Antioxidant activity of *Synechococcus* sp. Nägeli isolated from Cochin estuary, India

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In the present study, the strain *Synechococcus* sp. was isolated from the Cochin backwaters and its antioxidant activity was determined by *in vitro* antioxidant assays. Methanol fraction exhibited the highest phenolic content 44.33±0.433 µg GAE. Antioxidant capacity was found to be 83.63±0.50 µg AE. Ferric reducing antioxidant power and 2, 2 diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity was found to be concentration dependent. The de-oxy ribose free radical was scavenged at 22%. Methanol and dimethyl sulphoxide fractions were found to be effective for the extraction of antioxidant principle present in the strain.

**Keywords:** *Synechococcus* sp., phenolic content, antioxidant activity, ferric reducing antioxidant power, DPPH radical scavenging activity, de-oxyribose radical scavenging activity

**Introduction**

Cyanobacteria have turned out to be gold mines for obtaining novel compounds. Researchers have been carried out worldwide to employ cyanobacteria in various fields such as aquaculture, food, nutraceutical and pharmaceutical industries. Studies report that they have antimicrobial, antiviral, anticancerous, enzyme-inhibiting, immunomodulating, antioxidant activities etc. Commercially employed synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) cause side effects such as damage to nucleic acids. This may lead to carcinogenic conditions in the biological organism and are environmentally unfriendly. Natural antioxidants are safe and eco-friendly. In this context, the cyanobacteria which are primitive photoautotrophs have mechanism within them such as phenolics, pigments, vitamins, flavonoids etc to neutralize the effects of free radicals. The present study is an attempt to evaluate the antioxidant potential of *Synechococcus* sp. isolated from the Cochin estuary using various *in vitro* antioxidant assays.

**Materials and Methods**

*Synechococcus* sp. Nägeli, 1849 was isolated from Cochin estuary (9º 55’ 52” N 76º 16’ 22” E) by nutrient enrichment method followed by quadrant streaking on agar. Strain was identified morphologically and was subjected to purification procedures. The uni algal strain thus obtained was maintained in SN medium at 22±2°C with a light exposure of 21µmoles/m²/s photons in the marine algal culture laboratory of the Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology.

The cyanobacterium was harvested in the late logarithmic phase and the biomass was lyophilized. Approximately 100 mg of the biomass was extracted with 10 ml of five different solvents, acetone, chloroform and methanol (2:1), dimethyl sulphoxide (DMSO), ethanol and methanol at room temperature for 30 minutes followed by centrifugation at 10,000 rpm for 10 minutes. Supernatants were collected and the process was repeated twice. The supernatants were finally pooled and stored at -20°C for the antioxidant assays.

The phenolic content was estimated by Folin-ciocalteau method of Singleton and Rossi (1965). 300 µl of the extracts was treated with 1ml of Folin ciocalteau reagent (1:10). After 4 minutes of incubation, 0.8 ml of saturated sodium carbonate (7.5% w/v) was added and mixed well. The reaction mixture was kept undisturbed for 2 hrs. Absorbance was measured at 765 nm in a Hitachi U3900-spectrophotometer. Total phenolic content was expressed in terms of gallic acid equivalence (GAE) in microgram.
sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Sample was mixed well and was incubated for 90 minutes at 95°C in a water bath. Absorbance was measured at 695 nm. Results were expressed in terms of ascorbic acid equivalence (AE).

The reducing power assay was done according to Oyaizu14 (1986). 2.50 ml of 0.2 M phosphate buffer (pH 6.6) and potassium ferricyanide (1%) were added to the extracts (100-300 µl) and incubated at 50°C for 20 minutes followed by addition of 2.50 ml of 10% trichloroacetic acid and centrifuged at 1500 rpm for 10 minutes. 2.50 ml of solution was removed from the upper layer and was mixed with an equal volume of distilled water. 0.50 ml of ferric chloride solution (0.1%) was added and the absorbance of the reaction mixture was measured at 700 nm. The protocol of Yen and Chen15 (1995) was followed for the assay. 3.0 ml of 0.16 mM 2, 2-diphenyl-1-picryl hydrazyl (DPPH) prepared in ethanol was added to the extracts and incubated for 30 minutes at room temperature in dark. The absorbance of the sample was measured at 517 nm. The scavenging effect was calculated by the following formula:

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\text{% Scavenging effect} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100
\]

The deoxyribose radical scavenging activity was determined by the method described by Chung16 et al. (1997). To 1.0 ml of the sample 1.0 ml of FeSO₄·7H₂O(10 mM), 0.1 ml EDTA(10 mM) and 0.1 ml H₂O₂ (10 mM) was added and mixed well. 0.9 ml of phosphate buffer (0.1 M, 7.4 pH) and 0.1 ml H₂O₂ (10 mM) was added and incubated at 37°C for 4 hrs in dark. 0.5 ml of trichloroacetic acid (2.8%) and 0.5 ml of tertiary butyl alcohol (1%) were added and incubated in a boiling water bath for 10 minutes. Absorbance was measured at 532 nm. The scavenging ability was calculated as per Heo17 et al. (2005).

All the experiments were conducted in triplicates. The results were given as mean value with standard deviations. To determine whether there were any significant differences in the results of antioxidant activity depending on the solvents used, analysis of variance (uni variate) was done using SPSS 16.0 software package for windows. p < 0.05 were considered as significantly different.

**Results and Discussion**

Phenolic compounds are an integral part of the antioxidant defense mechanism of microalgae (Goiris18 et al., 2015). The solvents affect the phenolic extraction from the strains (Lopez19 et al., 2011). In the present study highest phenolic content was obtained in the methanol fraction 44.33±0.433µg GAE followed by ethanol and DMSO fractions 37.73±0.244 and 34.36±1.005µg GAE respectively (Fig. 1). Least phenolic content was obtained in the chloroform:methanol fraction 10.51±0.22 µg GAE. There was significant difference in the amount of phenolic content (p< 0.05) depending on the solvent used. Li20 et al. (2007) reported that the cyanobacteria *Synechococcus* sp. FACHB 283 and *Nostoc ellipsosporum* CCAP 1453/17 possessed high antioxidant activities and predicted that the strains could be potential candidates for source of natural antioxidants. Hajimahmoodi8 et al. (2010) observed that the phenolic compounds contributed significantly to the antioxidant properties of microalgae. Miranda6 et al. (1998) analysed the phenolic content in the methanolic extract of *Spirulina maxima* and found that the strain prevented oxidation both in vivo and in vitro systems. Goiris18 et al. (2015) suggested that growing the microalgae in nutrient replete conditions can increase the yield of phenolic antioxidants and carotenoids in the biomass.

The ability of the test strains to reduce the phosphomolydenum (Mo⁶⁺) to phosphate/ Mo⁵⁺ is measured in the assay. The DMSO extract of *Synechococcus* sp. exhibited the highest antioxidant activity of 83.63±0.503µg AE, and the methanolic extract showed 69.8±1.562 µg AE (Fig. 2). Karunamoorthy11 et al. (2012) determined the antioxidant activity of *Chlorella marina* and found that the diethyl ether extract showed highest activity than methanolic and hexane extracts. Ganesan21 et al. (2008) evaluated the antioxidant activity of crude methanolic as well as different fractions of red sea...
weeds, the ethyl acetate fraction of *Acanthophora spicifera* showed maximum activity whereas petroleum ether fraction of *Gracilaria edulis* and *Euchema kappaphycus* showed better activity than other fractions. The solvents used for the extraction affects the antioxidant activity of the strains.

Ferric reducing power is measured on the basis of the ability of the sample to reduce ferric (III) to ferrous (II) in a redox-linked colorimetric reaction. Reductones present are able to break the free radical chain reaction by donating hydrogen atoms (Gordon, 1990). Reducing ability is indicated by increase in absorbance value. The DMSO extract and methanol extract of the test strain had the highest reducing power (Fig. 3). Least reducing activity was observed for the chloroform:methanol fraction. The reducing power of all the algal extracts increased with increasing concentration, which means that the assay is concentration dependent. Earlier reports on the reducing power of seaweeds by Ganesan et al. (2008) and Kuda et al. (2005) indicate the same.

DPPH is extensively used to assess the free radical scavenging activity of compounds. Methanol extract of *Synechococcus* sp. had the strongest free radical scavenging potential, 55.83%±2.066 (Fig. 4). The DMSO, ethanol and acetone extracts of the strain also exhibited good free radical scavenging potential. Stronger scavenging activity at higher concentrations of the extracts clearly indicated the dose dependency. The concentration dependency of the antioxidant activity has been proven in the case of many seaweeds and higher plants (Wang et al., 2009, Kuda et al., 2005, Kumaran and Karunakaran, 2007). Athukorala et al. (2006) analysed the scavenging activity of *Ecklonia cava* which was higher than commercial antioxidants and the assay was concentration dependent. In another study by Maadane et al. (2015) the ethanolic extracts of microalgae exhibited higher DPPH free radical scavenging activity than the aqueous extracts.

The hydroxyl radical generated in the assay was neutralized by the DMSO fraction at 22.85%±0.250. Both ethanol and methanol fractions had similar activity (Fig. 5). According to Karunamoorthy et al. (2012) the hexane extracts of *C. marina* exhibited higher deoxyribose scavenging potential than the methanol extracts. Ganesan et al. (2008) reported higher scavenging activity in the Indian red seaweeds...
but Heo\textsuperscript{17} \textit{et al.} (2005) reported lower activity from enzymatic extract of brown seaweeds. The scavenging activity may be influenced by the solvent used for the extraction.

**Conclusion**

The present study indicates that the test strain has strong antioxidant activity. Free radical scavenging activity could be well utilized in the food industry as well as in the aquaculture field. Previous reports suggest that altering the nutrient conditions of the strains improves their antioxidant potential. The results also reveal that the solvent used for the extraction has significant influence on the antioxidant activity.

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**References**