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Phytochemical Evaluation, Mineral and Trace Element Analysis of Selected Brown Seaweeds from Thirumullavaram Coast, Kerala, India

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ABSTRACT: *Seaweeds are rich sources of structurally diverse bioactive compounds with various biological activities and their importance as a source of novel bioactive substances growing rapidly. In the current study Sargassum wightii Greville, Sargassum cristaefolium C.Agardh and Padina tetrastromatica Hauck from Thirumullavaram coastal region of Kollam, Kerala were selected to evaluate the phytochemical composition and mineral analysis. Various standard methods were used for the evaluation of phytochemical components. The results revealed that among the primary metabolites, quantity of Carbohydrate is more in Sargassum wightii Greville compared two other species. Sterol composition was recorded more in Padina tetrastromatica Hauck. Mineral analysis were done using ICP-AES method. Among the minerals, Calcium was found to be higher in all the three brown algal members compared to Sodium and Potassium. An appreciable quantity of Iron was found to be more in Padina tetrastromatica Hauck compared to other two species and all the other trace elements were found to be in low concentration. The results concluded that all the three brown algal species selected for the present study holds a good amount of primary and secondary metabolites and also these species are rich in important minerals of dietary values.*

Key Words: *Phytochemical components, Mineral analysis, ICP-AES, Brown seaweeds, Carbohydrates, Sterol, Calcium, Sodium, Potassium*

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I. INTRODUCTION

Seaweeds or marine macro algae are non -flowering primitive plants and are not differentiated into true roots, stems or leaves. Seaweeds are distributed in the intertidal and sub tidal regions of the sea and also estuaries and back waters and grow abundantly in the rocky or suitable substrata are available for their attachment. They are classified into green (Chlorophyceae), red (Rhodophyceae) and brown (Phaeophyceae) algae based on morphology, cell wall and pigment composition.

Marine organisms are rich sources of structurally diverse bioactive compounds with various biological activities and their importance as a source of novel bioactive substances is growing rapidly. Seaweeds serve as food stuff in the Asian diet for centuries especially in the coastal areas as it contains proteins, essential fatty acids, vitamins, minerals, carotenoids and dietary fibres. The main uses of seaweeds in India are the production of various phycocolloids namely agar, agarose, carrageenan and seaweed liquid fertilizers for commercial purposes (Ramalingam et al., 2000).

Marine algae or seaweeds are rich sources of bioactive compounds like phenol. flavonoid, terpenoid, sterols etc. with diverse biological activities. Nowadays, their importance as a source of novel bioactive substances is increasing rapidly and researchers have revealed that the compounds of marine algal origin shows various biological activities (Kim and Wijesekara, 2010). The primary and secondary metabolites of marine algae have always evoked interest in biochemists because of their diversity which can be compared to those present in the higher plants. Seaweeds are potentially good sources of proteins, polysaccharides and fibres. Indian seaweeds possessed great food value and some seaweed contain 16% to 30% protein on dry weight basis with all essential amino acids that are not available in vegetable food material. Bioactive components with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae (Chew et al., 2008). Like terrestrial plants marine algae also contain different organic and inorganic substances such as minerals, trace elements, polysaccharides, lipids etc. that make these resources as an important part in agriculture and horticulture (Kuda et al., 2002).The present study is focussed on the phytochemical evaluation and mineral and trace element analysis in selected brown seaweeds from Thirumullavaram coast , Kerala,

II. METHODOLOGY

A. Selection of Area and sample

Quilon or Kollam, an old sea port town on the Arabian Sea coast of India and is located on the southwest part of Kerala with a long coast line. Thirumullavaram beach is located at 6km north of Kollam town and includes a patch of sand overloaded by rocks and the beach forms a shallow inlet. Laterite rocks and scattered granite boulders were noticed in the intertidal and sub tidal zones which formed the habitat for rich algal growth. Random distribution of the seaweeds were found in this locality.

B. Collection of seaweeds

The samples were collected at the time of low tides mainly from the subtidal and intertidal regions by handpicking and using scalpels. The handpicked samples were thoroughly washed with seawater to remove all the impurities, sand particles and epiphytes and were brought to the laboratory in polythene bags and then washed again with fresh water followed by distilled water. The fresh samples were preserved in 4% formalin for authentication and for morphological studies. The samples were stored in a deep freezer at 4^{o}C for long term preservation. These brown algal members were then shade dried for 7-10 days, powdered and stored in glass bottles and kept in a desiccator for further investigation.

C. Sample Extract Preparation

For the algal extract preparation, 25gm powdered samples were extracted in a soxhlet extractor in 250ml hexane and methanol successively and this was repeated for several times. The extracts were then filtered using Whatman no.1 filter paper reduced and concentrated using a rotary vacuum evaporator. The final concentrates were weighed and was stored in airtight containers in a freezer at -18^{o}c for further phytochemical analysis.

D. Phytochemical Evaluation

The quantification of metabolites were done by standard procedures viz. carbohydrates by (Dubois et al., 1956), proteins (Lowry et al., 1951), lipids (Barnes and Blackstock, 1973), phenol (Turkmen et al., 2005), flavonoid (Zhishen et al.,1999), alkaloid (Manjunath et al., 2012) and terpenoid (Narayan et al., 2012) and sterol (Bartos and Pesez, 1976).

E. Mineral and Trace element Analysis By ICP-AES

Minerals and trace elements such as Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (K), Phosphorous (P), Iron (Fe), Aluminium (Al), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Manganese (Mn), Nickel (Ni), Lead (Pb) and Zinc (Zn) were analysed in the brown seaweed samples seasonally using ICP-AES **(**Inductive coupled plasma atomic emission spectrometry). It is one of the most common techniques for elemental analysis. An inductively coupled plasma (ICP) is used to produce excited atoms from a sample. These emit electromagnetic radiation at wavelengths characteristic of a particular element. A spectrometer separates and resolves these lines and measures their strength. (Aceto et al., 2002)

III. RESULTS AND DISCUSSION

A. Phytochemical Evaluation

Among the primary metabolites Carbohydrate content was more compared to protein and lipid. *Sargassum cristaefolium* holds the highest concentration of carbohydrate (72.75±0.13mg/g). Compared to both *Sargassum* sps, the quantity of lipid was high in *Padina tetrastromatica* (16.03±0.01mg/g). (**Fig:1**)

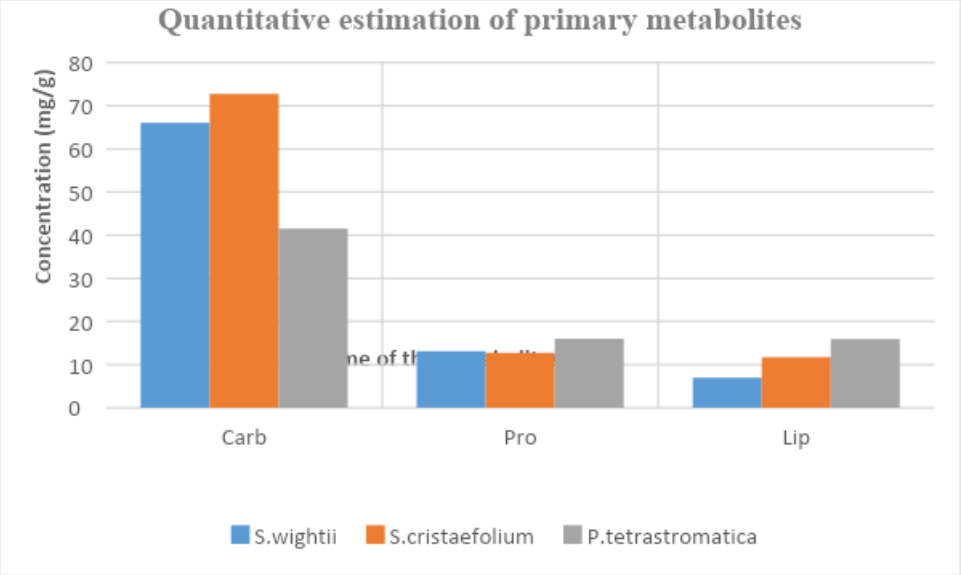


Fig:1 –Evaluation of primary metabolites

Among the secondary metabolites, the concentration of phenol (3.1±0.02mg/g dw) and sterol (5.6 ± 0,01mg/g dw) was high and *Padina tetrastroamtica* compared to other species. Among the *Sargassum* sps, *Sargassum cristaefolium* hold more phenol (2.5±0.03mg/g dw) and sterol (2.76±0.05mg/g dw) than that of *Sargassum wightii*. Flavonoid, alkaloid and terpenoid content was very less in all three species, but terpenoid content was more compared to flavonoid. **(Fig: 2)**

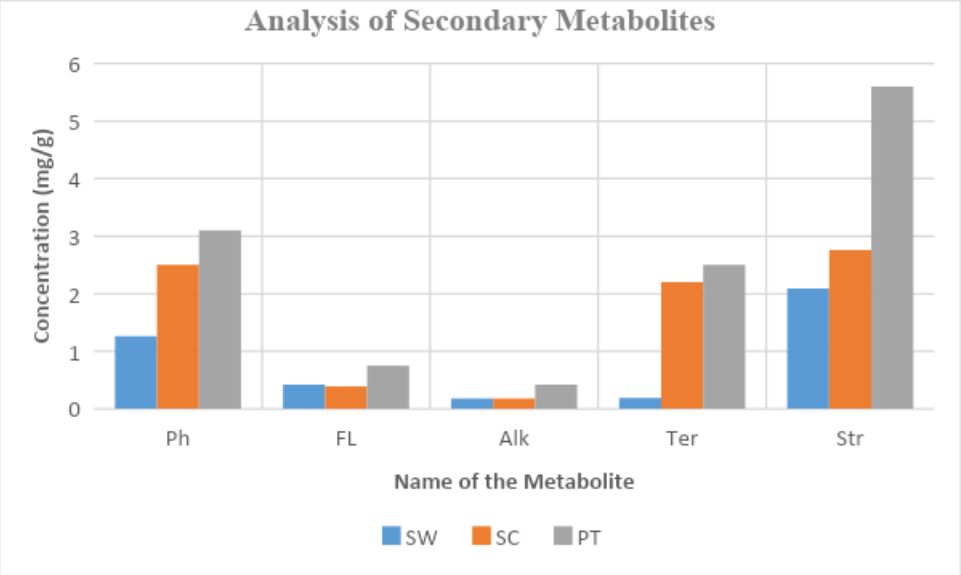


Fig:2- Evaluation of secondary metabolites

Seaweeds are potentially good sources of proteins, polysaccharides and fibre (Lahaye, 1991; Darcy-Vrillon, 1993). Studies on the biochemical constituents such as protein, carbohydrate and lipid in green and brown marine algae have been carried out from different parts of Indian coast (SumitraVijayaragavan et al., 1980; Muthuraman and Ranganathan, 2004; Dave and Parekh, 1975).

Quantitative estimation of the chemical constituents of seaweeds has shown that these are good sources of primary metabolites (carbohydrate, protein and lipid) and secondary metabolites (phenol, flavonoid, alkaloid, terpenoid and sterol). Marine resources are an incomparable resource of biologically active natural products, many of which display structural features that have not been found in terrestrial organisms (Saritha et al., 2013). Importance of incorporating sterols in food supplements particularly phytosterols are mentioned in various reports, as they are very effective in controlling the blood cholesterol levels (Dunford and King, 2000 and Rondanelli et al., 2013). Even though the ecological differences, geographical origin and developmental stage of Phaeophyta contribute enormously to the origin of unique phytosterol combinations,

distinctive biosynthetic pathway leads to a wide range side chain functionalization in these molecules (Kapetanovic et al., 2005).

C. **Mineral and Trace element Analysis**

Calcium is an important essential macro nutrient required by the plants for proper growth, nutrition and cell wall deposition. Among the minerals, Ca was found to be more in *Sargassum wightii* (5.351±0.02%) compared to other two species. Mg is an indispensable macro mineral for plant growth as it plays a major role in the formation of chlorophyll in plants and in animals it has an important role in body's functions such as cell replication, bone formation, hormone regulation, etc. Among the studied samples quantity of Mg in *Padia tetrastromatica* (1.144±0.05 %) was low compared to other two species. The accumulation of Na and their salts were observed to be more than K in seaweeds in general, however some seaweeds also accumulate K salts, as this is an essential macro nutrient required for their growth and metabolic activities. In the present investigation also same results were observed. Among the three brown algae, *Padina tetrastromatica* hold lower amount of macro minerals compared to *Sargassum wightii* and *Sargassum cristaefolium*. The order of macro minerals in the studied samples are as follows: *Sargassum wightii* – Ca>K>Mg>Na>P, *Sargassum cristaefolium* – Ca>Mg>Na>K>P and *Padina tetrastromatica* – Ca> K>Mg>Na>P. In contrast to this, the quantity of trace elements like Fe, Al and Mn were found to be more in *Padina tetrastromatica*. Among the trace elements, the quantity of heavy metals like Ni, Cd and Pb was found be either low or below detection level. **(Fig: 3 – Fig: 5 & Table 1)**

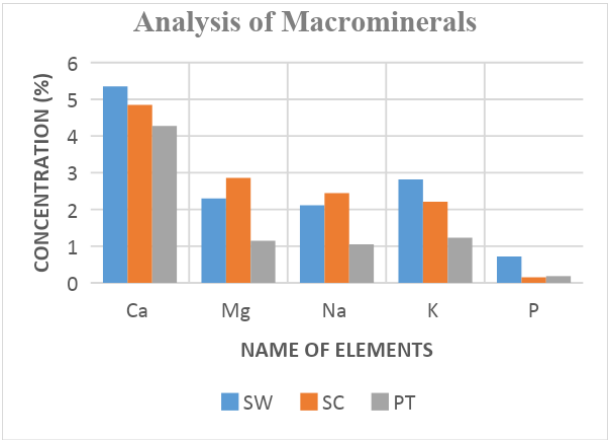
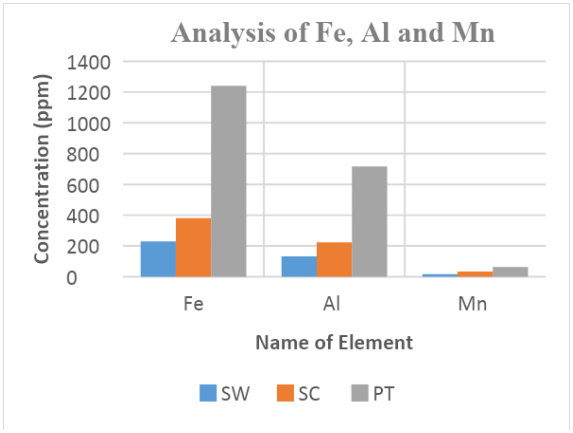


Figure : :3



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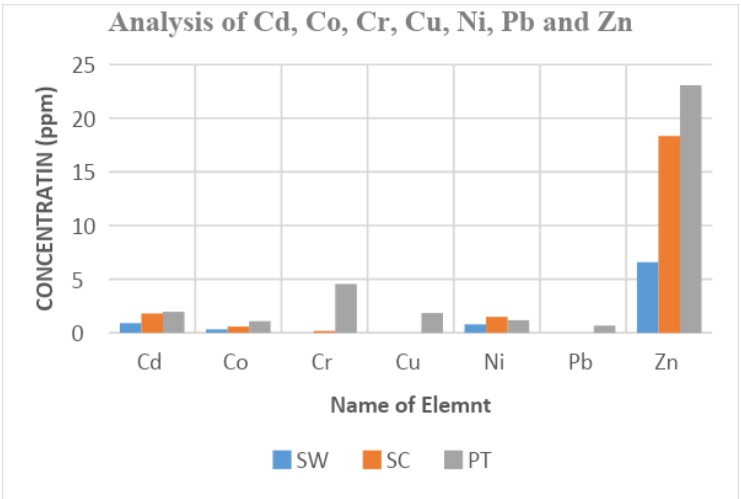

Fig:5

Table 1: Mineral and Trace element composition analysis in selected brown algal species (All the values are expressed in Mean±SD

S No.	Name of Element	*Sargassum wightii*	*Sargassum cristaefolium*	*Padina tetrasrtromatica*
1	Ca (%)	5.351±0.02	4.848±0.06	4.273±0.03
2	Mg(%)	2.299±0.07	2.856±0.03	1.144±0.05
3	Na(%)	2.11±0.01	2.443±0.05	1.232±0.04
4	K(%)	2.82±0.03	2.212±0.2	1.232±0.03
5	P(%)	0.719±0.1	0.152±0.09	0.184±0.31
6	Fe (ppm)	230±0.08	380±0.04	1240±0.04
7	Al(ppm)	380±0.08	222.933±0.13	716±0.09
8	Mn(ppm)	16.51±0.04	33.88±0.05	63.81±0.03
9	Cd(ppm)	0.9±0.01	1.8±0.06	1.96±0.01
10	Co(ppm)	0.32±0.05	0.58±0.03	1.09±0.18
11	Cr(ppm)	BD	0.173±0.1	4.553±0.03
12	Cu(ppm)	BD	BD	1.84±0.15
13	Ni(ppm)	0.787±0.11	1.49±0.01	1.18±0.01
14	Pb(ppm)	BD	BD	0.677±0.08
15	Zn(ppm)	6.6±0.07	18.347±0.09	23.087±0.03

The results revealed that, out of 15 minerals quantified, most abundant mineral was calcium in all the samples analysed. Calcium content in seaweeds may be as high as 7 % of the dry weight and up to 25 to 34 % in the chalky seaweed, *Lithothamnion* and suggested to consume by those people who are at a risk of calcium deficiency, like expectant mothers, adolescents, and older age people. (Burtin, 2003).Due to the higher polysaccharide content seaweeds are considered as a very good sorce of vitamins and minerals Seaweeds are known as an excellent source of vitamins and minerals, especially Sodium and Iodine that could also imply a high level of soluble and insoluble dietary fiber (Lahaye, 1991). Mineral deficiency may lead severe impairment of health. Calcium deficiency causes Osteoporosis and Iron deficiency causes anemia. (Reinhold, 1988; Martinez – Navareete et al., 2002). Seaweeds contain high amount of copper and Iron compared to other food sources such as meat and spinach (Holland et al., 1993). Other than this the presence of Fe in marine plants can be attributed the fact that they are one of the important micro nutrient that impart various metabolic activities of the plants. *Sargassum* sps and *Padina* were reported to be useful for biomonitoring and phytoremediation of Zn from the marine environment. High concentration of Mn in plants will help in controlling osmotic balance, enzyme regulation etc. (Clarkson and Hanson, 1980)

IV. CONCLUSION

Phytochemical analysis of selected members of brown algae showed that this class of seaweeds were packed with nutrients and several bioactive components. The phenolic contents and sterols can be attributed to their bioactive potential. Evaluation of mineral and trace elements revealed that calcium was the macro mineral found to be high in all the samples followed by potassium, magnesium, sodium and phosphorous. The level of lead and nickel was low or below detection limit in all the three samples.

It can be concluded from the present investigation that seaweeds are a potential health food in human diets and may be used to the food industry as a source of ingredients with high nutritional value. Thus these can be provided as a dietary alternative due to its significant nutritional value and its commercial value can be enhanced by improving the quality and expanding the range of seaweed – based products.

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