

## Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis*

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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<sup>•</sup>) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging methods at 100 and 200 µg/mL. 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) and these correlated with chemical constituents of extract from phenolic and phycobiliprotein compounds. It is elucidated 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<sup>1</sup>. 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<sup>2</sup>. Polyphenols constitute a large group of naturally occurring substances in the plant kingdom, which include the flavonoids. 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<sup>3</sup>. Evidence suggests that high intake of antioxidant nutrients from food sources offers health advantages<sup>4</sup>.

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<sup>5</sup> 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.).

Numerous methods are used to evaluate antioxidant activities of natural compounds in foods or biological systems with varying results. Two free radicals 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. 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. Reduction of blue-green ABTS radical colored solution by hydrogen-donating antioxidant is measured by the suppression of its characteristic long wave (734 nm) absorption spectrum<sup>6</sup>.

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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. DPPH method is widely used to determine antiradical/antioxidant activity of purified phenolic compounds as well as natural plant extracts<sup>7</sup>. Bondet *et al.*<sup>8</sup> found that most phenolic antioxidants react slowly with DPPH, reaching a steady state in 1-6 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<sup>9</sup>.

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<sup>10</sup>. 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 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; implying that the reactivity of the antioxidants in sorghums with these free radicals is somehow slowed in alcoholic media. Brand-Williams *et al.*<sup>11</sup> reported similar slow reaction of most antioxidants which were tested with the DPPH. The objective of this study was to compare the antiradical and antioxidant activities of alcoholic and aqueous extracts of *Spirulina platensis* using DPPH and ABTS radical methods.

## Materials and Methods

*Spirulina platensis* was obtained from the Culture Collection of Botany Dept., Faculty of Science, Cairo University, Giza, Egypt. 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 this raw materials, phycobiliproteins are the main soluble constituents in water.

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 three times with 100 mL of methanol at 50°C. After separation of the raw material by filtration, the methanol was evaporated under reduced pressure (at 40-50°C) weighted and was kept in deep freezer until use<sup>12</sup>.

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 weighted.

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 *et al.*<sup>13</sup>

### Yield of extraction (Y %)

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

$$Y\% = \frac{Cle}{W_R} \cdot 100\%$$

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. 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.*<sup>11</sup>.

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

$$AU_{515} = (A_0 - A_I) - (A_{0K} - A_{IK})$$

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),  $A_I$  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  $A_{IK}$  the absorbance of the control sample after incubation times (20-120 sec) of the reaction. Because  $A_{0K} - A_{IK}$  was always equal to 0, the above equation was simplified to:

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

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:

$$EAU_{515} = \frac{AU_{515}}{Ie}$$

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:

$$PAU_{515} = \frac{Cle}{Ie} AU_{515}$$

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:

$$TAU_{515} = \frac{PAU_{515}(a) + PAU_{515}(b) + PAU_{515}(c)}{W_R}$$

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

$wR$  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 (1).

#### Antioxidant activity

##### a. DPPH method

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) tests were carried out as described by Burits and Bucar<sup>14</sup>. One mL of algal extract (100 and 200 µg/mL) was mixed with 1 mL 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 515 nm (using Jenway 6130 spectrophotometer). This test was carried out in triplicate and the antioxidant activity was calculated as the following:

$$\text{Activity (\%)} = \frac{Ac - At}{Ac} \times 100$$

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

##### b. ABTS method

This assay was based on the ability of different substances to scavenge 2, 2'-azino-bis(ethylbenzthiazoline-6-sulfonic acid (ABTS<sup>+</sup>) 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<sup>+</sup> solution was diluted with ethanol to an absorbance of  $0.700 \pm 0.05$  at 734 nm for measurements. The photometric assay was conducted on 0.9 mL of ABTS<sup>+</sup> 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 antioxidative activity of the tested samples was calculated by determining the decrease in absorbance at different concentrations by using the following equation:  $E = ((Ac - At) / Ac) \times 100$ , where:

$At$  and  $Ac$  are the respective absorbance of tested samples and ABTS<sup>+</sup>, was expressed as µmol<sup>15</sup>.

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

The water soluble phycobiliproteins pigments including allophycocyanin (APC), phycocyanin (C-PC) and C-phycoerythrin (C-PE) were determined according to Bryant *et al.*,<sup>13</sup>. The absorbance (A) of the solution was recorded at the following wave lengths: 650 nm; 620 nm; 565 nm.

### Total phenolic contents

The phenolic content of each extract was determined by the method of Taga *et al.*,<sup>16</sup>. An aliquot of each extract was dissolved in a known amount of appropriate solvent and its absorption was measured at 720 nm. 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.

#### a. 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<sup>17</sup>. Briefly, in test tube contained algal extract one ml acetic anhydride was added followed by few ml of conc. H<sub>2</sub>SO<sub>4</sub> 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.

#### b. 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<sup>18</sup>; a positive result gave pinkish color.

#### c. 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<sup>19</sup>.

#### d. Test for glycosides and/or carbohydrates

Extracts were tested for carbohydrates and reducing sugars in the usual manner using Molishs and Fehling reagents<sup>19</sup>.

#### 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<sup>17</sup>.

#### 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 underlayered 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<sup>17</sup>.

Chromatographic condition for separation of phenolic compounds.

Phenolic compositions of different extracts were determined by HPLC, as already described by Rodriguez Delgado *et al.*,<sup>20</sup> with some modifications. Samples were filtered through a 0,45 µm membrane Millipore chromatographic filter before injection. Volume injected was 50 µL. 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). 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 (Fig. 1).

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).  $\bar{t}$  test at the 0.05 and 0.01 levels were determined according to the method of Snedecor and Cochran<sup>21</sup>.

## 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 1 g of raw material (TAU515) during the incubation period of

20-120 seconds. The antioxidant activity (%) was performed using two extract concentration (100, 200 ug/mL) during incubation periods 2-30 min.

Using DPPH radical scavenging method, the obtained results recorded in Tables 1-4, 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 ug/mL) and at 120 seconds of incubation (Tables 1, 2, 3).

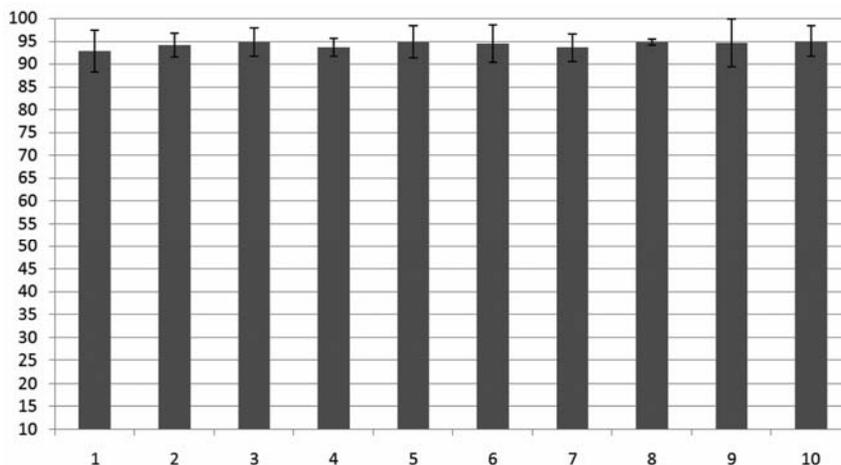


Fig.1—Blending of synthetic antioxidant (BHT) with natural extract (Promising extract from *Spirulina platensis*) 1- 100% methanol extract 2- 90% Methanol ext.: 10% BHT 3-80% Methanol ext.: 20% BHT 4-70% Methanol ext.: 30% BHT 5-60% Methanol ext.: 40% BHT 6- 50% Methanol ext.: 50% BHT 7-40% Methanol ext.: 60% BHT 8-30% Methanol ext.: 70% BHT 9-20% Methanol ext.: 80% BHT 10- 100% BHT

Table 1—Antiradical Unit (AU) against DPPH of *Spirulina platensis* at two different concentrations (100 and 200 ug/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<sub>515</sub>) and the number of antiradical units in 1g raw material (TAU<sub>515</sub>) against DPPH. At 100 ug/mL

Sample no.	Times (Sec)						The number of antiradical units in 1g raw material (TAU <sub>515</sub> )
	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	

Water extract recorded also the highest antioxidant activity (Table 4, 95.3%) at concentration of 200 ug/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 concentration and after the same incubation period.

The obtained results clearly showed that both the highest antiradical and antioxidant activity,

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

Sample no.	After 2 min		After 30 min	
	100 ug/mL	200 ug/mL	100 ug/mL	200 ug/mL
Water 100 %	65.73	72.58	79.4	95.3
Methanol 100 %	14.22	61.37	84.3	89.61
Water:	24.71	69.57	65.2	68.41
Methanol 50: 50				
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	0.80	0.891	0.891	0.892	0.892	0.892
Water 100 %						
Methanol	0.81	0.847	0.893	0.893	0.893	0.893
Methanol 100%						
Water:	0.81	0.84	0.847	0.848	0.852	0.872
Methanol 50: 50						
	200 µg/mL					
Water 100%	0.80	0.890	0.893	0.894	0.894	0.899
Methanol	0.82	0.850	0.899	0.901	0.908	0.912
Methanol 100%						
Water:	0.82	0.843	0.851	0.855	0.860	0.880
Methanol 50: 50						

Table 6—EAU of different extracts of *Spirulina platensis* against ABTS

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

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.

Table 7—Total number of antiradical activity units in 1 mg extracts (PAU<sub>515</sub>) and the number of antiradical units in 1g raw material (TAU<sub>515</sub>) against ABTS. At 100 µg/mL

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

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

Sample no.	After 2 min		After 30 min	
	100 ug/mL	200 ug/mL	100 ug/mL	200 ug/mL
Water 100 %	78.50	82.50	94.88	99.44
Methanol	80.65	85.91	99.11	99.55
Methanol 100 %				
Water: Methanol	77.60	81.74	96.11	97.44
50: 50				
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

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.

Total phycobilin pigments (CPC, APC, and 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%) as shown in Table 10.

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).

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

With DPPH method, water extract of *S. platensis* recorded higher antiradical and antioxidant activity which may be mainly due to the great content of phycobilinprotein pigments (8.23 mg/g) which was famously known by its potent antiradical activity<sup>13,22, 28</sup>.

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

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  $P \leq 0.01$  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  $P \leq 0.01$  according to Duncan's multiple range test

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

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

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

Sample no.	Steroids	Flavonoids	Reducing sugars	Tannins	Cardic glycosides	Saponin	Anthraquinone
Water 100 %	-	-	-	-	+	-	+
Methanol 100 %	+	+	+	+	-	+	-
Water: Methanol 50: 50	+	+	-	-	+	-	-

+: present; -: Absent.

by its great free radical scavenging, hydrogen donating and metal chelating efficiencies. These results were in accordance with those obtained by Cook and Samman<sup>3</sup>, Sroka (12) and Melichacova *et al.*<sup>26</sup>.

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 (Fig. 2).

Absolute methanol extract recorded the highest antiradical and antioxidant activities comparing with those of water extract (Table 13). Mixing (blending)

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

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
Protocatechuic	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

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. 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.* (23) and Tiriyitis and Bartosz<sup>5</sup> in the same context kaviarasan *et al.*<sup>24</sup> 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<sup>12</sup> 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.*<sup>26</sup> 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*<sup>27</sup> 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

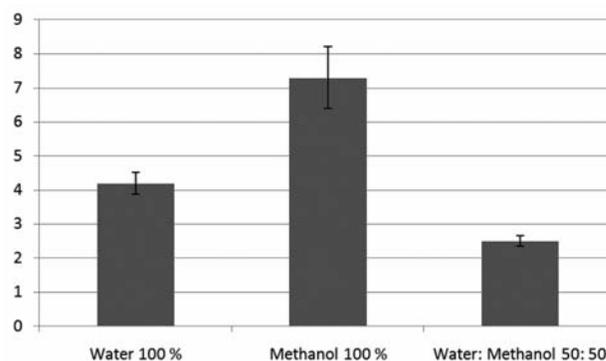


Fig. 2—The yield percentage of different extracts from *Spirulina platensis*

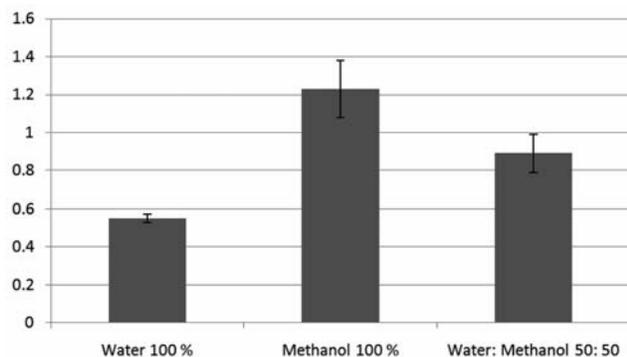


Fig. 3—Phenolic compounds percentage of different extracts from *Spirulina platensis*

(89%) as reported by Shalaby *et al.*<sup>28</sup> which correlated with both phycobilin pigments and phenolics (Fig. 3).

### Conclusion

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

*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.

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