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Antioxidant activity of methanolic extract of blue green algae *Anabaena* sp. (Nostocaceae)

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ABSTRACT

Blue green algae, Anabaena sp. (Nostocaceae), collected from rice field of Anandapuram, Visakhapatnam, AP, India was examined for antioxidant properties. Methanol extract was screened for the estimation of total phenolic content by Folin-Ciocalteau assay. Antioxidant potential of the extract was measured by 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay. Methanol extract exhibited 57.06 GAE mg/gm phenolic content and 48.62±0.29% DPPH radical scavenging activity at 100 µg/ml concentration with an IC_{50} value of 101.81 µg/ml. These results concluded that Anabaena sp. possess high antioxidant activity and can used for the isolation of antioxidant compounds.

Keywords: *Anabaena* sp.; Antioxidant; DPPH radical scavenging assay; phenolic compounds.

INTRODUCTION

Anabaena sp. is a filamentous cyanobacterium (blue-green algae) usually found in all kinds of water. *Anabaena* has uniseriate, straight, curved or coiled trichomes that may be constricted at the cell walls. The cells appears in spherical, ellipsoidal, cylindrical or bent shapes with blue-green to yellow-green colour because of phycocyanin pigment. *Anabaena* sp. is commonly known for its nitrogen fixing abilities [1]. In the recent years, *Anabaena* sp. has been reported for

some of the medicinal properties such as antimicrobial activity [2], antioxidant activity [3], antitumor activity [4], larvicidal activity [5, 6] etc.

Oxidation is a natural metabolic process in cell, which resulting in the formation of free radicals such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and ozone (O_3). Free radicals damage the cell membranes, proteins, fats, nucleic acid in the cells and cause several disorders such as cancer, Parkinson's disease, Alzheimer's disease and myocardial infarction [7, 8] etc. Some other factors such as cigarette smoke, automobile exhaust, radiation, pesticides and air pollution etc also resulting in to the formation of free radicals.

Antioxidant compounds are widely used compounds to counter the free radicals mediate oxidative stress in the cell [9]. These antioxidant compounds can be derived from natural and chemical sources. Natural sources are much safer to use due to less toxicity and side effects, so the production of antioxidant compound from the natural sources such as plants and algae is in great demand. Earlier a variety of algal species have been reported to possess antioxidant activity such as *Acanthophora spicifera, Cystoseira compressia, C. myrica, Sargassum furcatum, S. swartzii, Chlorella sp*, and *Colpomenia sinuosa* etc. [10-13]. This study was designed to investigate the total phenolic content and the DPPH radical scavenging activity of the *Anabaena* sp. collected from the rice field of Anandapuram village, Dist- Visakhapatnam, AP, India.

MATERIALS AND METHODS

Chemicals

Methanol, Folin- Ciocalteau reagent and Gallic acid were purchased from SRL (Mumbai, India). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). Sodium carbonate, ingredients of Chu 10 medium (ammonium heptamolybdate tetrahydrate, boric acid, calcium nitrate, cobaltous nitrate, copper sulphate, ferric chloride, potassium biphosphate, magnesium sulphate, manganese sulphate, sodium carbonate, sodium silicate, zinc sulphate) were purchased from Himedia Laboratories Pvt. Ltd. (Mumbai, India).

Collection of cyanobacterial strains

The filamentous and heterocystous cyanobacterial strains were collected from the rice field of Anandapuram Village, Dist- Visakhapatnam, AP, India during September 2009. The samples were collected in sterilized plastic bags and transferred to the ice box, immediately. The samples were brought to the Laboratory for further processing.

Isolation of *Anabaena* sp.

The cultures were isolated and purified by repeated plating on solid Chu 10 medium and colonies of different morphologies were identified according to morphological properties and pigment composition [14-19]. The microscopic structure was also observed.

Production of purified algal biomass

Pure culture was maintained by subculturing in 250 ml Erlenmeyer flasks containing 100 ml of sterile Chu 10 medium (liquid) and incubated under florescent light (3000 lux) at a temperature of 25 ± 1°C. The culture was harvested by centrifugation (4000 rpm for 15 minutes) after 15 days of inoculation.

Preparation of the extract

The algal biomass was harvested and washed thoroughly in sterilized distilled water. The algal biomass was shade dried at room temperature. Dried algal biomass was uniformly grinded using mechanical grinder to make fine powder. Ten gram of the powder was extracted in methanol using a Soxhlet apparatus. The extract was concentrated at 40°C under reduced pressure (72 mbar) with a rotary evaporator and dried using lyophilizer. Dried extract was collect in air tight container and stored at 4°C up for further use.

Estimation of total phenolic content

Total phenolic content of the methanol extract of filamentous cyanobacteria *Anabaena* sp. was determined using the Folin-Ciocalteau reagent method [20]. The crude methanol extracts were diluted in methanol to obtain different concentrations (125, 250, 500 and 1000 µg). 50 µl of each extract was mixed with 2.5 ml of Folin- Ciocalteau reagent (1/10 dilution in purified water) and 2 ml of 7.5% Na_2CO_3 (w/v in purified water). The mixture was incubated at 45°C for 15 min. The absorbance was measured at 765 nm. Na_2CO_3 solution (2 ml of 7.5% Na_2CO_3 in 2.55 ml of distilled water) was used as blank. The results were expressed as gallic acid equivalence in µg. Each experiment was performed in triplicates at each concentration.

DPPH radical scavenging activity

The DPPH radical scavenging activity was performed according to the method of Guha et al. 2010 [21]. The methanol extract of *Anabaena* sp. was diluted in distilled water to make 20, 40, 60, 80 and 100 µg/ml dilutions. Two millilitres of each dilution was mixed with 1 ml of DPPH solution (0.2 mM/ml in methanol) and mixed thoroughly. The mixture was incubated in dark at 20°C for 40 min. Absorbance was measured at 517 nm using UV–Vis spectrophotometer with methanol as blank. Each experiment was performed in triplicates at each concentration.

The percentage scavenging of DPPH by the extracts was calculated according to the following formula:

% DPPH Radical scavenging= [(Ac - At) / Ac] × 100
Here.
Ac is the absorbance of the control (DPPH),
At is the absorbance of test sample.

Statistical Analysis

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD). Results were analyzed statically by using Microsoft Excel 2007 (Roselle, IL, USA).

RESULTS AND DISCUSSION

Algae are found in soil as well as water (fresh and marine). Algae are a group of autotrophic organisms that synthesized there food by photosynthesis. Algae are a rich source of carbohydrates, protein, enzymes, fiber, vitamins and minerals etc. Algae offer a wide range of therapeutic possibilities such as antinociceptive, anti-inflammatory, antibacterial, hepatoprotective, antitumor, antioxidant, anti-proliferative, anticoagulant and nematicidal activity [13, 22-30].

In this study, the antioxidant potential of the blue green algae (*Anabaena* sp) was explored. The algal culture was collected from the rice field of Anandapuram Village, Dist- Visakhapatnam, AP, India. The culture was purified on Chu 10 medium (Figure 1A) and the microscopic structure was observed. The microscopic observation showed the presence of long chains of vegetative cell with the presence of heterocyst that confirms the presence of *Anabaena* sp. (Figure 1B).

Percentage yield

10 gm of dried algae (*Anabaena* sp.) powder was extracted in methanol to obtain the test extract. After drying the filtrate yielded 0.19 gm of extract that is 1.9% of the initial powder (10 gm).

Total phenolic content

Phenolic compounds are the major chemical with antioxidant potential. In the current study, total phenolic content of the methanol extract of *Anabaena* sp. was measured. Results are expressed as gallic acid exultance (GAE) in µg and reported in Figure 2. Phenolic content of the extract showed dose dependent increases.

Catechin, flavonols, flavonol, glycosides, phlorotannins and phenolic compounds have been reported from a variety of algae; among them phenolic compounds are the key compounds present in algae with high free radical scavenging activity. It has generated a great interest for the development of natural antioxidant compounds from the algae [31].

DPPH radical scavenging activity

Antioxidant potential of the methanol extract was measured by DPPH radical scavenging activity. The results are expressed as percentage inhibition of DPPH and reported in Figure 3. Methanol extract resulted in high DPPH radical scavenging activity with IC_{50} value of 101.81 µg/ml and the scavenging activity was found to be increasing with the dose. Earlier, the methanolic extract of *Anabaena* PCC 7119 and its fractions were reported to possess efficient antioxidant activity. The extracts also reported to prevent the oxidation in soybean oil [32]. Suhail et al (2011) reported the DPPH radical scavenging potential of the *A. variabilis.* The results showed that the methanolic extract of the *A. variabilis* resulted in to approximately 16% DPPH radical scavenging activity [3]. Medicinal plants also a valuable source of natural antioxidant compounds, earlier a variety of plants also reported for antioxidant activity such as *Syzigum cuminii, Crateva nurvula, Mentha piperita Asparagus racemosus* and *Camellia sinesis* etc [33-37].

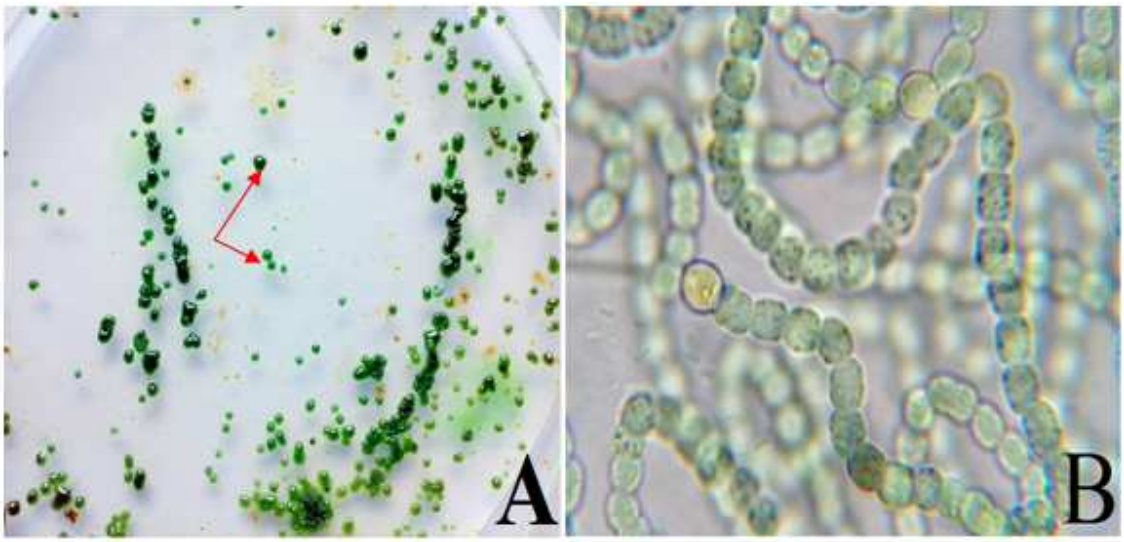

Figure 1: *Anabaena* sp. used during the study, A: Growth of *Anabaena* sp. on Chu 10 medium, B: microscopic structure of the purified *Anabaena* sp.

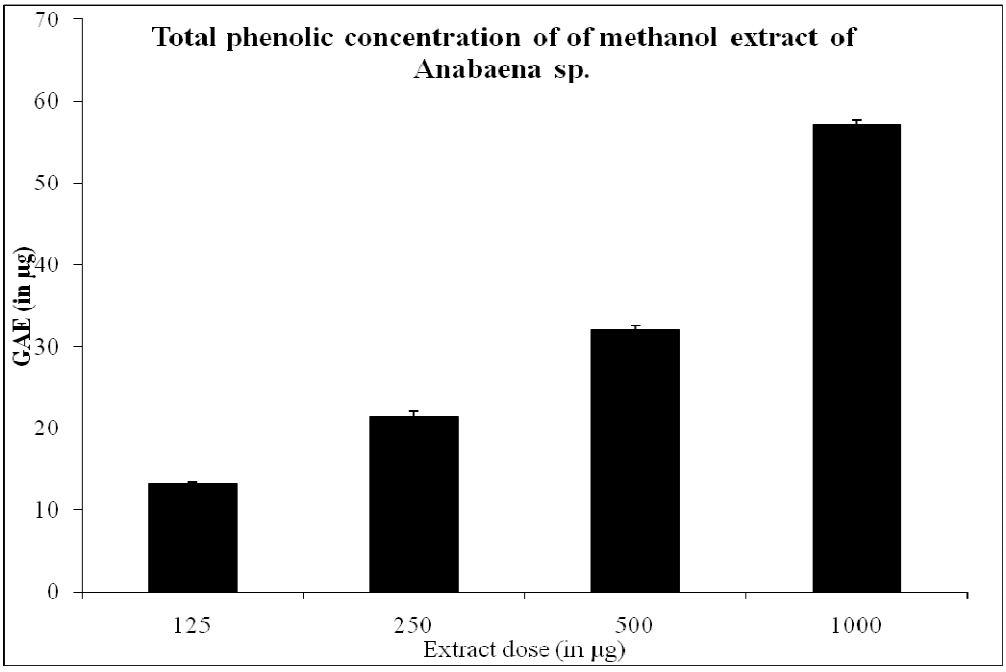

Figure 2: Total phenolic content in varying concentrations of methanol extract of *Anabaena* sp. Data is given in mean ± SD (n = 3) and expressed as Gallic acid equivalence (GAE) in µg.

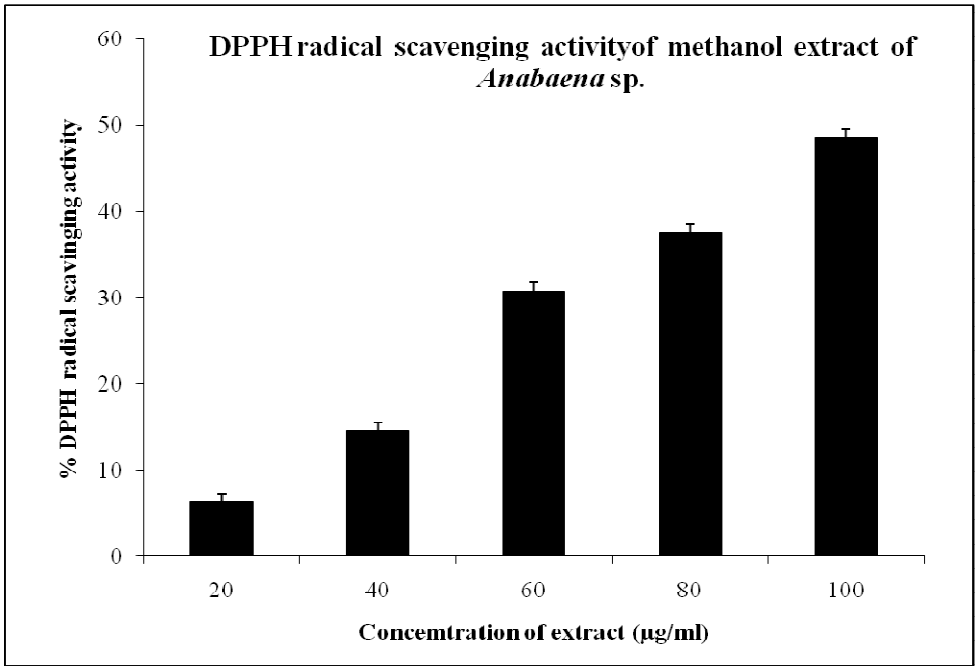

Figure 3: DPPH radical scavenging activity of the varying concentrations of methanol extract of *Anabaena* sp. Data is given in mean ± SD (n = 3)

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