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COMPARATIVE STUDY OF MARINE ALGAE ISOLATED FROM EAST COAST OF INDIA BY REDUCING POWER ASSAY

Leelavathi M S^{*1}, Prasad M P²

^{*1} Research Scholar, Bharathiar University, Coimbatore, India

^{*1} Faculty, VidyaSoudha PU College, Peenya, Bangalore, India

²Prasad M P, Senior Scientist, Sangenomics Research Labs, Bangalore, India

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Abstract

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, Pheophyta and Chlorophyta, in both the inter-tidal and deep water regions. Seaweed constitutes a commercially important marine renewable resource. 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 thus, in the present investigation the sea weed samples were collected in sterile condition and their antioxidant property for methanolic and petroleum ether extract was carried out by reducing power assay. The extraction process was carried out by drying and extracted the fine powder with different solvents. The extracted samples were then dissolved to a stock concentration of 1mg/ml and the reducing power assay was carried out with different concentrations of the sample. The methanolic extract of *Cymodoceaeserrulata*, *Gracillariafolifera* and *Turbinariaconoides* showed higher reducing power when compared to the standard. The petroleum ether extract of *Cymodoceaerotundata* and *Ulvareticulata* extracts showed higher activity when compared to the standard whereas *Kappaphycussps* showed the least activity when checked for its reducing power activity.

Keywords: Reducing Power Assay; Antioxidant; *Gracillaria* Species; *Cymodeacea* Species; *Ulvalactuca*.

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1. Introduction

Free radicals are responsible for aging and causing various human diseases. A study shows that 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 (Jadhav *et al.*, 1995; Yamaguchi *et al.*, 1998). This action helps in protecting the body from degenerative diseases. Natural antioxidants are not limited to terrestrial sources and reports have revealed seaweeds to be rich sources of natural antioxidant compounds (Lim *et al.*, 2002; Duan *et al.*, 2006; Kuda *et al.*, 2007).

Seaweeds belong to a group of plants known as algae. They are rich in polysaccharides, minerals, proteins and vitamins. They have been 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). The estimated range of seaweeds is probably around 45,000 species (Bequette & France, 1997). Most seaweeds are divided into three categories based on their colours such as red (4,500 species), green (900 species) and brown (1,000 species). It has been used as food, fertilizer and for medicinal purposes for a long time.

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.).

Seaweeds have become a major food ingredient in products especially in Japan, Korea and China. Boukhari & Sophie (1998) reported that most Europeans and Americans use processed seaweeds as additives in their food preparation. In Asia, seaweeds have been used for centuries in the preparation of salads, soups and also as low-calorie foods (Jiménez-Escrig & Sánchez-Muniz, 2000). Although most Malaysians exhibit little interest in consuming seaweeds, it is consumed by small pockets of the population along the coastal areas of Peninsular Malaysia and East Malaysia (Norziah & Ching, 2000).

2. Materials and Methodology

The samples were collected a depth of 5–10m by SCUBA diving at Mantapam to Kanyakumari South coast of India and 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 were authenticated by J. R. Ramalingam, Former technical officer, Mantapam Regional center of central Marine Fisheries Research Institute, Tamil Nadu, India as *Gracillariacrassa*, *Gracillariaedulis*, *Cymodoceaerotundata*, *Cymodoceaeserrulata*, *Ulvalactuca*, *Ulvareticulata*, *Gracillariafoliifera*, *Gelidiellaaccrosa*, *Turbinariaconoides*, *Kappaphycusalvarezii* and *Acanthoporaspicifera*. 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.

Solvent extraction:

5gm of each sea weed sample powder was added to 50ml of methanol and petroleum ether solvent separately and kept for 48hrs with slight shaking condition. Here, methanol and petroleum ether was used as a solvent. After 48hrs, the extract was filtered by using whattmann no1 filter paper. The filtrate collected was evaporated so remove all the solvent present and to obtain a dry powder. The evaporation process was carried out by placing the filtrate in water bath at the boiling temperature of the solvent until the solvent is completely evaporated. The powder was then redissolved in solvents to get a final concentration of 1mg/ml. These stocks extracts were refrigerated until further use.

Reducing power assay:

1 ml of different concentration (200 – 1000 µg/ml) of sample was mixed with 1.25 ml of potassiumferricyanide (1%, w/v), and incubated at 50°C for 30 min. Afterwards, 1.25 ml of TCA (10%, w/v) was added to the mixture to terminate the reaction. Allow the mixture to settle down and leave the residues. About 1 ml of supernatant was diluted with 1 ml of methanol, then the solution was mixed with 0.5 ml ferric chloride (0.1%, w/v) and the absorbance was measured at 700 nm. All tests were performed in triplicate. Graph was plotted with the average of three determinations. L- Ascorbic acid was taken as a standard antioxidant.

3. Results

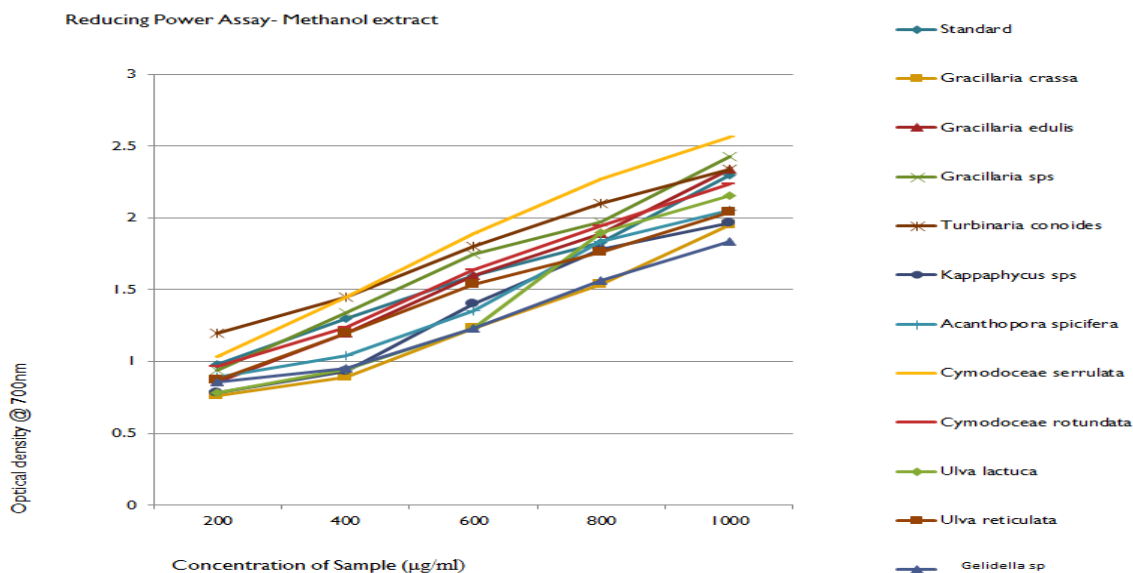


Figure 1: Reducing Power Assay for the methanolic extract of sea weeds.

The reducing power activity for the methanolic extracts was determined and compared with standard ascorbic acid. *Cymodoceaeserrulata*, *Gracillariafolifera* and *Turbinariaconoides* showed higher reducing power when compared to the standard. The activity was determined by using the optical density value at 700nm. The other samples showed similar kind of reducing power activity and were below the standard. *Geliedella sp* showed the least reducing power activity (Figure 1).

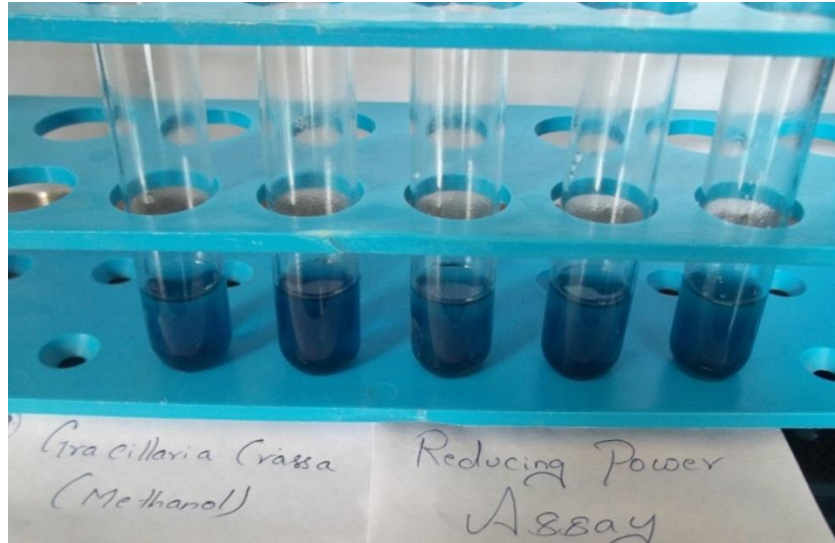


Figure 2: Reducing Power Assay for the methanolic extract of *Gracillariacrassa*.

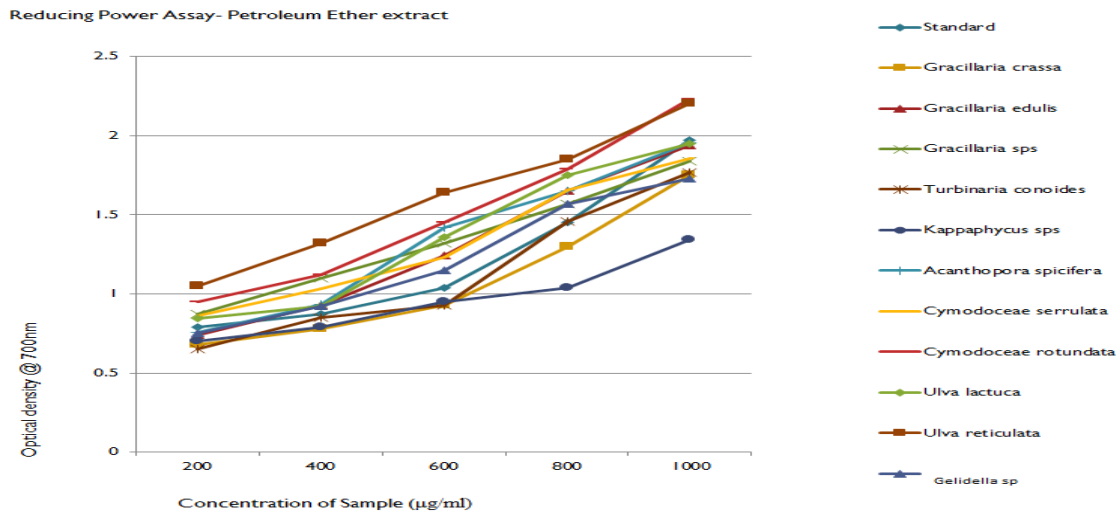


Figure 3: Reducing Power Assay for the Petroleum Ether extract of sea weeds.

The reducing power assay was carried out to determine the reducing power capacity of the petroleum ether extract of the samples in different concentration. The activity of the extracts increased with the increase in the concentration of the samples. The *Cymodocea rotundata* and *Ulva reticulata* extracts showed higher activity when compared to the standard. The petroleum ether extract of *Kappaphycus sps.* showed the least activity when checked for its reducing power activity (Figure 3).

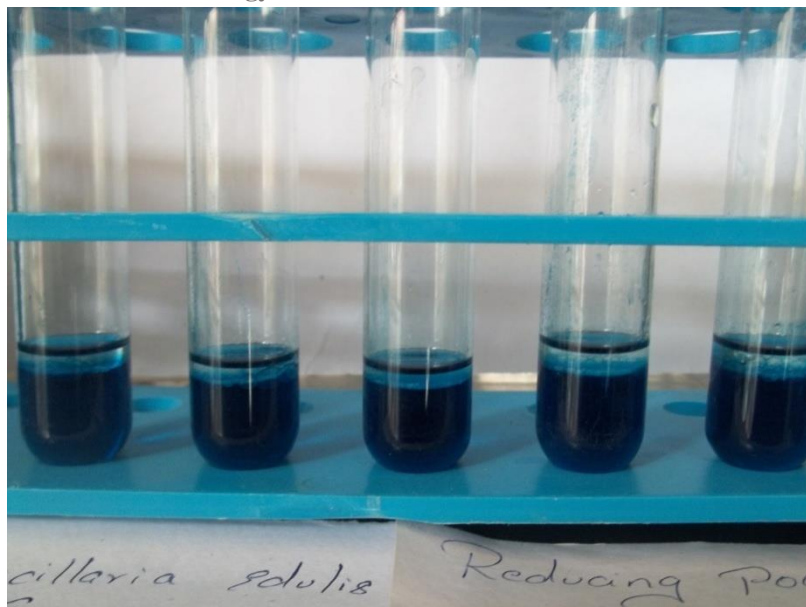


Figure 4: Reducing Power Assay for the Petroleum Ether extract of *Gracillaria edulis*.

4. Discussion

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized 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; Bansemiret *et al.*, 2006; Chew *et al.*, 2008).

Sargassum species might be a valuable source of natural antioxidants containing both water- and fatsoluble antioxidative components, preventing oxidative damage of food oils (Siriwardhana *et al.* 2003, 2004). Further antioxidative effects of compounds from macroalgae with potential in applications in human health and nutrition are described by Cornish and Garbary (2010).

Le Tutouret *et al.* (1998) demonstrated the ability of several brown seaweed extracts to scavenge peroxy radicals. Ruperézet *et al.* 2002 demonstrated that fucoidan from *Fucus vesiculosus* had the highest antioxidant activity in relation to the other fractions, with high levels of uronic acid. Several studies were subsequently performed to verify the antioxidant properties of algae (Zhang *et al.* 2003; Yuan *et al.* 2005). Recently, the antioxidant activity of polysaccharides from the chlorophyte *Ulva pertusa*, was also investigated. All of the compounds analyzed showed that molecular weight (MW) had a significant effect on antioxidant activity (Qi *et al.*, 2005).

Brown algae such as *Sargassum kjellmanianum* (Yan *et al.*, 1996; Yan *et al.*, 1997; Wei & Xu, 2003), *Eisenia bicyclis* (Nakamura *et al.*, 1996), *Cystoseira* sp. (Chkhikvishvili & Ramazanov, 2000), *Fucus* sp. (Jimenez-Escriget *et al.*, 2001), and *Ecklonia stolonifera* (Kang *et al.*, 2003a, 2004) have been reported with respect to free radical scavenging and the inhibition of total reactive oxygen species generation by phlorotannin compounds.

5. Conclusion

The Seaweeds are natural bioactive compounds which are used in Nutraceuticals which have chemical constituents of high therapeutic efficacy. Further studies are required to investigate the marine algae for potential pharmacological properties. The present study suggests that the seaweed extracts possessed antioxidant activity, promising a future scope for the use of marine seaweeds.

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*Corresponding author.

E-mail address: leelavathims@gmail.com