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IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF BLUE GREEN ALGAE *SPIRULINA PLATENSIS*

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ABSTRACT

The free radical scavenging potential of blue green algae *spirulina platensis* (Oscillatoriaceae) was studied by different antioxidant models. Free radicals are implicated for more than 80 diseases including Diabetes mellitus, arthritis, cancer, ageing etc. In the treatment of theses diseases, antioxidant therapy has gained an utmost importance. Current research is directed towards finding naturally occurring antioxidant of plant origin. In Indian system of medicine, blue green algae *spirulina platensis* is an important medicinal plant and it has been used in various ailments and as health tonic. To understand the mechanisms of pharmacological actions, the *in vitro* antioxidant activity of ethanolic extract of *spirulina platensis* was investigated for the activity of scavenging DPPH radical, nitric oxide radical. In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. These results clearly indicate that blue green algae *spirulina platensis* is effective against free radical mediated diseases. The data suggests that the extract contains compounds may be effectively utilized as a wide spectrum of antioxidant agent.

INTRODUCTION: Free- radical reactions have been implicated in the pathology of many human diseases/disease conditions like atherosclerosis, ischemic heart disease, aging process, aging process, inflammation, diabetes, immune - suppression, neurodegenerative disease etc.,[1-3]. Radicals and other reactive oxygen species are formed constantly in the human body and are removed by the enzymatic and non-enzymatic antioxidant defense [4]. The disturbance in 'redox homeostasis' occurring when antioxidant defenses are inadequate can damage lipids, proteins, carbohydrates and DNA. Drugs with multiple protective mechanisms, including antioxidant activity, may be one way of minimizing tissue injury [5]. In recent years, many studies evidenced that plants containing high content of antioxidant phytochemical can provide protection against various diseases [6]. In recent years the popularity of complementary medicine has increased. Dietary measures and traditional plant therapies as prescribed by ayurvedic and other indigenous systems of medicine are used commonly in India [7]. The World Health Organization has also recommended the evaluation of the plants effective in conditions where safe modern drugs are lacking [8].

The blue green algae *spirulina platensis* (Oscillatoriaceae) grows in fresh water in plank tonic form the major producer of the algae are USA, China, Thailand, Mexico and India. This group of algae is considered to be one of the remarkable groups of photosynthetic simple plank tonic forms. It represent link between green plants and bacteria. It has a soft cell wall made up of complex sugars and proteins and different from most algae, that it is easily digested.

Spirulina contains proteins (50-70%), protenious nitrogen (11.36%), total organic nitrogen (13.5%), nitrogen from nucleic acid (1.9%), it has net protein utilization (NPU) up to 62%. It contains lipids (5-6%) having mostly essential fatty acid (vitamin F), composed of oleic, linoleic, gamma linoleic, palmitic, palmitoleic, heptadecanoic acids. About 40% of the fats include glycolipids sulpholipids (2-5%) which have significant anti-HIV activity. Spirulina provides 8-14% of recommended daily allowance (RNA) of fats[9]. The *in vitro* antioxidant activity has not been reported for these algae. The objective of the present work to evaluate the *in vitro* antioxidant activity of algae *spirulina platensis* by different antioxidant models.

MATERIALS AND METHOD:

Plant materials: The algae was collected from the ezha edhilier maruvaazhvu kazhagam, veerappan sathiyrum, Erode, tamil nadu. The algae was identified and authenticated by joint director, botanical survey of india, southern circle, coimbatore. The filtered algae washed with tab water to remove the foreign matter. Gel form of algae was dried in the direct sun light. Then the dried algae were subjected to milling and powdered to get a coarse powder. The powdered mass was passed through sieve no 60 and used for extraction.

Preparation of extract: The powdered material (algae) was extracted with ethanol. The extract were concentrated under reduced pressure to get dry mass (yield 15.41% w/w respectively) the crude extract obtained were subjected to further studies.

Phytochemical screening: The ethanolic extract of algae *spirulina platensis* was subjected to phytochemical screening [10-11].

Antioxidant activity: The antioxidant activity was determined by using Diphenyl picryl hydrazyl (DPPH) radical scavenging, Nitric oxide (NO) radical scavenging [12-13].

1. **Dpph Radical Scavenging Activity**: The DPPH radical scavenging activity was measured by spectrophotometric method. 1mL of ethanolic solution of extract of various concentrations (25, 50, 75, 100 µg/mL) were mixed with 1mL of ethanolic solution of DPPH (200µM). Similarly 1mL ethanolic solutions of ascorbic acid (200 µg/mL) were mixed with 1mL of DPPH solution. A mixture of 1mL of ethanol and 1mL of ethanolic solution of DPPH (200 µM) served as control. After mixing, all the solutions were incubated in dark for 20 minutes and absorbance was measured at 517 nm. The experiments were performed in triplicate and percent scavenging activity was calculated as follows

$$\text{Scavenging \%} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2. **Nitric Oxide Radical Scavenging Activity:** The nitric oxide radical scavenging activity was measured by using Griess' reagent. 5mL each of extract solutions of different concentrations (25, 50, 75, 100 µg/mL) in standard phosphate buffer solution (pH 7.4) were incubated with 5mL of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4) at 25°C for 5 hours. In an identical manner 5mL of ascorbic acid solution (200 µg/mL) in standard phosphate buffer solution (pH 7.4) was also incubated with 5mL of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4). Control experiments without the test compound but with equivalent amount of buffer were also conducted. After incubation, 0.5mL of the incubation mixture was mixed with 0.5 mL of Griess' reagent (Sulphanilamide 1%, O-phosphoric acid 2% and naphthyl ethylene diamine dihydrochloride 0.1%) and the absorbance was measured at 546nm. From the absorbance the percent scavenging activity was calculated using the same formula as described above. The experiments were performed in triplicate.

RESULT AND DISCUSSION: The phytochemical screening of the ethanolic extract of algae *spirulina platensis* shows the presence of alkaloids, flavonoids, glycosides, tannins & phenolic compounds, steroids, proteins & amino acids and saponins. The result in **table 1** clearly demonstrates the *in vitro* antioxidant activity the ethanolic extract of algae *spirulina platensis* produced good antioxidant activity and was comparable with that of standard ascorbic acid.

TABLE 1: *IN-VITRO* EVALUATION OF ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF ALGAE *SPIRULINA PLATENSIS*

Treatment group	% inhibition (Mean ±SEM)	
	DPPH	NO
Algae *spirulina platensis* 25 µg/mL	10.30±0.80	1.84±0.44
Algae *spirulina platensis* 50 µg/mL	16.97±3.33	3.69±0.60
Algae *spirulina platensis* 75 µg/mL	17.27±1.05	9.72±0.17
Algae *spirulina platensis* 100 µg/mL	27.88±1.21	20.27±0.17
Ascorbic acid 200 µg/mL	87.57±1.98	94.97±1.90

Values represent the mean ± SEM; number of readings in each group = 3

CONCLUSION: Based on the results described, we may conclude that the ethanol extract of algae *spirulina platensis* posses significant free radical scavenging activity. The data suggests that the extract contains compounds may be effectively utilized as a wide spectrum of antioxidant agent. Further analysis including additional purification of extract and chemical characterization of isolated compounds, along with further antioxidant testing should be required for identification of compounds and possible mechanism of action of the above said activity.

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