A STUDY ON ANTIOXIDANT PROPERTIES OF SEAWEEDS BY HYDROGEN PEROXIDE ASSAY

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ABSTRACT:

Marine organisms are emerging as good candidates as an alternate source for bioactive substances in pharmaceutical industry and also as a source of food and other health aspects.Seaweeds belong to a group of plants known as algae. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health. In the present investigation, sea weeds samples were collected from Kanyakumari South coast of India and identified as Gracillaria crassa, Gracillaria edulis, Cymodoceae rotundata,Cymodoceae serrulata, Ulva lactuca, Ulva reticulata, Gracillaria foliifera, Gelidiella accrosa, Turbinaria conoides, Kappaphycus alvarezii and Acanthopora spicifera. The antioxidant property for the methanolic and petroleum ether extract of the samples was analysed by hydrogen peroxide assay and it was found that all the samples had antioxidant property. The methanolic extract showed better property then the petroleum ether extracts. The methanolic extract of Acanthopora spicifera showed the highest antioxidant activity where in 1mg/ml of the extract scavenged 18% of hydrogen peroxide followed by Ulva lactuca which showed 17% of scavenging activity. Gracillaria crassa showed least antioxidant activity.

Key Words: Antioxidant, Hydrogen Peroxide assay, Sea weeds, Gracillaria crassa.

1. INTRODUCTION

All living organisms contain complex systems of antioxidant enzymes. Some of these systems, e.g. the thioredoxin system, are conserved throughout evolution and are required for life. Antioxidants in biological systems have multiple functions, including defending against oxidative damage and participating in the major signalling pathways of cells. One major action of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species. Reactive oxygen species include hydrogen peroxide (H_2O_2), the superoxide anion (O_ 2), and free radicals, such as the hydroxyl radical (_OH). These molecules are unstable and highly reactive, and can damage cells by chain reactions, such as lipid peroxidation, or formation of DNA adducts that could cause cancer-promoting mutations or cell death. In order to reduce or prevent this damage, all cells invariably contain antioxidants. Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food and beverages. It is widely used as a bleaching agent in the textile, paper and pulp industries. Human beings exposed to H_2O_2 indirectly via the environment are estimated as 0.28 mg/kg/day with intake from leaf crops

contributing most to this exposure. Hydrogen peroxide enters the human body through inhalation of vapor or mist and through eye or skin contact. In the body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\cdot}$) that can initiate lipid peroxidation and cause DNA damage.

Seaweeds belong to a group of plants known as algae. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health (Kuda *et al.*, 2002).Seaweeds are known for their richness in polysaccharides, minerals and certain vitamins (Arasaki and Arasaki 1983), but they also contain bioactive substances like polysaccharides, proteins, lipids and polyphenols, with antibacterial, antiviral and antifungal properties, as well as many others (Kumar *et al*. 2008b). This gives seaweed great potential as a supplement in functional food or for the extraction of compounds.

The Gulf of Mannar is a Marine Biosphere Reserve situated along the east coast of India and Sri Lanka, an area of about 10,500 sq. km which has 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. Seaweed constitutes a commercially important marine renewable resource. *Sargassum, Padina, Dictyota* and *Gracilaria sps*. are used by common people as fertilizers, food additives and animal feed (Qasim 1998).Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterised by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae (Yuan *et al.*, 2005; Bansemir *et al.*, 2006; Chew *et al.*, 2008).

2. MATERIALS AND METHODOLOGY:

Collection of Samples:

The Seaweeds sp are collected a depth of 5–10m by SCUBA diving at Mantapam to Kanyakumari South coast of India. The samples were placed inside sterile ethyl polythene bags underwater and transferred to the lab aseptically in iceboxes. The sea weed samples were washed with tap water several times and shade dried, powdered with a blender and stored in an air tight container and kept in a room temperature for further study. The samples were authenticated by J. R. Ramalingam, Former technical officer, Mandapam regional center of central Marine Fisheries Research Institute, Tamil Nadu, India as *Gracillaria crassa, Gracillaria edulis, Cymodoceae rotundata, Cymodoceae serrulata, Ulva lactuca, Ulva reticulata, Gracillaria foliifera, Gelidiella accrosa, Turbinaria conoides*, *Kappaphycus alvarezii and Acanthopora spicifera.* The samples were cut into small pieces and sun dried. The dried samples were then powdered using a blender.

Solvent extraction:

2gm of each sample powder was added to 20ml of solvent and kept for 48hrs with slight shaking condition. Here, methanol and petroleum ether was used as a solvent. After 48hrs, extract were filtered by using whattmann no1 filter paper to get filtrate. The filtrate collected was evaporated at particular boiling point of the solvent to get dry powder. The powder was then

redissolved in respective solvents to get a final concentration of 1mg/ml. These stocks extracts were refrigerated until further use.

HYDROGEN PEROXIDE SCAVENGING CAPACITY:

The ability of the extracts to scavenge hydrogen peroxide was determined according to the standard method. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Extracts (200, 400, 600, 800, 1000 µg/ml) in the methanol solvent were added to a hydrogen peroxide solution (0.6ml, 40mM). Absorbance of hydrogen peroxide at 230nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both seaweeed extracts and standard compounds were calculated:

$$\% \text{ Scavenged } [H_2O_2] = [(A_C - A_S) / A_C] * 100$$

Where A_C is the absorbance of the control and A_S is the absorbance in the presence of sample extracts or standards.

3. RESULTS:

The sea weed samples *Gracillaria crassa, Gracillaria edulis, Cymodoceae rotundata, Cymodoceae serrulata, Ulva lactuca, Ulva reticulata, Gracillaria foliifera, Gelidiella accrosa, Turbinaria conoides*, *Kappaphycus alvarezii and Acanthopora spicifera* were collected from Kanyakumari South coast of India.

Figure 1: *Kappaphycus alvarezii*

Hydrogen Peroxide Assay:

Methanolic Extract:

The antioxidant property of the methanolic extract of the samples was checked by determining the hydrogen peroxide scavenging percentage along with ascorbic acid as the standard. The methanolic extract of all the samples showed scavenging activity against hydrogen peroxide, the activity increased with the increase in the concentration of the samples. Few extracts showed higher antioxidant property when compared to the standard L-ascorbic acid. *Acanthopora spicifera* showed the highest antioxidant activity where in 1mg/ml of the extract scavenged 18% of hydrogen peroxide followed by *Ulva lactuca* which showed 17% of scavenging activity. The methonlic extract of *Gracillaria crassa* showed the least antioxidant activity.

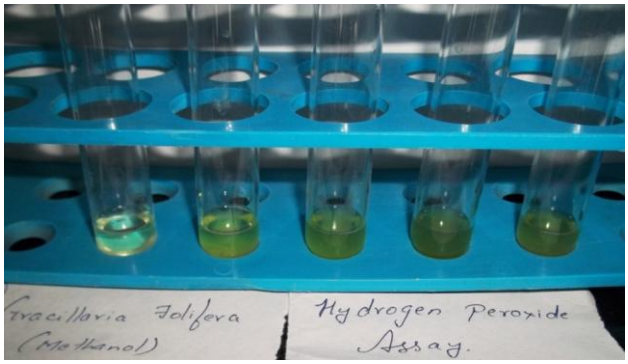

Figure 2: Hydrogen peroxide assay of methanolic *Gracillaria folifera* extract.

Petroleum Ether Extract:

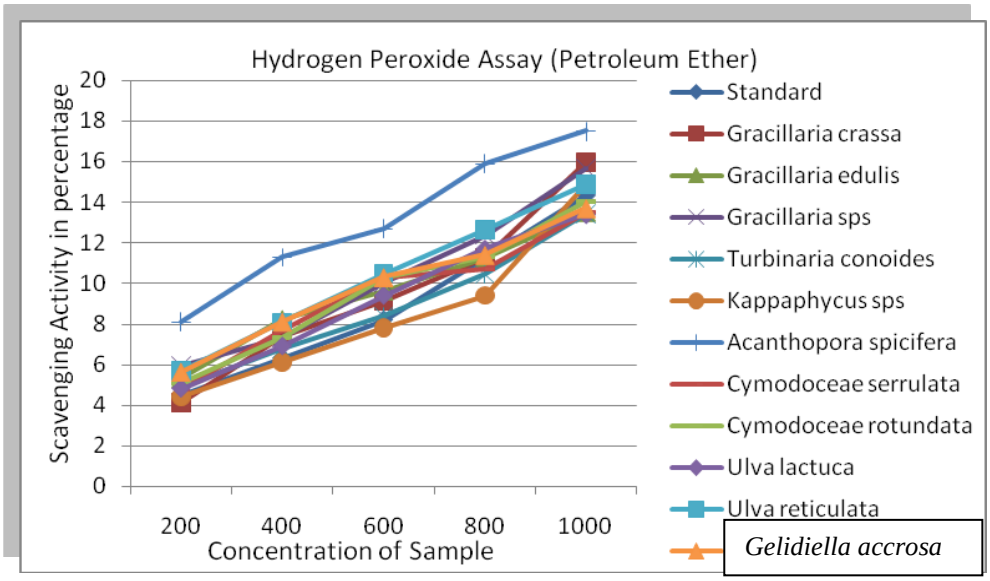

Figure 3: Hydrogen peroxide assay of Petroleum Ether extract.

The hydrogen peroxide assay was carried out to determine the antioxidant property of petroleum ether extract of the samples. *Acanthopora spicifera* showed the highest antioxidant activity followed by *Gracillaria crassa*. These samples showed higher antioxidant activity when compared to the standard. The least activity was seen by petroleum ether extract of *Gelidiella accrosa*. The antioxidant activity of all the samples showed increase in the activity with the increase in the concentration of the samples (Figure 3).

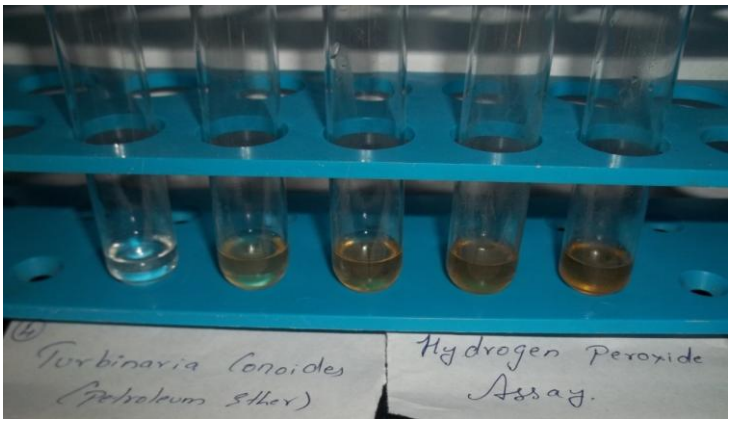

Figure 4: Hydrogen peroxide assay of Petroleum ether *Turbinaria conoides* extract.

4. DISCUSSION:

Seaweeds are rich in polysaccharides, minerals, proteins and vitamins. Documented antioxidant activity would elevate their value in the human diet as food and pharmaceutical supplements (Yan, Nagata, & Fan, 1998). Few reports are available on the antioxidant potential of seaweeds (Jimenez-Escrig *et al*., 2001). Ismail and Hong (2002) reported antioxidant activity of four commercial edible seaweeds, namely Nori (*Porphyra* sp.), Kumbu (*Laminaria* sp.), Wakame (*Undaria* sp.) and Hijiki (*Hijikia* sp). Zaragoza *et al*. (2008) investigated the antioxidant behaviour of two extracts from brown seaweed *F. vesiculosus* using either 30–35% ethanol or 50–70% ethanol. The 30–35% ethanol extract exhibited more potent antioxidant behaviour than the 50–70% ethanol extract, showing superior DPPH, superoxide, peroxyl and ABST radical scavenging ability. The 50–70% ethanol extract was found to increase the reducing power and superoxide scavenging ability in the plasma of laboratory rats. The 50–70% ethanol extract was also found to stimulate the immune system of the animals tested by increasing the Cu–Zn SOD activity by approx. 32% (Dembitsky and Srebnik 2002).

Investigations on crude extracts and phenol content show that the reducing power and hydroxyl radical scavenging activity of some seaweed species (*Eucheuma*, *Kappaphycus* and *Turbinaria conoides*) are found to be higher compared to standard antioxidant (a-tocopherol). In several experiments, the total phenol content of several seaweed species was significantly different in three out of six seaweed species tested. The in vitro antioxidant activities of methanol extracts of all six species tested exhibited dose dependency and increased with increasing concentration of the extract (Ganesan and CS Bhasker 2008; Kumar *et al*. 2008a).

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