Isolation of *oscillatoria* from fresh waterbodies of Mysuru city, characterization of bioactives: Relevance to antioxidant therapy.

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Abstract:

Cyanobacteria are among the first microorganisms to have inhabited the Earth. Throughout the last few billion years, they have played a major role in shaping the Earth as the planet we live in, and they continue to play a significant role in our everyday lives. Besides being an essential source of atmospheric oxygen, marine cyanobacteria are prolific secondary metabolite producers, often despite the exceptionally small genomes. Secondary metabolites produced by these organisms are diverse and complex; these include compounds, such as pigments and fluorescent dyes, as well as biologically active compounds with a particular interest for the pharmaceutical industry with antiviral, antibacterial, antifungal, and anticancer properties. In the present study, the cyanobacterium *Oscillatoria* was isolated from the local water bodies in and around Mysuru. Methanol crude extract of Oscillatoria was used to screen the phytoconstituents and assayed for antioxidants. The quantitative test of MEOs shows significant indication at the presence of metabolites such as flavonoids, terpenoids, alkaloids, steroids, saponins, and phytosterols, The total phenolic contents in the methanol extracts were determined using Folin-Ciocalteu's reagent method, and was found to be 144 mg GAEg. Free radical scavenging properties of the extract for 160µg/ml was 94.20% (DPPH). FRAP is the novel method for assessing antioxidant power the result indicate for MEOs sample at 1200µg/ml. The results obtained in the present study indicate that methanol extracts of *Oscillatoria* contains active compounds which have the potential for pharmaceutical applications.

Keywords: *Oscillatoria*, MEOs(Methanol extracted *Oscillatoria* sample) DPPH, Antioxidant.

Introduction:

Cyanobacteria have a significant attraction as natural source of bioactive molecules with a broad range of biological activities including antimicrobial, antiviral, anticancer, antioxidant and anti- inflammatory effects (Tuney et al. 2006; Patra et al. 2009). Cyanobacteria believed to be rich in antioxidants and phycobiliproteins (Mata et al. 2010) (PBPs) which are the unique photosynthetic pigments of cyanobacteria. Cyanobacteria are the potential source for food and pharmaceuticals. Phytonutrients and pigments present in the cyanobacteria act as antioxidants which facilitate the formation of the body's defense against free radical damage to cells. Reactive oxygen species (ROS) are often generated either as byproducts of biological reactions. Reactive oxygen species and free radicals formed during oxidation have been reported to contribute for diseases like cancer, diabetes, cardiovascular diseases and ageing. Antioxidants have the ability to protect the body from oxidative damage by scavenging the free radicals and inhibiting peroxidation and other radical mediated processes. In recent years, significant attention was given towards exploring plant-based natural antioxidants, especially the phenolics and tocopherols.

Natural antioxidants have the ability to neutralize reactive oxygen species which are implicated in the treatment of certain diseases. There is a great demand throughout the world in finding new natural sources for antioxidants to prevent oxidative damage to living cells. Cyanobacteria have a highly evolved antioxidant system that catalyzes the harmful oxy radicals produced during photosynthesis. Carotenoids of cyanobacteria take part in photosynthesis and possess potent antioxidant activity. Screening of cyanobacteria for antioxidants and other

pharmacologically active compounds has received increasing attention as a potential source for new drugs. Oscillatoria is blue green algae and consists of photosynthetic pigments which aid in performing the photosynthetic process. Oscillatoria is called as oxygenic photosynthetic bacteria and blue green algae otherwise called as cyanobacteria. Oscillatoria can also carry out anoxygenic photosynthesis. The organism consists of straight unbranched filament of vegetative cells. Each of the cells comprise of gas vacuoles and chlorophyll a. Due to the presence of chlorophyll a pigment, it appears green in colour. Oscillatoria is also rich in essential amino acids, minerals, essential fatty acids, and antioxidants normally, when Oscillatoria is used as a nutritional supplement, it is recommended that no more than 15 g per day is consumed (Small 2011). In recent years, Oscillatoria has attracted more and more attention as a potential source of pharmaceutical compounds. In the present study we are focusing on the potential antioxidant activity of Oscillatoria which can induce antioxidant enzyme activity, helps to prevent lipid peroxidation and DNA damage, and scavenges free radicals

Materials & Methods:

1. **Sample collection and preparation**: Water samples were collected from Mysore using a Ruttner sampler from the photic surface layer, filtered through 20 µm mesh size planktonic net, transferred into a screw cap plastic tube making the final volume into 25 mL by adding original water , further a quantity of 10 mL of the planktonic samples were transferred into 50 mL of BG11 (Stanier et al. 1971) media in 100 mL conical flask for culturing.
2. **Culturing and subculturing:** Samples with media were kept on the shaker in biological growth chamber under 2000 lux light intensity at pH 7.5 and 200 rpm of shaking. Growth appearance was expressed as days in each media for the cultures to appear the bluish green color. Frequent subculturing was practiced to isolate single colonies from mixed growths of microorganisms (Pulz and Gross 2004). Morphological identification of monoculture of the sample was put on a glass slide, covered with a cover slip and observed under the microscope. Identifications were carried out using morphological characteristics described by Desikachary.
3. **Biomass harvest:** Algal biomass was harvested by centrifuging the cultures in 1075 g for 5 min at 27°C temperature. The cell pellet was separated in order to be dried at 55°C for 24 h in oven to obtain dry matter (%).
4. **Preparation of extracts**: A quantity of 0.15 g of dry biomass was taken with 10 mL of methanol and cell disruptions is carried out using the process of sonication (Sonics, vibra- cell TM, USA) at 10 KHz for 5 min (Sharathchandra and Rajashekhar 2013). After cell disruption, any insoluble material was removed from the cell- free extract by centrifugation with 2000 rpm at 27°C for 5 min. The supernatant were obtained for analysis.
5. **Phytochemical screening**: The solvent extracted Oscillatoria was screened for the presence of Phytochemical compounds as per the standard methods followed by Prashant Tiwari *etal*., (2011).
6. **Determination of total phenolic content**:.The amount of total phenolics in extracts was determined according to the FolinCiocalteu's procedure. Samples (2mL, triplicates) were introduced into test tubes; 1.0 mL of Folin-Ciocalteu's reagent and 0.8 mL of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (Systronics UV-via spectrophotometer). The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per gram dry material
7. **DPPH free radical scavenging activity**: The scavenging activity was measured according to the method of Hou et al., 2001(Hou et al. 2001) with some modification. DPPH concentration was prepared as 0.06 mg/mL in methanol. A quantity of 60 µL of the different cell- free extract samples and 90 µL distilled water followed by 100 µL DPPH solution was added. Then, it was kept for 30 min under light protection. The absorbance at 517 nm was taken. An ascorbic acid (AA) standard curve was plotted. All determinations were carried out in triplicate.
8. **Ferric reducing antioxidant power (FRAP) assay:** The ferric reducing antioxidant power (FRAP) assay was performed according to the method of Benzie and Strain, 1999(Benzie and Strain 1999) with some modification. Briefly, 200 µL of the FRAP solution was mixed with 50 µL of sample. The reaction

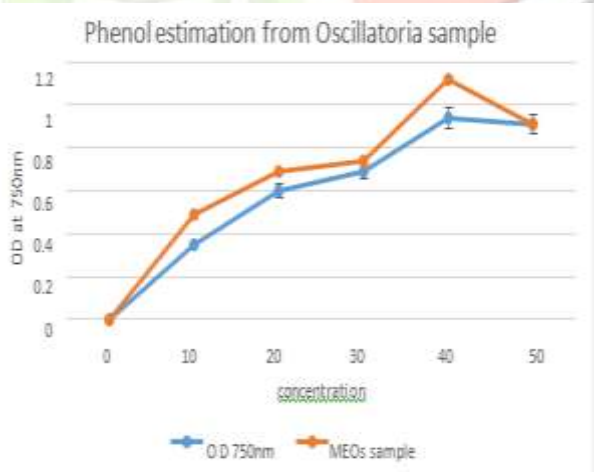
mixture was incubated at 37°C for 4 min and the absorbance was measured at 593 nm. FRAP values were expressed as micromoles of ferrous equivalent (µmol/L Fe) per g of sample

Results & Discussion:

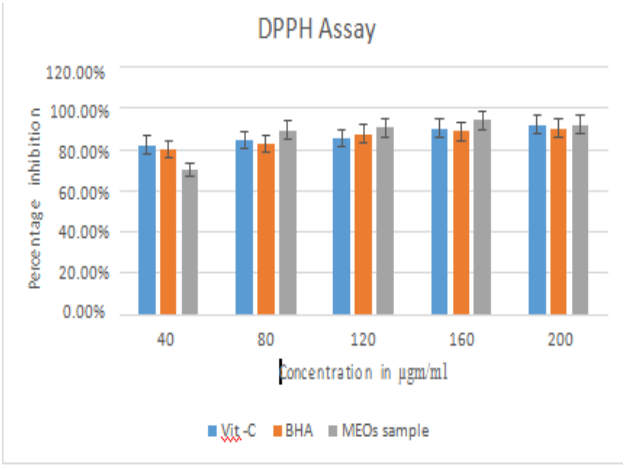
In the present study water samples were collected from local water bodies in and around Mysuru and then further subjected to isolation of individual strains from the mixture by pipette method followed by purification of the sample to get a single culture by antibiotic treatment method . The quantitative test of MEOs shows significant indication at the presence of metabolites flavonoids, terpenoids, alkaloids, steroids, saponins, and phytosterols thus reflecting its importance. The qualitative estimation is conducted using TLC system with suitable solvent namely, ethyl acetate, methanol, water in the ratio of 1:65:1.25:10 respectively. The result indicate the separation of wide range of compounds of biochemical interest. The three pigments namely chlorophyll, pycocyanin & Phycoerythrin are observed.

Fig.No.1: Collection, sub-culturing and isolation, TLC analysis of *Oscillatoria*

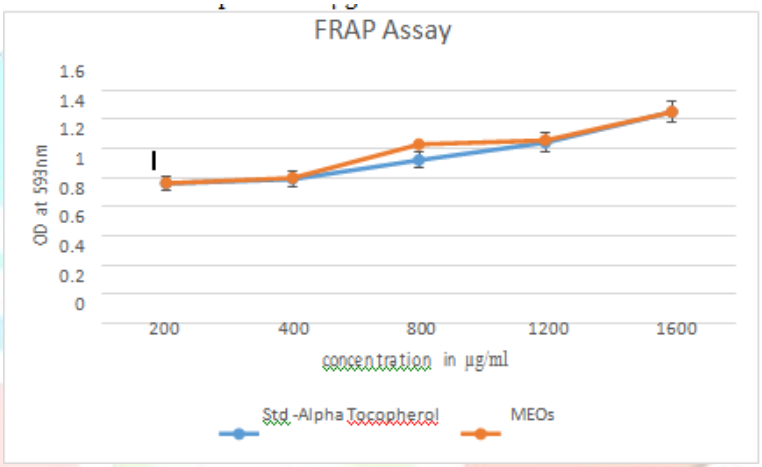
Phenol estimation: The total phenolic content is estimated using FC reagent and the result is expressed in the form of GAE/mg of sample and is found to be 144 GAE/ mg for MEOs sample.



DPPH Assay: DPPH has been used for radical scavenging assessment due to its ease and convenience. The free radical scavenging activity of MEOs is found to be high at 160µg/ml concentration showing 94.20% inhibition comparable to Vit-c and BHA, whose inhibition increased with increased concentration. Therefore these results indicate the outstanding scavenging effect of MEOs on DPPH



FRAP Assay: FRAP Assay is the novel method for assessing antioxidant power. The result indicate for MEOs sample at 1200µg/ml concentration.



Summary

Oscillatoria sp. are group of organisms which plays a major role in photoproduction of biofuels, ammonia, various metabolites, vitamins, toxins, therapeutic substances, aqua are animal feed. Cyanobacteria are also used as energy source and biofertilizers. As it is obtained from a novel natural resource steps for drug development can be taken into consideration., with most of the species actively possessing bioactive compounds.

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