



RESEARCH ARTICLE

Study of mineral and nutritional composition of some seaweeds found along the coast of Gulf of Mannar, India

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ABSTRACT

The presence of Algae on the Earth is ubiquitous. The industry that widely uses algae is food industry, where the algae are used as a food supplement and also as an addition to the nutrient rich food. This study emphasizes on the mineral and nutritional composition of the selected fourteen algal species which are abundantly found along the coast of the Gulf of Mannar. The selected species of algae belong to different algal families such as Chlorophyta, Phaeophyta and Rhodophyta. The amount of minerals such as Ca, Zn, Fe, K, Mg, Mn, and Cu were estimated by employing the method of acid digestion followed by atomic absorption spectroscopy. We estimated the nutritional content based on the assessment of total protein, carbohydrate, phenol, ash and moisture contents of the algal species. The results based on the analysis of the mineral content in the algal seaweeds depicted that the seaweeds comprised of high amount of the macro minerals and trace minerals. Estimation of nutritional composition revealed that these algal species are rich in protein and carbohydrate. The ash contents were found to be very high in *Jania rubens* (86.66%), *Padina boergesenii* (85%) and *Valoniopsis pachynema* (84%). Based on the present study we infer that the algal seaweeds contained high amount of the nutritional compounds, which might pave the way for a higher standard of nutritional supply to the humans in the future.

Introduction

Algae are one of the most copious aquatic organisms that are present across the Earth. The use of algae has been recorded for all time. They have been widely used as food, fodder, fertilizer and even as medicines. Because of their abundant nature, they can be witnessed in water bodies easily, such as in the form of algal blooms. This profusely available characteristic of the algae has made it as one of the most familiar groups of organism present. The widespread algal species which are found in the saline conditions, pond scum which seen as a mass growing on the surface of stagnant water and giant kelp which found in cold conditions, are considered commercially significant renewable resources (1).

The seaweeds also comprise various organic and inorganic compounds beneficial to human health. The most common ones are those belonging to the family of Rhodophyta, followed by the Chlorophyta and

Phaeophyta. It has been found that they encompass over 60 trace elements and various bioactive compounds which have economic importance based on the potential in the aspect of breeding and feeding of the fishes and other invertebrates (1). The mineral composition makes them significant also from the commercial perspective, as they are a form of perennial source of raw materials which can be employed in various industries such as pharmaceutical, food and cosmetic (2).

The consumption of the seaweeds as a dietary source has been seen in the Asian countries, especially in Japan, China and Korea. As per the studies it has been estimated that the total seaweed consumption by humans in these countries are depicted as green algae (5%), brown algae (66.5%) and red algae (33%) (3). The consumption of seaweeds as vegetable salads has now become a trend in certain developing countries. At present China has become the largest producer of the

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edible seaweeds. It is also noteworthy that the demand on the edible seaweeds is now broadened to North America, South Africa and Europe (4).

The mineral composition of the seaweeds is greatly influenced by various topographical factors which include temperature, the salinity of the water, amount of light and nutrient availability (5). These environmental conditions show variations based upon the season, thereby either stimulating or inhibiting several physiological and metabolic mechanisms in the seaweeds (6). The nutritional qualities of the seaweeds are not known, and they are generally evaluated based on their chemical composition (7). Based on the season, topography, species, as well as the temperature in the water, the composition, varies among different seaweeds (8).

It is a known fact that minerals play a vital role in the physiological mechanisms of the human body. For instance, for the proper maintenance and functioning of bones and teeth, the supply of minerals such as Ca, P and Mg is inevitable. Similarly, for the maintenance of homeostasis in the body by balancing the acid-base balance by monitoring the entry and exit of various fluids and nutrients in a cell is regulated by Na and K (9). The unavailability of the adequate amount of minerals would cause severe health disorders (10, 11).

Minerals that are commonly found in the seaweeds include iodine, magnesium, calcium, phosphorus, iron, potassium and copper. The seaweeds comprise the non-starch polysaccharides, minerals and vitamins that act as dietary fibres and food ingredients which, when intaking will prevent the occurrence of chronic disorders (12, 13). This is the point where the significance of this study lies as it emphasizes on the estimation of mineral composition of the seaweeds, thereby understanding how these compounds will be beneficial to the metabolic functions that are carried out in the body.

Materials and Methods

Collection of Seaweeds

The specimens were collected from the southeast coast of the Gulf of Mannar (8° 55'- 9° 15' N: 78° 08'- 79° 16' E). The algal species were 14 (Authentication/E2BPL/2019/01), and they belonged to 3 different divisions, namely Chlorophyta, Phaeophyta and Rhodophyta (Table 1) (Fig. 1). The handpicked samples were then subjected to cleansing initially by seawater to remove the sand and other epiphytic other substances. Seaweeds were immediately transported to the laboratory and cleaned thoroughly using tap water to remove salt present on the surface of the sample. They were then spread on blotting paper to remove excess water and they were shade dried at room temperature. All the samples were shade dried and were powdered to fine granules. All the voucher specimens were deposited at Plant Science laboratory, Department of Life

Sciences, CHRIST (Deemed to be University), Bengaluru.

Estimation of Mineral Content

From the prepared sample about 0.5 gm of the dried algal sample was treated with a mixture of 4 ml of HNO₃ and 1 ml H₂O₂ and left overnight. The sample was then subjected to heating at 220 °C for 5 minutes for digestion and then allowed to cool down. The volume of samples were made upto 25 ml by using distilled and deionized water (14). After filtration the analyzes of minerals such as Ca, Zn, Fe, K, Mg, Mn, and Cu were estimated by Flame Atomic Absorption Spectrometer (SHIMADZU AA-6880; Measurement Range: 185-900 nm, Detector: Photomultiplier tube, Photometric method: Optical Double Beam, Flame type: Air-C2H2).

Estimation of Protein

The protein content of the algal samples was estimated by Bradford method (15). The working standard of 100 µg/ml of BSA (HIMEDIA) was prepared. 5 ml of Bradford reagent were added. The samples were incubated at room temperature for 10 minutes. After the incubation, the absorbances of the samples were measured at 595 nm.

Estimation of Carbohydrate

The total carbohydrate content of the algal samples was estimated using the Phenol-Sulphuric Acid method (16). A working standard of D-Glucose (HIMEDIA) of concentration 100 µg/ml was prepared. 0.1 gm of the dried algal samples were taken into which 5 ml of 2.5N HCl was added and incubated in the water bath for 3 hrs. It was followed by the addition of sodium carbonate until the effervescence was ceased to neutralize the mixture. After centrifugation at 10,000 rpm for 10 minutes, 0.2 ml was taken and 1 ml of 2% Phenol and 5 ml of 96% Sulphuric Acid was added. After incubation at room temperature for 20 minutes, the absorbance was estimated at 490 nm.

Estimation of Total Phenol

Folin-Ciocalteu's Assay was employed for the estimation of the Total Phenol content of the algal samples (17, 18). Algal extract of 0.2 ml was taken into which about 0.5 ml of the Folin-Ciocalteu's reagent and 2 ml of sodium bicarbonate solution of 20% [w/v] were added. The absorbance was then read at 638 nm. For the quantification of the total phenol content present in the algal sample, a standard calibration curve was plotted using catechol as the standard.

Estimation of Ash Content

The dried algal samples were subjected to ashing by placing them in a muffle furnace at 525 °C for overnight for obtaining the ash content (19, 20).

Estimation of Moisture Content

About 2 gm of the algal samples were subjected to heating at 105 °C until the moisture content was removed (20). The samples were placed in a SHIMADZU moisture analyzer for the estimation of moisture content.

$$\text{Moisture content (\%)} = \frac{\text{Weight of substrate} - \text{Dry weight of substrate}}{\text{Dry weight of substrate}} \times 100$$

Statistical Analysis

The statistical analyses was carried out using the SPSS software. One-way ANOVA was employed to analyze the mean values to identify significant differences between the mineral compositions of the seaweeds. The nutritional content of the sample such as Protein, Carbohydrates and Phenol were acquired as a set of three replicates \pm SD. One-way ANOVA was also executed followed by the Duncan's multiple range test with $p < 0.05$ for the mean values of the biochemical components for finding out any significant differences between the values got under various parameters. To find out the relation between ash content and moisture content, Pearson's Correlation analysis was performed. Paired sample T-test was carried out to analyze the mean values to detect any significant differences between ash content and moisture content.

Results and Discussion

Estimation of Mineral Content

From the mineral analysis conducted, it was found that the mineral content of seaweed samples were high and are in turn beneficial as a nutrient supply for the human body. The macro-nutrients such as (Ca and K) as well as trace elements such as (Fe, Mg, Mn, Cu and Zn) were found in higher in the seaweeds (Table 2). Calcium and potassium were the most abundant elements, followed by manganese and magnesium. The highest Ca measured was 112.34 ppm in *Jania rubens* followed by 105.67 ppm in *Valoniopsis pachynema*. The lowest Ca measured was 53.83 ppm in *Kappaphycu alvarezii* followed by 59.22 ppm in *Graciola corticata*. The highest K measured was 14.84 ppm in *Chaetomorpha antennia* followed by 13.49 ppm in *Graciola corticata*. The lowest K measured was 5.7 ppm in *Padina boergesenii*. The highest Fe measured was 12.52 ppm in *Valoniopsis pachynema*, while the lowest was 1.29 ppm in *Graciola corticata*. Manganese contents were between 3.67 ppm in *Jania rubens* to 0.69 ppm in *Gratrloupia lithophila*. Magnesium contents were between 1.48 ppm in *Jania rubens* to 1.09 ppm in *Gelidiella acerosa*. Copper contents were between 1.53 ppm in *Valoniopsis pachynema* to 0.06 ppm in *Graciola corticata*. Zinc contents were between 1.00 ppm in *Gratrloupia lithophila* to 0.15 ppm in *Stoechospermum marginatum*. There is no significant difference (one-way ANOVA, $p > 0.05$) between different algal species and their minerals (Table 4). But there is a significant difference (one-way ANOVA, $p < 0.05$) between minerals and its algal species (Table 5).

Seaweeds possess a great amount of minerals which make up about 36% of their dry weight (21). Many research studies show that the mineral composition of seaweeds are not only dependent on the season and geography (22, 24), but also depend on the nutrient availability of the environment they

belong to (25). Data collected on analysis, it was found that *J. rubens* had the most amount of calcium, which was followed by *V. pachynema*. In a study it was reported that the calcium concentration was found higher in *P. gymnospora* which was about 4140 ppm (26). The advantages of calcium obtained from the seaweeds makes them commercially more important and valuable. The calcium got from seaweeds helps in enhancing the bone density irrespective of the age. The drawback of the conventionally derived calcium through calcium supplements would result in certain side effects such as bloating, nausea and constipation. The algal calcium also possesses 12 essential bone supporting minerals (magnesium, boron, copper, manganese, silicon, nickel, selenium, strontium, phosphorus, potassium, vanadium and zinc) besides vitamins like C, D3 and K2, resulting in the adequate supply of nutrients to the bones (27).

In the present study, it was shown that *C. antennia* and *G. corticata* had highest amount of potassium when compared to other species. According to another study (28) the potassium content was found to be higher in *G. corticata*. Potassium is one of the main intercellular cations, mainly involved in the maintenance of the membrane potential and electrical excitation of nerves as well as muscle cells. It also plays an important role in regulating the acid-base balance. The maintenance of the normal heart rhythm is supported by the controlled electrical activity of the heart monitored by the potassium. According to Institute of Medicine, an adult should consume 4700 mg of potassium per day (51). A study (29) indicates how the energy-dependent uptake of elements into the cells maintains the categorization of the potassium, and the associated extrusion of the sodium via a specific cell membrane-bound enzyme called as potassium adenosine triphosphatase.

Iron is another important nutrient that plays an important role in the metabolic activities of the human body. The deficiency in iron can be seen quite common irrespective of whether the country is developed or developing. From the research studies conducted, it is a known fact that deficiency in iron will pave way for anaemic conditions in the human body. The role played by iron in the physiological functions of the human body is vital as iron is utilized for the production of haemoglobin and myoglobin, which are entrusted with carrying oxygen throughout the body. From the statistics that Institute of Medicine had provided, an adult requires 16.3 mg to 18.2 mg of iron per day and an adult woman requires 12.6 to 13.5 mg of iron per day. According to a study it was found that iron is the most abundant micronutrient which was found about 199.45 g^{-1} to 67.35 g^{-1} (30) and the highest amount was found to be in *U. lactuca*. Another study (31) reported that iron content is high in *Antithamnion cruciatum* (3,949 $\mu g/g$). Iron deficiency can occur because of inadequate supply of iron to the body at the time when iron is needed in high amount such as pregnancy, high menstrual loss, growth etc (32). From this study, it was understood that iron content was high in *V. pachynema* followed by *P. boergesenii*.

One of the crucial minerals that is associated with metabolism, immune function and repairing the body cells is Zinc. According to the studies conducted, the amount of Zinc that a person should incorporate in the diet is approximately 8-9 mg per day. The amount consumed by the body should be increased to 11-12 mg in case of pregnant women per day and in case of lactating women it is around 12-13 mg per day. Since Zinc is involved in the repairing of the cells, a deficiency in the intake of zinc will result in the delayed wound healing. Other problems associated with the deficiency of zinc is hair loss and diarrhoea. According to a study (31) *Gelidium latifolium* has high amount of zinc, which is estimated to be 64.8 µg/g. Another study (33) reported that zinc content was high in *Chondrus crispus* which is estimated to be 7.14 mg/100 g. Although zinc presence is been found out in plants because of their phytate content the uptake of zinc from plants to the human body is confined to certain extent (34). In the present study *G. lithophila* showed the highest amount of zinc (1 ppm).

Magnesium has been found as a principal mineral which supports the muscle and nerve functions. It has other roles such as building up strength in bones and boosting the immunity of the body. According to the statistics reported by the Institute of Medicine, an adult woman is expected to consume 310-320 mg per day and an adult man should consume 400-420 mg per day. Based on a study (34) the magnesium content was high in *Ulva reticulata* which is estimated to be 181.5 ppm. Yet another study (14) stated that the high amount of magnesium was found out in *Ulva rigida* which is about 7.85 mg/g d.w. Another research reports (26) that the magnesium content in *U. lactuca* is 174.5 ppm. A report based on the study (35) stated that *Ulva ohnoi* can be consumed as a human food thereby an effective cultivation would bring a new commercial scope. The marine alga such as *Ulva ohnoi* has been employed as a fertilizer in various parts of the world. Earlier studies have revealed that the species of *Ulva ohnoi* possess high amount of magnesium content and it has also enabled to infer that *U. reticulata* has the second-highest magnesium content. The highest amount of magnesium was found in *J. rubens* (1.48 ppm) followed by *U. reticulata* (1.33 ppm).

Trace elements or micro minerals are also important for the proper metabolism in the human body. Although they are in a minimal quantity, they are required to perform various physiological activities. For instance, manganese plays a role in balancing the sugar levels in the blood, augments the absorption of calcium and for connective tissues and bones. Similarly, various enzymes produced in the body are controlled by the micro-minerals copper and zinc (36). The micro-minerals include copper, zinc and manganese. According to a study (34) manganese amount was high in the *Cladophora glomerata* which is approximately 4.30 ppm. In the study (14), reported highest manganese content in *Chaetomorpha linum* 29.4 mg/g d.w. Based on the data got from this study, the manganese amount was high in *Jania rubens* 3.67 ppm followed by *V. pachynema* 3.62 ppm. In another study (30), it has been found that copper content was high in *U.*

lactuca which was about 1.45 mg 100g⁻¹ d.w., while another study (31) reported highest copper content in *Antithamnion cruciotum* which is about 17.1 µg/g.

Based on the topographic factors such as temperature, light intensity and nutrient supply, the composition of various minerals present in the algal seaweeds vary. This variation can be witnessed across the species, exposure to seasons, physiological factors and mineralization methods in each species and the processing of these minerals by these species (6). From this study, it can be deduced that seaweeds can be a potential food supplement for the human diet; thereby the nutritional value is enhanced. It gives much scope to the food industry in producing more nutritional value in food products. Seaweeds can also be employed as nutritive diet product, keeping in mind of its commercial value. In future the application of seaweeds can be expanded by bringing more seaweed-based food products into the limelight.

Estimation of Protein

Present study showed the presence of protein in all the 14 seaweed studied. *A. spicifera* (0.89 mg/g) possess the highest amount of protein, followed by *G. lithophila* (0.74 mg/g). The *S. wightii* had (0.28 mg/g) of protein and the least amount of protein was estimated in *V. pachynema* (0.18 mg/g) (Table.3).

The protein content in the algal species varies based on the seasonal changes and topography (37). Several studies reveal that seaweeds contain almost 16-30 % of proteins in dry weight. It has been found that the proteins from algae contain many essential amino acids including iodine. These proteins can be extracted and can be added to food products that as such do not have high protein content (38). Based on the study (22), protein content was high in Rhodophyta which is about 35-47 % and the least was observed in Phaeophyta which make up about 5-10 %. From this study, it can be inferred that that highest amount of protein is estimated in *Acanthophora spicifera* which is about 0.89 mg/g which is a Rhodophyta followed by *G. lithophila* which is estimated to be 0.7428 mg/g.

Estimation of Carbohydrate

The total carbohydrate content of all 14 algal species was shown in Table 3. In case of carbohydrate content, highest value was recorded in *U. reticulata* (0.67 g⁻¹) followed by *G. lithophila* (0.66 g⁻¹). The lowest carbohydrate concentration was observed from *J. rubens* (0.06 g⁻¹) followed by *C. antenna* (0.07 g⁻¹).

Carbohydrates are defined as an immediate source of energy for the human body. It also plays an important role in respiration and other metabolic processes (19). One study (39) revealed that carbohydrate was found in high amount in the *Ulva* species which belong to the Chlorophyta, which was estimated to be about 63.04 g kg⁻¹d.w. Based on the data obtained from this study, high amount of carbohydrate was found in the *U. reticulata* (0.67 g⁻¹) followed by *G. lithophila* (0.66 g⁻¹). The algal seaweeds are consumed by humans after processing and drying it (40). The carbohydrates present in the

algae are not easily digestible and hence the value of algal food also depends on the minerals, trace elements, proteins and vitamins present in it (41). In Japan, Indonesia, China, Philippines and other Indo-Pacific regions of the world seaweeds such as *Caulerpa*, *Sargassum*, *Gracilaria* and *Acanthophora* are consumed as edible product (42).

Estimation of Phenol

The total phenol content in all the 14 algal seaweed samples are shown in Table 3. In case of Phenol content, highest phenol was recorded in *G. acerosa* (1.233 mg/g) followed by *U. reticulata* (0.65 mg/g). *Turbinaria conoides* and *P. boergesenii* has phenol content of about (0.11 mg/g). We observed the least total phenol concentration in *A. spicifera* (0.01 mg/g).

There was no significant difference (one-way ANOVA, $p > 0.05$) between nutritional composition and the amount of protein, carbohydrate and phenol (Table 6). There was also no significant difference (one-way ANOVA, $p > 0.05$) between algal species and its nutritional composition (Table 7).

The role of phenol in providing resistance to the seaweeds is important (18). The role of phenol is not only confined to radical quenching properties but also it has the role in the formation of the cell wall structure and the anti-microbial properties associated to the cell (43). Another report (44) showed that the maximum amount of phenol was present in the methanolic extract of the *E. intestinalis* which was estimated to be about 3.72 mg/g followed by *G. acerosa* which had about 3.50 mg/g phenol. In this study, the maximum amount of phenol was estimated in the species *G. acerosa* (1.233 mg/g) followed by the species *U. reticulata* (0.65 mg/g). Among the 14 species selected for estimation the least amount of phenol was estimated in the species *A. spicifera* (0.01 mg/g). The species *T. conoides* and *P. boergesenii* had 0.11 mg/g each.

Estimation of Ash Content

The ash content of all 14 algal species was shown in Table 3. In case of Ash concentration, highest ash value was observed in *J. rubens* 86.66% followed by *P. boergesenii* 85.00%. The lowest ash value was observed in *G. lithophila* 18.00% followed by *G. corticata* (20.86%).

Estimation of ash content of the algal sample is related to the durability of the product. It also helps to understand the nutritional value of the food. The analysis of the ash content of the sample is carried out by burning the organic content, leaving behind the inorganic minerals in the sample. This will pave the way to understand the type and the amount of minerals within the sample and its physiochemical properties and how well it retards the growth of microorganisms. The ash content of seaweeds is higher than the terrestrial vegetables, such as spinach (45). The ash content in the algal samples depends on the environmental factors and topography (46). In a previous study (19) the ash content in the species *J. rubens* as found to be 50.54% d.w. From the results analyzed, it has been found that *J. rubens* has the highest value of ash content which is about 86.66%, followed by *P. boergesenii* (85.00%).

The least amount of ash content was estimated in *G. lithophila* (18.00%). The species *G. corticata* was estimated to have 20.86% of ash content.

Estimation of Moisture Content

The moisture content of all 14 algal species was shown in Table 3. In the case of moisture concentration, highest moisture content was observed in *G. lithophila* (13.12%) followed by *K. alvarezii* (11.01%). The lowest moisture content was observed in *V. pachynema* (1.75%).

There is a negative correlation between ash and moisture content which implies whenever ash content increases moisture content decreases (Table 8). The student T-test showed that there is a significant difference ($P < 0.05$) between ash content and moisture content.

The shelf life of the food products relies on the moisture content present in it. From the consumer perspective moisture content has an important role as change in moisture will drastically affect the flavor and texture of the food product, making it inedible and not fit for consumption. It also affects the physical and chemical properties of the food product as the water molecules provide a conducive environment to catalyze the chemical reactions. The presence of free moisture enhances the water activity, which makes the food more prone to activities of microorganisms. The shelf life of the food is greatly determined by the packing materials used for covering the food. Similarly, the amount of food packed, type of packaging and the environmental conditions under which the packaged food is stored also plays a role in the change of moisture content throughout time (48). According to the study (47) highest moisture content was observed in *Turbinaria triquetra* (23.6%). Based on a study, the moisture content in the dried vegetables is about 12-18%, and in the case of dried fruits, it is about 18-25%. The dried seaweed has an advantage as it possesses a considerable amount of the Na and antimicrobial constituents that will prevent the growth of the microorganisms (47). In this study, the highest moisture content was found in the *G. lithophila* (13.12%) followed by *K. alvarezii* (11.01%). According to one study (50), highest amount of moisture was observed in *A. spicifera* (85%).

Conclusion

The fourteen species of algal seaweeds in the present study have proved to be effective as nutritional and mineral supplement. According to this research rhodophyceae alga has a good source of mineral. The algal species are a potential food product that gives the food industry a wide scope. Although this study points out the nutritional and mineral composition of the algal seaweeds, evaluation on the biological grounds based on humans and animals should be conducted to understand the digestibility and the bioavailability of the nutrients. This study discovers the possibility of using the seaweeds as a complimentary to the traditional dietary that can be beneficial to the third world people who are facing the situation of food scarcity and starvation.

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Authors' contributions

JJ and JX have contributed equally in this work.

Conflict of interests

Authors hereby declare that there is no conflict of interest.

Supplementary file

Table 1: Algal samples collected from Gulf of Mannar

Table 2: Mineral Analysis of Algal samples Collected from Gulf of Mannar

Table 3: Nutritional Composition of Algal samples Collected from Gulf of Mannar

Table 4: ANOVA table showing the statistical significance between the different algal species and the minerals

Table 5: ANOVA table showing the statistical significance between the minerals and the algal species

Table 6: ANOVA Statistics of the significance of Nutritional composition on different Parameter

Table 7: ANOVA Statistics of the significance of Algal species on different Nutritional Composition

Table 8: Correlation and Student T-test analysis between Ash Content and Moisture Content

Figure 1 -14: Figures of Algae

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