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Biochemical Characterization of Protein Isolated from Seaweed, *Gracilaria edulis*

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Abstract: The Seaweed, *Gracilaria edulis*, collected from Mandapam coast, was studied for Biochemical characterization of protein. Aqueous and methanol extracts yielded a total amount of 6.3 and 5.7 from 500 g of seaweed respectively. Crude protein extracts from the seaweed was 1.9 mg/mL in aqueous and 2.7 mg/mL in methanol extract respectively. The partial purification of protein is done by using DEAE cellulose. The aqueous and methanol extracts showed highest antioxidant activity in DPPH (95 and 82%). On SDS-PAGE the crude protein yielded four well defined bands at 31.4, 69.5, 92.7 kDa in both the extracts. The Fatty acid profile showed that myristic acid was dominant in chloroform extract and palmitic acid was dominant in aqueous extract.

Key words: Characterization, antioxidant, fatty acid, *Gracilaria edulis,* and palmitic acid

INTRODUCTION

Seaweeds offer a wide range of therapeutic possibilities both internally and externally. Seaweeds are extensive profile source of secondary metabolites. More than 600 secondary metabolites have been isolated from marine algae (Faulkner, 1986). Although a majority of these (about 60%) are terpenes, but some fatty acids are also common (20%) with nitrogenous compounds (Van Alstyne and Paul, 1988). Many of these compounds are bioactive and have been extensively studied using bioassays and pharmacological assays (Paul and Fenical, 1987). Potential antitumor promoting properties of 36 edible/common marine algae from sea near Maozuru, Kyoto, Japan were examined and strong inhibitory activities were found in *Undaria pinnatifida, Laminaria* and *Sargassum* species (Ohigashi *et al*., 1992). The production of inhibitory substances from seaweeds was noted as early as in 1917 (Harder and Oppermann, 1953). Since then, numerous studies have been carried out to detect and extra antimicrobial compounds from marine algae of all three groups *viz*. Rhodophyceae, Phaeophyceae and Chlorophyceae (Biard *et al*., 1980). Seaweeds provide an excellent source of bioactive compounds such as carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals). In the present investigation an attempt has been made here to find out the bioactivities of protein from sea weed, *Gracilaria edulis*.

MATERIALS AND METHODS

The study area was located at Mandapam coast, Tamil Nadu (Lat. 78º11' to 79º15' E ; Lat. 8º49' to 9º15' N) in the Gulf of Mannar (Janaurary to June 2008), Specimens of *G. edulis,* were collected at low tide and brought to the laboratory in seawater and identified by the method described by Umamaheshwara Rao (1987). The fresh seaweed was rinsed with fresh water and air dried in shade at 65ºC and subsequently pulverized into a fine powder.

Preparation of seaweed extract:

Aqueous extraction: The aqueous extract of seaweed was prepared by squeezing the sand – free specimens in triple distilled water. The resultant solution was filtered and dialyzed, using Sigma dialysis membrane-500 (Av Flat width-24.26 mm, Av. Diameter-14.3 mm and capacity approx-1.61 ml/cm) against D-glucose to remove the excess water. The supernatant so obtained was lyophilized and stored at 4 ºC in a refrigerator for the further use as crude aqueous extract.

Methanolic extraction: For methanol extraction, the seaweed was dried in air for 2 days and after complete drying, 10 g dried seaweed was put into 200 ml of chloroform, covered and kept standing for 5 hours. The solvent was then removed after squeezing the seaweed and filtered through Whattman filter No. 1 paper. The solvent was evaporated at low pressure by using a Buchi Rotavapor R-200 at 45ºC in refrigerator for further use as crude methanol extracts.

Partial purification of crude protein: Partial purification of the crude extract was carried out using DEAE Cellulose.

Protein estimation: Protein estimation was done as described by Lowry and Lopaz (1946), using Bovine serum albumin at the rate of 1mg/ml as the standard.

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Different concentrations of the standard ranging from 0.1 to 1 mg/ml were taken and made up to 1 mg/ml. Then 5ml of alkaline copper reagent was added, mixed well and allowed to stand for 10 min at room temperature. Then 0.5 ml of diluted Folins phenol reagent was added and mixed well. The mixture was incubated for 30 minutes at room temperature. The absorbance at 650 nm was read spectrophotometrically. The protein concentrations of *G. edilus* extracts were estimated.

Table 1: Protein estimation of seaweed, *G.edulis*

S.No	Type of extract	Absorbance at 750 nm	Concentration of protein In mg /ml
1	Methanol	1.6	2.7
2	Aqueous	1.4	1.9

Table 2: Fatty acid profile from the chloroform extract of *G.edulis*

Peak No.	Reten Time	Area (mV.s)	Amount (µ)	Amount (%)	Peak Type	Component Name
01	0.963	4.3753	4.3753	1.984	Free	Lauric
02	1.593	32.3586	32.3586	14.676	Free	Myristic
03	2.495	32.22083	32.2208	14.614	Free	Palmitic
04	3.637	22.5232	22.5232	10.215	-	-
05	4.600	17.3247	17.3247	7.858	Free	Stearic

Table 3: Fatty acid profile from the aqueous extract of *G.edulis*

Peak No.	Reten.	TimeArea (mV.s)	Amount (µ)	Amount (%)	Peak Type	Component Name
01.	1.063	2.8542	2.8542	4.426	Free	Lauric
02.	1.563	3.4139	3.4139	8.882	Free	Myristic
03.	2.405	17.5114	17.5114	45.561	Free	Palmitic
04.	3.578	14.6554	14.6554	38.131	Free	Stearic
Total	-	38.4349	38.4349	100.000	-	-

DPPH radical scavenging assay: DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging was assayed. 10 ml of the seaweed extract with 0.2 ml of DPPH (100 µM) (Sigma-Aldrich) in methanol solution was incubated at 37ºC for 30 min and the absorbance of the supernatant was measured at 490 nm using ELISA micro plate reader.

SDS-PAGE: One dimension sodium dodecyl sulphate (SDS) Polyacrylamide gel electrophoresis (PAGE) was carried out. SDS-PAGE was run on vertical slab gel system. Proteins were electrphorised on 12% separating gel (0.75 mm thickness) overlaid with 5% stacking gel. A 10 % (w/v) stock solution was prepared in deionized water and stored in room temperature.

Fatty acid profile: Gas chromatography was used for the fatty acid analysis. Identification of fatty acid was carried out on the basis of retention times of the standard mixtures of fatty acids.

RESULTS AND DISCUSSION

The seaweed identification was confirmed based on the morphological characters. Methanolic and aqueous extracts of seaweed *G. edulis* yielded a total amount of 5.7g and 6.3g of crude extracts respectively from 500 g of seaweed. The dry weight and wet weight ratio varied according to the biogeographical factors and its extraction yield differ from species to species of different algal groups (Lourdu Mariadoss *et al.,* 1998)

The protein contents of both methanolic and aqueous crude extracts of *G. edulis* was found to be 2.7mg/ml and 1.9 mg/ml (Table 1). Manivannan *et al.* (2009) described that the higher protein was found in *G. acerosa* (31.07 ± 0.33%) followed by *H. macroloba* (28.94 ± 0.68%), *H. tuna* (23.12 ± 0.86) and *C. glomerata* (20.38 ± 0.73%).

The increase in scavenging activity of *G. edulis* extracts on DPPH radicals was dependent on concentration. The methanol and aqueous extract exhibited a strong scavenging activity on DPPH (95 and 82 %) at 0.25 mg concentration. Lahaye and Kaffer (1997) explained that seaweeds are low in fats but contain vitamins and bioactive compounds, like terpenoids, sulfated polysaccharides and polyphenolic compounds, the latter being a potential natural antioxidant not found in land plants. Le Tutour *et al.* (1990) investigated the antioxidant activities of different seaweeds.

Gas chromatography indicates that palmitic acid is the most abundant fatty acid in aqueous extract and followed in decreasing order by stearic acid, myristic acid and Lauric acid. In methanol extract, myristic acid was found to be dominant and followed, in decreasing order, by palmitic acid, strearic acid and Lauric acid (Table 2 and 3).

Amal *et al.* (2008) comparing the data obtained from the GLC analysis of hydrocarbons, they found that docosane was the most commonly occurring hydrocarbons. It was detected in all the investigated species and found in predominant quantity, ranged from 10.61 to 39.99%.These data are not detected before.

The SDS-PAGE on gel, crude protein toxins yielded 4 bands in the methanol extract and 5 bands in the aqueous extract of *G. edulis,* ranging from 24.4 to 130 kDa molecular weight with 3 well-defined bands of 31.4, 69.5, 92.7 kDa in both the extracts.

Yoshihiro *et al.* (1987) showed that SDS- PAGE patterns consisting of many protein bands were obtained for the three seaweeds. In each seaweed, most fractions gave rise to patterns fairly similar to each other.

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