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Research Article

EFFECT OF ELECTRIC TREATMENT ON TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *ANABAENA VARIABILIS*

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ABSTRACT

In this study, *Anabaena variabilis* was isolated from industrial effluent samples by repeated subculturing on Chu 10 medium. The algal biomass was exposed to electricity in controlled conditions for increasing the production of secondary metabolites. Further, this study also compares the total phenolic content and free radical scavenging activity of *A. variabilis* before and after the electric treatment. Methanol extracts of control and treated *A. variabilis* were screened for the estimation of total phenolic content by Folin-Ciocalteau assay. Antioxidant potential of the extract was estimated by 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay. Methanolic extract from the treated algae exhibited higher total phenolic content and free radical scavenging activity than that of control. Results emphasize the positive effect of electric treatment on the antioxidant potential of *A. variabilis*.

Keywords: *Anabaena variabilis*, Industrial effluent, Electricity treatment, Antioxidant activity.

INTRODUCTION

Free radicles *viz*, hydrogen peroxide, hypochlorous acid, hydroxyl radical, hydroxyl ion and superoxide anion are generated during the cellular respiration. They are highly reactive in nature and react with cellular biomolecules such as lipids, proteins and DNA, resulting in their damage [1]. Free radicals mediated damage of cellular biomolecules causes several physiological disorders such as cancer, diabetes, ischemo-reperfusion cardiac injury, neuro degenerative disorders such as, Alzheimer's disease, Parkinsonism and neurological conditions like epileptic seizures, stroke, brain damage and neuro trauma [2, 3, 4]. Smoking, radiation, pesticides, heavy metals, drugs and xenobiotics are some factors that enhance the production of free radicals [5].

An antioxidant is a substrate that when present in small amounts significantly prevents or delays the cell damage caused by free radicals. A potential antioxidant therapy could efectively protect the body from the free radicles mediated oxidative damage by neutralizing free radicals. The use of synthetic antioxidants has been decreased due to their suspected activity as carcinogens as well as general consumer rejection of synthetic food additives [6]. Currently there is a renewed interest in finding new anti-oxidants from natural sources such as plants, algae and microorganisms [7, 8, 9].

Prokaryotic organisms such as cyanobacteria exhibit a more diverse array of antioxidant compounds compard to most terrestrial plants. Carotenoids, Phycobilin pigments, Catechin, flavonols, glycosides, sulphated polysaccharides, vitamins, phlorotannins and phenolic compounds are some major classes of antioxidant compounds reported from a variety of algae [10].

Anabaena sp. is a photosynthetic, heterotrophic, filamentous cyanobacteria, having feature of both Gram positive and Gram negative bacteria, as it contains an outer membrane with lipopolysaccharides and also possesses a thick, highly cross linked peptidoglycan layer [11]. Various species of *Anabaena* sp. synthesize a variety of primary and secondary metabolites, many of them exhibit antibacterial [12, 13], antifungal [14], antiviral [15, 16], antioxidant [17], immunosuppresser [18] and antioncogenic activity [19]. Few species of *Anabaena* are used as biofertilizer for rice crops to increases nitrogen content [20, 21]. Phycoremediation is another important application of *Anabaena* sp. which is responsible for removal of heavy metals from the polluted water bodies and industrial effluents [22].

Magnetic field generated by the electricity can effect the biological processes in living system and could influence the synthesis of the metabolites, however uncontrolled treatment may lead to the cell damage as well. earlier many studies reported the beneficial efect of electric treatment on various crops and vegetables [23, 24]. In this study the filamentous cynobacteria *A. variabilis* was treated with electric current in controlled conditions to influence the production of metabolites.

The present study is an attempt to screen the effect of electric treatment in *A. variabilis* with regard to total phenolic content and antioxidant activity.

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 and 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 and 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 Visakhapatnam Steel Plant effluent, Dist-Visakhapatnam, AP, India during September 2010. 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 variabilis*

Technique of repeated plating on the solid chu 10 medium was used for isolation and purification of cultures. Identification based on morphological properties, pigment production and microscopic observation [25].

Electric shock treatment method

Cyanobacterial culture (5%, OD: 0.15 at 560 nm) in liquid Chu 10 medium was treated with 10 amperes of current for 80 mins by the means of electrophoresis power supply as reported earlier [26]. After treatment 1 ml of treated sample was transferred aseptically in a test tube containing 4 ml of Chu 10 medium. The tubes were incubated under ideal growth conditions for 15 days and further studied morphologically.

Mass production of purified algal biomass

Pure cultures (control and treated) were inoculated in 1000 ml Erlenmeyer flasks containing 500 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, followed by drying in hot air oven at 50°C. Dried algal biomass was uniformly grinded to make fine powder. Ten grams 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 naturally. Dried extract was collected in air tight container and stored at 4°C for further use.

Estimation of total phenolic content

Total phenolic content of the methanol extracts of *A. variabilis* (control and treated) were determined using the Folin-Ciocalteau reagent method [27]. The crude methanol extracts were diluted in methanol to obtain different concentrations (125, 250, 500 and 1000 µg). Fifty microliters 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% Sodium carbonate (w/v in purified water). The mixture was incubated at 45°C for 15 min in a water bath. The absorbance was measured at 765 nm. Sodium carbonate 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 micrograms. Each experiment was performed in triplicates at each concentration.

DPPH radical scavenging activity

The methanol extracts of *Anabaena variabilis* (control and treated) were diluted in distilled water to make 10, 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 [28].

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

Micro algae represent an alternative, safe and cost effective source of natural medicines. In past many algal species have been reported to possess various medicinal properties such as antimicrobial, anti-viral, antioxidant, anti-diabetic, hepatoprotective, anticoagulant andi anti-inflammatory activities [29-35].

In this study, *A. variabilis* cells were exposed to electric current in controlled conditions (10 amperes for 80 mins). Algal cell adapted to the environment and survived, however, exposure of the algal cells to electricity caused changes in the general morphology of the algae. Microscopic examination showed increase in frequency and size of heterocysts with compression to control. The treated cells were mass multiplied and processed for extract preparation and the crude extract was subjected to antioxidant activity. Earlier many terrestrial plants also reported to possess antioxidant activity against a variety of free radicals [36-37].

Percentage yield of extract

Dry algal biomass (control and test) was extracted in methanol to get extract. Electricity treated algal biomass yielded more than that of control biomass; however the difference in yield was not very high. Control algal biomass yield 1.43 % of the extract, whereas test algal biomass yielded 1.6% of extract.

Total phenolic concentration

Total phenolic content of methanol extracts of *A. variabilis* (control and electricity treated) were measured by Folin-Ciocalteau reagent method. Results are expressed as gallic acid exultance (GAE) in µg as a response of three replicates (mean±sd). Total phenolic content in varying concentrations of control and treated extracts are graphically represented in Figure 1. Both extracts exhibited presence of high content of phenolic compounds. However, electrically treated sample exhibited higher amount of phenolic content than that of control sample. Phenolic content of both extracts showed dose dependent increases. Electricity treatment of *A. variabilis* in controlled conditions may lead to the expression of high quantity of phenolic compounds, which could be utilized for the development of natural antioxidant compounds.

Phenolic compound are one of the most abundant class of phytochemicals present in algae. Presence of phenolic compounds in any medicinal preparation may attribute to various medicinal properties, earlier many algae have been reported to possess a variety of phenolic compounds with antioxidant and hepatoprotective properties [38, 39].

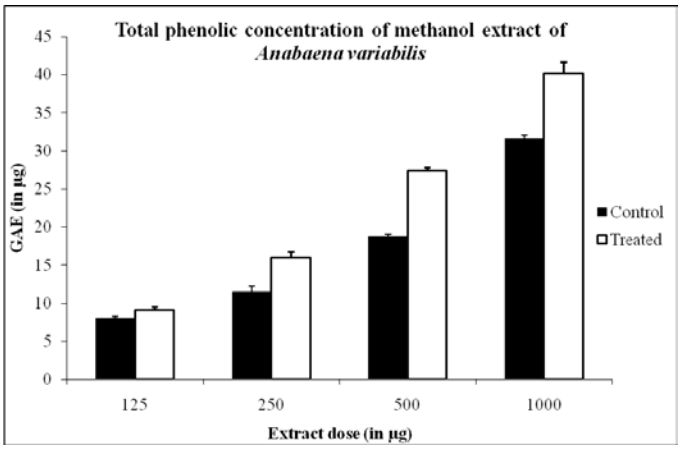

Fig. 1: Total phenolic content in varying concentrations of methanol extract of *Anabaena variabilis* (control and treated). Data is given in mean ± SD (n = 3) and expressed as Gallic acid equivalence (GAE) in µg.

DPPH radical scavenging activity

Antioxidant potential of the methanol extracts of *A. variabilis* (control and electricity treated) were measured by DPPH radical scavenging activity. The results are expressed as percentage inhibition of DPPH based on a response of three replicates (mean±sd) and reported in Figure 3. DPPH radical scavenging activity of varying concentrations of control and treated extracts are graphically represented in Figure 2. Both extracts exhibited high DPPH radical scavenging activity. However, electrically treated sample exhibited higher DPPH radical scavenging activity (IC_{50}= 107.53) than that of control (IC_{50}=116.95). Both extracts exhibited dose dependent increases in the DPPH radical scavenging activity.

Earlier, methanol extract of *Anabaena variabilis* was reported to possess DPPH radical scavenging activity. At 50 µg/ml concentration of the extract resulted in approximately 15% scavenging activity which is quite low when compared to our observations [40]. Antioxidant potential of the sample may vary according to the solvent choice, season of sample collection, geographical location where the sample was grown and stress factors present at the sample location.

In our study algal sample was collected from the industrial effluent of a steel plant. Presence of metals and other chemicals in effluent may enhance the production of certain secondary metabolites in order to protect the algae.

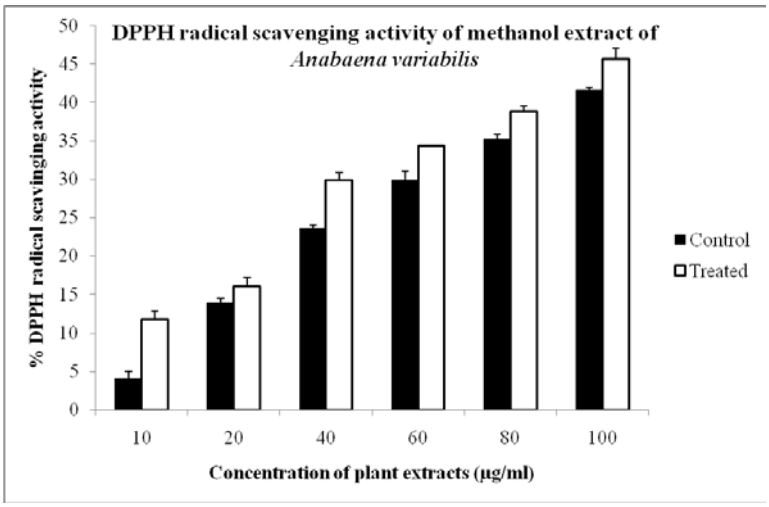

Fig. 2: DPPH radical scavenging activity of the varying concentrations of methanol extract of *Anabaena variabilis* (control and treated). Data is given in mean ± SD (n = 3)

CONCLUSIONS

Results of this study exhibited positive impact of electric treatment of *Anabaena variabilis* for the production of phenolic compounds and antioxidant activity. The methanol extract of the electric treated algal biomass showed higher amount of total phenolic content and free radical scavenging activity. We conclude that electric treatment can be utilized as a physical method to alter the production of specific metabolites; however more sophisticated studies are required before establishing the method.

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