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Comparison of nutrient compositions and calorific values of eight tropical seaweeds.

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Abstract

This work focuses on the proximate composition of the eight seaweeds belonging to Chlorophyta (*Chaetomorpha antennina, Enteromorpha prolifera* and *Ulva fasciata*) and Rhodophyta (*Acanthophora spicifera, Gracilaria corticata, G. corticata* var. *cylindrica, G. foliifera* and *Gelidium pusillum*), collected from the south west coast of India. The total protein, carbohydrate, lipid, iodine, ash, crude fiber, calorific value and moisture contents were estimated. Total protein content varied from 5.98 to 25.12% and total carbohydrate from 31.02 to 75.95%, where as total lipid levels were only 1.49 to 10.76 %. Greater sources of ions, minerals and metals in seaweeds were evidenced from 13.06 to 27.71 % of ash contents. Comparatively, higher levels of crude fiber content were observed (37.57 to 63.39%) in the seaweed species. Seaweeds also remarked with their iodine concentrations which vary from 41.46 to185.38 mg kg^{-1}. Calorific values ranged from 1.39 to 3.52 kcal g^{-1}. The water holding capacity of the seaweeds was observed between 72.52 and 89.15 %. Correlation study of biochemical constituents with the corresponding species/genus/division had determined the interdependence of compositional groups, which helped in judging the cross compositional relation. Recommended Daily Intake (RDI) values recommended by the Council for Responsible Nutrition (CRN), United States Food and Drug Administration (USFDA) and Electronic Code of Food Regulations (ECFR) showed its potential importance in diet with genus *Gracilaria* as the major contributor to the nutraceutical sectors.

Keywords: –Calorific value, Carbohydrate, Iodine, Lipid, Protein, RDI, Seaweeds

Abbreviations- Pt - Platinum; RDI – Recommended Daily Intake; CRN - Council for Responsible Nutrition; USFDA - United States Food and Drug Administration; ECFR – Electronic Code of Food Regulations; ASTA - American Spice Trade Association.

Introduction

The seaweeds are used in the food, manure, nutraceuticals, cosmetics and pharmaceutical sectors due to its contribution of important economical and dietary resources which lead to significant attention to the world. As a pronounced source for domestic and industrial applications, the seaweeds were getting harvested in the past years which have crossed millions of tons annually (Mc Hugh, 2003). The source as dietary requisites was the major use of seaweeds irrespective of its classification, and its commercial cultivation is carried out (Mc Hugh, 2003). Seaweeds which possess a valuable resource of protein, carbohydrates, lipids, vitamins, minerals, dietary fiber and iodine are also used up in animal nutrition (Sara-Marsham *et al.,* 2007). The enormous amount of seaweeds available in the world is not completely used up commercially which might be due to the lack of knowledge on its potentiality, harvesting negligence or incompatibility as a nutritional supplement (Sara-Marsham *et al.,* 2007).

In the past years, nutritional evaluation of seaweeds concentrated mainly on the red than the brown or green seaweeds (Siddique *et al.,* 2013). The previously analyzed seaweeds such as the *E. bicyclis, F. vesiculosus, D. antarctica, P. palmata, E. cava, G. edulis, S. fusiforme, S. japonica, S. vulgare, U. pinnatifida* etc., were been used as a food source in China, Japan, South Korea, Thailand, Vietnam and Taiwan. The seaweeds were generally observed with low calorific value, rich in vitamins, minerals, dietary fibers and were observed as a valuable source of protein, lipid, fiber, vitamins and minerals (Ortiz *et al.*, 2006). Seaweeds are used as a medium for toxic element absorbance (Chojnacka *et al.,* 2012). Being a marine macroalgae and a primitive type of plants, they flourish on all sources of attachments like the rocky, coral or any substrata for the growth, which make them a versatile product widely being used as a food for direct consumption (Ghosh *et al.,* 2012). As a renewable source, and on easier availability, seaweeds are being used as food, fodder and fertilizer from ancient times (El-Shafay, 2014).

The influence of seaweeds as a leading biological factor on the field crops has increased in recent years with its extracts being used as a seaweed liquid fertilizer (SLF) due to the immense quantity of biochemical constituents (Renuka Bai *et al.,* 2007). In food sector, they show their presence by contributing phycocolloids which act as thickening and gelling agents (El-Shafay, 2014). The use of seaweeds as fuel and in cosmetics products were already remarked (Mc Hugh, 2003).

Seaweeds are classified based on its chemical composition as Rhodophyta (red algae), Chlorophyta (green algae) and Phaeophyta (brown algae) (Dawczynski *et al.,* 2007) whose chemical composition varies temporally, seasonally and spatially and so the monitoring of the nutritional contents to select the better source is important.Seasonal, temporal and

spatial variation in chemical composition and growth were seen in the proximate compositional analysis (Khairy and El-Shafay, 2013) which could also be seen inter-specific, inter-annually or intra-annually.

It has been observed that the concentration of proteins and carbohydrates varies with species and has greater remarks on seasonal and temporal variations (Dawczynski *et al.,* 2007). Earlier reports in seaweeds show 6 to 44 % of total protein (Sara-Marsham *et al.,* 2007). The total carbohydrates in seaweeds were reported in previous studies as above 19% (Alberto Peña-Rodríguez *et al.,* 2011; El-Said and El-Sikaily, 2013). Lipid or general fat content is also varywith seasonal, temporal and spatial conditions and was accounted in range of 1-6% (Jurković *et al.,* 1995).The fibre content is found higher in seaweeds than in terrestrial fruits and vegetables. These fibres are found to be health promoting and the dietary fiber content was reported in range of 36-65% in seaweeds (Dawczynski *et al.,* 2007).

Seaweeds act as a good contributor to iodine (Apaydin *et al.,* 2010) which could be consumed by animals as a potential source of iodine in thyroid malfunction. Iodine content was found in the range of 30 to 2984 mg kg^{-1} in seaweeds (Jane-Teas *et al.,* 2004).Seaweeds are a greater source of ions, minerals and metals which might be absorbed or adsorbed during its dwelling on seashores and are collectively evidenced as its ash content. Ash content reported in seaweeds range from 9.3 to 77.8 % (Sara-Marsham *et al.,* 2007). The energy that could be generated by the organism utilising its biochemical composition upon ignition is derived as the calorific value and this value varied from 0.64 to 4.37 $kcalg^{-1}$ (Sara-Marsham *et al.,* 2007). Seaweeds are also appreciable sources of carotenoids, vitamins and flavonoids and these beneficial constituents make the use of seaweeds in pharmaceutical activities.

The current search for commercial raw material to extract biologically important chemical constituents and as a highly nutritive food source has increased tremendously. The seaweeds vary greatly in their colour, quality, consistency and constituents. The present study on the proximate composition analysis evaluated the total protein, carbohydrate, lipid, iodine, ash, fiber and calorific value on eight seaweeds from the south west coast of India, and compared their biochemical correlation. The study compared the composition with the recommended daily intake quantity (RDI) defined by the CRN, USFDA and ECFR for an average person, in order to make a concrete base to the thought of utilisation of the studied seaweeds in nutrition and nutraceuticals.

Materials and methods

Materials

Eight seaweeds, comprising of Chlorophyta (*Chaetomorpha antennina, Enteromorpha prolifera* and *Ulva fasciata*) and Rhodophyta (*Acanthophora spicifera, Gracilaria corticata, G. corticata* var. *cylindrica, G. foliifera* and *Gelidium pusillum*) were collected during the post monsoon season (September 2009) from two locations (Njarakkal (10°01'33.8"N 76°12'40.2"E) and Kayamkulam (9°08'31.4"N 76°27'37.5"E)) of the Kerala coast, south India. A common species *E. prolifera was* collected from both locations. The samples collected were washed with seawater and then with tap water to remove all the epifauna and epiphytes. The samples were then freeze dried, powdered and stored in glass bottles by maintaining the moisture content less than 2% for further analysis.

Proximate compositional analyses

Fresh seaweeds upon removal of epiphytes and epifauna were analysed for moisture content (Karl Fischer Moisture analysis - Peter-Bruttel and Regina-Schlink, 2003). The moisture content was estimated potentiometrically, with the anode solution consisting of methanol, imidazole, SO_2 and I_2.The titration cell has a smaller compartment with a cathode immersed in the anode solution of the main compartment. The two compartments are separated by an ion-permeable membrane, and the platinum anode generates I_2 when current is provided through the electric circuit. The net reaction commutes as one mole of I_2 is consumed for each mole of H_2O. In other words, 2 moles of electrons are consumed per mole of water. The end point is detected most commonly by a bipotentiometric method. A second pair of Pt electrodes are immersed in the anode solution known as the detector circuit which maintains a constant current between the two detector electrodes during titration. Prior to the equivalence point, the solution contains I^- and little I_2. At the equivalence point, excess of I_2 appears and an abrupt voltage drop denotes the end point. The amount of current needed to generate I_2 and reach the observed end point is be used to calculate the amount of water in the original sample.

The freeze dried samples were analysed for the total protein, carbohydrate, lipid, iodine, ash, crude fibre (dietary fibre) content and calorific value. All the analyses were done in triplicates with results expressed with standard deviations (n=3) against dry weight.

Total protein content was estimated by the UV-Vis spectrophotometric method (Lowry *et al.,* 1951). UV-Vis Cary 60 spectrophotometer was used at 750 nm. 100mg of the powdered sample was weighed into a 10 mL standard flask and made upto volume using double distilled water. 0.1 mL of this sample solution was mixed with 0.1 mL of 2N NaOH

solution. The mixture was hydrolyzed at 100 °C for 10 min in a boiling water bath. The hydrolysate was cooled to room temperature and 1 mL of freshly mixed complex-forming reagent (containing 2% Na_2CO_3, 2% sodium potassium tartarate and 1% $CuSO_4.5H_2O$) was added and the mixture was allowed to stand at room temperature for 10 min. To this mixture, 0.1 mL of Folin reagent was added, mixed using a vortex mixer and allowed to stand at room temperature for 30–60 min. The absorbance was measured. Bovin serum albumin was used as a standard with multi point calibration yielding the correlation factor r^2 as 0.999. Results are expressed as % to dry weight of the sample.

Total carbohydrates were estimated by the UV-Vis spectro-photometric method (Dubois *et al.,* 1956). Estimation was done upon the dehydration reaction between the carbohydrates with concentrated sulphuric acid which produces furfural derivatives. The reaction between furfural derivatives and phenol develops detectable colour. 100 mg of the sample was measured into a 10 mL standard flask and made up with double distilled water. A 2 mL aliquot of this sample solution was mixed with 1 mL of 5% aqueous solution of phenol in a test tube. Subsequently, 5 mL of concentrated sulphuric acid was added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they were vortexed for 30 s and placed for 20 min in a water bath at room temperature for colour development. The absorbance was measured at 490 nm. The phenol used in this procedure was redistilled and 5% phenol in water (w/w) was prepared immediately before the measurements. Glucose was used as a standard with multi point calibration yielding the correlation factor r^2 as 0.999. Results are expressed as % to dry weight of the sample.

Sulfophospho vanillin method (Barnes and Blackstock, 1973) was used to estimate the total lipids. 100 mg of the sample weighed into a 10 mL standard flask and made up to the volume using HPLC grade methanol. 0.5 ml of this solution was taken into a clean test tube and dried under vacuum in a desiccator loaded with silica gel. The dried extract was dissolved in 0.5 ml of concentrated sulphuric acid and mixed well. The tube was plugged with non-absorbent cotton wool and placed in a boiling water bath for 10 min. The tubes were cooled to room temperature. 0.2 ml of this acid digest was mixed with 5 ml of vanillin reagent in another test tube, mixed well and allowed to stand for half an hour. The developed colour was measured at 520 nm. Cholesterol was used as a standard with multi point calibration yielding the correlation factor r^2 as 0.999. Results are expressed as % to dry weight of the sample.

Total iodine content was determined spectrophotometrically (Saenko *et al.,* 1978). 50 to 100 mg of the sample was taken in a crucible and moistened with 30% K_2CO_3 solution. The crucible was kept in a muffle furnace for 4-5 hr at 400 to 500 ^oC. Upon complete ash formation, the contents in crucible were transferred to a 100 mL standard flask. To this, 25 mL 20 % NaCl solution was added and mixed for 1 hr. The mixture was filtered and the residue was placed in a calibrated test tube with a ground plug. The volume was increased to 10 mL with 20% NaCl solution. To this mixture, a freshly prepared 0.5 % $NaNO_2$ solution (0.25 mL), 20% NaBr solution (0.25 mL) and 0.5 % brilliant green solution (0.25 mL) were added. The mixture was then agitated for 2 min with 5 mL toluene and 1mL 5N H_2SO_4. After 20 min, blue green toluene layer was taken off using a separating funnel into a cuvette and absorbance at 680 nm was measured. Potassium iodide was the standard with multi point calibrations yielding r^2 value 0.999. Results are expressed as μgg^{-1} to dry weight of the sample.

Total ash content was estimated as per the method underlined in ASTA (ASTA, 1999). 2 to 3 g of the well mixed sample was placed in a pre-weighed crucible in the entrance of the open muffle furnace until the sample is well carbonized. The carbonized sample was placed in the furnace at 600 ^oC and incinerated for two hours, until light gray ash was obtained to constant weight. The carbon remains were leached with hot water, filtered through an ash less filter paper. The filter paper was washed thoroughly and the paper and contents were transferred to the original crucible. The crucible was dried and ignited in the muffle furnace at 600 ^oC until the ash was white and free from carbon. The dish was cooled and the weight of the ash was measured. The results are represented as % to dry weight of the sample.

Total crude fibre content was also estimated as per the method in ASTA (ASTA, 1999). 2 g of sample was extracted three times with methylene chloride. The extract was discarded and the residue together with 0.5 g of ceramic fibre was added onto a digestion flask. 200 mL of 1N H_2SO_4 solution was added and refluxed for 30 minutes with frequent rotation of the flask to ensure thorough wetting and mixing of the sample. Upon completion of the boiling, the solution was filtered through a filter cloth under suction. The residue was washed with boiling water until washings are no longer acid. The residue was then transferred into another digestion flask containing 200 mL of 1 N NaOH solution. The mixture was boiled for 30 minutes and upon completion, the mixture was filtered through a Gooch crucible. The residue was thoroughly cleaned with water and then with 15 mL of ethyl alcohol. The crucible was dried at 110^oC to a constant weight, cooled in a desiccator and weighed. The crucible was then ignited in an electric muffle furnace at 600^oC, for 20 min. The crucible was cooled in a desiccator and weighed to calculate the crude fibre content. The results are represented as % to dry weight of the sample.

The calorific value of the samples was measured using a bomb calorimeter (Dare and Edwtards, 1975). Small pellets of the dried sample (1 g) were placed in the bomb chamber, pressurized to 425 psi with pure oxygen, combusted and

the amount of difference of heat liberated was recorded. The bomb-calorimeter was calibrated against benzoic acid standards prior to the analysis of samples. The results are expressed as kcal g^{-1} to dry weight of the sample.

Correlation studies

The biochemical composition of the seaweeds was subjected to its inter-compositional Pearson correlation studies using the SPS 16.0 software for windows. The species wise, division wise (Chlorophyta and Rhodophyta), and genera wise (genus *Gracilaria* including the *G. corticata, G. corticata* var. *cylindrica* and *G. foliifera*) correlations were attempted. The compositional ratios were determined to evaluate the biochemical distribution pattern.

Evaluation of seaweed biochemical constituent's contribution to RDI

Recommended daily intake amount or the daily recommended intake values of protein, carbohydrate, lipid, iodine, crude fibre and calorific value stated by the CRN, USFDA and ECFR were compared with the corresponding values of the analyzed seaweeds. The contribution of the seaweeds to the daily dietary requirement of an average person was evaluated for deriving the potential seaweed source that require commercial attention. The results are reported as the amount of seaweeds to be consumed in gram per day. The seaweed with minimal intake quantity was concluded as the major contributor to the future dietary and commercial requirements.

Results and Discussion

Proximate compositional analyses

The nutritional composition data is given in Table 1. In general, the red and green algae showed differences in the concentrations of their chemical constituents. Biochemical compositional relation with corresponding seaweeds is given in Figure 1. *U. fasciata* and *E. prolifera* showed the highest protein contents. With respect to division, the green seaweeds had major concentration of protein (8.99 to 25.12%). In red seaweeds, *A. spicifera* (9.37%) had almost comparable protein content with the green seaweed *C. antennina* (8.99%). The least protein containing seaweeds were *G. pusillum* (5.98%) and *G. corticata* var. *cylindrica* (5.98%) with almost similar protein content which shows the similarity in the location of sampling.

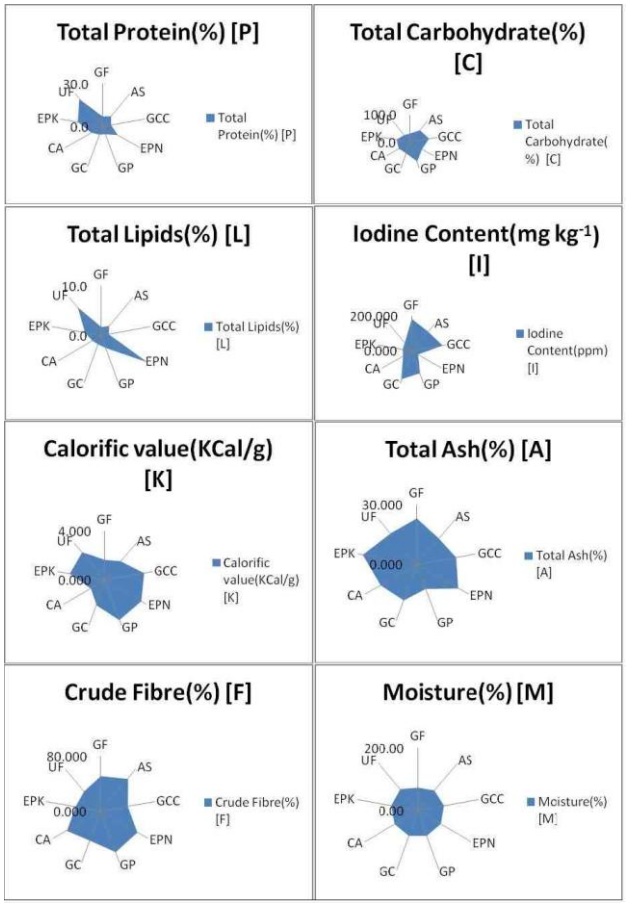
Table 1

Biochemical composition of analyzed seaweeds (mean ± SD, n=3).

Species	P	C	L	I	A	F	K	M
A. spicifera (N)	9.37 ± 0.14	55.81 ± 0.86	2.49 ± 0.04	145.51 ± 2.23	17.14 ± 0.26	61.89 ± 0.95	2.03 ± 0.03	83.65 ± 0.83
C. antennina (N)	8.99 ± 0.18	48.44 ± 0.74	2.09 ± 0.03	57.81 ± 0.89	21.03 ± 0.32	56.91 ± 0.87	1.39 ± 0.02	87.46 ± 1.34
E. prolifera (N)	12.26 ± 0.19	50.83 ± 0.78	10.76 ± 0.17	41.46 ± 0.64	24.12 ± 0.37	61.59 ± 0.94	3.49 ± 0.05	86.09 ± 0.72
G. pusillum (N)	5.98 ± 0.18	75.95 ± 1.16	2.65 ± 0.04	137.54 ± 2.11	13.06 ± 0.20	63.39 ± 0.97	3.52 ± 0.05	86.44 ± 0.99
G. corticata (N)	6.18 ± 0.09	43.85 ± 0.67	1.89 ± 0.03	175.41 ± 2.69	19.24 ± 0.29	45.58 ± 0.70	2.17 ± 0.03	86.69 ± 1.03
G. corticata var. *cylindrica* (N)	5.98 ± 0.09	70.56 ± 1.08	1.49 ± 0.02	179.40 ± 2.75	20.13 ± 0.31	40.07 ± 0.61	3.32 ± 0.05	86.06 ± 1.11
G. foliifera (N)	6.98 ± 0.11	31.02 ± 0.48	1.69 ± 0.03	185.38 ± 2.84	23.12 ± 0.35	51.43 ± 0.79	1.67 ± 0.03	72.52 ± 0.80
E. prolifera (K)	17.45 ± 0.27	50.33 ± 0.77	3.35 ± 0.05	52.83 ± 0.81	27.71 ± 0.42	37.57 ± 0.58	3.01 ± 0.05	87.79 ± 0.14

P- Total protein content (%), C- Total carbohydrate content (%), L- Total lipid content (%), I- Iodine content (mg kg^{-1}), A- Total ash content (%), F- Crude fiber content (%), K- Calorific value (kcal g^{-1}), M- Total moisture content (%), N- Njarakkal location and K- Kayamkulam location.

Figure 1 . Radar graph of biochemical compositions in relation with the seaweeds.



AS - *A. spicifera*, CA - *C. antennina*, EPN - *E. prolifera* from Njarakkal, GP- *G. pusillum*, GC- *G. corticata*, GCC- *G. corticata* var. *cylindrica*, GF- *G. foliifera*, EPK- *E. prolifera* from Kayamkulam, UF- *U. fasciata*.

Total carbohydrate contents which are the sources of agars, carrageenans and fucoidans were seen in dominance in seaweeds. *G. pusillum* (75.95%) and *G. corticata* var. *cylindrica* (70.56%) showed similar carbohydrate contents which explain the spatial adherence of seaweed dwelling. The lowest carbohydrate content among the analysed seaweeds was in the red seaweed *G. foliifera* (31.02%). In green seaweeds, carbohydrates varied from 33.17 to 50.83%, and *E. prolifera* from both locations comprised almost similar carbohydrate concentrations which showed their spatial relation.

Lipid content showed a divisional variation with green seaweeds having slightly higher concentrations in comparison with red seaweeds. The highest lipid containing seaweed species was *E. prolifera* (from Njarakkal site), possessing 10.76%. *U. fasciata* and *E. prolifera* were showing comparatively higher lipid levels (7.38 and 3.35 respectively). Lipid contents in green seaweeds varied from 2.09 to 10.76 % and in red seaweeds ranging from 1.49 to 2.65%.

Iodine content showed the species wise relation, ranged from 41.46 to 62.49 mg kg^{-1} in green seaweeds and 137.54 to 185.38 mg kg^{-1} in red seaweeds. The *U. fasciata* and *G. foliifera* dominated in the green and red seaweed species respectively. Iodine content was observed to be in deviation from the spatial similarities.

The total ash content which is the non combustible inorganic matter was observed to be in almost equal dispersion throughout the seaweeds. In green seaweeds, total ash content ranged from 20.43% in *U. fasciata* to 27.71% in *E. prolifera* and in red seaweeds, it ranged from 13.06% in *G. pusillum* to 23.12% in *G. foliifera*.

The crude fibre also termed as the dietary fibre demonstrated no similarities in the species or division wise. It had a varying pattern which ranged from 37.57 to 61.59% in green seaweeds and 40.07 to 63.39% in red seaweeds. The highest crude fibre was observed in *G. pusillum* and lowest in *E. prolifera*. The spatial similarity of *G. pusillum* and *G. corticata* var. *cylindrica* was also not observed in the case of crude fibre content.

The calorific value which is the energy released upon combustion of a sample showed a similarity in species of *E. prolifera* (3.01 kcal g^{-1}) and *U. fasciata* (2.99 kcal g^{-1}). The green seaweed, *C. antennina* exhibited the least calorific value

(1.39 ± 0.02 kcal g^{-1}). The highest calorific value was for *G. pusillum* with 3.52 kcal g^{-1}. With respect to red seaweeds, *G. pusillum* and *G. corticata* var. *cylindrica* showed similarity in calorific values (3.52 and 3.32 kcal g^{-1} respectively) which showed the adherence to spatial correlation.

The moisture content which determines the water content in fresh raw seaweed showed a range of 72.52 to 86.44% in red seaweeds and 86.09 to 89.15% in green seaweeds. The highest was observed in *G. pusillum* among the red seaweeds and *U. fasciata* in green seaweeds. The least water clogging inter species wise was observed in *G. foliifera (72.52%)*.

The results of current studies were aligning with the previous proximate compositional studies carried on other locations. Table 2 depicts the biochemical composition data of 54 seaweed varieties reported recently in comparison with the data of the 8 seaweeds in the present study.

Table 2. Biochemical composition data of seaweeds in comparison with present study.

Species	Sampling Location	P	C	L	I	F	K	A	M	Reference
A. spicifera	Mandapam coast, India	5.291	-	1.114	-	-	-	-	-	Sreenivasan *et al.*, 2012
A. spicifera	**Njarakkal, Kerala coast, India**	**9.37**	**55.81**	**2.49**	**145.51**	**61.89**	**2.03**	**17.14**	**83.65**	**Present study**
A. taxiformis	Brazil	11.7	22.9	4.8	-	-	-	-	-	Diniz *et al.,* 2011
C. adhaerens	Mandapam coast, India	5.219	-	1.213	-	-	-	-	-	Sreenivasan *et al.*, 2012
C. antennina	**Njarakkal, Kerala coast, India**	**8.99**	**48.44**	**2.09**	**57.81**	**56.91**	**1.39**	**21.03**	**87.46**	**Present study**
C. clavulatum	Brazil	11.3	27.1	2.78	-	-	-	-	-	Diniz *et al.,* 2011
C. rubrum	Egypt	9.273	88.76	0.0082	-	-	-	0.53	-	El-Shafay 2014
C.aerea	Brazil	16.1	29.4	5.49	-	-	-	-	-	Diniz *et al.,* 2011
E. prolifera	**Kayamkulam. Kerala coast, India**	**17.45**	**50.33**	**3.35**	**52.83**	**37.57**	**3.01**	**27.71**	**87.79**	**Present study**
E. prolifera	**Njarakkal, Kerala coast, India**	**12.26**	**50.83**	**10.76**	**41.46**	**61.59**	**3.49**	**24.12**	**86.09**	**Present study**
G. corticata	**Njarakkal, Kerala coast, India**	**6.18**	**43.85**	**1.89**	**175.41**	**45.58**	**2.17**	**19.24**	**86.69**	**Present study**
G. corticata* var. *cylindrica	**Njarakkal, Kerala coast, India**	**5.98**	**70.56**	**1.49**	**179.4**	**40.07**	**3.32**	**20.13**	**86.06**	**Present study**
G. fisheri	Pattani bay, Southern Thailand	11.6	-	1.7-2.7	-	57.5-64.0	-	21.2-22.9	-	Benjama and Masniyom, 2012
G. foliifera	**Njarakkal, Kerala coast, India**	**6.98**	**31.02**	**1.69**	**185.38**	**51.43**	**1.67**	**23.12**	**72.52**	**Present study**
G. pusillum	**Njarakkal, Kerala coast, India**	**5.98**	**75.95**	**2.65**	**137.54**	**63.39**	**3.52**	**13.06**	**86.44**	**Present study**
G. pusillum	St. Martin's Island, Bangladesh	11.31	40.64	2.16	-	24.74	-	21.15	-	Siddique *et al.,* 2013
G. tenuislipitata	Pattani bay, Southern Thailand	20.3-22.9	-	1.9-3.6	-	56.6-60.2	-	7.9-26.0	-	Benjama and Masniyom, 2012
H. musciformis	St. Martin's Island, Bangladesh	18.64	20.6	1.27	-	37.92	-	21.57	-	Siddique *et al.,* 2013
H. pannosa	St. Martin's	16.31	22.89	1.56	-	40.59	-	18.65	-	Siddique *et al.,*

	Island, Bangladesh									2013
J. rubens	Abu Qir bay, Egypt	9.76-12.93	34.57-42.18	1.47-2.39	-	-	-	39.25-50.54	-	Khairy and El-Shafay, 2013
Laminaria sp.	Taiwan, Japan, Thailand and Korea	-	-	-	241 - 4921.3	-	-	-	-	Yeh *et al.,* 2014
P. capillacea	Abu Qir bay, Egypt	17.35-23.72	47.98-50.96	1.76-2.71	-	-	-	13.02-23.68	-	Khairy and El-Shafay, 2013
P. pavonica	Egypt	8.35	90.5	0.006	-	-	-	0.51	-	El-Shafay, 2014
P. tenera	Taiwan, Japan, Thailand and Korea	-	-	-	29.3 - 45.8	-	-	-	-	Yeh *et al.,* 2014
Porphyra sp.	China, Japan and Korea	30.9 - 31.4		1.0 - 2.8		45.7 - 49.8	-	-	-	Dawczynski *et al.*, 2007
S. filipendula	Brazil	8.72	16.8	2.92	-	-	-	-	-	Diniz *et al.,* 2011
S. fusiforme	Egypt	8.85	90.71	0.0204	-	-	-	0.33	-	El-Shafay, 2014
S. hypnoides	Brazil	10.7	27.7	4.2	-	-	-	-	-	Diniz *et al.,* 2011
S. vulgare	Egypt	5.85	93.34	0.0403	-	-	-	0.19	-	El-Shafay, 2014
U. clathrata	Mexico	20-26	-	2.5-4	-	24-27	-	28-50	-	Rodriguez *et al.,* 2011
U. fasciata	**Kayamkulam. Kerala coast, India**	**25.12**	**33.17**	**7.38**	**62.49**	**37.97**	**2.99**	**20.43**	**89.15**	**Present study**
U. intestinalis	Thailand	16.4 - 19.5	-	7.3 - 8.7	-	51.3 - 62.2	-	26.9 - 28.4	-	Benjama and Masniyom, 2011
U. lactuca	Abu Qir bay, Egypt	16.78-20.12	42.09-46.42	3.14-4.09	-	-	-	17.56-23.19	-	Khairy and El-Shafay, 2013
U. lactuca	Algeria	15.3	-	-	-	22.8	-	39.1	-	Hind *et al.,* 2014
U. pertusa	Thailand	14.6 - 16.1	-	2.1 - 7.4	-	52.2 - 59.0	-	25.9 - 28.6	-	Benjama and Masniyom, 2011
U. pinnatifida	Taiwan, Japan, Thailand and Korea	-	-	-	93.9 - 185.1	-	-	-	-	Yeh *et al.,* 2014
C. glomerata *D. dichotoma* *E. compressa* *G. acerosa* *G. crassa* *H. macroloba* *H. musciformis* *H. tuna* *P. pavonica* *T.ornata* *U. reticulata*	Southeast coast, India	9.65 - 31.07	14.73 - 17.49	0.26 - 3.58	-	-	-	-	-	Manivannan *et al.,* 2009
U. pinnatifida	China, Japan and Korea	7.5 - 19.8	-	1.0 - 4.5	-	36.0 - 62.3	-	-	-	Dawczynski *et al.,* 2007
Laminaria sp.	China, Japan and Korea									
H. fusiforme	China, Japan and Korea									
C. lentillifera *C. racemosa*	Sabah	10.52 - 13.24	53.08 - 67.40	0.15 - 0.17	-	11.29 - 19.40	-	10.64 - 14.10	90.84 - 92.00	Ahmad *et al.,* 2012
E. denticulatum *G. verrucosa* *K. alvarezii* *K. striatum* var. *sacol*	Sabah	5.22 - 17.28	57.79 - 74.11	0.18 - 0.54	-	4.03 - 7.84	-	6.05 - 28.79	75.95 - 96.03	Ahmad *et al.,* 2012

Laurencia H. cuneiformis P. gymnospora S. polycystum T. conoides	Sabah	5.93 - 7.78	26.86 - 41.03	0.51 - 0.84	-	21.66 - 34.71	-	21.37 - 45.04	83.51 - 86.86	Ahmad *et al.*, 2012
C. rotundata	Palk Bay, Bay of Bengal, India	-	-	-	-	-	60.62 - 63.68	-	-	Pradheeba *et al.*, 2011
C. serrulata							37.33 - 43.40			
E. acoroides							68.82 - 77.84			
G. corticata							18.69			
G. edulis							19.41			
G. pusillum							27.46 - 29.16			
H. beccarii							38.84			
H. musciformis							12.08 - 24.08			
H. ovalis							37.03 - 28.23			
H. pinifoli							28.39 - 37.16			
H. uninervis							40.15 - 30.48			
S. isoetifolium							38.38			
S. wightii							22.46			
T. conoides							23.98			

P- Total protein content (%), C- Total carbohydrate content (%), L- Total lipid content (%), I- Iodine content (mg kg^{-1}), A- Total ash content (%), F- Crude fiber content (%), K- Calorific value (kcal g^{-1}) and M- Total moisture content (%).

Correlation studies

The correlation of the individual biochemical constituent to species was aided in the determination of the individual biochemical composition ratio (Table 3). Correlation with positive values exhibits direct progressive relation and negative values indicates regression in the concentrations. In the total biochemical compositional correlation analysis (Table 4 (a)), total protein content showed positive correlations with total lipid content (+ 0.586) and negative correlations with the iodine content (- 0.690), with higher significance of < 0.05 level. Total carbohydrate content showed positive correlations with calorific value (+ 0.528), and negative correlation with total ash content (- 0.513). Between total lipid content and iodine content, the correlation factor was – 0.682 i.e., significant to the level of < 0.05. No other correlations were observed in prominence between the biochemical compositions possessing significant relations.

Table 3. Biochemical composition ratio of the analyzed seaweeds.

Species	P:C:L:A:F Ratio
A. spicifera (N)	4:22:1:7:25
C. antennina (N)	4:23:1:10:27
E. prolifera (N)	1:5:1:2:6
G. pusillum (N)	2:29:1:5:24
G. corticata (N)	3:23:1:10:24
G. corticata var. *cylindrica* (N)	4:47:1:13:27
G. foliifera (N)	4:18:1:14:30
E. prolifera (K)	5:15:1:8:11
U. fasciata (K)	3:4:1:3:5

P- Total protein content (%), C- Total carbohydrate content (%), L- Total lipid content (%), A- Total ash content (%), F- Crude fiber content (%), N- Njarakkal location and K- Kayamkulam location.

The correlation studies upon extension to division wise analysis (Table 4 (b)) in Chlorophyta showed significant negative correlations between the total carbohydrate content and moisture content (- 0.841), crude fibre and moisture content (- 0.832), total protein content and carbohydrate content (- 0.822), total carbohydrate content and iodine content (- 0.727), and iodine content and crude fibre content (- 0.614). Positive correlations were observed between iodine content and moisture content (+ 0.921), total protein content and moisture content (+ 0.794), total lipid content and calorific value (+ 0.780) and total carbohydrate content and ash content (+ 0.645).

Table 4. Pearson correlation table.

(a) Total biochemical compositional correlation analysis.

	P	C	L	I	A	F	K	M
P	1							
C	-0.47	1						
L	0.586	-0.222	1					
I	-.690*	0.174	-.682*	1				
A	0.41	-0.513	0.296	-0.456	1			
F	-0.484	0.313	0.107	0.003	-0.476	1		
K	0.241	0.528	0.497	-0.212	-0.059	-0.129	1	
M	0.407	0.345	0.3	-0.553	-0.099	-0.208	0.449	1

(b) Division wise biochemical compositional correlation analysis- Chlorophyta

	P	C	L	I	A	F	K	M
P	1							
C	-0.822	1						
L	0.22	-0.159	1					
I	0.503	-0.727	-0.56	1				
A	-0.098	0.645	-0.072	-0.535	1			
F	-0.83	0.542	0.295	-0.614	-0.209	1		
K	0.482	-0.075	0.78	-0.514	0.452	-0.206	1	
M	0.794	-0.841	-0.335	0.921	-0.36	-0.832	-0.146	1

(c) Division wise biochemical compositional correlation analysis- Rhodophyta

	P	C	L	I	A	F	K	M
P	1							
C	-0.238	1						
L	0.39	0.39	1					
I	-0.337	-0.605	-.965**	1				
A	0	-0.751	-0.867	.933*	1			
F	0.505	0.193	.934*	-0.863	-0.675	1		
K	-0.564	.934*	0.179	-0.383	-0.62	-0.01	1	
M	-0.227	0.738	0.314	-0.458	-0.669	-0.036	0.663	1

(d) Family wise biochemical compositional correlation analysis- *Gracilaria*

	P	C	L	I	A	F	K	M
P	1							
C	-0.861	1						
L	0.189	-0.662	1					

I	0.826	-0.424	-0.398	1				
A	0.917	-0.586	-0.219	0.982	1			
F	0.95	-0.976	0.485	0.61	0.747	1		
K	-0.849	1.000*	-0.68	-0.403	-0.567	-0.971	1	
M	-0.974	0.723	0.039	-0.933	-0.984	-0.855	0.706	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

P- Total protein content (%), C- Total carbohydrate content (%), L- Total lipid content (%), I- Iodine content ($\mu g g^{-1}$), A- Total ash content (%), F- Crude fiber content (%). K- Calorific value (kcal g^{-1}) and M- Total moisture content.

Rhodophyta upon consideration showed significant positive correlation (Table 4 (c)) between total carbohydrate content and calorific value (+ 0.934), total lipids content and crude fibre content (+ 0.934), iodine content and total ash content (+ 0.933), total carbohydrate content and moisture content (+ 0.738), and between calorific value and moisture content (+ 0.663). Significant negative correlations were observed between total lipids content and iodine content (- 0.965), total lipids content and ash content (- 0.867), iodine content and crude fibre content (- 0.863), total carbohydrate content and ash content (- 0.751), total ash content and crude fibre content (- 0.675), moisture content (- 0.669) and calorific value (- 0.620) and between the total carbohydrate content and iodine content (- 0.605).

Gracilaria genus on its intra species correlation (Table 4 (d)), exhibited significant positive correlation between total carbohydrate content and calorific value (+ 1.000), iodine content and ash content (+ 0.982), total protein content and crude fibre content (+ 0.950) and between total protein content and ash content (+ 0.917). Significant negative correlations were also observed between ash content and moisture content (- 0.984), total carbohydrate content and crude fibre content (- 0.976), total protein content and moisture content (- 0.974), crude fibre content and calorific value (- 0.971) and iodine content and moisture content (- 0.933).

Some medicinal plants collected from Nigeria showed proximate correlations with *P. americana*, exhibiting positive correlation (+ 0.95) between its moisture content and protein content. Positive correlation of +1 was also seen between the moisture content and lipid contents of *P. americana*. *M. pudica* showed a positive correlation of + 0.95 between total ash content and moisture content. *C. zambesicus* showed positive correlation of + 0.98 between total protein and ash content (Olayiwola-Olajumoke-Abidemi, 2013).The total lipid content of *B. vulgaris* showed positive correlation (+ 0.87) with the total ash content of *P. americana* (Olayiwola-Olajumoke-Abidemi, 2013). Ten medicinal plants from Jordan showed positive correlations of + 0.48 between the total crude fiber and moisture content. + 0.40 was seen between total lipid content and protein contents. + 0.06 was seen between the moisture content and carbohydrates. Negative correlation between total carbohydrate and crude fiber (- 0.78), total lipids content and moisture (- 0.67), total lipid content and ash content (- 0.55), total lipids content and carbohydrates (- 0.55) showed significant correlations at the < 0.05 level (Khalil *et al.*, 2012).Twenty rice varieties collected from the Ebonyi state of Nigeria also showed notable correlation with respect to the proximate compositions. Correlations were observed in positive maxima relation between the calorific value and total carbohydrate contents (+ 0.933). Maxima negative correlation was observed between moisture content and carbohydrate content (- 0.978) and between calorific value and moisture content (- 0.951) (Oko *et al.*, 2012). Negative correlation was reported between the total ash content and calorific value on *Chaetomorpha* species and *Sargassum* species (Nirmal Kumar *et al.*, 2009).

Contribution of seaweed biochemical constituents to RDI

Evaluation of seaweed biochemical constituents and their contribution to the RDI is important. The RDI levels and the amount of seaweeds required to be included in the daily food intake are described in Table 5. As per the stated standards, for an average person, a daily intake of 50 g protein, 300 g carbohydrate, 65 g lipid, 150 mg iodine, 25 g crude fiber and 2000 calories of energy is recommended. In order to meet the values, food has to be supplemented with other sources of nutritional inputs which are generally stated as the nutraceutical components. In the present study, the seaweeds *U. fasciata* and *E. prolifera* were observed to be a potential source that could be suggested as a protein supplement. A daily intake of 199 g and 286 g would be enough to meet the required levels. *G. pusillum* and *G. corticata* var. *cylindrica* were observed to be a promising source of carbohydrates. *E. prolifera* and *U. fasciata* also proved to be an alternate source of lipids even though the consumption quantity was observed to be on a higher side. Hence with these could be utilized in the natural extraction sectors so as to develop an alternate mode of nutritional products in the form of value added food or health supplements. All the seaweeds were observed as a good source of iodine content and these could be taken along with food in the range of 0.81 to 3.62 g per day. The genus *Gracilaria* was observed to be the major contributor to iodine. Similarly all

the seaweeds were observed as a rich source in dietary fibers and a daily consumption of 39 to 67 g per day could meet the daily requirements. Obesity is the current adverse impact on the changed food consumption patterns. Seaweeds which provide the recommended energy levels with minimum consumption could result in the declination of the risk of obesity. All the analyzed seaweeds were observed to be a good contributor to energy.

Table 5. Contribution of seaweed biochemical constituents to RDI

	Dietary inputs					
Species	**Protein**	**Carbohydrate**	**Lipid**	**Iodine**	**Crude fibre**	**Calorific value**
RDI	50 g	300 g	65 g	150 mcg	25 g	2 kcal
	Recommended daily intake of seaweed (g)					
A. spicifera (N)	533.62	537.54	2610.44	1.03	**40.39**	0.99
C. antennina (N)	556.17	619.32	3110.05	2.59	43.93	1.44
E. prolifera (N)	407.83	590.20	**604.09**	3.62	**40.59**	**0.57**
G. pusillum (N)	836.12	**395.00**	2452.83	1.09	**39.44**	**0.57**
G. corticata (N)	809.06	684.15	3439.15	**0.86**	54.85	0.92
G. corticata var. *cylindrica* (N)	836.12	**425.17**	4362.42	**0.84**	62.39	**0.60**
G. foliifera (N)	716.33	967.12	3846.15	**0.81**	48.61	1.20
E. prolifera (K)	**286.53**	596.07	1940.30	2.84	66.54	0.66
U. fasciata (K)	**199.04**	904.43	**880.76**	2.40	65.84	0.67

N- Njarakkal location and K- Kayamkulam location

The correlation studies done were capable to explain how nutrition supplements can affect the concentrations of the corresponding counterparts. Compositions with positive correlation increases up with the supplement intakes and those with negative correlation decreases up. The process is dynamic. Correlations zero were those which couldn't exhibit any sort of correlation factors or those whose actions were at par with positive and negative effects. Water content which was considered upon in the study showed the promotional inputs on nutritional composition of the organism. The comparative analysis to determine the acceptance in nutrient levels, as a source of food or commercial attitudes, screened out the analysed seaweeds and determined that the class of green seaweeds would be advisable for further exploitation. The overall results under consideration with total ash content suggests *G. pusillum* as the best source of food and commercial exploitations, even though all the eight seaweeds studied could be considered as nutritional supplements. Energy contribution was found to be highest in *G. pusillum* and *E. prolifera*, where the source could act as food and energy alternatives. The study focused on the analytes importance to be used as a potential food supplement so as to boost up the dietary requirements by providing adequate nutritive inputs. Seaweeds being one of the vital components of almost all the ecosystems have high potential for use as food and bio fuels.

The seaweeds discussed in the current study, are remaining untouched and unattended which could be advised to be taken over by the commercial food sectors or value added nutraceutical sectors to produce economical food sources that could be evolved as a remedy to the growing food scarcity faced all over the world. Adequate nutrition with minimal investments could be the other side of the coin as the seaweed growth requires very less human intervention.

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