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Nutritional composition of two species of a genus ulva collected from Rameswaram to Kanyakumari coasts, Tamilnadu, India

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Abstract
The present quantitative nutrition on *U. lactuca* and *U. rigida* was evaluated and compared. Data reveals that proximate analysis such as moisture, ash, dietary fiber, carbohydrate, protein, lipid, flavonoids, and phenol content was significantly higher in *Ulva rigida*. Analysis of variance showed significant differences in the proximate compositions of the rice varieties studied. *Ulva rigida have* 68% carbohydrate, 70% Dietary fibre, 17% protein and 2% lipid whereas *Ulva lactuca contain* 65% carbohydrate, 63 % Dietary fibre, 15% protein and 1.2% of lipid. The concentration of vitamin A is 0.5 and 0.6 IU, vitamin B1 is 5.22 and 5.85 mg/Kg and Vitamin B2 is 0.97 and 1.22 mg/kg respectively for *Ulva lactuca* and *Ulva rigida*. Photosynthetic pigments and mineral contents also higher in *U. rigida.* The seaweed *U. rigida* and *U. lactuca* were examined in this study has high protein, carbohydrate, and dietary fiber content but low in fat. Among the two species, *U. rigida* had a higher quantity of the nutrient contents than *U. lactuca*. The result of this study showed that both the species may be considered to be developed as an alternative source of healthy food for humans in the future.

Keywords: proximate, vitamin, pigments, heavy metal, ulva lactuca, ulva rigida

Introduction
Worldwide, seaweeds are traditionally consumed as daily diet either fresh or dried especially by people living in and around the coastal areas. The earlier reports showed that certain edible algae contain an adequate amount of essential biochemical like protein, vitamins, and minerals for human nutrition. In the period of late 1990s reports stated that the human consumption of algae has less in green algae, little higher in red algae and the highest in brown algae with 5 %, 33%, and 66.5 % respectively in Asia countries like China, Japan, and Korea (Dawes *et al*, 1998) [11]. The marine macroalgae had been withstood as a staple and vital source for Chinese, Japanese, and Korean diet. Particularly, China and Japan are the main contributors to the world production-consumption scenario. About 20% of the Asian diet has comprised of seaweeds that are being relished not only for their nutritional point of view but also of unique and enchanting flavor compounds. While in the Western diet with the seaweeds are being just used as food additives and extracts (Carvalho *et al*., 2009) [8]. Apaydin *et al*., (2010) [4] reported that the seaweeds have been simply categorized into different parts based upon their uses and applications like agriculture, horticulture, and agronomy, in animal aquaculture, aesthetics, cosmetics, environmental health, monitoring, and remediation, food, health, thalassic and wellness, industry, pharmaceutical and pharmacology and science, technology, and biomedicine.
Seaweeds are excellent dietary sources of vitamins, proteins, carbohydrates, and trace elements (Rohani-Ghadikolalel *et al*., 2012) [21] and they are getting an essential rank in different fields like food, medicine, etc. The *Ulva* is a genus of many species under the family of Ulvaceae, which are functionally and nutritionally the edible green algae. They have widely occurred in the coasts of seas and oceans worldwide, they are also considered as interesting candidates for commercial exploitation in food industries for producing functional and health-promoting foods, because due to their versatility of biochemical compositions (Wolf *et al*., 2012) [25]. The *U. lactuca*, *U. pertusa*, *U. compressa*, and *U. clathrata* have been studied their uses in food industries that they are exhibiting a wide range of nutritional constituents which makes them excellent sources for a portion of healthy food for human nutrition with a higher % of protein comparatively, dietary fiber, low total lipid contents, relatively high levels of essential amino acids and fatty acids, polyunsaturated fatty acids such as oleic, linoleic and linolenic acids, essential vitamins and minerals (Tabarsa *et al*., 2012) [22]. Among the useful species of *Ulva*, *U. lactuca* is being used in soups, cookies, salads (Tan, 2007) [23] and it could be exploited in the form of food, energy, medicine, and biotechnological tools.
The present study is the first published data on the proximate composition, vitamins, minerals, and heavy metals content of *U. lactuca* and *U. rigida* collected from Rameswaram to Kanyakumari coasts, Tamil Nadu, India. As edible seaweeds of *U. lactuca* and *U. rigida* by coastal communities of Rameswaram to Kanyakumari generally used as delicious fresh vegetables. The purpose of this present research work is to know the nutritional constituents of the edible freshly dried seaweeds *U. lactuca* and *U. rigida* with a view to utilization in human nutrition.

Materials and Methods
Sample Collection and Preparation
Seaweed sample was collected from Rameswaram to Kanyakumari coasts, Tamil Nadu, India. The collected seaweed sample was cleaned and washed with seawater to remove unwanted extraneous matter attached to the thalli and immediately transported to the laboratory. In the laboratory, the sample was thoroughly cleaned by rinsing

with distilled water to remove the surface salty materials. And then, it was air-dried under shadow for five days and later ground in a blender. The powdered samples were air tightly kept in dark container and stored at room temperature for further analysis.

Proximate analysis (Chakraborty and Santra, 2006) [9]

Measurement of Moisture Content

The known quantity of the sample was dried to find out its moisture content. The sample was kept into an oven at 105°C for 3 hrs. Immediately after the sample dried, transferred into desiccator, allowed to cool and reweighed. The result of the moisture content of the sample was expressed in percentage.

Determination of Ash Content

Ash content of the sample was determined by heating for 4hrs in a muffle furnace at 550°C until it turned white and free of carbon. The sample was then taken from the furnace, transferred into a desiccator, allowed to cool, and reweighed immediately. The result of the ash content of the sample was expressed in percentage.

Determination of Fat Content

The total fat content of the selected sample was determined by loosely wrapping with a filter paper and put into the thimble which was fitted to a clean round bottom flask, and then it has been cleaned, dried, and weighed. The flask contained 120 ml of petroleum ether. The flask was heated with a heating mantle and allowed to reflux for 5 hrs. After the heating over, it was thimble with the spent samples kept and later weighed.

Determination of Protein

Total protein was calculated from the elemental N determination using the nitrogen-protein conversion factor of 6.25

Determination of Carbohydrate and Dietary Fibre

The carbohydrate content was estimated by difference: 100 – (moisture + ash + protein + fat) %. The dietary fiber content was determined by weighing 2 g of sample and 1 g asbestos and put into 200 mL of 1.25% of H_2SO_4 and It was boiled for 30 min. Solution and content were filtered by Buchner funnel and residues were put into 200 ml boiled NaOH. Boiling continued for 30 min, then transferred to the Buchner funnel and filtered. It was washed twice with alcohol, and then again washed twice with petroleum ether. The residue obtained was put in a clean dry crucible and dried in the oven to a constant weight.

Determination of Vitamin – A. (Eitenmiller and Landen, 1998)

About 2.5 g of the sample was dissolved with an accuracy of 0.1% in 5 ml of pentane R and dilute with 2-propanol R1 to a presumed concentration of 10 µ/ml to 15 µ/ml. O.D was read at 225 nm using UV-VIS Spectrometer SL 150 at the absorption maximum of 326 nm. The amount of vitamin A was calculated as below:

Amount of Vitamin A = A225 × V ×1900 / 100 × m.

Where A225 = absorbance at 326nm. m = mass of the substance to be examined in grams.

V = total volume to which the substance to be examined is diluted to give 10 µ/ml to 15 µ/ml.

1900 is the value number for the factor to convert the specific absorbance of esters of retinol into international units per gram.

Determination of Vitamin B1

About 5 ml of the standard and sample was taken in marked test tubes. In each test tube, 5 ml NH_4OH (0.1M) and 0.5 ml 4-Amino phenol solution added and mixed well, then kept for 5 minutes added 10 ml chloroform and separate of chloroform layer. The absorbance recorded the chloroform layer at 430 nm against a blank (Tripathi, 2008) [24].

Determination of Vitamin B2

Five ml of the standard and sample solution were taken in marked test tubes. In each test tube, 2 ml hydrochloric acid (1 M), 2 ml glacial acetic acid, 2 ml hydrogen peroxide, 2 ml potassium permanganate (15% w/v) and 2 ml phosphate buffer (pH 6.8) added and mixed well and absorbance recorded at 444 nm against a blank (Indian Pharmacopoeia, 2010) [13].

Determination of total phenolic content

The amount of total phenol content in crude methanolic extracts of selected algal samples was determined spectrophotometrically using Folin–Ciocalteu reagent (McDonald *et al*., 2001) [17] with slight modifications. To 0.5 ml of each sample (0.1 mg/ml), 2.5 ml of 1/10 dilution of Folin-Ciocalteau's reagent and 2 ml of 20% Na_2CO_3 were added and incubated at 37 °C for 15 min. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. For standard reading Gallic acid (standard phenolic compound 0.1 mg/ml) was used. The standard graph was compared with a sample reading. The total phenolic content of extracts was noted in µg/g (on a dry weight basis). All samples were analyzed in triplicates.

Determination of total flavonoid content

The aluminium chloride colorimetric method (Chang *et al*., 2002) [10] was used to determine the flavonoid content in crude methanolic extracts of selected macroalgae. 1.0 ml of crude methanolic extracts (1mg/ml) were mixed with 1.5 ml of methanol, 0.1ml of 10% aluminium chloride, 0.1 ml of 1M sodium acetate, and 7.3 ml of distilled water was added then the mixture was allowed to stand for 30.0 minutes at room temperature. Its absorbance was taken at 415 nm. Rutin (Standard flavonoid compound 0.1 mg RUE/ml) was used as the standard for the calibration curve. The standard graph was compared with sample readings. The total flavonoid content of samples was expressed in µg/g (on a dry weight basis). All samples were analyzed in triplicates.

Determination of Minerals Content

The mineral content like calcium, potassium, iron, and sodium was determined by the standard AOAC method (2000) [3]. Phosphorus was determined by the spectrophotometric method. While heavy metals such as mercury, cadmium, arsenic, and lead content were determined by the standard Atomic absorption method

Estimation of Chlorophyll (Saranya and Girija, 2013) [5]

About 500 mg of a fresh sample of seaweed was kept in a pestle and mortar with an adequate amount of 80% acetone and then it was ground well. The homogenate liquid was centrifuged at 3000 rpm for 10 minutes and the supernatant

was stored. The pell*et al*so was extracted by repeated washing with 80 % acetone till it turned into colourless. The extracts were pooled and subjected to determining the chlorophyll content. The extract absorbance was observed at 645 nm and 663 nm in a spectrophotometer. The chlorophyll content was calculated using the following formula.

$$\text{Chlorophyll 'a' (mg/g. fr.wt.)} = \frac{12.7 \times A663 - 2.69 \times A645}{a \times 1000 \times W} \times V$$

$$\text{Chlorophyll 'b' (mg/g. fr.wt.)} = \frac{22.9 \times A645 - 4.68 \times A663}{a \times 1000 \times W} \times V$$

$$\text{Total Chlorophyll (mg/g. fr.wt.)} = \frac{20.2 \times A645 + 8.02 \times A663}{a \times 1000 \times W} \times V$$

Where A = Absorbance at respective wavelength
Volume of extract (ml),
W = Fresh weight of the sample (g).

Estimation of Carotenoid

The same algal chlorophyll extract was used to measure carotenoid. The extract OD was taken at 480 nm.
Carotenoid: µg/g.fr.wt. = A.480 + (0.114 X A. 663) – (0.638 X A. 645).
Where A = Absorbance at the respective wavelength.

Results and Discussion

The dried green algae *Ulva lactuca* and *U. rigida* from Rameswaram to Kanyakumari coasts, Tamil Nadu, India were studied for proximate, vitamins, pigments, minerals and heavy metals analysis. Proximate analysis was done for moisture, ash, dietary fiber, carbohydrate, protein, lipid, flavonoids, and phenol content were given in table 1. The ash content was higher in *U. rigida* with 15.66% then the *U. lactuca* (11.5%) This result was 3-5 % higher than reported by Ortiz *et al.* (2006) [20] and was but lower than report of Khairy and El-Shafay (2013) [14]. The dietary fiber content was 70.25% in *U. rigida*, which was higher than *U. lactuca* (63.15%). According to research data the levels of the dietary fiber content of both species of *Ulva is estimated as 60* %. Suggest it might be a alternative source of dietary fiber. Fibre-rich foods facilitate better bowel function and reduce risk of intestinal disorders. Moisture content of *U. lactuca* and *U. rigida* was 16.8 and 18.9% respectively. Moisture content is important for determining the shelf-life and quality of processed seaweed meals as high moisture may stimulate the growth of microorganisms. In this study, the moisture content was 16.9%. This result was higher than the previous study reported as 10.5% (Abirami and Kowsalya 2011) [2]. The carbohydrate level was higher in *U. rigida* (68.75%) than *U. lactuca* (65.11%). The protein content in *U. rigida and U. lactuca was* examined in this study with 17.15 and 15.75% respectively. This result was higher than the protein content in the *U. lactuca* was 10.89%. The fat content detected was 2.15 and 1.25% with *U. rigida* and *U. lactuca* respectively which has higher than the report of Xiao-Ling *et al.* (2003) [26], and Abdel-Khaliq *et al.* (2014) [1]. The correlation coefficients among the proximate values for carbohydrate, moisture, fat, protein, fibre, and ash were quite high and positively correlated (r = 0.96, p < 0.0001). A negative correlation between carbohydrate and moisture, moisture and energy, fibre with protein and carbohydrate with fibre was also reported by Oko *et al*., (2012) [19]. The important phytochemicals of vitamins, flavonoid and phenol were analyzed in the algal samples (table 2). Vitamin analysis was done for vitamin A, vitamin B1(thiamine) and vitamin B2(riboflavin), the values of which were found to be less than 0.6 (IU/100 g), 5.85 mg/kg, 1.22 mg/kg in *U. rigida* respectively and also 0.5 (IU/100 g), 5.22 mg/kg, 0.97 mg/kg in *U. lactuca* respectively. Vitamins are essential organic micronutrients, which cannot be synthesized directly in sufficient quantities by organisms and so instead must obtain from the diet. Well-known human vitamin-deficiency diseases like beriberi, pellagra, pernicious anemia and scurvy are due to the lake of thiamine (vitamin B1), niacin, cobalamin (vitamin B12), and ascorbic acid (vitamin C) respectively (Martin *et al.*, 2011) [16]. The vitamins such as vitamin A, Vitamin B1, and Vitamin B2 were done the two algal species samples. The photosynthetic pigments such as chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid also were carried out with the two samples, there are five different mineral contents were estimated with the selected algae. The photosynthetic pigments were estimated and the data is represented in figure 1. The concentration of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were 0.41, 0.19, and 0.65 mg/g, and 0.37µg/g with *U. rigida* and 0.39, 0.09, and 0.49 mg/g., and 0.32µg/g with *U. lactuca* respectively. The chlorophyll content of *U. rigida* and *U. lactuca* was similar to green alga *Caulerpa scalpeliformis*, and less than to red alga *Acanthafera spicifera*. Muthuraman and Ranganathan (2004) [18] reported that the maximum carotenoid content in the brown seaweed which was higher than the selected species of *U. rigida* and *U. lactuca.* The flavonoid was higher in *U. rigida* with 1.97 mg RUE/g dry wt., than the *U. lactuca* with 1.76 mg RUE/g dry wt. Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants and blue-green algae. The content of flavonoid compounds in ethanol extract of marine water algal sample was determined from the standard calibration curve of Rutin equivalents and expressed in mg RUE/ g-1 dry wt. mass. The flavonoid content of ethanol extract of marine water algal samples was found to be 1.97 ± 0.02 and 1.76 ± 0.05 mg RUE/ g-1 dry wt., mass in *U. rigida* and *U. lactuca* respectively. Early studies have found marked changes in the chemical compounds with a change of seasons and environmental conditions (Manivannan *et al*., 2009) [15].

The phenol content was higher in *U. rigida* with 2.14mg GAE /g dry wt., than 2.05 mg GAE /g dry wt., of *U. lactuca* (Table-1). The content of phenolic compounds varied from 2.14 ± 0.04 (*U. rigida*) to 2.05 ± 0.05 (*U. lactuca*) mg GAE/g $^{-1}$ dry wt. The phenolic content in the *U. rigida* and *U. lactuca* extracts was significantly differed as compared with those of the other species phenolic compounds which are considered to be the most significant and biologically active compounds with various health beneficial properties. Finally, four heavy metals like mercury, arsenic, cadmium, and lead were analyzed with *U. lactuca* and *U. rigida* samples (Table 3). Mineral contents are concerned, sodium, calcium, iron, potassium and phosphorus were determined in *U. rigida* with 347.0 mg/100 g, 1668.0 mg/100 g, 170.12 mg/100 g, 415.0 mg/100 g and 81mg/100 g, and *U. lactuca*

with 310.0 mg/100 g, 1428.0 mg/100 g, 130.12 mg/100 g, 367.0 mg/100 g and 73mg/100 g, respectively. Calcium contents were 240 mg higher *and iron content is 40 mg higher* in *U. rigida* but phosphorus content 8% is greater than *U. lactuca*.

Heavy metals like mercury, arsenic, cadmium and lead were determined in which the values were found to be higher in *U. rigida* with 0.018mg/kg, 0.12mg/kg, 0.67mg/kg and 0.24mg/kg in *U. rigida* and 0.006mg/kg, 0.08mg/kg, 0.49mg/kg, and 0.39mg/kg in *U. lactuca* respectively. According to Burtins (2003) [7] seaweeds must meet safety regulations in terms of toxicological and bacteriological criteria.

This regulation allows the food industry to include seaweeds as raw or semi-processed materials in the formulation of seafood products because seaweeds are having potential nutritional properties. The heavy metal content reported in this study was within the tolerable based on the qualification criteria for edible seaweeds in India.

Conclusion

This study concluded that nutrient composition of marine green algae namely *Ulva rigida and Ulva lactuca* were quantitatively analyzed. The seaweed *U. rigida* were examined in this study has high protein, carbohydrate, and dietary fiber content but low in fat. The result of this study shows that both the species may be considered to be developed as an alternative source of healthy food for humans in the future.

Table 1: Major biochemical values of genus *Ulva* spp

S.No.	Nutrient Contents	*Ulva lactuca*	*Ulva rigida*
1	Moisture (%)	16.8 ± 0.11	18.9 ± 0.05
2	Ash (%)	11.5 ± 0.03	15.66 ± 0.02
3	Dietary fibre (%)	63.15 ± 0.02	70.25 ± 0.01
4	Carbohydrate(%w/w)	65.11 ± 0.14	68.75 ± 0.12
5	Protein(%w/w)	15.75 ± 0.02	17.15 ± 0.12
6	Lipid (%w/w)	1.25 ± 0.05	2.15 ± 0.04

Table 2: vitamins and pigment values of genus *Ulva* spp

Parameter	*Ulva lactuca*	*Ulva rigida*
Vitamin A (IU/100g)	> 0.5 ± 0.11	> 0.6 ± 0.12
Vitamin B1 (thiamine) (mg/kg)	5.22 ± 0.06	5.85 ± 0.04
Vitamin B2(riboflavin) (mg/kg)	0.97 ± 0.01	1.22 ± 0.01
Chlorophyll a	0.39 ± 0.05	0.41 ± 0.52
Chlorophyll b	0.09 ± 0.03	0.19 ± 0.12
Total Chlorophyll	0.49 ± 0.08	0.65 ± 0.07
Carotenoid (µg /g. fr. wt)	0.32 ± 0.11	0.37 ± 0.55
Flavonoid* (mg RUE/g dry wt.)	1.76 ± 0.05	1.97 ± 0.02
Phenol** (mg GAE/g dry wt.)	2.05 ± 0.05	2.14 ± 0.04
*RUE - Rutin Equivalent. **GAE - Gallic Acid Equivalent		

Table 3: Minerals and metals concentration of genus *Ulva* spp

Elemental test	*Ulva lactuca*	*Ulva rigida*
Sodium(mg/100g)	310.0 ± 0.02	347.0 ± 0.03
Calcium(mg/100g)	1428.0 ± 0.01	1668.0 ± 0.02
Iron (mg/100g)	130.12 ± 0.11	170.12 ± 0.12
Potassium (mg/100g)	367.0 ± 0.12	415.0 ± 0.11
Phosphorus (%)	73 ± 0.05	81 ± 0.04
Mercury (mg/kg)	0.006 ± 0.12	0.018 ± 0.13
Arsenic (mg/kg)	0.08 ± 0.03	0.12 ± 0.04
Cadmium (mg/kg)	0.49 ± 0.02	0.67 ± 0.12
Lead (mg/kg)	0.19 ± 0.11	0.24 ± 0.01

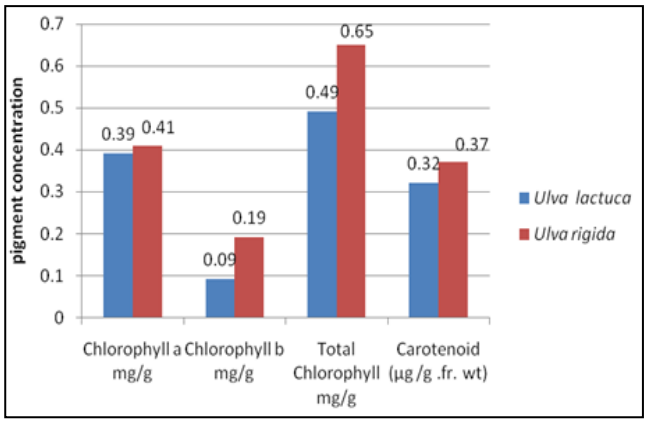

Fig 1: chlorophyll and carotenoid Pigment quantification

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