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Original Research Article

FT-IR and EDS analysis of the seaweeds *Sargassum wightii* (brown algae) and *Gracilaria corticata* (red algae)

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A B S T R A C T

Keywords

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An Intensive spectroscopic (FT-IR and EDS) analyses were made on both brown algae Sargassum wightii and red algae Gracilaria corticata. The samples were collected from Vedalai, Gulf of Mannar, near Rameswaram coastal region, Tamil Nadu. The collected samples were stored in a cool place and the SEM diagrams for cross sectional area of the samples were taken. To identify the frequency of functional groups in the samples, FT-IR technique was performed. The bands at 3371 cm^{-1}, 2924 cm^{-1} and 2358 cm^{-1} corresponding to N-H / O-H, C-H and C-O stretching vibrations respectively in various amines, hydroxyl and Carboxylic groups. Elements such as S, Cl, Fe, Zn were found to occur in largest amount in Sargassum wightii while Fe, Si, Cl, S, and Na are in highest proportion in the Gracilaria corticata. The main functional groups involved in the seaweeds uptake are Carboxyl, Sulfydryl and Hydroxyl which are the prime constituents of the seaweeds. The estimation of trace elements in the seaweeds is of great value so that the seaweeds can be treated with necessary nutrient materials in order to ensure proper growth, yields and make them resistant to disease and toxicity.

Introduction

Seaweeds are macrophytic marine algae, both wild and cultivated, growing in saltwater. They are available largely in shallow coastal waters. Seaweeds offer a wide range of therapeutic possibilities both internally and externally. Seaweeds are harvested by man for centuries particularly in Japan and China where they form a part of staple food. The use of seaweeds as food, fodder and manure are well-known in many countries. Marine algae contain more than 60 trace elements in a concentration much higher than in terrestrial plants. They also contain carbohydrates, protein, amino acids, iodine, bromine, vitamins and substance of stimulatory and antibiotic nature. Earlier, marine algae were considered to be the major source for extraction of iodine and potash. It has been estimated that the seaweeds resources of the world comprise 1460 million tons wet weight brown algae and 261 million tons wet weight red algae. The total seaweeds production is about 1721 x 104 tons wet weight annually (Michanek, 1975). The major source of seaweeds is in the northeast, western, central and southeast Atlantic and the eastern central and northwest Pacific areas. India with long coastlines (6100 km) has a vast resource of seaweeds along many open coasts and estuarine areas. The Lakshadweep and Andaman – Nicobar Islands have rich seaweeds vegetation; surveys have been conducted by various organizations in India to assess the occurrence and distribution of seaweeds along our coasts. Many of the seaweeds possess bioactive components which contain low calories, and they are rich in vitamins, minerals, proteins, polysaccharides, steroids and dietary fibers (Govindasamy *et al.*, 2012).

Seaweeds are one of the most important marine resources of the world and being used as human food, animal feed and raw material for many industries. For centuries, seaweed has been of botanical, industrial and pharmaceutical interest. In recent years, research on the chemistry of seaweeds has experienced a tremendous increase due to the need for compounds possessing bioactivities of possible pharmaceutical applications or other potential economic properties (Rajasekar *et al.*,2013) . Biological compounds extracted from some seaweed species namely Phaeophyceae, Rhodophyceae and Chlorophyceae were proven to have potential medicinal activities such as antibacterial, antiviral, antitumour, antifungal, antiprotozoal, antioxidant and mosquito and larva control (Taskin *et al.*, 2007; Thillairajasekar *et al.*, 2009; Patra *et al.*, 2008; Manivannan *et al.*, 2008). Several studies have demonstrated that seaweeds are an excellent source of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols and carotenoids have exhibited different biological activities (Rodriguez *et al.*, 2010).

In the present study, an interest is evinced in seaweeds both brown algae (Sargassum wightii) and red algae (Gracilaria corticata) to study spectroscopically to estimate the elemental concentration of Na, Mg, Si, P, S, Cl, K, Ca, Mn, Fe and Zn by SEM-EDS and to identify the frequency of functional groups by FT-IR method.

Materials and Methods

Seaweeds collection

The Gulf of Mannar Biosphere Reserve (GOMBR) is located in the Southeastern tip of Tamil Nadu extending from Rameswaram in the North to Kanyakumari in the south. The extent of GOMBR is 10,500 sq.km with the core area covering 560 km and it covers the coast of Rameswaram, Tuticorin, Tirunelveli and Kanyakumari. It is one of the world's richest regions from marine biodiversity perspective and the first marine biosphere reserve in SouthEast Asia. The seaweeds Sargassum wightii and Gracilaria corticata were collected from Vedalai, Gulf of Mannar, near Rameswaram coastal region, Tamil Nadu.The fresh plants were thoroughly washed with ambient water to remove clay sands, dusts, associated algae, sediments and debris. The cleaned algae were placed separately in polythene plates.

Powder sample preparation

Then the seaweeds are shade dried for 10 days in a clean environment to avoid the contamination and they were dried in the oven at 60 °C for four hours to remove the moisture content. The oven-dried seaweeds were ground into a fine powder by using an agate mortar. Two milligrams of the sample were mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The pellet was immediately put into the sample holder and FT-IR spectra were recorded in the range 4000–400 cm^{-1}.

The microphotographs were recorded using SEM JEOL model, JSE-5610LV with an accelerating voltage of 20 keV, at high vacuum mode and Secondary Electron Image (SEI). The semi-quantification elemental analysis to identify the weight percentage of major and minor elements present in the samples was done using the OXFORD INCA Energy Dispersive X-ray Spectrometer (EDS). This technique is being used in numerous applications for environmental science and technology. The SEM diagrams for cross sectional area of the samples were taken and the elements like Na, Mg, Si, P, S, Cl, K, Ca, Mn, Fe and Zn were identified.

Results and Discussion

Figure 1 and 2 shows the FT-IR spectrum of Sargassum wightii and Gracilaria corticata respectively which exhibits the characteristic finger print band features. The characteristic absorbed bands are listed in Table 1. The strong absorption bands at 3371 and 3408 cm^{-1} in both algae are representing the C-H, O-H and N-H stretching. The weak band at 2924 and 2854 cm^{-1} are due to the stretching vibration of –CH3 and –CH2 groups. The absorption peak of weak band 2344-2365 cm^{-1} observed in the samples of both species may correspond to the C-O stretching band.

The strong absorption peak around 1662-1653 cm^{-1} of the spectrum of both species is due to the C-O stretching and N-O asymmetric stretching. The weak bands around 1558 cm^{-1} represent C=C stretching vibration. A weak absorption band occurs around 1457 cm^{-1} both in Sargassum wightii and Gracilaria corticata shows the presence of C-O stretching and O-H bending vibration (Carboxylic acid). The distinctive weak band at 1374 cm^{-1} indicative of S-O stretching vibration (sulfonamide) can be observed only in the seaweed Gracilaria corticata, but it is absent in Sargassum wightii. The absorption band at 1246 cm^{-1} and 1258 cm^{-1} are due to S=O. The Seaweeds both Sargassum wightii and Gracilaria corticata contain a strong absorption band at 1034 cm^{-1} due to S=O stretching vibration.

The peak of weak band observed near 820 - 900 cm^{-1} in the spectrum of two samples can be assigned to out of plane C-H bending vibrations. The absorption peak around 800 - 860 cm^{-1} may correspond to the S=O. The weak absorption band observed near 600 - 670 cm^{-1} due to C-S and C=S stretching vibrations. The red algae Gracilaria corticata contains weak absorption band at 531 cm^{-1} and 504 cm^{-1} due to brominated and iodo components present in it. But this absorption band is not present in Sargassum wightii. Weak band near 415 - 480 cm^{-1} is due to S-S stretching (disulfides).

Figures 3(a) and 4(a) shows the scanning electron micro photograph of the cross-sectional area of the Sargassum wightii and Gracilaria corticata typically setting at a magnification of X1,000 (10 μm) for the sample of study and the corresponding EDS spectrum is shown in Figures 3(b) and 4(b). Table 2 shows the concentration of macro and micro elements like Sodium, Magnesium, Silicon, Phosphorus, Sulphur, Chloride, Potassium, Calcium, manganese, Iron and Zinc .

The concentration of Sodium present in Sargassum wightii is 3.11% whereas its presence in Gracilaria corticata is 10.99%. The Gracilaria corticata only contains 2.49% of Magnesium. The Silicon percentage in Gracilaria corticata is 18.16 %. But it is somewhat low (10.66 %) in Sargassum wightii. The concentration of Phosphorous in *Gracilaria corticata* is 6.85%. But it is nil in Sargassum wightii. The concentration of Sulphur is very high in Sargassum wightii when comparing with other elements present in it. But its presence in red algae is 12.50%. The percentage of Chloride in Sargassum wightii is 19.29% whereas its percentage in Gracilaria corticata is 12.82%. The concentration of Potassium seems to be low in both samples ie 2.27% and 5.74% respectively.

The element Calcium present in Sargassum wightii and Gracilaria corticata is 23.72% and 9.47% respectively. The concentration of Manganese present in the Sargassum wightii is very low and that is of 0.30%. But its presence in Gracilaria Corticata is nil. The Sargassum Wightii contains 15.75% of Iron. But the percentage of iron in Gracilaria corticata is very high when comparing with other elements present in it and its percentage is 25.69%. The value of Zinc in Sargassum wightii is 12.37 %. But its value is nil in the other sample Gracilaria corticata.

FT-IR analysis

Marine environment contains a source of functional materials, including polyunsaturated fatty acids, polysaccharides, essential minerals, vitamins, antioxidants, enzymes and bioactive peptides (Kim and Wijesekara, 2010). FT-IR is a valuable tool for measuring many chemical constituents in plants (Kristin Lammers, 2009) and seaweeds and it is used to reveal some qualitative aspects regarding the organic compounds. Several indicator bands that are pertained to functional groups represent chemical components or metabolic products. The application of infrared (IR) spectroscopy in plant analysis is still limited compared to its applications in other areas. In this article, FT-IR spectrum is able to predict the main chemical constituents in Sargassum wightii and Gracilaria Corticata.

The strong absorption bands at 3371 and 3408 cm^{-1} in both algae C-H, O-H and N-H stretching vibrations, characteristic of the presence of amino acids (Rao, 1963). $-CH_3$ and $-CH_2$ groups (Socrates, 1994 and Bellamy, 1975) indicative of the chlorophyll groups at 2924 and 2854 cm^{-1}. The weak band 2344-2365 cm^{-1} observed in the samples of both species may correspond to the C-O stretching band which is a characteristic peak for carboxylic group (Rajasekar *et al.*, 2013).The peak around 1662-1653 cm^{-1} of the spectrum of both species is due to the C-O stretching and N-O asymmetric stretching indicative ester group (Stewart, 1995). The weak bands around 1558 cm^{-1} represent C=C stretching vibration indicative of the lignin (Kubo and Kadla, 2005;Chatjigakis,1998). The absorption band at 1246 cm^{-1} and 1258 cm^{-1} are due to S=O (sulfate esters) (Silva *et al*.,2005; Boeriu *et al*., 2004). The absorption bands at 1100 – 1000 cm^{-1} in the fingerprint region indicate several modes such as C-H deformation or C-O or C-C stretching, pertaining to carbohydrates (Li *et al*., 2004) and polysaccharides (Nakamoto,

Table.1 FT-IR absorption frequencies (cm^{-1}), intensity estimation and functional group of seaweeds *Sargassum wightii and Gracilaria corticata*.

Sargassum wightii		**Gracilaria corticata**		**Functional Groups**	**Compound**
Abs. Fre cm^{-1}	**I.E**	**Abs. Fre cm^{-1}**	**I.E**		
3371	S	3408	S	N-H Stretching O-H Stretching	Polysaccharides Amino acids
2924	W	2924	W	N-H Stretching CH_3 and CH_2 stretching	Aliphatic Compounds
2854	W	2854	W	C-H Symmetry stretching (aliphatic)	Aliphatic Compounds
2358, 2342	W	2365, 2344	W	C-O Stretching band, P-H stretching	Phosphine
1651, 1634, 1622	S	1662, 1653, 1645	S	C=O Stretching, N=O asymmetric Stretching (Nitrate)	Ester, Pectin
1558, 1539	W	1560	W	C=C Stretching	Lignin
1477, 1455 1423	W	1457, 1430	W	C- O Stretching O-H bending	Cutin
-	-	1374	W	S=O Stretching (Sulfonamides)	Lignin
1324	W	1326	W	S=O Stretching (sulfone)	Alkanes
1258	W	1246	W	C-C-O, S=O Stretching	Lignin
-	-	1193	W	C-O Stretching (phenols) C-F Stretching	Cellulose
1058	M	1102	M	C-F Stretching Si-O	Cellulose, Carbohydrates
1034	**S**	1034	M	S=O Stretching (sulfonides)	Starch and polysaccharides
877, 862, 820	W	876, 861, 824	W	Out of plane C-H bending	Glucose, Galactose
-	-	779	W	Out of plane N-H Wagging	Fatty acids
668, 615 606	W	657, 617 606	W	C-S Stretching C=S Stretching (sulfides)	Sulfates
-	-	531, 504	W	Brominated and Iodo Components P-O Stretch	Phosphates
478, 415	W	467	W	S-S Stretching	Disulfides

I. E. - Intensity Estimation, S – Strong, M – Medium, W = Weak

Table.2 The percentage of trace elements presents in the seaweeds *Sargassum wightii and Gracilaria corticata.*

Characteristics	Elemental Concentration in %	
Elements	**Sargassum wightii**	**Gracilaria corticata**
Na	03.11	10.99
Mg	-	02.49
Si	10.66	18.16
P	-	06.85
S	21.90	12.50
Cl	19.29	12.82
K	02.27	05.74
Ca	03.72	09.47
Mn	00.30	-
Fe	15.75	25.69
Zn	12.37	-

Figure.1 FT-IR Spectrum of *Sargassum wightii (Brown algae)*

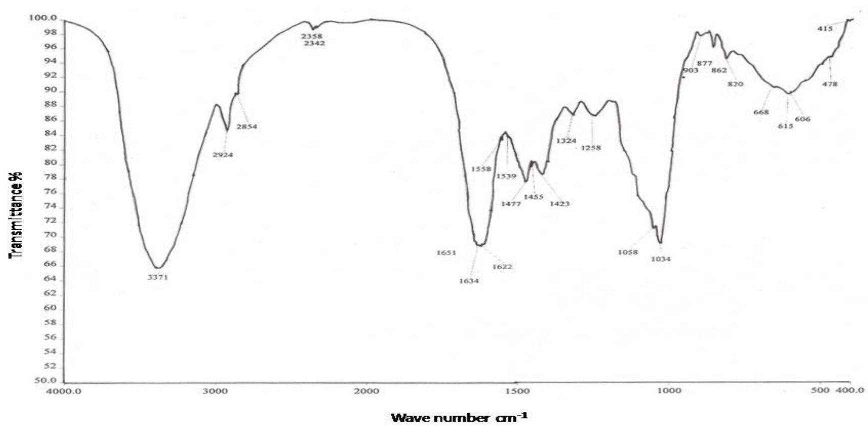


Figure.2 FT-IR Spectrum of *Gracilaria corticata (Red Algae)*

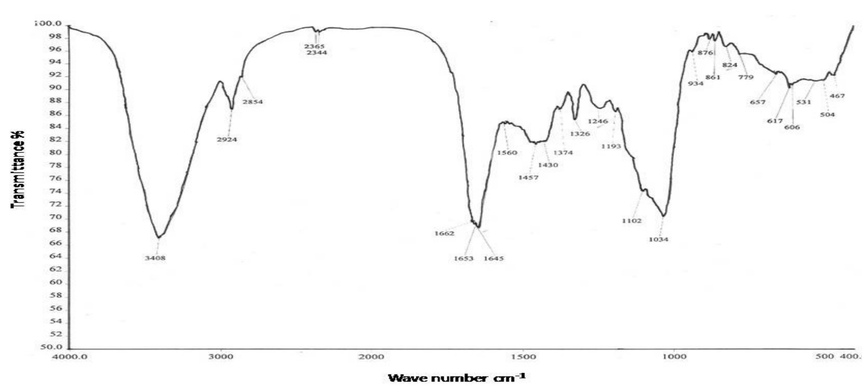


Figure.3 (a)Scanning Electron Micro photo Graph of *Sargassum wightii* with cell wall enlarged view (b) EDS spectrum of *Sargassum wightii*

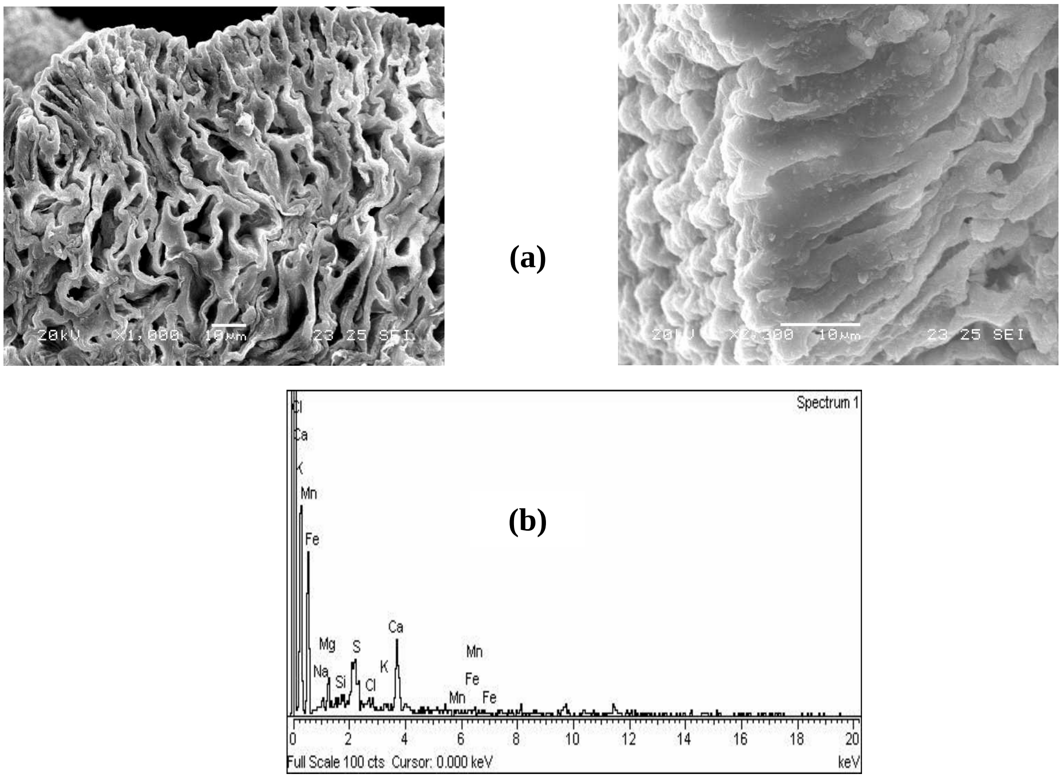
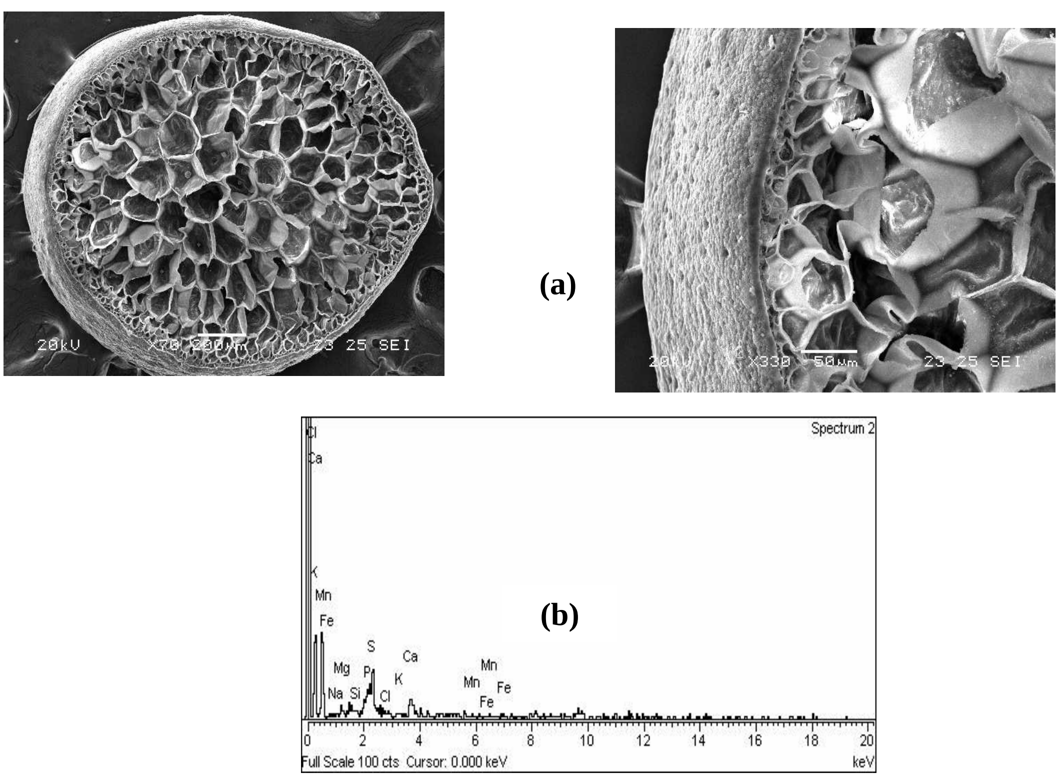


Figure.4 (a) Scanning Electron Micro photo Graph of *Gracilaria corticata* with cell wall enlarged view (b) EDS spectrum of *Gracilaria corticata*



1986). The Seaweeds both Sargassum wightii and Gracilaria corticata contain a strong absorption band at 1034 cm^{-1} due to S=O stretching vibration also indicates the starch and polysaccharides content in the sample. The absorption peak around 800 - 860 cm^{-1} may correspond to the S=O, which indicates the presence of the sulfonate group, observed generally in seaweeds (Figueira, 1999). The weak absorption band observed near 600 - 670 cm^{-1} due to C-S and C=S stretching vibrations (sulfides). The red algae Gracilaria corticata contains weak absorption band at 531 cm^{-1} and 504 cm^{-1} due to brominated and iodo components present in it. But this absorption band is not present in Sargassum wightii.

Impressive developments in the field of mineral elements have taken place in the chemical, biochemical and immunological areas of research. Deficiency of trace elements in human subjects can occur under the most practical dietary conditions and in many diseased statuses. In recent years, scientists and nutritionalists have started believing in the therapeutic role of metals in human health (Udayakumar and Begum, 2004). The imbalance in human health has been linked with the excess or deficiency of trace elements in soils, water, plants and animals (Khan *et al*., 2011).

It has been known that for many years the concentration of heavy metals is significantly higher in the marine biosphere than in the hydrosphere. The estimation of trace elements in the seaweeds is of great value so that the seaweeds can be treated with necessary nutrient materials in order to ensure proper growth, yields and make them resistant to disease and toxicity. Also, the identification of useful and toxic elements can be made.

Sargassum wightii and Gracilaria corticata contain all organic compounds, amino acids, chlorophyll, amides, lignin, carbohydrates and starch pertaining to a healthy plant. The main functional groups involved in the seaweeds uptake are Carboxyl, Sulfydryl and Hydroxyl (mainly those from polysaccharides), which are the prime constituents of the seaweeds.

EDS analysis

Trace elements play both curative and preventive role in combating diseases. Elements research has definitely been part of this explosion of scientific knowledge (Said *et al*., 1996).

Sodium is considered to be non-essential for seaweeds. The presence of Magnesium helps to regulate blood sugar levels, promotes normal blood pressure and is known to be involved in energy metabolism and protein synthesis (Broome, 1998). Phosphorous plays a vital role in a large number of enzymatic reactions that depend on phosphorylation. Sulfur required for the formation of certain amino acids, used to make hair and nails and is important in activating many enzymes and important in metabolism.

Chloride and Potassium, the macro elements maintain the osmotic gradients, ionic exchange and normal neural functions. Potassium is a very important mineral for the proper function of all cells, tissues, and organs in the human body. It has an important part in regulation of water balance of the body (Anderson *et al*., 2008). High concentration of Calcium is considered important in medicinal plants because of its role in bones, teeth, muscles system and heart functions (Brody, 1994). Manganese promotes growth, development and cell function. It helps many body enzymes to generate energy. It is needed for bone growth. It is needed for protein and fat metabolism, healthy nerves, healthy blood sugar regulation, and a healthy- immune system. Iron plays a vital role in human health since it is an essential component of hemoglobin of the blood and active in maintaining healthy immune system (Heydorn *et al*., 1988). Zinc is an essential trace element for the normal functioning of cells, because it takes part in the synthesis of DNA and thus cell growth. Zinc is one of the essential nutrients for plant growth. Hence, Zinc is essential for the normal functioning of the cells including protein synthesis, carbohydrates metabolism, cell growth and cell division.

Many elements such as Na, Mg, Si, P, S, Cl, K, Ca, Mn, Fe, Zn and Zr were determined. Among them S, Cl, Fe and Zn were found to occur in largest amount in brown seaweed (Sargassum wightii) while Fe, Si, Cl, S and Na is highest proportion in the investigated red seaweed (Gracilaria corticata). Trace quantities of these elements are essential for enzyme catalyzed biological processes. These elements are made available to human beings by the plants. Hence, their presence is vital for the health and to cure diseases.

As a result the availability of main functional groups involved in the seaweeds uptake are Carboxyl, Sulfydryl and Hydroxyl (mainly those from polysaccharides), which are the prime constituents of the seaweeds. It may be used as medicine in future for the treatment of various ailments. The presence of trace element indicates the medicinal value of the seaweeds. Sometimes different combinations of the elements present in medicinal plants helps to cure the ailments.

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