BIOACTIVE POTENTIALS OF BROWN SEAWEEDS, SARGASSUM MYRIOCYSTUM J. AGARDH S.PLAGIOPHYLLUM C.AGARDH AND S.ILICIFOLIUM (TURNER) J. AGARDH

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INTRODUCTION:

Brown seaweeds are widely distributed on the southern coast of Tamil Nadu, India and used as animal feed, food ingredients and fertilizer. Also, they are good sources of proteins, carbohydrates, vitamins and minerals in human nutrition. The phenolic compounds are the most effective antioxidant present in the brown alga. The phenolic content of brown algae varies from 20-30% dry weight. Antioxidant compounds play an important role against various diseases such as atherosclerosis, chronic inflammation, cardiovascular disorders, cancer and aging processes. Among brown seaweeds, Sargassum had been studied extensively for its high antioxidant potential in vitro.

Alginates are a family of linear polysaccharides occurring as a structural component of the cell wall in marine brown algae. It is a linear 1,4-linked copolymer of β-D- mannuronic acid (M) and α-L- guluronic acid (G). In a wide range of industrial applications, alginates are essential compounds as thickening, gelling or stabilizing agents. In temperate areas, the alginates are mainly extracted from the brown seaweeds such as Ascophyllum nodosum, Macrocystis pyrifera and Laminaria sp., whereas in tropical regions (China, Philippines, Vietnam and India) Sargassum, Turbinaria, and Padina are the major sources of extraction. The annual alginate production is reaching globally to regions (China, Philippines, Vietnam and India)

Total protein was estimated using the Lowry method. The protein was calculated by using BSA as a standard and expressed in percentage.

Carbohydrate estimation: The total carbohydrate was estimated by following the phenol-sulphuric acid method. The carbohydrate content was calculated by referring to a standard D-glucose and the results are expressed in percentage.

Lipid estimation: The extraction of lipid was done by the chloroform-methanol mixture by using Folch method and it is expressed in percentage.

Total phenolic contents: The amount of total phenolics in methanol extract was determined with Folin–Ciocalteu reagent according to

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Abstract: The use of macroalgae has grown exponentially during the last decade. In addition to their value for human nutrition, seaweeds have multiple therapeutic applications. This work is aimed at providing information on the three least studied species of Sargassum so as to promote these algae to be potentially profitable from biotechnology and commercial perspectives, and also benefit public health. The proximate composition, total phenolic content, total antioxidant activity, alginic acid yield of three brown seaweeds, Sargassum myriocystum, S. plagiophyllum and S. ilicifolium were studied. The seaweeds were high in carbohydrate (33.38-40.21%) and low in lipid content (0.17-0.4%) on dry wt. basis. Total phenolic content and total antioxidant activity were high in Sargassum plagiophyllum (28.66 ±3.05 mg /g) and S. ilicifolium (36.66 ± 9.86 mg /g). These seaweeds showed (12.6-15.0%) alginic acid yield. The characteristic peaks of alginic acid appeared at 3414.00, 3491.16, 3414.00, 1635.64, 1633.71 and 1413.82 cm⁻¹, 1409.96 cm⁻¹, corresponding to hydroxyl (OH), carbonyl (C=O) and carboxyl (COOH), respectively.

Keywords: Biochemical composition, total phenol, total antioxidant, alginic acid yield, FT-IR, brown seaweeds

MATERIALS AND METHODS:
The brown algae viz., Sargassum myriocystum, S. plagiophyllum and S. ilicifolium were collected from Mandapam coastal region during low tide in January 2012. The collected seaweeds were washed thoroughly with seawater to remove all the unwanted impurities, adhering sand particles and epiphytes. The seaweeds were shade dried and then kept in an oven 60°C for 4 hrs and then the dried seaweeds were ground to raw material.

Biochemical properties

Protein estimation: Total protein was estimated using the Lowry method. The protein was calculated by using BSA as a standard and expressed in percentage.

Carbohydrate estimation: The total carbohydrate was estimated by following the phenol-sulphuric acid method. The carbohydrate content was calculated by referring to a standard D-glucose and the results are expressed in percentage.

Lipid estimation: The extraction of lipid was done by the chloroform-methanol mixture by using Folch method and it is expressed in percentage.

Total phenolic contents: The amount of total phenolics in methanol extract was determined with Folin–Ciocalteu reagent according to
the method of Singleton and Rossi with Gallic acid as the standard. Briefly standard stock solution of 10 mg/10 ml of gallic acid was prepared in distilled water. From this, various concentrations ranging from 200-1000 μg/ml were prepared. To this, 1 ml Folin and Ciacalteau reagents (1:2 with water) was added and kept at room temperature for 5 min and then 1 ml of 7% sodium carbonate solution was added to the reaction mixture and incubated at room temperature for 90 minutes. The colour developed was read at 750 nm. A 100 µl of methanol extract of sample was mixed with the same reagents. Gallic acid was used as the reference standard and the results are expressed as milligram gallic acid equivalent mg/g dry weight of seaweed material. All samples were analyzed in triplicate.

**Total antioxidant activity:**
Total antioxidant activity of seaweed extracts was determined according to the standard method. Briefly 0.3 ml of sample was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Then reaction mixture was incubated at 95°C for 90 min. Absorbance of all the sample was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalence of ascorbic acid in milligram per gram of extract.

**Alginic acid yield:**
The method of Suzuki was adopted for estimation of alginic acid. Alginic acid is expressed as percentage dry weight of seaweed.

**FTIR spectra of alginic acid:**
The alginic acid of *Sargassum myriocystum*, *S. plagiophyllum* and *S. ilicifolium* (10 mg) was mixed with 100 mg of dried potassium bromide and compressed to prepared as a salt disc. The disc was then read spectrophotometrically (Bio-Rad FTIR-40-model, USA). The frequencies of different components present in each sample were analyzed.

**RESULTS AND DISCUSSION**
Scientific research on bioactive compounds from seaweeds is available for just a few commercially important species and compounds. Bioactive compounds that are most extensively researched are sulfated polysaccharides, phlorotannins and diterpenes from brown algae. These compounds have been reported to possess strong antibacterial, anti-viral and anti-cancer properties. The pharmaceutical, health and research potentials of different bioactive compounds present in brown seaweeds have been reviewed. Studies on the chemical composition of seaweeds have shown that these are good sources of proteins, lipids and carbohydrates.

In the present study, the bioactive potentials of *Sargassum* speciee, viz., *Sargassum myriocystum*, *S. plagiophyllum* and *S. ilicifolium* were studied. *Sargassum myriocystum* showed highest percentage of protein (13.21±0.024%) followed by *S. plagiophyllum* (12.30±0.11%) while lowest percentage was observed in *S. ilicifolium* (9.71 ± 0.08 %). The protein values were significantly different between the three seaweeds (P < 0.05). The highest carbohydrate content was found in *Sargassum myriocystum* (40.21± 1.33%) followed by *S. ilicifolium* (38.72 ± 0.96 %) while the lowest percentage was observed in *S. plagiophyllum* (33.38 ± 0.80 %). The carbohydrate values were significantly different between the three seaweeds (P < 0.05). The highest lipid content was found in *Sargassum ilicifolium* (0.420 ± 0.009 %) followed by *S. myriocystum* (0.171 ± 0.001%) while the lowest lipid content was found in *S. plagiophyllum* (0.165  ± 0.006 %). The lipid values were significantly different between the three seaweeds (P < 0.05) (Graph.1).

![Graph 1: Biochemical composition of *Sargassum* species collected from Mandapam region.](image-url)
The biochemical analysis of Indian seaweeds has been carried out by many workers. The protein content of brown seaweeds is generally small (average: 5-15 % of the dry weight). Most of seaweeds contain large amounts of polysaccharides, which are mostly concentrated in algal cell walls, e.g. alginites predominate in brown seaweeds whereas lipid content of seaweeds accounts for 1-6 g/100 g dry wt. Similarly in the present study protein was in the same range as reported earlier for other Sargassum species but lipid was comparatively very low. Also the carbohydrate content was high in Sargassum myriocystum when compared to other Sargassum species collected from same region but it is less than S. vulgare (67.8%). In general, all the three brown seaweed species viz., Sargassum myriocystum, S. plagiophyllum and S. ilicifolium displayed little intra specific variation in carbohydrate, protein and lipid content.

It has been earlier reported that some major active compounds such as phlorotannins and fucoxanthin from brown seaweed have antioxidantive properties. Tropical brown algae have proven to produce a very effective antioxidant defence system due to the strong UV radiation in tropical environment. In the present study Sargassum plagiophyllum extract showed the highest total phenolic content (28.66 ± 0.5 mg galic acid equivalent/g) followed by S. myriocystum (22 ± 2 mg/g) and S. ilicifolium showed the lowest phenolic content (19.33 ± 3.05 mg/g), whereas highest antioxidant activity was observed in S. ilicifolium (36.66 ± 9.6 mg ascorbic acid equivalent/g), followed by S. myriocystum (32.66 ± 1.15 mg/g) and S. plagiophyllum had lowest antioxidant activity (24.66 ± 1.15 mg/g) (Graph 2).

The total phenolic content is influenced by types of extracts, seasons, sites and species. It is assumed that the antioxidant properties of phenolics are related to the number of phenol rings that makes them more effective hydrogen donors and quenchers. Similarly in the present study, significant differences were observed both in total phenolic contents and antioxidant activities of extracts. No correlation was observed between the total phenolic content and antioxidant activity. Some other bioactive compounds present in the extract were responsible for the antioxidant activity. The total antioxidant activities are higher in the present study when compared to other Sargassum species.

Alginic acid content varies from 22.3 to 30.8% in S. ilicifolium and from 15.9 to 34.5% in S. myriocystum whereas in the present study maximum alginic acid yield was obtained from S. plagiophyllum (15.0%) followed by S. ilicifolium (14.26%) and least in S. myriocystum (12.6%). Similarly the analysis of other brown algae such as species of Padina, Cystophyllum muricatum Cystoseira indica and S. tenenimum growing in different parts of the Indian coast, showed more or less same value.

The FTIR spectrum is used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The crude powder (alginic acid) of Sargassum myriocystum, S. plagiophyllum and S. ilicifolium was passed into the FTIR and the functional groups of the components were separated based on the peak value. The FTIR spectra of alginic acid obtained from Sargassum myriocystum showed a peak at 3414.00, 2927.94, 2634.76, 1735.93, 1635.64, 1265.30, 1409.96, 1037.70, 800.46 and 665.44 cm⁻¹ respectively (Fig.1). The FTIR spectra of alginic acid of S. plagiophyllum showed a peak at 3491.16, 2655.98, 1705.07, 1616.35, 1539.20, 1442.75, 1253.73, 1028.06, 866.04 and 700.16 cm⁻¹ respectively (Fig.2). The FTIR spectra of alginic acid of S. ilicifolium showed a peak at 3491.16, 2655.98, 1705.07, 1616.35, 1539.20, 1442.75, 1253.73, 1028.06, 866.04 and 700.16 cm⁻¹ respectively (Fig.3). The characteristic peak at 3414.00, 3491.16, 3414.00, 1635.64, 1633.71 and 1413.82 cm⁻¹, 1409.96 cm⁻¹ is corresponding to hydroxyl (OH), carbonyl (C=O) and carboxyl (COOH), respectively. In seaweeds, different types of alginate present in different proportions and different alternating patterns of guluronic and mannuronic units. The acids can be identified from their characteristic bands such as guluronic units originate a band at approximately 1025 cm⁻¹, the mannuronic units originate a band at approximately 1100 cm⁻¹. In the
present work the FTIR spectrum of alginic acid, the peaks around 3429, 1630 and 1428 cm$^{-1}$, corresponds to hydroxyl (OH), carbonyl (C=O) and carboxyl (COOH) respectively. Due to commercial and scientific interest in seaweed phycocolloids, the FTIR spectra provides more information on characteristic bands and the type of polysaccharide. FTIR is more easily applied method for studying natural product.

Figure 1: FT-IR spectrum of Sargassum myriocystum

Figure 2: FT-IR spectrum of Sargassum plagiophyllum
Conclusion:

Bioactive potentials of *Sargassum* species collected from Mandapam region was evaluated. All the three species were found to have good antioxidant capacities and thus could be potential rich sources of natural antioxidants. Therefore it is suggested that further works may be performed on these least studied species for its industrial applications.

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