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Research Article

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Evaluation of Antioxidant properties of Marine Sea Weed samples by DPPH method

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ABSTRACT

Marine resources in the recent years have attracted attention in the search for bioactive compounds to develop new drugs and healthy foods. In particular, seaweeds are a very important and commercially valuable resource for food, fodder, soil conditioners and pharmaceuticals thus this study aims to evaluate the antioxidant activity of seaweeds. Seaweeds belong to a group of plants known as algae. Seaweeds are classified as Rhodophyta, Phaeophyta or Chlorophyta depending on their nutrient and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health. Ten types of seaweeds namely Cymodeace rotundata, Acanthopora spicifera, Ulva lactuca, Ulva reticulate, Turbinaria conoides, Gracillaria edulis, Kappaphycus alvarezii, Gracillaria crassa, Gracillaria foliifera and Cymodeace serrulata were used in the study. The extracts were prepared with Methanol and petroleum ether. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays were used to determine antioxidant properties of seaweeds by measuring the decrease in absorbance at 517 nm. In methanol extract extract Cymodeace rotundata, Gracillaria crassa and Cymodeace serrulata showed the highest total antioxidant activity of compared with other samples. Ulva lactuca exhibited the highest antioxidant and free radical scavenging activities in petroleum ether extract.

Keywords: *DPPH, Sea weeds/Marine Algae, Antioxidant Compounds, Solvent Extracts.*

INTRODUCTION

The ocean is considered to be a great source of potential drugs. As a result of the potential for new drug discovery, Marine natural products have attracted the attention of biologists and chemists the world over for the past five decades. Marine organisms are a rich source of structurally novel and biologically active metabolites. Seaweeds refer to any large marine benthic algae that are multicellular, macrothallic, and macroscopic anchored to the bottom of the ocean or on to solid substrata and thus differentiated from most algae that are of microscopic size. Seaweeds are classified into brown algae (Phaeophyta), red algae (Rhodophyta) and green algae (Chlorophyta).

The ability of Seaweeds to produce secondary metabolite of potential interest has been extensively documented. Seaweeds have shown to exhibit a broad range of biological activities, such as Antibacterial, Antifungal, Antiviral, Antitumor, Anti helminthic, Anti inflamatory and even Neurotoxin properties[1,2]. Antioxidant substances which scavenge free radicals play an important role in the prevention of free radical-induced diseases. By donating hydrogen radicals, the primary radicals are reduced to nonradical chemical compounds and are then converted to oxidize antioxidant radicals[3,4,5,6].

Seaweeds are rich source of variety of nutrients and bioactive components [7]. Seaweeds constitute one of the major components of diet in several Asian countries. The consumption of seaweed as a part of diet has been shown to be one of the reasons for low incidence of breast and prostrate cancer in Japan and China

compared to North America and Europe[8]. Seaweeds are commonly used as sushi wrappings, seasonings, condiments and vegetables and can thus constitute between 10% and 25% of food intake by most Japanese[9,10]. *In vivo* studies of sea weeds have demonstrated the anti-cancerous, anti-obesity, anti-infl ammatory and anti-proliferative effects from their components[11,12].

The sea weeds samples for the present investigation were collected from Gulf of Mannar as it is a Marine Biosphere Reserve situated along the east coast of India and Sri Lanka, with an area of about 10,500 sq. km with a luxuriant growth of about 680 species of seaweed belonging to the Rhodophyta, Pheaophyta and Chlorophyta, in both the inter-tidal and deep water regions.

MATERIALS AND METHODOLOGY

Sample collection:

The samples were collected from Gulf of Mannar during low tides and also by deep sea divers in clean containers and were transported to the lab in refrigerating condition for further processing and investigation. The specimens were identified at Mandapam Regional Centre Marine Fisheries Research Institute, Tamilnadu.

Solvent Extraction:

The samples were washed thrice in fresh water and rinsed in distilled water and shade dried and blended into fine powder and 10grams of the powder was mixed with 100ml of solvents like methanol and petroleum ether. The samples were then kept in shaking condition for 48-72 hours. After extraction the solvents were evaporated in hot water bath at the boiling temperature of the particular solvent. The residual extract was used for further analysis.

DPPH Assay:

The free radical scavenging activity was measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH). This method was based on the reduction of stable DPPH radical antioxidants in a methanol solution. In the presence of antioxidants, the reduction of the DPPH radical solution was monitored. 1 ml of 0.1 mM DPPH solution was taken and 1 ml of different concentration (100 – 1000 µg/ml) of sample was added. The reaction mixture was incubated on dark for 30 minutes. The change of colour from purple to yellow was read at $A_{517nm.}$ 0.1 mM DPPH + methanol solution was taken as control. L- Ascorbic acid was taken as a standard antioxidant. Each antioxidant activity assay was done in triplicates from the same extract in order to determine their reproducibility.

The percentage of scavenging was calculated as follows;

Radical scavenging activity (%) = $[(A_0-A_1/ A_0)* 100]$

Where A_0= Absorbance of control; A_1= Absorbance of sample.

RESULTS

The specimens were identified as *Cymodeace rotundata, Acanthopora spicifera, Ulva lactuca, Ulva reticulate, Turbinaria conoides, Gracillaria edulis, Kappaphycus alvarezii, Gracillaria crassa, Gracillaria foliifera* and *Cymodeace serrulata.*

Acanthopora spicifera

Cymodoceae rotundata

Cymodoceae serrulata

Gracillaria foliifera

Gracillaroa crossa

Gracillaroa edulis

Kappaphycus alvarezii

Turbinaria conoides

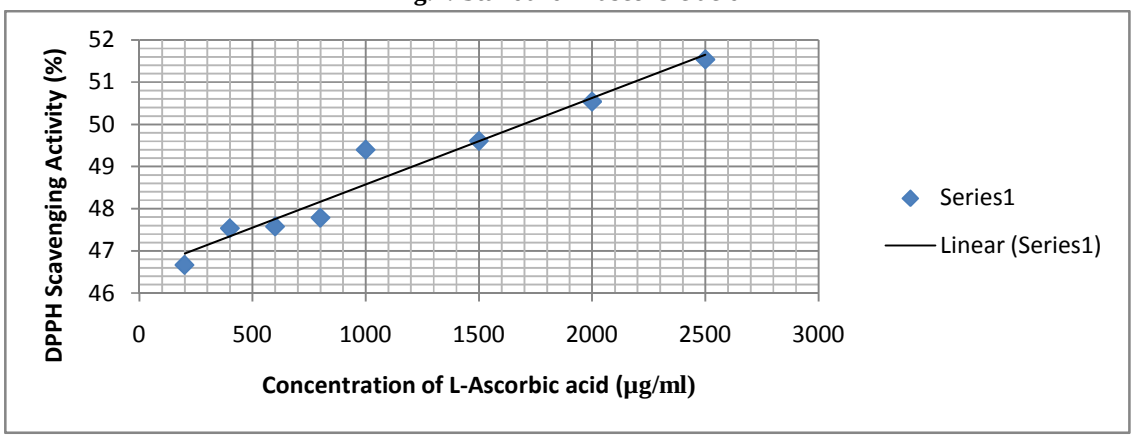
Ulva lactuca

Ulva reticulate

Antioxidant assay:

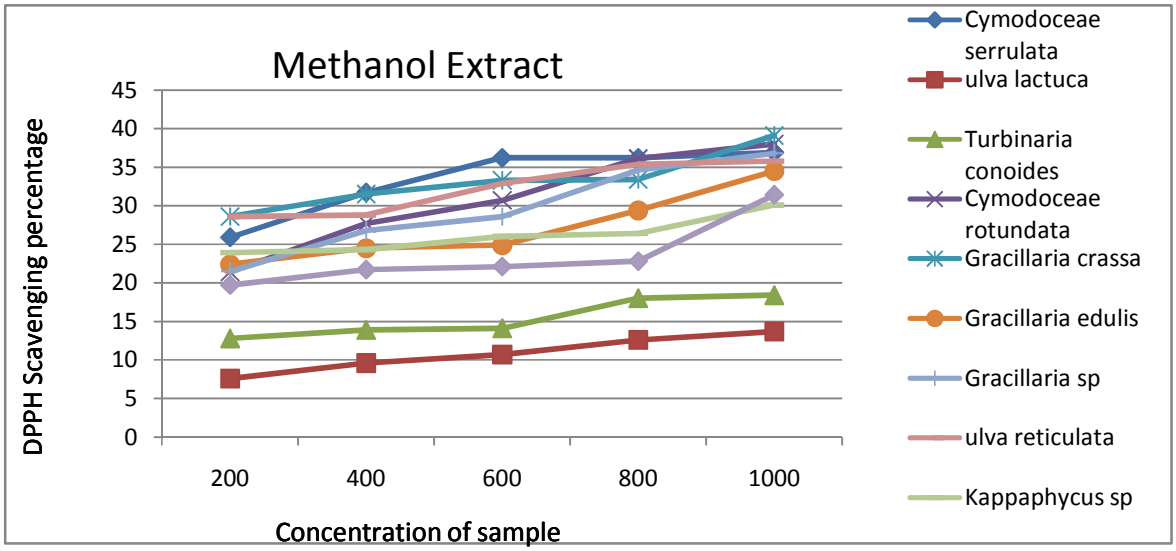
The results of the antioxidant assays are depicted in the figures-1 for the DPPH standard L-Ascorbic acid figure-2 for methanolic extract and figure-3 for petroleum ether extract.

Fig.1: Standard L-ascorbic acid



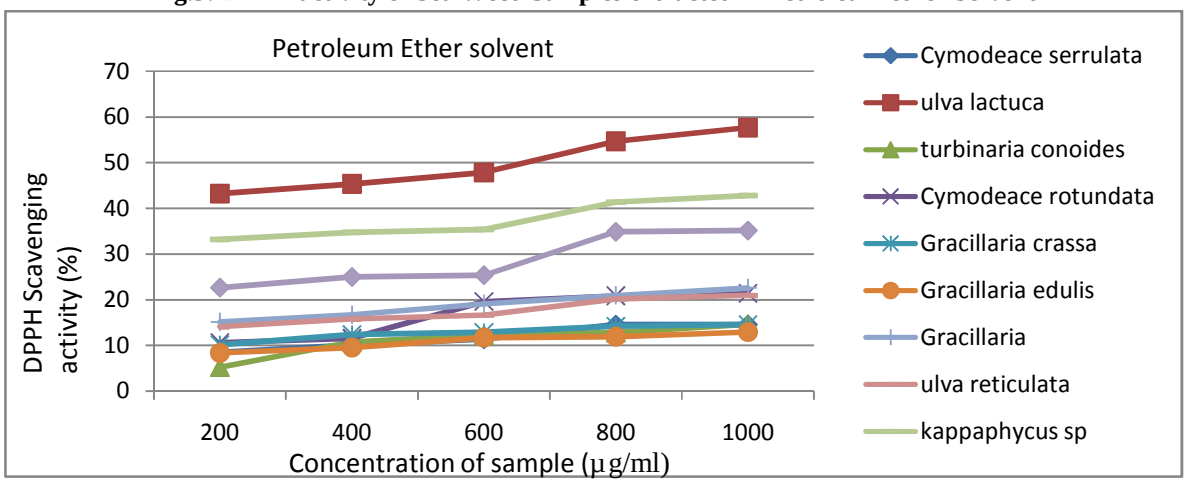
L-ascorbic acid was used as the standard for DPPH reduction in different concentrations and it showed reduction from 46-52%. The reduction was directly proportional to the concentration of the standard.

Fig.2: DPPH activity of Sea Weed Samples extracted in Methanol solvent



The marine sea weed samples were checked for its antioxidant activity by DPPH method, the methanolic extracts of the samples were checked in different concentrations from 200-1000µg. The DPPH reduction took place with increase in concentration of the sample indicating that the samples had antioxidant property. Cymodeace rotundata, Gracillaria crassa and Cymodeace serrulata showed higher DPPH activity with methanolic extract. Cymodeace rotundata, Gracillaria crassa and Cymodeace serrulata reduced DPPH upto 35% from 23%. Ulva lactuca showed least antioxidant activity with a maximum reduction of 14% with 1mg of concentration. Acanthopora spicifera, Gracillaria edulis, Kappaphycus *alvarezii* showed reduction upto 32%. Turbinaria conoides showed DPPH reduction from 12-19%. The methanolic extract of the samples are required above 1mg concentration to reduce DPPH to its 50% concentration.

Fig.3: DPPH activity of Sea Weed Samples extracted in Petroleum ether Solvent



The petroleum ether solvent extracted samples in different concentration were checked for antioxidant property by DPPH assay. DPPH reduced with increase in the concentration of all the samples. Ulva lactuca sample showed highest DPPH reduction followed by *Kappaphycus alvarezii, Gracillaria edulis* showed the least DPPH reduction. Ulva lactuca showed 50% of DPPH reduction above 750µg of sample. Other extracts showed DPPH reduction below 50% for even 1mg concentration. Ulva lactuca showed reduction from 42-60%, kappaphycus sp., showed from 33-42%, Acanthopora spicifera showed reduction ranging from 22-36%, the other samples showed reduction from 8-22% for different concentrations.

DISCUSSION

The antioxidant property of seaweed *Sargassum hemiphyllum* was evaluated and was found that the antioxidant activity was increasing in correlating with the concentration below 3.5 mg/ml [13]. Similar results were observed in the present study with antioxidant activity increasing in the lower concentrations. [14] studied the antioxidant property of *Gelidiella acerosa* (Rhodophyta), *Gracilaria edulis* (Rhodophyta), *Turbinaria conoides* (Phaeophyta), *Padina gymnospora* (Phaeophyta), *Chondrococcus hornemanni* (Rhodophyta), *Hypnea pannosa* (Rhodophyta), *Dictyota dichotoma* (Phaeophyta), *Jania rubens* (Rhodophyta), *Sargassum wightii* (Phaeophyta) and *Haligra* sps and showed that the methanolic extracts of all the seaweeds exhibited DPPH scavenging activity, the most effective being *G.acerosa* which exhibited significantly higher DPPH scavenging activity of 72.5% inhibition followed by *Haligra* species with DPPH inhibition of 55%. In the present investigation methanolic extracts showed antioxidant activity for all the samples analysed.

Antioxidant activity of extracts from brown and red seaweeds of Indian origin was studied and found that brown seaweeds showed higher peroxyl radical scavenging activity compared to red seaweeds [15]. In fractions from brown seaweed extracts, highest activity was observed in ethyl acetate fraction followed by hexane fraction. [16] studied in vitro antioxidant activities of sulfated polysaccharides from *Fucus vesiculosus* and *Padina gymnospora.*

REFERENCES

1. Ito K, Hori K. Seaweed: Chemical composition and potential food uses. *Food Rev Int* **5**:101–144 (1989)
2. Pisani, P. Bray, F. Parkin, D.M. Estimates of the world-wide prevalence of cancer for 25 sites in the adult population. *Int J Cancer* **97**:71–81.
3. Jadhav, S.J. Nimbalkar, S.S. Kulkarni, A.D. & Madhavi, D.L. Lipid Oxidation In Biological and Food Systems. In: Food Antioxidants. Madhavi DL, Deshpande SS & Salunkhe DK (eds). New York. (1995)
4. Yamaguchi, T. Takamura, H. Matoba, T. & Terao, J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1, 1-diphenyl-2-picrylhydrazyl. *Biosci Biotechnol Biochem* **62**: 1201-1204 (1998)
5. Pai-An Hwang, Chwen-Herng Wu, Shu-Yun Gau, Shih-Yung Chien and Deng-Fwu Hwang, "Antioxidant and immune-stimulating activities of hot-water extract from seaweed *Sargassum hemiphyllum"*, *Journal of Marine Science and Technology*, **18(1)**: pp. 41-46 (2010)
6. Kasi Pandima Devi, Natarajan Suganthy, Periyanaina Kesika and Shanmugaiahthevar Karutha Pandian, "Bioprotective properties of seaweeds: *In vitro* evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content", *BMC Complementary and Alternative Medicine*, **8**:38. (2008)
7. Sachindra, N.M. Airanthi, M.K.W.A, Hosokawa, M. Miyashita, K.,"Radical scavenging and singlet oxygen quenching activity of extracts from Indian seaweeds", *J Food Sci Technol*, **47(1)**: 94–99 (2010)
8. Skibola, C., The effect of Fucus vesiculosus, an edible brown seaweed, upon menstrual cycle length and hormonal status in three pre-menopausal women: a case report. *BMC Complementary and Alternative Medicine* **4**, 10–17 (2004)
9. Teas, J., The consumption of seaweed as a protective factor in the etiology of breast cancer. *Medical Hypotheses* **7**: 601–613 (1981)
10. Micheline Cristiane Rocha de Souza, Cybelle Teixeira Marques, Celina Maria Guerra Dore, Fernando Roberto Ferreira da Silva, Hugo Alexandre Oliveira Rocha and Edda Lisboa Leite, "Antioxidant activities of sulfated polysaccharides from brown and red seaweeds", *J Appl Phycol* **19**:153–160 (2007)

11. Miyashita, K. Seaweed carotenoid fucoxanthin with highly bioactive and nutritional activities. *J Mar Biosci Biotechnol* **1**: 48–58 (2006)
12. Yuan, Y.V. Walsh, N.A. Antioxidant and antiproliferative activities of extracts from variety of edible seaweeds. *Food Chem Toxicol* **44**:1144–1150 (2006)
13. Fujimoto, K. and Kaneda, T. Separation of antioxygenic (antioxidant) compounds from marine algae. *Hydrobiologia*, **116**: 111–3 (1984)
14. Matsukawa, R., Z. Dubinsky, E. Kishimoto, K. Masaki, Y. Masuda, T. Takeuchi, M. Chihara, Y. Yamamoto, E. Niki and I. Karube, A comparison of screening methods for antioxidant activity in seaweeds. *J. Appl. Phycol*., **9:** 29–35 (1997)
15. Lim, S.N., P.C.K Cheung, V.E.C. Ooi and P.O. Ang, Evaluation of antioxidant activity of extracts from a brown seaweed, *Sargassum siliquastrum. J. Agric. Food Chem.,* **50**: 3862–6 (2002)
16. Xue, Z., C.H. Xue, Z.J. Li, Y.P. Cai, H.Y. Liu and H.T. Qi, Antioxidant and hepatoprotective activities of low molecular weight sulfated polysaccharide from *Laminaria japonica*. *J. Appl. Phycol.*, **16**: 111–5 (2004)