Original Research Article

Antioxidant Activities of Phycocyanin: A bioactive compound *from Spirulina platensis*

ABSTRACT

Background and Objectives

The cyanobacterium *Spirulina* also called blue green algae is a class of gram negative bacteria which possesses wide range of bioactive colored components as Phycocyanin, carotenoids and chlorophyll. *Spirulina* is one of the microalgae containing nutrients that has been used as a functional food in addition to therapeutic and pharmaceutical applications. This study aimed to evaluate the biochemical composition of *Spirulina platensis* biomass and its ethanolic and aqueous extracts, as well as, evaluate the antioxidant activities of the biomass, ethanolic, aqueous and the purified Phycocyanin.

Materials and Methods:

The chemical compositions of *Spirulina platensis* were determined, as well as the antioxidant activity of extracts, Phycocyanin, Phycocyanopeptide and Phycocyanobilin using (DPPH) radical-scavenging activity.

Results:

Results show that biomass has higher total proteins (49.72±0.508%), total carbohydrates (10.3±0.330%), moisture content (7.5±0.685%), lipids (7.2±0.105%) and Minerals (6.9±0.130%). In biomass, the total phenols (51.20±0.25µg/mL) and flavonoids (97.73±1.858 µg/mL) were high compared to the ethanolic (49.48±0.130 and 69.07±1.814 µg/mL) and aqueous (15.27±0.639 and 4.67±0.611 µg/mL) extracts respectively. In the phenolic compounds, pyrogallol was identified as the major compound in biomass and aqueous extract (63.85 and 12.33%) respectively, E-vanillic acid in ethanolic (18.20 %), whereas, hespirdin (3.517 and1.639%) were major flavonoids found in aqueous and ethanolic extracts respectively. The DPPH scavenging activity was found higher in ethanolic extract compared to aqueous while in bioactive, the order of antioxidant activity was Phycocyanin > Phycocyanobilin > Phycocyanopeptide.

Conclusions:

The study data regarding to *Spirulina* nutritional value, making *Spirulina* an excellent choice when formulating diets and combating malnutrition. In addition to, it is strong antioxidant and could be used as alternative treatments as anticancer, antidiabetic, and anti-inflammatory agent.

Key words: *Spirulina platensis*; antioxidants; chemical composition; phycocynin.

1. INTRODUCTION

Spirulina, a microscopic, filamentous, dried biomass of *Arthrospira plantesis*, is an oxygenic photosynthetic cyanobacterium found in fresh and marine waters worldwide [1]. It is consumed by both humans and animals as a food supplement [2,3]. Spirulina has been labeled as a superfood because of its richness in carbohydrates, proteins, sterols, polyunsaturated fatty acids and minerals such as selenium, calcium, chromium, iron, manganese, zinc and magnesium [4]. It is also one of the natural source of provitamin A and vitamin B_{12}, E, C, phenolic acids, linoleic acid, and xanthophylls phytopigments [5]. Multiple reported activities of *spirulina* such as anti-inflammatory, anticancer, cholesterol-lowering and antiviral, are attracting the attention of scientists for using *spirulina* as a nutraceutical and pharmaceutical sources [6-8]. Phycocyanin (PC), an extracted pigment from *Spirulina platensis*, has been widely used as a natural blue colour for multi-purposes [9]. In addition, PC being natural antioxidant is useful for treatment of many diseases such as Alzheimer's, Parkinsons and Huntingtons diseases [10]. Phycocyanobilin is one of the two compounds extracted from PC, it has free radical scavenger and thus protecting the living cells from oxidative stress [11, 12]. The current study aimed to evaluate the biochemical components of *Spirulina* alga biomass, its ethanolic and aqueous extract, as well as to determine their and Phycocyanin antioxidant activities.

2. MATERIALS AND METHODS

2.1. Microalgae Preparation

Spirulina (Arthrospira) platensis strain was cultured at National Research Centre (NRC), Unit algae technology, Giza, Egypt. The cultures were allowed to grow for eighteen days in shaking incubator with a light intensity of 50 μmol photons m^{-2} s^{-1} and 12:12 light/dark regime, at 30 ±1°C, pH 9.0 [13].

2.2. Drying of fresh biomass of *Spirulina platensis.*

The biomass of *spirulina platensis* was collected by filtration through a nylon cloth, then the adhered salts were removed by washing with distilled water and stored at -20 °C [14]. The harvested fresh biomass was subjected to different methods of drying including drum drying, spray drying, sun drying and oven drying [15].

2.3. Preparation of ethanolic and aqueous extracts of *Spirulina platensis.*

2.3.1. Ethanolic extract.

Ten grams of *Spirulina platensis* dried powder were extracted by adding 100 ml of 80% ethanol and kept overnight on a rotary shaker at room temperature. The mixture was filtered and centrifuged at 5,000×g for 15 min., the supernatant collected and stored for further investigations [14].

2.3.2. Aqueous extract.

Ten grams of dried *Spirulina platensis* powder (by spray drying which give the best method of drying) were extracted in 100 mL distilled water kept on a rotary shaker for 2h and then kept at 4°C for 24h according to the method of Anwer et al. [14].

2.4. Chemical composition of *Spirulina platensis*

2.4.1. Determination of total soluble proteins.

The concentration of the total soluble protein was determined in the spirulina biomass according to Bradford [16] using bovine serum albumin as a standard.

2.4.2. Determination of free amino acids.

The free amino acids were extracted by mixing 5g of *Spirulina platensis* powder with 50 ml ethanol 80% at 70ºC and refluxing for 1h. This method was repeated three times, the extracts were collected, filtered and the supernatant was evaporated at 55ºC. The dried film was dissolved

in 10 ml of isopropanol (10%) and the total volume was completed to 50 ml. The isopropyl extracts were kept at -20ºC until further analysis [17].

2.4.3. Determination of total carbohydrates.

Total carbohydrates were determined according to the method of A.O.A.C [18]. Approximately 0.1 g of sample dried by air and hydrolyzed by 1 N HCl in boiling water bath for 6 h. The resultant solution was filtered, neutralized and the final volume was made up to 100 ml with distilled water. Total reducing sugars was measured calorimetrically in 1ml of sample by alkaline potassium ferricyanide reagent at 420 nm using UV-VIS spectrophotometer.

2.4.4. Determination of moisture content.

Moisture content was measured by drying approximately 2g of sample in a ventilated oven at 70°C for 24h, then at 105°C for 3h. Samples cooled over $CaCl_2$ in glass desiccator, the weighed samples were heated many times at the same temperature until constant weight was achieved and moisture content was calculated according to A.O.A.C [18].

2.5. Determination of pigments

Chlorophyll-a and carotenoids were extracted and estimated according to A.O. A.C [18]. 0.5g samples (fresh alga biomass and both extracts) were homogenized in a mortar with 85% acetone in the presence of washed dried sand and a little amount of $CaCO_3$ (0.1g) in order to neutralize organic acids in the homogenate of the fresh alga biomass. The homogenate was then filtered through sintered glass funnel. The residue washed several times with acetone until the filtrate became colorless. The optical density of this extract was determined using a spectrophotometer (UV-Vis spectrophotometer UV 9100 B, LabTech) at 662, 644 nm for (Chl. a, and Chl. b, respectively) and at 440.5 nm for carotenoids. The extraction and purification of PC from *Spirulina platensis* was followed by earlier method mentioned in our previous study [19].

2.6. Determination of total flavonoids.

Total flavonoid content of spirulina biomass and both extracts were determined by the aluminum chloride colorimetric assay as described by Marinova et al. [20]. One ml of each sample was added to 10 ml volumetric flask containing 4 ml of distilled water. 0.3 ml 5% $NaNO_2$ was added then 0.3 ml 10% $AlCl_3$ after 5 min. At the 6^{th} min, 2 ml of 1M NaOH was added and total volume was completed to 10 ml with dH_2O. The solution was well mixed and the absorbance was reading at 510 nm. The concentration of total flavonoids was calculated using quercetin standard curve.

2.7. Determination of total phenolic compounds.

Total soluble phenolics of spirulina biomass and both extracts were determined using the method described by Shahidi and Naczk [21]. A known weight of each sample was immersed in 10 ml ethanol 80% and kept in a dark bottle for 24 h at 0˚C. The samples were re-extracted 3 times then the clarified extract was completed to 50 ml with ethanol 80%. Then 1 ml of the extract was mixed with 0.5 ml Folin-Denis reagent in a test tube and thoroughly shaken. After 3 min, 1 ml of saturated Na_2CO_3 (33g anhydrous salt were dissolved in 100 ml dH_2O at 70-80˚C, then cooled and kept overnight at room temperature then filtered. The absorbance was measured at 725 nm using spectrophotometer (UV-Vis spectrophotometer UV 9100 B, LabTech). The concentration of total soluble phenols was calculated using gallic acid standard curve. The total phenols concentration was expressed as µg equivalents of gallic acid per g DW of the sample.

2.8. Identification and quantification of phenolic compounds of *Spirulina platensis* biomass and both extracts by HPLC.

The Phenolic compounds in *Spirulina* biomass and both extract were identified by HPLC [22]. 5 g of sample were homogenized with methanol and centrifuged at 10000 rpm for 10 min. The supernatant was filtered through 0.2 µm Millipore filter membrane, 3 ml of filtrate was collected in a vial for HPLC injection. The stationary phase is octadecylsilyl (reversed phase) and the

gradient separation was carried out with ethanol and acetonitrile as a mobile phase at flow rate of 1ml/min. The standard of phenolic compounds purchased from sigma were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculate the concentration of phenolic compounds by using the data analysis of Hewlett Packard software.

2.9. Antioxidant activity of extracts, Phycocyanin, Phycocyanopeptide and Phycocyanobilin.

The antioxidant activity of the ethanolic and aquatic extracts of *spirulina platensis* and its bioactive compounds phycocyanin, phycocyanopeptide and phycocyanobilin were determined based on 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radical-scavenging activity [23]. In the DPPH assay, 1 ml of sample was mixed with 0.5 ml of 50 μM DPPH in ethanol and kept in dark for 30 min. The mixture absorbance was measured at 517 nm., and vitamin C standard (5–30 μg/ml) was used as positive controls. The radical scavenging activity was determined based on percentage of inhibition by using the following formula:

$$\% \text{ inhibition} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100.$$

3. Results and Discussion

3.1. Major chemical components of *Spirulina platensis* biomass and its both extracts.

The chemical composition of *Spirulina platensis* biomass results show, *Spirulina platensis* have the following chemical composition: high protein content, 49.72 (% w/w), total lipids 7.2% (w/w), total carbohydrates 10.3% (w/w), moisture 7.5% (w/w) and minerals 6.9 % (w/w) (Fig. 1). These results were supported by El-Baz et al. [24] who found the protein content of *Spirulina platensis* was (35–65%) while, Wong and Chan [25] reported *spirulina* has 10% (w/w) of minerals content, in addition, Aly and Gad [26] found the *Spirulina platensis* biomass contain fat and total carbohydrates contents were 8 and 10%, respectively. Different studies reported that the

Spirulina platensis is a rich protein source, essential amino acids, polyunsaturated fatty acids γ-linolenic acid (GLA), sterols and some more vital elements like magnesium, calcium, zinc, iron, manganese, selenium and chromium [26, 27].

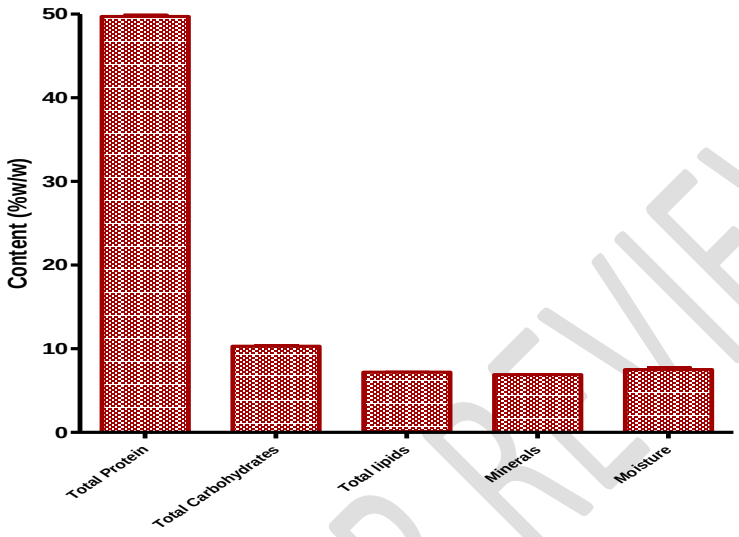

Fig. 1. Major chemical composition of *Spirulina platensis*

Data presented as the means of three replicates ± SD.

The obtained data show the ethanolic extract of *Spirulina platensis* contained higher concentrations of carotenoids, chlorophyll-a, total phenols and flavonoids and lower concentration of PC, free amino acids, total proteins and carbohydrates (Table 1). On the other hand, the aqueous extract of *Spirulina platensis* contained higher contents of phycocyanin, free amino acids, total proteins and carbohydrates, while it contained lower concentration of carotenoids, chlorophyll-a, total phenols and flavonoids. These results show the main constituent of biomass alga and its water extract contain high percentage of total proteins (49.72 and 48.01% w/w), total carbohydrate (10.3 and 9.4 %), total free amino acids (4.71 and 4.68%) respectively compared to ethanolic extract (Table 1).

Multiple studies show that the phytopigments such as carotenoids, chlorophyll-a and phycocyanin play an important role as antioxidant and anticancer agents [28, 29]. The results

indicated the *Spirulina platensis* have the highest content of carotenoids and chlorophyll-a (4.69 and 25.59 mg/g dw) followed by the ethanolic extract (4.25 and 25.43 mg/g dw). On the other hand, the concentration of PC in *Spirulina platensis* (0.335 mg/mL) was higher than aqueous extract (0.185 mg/mL) followed by ethanolic extract (0.007 mg/mL).

The total phenols in *Spirulina* alga (51.20 µg/mL) were higher than ethanolic extract (49.48 µg/mL), while water extract show the lowest total phenols content (15.26 µg/mL). *Spirulina* significantly contained the highest content of total flavonoids (97.73 µg/mL), followed by its ethanolic extract (69.07 µg/mL) and the lowest content was observed in its water extract (4.67 µg/mL).

Table 1. Major chemical components of Spirulina alga and its water and ethanolic extracts

	Phytopigments			Phenolic compounds (µg/mL)	
	chlorophyll-a (mg/g DW)	Carotenoids (mg/g DW)	Phycocynin (mg/ml)	Total Phenols	Total Flavonoid
Spirulina platensis	25.59^{a}±0.025	4.69^{a}±0.048	0.335^{a}±0.00	51.20^{a}±0.025	97.73^{a}±1.858
Ethanolic Extract	0.652^{c}±0.017	0.50^{c}±0.007	0.185^{b}±0.00	15.27^{c}±0.639	4.67^{c}±0.611
Aqueous Extract	25.43^{b}±0.021	4.25^{b}±0.104	0.007^{c}±0.00	49.48^{b}±0.130	69.07^{b}±1.814

Each value represents the means ± SD. Different letters refer to significant differences ($P \leq 0.05$).

3.2. Identification and quantification of phenolic compounds of *Spirulina platensis* by HPLC.

Spirulina platensis and its ethanolic and aqueous extracts were subjected to HPLC analysis to investigate the types of phenolic compounds responsible for possible antioxidant activities. In this investigation, thirty-three phenolic compounds were identified and quantified in *Spirulina platensis* and its extracts by comparing its HPLC chromatograms with the standard compounds

based on the retention time. The identified phenols were pyrogallol, gallic, chlorogenic, caffeine, vanillic, p-Coumaric and other phenols. Flavonoids were naringin, hespirdin, rutin, quercetrin, naringenin, catechein, hespirtin and other flavonoids (Tables 2 and 3). The results show low amounts of phenol and flavonoid compounds in water extract of alga compared with ethanolic extract. Pyrogallol was identified as the major phenol compound in *Spirulina platensis* (638.50 mg/100g) and its water extract (12.33 mg/100g), while, E-Vanillic was the major compounds in ethanolic extract (18.20 mg/100g). Also, water extract contained high amount of Ellagic and not detected in ethanolic extract and the major flavonoid compound in *Spirulina platensis* biomass was Hespirdin (9.013 mg/100g). Also, *Spirulina* exhibited highest amount of naringin (4.743 mg/100g) followed by ethanolic extract (0.652mg/100g) and water extract (0.359mg/100g). The quercetin content was 0.922 mg/100g in *Spirulina* biomass followed by alcoholic extract (0.278 mg/100g), it was not detected in the water extract. Generally, HPLC analysis show the content of phenolic compounds of *Spirulina platensis* and its extracts have the following order, *Spirulina platensis* biomass > ethanolic extract > water extract.

Table 2. Types, retention time (min) and the content of phenols (mg/100g) identified by HPLC analysis of *Spirulina platensis* biomass and its aqueous and ethanolic extracts

	Compound	*Spirulina platensis*		Aqueous extract		Ethanolic extract	
		(mg/100g)	min	(mg/100g)	min	(mg/100g)	min
1	Pyrogallol	638.5	6.875	12.33	6.841	8.50	6.896
2	Gallic	4.64	7.011	1.63	7.003	0.36	7.065
3	4-Amino-benzoic	0.32	8.246	0.05	8.342	0.02	8.319
4	Protocatchuic	1.26	8.694	0.27	8.509	0.78	8.467
5	Catechol	7.62	8.636	2.55	8.656	0.49	8.612
6	P-OH- benzoic	1.90	9.136	0.13	9.048	0.18	9.027
7	Caffeine	0.75	9.762	0.15	9.655	0.21	9.653
8	Chlorogenic	3.06	9.859	0.52	9.840	0.94	9.807
9	Vanillic	2.62	9.875	1.31	9.880	0.57	9.907

10	Caffeic	5.97	10.324	0.28	10.246	0.43	10.102
11	P-Coumaric	0.28	10.900	0.11	10.349	0.33	10.322
12	Ferulic	0.39	11.657	0.11	11.586	0.21	11.601
13	Iso-Ferulic	0.22	11.938	0.04	11.820	0.09	11.839
14	E-Vanillic	31.53	12.107	4.47	12.209	18.20	12.196
15	Alpha-Coumaric	1.26	12.524	0.18	12.280	0.24	12.292
16	Benzoic	6.82	13.268	0.34	13.241	1.06	13.283
17	Ellagic	19.83	13.488	2.59	13.393	ND	ND
18	3,4,5-methoxy-cinnamic	1.40	14.183	0.53	14.181	2.91	14.210
19	Rosmarinic	0.18	14.282	0.04	14.380	0.03	14.197
20	Coumarin	1.08	14.552	0.11	14.385	0.27	14.373
21	Cinnamic	0.12	15.285	0.02	15.262	0.04	15.238
22	salycilic	3.89	16.258	0.82	16.480	1.01	16.370

Table 3. Quantity of flavonoids (mg/100g) identified by HPLC analysis of *Spirulina platensis* biomass and its extracts.

	Compound	*Spirulina platensis*		Aqueous extract		Ethanolic extract	
		(mg/100g)	min	(mg/100g)	min	(mg/100g)	min
1	Catechein	3.77	8.246	0.86	8.425	0.47	8.469
2	Epicatechein	1.68	10.324	0.09	9.597	0.46	9.663
3	Naringin	4.74	12.340	0.36	12.087	0.65	12.239
4	Hespirdin	9.01	12.438	3.52	12.514	1.64	12.538
5	Rutin	1.23	12.575	0.12	12.780	1.15	12.806
6	Quercetrin	2.26	13.482	0.09	13.680	0.65	13.709
7	Quercetin	0.92	14.887	ND	ND	0.28	14.655
8	Naringenin	0.89	15.034	0.14	15.192	0.15	15.196
9	Hespirtin	5.16	15.324	0.34	15.634	0.41	15.431
10	Kampferol	1.20	16.243	0.04	15.927	0.26	16.224
11	Apigenin	0.54	16.600	0.01	16.641	0.02	16.683

These results were supported by the studies of Syarina et al. [30] on *S. platensis* aqueous extract. They recorded that cinnamic acid, narigenin and kaempferol have identified in *S. platensis*

aqueous extract by LC-MS analysis. Also, El-Baky et al. [31] showed the presence of large number of phenolic acids and few numbers of flavonoids which includes gallic, cinnamic, chlorogenic, *p*-OH-benzoic, ferulic acids, vanillic, quimic and caffeic in *Spirulina platensis*.

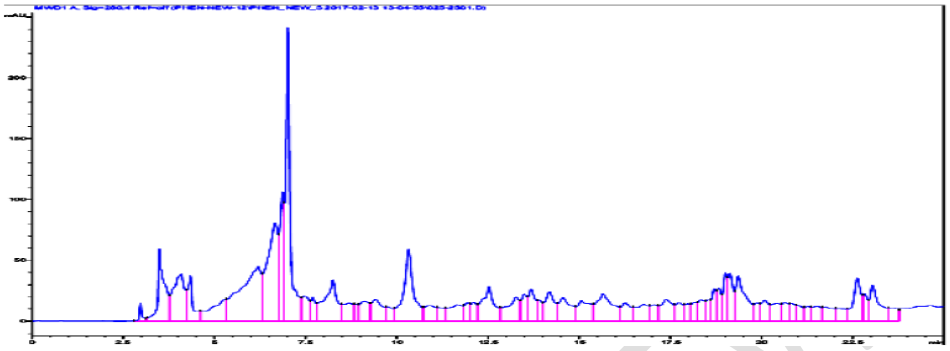

Fig 2. HPLC -UV chromatogram of phenolic compounds of *Spirulina platensis* alga.

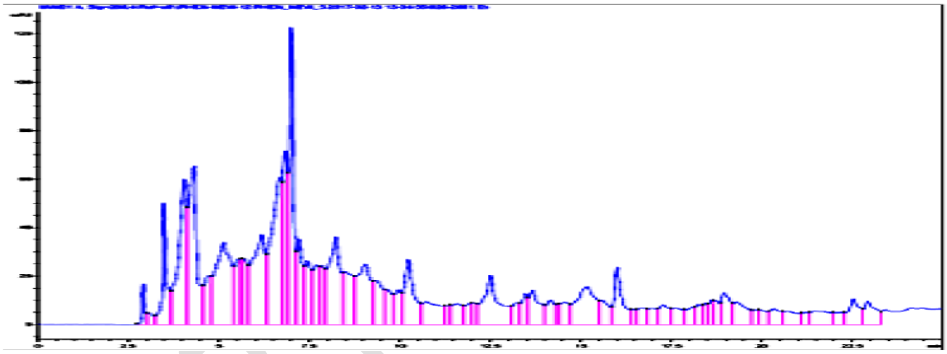

Fig 3. HPLC -UV chromatogram of phenolic compounds of aqueous extract of *Spirulina platensis*.

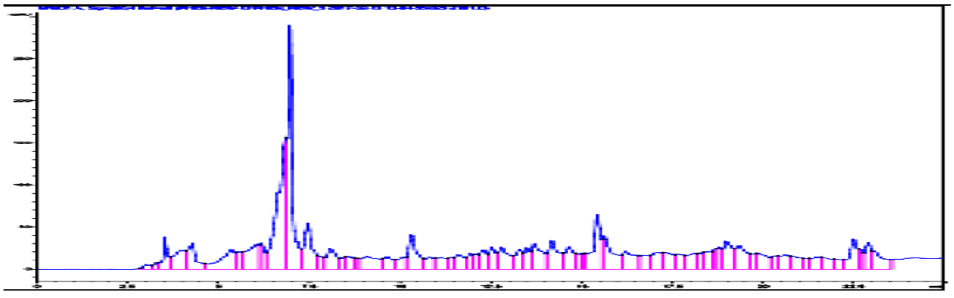

Fig 4. HPLC -UV chromatogram of phenolic compounds of ethanolic extract of *Spirulina platensis*.

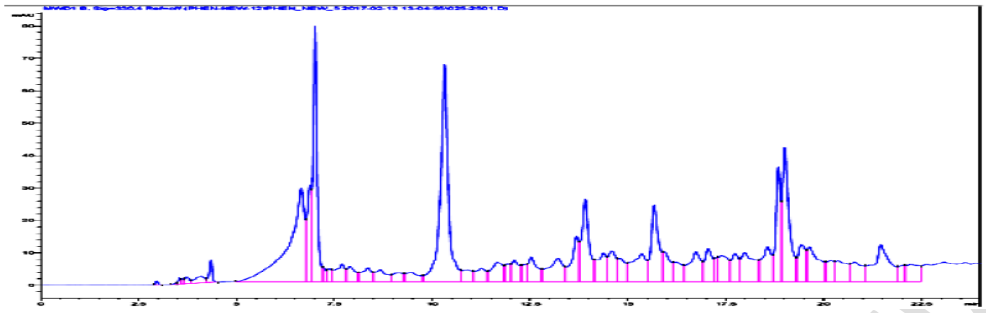

Fig 5. HPLC -UV chromatogram of flavonoids of *Spirulina platensis*.

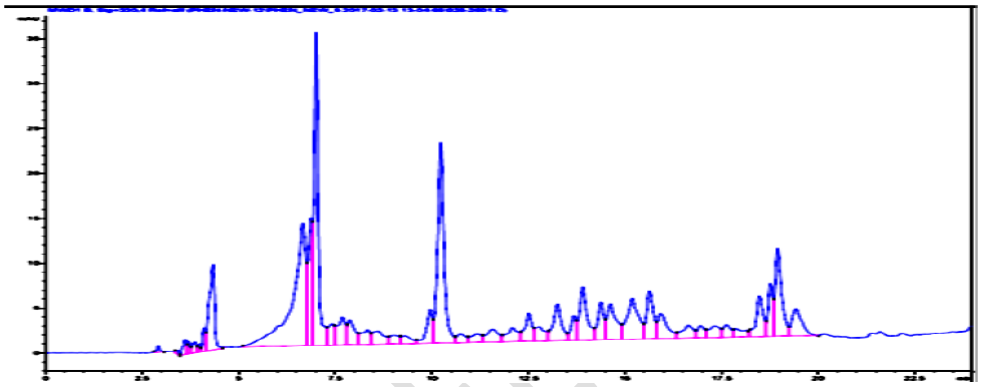

Fig 6. HPLC -UV chromatogram of flavonoids of aqueous extract of *Spirulina platensis*.

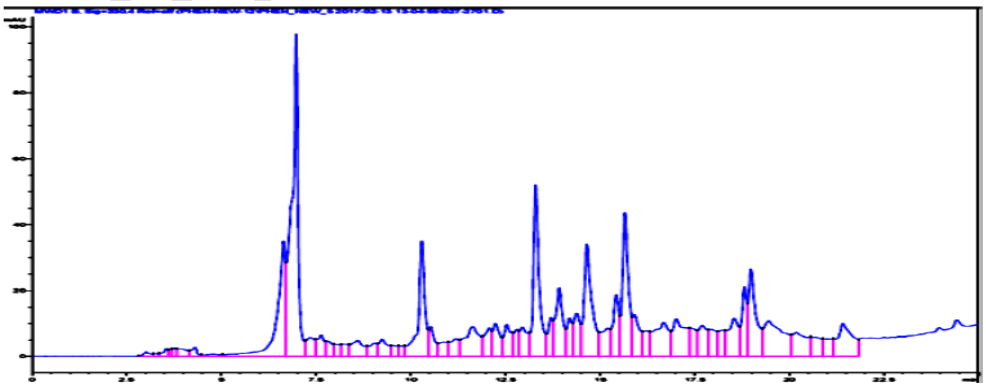

Fig 7. HPLC -UV chromatogram of flavonoids of ethanolic extract of *Spirulina platensis*.

Colla et al. [32] mentioned that phenolic compounds in *Spirulina* such as organic acids (chlorogenic, synaptic, quimic, salicylic, trans-cinnamic and caffeic) can act as antioxidants synergistically or individually. Also, Miranda et al. [33] identified the main phenolic compounds in *Spirulina*, which include *trans*-cinnamic, chlorogenic, salicylic, synaptic and caffeic acids. In addition, the antioxidant and pharmacological activities of phenols and flavonoids in *Spirulina* were reported by many studies, such as cinnamic acid and their derivatives inhibit diabetic complications and moderately induce corneal epithelial wound healing by inhibiting protein tyrosine phosphatases (PTP)-1β gene [34]. Also, Narigenin has inhibitory effect on the pro-inflammatory cytokine (IL-6, IL-1β, IL-8 and TNF-α) response stimulated by lipopolysaccharide in both whole blood and macrophages [35]. Kaempferol has been reported to possess antioxidant and anti-inflammatory activity [36]. Chlorogenic acid (CGA) has many biological activities as antibacterial, antioxidant, anticarcinogenic, hypoglycemic and hypolipidemic [37-39].

3.3. Antioxidant activities

3.3.1. DPPH assay of *Spirulina platensis* ethanolic and aqueous extracts.

The free radical scavenging ability of aqueous and ethanolic of *Spirulina platensis* biomass was assessed on the basis of the radical scavenging effect on the 1,1-diphenyl-2-picryl-hydrazil (DPPH) free radical. The antioxidant activity increased with increasing the concentration of sample solution [40]. The highest antioxidant activity in term of % inhibition was detected in ethanolic extract followed by vitamin C and water extract. The obtained results show that the ethanolic extract of *Spirulina platensis* has higher scavenging activity (96.33%) than water extract (73.58%) at 30 μg/ml, while the activity in biomass was (90.60%) (Fig. 8).

Generally, it could be concluded that ethanolic extract possessed the highest antioxidant activity comparing with aqueous extract of *Spirulina platensis* due to the presence of high content of antioxidant phenols or flavonoids in the ethanolic extract. The ethanolic extract of *Spirulina*

maxima possessed a great ability of scavenging DPPH radical activity compared to positive synthetic antioxidants butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) depending on phenolic contents in extract [41, 42].

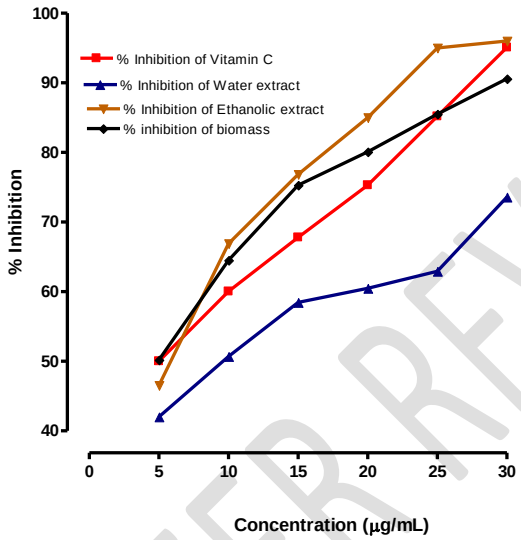

Fig. 8. DPPH free radical scavenging activity of biomass, aqueous and ethanolic extracts of *Spirulina platensis* compared with vitamin C.

The carotenoids in *Spirulina platensis* act as antioxidant and it has the ability to quench singlet oxygen and scavenge of the free radicals, also, α- tocopherol act as antioxidant compound in brown algae [43].

3.3.2. DPPH assay of Phycocyanin and its two bioactive components.

Both of the phycocyanopeptide and phycocyanobilin had high radical scavenging activity in DPPH assay. The antioxidant activity in term of % inhibition was detected in phycocyanopeptide and phycocyanobilin were (93.59 %) and (95.48 %) respectively compared to phycocyanin (97.32 %). The antioxidant capacity of phycocyanin and its bioactive components (phycocyanopeptide and phycocyanobilin) was showed the following descending order,

phycocyanopeptide < phycocyanobilin < phycocyanin and the increased activity as their concentrations were increased. Based on the DPPH assay, the antioxidant activity of phycocyanin, phycocyanobilin and phycocyanopeptide at 5 µg/mL was about 46.71, 42.03 and 40.00%, respectively (Fig. 9). These results are in agreement with Estrada et al. [44] who observed that an increase in the amount of phycocyanin caused an increase in the antioxidant activity.

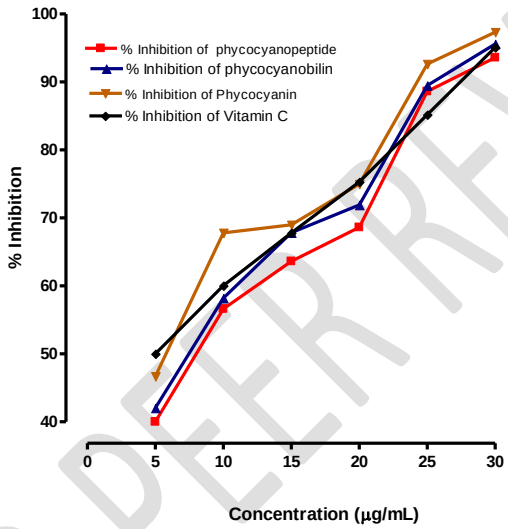

Fig. 9. Antioxidant activity of phycocyanin and its bioactive components (phycocyanopeptide and phycocyanobilin) as function of concentration.

Phycocyanin is used as a food coloring and has a great antioxidant [45, 46]. It is the major water-soluble antioxidant constituent in *Spirulina*; its activity was about 20 times more efficient than vitamin C [47]. The covalently-linked tetrapyrole chromatophore phycocyanobilin is suggested to be involved in the scavenging activity of phycocyanin [48]. Also, Cherdkiatikul & Suwanwong [49] mentioned that the two components of phycocyanin (phycocyanopeptide and phycocyanobilin) were possessed strong antioxidant activity. For instance, selenium-containing phycocyanin from Spirulina has been shown to have strong alkoxyl, superoxide and hydrogen

peroxide radical scavenging activities [50]. In addition to, the selenium possessed antioxidant effect and plays an important role in protecting against oxidative stress [51]. Also, Cherdkiatikul and Suwanwong [49] found that the apophycocyanin possessed strong antioxidant. The strong antioxidant activity is related to selenium content. Selenium binds to phycobiliproteins, increasing their antioxidant properties [52]. The antioxidant activity of free phycocyanobilin was higher than phycocyanobilin bound in phycocyanin (PC) when denaturated or trypsin digestion of PC [53].

Conclusion

Total biochemical were found higher in *Spirulina* biomass whereas its ethanolic extract contained the highest total phenols and flavonoids while total proteins and phycocyanins were found higher in the aqueous extract of Spirulina. The antioxidant activity of phycocynin was found highest followed by ethanolic extract whereas aqueous extract was found least potent.

References

1. Okechukwu PN, Ekeuku SO, Sharma M, Nee CP, Chan HK, Mohamed N, Anisah Froemming GR. In vivo and in vitro antidiabetic and antioxidant activity of *spirulina*. Phcog Mag. 2019; 15: 17-29.
2. Deepa J, Aleykutty NA, Jyoti H. Effect of combination of two plant extracts on diabetes mellitus. Int J Pharm Pharm Sci. 2018;10: 49-52.
3. Zheng J, Inoguchi T, Sasaki S, Maeda Y, McCarty MF, Fujii M, Ikeda N, Kobayashi K, Sonoda N, Takayanagi R. Phycocyanin and phycocyanobilin from *Spirulina platensis* protect against diabetic nephropathy by inhibiting oxidative stress. Am J Physiol Regul Integr Comp Physiol. 2013; 304: 110–120.
4. Ojiakoi OA, Nwanjo HU. Effects of pioglitazone on atherogenic risk predictor indices of alloxan-induced diabetic rabbits. Biokemistri. 2005; 17:179-84.

5. Suzery M, Majid D, Setyawan D, Sutanto H. Improvement of Stability and Antioxidant Activities by Using Phycocyanin - Chitosan Encapsulation Technique. Earth Environ. Sci. 2017; 55: 1-7.
6. Jensen GS, Attridge VL, Beaman JL, Guthrie J, Ehmann A, Benson KF. Antioxidant and anti-inflammatory properties of an aqueous cyanophyta extract derived from *arthrospira platensis*: contribution to bioactivities by the non-phycocyanin aqueous fraction. Journal of Medicinal Food. 2015; 18(5): 535–541.
7. Banji D, Banji OJF, Pratusha NG, Annamalai AR. Investigation on the role of *Spirulina platensis* in ameliorating behavioral changes, thyroid dysfunction and oxidative stress in offspring of pregnant rats exposed to fluoride. Food Chemistry. 2013; 140(1-2): 321–331.
8. Simpore J, Zongo F, Kabore F. Nutrition rehabilitation of HIV-infected and HIV-negative undernourished children utilizing spirulina. *Annals of Nutrition and Metabolism*. 2005; 49(6): 373–380.
9. Wu Q, Liu L, Miron A, Klímová B, Wan D, Kuˇca K. The antioxidant, immunomodulatory, and anti-inflammatory activities of *Spirulina*: An overview. Arch. Toxicol. 2016; 90: 1817–1840.
10. Avila J, Magesh D. Purification, characterization and antioxidant properties of C-Phycocyanin from *Spirulina platensis.* SIRJ-APBBP. 2015; 2(1): 1-15.
11. Ismaiel MMS, El-Ayouty YM, Piercey-Normorea M. Role of pH on antioxidants production by *Spirulina (Arthrospira) platensis*. Braz. J. Microbiol. 2016; 47: 298–304.
12. Hema A, Setyo W, Kustiariyah T, Mega S, Iriani S. Antioxidant activity of *Spirulina platensis* and sea cucumber *Stichopus hermanii* in streptozotocin-induced diabetic rats. Tropical Life Sciences Research. 2019; 30(2): 119–129.
13. Dhiab RB, Ouada HB, Boussetta H, Franck F, Elabed A, Brouers M. Growth, fluorescence, photosynthetic O2 production and pigment content of salt adapted cultures of Arthrospira (Spirulina) platensis. J. Appl. Phycol. 2007; 19: 293–301.
14. Anwer R, Alam A, Khursheed S, Kashif SM, Kabir H, Tasneem F. *Spirulina*: possible pharmacological evaluation for insulin-like protein. J. Appl. Phycol. 2012; 25(3): 883-889.

15. Sarada R, Pillai GM, Ravishankar GA. Phycocyanin from *Spirulina* sp: influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin. Process Biochemistry. 1999; 34: 795–801.
16. Bradford MM. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of Protein-Dye binding. Analytical Biochemistry. 1976; 72: 348-363.
17. Biancarosa I, Espe M, Bruckner CG, Heesch S, Liland N, Waagbo R, Torstensen B, Lock EJ. Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters. J. Appl. Phycol. 2017; 29: 1001–1009.
18. AOAC. Official methods of analysis of the Association of Agricultural Chemists. Published by AOAC: 18th ed Washington, DC, USA. 2005.
19. Salwa ME, Hikal MS, Abo El- Khair BE, El-Ghobashy RE, El-Assar AM. Hypoglycemic and hypolipidemic effects of *Spirulina Platemsis*, phycocyanin, phycocyanopeptide and phycocyanobilin on male diabetic rats. Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo Special Issue. 2018; 26 (2A): 1121-1134.
20. Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in bulgarian fruits and vegetables. J. of the University Chemical Technology and metallurgy. 2005; 40(3): 255-260.
21. Shahidi F, Naczk M. Methods of Analysis and Quantification of Phenolic Compounds. Food phenolic: sources, chemistry, effects and applications (pp. 287-293). Technomic Publishind Company, Inc: Lancaster. 1995.
22. Goupy P, Hugues M, Biovin P, Amiot MJ. Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. J. Sci. Food Agric. 1999; 79: 1625-1634.
23. Elzaawely AA, Xuan TD, Tawata S. Essential oils, kava pyrones and phenolic compounds from leaves and rhizomes of *Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm. and their antioxidant activity. Food Chem. 2007; 103: 486–494.
24. El-Baz F, Aly HF, El-Sayed AB, Mohamed AA. Role of *Spirulina platensis* in the control of glycemia in DM2 rats. Int. J. Sci. Eng. Res. 2013; 4: 1731–1740.
25. Wong, PK, Chan KY. Growth and value of *Chlorella S. alina* grown in highly saline sewage effluent. Agric ecosystem and Environment. 1990; 30: 235-250.

26. Aly MS, Gad AS. Chemical Composition and Potential Application of *Spirulina Platensis* Biomass. Journal of American Science. 2010; 6 (9): 819-826.
27. Rajaa S, Mourad K, Azzouz E. Functional Composition, Nutritional Properties, and Biological Activities of Moroccan *Spirulina* Microalga. Journal of Food Quality 2019; Volume 2019, Article ID 3707219, 11 pages,
28. Plaza M, Santoyo S, Jaime LG, Reina GB, Herrero M, Señoráns FJ, Ibáñez E. Screening for bioactive compounds from algae. Journal of Pharmaceutical and Biomedical Analysis. 2010; 51(2): 450–455.
29. Konícková R, Vanková K, Vaníková J, Vánová K, Muchová L, Subhanová I, Vítek L. Anti-cancer effects of blue-green alga Spirulina platensis: A natural source of bilirubinlike tetrapyrrolic compounds. Ann. Hepatol. 2014; 13: 273–283.
30. Syarina PN, Karthivashan G, Abas F, Arulselvan P, Fakurazi S. Wound Healing Potential of *Spirulina Platensis* Extracts on Human Dermal Fibroblast Cells. EXCLI Journal. 2015; 14: 385-393.
31. El-Baky HHA, El Baz FK, El-Baroty GS. Production of phenolic compounds from *Spirulina maxima* microalgae and its protective effects. Afr. J. Biotechnol. 2009; 8 (24): 7059-7067.
32. Colla LM, Reinehr CO, Reichert CJ, Costa AV. Production of biomass and nutraceutical compounds by Spirulina platensis under different temperature and nitrogen regimes. Biores. Technol. 2007; 98: 1489-1493.
33. Miranda MS, Cintra RG, Barros SBM, Mancini-Filho J. Antioxidant activity of the microalga Spirulina maxima. Braz. J. Med. Biol. Res. 1998; 31: 1075-1079.
34. Adisakwattana S, Pongsuwan J, Wungcharoen C, Yibchok-anun S. In-vitro effects of cinnamic acid derivatives on protein tyrosine phosphatase 1B. J. Enzyme Inhib. Med. Chem. 2013; 28: 1067-1072.
35. Bodet C, La VD, Epifano T, Grenier FD. Narigenin has anti-inflammatory properties in macrophage and ex vivo human whole-blood models. J. Periodontal. Res. 2008; 43: 400-407.
36. Karthivashan G, Tangestani M, Arulselvan P, Abas F, Fakurazi S. Identification of bioactive candidate compounds responsible for oxidative challenge from hydroethanolic extract of *Moringa oleifera* leaves. J. Food Sci. 2013; 78: 1368–1375.

37. Bassoli BK, Cassolla P, Borba-Murad GR. Chlorogenic acid reduces the plasma glucose peak in the oral glucose tolerance test: effects on hepatic glucose release and glycaemia. Cell Biochemistry and Function. 2008; 26 (3):320–328.
38. Sotillo DV, Hadley M. Chlorogenic acid modifies plasma and liver concentrations of: cholesterol, triacylglycerol, and minerals in (fa/fa) Zucker rats. Journal of Nutritional Biochemistry. 2002; 13 (12): 717–726.
39. Santos MD, Almeida MC, Lopes NP, Souza GEP. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. Biological and Pharmaceutical Bulletin. 2006; 29 (11): 2236–2240.
40. Babita R, Pandit HM. Quantitative Analysis of Flavonoid 'Naringin' A Natural Product and Its Correlation with Antioxidant Activity in Different Fruit Fractions of Grapefruit, Citrus Decuana Var. Paradisi (Macfad.) H.H.A Nicholls: Use of Industrial Waste. Int. J. Pharm. Bio. Sci. 2017; 8(2): 715-721.
41. El-Baky HHA, El Baz FK, El-Baroty GS. Enhancement of antioxidant production in *Spirulina plantensis* under oxidative stress. American-Eurasian Journal of Scientific Research. 2007; 2(2): 170–179.
42. Thomas NV, Kim SK. Potential pharmacological applications of polyphenolic derivatives from marine brown algae. Environ. Toxicol. Pharmacol. 2011; 32: 325–335.
43. Susanto E, Fahmi AS, Agustini TW, Rosyadi S, Wardani AD. Effects of Different Heat Processing on Fucoxanthin, Antioxidant Activity and Colour of Indonesian Brown Seaweeds. Earth and Environmental Science. 2017; 55: 1-10.
44. Estrada JE, Bescos PB, Fresno AMV. Antioxidant activity of different fractions of *Spirulina platensis* protean extract. Il Farmaco. 2001; 56: 497-500.
45. Patel A, Mishra S, Ghosh PK. Antioxidant potential of C-Phycocyanin isolated from three cyanobacterial species: *Lyngbya, Phormidium* and *Spirulina* spp. Indian Journal of Biochemistry & Biophysics. 2006; 43(1): 31-35.
46. Sonani RR, Rastogi RP, Madamwar D. Antioxidant potential of phycobiliproteins: role in anti-aging research. Biochemistry and Analytical Biochemistry. 2015; 4 (4):1-8.
47. Chopra K, Bishnoi M. Antioxidant profile of Spirulina: a blue-green microalga. In Spirulina in Human Nutrition and Health. Boca Raton: CRC Press: 2008; pp. 101-118.

48. Zhou SS, Li D, Sun WP, Guo M, Lun YZ, Zhou YM. Nicotinamide overload may play a role in the development of type 2 diabetes. World J. Gastroenterol. 2008; 7 (1545): 5674-5684.
49. Cherdkiatikul T, Suwanwong Y. Production of the α and β Subunits of Spirulina Allophycocyanin and C-Phycocyanin in *Escherichia coli*: A Comparative Study of Their Antioxidant Activities. Journal of Biomolecular Screening. 2014;19(6): 959–965.
50. Sangeetha P, Babu S, Rengasamy R. Antioxidant Properties of R-Phycoerythrin from *Kappaphycus alvarezii* (Doty) Doty EX SILVA. Journal of Innovative Research and Solutions (JIRAS). 2017; 3(1): 47-56.
51. Zhai X, Zhang C, Zhao G, Ran F, Leng X. Antioxidant capacities of the selenium nanoparticles stabilized by chitosan. J. Nanobiotechnol. 2017; 15 (4): 1-12.
52. Belokobylsky A, Ginturi E, Kuchava N, Kirkesali E, Mosulishvili L, Frontasyeva M, Pavlov SS, Aksenova NG. Accumulation of selenium and chromium in the growth dynamics of *Spirulina platensis*. J. Radioanal. Nucl. Chem. 2004; 259 (1): 65-68.
53. Zhou ZP, Liu XL, Chen JX, Wang M, Chen Y, Zhang Z, Zhou BC. Factors that effect antioxidant activity of c-phycocyanins from Spirulina platensis. Journal of Food Biochemistry. 2005; 29(3):313–322.