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Analysis of seaweed elements and potential bioactive chemical estimate using GCMS in powered seaweed samples

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Abstract
Seaweeds are macroscopic algae that thrive in marine habitats, shallow coastal areas, and on rocky coasts. The primary industrial products derived from seaweeds include agar, agarose, and carrageenan. Seaweeds contains various pharmacologically important bioactive compounds like carotenoids, flavonoids, essential fatty acids, fibre, protein, vitamins and minerals. The present study mainly focus with the analysis of elemental composition of three species red seaweeds (Rhodophyceae) and three species of brown seaweeds (Phaeophyceae) using SEM-EDS. The collected seaweed species were washed, shade dried and thengrinded into a fine powder. The elements present in the seaweed species were determined by SEM-EDS in Central instrumentation laboratory, Department of Physics. Various element present in the seaweed are N>Na>Mg>Al>Si>Cl>S>B>K>Ca>Mn>Fe>Cu>Zn. Among the elements the seaweeds, maximum amount of nitrogen and potassium are observing in the *Acanthophora spicifera* and *Sargassum wightii* followed by *Kappaphycus alverzii*. From the results of SEM-EDS *Sargassum wightii* showed best result. Hence, the species was chosen for analysis of bioactive compounds by using the technique Gas Chromatography Mass Spectroscopy (GC- MS). Based on the results, the *Acanthophora spicifera* and *Sargassum wightii* can be used as a liquid fertilizer, which will improve the crop growth and productivity and hence these two seaweeds namely *Acanthophora spicifera* and *Sargassum wightii* are analysed for further studies.

Keywords: Seaweeds, bioactive compounds, SEM-EDS, elements, compounds

Introduction
Seaweeds are widely recognized as plant bio-stimulants and are known to promote diverse positive effects on plant growth and development (Porchelvan *et al*., 2024; Porchelvan *et al*., 2025) [10, 11]. They are a kind of photosynthetic, non-flowering organisms that resemble plants and are found in the ocean. They belong to three primary groupings based on their dominant pigmentation: red (Rhodophyta), brown (Phaeophyta) and green (Chlorophyta) (Rao *et al*., 2018; Ashwal and Abdelbary, 2021) [12, 1]. In China, Japan and the Republic of korea, seaweeds have long been utilized as food. There are currently, 844 species of seaweeds are known to exist in Indian seas and their standing stock is estimated to be around 58,715 tonnes on a wet weight basis. Among these total 844-seaweed species, India own to have216 species of Green algae, 434 species of Red algae and 194 species of Brown algae. Agar, Agarose and carrageenan are some of the major industrial products of seaweeds which is used in laboratories. Macroalgae are rich in variety of minerals and trace elements, making them valuable source of nutrition (Circuncisao *et al.*, 2018) [2]. Significant concentrations of important minerals like Na, K, Ca, and Mg, and trace elements suchas Fe, Zn, Mn, and Cu are also present in seaweeds. Changes in ecological conditions and seasons may cause the environmental parameters to vary, which can either stimulate or impede the production of certain nutrients. Hence, the current research mainly focus with the analysis of elemental content of three species of red algae (Rhodophyceae) and three species of brown seaweeds (Phaeophyceae) using SEM- EDS (Energy Dispersive Spectroscopic) by standard protocol. *Sargassum wightii*, a brown algae contains significant bioactive chemicals that were identified using the Gas Chromatography Mass Spectrometry (GS MS) technique.

Materials and Methods

Isolation and processing of macroalgae

Seaweeds of different species were collected from different coastal regions in Tamilnadu, South India. Three seaweed samples from different species of red algae *viz., Acanthophora spicifera, Halymenia dialata, Kappaphycus alverzii* and three species from brown algae *viz., Padina gymnospora, Steochospermum marginatum and Sargassum wightii* were collected. The collected seaweed samples were washed thoroughly in seawater and again rinsed with running tapwater to get rid of the salt, sand and other impurities. Seaweeds that had been cleaned were then shade dried and ground into a fine powder,which was kept at room temperature and used for more detailed study.

SEM studies in seaweed

The samples of seaweed were scrutinized using JOELJSM-56010 LVwithINSA-EDS and a selective photo micrograph was obtained from the computer screen in Central instrumentation laboratory at the Department of Physics. The components found in the cell wall of six seaweeds were examined using EDS. Energy dispersive x-ray microanalysis, according to Sundari and Selvaraj (2009) [14], offers a special method for obtaining qualitative and quantitative compositional analysis of individual cells and intracellular compartments to localize distribution of elements of leaves that differ on both a quality and quantity level.

Sample preparation for GC-MS analysis

The powdered sample of seaweed *Sargassum wightii* ten gram (10g) was taken and extracted with 30ml methanol over night and filtered through filter paper containing 2 g of sodium sulphate. The extract was then concentrated to 1 ml by bubbling nitrogen into the solution. The components were separated using helium (1ml/min) as the carrier gas, and the clarus 500 GC used in the analysis used a column packed with Rtx-5MS (5% Diphenyl/ 95%Dimethyl Poly Siloxane) 30m×0.25mm ID×0.25µmdf. The Mass Hunter Workstation Software helped the Single Quardrupole Mass spectrometer to detect the 1µl of sample extract that was put into the device. The oven was kept at 110°C with a holding time of 3.50 minutes for the 36 minute GC extraction operation. The mass analyzer's injector temperature was set to 280°C. In 40.50 minutes, the MS detection was finished, and each component's relative proportion was determined by comparing its average peak to the total regions. The NIST(National Institute of Standard and Technology) version 2.0 year 2020 library was used for the detection process. The compound prediction is based on Duke's Phytochemical and Ethno botanical databases (Duke,1992) [5].

Results and Discussion

Location and designation of Isolated seaweeds

The seaweed species were collected from the different coastal regions *viz*., Mandapam, Thoothukudi, Rameshwaram, Pamban, Kanyakumari in Tamil Nadu and designated as SWr1, SWr2, SWr3, SWb4, SWb5, SWb6 were represented in the table 1.

Identification of the collected seaweeds

The seaweed samples obtained were identified based on their morphological characters such as size, shape, colour, growthetc. Out of these six isolates three of the seaweeds (*Acanthophora spicifera, Halymenia dilata, kappaphycus alverzii)* were belong to red algae and remaining three seaweeds *(Padina gymnospora, Steochospermum marginatum, Sargassum wightii)* are coming under brown algae. SWr and SWb indicates the seaweeds belongs to red algae and brown algae and the results were interpreted in the table-2.

Analysis of different element components in seaweed

The presence of various elements in collected seaweeds are analysed by SEM-EDS and the results were represented in the table-3. The elements present in the cell wall of all seaweeds are normally in the order of N>Na>Mg>Al>Si>Cl>S>B>K>Ca>Mn>Fe>Cu>Zn. The mass of the elements in sample are given as percentage (%). The red algae *Acanthophora spicifera* showed nine elements and they are N, Na, Mg, Al, S, K, Ca, Mn, Cu. Among these various elements 'Nitrogen'(76.65%) shows the maximum amount followed by 'Calcium'(16.54%). The minimum amount of the element is 'Manganese'(0.02%)_

Among the brown alagae, *Sargassum wightii* showed the elements *viz*., Na, Mg, Si, Cl, K, Ca, Fe, Cu. Among these elements, the highest amount was recorded in 'Nitrogen'(37.51%). and the lowest value was recorded in 'Silicon' (2.70%). The 'Potassium' content in *Sargassum wightii* shows the maximum amount when compared to other brown algal species. It shows (23.92%) amount of 'Potassium'.

GC-MS analysis of Bioactive Compounds in Brown Algae *Sargassum wightii*

The brown algae *Sargassum wightii*(SWb6) were studied to identify the important bioactive compounds naturally present in it. Therefore, it was analysed technically by using Gas Chromatography Mass Spectrometry (GC- MS). The results obtained from the GC-MS shows that it contains four important compounds *viz*., Nonadecane, Desonide, Tricyclo4-3-1-1(3,8) undecane3-carboxylicacid and Dihydroxanthin. The role of activity for each compounds were obtained from duke's phyto chemical databases. The results were interpreted in the Table 4.

Table 1: Location and designation of isolated seaweeds

S. No.	Name of the Seaweeds	Locations	Designation
1.	*Acanthophora spicifera*	Mandapam	SWr1
2.	*Halymenia dilata*	Thoothukudi	SWr2
3.	*Kappaphycus alverzii*	Rameswaram	SWr3
4.	*Padina gymnospora*	Pamban	SWb4
5.	*Steochospermum marginatum*	Kanyakumari	SWb5
6.	*Sargassum wightii*	Mandapam	SWb6

Table 2: Morphological identification of different seaweeds

S. No.	Designated Code	Morphological characters	Tentative identification of seaweeds	Type
1.	SWr1	Plantbushy, erect, 15 and 20 cm tall, cartilaginous, reddish to greenish.	*Acanthophora spicifera*	Red algae
2.	SWr2	Plants are erect, rose to red in colour and gelatinous in consistency, smalldisc, variable in shape.	*Halymenia dilate*	Red algae
3.	SWr3	Plants are fleshy, firm upto 2 meter tall, thallus coarse with axes and branches1-2cm diameter	*Kappaphycus alverzii*	Red algae
4.	SWb4	Plants are brown, darker at the base, robust, upto 200 mm across, fan shaped lobes are present.	*Padina gymnospora*	Brown algae
5.	SWb5	Plants are large tufts,15-25cm or high. Thallus erect, segments of thallus1-2cm broad.	*Steochospermum marginatum*	Brown algae
6.	SWb6	Root expanded and disc like, stem erect, 30-60cm high or more, branches are several, leaves 2.5 to 5cm or more long.	*Sargassum wightii*	Brown algae

Table 3: Elemental composition (%) of Seaweeds by Scanning Electron Microscopy- Energy Dispersive Spectroscopy

Seaweed species	N	Na	Mg	Al	Si	Cl	K	Ca	Mn
Acanthophora spicifera	16.54	1.65	0.83	0.40	0.59	0.70	0.72	76.65	0.02
Halymenia dilate	-	16.57	11.17	-	-	-	8.54	7.61	0.20
Kappaphycus alverzii	10.11	12.35	0.46	0.61	-	37.21	23.02	1.31	0.15
Padina gymnospora	-	-	10.13	-	7.81	-	2.73	35.08	-
Steochospermum marginatum	3.48	1.81	0.95	8.80	7.48	-	5.54	28.31	-
Sargassum wightii	37.51	5.05	3.90	-	2.70	9.74	23.92	27.31	-

Table 4: GC-MS analysis of Bioactive Compounds in Brown Algae *Sargassum wightii*

S. No.	RT	Name of the compound	Molecular Formulae	MolecularWeight g/kg	Peak Area%	Role of activity
1.	12.33	Nonadecane	C19H40	268	69.12	Root inducer
2.	14.02	Desonide	C24H32O6	416	12.84	Medicinal properties
3.	15.52	Tricyclo [4.3.1.1(3,8)] undecane-3-carboxylicacid	C12H18O2	194	5.98	Leaf inducer
4.	17.64	Dihydroxanthin	C17H24O5	308	12.05	Medicinal properties

Discussion

Seaweeds are traditionally consumed in different parts of the world. Nowadays, human consumption of brown algae (64.5%), red algae (34%) and green algae (6%). Seaweeds contain many nutrients and elements. The technique in EDS can give a valuble input in estimating the presence of various elements over the surface of seaweeds are reported by Figueria *et al.*, 1999.Current scenario a best-practice guide for regional agricultural to improve harvesting quantity and quality in agriculture and horticulture. It has been proved that seaweed extracts enhance seed germination, increase the uptake of plants nutrition, degree of frost resistance and make the plants better able to with stand fungi and insect pests. There were various reports on the distribution of seaweeds at different areas of Gulf of Manner, South East Coast of Tamil Nādu, like Mandapam Umamaheswara Rao, (1972) [15], Pudumadam, Nochiyurani, Kilakkari, Tuticorin, Tiruchendur Krishnamurthy and Balasundaram, (1990) [8].

Satheesh and Wesley (2012) [13] revealed that the rich seaweed beds occur around Visakhapatnam in the eastern coast, Mahabalipuram, Gulf of Mannar, Thiruchendur, Tuticorin, Kanyakumari and Kerala in the southern coast, Veravel and Gulf of Kutch in the western coast, Andaman and Nicobar Islands and Lakshadweep. In the present study, totally six seaweeds were collected from different coastal belts of Tamil Nadu. Most of them belongs to red and brown algae. The red algae were designated as SWr_1 to SWr_3 and the brown algae were designated as SWb_4 toSWb_6.

Deepika (2018) [4] have reported that the EDS spectrum helps in the identification of the concentration of elements such as sodium, magnesium, silicon, phosphorus, Sulphur, chloride, potassium, calcium, manganese, iron and zinc thus aiding in creation of quantitative compositional profile for *sargassum wightii*. The present study detected the elemental composition in the cell wall of selected seaweeds by SEM-EDS and reported the elements such as N, K, Ca, Si, Mg, Mn, Cl, Ca, Fe, Cu. Kannan (2014) [7] reported that the seaweed *Sargassum wightii* contains various element *viz*., Na, Mg, Si, S, P, Cl, K, Mn, Zn, Fe, Ca. The concentrations of each element in *Sargassum wightii* are 3.11% Na, 10.66% Si, 19.29%Cl, 5.74% K, 23.72% Ca, 0.30% Mn, 15%Fe and 12.37% Zn. The elements Mg and P are absent in *Sargassum wightii*.

In this present study, the *Sargassum wightii* found to have the elements *viz*., N (37.51%), Na (5.05%), Mg (3.90%), Si (2.70%), Cl (9.74%), K (23.92%), Ca (27.31%). The present study detected the elemental composition in the cell wall of selected seaweeds by SEM-EDS and reported the elements such as N, K, Ca, Si, Mg, Mn, Cl, Ca, Fe, Cu. Hannan *et al.*, have revealed that the *Ulva lactuca* species has huge amount of calcium. The major elements found in seaweed (*Acanthophora spicifera & Sargassum wightii)* are Na, Mg, Si, Cl, K, Mn,N, Ca, Fe, Cu. The macro molecules such as nitrogen, potassium, calcium, sodium, magnesium are present in significant amounts in marine algae (Nisizawa, 2006). The seaweeds show great variation in nutrient contents which are related to several environmental factors such as water, temperature, salinity, light and nutrients (Dawes,1998) [3]. The macro molecules such as nitrogen, potassium, calcium, sodium, magnesium is present in significant amounts in marine algae. The seaweeds show various differences in nutrient contents which are related to several environmental factors such as salinity, temperature, water light and nutrients (Dawes,1998) [3]. Brown algae are found to have rich in bioactive compounds such as polysaccharides, peptides, omega-3-fattyacids, carotenoids, phenolics, vitamins, minerals and consist of mainly 90% water Shekhar *et al.* (2014).

Bioactive compounds of *Sargassum wightii* (SWb6) was identified by GC- MS. Totally undecane 3- carboxylic acid, Dihydroxanthanin with their peak area, molecular weight and molecular formula. Deepika (2018) [4] reported that the *Sargassum wightii* analysed by the GC-MS were observed the compounds such as 8,10-Dodecadien-1-ol acetate, Cumarin-3-carboxylic acid-7-methoxy, Z-(13,14-Epoxy)-tetradec-11-en-1-ol acetate, Hexadecanoic acid methyl ester, etc with their retention time, molecular weight and molecular formula. In these study *Sargassum wightii* were analyzed technically by using GC-MS result shows that it contain four important compounds *viz*., Nonadecane, Desonide, Tricyclo 4-3-1-1 (3,8) undecane3-carboxylic acid and Dihydroxanthin.

Conclusion

The results of the present study conclude that the brown algae *Sargassum wightii* and red algae *Acanthophora spicifera* showed many nutrients especially large quantity of nitrogen and potassium. So, it may be used as liquid biofertilizers which will improve the crop productivity and quality. The seaweed extract formulation with the addition of PGPR microorganisms will increase the nutrient uptake of crop plants and enrich the soil in eco- friendly way.

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