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**Research Article**

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

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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.
Key**words:** Biochemical composition, total phenol, total antioxidant, alginic acid yield, FT-IR, brown seaweeds

## 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 algae.[1] The phenolic content of brown algae varies from 20-30% dry weight.[2] Antioxidant compounds play an important role against various diseases such as atherosclerosis, chronic inflammation, cardiovascular disorders, cancer and aging processes.[3] Among brown seaweeds, *Sargassum* had been studied extensively for its high antioxidant potential *in vitro*.[4]

Alginates are a family of linear polysaccharides occurring as a structural component of the cell wall in marine brown algae.[5] 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.[6,7] 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.[8] The annual alginate production is reaching globally to 30.000 tonnes and it requires an additional 50,000 tonnes of dry *L. japonica* seaweed as raw material.[9]

Brown algae *Sargassum* and *Turbinaria* are potential source of alginate in India. A standing crop of 16,000 tonnes of algin yielding *Sargassum* and *Turbinaria* was reported from Indian waters.[10] The seaweeds belonging to the order Fucales *Sargassum myriocystum J.Ag, Sargassum plagiophyllum* C.Ag *and Sargassum ilicifolium(Turn.) J.Ag.* are available in appreciable amount in the Indian coastal regions, thus making them highly suitable for industrial exploitation as a source of alginates and secondary metabolites. However, little information about their biological activity and polysaccharides is available compared with those of other *Sargassum* species. The present study was undertaken to evaluate the potential uses of three *Sargassum* species i.e *Sargassum myriocystum, S. plagiophyllum and S. ilicifolium* .

## 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 fine powder.

### Biochemical properties

### Protein estimation:

Total protein was estimated using the Lowry method.[11] 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.[12] 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.[13]

### 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.[14] 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.[15] 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.[16] Alginic acid is expressed as percentage dry weight of seaweed.

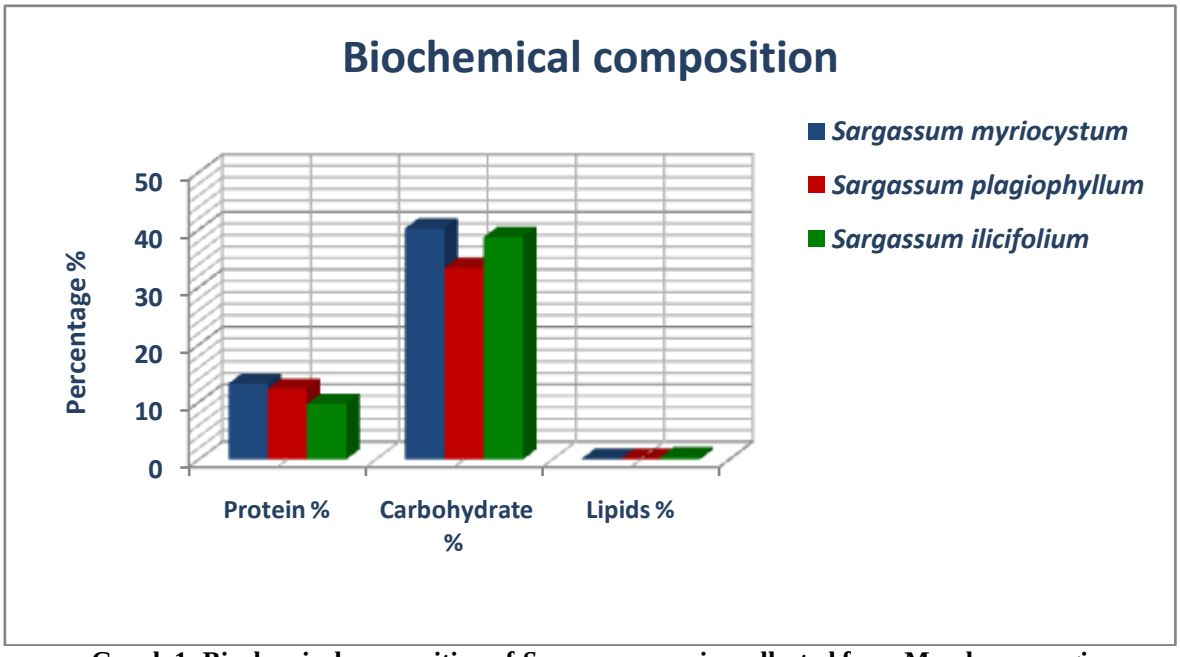
**FTIR spectra of alginic acid:**

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.[17]

**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.[18] Studies on the chemical composition of seaweeds have shown that these are good sources of proteins, lipids and carbohydrates.

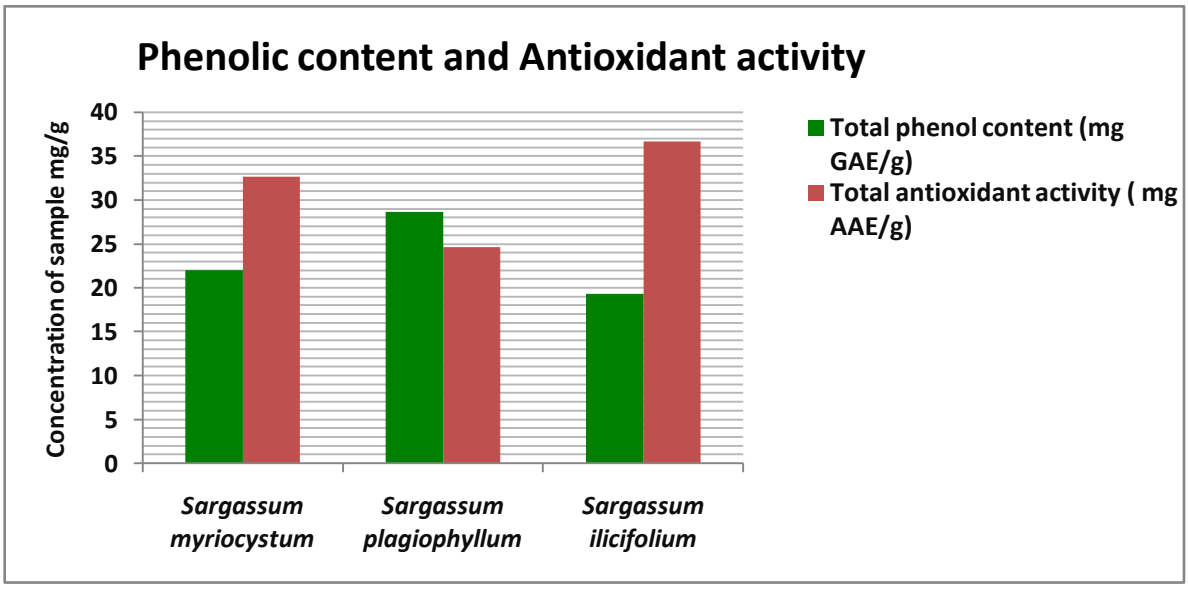
In the present study, the bioactive potentials of *Sargassum* speciec, *viz.*, *Sargassum myriocystum, S. plagiophyllum* and *S.ilicifolium* were studied. *Sargassum myriocystum* showed highest percentage of protein (13.21±0.024%) followed by *S. plagiophyllum* (12.30±0.11%) while lowest percentage was 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.**

The biochemical composition of Indian seaweeds has been carried out by many workers.[19,20,21,22,23,24] The protein content of brown seaweeds is generally small (average: 5-15 % of the dry weight).[25] Most of seaweeds contain large amounts of polysaccharides, which are mostly concentrated in algal cell walls, e.g. alginates predominate in brown seaweeds whereas lipid content of seaweeds accounts for 1-6 g/100g dry.wt.[26] Similarly in the present study protein was in the same range as reported earlier for other *Sargassum* species but lipid was comparatively very low. [27,28,29,30,31] 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* (67.80%).[32] 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 antioxidative properties.[33] Tropical brown algae have proven to produce a very effective antioxidant defence system due to the strong UV radiation in tropical environment.[34] In the present study *Sargassum plagiophyllum* extract showed the highest total phenolic content (28.66 ±3.05 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 ± 9.86 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).




**Graph 2: Total phenolic content and total antioxidant activity of *Sargassum* species collected from Mandapam region.**

The total phenolic content is influenced by types of extracts, seasons, sites and species.[35] 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.[36,37,38]

Alginic acid content varies from 22.3 to 30.8% in S. *ilicifolium* and from 15.9 to 34.5% in S. *myriocyslum*.[39] 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. [40,41,42,43,44]

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 *Sargassum ilicifolium* showed a peak at 3414.00, 2927.94, 2632.83, 1737.86, 1633.71, 1413.82, 1261.45, 1097.50, 1035.77, 802.39 and 665.44 $cm^{-1}$ 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 orginate a band at approximately 1025 $cm^{-1}$, the mannuronic units orginate a band at approximately 1100 $cm^{-1}$. [45] 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 phycolloids, the FTIR spectra provides more information on characteristic bands and the type of polysaccharide. FTIR is more easily applied method for studying natural product .[46]

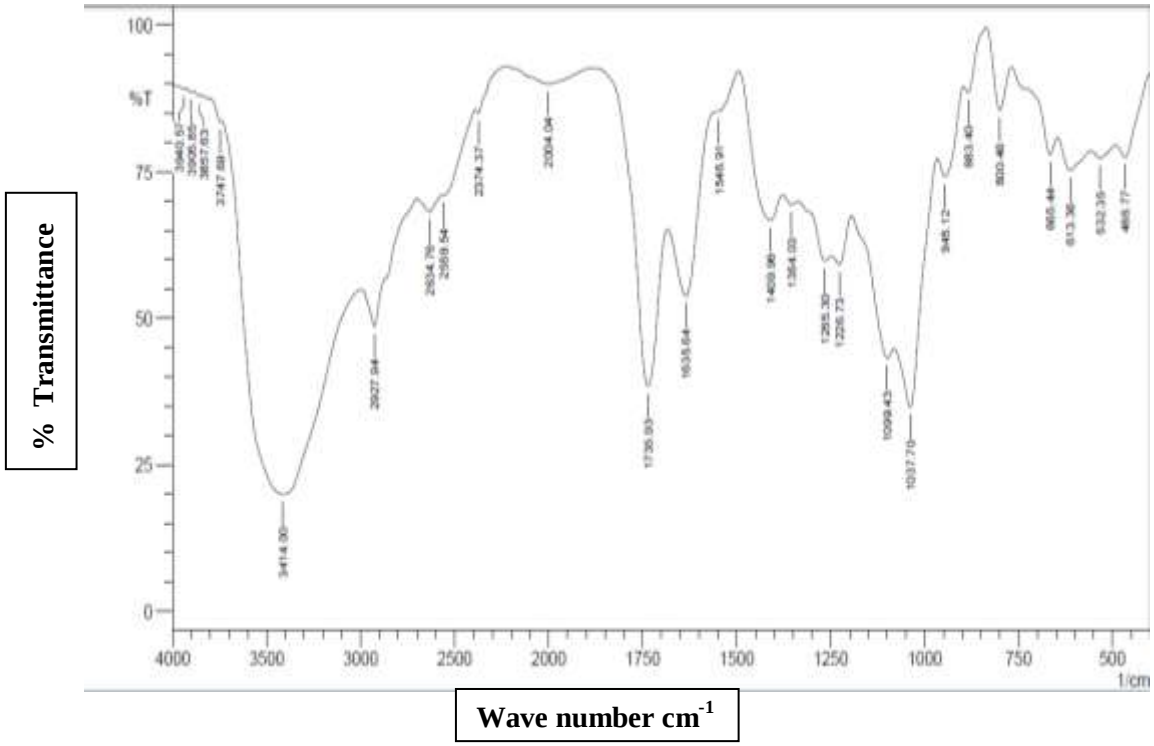



**Figure 1:FT-IR spectrum of *Sargassum myriocystum***

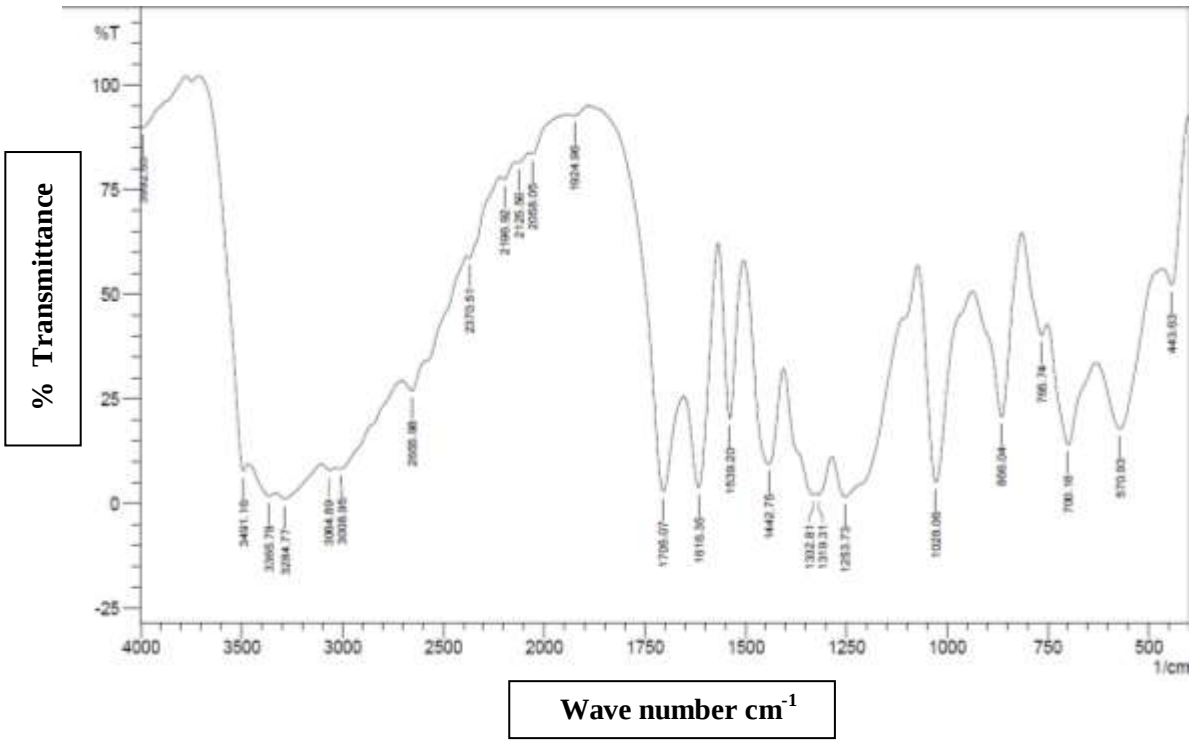



**Figure 2: FT-IR spectrum of *Sargassum plagiophyllum***

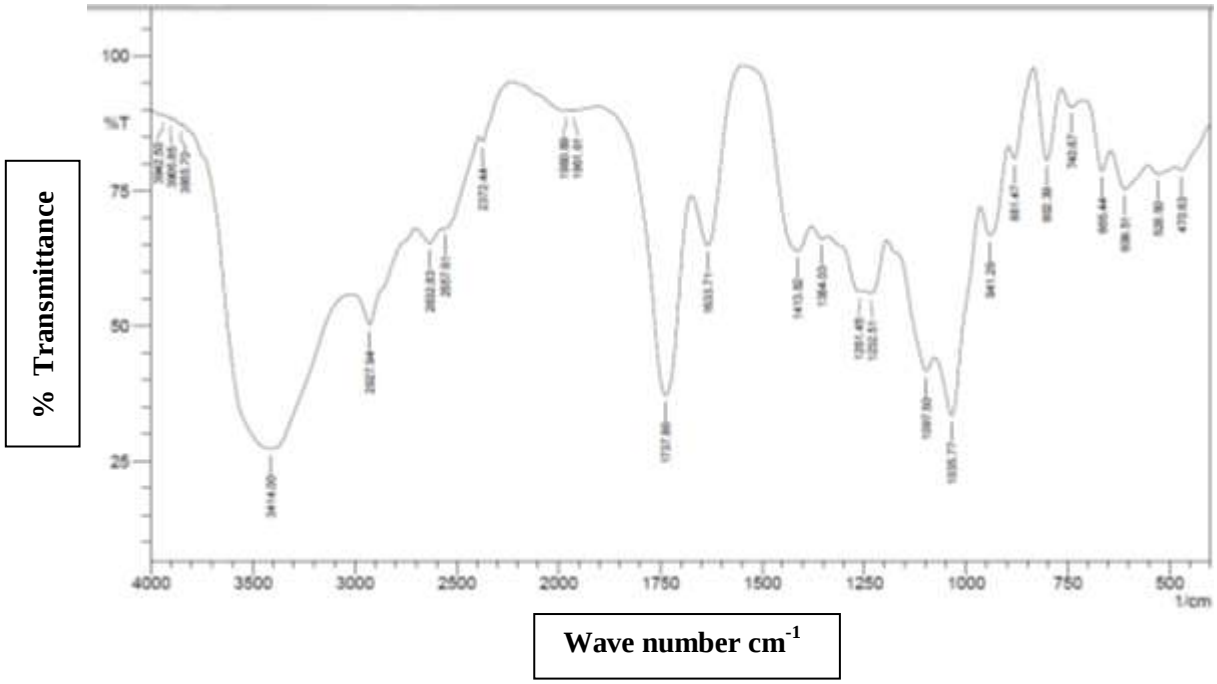



**Figure 3:FT-IR spectrum of *Sargassum ilicifolium***

**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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**REFERENCES:**

1. Nagai T, Yukimoto T. Preparation and functional properties of beverages made from sea algae, *Food Chemistry*, **2003**; 81: 327-332
2. Ragan MA, Glombitza KW. Phlorotannins Brown Algal Polyphenols. InProgress in Phycological Research Bio press, Round, F.E. and D.J. Chapman (eds.), Bristol, UK,**1986**; 129-241.
3. Kohen R, Nyska A. Invited review :Oxidation of biological systems: Oxidative stress phenomena, antioxidants, redox reactions and methods for their quantification, *Toxicologic Pathology*, **2002**; 30: 620-650.
4. Khotimchenko SYu. The antitumor properties of non starch polysaccharides: carrageenans, alginates, pectins, *Russian Journal of Marine Biology*, **2010;** 36(6):401-412.
5. Kalimuthu S, Kaliaperumal N , Ramaligam JR. Standing crop, algin and mannitol of some alginophytes of Mandapam coast, *Journal of the Marine Biological Association of India*, **1991**; 33 (1&2);170-174.
6. McHugh DJ. Production, properties and uses of alginates. FAO Fisheries Technology, **1987**; 288: 58-115.
7. Pérez R, Kaas R, Campello F, Arbault S, Barbaroux O. Ed. La culture des algues marinesdans le monde, IFREMER, Plouzane, France, **1992**; 614.
8. Critchley AT, Ohno M. Seaweed resources of the world. (eds). Japan: Japan International Cooperation Agency, Yokosuka, **1988**; 429.
9. Bixler HJ, Porse H. A decade of change in the seaweed hydrocolloids industry, *Journal of Applied Phycology*, **2010**; 23:321-335.
10. Kaladharan P, Kaliaperumal M, seaweed industry in India, ICLARM (NAGA), **1999**; 22:11-14.
11. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the phenol regent, *Journal of Biological Chemistry*, **1951**; 193: 265-275.(original method).
12. Dubois M, Giles KA, Hamilton JK, Reborsand PA, and Smith F. Calorimetric method for determination of sugars and related substances, *Analytical Chemistry*, **1956**; 28: 350-356.
13. Folch J, Lees M, Solane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues, *Journal Biological Chemistry*, **1956**; 226: 497-509, (1956).
14. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents, *American Journal of Enology and Viticulture*, **1956**; 16: 144-158.
15. Prieto P, Pineda M, Aguilar MM. Spectrophotometric quantification of antioxidant capacity through the formation of a

phoshomolybdenum complex; specific application to the determination of vitamin E, *Analytical Biochemistry*, **1999**; 269: 337-341.

**16.** Suzuki N, Studies on the manufacture of algin from brown algae. Memoirs of the Faculty of Fisheries, Hokkaido University,**1955**; 3: 93-158.

**17.** Pathak TS, Kim JS, Lee JS, Baek DJ, and Paeng KJ. Preparation of alginic acid and metal alginate from algae and their comparative Study, *Journal of Polymer and the Environment*, **2008**; 16:198-204.

**18.** Gupta S, Abu-Ghannam N. Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods, *Innovative Food Science and Emerging Technologies*, **2011**; 12: 600-609.

**19.** Neela MV. Analysis of seaweeds. Home. Science. Bull. Women's Christian Coll. Madras,**1956**.

**20.** Pillai VK. Chemical studies on Indian seaweeds. II: Partition of Nitrogen Proceedings on Indian Academic Science, **1957** a; B 45: 43-63.

**21.** Sitakara Rao V, Tipnis UK . Protein content of marine algae from Gujarat coast, *Current Science*, **1964**; 33: 16-17.

**22.** Sitakara Rao V, Tipnis UK , Chemical constituents of marine algae from Gujarat coast. Proceedings and Seminar an sea salt and plants, CSMCRI, Bhavanagar, **1967**; 277-288,

**23.** Parekh RG, Maru LV, Dave MJ. Chemical composition of green seaweeds of Surashtra Coast, *Botanica Marina*, **1977**; 20: (6): 359-362.

**24.** Murthy MS, Radia . Eco-biochemical studies on some economically important intertidal algae from Port Okha (India). *Botanica Marina*,**1978**; 21(7): 417-422

**25.** Burtin P. Nutritional value of seaweeds. Electronic Journal of Environmental, *Agricultural and Food Chemistry*, **2003**; 2: 498-503.

**26.** Fleurence J, Gutbier F, Mabeau S, Leray C. Fatty acids from 11 marine macro algae of the French Britanny coast, *Journal of Applied Phycology*, **1994**; 6:527-532.

**27.** Chidambaram K, Unny MM. Note on the value of seaweeds as manure. Ist *Int*. Seaweed Research and Utilizations, **1953**; 67-68.

**28.** Pise NM, Sabale AB. Biochemical Composition of seaweeds along central west coast of India. *Pharmacognosy Journal*, **2010**; 2:7.

**29.** Hossain Z, Kurihara H, Takahashi K. Biochemical composition and lipid compositional properties of the brown alga *Sargassum horneri*, *Pakistan Journal of Biological Sciences*, **2003**; 6 (17): 1497 -1500.

**30.** Ambreen, Hira, k. Amna Tariq, Ruqqia1, Vqar sultana1 Andjehan ara. Evaluation of biochemical component and antimicrobial activity of some seaweeds occurring at Karachi coast, *Pakistan Journal of Botany*, **2012**; 44(5): 1799-1803.

**31.** Manivannan K, Thirumaran G, Karthikai Devi G, Hemalatha A, Anantharaman P. Biochemical composition of seaweeds from Mandapam coastal regions along southeast coast of India, *American -Eurasian Journal of Botany*, **2008**; 1(2); 32-37.

**32.** Marinho-Soriano E, Fonseca PC, Carneiro MAA, Moreira WSC. Seasonal variation in the chemical composition of two tropical seaweeds, *Bioresource Technology*, **2006**; 97:2402-2406.

**33.** Kuda T, Ikemori T. Minerals, polysaccharides and antioxidant properties of aqueous solutions obtained from macroalgal beach-casts in the Noto Peninsula, Ishikawa, Japan, *Food Chemistry*, **2009**;112, 575–581.

**34.** Pavia H, Cervin G, Lindgren A, Aberg P. Effects of UV-B radiation and stimulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. *Marine Ecology Progress Series*,**1997**; 157:139-146.

**35.** Budhiyanti SA, Raharjo S, Marseno DW, Lelana IYB. Free radical scavenging, metal chelating and singlet oxygen quenching activity of fractionated brown seaweed *Sargassum hystri*x extract, *Journal of Biological Sciences*,**2011**; 11: 288-298.

**36.** Simpi CC, Nagathan CV, Karajgi SR, Kalyane NV. Evaluation of marine brown algae *Sargassum ilicifolium* extract for analgesic and anti-inflammatory activity, *Pharmacognosy Research*,**2013**; 5 (3) : 146-149.

**37.** Suresh V, Senthil Kumar N, Murugan P, Palani P, Rengasamy R, and Anbazhagan C. Antioxidant properties of sequential extracts from brown seaweed, *Sargassum plagiophyllum*, C. Agardh, *Asian Pacific Journal of Tropical Disease*, **2012**; S937-S939.

**38.** Kumar M, Kumar P, Trivedi N, Shukla MK, Gupta V, Reddy CRK, and Jha B. Minerals ,PUFAs and antioxidant properties of some tropical seaweeds from Saurashtra coast of India, *Journal Applied Phycology*, **2010**; 23:797–810.

**39.** Chennubhotla VSK, Kaliaperumal N, Kalimuthu S, Selvaraj M, Ramalingam JR, and Najmuddin M. Seasonal change in growth & alginic acid & mannitol contents in *Sargassum ilicifolium* (Turner) J.Agardh & *S.myriocystum* J.Agardh, *Indian Journal of Marine Science*, **1982**; 11.

**40.** Valson A P. Alginic acid content of some of the common seaweeds of the Gulf of Mannar area, *Current Sciences*, **1955**; 24: 343-345.

**41.** Kappanna AN, Visweswara Rao A, Mody IC. Alginic acid content of some of the brown seaweeds of Sourashtra coast, *Current Sciences*,**1962**; 31: 463-46.

**42.** Chauhan VD. Variation in alginic acid content with growth stages in two species of *Sargassum. Botanica Marina*,**1970**; 13 (1): 57-58.

**43.** Solimabi, Naqvi SWA. Alginic acid content of some brown seaweeds of Goa. *Mahasagar*, **1975**; 8 (1& 2): 97-99.

**44.** Mairh O P. Seasonal variation in alginic acid and viscosity of sodium alginate from a brown alga *Cystoseira indica* (Thivy. et Doshi) Mairh from Port Okha. *Seaweed Research. Utilization*, **1982**; 5 (1): 43-46.

**45.** Janakiraman N, Sahaya Sathish S, Johnson M. UV-VIS and FTIR spectroscopic studies on Peristrophe bicalyculata (Retz.) Nees. *Asian Journal of Pharmaceutical and Clinical Research*, **2011**; 4(4): 125-129.

**46.** Pereira L, Sousa A, Coelho H, Amado MA, Ribeiro-Claro AJP. Use of FTIR, FT-Raman and $^{13}$C-NMR spectroscopy for identification of some seaweed phycocolloids. *Biomolecular Engineering*, **2003**; 20:223-228.

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