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Evaluation of Phytochemical and Biochemical Profiling of Marine Red Algae *Gracilariacrassa*

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Abstract

The aim of the present study sought to evaluated that biochemical constituents like protein, carbohydrate, total lipids, total phenols and phytochemical were analyzed from the marine red algae *Gracilariacrassa*. The present observation *G.crassa* showed the flavonoids, alkaloid, phenol, chlorogenic acid and carbohydrates were presented all the extracts. The biochemical content, fatty acid profile and mineral compositions were also recorded from the *Gracilariacrassa* extract. In this study, the biochemical constituents such as total carbohydrate (19.34±0.10%), total protein (23.13±0.005%), total phenols (7.81±0.23 mg/g) and total lipids (0.27±0.5%) were observed from the extract of *G.crassa*. The fatty acid profile showed that the higher concentration of saturated fatty acid and poly unsaturated linoleic acids were recorded. In mineral composition, the Ca (135.4±0.20 mg/100^{-1}) level was high when compared with other elements. In view of the results, the present study suggests that *G.crassa* contains important nutrients for human health and is possible natural functional foods.

Key words: phytochemical, fatty acid, *Gracilariacrassa*, protein, mineral and total lipids.

INTRODUCTION

Seaweeds are primitive non-flowering plants without true roots, stem and leaves. They grow in the intertidal, shallow and deep sea areas up to 180 meter depth and also in estuaries, backwaters and lagoons on solid substrates such as rocks, dead corals, pebbles, shells, mangroves, and other plants (Anantharaman*et al.*,2007). Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) depending on their nutrient and chemical composition. It was estimated that about 90% of the species of marine plant are algae and about 50% of the global photosynthesis is contributed from algae

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(Dhargalkar*et al.,* 2005).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 (Chew *et al.*, 2008). The environment in which seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidising agents but seaweeds seldom suffer any serious photodynamic damage during metabolism. This fact implies that seaweed cells have some protective mechanisms and compounds (Matasukawa*et al.*, 1997). Many metabolites isolated from marine algae possess bioactive effects. The discovery of metabolites with biological activities, from macro algae, has increased significantly in the past three decades; on the other hand, seaweeds have recently received significant attention for their potential as natural antioxidants. Marine organisms are a rich source of structurally novel and biologically active metabolites (Perry *et al.,* 1991).

Seaweeds are very important natural resources from the oceans that are employed as human foods and animal feeds in their whole form, and as sources of polysaccharides (mainly alginates, carrageenans and agar), carotenoids, lipids, vitamins, minerals, dietary fiber, proline and amino acids for use in food and pharmaceutical industry (Debbarama*et al.*, 2016). Seaweeds have been included for a long time in the traditional diet of East Asian countries such as Japan, Korea and China; more recently, their presence in all forms in the diet of Western countries has been progressively increasing (Torres *et al.,* 2019). Sumitra Vijayaragavan *et al.,* (1980) analyzed the seasonal variations in biochemical composition of some seaweed from Goa coast. Muthuraman and Ranganathan, (2004) investigated protein, amino acids, total sugars and lipid contents of *Caulerpascalpelliformis, Cladophoravagabunda, Enteromorphacompressa*, *Halimedamacroloba, Ulvafasciata and Chaetomorphaantennina*. The biochemical composition of seaweeds differs and is affected by inflow of land sources, geographic area and season of the year and temperature of water (Jenson, 1993).

However, the nutrient profile of seaweeds such as Gracilaria is influenced by different factors such as seaweed species, habitat, maturity stage, season, water temperature and the sampling conditions and method employed in the determinations (Torres *et al.,* 2019). *Gracilariacrassa* is exclusively marine red algae. It varies in size and shape. They are either epiphyte, grow as crust on the rocks or shells as a large fleshy, and branched like thalli. There are several benefits arises from this species such as medical and food wise. In the present study, the phytochemical screening from marine red algae and evaluate their antimicrobial potential. Thus, the present study sought to evaluate the phytochemical screening and chemical composition of *Gracilariacrassa*from the Muttam coast of Palk Bay, Kanyakumari.

MATERIALS AND METHODS

Collection of seaweeds

The seaweed*Gracilariacrassa* was collected from coastal area of Muttam, Kanyakumari district, Tamil

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Nadu, India. Macro algae samples were collected manually using transects method from the submerged marine rocks, soft substratum, during low tide in the intertidal and sub-tidal regions.After collection of sample, it was brought to the laboratory. Algal samples were washed in running tap water to remove any associated debris and then with the distilled water. After washing the samples were dried in a blotting paper for two weeks. After drying the sample was grinded in to powder form which was then stored in 4 °C for further studies. The algal specimen was identified at central salt and marine algal research station (CSMARS), Mandapam.

Preparation of extract

One gram of algal sample was extracted with 10 ml of different solvents systems such as methanol, chloroform and aqueous in a beaker for 24 hours at room temperature. Then the solvent portion was centrifuge at 5000rpm for 10minutes. The supernatant was collected from the centrifuge tube and the solvent were evaporated. Finally crude extract was obtained. The extracts were collected in separate plastic vials and stored in the refrigerator for further studies.

Phytochemical analysis

Phytochemical screening was carried out to assess the qualitative chemical composition of different solvent extracts using commonly employed precipitation and coloration to identify the major natural chemical groups such as alkaloids, saponin, phenols, carbohydrates, flavonoids, glycosides, coumarins, steroids, tannins, chlorogenic acid and anthocyanin were performed by the standard procedure as described by Harborne,1973.

Biochemical analysis

Estimation of Carbohydrate

The total carbohydrate content of the powdered *Gracilariacrassa* was estimated by phenol-sulphuric acid method (Dubois, *et al,* 1956). The Phenol - Sulfuric Acid method is an example of a colorimetric method that is widely used to determine the total concentration of carbohydrates present in foods. A clear aqueous solution of the carbohydrates to be analyzed is placed in a test-tube, then phenol and sulfuric acid are added. The solution turns a yellow-orange color as a result of the interaction between the carbohydrates and the phenol. The absorbance at 420 nm is proportional to the carbohydrate concentration initially in the sample. The sulfuric acid causes all non-reducing sugars to be converted to reducing sugars, so that this method determines the total sugars present. This method is non-stoichemetric and so it is necessary to prepare a calibration curve using a series of standards of known carbohydrate concentration.

Estimation of Protein

The total protein content in the crude extracts of *Gracilariacrassa*was estimated by Biuret method. Seaweeds powders 250 mg were taken in a test tube and 2 ml distilled water was added to it. The mixtures

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were mixed thoroughly by shaking for 1 minute by CM 101 Cyclo mixer, REMI and 4 ml Biuret reagent (9 g of sodium potassium tartrate, 3 g of copper sulphate, 5H2O and 5g of potassium iodide, in 400 ml of 0.2N sodium hydroxide solution and make up the volume to 1000 ml) was added to each seaweeds solution which were incubated for 30 minutes in room temperature and after incubation, mixtures were centrifuged at 4000 rpm for 10 minutes, supernatants were collected and the observance of all supernatants were taken at 540 nm with UV/Vis Spectrophotometer (Goshev and Nedkov, 1979). Bovine serum albumin (BSA) solution was used as standard. From 0-10 mg/ml of different concentration of BSA solutions was prepared and from each working standard 1 ml of solutions was taken and 4 ml Biuret reagent were added to it and incubated for 30 minutes and observance of OD value was taken at 540 nm. The standard calibration curve was made by using the estimated absorbance at y axis and concentration at x axis. From this calibration standard curve protein content of seaweeds were estimated.

Estimation of total Lipids

The lipid content of the *Gracilariacrassa* was estimated by using chloroform methanol mixture as described by Folch *et al*, (1957). The extract of seaweed was homogenized with chloroform / methanol (2:1) to a final volume 20 times the volume of the sample. After dispersion, the whole mixture was agitated for 15-20 mins in an orbital shaker at room temperature. The homogenate was then centrifuged to recover the liquid phase. Then solvent was washed with 0.9% sodium chloride solution and vortexed for few seconds and the mixture was centrifuged at low speed (2000rpm) to separate the 2 phases. The upper layer was siphonized without mixing the whole preparation. The lower chloroform phase containing lipids was evaporated under vacuum using a rotary evaporator.

Estimation of total Phenol

Total phenolic compounds were determined in sample extracts using the Folin– Ciocalteu reagent (Dewanto *et al.*, 2002). An aliquot of 0.125 ml of diluted extracts were mixed with 0.5 ml of distilled water and 0.125 ml of the Folin–Ciocalteu reagent. After 6 min, 1.25 ml of Na2CO3 (7 %) and 1 ml of distilled water were added and the obtained preparation was mixed thoroughly then incubated. After 90 min, the absorbance was monitored at 760 nm and the results are expressed as mg of gallic acid equivalents per gramme of dry residue (mg GAE/g). The assay was done in triplicate.

Estimation of fatty acid

Fatty acids in the sample were identified and quantified methyl esters in NEON II gas chromatography instrument following the procedure outlined by Niller and Berger (1985).

Estimation of mineral content

For mineral composition, 100 mg ground dried samples were treated with 10 mL of concentrated HNO3

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overnight (Santoso*et al.*, 2006). Thereafter, 2.5 mL concentrated HClO4 and 250 µL H2SO4 were added to the samples followed by heating until no white smoke was emitted. One hundred milliliters of 2 % HCl was added in the digested sample and filtered with a 0.22-µm membrane filter. The samples were analyzed using inductively coupled plasma atomic emission spectroscopy (PerkinElmer, Optima 2000, USA).

RESULTS AND DISCUSSION

Phytochemical analysis

Preliminary phytochemical tests of *Gracilariacrassa* were done by using methanol, chloroform and water. The each extracts confirmed the presence of important active chemical constituents such as flavonoids, alkaloid, phenol, chlorogenic acid and carbohydrates. The saponinand tannin are presented in the methanol extract only and coumarine and anthocyanin were absent in all the extracts. Glycosides are presented in both chloroform and methanol extracts. The results of the phytochemical analysis are compiled in Table.1.Phytochemicals are naturally present in the seaweeds and are biologically significant and plays an essential role in defending themselves against various pathogenic microbes. The phytochemical screening of plants reveals the presence of primary and secondary metabolites that suggest the plant might be of medicinal orindustrial importance. Rajakumar and Alwin PremSingh (2017) reported that the methanol and aqueous extracts of *Gracilariaedulis*showed the presence of a number of metabolites such as alkaloids, saponin, phenols, terpenoids, proteins, flavonoids, glycosides, coumarins and tannins.The present observation *G.crassa*showed the flavonoids, alkaloid, phenol, chlorogenic acid and carbohydrates were presented all the extracts. This was correlated with the previous work done by Harold Peter (2011), reported that the whole plant extracts of *Canthiumparviflorum* revealed the presence of phytochemicals such as alkaloids, oils, flavanoids, gums, phenols, saponins, steroids, tannins and terpenoids. Flavonoids possess anti-allergic, anti-inflammatory, antiviral and antioxidant activities (Bbosa, 2010).

Biochemical analysis

The determined for various chemical constituents like, carbohydrate, protein, total lipids and total phenols were recorded in *G. crassa*are shown in the Table.2. The biochemical constituents such as total carbohydrate (19.34±0.10%), total protein (23.13±0.005%), total phenols (7.81±0.23 mg/g) and total lipids (0.27±0.5%) were observed from the extract of *G.crassa*. Among the biochemical constituents, the protein content was higher compared to other content. In previous study, Dhamotharan (2002) investigated the level of the total lipid and protein content in brown algae and found that the levels of lipid and protein were high in *stoechospernummarginatum* as compared to *padina*.Mumtaj (2015) reported that the protein contents differ according to the species and seasonal conditions. It is confirmed in this study brown algae *Turpanariagonaidae*showing maximum concentration of protein, DNA and amino acid and it will be followed by *Gracilariafolifera*and *Bryopsis*. Impellizzeri *et al.*,(1975) reported the occurrence of amino

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acids and low-MW carbohydrates in 18 macroscopic marine algae belonging to the division *Rhodophyta*, class *Florideophyceae*. Both biotic and abiotic factors form the micro and macro environment of the organism and the strong or less perceivable variations in the environmental parameters influence such distinct differential amount of carbohydrates. Chemical composition of green, brown seaweeds and protein contents of red seaweeds of Sourashtra coast were reported by Dave *et al.*,(1977). Dhamotharan (2002) estimated the total phenol content in brown algae and found that the levels of total phenol content in *stoechospernummarginatum* (920μg/g dry wt.*)* is three folds that of padina (280 μg/g dry wt). Therefore, a number of studies have focused on the biological activities of phenolic compounds. As one of the major utilizable algal resources of the sea, they are known to contain carbohydrates, proteins, vitamins and minerals and micro nutrients (Chapman, 1980). The present observation *G.crassa*showed the higher concentration protein.

Fatty acid profile

The fatty acid profile was recorded in *G. crassa.* Among the fatty acid content, the omega fatty acid (0.9832±0.002%) was presented in higher and followed by linolenic acid (0.2834±0.0020%). The moderate amount of alpha linolenic acid (0.1184±0.0010%), margaric acid (0.1137±0.0010%) and oleic acid (0.1134±0.0010%) were also presented. The least composition moroctic acid (0.0096±0.0001%) was recorded (Table.3). Red seaweeds are particularly rich in SFAs and PUFAs which have nutritional applications that lead to their extensive use in food, feed, cosmetic, biotechnological and pharmaceutical applications (Kumari*et al.,* 2010).Variation in fatty acid content may also be due to the season of collection as well as other abiotic factors such as nutrition, salinity, light and temperature (Francavilla*et al.,* 2013). According to this work (Sakthivel and Devi, 2015), the most abundant fatty acids in both seaweeds were palmitic, stearic and α-linoleic acid acids. The same fatty acids were also found abundant in *G. changii* (Francavilla*et al.,* 2013).In the present study, omega fatty acid (0.9832±0.002%) was presented in higher and followed by linolenic acid (0.2834±0.0020%). The presence of this n-3 fatty acid in *Gracilaria*spp. is inconstant, because it was found in *G. gracilis* (Francavilla*et al.,* 2013), but it was not detected in *G. changii*(Chan and Matanjun,2017) or *G. edulis* (Sakthivel and Devi, 2015). Fatty acids overall profile obtained in this work were significantly different than 57.5% SFAs, 18.3% MUFAs and 18.4% PUFAs reported for *Gracilaria* sp. (Da Coasta*et al.,* 2017) or the 7.5% SFAs, 38.3% MUFAs and 51.2% PUFAs 18.4% reported for *Gracilariachangii* (Chan and Matanjun,2017).

Mineral content

The mineral such as, calcium, magnesium, iron, sodium, potassium, copper, zinc, phosphorus, manganese, chromium, lead, cadmium, iodine, nickel and molybdenum were estimated in *G.crassa* extract. Calcium (135.4±0.20 mg/100^{-1}) was the major mineral constituent in *G.crassa.* The moderate amount of potassium

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(34.43±0.152mg/100^{-1}), sodium (25.56±0.152mg/100^{-1}) and phosphorus (14.65±0.020mg/100^{-1}) were also presented. The level of inc (5.653±0.0153mg/100^{-1}), iodine (3.110±0.021mg/100^{-1}), chromium (2.440±0.200mg/100^{-1}), magnesium (2.343±0.015mg/100^{-1}) and manganese (1.960±0.0152mg/100^{-1}) were also recorded. The least level of iron (0.119±0.002mg/100^{-1}), copper (0.119±0.0012mg/100^{-1}), lead (0.454±0.200mg/100^{-1}), cadmium (0.214±0.201mg/100^{-1}), molybdenum (0.68±0.15mg/100^{-1}) and nickel (0.342±0.05mg/100^{-1}) were observed (Table.4). Seaweeds are one of the richest sources of minerals and trace elements, because the cell-wall polysaccharides and proteins of seaweed contain sulfate, anionic carboxyl and phosphate groups which act as binding sites for metal retention (Ródenas de la Rocha *et al.*, 2009). With respect to the mineral content, *G.crassa* showed a higher content of Ca (135.4±0.20 mg/100^{-1}) in previous work, seaweeds had a higher or similar content of minerals like Zn, Cu, Mg and Fe when compared with the content of *G. acerosa* (Syad et al., 2013), *G. edulis* (Sakthivel and Devi, 2015), *G. fisheri* and *G. tenuistipidatata* (Benjama and Masniyom*et al.,* 2012), with the exception of Mg in *G. edulis* which were lower than those found for other previous works as *G. changii*(Chan and Matanjun,2017). The ability of seaweeds to accumulate metals will depend on a variety of factors such as location, exposure, salinity, temperature, pH, light, nitrogen content, season, plant age, metabolic processes or the affinity of the plant for each element among others (Sánchez-Rodríguez *et al.,* 2001). In view of the present results, *G.crassa* contain an adequate amount of minerals, which suggests that these seaweeds could act as important sources of mineral supplements which are essential for human nutrition.

Taple.1 Preliminary phytochemical analysis of various solvents extract of *G.crassa*

S.No	Phytochemical content	Name of the test	Chloroform	Methanol	Aqueous
1	Alkaloid	Mayers test	+	+	+
		Dragendroffs test	+	+	+
		Wagner test	-	+	-
2	Saponin	Foam test	-	+	-
3	Flavonoids	Ammonia test	+	+	+
4	Phenol	Phenol reagent	+	+	+
5	Carbohydrate	Molish test	+	+	+
		Fehling test	-	-	-
		Benedicts test	-	-	-
6	Glycosides	Burchard test	+	+	-
7	Coumarins	NaCl test	-	-	-
8	Tannin	Lead acetate test	-	+	-

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9	Chlorogenic acid	Ammonia test	+	+	+
10	Anthocyanin	NaOH test	-	-	-

Table.2 Biochemical analysis of *G.crassa*

S.No	Biochemical content	Composition (%)
1	Carbohydrates	19.34±0.10
2	Protein	23.13±0.05
3	Lipid	0.27±0.5
4	Phenol	7.81 mg/g

Table.3 Fatty acid profile of *G.crassa*

S. No	Fatty acid	Composition (%)
1	Palmitic acid	0.0816 ±0.1000
2	Margaric acid	0.1137±0.0010
3	Stearic acid	0.0834±0.010
4	Oleic acid	0.1134±0.0010
5	Linolenic acid	0.2834±0.0020
6	Alpha linolenic acid	0.1184±0.0010
7	Moroctic acid	0.0096±0.0001
8	Omega fatty acid	0.9832±0.002
9	DHA	0.0871±0.001
10	EPA	0.1948±0.003

Table.4 Mineral composition of *G.crassa*

S.No	Minerals	mg/100g^{-1}
1	Ca	135.4±0.20
2	Mg	2.343±0.015
3	Fe	0.119±0.002
4	Na	25.56±0.152
5	K	34.43±0.152
6	Cu	0.119±0.0012
7	Zn	5.653±0.0153
8	P	14.65±0.020
9	Mn	1.960±0.0152

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10	Cr	2.440±0.200
11	Pb	0.454±0.200
12	Cd	0.214±0.201
13	I	3.110±0.021
14	Mo	0.68±0.15
15	Ni	0.342±0.05

CONCLUSION

The alga is rich source of omega fatty acid, calcium and phosphorus formed the major bulks in the minerals as well as in fatty acid content. The investigation revealed the richness of alga in protein and carbohydrate content; the lipid content being least. Thus, the overall observation of the present study suggests that shows nutritive biochemical properties and promising as a source of pharmacognosical value. Hence, it can be concluded that the *G.crassa* was used in food industry for nutritional purpose and pharmaceutical industry as a source of basic materials in the preparation of nutrient supplement products and fine chemical synthesis.

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