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C-Phycocyanin, Anticancer Activity and Nutritional Value of Mass-Produced *Spirulina platensis*

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Abstract

Spirulina platensis, a blue-green alga of Cyanophyta family, was isolated from Wady El-Natron district, El-Behara Governorate, 30.58333°N 30.33333°E. The dried biomass was determined to have a nutritional value of 61.0% protein, 23.5% carbohydrates, and 6.21% lipids. HPLC analysis revealed presence of eight essential amino acids (23.7 g·100 g^{-1}) and nine non-essential amino acids (28.7 g·100 g^{-1}). The major essential amino acid is methionine (4.8 g·100 g^{-1}). However, glutamic acid is a significant non-essential amino acid in dried powder (7.8 g/100 g). Additionally, three major sugars totaling 3.108 g·100 g^{-1} of dried *S. platensis* are identified. Along with minerals and a few polyphenolic compounds, various forms of vitamin B (B-1, 2,3,6,9, and 12) were detected. C-phycocyanin was extracted from wet biomass using organic acid extraction and cold maceration methods, followed by purification with various concentrations of ammonium sulfate. The two procedures were compared to determine the most efficient one for maximum C-PC recovery, and then the extract was screened an antioxidant using DPPH & ABTS beside to anticancer agents against the human breast (MCF-7), human colon (HCT-116), and human liver (HepG 2) carcinoma cell lines by 5.1%, 14.3%, and 31.2% inhibition, respectively, with cold maceration being the most efficient and effective process.

Keywords: *Spirulina platensis*; nutritional; phycocyanin; antioxidant; anticancer

Introduction

The blue green alga *Spirulina platensis* (Cyanophyta) has been cultivated worldwide for its nutritional and therapeutic properties. It is widely used in the health food industry and has numerous medicinal applications due to its valuable active ingredients such as proteins, carbohydrates, and vitamins [1,2] as well as natural pigments such as carotenoids, chlorophyll, and phycocyanin (PC) that account for 0.4, 1.0, and 14% of the dry weight, respectively [3]. In 1978 (NRC), Egypt achieved mass production of algae using a variety of algae species, including *Scenedesmus*, *Chlorella*, *Anabaena*, *Nostoc*, *Dunaliella*, *Haematococcus*, *Amphora*, *Nannochloropsis*, and *Spirulina*-spp. [4]. All technical and economic data were comprehended completely. *S. platensis* has received more attention and has a more promising future use due to its potential for ambient growth [5]. Mass production and beneficial applications have been carried out as a functional food [6]; plant bio-stimulators [7]; antiviral and antibacterial agents [8,9]; and as a

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source of polysaccharides in pharmaceuticals and food supplements [10,11].

In contrast to terrestrial crops, which are grown in soil, *Spirulina* grows in mineral-rich water that is free of nutrient-inhibiting substances. Thus, nutrient-enriched water promotes nutrient uptake, which in turn promotes microalgal growth and intracellular substance accumulation [12].

Spirulina is composed of 13.6% carbohydrate and 55% protein. Mannuronic acid was identified as the acidic sugar in the algal hydrolysates and water-soluble polysaccharides were glucuronic, galacturonic, and mannuronic acids [13].

In accordance with the findings of [14–16], GLC analysis of *S. platensis* using cold and hot aqueous extraction methods (SCEM and SHEM) revealed the presence of 12 and 11 monosaccharides, respectively. The two polysaccharides fraction (SCEM and SHEM) were found to be particularly rich in neutral sugars, accounting for 71.24% and 79.97%, respectively. The neutral sugars that predominated were glucose (21.80% and 24.08%), galactose (9.24% and 12.12%), and mannose (8.88% and 12.28%); while galactose being the most abundant. In addition, the presence of neutral sugars was also determined in both glycoproteins. Glucuronic and galatouronic-acids were found in trace amounts in SCEM (0.13% and 0.08%, respectively), whereas glucuronic acid was found only in SHEM (0.13% and 0.08%) (0.15%).

Phycobiliproteins are a type of protein produced by *S. platensis* that are essential photosynthetic pigments for energy transfer during the photosynthesis process. They are divided into three main groups based on their colors and absorbance properties: phycocyanin (deep blue), phycoerythrin (deep red), and allophycocyanin (bluish green) [15,17]. Phycocyanine is the most intensely colored of the three. Thioether bonds are used to covalently attach PC to the apoprotein, which results in a metal-free tetrapyrrole consisting of a chromophore. phycocyanobilin (PCB) covalently attached to the apoprotein by the thioether bond [16,18]. The bilin chromophore is responsible for the strong UV absorption peak at 615 nm and a weak absorption peak at 360 nm [19], which are both observed in the presence of neutral pH [20]. Denaturation of the secondary, tertiary, and quaternary structures of the protein results in a decrease in the intensity of the visible absorption and fluorescence [21], which is observed in the presence of neutral pH.

S. platensis [22] contains phycocyanin, which is found in the thylakoid system or in the photosynthetic lamellas in the cytoplasmic membrane and is used as a food colorant in the production of gums and dairy products, as a nutraceutical [23], and in cosmetic applications such as eyeliners and lipsticks in China and Japan, in addition to its profitable beneficial uses as an antioxidant and anti-inflammatory [24]. Furthermore, it has the ability to scavenge alkoxyl, hydroxyl, and peroxyl radicals as well as react with peroxynitrite ($ONOO^-$) and hypochlorous acid (HOCl). It also has the additional effect of inhibiting microsomal lipid peroxidation induced by Fe^{+2}, ascorbic acid or the free radical initiator 2,2′azobis(2-amidinopropane) hydrochloride (AAPH) [21].

Due to the incredible significance of *S. platensis* alga and the numerous applications of phycocyanin, this work was achieved aimed to investigate the nutritional profile of *Spirulina* extract PC under various pH ranges, and determine its concentration and purity in each range in order to determine the optimal conditions for PC isolation and to evaluate its antioxidant and anticancer activity in various cell lines.

2. Materials and Methods

2.1. Alga Production and Aqueous Extract Preparation

The locally isolated alga *S. platensis* alga (Algal Biotechnology Unit, National Research Centre, Egypt) was used to achieve the current work. Mass production was performed to obtain the algal biomass through three open ponds totaling 75 m^3 in volume. Based on Zarrouk growth medium, nitrate was substituted by the equivalent amount of nitrogen as urea (0.89 $g.l^{-1}$) and other culture maintenance was performed. Continuous centrifugation allowing the biomass harvesting which followed drying at 45°C for 24 h in a hot air oven before being ground into fine powder [4]. The algal extract was obtained using distilled water. After being separated and filtered through Whatman No. 1 filter paper. The extract was fully dewatering under vacuum rotary evaporated (50^oC). All the used reagents were analytical grade (Sigma and MERK, Germany).

2.2. Nutritional Profile

Quantitative determination of total protein was done via determination of total nitrogen and calculated as crude protein % = total nitrogen x 6.25 [25]; while white total carbohydrates were spectrophotometrically determined at 490 nm according to phenol- sulfuric method using glucose as standard [26].

2.3. HPLC Analysis of the Total Amino Acids and Sugars

The HPLC analysis was carried out on an Eppendorf-Germany LC 3000 amino acid analyzer with a high-pressure stainless-steel column (120 × 4 mm), and the results were reported. Elusion was accomplished through the use of buffers. The flow rate was 0.2 ml/min, the buffer pressure ranged from 0 to 50 bars, and both the buffer and reagent pressure ranged from 0 to 150 bars. The reaction temperature was 123 °C. The HPLC analysis was performed with a Shimadzu Class-VPV 10 AVP equipped with a refractive index detector, an LC-16 ADVP binary pump, a Dcou-14A degasser, and Shodex PL Hi-PlexPb column (Sc 1011 No. H706081), a guard column Sc-LcShodex, and a heater set at 80°C.

2.4. Determination of Vitamins and Minerals

According to the method suggested by [27]; the presence of different types of vitamin B (B1, B2, B3, B6, B9, and B12) in the powder of dried vegetative *S. platensis* was ascertained using a High-Performance Liquid Chromatographic (HPLC) system (Shi-Shimadzu-UFLC Prominence), equipped with an auto-sampler (Model-SIL 20AC HT) and a UV-Visible detector (Model-SPD 20A). While the mineral contents of dried vegetative *S. platensis* (Phosphorus, Potassium, Calcium, Magnesium, Sodium, Iron, Manganese, Zinc, and Copper) were ascertained using the procedure described in [28].

2.5. Total Polyphenolic Compounds

The total amount of polyphenolic compounds was calculated in the aqueous extract of *S. platensis* alga according to [29] using folin ciocalteu. In accordance with the calibration curve of gallic acid standard solutions which covering the concentration range between 0.2 and 1.0 $mg \cdot ml^{-1}$. The total polyphenols concentration was calculated as a gallic acid equivalent concentration [30].

2.6. Extraction of C-Phycocyanin

Each microorganism exhibits unique characteristics depending on the location of the proteins produced within the cells, which means that proteins can be found in the cytoplasm, periplasm, or mitochondria. As a result, the extraction methods may vary depending on the required protein [31]. Thus, the extraction protocol is critical for maximizing PC production in the natural form of *S. platensis* alga, where the steps in PC extraction are dependent on cell rupture and PC release from the cells [32]; however, the algae's cell walls are tough and may resist rupture. As a result, extreme conditions such as mechanical cell rupture are now desired in large-scale applications to ensure complete disintegration of the algal biomass while maximizing PC production yields [31]. Thus, wet algal biomass was used for C-Phycocyanin extraction using two different methods in this study including organic acid extraction (OAE) and cold maceration (CM).

2.6.1. Organic acid Extraction

Here, the wet biomass of *S. platensis* was soaked in 1.0 M acetic acid (5:1, w: v); allow to stand (24h at room temperature) [31] before being extracted. All extracts were centrifuged and the yield of extraction was determined through supernatant determination.

2.6.2. Cold Maceration

Phycocyanin was extracted from *S. platensis* wet biomass after soaking in distilled water 1:25 (*w*/*v*) for 24 h. For the purpose of removing cell debris, the resulting slurry was centrifuged at 10,000× *g* for 15 min at 4 °C. The precipitate was discarded and the crude extract obtained from the supernatant was collected. The pH of the crude extract was adjusted to 7.0 by adding 10% potassium hydroxide (KOH). Using ammonium sulfate, purification of C-PC was carried out using 100 ml crude extract for achieving 10%, 15%, and 40% saturation, and the yield was compared to that reported by Kamble *et al.* [33]; who used 25% ammonium sulfate. The resulting solution was allowed to stand for 2 h before being centrifuged (12000xg/30 mins). According to [34], the blue precipitate was dissolved in 0.005 M Na-phosphate buffer (pH 7.0) to obtain a clear solution.

2.7. Spectrophotometric Measurement of C-Phycocyanin

C-phycocyanin absorbance was spectrophotometrically measured at 620 and 652 nm and calculated as CPC ($mg.g^{-1}$) = OD620 − 0.474(OD 652)/5.34 [35]. Extraction Yield = (CPC) V/W, which was previously reported. As for phycocyanin spectra, it was measured between 400 and 800 nm. At room temperature, the UV/V spectra of *S. platensis* aqueous extract and extracted C-phycocyanin were recorded using a Shimadzu spectrofluorometer model RF-1501 [37].

2.8. Free Radical Scavenging Activity

S. platensis aqueous extract and isolated phycocyanin were tested for antioxidant activity [38] using DPPH-free radical as a test substrate (40 mg of DPPH in 10 mL of 70% ethanol). A spectroscopic assay was used to determine the *in vitro* inhibition percentages of DPPH radical, which was dependent on the estimation of color intensity at 518 nm in comparison to a blank solution [39]. With a few minor changes, ABTS assay was performed. The absorbance at 734 nm was determined spectrophotometrically after the samples were allowed to react with the ABTS solution for 7 minutes. The samples were compared to ascorbic acid's ability to scavenge ABTS [40].

2.9. Anticancer Activity

a. Cell lines

Human breast (MCF-7), human colon (HCT-116), and human liver (HepG 2) carcinoma cell lines were obtained from the Karolinska Center, Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden, and used in this study.

b. Cell culture

Laminar air flow cabinet with biosafety class II level was used, the procedure was carried out in an isolated sterile environment. It was necessary to maintain the culture in RPMI 1640 medium supplemented with 1% antibiotic-antimitotic mixture (10,000 U/ml potassium penicillin, 10,000 g/ml streptomycin sulfate, and 25 g/ml amphotericin B), 1% L-glutamine, and 10% heat-inactivated fetal bovine serum. According to [41], cultivation and sub-culturing were carried out in the laboratory. The chemotherapy drug doxorubicin was used as a positive control. In addition, a negative control consisting of DMSO was used.

c. Cell viability assay

The cells were seeded at a density of 10 × 103 cells per well in the case of MCF-7 and HepG2 cell lines, and at a density of 20 × 103 cells per well in a fresh complete growth medium in the case of HCT-116 using 96-well microtiter plastic plates at 37 °C for 24 h under 5% CO_2 in a water jacketed carbon dioxide incubator. Following the addition of 40 ml MTT salt (2.5 mg/ml), 200 ml sodium dodecyl sulfate (10%) was added (SDS). 595 nm was used as the absorbance measurement wavelength, with a wavelength of 690 nm used as the reference [42].

d. Determination of IC_{50} values

Probity analysis was used for IC50 calculation and SPSS software for statistical analysis (version 9, 1989, SPSS Inc., Chicago, IL, USA).

e. DNA fragmentation quantification

The degree of DNA fragmentation was determined in the manner. A diphenylamine reagent was used to determine the amount of DNA present in the pellet and the supernatant of the culture. At a wavelength of 600 nm, the optical density is measured colorimetrically. The percentage of DNA fragmentation was calculated by dividing the amount of DNA in the supernatant by the amount of DNA present in the pellet [43].

f. Human Caspase 3 estimation

In each of the microtiter strips provided, a monoclonal antibody specific for human caspase-3 has been coated onto the wells of the strip in question. A rabbit antibody specific for human active caspase-3 is pipetted into each of these wells, and then the wells are incubated with the samples. At the beginning of the incubation, the human caspase-3 protein binds to the immobilized (capture) antibody. At the end of the incubation, the specific active caspase-3 antibody serves as a detection antibody by binding to the immobilized active caspase-3 protein. An anti-Rabbit IgG (Anti-Rabbit IgG HRP) labeled with horseradish peroxidase is added after the first incubation step and after washing to remove excess protein and detection antibody. This antibody binds to the detection antibody and causes it to fluoresce. It is followed by a third incubation and washing to

remove all of the excess Anti-Rabbit IgG HRP before adding a substrate solution, which is then acted upon by the bound enzyme to produce color. In this study, the optical density was spectrophotometrically measured at 450 ± 2 nm .The intensity of this colored product is directly proportional to the amount of human active caspase-3 present in the original specimen [44], indicating that the original specimen contained a high concentration of active caspase-3.

g. Human Caspase 9 estimation

Microwells are coated with an anti-human Caspase-9 coating antibody that has been adsorbed onto them. This antibody (rabbit) recognizes and binds to human Caspase-9 that has been captured in the laboratory. Anti-rabbit IgG-HRP solution was added to the wells, and the substrate solution reactive with HRP is added to the wells as well. The amount of human Caspase-9 present in the sample or standard is reflected in the color of the product produced. The reaction is brought to a close by the addition of acid, and the absorbance at 450 nm is measured according to the manufacture's protocol [45].

h. Measurement of Bcl-2 levels

Samples and standard of BCL-2 binding to the antibody coated on the plate, indicating that the samples and standards are positive. It is then necessary to add a biotin-conjugated antibody, which recognizes the protein that was captured by the first antibody. The biotin-conjugated antibody is bound to the streptavidin-HRP that has been added. After the wells have been filled with the substrate solution, the colored products are formed. The reaction is then brought to a close with acid addition, and the absorbance at 450 nm is measured. It is necessary to prepare a standard curve in order to calculate the protein concentration [46].

i. Human P53 Estimation using ELIZA

Human p53 in the sample or standard binds to antibodies in the microwells, forming a complex. A biotin-conjugated antibody was added to the reaction and allowed to incubate. Just after dispensing unbound biotin-conjugated streptavidin, HRP is added to the reaction. The process is stopped by adding acid and measuring the absorbance at 450 nm [47].

3. Results and Discussion

3.1. Spirulina Nutritional Profile

In the chemical analysis of *Spirulina*, a wide range of nutritional constituents were found to be present. These included protein, vitamins, essential amino acids, dietary minerals, essential fatty acids, and growth factors, among other things. In addition, immunomodulatory, anticancer, antioxidant, antiviral, and antibacterial properties are all possible health benefits [48–50].

In its dried state, *Spirulina* is predominantly constituted of 55–70% protein and 5–6% fat (weight-for-weight dry cell). Polyunsaturated fatty acids (PUFAs) represent approximately 1.5 - 2% of the overall lipid content in this alga (fats and oils). *Spirulina* species are rich in linolenic acid (36% of overall PUFAs), vitamins (thiamine, riboflavin, niacin, B6, folate, cobalamin, ascorbic acid, D, and E), minerals (potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc), and pigments (chlorophyll a, xanthophyll, beta carotene. As a result, biomass derived from this plentiful supply of components is used as feed and food additives in a variety of sectors around the world *e.g.*; agriculture, food, pharmaceutics and perfumery. While the chemical characteristics of two species belonging to the same microalgal category are generally similar, their chemical characteristics differ depending on the specific source of the algae, the culture conditions, the harvest time, and the extraction method used. The following is a brief summary of the general composition (in percent of dry weight): Proteins account for 50–70% of the total, carbohydrates account for 15–25%, lipids account for 6–13%, nucleic acids account for 4.2–6% and minerals account for 2.2–4.8% [45,51,52].

In this study, total carbohydrates and protein content of the dried biomass were determined and reached 23.50% and 61.00% (on dry weight basis); respectively. Proteins are made up of a large number of amino acids that are held together by peptide bonds, which are covalently bonded to one another. The number and arrangement of these amino acids were used to determine the type of protein that is present.

Sugars and amino acids were analyzed using HPLC, and the results are shown in Fig. (1 and 2), respectively. Aside from that, *S. platensis* contains eight essential amino acids, which amount to 23.7 g

per 100 g of dried biomass in addition to nine non-essential amino acids totaling 28.7 g·100 g^{-1} dried biomass. Among the essential amino acids, methionine and lysine are presented as the most abundant (4.8 and 4.2 g·100 g^{-1} dried biomass, respectively).

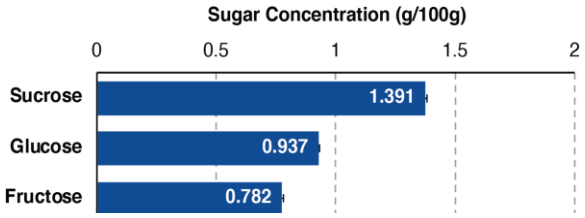

Fig 1. HPLC analysis of sugar content in dried *S. platensis.*

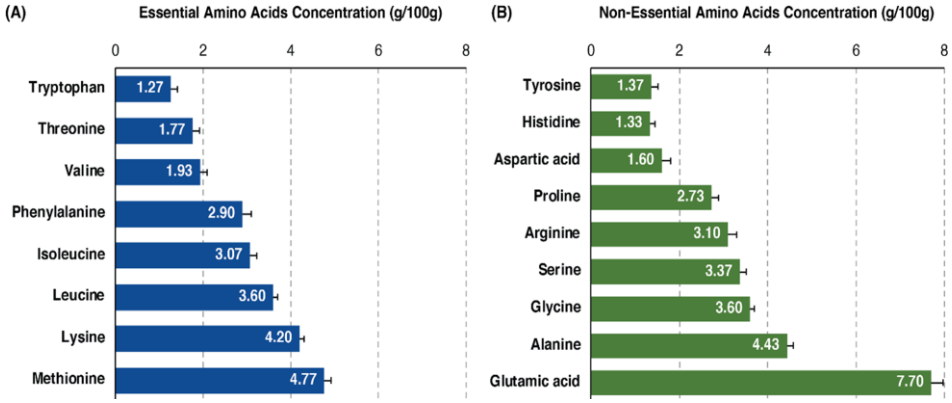

Fig 2. Analysis of *S. platensis* amino acids (g·100 g^{-1}).

Glutamic acid, on the other hand, was discovered to be a significant non-essential amino acid, accounting for 7.8 g·100 g^{-1} dried biomass. Furthermore, three major sugars were discovered in dried *S. platensis* biomass, with a total weight of 3.108 g·100 g^{-1}. 1.392 sucrose, 0.931 glucose, and 0.785 fructose are found in one hundred grams of sucrose (1.100 g^{-1} of sucrose).

The aforementioned chemical composition is extremely sensitive to both environmental conditions, nutritional status and technical practice. The dried *S. platensis* biomass passed the carbohydrate and protein tests, indicating that it contains both [53]. The functions of cell wall polysaccharides have traditionally been considered to include energy storage, contribution to structural integrity and mechanical strength, control of osmotic pressure, the buffer layer that protects against drought and infective organisms such as viruses, bacteria, and fungi [54–56] and the control of osmotic pressure. Previously and under the same conditions, both the cold and hot aqueous extraction methods of *S. platensis* revealed that the most abundant sugars i were glucose, galactose, and mannose [14].

Protein content and amino acid composition of hot air-dried *Spirulina* biomass were found to be approximately 61% crude protein with low soluble protein content (0.8%) on a dry weight basis with presence of 17 different amino acids, including 8 essential amino acids and 9 non-essential amino acids. Among the most potent amino acids, methionine (4.8%), lysine (4.2%), leucine (3.7%) and glutamic acid (7.8%) of total amino acid content were detected.

The results of amino acid analysis were found to be in agreement with those obtained by [14], who reported that amino acid analysis revealed the presence of 16 amino acids, with aspartic and glutamic acids being detected as the most prevalent non-essential amino acids, and leucine, phenylalanine, and valine being presented as the most prevalent essential amino acids. This range of amino acids demonstrates that the biological value of proteins in *Spirulina* is high and that an ideal item could be achieved by supplementing with a good source of sulfur-containing amino acids as well as lysine and/or histidine to name a few nutrients. Rice, wheat, and millet are examples of cereals that should be considered excellent supplements, as should

certain oil seeds such as sesame and flaxseed. It should be noted that the populations of Chad that consume *Spirulina* do so in conjunction with millet, which is particularly high in methionine and cysteine [57].

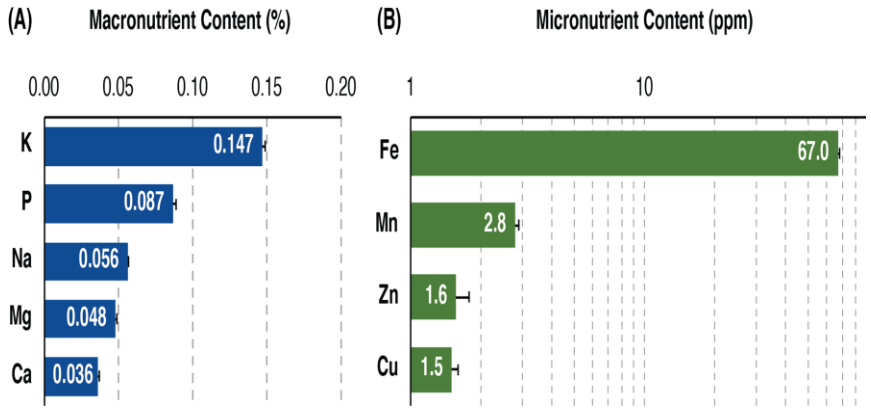

Fig 3. Some minerals content of dried *S. platensis*.

As shown in Fig. 3, alga has been shown to be highly effective in the accumulation of macro and micro-elements [58]. It was previously believed that algae were a rich source of minerals and other supplements, among other things. *Cladophora* has an ash content ranging from 12% to 44% in some cases, depending on the species. The ash content of the *Cladophora* can range from 12% to 44% in some cases. Some edible algae contain a high concentration of macro and other trace-elements, while others do not. For this reason, macro and micro-algae are considered as supplement sources of minerals in addition to the other supplements of food.

Micronutrients deficiency is a type of malnutrition that resulted in physiological disorders in humans as a result of the consumption of crops grown in soils that are depleted of nutrients. A significant increase in the risk of sterility, fetal structural defects and long-term illnesses has been demonstrated in association with micronutrient deficiencies [59]. The role of trace elements in the activity of virtually all enzymes, whether within the active site of the enzyme or as co-factors; as well as the role of vitamins as coenzymes; has been well documented in the literature. Micronutrients are critical for the proper functioning of the metabolism and the preservation of tissue capacities [60].

Supplementation with *Spirulina* is one method of alleviating malnutrition caused by a lack of nutrients in the food supply (*Arthrospira*). Despite the fact that it has a high protein content and is digestible, *Spirulina* is widely used as a food source due to the presence of high-value nutritional components such as vitamin A, chlorophyll, essential fatty acids, and so on [60]; which make it a popular choice.

Aside from that, *Spirulina* is considered by NASA to be an ideal food for astronauts and has been dubbed “the food of the future” [61]. Also, noteworthy is the fact that S*pirulina* is the second most important commercial microalgae in the production of nutritious food and livestock feed [62].

Vitamin B types (B1, B2, B3, B6, B9, and B12) were determined in *S. platensis* biomass with B2 being the most abundant (7.37 mg·100 g^{-1}); while the total content was 13.52 mg·100 g^{-1} (Table 4). Certain algae species contain a high concentration of vitamins and can be used as a complete source of vitamins supplements as shown in Fig. 4 [58].

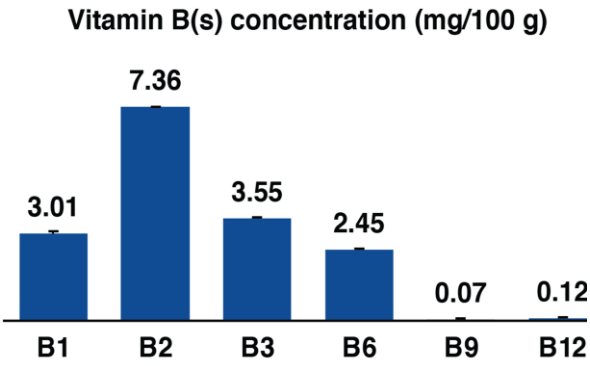

Fig. 4. Vitamin $B_{(s)}$ concentration (mg/100 g) of *S. platensis*

Polyphenols; on the other hand; are the most well-known biologically active compounds in existence. They are primarily distinguished by the presence of one or more phenol units in their structure. They are regarded as one of the most important classes of secondary metabolites, and their antioxidant properties have been shown to play a critical role in the prevention of chronic diseases [63,64]. Using quantitative spectrophotometric analysis of polyphenolic compounds in the aqueous extract of *S. platensis* revealed that the plant contains a high concentration of gallic acid (865.43 ± 2.13 mg gallic acid.100 gm^{-1}), which was the subject of the current investigation.

3.2. Extraction and Purification of C-Phycocyanin

In order to determine the best technique for maximum C-phycocyanin recovery from *S. platensis* wet biomass, a comparison between two techniques (Organic acid extraction and cold maceration in distilled water with different concentrations of ammonium sulfate for extraction and purification of C-phycocyanin from *S. platensis* wet biomass was carried out separately and repeated three times.

It was found that water extraction method surpasses organic acid extraction in concern C-phycocyanin ($mg.ml^{-1}$) and yield ($mg.g^{-1}$). Furthermore, cold maceration method followed by purification using 10% ammonium sulfate would be the most straight forward and efficient protocol (Fig. 5). It was reached 96.5 ± 0.14 $mg{\cdot}g^{-1}$ for extraction of C-PC in higher yield (0.193 ± 0.15 $mg{\cdot}ml^{-1}$).The absorption spectra of *S. platensis* aqueous extract and the extracted C-phycocyanin were recorded between 400 and 800 nm (Fig. 6). It was revealed that the aqueous extract of *S. platensis* gave three absorption peaks (553, 620, and 665 nm); which corresponded to phycoerythrins (PEs, λ_{max}540–570 nm), phycocyanins (PCs, λ_{max}610–620 nm) and allophycocyanins (APC, λ_{max}650–670 nm) [65]; while the isolated C-phycocyanin recorded one absorption peak at 620 nm confirming its purity.

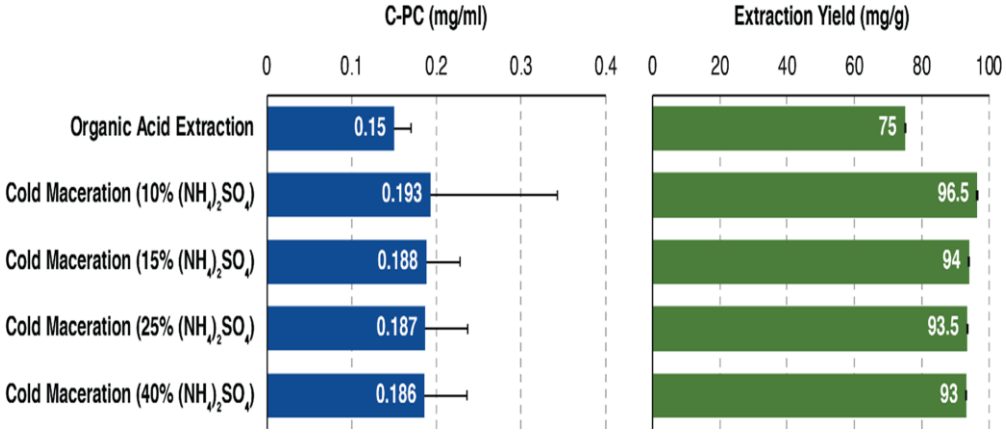

Fig. 5. C-PC concentration and extraction yield of two extraction methods from *S. platensis* wet biomass (mean values ± SE).

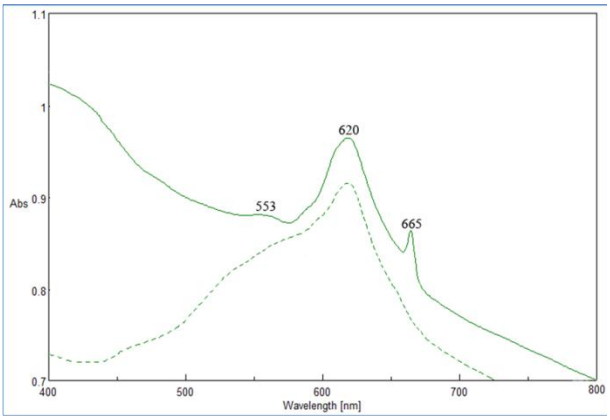

Fig. 6. Absorption spectrum of the aqueous extract of *S. platensis* (—) and the extracted C-phycocyanin (- - -).

3.3. Free Radical Scavenging Activity

It has been demonstrated that C-phycocyanin is a powerful antioxidant [66]. Aqueous extract of *S. platensis* and its isolated C-PC were tested for their radical scavenging activity against the DPPH and ABTS radicals in the present study. Despite the fact that both the aqueous extract and the isolated phycocyanin from *S. platensis* demonstrated significant antioxidant activity, the results in Fig 7 clearly demonstrated that C-PC has a more potent effect, which is consistent with the findings of [67,68].

According to Anandjiwala *et al.* [69], in the presence of a hydrogen-donating antioxidant (AH), the concentration of alcoholic DPPH solution decreases due to the formation of the non-radical form DPPHH by the reaction of DPPH + AH DPPHH + A, and the amount of alcoholic DPPH solution remaining after a specific time correspond inversely to the antioxidant's radical scavenging activity.

The present study's findings have revealed the potential of a cyanobacterial strain that contains antiradical metabolites that decolorize the stable DPPH radical, which was previously unknown. The cyanobacterial strain and the isolated C-PC, on the other hand, demonstrated the ability to scavenge ABTS radicals. According to the findings, high molecular weight phenolics have greater ability to reduce free radicals (ABTS•+), and their efficiency is more dependent on the molecular weight, the number of aromatic rings, and the nature of hydroxyl group substitutions than on the specific functional groups [70]. The present findings suggested that the free radical scavenging activity of *S. platensis* extract may be attributed to the presence of high molecular weight phenolics and derivatives.

Spirulina contains Beta carotenes in concentrations of 33%, and the carotenes' nature is fundamentally the same as that of carrot carotene and the carotene found in the other angiosperm sources. The *Spirulina* genus contains a variety of vitamins and minerals, including vitamin E, thiamine, cobalamine, biotin, and inositol, among others. Tocopherols are produced in significant quantities by a number of microalgae. Algae are a rich source of a variety of minerals with varying chemical compositions, which are found in abundance. In the animal feed industry, they are employed [71]. Algae as a source of antioxidants and are also extremely rich sources of antioxidants, as well as a variety of other compounds. In some cases, the antioxidants found in certain genera can be used to make pharmaceutical products [72].

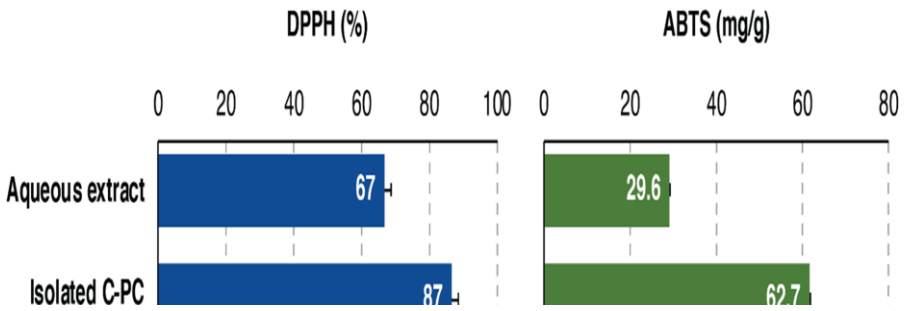

Fig. 7. Free radical scavenging activity of the aqueous extract and isolated phycocyanin from *S. platensis*.

3.4. Anticancer Activity

The aqueous extract of *S. platensis* and the isolated C-PC were screened for anticancer activity using the MCF-7, HCT-116, and HepG 2 cell lines. The aqueous extract exhibited only 5.1%, 14.3%, and 31.2% anticancer activity against MCF-7, HCT-116, and HepG 2, respectively (Fig. 8). On the other hand, because the isolated C-PC demonstrated significant anticancer activity against the three tested cell lines, it was subjected to a dose study assay against the three cell lines in order to determine the IC50 value for each cell line. On MCF-7, HCT-116, and HepG 2, phycocyanin had IC50 values of 58.9, 48.1, and 44.7 $g \cdot ml^{-1}$, respectively. These findings are consistent with [73]. Thus, for the purpose of comparison, the specific anticancer pathway in each cell line was investigated. The effect of C-PC prompt-hepatotoxicity on caspase 3 level was determined, and it was discovered that it increased caspase 3 protein level from 46.8 to 255.4 pg/mL in comparison to untreated hepatocarcinoma cells. These findings, in conjunction with previous research, confirmed that phycocyanin activates proapoptotic effect in hepatocarcinoma cells via elevation of caspase 3 level among other apoptotic markers [74–76].

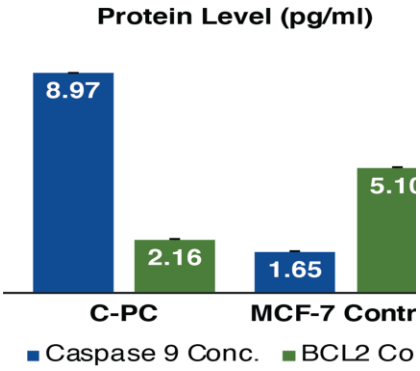

Fig. 9. Bcl2 and caspase-9 protein level of MCF-7 treated with C-PC compared to untreated cells.

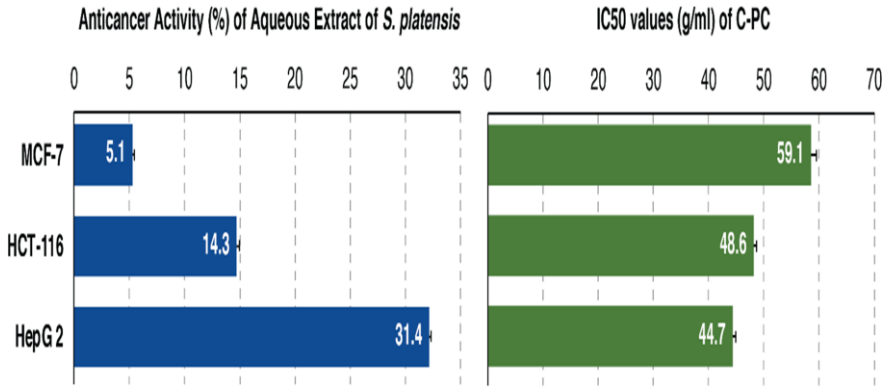

Fig. 8. Anticancer activity of aqueous extract of *S. platensis* and IC50 values of C-PC for the MCF-7, HCT-116, and HepG2 cell lines.

Three parameters were examined to determine the effect of C-PC cytotoxicity on breast anticancer activity: BCL2 antiapoptotic level, Caspase 9 level and P53 protein level. As illustrated in Fig 9, C-PC induced apoptosis in breast cancer cells by down-regulating BCL2 and up-regulating Caspase 9 and P53 protein levels in comparison to untreated control breast cancer. Additionally, phycocyanin significantly increased the tumor suppressor protein P53 from 43.55 to 469.9 Pg/ml, consistent with [77,78]. Additionally, the anticancer effect of C-PC on HCT-116 cells was evaluated by examining its effect on DNA, which revealed that it increased DNA fragmentation within colon carcinoma cells by 5.632184%, compared to untreated HCT-116 cells, which was 27.76%, implying that it mediates distinct apoptotic cascades.

While *S. platensis* is the most widely used commercial microalga in the world, it has also been recognized by international accreditation for its safe nutritional and dietary supplement properties, as well as its use in medical applications [52,79]. Due to the high concentration of carbohydrate and protein in dried vegetative *S. platensis* powder, it is considered

a potential source of health food in the human diet, as it helps to maintain consistent energy levels [80]. The study also discovered that different types of vitamin B and minerals could be found in the powder of dried vegetative *S. platensis*, indicating that *S. platensis* could be a good source of vitamins and minerals.

It may provide an adequate and safe source of iron for anemic pregnant women because it promotes the formation of hemoglobin, the oxygen-carrying blood pigment found in healthy red blood cells. It also contains potassium, a critical mineral for maintaining electrolyte balance in the body, and phosphorus, the second most abundant mineral in the human body, which helps to maintain bone density in conjunction with calcium, among other nutrients. The presence of polyphenolics in *S. platensis*, on the other hand, was found to be in high concentration. Polyphenolics are the most common biologically active compounds and, as a result of their antioxidant properties, they play an important role in the prevention of chronic diseases.

The organic acid extraction method and the cold maceration method, followed by purification with different concentrations of ammonium sulfate, were both employed in order to obtain pure C-phycocyanin from wet biomass of *S. platensis*. The concentration of extracted CPC (mg/mL) and the extraction yield (mg/g) of the two procedures were compared, and the extraction yield (mg/g) was calculated for each procedure. The cold maceration method, followed by purification with 10% ammonium sulfate, was found to be the most efficient procedure for maximum CPC recovery, and it was also found to be the most effective procedure for screening as an anticancer agent over MCF-7, HCT-116, HepG 2 cells.

Conclusions

The findings of this study demonstrated that *S. platensis* is a potential health food in the human diet and is used in the food industry as a source of ingredients, particularly those containing high levels of carbohydrates, protein, vitamins, and minerals. It can be used beneficially for people on low-calorie diets because it is considered an important source of food, and as a health tonic in addition to its antioxidant and anticancer properties.

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