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Research Article

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Antioxidant potential of eight species of cyanobacteria isolated from Arabian Sea coast of Karnataka

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ABSTRACT

In the present study, eight species of marine cyanobacteria species namely, Lyngbya confervoides, Nostoc commune, Oscillatoria fremyii, O. geminata, O. sancta, Phormidium corium, P. tenue and Spirulina major were evaluated for their antioxidant activities by in vitro assays. The cyanobacterium, Oscillatoria fremyii (209.49 µmol AAE/g extract) exhibited high total antioxidant activity. Low value was found in Phormidium corium (45.20 µmol AAE/g extract). The radical scavenging capacity, reducing power capacity and chelating activity of the culture extracts varied from 2.29 to 8.62 µmol GAE/g extract, 6.21 to 44.67 µmol GAE/g extract and 2.15 to 30.29 µmol EDTAE/g extract, respectively. The study illustrates that methanol extracts of cyanobcateria isolates have effective antioxidant activities and could be used as natural sources of antioxidants.

Keywords: Cyanobacteria; Arabian Sea; Antioxidant activity; DPPH radical scavenging activity

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INTRODUCTION

Marine phytoplankton represent an almost untapped resource of natural antioxidants, due to their enormous biodiversity, much more diverse than higher plants [1-4]. They are photoautotrophic organisms that are exposed to high oxygen and radical stresses and consequently have developed defiance system against photo-oxidative damage by oxidative mechanisms to detoxify and eliminate highly reactive oxygen species [5]. They exhibit antioxidant capacities by different mechanisms such as scavenging, reducing and chelating of free radical ions [2, 4, 6].

Cyanobacteria are one of the important group of marine phytoplankton. They synthesize diverse of novel biologically active compounds with potential for pharmaceutical applications [2, 7-10]. Several studies have been carried out on the antioxidant activities of cyanobacterium *Spirulina* [11-16]. Nowdays, synthetic antioxidants such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) are commonly used. As these components are suspected as promoters of carcinogenesis [17, 18]. Recent reserachers have been interested in finding novel antioxidants from natural sources to prevent reactive oxygen species mediated diseases [19]. Thus, the present study was carried out to investigate *in vitro* antioxidant activities of eight species of marine cyanobacteria isolated from Arabian Sea coast of Karnataka (West coast of India).

EXPERIMENTAL SECTION

Cultivation of cyanobacteria

The eight species of cyanobacteria namely, *Lyngbya confervoides*, *Nostoc commune*, *Oscillatoria fremyii*, *O. geminata*, *O. sancta*, *Phormidium corium*, *P. tenue* and *Spirulina major* were isolated from rocks surfaces, puddles and filtered surface sea water of Arabian Sea coast, Karnataka. The cyanobactria samples were initially washed with sterilized sea water in the Petri plate to remove the impurities. The samples were diluted and single filament was isolated under the microscope by using micropipette, inoculated into the sterilized natural sea water f/2 culture media and incubated under 1000 lux illumination at 28±2˚C with 8 : 16 h light and dark regime [20]. After a period of 10 to 14 days of incubation,

cultures were microscopically examined for the assessment of growth and contamination. The successful axenic cultures were diluted, subcultured and used for the study.

Preparation of extracts

Cyanobacteria cultures in stationary growth phase were harvested by centrifugation at 3000 rpm for 10 min and washed in physiological saline (0.85% NaCl). The biomass was dried at 60˚C to reach a fixed weight and extracted with methanol. The extracts were dried under reduced pressure and preserved at 4˚C for further investigation.

Total antioxidant activity

The antioxidant activity of methanol extracts was determined by Phosphomolybdenum method [21]. Hundred microlitres of sample was mixed with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95˚C for 90 min under water bath. Absorbance was measured at 695 nm and result was expressed as µmol ascorbic acid equivalent (AAE)/g dry weight.

DPPH radical scavenging activity

The free radical scavenging activity was determined by DPPH scavenging assay [22]. Aliquotes of 100 µl methanolic extract and 4 ml of freshly prepared 0.1 mM DPPH (1,1-Diphenyl-2-picrylhydrazyl) methanolic solutions were thoroughly mixed and kept for 20 min in the dark. The decrease of absorbance at 517 nm was read and expressed as µmol gallic acid equivalent (GAE)/g dry weight.

Ferric reducing power capacity

The reducing power of the extracts was determined by ferric reducing power assay [23]. One ml of extract was mixed with 2.5 ml of 0.2 M phosphate buffer and 2.5 ml of 1% potassium ferricyanide. The reaction mixture was incubated at 50˚C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. One ml of supernatant was mixed with 1 ml of distilled water and 0.2 ml of 0.1% ferric chloride. Absorbance at 700 nm was measured and expressed as µmol gallic acid equivalent (GAE)/g dry weight.

Ferrous ion chelating capacity

The chelating activity was determined by ferrous ion chelating assay [22]. One ml of extract was mixed with 0.1 ml of 2 mM ferrous chloride tetrahydrate and 0.2 ml of 5 mM ferrozine. Volume in all tubes made up to 5 ml with methanol and reacted for 10 min. Absorbance was read at 562 nm and expressed as µmol EDTA equivalent (EDTAE)/g dry weight.

RESULTS AND DISCUSSION

Marine cyanobacteria have gained a lot of attention in recent years because of their ability to synthesize variety of bioactive compounds [10]. There is an increasing interest in the use of cyanobacteria as natural antioxidant source [24]. In the presents study, cyanobacteria isolates showed diverse antioxidant activities (Fig. 1). The cyanobacterium, *Oscillatoria fremyii* (209.49 µmol AAE/g extract) exhibited high total antioxidant activity followed by *Lyngbya confervoides* (187.16 µmol AAE/g extract), *Oscillatoria geminata* (148.06 µmol AAE/g extract), *Nostoc commune* (118.99 µmol AAE/g extract), *Phormidium tenue* (96.61 µmol AAE/g extract), *Spirulina major* (73.88 µmol AAE/g extract), *Oscillatoria sancta* (56.45 µmol AAE/g extract) and *Phormidium corium* (45.20 µmol AAE/g extract), respectively.

There are many reports on the evaluation of antioxidant activity of cyanobacteria species belonging to genera *Spirulina* [1], *Phormidium* [9], *Nostoc* [25] and *Oscillatoria* [26]. Their studies concluded that several cyanobactreria genera contain potent antioxidants, both from lipophilic and hydrophilic nature. They exhibit antioxidant capacities by different mechanisms such as scavenging, reducing and chelating of free radical ions [6, 24, 27].

Antioxidants in the extracts of cyanobacteria isolates reduced DPPH radical to DPPH-H which indicated the potential to be an H-atom donor [28]. The radical scavenging capacity of the culture extracts varied between 2.29 µmol GAE/g extract in *Oscillatoria sancta* and 8.62 µmol GAE/g extract in *Oscillatoria fremyii* (Fig. 2). These values are comparable with the values reported by Li et al. [25] for radical scavenging capacity of 23 species of phytoplankton which were analyzed by Trolox equivalent antioxidant capacity (TEAC) assay and values ranged from 1.33 to 29.56 µmol trolox/g. The high radical scavenging capacity was obtained for phytoplankton species evaluated by Goiris et al. [4] which ranged from 4.55 to 69.40 µmol trolox/g. The study conducted by Natrah et al. [6] showed that species of *Oscillatoria* were inactive in the DPPH radical scavenging assay, but the same species exhibited radical scavenging activity in the present study.

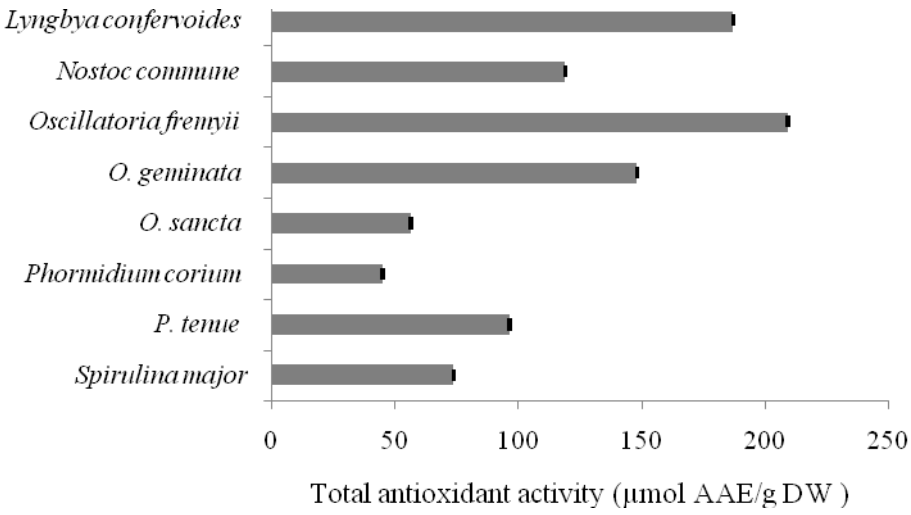

Fig. 1: Total antioxidant activity (µmol AAE/g DW) of cyanobacteria extracts

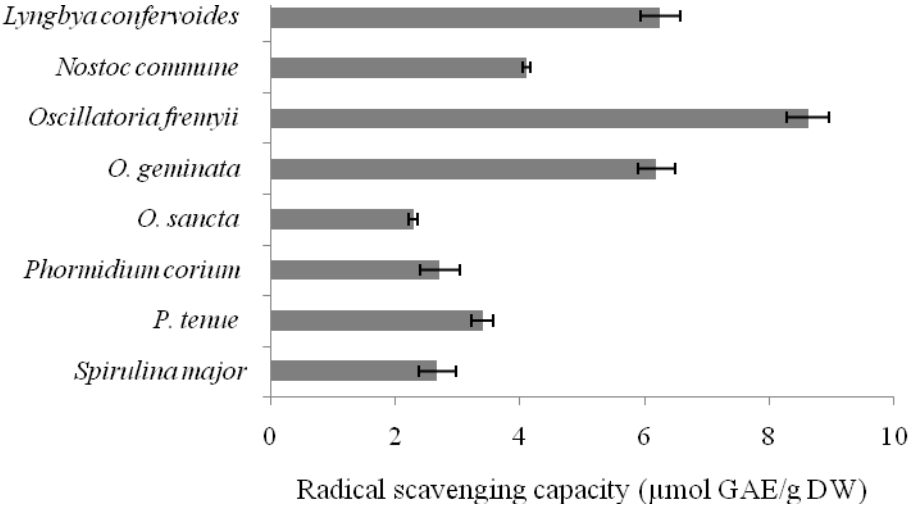

Fig. 2: DPPH radical scavenging activity (µmol GAE/g DW) of cyanobacteria extracts

The DPPH radical scavenging activity of four thermophilic cyanobacteria namely, *Phormidium* sp. PD40-1, *Leptolyngbya* sp. KC45, *Scytonema* sp. TP40 and *Cyanosarcina* sp. SK40 was reported by Pumas et al. [9] and values were 6.16, 7.44, 3.20 and 2.36 mg GAE/g dry weight, respectively. Hajimahmoodi et al. [3] evaluated the radical scavenging potential in cell masses and extracellular substances of 12 phytoplankton species by DPPH-HPLC assay and expressed in per cent of radical scavenging activity which varied between 0.64% and 113.23% in extracellular substances of *Chroococcus dispersus* and *Chlorella vulgaris*, respectively.

Reducing power is an ability to reduce or give electrons to free radicals and change them into the stabilized form, which can halt the free radical chain reaction [23]. The reducing power capacity of isolates determined by ferric reducing antioxidant assay [23]. In this method, the extracts donate an electron to $Fe^{3+}(CN^-)_6$ and changes it to be $Fe^{2+}(CN^-)_6$. In the present study, the reducing power capacity of isolates ranged from 6.21 to 44.67 µmol GAE/g extract (Fig. 3). These values are similar to the values reported by Hajimahmoodi et al. [3] and Goiris et al. [4] for different species of phytoplankton. The reducing power capacity of 12 phytoplankton species analyzed by Hajimahmoodi et al. [3] varied from 1.33 to 74.34 µmol trolox/g, whereas that of 32 species of phytoplankton evaluated by Goiris et al. [4] varied from 3.30 to 89.70 µmol trolox/g.

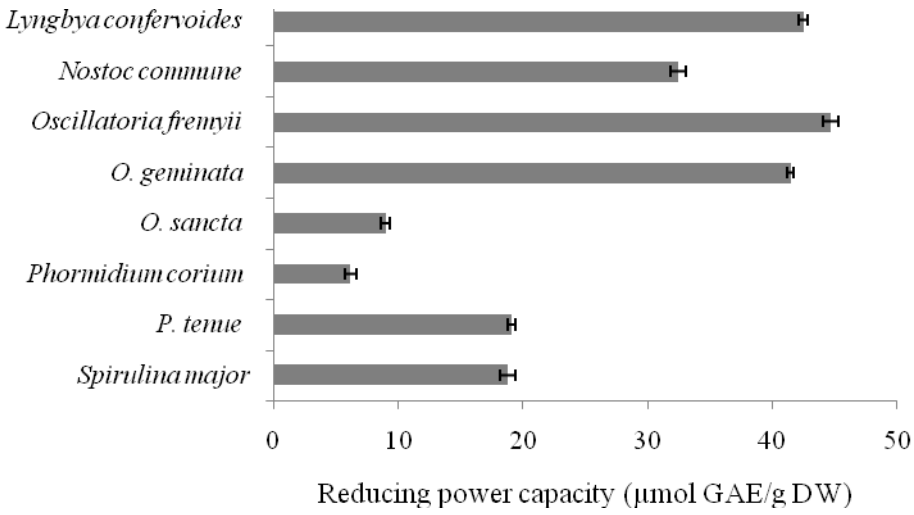

Fig. 3: Ferric reducing power capacity (µmol GAE/g DW) of cyanobacteria extracts

In the present study, *Oscillatoria fremyii* (44.67 µmol GAE/g), *Lyngbya confervoides* (42.51 µmol GAE/g), *Oscillatoria geminata* (41.45 µmol GAE/g), *Nostoc commune* (32.44 µmol GAE/g), *Phormidium tenue* (19.06 µmol GAE/g) and *Spirulina major* (18.81 µmol GAE/g) showed significant reducing power capacity. These values are comparable with the values obtained for reducing power capacity in other species of cyanobacteria namely, *Anabaena cylindrica*, *Chroococcus dispersus*, *Fischerella ambigua*, *F. musicola*, *Microchaete tenera*, *Nostoc ellipsosporum*, *N. muscorum*, *N. piscinale* and *Tolypothrix tenuis* [3].

The radical scavenging activity and reducing power capacity were proved that the crude extracts of cyanobacteria contained both the electron and hydrogen atom donating ability. The extracts containing antioxidants such as phenolic compounds are able to donate hydrogen atom to the free radical thus stopping the propagation chain reaction during lipid oxidation process [29]. It is known that prevention of the chain initiation step by scavenging various reactive species such as free radicals is considered to be important mode of action [30].

The ability to chelate off the ferrous ion is important to avoid a Fenton reaction, which would produce the most harmful radical, the hydroxyl radical. The production of the hydroxyl radical is a key initial step to producing other harmful radicals, which should be avoided. The chelating activity of cyanobacteria extracts shown in Fig. 4.

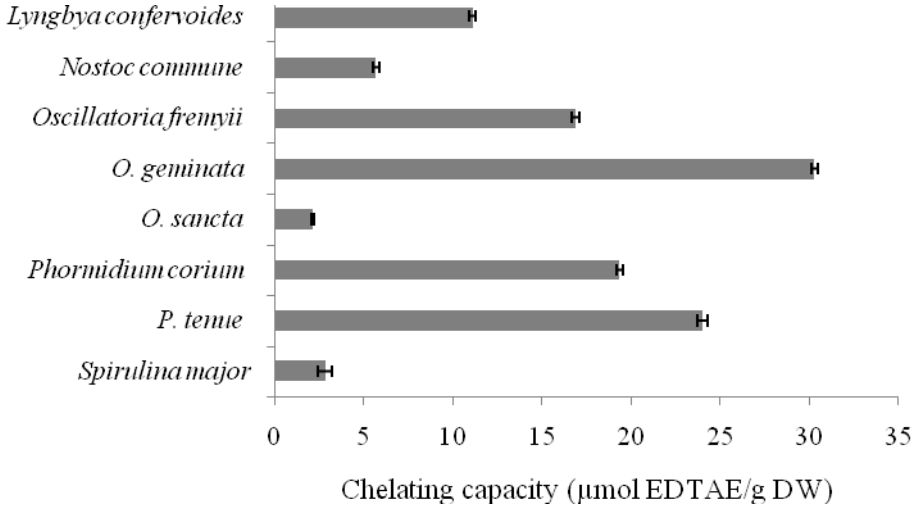

Fig. 4: Ferrous ion chelating capacity (µmol EDTAE/g DW) of cyanobacteria extracts

In the study, all isolates exhibited the chelating ability in which cyanobacterium, *Oscillatoria geminata* showed high chelating ability (30.29 µmol EDTAE/g extract). Goh et al. [2] reported that if most compounds found in the extract contained the protonated form, higher the chelating ability. This suggested that most compounds found in the extract of

Oscillatoria geminata contained the protonated form, thus with high chelating ability, which was found least in *Oscillatoria sancta*.

CONCLUSION

The present study concluded that cyanobacteria isolates contain potent antioxidants, which exhibit antioxidant activities by different mechanisms such as scavenging, reducing and chelating of free radical ions. Hence, they can be used as natural antioxidants source for cosmetics and nutraceuticals. The characterization of these antioxidant compounds give a clear picture about their biological activity. Further investigations need to be carried out on the *in vivo* activity of these cyanobacteria extracts.

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