Characterization of Isolated Polysaccharide and Biochemical Attributes of Red Algae, *Grateloupia filicina*

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Abstract: ***The polymer extracted from the red alga Grateloupia filicina finds its use as an alternate and potential source of polysaccharide and other biochemical constituents for nutraceutical functions. Presently in this study, an attempt was made to analyze the carbohydrate, protein and lipid content in G. filicina. The total carbohydrate, protein and lipid contents varied from 40.1 to 58%, 10.1 to 8.65% and 0.21 to 1.0% on its dry weight basis, respectively. Seasonal variation was studied with respect to the yield and chemical constituents of the extracts like apparent viscosity, 3,6-anhydrogalactose, galactose and sulphate contents. FT-IR study revealed that the functional groups of the sulphated galactose from Grateloupia filicina resembles with that of the λ-carrageenan. The results obtained in this study indicated that the seaweed can be treated as an important indigenous resource for use in phycocolloid and nutraceutical industries.***

Keywords: *Grateloupia filicina*, phycocolloid, carbohydrate, protein, lipid

1. Introduction

Marine algae (popularly known as seaweed) are renewable source of polysaccharides as well as biochemical constituents such as carbohydrate, protein and lipid contents [15],[16],[19],[20]..Sulphated polysaccharides are widespread in nature, usually occurring in seaweed, some plants and in a great variety of other organisms [14]. In seaweed, they are present as sulfate galactans (carrageenans and agars) and as sulfate fucose (fucoidans). Sulphated polysaccharides show various chemical and biological functions. These polysaccharides serve a number of structural and protective roles in plants.

The present study originates primarily from the availability of enormous data on the extracts of sulphated polysaccharides from red seaweed, *Grateloupia filicina* ressembling to λ- type carrageenan. Traditionally, about fifteen carrageenan structures have been identified by Greek letters [6]. Carrageenans are hydrophilic phycocolloids which occur as natural matrix material in various species of red seaweed. It is made up of repeating galactose and 3,6-anhydrogalactose (3,6-AG) units of both sulfated and non-sulfated type. The units are joined by alternating α-1→3 and β-1→ 4 glycosidic linkages. The carrageenans of commercial interest are *kappa* (κ-), *iota* (ι-) and *lambda* (λ-). All these three types have common structural features which differ in degree and positions of sulphur group, conformation of pyranose ring and the presence of 3,6-AG. In κ -carrageenan, 1, 3 and 1, 4 linked units are D-galactose-4-sulphate and 3, 6-AG, respectively. λ-carrageenan is highly sulfated and does not gel, while κ - carrageenan has high gel strength. ι-carrageenan is a type of carrageenan with a sulfate content intermediate between κ- and λ- carrageenan [3].

The red seaweed *Grateloupia filicina* (Lamouroux) C. Agardh (Halymeniaceae, Cryptonemiales, Rhodophyta) is the type species of genus *Grateloupia*. The alga, *G. filicina*, grows in considerable quantities in the southeast coast of India [2], [23]. This red seaweed is an important source of a sulphated polysaccharide (SP) which is similar to λ-type carrageenan but is distinct in its properties and proportions of sulfate and 3, 6-AG [2, 27] . The λ-type of carrageenan has a property that is highly desirable in certain applications for the food industry [11]. The present report details the analysis of the SP from *G. filicina* and its biochemical constituents like carbohydrate, protein and lipid content. The present information finds the properties of SP from this native alga show similar to the λ-type carrageenan and the use of other biochemical constituents for nutritional applications.

2. Materials and Methods

Carrageenan standards were purchased from Sigma-Aldrich Chemicals Inc., St. Louis, USA. All other chemicals used were of analytical grade or better. Fresh and healthy plants of *G. filicina* were collected periodically in 2008 (pre-monsoon, monsoon and post-monsoon) during the low tide from Mandapam (09°17'N, 79 °2'E), south-east coast of India. Selected fronds (Figure 1) were washed thoroughly in sea water to remove the visible epiphytes and were shade-dried. The shade-dried seaweed was powdered and estimated for the yield and properties of polysaccharide for sulphate, galactose, carrageenan, lipid, protein, and carbohydrate.

Figure 1: Thalli of *Grateloupia filicina*

Using the modified protocol of Craigie and Leigh [4], the polymer was extracted from the dried plants of *G.filicina*. In brief, the dry algal powder was soaked in distilled water for 1/2 h at room temperature then autoclaved at 107°C for 1 h. Filtration of the viscous solution as done. Like carrageenan extraction the polymer was then obtained by precipitation of the cleared extractives in isopropanol (IPA) and water in the ratio of 1:2 (v/v), followed by drying, grinding and storage for further analysis. The percentage yield was calculated. This powdered sample was used for measurements of various parameters like galactose, sulphate, carbohydrate, viscosity, protein and lipid contents. Gel samples (1.0% gel) were prepared by dissolving in 1% KCl solution and then autoclaved at 107°C for 15 min. Apparent viscosity was measured for 0.1% sample at 30ºC on a Brookfield Viscometer, using Spindle No.1 at a speed of 60 rpm.

The galactose content was estimated by improved phenol-resorcinol method using fructose as standard [26]. Sulphate content was determined using K_2SO_4 as standard by the method of Verma [25]. The lipid content by Folch method [9], protein content by Lowry method [18], and carbohydrate content was estimated by phenol-sulfuric acid method [8]. Absorbance for measurements of lipid, protein, and carbohydrate were recorded at 520, 500, and 490 nm, respectively. Ash content was also determined in a furnace.

The FT-IR analysis of the powdered sample and the carrageenan from Sigma was performed respectively by grinding with infrared grade KBr (1:10) and pressed into disks under vacuum using a Spectra Lab Pelletizer. FT-IR spectrum in the KBr disc was recorded in the wavenumber region of 4,000–500 cm^{-1} using a Thermo Nicolet Nexus 670 FT-IR spectrophotometer. Tests for carrageenan such as KCl solubility, methylene blue test, milk reactivity and gel formation were also carried out.

3. Results and Discussion

The yield and SP constituents of the alga *G. filicina* were found to vary at different seasons (Figure 2). The maximum yield of SP was 35.12% which was obtained during pre-monsoon season, i.e. the summer period. The galactose content of SP was also found high during the pre-monsoon season, while 3, 6-AG and sulphate content was found higher of 4.76% and 31.36%, respectively, for the period of post-monsoon season. The high sulphate and low 3,6-AG in the phycocolloid may be the reason for the presence of non gelling property of λ- Carrageenan [10]. There was not much variation observed in the viscosity values (0.67 to 1.06%). Seasonal variation observed in carbohydrate, protein and lipid contents is depicted in

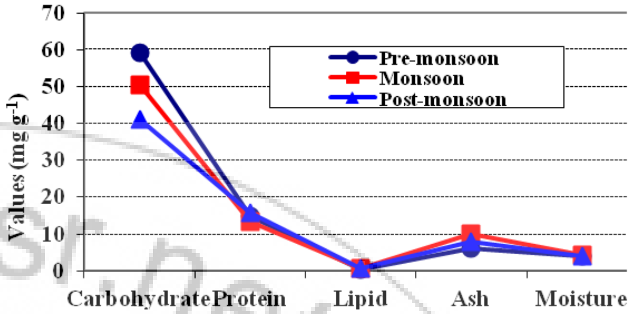

Figure 2: Seasonal variation in biochemical constituents in *Grateloupia filicina*

The order of concentration was carbohydrate > protein > lipid. The total carbohydrate, protein and lipid contents ranged between 40.1– 58%, 10.1–8.65% and 0.21–1.0% (on dry weight basis), respectively. All these content varied in different season (Figure 3). Carbohydrate content recorded was maximum during the pre-monsoon season; protein content was maximum during the post- monsoon season, whereas maximum lipid content was observed during the monsoon season. The changes in sea water quality in different seasons could be the major factor for the variations found in lipid protein, and carbohydrate content [22]. The physicochemical properties of the galactans from *Grateloupia filicina* have been characterized as a gelling agar viscous λ-type carrageenan by Lai and his co-author [17].

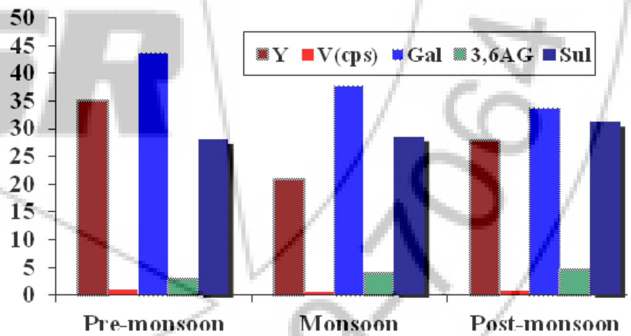

Figure 3: Seasonal variation in biochemical constituents in *Grateloupia filicina*

The FT-IR spectra of commercial carrageenan and extracted are shown in Figure 4. The O-H stretching at around 3400 cm^{-1} with very strong intensity of bands was observed in the commercial and extracted carrageenan from *G. filicina* of the present study corroborates the earlier observations made by Gunasekaran and co-workers [12]. The peaks in the region 2930 cm^{-1} and a weak intensity of bands at or around 1450 cm^{-1} is characteristic of the presence of a methyl group. The ester sulphate link vibrations exhibited correspond to the wavenumbers 1060, 1180, 1250 and 1370 cm^{-1} as reported earlier by Alkahane and Izumi [1]. The present study also confirms the peaks at similar region at or around 1030, 1070, 1150, 1240 and 1380 cm^{-1}. The polysaccharide studied in this work showed an absorbance band at 1250 cm^{-1} which is due to the sulphate content [1]. The presence of sulphate positions is attributed to the bands appearing at 830 and 820 cm^{-1} in the λ-spectrum corresponding to galactose-2-sulphate and galactose-6-sulphate, respectively [5]. The present study also confirms the peak at around 830 cm^{-1} which is due to the presence of sulphate on the C- 2 galactose. The presence of carboxylic acids and carboxylic ester showed strong C=O stretching bands in the region of 1700-1540 cm^{-1}[12]. Confirmation of these peaks were also seen in the present investigation. The spectra of the suphated polysaccharide from this investigation showed a strong intensity at 1640 cm^{-1} and 1540 cm^{-1}, which indicate the existence of N-H band thus confirming with the earlier report of Christiaen and Bodard[6]. These bands also confirm the presence of protein in the sulphated polysaccharide (SP). Further, the presence of low content of 3, 6-AG in the extracted SP from *G. filicina* was confirmed by the presence of a weak band at 930c m $^{-1}$.

wavenumbers (cm-1)

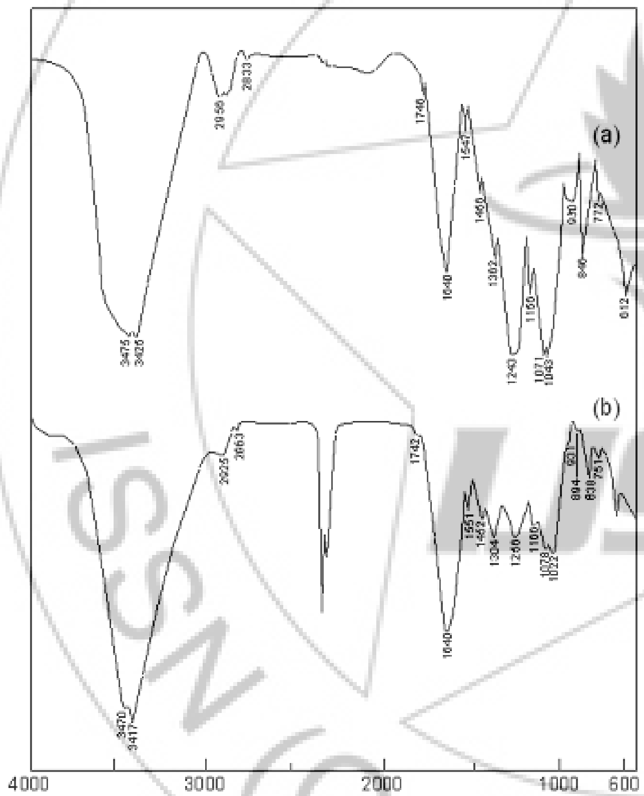

Figure 4: FT-IR spectrum of (a) commercial carrageenan, and (b) extracted carrageenan

However, recently study done by Yu and his co-author [27] confirmed the presence of homogenous sulphated polysaccharide with an agaran-type backbone in *G. filicina* (GFP) using IR and ^{13}C NMR and linkage analysis. There was no gel found in the present study.

Properties like positive methylene blue test, milk reactivity, solubility in 3M KCl solution and no gel formation indicated the λ- carrageenan nature of the colloid *from G. filicina*. Its capacity to agglomerate casein suggested the use of this alga as an alternate and potential source of λ-carrageenan as well as in the nutraceutical industries.

4. Conclusions

The current investigation suggests that the SP extracted from red alga, *Grateloupia filicina* could as a type of carrageenan but an in-depth analysis is needed on its rheological properties. Considering the increased global demand of sulphated galactans, this study holds tremendous scope as an elite biomass for the seaweed as well as polymer industries.

5. Scope for Future Study

Future studies on the alga *Grateloupia filicina,* is needed to elucidate the characterization of its polysaccharide by using various spectral analysis like NMR, GC, NOSY etc. These data will generate more information's oriented applications in various fields like food, pharma, and cosmetics.

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References

[1] Alkahane, T. and Izumi, S. 1976. "Sulphate groups of the mucilage of red seaweed", Agricultural and Biological Chemistry, 40: 285–289
[2] Baweja,P. and Sahoo, D. 2002. Structure and Reproduction of *Grateloupia filicina* (Halymeniaceae, Rhodophyta) from Indian coast. Algae 17: 161-170
[3] Bixler H. J. and Porse, H. 2011. "A decade of change in the seaweed hydrocolloids industry," Journal of Applied Phycology, 23: 321–335
[4] Craigie, J. S. and Leigh, C. 1978."Carrageenans and Agars". In: Hellebust JA, Craigie JS (eds), Handbook of Phycological Methods, Cambridge University Press, Cambridge, 109–131
[5] Chopin, T. and Whalen, E. 1993. Carbohydrate Research, 246: 51–59
[6] Chopin, T., Kerin, B. F. and Mazerolle, R. 1999. "Phycocolloid chemistry as a taxonomic indicator of phylogeny in the Gigartinales, Rhodophyceae: a review and current developments using Fourier transform infrared diffuse reflectance spectroscopy," Phycological Research, 47: 167–188
[7] Christiaen, D. and Bodard, M. 1983. "Spectroscopie infrarouge de films d' agar de *Gracilaria verrucosa* (Huds.) Papenfus", Botanica Marina, 26: 425–427
[8] Dubois, M. , Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. "Colorimetric method for determination of sugars and related substances", Analytical Chemistry, 28: 350–366
[9] Folch, J., Lees, M. and Sloane-Stanley, G. H. 1957. "A simple method for the isolation and purification of total lipides from animal tissues", Journal of Biological Chemistry, 226: 497–509
[10] Freile-Pelegrin, Y., Azamar, J.A.and Robelodo, D. 2011. Preliminary Characterization of Carrageenan from the

red seaweed *Halymenia floresii*. Jr. of Aquatic Food Product Technology, 20: 73-83

[11] Guist, G.G. 1990. "Application for seaweed hydrocolloids in prepared foods". In: I. Akatsuka, (ed.) Introduction to Applied Phycology, SPB Academic Publishing, The Netherlands, 391–400

[12] Gunasekaran, S., Varadhan, S. R. and Karunanidhi, N. 1996. "Qualitative analysis on the infra red bands of tetracycline and ampicillin", Proceedings of Indian National Science Academy, 62: 309–316

[13] Hill, R. R. and Rendell,1975. "The Interpretation of infra red spectra", Heyden, New York.

[14] Hooper, L.V. , Manzella, S.M. and Baenziger, J.U. (1996), From legumes to leukocytes: biological roles for sulfated carbohydrates. FASEB J 10: 1137-1146.

[15] Kaliaperumal, N. , Kalimuthu, S. and Ramalingam, J. R. 1995. "Economically important seaweeds", *CMFRI Special Bulletin*, Central Marine Fisheries Research Institute, 62:1- 40

[16] Kaliaperumal, N. , Kalimuthu, S. and Ramalingam, J. R. 1998. In: R. M. Anand, K. Dorairaj, and A. Parida, Eds. *Biodiversity of Gulf of Mannar Marine Biosphere Reserve*, M.S. Swaminathan Research Foundation, Chennai, 92–101

[17] Lai, M., Li, C.F., and Li,C.Y. 1994. Characterization and thermal behavior of six sulphated polysaccharides from seaweeds. Food Hydrocolloids 8: 215–232

[18] Lowry, O.H., Rosebrough, N.J., Farr, A. L. and Randall, R. J. 1951. "Protein measurement with the Folin-Phenol reagents", Journal of Biological Chemistry, 193: 265–275

[19] Mc Hugh, D. J. 1987. "FAO Fisheries Technical paper", 288 (www.fao.org/docrep).

[20] Mc Hugh, D. J. 2003. "Seaweeds used as a source of carrageenan". In: A Guide to the Seaweed Industry, FAO Fisheries Technical Report, 441, FAO of United Nations, Rome,1–105

[21] Rincones, R.E. 2010. "The Introduction and cultivation of the red alga *Kappaphycus alvarezii* for the production of carrageenan in the Caribbean and the Western Atlantic: An alternative livelihood for coastal communities". XX International Seaweed Symposium 76,1-93

[22] Roslin, A.S. 2001. "Seasonal variations in the carrageenan content of some marine algae inrelation to environmental parameters in Arockiapuram coast", Seaweed Research and Utilization, 23:129–132

[23] Sahoo, D., Nivedita and Debasish 2001."Seaweeds of Indian Coast". APH Publishing Corporation, New Delhi, 1-282

[24] Stanley, N.F. 1990. Carrageenan. In: Harris, P. (Ed.), "Food Gel", Elsevier Science Publishers, Ltd., London, England, UK, 79–119

[25] Verma, B.C. 1977. "An improved tubidimetric procedure for the determination of sulphate in plants and soils", Talanta, 24: 49–50

[26] Yaphe, W. and Arsenault, G. P. 1965. "Improved resorcinol reagent for the determination of fructose, and 3,6-anhydrogalactose in polysaccharide," Analytical Biochemistry,13: 143–148

[27] Yu, Q., Yan, J., Wang, S., Ji, L., Ding, K., Vella, C., Wang, Z. and Hu, Z. 2012. Antiangiogenic effects of GFP08, an agaran-type polysaccharide isolated from *Grateloupia filicina* . Glycobiology, 22:1343–1352

[28] Zablackis, E. and Perez, J. 1990. "A partially pyruvated carrageenan from Hawaiian *Grateloupia filicina* (Cryptonemiales, Rhodophyta)", Botanica Marina, 33: 273–276

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