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М.М. ІСМАЇЛ,
Національний Інститут Океанографії та Рибного Господарства,
Каїр, Єгипет
mona_es5@yahoo.com
ORCID 0000-0001-7029-6802

Н.Х. НОАМАН,
Олександрійський Університет,
Олександрія, Єгипет

БІОХІМІЧНИЙ ПРОФІЛЬ, ХАРЧОВА ЦІННІСТЬ ТА БІОЛОГІЧНА АКТИВНІСТЬ *ARTHROSPIRA PLATENSIS* GOMONT

Arthrospira platensis (= Spirulina platensis) є перспективним джерелом біологічно активних сполук і тому традиційно використовується для лікування різних хвороб. Метою даної роботи було охарактеризувати харчові та біологічні властивості Arthrospira platensis in vitro. Визначена калорійність (428,9 ккал/100 г) та чистота фікоціаніну (0,22 %) дають можливість рекомендувати цей вид водоростей, як альтернативне джерело здорової їжі. Розрахована добова доза Arthrospira platensis є нижчою за встановлений ВООЗ рівень, що свідчить про те, що вона не спричинює негативний вплив на здоров'я людини. В процесі досліджень визначали антиоксидантну, протиартритну, протизапальну, антидіабетичну та протиацетилхолінестеразну активність семи різних екстрактів водорості. Високий вміст фенольних сполук та високу біологічну активність спостерігали в екстрактах хлороформу та етанолу, які проявляли різні біологічні властивості у порівнянні зі стандартними ліками. Газово-хроматографічний та мас-спектрометричний аналіз обох активних екстрактів дав змогу виділити 21 біологічно активну речовину, включаючи жирні кислоти, терпеноїди, феноли та алкани, в залежності від використаного розчинника. Можна зробити висновок, що Arthrospira platensis може бути використана в комерційних цілях як важливе джерело багатьох біологічно активних сполук. Наголошується на необхідності подальшого вивчення цього виду водоростей з метою виділення та очищення ефективних сполук, що можуть бути використані у фармацевтичній галузі.

Ключові слова: *Arthrospira platensis, біологічно активні речовини, харчова цінність, комерційне застосування, медичне використання.*

In recent years, the interest in obtaining bio-functional compounds from natural sources increased all over the world. Microalgae and cyanobacteria are thought to be among the best natural resources due to their ability to grow efficiently in large-scale raceway ponds or photobioreactors. *Arthrospira* species are common filamentous free floating cyanobacteria occurring in different fresh and marine habitats. This genus is commercially cultivated all over the world as an essential source of nutrients in the traditional diets in many count-

ries with no risks to health. It is marked as bio-function food due to a high content of valuable proteins (60—70 % of its dry weight (DW)), including the most essential amino acids like tryptophan, threonine, histidine, lysine, isoleucine, leucine, and phenylalanine [10]. In addition, this alga contains respectively 8—16 % and 4—9 % DW of carbohydrates and lipids, and also other nutrients, including vitamins A, B, D, E, and harmless β-carotene [70]. *Arthrospira platensis* (*Spirulina platensis*) is characterized by a high content of microelements like Fe, P, Mg, Zn, K, and Cu [23]. These compounds have the ability to quench free radicals and chelate catalytic metals and scavenge oxygen [84]. These natural antioxidants are known as potential anti-inflammatory agents, which safely protect human body against inflammation, thus preventing diseases and disorders caused by inflammation [50].

Moreover, *Spirulina platensis* contains distinctive natural green, orange, and blue pigments, namely chlorophylls, carotenoids, and phycocyanins, respectively. Phycocyanin, especially C-phycocyanin (C-PC), is widely considered as a precious food-dye because of its protein-based structure and rare intense-blue color [48]. These phytopigments possess nutritional and pharmaceutical properties such as antioxidant, anti-inflammatory, anticancer, and cholesterol-lowering effects. Furthermore, the cells of this microalga are characterized by high digestibility (75—83 %) due to the lack of cellulose, which facilitates their use for human consumption [6].

Globally, this commercially important algal species is produced by many companies for selling as food supplement in 20 countries all over the world. It has been documented as GRAS (Generally Recognized as Safe) with no risks to health by the FDA (USA Food and Drug Administration). Generally, *Spirulina platensis* and *Spirulina maxima* are the most important species used for consumption [2]. Many studies support the nutritional value of *Spirulina* powder as bio-function food or as natural colorant [27, 56].

Recently, *Spirulina* plays a unique role in medicine and pharmaceutical field as antimicrobial agent in the treatment of arthritis, anemia, cardiovascular diseases, diabetes, and cancer [30, 50]. In addition, *S. platensis* can be used as an alternative medicine in the treatment of debilitating diseases such as Alzheimer's, diabetes, and hepatic damage [13].

The objective of the present work was to estimate the biochemical content and nutritional value of *Arthrospira platensis*, to evaluate diabetic, antioxidant, anti-arthritic, anti-inflammatory, and anti-AChE properties of different algal extracts using various techniques *in vitro*, and also to establish relationship between the estimated biological activities and phenol content.

Material and Methods

Chemicals

All chemicals were purchased from the Sigma-Aldrich Co. (Darmstadt, Germany).

Algal samples

Arthrospira platensis Gomont (formerly *Spirulina platensis* (Gomont) Geitler) was cultivated on the F/2 marine enrichment medium for cyanobacterial cultures, aerated by air pumps, and incubated at 28±2 °C under 16 : 8 light/dark conditions with light intensity of 120 μmol/m^2·s. Algal cells were harvested after 12 days, freeze-dried, and then grounded into a fine powder for further analyses.

Preparation of different extracts

A precisely weighed ~1 g of the grounded freeze-dried *Arthrospira platensis* was extracted with 10 mL of different solvents, including acetone, ethanol, methanol, chloroform, diethyl ether, ethyl acetate, and water, overnight at room temperature (25 °C±2). The tube was centrifuged at 4 500 rpm for 10 min and the supernatant was recovered. The extraction was repeated with 2 mL of different solvents and two supernatants were combined. The residue was subsequently extracted twice for 30 min at room temperature and supernatants were combined for further analyses [14].

Qualitative analysis for phytochemical screening of different extracts

On the whole, nine qualitative analyses were carried out for phytochemical screening of different extracts of the tested alga. The presence or absence of such substances as steroids, terpenes, saponins, quinones, coumarins, tannins, flavonoids, phenols, and cardiac glycosides was revealed in various extracts according to standard procedures [82].

Nutritional content

The total content of carbohydrates and proteins in the tested alga was estimated according to [20, 45] using d-glucose and bovine serum albumin (BSA) as standards, respectively. The total content of lipids was assessed following [5]. The data were expressed in percent of algal dry weight.

The calorie content of the alga was determined by the following equation [21]:

$$\text{Calories (Kcal/100 g)} = 4 \times \text{proteins} + 9 \times \text{lipids} + 4 \times \text{carbohydrates}.$$

Phytochemical analyses

The total content of phenols in the studied extracts was estimated by the Folin-Ciocalteu method [37]. The obtained results were expressed as gallic acid equivalent (GAE)/g of dry weight (DW). The total content of flavonoids was estimated as described [25] and expressed as mg of quercetin equivalent (QE)/g of extract. The content of tannins in various extracts was measured using the Folin-Ciocalteu reagent assay according to [80] and expressed in mg of GAE /g of extract.

Estimation of vitamin content

Vitamin C (ascorbic acid) content in the tested alga was determined following [59] and expressed as mg of ascorbic acid (AA) per 100 g of dry weight. Vitamin E content was estimated according to the method [66] and expressed as α-tocopherol equivalents per gram of dry weight.

Pigment content

The extraction of pigments was carried out in triplicate using 10 ml of methanol 100 %, at room temperature and under dark conditions. The extracts were then centrifuged at 2500 rpm for 10 min and the collected supernatant was subjected to another centrifugation (5000 rpm for 5 min). The absorbance of the supernatant was read using a UV-1800 ultraviolet-visible spectrophotometer (Shimadzu, Japan) at 480, 632, 652, 665, 696, 750 nm wavelengths and calculated using the following equation. The obtained results were expressed in mg/g DW [16, 58].

$$\text{Chl } a \text{ (mg/g)} =$$

$$= \frac{[-2.0780 \times (A632 - A750) - 6.5079 \times (A652 - A750) + 16.2127 \times (A665 - A750) - 2.1372 \times (A696 - A750)]}{wt}$$

$$\text{Chl } c \text{ (mg/g)} =$$

$$= \frac{[34.0115 \times (A632 - A750) - 12.7873 \times (A652 - A750) + 1.4489 \times (A665 - A750) - 2.5812 \times (A696 - A750)]}{wt}$$

$$\text{Chl } d \text{ (mg/g)} =$$

$$= \frac{[-0.3411 \times (A632 - A750) + 0.1129 - (A652 - A750) - 0.2538 \times (A665 - A750) + 12.9508 \times (A696 - A750)]}{wt}$$

$$\text{Carotenoids (mg/g)} = \frac{[4 \times (A480 - A750)]}{wt}$$

β-carotene and lycopene were determined by colorimetric assay according to [53] and estimated by the following equations.

$$\beta\text{-carotene (mg/100 mL)} = 0.216 \times A663 - 0.304 \times A505 + 0.452 \times A453$$

$$\text{Lycopene (mg/100 mL)} = (-0.0458 \times A663) + 186\ (0.372 \times A505) - (0.0806 \times A453).$$

The concentration of phycobiliproteins (mg/g DW) in the algal buffer extract (pH 7) were measured at 562, 620, and 652 nm and then calculated using the following formulae [61]:

$$C - PC = \frac{A620 - (0.474 \times A652)}{5.34 \cdot wt}$$

$$APC = \frac{A652 - (0.208 \times A620)}{5.09 \cdot wt}$$

$$PE = \frac{A562 - 2.41 \times [PC] - 0.849 \times [APC]}{9.62 \cdot wt}$$

The final results were expressed in mg of the individual phycobiliproteins/g of algal DW.

The purity of phycocyanin was estimated by the A615/A280 ratio.

Estimation of multi-minerals

According to [73], algal samples were digested to determine the element content. The concentration of Cu, Fe, Zn, Ca, Mg, Na, K, S, and Mn was determined using atomic absorption spectrophotometer AAS/flame mode (Savant AAGBC).

The ratio of ion quotient was estimated using the following equation with the concentrations given in moles [39].

$$\text{Ion quotient} = \frac{(\text{Ca}+\text{Na})}{(\text{K}+\text{Mg})}$$

Estimated daily intake for adult

The estimated daily intake (EDI) was calculated using the following equation (Health Consultation, Land Crab Evaluation, National Oceanographic Atmospheric Administration Data 2006):

$$\text{EDI [mg/kg/day]} = \frac{\text{C}\times\text{IR}\times\text{EF}\times\text{ED}}{\text{BW}\times\text{AT}}$$

where C — the average concentration of the contaminant (mg/kg), IR — ingestion rate (0.227 kg/day (8-oz. meal) for adult), EF — exposure frequency or the number of exposure events per year of exposure (365 days/year), ED — exposure time (70 years), BW — body weight (70 kg), and AT — averaging time (non-cancer/lifetime — ЕДЧ365 days/year).

Biological activities of algal extracts

Antioxidant activity

Total antioxidant activity

The total antioxidant activity (TAC) of crude extracts was determined according to [66]. The total antioxidant activity is expressed as the number of equivalents of ascorbic acid. A calibration curve of ascorbic acid was prepared and the total antioxidant activity was standardized against ascorbic acid equivalents per gram of sample on dry weight basis (mg/g ASA).

Hydrogen peroxide radical scavenging activity

The capability of the extracts to scavenge H_2O_2 was determined based on [26] and calculated by the formula:

$$\text{Free radical scavenging } (H_2O_2)\ (\%) = \frac{A_C - A_S}{A_C}\times 100,$$

where A_C is the absorbance of control (ascorbic acid) and A_S is the absorbance in the presence of sample or standard.

Anti-inflammatory activity (15-lipoxygenase inhibitory assay)

Anti-lipoxygenase activity assay was done by [63] technique with minor modifications. The assay was based on measuring the formation of the complex Fe^{3+}/xylenol orange in a spectrophotometer at 560 nm.

$$\% \text{ inhibition} = \frac{(\text{A control} - \text{A sample})}{(\text{A control})} \times 100$$

Anti-arthritic activity (protein denaturation assay)

Anti-arthritic activity of the tested extracts was done by [72] method with slight modifications. Diclofenac sodium and distilled water were used as the positive and negative controls, respectively. The inhibition percentage was measured at 416 nm and calculated according to the following formula:

$$\% \text{ inhibition} = \frac{(\text{A control} - \text{A sample})}{(\text{A control})} \times 100$$

where A control (doubled-distilled water) is the absorbance of control well and A sample is the absorbance of the sample well or standard (diclofenac sodium).

Anti-diabetic activity

Inhibition of α-amylase activity

In vitro anti-diabetic activities by inhibition of α-amylase were measured according to [28]. The extract samples (500 μL) and standard drug acarbose (100—1000 μg/mL) were added to phosphate buffer (500 μL, 0.20 mM, pH 6.9) containing α-amylase (0.5 mg/mL) solution and incubated at 25 °C for 10 min. Thereafter, starch solution (500 μL, 1 % w/v in 0.02 M sodium phosphate buffer pH 6.9) was added to the reaction mixture, which was incubated at 25 °C for 10 min. The reaction was quenched with 3, 5 dinitrosalicylic acid reagent (1.0 mL) by heating in a boiling water bath for 5 min before being cooled at room temperature. The reaction mixture was then diluted with distilled water (10 mL) and the absorbance was measured at 540 nm.

Inhibition of α-glucosidase activity

The α-glucosidase inhibition assay was performed according to the modified method [19]. In brief, a solution of starch substrate (2 % w/v sucrose, 1 mL in 0.2 M Tris buffer pH 8.0) and various concentrations of algal extracts were incubated for 5 min at 37 °C. The reaction was initiated by adding α-glucosidase enzyme (1 mL, 1 U/mL) to the reaction mixture, followed by incubation for 10 min at 37 °C. The reaction was stopped with 3, 5 dinitrosalicylic acid reagent (1 mL) by heating for 2 min in a boiling water bath before being cooled at room temperature. The reaction mixture was then diluted with distilled water (9 mL), and the absorbance was measured at 540 nm.

Anti-cholinesterase inhibitory activities

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities using galantamine as positive control were determined according to [22].

Acetylcholinesterase (AChE) inhibitory activity

AChE assay was carried out as follows: 0.8 mM in 2 ml of assay solutions with 100 mM of phosphate buffer (pH 7.5) and 1.0 mM 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) were mixed and incubated at 25 °C. The hydrolysis was

monitored by the formation of thiolate dianion of DTNB at 412 nm for 2 min (intervals of 30 s).

Butyrylcholinesterase (BChE) inhibitory activity

BChE assay was carried out as follows: 10 μL of each algal extract in 0.2 % DMSO, 79 μL of 20 mM sodium phosphate buffer (pH 7.6) and 1 μL enzyme preparation (with final concentrations of 0.035 unit/mL for BChE and final concentrations of 1 to 500/1000 μM for the compounds tested) were mixed and incubated for 15 min. Then, 10 μL of substrate solutions were added to the mixture (4 mM for butyrylthiocholine iodide) and incubated for 30 min. The reaction was stopped by adding 900 μL DTNB-phosphate-ethanol reagent. The absorption was read immediately at 412 nm.

The inhibition percentage of both activities was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{A control} - \text{A sample})}{(\text{A control})} \times 100$$

GC-MS analysis of chloroform and ethanol extracts

GC-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) was used to identify bioactive compounds in ethanol and chloroform extracts at the City of Scientific Research and Technological Applications. GC- ISQ mass spectrometer included a capillary column TG—5MS (30 m × 0.25 mm × 0.25 μm film thickness). The initial temperature was 55 °C, then increased by 5 °C/min to 250 °C, withhold 2 min and then increased to 300 °C with 25 °C/min. The column oven temperature was initially held at 55 °C and then increased by 5 °C/min to 250 °C, withhold 2 min and then increased to 300 with 25 °C/min. The injector temperature was kept at 270 °C. Helium was used as gas carrier at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 μl were injected automatically using the Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50—650 in full scan mode. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST14 mass spectral database [12].

Statistical Analysis

All experiments were estimated in triplicate and mean values were presented ± standard deviation. For comparing means between extracts, ANOVA — on way was established using SPSS base 15.0 software (Chicago, USA: Users guide SPSS Inc., 2006) at $p < 0.05$ level of significance. Pearson's correlation analysis was used to establish relationship between the content of phenol compounds and the estimated biological activities using SPSS software.

Results and Discussion

Qualitative analyses of phytochemical content

Qualitative screening of bioactive constituents is important for medical uses since the presence of these compounds of interest may lead to their further purification and identification. As it is evident from Table 1, the highest per-

cent yield was obtained in ethanol extract (24.84 %) followed by methanol (17.73 %) and chloroform (17.62 %) extracts. Ethyl acetate extraction resulted in minimum percent yield with 8.19 %. This highlights that ethanol is efficient in extracting phytochemicals from *Arthrospira platensis*. This difference may be related to the variances in the polarity of the used solvents, which could cause a wide variation in the concentration of bioactive compounds in the extract. Moreover, the nature of extracting solvent plays a master role in extraction of vital compounds of antioxidant capability since the compounds differ in chemical characteristics, polarities and solubility [58].

As a result of preliminary phytochemical screening nine secondary metabolites (steroids, terpenes, saponins, quinones, coumarins, tannins, phenols, flavonoids, and cardiac glycosides) were tested in seven different extracts of *Arthrospira platensis* such as acetone, ethanol, ethyl acetate, chloroform, diethyl ether, water, and methanol (Table 1). The ethanol and methanol extracts exhibited the presence of all bioactive compounds excepting cardiac glycosides. In this case, saponins, quinones, and glycosides were absent in acetone, ethyl acetate, and chloroform extracts. While terpenes and steroids were completely absent in acetone extract. In addition, coumarins were also absent in chloroform extract. Diethyl ether extract showed the presence of most

Table 1

Qualitative analyses of phytochemicals in different *A. platensis* extracts and yield

Extracts	Yield, %	Steroids	Terpenes	Saponins	Quinones	Coumarins	Tannins	Phenols	Flavonoids	Cardiac glycosides
Acetone	9.72	nd	nd	nd	nd	*	*	*	*	nd
Ethyl acetate	8.19	**	**	nd	nd	*	*	*	*	nd
Chloroform	17.62	*	*	nd	nd	nd	**	**	**	nd
Ethanol	24.84	**	**	*	*	**	**	**	**	nd
Diethyl ether	16.26	*	*	nd	*	**	*	*	*	nd
Water	16,81	**	**	*	nd	nd	*	**	**	nd
Methanol	17.73	**	**	*	*	**	**	**	**	nd

Note. * moderate and ** high content based on the intensity of the color produced from the reactions. nd — not detected.

bioactive compounds except saponins and glycosides. The aqueous extract showed negative results for coumarins, quinones, and glycosides. These phytochemicals are biologically significant and play a vital role in medicinal applications due mainly to a high efficiency in suppression the oxidative stress as demonstrated by [13]. Previously, it has been found [11] that phytochemicals from *Spirulina* can be used in treating dreadful diseases like cancer, tuberculosis, inflammation, and many other blood-related diseases *in vitro*.

Nutritional value

Proteins, carbohydrates, and lipids are the main nutritional components in algal biomass [67]. *Spirulina* species are characterized by the presence of vital biochemical components, the content of which depends on the composition of nutrients in the cultural medium [79]. The proximate nutritional composition of the tested alga is presented in Table 2. Carbohydrates are essential components of primary metabolism, as they provide the energy needed for the development and other metabolic processes [35]. The estimated carbohydrates (31.24 %) and proteins (49.28 %) ratio was similar to that given in literature — 45.24 and 33.90 %, respectively [23]. However, protein content was lower than that in Moroccan *Spirulina* (76.65 %) [74]. Protein ratio is important information because the majority of plant-based foods, which are considered as acceptable protein sources, contain about 35 % DW [44].

The estimated lipid content (11.88 %) was lower than that in the same species from Alexandria water (16.45 and 15 %) [23, 33]. However, the variation in nutritional content (proteins, carbohydrates, vitamins, and lipids) between studies could be attributable to a variety of factors, including algal strains, cultural medium and conditions, and analytical method used [46].

The detection of calorie amounts in algal species is essential for recommendation it to use as a source of food or feed. It is well known that calorie value is related to food quality and can be estimated depending on the content of carbohydrates, proteins, and lipids. The calorie value was about 428.98

Table 2

Proximate and nutritional content of *A. platensis*

Biochemical components	Content
Carbohydrates, %	31.24±2.56
Proteins, %	49.28±3.43
Lipids, %	11.88±1.21
Crude fibers, %	3.01±0.52
Calorie (kcal/100 g)	428.98±0.87
Vit. C (mg ascorbic acid/100 g DW)	0.47±3.52
Vit. E (mg α-tocopherol equivalent/g DW)	6.79±1.98
β-carotene (pro-vitamin A) (mg/100 ml)	0.55±0.01
Lycopene (mg/100 ml)	7.56±1.28

(kcal/100 g). This value is similar to that reported by [74], which estimated about 436.18 (kcal/100 g) energy in Moroccan *Spirulina*.

The crude fiber ratio was 3.01±0.52 %, which correlates well with literature data [6, 74]. In other studied algal species it varied from 1.36 to 7.73 % [47].

Vitamins are nutritional substances required in trace amounts, but essential in maintaining human and animal health. The dried powder of *Spirulina* contains many vitamins like Vitamin E, which is a powerful antioxidant substance with anti-inflammatory activity [42]. The concentration of vitamin E in the tested species was higher (6.79±1.98 mg/g DW) than that (0.41 mg/g DW) determined by [44].

Vitamin C is an antioxidant substance and plays a crucial role in hormones and deoxyribonucleic acid biosynthesis. In addition, it protects the photosynthetic apparatus from oxygen free radicals and H_2O_2 formed during photosynthesis through the Mehler reaction. The determined vitamin C content of the tested species was lower than that determined by [4].

β-carotene (pro-vitamin A) and lycopene are the types of carotenoids with nutritional and antioxidant properties, which are used for chronic diseases prevention [57]. The concentration of lycopene was similar to that detected in other microalgae, including *Scenedesmus armatus* (7.08 mg/100 ml) [71]. At the same time, the detected content of β-carotene (0.547±0.01 mg/100 ml) was lower than that (57.38 ± 9.98 mg/100 ml) detected by [8].

Multi-minerals content

Minerals are essential nutrients, which are required in minute quantity by humans to preserve good health. While human bodies are unable to produce minerals, thus they are completely reliant on the food they consume. Among the analyzed minerals (Table 3), calcium and potassium represented a large content in *Arthrospira* biomass (13.51 and 8.35 mg/g DW, respectively). Comparing to other studies, the concentration of the tested minerals was similar or lower than that estimated by [23, 44]. It has been known that K, Na, Mg, and Ca

Table 3

Element content and estimated daily intake of the tested *A. platensis*

Elements	Concentration (mg/g DW)	EDI	International accepted daily intake
Mn	0.16	0.525	4.2
Cu	0.53	1.709	2
Fe	5.19	16.853	18
Zn	1.39	4.534	15
S	0.20	0.649	0.18—1.07
Ca	13.51	43.811	100
Mg	1.12	3.632	5
Na	2.98	9.660	2400
K	8.35	27.078	3500

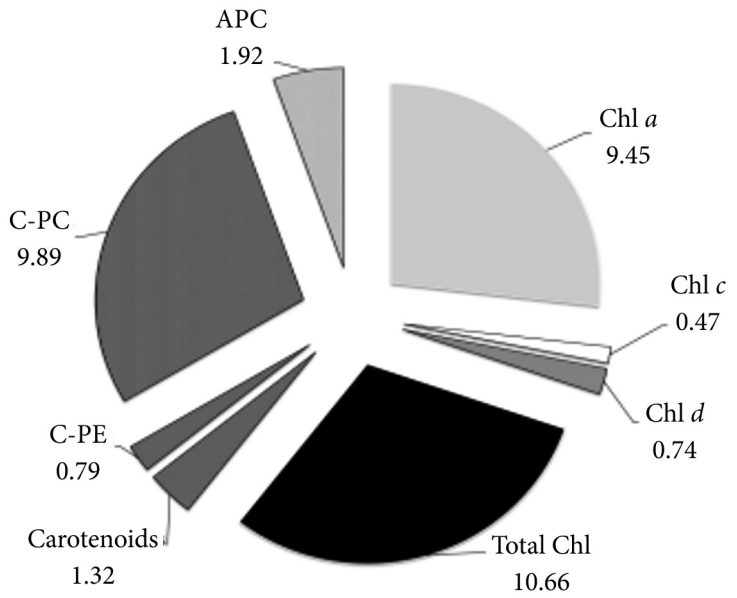

Fig. 1. Phytopigment composition of the tested alga (mg/g DW) with phycocyanin purity of 0.22 %

are essential minerals for mental and physical health [40]. The ratio of K/Na 2.83 is important for persons, who take diuretics to manage their blood pressure and suffer from excessive K excretion [35]. This variation may be related to environmental conditions, processing, and methods of mineralization [74]. As a result, the study demonstrates that the benefits of *Arthrospira* can be related to its mineral and trace element content. The estimated molar ratio was lower (1.74) than that on quotient range in human body (2.5—4.0), which suggests that the feeding on *Arthrospira* can decrease hypertension, preeclampsia, and heart disease.

Estimated daily intake

Estimated daily intake is one of the most important nutritional values, it reflects the safety of metal intake due to its direct effects on human health. Based on the BBC Health values, Food and Drug Administration (FDA), World Health Organization (WHO) [17, 35], the daily intake of the measured elements is lower than the international accepted daily intake, which confirms that this species is safe for human food and can be utilized as food supplements to help meet daily mineral and trace element needs.

Pigment content

Spirulina is known to be a good pigment source since it contains chlorophylls, carotenoids, and phycocyanin (Figure 1) belonging to nutritional compounds and greatly influencing its antioxidant effects [44]. Chlorophyll *a* and carotenoids content accounted for (9.45±1.12 and 1.32±0.13 mg/g DW, respectively) similar in the quantity obtained by [81]. The estimated Chl*c* (0.47±

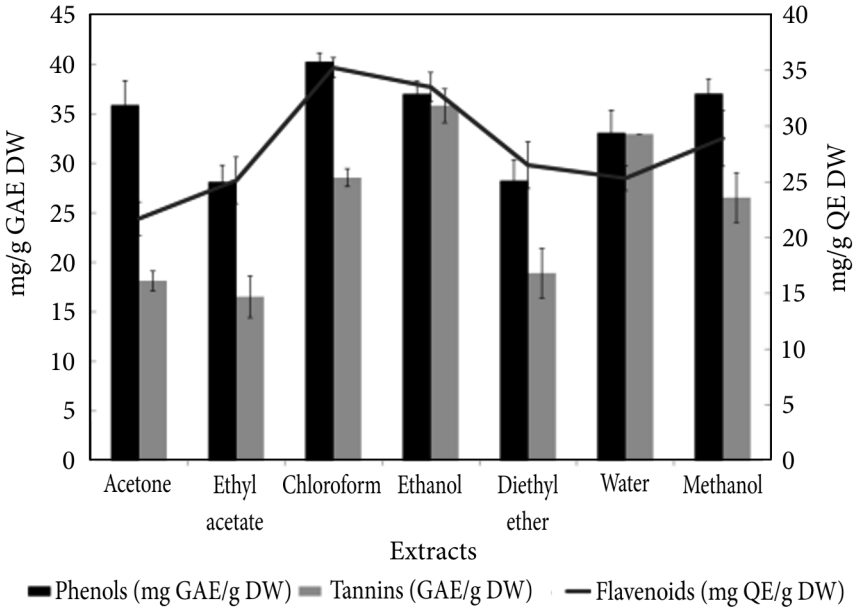

Fig. 2. Phytochemical content of *A. platensis* extracts

0.03 mg/g) and *d* (0.74±0.07 mg/g) content was higher than Chl*c* and *d* content registered in the edible *Spirulina* — 0.123 and 0.155 mg/g, respectively [58]. Within the class of phycobiliproteins, phycocyanin (C-PC) represented the highest content (9.89±1.15 mg/g DW) followed by allophycocyanin (APC) (1.92±0.68 mg/g DW) and phycoerythrin (C-PE) (0.79±0.03 mg/g DW). These accessory pigments have different economical applications. It is especially true of C-PC, which has the widest use as a natural dye in food due to its favorable antioxidant, anti-diabetic, and anti-inflammatory properties [65].

The purity level of the extracted phycocyanin is an essential property for a specific application (0.22), which lies in the food grade according to [69], which stated that phycocyanin preparations with A620/A280 lower than 0.7 are considered to be food grade, while those with A620/A280 between 0.7 and 3.9 are reagent grade, and those with A620/A280 greater than 4.0 are considered to be analytical grade, which is enough to satisfy the needs of a specific application.

Bioactive compounds content

Significant difference ($p<0.05$) was found in the content of the bioactive components (phenols, flavonoids, and tannins) extracted from *A. platensis* using different solvents (Figure 2). These variations depended on the polarity of the used solvent. The phenols extracted from the alga have been documented to have various useful activities, including anti-inflammatory, anti- allergic, antioxidant, vascular, cytotoxic antitumor activities, and enzyme inhibition [7, 34, 68]. Chloroform extract gave the best result overall along with the highest value of the total phenol and flavonoid content (40.25±3.41 mg GAE/g DW) and (35.23±2.23 mg QE/g DW) followed by ethanol (37.00±2.65 GAE/g DW)

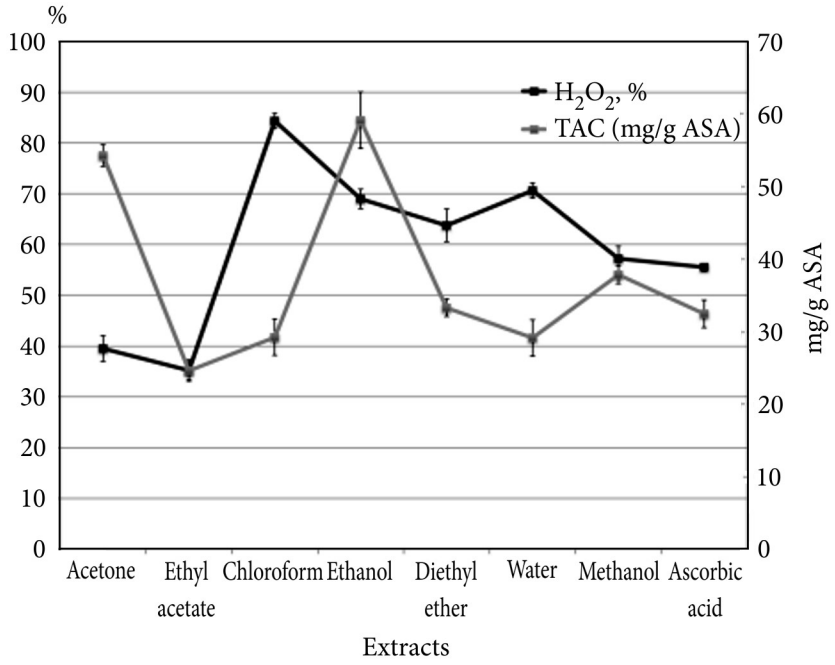

Fig. 3. Antioxidant activity of different *A. platensis* extracts

and (33.51±1.63 mg QE/g DW), respectively. Ethanol exhibited high efficiency in the extraction of tannins with 35.81 mg/g DW. By contrast, low concentrations of phenols, flavonoids, and tannins were obtained in the ethyl acetate extracts.

Biological activities

Several scientific investigations have shown that *Spirulina* species could be used in the treatment of different diseases due to synergetic impact of various phytochemicals in their cells. The present study was designed to compare the effects of different solvent types on the content of phytochemicals and their relevant antioxidant scavenging, anti-inflammatory, anti-arthritic, anti-diabetic, and anti-acetyl cholinesterase abilities.

Antioxidant activity

Two different methods were used to evaluate the antioxidant activity of *A. platensis* extracts like total antioxidant capacity and hydrogen peroxide scavenging assay. The difference in antioxidant efficiency based on the two methods might be due to the different mechanisms in scavenging radicals. *A. platensis* chloroform extract had the most efficient hydrogen peroxide radical scavenging capacity (84.33 %) as illustrated in Figure 3. While, ethanol extract exhibited a higher total antioxidant capacity (59.11 mg/g ASA equivalent/g extract) than other extracts and ascorbic acid (standard). Among the used solvents, ethyl acetate exhibited a lower antioxidant activity than ascorbic acid. It has been shown that chloroform extract of the plant *Vanda roxburghii* had a

strong scavenging activity against hydroxyl free radicals and DPPH compared to other extracts and catechin due to its phenol compounds [54].

As it is evident from Figure 3, positive correlation was established between the total antioxidant activity (TAC) and hydrogen peroxide radical scavenging activity and the total content of phenols ($r = 0.74$ and $r = 0.92$). Many publications confirm antioxidant properties of phenol compounds [7, 23, 32], their ability to be donors of hydrogen atoms or electrons and to capture free radicals [29]. Moreover, a high content of flavonoids and tannins was detected using both antioxidant techniques. It has been known that flavonoids react directly with free radicals, which results in their elimination [3]. It should be noted that antioxidant effect of the tested alga is not restricted to its phenol components, but also includes the presence of other antioxidant metabolites like carotenoids, phycobiliproteins, and vitamins that contribute significantly to the activity directly or indirectly [4, 48].

Anti-inflammatory and anti-arthritic activity

Inflammation is the first response of the immune system to infection and plays a pivotal role in various diseases. The highest anti-inflammatory and anti-arthritic activity (66.083±2.69 % and 96.051±1.87 %) was observed in ethanol extract of *A. platensis*, followed by water extract (64.850±1.54 % and 94.157±0.54 %), respectively (Table 4).

The obtained results indicate that the anti-inflammatory and anti-arthritic potentials significantly correlated with the content of tannins (r = 0.92 and 0.66) and flavonoids ($r = 0.71$ and 0.61). Tannins and flavonoids represent the complex of phenol substances possessing anti-inflammatory ability via controlling all indications of gastritis, esophagitis, enteritis, and other irritable bowel illnesses [13]. Significant relationship was established between H_2O_2 radical scavenging activity and both anti-inflammatory ($r = 0.88$) and anti-arthritic activity (r = 0.68) relating to the ability of antioxidant compounds to prevent various diseases like inflammatory disorders, tumor diseases, and neurological degenerations.

Table 4

Anti-arthritic and anti-inflammatory activity of *A. platensis* extracts, %

Extracts	Anti-arthritic	Anti-inflammatory
Acetone	50.587	46.484
Ethyl acetate	91.942	63.408
Chloroform	90.115	62.218
Ethanol	96.051	66.083
Diethyl ether	90.181	62.262
Water	94.157	64.850
Methanol	85.059	58.927
Standard	Diclofenac sodium 77.50	Quercetin 54.54

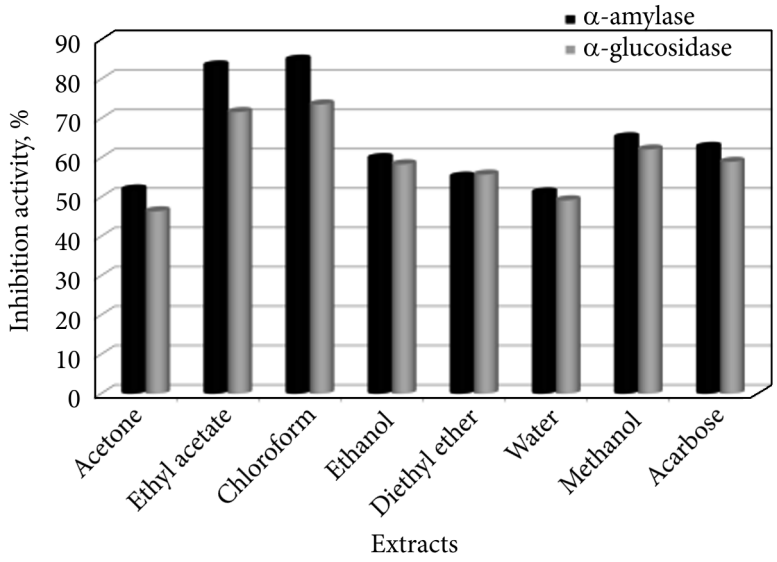

Fig. 4. α-amylase and α-glucosidase inhibitory activity of different *A. platensis* extracts

Anti-diabetic activity

The intensity of α-amylase and α-glucosidase activity inhibition by different types of *S. platensis* extracts varied (Figure 4). Chloroform extract inhibited α-amylase and α-glucosidase activity more intensively compared to other extracts with the maximum efficiency of 84.96 and 3.58 %. The minimum α-amylase (50.03 %) and α-glucosidase (42.40 %) inhibitory activity was recorded by acetone extract. The inhibition of α-amylase and α-glucosidase activity significantly correlated with the content of phenols ($r = 0.72$ and $r = 0.61$), flavonoids ($r = 0.95$ and $r = 0.90$), and also with hydrogen peroxide radical scavenging activity ($r = 0.75$ and $r = 0.77$) in all extracts. Recent publications [56] demonstrated the important role of antioxidant compounds of *Spirulina* in suppression of α-glucosidase and α-amylase enzymes via impairment of pancreas β-cells. It is thought [55] that the addition of antioxidant substances to dietary meals may be effective in governing diabetic complications. Other bioactive compounds of *Spirulina* cells like carotenoids also exhibited anti-diabetic activity. It has been shown that the risk of acquiring type 2 diabetes mellitus (T2DM) decreased in consuming carotenoids [78].

Cholinesterase inhibitory activity

The capability of the tested extracts of *A. platensis* to inhibit both AChE and BChE is shown in Figure 5. All tested extracts inhibited the activity of both enzymes to varying degrees depending on the used solvents. Chloroform extract exhibited the maximum inhibitory activity toward AChE (67.25 %) and BChE (75.91 %), which was closely similar to that of the reference standard donepezil and galantamine currently utilized as Alzheimer's disease (AD) drugs. The activity of ethanol extract was found to be moderate comparing with both

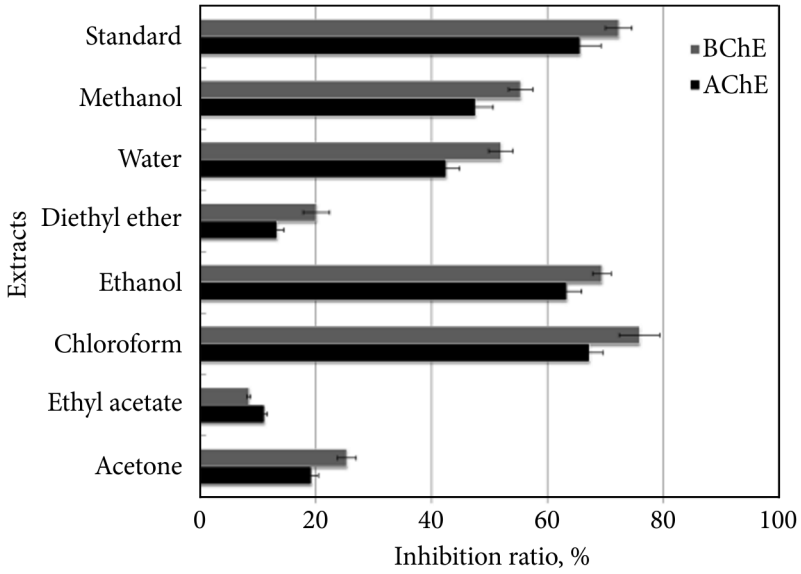

Fig. 5. Inhibition percentage of acetylcholinesterase and butyrylcholinesterase activity of the tested *A. platensis* extracts

standards. Due to the critical role of BChE in acetylcholine hydrolysis, dual inhibition of AChE and BChE may improve the signs and symptoms of Alzheimer's disease [54]. Both TAC and hydrogen peroxide radical scavenging activity showed significant correlation with both cholinesterase enzymes as it is evident from Table 5. Thus, relationship was established between TAC and H_2O_2 radical scavenging activity and AChE (r = 0.90 and 0.98) and BChE (r = 0.90 and 0.97), respectively. The content of phenols, flavonoids, and tannins significantly correlated with both activities (r = 0.8). This direct relationship may be related to their antioxidant activity improving mental disorders [43]. It has been shown [3] that phenols and flavonoids possess antioxidant properties improving neurodegenerative disorders like Alzeheimer's disease.

Significant relationship was established between both tested cholinesterase enzymes and anti-inflammatory activity (r = 0.81). The inflammation plays a crucial part in the etiology of Alzheimer's disease. Due to the capability of chloroform extract of *Spirulina* to scavenge free radicals and reduce inflammation and arthritis, it may be a viable candidate medicine in the treatment of Alzheimer's disease and thus deserves to be tested in an animal model.

GC-MS

GC-MS chromatogram of ethanol and chloroform extracts of *A. platensis* identified different compounds, which might be responsible for their tested biological activities. The retention time, molecular weight, molecular formula, peak area, and bioactive properties of these compounds are given in Table 6.

It has been found that ethanol and chloroform extracts contain fifteen and twelve compounds, respectively. The major bioactive constituents were phytol (19.08 % and 21.12 %) and hexadecanoic acid (12.84 % and 27.12 %) in both et-

Table 5

Matrix of simple linear correlation coefficient (*r*) between phytochemicals and the tested biological activities

	Phenols	Flavonoids	Tannins	Anti-arthritic	Anti-inflammatory	α-amylase	α-glucosidase	H_2O_2	TAC	AChE	BChE
Phenols	—										
Flavonoids	0.60	—									
Tannins	0.58	0.64	—								
Anti-arthritic	0.22	0.61	**0.66**	—							
Anti-inflammatory	0.38	**0.71**	**0.92**	**0.84**	—						
α-amylase	**0.72**	**0.95**	0.50	0.48	0.54	—					
α-glucosidase	**0.61**	**0.90**	0.48	**0.63**	**0.62**	**0.95**	—				
H_2O_2	**0.77**	**0.79**	**0.91**	**0.68**	**0.88**	**0.75**	**0.77**	—			
TAC	**0.80**	**0.70**	**0.88**	0.34	**0.69**	**0.64**	**0.49**	**0.85**	—		
AChE	**0.84**	**0.85**	**0.87**	0.62	**0.80**	**0.84**	**0.80**	**0.98**	**0.90**	—	
BChE	**0.83**	**0.85**	**0.87**	0.64	**0.81**	**0.83**	**0.79**	**0.97**	**0.90**	**0.99**	—

Note. The values of *r* significant at $p \leq 0.05$ are given in bold face.

Table 6

GC-MS analysis of ethanol and chloroform extracts of *A. platensis*

Bioactive compounds	Retention time	Ethanol extract	Chloroform extract	Molecular formula	Molecular weight	Biological activities
1-Butanol, 3-methyl-	3.680	3.61		$C_5H_{12}O$	88	Antioxidant and antimicrobial [24]
Benzene, 1,3-dimethyl-	5.390	17.02		C_8H_{10}	106	Antioxidant and anti-diabetic [83]
Decane	6.951	1.65		$C_{10}H_{22}$	142	Antioxidant and antimicrobial [64]
Undecane	8.110	2.79		$C_{11}H_{24}$	156	Antioxidant and antimicrobial [9]
Dodecane	9.187	2.6		$C_{12}H_{26}$	170	Antioxidant and ant-inflammatory [62]
3,5-*bis* (1,1-dimethylethyl)-phenol	13.220	4.52	7.04	$C_{14}H_{22}O$	206	Antioxidant and anti-inflammatory [51]
2,6-*bis* (1,1-dimethylethyl) phenol	13.290	5.25	4.7	$C_{14}H_{22}O$	206	Antioxidant and anti-inflammatory [51]
Heptadecane	13.600	3.96	9.34	$C_{17}H_{36}$	240	Various biological activities [38]
1-Hexacosene	15.273		1.77	$C_{26}H_{52}$	364	Antioxidant [18]
n-Hexadecanoic acid	15.524	12.84	27.12	$C_{16}H_{32}O_2$	256	Antioxidant, anti-inflammatory and anti-acetyl cholinesterase [1, 77]
Nonadecanoic acid, ethyl ester	15.717		1.03	$C_{21}H_{42}O_2$	326	Antimicrobial [15]

Table 6 (continued)

Bioactive compounds	Retention time	Ethanol extract	Chloroform extract	Molecular formula	Molecular weight	Biological activities
Cyclohexane, 1,5-diethyl-2,3-dimethyl-	16.462		9.02	$C_{12}H_{24}$	168	Antimicrobial and antioxidant [41]
11-Octadecenoic acid, methyl ester	16.606	1.15		$C_{19}H_{36}O_2$	296	Antioxidant and antimicrobial [49]
Phytol	16.750	19.08	21.12	$C_{20}H_{40}O$	296	Various biological activities [76]
cis-5-Dodecenoic acid	16.983		5.72	$C_{12}H_{22}O_2$	198	Various biological activities [31]
cis-10-Heptadecenoic acid	17.098	14.92		$C_{17}H_{32}O_2$	268	Antioxidant and antitumor [77]
1-Heptadecanol, acetate	17.220		1.86	$C_{19}H_{38}O_2$	298	Antioxidant [60]
Octadecanoic acid	17.232	4.91		$C_{18}H_{36}O_2$	284	Various biological activities [77]
2-Propenoic acid, pentadecyl ester	17.993		1.03	$C_{18}H_{34}O_2$	282	Various biological activities [75]
cis-11-Eicosenoic acid, methyl ester	18.762	1.18		$C_{21}H_{40}O_2$	324	Antioxidant and antitumor [83]
Cholestan-3-ol, 2-methylene-, (3β,5α)-	19.39	4.52	10.25	$C_{28}H_{48}O$	389	Antioxidant and anti-diabetic [52]

hanol and chloroform extracts. In addition, benzene, 1,3-dimethyl- (17.02 %) and cis-10-heptadecenoic acid (14.92 %) were found in ethanol extract.

All these compounds have been reported as antioxidant and anti-inflammatory agents like 3,5-*bis* (1,1-dimethylethyl)-phenol, 2,6-*bis* (1,1-dimethylethyl) phenol, heptadecane, and octadecanoic acid [51, 77]. In this case, n-hexadecanoic acid was detected in ethanol (12.84 %) and chloroform (27.12 %) extracts possessing anti-acetyl cholinesterase, antioxidant, and anti-inflammatory properties [1, 77].

Few of the detected compounds possessed anti-diabetic activities, including cholestan-3-ol, 2-methylene-, (3β,5α)- in both extracts and benzene, 1,3-dimethyl- in ethanol extract only [36, 52, 83].

Heptadecane was identified in ethanol and chloroform extracts (3.96 % and 9.34 %), which comprises high ratios of amino acids, vitamins, β-carotene, and pigments [36]. It is characterized by high biological activities, including antioxidant, anti-proliferative and antitumor efficiency [38].

Conclusion

The obtained results suggest that *Arthrospira platensis* is a promising antioxidant agent for food industry relating to its bioactive compounds, calorie content, ion quotient, and estimated daily intake values. Especially chloroform and ethanol extracts of *A. platensis* may replace the current synthetic drugs used in treating a variety of disorders such as inflammation, diabetes, etc. Further studies are needed to detect the mode of action of these biocompounds *in vivo* using different animal models to illustrate the exact action mechanism of these compounds.

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Mona M. Ismail,
National Institute of Oceanography and Fisheries (NIOF),
Cairo, Egypt
mona_es5@yahoo.com
orcid 0000-0001-7029-6802
Nadia H. Noaman,
Alexandria University,
Alexandria, Egypt

BIOCHEMICAL PROFILE, NUTRITIONAL VALUE, AND BIOLOGICAL ACTIVITIES OF *ARTHROSPIRA PLATENSIS* GOMONT

Arthrospira platensis (formerly *Spirulina platensis*) is a promising source of biological compounds since it has been traditionally used for the treatment of various diseases. The aim of the present study was to characterize the nutritional and biological properties of *A. platensis in vitro.* The detected calorie content (428.98 kcal/100 g) and phycocyanin purity (0.22 %) make it possible to recommend this species as an alternative source of healthy food to reduce obesity. The estimated daily intake of *A. platensis* was below the acceptable WHO/FAO level so it had no adverse impacts on human health. The antioxidant, anti-arthritic, anti-inflammatory, anti-diabetic, and anti-acetyl cholinesterase activities of seven different algal extracts were detected. Relationship was established between the estimated biological activities and phenol content. Chloroform and ethanol algal extracts exhibited different biological properties compared to standard drugs. On the whole, 21 bioactive compounds, including fatty acids, terpenoids, phenols, and alkanes, were revealed in algal extracts as a result of gas chromatography-mass spectrometry analysis. It can be concluded that *A. platensis* can serve as a very important potential source of many bioactive compounds with commercial impact depending on the used solvent. Further study should be done for isolating and purifying the effective compounds, which can be used in pharmaceutical and biological manufacturing.

Keywords: *Arthrospira platensis, biologically active substances, nutritional value, commercial use, medical application.*