Shalaby and Shanab, J Mar Biol Oceanogr 2013, 2:1
http://dx.doi.org/10.4172/2324-8661.1000105

Journal of Marine Biology & Oceanography

Research Article

A SCITECHNOL JOURNAL

# Antiradical and Antioxidant Activities of Different Spirulina platensis Extracts against DPPH and ABTS Radical Assays

**Emad A. Shalaby[1]* and Sanaa M. M. Shanab[2]**

## Abstract

The aim of the present research was to evaluate and compare the antiradical and antioxidant activities of extracts from *Spirulina platensis*. In the present study, Three extracts (Water, absolute methanol and 50% methanol in water) were analyzed for the total Phenolic compounds, phycobiliprotein content: Antiradical and antioxidant activities were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging methods.

The obtained results revealed that, absolute methanol extract recorded the highest number of antiradical units 1 mg extract followed in descending order by that of water while the lowest number was that of aqueous methanol (against DPPH and ABTS radical methods). The results indicated that selection of the cyanobacterium (*Spirulina platensis*) very important for consumer's health, as it is considered as potential sources of dietary antioxidants.

## Keywords

*Spirulina platensis*; Antioxidant; Antiradical; DPPH; ABTS; Phenolic compounds

## Introduction

Among several alga genera, *Spirulina* and *Chlorella* deserve special attention due to their importance as human food and their *in vitro* and/or *in vivo* antioxidant potential [1]. *Spirulina* is an important source of nutrients in the traditional diet of some populations of Africa and Mexico. These algae can be extensively grown to obtain a protein-rich material of alimentary (foodstuff for diet complementation) or industrial use (blue pigments, emulsifiers, thickening and gelling agent). The chemical composition of *Spirulina* indicates that it has a high nutritional value due to a wide range of essential nutrients, such as vitamins, minerals and proteins [1]. Moreover, it contains other components such as ω-3 and ω-6 polyunsaturated fatty acid, provitamins and phenolic compounds. In addition, this alga can be cultivated in large-scale systems [2].

Polyphenols constitute a large group of naturally occurring substances in the plant kingdom, which include the flavonoids. The plant phenolics are commonly present in fruits, vegetables, leaves, nuts, seeds, barks, roots and in other plant parts. These substances have considerable interest in the field of food chemistry, pharmacy and medicine due to a wide range of favorable biological effects including antioxidant properties. The antioxidant property of phenolics is mainly due to their redox properties. They act as reducing agents (free radical terminators), hydrogen donors, singlet oxygen quenchers and metal chelators [3]. Evidence suggests that high intake of antioxidant nutrients from food sources offers health advantages [4].

It should be emphasized that there is a great difference between "antiradical" and "antioxidant" activity and that they do not necessarily coincide. According to Tirzitis and Bartosz [5] the antiradical activity characterizes the ability of compounds to react with free radicals (in a single free radical reaction), but antioxidant activity represents the ability to inhibit the process of oxidation (which usually, at least in the case of lipids, involves a set of different reactions). Consequently, all test systems using a stable free radical (for example, DPPH, ABTS, etc) give information on the radical scavenging or antiradical activity, although in many cases this activity does not correspond to the antioxidant activity. In order to obtain information about the real antioxidant activity with respect to lipids or food stabilization, it is necessary to carry out the study on the real product (plant oil, lipoproteins, etc.).

Two methods that are commonly used to assess antioxidant activity in vitro are 2, 2-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS) and 2, 2-diphenyl- 1-picrylhydrazyl (DPPH). However, both of these radicals are foreign to biological systems. The ABTS assay measures the relative ability of antioxidant to scavenge the ABTS generated in aqueous phase, as compared with a Trolox (water soluble vitamin E analogue) standard. The ABTS is generated by reacting a strong oxidizing agent (e.g., potassium permanganate or potassium persulfate) with the ABTS salt. The reduction of the blue-green ABTS radical colored solution by hydrogen-donating antioxidant is measured by the suppression of its characteristic long wave (734 nm) absorption spectrum [6]. The method is usually expressed as Trolox equivalent antioxidant capacity (TEAC). The method is rapid and can be used over a wide range of pH values, in both aqueous and organic solvent systems. It also has good repeatability and is simple to perform; hence, it is widely reported. The method, however, has not been correlated with biological effects; hence, its actual relevance to in vivo antioxidant efficacy is unknown.

The DPPH is a stable free radical with an absorption band at 515 nm. It loses this absorption when reduced by an antioxidant or a free radical species. The DPPH method is widely used to determine antiradical/antioxidant activity of purified phenolic compounds as well as natural plant extracts [7]. Bondet et al. [8] found that most phenolic antioxidants react slowly with DPPH, reaching a steady state in 1-6s h or longer. This suggests that antioxidant activity using DPPH should be evaluated over time. The method also has good repeatability and is used frequently. However, like ABTS, it has limited, if any, relevance to biological systems. Also, color interference of DPPH with samples that contain anthocyanins leads to underestimation of antioxidant activity [9].

***Corresponding author:** Emad A. Shalaby, Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt-12613, E-mail: dremad2009@yahoo.com

**Received:** December 11, 2012 **Accepted:** January 02, 2013 **Published:** January 09, 2013

The ABTS method has the extra flexibility in that it can be used at different pH levels (unlike DPPH, which is sensitive to acidic pH) and thus is useful when studying the effect of pH on antioxidant activity of various compounds [10]. It is also useful for measuring antioxidant activity of samples extracted in acidic solvents. Additionally, ABTS is soluble in aqueous and organic solvents and is thus useful in assessing antioxidant activity of samples in different media and is currently most commonly used in simulated serum ionic potential solution (pH 7.4 phosphate buffer containing 150 mM NaCl) (PBS). Another advantage of ABTS method was that samples reacted rapidly with ABTS in the aqueous buffer solution (PBS) reaching a steady state within 30 min. The DPPH reacted very slowly with the samples, approaching, but not reaching, steady state after 8 h. This slow reaction was also observed when ABTS was reacted with samples in alcohol (data not shown); implying that the reactivity of the antioxidants in sorghums with these free radicals is somehow slowed in alcoholic media. Brand-Williams et al. [11] reported similar slow reaction of most antioxidants which were tested with the DPPH.

The objective of this study was to estimate the antiradical and antioxidant activities of alcoholic and aqueous extracts of *Spirulina platenis* using DPPH and ABTS radical methods.

## Materials and Methods

### Algal source

*Spirulina platensis* was obtained from the Culture Collection of Botany Dept., Faculty of Science, Cairo University, Giza, Egypt.

### Extracts preparation

Three methods of extraction were used *I, II* and *III*. Method *I* was used to extract raw materials. In this method, absolute methanol was used as the first solvent. In raw materials, flavonoids and phenolic acids, which are soluble in methanol, were recognized as the main phenolic fraction with antiradical activity. Method *II* was applied to extract raw materials with 50% methanol in water. In these raw materials, tannins were considered as the important fraction with antiradical activity. Tannins are insoluble in methanol, but well soluble in a mixture of methanol: water (1:1). Method *III* was applied to extract raw materials with Dist. Water. In these raw materials, phycobiliproteins are the main soluble constituents in water.

### Method I of extraction

In method *I*, absolute methanol was chosen as the first solvent in order to extract a wide range of phenolic compounds with a limited amount of tannins. Ten grams of raw material (*Spirulina platensis*) was extracted with 100 ml of methanol at 50°C. After separation of the raw material by filtration, the methanol evaporation under reduced pressure weighted and was kept in deep freezer until use [12].

### Method II of extraction

In method *II*, 50% methanol in water was used to obtain an extract with a large quantity of tannins. Ten grams of raw material was extracted with 100 ml of a solution of methanol-water (1:1 v/v) at 50°C, then filtration followed by solvent was evaporated using rotary evaporator and residue was weighted.

### Method III of extraction

In method *III*, dist. water was used to obtain an aqueous extract with a large quantity of phycobiliproteins, ten grams of the alga raw material was extracted with 100 ml of water (1:1 v/v) and residue was weighted as recorded by Bryant [13].

### Yield of extraction (Y %)

(Y %) was calculated for the three methods (I, II and III) accordingly:

$$Y\% = \frac{Cle}{w_R} 100\%$$

Where $w_R$ is the weight of algal raw material used for extraction (g), *Cle* is total amount of extracts (g).

### Measurement of antiradical activity

DPPH and ABTS radicals in its radical form has a characteristic absorbance at 515 and 734 nm respectively, which disappears after its reduction by an antiradical compound (AH). The reduction of DPPH and ABTS can thus be monitored by measuring the decrease in its absorbance at 515 and 734 nm during the reaction. The methods are simple, precise, and inexpensive, which is important in a screening investigation. All details related to the method are described by Brand-Williams et al. [11].

The antiradical activity ($AU_{515}$) was calculated according to the equation:

$$AU_{515} = (A_0 - A_1) - (A_{0K} - A_{1K})$$

where $AU_{515}$ is the antiradical activity of the extract, $A_0$ the absorbance of the sample at the beginning of the reaction (0 min), *A1* the absorbance of the sample after incubation times (20-120 sec) of the reaction, $A_{0K}$ the absorbance of the control sample at the beginning of the reaction, and $A_{1K}$ the absorbance of the control sample after incubation times (20-120 sec) of the reaction. Because $A_{0K} - A_{1K}$ was always equal to 0, the above equation was simplified to:

$$AU_{515} = A_0 - A_1$$

The absorbance of the samples was measured three times and the standard deviation was calculated. The antiradical activity unit was defined as the activity decreasing the absorbance of a sample at 515 nm after incubation times (20-120 sec) of reaction at 20°C under the defined test conditions. The number of antiradical activity units ($EAU_{515}$) was calculated per 1 mg of each extract according to the following equation 1:

$$EAU_{515} = \frac{AU_{515}}{Ie} \quad (1)$$

Where *Ie* is the amount of extract in the sample (mg) and $AU_{515}$ the antiradical activity of the extract. Then the total number of antiradical activity units extracted from each raw material was calculated per 1 g of raw material ($TAU_{515}$) as described below. For raw materials extracted according to method *I*, $PAU_{515}$ (total number of antiradical activity units in the extract) was calculated separately for each extract a, b, and c according to the equation 2:

$$PAU_{515} = \frac{Cle}{Ie} . AU_{515} \quad (2)$$

Where *Cle* is the total amount of extract (mg) and *Ie* the amount of extract in the measured sample (mg). Then the number of antiradical activity units ($TAU_{515}$) isolated from 1 g of raw materials was calculated as (equation 3):

$$TAU_{515}=\frac{PAU_{515}(a)+PAU_{515}(b)+PAU_{515}(c)}{w_R} \quad (3)$$

Where $PAU_{515}$(a) is $PAU_{515}$ calculated for extracts a, $PAU_{515}$(b) is $PAU_{515}$ calculated for extracts b, $PAU_{515}$(c) is $PAU_{515}$ calculated for extracts c, and $w_R$ is the weight of raw material taken for extraction (g). For raw materials extracted with method *II*, $PAU_{515}$ was calculated separately for extracts A, B, and C according to equation 2.

## Measurement of antioxidant activity

**DPPH method:** The 2, 2 diphenyl-1-picrylhydrazyl (DPPH) test was carried out as described by Burits and Bucar [14]. One ml of algal extract (100 and 200 µg/ml) was mixed with 1ml DPPH reagent (0.002% (w/v) /methanol solution). After an incubation in the dark at room temperature for 30 min., the absorbance was measured at 515nm (using jenway 6130 spectrophotometer). This test was carried out in triplicate and the antioxidant activity was calculated as the following:

Activity (%) =Ac-At / Ac × 100

Where At was the absorbance of samples and Ac the absorbance of methanolic DPPH solution.

**ABTS method:** This assay was based on the ability of different substances to scavenge 2,2'- azino-bis (ethylbenzthiazoline-6-sulfonic acid ($ABTS^{\cdot+}$) radical cation. The radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and leaving the mixture for 4-16 h until the reaction was complete and the absorbance was stable. The $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.700 ± 0.05 at 734 nm for measurements. The photometric assay was conducted on 0.9 ml of $ABTS^{\cdot+}$ solution and 0.1 ml of tested samples (100 and 200 µg/ml) and mixed for 45 sec; measurements were taken immediately at 734 nm after 15 min. The antioxidant activity of the tested samples was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

E= ((Ac-At)/ Ac) × 100

Where At and Ac are the respective absorbance of tested samples and $ABTS^{\cdot+}$, was expressed as µmol [15].

## Extraction and determination of water soluble pigments (phycobiliprotein)

The water soluble phycobiliproteins pigments including allophycocyanin (APC), phycocyanin (C-PC) and C-phycoerytherine (C-PE) were determined according to Bryant [13]. The absorbance (A) of the solution was recorded at the following wave lengths: 650nm; 620nm; 565nm.

## Total phenolic contents

The phenolic content of each extract was determined by the method of Taga et al. [16]. An aliquot of each extract was dissolved in a known amount of appropriate solvent and its absorption was measured at 720nm. The phenolic contents were expressed as gallic acid equivalents per gram of sample (GAE/g).

## The preliminary phytochemical screening

The algal sample was subjected to preliminary phytochemical screening include, testing for tannins, sterols, flavonoids, glycosides and reducing sugar.

**Test for sterol and terpenes:** Two milliliters from each extract were evaporated to dryness and the residue was dissolved in 2 ml chloroform and filtered. The filtrate was detected by libermann-Burchards test [17]. Briefly, in test tube contained algal extract one ml acetic anhydride was added followed by few ml of conc. $H_2SO_4$ poured carefully down the side of tube until the solution formed two separate layers. The formed red ring indicated the presence of sterol or terpene.

**Test for flavonoids:** The test was carried out by adding conc. HCl drop wise to one ml of solution containing a fragment of magnesium ribbon [18]; a positive result gave pinkish color.

**Test for tannins:** Two ml of distilled water were added to 5 ml of extract, and filtrate. Ferric chloride solution (5%) was then added to the filtrate. The formations of yellowish green color indicate the probable presence of tannins [19].

**Test for glycosides and/or carbohydrates:** Extracts were tested for carbohydrates and reducing sugars in the usual manner using Molishs and Fehling reagents [19].

## e-Test for saponin

2 ml of the filtrate was mixed with 1 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion [17].

## Test for cardiac glycosides (Keller-Killani test)

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer [17].

## Chromatographic condition for separation of phenolic compounds

The phenolic compositions of different extracts were determined by HPLC, as already described by Rodriguez Delgado et al. [20] with some modifications. Samples were filtered through a 0.45 µm membrane Millipore chromatographic filter before injection. Volume injected was 50 µl. The separation was performed using an ODS Hypersil 5 µm column (250×4 mm) protected by a guard column of LiChrospher 100 RP-18, 5 µm (4×4 mm). The chromatographic separation was carried out using as mobile phase methanol-acetic acid-water (10:2:88, v/v) as solvent A and methanol-acetic acid-water (90:2:8, v/v) as solvent B programmed in gradient. Duplicate analyses were performed for each sample. The identification of each compound was established by comparing the retention time and UV-Vis spectra of the peaks in wine with those previously obtained by the injection of standards. The quantification was performed by external calibration with standards.

## Blending of algal extract and BHT as synthetic standard

The promising extract of *Spirulina platensis* was used for the determination of its antioxidant activity using DPPH method (as mentioned before) after blending (mixed) with synthetic antioxidant standard (BHT) from 100% methanol extract to 100 % BHT.

### Statistic analysis

Data were subjected to an analysis of variance, and the means were compared using the Least Significant Difference (LSD). ″test at the 0.05 and 0.01 levels were determined according to the method of Snedecor and Cochran [21].

## Results and Discussion

Antiradical and antioxidant activities of the three *Spirulina platensis* extracts (water, absolute methanol, 50% aqueous methanol) were determined using both DPPH and ABTS radical scavenging methods.

Antiradical activity was recorded as the number of antiradical units (AU), the number of units in 1 mg extract (EAU), the total number of units in 1 mg extract (PAU515), the number of antiradical units in 1g of raw material (TAU515) during the incubation period of 20-120 seconds. The antioxidant activity (%) was performed using two extract concentrations (100, 200 µg/ml) during incubation periods 2-30 min.

Using DPPH radical scavenging method, the obtained results recorded in Tables 1-3, revealed that, both the antiradical and antioxidant activities go parallel and were shown to be concentration of extract and incubation time dependent.

Water extract showed the highest antiradical activity represented as AU (0.963), EAU (17.40), PAU 515 (0.133) and TAU 515 (172.8) at higher extract concentration (200 µg/ml) and at 120 seconds of incubation (Tables 1-3).

Water extract recorded also the highest antioxidant activity (Table 4, 95.3%) at concentration of 200 µg/ml; and after 30 min of incubation followed by absolute methanol 89.61% and aqueous methanol 68.41%. The greatest antiradical activity of water extract was followed in descending order by those of 50% aqueous methanol and absolute methanol at the same extract conc. and after the same incubation period.

The obtained results clearly showed that both the highest antiradical and antioxidant activity, determined by DPPH method, were highly correlated with water extract of *S. platensis*.

Using ABTS radical scavenging method, for the determination of antiradical and antioxidant activities, the obtained results recorded in Tables 5-8, illustrated that both activities have the same trend and were extract concentration and time of incubation dependent.

Contrary to the DPPH method and the higher activities of water extract, the ABTS method revealed that, absolute methanol extract showed the highest antiradical (AU, 0.912, EAU 16.84, PAU 515 0.434 and TAU515 1168) as well as antioxidant (99.55%) activities. These were followed in descending order by those of water and aqueous method.

These results clearly showed that both activities were highly correlated with absolute methanol extract of *S. platensis* to get an explanation of these results, we have to detect and determine the active substances in the three tested *S. platensis* extract which may interfere or participate in both antiradical and antioxidant activities.

Phycobilinprotein pigments, phenolic contents and phytochemical screening of active constituents were performed.

**Table 1:** Antiradical Unit (AU) against DPPH of *Spirulina platensis* at two different concentrations (100 and 200 µg/ml).

| Sample no. | 100 µg/ml | | | | | |
|---|---|---|---|---|---|---|
| | Times (Sec) | | | | | |
| | 20 | 40 | 60 | 80 | 100 | 120 |
| **Water 100 %** | 0.553 | 0.577 | 0.595 | 0.614 | 0.624 | 0.633 |
| **Methanol 100 %** | 0.021 | 0.053 | 0.075 | 0.101 | 0.123 | 0.137 |
| **Water:Methanol 50: 50** | 0.156 | 0.180 | 0.203 | 0.213 | 0.228 | 0.238 |
| **200 µg/ml** | | | | | | |
| **Water 100 %** | 0.693 | 0.963 | 0.963 | 0.963 | 0.0.963 | 0.963 |
| **Methanol 100 %** | 0.420 | 0.470 | 0.507 | 0.543 | 0.567 | 0.591 |
| **Water: Methanol 50: 50** | 0.57 | 0.602 | 0.625 | 0.645 | 0.658 | 0.670 |

**Table 2:** The number of antiradical activity unites in 1 mg of extract (EAU) against DPPH.

| Sample no. | 100 µg/ml | | | | | |
|---|---|---|---|---|---|---|
| | Times (Sec) | | | | | |
| | 20 | 40 | 60 | 80 | 100 | 120 |
| **Water 100 %** | 9.99 | 10.43 | 10.75 | 11.09 | 11.27 | 11.44 |
| **Methanol 100 %** | 0.37 | 0.95 | 1.35 | 1.82 | 2.22 | 2.47 |
| **Water: Methanol 50: 50** | 2.81 | 3.25 | 3.66 | 3.85 | 4.12 | 4.30 |
| **200 µg/ml** | | | | | | |
| **Water 100 %** | 12.52 | 17.40 | 17.40 | 17.40 | 17.40 | 17.40 |
| **Methanol 100 %** | 7.59 | 8.49 | 9.16 | 9.81 | 10.24 | 10.68 |
| **Water: Methanol 50: 50** | 10.30 | 10.88 | 11.29 | 11.65 | 11.89 | 12.11 |

**Table 3:** Total number of antiradical activity units in 1 mg extracts ($PAU_{515}$) and the number of antiradical units in 1g raw material ($TAU_{515}$) against DPPH. At 100 µg/ml.

| Sample no. | Times (Sec) | | | | | | The number of antiradical units in 1g raw material ($TAU_{515}$) |
|---|---|---|---|---|---|---|---|
| | 20 | 40 | 60 | 80 | 100 | 120 | |
| **Water 100 %** | 0.0116 | 0.121 | 0.1249 | 0.1289 | 0.131 | 0.133 | 172.8 |
| **Methanol 100 %** | 0.0038 | 0.00967 | 0.01368 | 0.184 | 0.0224 | 0.025 | |
| **Water: Methanol 50: 50** | 0.00975 | 0.0113 | 0.0126 | 0.0133 | 0.0143 | 0.0148 | |

**Table 4:** Antioxidant activity (%) of different extracts of *Spirulina platensis* against DPPH.

| Sample no. | After 2 min | | After 30 min | |
|---|---|---|---|---|
| | 100 µg/ml | 200 µg/ml | 100 µg/ml | 200 µg/ml |
| **Water 100 %** | 65.73 | 72.58 | 79.4 | 95.3 |
| **Methanol 100 %** | 14.22 | 61.37 | 84.3 | 89.61 |
| **Water: Methanol 50: 50** | 24.71 | 69.57 | 65.2 | 68.41 |
| **L.S.D** | 0.041 | 0.0413 | 0.079 | 0.058 |

Each value is presented as mean of triplet treatments, LSD: Least significantly different at P ≤ 0.01 according to Duncan's multiple range test

**Table 5:** Antiradical Unit (AU) of different extracts of *Spirulina platensis* against ABTS.

| Sample no. | 100 µg/ml | | | | | |
|---|---|---|---|---|---|---|
| | Times (Sec) | | | | | |
| | 20 | 40 | 60 | 80 | 100 | 120 |
| **Water 100 %** | 0.80 | 0.891 | 0.891 | 0.892 | 0.892 | 0.892 |
| **Methanol 100 %** | 0.81 | 0.847 | 0.893 | 0.893 | 0.893 | 0.893 |
| **Water: Methanol 50: 50** | 0.81 | 0.84 | 0.847 | 0.848 | 0.852 | 0.872 |
| **200 µg/ml** | | | | | | |
| **Water 100 %** | 0.80 | 0.890 | 0.893 | 0.894 | 0.894 | 0.899 |
| **Methanol 100 %** | 0.82 | 0.850 | 0.899 | 0.901 | 0.908 | 0.912 |
| **Water: Methanol 50: 50** | 0.82 | 0.843 | 0.851 | 0.855 | 0.860 | 0.880 |

**Table 6:** EAU of different extracts of *Spirulina platensis* against against ABTS.

| Sample no. | 100 µg/ml | | | | | |
|---|---|---|---|---|---|---|
| | Times (Sec) | | | | | |
| | 20 | 40 | 60 | 80 | 100 | 120 |
| **Water 100 %** | 14.46 | 16.11 | 16.11 | 16.12 | 16.12 | 16.12 |
| **Methanol 100 %** | 14.64 | 15.31 | 16.14 | 16.14 | 16.14 | 16.14 |
| **Water: Methanol 50: 50** | 14.64 | 15.18 | 15.31 | 15.32 | 15.40 | 15.76 |
| **200 µg/ml** | | | | | | |
| **Water 100 %** | 14.46 | 16.08 | 16.14 | 16.16 | 16.16 | 16.25 |
| **Methanol 100 %** | 14.82 | 15.36 | 16.25 | 16.28 | 16.41 | 16.48 |
| **Water: Methanol 50: 50** | 14.82 | 15.23 | 15.38 | 15.45 | 15.45 | 15.90 |

**Table 7:** Total number of antiradical activity units in 1 mg extracts ($PAU_{515}$) and the number of antiradical units in 1g raw material ($TAU_{515}$) against ABTS. At 100 µg/ml.

| Sample no. | Times (Sec) | | | | | | The number of antiradical units in 1g raw material ($TAU_{515}$) |
|---|---|---|---|---|---|---|---|
| | 20 | 40 | 60 | 80 | 100 | 120 | |
| **Water 100 %** | 0.275 | 0.280 | 0.283 | 0.321 | 0.345 | 0.370 | 1168 |
| **Methanol 100 %** | 0.284 | 0.391 | 0.394 | 0.405 | 0.430 | 0.434 | |
| **Water: Methanol 50: 50** | 0.264 | 0.268 | 0.301 | 0.325 | 0.342 | 0.364 | |

Total phycobilin pigments (CPC, APC, CPE) content in water extract recorded the highest values followed by those of absolute methanol and then aqueous methanol (8.23, 3.583, 1.090 mg/g respectively) as illustrated in Table 9. The weight of these extracts represented as % yield showed that absolute methanol have about two folds (7.3%) that of water (4.2%) and three folds that of aqueous methanol (2.5%) (Table 10 and Figure 1).

Phytochemical screening recorded that absolute methanol contained five active substances, then aqueous methanol (three substances) and water contained only two substances (in order absolute methanol (5 substances) < aqueous methanol (3 substances) < water (2 substances)) as illustrated in Table 11.

Determination of phenolic compounds in the three extracts revealed that, absolute methanol recorded the highest percentage (1.23%) then aqueous methanol (0.89%) and finally water extract with 0.55% (Table 12 and Figure 2).

Analysis of phenolic compounds in both water and absolute methanol (By HPLC) which recorded higher activities revealed that, absolute methanol contained the gretest of total phenolic compounds (282.76 mg/100g) with maximum peak of pyragallol (182.15 mg/g). While, water extract showed total phenolic compounds of 169.15 mg/100g, with two moderate peaks of gallic (42.71 mg/100g) and pyragallol (37.2 mg/100g).

**Table 8:** Antioxidant activity (%) of different extracts of *Spirulina platensis* against ABTS.

| Sample no. | After 2 min | | After 30 min | |
|---|---|---|---|---|
| | 100 µg/ml | 200 µg/ml | 100 µg/ml | 200 µg/ml |
| **Water 100 %** | 78.50 | 82.50 | 94.88 | 99.44 |
| **Methanol 100 %** | 80.65 | 85.91 | 99.11 | 99.55 |
| **Water: Methanol 50: 50** | 77.60 | 81.74 | 96.11 | 97.44 |
| **L.S.D** | 20.37 | 3.040 | 2.13 | 1.98 |

Each value is presented as mean of triplet treatments, LSD: Least significantly different at P ≤ 0.01 according to Duncan's multiple range test

**Table 9:** Phycobiliprotein content as mg/g of different extracts of *Spirulina platensis*.

| Algae sample | CPC | APC | CPE |
|---|---|---|---|
| **Water 100 %** | 1.3 | 4.1 | 2.83 |
| **Methanol 100 %** | 1.1 | 2.42 | 0.063 |
| **Water: Methanol 50: 50** | 0.3 | 0.65 | 0.14 |
| **L.S.D** | 0.041 | 0.058 | 0.024 |

CPC: C0phycocyanin; APC: Allophycocyanin; CPE: C-phycoerytherin.
Each value is presented as mean of triplet treatments, LSD: Least significantly different at P ≤ 0.01 according to Duncan's multiple range test

**Table: 10.** Yield % of different extracts of *Spirulina platensis*.

| Sample no. | Y % |
|---|---|
| **Water 100 %** | 4.2 |
| **Methanol 100 %** | 7.3 |
| **Water: Methanol 50: 50** | 2.5 |
| **L.S.D** | 0.2408 |

Each value is presented as mean of triplet treatments, LSD: Least significantly different at P ≤ 0.01 according to Duncan's multiple range test

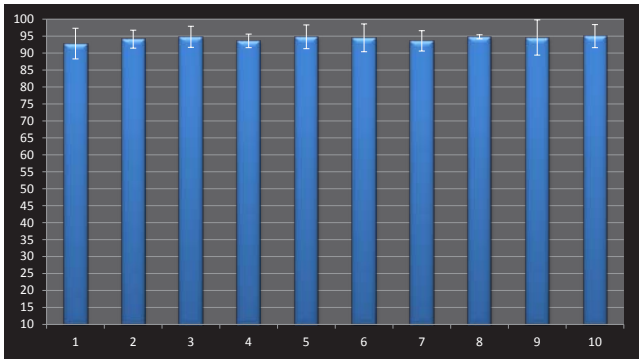


**Figure 1:** Blending of synthetic antioxidant (BHT) with natural extract (Promising extract from *Spirulina*).

**Table: 11**: Phytochemical screening of different extracts from *Spirulina platensis*.

| Sample no. | Ste-roids | Flavo-noids | Reduc-ing sugars | Tannins | Cardic glyco-sides | Sapo-nin | Anthra-qui-none |
|---|---|---|---|---|---|---|---|
| **Water 100 %** | - | - | - | - | + | - | + |
| **Methanol 100 %** | + | + | + | + | - | + | - |
| **Water: Methanol 50: 50** | + | + | - | - | + | - | - |

+: present; -: Absent.

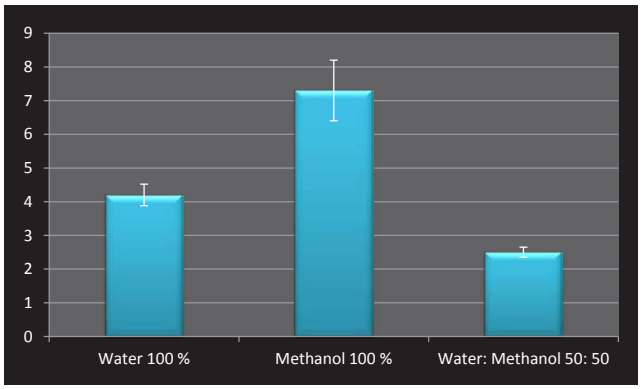


**Figure 2:** The yield percentage of different extracts from *Spirulina platensis*.

**Table: 12:** Total phenolic compounds of *Spirulina platensis*

| Sample no. | Phenolic content % |
|---|---|
| **Water 100 %** | 0.55 |
| **Methanol 100 %** | 1.23 |
| **Water: Methanol 50: 50** | 0.89 |
| **L.S.D** | 0.0041 |

With DPPH method, water extract of *S. platensis* recorded higher antiradical and antioxidant activity which may be mainly due to the great content of phycobiliprotein pigments (8.23 mg/g) which was famously known by its potent antiradical activity [13,22,23].

Also, water extract have lower contents of both phenolic compounds (about half that in methanol) and the phytochemical

substances (saponin and anthraquinone) which participate synergistically with phycobilin the pronounced higher antiradical and antioxidant activity of water extract.

On the other hand, using ABTS method, greater antiradical and antioxidant activities were recorded in absolute methanol (water extract in the second order) extract. These results may be due to the pronounced contents in this extract of total phenolic content and phenolic compounds (HPLC) which were characterized by its great free radical scavengting, hydrogen donating and metal chelating efficiencies. These results were in accordance with those obtained by Cook and Samman [3], Sroka [12] and Melichacova et al. [24].

Methanol extract also contained large quantity of biologically active phytochemical substances (sterols, flavonoids, reducing sugar, tannins and anthraquinone) which may exhibit great additional antiradical and antioxidant activity to those exerted by phenolics.

The lower content of phycobilins in methanol extract (3.583 mg/g) may also participate synergistically in the potent activity excreted by absolute methanol extract (have the highest % yield of 7.3%).

Aqueous methanol extract either with DPPH or ABTS methods, recorded moderate antiradical and antioxidant activities which were attributed its moderate contents of phycobilin pigments, total phenolic content, phytochemical substances which coincided with the lower % yield of this extract (2.5%).

So antiradical and antioxidant activities by DPPH method was correlated with water extract while those recorded with ABTS method correlated with absolute methanol (Table 13).

The % yield (quantity) of both extracts affects the calculated PAU and TAU on using both DPPH and ABTS.

Absolute methanol extract recorded the highest antiradical and antioxidant activities comparing with those of water extract (Table 13). Mixing (blending) the promising absolute methanol extract (gave 99.55% antioxidant activity) with the standard synthetic antioxidant BHT in gradual proportion (from 100% methanol extract to 100% BHT), including closely similar activities (with very few differences) in all extracts and BHT proportions (Figure 3). This means that the activity extract by the standard BHT was increased by each proportion of extract with an obvious synergism between them.

The obtained results were found to be in agreement with those recorded by Nivas et al. [25] and Tiryitis and Bartosz [5] in the same context Kaviarasan et al. [26] studied the antiradical and antioxidant activity (by ABTS and DPPH) using aqueous methanolic extract (80%) of fenugreek seeds and they found that the activities could be correlated with the polyphenolic compounds in the extract.

Meanwhile, Sroka [12] determined the antiradical and antioxidant activities in absolute and 50% methanol extracts of green and black tea leaves. He reported that absolute methanol extracted the tannins from tea leaves in addition to other sources. Higher antiradical activity unit TAU/g was those of green and black tea leaves in ethyl acetate fraction of aqueous methanol extract.

In the same context Melichacova et al. [24] reported that the antioxidant activity of 50% methanol extract of both sweet cherry and tart cherry fruit were due to and correlated with the soluble phenolics in this solvent.

On the other hand, the potent antioxidant activity of ethyl acetate fraction of 70% ethanol extract of the green seaweed *Enteromorpha compressa* [27] was found to be not correlated with phenolic content.

Also, higher concentration of ethanol extracts of the salt stressed *Spirulina platensis* recorded potent antioxidant activity with both DPPH (85%) and ABTS (89%) as reported by Shalaby et al. [23] which correlated with both phycobilin pigments and phenolics

**Table 13:** HPLC profile of phenolic compounds (as mg/100 g) of water and methanol extracts from *Spirulina plantensis*.

| Phenolic compounds | Extracts | |
|---|---|---|
| | Water | Methanol |
| **Pyrogallol** | 37.2 | 182.15 |
| **Gallic** | 42.71 | — |
| **P-OH Benzoic** | 4.83 | 2.45 |
| **Catechol** | 10.6 | 5.79 |
| **Caffeic** | 2.61 | 5.24 |
| **Protocatechouic** | 12.59 | 17.76 |
| **Catechin** | 17.19 | 32.32 |
| **Chlorogenic** | 4.05 | 16.14 |
| **Vanillic** | 4.88 | 2.59 |
| **Synergic** | 2.59 | 3.73 |
| **Caffeine** | 9.49 | 2.54 |
| **Ferulic** | 4.61 | — |
| **Salicylic** | 12.08 | 8.68 |
| **Coumarin** | 3.78 | 3.37 |
| **Total** | 169.15 | 282.76 |

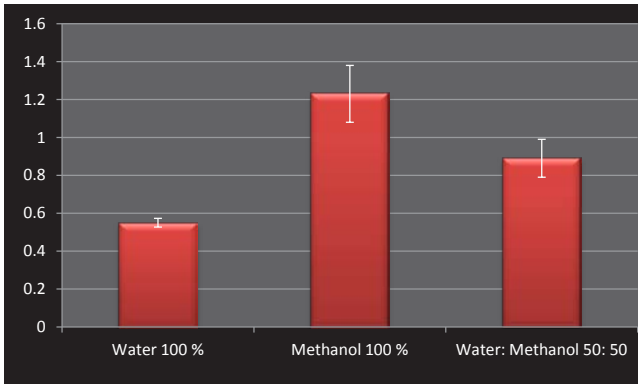


**Figure 3:** Phenolic compounds percentage of different extracts from *Spirulina platensis*.

## Conclusion

Antiradical activity of plant extracts was concentration of extract and incubation timed dependent.

As, the antiradical activity defined as the ability of a compound to react with free radicals in a single free radical reaction, calculating the antiradical activity units showed the highest values after 120 seconds (2 minutes) comparing with antioxidant activity (30 min) which is important in time saving and considered more sensitive especially with the electron reacting ABTS radicals.

*Spirulina platensis* showed higher antiradical and antioxidant activity (99.55%) with ABTS and absolute methanol extract, while the water extract recorded slightly lower activities (95.3%) when tested with DPPH hydrogen reacting radicals.

## References

1. Dillon JC, Phan PA (1993) Spirulina as source of protein in human nutrition. Bulletin de l'Institut Oceanographique 12: 103-107.
2. Pascaud M (1993) The essential polyunsaturated fatty acids of Spirulina and our immune response. Bulletin de l'Institut Oceanographique 12: 49-57.
3. Cook NC, Samman S (1996) Flavonoids: Chemistry, metabolism, cardioprotective effects, and dietary sources. Nutritional Biochemistry 7: 66–76.
4. Aruoma OI (1998) Free radicals, oxidative stress, and antioxidants in human health and disease. Journal of the American Oil Chemists' Society 75: 199–212.
5. Tirzitis G, Bartosz G (2010) Determination of antiradical and antioxidant activity: basic principles and new insights. Acta Biochemica Polonica 57: 139-142.
6. Miller NJ, Rice-Evans CA (1997) Factors influencing the antioxidant activity determined by the ABTS.+ radical cation assay. Free Radic Res 26: 195-199.
7. Fukumoto LR, Mazza G (2000) Assessing antioxidant and prooxidant activities of phenolic compounds. J Agric Food Chem 48: 3597-3604.
8. Bondet V, Brand-Williams W, Berset C (1997) Kinetics and mechanisms of antioxidant activity using the DPPH• free radical method. Lebensm.-Wiss. u.-Technol 30: 609-615.
9. Arnao MB (2000) Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case. Trends Food Sci Technol 11: 419-421.
10. Ou B, Huang D, Hampsch-Woodill M, Flanagan JA, Deemer EK (2002) Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. J Agric Food Chem 50: 3122-3128.
11. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technol 28: 25-30.
12. Sroka Z (2006). The screening analysis of antiradical activity of some plant extracts. Postepy Hig Med Dosw 60: 563-570
13. Bryant DA (1979) Phycoerythrin and phycocyanin properties and occurance in cyanobacteria. J Gen Microbiol 128: 835-844.
14. Burits M, Bucar F (2000) Antioxidant activity of Nigella Sativa esserntial oil. Phytother Res 14: 323-328.
15. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M , et al. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26: 1231-1237.
16. Taga MS, Miller EE, Pratt DE (1984) Chia seeds as a source of natural lipid antioxidants. J Am Oil Chem Soc 61: 928–931.
17. Berieskern CH, Klinger-Handpolonius W (1961) Triterpene and sterol in leaves of Saliva trilopa and Pyrus malus. Arch Pharm 294: 380-391.
18. Wall ME, Krider MM, Krewson CF, Eddy CR, Williaman JJ, et al. (1954) Steroidal sapogenins. VII. Survey of plants for steroidal sapogenins and other constituents. J Pharm Soc 43: 1-3.
19. Claus ER (1967) Review of physiological chemistry, (15thedn). Long Medicinal publication. Los Angles, California.
20. Rodriguez-Delgado MA, González-Hernández G, Conde-González JE, Pérez-Trujillo JP (2002) Principal component analysis of the polyphenol content in young red wines. Food Chemistry 78: 523-532.
21. Snedecor GW, Cochran WG (1982) Statistical Methods. The Iowa State Univ. Press., Ames., Iowa, USA 507.
22. 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.
23. Shalaby EA, Shanab SMM, Singh V (2010) Salt stress enhancement of antioxidant and antiviral efficiency of *Spirulina platensis*. J Med Plants Res 4: 2622-2632.
24. Melichacova S, Timoracka M, Bystrick J, Vollmannova A, Cery I (2010) Relation of total antiradical activity and total polyphenol content of sweet cherries (*Prunus avium* L.) and tart cherries (*Prunus cerasus* L.). Acta agriculturae slovenica 95: 21-28.
25. Nivas D, Gaikwad DK, Havan PD (2010) Antiradical activity of radically important *Morinda pubescens* fruits. International Journal of Pharma and Bio Sciences 1: 1-4.
26. Kaviarasan S, Naik GH, Gangabhagirathi R, Anuradha CV, Priyadarsini KI (2007) In vitro studies on antiradical and antioxidant activities of Fenugreek (*Trigonella foeveem*) seeds. Food Chemistry 103: 31-37.
27. Shanab SMM, Shalaby EA, El-Fayoumy EA (2011) *Enteromorpha compressa* exhibits potent antioxidant activity. J Biomed Biotechnol 1-11.

## Author Affiliations

Top

[1]*Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt-12613*
[2]*Department of Botany, Faculty of Science, Cairo University, Giza, Egypt-12613*