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Antioxidant activity of cyanobacterium *Nostoc spongiaeforme* C.Agardh *ex* Born. & Flah.

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Abstract

The phytochemicals and pigments present in cyanobacteria act as antioxidants. In the present study *Nostoc spongiaeforme* possess higher amount of phenolic content. The DPPH assay showed that scavenging activity increases with increase in concentration of DPPH. The *Nostoc spongiaeforme* possess significant antioxidant activity with high phenolic content, phycobiliprotein content and ability to scavenge free radicals and shows that it is used as a potential source of antioxidants.

Key words: Cyanobacteria, antioxidant, phenolic content, Phycobiliproteins, DPPH.

INTRODUCTION

Cyanobacteria are a group of gram negative, photosynthetic and nitrogen fixing organisms. They evolved during protozoic era and consisting of nearly 2000 species. They are cosmopolitan in distribution, found in aquatic, terrestrial habitat and even in extreme and inhospitable places like glaciers, desert and hot springs. Some members show symbiotic association with algae, fungi, bryophytes, pteridophytes, gymnosperms and angiosperms. They are morphologically and physiologically diverse organisms, showed wide range of organization from unicellular forms to colonial forms.

There is world-wide curiosity in finding novel and non- toxic antioxidants from natural sources such as plants, algae and microorganisms (Pant et al. 2011). Antioxidants are the substances that reduce damage due to oxygen, such as caused by free radicals. Cyanobacteria have a highly evolved antioxidant system that catalyzes the harmful oxy radicals produced during photosynthesis (Padmapriya and Anand 2010). The phytonutrients and pigments present in cyanobacteria act as antioxidants. The antioxidant activity of cyanobacteria is a co- responsibility of pigments such as phycobiliproteins, carotenes, phenolic compounds and other oxidative substances present in the cells.

MATERIALS AND METHODS

The fresh water cyanobacterium *N. spongiaeforme* was used for antioxidant t studies. The sample was collected from fresh water bodies of Sholayar , Kerala state, India . The collected sample was deposited in Cyanobacterial Culture Collection, Department of Botany, University of Calicut for isolation, purification and identification purposes. It was cultured in BG-11 medium (Rippka et al. 1979) and the isolation of cyanobacterial strain was done by repeated sub culturing on agar plate by streak plate method. Inoculated plates were kept in cyanobacterial culture room under white fluorescent tubes (2500 Lux) at 25±2^{o}C, 16/18 light/dark photoperiod fo r 1 - 2 weeks. Visibly distinct cyanobacterial colonies were re-inoculated for further purification, the pure cultures were maintained in BG-11 medium.

Preparation of extract

About 30 days old cyanobacterial strain grown in BG-11 medium were harvested and used for preparation of cyanobacterial extract. The biomass was dried at a temperature of 60^{0}C, weighed and homogenized in 90% methanol. The homogenate was centrifuged at 5000 rpm for 15 minutes at 4^{0}C. The supernatant was used for estimation of phenolic compounds and antioxidant assays.

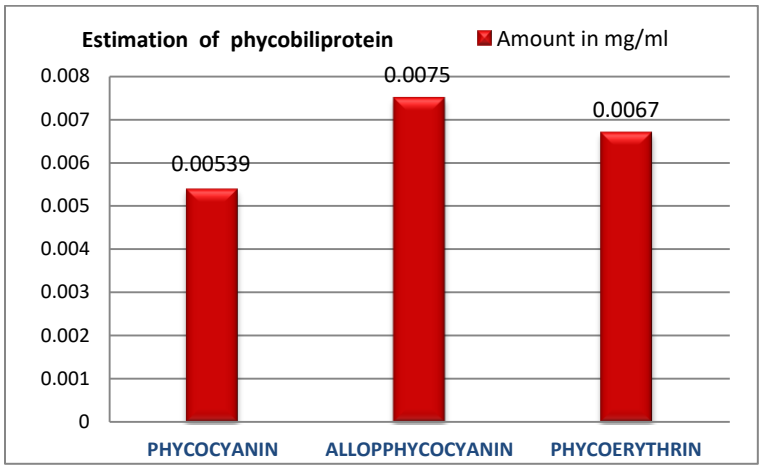
Phycobiliproteins were estimated as per the protocol described by Bennett and Bogorad (1973). The phenolic content was estimated according to Folin-ciocalteu method. The free radical scavenging activity of cyanobacteria was determined according to the method described by Shimada *et al.* 2010 (DPPH assay).

RESULT AND DISCUSSION

Cyanobacteria are well adapted to different environmental conditions. The phytochemicals and pigments present in it act as potential antioxidants. There are many reports on evolution of antioxidant activity of cyanobacterial genus *Nostoc.* The antioxidative activity of cyanobacteria is a co- responsibility of phycobiliproteins, phenolic compounds and other antioxidative substances present in the cells.

Estimation of phycobiliprotein

Phycobiliproteins are water soluble pigments and have been described as strong antioxidants (Bhat and Madyastha 2001; Estrada *et al*. 2001; Ge *et al.* 2006). The result of present study showed that allophycocyanin was major pigment in the *N . spongiaeforme* followed by phycoerythrin and phycocyanin. All the three pigments possess the ability to quench the hydroxyl radicals, superoxide and alkoxy radicals. The amount of the phycocyanin, allophycocyanin and phycoerythrin were presented in graph I. Several previous works revealed that C- Phycocyanin act as a strong antioxidant. Allophycocyanin is the main component in cyanobacteria and their antioxidant property was evaluated, (Ge *et al.* 2006). Phycocyanin and allophycocyanin are major phycobiliproteins in *Oscillatoria limosa* and *Scytonema aquatilis* whereas phycoerythrin is major one in *Scytonema elongatus*, (Rajisha mol *et al*. 2016).

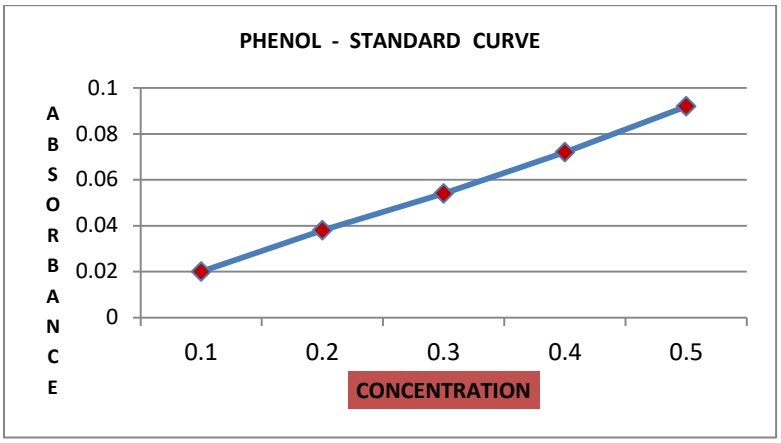


Graph I

Estimation of phenol

Phenolic compounds are known to be powerful chain breaking antioxidants. They may contribute directly to antioxidative action. They are one of the most abundant classes of phytochemicals and are potential antioxidants as they scavenge singlet oxygen, super oxides and peroxyl radicals (Shanab *et al.* 2011). The total phenolic content would be used as a basis for rapid screening of antioxidant activity. The phenol reacts with phosphomolybidic acid in Folin-ciocalteau reagent to form a blue colour. In the present study phenolic content of the sample (Methanolic extract) was estimated and the standard curve of phenol estimation was shown in the graph II. It showed that *N. spongiaeforme* express antioxidant activity. They possess high amount of phenolic compounds. Several studies showed a high correlation between antioxidative activity and phenolic content (Demirel *et al*. 2011; Sivakumar *et al.* 2011). So that the antioxidative nature of methanol extract of *N. spongiaeforme* might depends on its phenolic content. The values of total phenolic content showed dose dependent increase. *Oscillatoria limosa* showed the highest phenolic content.

Antioxidative nature of methanol extract of *Scytonema elongatus, O. limosa and Scytonema aquatilis* might depend on its phenolics (Rajisha mol *et al.* 2016).



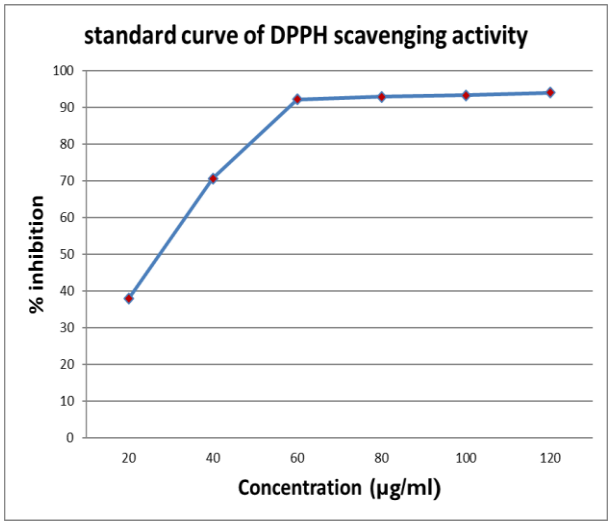
Graph II

DPPH Assay

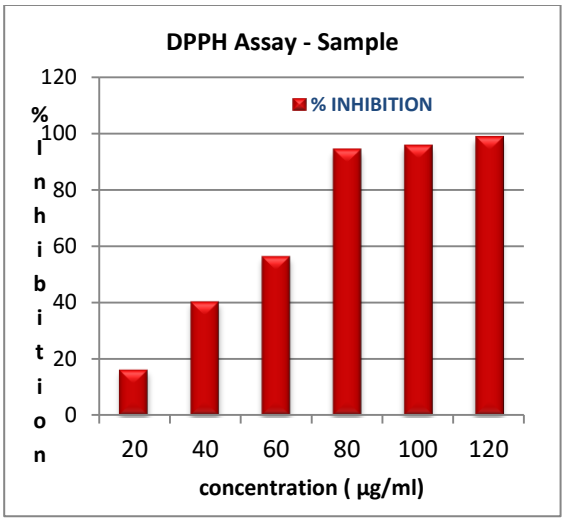
The radical scavenging activity of *N. spongiaeforme* extract was determined by DPPH method. DPPH is a stable nitrogen centered radical that is widely used to test the free radical scavenging activity of sample. Antioxidants in the sample reduced DPPH* radical (violet colour) to DPPH-H (yellowish colour) and that indicated the potential to be a hydrogen atom donor. The effects on antioxidants on DPPH are thought to be due to their hydrogen donating ability (Shirwaikar *et al*. 2006).

The result of DPPH assay was expressed as % inhibition of DPPH reported in the graph III and IV. The graph III showed the standard curve for DPPH scavenging activity in which ascorbic acid was used as standard. The reduction capacity of DPPH radical was estimated by increase in its absorbance at 517 nm, which is induced by antioxidants present in the extract. The absorbance value obtained for ascorbic acid at different concentrations were found to be decreasing with concentration, also it was lower than the absorbance value of control and the colour change from violet to yellow. The graph IV shows the dose dependent curve of DPPH scavenging activity of methanolic extract of *N. spongiaeforme*. The absorbance of sample at different concentrations was found to be decreasing with increasing concentration. The scavenging activity recorded was found to be increasing with concentrations. The highest value (98.65 %) was obtained at higher concentration (120µg).

The result showed that antioxidant activity is concentration dependent and the colour change from violet to yellow was also observed. This indicates that methanolic extract of *N. spongiaeforme* as a potential hydrogen atom donor and it possess antioxidant activity. In the present study the scavenging activity of *N. spongiaeforme* extract increases with concentration and the colour changes from violet to yellow indicates the proton donating ability. The antioxidant potential of methanolic extract of different cyanobacteria was determined by Shazia *et al.* (2011), among the extract of different cyanobacteria, *Plectonema boryanum* and *Scytonema* sp exhibit greater antioxidant activity as it was 30% and 27% inhibition of DPPH than positive control ascorbic acid (25%) at 50 mg/ml. Likewise in the present study the scavenging activity was found to be increases with concentration. Shirwaikar *et al*. (2006) studied the scavenging activity of methanol extract of *Oscillatoria terebriformis* and the result showed that at a concentration of 250 µg, the scavenging activity of methanol extract of *O. terebriformis* was 58.1 %. The result of DPPH scavenging activity in present study proved that the methanolic extract of *N. spongiaeforme* contained hydrogen atom donating ability and could be served as a free radical inhibitor or scavenger, acting possibly as a primary antioxidant.



Graph III



Graph IV

CONCLUSION

The antioxidant activity of cyanobacteria is a co-responsibility of phycobiliproteins, phenolic compounds and other antioxidant substances present in the cell. The present study indicated that the tested cyanobacterium *N. spongiaeforme* possess significant antioxidantt activity with high phenolic content, phycobiliprotein content and ability to scavenge free radicals. Thus the methanolic extract of *N. spongiaeforme* is a significant source of antioxidants, which might be helpful in preventing the progress of variouss oxidative stresses and used as a natural source of antioxidants for cosmetics and pharmaceuticals.

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