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In vitro antioxidant activity of isolated beta glucan from *Chroococcus turgidus*

K.Chinnu, S. Mukund, M. Muthukumaran and V. Sivasubramanian
Department of Plant Biology and Plant Biotechnology, RKM Vivekananda College, Chennai, India

Abstract
In this study, the antioxidant activity of isolated beta glucan extract of *Chroococcus turgidus* was studied using various *in vitro* assays. The antioxidant activity of *Chroococcus turgidus* was evaluated by using the free radical scavenging activity assay (DPPH method) the enzymatic antioxidant activity of *Chroococcus turgidus* was also studied using Superoxide dismutase and catalase.The results of the study show that isolated beta glucan extract of *Chroococcus turgidus* possesses significant antioxidant properties

Keywords: Betaglucan, DPPH, Nitric Oxide Scavenging Assay , SOD,Catalase.

1. Introduction

Cyanobacteria, a group of Gram-negative photoautotrophic prokaryotes, are capable of performing oxygenic photosynthesis. This unique class of microorganisms has many names, like cyanoprokaryotes, cyanophytes and blue–green bacteria. These names are derived because of the presence of a blue–green coloured pigment, c-phycocyanin (C-PC), which is a pigment used for photosynthesis. These bacteria have a fossil record from 3.3 to 3.5 billion years, and are still among the most successful organisms on earth (Schopf, *etal.,* 1987).Cyanobacteria have many more biotechnological applications that await possible uses in mariculture, food, fuel, fertilizers, colorants and production of various secondary metabolites including toxins, vitamins, enzymes and pharmaceuticals. Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage-free radicals might otherwise cause .Antioxidant acts as cell protectors. Oxygen, an essential element for life, can create damaging by, by-products during normal cellular metabolism. Antioxidants counteract these cellular by-products during normal cellular metabolism.

Thus, antioxidants play an important role in the protection of cells against oxidative damage caused by ROS (Khan *et al*.2005).At present, cyanobacteria generally remain as potential sources for further investigations as prospective and excellent sources of biologically active constituents produced during primary and especially secondary metabolism (Skulberg 2000). The aim of the present study was to evaluate antioxidant and enzymatic antioxidant properties of isolated beta glucan extract of *Chroococcus turgidus*

2.1 Cyanobacterial culture

Chroococcus turgidus, a cyanobacterium was obtained from the culture collection of Vivekananda Institute of Algal technology (VIAT) Chennai. Biomass was obtained by growing algal cultures in 20L of water and 0.25g / L of NPK fertilizer was added with a facility to pump the culture with aeration pump. The algae was grown for 20 days and harvested.

2.1.Extraction and Estimation of Beta-glucan (Erick Reyes Suárez *et al.,* 2008)
Extraction and Drying

The *Chroococcus turgidus* were air-dried at room temperature (30°C) for two weeks, after which it was ground to a uniform powder. The extracts of the dried samples were prepared in a sequential procedure by soaking 20 g of dried powder in 60 ml of 80%methanol for 48 h. procedure was repeated. At the end of each respective extraction, the extracts were filtered using Whatma1 filter paper. The filtrate was concentrated under reduced pressure in vacuum at 40°C for 25 min using a rotary evaporator (Super fit-rotavap, India). The percentage yield of extracts was calculated.

2.2 Estimation of DPPH free Radical scavenging assay

Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by Shimada *et al* (1992).various concentrations of methanol extract of the sample (4.0 ml) was mixed with 1.0 ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM.The Mixture was shaken vigorously and left to stand for 30 minutes, and the absorbance was measured at 517 nm. BHA was used as control. The Percentage of DPPH decolourization of the sample was calculated according to the equation: Scavenging activity (%) = [$Abs_{(control)} - Abs_{(standard)}$] ×100**.**

2.3. Estimation of Nitric oxide scavenging activity

Scavenging activity of the extracts was tested by Nitric oxide radical scavenging activity as described by Govindarajan *et al* (2003). Various concentrations of the extract were mixed with 1.0 ml of 1mM sodium nitrite. Then the mixture was added to 8ml of 0.2 M citrate buffer, pH 4.2. The mixture was incubated for1 hour at 37 *C.1.0 ml of the solution was withdrawn and added to 2.0 ml of 2% acetic acid and 0.4 ml of Griess reagent. The mixture was incubated at room temperature for 15 minutes and the absorbance was measured at 520 nm.
Nitric oxide scavenged (%) = [Abs (control) – Abs (standard) / Abs (control)] × 100.

2.4 Enzymatic Antioxidants of *Chroococcus turgidus*

2.4.1 Estimation of Superoxide dismutase (SOD) activity
SOD activity was assayed using the nitroblue tetrazolium (NBT) method of Beauchamp and Fridovich (1971). NBT was reduced to blue formazan by O2−, which has a strong absorbance at 560 nm. However, the presence of SOD inhibits this reaction. The cells were homogenized in 0.05 M sodium carbonate buffer (pH 10.2). The assay mixture consisted of 0.05 M sodium carbonate buffer (pH 10.2) containing 3 mM xanthine, 0.75 mM NBT, 3 mM EDTA, 1.5 mg/ml BSA and 50 µl of homogenate. The reaction was initiated by adding 50 µl of xanthine oxidase (0.1 mg/ml) and incubated for 30 min at room temperature. The reaction was stopped by adding 6 mM of copper (II) chloride and centrifuged at 1500 rpm for 10 min. The absorbance of formazan at 560 nm was then measured in the supernatants. The absorbance of the mixture was measured at 560 nm. The scavenging activity on superoxide radical (%) = (1 – A sample/A blank) x 100. Deionized water and ascorbic acid were used as the blank and control, respectively.

2.4.2 Estimation of catalase activity

Catalase activity was measured by the method of Jin *et al*., (1996). The hydroxyl radical was generated in the mixture of 1 ml of 0.75 mM 1,10-phenanthroline, 1.5 ml of 0.15 M sodium phosphate buffer (pH 7.4), 1 ml of 0.75 mM FeSO4 and 1 ml of H2O2 (0.01%, v/v). After addition of 1 ml sample (0.05–1 mg/ml), the mixture was incubated at 37 °C for 30 min. The absorbance of the mixture was measured at 536 nm. The scavenging activity on hydroxyl radical (%)= (Asample - Ablank)/ (A0 -Ablank) x 100, where A0 was the absorbance of the deionised water instead of H_2O_2 and sample in the assay system. Deionised water and ascorbic acid were used as the blank and control, respectively.

Results

3.1. Extraction of Beta-glucan from *Chroococcus turgidus*

Using the above mentioned procedure, the cyanobacterium chrococcus turgidus dried biomass from was analyzed. The extract was used for further analysis. The percentage yield of extracts was calculated. The yield beta-glucan obtained (0.0344g/10g) of 80% methanol extract from the *Chroococcus turgidus* algal dried biomass.

Antioxidant activity of Isloated beta-glucan from *Chroococcus turgidus*

3.1.2 DPPH Activity of *Chroococus turgidus*

Beta-glucan extract of *Chroococus turgidus* were shown significant radical scavenging activity at higher dose. At a concentration of 250 µg, the scavenging activity of methanol extract of *Chroococus turgidus* was 70%. The Table 1 and Figure 1 shows the dose-response curve of DPPH radical scavenging activity of the *Chroococus turgidus.*

3.2.2. Nitric Oxide Scavenging Assay of *Chroococus turgidus*

Beta-glucan extract of *Chroococus turgidus* showed moderately good nitric oxide scavenging activity between 50 and 250µg/ml. At a concentration of 250 µg, the scavenging activity of *Chroococus turgidus* was 78 %. The percentages of inhibitions were increased with increasing concentration of the extracts. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological condition. The Table 1 and Figure 1 showed the dose-response curve of Nitric Oxide Scavenging activity of the *Chroococus turgidus.*

Table 1: Antioxidant activity of *Chroococcus turgidus*

Concentration (µg/ml)	DPPH inhibition (%)	NO_2 inhibition (%)
50	14	16
100	26	30
150	39	45
200	52	67
250	70	78

Figure 1:Antioxidant activity of *Chroococcusturgidus*

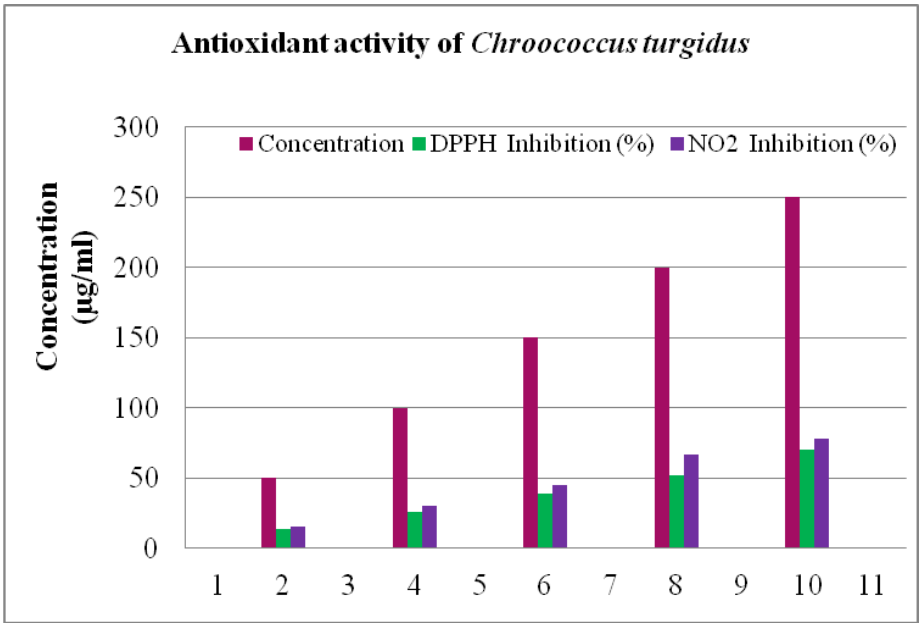

Figure 2:Enzymatic Antioxidant activity of *Chroococcusturgidus*

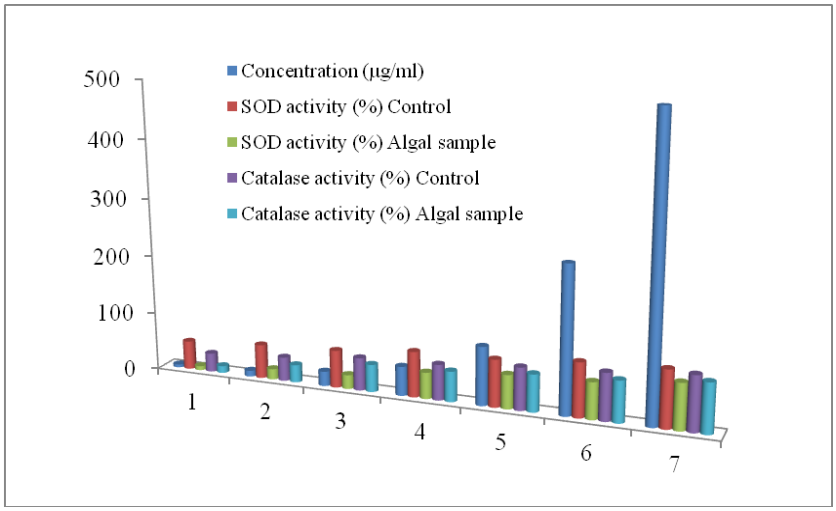

Table 2: Enzymatic Antioxidant activity of *Chroococcus turgidus*

Concentration (µg/ml)	SOD activity (%)		Catalase activity (%)	
	Control	Algal sample	Control	Algal sample
5	49	07	32	12
10	58	18	41	30
25	64	24	56	47
50	78	45	61	52
100	81	58	73	64
250	93	63	81	71
500	98	79	94	85

3.3. Enzymatic Antioxidant of isolated beta-glucan from *Chroococus turgidus*

3.3.1. Superoxide Dismutase of *Chroococus turgidus*

The SOD activity of beta-glucan extract from *Chroococus turgidus* was estimated by using Nitroblue tetrazolium (NBT) method of Beauchamp and Fridovich (1971), were compared with the standard ascorbic acid at different concentrations viz. 5,10,25,50,100,250 and 500µg/ml (Table 2 & Figure 2), the results indicated that, activity of SOD of *Chroococus turgidus*. The levels were found to be 98% super oxide inhibition at concentration of 500 µg/ml.

3.3.2. Catalase Activity of *Chroococus turgidus*

The Catalase activity of beta-glucan extract from *Chroococus turgidus* was estimated by using hydroxyl radical scavenging method of Jin *et al.*, (1996) and were compared with the standard ascorbic acid at different concentrations viz. 5,10,25,50,100,250 and 500µg/ml (Table 2 and Figure 2); the results indicated that, activity of Catalase of *Chroococus turgidus*. The levels were found to be 85(%) hydroxyl inhibitions at concentration of 500µg/ml.

Discussion

The potential antioxidant activity of beta-glucan extracts of *Chroococcus turgidus* was analysed using above mentioned procedure.

Antioxidant activity

The 2, 2-diphenyl-2-picryl hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic and anthocyanins or crude mixture such as the ethanol extract of plants. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The electrons becomes paired off and solution losses colours stochiometrically depending on the number of electrons taken up (**Baskar *et al* 2007).**

Nitric oxide (NO) is a very unstable species, under aerobic condition it reacts with O_2 to produce stable product nitrate & nitrite through intermediates NO_2, N_2O_4 & N_3O_4. In the present study**,** the nitrite produced by the reaction mixture was reduced by the beta glucan extract of *chroococus turgidus***.** This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxi**de (Lalenti *etal.*, 1993**).Nitric oxide (NO) is a potent pleiotropic mediator generated from amino acid L-arginine by vascular endothelial cells, phagocytes and certain cells of brain. The toxicity of NO becomes adverse when it reacts with super oxide radical, forming a highly reactive peroxynitrite anion (ONOO–). The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite (**Tylor *et al* 1997).**

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide.(Zelko et. al.,2002, Banniste et al.,1987).SOD enzymes are present in almost all aerobic cells and in extracellular fluids.((Johnson *et al.*, 2005). There are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese), and finally the Ni type which binds nickel (Wuerges *et al* ,2004).In higher plants, SOD isozymes have been localized in different cell compartments. Mn-SOD is present in mitochondria and peroxisomes. Fe-SOD has been found mainly in chloroplasts but has also been detected in peroxisomes, and CuZn-SOD has been localized in cytosol, chloroplasts, peroxisomes, and apoplast. (Wuerges 2004 and Corpas 2006). According to Journal of Translational Medicine, SOD is an important antioxidant. It not only rejuvenates skin to prevent wrinkles, but also checks the onset of cancer by preventing precancerous cells from becoming aggressive. Superoxide dismutase tends to inactivate free radicals. Free radicals are produced in the body by natural biological processes and can damage cells, proteins and DNA by altering their chemical structure. Researchers have taken up a new quest to use this enzyme as an age-blocking agent and have made some progress in this respect. The human body remains fit and healthy with the help of natural antioxidants. These are derived from the food. It is important that we have a balanced diet so the body develops the strength to quell diseases. 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).Catalase is an antioxidant enzyme that, like superoxide dismutase (SOD) and glutathione peroxidase, is produced naturally within the body. It helps the body to convert hydrogen peroxide into water and oxygen, thus preventing the formation of carbon dioxide bubbles in the blood. Catalase also uses hydrogen peroxide to break down potentially harmful toxins in the body, including alcohol, phenol, and formaldehyde.

The present study showed that the beta-glucan extract of *Chroococus turgidus* has shown significant radical scavenging activity at higher dose. At a concentration of 250 µg, the scavenging activity of beta-glucan ol extract of *Chroococus turgidus* was 70%. This was depicted in the dose-response curve of DPPH radical scavenging activity of the *Chroococus turgidus*.The moderately good nitric oxide scavenging activity was observed between 50 and 250µg/ml. At a concentration of 250 µg, the scavenging activity of *Chroococus turgidus* was 78 %. The results indicated that, activity of SOD of *Chroococus turgidus*, the levels were found to be 98% super oxide inhibition at concentration of 500 µg/ml.

Conclusion

In this study, we examined the antioxidant potentials of Chrocoocus turgidus was examined .the results showed isolated beta glucan from Chrocoocus turgidus showed good activity . Therefore, further investigations need to be

carried out to isolate and identify the antioxidant compounds present in the cyanobacterial extract. Furthermore, the in vivo antioxidant activity of this extract needs to be assessed prior to clinical use.

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