

Full Length Research Paper

Production of phenolic compounds from *Spirulina maxima* microalgae and its protective effects *in vitro* toward hepatotoxicity model

Hanaa H. Abd El-Baky^{1*}, Farouk K. El Baz¹ and Gamal S. El-Baroty²

¹Plant Biochemistry Department, National Research Centre, Dokki, Cairo, Egypt.

²Department of Biochemistry, Faculty of Agriculture, Cairo University, Egypt.

Accepted 20 March, 2009

This study illustrates the process of enhancing phenolics synthesis in *Spirulina maxima* grown in Zarrouk's medium supplemented with sodium nitrate (NaNO₃) and or phenylalanine (L-PA), attaining highest production obtained in medium containing 3.77 g.L⁻¹ NaNO₃ and 100 mgL⁻¹ L-PA. HPLC profile showed the presence of phenolic acids and flavonoids predominantly with gallate, chlorogenate, cinnamate, pinostrobate and p-OH-benzoates. The protective action of *Spirulina* phenolic compounds (SPC) against CCl₄-induced *in vitro* hepato-toxicity symptoms like microsomal lipid peroxidation and hydroxyl radical formation was studied. SPC exhibited antioxidant effects on DPPH radical scavenging with IC₅₀ values ranging from 23.22 to 35.62 µg mL⁻¹ and inhibit CCl₄ induced lipid peroxidation in hepato-microsoms model, in dose-depended manner. Their protective potential was comparable to that of standard phenolic antioxidants such as BHT, BHA and α – tocopherol (IC₅₀ values ranged from 13.22 to 23.62 µg mL⁻¹).

Key words: *Spirulina maxima*, antioxidant activity, carbon tetrachloride, hepatoprotective effects.

INTRODUCTION

Recently, much attention has been focused on the microalgae as sources of novel, biologically active compounds such as phycobiline, phenols, terpenoids, steroids and polysaccharide (Li et al., 2007; Abd El Baky et al., 2008). However, the occurrence of phenolic compounds in blue green algae is less documented than that in higher plants (Colla et al., 2007). Algal phenolic compounds were reported to be a potential candidate to combat free radicals, which are harmful to our body and food systems (Estrada et al., 2001). *In vitro* studies demonstrated that *spirulina* and *nestoc* species have several therapeutic properties, due to their ability to scavenge superoxide and hydroxyl radicals and inhibit lipid peroxidation (Li et al., 2007).

The influence of growth conditions on the chemical composition of *spirulina* have been studied by many researchers with the purpose of optimizing the production of economically and nutritionally interesting compounds, such as pigments, antioxidants vitamins and phycocyanin (Abd El-Baky, et al., 2006). However, the mani-

the manipulating growth conditions for biomass production and productivity are usually used in commercial production of potentially useful compounds such as ω-3 fatty acids, carotenoids and phenolics (Abd El-Baky et al., 2007).

In the present study, an effort is made to enhance the production of phenolics by growing *Spirulina maxima* in Zarrouk's medium supplemented with NaNO₃ and or L-phenylalanine (L-PA as a precursor for phenylpropanoid synthesis). The protective action of *spirulina* phenolic compounds (SPC) against CCl₄ induced *in vitro* hepato-toxicity symptoms like microsomal lipid peroxidation and hydroxyl radical formation was studied.

MATERIALS AND METHODS

Spirulina maxima growth

The blue green algae, *S. maxima* were obtained from the culture collection of Texas University, Austin, Texas, USA. Strain was maintained in Zarrouk's medium containing 2.5 gL⁻¹ sodium nitrate as nitrogen source (Zarrouk, 1966). Large-scale cultivation on Zarrouk's medium containing four nitrogen concentrations (2.5, 3.38 and 3.75 gL⁻¹, as NaNO₃) at pH 10.5 was done in five

*Corresponding author. E-mail: abdelbakyh@hotmail.com.

aquariums (30 L, each). The cultures were gassed with air containing 0.3% CO₂ (v/v), and continuously illuminated with ten cool white fluorescent lamps (40 W each, Philips). The culture temperature was maintained at 28± 2°C. The algal growth (biomass gL⁻¹) was spectrophotometrically determined at 750 nm. The calculated biomass (the average of three experiments) was used to obtain maximum specific growth rates (μ_{max}) from the log phase of the growth curves by exponential regression. Productivities were calculated from the equation $P = (X_i - X_0) / t_i$, where P = productivity (mg L⁻¹ day⁻¹), X_0 = initial biomass density (mg L⁻¹), X_i = biomass density at t_i (mg L⁻¹) and t_i = time interval (h) between X_0 and X_i (Colla et al., 2007). Ten ml from different cultures were filtered under vacuum into filter membrane (0.45 μ m), washed several times with distilled water, dried at 80°C for 30 min, and weighed.

Extraction and estimation of phenols and flavonoids content

The algae cells were harvested by continuous flow centrifugation (3 L/h) at 2000 xg for 30 min at 4°C and then the resulting whole cell pellet was weighed. Four grams of pellet were resuspended in ethanol (20 mL), sonicated to disrupt cells and homogenized for 3 min at 4°C. The homogenate was centrifuged at 2000 xg for 15 min (at 4°C), the resulting supernatant was centrifuged again (2000 xg for 10 min). Then, the supernatant was filtered through Millipore filters (0.45 μ m pore size), and the filtrate was evaporated to dryness to give a crude algal ethanolic extracts (enrich in phenolic compounds).

Determination of phenols content

The total phenolic content was estimated by the Folin-Ciocalteu method using gallic acids (10-200 μ g mL⁻¹) as a standard and the absorbance measured at 720 nm (Singleton et al. 1999).

Determination of flavonoids content

The total flavonoid content (TFC) was estimated spectrophotometrically by the aluminum chloride method based on the formation of complex flavonoid aluminum (Djeridane et al., 2006). One milliliter of sample was mixed with 1 mL of AlCl₃ methanolic solution (2%, w/v). After incubation at room temperature for 15 min, the absorbance was read at 430 nm. The amount of TFC was estimated from the standard calibration curve of 10-100 μ g mL⁻¹ quercetin.

HPLC analysis

The chemical constituents of the *S. maxima* extracts were identified by HPLC method reported by Mendiola et al. (2005). Dionex Summit IV HPLC system consisted of a Dionex P680 dual gradient pump, an ASI-100 auto-sampler equipped with a 20- μ L loop and PDA-100 photodiode array detector were used. A reversed phase column C18 (250 x 4.6 mm, 5 μ m particles) was used. The mobile phase was a mixture of solvent A (methanol / ammonium acetate 0.1 N; 7:3, v/v) and solvent B (100% methanol) at rate of 0.9 ml min⁻¹ as 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. 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 compared with data appearing in the literature.

Antioxidant assays

The antioxidant activity of the phenolics *S. maxima* extracts was determined by two methods namely DPPH free-radical scavenging and inhibition of lipid peroxidation of hepatic microsomes induced by CCl₄.

DPPH free-radical scavenging assay: DPPH radical scavenging by SPC was assayed as by Tagashira and Ohtake method (1998). Twenty-five ml of DPPH (0.004%) reagent was incubated in darkness at 30 ± 1°C, with one mL of SPC (5 to 100 μ g mL⁻¹) and the absorbance was monitored at 517 nm at 15 min intervals for 90 min, against methanol as blank. The value of IC₅₀ was calculated. All samples were carried out in triplicate. The BHT, BHA and α -tocopherol (100 μ g mL⁻¹) were used as reference standards.

Microsomal lipid peroxidation assay in vitro: The hepatic microsomes were prepared from livers of male Wistar rats (body weight 120 ± 5 g) by differential centrifugation (Kornbrast and Mavis, 1980). Five rats were decapitated and allow exsanguinations. Then, the liver was quickly excised, washed several times with ice cold 0.15 M KCl, then homogenized in the same saline solution, filtered and centrifuged at 3000 xg at 4°C for 10 min. The supernatant was centrifuged at 20,000 xg for 10 min. The resulted supernatant was centrifuged at 105,000 xg for 1 h at 4°C, and the microsomal pellet was suspended in ice cold Tris-buffer (pH 7.4), kept frozen at -30°C until use. Lipid oxidation in hepatic microsomes was induced by added 10 μ L of carbon tetrachloride (CCl₄, as oxidizing reagent) to 200 μ L microsomes extracts (containing 10 mg protein) and the volume was completed to 3 mL with phosphate buffer (pH 7.2). Then, 1 mL of SPC of different extracts (containing 50 and 100 μ g of SPC/ mL) was added to the reaction mixture. After incubation for 45 min at 37±1°C, the concentration of TBARS (as a product of lipid peroxidation) was measured according to Haraguchi et al. (1997). Briefly, the malondialdehyde (MDA) contents in 1 mL of the reaction mixture were quantized by reaction with 2.5 mL of TBA-TCA reagent (consisted of 1 ml thiobarbituric acid (0.38%, w/v) and 1.5 mL of 15% trichloroacetic acid). Then, the mixture was incubated for 30 min at 90°C. After centrifugation (1000 xg for 5 min), the absorbance of the supernatant was measured at 532 nm. TBARS content was expressed as μ mol. mL⁻¹. The butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) was used as antioxidant standards (at 50 and 100 μ g mL⁻¹).

Statistical analysis

All analyses were performed in triplicate and data reported as mean ± standard deviation, unless otherwise stated. Analysis of variance was done using the COSTAT computer package (Cohort Software, CA, and USA). The mean values were compared with LSD test.

RESULTS AND DISCUSSION

Table 1 shows the effect of NaNO₃ and L-PA on the specific growth rates (μ_{max}), productivity (P_{432} , mg L⁻¹ day⁻¹) and yield of ethanolic extract of *S. maxima* cultures. NaNO₃ (2.5 - 3.77 g.L⁻¹) combined with L-A (50 - 100 mg.L⁻¹) enhanced the productivity to 42.6- 65.4 mg.L⁻¹ and μ_{max} to 0.13-0.25 day⁻¹. In addition, the yield of extracts differed among algal cultures with values of 10.4 – 16.35% (based on dry weight, d.w). Our results

Table 1. Growth parameter of *Spirulina maxima* grown under sodium nitrate (NaNO₃) and phenylalanine (L-PA) concentrations

Treatments	μ_{\max} (day ⁻¹)	Δt (h)	P_{432} (mg L ⁻¹ day ⁻¹)	Extraction yield %
2.5 (g L ⁻¹) NaNO ₃	0.099 ±0.003	24-432	37.50 ±0.11	10.14
+ 50 mg L ⁻¹ L-PA	0.130 ±0.003	24-432	42.60 ±0.14	11.65
+ 100 mg L ⁻¹ L-PA	0.151 ±0.003	24-432	46.70 ±0.13	13.63
3.125 (g L ⁻¹) NaNO ₃	0.111 ±0.004	24-432	45.50 ±0.14	11.25
+ 50 mg L ⁻¹ L-PA	0.183 ±0.005	24-432	51.80 ±0.12	13.58
+ 100 mg L ⁻¹ L-PA	0.201 ±0.003	24-432	57.70 ±0.15	14.51
3.777 (g L ⁻¹) NaNO ₃	0.156 ±0.003	24-432	51.20 ±0.13	12.64
+ 50 mg L ⁻¹ L-PA	0.192 ±0.004	24-432	56.80 ±0.12	14.98
+ 100 mg L ⁻¹ L-PA	0.251 ±0.004	24-432	65.40 ±0.13	16.35

Except for Δt (24-432 h), all values show mean \pm standard deviation. μ_{\max} = maximum specific growth rate; Δt =start end of the exponential growth phase; P_{432} =productivity at 432 h.

Table 2. Influence of sodium nitrate and phenylalanine concentrations on phenolics and flavonoids content of *Spirulina maxima*

Treatments	Total henol (mg g ⁻¹ d.w)	Phenolic %	Ratio ^a	Total flavonoids (mg g ⁻¹ d.w)	Flavonoids %	Ratio ^a
2.5 (g. L⁻¹) NaNO₃	4.51 ±0.23	0.45	1.00	1.32 ±0.03	0.13	1.0
+ 50 mg.L ⁻¹ L-PA	5.68 ±0.33	0.57	1.26	1.54 ±0.08	0.15	1.2
+ 100 mg L ⁻¹ L-PA	7.36 ±0.36	0.74	1.63	1.94 ±0.06	0.19	1.5
3.125 (g.L⁻¹) aNO₃	5.19 ±0.35	0.52	1.15	1.45 ±0.07	0.14	1.1
+ 50 mg L ⁻¹ L-PA	8.64 ±0.27	0.86	1.91	2.25 ±0.13	0.22	1.7
+ 100 mg L ⁻¹ L-PA	10.95 ±0.47	1.09	2.42	3.31 ±0.21	0.33	2.5
3.777 (g.L⁻¹) aNO₃	6.54 ±0.54	0.65	1.45	1.93 ±0.05	0.19	1.5
+ 50 mg L ⁻¹ L-PA	12.94 ±0.93	1.29	2.86	4.65 ±0.14	0.46	3.5
+ 100 mg L ⁻¹ L-PA	16.96 ±1.24	1.69	3.75	5.12 ±0.22	0.51	3.9

All values show mean of three replicates, \pm standard deviation, values are significant at (P=0.01), \pm SD

corroborate the reports on a positive interaction between NaNO₃ concentrations with an increase of biomasses in three species of *spirulina*: *Spirulina platensis*, *Spirulina lonar* and *Spirulina laxissima* (Colla et al., 2007; Susinjan and Shivaprakash, 2005).

Furthermore, L-PA showed an important effect on the productivity and μ_{\max} of *S. maxima* culture. This is to be expected, that L-PA like other amino acid may be play an impotent role as growth factors, which leads to an enhance synthesis of protein and other cellular components and/or an increases rate of photosynthesis. Therefore, biomasses of algal cells increased as compared with that in control. Table 2 depicts the changes in phenolic (TPC) and flavonoid (TFC) contents of *S. maxima* grown in medium supplemented with NaNO₃ and L-PA at different NaNO₃. Addition of L-PA to the medium enhanced the production of TPC and TFC. The highest amounts of TPC (16.96 mg/g d.w) and TFC (1.69 mg.g⁻¹ d.w) was obtained in culture grown in medium containing 100 mg L⁻¹ L-PA and 3.77 g.L⁻¹ NaNO₃ (highest level). While, 5.68 mg.g⁻¹ d.w TPC and 0.57 mg.g⁻¹ d.w TFC was recorded in culture grown with 2.5 gL⁻¹ NaNO₃ with 50 mgL⁻¹ L-PA (Table 2). However, this results revealed that the levels TPC and TPC

increased markedly by the increase of NaNO₃ combined with L-PA in growth medium. For instance, the amounts of TPC and TFC (in parenthesis) in the *S. maxima* cells grown in medium contained 2.5, 3.12 and 3.77 gL⁻¹ NaNO₃ combined with 100 mgL⁻¹ L-PA were being 1.26 (1.63), 1.91 (2.42) and 2.86 fold (3.75 fold), respectively as great as was seen in the *S. maxima* culture grown in L-PA free Zarrouk's medium. This result clearly indicates that the L-PA has a stimulatory role on an accumulation of phenolics in *Spirulina* cultures. Dixon and Paiva (1995) reported that L-PA act as a precursor compounds for phenyl-propanoid pathway, which is responsible for theg⁻¹ (d.w) flavanoids was recorded compared to that of 4.51 mg g⁻¹ TPC and 1.32 mg g⁻¹ (d.w) TFC with 2.5 g L⁻¹ concentration. At 3.12 and 3.77 gL⁻¹ NaNO₃, 5.12 and ¹ synthesis of phenolics. The present findings corroborate reports with the enhancement in the accumulation of phenolics by L-PA (Shetty and Randhir, 2005; Rechner et al., 2001).

Identification of phenol compounds

The HPLC-DAD profile of phenolic extracts showed the presence of phenolic acids and few flavonoid com-

Table 3. Influence of sodium nitrate and phenylalanine concentrations on phenolics composition (relative area %) of *Spirulina maxima*

Phenylalanine Phenolic compounds	2.50 (g L ⁻¹)			3.125 (g L ⁻¹)			3.777 (g L ⁻¹)		
	0.0	50 mg L ⁻¹	100 mg L ⁻¹	0.0	50 mg L ⁻¹	100 mg L ⁻¹	0.0	50 mg L ⁻¹	100 mg L ⁻¹
Gallic acid	19.82	16.0	12.15	17.91	13.3	6.94	15.5	8.23	4.78
P-OH benzoic acid	14.18	12.31	10.27	12.18	8.27	7.62	11.7	6.12	3.32
Chlorogenic cid	13.21	10.21	7.21	9.21	3.24	3.54	13.7	6.23	1.14
Vanillin acid	7.12	5.25	3.51	5.31	4.33	3.85	1.21	0.57	0.23
Caffeic acid	6.36	4.31	2.22	4.95	3.61	2.97	7.92	2.44	1.78
Syringic acid	5.21	4.25	2.85	1.75	1.54	0.84
Salicylic acid	4.12	2.37	1.82	3.00	2.85	1.49	1.22	0.34	0.19
O-Coumaric cid	4.13	3.21	2.47	2.66	1.92	1.21	2.79	1.21	0.11
Fuerulic acid	3.12	3.02	3.11	2.96	4.51	5.67	3.14	5.31	7.22
Cinnamic acid	1.05	4.57	7.54	3.85	10.99	15.77	8.25	15.65	18.14
Quercetin	1.14	4.25	6.32	1.85	2.45	3.54	2.35	3.21	4.23
Genstein	1.22	0.52
Unknown	1.15	2.11
Unknown	1.35	2.98	0.73	2.39	1.69
Unknown	1.92	2.51	2.11	1.48	2.13	1.05	2.39	1.98
Kaermphereol	1.33	2.81	3.51	3.34	4.51	5.21	3.51	6.52	7.38
Euganol	1.09	1.89	3.53	5.11	5.39	3.85	1.13	4.31	7.25
Unknown	1.25	1.81	2.33	3.55	4.81	1.35	1.8	2.45	2.71
Chrysin	1.02
Galangin	0.54	0.65	0.89	1.14	1.87	2.11	1.57	2.65	3.25
Unknown	1.53	1.83	2.13	2.06	3.35	0.53
Unknown	1.52	1.98	2.28	1.95	0.97	1.17	3.64	1.35	2.17
Unknown	2.29	3.11	4.51	3.62	2.65	1.35	1.10	1.98	2.65
Pinostrobin	7.28	10.25	15.31	11.1	15.54	29.73	15.8	25.98	33.61
.....	1.17	0.82	1.31	1.28	2.83
Total identified	86.45	86.78	84.11	80.71	85.63	95.25	96.1	97.78	99.77

pounds (Table 3). Gallic, chlorogenic, cinnamic, p-OH-benzoic, quimic, caffeic, vanillic and ferulic acids were the most abundant constituents (>1 to <10 % of total chromatographic area) of phenolic acids. The values of caffeic, vanillic and ferulic acids in cultures grown at 3.77 g L⁻¹ NaNO₃ combined with 50 mg L⁻¹ L-PA was 2.44, 0.57 and 5.31%, respectively. Their values were 1.78, 0.23 and 7.22 %, respectively in culture containing 3.77 g L⁻¹ NaNO₃ combined with 50 mg L-PA. In sharp contrast, these components were 6.36, 7.12 and 3.12 % in *spirulina* grown 2.5 gL⁻¹ NaNO₃ only. This finding revealed that phenolic acids constituent in *spirulina* culture decreased by an increase in the levels of L-PA and NaNO₃ in growth medium. An opposite trend was observed for flavonoids constituents. However, quaristin (1.14- 6.32%) was identified as the main constituent. However, salicylic, trans-cinnamic, synaptic, chlorogenic and caffeic acids are the main phenolic acids found in *spirulina* (Miranda et al., 1998).

Antioxidant activity

The potential health benefits of algal bio-active compounds such as phenolics are widely investigated. In humans, they have been shown to be strong antioxidants, which might prevent oxidative damage to biomolecules like DNA, lipids and proteins that play role chronic diseases such as cancer and brain dysfunction (Droge, 2002). However, antioxidants capable of inhibiting lipid peroxidation in model system may suppress lipid peroxidation in the biological system and prevent life important molecules such as DNA and membrane lipid and reduce the risk of several aging associated health problems such as liver and heart diseases. Thus, the antioxidant activity of the phenolic extract (enrich in phenolic antioxidant) of the different *spirulina* culture was evaluated by two different methods (Tables 4 and 5). Free radical scavenging and inhibiting lipid peroxidation of hepatic microsomes induced by CCl₄ are use

Table 4. Scavenging activity of *Spirulina maxima* phenolic extracts on DPPH• radical

Treatments	IC ₅₀ ^b (mg mL ⁻¹)
TBA	14.8
BHT	16.6
α-Tocopherol	18.8
2.50 Na NO ₃ (g L ⁻¹)	35.62
+ 50 mg L ⁻¹ phenylalanine	31.27
+ 100 mg L ⁻¹ phenylalanine	29.35
3.125 Na NO ₃ (g L ⁻¹)	33.51
+ 50 mg L ⁻¹ phenylalanine	30.25
+ 100 mg L ⁻¹ phenylalanine	27.31
3.777 Na NO ₃ (g L ⁻¹)	30.54
+ 50 mg L ⁻¹ phenylalanine	25.14
+ 100 mg L ⁻¹ phenylalanine	23.22
LSD at level (P< 0.01)	1.36

IC₅₀^b: Concentration (μg ml⁻¹) for a 50% inhibition was calculated from the plot of *Spirulina maxima* extract concentration against inhibition % of lipid peroxidation

Table 5. Effect of algal phenolic extracts on Inhibition (%) of lipid peroxidation of rat liver microsomes induced by CCl₄

Treatments	TPC, ppm	Incubation period (min)						Mean of Inh. (%)
		15		30		45		
		TBARs μmol.mL ⁻¹	Inh. (%)	TBARs μmol.mL ⁻¹	Inh. (%)	TBARs μmol.mL ⁻¹	Inh. (%)	
CCl ₄ (control)		7.53	0.00	11.29	0.00	14.59	0.00	0.00
BHT	50	5.23	30.54	6.35	43.75	7.82	46.40	40.23
	100	2.82	62.54	3.25	72.68	3.85	73.61	69.61
2.5 Na NO ₃ (g L ⁻¹)	50	6.23	17.26	8.35	26.04	9.82	32.69	25.33
	100	5.33	29.22	7.32	35.16	8.25	43.45	35.94
2.5 Na NO ₃ (g L ⁻¹) + 50 mg L ⁻¹ phenylalanine	50	5.44	27.76	7.45	34.01	8.26	43.38	35.05
	100	4.39	41.69	5.22	53.76	6.52	55.31	50.25
2.5 Na NO ₃ (g L ⁻¹) + 100 mg L ⁻¹ phenylalanine	50	4.82	35.98	6.23	44.82	7.35	49.62	43.47
	100	3.11	58.68	4.33	61.64	5.21	64.29	61.53
3.125 Na NO ₃ (g L ⁻¹)	50	5.85	22.31	7.83	30.64	8.32	42.97	31.97
	100	4.98	34.06	6.34	43.84	7.31	49.89	42.59
3.125 Na NO ₃ (g L ⁻¹) + 50 mg L ⁻¹ phenylalanine	50	5.11	32.14	6.35	34.75	8.27	43.33	36.74
	100	3.21	57.37	4.15	63.24	4.84	66.82	62.47
3.125 Na NO ₃ (g L ⁻¹) + 100 mg L ⁻¹ phenylalanine	50	4.15	44.88	5.28	53.23	6.34	56.54	51.55
	100	2.22	70.52	2.55	77.41	3.11	78.68	75.53
3.777 Na NO ₃ (g L ⁻¹)	50	5.45	27.62	6.82	39.59	7.33	49.76	38.99
	100	4.35	42.23	5.21	53.85	5.53	62.09	52.72
3.777 Na NO ₃ (g L ⁻¹) + 50 mg L ⁻¹ phenylalanine	50	2.42	67.86	4.31	61.82	5.94	59.29	62.99
	100	1.81	75.96	3.22	71.48	4.11	71.83	73.09
3.777 Na NO ₃ (g L ⁻¹) + 100 mg L ⁻¹ phenylalanine	50	1.01	86.58	1.21	89.28	1.23	91.57	89.14

for an estimate of antioxidants. The effect of antioxidants on DPPH radical scavenging is due to their hydrogen donating ability (Abd El-Baky et al., 2007). *Spirulina* phenolic extracts have the ability to scavenge DPPH radical (RSA), with various degrees (Table 4). The IC₅₀ values for *spirulina* phenolic extracts ranged

from 23.22 to 35.62 μg/ml. Where as their values was 18.8, 16.6 and 14.80 μg/ml for α-TOC, BHT and BHA, respectively. Thus, *S. maxima* phenolic extracts showed significant DPPH radical scavenging activity comparable with the commercial antioxidant. However, the DPPH scavenging activity of *S. maxima* extracts is in

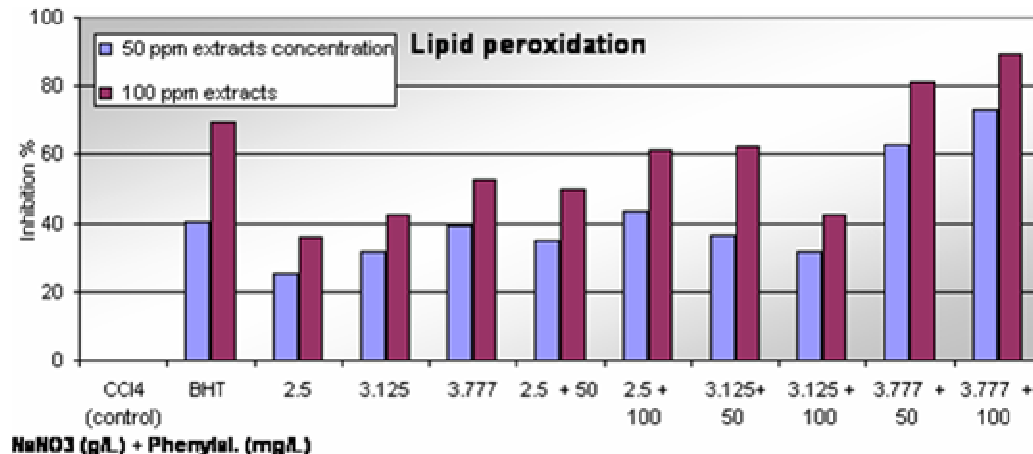


Figure 1. Effect of algal phenolic extracts on inhibition (%) of lipid peroxidation of rat liver microsomes induced by CCl₄

positive correlated to the phenolic content of the extracts, as increase in extract concentration also increases the concentration of phenolic compounds (Tables 4 and 2). Thus, the antioxidant nature of *S. maxima* extracts can be attributed to phenolic contents (Table 2), which are widely accepted antioxidants in plants with free radical scavenging activity (Halliwall and Gutteridge, 1989).

In order to exploit the antioxidant activity of *spirulina* phenolics for pharmaceutical use in hepato-protective effect through the inhibition of oxidative stress, the level of TBA-MDA complex as oxidative marker was determined in rat hepatic homogenate (Santoyo et al., 2006). As regard to TBARS formation, the amount of MDA contents were significantly elevated from 0.53 to 14.59 $\mu\text{mol mL}^{-1}$, after 45 min of incubation of *in vitro* hepatotoxicity model (CCl₄ induced lipid peroxidation of rats liver microsomes) (Table 5 and Figure 1). The catalyzed of hepatic microsomes with *S. maxima* phenolic extracts at 50 and 100 (in parentheses) mg mL^{-1} caused an inhibition of MAD formation ranging from 25.33 to 73.09 % of control (42.42 to 80.5% of control), respectively. BHT at 50 and 100 mg mL^{-1} were inhibited MAD formation by 40.23 and 69.61% of control, respectively. The algal phenolic extracts caused a significant decrease in the level MDA contents with increase in phenolic concentration (50-100 $\mu\text{g mL}^{-1}$), that led to a progressive inhibition the CCl₄ induced MDA formation with a concomitant increase of hepatic microsomal resistance to oxidation. These results clearly suggested that inhibition of CCl₄ induced lipid peroxidation in rat liver microsomes (*in vitro* hepato-toxicity model) were related to their abilities of phenolic and flavonoid compounds presence in *spirulina* extracts to inhibited hepatic oxidative enzymes (cytochrome P450 system). Droge (2002) reported that certain flavonoids and phenols have protective effect on liver damage due to its antioxidant properties. The experimental results in

DPPH assay demonstrate that *S. maxima* phenolic extracts exercises free radical scavenging activity. Therefore, inhibiting the TBA formation corresponds to their antioxidant activity (Figure 1). It is well established that CCl₄ induces hepato-toxicity by cytochrome P450 mediated reactions to produce CCl₄ derived radicals such as trichloromethyl ($\cdot\text{CCl}_3$) and trichloromethyl peroxy ($\cdot\text{OCCl}_3$) (Santoyo et al., 2006). These active-radicals bind covalently to the macromolecules (lipids and proteins) and induce peroxidative degradation of membrane lipids (rich in polyunsaturated fatty acids) and gradually leads to cell death (Johnston and Kroening, 1998). Moreover, this oxidative process is one of the principal causes of hepatotoxicity of CCl₄ (Cotran et al., 1994; Opoku et al., 2007). Therefore, the antioxidant activity and/or the inhibition of free radical generation are important in terms of protecting the liver from CCl₄ induce damage (Opoku et al., 2007).

It is concluded from the present study that algal phenolic extracts could protect the liver against CCl₄ induced lipid peroxidation *in vitro*. Therefore, *Spirulina maxima* extracts enrich in phenolic antioxidants may provide a promising source of natural antioxidant. Furthermore, the production of algal antioxidants can be enhanced by amendment of culture conditions.

REFERENCES

- 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.
- Abd El-Baky HH, El Baz FK, El-Baroty GS (2007). Production of carotenoids from marine microalgae and its evaluation as safe food colorant and lowering cholesterol agents. *Am. Eurasian J. Agric. Environ. Sci.* 2: 792-800.
- Abd El-Baky HH, El Baz FK, El-Baroty GS (2008). Evaluation of Marine Alga *Ulva lactuca* L. as A Source of Natural Preservative Ingredient. *Am. Eurasian J. Agric. Environ. Sci.* 3: 434-444.
- Athukorala Y, Kim K, Jeon Y (2006). Antiproliferative and antioxidant

- properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. Food Chem. Toxicol. 44: 1065-1074.
- Cervato G, Carabelli M, Gervasio S, Cittera A, Cazzola R, Cestaro B (2000). Antioxidant properties of oregano (*Origanum vulgare*) leaf extracts. J. Food Biochem. 24: 453-465.
- Colla LM, Reinehr CO, Reichert CJ, Costa AV (2007). Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. Bioreso. Technol. 98:1489-1493.
- Cotran RS, Kumar V, Robbins SL (1994). Cell injury and cellular death. In, Robbin's Pathologic Basis of Disease, 5th Edition, Prism Book Pvt. Ltd., pp. 379-430.
- Dixon RA, Paiva NL (1995). Stress-induced phenylpropanoid metabolism. Plant Cell, 7:1085-1097.
- Djeridane A, Yous M, Nadjemi B, Boutassouna D, Stocker P, Vidal N (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry, 97, 654-660.
- Droge W (2002). Free radicals in the physiological control of cell function. Physiol. Rev. 82: 47-95.
- Estrada JEP, Bermejo Bescos P, Villar del Fresno AM (2001). Antioxidant activity of different fractions of *Spirulina platensis* protean extract. Farmaco, 56: 497-500.
- Haraguchi H, Ishikawa H, Kubo I (1997). Antioxidative action of diterpenoids from *Podocarpus nagi*. Planta Medica. 63: 213-215.
- Halliwell B, Gutteridge JM (1989). Free radicals, other reactive species and disease. In Free radicals in biology and medicine Oxford: Clarendon Press, pp. 617-623
- Johnston DE, Kroening C (1998). Mechanism of early carbon tetrachloride toxicity in cultured rat hepatocytes. Pharmacol. Toxicol. 83: 231-39.
- Kornbrast JD, Mavis DR (1980). The effect of paraquat on microsomal lipid peroxidation *in vitro* and *in vivo*. Toxicol. Appl. Pharmacol. 53: 323-332.
- Li AH, Cheng K, Wong C, King-Wai F, Feng C, Yue J (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. 102: 771-776.
- 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. Biol. Res. 31: 1075-1079.
- Opoku AR, Ndlovu IM, Terblanche SE, Hutchings AH (2007). *In vivo* hepatoprotective effects of *Rhoicissus tridentata* sub sp. *cuneifolia*, a traditional Zulu medicinal plant, against CCl₄-induced acute liver injury in rats. South Afr. J. Bot. 73: 372-377.
- Rechner AR, Spencer JPE, Kuhnle G, Hahn U, Rice-Evans CA (2001). Novel biomarkers of the metabolism of caffeic acid derivatives *in vivo*. Free Rad. Biol. Med. 30: 1213-1222.
- Santoyo S, Herrero M, Javier F, Cifuentes A, Ibanez E, Jaime L (2006). Functional characterization of pressurized liquid extracts of *Spirulina platensis*. European. Food Res. Technol. 224: 75-81.
- Shetty K. (1997). Biotechnology to harness the benefits of dietary phenolics focus on Laminaceae. Asia Pacific J. Clin. Nutr. 6:162-71.
- Shetty K, Randhir R (2005). Developmental stimulation of total phenolics and related antioxidant activity in light- and dark-germinated corn by natural elicitors. Process Biochem. 40: 1721-1732
- 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 Enzymol. 299:152-178
- Susinjan B, Shivaprakash MK (2005). Evaluation of three spirulina species grown under similar condition for their growth and biochemicals. J. Sci. Food Agric. 85: 333-336.
- 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.