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Antioxidant and Anticancer Activity of *Spirulina platensis* Water Extracts

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ABSTRACT
Spirulina, a filamentous cyanobacterium, botanists classify it as a micro alga belonging to cyanophyceae, it has a simple structure but a complex composition. *Spirulina* and its components have been shown to have positive benefits across a range of human health indications from malnutrition to antioxidant properties. One of its species *Spirulina platensis* or its extract showed therapeutic properties, such as the ability to prevent cancers, decrease blood cholesterol level, reduce nephrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effect of radiation. Therefore, the aim of the present study is to determine antioxidant activity of *Spirulina platensis* water extracts and their cytotoxicity against cell lines {colon carcinoma cells (HCT116) and hepatocellular carcinoma cells (HEPG2)}. Results showed that at *S. platensis* water extract of 1.5 g/100 mL, highest antioxidants percentage (81.1%) and total phenolic compounds (40.45 mg g^{-1}) were recorded. For cytotoxicity assay, the IC_{50} concentrations (the concentration of *S. platensis* that is required to cause 50% inhibition in cell lines viability) were 18.8 and 22.3 μg mL^{-1} for HCT116 and HEPG2 cell lines, respectively. In addition, the correlation between HCT116, HEPG2 and *S. platensis* water extracts was negative and significant.

Key words: Antioxidant, anticancer activity, *Spirulina platensis*, water extracts

INTRODUCTION

The food industry has a challenge to produce low cost, nutritive and convenient foods. In order to meet this demand, it is important to study sources of fibers and antioxidants compounds that are technology viable, with positive environmental and economic impacts (Bolanho *et al*., 2014). In recent years, there has been an explosive interest in the use of antioxidants nutritional supplements (Gigante *et al*., 2007). Epidemiological evidence suggests that intake of some vitamins, minerals and other food constituents may help to protect the body against heart disease, cancer and the aging process and that antioxidants may have a protective effect, either in preventing these diseases or lessening their severity (Hsia *et al*., 2007).

Several studies found that antioxidants which are immensely present in edible green plants work by significantly slowing or preventing the oxidative or damage from oxygen process caused by free radicals such as superoxide radical, hydroxyl radical (OH) and non-free radical species such as H_2O_2 and singlet oxygen ($^1O_2$) is associated with cellular and metabolic injury, accelerating aging, cancer, cardio-vascular diseases, neurodegenerative diseases and inflammation (Sahu *et al*., 2013).

Spirulina is free floating filamentous blue-green microalga growing in alkaline water bodies, which has a simple structure but a complex composition. It is known to have appeared 3.6 million years ago as an evolutionary bridge between bacteria and green plants. It has been a common dietary substance around the world from ancient times.

Although, dietary usage and supplementation continues to be popular, there was for a long time no strong scientific evidence of *Spirulina*'s nutritive and health benefits. In recent years, *Spirulina* has attracted scientific attention, not only for its various health benefits, but also at a micro level of understanding the mechanisms of action of its various components. From being a 'complete' protein source, *Spirulina* and its components have been shown to have positive benefits across a range of human health indications from malnutrition to antioxidant properties (Ravi *et al*., 2010).

Deng and Chow (2010) mentioned that the nutritional value of *Spirulina* is well recognized with its unusual high protein content (60-70% by dry weight) and its richness in vitamins, minerals, essential fatty acids and other nutrients. Because of its unusual high nutritional values, *Spirulina* was recommended by both National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA) as one of the primary foods during long-term space missions.

It has been reported that consumption of *Spirulina* as diet supplement has health benefits in preventing or managing hypercholesterolemia, hyperglycerolemia, certain inflammatory diseases, allergies, cancer, environmental toxicant and drug-induced toxicities, viral infections, cardiovascular diseases, diabetes and other metabolic disease among others (Khan *et al*., 2005).

Among large number of *Spirulina* species, three species of *Spirulina*, including *Spirulina platensis* (*Arthrospira platensis*), *Spirulina maxima* (*Arthrospira maxima*) and *Spirulina fusiformis* (*Arthrospira fusiformis*) are most intensively investigated as those *Spirulina* species are edible with high nutritional as well as potential therapeutic values (Deng and Chow, 2010). *Spirulina platensis* or its extract show therapeutic properties, such as the ability to prevent cancers, decrease blood cholesterol level, reduce nephrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effect of radiation (Kumar *et al*., 2011).

Some studies reported that cancer was prevented by algae extracts because of their antioxidant properties (Mirada *et al*., 1998). Substances known as antioxidants are bioactive compounds that are able to inactivate free radicals, which are instable molecules that may cause several deleterious effects to human health (Bolanho *et al*., 2014). Several activities of the antioxidants are mediated by inhibition of Reactive Oxygen Species (ROS), which are generated during the oxidative burst. Thus the usefulness of antioxidants in protecting cellular components against oxidative stress is well established (Bermejo-Bescos *et al*., 2008).

Spirulina platensis was found to have free radical scavenging properties and antioxidant activity, as it contains a number of natural pigments such as chlorophyll, beta-carotene, phycoerythrin and phycocyanin (Gad *et al*., 2011). In the same aspect Bermejo-Bescos *et al*. (2008) mentioned that *S. platensis* contains phycobilisomes as light harvesting protein pigment complexes. Phycobilisomes are mainly composed of polypeptides named phycobiliprotiens. The two more important phycobiliprotiens which occur in this microalgae are phycocyanin and allophycocyanin; both of them have the same chromophore group. Moreover, *Spirulina* contains a spectrum of natural mixed carotene and xanthophyll phytopigments that together with phycocyanin seem to be related to its antioxidant activity.

The objective of the present study was to evaluate the antioxidant activity of *Spirulina platensis* water extracts and studying their cytotoxicity against cell lines {colon carcinoma cells (HCT116) and hepatocellular carcinoma cells (HEPG-2)}.

MATERIALS AND METHODS

Source of *Spirulina platensis* used in this study: *Spirulina platensis* food supplement tablets were obtained from DXN Company; the first Multi-Level Marketing (MLM) company in Malaysia producing *Spirulina* from the cultivation process to finished goods, where only the selected best species are naturally cultivated in a clean pond with no pesticides or herbicides applied.

Determination of Total Phenolic Compounds (TPC) in *Spirulina platensis* water extracts: Chemicals used were purchased from Sigma (St. Louis, MO) and Merck (Darmstadt, Germany). Cold water extracts from *Spirulina platensis* were prepared as follows; *Spirulina* powder 0.5, 1.0 and 1.5 g were soaked in 100 mL of ultrapure water and shaken continuously for 24 h at room temperature. The mixtures were then centrifuged at 5,000 rpm for 10 min (4°C) and the supernatant was filtered (Whatman No. 1) to remove the cell debris. The samples were then freeze-dried and the dried extracts were stored at 4°C before use for the experiments. The amount of total phenolic compounds in extracts was determined according to the Folin-Ciocalteu procedure (Kahkonen *et al*., 1999). Samples (200 μL, two replicates) were introduced into test tubes; 1.0 mL of Folin-Ciocalteu's reagent and 0.8 mL of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured by using Jenway 6705 UV/Vis spectrophotometer. Total phenolic content was expressed as Gallic Acid Equivalents (GAE) in milligrams per gram dry material.

Antioxidant activity of *Spirulina platensis* water extracts determined by DPPH radical scavenging assay: The DPPH (1, 1-diphenyl 2-picrylhyorazyl) free radical scavenging activity of *S. platensis* water extracts was determined according to the method described by Choi *et al*. (2000). Butylated hydroxytoluene (BHT) was used as a reference result. The radical scavenging activity of *S. platensis* water

extracts (0.5, 1.0 and 1.5 g/100 mL) were expressed as (%) inhibition of DPPH. Reading was calculated according to the following equation of Yen and Duh (1994):

$$Ip = \frac{A_B - A_A}{A_B} \times 100$$

Where:

Ip : Inhibition percentage

A_B : Absorbance values of the blank samples checked after 70 min

A_A : Absorbance values of *S. platensis* solutions checked after 70 min

Cytotoxic assay of *Spirulina platensis* water extracts on HCT and HEPG2 cell lines: Colon carcinoma cells (HCT116) and hepatocellular carcinoma cells {human liver cancer cell lines (HEPG2) involved in the present study were obtained from National Cancer Institute (NCI), Cairo-Egypt. Testing of the cytotoxic effect was performed in the Pharmacology Unit, Department of Cancer Biology, National Cancer Institute, Cairo University, Egypt.

Various concentrations of *S. platensis* water extracts (from 0-50 μg mL^{-1}) were prepared and incubated for 48 h with both viable cell lines yield using colon carcinoma cells (HCT116) and hepatocellular carcinoma cells (HEPG2). The cytotoxicity of *Spirulina* against cell lines was determined by a colorimetric method described by Vijayan *et al*. (2004). Calculations of half-maximal inhibitory concentration (IC_{50}) of HCT116 and HEPG2 cell lines were done by using GraphPad prism 5 program best fitting line.

Statistical analysis: SPSS 16.0 software package was used for statistical analysis. Differences among means were determined using a two-tailed t-test. Differences were considered significant at $p<0.05$ and highly significant at $p<0.01$. The relationship between concentration of Total Phenolic Compounds (TPC) and percentage of antioxidant activity of *S. platensis* were described using correlation bivariate statistics. For cytotoxic effect of *S. platensis* water extracts on HCT116 and HEPG2 cell lines; trend line equations were estimated according to coefficient of determination (R^2).

RESULTS AND DISCUSSION

The DPPH radical scavenging activity is one of the most widely used methods for screening the antioxidant activity of plant extracts. The antioxidant activity of *Spirulina* water extracts was measured based on the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Choi *et al*. (2000).

Data in Table 1 showed that the scavenging activity increased with increasing concentration of *S. plantensis* extract. The highest scavenging ability of *Spirulina* observed at 1.5 g/100 mL, this was in line with Sahu *et al*. (2013), who studied DPPH free radical scavenging activity of some leafy vegetables and found that the antioxidant properties of plant extracts were concentration dependent. In the same trend Gad *et al*. (2011) concluded that the DPPH scavenging capacity of *Spirulina* solution was higher than that of Why Protein Concentrate (WPC); moreover the scavenging capacity of *Spirulina* as a natural product at 100 mg/100 mL was found to be higher than that of other concentrations. *Spirulina platensis* contains carotenoid, vitamin E, phycocyanin and chlorophyll (Nakaya *et al*., 1998). These compounds are well known to decrease DPPH radicals by their hydrogen-donating ability (Li *et al*., 2009). Bolanho *et al*. (2014) found that supplementation of *S. plantensis* to cookies increased their total phenolic compounds and antioxidant potential; in this aspect they used different methods for determining *Spirulina* antioxidant potential including DPPH free radical scavenging assay, they noticed a significant increase in the antioxidant potential towards the DPPH free radical method; their results were in accordance with the present study.

Table 1: Antioxidant activity of *Spirulina plantensis* water extracts

Concentration of *Spirulina plantensis* water extracts (mg/100 mL)	*DPPH inhibition (%)	**BHT 0.2 mg mL^{-1} (200 ppm)
500	72.46	
1000	77.47	80.40%
1500	81.01	

*Inhibition (%) determined by DPPH as free radical scavenging activity producer; **BHT is synthetic reference

Table 2: Total phenolic compounds determined in *Spirulina plantensis* biomass

	Sample		
Biomass concentration (g)	First	Second	Third
TPC (mg g^{-1})	0.50	1.00	1.50
	26.75	31.90	40.45

TPC: Total phenolic compounds

Phenolic compounds have been extensively studied for their antioxidant properties not only in fruits and vegetables but also in cyanobacteria (Colla *et al*., 2007).

According to Table 2, *S. platensis* biomass showed considerable content of Total Phenolic Compounds (TPC) ranged between (26.75 and 40.45 mg g^{-1}). Results revealed that the highest content of phenolic compounds (40.45 mg g^{-1}) was determined in *Spirulina* biomass of 1.5 g, which is the highest concentration examined in the present study following by that the same pattern of the antioxidant capacity. Several studies are in accordance with our findings; Collado *et al*. (2006) found that the content of phenolic compounds in *Spirulina* prepared by different conditions ranged between 2.4 and 5.0 g kg^{-1}. Bolanho *et al*. (2014) scored a high content of total phenolic compound (12.2 g kg^{-1}) in *Spirulina*, they found that adding *Spirulina* for cookies formulation contributed to high content of phenolic compounds.

Statistical analysis (Fig. 1) showed significant positive correlation ($p<0.05$) between phenolic contents and

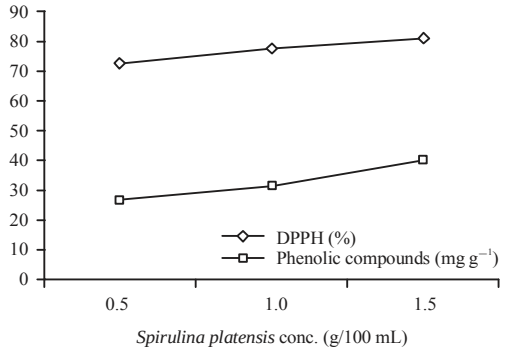
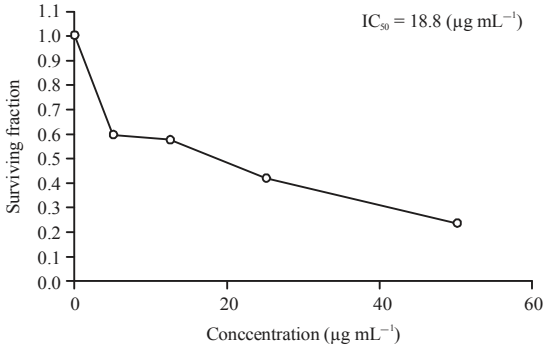


Fig. 1: Antioxidant activity (% DPPH radical inhibition) and total phenolic compounds of *Spirulina platensis* water extracts



Drug cytotoxicity

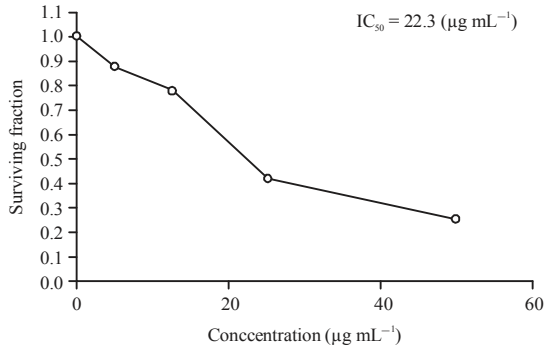
Concentration (µg mL^{-1})	HCT-ABEER
0	1.000
5	0.596
12	0.577
25	0.423
50	0.237

Fig. 2: Cytotoxic effect of *Spirulina platensis* water extracts on HCT116 cell lines

antioxidant activity of *S. platensis* (% DPPH radical inhibition); in this aspect Sahu *et al*. (2013) observed a significant correlation between phenolic content and the scavenging of DPPH radical in all examined leafy vegetables ($r = 0.993$, $p<0.5$) indicate that the radical scavenging capacity of each extract might be related to their concentration of phenolic hydroxyl groups, they found that many studies have shown that many polyphenols contribute significantly to the antioxidant activity and act as highly effective free radical scavengers, which are mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. From these studies; Colla *et al*. (2007) mentioned that phenolic compounds can not only increase the shelf-life of foods but also act as antioxidants in many biological systems. Gershwin and Belay (2007) recorded that phenolic compounds are known to have antioxidant capacity and to interact with free radicals; they are able to inhibit lipid peroxidation *in vitro* by means of their ability to sequester free radicals and act as metal chelators. For example, Mirada *et al*. (1998) studied the antioxidant activity of carotenoids, phenolics and tocopherols extracted from *Spirulina maxima* and found that the phenolic compounds responsible for the antioxidant properties of the *S. maxima* extracts were organic acids (caffeic, chlorogenic, quimic, salicylic, synaptic and trans-cinnamic) which acted individually or synergistically, in the same line Estrada *et al*. (2001) demonstrated the antioxidant activity of the phycobiliproteins; phycocyanin and allophycocyanin present in *Spirulina* biomass. Colla *et al*. (2007) observed that phenol-containing methanol extracts of lyophilized *S. platensis* reduced the amount of peroxidase-induced browning of guayacol; in addition the quantity of phenolic compounds produced by *S. platensis* was related to the Antioxidant Potential (AP) of the phenol-containing *S. platensis* methanol extracts, they concluded that higher concentrations of phenols resulted in less browning and higher Antioxidant Potential (AP). Results from above mentioned studies were in compliance with our findings.

In the present study, anticancer activity of *S. platensis* water extracts with different concentrations were determined by cell lines viability assay using colon carcinoma cells (HCT116) and hepatocellular carcinoma cells (HEPG2). Some studies reported that cancer was prevented by *Spirulina* extracts, because of their antioxidant properties (Mirada *et al*., 1998). Mohd-Syahril *et al*. (2011) reported that chemotherapy is one of the main treatments used to cure cancer. Besides that, a group of drugs are used to kill or inhibit the growth of cancer cells. These drugs are associated with toxicity, which at best is unpleasant and at worst may threaten life. Many side effects of chemotherapeutic drugs include hair loss, mouth sores, diarrhea, nausea, vomiting, loss of appetite and fatigue. Hence, new anticancer agents should be investigated from various resources. A great number of antitumor compounds are natural products or their derivatives, mainly produced by blue-green algae.

Cytotoxic effect of *S. platensis* water extracts on HCT116 and HEPG2 cell lines viability and (IC_{50}) values were shown in Fig. 2 and 3. *Spirulina platensis* water extracts was found to inhibit proliferation of human colon (HCT116) and liver (HEPG2) cancers. The half-maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. It represents the concentration of a substance or drug that is required for 50% inhibition *in vitro*. Results showed that water extracts of *S. platensis* exhibited 50% inhibition (IC_{50}) of HCT116 and HEPG2 cell lines at18.8 and 22.3 µg mL^{-1}, respectively. On the same line Mohd-Syahril *et al*. (2011) tested *Chlorella vulgaris* and *Spirulina platensis* crude extracts for their effectiveness as anticancer agent on breast cancer cell lines MCF-7 and liver cancer cell lines HEPG2; they found that these microalgae display anticancer properties towards tested kinds of cancers, suggesting that new anticancer natural products from unicellular green algae and filamentous microalgae are possible. It has recently been shown that



Drug cytotoxicity

Concentration (µg mL^{-1})	HEPG2-ABEER
0	1.000
5	0.870
12	0.777
25	0.420
50	0.254

Fig. 3: Cytotoxic effect of *Spirulina platensis* water extracts on HEPG2 cell lines

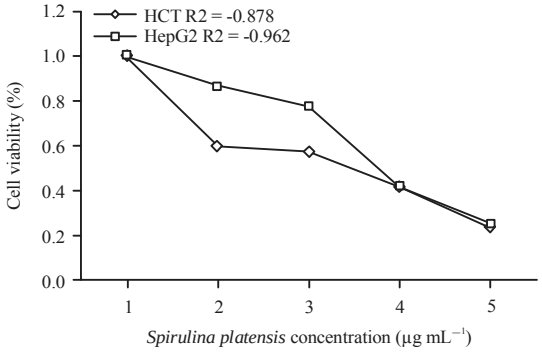


Fig. 4: Inhibitory activity of *Spirulina platensis* against HCT116 and HEPG2 cell lines and trend line equations

selenium enriched *S. platensis* inhibited the growth of MCF-7 human breast cancer cells (Hoseini *et al*., 2013). Similarly Ravi *et al*. (2010) reported that selenium-containing phycocyanin (Se-PC) showed potent antiproliferative properties in human melanomaA375 cells and human breast adenocarcinoma MCF-7 cells.

Mechanisms of anticancer, antiviral and antimicrobial effects of *Spirulina* are due to its content of endonuclease (which repair damaged DNA), calcium sulfated polysaccharide (which inhibits *in vitro* replication of viruses) and fatty acids (specially high content of γ-linolenic acid), respectively. In addition, the metalloprotective role of *Spirulina* may be attributed to the presence of beta-carotene, vitamins C and E, enzyme superoxide dismutase, selenium and brilliant blue polypeptide pigment phycocyanin (Hoseini *et al*., 2013).

Statistical analysis (Fig. 4) revealed that the correlation between HCT116, HEPG2 and *S. platensis* water extracts is negative and significant. The inhibitory activity of *S. platensis* against HEPG2 and HCT116 cell lines was best described by the following Eq:

HCT116 (R^2) = -0.878

HEPG-2 (R^2) = -0.962

CONCLUSION

It can be concluded from this study that *S. platensis* biomass showed considerable content of Total Phenolic Compounds (TPC); explaining the high antioxidant capacity of its water extracts, in addition *S. platensis* water extracts showed antiproliferative properties in human colon carcinoma cells (HCT116) and hepatocellular carcinoma cells (HEPG2) suggesting that new promising anticancer natural products from blue-green algae are possible. However, further studies are needed to display *S. platensis* anticancer properties towards other kinds of cell lines and to fully discover the mechanisms by which its extracts cause cell death; this will be the subject of interest in our future researches.

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