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Biochemical Composition of Seaweeds along the Southwest Coast of India

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Seaweeds have been utilized traditionally for food, fertilizer, and various industrial applications. In recent years, there has been growing interest in their potential as a source of bioactive compounds with applications in the food, pharmaceutical, and cosmetic industries. Additionally, seaweeds are being explored for their environmental benefits, such as their role in carbon sequestration and wastewater remediation. In the present study, three biochemical components: Protein, Carbohydrate, and Lipids were tested in five seaweed species (*Grateloupia lithophila*, *Gelidium pusillum*, *Ulva flexuosa*, *Gracilaria corticata*, and *Ceratodictyon variabile*) belonging to Phylum-Rhodophyta and Chlorophyta. It was found that the Carbohydrate content was higher in species belonging to Phylum- Rhodophyta, ranging from 63.6 mg/g to 376.4 mg/g, and lower in Species belonging to Phylum-Chlorophyta with a value of 56.68 mg/g. The protein content showed a similar pattern of data, with Protein content higher in species belonging to Phylum-Rhodophyta and lower in Species belonging to Phylum-Chlorophyta. The protein content ranged from 28.05 mg/g to 58.32 mg/g. whereas, the opposite was seen in the case of lipids. Lipid content was recorded to be higher in Chlorophyta and lower in Rhodophyta, with values ranging from, 5.1 mg/g to 62.6 mg/g.

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1. INTRODUCTION

"Seaweeds refer to any large marine benthic algae that are multicellular, macrothallic, and thus differentiated from most algae that are microscopic. These plants form an important renewable resource in the marine environment and have been a part of human civilization from time immemorial" [1]. "Marine algae contain more than 60 trace elements in a concentration much higher than in terrestrial plants" [2]. "The marine algae and the isolated seaweed have the possible benefit to both health and improve food acceptability and also offer exciting potential as a constituent in the expansion of the latest foodstuff products" [3]. "Marine algae are one of the largest producers of biomass in the marine environment" 3-6" [4].

"Seaweeds are reservoirs of many Bioactive Compounds with immense medicinal potential which have attracted the attention of pharmaceutical industries. They usually grow in all seas except in the Polar Regions and produce biologically active compounds" [5]. "Seaweeds have been included for a long time in the Traditional diet of East Asian countries such as Japan, Korea and China; more recently, their presence in all forms in the diet of Western countries has been progressively increasing" [6]. "Many reports have been stated regarding the Anti-microbial nature of Seaweeds. Every species of seaweed is found to be beneficial in some or another way [7-10]. Still very little is known about the seaweed and its characteristics as a whole. It's a colossal world yet to be explored. Since ancient times, macroscopic marine algae has been closely associated with human life and has been exhaustively used in numerous ways as a source of food, feed, fertilizer, and medicine, and chiefly used for economically important phycocolloids" [11,12,13]. "The phenolic compounds also contribute to ascertaining the nutritional value and quality of the respective foods in terms of renovating the taste, color, flavor, and aroma and thereby promoting health-beneficial effects" [14]. "Seaweeds are also an excellent source of both soluble and insoluble dietary fibre. Now-a-days sea weeds have been widely accepted by the people of coastal region throughout the world due to their important sources of nutrients" [15]. "Among red algae, the genus Gracilaria contains a broad diversity of valuable contents for human nutrition and are one of the world's most cultivated and valuable marine seaweed" [16]. "The genus Gracilaria and Hypnea (Rhodophyta) befalls naturally in tropical and subtropical coastal areas of the world and it has been regularly considered as one of the best auspicious candidates as an alternative source of nutrients for aqua feeds, mostly herbivorous gastropods" [17,18,19]. "Thus, they have a great potential as potential food supplements and may be used in the food industry as a source of ingredients with an appreciable amount of nutritional value" [20-23]. "Phytochemicals are responsible for the medicinal activity of plants. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties" (www.iomcworld.org, As on- 18-12-2023). "So, phytochemical analysis of the seaweeds will be a good preliminary approach to reveal their secondary metabolite constituents and the resultant medicinal values" [24]. Not much work has been done on the selected sites. Few sites are still untouched from the Biochemical point of view. The present study is an attempt to cover the information gap present till now. To the best of Knowledge it is the Pioneer work on Elathur, Kappad and Beypore.

2. MATERIALS AND METHODS

2.1 Sample Collection

Seaweed samples were collected from the coastline of Beypore, Kappad, Elathur, and Thikkodi from the Northern coast of Kerala during 2021-2022, encompassing all four seasons: Southwest Monsoon, Northeast Monsoon, Post monsoon, and summer. Samples were collected based on the abundance and availability during the season. The samples from all the seasons of the species found abundant throughout all seasons were considered and processed further. Samples were washed thoroughly in the habitat water to remove debris like, sand particles, associated organisms, epiphytes, and other impurities. Samples were kept in labelled zip lock covers and brought to the laboratory. Seaweeds were further washed properly under tap water to remove the saltwater content and other remaining debris from the surface of seaweeds. They were dried in the shade for one week. The dried seaweeds were then pulverized in a sample grinder and kept in zip-lock covers for further analysis. The seaweeds were identified using various taxonomic identification manuals. (Refer to Table 1).

Table 1. Taxonomic details of seaweed species taken for Biochemical Analysis.

Name of the species	Author	Phylum	Oder	Class	Family
Grateloupia lithophila	Børgesen, 1938	Rhodophyta	Halymeniales	Florideophyceae	Grateloupiaceae
Gelidium pusillum	(Stackhouse) Le Jolis, 1863	Rhodophyta	Gelidiales	Florideophyceae	Gelidiaceae
Ulva flexuosa	*Wulfen, 1803*	Chlorophyta	Ulvales	Ulvophyceae	Ulvaceae
Gracilaria corticata	*(J.Agardh) J.Agardh, 1852*	Rhodophyta	Gracilariales	Florideophyceae	Gracilariaceae
Ceratodictyon variabile	*(J.Agardh) R.E.Norris, 1987*	Rhodophyta	Rhodymeniales	Florideophyceae	Lomentariaceae

2.2 Carbohydrate Estimation

The presence of carbohydrates in the collected seaweeds was tested using the standard Anthrone method [25]. 250 mg of powdered seaweed was taken in a boiling tube, to which 5 ml of 2.5 N HCL was added and the mouth of the tubes was covered with glass stoppers and hydrolysed for 3 hours in a boiling water bath. After 3 hours, the boiling tubes were taken out of the water bath and allowed to cool down completely at room temperature. Once cooled, amorphous Sodium carbonate (Na_2CO_3) was added little by little to the respective boiling tube until the effervescence ceased. To this Distilled water (DW), was added and the volume was made up to 100ml, this one was then centrifuged for 20 minutes at 5000 rpm. The supernatant was then carefully collected and transferred to 100 Tarsons sterile storage vials for further use. 1 ml of sample was taken from this in a boiling tube, to this 4 ml of Anthrone agent was added and vortexed for 3 minutes and allowed to cool down. Boiling tubes were then covered with caps and incubated in a Boling water bath for 10 minutes. After 10 minutes they were taken out and cooled rapidly by keeping them in the icebox and the absorbance of the Green to dark green solution was measured at 620nm against a blank Anthrone Reagent using UV-VIS Spectrophotometer, precisely Aligent technologies Cary series 100 UV-Vis Spectrophotometer. The D-glucose solution was made by mixing 100mg Dextrose in 100 ml DW, this was considered as a stock solution. 10ml of stock solution was taken and to that 100 ml DW was added, this was considered as the working standard Solution. 9 different concentrations (50µL, 100 µL, 200 µL, 300 µL, 400 µL, 500 µL, 600 µL, 700 µL, 800 µL, 900 µL, and 1000 µL) of Working standard solution of D-glucose was taken in 9 different test tubes, to this 4 ml of Anthrone Reagent was added and the earlier procedure was repeated. The absorbance of all the 9 concentrations was measured at 620nm. Standard graphs were plotted for both the sample and Glucose solution with concentrations on the X-axis and Absorbance on the Y-axis. Carbohydrate was calculated by these calibration curves.

2.3 Protein Estimation

Protein content from the collected seaweeds was tested using the Lowry method of Protein estimation [26]. 2 grams of seaweed powder was taken and homogenized in a motor pestle by adding 3 ml of DW to it. The homogenate was taken in vials and centrifuged at 3000rpm for 15 minutes. The supernatant was collected carefully in 5 ml vials. 1 ml of the aliquots of all samples were taken in different test tubes, to this 4.5 ml of Reagent I was added, which was comprised of 48 ml of 2% Na_2CO_3 in 0.1 N NaOH, 1ml of NaK Tartarate in DW, 1ml 0.5% $CuSO_4$ $5H_2O$ in DW and was incubated at room temperature in a dark chamber for 10 minutes. After 10 minutes 0.5 ml of Folin's Reagent (1:1 ratio with DW) was added to each test tube and incubated again at room temperature for 30 minutes. After 30 minutes, samples turned blue, showing the presence of proteins, Absorbance was measured at 720nm. Likewise, different concentrations (0.1 ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml, 0.7ml, 0.8ml, 0.9ml, 1 ml) of standard BSA solution (1mg/1ml DW) was taken in different test tubes and made up to 1 ml by adding DW to each of them. 1 ml of DW was taken in a test tube, which was considered as blank. To all these test tubes 1 ml of Reagent 1 was added and incubated for 10 minutes, and the earlier method was followed. Absorbance was measured for all the concentrations, and graphs were plotted separately for sample and BSA with concentrations on the X-axis and Absorbance in Y-axis. Protein was calculated using the standard curve.

2.4 Lipid Estimation

Lipids were estimated by following Bligh and Dyer's method [27]. 10 grams of powdered seaweed was taken and homogenized with 10ml of chloroform and 20ml of methanol. To this mixture, 10 ml of chloroform was added and homogenized for another 30 seconds. To this 10 ml of DW was added and homogenized for another 30 seconds. The homogenate was filtered using Whatman's 42 gauze ashless 110nm Diameter filter paper with the help of a vacuum pump to get maximum filtrate. The filtrate was transferred to a 100 ml graduated cylinder and was allowed to sit for some time, for the Chloroform and Methanol layer to separate. The top methanol layer was pipetted and discarded, and a little bit of chloroform layer was pipetted out to ensure no methanol remained. The purified lipids are present in this Chloroform Layer. This chloroform was then taken in respective pre-weighed Petri dishes and kept in a Hot air Oven for 1 hour at 60°C for the evaporation of chloroform. After 1 hour the Petri dishes were kept in desiccator for 20 minutes to ensure no Chloroform remains. The petri dishes were left with pure lipids. The weight of Petri dishes was measured and the lipid content was estimated.

3. RESULTS AND DISCUSSION

3.1 Protein Content

A discrepancy in values was observed during the quantitative screening of protein content in five aforementioned (Table 2.) seaweeds. The protein content ranged from 28 mg/g to 58.32 mg/g. The lowest protein content was recorded in *Ulva flexuosa* belonging to Phylum-Chlorophyta. The protein content of green seaweed *Ulva rigida* collected from the coastal region of Chilka Lake of India is 6.64% [28]. In addition, the highest values were recorded in *Grateloupia lithophila* with 58.32 mg/g, which belongs to Phylum-Rhodophyta (Refer to Fig. 1). The rest 3 species, which showed higher values, belonged to the same Phylum-Rhodophyta, with *Gelidium pusillum* with a protein content of 35.04 mg/g, *Gracilaria corticata*- 38.09 mg/g, *Ceratodictyon variabile* - 33.56 mg/g (Refer to Table 2).

3.2 Carbohydrate Content

Among all Biochemical components, carbohydrates showed 4-5 times the highest values. Among which species belonging to Phylum-Rhodophyta showed maximum carbohydrate content (Refer to Fig. 1). The carbohydrate content ranged from 56.68 mg/g to 376.4 mg/g, among both the Phylum- Rhodophyta and Chlorophta. The highest values were recorded in *Gelidium pusillum* with 376.4 mg/g of carbohydrate, followed by it was *Gracilaria corticata* with 216.95 mg/g, *Ceratodictyon variabile* with 146.61 mg/g, *Grateloupia lithophila* with 63.6 mg/g of carbohydrate, and the lowest carbohydrate content was recorded in *Ulva flexuosa* with 56.68 mg/g (Refer Table 2.).

3.3 Lipid Content

Lipid content was the lowest among all 3 Biochemical components. The values ranged from 5.1 mg/g to 62.6 mg/g. The highest Lipid content was recorded in the case of *Ulva flexuosa* at 62.6 mg/g, followed by it was *Gracilaria corticata* with 57.1 mg/g, *Gelidium pusillum* at 23.4 mg/g, *Grateloupia lithophila* with 11.9 mg/g, and the lowest lipid content was recorded in case of *Ceratodictyon variabile* with 5.1 mg/g. (Refer to Table 2. & Fig. 1.). In case of Lipid content in the Green macroalgae, *Ulva flexuosa*, belonging to Phylum- Chlorophyta showed highest values, which was not the same in case of Carbohydrate and Protein content.

Table 2. Representing Biochemical contents of different seaweed species

Sl no.	Species	Carbohydrate (mg/g)	Protein(mg/g)	Lipids(mg/g)
1	*Grateloupia lithophila*	63.6	58.32	11.9
2	*Gelidium pusillum*	376.4	35.04	23.4
3	*Ulva flexuosa*	56.68	28.05	62.6
4	*Gracilaria corticata*	216.95	38.09	57.1
5	*Gelidiopsis variabilis*	146.61	33.56	5.1

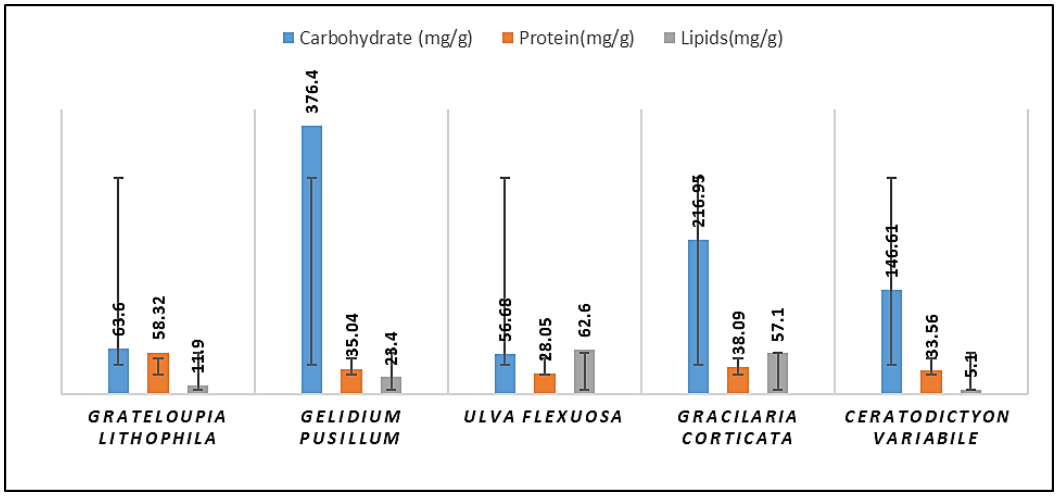

Fig. 1. Shows variations in biochemical contents among different seaweed species

4. DISCUSSION

Chlorophycean members contain high carbohydrate content than Rhodophycean and Phaeophycean members [29,30], (Kumar et al., 2014). The current study shows contrasting values.

In the current study on the Biochemical analysis of seaweeds of the Northern Kerala coast, it was found that Carbohydrate content was highest compared to Protein and Lipid content in seaweeds taken for the study. Whereas, Lipid was found to be the lowest among all three Biochemical components. It was also observed that Carbohydrate and Protein content was higher in Red seaweeds and lower in Green seaweeds. Whereas, the opposite was seen in the case of Lipid content. Lipid was found to be higher in green seaweed and lower in all the species of red seaweeds. The observation that Carbohydrate content was higher in Red seaweeds, coincided with the work done by Roy and Anantharaman [31], Dhargalkar et al. [3]. But it did not coincide with the data of Chakraborthy and Santra [32], Kaliaperumal et al. [33], according to them the Carbohydrate content was more in green seaweeds and less in Red seaweeds. The seasonal study done by K.Murugaiyan, 2020 [34] on macroalgae also stated that the percentage of Carbohydrate content observed maximum in *Gracilaria verrucosa* (72.25±3.15) summer season and minimum in *Turbinaria ornata* (4.50±0.12% of DW) during the summer season. The high content of carbohydrates in red algae might be due to higher phycocolloid content in their cell walls [3]. The protein content was also found to be higher in the case of Red seaweeds and lower in Green seaweeds. Selvi et al. [35] Renaud et al. [36], Kumar V. [37], Manivannan et al. 2008, reported the same in the case of *Hypnea valentiae,* which is a Red seaweed. which showed that Red seaweeds have higher protein content than green seaweeds. Protein content varied among different genera and also in different species of the same genus [3]. It is largely attributed to the surrounding water quality as reported by Dave and Parekh [38]. Lipid content in the present study was found to be low in Red seaweeds and higher in Green seaweeds. This coincided with the work done by Anantharaman P. et al. [30], Chakraborthy and Santra [32], Verma et al. [39], and Muthuraman and Ranganathan [40]. In general, seaweeds exhibit low lipid contents [38 ,41].

5. CONCLUSION

From the present study, it can be concluded that seaweeds are a tremendous source of proteins and carbohydrates. It is a very good source of essential vitamins and minerals and has high nutritional value. As it is high in its nutritional value it can play an important role in the diet of human beings and other organisms. It can also provide a wide array of options in the field of Food and Pharmaceutical sectors. More detailed work needs to be done in the area connected to the Food, Pharmaceutical, and Agricultural sectors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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