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Full Length Research Paper

Characterization of nutraceutical compounds in blue green alga *Spirulina maxima*

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This work was conducted to evaluate the influence of culture conditions (nitrogen concentrations in growth medium) on characterization of some nutraceutical compounds in algal *Spirulina maxima*. Antioxidant and antibacterial activities as well as chemical composition of organic extracts obtained from different cultures were examined. The amounts of total carotenoids, chlorophylls-derived and phenolic compounds were associated inversely with concentration of nitrogen in growth media. The antibacterial results showed that all *S.maxima* extracts exhibited great potential antibacterial activities against six bacterial strains with inhibition zones ranged 7 - 18 mm and MICs values ranged 30 - 40 µg/mL. Also, all *S.maxima* extracts possessed potent antioxidant properties as comparable to commercial antioxidants. The chromatographic analyses of *Spirulina* organic extracts with TLC and HPLC showed that carotenoids, chlorophyll-derived and phenol compounds were presence as main constituents and their quantities was significantly changed dependant on culture conditions. Thus, it could be suggested that the *Spirulina* is useful bio-system for production bioactive compounds possess an antioxidants and antimicrobial principles and as natural pigments.

Key words: *Spirulina maxima*, organic extract, antioxidant activity, antimicrobial activity.

INTRODUCTION

Several studies have focused on physiological properties of some valuable antiviral or antioxidant compounds in blue green alga *Spirulina* (Ozdemir et al., 2004; Khan et al., 2005 and 2006). The occurrence of many compounds possess antioxidant activity in biological systems in higher plants is well documented, while in microalgae are less documented (Colla et al., 2007). *Spirulina platensis* or its extracts show therapeutic properties, such as the ability to prevent the incidence of cancers, decrease blood cholesterol levels, stimulate the immunological system, reduce the nephrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effect of radiation (Belay et al., 1993; Abd El-Baky et al., 2006). Meanwhile, *Spirulina* deserves special attention as a source of protein-rich material and for its health-enhancing properties (Miranda et al., 1998). These properties have been attributed to different compounds such as phycocyanins, carotenoids, organic acids, sulfated polysaccharide and polyunsaturated fatty acids (Estrada et al., 2001, Abd El-Baky 2003; Ozdemir et al., 2004; Khan et al., 2006).

Cancer and cardiovascular as chronic diseases are the main causes of death in the world, where oxidative stress induced by reactive oxygen species (ROS) is one of the foci related to these diseases. ROS are highly reactive oxidant molecules that are endogenously generate through regular metabolic activity, lifestyle activity and diet, and they are reacting with cellular components, causing oxidative damage to such critical cellular bio-molecules such as lipids, proteins and DNA. Hence, there is strong evidence that this damage may play a significant role in causation of several chronic diseases (Halliwell, 1994). The presence of certain substances act as antioxidant or free radical scavenger may protect the body from the consequences of oxidative stress. Thus, antioxidants play an important role in the protection of cells against oxidative damage caused by ROS (Qi et al., 2006; Khan et al., 2005).

Nowadays, food industries need new food ingredients obtained from natural sources, to develop novel functional foods or nutraceuticals. Algal species are an important alternative material to extract natural antioxidant

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compounds to delay or prevent the oxidative damage caused by ROS molecules (El-Baz et al., 2002; Athukorala et al., 2006). Among algal species, *Spirulina* has been reported to prevent oxidative damage by scavenging free radicals and active oxygen, and hence indirectly reduce cancer formation in human body. In this respect, the increased consumption of foods characterized by free radical scavenging activity, leads up to a doubling of protection against many common types of cancer formulation (Cooke et al., 2002).

The present work was conducted to study the influence of different nitrogen concentrations in growth media for production some nutraceutical compounds by *S.maxima*. In addition, the crude organic extracts of different algal cultures were assessing for their antioxidant and antimicrobial activities as well as the chemical constituents of various extracts.

MATERIALS AND METHODS

Algal source: The blue green alga, *Spirulina maxima* was obtained from the Culture Collection of Texas University, Austin, Texas, USA. Strain was maintained in Zarrouk's medium, a standard synthetic medium containing 2.5 g L^{-1} sodium nitrate as nitrogen source (Zarrouk, 1966). This medium was used in this work while the concentrations of the sodium nitrate were altered.

Growth conditions: Large-scale cultivation on Zarrouk's medium containing five nitrogen concentrations (2.5, 1.78, 1.25, 0.624 and zero g/L, as $NaNO_3$) at pH 10.5 were done in five aquariums (30 L, each). The cultures were gassed with air containing 0.3% CO_2 (v/v), and continuously illuminated with ten cool white fluorescent lamps (40 W each, Philips). The culture temperature was maintained at 28 ± 2ºC.

Growth measurements: Algal growth was spectophotometrically measured as described by Payer (1971). The calculated biomass (the average of three experiments) was used to obtain maximum specific growth rates (µmax) from the log phase of the growth curves by exponential regression. Productivities was calculated from the equation P= (Xi -X0) / ti, where P = productivity (mg $L^{-1}day^{-1}$), X0 = initial biomass density (mg L^{-1}), Xi = biomass density at time i (mg L^{-1}) and ti =time interval (h) between X0 and Xi (Colla et al., 2007).

Determination of dry weight: Ten ml from different cultures were filtered under vacuum using filter membrane (0.45 µm) and washed several time with distilled water. Then, the algae cells was dried at 80ºC for 30 min and weighed.

Preparation of organic extracts (COEs): The fresh and filtered *S.maxima* samples (ca. 5 g) were homogenized with 50 mL dichloromethane/ methanol (1:1, v/v), then the homogenate was filtered and the filtrate was evaporated under vacuum to dryness at 40°C. The extraction yields were based on dry weight from cells (11.0, 12.0 12.3, 13.0 and 13.9%) grown in media containing five different sodium nitrate levels (2.5, 1.785, 1.25, 0.625 and zero g/L). All extraction were done in duplicates. The dry extracts were kept at 4°C under N_2 when not in use.

Determination of total phenol content: The concentrations of phenolics in *S.maxima* samples were estimated by the Folin-Ciocalteu procedure of Singleton et al. (1999) and the results were expressed in gallic acid equivalent, a naturally occurring phenol.

Determination of chlorophyll: One gram of *S.maxima* was homogenized in acetone (20 ml, 80%) and allowed to stand overnight in dark at 4°C for complete extraction followed by centrifugation at 10,000 xg for 5 min. The contents of total chlorophyll (T-Chl), chlorophyll a (Chl-a) and chlorophyll b (Chl-b) in the supernatant were spectophotometrically determined according to Lichtenthaler (1987) method.

Determination of carotenoids: The total carotenoids in *Sp. maxima* samples were spectophotometrically determined at 450 nm according to AOAC standard methods AOAC (1995). β-carotene, as a standard compound, was used for preparation of the calibration curve.

Determination of algal tocopherols: Tocopherols were determined by HPLC equipped with Spectra System UV2000 detector at 290 nm and separated on a 250 x 4.6 mm (i.d) column packed with Vydac and eluted with 90:10 acetonitrile: methanol (v/v) at a flow rate of 1 mL min^{-1}. Standard of □tocopherol was run under the same conditions (Abd El-Baky et al., 2004).

Extraction and determination of phycocyanin: The concentration of total blue pigment phycocyanin was spectophotometrically determined at 280, 615 and 652 nm; respectively as reported by Silverira et al. (2007). Phycocyanin concentration (PC) and extraction purity (EP) were calculated by the following equation: (PC) = OD615 – 0.474 (OD 652) / 5.34 mg ml^{-1} and (EP) = OD615 / OD280, respectively.

Chromatographic analysis

TLC analysis: Two µL of different organic extract solutions (20 mg mL^{-1}) were subjected to thin layer chromatography (TLC) on 10 × 20 cm glass plates, coated with 0.2 mm silica of gel 60 F254 (Merck, Germany), and allowed to dry for a few minutes, then developed with hexane/acetone (75:25, v/v). The separated compounds were located and identified by visualizing TLC plats either with solution consisting of 1% $FeCl_3^{-1}$% $K(CN)_6$, and under UV and without reagents to identified their chemical constituents.

HPLC analysis: The chemical constituents of *S.maxima* extracts were identified by HPLC method as reported by Mendiola et al. (2005), using Dionex Summit IV HPLC system consisting of a Dionex P680 dual gradient pump; a ASI-100 auto-sampler equipped with a 20 mL loop and PDA-100 photodiode array detector. A reverse phase column C18 (250 x 4.6 mm, 5 mm partials) was used. The mobile phase was a mixture of solvent A (methanol / ammonium acetate 0.1 N; 7:3, v/v) and solvent B (pure methanol) at 0.9 ml min^{-1} according to a step gradient, lasting 35 min, which started from 25% B, changing at 50% in 1 min, rising up to 100% B at 10 min. Then, the mobile phase composition was kept constant until the end of the analysis. Total acquisition time was 35 min and the temperature was set at 25°C. The identification of the peaks was performed, when possible, using standards. When no standards were available, tentative identification was done based on UV-Vis spectra characteristics and comparing with data reported in the literature.

Determination of antioxidant capacity

Rapid screening of antioxidant capacity with TLC-autography methods: The TLC-autography technique was used for detecting antioxidant activity of crud organic extract (COE) of *S.maxima*. In which, the separated compounds on TLC plates were sprayed with a oxidizing solution of β-carotene/ linoleic acid mixture (contained 9 mg β-carotene and 27 µl linoleic acid in 90 ml chloroform/ ethanol, 2:1 v/v) and/or with 0.5 mmol/L 2,2-diphenyl-1-picrylhydrazyl

Table 1. Growth parameters and extraction yields of *Spirulina maxima* grown at various sodium nitrate concentrations

Na NO_3 (g L^{-1})	μ max (day^{-1})	P_{432} (mg L^{-1} day^{-1})	Extraction yield %
2.500	0.094 ±0.003	36.5 ±0.12	11.0
1.875	0.089 ±0.005	34.33 ±0.21	12.1
1.250	0.081 ±0.004	32.35 ±0.32	12.3
0.625	0.074 ±0.004	30.15 ± 0.71	13.2
0.000	0.069 ±0.003	28.91 ±0.53	13.9

The data refer to mean value ± standard deviation.
μmax =maximum specific growth rate
P432=productivity at 432 h = 18 days.

(methanolic DPPH) radical to detect antioxidant active compounds as reported by Pratt and Miller (1984) and Jaime et al. (2005) , respectively. The protecting against the bleaching β-carotene gave orange spots, and scavenging DPPH radical gave yellow colored spots were taken as positive results (Jaime et al,. 2005).

ββ-carotene/linoleic acid bleaching in aqueous media: The procedure is based on a previously reported method (Farag et al., 1984) with slight modifications. Five mg of β-carotene dissolved in 2 ml chloroform were added into flask containing 27 µL linoleic acid and 500 mg Tween-20, then the solvent was evaporated under vacuum. Ultra-pure water (250 mL) was added and emulsification was achieved by agitation in ultrasonic bath for 15 min. A 200 µL of each *S.maxima* extracts (containing 50 - 200 µg/ml of 0.5% Tween-20 solution) was added into flask containing 50 ml of reaction mixture, which were subjected to thermal auto-oxidation at 50 ± 2ºC for 7 h. The absorbance of 4 ml from reaction mixture at 20 min intervals was measured at 470 nm against an appropriate blank. All samples were assayed in triplicate. Antioxidant capacities of algal extracts were compared with those of BHT, BHA and α-tocopherol (200 ppm) and control. Inhibition of bleaching β-carotene (I%) was calculated as following: I% = (A initial –A sample / A initial) x 100, where A initial = absorbance at zero time and A samples= absorbance after 2 h.

DPPH• free radical-scavenging assay: The DPPH assay was carried out as mentioned by the method of Tagashira and Ohtake (1998). The four different concentrations of the respective *S. maxima* extracts were added to 25 ml of 0.004% DPPH radical. The reaction mixture was vigorously shaken, and kept in the dark at 30 ± 1ºC. At 15 min intervals up to 180 min, the absorbance of the resulting solution was measured at 517 nm, against an appropriate blank. The differences in absorbance between a test sample and a control (methanolic DPPH) was considered as an antioxidant activity. The methanol was used to adjust zero and BHT, BHA and a-tocopherol (at 200 µg/mL) were used as reference standard. The radical scavenging activity of *S.maxima* extracts in the reaction mixture was calculated from a calibration curve at 517 nm. All tests were run in triplicate and averaged.

Antimicrobial activity

Microorganisms: Bacterial strains from the Fisher Scientific Co. (Texas, USA) were used (*Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Micrococcus luteus, Klebsiella pneumoniae, Serralia marcescencs*) for antibacterial evaluation.

Antibacterial testing

Preparation of inoculums: The bacteria strains were inoculated on nutrient broth (Difco) and incubated for 24 h at 30 ± 0.1°C. Adequate amounts of autoclaved Difco Agar medium were dispensed into sterile plates, and allowed to solidify under aseptic conditions. An aliquot (0.1 mL) of the test organisms was inoculated with a sterile swab on the surface of appropriate solid medium in plates and then was incubated at 27°C for 1 h.

Antibacterial assay: The antibacterial activity was evaluated with the paper disk diffusion method Bradshow, (1992). Chloramphenicol was included in the assays as a positive control (10 and 20 µg $disk^{-1}$) and the inhibitory activity of DMSO was also tested.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration tests were carried out according the methods of the European Pharmacopoeia (2000). MIC was defined as the lowest algal extracts concentration showing no visible bacterial growth after incubation for 24 h at 37ºC.

Statistical analyses: Data obtained from measurements for each variable were subjected to analysis of variance using the COSTAT computer package (Cohort Software, CA, USA).

RESULTS AND DISCUSSION

Table 1 shows the values of maximum specific growth rate (μ max) and productivity P432 at 18 days for *S.maxima* grown at different $NaNO_3$ concentrations. The results revealed that the concentrations of $NaNO_3$ ranged from 1.25 to 2.50 g/L^{-1} had significant influences on u max and productivity. Lower $NaNO_3$ level (0.625 g/L^{-1}) did not show any significant affect on the growth parameters. Similar to that was obtained by Colla et al. (2007) , who demonstrated nitrogen concentration in the Zarrouk,s medium (2.50 g/L) could be reduced to 0.625 g L^{-1} without loss of productively and leads to decrease production cost in large-scale cultivation.

Some microalgae species are well known by its ability to accumulate higher amounts of carotenoids, phycocynin and a □tocopherol when grown under stress conditions. It seems that, the *S.maxima* algal growth was negatively associated with enhancement of carotenoids biosynthesis (Abd El-Baky et al., 2004, 2006). Table 2 shows the influence of N concentration on the growth and concentrations of total carotenoids (TCOR), α-tocopherol (TOC), total phenol compounds (TPC), total chlorophyll (T-Chl) and phycocynin (Phy). The nitrogen starvation led to

Table 2. Influence of varied sodium nitrate concentration on the levels of total chlorophylls, total carotenoids, total phenol compounds, α–tocopherol and total phycocyanin contents in *Spirulina maxima*

Na NO_3 (g L^{-1}) concentrations	Total Chl. (mg g^{-1})	Total carotenoids (mg g^{-1})	Total phenol compounds (mg g^{-1})	Tocopherol gKg^{-1}	Total phycocyanin (mg g^{-1})
2.500	16.23 ±1.24 [a]	7.32 ± 0.46[a]	4.98 ± 0.25[a]	0.266 ± 0.04[a]	106.4 ±2.15[a]
1.875	14.35 ±1.21[b]	8.25 ± 0.35[b]	4.23 ± 0.21[a]	0.44 ± 0.01[b]	92.9 ±1.35[b]
1.250	12.63 ±1.11[c]	12.35 ± 0.94[c]	3.22 ± 0.22[b]	0.501 ± 0.03[b]	70.1 ±0.147[c]
0.625	8.12 ±0.94[d]	16.53 ± 0.98[d]	3.55 ± 0.27[b]	0.978 ± 0.7[c]	55.7 ±1.67[d]
0.000	7.69 ±0.54[e]	20.00 ± 1.11[e]	3.08 ± 0.25[b]	1.32 ± 0.05[c]	45.3 ±1.23[d]
LSD at level (P<0.01)	1.22	1.13	1.21	0.65	7.58

Values are significant at P = 0.01.

accumulate high amounts of carotenoids and tocopherol, which was found in compatible with significant decreased of the biomass weight, growth rate, and Phy, TPC and Chl contents. Thus, the concentration of sodium nitrate in Zarrouk's medium exhibited a significant effect on the production of all phytochemical constituents. For instance, the highest concentration of carotenoid contents was obtained in culture grown either at lowest (0.625 g L^{-1}) or in free NaNO3 medium (zero g L^{-1}), with values of 16.53 and 20.0 mg g^{-1} (d. w), respectively. In contrast, lowest TCR content (7.32 mg g-1 d w) was found in culture grown at highest NaNO3 (2.5 g/ L^{-1}). Previous workers (Athukorala et al., 2006; Colla et al., 2007) have reported this finding, which the production of carotenoids occurred in microalgae cells grown under specific conditions, including high light intensity and high concentration of salt coupled with imbalanced nutrient. It seems to that the division of algae cells grown under N starvation are blocked, while photosynthesis continues leading to storage specific compounds such as carotenoids and triglycerides. The accumulation of these compounds do not require nitrogen for their biosynthesis and synthesizing enzymes and/or less susceptible to disorganization than in the system responsible for synthesis other compounds (Fogg, 1975; El-Baz et al., 2002).

S maxima grown at lower N concentration (0.652 and zero NaNO3 g L^{-1}), produced lower cellular contents of TPC and T-Chl, with values of 3.55 and 3.08 mg g^{-1} and 8.12 and 7.69 mg g^{-1}, respectively. In contrast, the concentrations of these substances were significantly increased in cells grown in media containing higher sodium nitrate level (2.5 g L^{-1}) with values of 4.98 and 16.23 mg g^{-1}, respectively. Thus, higher quantities of TPC and T-Chl are synthesized in *S.maxima* grown in media containing higher sodium nitrate level. For phycocyanin production, at lower sodium nitrate levels (0.625 and zero g L^{-1}), *S.maxima* had lower con-centrations of phycocynine with values of 55.7 and 45.3 mg g^{-1} as compared with the values of 106.4 mg g^{-1} in This finding might explain with that the sodium nitrate is grown cells under higher sodium nitrate level (2.5 g L^{-1}) required for synthesis of the amino acids, which make up proteins and other cellular components such as chlorophylls and phycocyanin (Abd El-Baky 2003; Colla et al., 2007). However, higher concentrations of NaNO3 led to an increase the levels of T-Chl, TPS and Phy compounds. These results were in agreement with those reported by several authors (Abd El-Baky 2003; Colla et al., 2007).

Identification of the chemical composition of *Spirulina* organic extracts by chromatographic methods

TLC and HPLC techniqunics were used for the identification of, chlorophyll-derived and carotenoids in organic extracts of *S.maxima* grown in different culture conditions (Tables 3). TLC chromatogram of organic extracts of *S.maxima* indicated the presence of several coloring pigment bands. Blue, red, orange, yellow green, and others, were observed corresponding to photosynthetic pigments such as: phycocynine, carotenoids and chlorophyll-derived in all *S.maxima* culture extracts. The main pigments zeaxanthin, violaxanthin, Chl-a, chl-*b*, and α and β-carotene were found at different hR_f ($hR_f = R_f \times 100$) values of 50, 18, 31, 41, 81 and 94, respectively. These coloring bands on TLC plats were found in agreement with colors of main pigments described in *Spirulina* (Abd El-Bakyet al., 2006; Kay 1991). In addition, phenolic compounds were located on TLC plates at the starting line or at less than hR_f <10, these bands gave blue color after spraying with either $FeCl_3$-FeK_3 $(CN)_6$ and Folin-Ciocalteu reagents. However, the relative density of bands *Spirulina* coloring compounds were varied as a result of N levels in Zarrouk's medium.

The organic extracts of *Spirulina* grown at different N concentrations were separated and identified by using HPLC-DAD (Table 4). Considerable variations were found among all *Spirulina* extracts. HPLC profile of these extracts showed that there were several pigments with in significant relative percentages (% of total area). At low N levels, α- and β-carotene, carotene-isomers and Chl-b were the most abundant constituents in the *Spirulina*, as compared with that did in cells grown at optimal N level.

Table 3. HPLC profile of photosynthetic pigments in *Spirulina maxima* organic extracts as affected by different sodium nitrate concentrations.

Rt	Compound	Relative area peak %				
(Min)		***S .mx.*COE grown in 2.5 g L^{-1} $NaNO_3$**	***S.mx.*COE grown in 1.875 g L^{-1} $NaNO_3$**	***S. mx.*COE grown in 1.250 g L^{-1} $NaNO_3$**	***S. mx.*COE grown in 0.625 g L^{-1} $NaNO_3$**	**S. mx.COE grown in zero $NaNO_3$**
3.46	Violaxaxanthin	27.02	0.31	1.26	0.64	2.02
4.50	Unknown	2.04	4.49	2.85	1.10	5.06
4.92	Unknown			0.14		9.81
5.20	Antheraxanthin	16.77	0.30	39.48	2.37	
5.70	Unknown		0.48			0.43
6.27	Unknown		1.12	1.08	3.32	2.57
6.46	Unknown					0.51
7.27	Astazanthein	0.06	11.69	0.13	2.29	1.51
7.98	Lutein	0.03	1.08	0.30	1.10	2.93
8.62	Unknown				0.93	0.54
8.81	Zeaxanthin	0.04	1.16	0.16	1.02	
9.57	Cryptoxanthin	0.26	3.26	0.28	1.34	1.93
10.57	Chlorophyll a	10.19	2.56	6.34	14.83	12.87
11.22	Chlorophyll b	0.41	39.22	13.30	0.16	9.19
12.67	b- carotene	5.13	6.96	5.29	2.16	8.36
13.68	Carotene- isomers	0.55	0.50	0.60	0.94	35.97
15.10	9 cis □b-carotene	35.75	22.90	24.16	63.77	0.14
15.63	Unknown	0.24	0.30	0.31	0.40	0.47
16.88	Carotenoids compounds	0.18	0.20	0.27	0.35	0.58
17.66	Unknown	0.27	0.37	0.36	0.54	0.22
18.55	Unknown	0.13	0.15	0.12	0.23	
						95.11
Total identified compounds		98.52	97.05	96.41	97.28	4.89
Unknown		1.48	2.95	3.59	2.72	
Rt refers to retention time						

The higher proportions of carotenoids in *S. maxima* were obtained at lower N concentrations (1.25, 0.625 and zero g/L of $NaNO_3$) compared with those obtained at optimal N level (2.5 g L^{-1} of $NaNO_3$). These results are in agreement with the previous studies with *S. plantensis*, which indicate an inverse relationship between the increased carotenoids levels and N concentration in growth medium (Abd El-Baky et al., 2004).

Antibacterial activity

The antibacterial activity of *S.maxima* organic extracts were assayed against six bacteria strains (*B. subtilis, B. cereus, S. aureus, M. luteus, K. pneumoniae, S. marcescencs*) by evaluating the inhibition zones, zone diameter and MIC values. Generally, all *S.maxima* extracts were found to be effective against all tested bacteria and these antibacterial activities was found to be dose depended. This phenomenon was in agreement with that found by Ozdemir et al. (2004) and Mundt et al. (2003). The data in Table 4 showed that the most susceptible bacteria were *K. pneumoniae* and *S. marcescence* to the organic-extracts of *S.maxima* with highest inhibition zones ranged 4 - 13 mm at concentrations 2 - 8 mg/disk. It is of interest to note that all *S.maxima* organic extracts manifested similar degrees of susceptibility towards both gram-positive and gram-negative bacteria. Thus, the antibacterial activity may be attributed to presence of some active components in all organic extracts such as lipophilic and phenol compounds. Organic extracts of *S.maxima* grown at high N level (2.5 g/L of $NaNO_3$) showed lower inhibition zones ranged from 4 to 14 mm, compared to the values ranged from 4 and 12 mm of organic extracts obtained for *Spirulina* grown at low $NaNO_3$ level (0.652 and zero g l^{-1}). On the other hand, *Spirulina* extracts showed good potential of antibacterial activities against all of 6 bacteria with MICs ranged from 30 - 50 µg/mL. Among all extracts of the *S.maxima*, extract of cells grown at higher N levels (0.625 and zero $NaNO_3$ g L^{-1}) were the most potent against all bacteria with MIC values of 30 µg mL-1, as compared with the value 40 µg mL^{-1} for extracts of algal grown in N free media. The maximal inhibition zones and MIC values for

Table 4. Antibacterial activities (inhibition zone in diameter (mm) around the disks [a] and MIC[b]) of *Spirulina maxima* organic extract organic extract

Bacterial strain	Chloramphenicol antibiotic			*S .mx.COE* grown in 2.5 g L-^{1} $NaNO_3$				*Sp .mx.COE* grown 1.875 Na NO_3 *(g L^{-1})*				*Sp .mx.COE* grown 1.25 Na NO_3 (g L^{-1})				*Sp .mx.COE* grown 0.625 Na NO_3 (g L^{-1})				*Sp .mx.COE* grown zero Na NO_3 (g L^{-1})			
	10	20	MIC	2	4	8	MIC	2	4	8	MIC	2	4	8	MIC	2	4	8	MIC	2	4	8	MIC
	(µg/disk)		µ g/ml	(µg/disk)			µg/ml		(µg/disk)		µg/ml	(µg/disk)			µg/ml		(µg/disk)		µg/ml		(µg/disk)		µg/ml
B cereus	15	20	20	7	7	13	50	5	8	12	45	5	9	12	40	4	8	12	30	4	8	12	30
B. substilis	16	21	20	5	7	13	50	4	7	13	45	4	8	12	40	4	8	12	30	5	7	11	30
Kl. pneumoniae	17	22	20	4	8	13	50	4	7	12	45	4	8	12	40	4	7	12	30	5	7	11	30
M. luteus	18	23	20	4	8	13	50	4	7	12	45	4	8	12	40	4	8	11	30	5	7	10	30
S.marcescencs	13	18	20	4	8	13	50	4	7	11	45	4	8	12	40	4	8	11	30	5	7	11	30
Staph. aureus	14	19	20	4	9	14	50	4	7	11	45	4	8	12	40	3	7	11	30	5	7	11	30

[a]Values represent the mean of three replicates
[b]MIC: Minimum inhibition concentration, values given as □g/ml for samples and for chloramphenicol.

bacterial strains sensitive to chloramphenicol as a standard antibiotic were in ranged of 13 - 23 mm and 20 µg mL^{-1}, respectively (Table 4).

It seems that the antimicrobial activity is related to the amounts of lipophilic and lipid soluble phenol compounds containing in *Spriulina* organic extracts. There are an agreement between the present results and with those published by Ozdemir et al. (2004), Mundt et al. (2003) and Sokmen et al. (1999). In addition, Prindle and Wright (1977) mentioned that the antibacterial effects of phenolic compounds are in concentration dependent.

Antioxidant capacity of *Sp. maxima* crude organic extracts

Preliminary TLC assay

The TLC screening assay of Shimada et al. (1992) was used to detect the antioxidant activity of organic extracts from *S.maxima* grown at different N levels.

Bleaching β-carotene: linoleic acid-TLC (BC-TLC) assay: The separated organic extracts of *S.maxima* on TLC plats were sprayed with β-carotene/ linoleic acid mixture, after 2 h, the back-ground was bleached and yellow colored bands were considered as antioxidant compound. As shown on TLC chromatoplat4 the bands with hR_f = 95, 81, 65, 41, 31 and >10 corresponded to lipid-soluble compounds include: carotenoids and phenol compounds were appeared to posses anti-oxidant activity.

DPPH -TLC (DPPH-TLC) assay: The fast DPPH-TLC assay was used to detect the antioxidant activity of *S.maxima* organic extracts. In this assay, bands with antioxidant activity reduced the stable radical DPPH to the pale yellow colored diphenyl-pirylhydrazyl on a violet background, within few min (<3 min). Bands with R_f = 0.95, 0.81, 0.65, 0.41, 0.31, and 0.10 exhibited potent DPPH radical scavenging phenomenon. All organic extracts of S.maxima were found to posses the approximately similar results as potent anti-oxidant. Therefore, antioxidant capacity coincided with carotenoids and phenol components containing in organic extracts of *S. maxima* grown under different levels of nitrogen (Figure 1).

Antioxidant activity of *S.maxima* organic extracts in aqueous model system

This set of experiment was conducted to indicate the antioxidant activity of algal extract in biological system. The antioxidant activity of *S.maxima* organic extracts in aqueous emulsion was eva-luated by measured the rate of □-carotene bleaching method (Table 5 and Figure 2). As compared with the control (without-antioxidant). All *Spirulina* organic extracts and BHT and BHA were able to inhibit the β-carotene degradation due to induction of free peroxyl radicals (LOO.) generated from linoleic acid auto-oxidation. Thus, these extracts exhibited antioxidant properties. In order to compare the antioxdiative capacity of the *S.maxima* extracts values of the relative inhibition percentage (I%) of β-carotene oxidation was cal-culated. The values for extracts obtained from *S. maxima* grown in media containing 2.5, 1.875, 1.25, 0.625 and zero $NaNO_3$ g L^{-1}, BHT and BHA

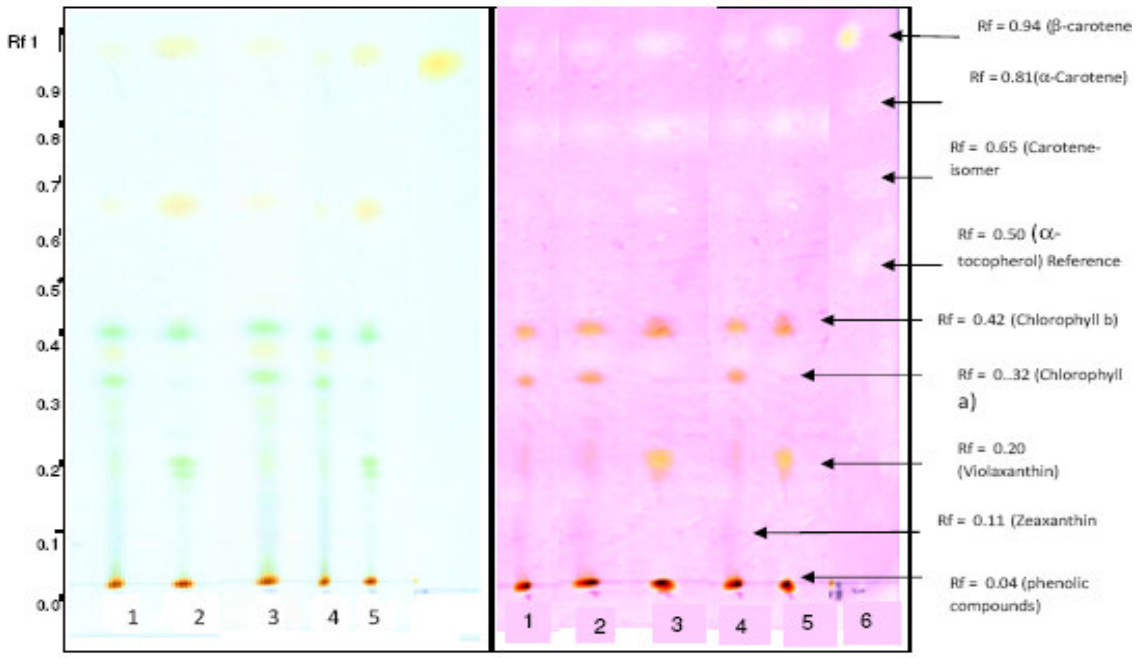

Figure 1. TLC chromatograms of *Spirulina maxima* organic extracts, (a) without staining, (b) stained with 0.004 %DPPH
1: *Sp .mx.*COE grown in 2.5 g L^{-1} $NaNO_3$
2: *Sp .mx.*COE grown in 1.875 g L^{-1} $NaNO_3$
3: Sp .mx.COE grown in 1.250 g L^{-1} $NaNO_3$
4: *Sp .mx.*COE grown in 0.625 g L^{-1} $NaNO_3$
5: *Sp .mx.*COE grown in 0.0 g L^{-1} $NaNO_3$
6: β-carotene and α-tocopherol reference

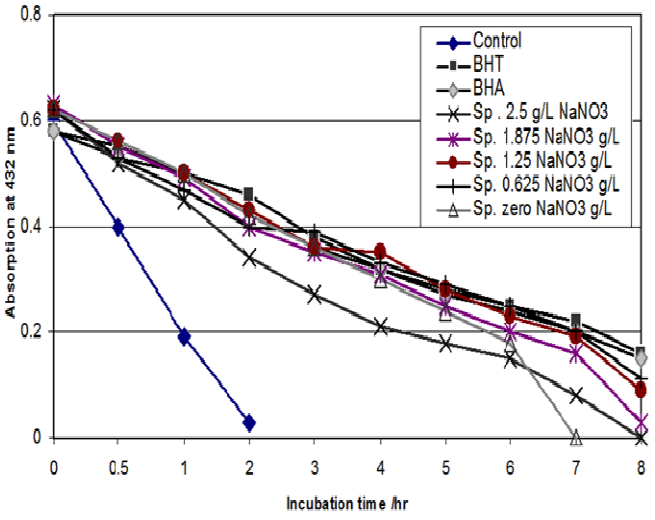

Figure 2. Effect of *Spirulina maxima* organic extracts grown under varied sodium nitrate on β-carotene bleaching

(at 100 µg/ ml) were 80.76, 85.57, 88.23, 85.85, 87.43, 92.18 and 89.58%, respectively. The inhibition % values revealed that the *S. maxima* organic extracts induced significant antioxidant activity as that found for commonly synthetic antioxidant BHT and /or BHA.

Free radical scavenging activity (FRSA)

Free radical scavenging activity (FRSA) of different organic extracts of *S.maxima* and reference substances: BHA, BHT and α-tecopherol to reduce the stable free radical DPPH are shown in Table 4. The most potent effect were observed for all organic extracts derived from *S.maxima* grown in media containing 2.5, 1.875, 1.25, 0.625 and zero $NaNO_3$ g/ L, with an IC50 value of 30.0, 28.0, 26.0, 23.0, and 22.0 µg/ml, respectively. These values were comparable with the synthetic antioxidant: TOC, BHT and BHA, that possessed high inhibitory activity, with an IC_{50} values of 14.6, 13.8 and 16.0, respectively. Thus, crude organic extracts of *S.maxima* possessed strongest radical scavenging activity. This finding suggests that the presence of electron and/or hydrogen donating constituent in algal organic extracts such as phycocyanin, carotenoid and phenol compounds. However, the positive correlations between phenol, phytosterol and carotenoids contents in *Spirulina* and its antioxidant activity is well documented as reported by Abd El-Baky (2003), Khan et al. (2005) and Athukorela et al. (2006). Total phenolic, phycocynin, carotenoids and chlorophyll-derived present in the organic extracts might

Table 5. Scavenging activity of *Spirulina maxima* organic extracts on DPPH radical

Samples	Inhibition %[a]	IC_{50}[b] (g ml^{-1})
Sp .mx.COE grown in 2.5 g L^{-1} $NaNO_3$	60.33 ±1.12	30.0
Sp .mx.COE grown in 1.875 g L^{-1} $NaNO_3$	63.65 ±0.92	28.0
Sp .mx.COE grown in 1.250 g L^{-1} $NaNO_3$	71.22 ±1.11	26.0
Sp .mx.COE grown in 0.625 g L^{-1} $NaNO_3$	81.14 ±1.25	23.0
Sp .mx.COE grown in 0.00 g L^{-1} $NaNO_3$	75.36 ±1.45	22.0
TBA	93.00 ±1.68	13.8
BHT	95.58 ±1.87	14.6
a-Tocopherol	91.54 ±1.95	16.0
LSD at level (P< 0.01)	**2.36**	**2.14**

a: Percentage of antioxidant inhibition was calculated from following equation:
%a = (A blank - A sample / A blank) X 100
A sample = absorbance of DPPH. radical + samples
IC50b: Concentration (µg/ml) for a 50% inhibition was calculated from the plot of inhibition concentration, Tests were carried out in triplicate

explain their high antioxidant activity. It is worth mentioning that, antioxidant activity of phenol compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizer free radical, quenching singlet and triplet oxygen, or decomposing peroxides (Ruberto et al., 2001; Zheng and Wang, 2001). Similar antioxidant properties were found for carotenoid compounds that is capable to scavenging of free radicals and quench singlet oxygen primarily by physical mechanism (Murthy et al., 2005). In addition, phycocynine, one of the major pigments in Spirulina, possesses significant antioxidant and radical scavenging properties as well as can inhibits lipid peroxidation mediated by ROS and could be act as chelating agent (Khan et al., 2005).

The present data indicated that the organic-extracts of *S.maxima* grown at different N concentrations possessed strongest antibacterial and antioxidant properties and may be considered as promising alternative source to synthetic substances. In addition, their activities can be improved by changing the culture conditions.

REFERENCES

Abd El-Baky, HH (2003). Blue green alga *Spirulina Sp.* and its inhibitory effect on growth. Ehrlich ascites carcinoma cells. J. Med. Sci. 3:314-324

Abd El-Baky HH, El Baz FK, El-Baroty GS (2004). Production of antioxidant by the green alga *Dunaliella salina*. Int. J. Agric. Biol., 6: 49-57.

Abd El-Baky HH, El Baz FK, El-Baroty GS (2006). Over-production of Lipid Rich in γ-Linolenic Acid by Blue Green Alga *Spirulina maxima* and its Inhibitory Effect on Carcinoma Cells. Ad. Food Sci., 4: 206-212.

AOAC (1995). Official Methods of Analysis. Association of Official Analytical Chemists, 16 th edn., K hlrich. Arlington, Virginia.

Athukorala Y, Nam K, Jeon Y (2006). Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, Ecklonia cava. Food Chem. Toxicol., 44: 1065-1074.

Belay A, Ota Y, Miyakawa K, Shimamatsu H (1993). Current knowledge on potential health benefits of *Spirulina*. J. Appl. Phycol.., 5: 235-241.

Bradshow L j (1992). Laboratory Microbiology. 4th edn., Saunders College Publishing.

Colla LM, Reinehr CO, Reichert CJ and Costa A V (2007). Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. Bioreso. Technol., 98: 1489–1493.

Cooke MS, Evans MD, Mistry N, Lunec J (2002). Role of dietary antioxidants in the prevention of in vivo oxidative DNA damage. Nut. Res. Rev., 15: 19-41.

El-Baz FK, Aboul-Enein AM, El-Baroty GS, Youssef AM, Abd El-Baky H (2002). Anticarcinogenic Anticarcinogenic activity of algal extracts. J. Med. Sci., 2: 243-251.

Estrada JEP, Bermejo B.P, Villar del Fresno A M (2001). Antioxidant activity of different fractions of *Spirulina platensis* protean extract. Farmaco, 56, 497-500.

Farag RS, Badei AZ M, EL-Baroty GS (1989). Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. Am. Oil. Chem. Soc., 66: 792-799.

Fogg GE (1975). Metabolic pattern and growth in alga cultures and phytoplankton ecology. 2nd ed, Univ. Wisconsin Press, pp: 52–61.

Halliwell B (1994). Free radical, antioxidants and human disease: Curosity, cause or consequence. Lancet, 344: 721-4.

Jaime L, Mendiola JA, Herrero M, Soler-Rivas C, Santoyo S, Senorans FJ, Cifuentes A, Ibanez E (2005). Separation and characterization of antioxidants from *Spirulina platensis* microalga combining pressurized liquid extraction, TLC, and HPLC-DAD. Sep. Sci., 28:2111–2119.

Kay AR (1991). Microaglae as food and supplement. Critical Reviews food Sci. Nut., pp: 30-55.

Khan M, Shobha CJ, Mohan JI, Rao UM, Prayag AN, Kutala KV (2006). Spirulina attenuates cyclosporine-induced nephrotoxicity in rats. J. Appl. Toxicol., 26: 444 –451

Khan M, Shobha CJ, Rao UM, Sundaram CM, Singh S, Mohan J.I, Kuppusamy P and Kutala KV (2005). Protective effect of *Spirulina* against doxorubicin-induced cardiotoxicity. Phytother. Res. 19, 1030–1037.

Lichtenthaler HK (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembrane, Methods Enzymol., 147: 350-382.

Mendiola JA, Marin FR, Hernandez SF, Arredondo BO, Senorans FJ, Ibanez E, Reglero G (2005). Characterization via liquid chromatography coupled to diode array detector and tandem mass spectrometry of supercritical fluid antioxidant extracts of *Spirulina platensis* microalga. J. Separa. Sci., .28: 1031-1038.

Miranda MS, Cintra RG, Barros SBM, Mancini-Filho J (1998). Antioxidant activity of the microalga Spirulina maxima. Braz. J. Med. Biological Res., 31: 1075-1079.

Mundt S, Kreitlow S, Jansen R (2003). Fatty acids with antibacterial activity from the cyanobacterium *Oscillatoria redekei* HUB 051. J. Appl. Phycol., 15: 263-267.I

Murthy KN, Vanitha A, Rajesha M, Swamy M, Sowmya PR, Ravishankar AG(2005). In vivo antioxidant activity of carotenoids from *Dunaliella salina*-a green microalga. Life Sci., 76: 1381-1390.

Ozdemir G, Karabay NU, Dalay CM, Pazarbasi B (2004). Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. Phytother. Res. 18, 754–757.

Payer HD (1971). First report upon the organization and experimental work of the Thailand German project on the production and utilization of single cell green algae as a protein source for human nutrition. Inst. Food Res. Product Development Kasetsar Univ., Bangkok, Thailand.

Pharmacopoeia European (2000). Nachtrag. Bologische Wertbestimmungen. Deutscher Apotheker Verlag Stuttgart, Govi-Verlag-pharmazeutischer Verlag GmbH Eschborn, pp 87-90.

Pratt DE, Miller EE (1984). A flavonoid antioxidant in Spanish peanuts (*Archia hypogoea)*. J. Am. Chem. Soc. 61:1064-1067.

Prindle RF, Wright ES (1977). Phenol compounds. In "Disinfection, Sterilization and Preservation (S.S. Block, ed.), Lea and Fibiger, Philadelphia, PA. pp. 115-118.

Qi, H, Zhang Q, Zhao,T., Hu, R., Zhangc, K., Li, Z. (2006). *In vitro* antioxidant activity of acetylated and benzoylated derivatives of polysaccharide extracted from Ulva pertusa (Chlorophyta). Bioorganic Med. Chem. Let., 2441–2445.

Ruberto G, Baratta MT, Biondi DM, Amico V (2001). Antioxidant activity of extracts of the marine algal genus Cystoseira in a micellar model system. J. Appl. Phycol., 13: 403-407.

Shimada K, Fijikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40: 945-948.

Silverira ST, Burkert MFJ, Costa JAV, Burkert CAV, Kalil SJ (2007). Optimization of phycocyanin extraction from *Spirulina platensis* using factorial design. Biosource Technolo., 98: 1629-1634.

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. Methods of Enzymol., 299: 152- 178.

Sokmen A, Jones BM, Erturk M (1999). The in vitro antibacterial activity of Turkish plants. J. Ethnopharmacol., 67: 79-86.

Tagashira M, Ohtake Y (1998). A new antioxidative 1,3- benzodioxole from Melissa officinalis. Planta Med., 64: 555-558.

Zarrouk C (1966). Contribution a l'etude d; une cyanophycee. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de *Spirulina maxima* (Setch. et Gardner) Geitler. Ph.D. Thesis, University of Paris, *France*.

Zheng W, Wang Y (2001). Antioxidant activity and phenol compounds in selected herbs. J. Agric. Food Chem., 49: 5165- 5170.