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ORIGINAL RESEARCH ARTICLE

Antioxidant and Radical Scavenging Effect of Blue-Green Alga *Spirulina platensis*

R. Shanmugapriya and T.Ramanathan*

Centre of Advanced study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608502, Tamil Nadu, India

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ABSTRACT

The Cyanobacterium *Spirulina platensis* is rich in nutrients, such as proteins, vitamins, minerals, carbohydrates, and γ-linolenic acid. *Spirulina* or its extracts could prevent or inhibit cancer in humans and animals, and has immuno-promoting effects. Preliminary phytochemical screening of the *Spirulina platensis* showed the presence of large amounts of phenolics compounds and flavonoids. Quantification showed the presence of 0.71% (*m/m*) phenolics (calculated as gallic acid) and 0.24% (m/m) flavonoids calculated as catechin equivalents per 150 g of fresh mass. The presence of phenolic compounds prompted to carry out this work to evaluate its antioxidant activity. Methanolic extract of *Spirulina platensis* was screened to evaluate its highest antioxidant and free radical scavenging ability. These finding was observed at a concentration of 2000 mg mL^{-1}.

Keywords: *Spirulina platensis*, Cyanobacteria, Methanolic extract, Prokaryotes, Antioxidant activity, Free radical scavenging activity.

INTRODUCTION

The Cyanobacteria (blue-green algae) are the Gram negative photosynthetic prokaryotes found in almost all the ecological habitats. It represent a large group within the prokaryotic kingdom They are the oldest oxygenic photosynthetic organisms known so far and they also serve as a rich source of novel bioactive metabolites, including many cytotoxic, antifungal and antiviral compounds [1]. Cyanobacteria of the genus *Spirulina* have been studied not only because of the potential as a protein source but also because of their therapeutic properties, which include reports of the ability of preparations of this cyanobacterium to prevent and inhibit cancers, to decrease blood cholesterol levels, stimulate the immunological system, to reduce the nefrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effects of radiation [2]. Genus *Spirulina* has gained an importance and international demand for its high phytonutrients value and pigments which have applications in healthy foods, animal feed, therapeutics and diagnostics [3, 4]. *Spirulina* has been used as food and nutritional supplements since long time [5]. It is generally a rich source of vitamins, essential amino acids, minerals, essential fatty acids such as γ-linolenic acid and sulfolipid [6]. Moreover, in addition to ω-3 and ω-6- polyunsaturated fatty acids, it has also phytocyanin and other phytochemical [7]. The search for cyanobacteria with antimicrobial activity has gained importance in recent years due to growing worldwide concern about alarming increase in the rate of infection by antibiotic-resistant micro-organisms. Various active substances with antibacterial, antiviral, fungicide, enzyme inhibiting, immunosuppressive and cytotoxic and algicide activity have been isolated from cyanobacterial biomass [8-11].

Arthrospira platensis, previously known as *Spirulina platensis*, a blue green microalga, has been used since ancient times as a source of food because of its high nutritional value [5]. The Cyanobacterium *Spirulina platensis* is rich in nutrients, such as proteins, vitamins, minerals, carbohydrates, and γ-linolenic acid. It is gaining more and more attention, not only for the foods aspects but also for the development of potential pharmaceuticals [12]. Several studies have shown that *Spirulina* or its extracts could prevent or inhibit cancer in humans and animals, and recent works have indicated that this species has immuno-promoting effects [13, 14, and 15]. Antioxidants act as a major defense against radical mediated toxicity by protecting the damages

***Corresponding Author:** Dr.T.Ramanathan**, Email:** drtrcasmb@gmail.com, **Phone No:** +91- 9894175200

caused by free radicals. Antioxidant-based drugs/ formulations for the prevention and treatment of complex diseases, like atherosclerosis, stroke, diabetes and cancer, have appeared in the last three decades [16]. The aim of the present investigation was to evaluate in vitro antioxidant and free radical scavenging activity of the methanolic extract of *Spirulina platensis.*

MATERIALS AND METHODS

Chemicals

Chemicals used in this study were 1, 1 –diphenyl 1-2-picrylhydrazyl (DPPH) obtained from Sigma-Aldrich, India, NADH and sulphanilamide obtained from Himedia, Laboratories Pvt. Ltd., India, Folin-Ciocalteu reagent, potassium ferricyanide and sodium nitroprusside obtained from Qualigens Fine Chemicals, Glaxo Smithkline Pharmaceutical Ltd., India, naphthylethylenediamine dihydrochloride, N-1-naphthylethylenediamine dihydrochloride, sodium nitrate, trichloroacetic acid, butylated hydroxyl anisole (BHA), ascorbic acid, a-tocopheryl acetate, ethylenediamine tetraacetic acid, phosphoric acid, nitro blue tetrazolium, phenazine methosulfate, ferrous ammonium sulphate, DMSO are obtained from Sd Fine Chemicals Ltd, India. All reagents used in the study were of analytical grade.

Culturing method

Spirulina platensis was obtained from the Parangipettai, Cuddalore District, Tamil Nadu. The algal was cultivated on liquid Zarrouk medium [17]. Cultures were incubated at 25 ± 1°C, light intensity of 40 µE / m²/s (Cool white fluorescent lamps), photoperiod of 16/8 light, dark cycles, for 20 days after which algal cells were harvested by centrifugation at 10,000 rpm / 5 min, frozen by liquid nitrogen and stored at -20°C till use. Then, algal cells were dried at 100°C for 30 min and weighed [18].

Preparation of the Extract

Spirulina platensis (150 g) in powdered from were extracted with methanol using a Soxhlet assembly for 48 h, filtered and last traces of the solvent were evaporated under reduced pressure in a rotary evaporator. The yield was 3.15 g of dry extract.

Total Phenolic Content

The total phenolic content of *Spirulina platensis* methanolic extract was determined Spectrometrically [19]. Follin-Ciocalteu's reagent, 1 mL previously diluted with 20 mL distilled water, was added to 1 mL of sample (250 mg mL^{-1}) and mixed thoroughly. To the mixture, 4 mL of sodium carbonate (75 g L^{-1}) and 10 mL of water were added and mixed well. The mixture was allowed to stand for 2 h at room temperature. Contents were then centrifuged at 2000 g for 5 min and the absorbance of the supernatant was taken at 760 nm using a double beam spectrophotometer 2202 (Systronics, India). Astandard curve was obtained using various concentrations of gallic acid. Results were expressed as percentage of gallic acid equivalents (GAE) per 150 g fresh mass.

Total Flavonoid Assay

Total flavonoid contents were measured with the aluminium chloride colrimetric assay [20]. Methanolic leaves extracts or standard solution of catechin (500 mg mL^{-1}) was added to 10 mL volumetric flask containing 4 mL of water. To the above mixture, 0.3 mL of 5% $NaNO_2$ was added. 2 mL of 1 mol L^{-1} NaOH was added and the total volume was made up to 10 mL with water. The solution was mixed well and the absorbance was measured against a prepared reagent blank at 510 nm. Total flavonoid content of the leaves was expressed as percentage of catechin equivalent per 150 g fresh mass.

DPPH Free Radical Scavenging Activity

The free-radical scavenging activity of *Spirulina platensis* extract was measured by the decrease in absorbance of methanolic solution of DPPH [21]. A stock solution of DPPH (33mg L^{-1}) was prepared in methanol and 5 mL of this stock solution was added to 1 mL of *Spirulina platensis* extract solution was added to 1 mL of the *Spirulina platensis* extract solution at different concentrations (250, 500, 1000, 1500, 2000 mg mL^{-1}). After 30 min, absorbance was measured at 517 nm and compared with the standards, i.e., ascorbic acid, BHA and a-tocopherol (10-50 mg mL^{-1}). Scavenging activity was expressed as the percentage inhibition.

Hydroxyl Radical Scavenging Activity

Methanolic extract at different concentrations was placed in a test tube and evaporated to dryness. One mL of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA) 0.5 mL of 0.018% EDTA, 1mL of DMSO-0.85%, *V/V*, in 0.1 Ml^{-1} phosphate buffer, pH 7.4 and 0.5 mL of 0.22% ascorbic acid were added to each tube [22]. The tubes were capped tightly and heated in a water bath at 80-900C for 15 min. The reaction was terminated by adding 1 mL if ice-cold TCA (17.5% *m/V*). Three ml of Nash reagent (75.0 g ammonium acetate, 3 mL glacial acetic acid and 2mL acetyl acetone were mixed and water was

added to a total volume of 1 L) was added to each tube; the tubes were left at room temperature for 15 min for colour development. The intensity of the yellow colour formed was measured at 412 nm against a blank of the reagent. Percentage inhibition was determined by comparing the results of the test and standard compounds.

Nitric Oxide Scavenging Activity

Nitric oxide scavenging activity was measured Spectrophotometrically [23]. Sodium nitroprusside (5 mM L^{-1}) in phosphate buffered saline pH 7.4, was mixed with different concentrations of the extract (250-2000 mg mL^{-1}) prepared in methanol and incubated at 25^0 C for 30 min. A control without the test compound, but with an equivalent amount of methanol, was taken. After 30 min, 1.5 mL of the incubated solution was removed and diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was measured with reference to the standard.

Statistical Analysis

Values were represented as mean ± SD of three parallel measurement and data were analyzed using the t-test.

RESULTS

From the results on the total phenolics content, it was found that there were 0.79% of gallic acid equivalents of phenolic compounds while the total flavonoid content was 0.14% of catechin equivalent of fresh mass of *Spirulina platensis* extract. The results of antioxidant and free radical scavenging activity are given in (**Table 1**). The free radical scavenging activity was evaluated by using various in vitro assays. DPPH radical was used as a substrate to evaluate the free radical scavenging activity of *Spirulina* extract. The scavenging effect of *Spirulina platensis* extract on the DPPH radical was 90.11±1.1, at a concentration of 2000 mg mL^{-1} compared to the scavenging effects of ascorbic acid, BHA and a-tocopherol at 50 mg mL^{-1} of 91.14±1.1, 84.12±1.2 and 70.11±0.5 respectively.

DISCUSSION

Hydroxyl radicals are the major active oxygen species that cause lipid oxidation and enormous biological damage [24]. Tocopherols level was high (523 mg/kg oil) in the *Spirulina platensis* lipids, wherein α-tocopherol constituted ca. 73% of the total analytes, the rest being γ-tocopherol (ca. 27%). α-Tocopherol is the most efficient antioxidant of these compounds. β-Tocopherol has 25–50% of the antioxidative activity of α-tocopherol, γ-tocopherol 10–35% [25]. The percentage inhibition of nitric oxide generation by *Spirulina platensis* at 2000 mg mL^{-1} concentration was found to be 61.3 ±3.5%. On the other hand, ascorbic acid at 50 mg mL^{-1} concentration showed 84.2±3.1 inhibition of nitric oxide.

Table 1: Antioxidant profile of *Spirulina platensis* methanolic extract

Sample	Concentration	DPPH radical Scavenging (%)	Hydroxyl radical Scavenging (%)		Nitrite radical scavenging (%)	
Spirulina platensis	2000	90.11±1.1	79.3	2.3	61.3	3.5
Ascorbic acid	50	91.14±1.1	_	_	84.2	3.1
BHA	50	84.12±1.2	89.2	2.2	_	_
Tocopherol	50	70.11±0.5	_	_	_	_

A Mean ± SD, n = 3.

CONCLUSION

In conclusion, free radical scavenging effect of *Spirulina platensis* extract increases with increasing concentration and maximum antioxidant activity was observed at 2000 mg mL^{-1}. Antioxidant activity may be due to phenolic compounds in *Spirulina platensis* but further work should be carried out.

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