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Proximate composition and amino acid profile of five green algal seaweeds from Mandapam Coastal regions, Tamil Nadu, India

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Abstract
Seaweeds one of the important marine living, renewable resources and are used for human consumption, animal feed and as manure in various states. Thus, some of the representatives from Chlorophyta were investigated in the present field of proximate composition and amino acid profile. The macroalgae showed varied quantities of biochemical constituent are namely Amino acids, Proteins, Lipids, Carbohydrates and Phenol. The highest concentration of Glutamic acid and Aspertic acid was recorded in alga *Caulerpa sertularioides* 16.3% and 15.6%, respectively, and lowest value of Methionine was recorded in all species except *Ulva reticulata* (3.0%). Higher protein was recorded in *Caulerpa sertularioides* 17.9% and low value was recorded in *Condium tomentosum* (9.5%). Lipid content was high in *Caulerpa racemosa* (18.1%) and low value in Ulvalactuca (0.4%). Carbohydrate content was high in *Caulerpa racemosa* 81.2% and low in *Ulva reticulata* 68.1% in phenol the highest value in *Caulerpa sertularioides* (32.6%) and lowest in *Caulerpa racemosa* (13.2%).

Keywords: Proximate composition, amino acids, green algal seaweeds, Mandapam

Introduction
Marine macroalgae, commonly referred to seaweeds, are categorized by their pigmentation, morphology, anatomy, and nutritional composition as red (Rhodophyta), brown (Phaeophyta) or green seaweeds (Chlorophyta) [1]. About 250 macro algal species have been commercially utilized. Worldwide and about 150 species are favorably consumed, as human food [2]. Seaweeds are valuable sources of protein, fiber, vitamins, polyunsaturated fatty acids and trace elements, as well as important bioactive compounds [3]. Therefore, they have been known as being beneficial for human and animal health [4]. Nevertheless, the nutrient compositions of seaweeds are different depending on species, habitats, maturity and environmental conditions [5].

Generally, green and red seaweeds contain higher protein contents (10-30% dry weight) than brown seaweeds (5-15% dry weight). Proteins are composed of several amino acids and their nutritional quality can be measured against the recommended amino acid pattern [6-7]. The lipid content of marine seaweeds accounts for 1-6% dry weight and provides a low amount of energy. Most seaweed has more ash contents than terrestrial plants and animal products. Some of the trace elements in seaweeds are rate or absent in terrestrial plants [8]. Thus, seaweeds are important sources of elements vital for the metabolic reactions in the human and animal health, such as enzymatic regulation of lipid, carbohydrate and protein metabolism [9].

In the Southern coast of Thailand, especially in the Pattani Bay, twelve seaweed species have been found [10]. Red seaweed (*Gracilaria* spp.) and green seaweed (*Ulva* spp.) have been abundant in the coastal region. However, the utilization of seaweeds is restricted to communities living in the coastal area. *Gracilaria tenuistipitata* and *G. fisheri* has been serving fresh or used in dried products for both human beings and animals, whereas *Ulva* species are still under-utilized because in Thailand the knowledge about under-utilized because in Thailand the knowledge about their nutritional composition is still limited. Therefore the present study aimed to determine the chemical composition, amino acids, and element contents of *Ulva pertusa* and *U. intestinalis* collected from the Pattani Bay in rainy and summer seasons in order to gain extensive information about their nutritional value. Furthermore, this research also investigated some physicochemical properties in order to measure their physiological effects in functional and health food.

Seaweeds are generally macroscopic algae and are used in many ways. They are harvested for food, fodder, fertilizer, medicine and chiefly for economically important phycocolloids [11]. The nutritional property of seaweeds from some regions of the world and Indian coast has been well documented [12-13]. Climate and sea conditions may cause variations in nutrient composition of seaweeds [14, 15]. The purpose of the present investigation was to study the proximate constituents of five species of seaweeds with potential economic value for use in human and animal nutrition.

Materials and Methods

Collection of seaweeds

The seaweeds *Caulerpa racemosa*, *Codium tomentosum* and *Caulerpa sertulariodies*, *Ulva lactuca* and *Ulva reticulata* were collected from Mandapam coastal regions, Southeast coast of India. Seaweed samples were picked by hand and now washed with seawater to remove the foreign particles, grit particles and epiphytes. Then it was restrained in an ice box containing slush ice and immediately sent to the laboratory and washed thoroughly using tap water to transfer the saltiness along the airfoil of the sample. Then the seaweeds were spread on blotting paper to remove excess water. Samples were dried in over at 37 °C, till constant weight and obtained and ground in an electric mixer [16]. The powdered samples were then stored in refrigerator.

Preliminary Phytochemical screening

The dried, powdered samples were subjected to qualitative tests for the identification of proximate constituents according to standard procedures [17-19].

Estimation of amino acids

Amino acid analysis was borne out by ion-exchange chromatography under the experimental conditions recommended for protein hydrolysates. Samples containing 5.0 mg of protein were acid hydrolyzed with 1.0 ml of 6 NHCl in vacuum-sealed hydrolysis vials at 110 °C for 22 h. Norelucine was added to the HCl as an internal standard. Tryptophan, cystine and cysteine are completely lost by acid hydrolysis, and methionine can be put down to varying points of this process. Hydrolyzates were suitable for analysis of all other amino acids. The tubes were cooled after hydrolysis, opened, and placed in desiccators containing NaOH pellets under vacuum until dry (5-6 days). The residue was then dissolved in a suitable volume of a sample dilution Na-S buffer, pH 2.2 (Beckman Instr.), filtered through a Millipore membrane (0.22 μm pore size) and analyzed for amino acids by ion-exchange chromatography in a Beckman (model 7300) instrument, equipped with an automatic Integrator. Nitrogen in amino acids was determined by multiplying the concentration of individual amino acids by corresponding factors calculated from the percentage N of each amino acid [20]. The ammonia content was included in the computation of protein nitrogen retrieval, as it does from the debasement of some amino acids during acid hydrolysis [21].

Estimation of Lipid

The lipid was estimated by using a chloroform, methanol mixture as described by [22]. 10 mg of dried powder sample taken in a test tube, 5 ml of chloroform-methanol (2:1) mixture was added. The mix was incubated at room temperature for 24 hrs after closing the oral cavity of the test tube with aluminum foil.

After the incubation, the mixture was filtered using a filter paper. The filtrate was collected in 10 ml pre weighed breaker, which was held on a hot plate. The chloroform, methanol mixture was evaporated, leaving a residue at the bottom of the beaker. The beaker with the residue and the weight of the empty beaker was calculated to know the weight of the lipid present in the sample.

Estimation of Proteins

The protein was estimated by Biurette method [23].

Estimation of Carbohydrates

The total carbohydrate was estimated by following the Phenol-sulphuric acid method by [24].

Estimation of Phenols

Total phenolic assay was determined by using Follin-Ciocalteu assay [25].

Results and Discussion

Totally five seaweeds were collected from the east coast of India, during the month of January 2014 and estimated their nutritive properties viz. Amino acids, proteins, lipids, carbohydrates and phenol (Table 1). Measurable differences in nutritional composition were apparent among the five species studied. Amino acids, protein, lipid, carbohydrates and phenol are the most important biochemical components in marine algae and their results are given in the (Table 1) proteins have crucial functions in all the biological processes.

Table 1: Amino acid composition of seaweeds

S. No.	Amino acids	*Caulerpa racemosa*	*Ulva lactuca*	*Ulva reticulate*	*Codium tomentosum*	*Caulerpa sertularioides*
1.	Aspartic acid	7.3 ± 0.2	10.6 ± 0.3	10.8 ± 0.2	12.6 ± 1.3	15.6 ± 0.6
2.	Alanine	5.9 ± 0.5	9.1 ± 1.3	8.4 ± 0.5	6.6 ± 0.3	5.4 ± 0.3
3.	Arginine	4.9 ± 0.7	6.2 ± 0.7	3.9 ± 0.1	4.6 ± 0.2	5.6 ± 0.3
4.	Glutamic acid	14.3 ± 0.6	11.8 ± 0.5	13.1 ± 0.4	13.8 ± 1.2	16.3 ± 0.6
5.	Glycine	7.1 ± 0.9	6.1 ± 0.2	5.5 ± 0.3	6.9 ± 0.7	4.2 ± 0.2
6.	Histidine	3.2 ± 1.0	3.2 ± 0.5	2.5 ± 0.2	2.6 ± 0.5	2.1 ± 0.4
7.	Isoleucine	3.7 ± 0.2	3.1 ± 0.2	4.5 ± 0.3	5.2 ± 0.6	3.5 ± 0.2
8.	Leucine	7.7 ± 0.4	8.5 ± 1.0	7.2 ± 0.3	7.8 ± 0.4	7.7 ± 0.6
9.	Lysine	7.0 ± 0.9	5.8 ± 0.7	6.3 ± 0.5	6.1 ± 0.4	8.5 ± 0.8
10.	Methionine	1.7 ± 0.9	1.2 ± 0.4	3.0 ± 0.3	1.4 ± 0.5	1.1 ± 0.3
11.	Phenylalanine	5.1 ± 0.2	5.4 ± 0.5	4.6 ± 0.2	5.2 ± 0.3	5.2 ± 0.4
12.	Proline	5.7 ± 1.1	3.8 ± 0.1	4.2 ± 0.3	3.9 ± 0.5	5.6 ± 0.7
13.	Serine	7.1 ± 1.1	6.6 ± 0.5	6.6 ± 0.7	6.6 ± 0.4	4.2 ± 0.1

14.	Threonine	6.6 ± 1.5	4.8 ± 0.4	5.3 ± 0.3	5.6 ± 0.3	5.8 ± 0.1
15.	Tyrosine	2.1 ± 0.4	4.6 ± 0.5	2.4 ± 0.4	2.8 ± 0.6	3.6 ± 0.3
16.	Valine	6.8 ± 0.8	6.2 ± 0.6	5.2 ± 0.5	6.4 ± 0.6	6.0 ± 0.5

Note: Dry weight basis as mean values (Mean ± standard deviation).

Their activities can be described by enzymatic catalysis, transport and storage, mechanical sustentation control. In the present study, Data of total amino acids are presented in Table-1. In all, 16 amino acids have been detected in the protein hydrolysate of seaweeds. These amino acids may occur as combined or in a free state [26, 28]. The distribution pattern of these amino acids reveals some pronounced differences among the species of Chlorophyceae. Glutamic acid and aspartic acid were the most abundant amino acid in all species. The percentage of methionine was found to be low in all species. When focusing on individual species, wider differences were found. For example, the highest concentration of glutamic acid and aspartic acid was found in alga *Caulerpa sertularioides* 16.3% and 15.6%, respectively, while the algae, *Ulva lactuca* 11.8% and 10.6%, and *Caulerpa racenisa* 14.3% and 7.3% had the lowest acids in all species except in *Ulva reticulate* (3.1%) which the lowest value was tyrosine 2.4% Protein content showed remarkable variation, with hgher values of 17.9% in *Caulerpa sertularioides* followed by 17.3% in *Caulerpa racemosa* and 13.7% in *Ulva lactuca.* The protein content of algae (*Ulva reticulata* and *Codium tomentosum*) was low. This is in agreement with the findings of higher protein content in species of in Chlorophyta and the lowest in Phaeophyta [29]. Also observed maximum protein content in some of the green algal species belonging to the genus of *Ulva*.

Lipids are rich constitute a convenient storage material for living organisms. In macro algae, the lipids are widely distributed, especially in several resistance stages [30]. In the present study 18.1%, 10.4% and 1.1% of lipid was recorded in *Caulerpa racemosa*, *Codium tomentosum* and *Caulerpa sertularioides* respectively.

Carbohydrate is one of the important components for metabolism and it supplies the energy needed for respiration and other most important processes. The concentration of carbohydrate was higher in most the selected species of green algae. The carbohydrate content was 81.2% in *Caulerpa racemosa* followed by *Ulva lactuca* (68.1%) and *Caulerpa sertularioides* (64.6%) [31]. Studied the species of green algae *Enteromorpha intenstinialis* and reported the highest average total carbohydrate content from *Ulva rigida* (63.04 ± 29.15 g/kg dry weight) [32].

Phenols are the aromatic metabolites which trigger various biochemical processes of the organisms. They consist of hydroxide groups which are widespread in photosynthetic organisms. The highest phenol content was observed in *Caulerpa sertularioides* (32.6%) followed *Codium tomentosum* (31.3%) and *Ulva reticulate* (18.7%). Similar results were also observed [33].

Seaweeds can be considered as promising plants of the future forming one of the important marine living resources of high nutritional value. Being plants with unique structure and biochemical composition, seaweeds could be exploited for their various properties in the form of food, energy, medicine and cosmetics and as biotechnological tools.

Conclusion

From the present study, it is evident that marine macro green algal seaweeds like *Caulerpa racemosa*, *Ulva lactuca*, *Ulva reticulate*, *Codium tomentosum* and *Caulerpa sertularioides* are rich in nutritive properties. The values obtained from protein and carbohydrate contents in the present study are similar to the earlier findings by [12, 32]. The value of protein content is high in *Caulerpa sertularioides* and *Caulerpa racemosa* and the carbohydrate concentration is high in *Caulerpa racemosa, Ulva lactuca* and *Caulerpa sertularioides*. The high lipid value was observed in the algae *Caulerpa racemosa* and *Codium tomentosum.* The present findings will be useful to collect the selected seaweeds from the South east coast of India and use them in the food and pharmaceutical industries for various purposes.

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