Biochemical Compositions of Seaweeds Collected from Olaikuda and Vadakkadu, Rameshwaram, Southeast Coast of India

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Abstract

Some seaweed has been used as an important source of commercial application of phycocolloids, agar, and alginate consequently some seaweeds have also been utilized as food and food ingredients due to their high nutritive compositions such as carbohydrates, protein, lipid, amino acids, fatty acids, fibre and minerals. In different countries, including India, seaweeds are gradually taking place as food items in the market, so their biochemical components analysis will decipher their nutritional implications. In this investigation, total 33 species of seaweeds were collected from the southeast coast of India and analyzed for their biochemical composition such as carbohydrate including glucose and starch, protein and lipid. The carbohydrate content varied from 15.20 ± 0.69 mg/gm dry weight (Padina boergeseni) to 97.69 ± 2.3 mg/gm dry wt (Gracilaria edulis). The carbohydrate content of red seaweeds was comparably higher than green and brown seaweeds. The protein content was minimum in Cystoseira indica (76.23 ± 0.21 mg/gm dry wt) and maximum in Amphiroa anceps (96.06 ± 0.95 mg/gm dry wt) and lipid content was comparatively high in Valoniaopsis pachynema (82.33 ± 2.51 mg/gm dry wt) and Caulerpa racemosa (81.06 ± 0.37 mg/gm dry wt). The glucose content was high in Dictyota setchelliae (0.78 ± 0.004 mg/gm dry wt) and Valoniaopsis pachynema (0.72 ± 0.01 mg/gm dry wt) likewise starch content was also high in Dictyota setchelliae (0.77 ± 0.08 mg/gm dry wt) and Laurencia papillosa (0.54 ± 0.00 mg/gm dry wt). From this study, it was concluded that the above mentioned seaweeds will be used as food after further more detailed analysis of the other biochemical components.

Keywords Seaweeds; Biochemical compositions; Rameshwaram; Southeast coast; India

Introduction

Some seaweed had been reported as an excellent source of carbohydrates, proteins, lipids, vitamins, trace minerals and other bioactive compounds [1]. During the last decades, some seaweed had become a major focus of industrial and commercial interest as potential, functional and health-promoting food ingredients and food items [2]. Presently, some edible seaweed were commonly used as additives to make snacks and first food items, so gradually seaweeds have been taken as food by peoples every day [3]. In recent years, due to the highly nutritional composition of seaweeds, they were exploited as food, so, several studies had been conducted by different authors on nutritional compositions and some physiological properties of marine macro algae in different parts of the World [4-11]. Some researchers worked on the biochemical compositions of some seaweed collected from southeast coasts of India [12-14]. The biochemical compositions of seaweeds had been also influenced by geographical location, season, physico-chemical parameters of water, local environmental condition and the land effluents [15,16]. As far as we know, there were no published data on biochemical compositions, including glucose and starch content of seaweeds from Olaikuda and Vadakkadu coasts, southeast coast of India. Depending on locations, especially seaweeds from these two coasts were not yet explored for their biochemical compositions. So, this work is the first time report of the exploration of the biochemical compositions of Olaikuda and Vadakkadu seaweeds.

The seaweeds have been gradually becoming an important food item and their commercial demand has been increasing in various industries. Attending to this, we analyzed the biochemical compositions such as carbohydrate including glucose and starch, protein and lipid, of 33 seaweeds.

Materials and Methods

Sampling description

Seaweeds were collected along the coast randomly during 2016 from Olaikuda and Vadakaadu at Rameshwaram, southeast coast of India (Figure 1). All the species were identified with the help of CMFRI taxonomy key and available taxonomic manuals (Table 1). The algal samples were washed thoroughly with seawater to remove all the impurities, sand particles, epiphytes and also cleaned with seawater to remove the salt and unwanted material on the surface of the sample. Seaweeds were a shade dried for one week. The dried seaweeds were finally pulverized in the commercial grinder and the powdered seaweed samples were stored and used for further analysis.

Description of study area

Olaikuda coast is continuously affected by anthropogenic activities (tourists) as it is situated near the principal shrine of Rameshwaram but Vadakkadu is comparatively undisturbed and situated away from local population with the natural vegetation of seaweeds, including Kappaphycus alverigea cultivation.
| S. No | Species Name                  | Division    | Location |
|-------|------------------------------|-------------|----------|
| 1     | Aghardhiella subulata        | Rhodophyta  | Olaikuda |
| 2     | Amphiroa aniceps             | Rhodophyta  | Vadakkadu|
| 3     | Amphiroa fragilissima        | Rhodophyta  | Olaikuda |
| 4     | Caulerpa racemosa            | Chlorophyta | Olaikuda |
| 5     | Caulerpa racemosa var. macrophysa | Chlorophyta | Olaikuda |
| 6     | Caulerpa scalpelliformis     | Chlorophyta | Olaikuda |
| 7     | Chaetomorpha antennina       | Chlorophyta | Olaikuda |
| 8     | Chlorodesmis hildebrandii    | Chlorophyta | Olaikuda |
| 9     | Cladophora vagabunda         | Chlorophyta | Olaikuda |
| 10    | Cystoseira indica            | Phaeophyta  | Vadakkadu|
| 11    | Digenea simplex              | Rhodophyta  | Olaikuda |
| 12    | Fucus vesiculosus            | Phaeophyta  | Vadakkadu|
| 13    | Gelidium acerosa             | Rhodophyta  | Vadakkadu|
| 14    | Gracilaria edulis            | Rhodophyta  | Vadakkadu|
| 15    | Gracilaria foliifera         | Rhodophyta  | Vadakkadu|
| 16    | Gracilaria opuntia           | Rhodophyta  | Vadakkadu|
| 17    | Halimeda gracilis            | Chlorophyta | Vadakkadu|
| 18    | Hydroclathrus clathratus     | Phaeophyta  | Olaikuda |
| 19    | Hypnea valentiae             | Rhodophyta  | Olaikuda |
| 20    | Kappaphycus alvarezii        | Rhodophyta  | Vadakkadu|
| 21    | Laurencia papillosa          | Chlorophyta | Olaikuda |
| 22    | Padina boergesenni           | Phaeophyta  | Vadakkadu|
| 23    | Padina tetrastromatica       | Phaeophyta  | Vadakkadu|
| 24    | Sargassum cinereum           | Phaeophyta  | Vadakkadu|
| 25    | Sargassum cinctum            | Phaeophyta  | Vadakkadu|
| 26    | Sargassum cristaeolium       | Phaeophyta  | Vadakkadu|

**Figure 1:** Map showed the sampling location.
The powdered seaweeds were taken in a boiling tube and hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and cooled to room temperature. After taking from water bath, the seaweed mixture was neutralised with solid sodium carbonate (Na$_2$CO$_3$) until the effervescence ceases and the volume made up to 100 ml and the solutions were centrifuged. The supernatant was collected and 1 ml of the aliquots was taken for analysis. To these samples 4 ml of Anthrone reagent was added and again heated for eight minutes in a boiling water bath. The samples were cooled rapidly and read the green to dark green colour at 630 nm using spectrophotometer Perkin Elmer precisely Lambda 25 UV/V is Spectrophotometer (UV-2600 SHIMADZU). The concentration of standard was prepared with D-glucose solution and 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of standard solution were taken in the test tube and water were added to make the volume to 1 ml and 4 ml of Anthrone reagent were added to it, the observance was taken at 630 nm. The standard graphs were plotted with concentration on x-axis versus absorbance on the y-axis. From this calibration curve carbohydrates content were calculated.

**Estimation of carbohydrates:** The standard Anthrone method [17] was used to estimate the carbohydrate content of selected seaweeds. The powder seaweeds were used for carbohydrate estimation and 250 mg of powder seaweeds were taken into a boiling tube and hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and cooled to room temperature. After taking from water bath, the seaweed mixture was neutralised with solid sodium carbonate (Na$_2$CO$_3$) until the effervescence ceases and the volume made up to 100 ml and the solutions were centrifuged. The supernatant was collected and 1 ml of the aliquots was taken for analysis. To these samples 4 ml of Anthrone reagent was added and again heated for eight minutes in a boiling water bath. The samples were cooled rapidly and read the green to dark green colour at 630 nm using spectrophotometer Perkin Elmer precisely Lambda 25 UV/V is Spectrophotometer (UV-2600 SHIMADZU). The different concentration of standard was prepared with D-glucose solution and 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of standard solution were taken in the test tube and water were added to make the volume to 1 ml and 4 ml of Anthrone reagent were added to it, the observance was taken at 630 nm. The standard graphs were plotted with concentration on x-axis versus absorbance on the y-axis. From this calibration curve carbohydrates content were calculated.

**Estimation of proteins:** The protein 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 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, 5H$_2$O and 5 g 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 [18]. 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 lipids:** The powder seaweeds of 250 g were taken in a test tube and 4 ml ethanol were added to it and kept in a water bath for 30 minutes at 30°C, centrifuged at 5000 rpm for 15 minutes and the supernatant with ethanol and lipid were collected. The supernatant were concentrated and hexane: water (4:1) 4 ml was added to the concentrated supernatant, mixed well and again centrifuged at 5000 rpm for 15 minutes. The hexane with lipid and ethanol in water were separated, and then hexane with lipid was collected and by gravimetric methods lipid was estimated [19].

**Estimation of glucose and starch by Anthrone method:** The seaweed powders of 0.5 g were homogenized with ethanol to remove sugars. After centrifugation, the residues were retained and residues were repeatedly washed with hot 80% ethanol till the washing did not give colour with Anthrone reagent. The residues were dried over water bath. To each residue 5 ml water and 6.5 ml of 52% Perchloric acid was added. The mixtures were centrifuged at 4000 rpm, 4°C for 20 minutes and the supernatants were collected and the volume made up to 100 ml with distilled water. The 0.2 ml of each supernatant solution was taken in a test tube and the volume adjusted to 1 ml with distilled water and to each sample supernatant 4 ml of Anthrone reagent was added. The mixtures were heated for eight minutes in a boiling water bath, cooled rapidly within icebox and the OD value of green to dark green were taken at UV-Visible Spectrophotometer at 630 nm (Hodge et al. 1962). The glucose content was estimated from the standard D-glucose calibration curve and the values were multiplied by 0.9 to determine the starch content of seaweeds.

**Results and Discussions**

**Carbohydrates content**

Among thirty three seaweeds, the carbohydrate was varied from 15.20 ± 0.69 mg/gm dry weight to 97.69 ± 2.3 mg/gm dry wt. The level of carbohydrate was high in some of the red algae such as Gracilaria edulis (97.69 ± 2.31 mg/gm dry wt), Gelidiella acerosa (96.29 ± 1.05 mg/gm dry wt) and Laurencia papillosa (92.22 ± 0.70 mg/gm dry wt) (Figure 2). It was shown that carbohydrate content was high in Ulva lactuca (35.27%) and Enteromorpha intestinalis (30.58%) from Sundarban [8]. Some seaweeds from Mandapam coast also contained high carbohydrates such as Hypnea valentiae (23.60%), Enteromorpha intestinalis (23.84%), Turbinaria conoides (23.9%), Sargassum tenerimum (23.55%), Sargassum wightii (23.50%) and Acanthophora spicifera (23.54%) and minimum carbohydrates content was recorded from some of the species Padina gymnospora (21.88%), Codium tomentosum (20.47%), Calpomenia sinuosa (22.46%) and Gracilaria foliata (22.32%) [12,13,20]. The high carbohydrate content (64.00% dry weight) in Caulerpa lentillifera was reported by Nguyen [21].

**Protein content**

Protein was adequately present in all the 33 species of seaweeds. Protein content varied from 76.23 ± 0.21 mg/gm dry wt to 96.06 ± 0.95 mg/gm dry wt. Some of the seaweed contained the highest amount of...
protein such as Amphiroa anceps (96.06 ± 0.95 mg/gm dry wt) and Chaetomorpha antennisna (92.22 ± 0.58 mg/gm dry wt).

Some seaweed had been reported for high protein content such as Turbinaria ornata from Gulf of Mannar region [22]; 22.22% of crude protein in Ulva fasciata [23]; 35% of dry mass of protein in Palmaria palmata and 47% of dry mass of protein in Porphyra tenera [24]. The proximate biochemical composition of some seaweed from Mandapam coast had been evaluated by Dharagalark [25] which revealed that Padina gymnospora contained maximum protein (17.08 ± 0.28%) including Enteromorpha intestinalis (16.38 ± 0.50%) and Sargassum tenerimum (12.42 ± 0.63%).

### Lipid Content

But the lipid content was highly variable among three divisions (Figure 2). Some of the species content high amount of crude lipid Valoniopsis pachynema (82.33 ± 2.51 mg/gm dry wt) and Caulerpa racemosa (81.06 ± 0.37 mg/gm dry wt) and some of the species contained minimum lipid such as Caulerpa racemosa var. macrophysa (5.43 ± 0.20 mg/gm dry wt.) and Digenea simplex (10.91 ± 0.92 mg/gm dry wt). Manivannan [12,13] and Patia [20] investigated some seaweed for their lipid content which recorded that maximum lipid was present in Enteromorpha clathratus (4.6%) and minimum lipid content was in Enteromorpha intestinalis (1.33%). Some of the seaweeds also contained adequate amount of lipid such as Gracilaria folifera (3.23%), Sargassum wightii (2.337%), Codium tomentosum (2.53%), Ulva lactuca (1.6%), and Sargassum tenerimum (1.46%).

### Glucose content

Glucose and starch content were investigated to know the calorie value of the studied seaweeds. The glucose content was comparatively high in Digenea simplex (0.78 ± 0.004 mg/gm dry wt) and Laurencia papillosa (0.61 ± 0.008 mg/gm dry wt). The fifty individual thallus of Saccharina, Fucus (serratus and spiralis) and Ascophyllum had been analysed for their glucose content which was 65%, 30% and 20% of the total sugars respectively [26].

### Starch content

The starch content was high in some of the seaweeds such as Digenea simplex (0.78 ± 0.004 mg/gm dry wt), Laurencia papillosa (0.61 ± 0.008 mg/gm dry wt), as well as Chaetomorpha antennisna (0.405 ± 0.01 mg/gm dry wt).

The one-way ANOVA were applied for division wise carbohydrate, protein and lipid which showed significant (P<0.05) estimation and observation. The Pearson correlation studies showed the positive relation between biochemical components. The significant positive correlation between carbohydrate content with starch (r=0.44) and carbohydrate with glucose (r=0.37) statistically confirm our estimation [27].

### Conclusions

The one year survey showed that Olaikuda was flourished with green seaweeds and Vadakkadu was with brown seaweeds. The biochemical compositions of some of the studied species indicated its importance for used as food items and it may be further considered as food items in the future. The starch provides energy to our body. The content of starch was high in some of the seaweeds. So, the uptake of seaweeds as a diet may increase significant calorie intake in our body. Consequently, glucose is a soluble monosaccharide which gives direct power to our bodily function and the main source of energy for the
brain and nervous system. Some of the seaweeds contained adequate amounts of glucose, so use of these seaweeds as food items may boost the brain development and impulse to the nervous system. Due to the gradual growth of the human population, to avoid food scarcity and adjust the increase population with adequate food, some more new source of food should be identified and included in the food list [28]. The marine environment is a natural source of so many marine organisms and marine plants. So, the details biochemical composition of seaweeds will be useful to easily identify some of the seaweeds as food items. Furthermore, globally in some countries, algal aquaculture may become another source of income in developed and undeveloped coastal area. In fish aquaculture, some of the seaweeds are used as fish feed, so biochemical composition analysis of seaweeds will help to identify the nutritious seaweeds for fish feed.

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Conflicts of Interest

There are no conflicts of interest to be declared.

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