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EVALUATION OF ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANTS OF *OSCILLATORIA TEREBRIFORMIS*

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Keywords:

Oscillatoria terebriformis, Enzymatic antioxidants, Ascorbic acid, Total reduced glutathione

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ABSTRACT

Antioxidants are free radical scavengers which protect the human body against free radicals that cause various pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation and aging process. Some of the antioxidant enzymes and non-enzymatic molecules widely distributed in the biological system which are capable of scavenging free radicals. The present study was conducted to evaluate the antioxidant potential of *Oscillatoria terebriformis* C.A. Aged *ex* Gomont by assessing enzymatic and non enzymatic parameters. The enzymatic antioxidants (Superoxide dismutase, Catalase, Glutathione peroxidase,) and non-enzymatic antioxidants (Ascorbic acid, and α-Tocopherol) activities were determined. The results obtained in the present study showed significant levels of enzymatic and non-enzymatic antioxidants in *Oscillatoria terebriformis*. From above study it is concluded that, the ***Oscillatoria terebriformis*** showed promising antioxidant activity which can be used as effective protecting agent against oxidative stress and various related diseases.

INTRODUCTION

Cyanobacteria, a group of photosynthetic organisms, supposed to have evolved around3.5 billion years ago are the first oxygen evolving organisms to release oxygen into the then oxygen free atmosphere leading to the development of aerobic metabolism and the subsequent rise of higher plants and animal forms (Walter *etal.,* 1992).

Antioxidants can be divided into three general classes; (i) lipid soluble and membrane associated tocopherols, (ii) water soluble reductants such as ascorbic acid and glutathione and (iii) enzymes such as superoxide dismutase (SOD), catalase, peroxidase, ascorbate peroxidase and glutathione reductase(Foyer*etal.,*1993). Recent research in natural products of cyanobacteria has made significant advances in aquaculture and they have been shown to produce a variety of compounds and some of them have been proved to possess biological activity of potential medicinal value (Kumar *et al.,* 2003).

The aim of the present work was. to investigate the antioxidant potentials of ***Oscillatoria terebriformis,*** which might be used as a possible source for antioxidative substances for commercial or pharmaceutical purpose.

MATERIALS AND METHODS

Collection and culturing of algae

Oscillatoria terebriformis, a thermophilic cyanobacterium was obtained from the culture collected from the culture collection of Vivekananda Institute of Algal Technology (VIAT), Chennai, Improvised CFTRI medium was used for cultivating thermophilic cyanobacterium and the composition is as follows (Venkataraman *etal*.,1985).

The algal samples were dried and the 0.5g of dried algal material was extracted in 20ml of methanol kept in an orbital shaker for overnight. The obtained extracts were filtered with Whatman no.1 filter paper and the filtrate was collected. The solvents were removed under reduced pressure at 50°C to yield a concentrated extract (15%)(Sivasubramanian *et.al*.,2011).

Estimation of Enzymatic and Non-enzymatic antioxidants in *Oscillatoria* *Terebriformis:*

The enzymatic antioxidant activities of Superoxide dismutase, Catalase, Glutathione peroxidase, Hydrogen peroxide radical scavenging (H2O2) assay were determined spectrophotometrically by using the methods of *Beauchamp and Fridovich (1971),* Jin, Cai, Li, and Zhao (1996), Paglia and Valentine (1967). Ruch *et al*., (1989). The non-enzymatic antioxidants like chlorophyll ‘a’ (Jeffrey and Humphrey (1975),β-Carotene(Shaish *etal*.,1992) ,Ascorbic acid, α-tocopherol, Thiamine, Pyridoxine and Cobalamin were studied by the method of (*Roe and Keuther et a*l., (*1953), Emmerie-Engel method et al., 1938* , Lawrence Evans *etal* ., 2004).

RESULTS AND DISCUSSION

Cellular systems scavenge these active oxygen species by invoking increased antioxidative machinery such as enzymatic and non enzymatic mechanism. Enzymatic defense mechanism includes superoxide dismutase, catalase and peroxidase etc., and the non-enzymatic mechanism includes organic chemicals like proline, ascorbate and carotenoids etc.Superoxide dismutase (SOD) and catalase (CAT) are widely distributed in aerobic organisms. Since the first discovery of these metalloenzymes in 1969 (Fridovich,1975), SOD and CAT has been considered as a key enzyme defending against oxidative stress. SOD can catalyzes peroxide anions to oxygen and hydrogen peroxide rapidly; then hydrogen peroxide is decomposed to water and oxygen by catalase. For many bacteria in natural environments, resistance to hydrogen peroxide and superoxide anions is mainly due to the presence of SOD and CAT (Mandell, 1975). Changes in antioxidant enzyme activity, along with protection against pollution, are the most important characteristics of an antioxidant system (Zhao *et al*., 2002).

The super oxide dismutase activity of methanol extract from *Oscillatoria terebriformis* was estimated and were compared with the Butylated hydroxytoluene. **Figure 1** shows the levels of superoxide dismutase were found to be 74.56 ±0.56% super oxide inhibitions at maximum concentration of 500 µg/ml.

Srivastava *et al*., (2005) described that every cyanobacterial cell possesses a complex array of enzymatic antioxidant defense system which comprises mainly the enzymes super oxide dismutase (SOD), catalase (CAT),) glutathione reductase (GR) (Wiktelius and Stenberg, 2007). Some Cyanobacteria have been shown to have both chemo preventive and or therapeutic effects on human diseases; SOD is one of the antioxidant enzymes that play a key role in cellular defense against ROS (Bowler *et al*., 1992)· Bandyopadhyay *et al*., (1999) stated that singlet oxygen ($O2^{-}$) is one of the active oxygen species, which plays a central role in oxidative damage of biological system (Kalai *etal*., 1998). The formation of O2 in different organisms under various stressful conditions such as drought, salt, low temperature and freezing, nutrient limitations, heavy metal toxicity, air pollutants, herbicides, UV-exposure etc., has been well reported (Alia *et al*., 2001). The first enzyme involved in the antioxidant defense is the superoxide dismutase: a metalloprotein found in both prokaryotic and eukaryotic cells. SOD catalyzes the dismutation of super oxide free anions (O2•−) to oxygen and hydrogen peroxide, and are rather considered the first line of defense against damage by O2•−.SODs are ubiquitous metalloenzymes that exist in four forms depending upon the nature of their catalytic metals: FeSOD, MnSOD, Cu/ZnSOD and NiSOD (Fridovich, 1997).

The Catalase activity of methanol extract from *Oscillatoria terebriformis* was estimated and were compared with the Butylated hydroxytoluene .**Figure 2** shows the activity of CAT of *Oscillatoria terebriformis.* The levels were found to be 81.25 ±0.41% Hydroxyl inhibitions at concentration of 500 µg/ml.Tichy and Vermaas, 1999 reviewed that the catalase exclusively dismutates hydrogen peroxide (H2O2), whereas peroxidases use a broad range of peroxides (ROOH) as substrates (Chelikani *et al*., 2004). Hydrogen peroxide is well known to have an inhibitory effect on the phototrophic growth of cyanobacteria. Catalases are one of the most-studied enzymes, and the availability of a huge number of their sequences has allowed a detailed understanding of their structure–function relationship. CAT is also one of the principal antioxidant enzymes; it eliminates H_2O_2 by transforming it into H_2O and O_2. The stimulation of SOD activity along with CAT seemed to play a protective role against membrane damage as Cu is particularly toxic to membranes (Ahmed *et al*.,2010).

The Glutathione peroxidase (GPX) activity of methanol extract from *Oscillatoria terebriformis* was estimated and were compared with the Butylated hydroxytoluene. **Figure 3** shows Glutathione peroxidase (GPX) activity *of Oscillatoria Terebriformis.* The levels were found to be 81.23 ±0.94 % at concentration of 500µg/ml. Gaber *et al*., (2004) experimented on Glutathione peroxidase (GPX) is the general name for a family of multiple isozymes that also catalyse the reduction of H2O2 or organic hydro peroxides to water or corresponding alcohols using reduced glutathione (GSH) as an electron donor (Hydrogen peroxide (H2O2) + 2 Reduced glutathione (GSH) Oxidized glutathione (GSSG) + 2 water (H2O)). A recently performed phylogenetic analysis demonstrates the occurrence of GPXs in cyanobacteria as well as their scattered distribution inside the group of proteobacteria (Margis *et al*., 2008). Glutathione peroxides utilize the reducing equivalents of glutathione to reduce hydrogen per oxide and it may be the main mechanism for protection against the deleterious effects of hydro peroxides. Glutathione peroxidase (Halliwell *et al.,* 1994).

Figure 1.Superoxide Dismutase activity of *Oscillatoria terebriformis*

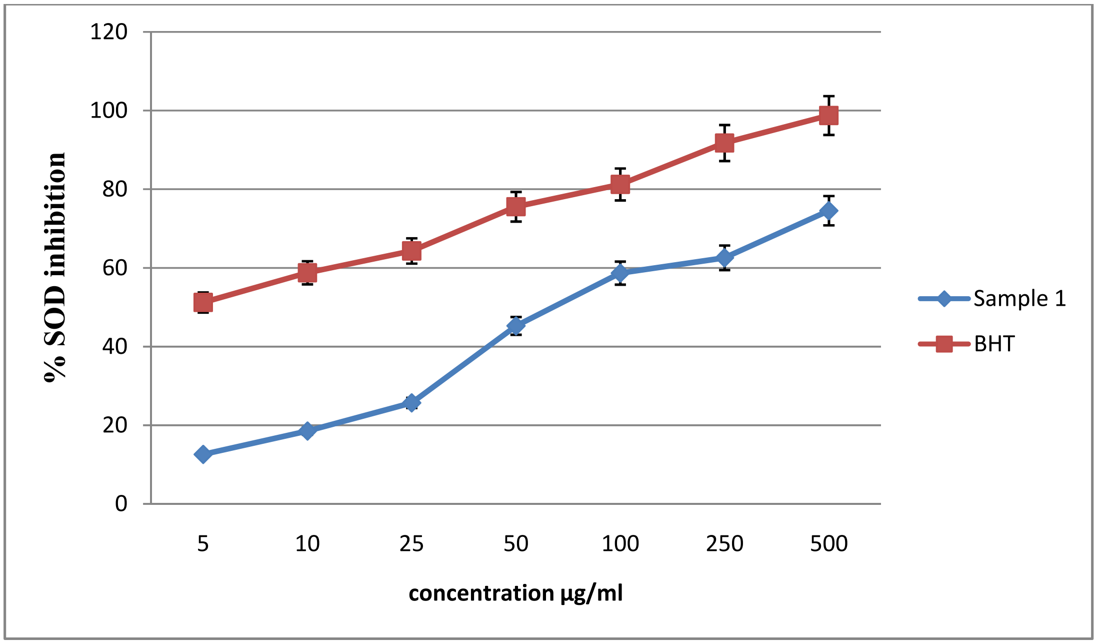


Figure 2. Catalase Activity of *Oscillatoria terebriformis*

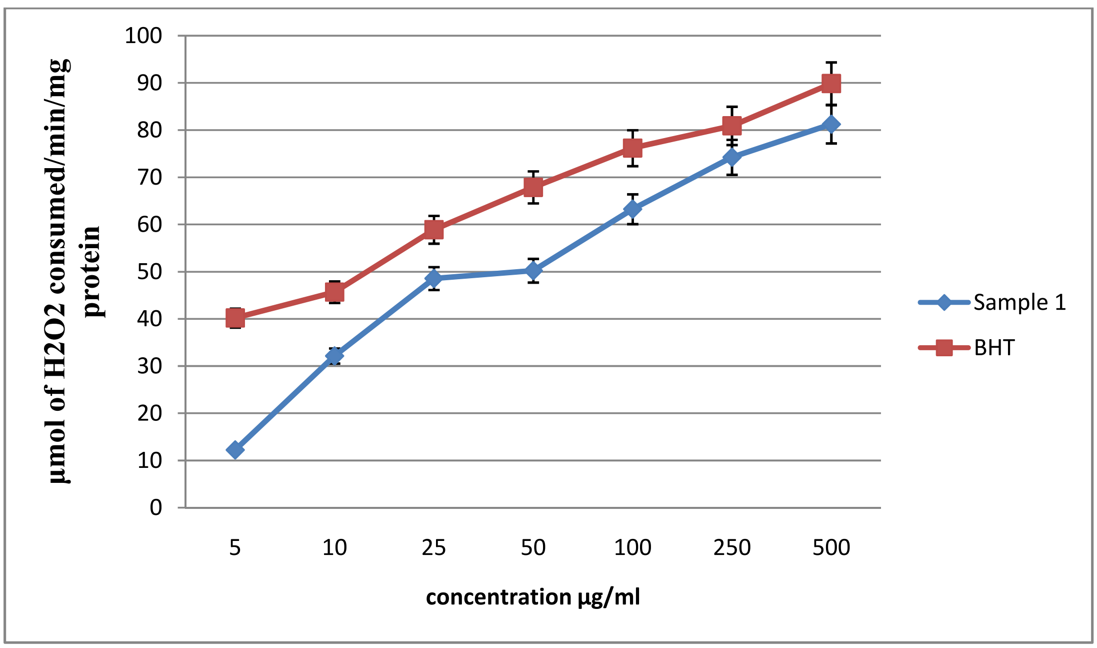


Figure 3. Glutathione peroxidase (GPX) activity of *Oscillatoria Terebriformis.*

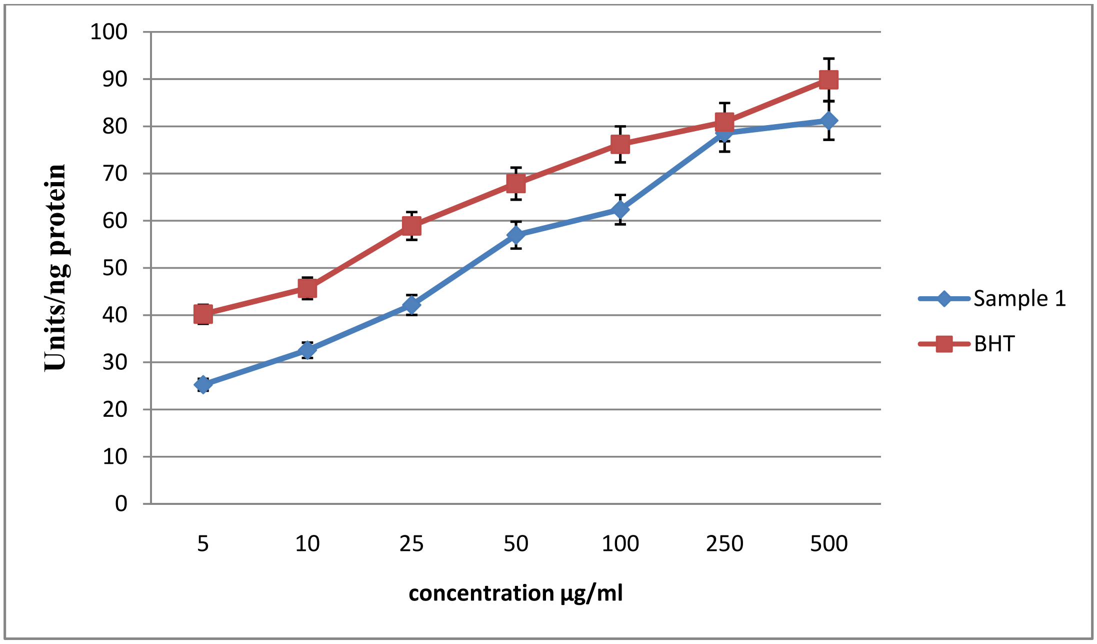
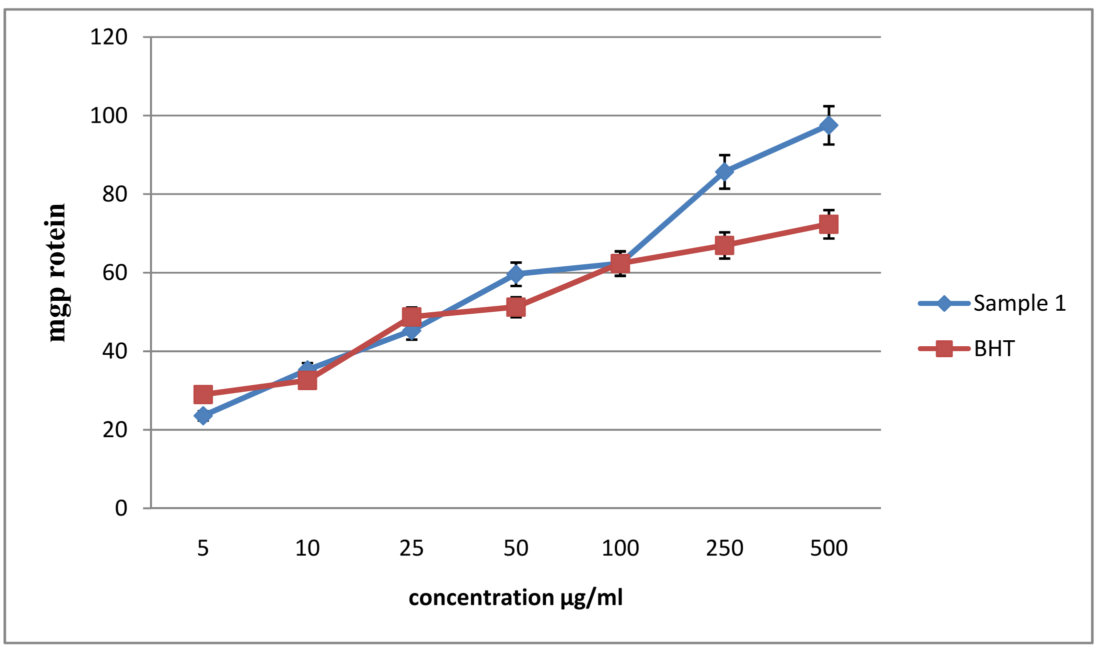


Figure 4: Hydrogen peroxide radical scavenging (H2O2) activity of *Oscillatoria Terebriformis.*



The cosmopolitan distribution of cyanobacteria indicates that they can cope with a wide spectrum of global environmental stresses such as heat, cold, desiccation, salinity, nitrogen starvation, photo-oxidation, anaerobiosis and metal stress etc. (Fay 1992, Tandeau de Marsac, Sinha and Häder, 1996). They have developed a number of mechanisms by which cyanobacteria defend themselves against environmental stressors. Important among them are enzymes such as superoxide dismutase, catalases and peroxidases (Burton and Ingold, 1984, Canini *et al*., 2001)

The Hydrogen peroxide radical scavenging (H_2O_2) activities of methanol extract from Oscillatoria terebriformis was estimated and were compared with the Butylated hydroxytoluene .**Figure 4** shows the activity of Hydrogen peroxide radical scavenging (H_2O_2) assay *of Oscillatoria terebriformis.* The levels were found to be 97.56 ±0.21 (%) at concentration of 500 µg/ml.Maintenance of a high antioxidant capacity in cells has been related to increased tolerance against different kinds of environmental stress (Okamoto *et al*., 2001). All of these factors lead to increasing number and accumulation of ROS in Cyanobacteria Scientific research shows that ROS are harmful to the cell because they can raise the oxidative level through loss of cellular structure and function (Lee *et al.,* 2001). The presence of antioxidant enzyme was observed in control due to presence of ROS. Normally the ROS was produced in the aerobic organism, which is involved in signal transduction etc., but level of ROS was increased during stress conditions. The organism also increased the production of antioxidant enzymes in order to protect themselves from oxidative damage caused by ROS and to maintain the low rate of generation of reactive oxygen species. Catalase decomposed the hydrogen peroxide into water and oxygen. Similarly increased production of catalase was observed in endosulfon treated *Plectonema boryanum* (Prasad *et al*., 2005).

Figure 5. Concentrations of Chlorophyll and Beta-carotene (in mg/g) of *Oscillatoria Terebriformis*

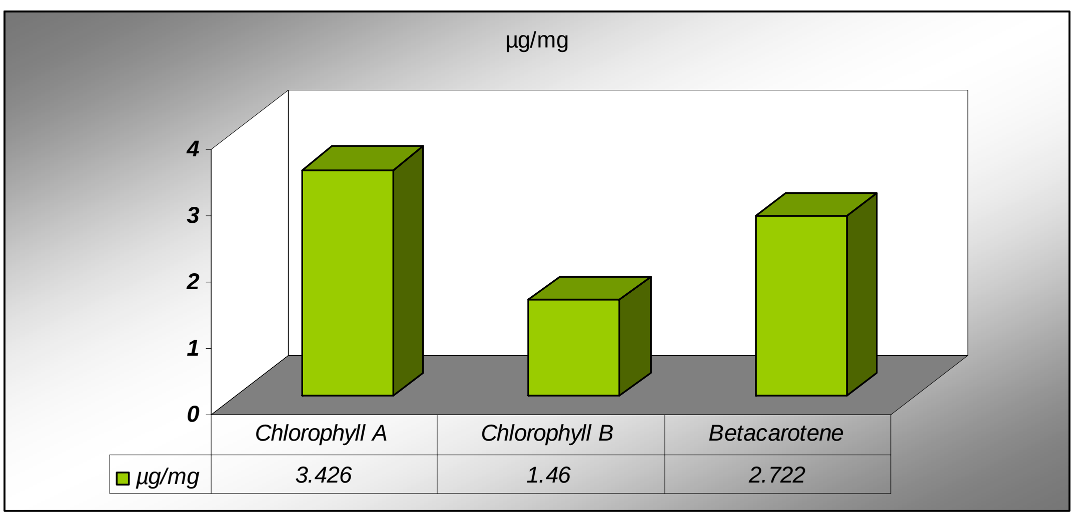


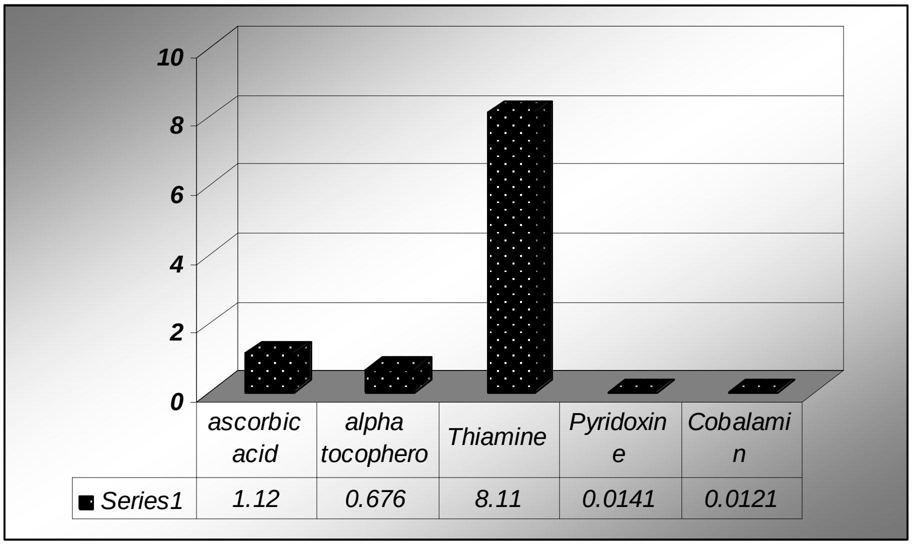
Figure 5 shows that levels of the biochemicals such as chlorophyll a& b and β -carotene. For photosynthesis, both carotenoids and chlorophylls are necessarily bound to peptides to form pigment-protein complexes in the thylakoid membrane. In cyanobacteria, some carotenoids are located in cytoplasmic membrane for protection from high-light (Kana, 1998, Masamoto, 1999).

Chlorophyll consists of two forms, A and B.A: **$C_{66}H_{72}O_5N_4Mg$,** B: **$C_{66}H_{70}O_6N_4Mg$**

Chlorophyll is the chemical in plants accomplishes photosynthesis and it is responsible for the green color of the plant. Chlorophyll can be found in dark green leafy vegetables, some algae, wheat grass and barley grass. Historically, chlorophyll was used to improve bad breath, and reduce the odors of urine and feces. It was also believed that it might have health benefits on constipation and anemia.Some preliminary evidence suggests that chlorophyll might have anti-inflammatory, antioxidant, and wound-healing activities. (Rudolph *et al*., 1930). Chlorophyll has also been shown to have benefits on chemoprevention.(Chernomorsky *et al.,*1988). A few studies have shown that chlorophyll prevents the detrimental, cytotoxic and hyperproliferative colonic effects of dietary haem. Diets high in red meat are associated with increased colon cancer risk. This association might be partly due to the haem content of red meat. In rats, dietary haem is metabolized in the gut to a cytotoxic factor that increases colonic cytotoxicity and epithelial proliferation. Chlorophyll is magnesium porphyrin structurally analogous to haem. (De Vogel *et al.,* 2005).

Beta-carotene belongs to a group of plant compounds called carotenoids. To date, over 500 carotenoids have been found to occur in nature. Carotenoids are the pigments that provide the yellow, orange, and red coloration in fruits and vegetables. Beta-carotene is the most abundant carotenoid in human foods and is generally thought to be the most important carotenoid for humans. Beta-carotene, which is also known as pro-vitamin A, consists of two molecules of vitamin A linked together (A-A). Enzymes in the epithelial lining of the intestinal tract split beta-carotene into two molecules of vitamin A whenever the body needs it. (Nagao A *et al.,* 1996) Beta-carotene is the most abundant precursor of vitamin A in fruits and vegetables.Beta-carotene functions as a chain-breaking antioxidant. This means it does not prevent the initiation of lipid peroxidation, but rather, it stops the chain reaction by trapping free radicals, which halts the progression of free radical activity.(Mathews-Roth *etal.,*1990) Beta-carotene is capable of quenching singlet oxygen free radicals in humans., (Iannone *et al*. ,1998).Chemotherapy stresses the antioxidant defense system and may lead to lower antioxidant levels which could cause an increase in the adverse side effects of the therapy. A study was conducted involving children with acute lymphoblastic leukemia who were undergoing chemotherapy were administered greater intakes of antioxidants. The increased consumption of beta-carotene at 6 months decreased the risk of toxicity. (Kennedy *etal*, 2004.).**Figure 6** shows that levels of the essentials vitamins such as ascorbic acid, α-tocopherol, vitamin B_1, Vitamin B_6,

Figure 6. Concentration of Ascorbic acid, Alpha-tocopherol, Thiamine, Pyridoxine, Cobalamin (in mg/g) of *Oscillatoria Terebriformis*



Vitamin B_{12}. Ascorbic acid or vitamin C is a water-soluble antioxidant that can reduce radicals from a variety of sources. It also appears to participate in recycling vitamin E radicals. Interestingly, vitamin C also functions as a pro-oxidant under certain circumstance (Ranjith kumar *etal.,* 2011). Vitamin C is an antioxidant, a compound that helps block the action of unstable molecules known as free radicals, which can damage cells. Vitamin C is thought by some to enhance the immune system by stimulating the activities of natural killer cells (a type of white blood cell) and anti-cancer agents. Some claim that the vitamin can prevent a variety of cancers from developing, including lung, prostate, bladder, breast, cervical, intestinal, esophageal, stomach, pancreatic, and salivary gland cancers, as well as leukemia and non-Hodgkin's lymphoma. Vitamin C is also said to prevent tumors from spreading, help the body heal after cancer surgery, enhance the effects of certain anti-cancer drugs, and reduce the toxic effects of other drugs used in chemotherapy. It also appears to participate in recycling vitamin E radicals. Interestingly, vitamin C also functions as a pro-oxidant under certain circumstances Neely *et al*., (1988) described that the α-Tocopherol (vitamin E) is a lipid-soluble organic molecule exclusively synthesized by oxygenic phototrophs, including all green algae, plants and some cyanobacteria. Tocopherols can undergo two oxidation reactions: they maybe oxidized by ROS to a tocopheryl radical and convert singlet oxygen to hydroperoxide. Both reactions can be reversed by ascorbate, recycling tocopherol Vitamin E is one of the few nutrients for which supplementation with higher than recommended levels have been shown to enhance immune response and resistance to diseases (Bendich A et al 1997). All B vitamins are water-soluble, meaning that the body does not store them .Thiamine is a vitamin, also called vitamin B_1.Thiamine is also used for digestive problems including poor appetite, ulcerative colitis, and ongoing diarrhea. Thiamine is also used for AIDS and boosting the immune system, diabetic pain, heart disease, alcoholism, aging, a type of brain damage called cerebellar syndrome, canker sores, vision problems such as cataracts and glaucoma, motion sickness, and improving athletic performance. Other uses include preventing cervical cancer and progression of kidney disease in patients with type 2 diabetes (Raschke *et al*., 2007).

Vitamin B_6, also called pyridoxine, is one of 8 B vitamins. All B vitamins help the body convert food (carbohydrates) into fuel (glucose), which is used to produce energy. B complex vitamins are needed for healthy skin, hair, eyes, and liver. They also help the nervous system function properly.B6 helps control levels of homocysteine in the blood. Homocysteine is an amino acid that may be associated with heart disease. Your body needs B6 in order to absorb vitamin B12 and to make red blood cells and cells of the immune system (McCormick *et al.,* 2006).

Vitamin B_{12}, also called cobalamin, is one of 8 B vitamins. All B vitamins help the body convert food (carbohydrates) into fuel (glucose), which is used to produce energy. These B vitamins often referred to as B complex vitamins, also help the body use fats and protein. B complex vitamins are needed for healthy skin, hair, eyes, and liver. They also help the nervous system function properly (Robert Clarke *et al.,* 2007).

CONCLUSION

The results of the above study clearly indicated the presence of enzymatic and non – enzymatic antioxidant in *Oscillatoria terebriformis* that could protect against oxidant and other free radical diseases.Thus the source of *Oscillatoria terebriformis* can be employed in all medicinal preparations to cure disease associated with oxidative stress like cancer and other related diseases.

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REFERENCES

American Journal of Clinical Nutrition": Homocysteine, B vitamins, and the risk of dementia; Robert Clarke; Feb.2007.

Ahmed.A, Hasnain A, Akhtar S, Hussain A, Yasin AUG, Wahid A et al.Antioxidant enzymes as bio-markers for copper tolerance in safflower (*Carthamus tinctorius.L)* African Journal of Biotechnology. 2010; 9 Suppl 33: 5441-5444.

Alia, Pardha Saradhi, P., 1993.Suppression in mitochondrial electron transport is the prime cause behind stress induced proline accumulation. Biochem. Biophys. Res. Commun. 193, 54–58.

Beauchamp, C. and Fridovich, I. (1971) Anal. Biochem. 44, 276.

Bendich A, Gabriel E & Machlin LJ (1986) Dietary vitamin E requirement for optimum immune responses in the rat. Journal of Nutrition 16, 675–681

Bowler C, Montagu MV, Inze D. Superoxide dismutase and stress tolerance. Annu.Rev. Plant Physiol. Plant Mol. Biol 1992; 43: 83- 116.

Burton G. W., Ingold K. U. (1984) β-Carotene: an unusual type of lipid antioxidant. Science 224: 569-573

Canini A., Leonardi D., Caiola M.G. (2001) Superoxide dismutase activity in the cyanobacterium Microcystis aeruginosa after surface bloom formation. New Phytol. 152: 107-116

Chernomorsky SA, Segelman AB. Biological activities of chlorophyll derivatives. N J Med 1988; 85:669-73.

Chelikani, P., Fita, I. and Loewen, P.C. (2004) Diversity of structures and properties among catalase. Cell. Mol. Life Sci. 61 (2), 192–208.

De Vogel J et al, Green vegetables, red meat and colon cancer: chlorophyll prevents the cytotoxic and hyperproliferative effects of haem in rat colon. Carcinogenesis. 2005 Feb; 26(2):387-93. Epub 2004 Nov 18

Emmerie A., Engel C., 1938.Colorimetric determination of tocopherol (vitamin E). Rec. Trav. Chim. 57, 1351.

Fay P. (1992) Oxygen relations of nitrogen fixation in cyanobacteria.Microbiol. Rev. 56: 340-373

C. H. Foyer. Ascorbic acid. In: R.G. Alscher, and J.L. Hess (eds.), Antioxidants in Higher Plants, CRC Press, Boca Raton, 1993, pp. 31-58.

Fridovich 1. (1975). Superoxide dismutase.A.Rev.Biochem. 44, 147-1 59

Gaber, A., K. Yoshimura, M. Tamoi, T. Takeda, Y. Nakano, and S. Shigeoka.2004. Induction and functional analysis of two reduced nicotinamide adeninedinucleotide phosphate-dependent glutathione peroxidase-like proteins inSynechocystis PCC 6803 during the progression of oxidative stress. PlantPhysiol. 136:2855–2861

Halliwell B.Antioxidants and human disease- A general introduction.Nutr Reviews 1994; 55: 522-544.

Iannone A, et al. Antioxidant activity of carotenoids: an electron-spin resonance study on beta-carotene and lutein interaction with free radicals generated in a chemical system. J Biochem Mol Toxicol. 1998; 12(5):299-304.

Jin M, Cai YX, Li JR and Zhao H. [J] Prog Biochem Biophy, 1996; 23:553.

Jeffrey, S.W. and Humphrey, G.F. 1975. New spectrophotometric equation for determining chlorophylls a, b, c and c2 in higher plants, algae and natural phytyoplankton. Biochem. Physiol. Pflanz., 167: 191-194

Kennedy DD, Tucker KL, Ladas ED, Rheingold SR, Blumberg J, Kelly KM. Low antioxidant vitamin intakes are associated with increases in adverse effects of chemotherapy in children with acute lymphoblastic leukemia.Am J ClinNutr.Jun2004; 79(6):1029-36.

Kalai, T., Hideg, E., Vass, I. & Hideg, K. (1998). Double (fluorescent and spin sensors for detection of reactive oxygen species in the thylakoid membrane).Free Radical. Biol. Med. 24, 649-652.

Kana, T.M.; Glibert, P.M.; Goericke, R.; Welschmeyer, N.A. Zeaxanthin and β-carotene in *Synechococcus* WH7803 respond differently to irradiance. *Limnol. Oceanogr.* 1998, *33*, 1623–1627.

Kumar, K., Lakshmanan, A. and Kannaiyan, 5. 2003. Bioregulatory and therapeutic effects of Blue green algae. Indian J. Microbiol., 43 (1): 9-16.

Klisch M., Sinha R.-P., Richter R. P., Häder D.-P. (2001) Mycosporinelike amino acids (MAAs) protect against UV-B-induced damage in Gyrodinium dorsum. J. Plant Physiol. 158: 1449-1454

Lawrence Evans, Ph.D. (2004), Senior Scientific Associate Expert Committee: (DSN) Dietary Supplements: Non-Botanicals USP27–NF22 Page 2063 Pharmacopeial Forum: Volume No. 28(5) Page 1545.

Lee DH, Young SK, Lee CB. The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). J. Plant Physiol 2001; 158: 737–745.

Mathews-Roth MM. Carotenoid functions in photoprotection and cancer prevention. J Environ Pathol Toxicol Oncol. Jul1990; 10(4-5):181-92.

MeisterA.Glutathione-ascorbic acid antioxidant system in animals.J.Biolchem.1994;269:9397–400.

McCormick D.Vitamin B6. In: Bowman B, Russell R, eds. Present Knowledge in Nutrition. 9th ed. Washington, DC: International Life Sciences Institute; 2006.

Margis R, Dunand C, Teixeira FK, Margis-Pinheiro M. 2008.Glutathion peroxidase family—an evolutionary overview. FEBS Journal275, 3959–3970.

Mandell, G. L. 1975. Catalase, superoxide dismutase, and virulence of *Staphylococcus aureus*. *In vitro* and *in vivo* studies with emphasis on staphylococcal-leukocyte interaction. J. Clin. Investig. 55**:** 561-566.

Masamoto, K.; Zsiros, O.; Gombos, Z. Accumulation of zeaxanthin in cytoplasmic membranes of the cyanobacteirum *Synechococcus* sp. Strain PCC 7942 grown under high light condition. *J. Plant Physiol.* 1999, *155*, 136–13 .

Mukund,S**,** N.S.Senthilkumar M.Palanisamy and V.Sivasubramanian Evaluation of enzymatic and non-enzymatic potential of Phormidium fragile. Algal Biomass Utln. 2014, 5(2): 58-65 ISSN: 2229- 6905

Nagao A, et al. Stoichiometric Conversion of All Trans-beta-carotene to Retinal by Pig Intestinal Extract. Arch Biochem Biophys. Apr1996; 328(1):57-63.

Neely W. C., Martin J. M., and Barker S. A. (1988), Products and relative reaction rates of the oxidation of tocopherols with singlet molecular oxygen. Photochem.Photobiol. 48, 423-428.

Okamoto, K.O., Pinto, E., Latorre, L.R., Bechara, E.J.H., Colepicolo, P., 2001a.Antioxidant modulation in response to metal-induced oxidative stress in algal chloroplasts. Arch. Environ. Contam. Toxicol. 40, 18–24

Priya B, Premanandh J, Dhanalakshmi RT, Seethalakshmi T, Uma L,Prabaharan D, Subramanian G: Comparative analysis of cyanobacterial superoxide dismutases to discriminate canonical forms. BMC Genomics 2007, 8:435

Paglia D and Valentine W 1967 Studies on the quantitative and qualitative characterization of erythtrocyte glutathione peroxidase; J. Lab. Clin. Med. 70 158–169

Prasad, S.M., D. Kumar and M. Zeeshan: Growth, photosynthesis, active oxygen species and antioxidants responses of paddy field cyanobacterium Plectonema boryanum to endosulfan stress. Gen.Appl. Microbiol., 51, 115-123 (2005

Raschke M, et al. Vitamin B1 biosynthesis in plants requires the essential iron sulfur cluster protein, THIC. *Proc Natl Acad Sci. USA*. 2007; 104(49):19637-42.

Roe, J. H and, C. A. Keuther, 1953.The determination of ascorbic acid in whole blood and urine through 2,4-dinitrohenylhydrazinederivatives of dehydroascorbic acid.J Biol.Chem.,147:399-407.

R.RanjithKumar, P.Hanumantha Rao, V .V .Subramanian & V.Sivasubramanian.2011. Enzymatic and non-enzymatic antioxidant potentials of *Chlorella vulgaris* grown in effluent of a confectionery industry.*J Food Sci Technol*,DOI 10.1007/s13197-011-0501-2

Ruch RJ, Cheng SJ and Klaunig JE.Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea, Carcinogen. 1989; 10:1003-1008.

Rudolph C. The therapeutic value of chlorophyll.Clin Med Surg 1930; 37:119-21

Tichy, M., andVermaas, W. (1999) The invivo role of catalase-peroxidase in s*ynechocystis*sp.strain PCC 6803. *J. Bacteriol.* 181, 1875-1882.

Tichy, M., and Vermaas, W. (1999) Accumulation of pre-apocytochrome in a *Synechocystis* sp. PCC 6803 mutant impaired in cytochrome *c* maturation. *J. Biol. Chem.* 274, 32396-32401.

Tandeau de Marsac N. Houmard J. (1993) Adaptation of cyanobacterial to environmental stimuli: new steps towards molecular mechanisms. FEMS Microbiol. Rev. 104: 119-190.

Shaish, A; Ben Amotz A. and Avron, M. 1992. Biosynthesis of beta carotene in Dunaliella Methods in Enzymology 213:439-444

Srivastava, A. K., Bhargava, P. & Rai, L. C. (2005). Salinity and copper-induced oxidative damage and changes in the antioxidative defense system of Anabaena doliolum. World J Microbiol Biotechnol 21, 1291–1298.

Venkataraman, L.V. and Becker, E. W. 1985. Biotechnology and Utilization of Algae: The Indian Experience. Department of Science and Technology, New Delhi, 257

M.R. Walter, J. Bauld, D.J. Des Maraisand J.W. Schopf. A general comparison of microbial mats and microbialstromatolites: bridging the gap between the modern and the fossil. In: J. Schopfand C. Klein, (eds), *The Proterozoic Biosphere*, Cambridge University Press, New Delhi, India (1992), pp. 335-338.

Wiktelius E, Stenberg G (2007) Novel class of glutathione transferases from cyanobacteria exhibit high catalytic activities towards naturally occurring isothiocyanates. Biochem J 406: 115–123.

Zhao, Y., Christensen, S.K., Fankhauser, C., Cashman, J.R.,Cohen, J.D., Weigel, D., and Chory, J. 2001. A role for flavinmonooxygenase-like enzymes in auxin biosynthesis. Science 291: 306–309.