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ANTI OXIDANT POTENTIAL OF FOUR CHLOROPHYCEAN MEMBERS FROM KERALA COAST

Biochemistry

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ABSTRACT

This study aims to evaluate the antioxidant activity (DPPH radical scavenging assay**,** Nitric oxide scavenging assay (NO assay), Superoxide radical scavenging assay) of seaweeds available in selected regions of Kerala coast. Four seaweeds of the class Chlorophyceae *viz., Chaetomorpha antennina, Chaetomorpha area, Ulva lactuca* and *Valoniopsis pachynema* were selected for the present work. The algal extracts were prepared in methanol for doing these assays. The antioxidant property was analyzed as percentage of inhibition. It was found that *Ulval actuca* showed highest radical scavenging activity in DPPH assayand also in NO scavenging assay.*Chaetomorpha antennina* exhibited lower percentage of inhibitionin all the three assays compared to the other three algal samples.

KEYWORDS:

Antioxidant Activity, DPPH Radical Scavenging Assay, Nitric Oxide Scavenging Assay, Superoxide Radical Scavenging Assay

INTRODUCTION

Seaweeds or benthic marine algae and are the group of plants that live either in marine or brackish water. Like the land plants seaweed contains photosynthetic pigments and with the help of sunlight and nutrient present in the seawater, they photosynthesize and produce food. Seaweeds are found in the coastal region between high tide to low tide and in the sub-tidal region up to a depth where 0.01 % photosynthetic light is available. Plant pigments, light, exposure, depth, temperature, tides and the shore characteristics combine to create different environment that determine the distribution and variety among seaweeds. They are basically classified according to their pigmentation into three main groups i.e. green (Chlorophyta), brown (Phaeophyta) and red (Rhodophyta). It has been used as food, fertilizer and for medicinal purposes for a long time. Like other plants, seaweeds contain various kinds of inorganic and organic substances which probably benefit human health. It has been reported that seaweeds contain high levels of minerals, vitamins, essential amino acids, indigestible carbohydrates, and dietary fiber (Jiménez-Escrig and Goni, 1999). In food manufacturing, seaweeds have been developed as raw or semi-processed food products (Mabeau and Fleurence, 1995). Now emerging interest is observed in the field of nutritional sciences due to the presence of antioxidant substances in fresh and processed foods.

The use of seaweed as food and medicine prior to 2000 BC found mention in ancient Chinese medicinal literature (Abbott, 1996). Seaweeds also have a number of secondary metabolites that serve as chemical defense mechanisms against herbivores and fouling (De Nys *et al.,* 1998, De Lara-Isassi *et al.,* 2000). It is thus highly probable that algae have the potential to provide an alternative source of leads in solving many biomedical problems, including oxidative damage (Ruberto *et al.,* 2002). Phenolic compounds play an important role in the antioxidative properties of many plant derived antioxidants (Canadanovic-Brunet *et al.,* 2006; Farombi *et al.,* 2000; Kaur and Kapoor 2001) and phenolic substances were also reported to possess a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory and vasodilator actions. The antioxidant effect of naturally occurring phenolic components has previously been studied in relation to the prevention of coronary diseases and cancer, as well as for age related degenerative brain disorders (Stoclet *et al.,* 2004; Stevenson and Hurst, 2007). The present study is an attempt to assess the antioxidant potential of four Chlorophycean members from Kerala coast.

MATERIALS AND METHODS

Sample collection and preparation

The algal samples collected for the present study was *Chaetomorpha antennia*, *Chaetomorpha area*, *Ulva lactuca* and *Valoniopsis pachynema* which are collected from two coastal regions of Kerala at the time of low tides *viz*., Thikkody and Thirumullavaram. *Chaetomorpha area* and *Ulva lactuca* were collected from Thikkody and *Chaetomorpha antennina* and *Valoniopsis pachynema* were from Thirumullavaram. The collected seaweeds were washed thoroughly with seawater to remove all the unwanted impurities, adhering sand particles and epiphytes and brought to the laboratory. They were shade dried for 7-10 days, and grounded to fine powder. The powdered samples were subjected for different antioxidant assays.

Preparation of extract

10 gm of the powders were extracted with methanol in a soxhlet extractor and were concentrated under reduced pressure and the resultant residue was stored in dark at 4ºC until further use. The antioxidant potential of selected members of chlorophyceae were determined using DPPH radical scavenging activity (1, 1-diphenyl-2 picrylhydrazyl**),** NO scavenging assay (Nitricoxide Radical), SOR scavenging assay (Superoxide Radical).

DPPH radical scavenging Assay

The radical scavenging activity of the seaweeds against free radical DPPH was analyzed using methanol extracts. DPPH are stable free radicals that accept hydrogen to become a stable diamagnetic molecule. Hence DPPH is usually used as a substrate to evaluate the antioxidant activity (Elimastas *et al*., 2006). Stock solutions of the crude extract were prepared by dissolving the extract using methanol as a solvent. Different concentrations of the extract used were 0.2, 0.4, 0.6 and 1.0 mg/ml for the assay. An aliquot of 0.1ml of various concentrations of methanol fraction was added to 0.9ml of freshly prepared 1.5mM DPPH solution. The reaction mixture was incubated in dark for 20 minutes at room temperature. Absorbance was taken at 517nm on UV-VIS Spectrophotometer, using methanol as blank. The addition of samples to the DPPH solution will induce a decrease in the OD value accordingly. The decrease in absorbance was converted to percentage radical scavenging antioxidant activity (%RSA). Percentage of inhibition was calculated using the formula: Absorbance of blank – Absorbance of sample / Absorbance of blank X 100 (Wang *et al.,* 2011).

Superoxide Radical Scavenging Activity

The superoxide radical scavenging activity of the test sample was studied using the method of Liu *et al.* (1997) with slight modifications. Superoxide radicals are generated in phenazinemethosulphate (PMS) - (Nicotinamide adenine dinucletide (NADH) systems by oxidation of NADH and assayed by the reduction of Nitro Blue Tetrazolium (NBT). 200.0 µl of test samples of different concentrations were taken in a series of test tube. Superoxide radicals were generated by 1.0 ml of Tris-HCl buffer (16.0 mM, pH-8.0), 1.0 ml of NBT (50.0 µM), 1.0 ml NADH (78.0 µM) solution and 1.0 ml of PMS (10 µM). The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560 nm was measured. A control tube containing Tris-HCl buffer was also processed in the same way without test sample.

Nitric Oxide Radical Scavenging Assay

Nitric oxide radical scavenging activity was measured spectrophotometrically (Govindharajan *et al.,* 2003). 1.0 ml of Sodium

nitroprusside (5 mmol) in phosphate buffer (pH 7.4, 0.1 M) was mixed with different concentrations of the extract (0.2- 1 mg/ml) in phosphate buffer (pH 7.4, 0.1 M). The tubes were then incubated at 25^{0}C for two hours. At the end of second hour 1.5 ml of reaction mixture was removed and diluted with 1.5 ml of Greiss reagent (1% sulphanilamide, 2% o-phosphoric acid, 0.1% of naphthylethylene diamine dihydrochloride) The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm. Control tube contain all chemicals except plant extract.

RESULTS AND DISCUSSION

DPPH Radical Scavenging Assay

The antioxidant potential of themethanolic extracts of the algaewere analyzed by DPPH method, in different concentrations from 0.2-1.0mg/ml. It was observed that percentage of inhibition increased with increase in concentration of the extract indicating that the radical scavenging activity is concentration dependent. The effect of antioxidants on DPPH radical scavenging was thought to be due to hydrogen donating ability. DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule. When a DPPH solution is mixed with a substrate acting as a hydrogen atom donor, a stable non-radical form of DPPH is obtained with simultaneous change of the violet colour to pale yellow (Molyneux, 2004). Hence DPPH has been used extensively as a free radical to evaluate reducing substances and is a useful reagent for investigating the free radical scavenging activities of compounds (Duan *et al*., 2006). In the present study *Ulva lactuca* showed a maximum percentage of inhibition (79.99%) and a minimum was shown by *Chaetomorpha antennina* (46.19%). It was observed from the present study that *Chaetomorpha area* showed a better percentage of inhibition (63.37%) at 1mg/ml, indicating a difference in radical scavenging property at genus level. *Valoniopsis pachynema* gave maximum percentage of inhibition of 69.58%. All the datas were represented graphically in Fig: 1

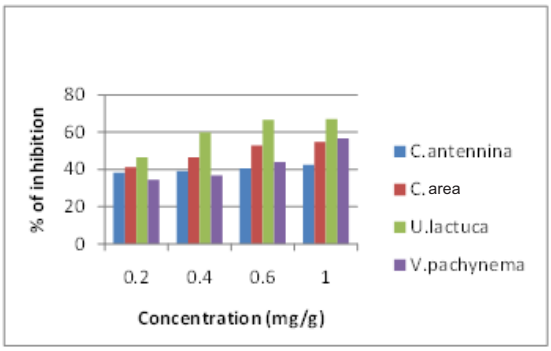

Fig:1. DPPH Radical scavenging activity of selected green seaweeds

Nitricoxide Radical scavenging assay

The nitrite oxide radical scavenging activity of the methanolic extracts of the selected samples were increased up to 85.95% (*Valoniopsis pachynema*) at the concentration of 1.0 mg/ml. *Chaetomorpha antennina* showed a maximum scavenging activity of 40.41% whereas *Chaetomorpha area* was found to be 83.77%. Seaweeds inhibit nitrite formation by competing with oxygen to react with nitric oxide directly. These compounds alter the structure and function of many cellular components. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this damage (Sanja*et al*., 2009).*Ulval actuca* showed radical scavenging activity up to 76.85% from 55.71%.Minimum percentage of inhibition obtained for *Valoniopsis pachynema* was 54.88%. Active oxygen species and free radicals are involved in a variety of pathological events nitric oxide radicals play an important role in inducing inflammatory response and their toxicity multiplies only when they react with O2- radicals to form peroxynitrite, which damages biomolecules like proteins, lipids and nucleic acids(Moncada *et al*., 1991). Graphical representation of the data was presented in Figure: 2

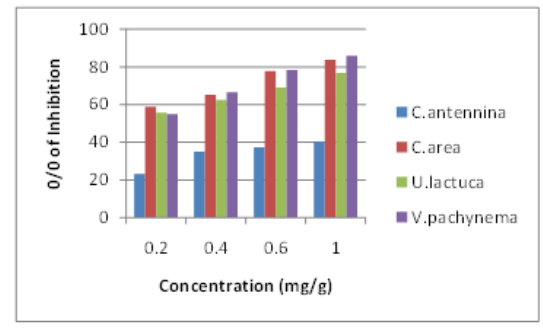

Fig 2. Nitric Oxide Radical scavenging activity of selected green seaweeds

Superoxide Radical scavenging Assay

Superoxide anion is the reduced form of molecular oxygen produced from the mitochondrial electron transport system upon the acceptance of a single electron. Energy is generated from mitochondria using electron transport chain reactions. Any loose electrons from the electron transport chain reactions will react directly with molecular oxygen, forming superoxide anion, which is the precursor for the formation of other ROS, including hydrogen peroxide, hydroxyl radicals and singlet oxygen (Lee *et al*., 2004). This assay was based on the reduction of nitro blue tetrazolium (NBT) in the presence of nicotinamide adenine dinucleotide (NADH) and phenazinemethosulfate (PMS) under aerobic condition (Chou *et al*., 2009). Compared to *U. lactuca*, *Chaetomorpha area* and *Valoniopsis pachynema*, scavenging activity of *C. antennina* was found to be very low (46.19%) even at 1mg/ml concentration of the extract. The decreasing order of scavenging activity in the four samples studied was *U. lactuca* (79.99%) > *V. pachynema* (69.58%) > *C. area* (63.37%) > *C. antennina* (46.19%) (Fig:3).

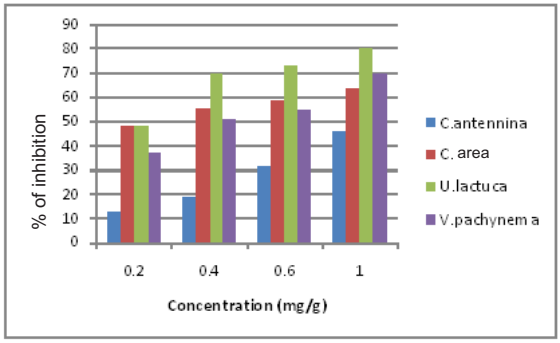

Fig 3. Superoxide anion Radical scavenging assay of selected green seaweeds

CONCLUSION

From the present study it can be concluded that the methanolic extracts of marine macro algae exhibit potent antioxidant activity. This study showed *Chetomorpha antennina,Chaetomorpha area, Ulva lactuca* and *Valoniopsis pachynema* that possessed varying degrees of radical scavenging activity in different concentrations. The results also indicated that there was difference in the radical scavenging property of the algae in different assays. The antioxidant property may be due to the presence of various bioactive compounds like Phenol, Flavonoid, Terpenoid, Sterol etc. Further investigation is needed to isolate and identify the specific class of compound that is responsible for the antioxidant activity so that these seaweeds can be used as a source of natural antioxidant with less side effects.

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