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ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *C.MINUTUS* AND *C.SACCHAROPHILA*

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ABSTRACT

The potential antioxidant activity of methanolic extracts of microalgae, *Chroococcus minutus* and *Chlorella saccharophila* were evaluated *in vitro*. The free radical scavenging activity of the extracts was measured by DPPH and ABTS indicate the antioxidant potential of *C. minutes* and *C. saccharophila*. The antioxidant potential of these microalgal extracts were compared with commercial antioxidants, such as Butylated Hydroxy Toulene (BHT) and L-ascorbic acid. Free radical DPPH and ABTS scavenging potential was higher in *C.minutus* than *C.saccharophila*. From the present study, it can be concluded that the extracts of the microalgae has the antioxidant activity.

Key Words: Antioxidant, Chroococcus minutus, Chlorella saccharophila.

INTRODUCTION

In recent years, the use of photosynthetic microorganism, such as microalgae, in life sciences, has received increasing attention due to their diverse phytometabolic contents with various chemical structures and biological activities (Skulberg, 2006). Antioxidant compounds play an important role against various diseases (e.g., chronic inflammation, antherosclerosis, cancer and cardiovascular disorders) and ageing processes, which explains their considerable commercial potential in medicine, food production and the cosmetic industry (Singh *et al.*, 2005). The use of antioxidants to prolong the shelf life of foodstuffs is ubiquitous. Most of the synthetic antioxidant [butylated hydroxytoulene (BHT)] used today were suspected to be carcinogenic (Namiki, 1990), and there has been a search in recent years to replace these synthetic antioxidants with natural antioxidants. Natural

sources are much safer to use due to less toxicity and side effects, so the production of antioxidant compound from natural sources such as microalgae is in great demand. The present study was to evaluate the antioxidant activity of methanolic extracts of microalgae, *C.minutus* and *C.saccharophila* by measuring their scavenging activity against free radicals.

MATERIALS AND METHODS

Freshwater microalgae *C.minutus* and *C.saccharophila* were obtained from the culture collection centre of Pachaiyappa's College, Chennai. Microalgal biomass was obtained by growing microalgal cultures media (BG11 for *C.minutus* and BBM for *C.saccharophila*). The microalgae was grown for 15 days and harvested.

Antioxidant activities assays

The antioxidant activity of the methanolic extracts of laboratory cultured microalgae was examined. The two free radical systems like DPPH and ABTS were used for assessing the free radical scavenging activity of the test extracts and the results were compared with the standard antioxidant Butylated hydroxytoluene and L-ascorbic acid.

DPPH radical activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging activity was measured by the spectrophotometric method. To a methanolic solution of DPPH (200 µM), 0.05 ml of the test compounds dissolved in methanol were added at different concentrations (100-900 µg/ml). An equal amount of methanol was added to the control. After 20 minutes, the decrease in the absorbance of the test mixture (due to quenching of DPPH free radicals) was read at 517 NM and the percentage inhibition was calculated by using the formula (Prasanth Kumar *et al.*, 2000). The experiment was repeated in triplicates.

$$\text{Inhibition (\%)} = \frac{(\text{Control- test})}{\text{Control}} \times 100$$

ABTS radical cation decolonization assay (Sun *et al*., 2007).

In this improved version, $ABTS^{\cdot-}$, the oxidant is generated by persulfate oxidation of 2, 2-azinobis (3-ethylbenzoline-6-sulfonic acid) - ($ABTS^{2-}$). ABTS radical cation was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulphate and the mixture were allowed to stand in the dark at room temperature for 12-16 hrs before use (Sun *et al.*, 2007). For the study, different concentrations (100-900 µg/ml) of methanolic extract (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with ethanol

to make 1.0 ml. The absorbance was read at 745 nm and the percentage inhibition was calculated using the formula

$$\text{Inhibition (\%)} = \frac{(\text{Control- test})}{\text{Control}} \times 100$$

All the determinations were carried out in triplicates. The IC_{50}, the antioxidant activity in terms of the amount (µg/ml) of the extracts necessary for inhibiting 50% of the cell growth.

RESULTS AND DISCUSSION

DPPH radical scavenging assay

Regarding DPPH, the methanolic extracts of laboratory cultured *C.minutus* and *C.saccharophila* were shown 49.8% and 48.2% of inhibition at 900 µg/ml concentration. The IC_{50} values using DPPH for the methanolic extracts of laboratory cultured microalgae *C.minutus* was 903 µg/ml, *C.saccharophila* was 933 µg/ml, standard BHT was 33 µg/ml and L-Ascorbic acid 73 µg/ml (Table.1). Uma *et al.*, (2011) reported the DPPH radical scavenging action in *Desmococcus* (95.8%) and *Chlorococcum* (93%). Microalgae like *Scenedesmus* sp and *Chlorella* sp contain a rich source of active metabolites with the anticarcinogenic effects (Farouk *et al.*, 2002). In DPPH assay, the antioxidant effect was likely to be due to the hydrogen donating ability of the extract (Conforti *et al.*, 2005). The results showed that the extract has effective radical scavenging activity against DPPH induced radicals. The activities of the laboratory culture of microalgal biomass extracts were dose dependent and characterized by increasing scavenging activity with a rise in sample concentration

ABTS radical scavenging assay

Regarding ABTS assay, the laboratory cultured *C.minutus* exhibited 90.78% and *C.saccharophila* exhibited 91.22% at 900 µg/ml. The IC_{50} values using ABTS for the methanolic extracts of laboratory cultured microalgae *C.minutus* was 278 µg/ml, *C.saccharophila* was 607 µg/ml, standard BHT was 32.5 µg/ml and L-Ascorbic acid 45.1 µg/ml (Table.1).

Table.1 IC_{50} values of standard and experimental microalgae on free radical scavenging system.

S.No.	Standard/ Experimental Microalgae	Free radical scavenging IC_{50} value (µg/ml)	
		DPPH	ABTS
1	BHT	33 ± 0.01	32.5 ± 0.01
2	L-Ascorbic acid	73 ± 0.01	45.1 ± 0.01
3	*C.minutus*	903 ± 0.01	278 ± 0.01
4	*C.saccharophila*	933 ± 0.01	607 ± 0.01

Values are expressed as mean ± SD of triplicates.

The ABTS assay is a measure of the activity of the antioxidant in scavenging proton radicals through a donation of electrons (Mathew *et al.*, 2004). Kovacik *et al.*, (2010) indicate that a variety of phenolic classes are present in the microalgae, but further identification of phenolic components is needed to understand better the differences in the contribution of phenolics to the antioxidant activity of the microalgae. Sanaa Shanab *et al.*, (2011) reported the antioxidant activity of *Oscillatoria* sp. Jimenez-Escrig *et al.*, (2001) described a significant relationship between total antioxidant capacity and total phenolic compounds. However, the report of Li *et al.*, (2007) showed opposite results for the three solvent fractions. The results observed with the methanolic extracts regarding the free radical scavenging potential are advantageous because of their richness in hydrophilic antioxidants and it may be considered as an antioxidant supplement for fish inhabiting contaminated areas and aquaculture farms to alleviate pollutant stress. Therefore, yet, no definite conclusion has arrived at on the role of the antioxidant capacity and therefore further studies are required.

CONCLUSION

The results revealed that the methanolic extracts of the laboratory cultured *C.minutus* and *C.saccharophila* had active radical scavenging activity against DPPH and ABTS. It is suggested that these microalgae (*C.minutus* and *C.saccharophila*) may be used as good sources of natural antioxidant agents. Present findings encourage for further studies for isolating and identifying of active components in microalgae and also *in vivo* studies for their mechanism of action.

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