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ANTI-OXIDANT ACTIVITY OF METHANOL EXTRACT OF *CHAETOMORPHA LITOREA* HARVEY (GREEN SEAWEED) IN KOOTHANKUZHI COAST, TAMIL NADU, INDIA

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ABSTRACT

The present study was undertaken to assess the antioxidant activity of methanol extract of *Chaetomorpha litorea* Harvey collected from Koothankuzhi coast in the south east coast of Tamil Nadu, India. Free radical scavenging activity was evaluated using 1,1-diphenyl2-picryl hydrazyl (DPPH) free radical by the method of Mensor *et al.* and a reducing power by Cupric Reducing Antioxidant Capacity (CUPRAC) assay. The percentage of scavenging activity of DPPH by methanol extract at 100µg, 200µg, 300µg, 400µg and 500µg were 48.14, 59.38, 76.58, 87.27 and 94.64% respectively. At a concentration of 100µg, 200µg, 300µg, 400µg and 500µg of methanol extract, the absorbance were 0.215, 0.272, 0.312, 0.329 and 0.349 respectively. These results similar to those obtained from the DPPH assay in which 500µg showed the highest total antioxidant capacity, followed by 400µg, 300µg and 200µg, and lastly 100µg. The results showed that both DPPH scavenging activity and absorbance were increased when the concentration of methanol extract was also increased. Vitamin C, a strong anti-oxidant was also used as control, and the anti-oxidant potential was compared to all tested samples.

Keywords: *Chaetomorpha litorea*, DPPH, Antioxidant Capacity.

INTRODUCTION

There is ample evidence that reactive oxygen species (ROS) generated in the human body can cause oxidative damages associated with many degenerative diseases such as atherosclerosis, coronary heart diseases, aging and cancer [1]. Reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydroxyl radical (OH), peroxyl radical (ROO) and nitric oxide radical (NO) attack biological molecules such as lipids, proteins, enzymes, DNA and RNA which leads to cell or tissue injury. ROS induce peroxidation of lipids (polyunsaturated fatty acids) generating secondary oxidants like heptanol and hexanal which contributes to oxidative rancidity, deteriorating the flavor of the food. These not only cause a loss in food quality but are also believed to be associated with carcinogenesis, mutagenesis, arthritis, diabetes, inflammation, cancer and genotoxicity [2]. To overcome these problems a wide range of synthetic antioxidants (butylatedhydroxytoluene (BHT), butylated hydrox yanisole (BHA), propyl gallate (PG) and butylated hydroquinone have been used as food preservatives. However, these synthetic antioxidants have side effects such as liver damage and are suspected to be mutagenic and neurotoxic. Hence, most consumers prefer additive-free foods or a safer approach like the utilization of more effective antioxidants of natural origin [3,4]. Recently, various phytochemicals like polyphenols, which are widely distributed in plants, have been reported to act as free radical scavengers [5]. Marine plants like seaweeds also contain high amount of polyphenols. Seaweed constitutes a commercially important renewable resource.

Sargassum, *Padina*, *Dictyota* and *Gracilaria* species can be used as fertilizers, food additives and

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animal feed [6]. Although seaweeds possess wide application in food and in the pharmaceutical industry, the antioxidant activities of many types of seaweed in the South Indian coastal area are still unexplored. The main objective of the present study is to evaluate the antioxidant activity of *Chaetomorpha litorea* Harvey, green seaweed obtained from Koothankuzhi coast in the south east coast of Tamil Nadu, India.

MATERIALS AND METHODS

Collection of Plant Materials

The collection of *Chaetomorpha litorea* Harvey (Figure 1) was made during the low tidal and subtidal regions (up to 1m depth) by hand picking from Koothankuzhi coast in the south east coast of Tamil Nadu, India. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. Then the samples were packed separately in polythene bags in wet conditions and brought to the laboratory, then thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the thalli. They were stored in 5% formalin solution. For drying, washed specimens were placed on blotting paper and spread out at room temperature in the shade. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use 30g powdered samples were packed in Soxhlet apparatus and extracted with chloroform for 8h separately [7].

Antioxidant activity

DPPH Free Radical Scavenging Assay

Methanol extract of *Chaetomorpha litorea* Harvey was analyzed for the antioxidant activity based on the scavenging activity of the 1,1-diphenyl2-picryl hydrazyl (DPPH) free radical using the method of Mensor *et al.* [8]. DPPH is a stable free radical and acts as a scavenger for other radicals. Rate reduction of a chemical reaction using DPPH is a useful indicator of the radical state of a reaction. Methanol extract were prepared in triplicates at different concentrations (100-500µg/ml) and transferred into 1ml of 0.3mM methanolic DPPH solution (Sigma Aldrich). Samples were left to stand for 30 minutes in the light and the absorbance was measured at 517nm, zeroing the spectrophotometer with a methanol blank. The DPPH radical had a dark violet colour solution, and once neutralized, became pale yellow allowing visual monitoring of the radical reaction. Ascorbic acid was used as a positive control from Sigma was also used for a comparison. The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition Percentage} = 1 - \frac{\text{Absorbance of Sample} - \text{Absorbance of Blank}}{\text{Absorbance of Control}} \times 100$$

CUPRAC Assay

The CUPRAC (Cupric Reducing Antioxidant Capacity) method was also applied for the determination of anti-oxidant activity of methanol extract of *Chaetomorpha litorea* Harvey. Copper chloride ($CuCl_2$) solution (0.01M) was prepared by dissolving 0.426g $CuCl_2$ in water and diluting the solution to 250ml. Ammonium acetate (NH_4Ac) buffer (pH 7, 1.0M) was made by dissolving 19.27g of NH_4Ac in water, and diluting this solution to 250ml. Neocuproine (Nc) solution (0.075M) was prepared fresh by dissolving 0.039g Nc in 96% ethanol and diluting to 25ml with ethanol. Methanol extract was prepared in triplicates at different concentrations (100-500µg/ml) and added into a solution containing 1ml $CuCl_2$, 1ml NH_4Ac, 1ml neocuproine and 0.1ml water. Test samples were incubated for 10 minutes at room temperature and the final absorbance was measured at 450nm, zeroing the spectrophotometer with water blank [9].

RESULTS AND DISCUSSION

DPPH Free Radical Scavenging Assay

Crude methanol extract of *Chaetomorpha litorea* Harvey at various concentrations (100-500µg) were tested for antioxidant activity via the DPPH and CUPRAC assays. The experimental results are illustrated in Tables and Figures, where methanol extract was established to possess antioxidant activity. Vitamin C was used as a positive control for the DPPH assay. Antioxidant activity was determined by assaying the reduction of DPPH radicals. The inhibition percentage of all tested samples showed a concentration dependent pattern as shown in Table 1 and Figure 2. The percentages of anti-oxidant property of the methanol extracts at concentrations ranging from 100-500µg, however, were lower than vitamin C. Vitamin C had over 90% scavenging activity at a concentration of 100µg, whereas the tested methanol extract required a concentration of 500µg to reach a similar percentage. The percentage of scavenging activity of DPPH by methanol extract of *Chaetomorpha litorea* Harvey at 100µg, 200µg, 300µg, 400µg and 500µg were 48.14, 59.38, 76.58, 87.27 and 94.64% respectively. Among the various concentration of methanol extract used, 500µg methanol extract of *Chaetomorpha litorea* Harvey had the strongest scavenging ability while 100µg methanol extract of *Chaetomorpha litorea* Harvey had the lowest. The results showed that the scavenging activity was increased when the concentration of methanol extract was also increased. Vitamin C, a strong anti-oxidant was also used as control, and the anti-oxidant potential was compared to all tested samples.

CUPRAC Assay

Table 2 showed the reducing power of methanol extract of *Chaetomorpha litorea* Harvey on copper ions

using the CUPRAC assay. Higher absorbance readings indicated higher reducing ability of the samples. All samples exhibited the ability of reducing coppers ions from Cu(II) to Cu(I) in a concentration dependent manner. 500µg methanol extract showed the highest reducing activity when compared to the other concentration of methanol extract. At a concentration of 100µg, 200µg, 300µg, 400µg and 500µg, the absorbance were 0.215, 0.272, 0.312, 0.329 and 0.349 respectively. These results similar to those obtained from the DPPH assay in which 500µg showed the highest total antioxidant capacity (TAC), followed by 400µg, 300µg and 200µg, and lastly 100µg. The results showed that the absorbance and anti-oxidant activity was increased when the concentration of methanol extract was also increased. Vitamin C, a strong anti-oxidant was also used as control, and the anti-oxidant potential was compared to all tested samples.

Table 1. Scavenging effects on DPPH free radical by various concentrations of methanol extract of *Chaetomorpha litorea* Harvey and Vitamin C

Concentration (µg)	Percentage of anti-oxidant effect on DPPH	
	Vitamin C	Methanol Extract
100	90.69±2.11	48.14±0.29
200	93.24±1.43	59.38±0.38
300	99.57±2.99	76.58±1.73
400	99.92±1.76	87.27±0.82
500	99.98±2.34	94.64±0.35

Table 2. CUPRAC assay by various concentrations of methanol extract of *Chaetomorpha litorea* Harvey and Vitamin C

Concentration	Wave length (nm)	ABSORBANCE(nm)	
		Vitamin C	Methanol Extract
100µg	450	0.318±0.002	0.215±0.011
200µg	450	0.356±0.001	0.272±0.017
300µg	450	0.415±0.001	0.312±0.009
400µg	450	0.446±0.003	0.329±0.054
500µg	450	0.569±0.002	0.349±0.028

CONCLUSION

The methanolic extract of *Chaetomorpha litorea* Harvey, an important green seaweed (Chlorophyceae) showed significant anti-oxidant efficacy which is evident by the results obtained. Among the various concentrations of methanolic extracts investigated, 500µg methanolic extract had the highest effect than other concentrations such as 400, 300, 200 and 100µg. However further studies required to predict the exact mechanism of action and the structure of the secondary metabolites which is responsible for anti-oxidant activity for the development as potent anti-oxidant drug.

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