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*Full Length Research Paper*

# Phenolics from *Spirulina maxima*: Over-production and *in vitro* protective effect of its phenolics on $CCl_4$ induced hepatotoxicity

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**This study illustrates the process of enhancing phenolics synthesis in *Spirulina maxima* grown in Zarrouk's medium supplemented with sodium nitrate ($NaNO_3$) and/or phenylalanine (L-PA); attaining highest production were obtained in medium containing 3.77 $gL^{-1}$ $NaNO_3$ 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 (*S*PC) against $CCl_4$-induced *in vitro*, hepato-toxicity symptoms like microsomal lipid peroxidation and hydroxyl radical formation were studied. SPC exhibited antioxidant effects on DPPH radical scavenging with $IC_{50}$ values ranging from 23.22 to 35.62 $\mu g.mL^{-1}$ and inhibited $CCl_4$-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 –tocopherol ($IC_{50}$ values ranged from 13.22 to 23.62 $\mu g.mL^{-1}$).**

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

## INTRODUCTION

Recently, much attention has been focused on micro-algae as sources of novel and 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 the *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* has 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 manipulating growth conditions for biomass production and productivity are usually used in the commercial production of potentially useful compounds such as - 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 $NaNO_3$ and/or L-phenylalanine (L-PA as a precursor for phenylpropanoid synthesis). The protective action of *Spirulina* phenolic compounds (*S*PC) against $CCl_4$-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. The strain was maintained in Zarrouk's medium containing 2.5 g $L^{-1}$ sodium nitrate as

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nitrogen source (Zarrouk, 1966). Large-scale cultivation on Zarrouk's medium containing four nitrogen concentrations (2.5, 3.38 and 3.75 g $L^{-1}$, as $NaNO_3$) at pH 10.5 was 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 ± 2°C.

The algal growth (biomass $gL^{-1}$) was spectophotometrically determined at 750 nm. The calculated biomass (the average of three experiments) was used to obtain maximum specific growth rates ($\mu_{max}$) 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). 10 mL from different cultures were filtered under vacuum into filter membrane (0.45 µm), 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. 4 g 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) and the resulting supernatant was centrifuged again (2000 xg for 10 min). Then, the supernatant was filtered through Millipore filters (0.45 m pore size) and the filtrate was evaporated to dryness to give a crude algal ethanolic extract (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 falvonoids content**

The total flavonoid content (TFC) was estimated spectrophotometercally by the aluminum chloride method based on the formation of complex flavonoid- aluminum (Djeridane et al., 2006). A 1 mL of the sample was mixed with 1 mL of $AlCl_3$ methanolic solution (2% w/v). After incubation at room temperature for 15 min, the absorbance was read at 430 nm. The amounts of TFC were 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 consisting 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 was used. A reversed phase column C18 (250 x 4.6 mm, 5 µ m partials) was also 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 a rate of 0.9 mL $min^{-1}$ as a steep gradient, lasting 35 min, which started from 25% B changing at 50% in 1 min and 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 $CCl_4$.

**DPPH• free- radical scavenging assay :** DPPH radical scavenging by SPC was assayed as by Tagashira and Ohtake (1998) method. 25 mL of DPPH (0.004%) reagent was incubated in darkness at 30 ± 1°C, with 1 ml of SPC (5 to 100 µ $gmL^{-1}$) and the absorbance was monitored at 517 nm at 15 min intervals for 90 min against methanol as blank. The value of $IC_{50}$ was calculated. All samples were carried out in triplicate. The BHT, BHA and -tocopherol (100 µg. $mL^{-1}$) were used as reference standards.

**Microsomal lipid peroxidation assay *in vitro*:** The hepatic microsomes were prepared from the livers of male Wister rats (body weight 120 ± 5 g) by differential centrifugation (Kornbrast and Mavis, 1980). Five rats were decapitated and allowed exsanguinations. Then the liver was quickly excised, washed several times with ice cold 0.15 M KCl, homogenized in the same saline solution, filtered and then 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) and kept frozen at - 30°C until use.

Lipid oxidation in hepatic microsomes was induced by addition of 10 µ L carbon tetrachloride ($CCl_4$, 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 µg 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 (consisting 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 µmoL $mL^{1}$. The butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) was used as antioxidant standards (at 50 and 100 µ $gmL^{-1}$).

**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, USA). The mean values were compared with LSD test.

## RESULTS AND DISCUSSION

Table 1 shows the effect of $NaNO_3$ and L-PA on the specific growth rates ($\mu_{max}$), productivity ($P_{432}$, mg $L^{-1}$ $day^{-1}$) and yield of ethanolic extract of *S. maxima* cultures. $NaNO_3$ (2.5 - 3.77 $gL^{-1}$) combined with L-PA (50 - 100 mg.$L^{-1}$) enhanced the productivity to 42.6- 65.4 mg.$L^{-1}$ and $\mu_{max}$ 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 corroborate the reports on a positive interaction between $NaNO_3$ concentration with an increase of biomasses in three species of *Spirulina: S. platensis, S. lonar and S. laxissma* (Colla et al., 2007; Susinjan and Shivaprakash, 2005).

Furthermore, L-PA showed an important effect on the

**Table 1.** Growth parameter of *S. maxima* grown under sodium nitrate ($NaNO_3$) and phenylalanine (L-PA) concentrations.

| Treatments | μ max ($day^{-1}$) | t (h) | $P_{432}$ ($mg\ L^{-1}\ day^{-1}$) | Extraction yield (%) |
|---|---|---|---|---|
| 2.5 ($gL^{-1}$) $NaNO_3$ | 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 t (24-432 h), all values show mean ± standard deviation.
μmax = maximum specific growth rate; t=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 *S. maxima.*

| Treatments | Total phenol ($mg\ g^{-1}$ d.w) | Phenolic (%) | Ratio[a] | Total flavonoids ($mg\ g^{-1}$ d.w) | Flavonoids (%) | Ratio[a] |
|---|---|---|---|---|---|---|
| 2.5 (g. $L^{-1}$) $NaNO_3$ | 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.$L^{-1}$) $NaNO_3$ | 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.$L^{-1}$) $NaNO_3$ | 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, ± standard deviation.
Values are significant at ($P=< 0.01$), ± SD.

productivity and $\mu_{max}$ 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 leading to an enhance synthesized 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 $NaNO_3$ and L-PA at different concentration. At 3.12 and 3.77 g $L^{-1}$ $NaNO_3$, 5.12 and 6.54 mg $g^{-1}$ (dw) total phenolics and 1.45 and 1.93 mg $g^{-1}$ (dw) flavanoids was recorded compared to that of 4.51 mg.$g^{-1}$ TPC and 1.32 mg $g^{-1}$ (dw) TFC with 2.5 g $L^{-1}$ $NaNO_3$. Addition of L-PA to the medium enhanced the production of TPC and TFC. The highest amounts of TPC (16.96 mg/g dw) and TFC (1.69 mg.$g^{-1}$ dw) were obtained in culture grown in medium containing 100 mg $L^{-1}$ L-PA and 3.77 g.$L^{-1}$ $NaNO_3$ (highest level). While, 5.68 mg $g^{-1}$ dw TPC and 0.57 mg.$g^{-1}$ dw TFC were recorded in culture grown with 2.5 g L- 1 $NaNO_3$ with 50 mg $L^{-1}$ L-PA (Table 2). However, these results revealed that the levels TPC and TFC increased markedly by increasing $NaNO_3$ 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 containing 2.5, 3.12 and 3.77 g $L^{-1}$ $NaNO_3$ combined with 100 mg $L^{-1}$ L-PA were 1.26 (1.63), 1.91 (2.42) and 2.86 -fold (3.75 -fold) respectively, greater than 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 acts as a precursor compound for phenyl-propanoid pathway, which is responsible for the 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).

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

| Na $NO_3$ | 2.50 (g $L^{-1}$) | | | 3.125 (g $L^{-1}$) | | | 3.777 (g $L^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| Phenylalanine<br>Phenolic compounds | 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 acid | 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 acid | 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 |

## Identification of phenol compounds

The HPLC-DAD profile of phenolic extracts showed the presence of phenolic acids and few flavonoid compounds (Table 3). Gallic, chlorogenic, cinnamic, *p*-OH-benzoic, quimic, caffeic, vanillic and ferulic acids were the most abundant constituents (>1% to <10 % of total chromatogramphic area) of phenolic acids. The values of caffeic, vanillic and ferulic acids in cultures grown at 3.77 g L$^{-1}$ $NaNO_3$ combined with 50 mg L$^{-1}$ L-PA were 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$^{-1}$ $NaNO_3$ combined with 50 mg L-PA. In sharp contrast, these components were 6.36, 7.12 and 3.12 % in S*pirulina* grown in 2.5 g L$^{-1}$ $NaNO_3$ only. This finding revealed that phenolic acids constituent in *Spirulina* culture decreased by increasing the levels of L-PA and $NaNO_3$ 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 shown to be strong antioxidants, which might prevent oxidative damage to biomolecules like DNA, lipids and proteins that play roles in 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).

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

| Treatments | $IC_{50}^{b}$ ( mg $mL^{-1}$) |
|---|---|
| TBA | 14.8 |
| BHT | 16.6 |
| -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 |

$IC_{50}^{b:}$ Concentration (µg $ml^{-1}$) for a 50% inhibition was calculated from the plot of *Spirulina maxima* extracts concentration against inhibition % of lipid- peroxidation.

Free radical scavenging and inhibiting lipid peroxidation of hepatic microsomes induced by $CCl_4$ are used 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) at various degrees (Table 4). The $IC_{50}$ values for *Spirulina* phenolic extracts ranged from 23.22 to 35.62 g/ml. Whereas, their values were 18.8, 16.6 and 14.80 µg.$ml^{-1}$ for -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 positive correlation to the phenolic content of the extracts, as increase in extract concen-tration also increases the concentration of phenolic compounds (Tables 4 and 2). Thus, the antioxidant nature of *S. maxima* extracts can be attributed to phe-noilc contents (Table 2), which are widely accepted anti-oxidants 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 µmol $mL^{-1}$, after 45 min of incubation of *in vitro* hepato-toxicity model ($CCl_4$-induced lipid peroxidation of rats liver microsomes) (Table 5 and Figure 1). The catalyzed heaptic 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 by 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 increasing phenolic concentration (50-100 $gmL^{-1}$), that led to a progressive inhibition of the $CCl_4$-induced MDA formation with a concomitant increase of hepatic microsomal resistance to oxidation. These results clearly suggested that inhibition of $CCl_4$-induced lipid peroxidation in rat liver microsomes (*in vitro* hepatotoxicity model) were related to their abilities of phenolic and flavonoid compounds present in *Spirulina* extracts to inhibit hepatic oxidative enzymes (cytochrome *P*450 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 $CCl_4$ induces hepato-toxicity by cytochrome *P*450 mediated reactions to produce $CCl_4$– derived radicals such as trichloromethyl (•$CCl_3$) and trichloromethyl peroxyl (•$OOCCl_3$) (Santoyo et al., 2006). These activated 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 $CCl_4$ (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 $CCl_4$– induce damage (Opoku et al., 2007). It is concluded from the present study that algal phenolic extracts could protect the liver against $CCl_4$- induced lipid peroxi-

**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) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | TPC, ppm | 15 TBARs μmol.mL$^{-1}$ | Inh. (%) | 30 TBARs μmol.mL$^{-1}$ | Inh. (%) | 45 TBARs μmol.mL$^{-1}$ | Inh.(%) | Mean of 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 |
| 3.777 Na $NO_3$ (g L$^{-1}$) + 100 mg L$^{-1}$ phenylalanine | 50 | 1.81 | 75.96 | 3.22 | 71.48 | 4.11 | 71.83 | 73.09 |
| | 100 | 1.01 | 86.58 | 1.21 | 89.28 | 1.23 | 91.57 | 89.14 |

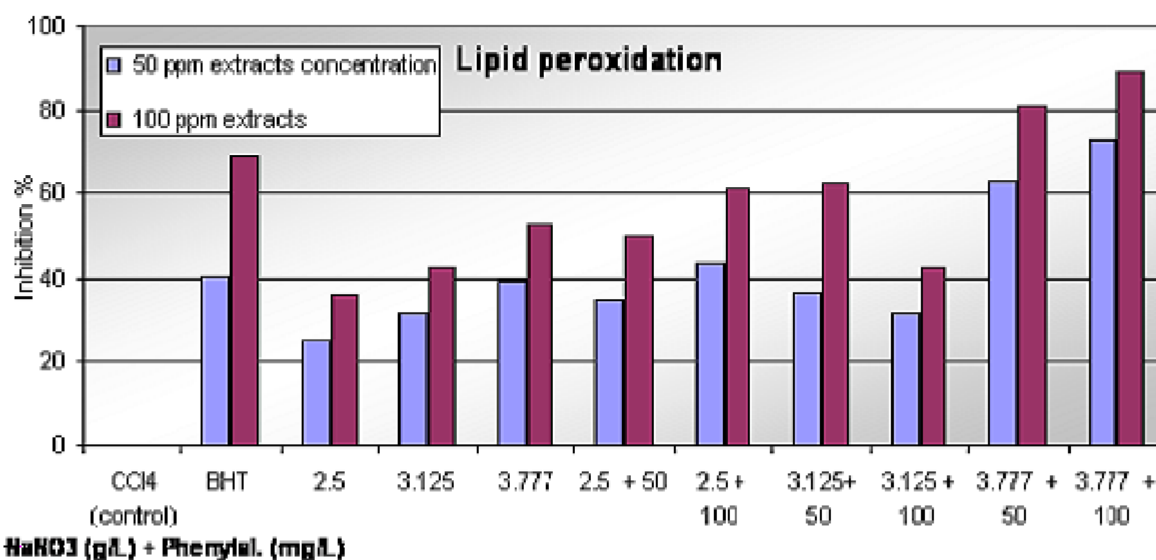


**Figure 1.** Effect of algal phenolic extracts on inhibition (%) of lipid peroxidation of rat liver microsomes induced by $CCl_4$.

dation *in vitro*. Therefore, *S. 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.

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