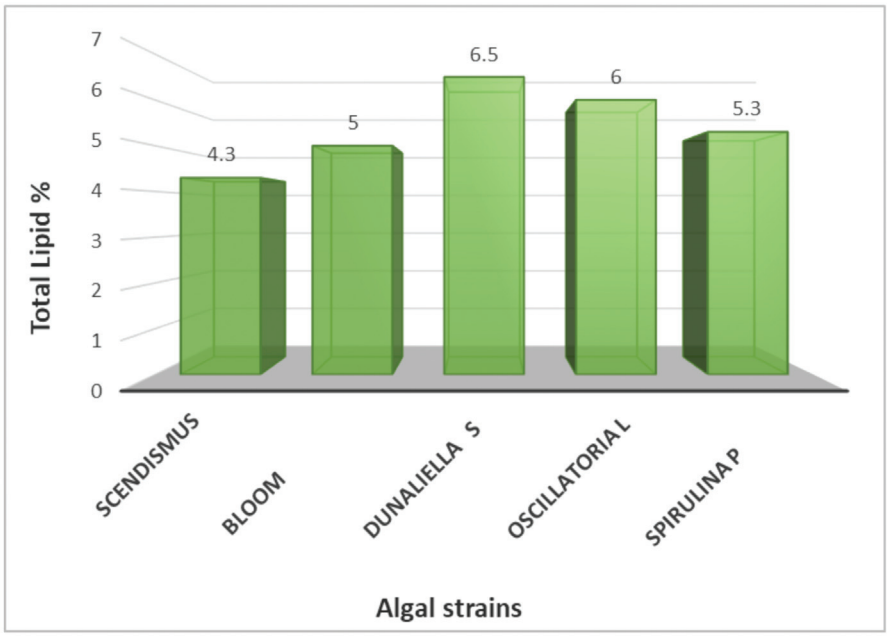
Polyunsaturated FA were present in amounts higher than the monounsaturated FA, as evidenced in Fig. 3,

Figure 1



Biological uses and applications of natural carotenoids in examined microalgae.

Figure 2



Total lipid percentage in examined algal strains.

Table 2 Fatty acids composition of microalgae lipid

Fatty acid	Common name	*Oscillatoria limnetica*	*Dunaliella salina*	*Scenedesmus* spp.	*Spirulina platensis*	Bloom
C:10	Caprice A.	5	1.4	0.5	–	–
C:12	Lauric A.	1.6	2.7	2.1	1	2.4
C:14	Myristic A.	2.4	17.8	3.0	1.2	1.5
C1:6	Palmitic A.	30.6	27.6	40	38	36
C:17	Margaric A.	1.5	3.9	1.3	1.1	1.5
C:18	Stearic A.	1	1	5.5	9.5	4.0
C:22	Behenic A.	11.6	2.3	–	–	–
C:24	Lignoceric A.	–	1.4	0.4	0.5	0.5
C16:1 (ω-7)	Palmitoleic A.	–	3.6	4.6	6.0	5.1
C18:1 (ω-9)	Oleic A.	6.5	8.4	11.5	10	12
C18:2 (ω-6)	Linoleic A.	17.8	10.5	13.2	12.0	11.5
C18:3 (ω-3)	Linolenic A.	11	12.1	8.0	9.5	14.7
C20:1 (ω-9)	Gondoic A.	3.5	4.2	–	–	–
C20:4 (ω-3)	Arachidonic A.	4.5	–	–	–	–
C22:5 (ω-3)	Docosapentaenoic	–	–	1.0	0.8	2.0
Saturated FA		53.7	58.1	52.8	51.3	45.9
Monounsaturated FA (ω-9)		10.0	16.2	16.1	16.0	17.1
Polyunsaturated F A (ω-3,6)		33.3	22.6	22.2	22.3	28.2
Total FA		97.0	96.9	91.1	92.7	95.4

FA, fatty acid.

and because they cannot be manufactured by the body, they are considered as EFAs.

The EFAs, ω-3, and ω-6 in particular, which are found in large amount, are very important for the integrity of tissues. γ-Linolenic acid has some cosmetic applications like revitalizing the skin and slowing aging. Linoleic and linolenic acids are essential nutrients for the immune system and tissue regeneration processes. Linoleic acid is also used for the treatment of hyperplasia of the skin [64]. Most studies showed that dietary ω-3 polyunsaturated fatty acids from microalgae have a protective effect against heart diseases besides reducing hypertension, and also important in the development and functioning of the nervous system [63].

Total phenolic content

The total phenolic concentrations of investigated algal cells based on Folin method were varied from one strain to another (Fig. 4). This agrees with previous studies, which demonstrated that the growth conditions and the type of species are affected in the concentration and composition of phenolic compounds of microalgae biomass [38]. Algal biomass of Bloom was higher phenolic content (5.36 mg/g) and *Scenedesmus* spp. had a lower value (2.9 mg/g). The studies also showed that there is a significant relationship between total antioxidant activity and total phenolic compounds [38,65].

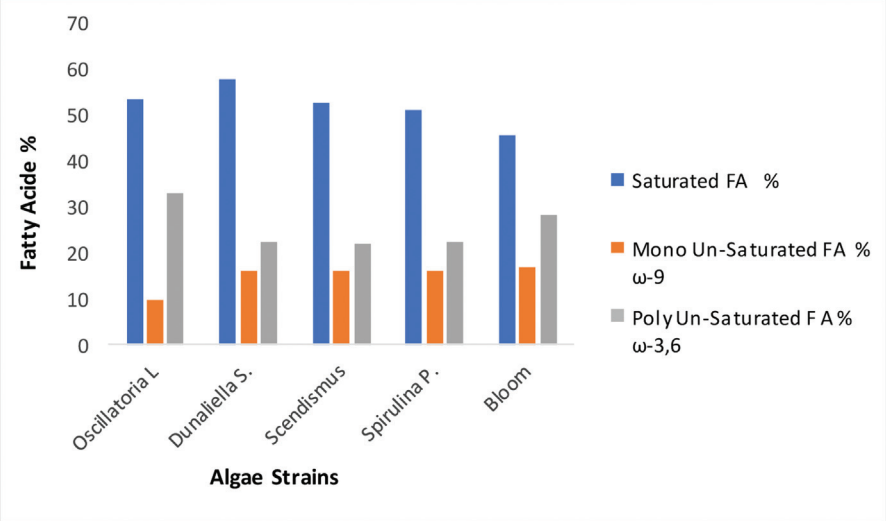
Microalgae contain a variety of phenolic classes [66]. Phenolic compounds are considered to be most significant and biologically active compounds with various health beneficial properties [66].

Many researches proved that a high dietary with natural phenolics is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, various types of cancer, diabetes, obesity, improved endothelial function, and reduced blood pressure [67–70].

Total flavonoid compound

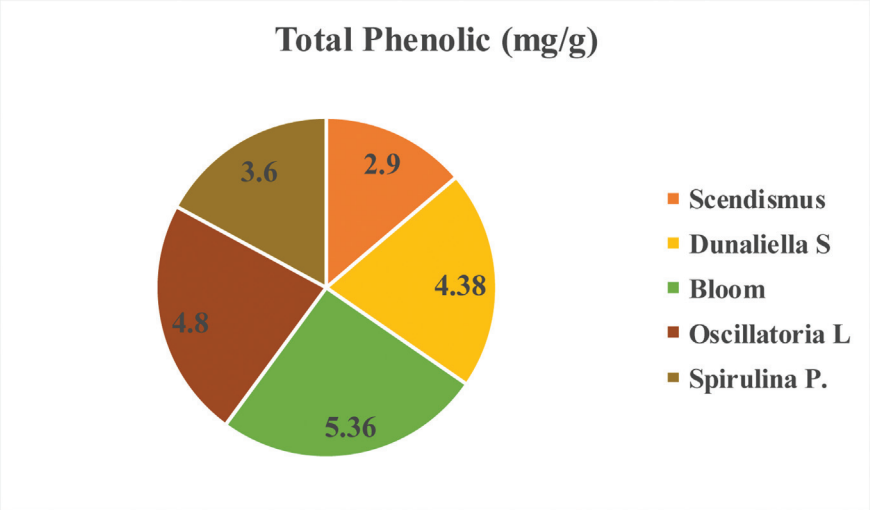
The colorimetric determination of FC in microalgae biomass, as shown in Fig. 5, clarifies a variation in FC, which may be because of the difference in physicochemical parameters of cultivation between the

Figure 3



Percentage of different fatty acids profile in microalgae strains of study. FA, fatty acid.

Figure 4



Total phenolic determined in algal strains.

selected strains. *S. platensis* recorded the highest value of FC (2 mg/g) and *O. limnetica* showed the lowest (1.3 mg/g). Flavonoids are secondary metabolites and have the capacity to act as strong antioxidants capable to scavenge free radicals, which are harmful to cell of human body and food products [71].

With the increasing demand of natural products as antiaging agents in pharmaceuticals, nutraceuticals, and cosmetic industries, flavonoids are gaining vital importance with their multiple activities such as antimicrobial, anticancer, and antidiabetic.

Total protein content

Determination of protein content in studied microalgae biomass showed (Table 3) that there was a significant difference between the protein values of the studied strains as follows: *Scenedesmus* spp. have the highest value of protein (210 mg/g), followed by *S. platensis* (177.9 mg/g), *O. limnetica* (67.3 mg/g), Bloom (54.3 mg/g), and *D. salina* (21.5 mg/g).

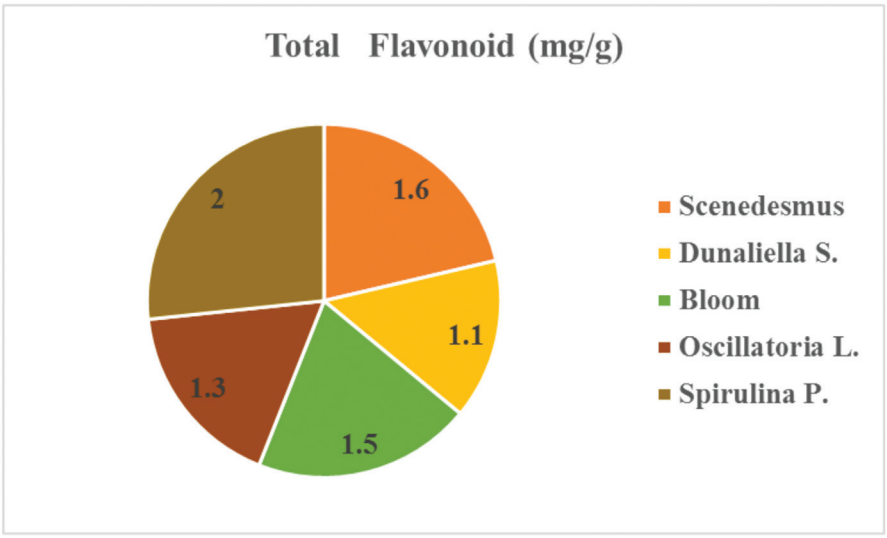
The high protein content of various microalgal species is one of the main reasons to consider them as an unconventional source of protein [12]. The nutritional quality of proteins is determined basically by their amino acids content where, proteins are composed of different amino acids [72]. Microalgae are able to enhance the nutritional value of conventional food preparations and hence have a positive effect on the health of humans and animals.

Total carbohydrates content

The microalga biomasses contain different amounts of carbohydrates (Table 4) because of the various growth conditions. As in all green plants, one of the most important cell components of microalgae is carbohydrates, which are found in the form of starch, glucose, sugars, and other polysaccharides. Certain polysaccharides have medical effects and functions such as protection against oxidative stress and efficacy in gastric ulcers, wounds, and constipation [73]. From the economical point of view, algal polysaccharides are the most important products manufactured by algae [74].

Carbohydrates are considered active raw materials that play an important role in cosmetics products such as deodorant, hair conditioning, hair waving or straightening, emulsifying, binding agent, viscosity controller, and stabilizing for gel forming, and also

Figure 5



Total flavonoids detected in algal strains.

Table 3 Total protein content of algal biomass

Algal species	Total protein (mg/g)
Scenedesmus spp.	210
Bloom	54.3
Dunaliella salina	21.5
Oscillatoria limnetica	67.3
Spirulina platensis	177.9

Table 4 Total carbohydrates of algal biomass

Algae strains	Carbohydrates (mg/g)
Scenedesmus spp.	6.6
Bloom	1.5
Dunaliella salina	2.3
Oscillatoria limnetica	3.5
Spirulina platensis	6.0

for skin products such as skin conditioning and emollient. Natural carbohydrates are characterized by antibacterial, antioxidative, anti-inflammatory, antitumor, and antiviral properties [75,76].

Conclusion

The microalgae are promising agents owing to increased demand for bioactive compounds like carotenoids, phenolic, flavonoids, proteins, and carbohydrates. Isolation and identification of novel metabolites from microalgae will help to the development of new therapeutic agents, nutraceutical, and food industries. The culture conditions of microalgae are strongly species dependent and significantly affect their metabolic pathways. Carotenoids in algal cells that are cultivated under salt stress conditions are present at very high concentrations than extracted from freshwater microalgae. The community of microalgae cells that is collected from municipal wastewater plant contains relatively high amount of different types of carotenoids. Carotenoids are soluble in fatty oils, so jojoba oil can extract carotenoids sufficiently. The extraction of carotenoids should be done before lipid extraction to obtain a higher amount of valuable carotenoid pigments. The natural active products are finding an extent range of applications in the cosmetics, pharmaceuticals, food, and feed industries.

Carotenoids, phenolic, and flavonoid are strong antioxidants, capable of reacting with scavenging oxygen species because of their hydroxyl groups. Several compounds extracted from algae are used in cosmetic industry as thickening agents, water-binding agents, and antioxidants in facial and skin care products. Natural carotenoids are preferred than synthesized carotenoids and have good applications in the food and feed industries, as well as in cosmetics and pharmaceuticals.

It is well known that microalgae contain valuable products including proteins, β-carotene, lutein, carbohydrate, phenolic, and flavonoids compounds that are useful for various industrial applications.

Carotenoids from microalgae are an essential class of antioxidants which play an important role in quenching reactive oxygen species generated during photosynthesis.

Recommendations

Further studies are required in the future to develop the beneficial uses of algae products.

The process of production of the algal bioactive compounds should be performed on a pilot plant scale to obtain chemical engineering data to design the scaling-up process.

Technoeconomic evaluation should be achieved to prove the feasibility of using wastewater microalgae in bioactive compounds production.

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Conflicts of interest

There are no conflicts of interest.

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