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Effect of environmental factors and biological parameters of *gracilaria edulis* and *kappaphycus alvarezii* from Rameshwaram Mandapam Coast, India

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Abstract

Seaweeds plays a major role in marine living organisms. They are marine macroscopic forms. The two species of red algae namely K. alvarezii and G. edulis was collected from Mandapam coast, Rameswaram. The present study was carried out to investigate the environmental parameters such as pH, nitrate and phosphate and its biochemical parameters namely carbohydrates, proteins and lipids from Gracilaria edulis and Kappaphycus alvarezii. The results revealed under environmental parameters chlorophyll showed the highest peak level compared to carbohydrates, proteins and lipids. In biochemical parameters Carbohydrate, protein and lipid content recorded the maximum peak value of 1.47 at 20th day. Protein and lipid content attains the highest peak value at 10th and 15th day with 1.88 and 1.69 respectively.

Keywords: K. alvarezii, G. edulis, nitrate, phosphate, environment.

Introduction

Seaweeds are sunlight dependent, aquatic plants which belongs to the kingdom, Plantae (Green Red and Brown algae). According to their pigmentation they can be divided into three major groups Green Red and Brown. The Green algae includes phylum (Chlorophyta) pigmentation to that of terrestrial plants. (Chlorophyll a,b and Carotenoids). Red algae includes photosynthetic pigment chlorophyll a phycobilins, R-Phycocyanin, R- Phycoerthrin, Carotenoids, β–carotene, lutein and zeaxanthin. Brown algae pigments include Chlorophyll a, c and as well as carotenoids were dominated by fucoxanthin[1]. The level of pH, Salinity, Phosphate, and Nitrate changes are the major part of marine environment. In benthic algae an optimum alkaline pH ranged from 8.2–9.0[2]. In some organisms the optimum pH level is known depending on the substrate concentration.

Enzymatic activities showed very low concentration under low salinity and increasing salinity increases up to 45-50%. The effect of salinity includes variation of osmotic compounds its concentration chemistry and its affinity with the part it is exposed. Phosphate plays a major role in development of nucleic acids, proteins and lipids. Energy gets mainly transferred mainly by ATP and through photosynthesis and respiration by the presence of high energy compounds[3]. In marine ecosystem nitrate acts as a primary limiting nutrient for the algal growth[4]. They are rich in protein and serves as human food in countries all over the world. Based on their geographical location, season, humidity and temperature the nutrient content differs from one species to other. In certain species proteins, lipids, minerals and vitamins were present in significant amounts[5].

Materials and methods

Collection and authentication of sample: *Kappaphycus alverizii* and *Gracilaria edulis* were collected from Rameshwaram coast, Tamilnadu. The samples were fixed in herbarium sheats. Algal material were washed well with tap water dried under shade till they dry at room temperature and were made into fine powder and kept in a air tight container and stored. (The samples were authenticated from BSI (Botanical Survey of India, Coimbatore).

CHU 13 medium[6]: The seaweeds collected were filtered and sterilized by autoclaving at 121°C for 15 minutes and the sterilized seawater was further used for medium preparation. Modified CHU 13 medium in one litre of seawater.

Preparation of algal extract: Ten gram of dried algal powder of *Kappaphycus alverizii* and *Gracilaria edulis* was extracted separately with 50mL of ethanol for 24 hours in 3 days. At overnight room temperature the algal samples were soaked and filtered through buchmann funnel. Under rotary evaporator at 40ºC the filtered solution was evaporated and weighed and dissolved in ethanol. It was stored under refrigerator for further use.

Biochemical Studies: Estimation of carbohydrate: Estimation of carbohydrate content was done according to DuBois M. et. al.[7]. 5% of 1mL phenol solution followed by 5mL of

concentrated sulphuric acid was added. It was placed under dark room for 30 min. Absorbance was taken at 490nm.

Estimation of protein: According to Lowry O. H. et al.[8] the protein level was studied. 0.5g of algal biomass was extracted and centrifuged. To the supernatant, 5mL of solution was added to 1mL of sample followed by 0.5mL of Folin phenol reagent was added. Absorbance was read at 650 nm.

Estimation of lipid[9]: 0.5g of fresh thallus was weighed ground well to that 1mL of the sample were homogenized. To the separating funnel the algal extract with 6mL chloroform and 2:1 methanol was transferred and 0.9% of 2mL Nacl was added and thoroughly mixed. From the chloroform phase the mixture was left undisturbed for 12 hrs. In a clean vial 50mL solvent was collected and allowed to evaporate at room temperature. After evaporation the pellet was collected to that 0.5mL of concentrated H_2SO_4 was added and mixed well. The samples were tightly closed and placed in boiling water bath for 10 min and further allowed to cool at room temperature. From 0.2mL of sample 5mL of vanillin reagent was added and mixed well and allowed to stand for 30 minutes. Absorbance was read at 520 nm.

Estimation of chlorophyll pigment: 0.5g algal tissue were kept in mortar and pestle. To that 10mL of 80% acetone was added and macerated. At 3000rpm for 15min the fragmented cell tissues were centrifuged and supernatant was stored. Repeated washing of pellet was done and 80% of 5mL acetone showed no colour.

Results and discussion

Collection of seaweeds: The seaweeds such as *Kappaphycus alvarezii* (*Doty*) *ex silva* and *Gracilaria edulis P.C. Silva* was collected from Rameswaram Mandapam coast Tamilnadu, India. The collected seaweeds were washed to remove the debris and identified from morphological details of the seaweeds using standards books.

Effect of environmental parameters such as pH, nitrate, phosphate and salinity and its biochemical estimation: Seaweeds have enormous resources of primary and secondary metabolites. As India is a rich in seaweeds which are exported as raw materials. The main reason is their potentials are not fully exploited. The present study various parameters such as pH, salinity, nitrate and phosphate.

pH is an important parameter owing to its relationship with various chemical characteristics and biochemical reactions. High primary productivity, respiration and mineralisation leads to the changes in pH. Increase in pH may also be due to utilization of carbon dioxide from carbonates by algae and higherplants[10]. As the absorption of metal increases the pH also increases from 0–100%[11]. According to Benjamin M.M. et al.[11], within tidal reaches, pH seems to be directly related to salinity. The greatest production for macro algae was found in pH values of 8.0-8.2.

In the present study pH level from 2-10 was carried out from carbohydrate, protein, and lipid. In *K. alvarezii* pH 8 the maximum carbohydrate content was reached with 0.42µg/mL. Protein and lipid content showed the maximum at pH 6 as 0.98µg/mL and 1.13µg/mL respectively. In *G.edulis* at pH 2 carbohydrate attained the maximum peak with 1.55 µg/mL followed by protein and lipid as 1.32µg/mL and 0.83µg/mL at pH 4 and 6 respectively (Figure-1,2).

Nitrate is the thermodynamically stable and most oxidized form of inorganic nitrogen and played a significant role in the nitrogen cycle. It is an important nutrient source for phytoplankton. The importance of nitrate lied in its ability to regulate primary production as a new nitrogen source for primary producers. Nyarko A.A. et al.[12] reported a maximum of 7.80 mM of nitrate in Australian Great Barrier Reefs after the cyclone events occurred. Nitrate profiles in coastal marine sediments were studied by Eppley, R.W. et al.[13] that the vertical distribution of nitrate depends on the availability of oxygen.

In the present study *K. alvarezii* carbohydrate content showed the highest peak level with 59.8µg/mL at 400g/L concentration followed by protein with 63.7µg/mL at 300g/L concentration and lipid content with 55.6µg/mL at 300g/L concentration. Chlorophyll content was noted to be highest at 500g/L concentration with 0.156 respectively. In *G. edulis* the carbohydrate content was found to be 66.1µg/mL at 100g/L concentration followed by protein with 68.9µg/mL at 400g/L concentration. Lipid content was found to be high with 300g/L concentration with 43.7µg/mL and chlorophyll content was found to be highest with 0.163 at 500g/L concentration. Compared with two algal species *K. alvarezii* was found to be highest in carbohydrate, protein and lipid content (Figure-3,4).

The fluctuation in phosphate changes the fresh weight and biochemistry of seaweeds. Henriksen K. et al.[14] showed that the fluctuations were erratic and the release of phosphate and its utilization occurred at short intervals of time. Regeneration of phosphate in the shallow mud bottoms was suggested by Jayaraman R. et al.[15]. Cooper L.H.N.[16] suggested release of phosphate in the interstitial sediments in considerable amounts is through autolysis of bacteria. In the present study, variation in the phosphate concentration *K. alvarezii* carbohydrate content was noted to be high at 45.5µg/mL with 40g/L concentration. Protein content was noted to be high at 48.6µg/mL with 80g/L concentration. At 60g/L concentration lipid content was recorded with 31.1µg/mL and at 80g/L concentration the chlorophyll content was found to be 0.146µg/mL. In *G. edulis* carbohydrate content was noted to be high at 40g/L concentration at 46.9µg/mL. At 80g/L concentration protein and chlorophyll content were recorded to be 48.6µg/mL and 0.176µg/mL. Lipid content recorded 31.1 at 60g/L concentration (Figure 5-6).

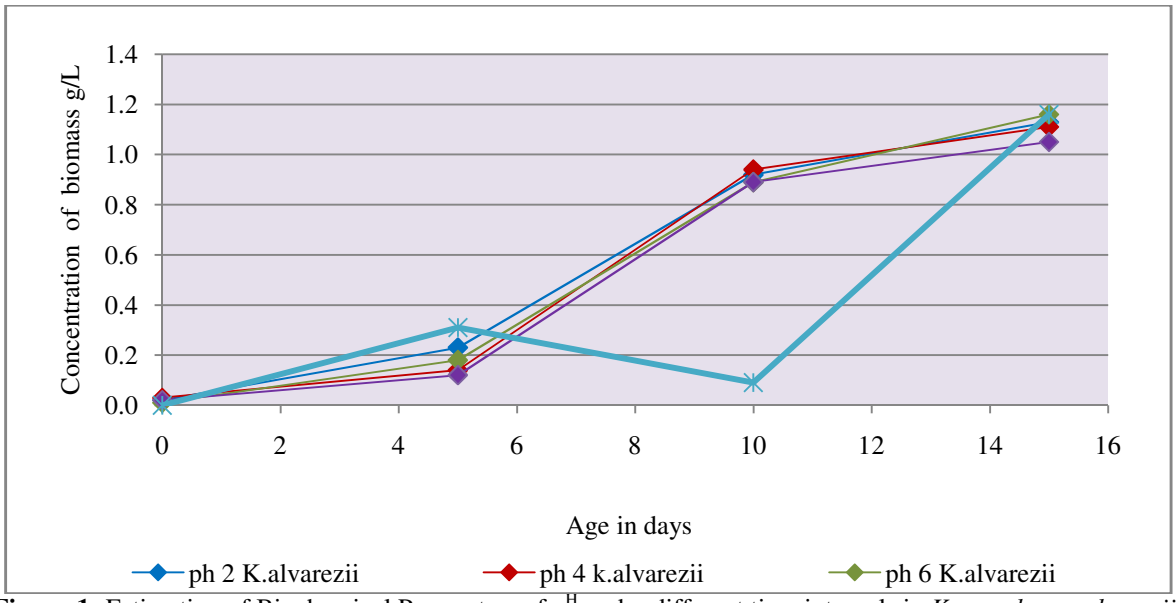

Figure-1: Estimation of Biochemical Parameters of p^H under different time intervals in *Kappaphycus alvarezii.*

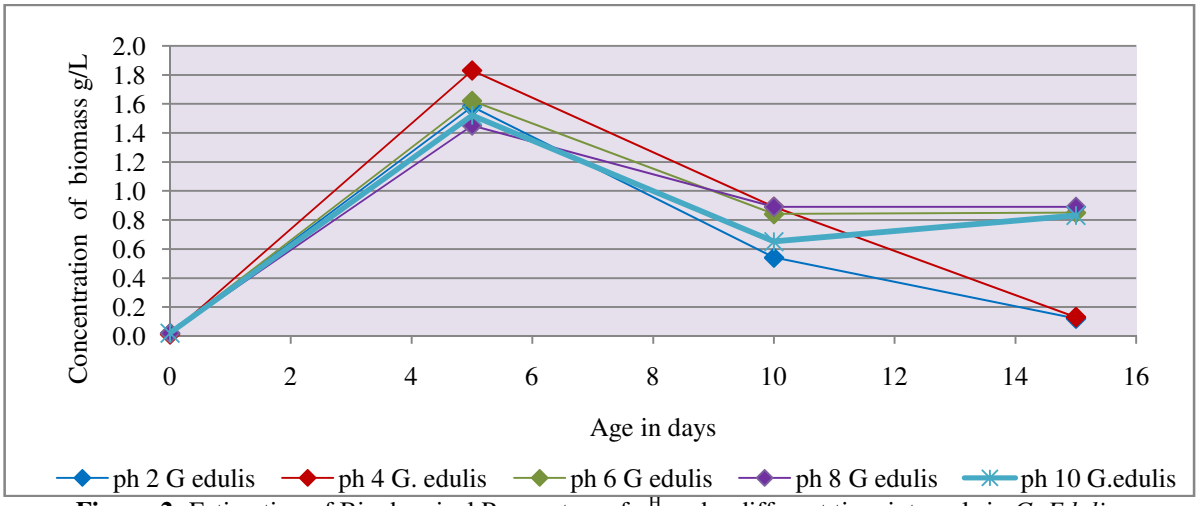

Figure-2: Estimation of Biochemical Parameters of p^H under different time intervals in *G. Edulis.*

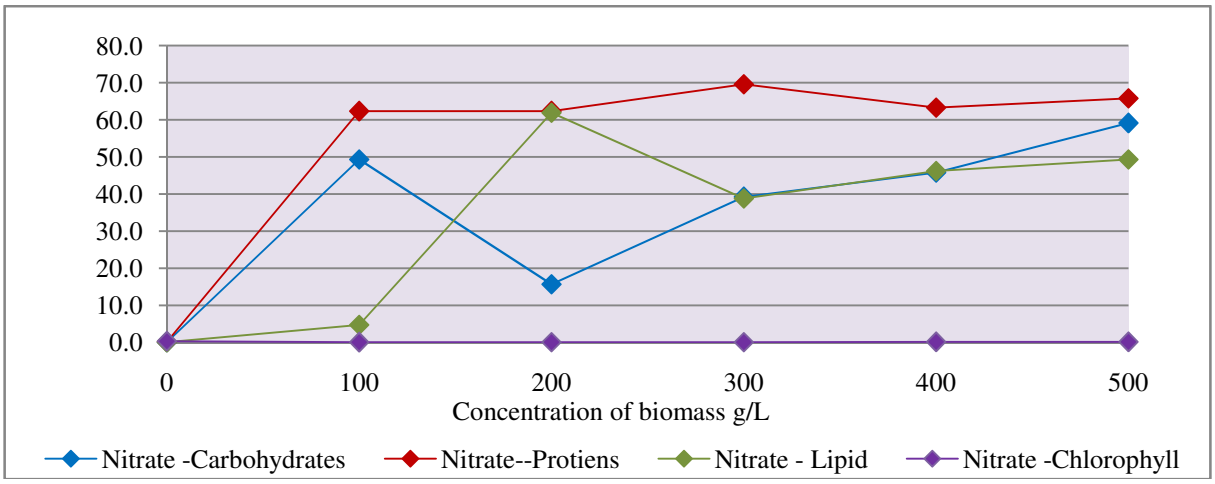

Figure-3: Estimation of Biochemical Parameters of Nitrate under different time intervals in *Kappaphycus alvarezii.*

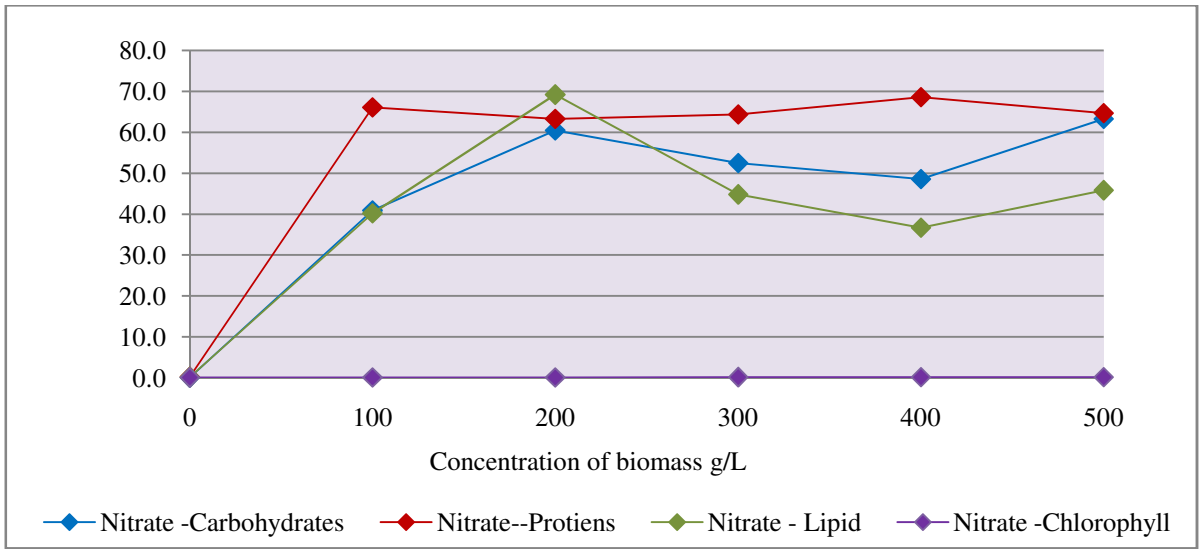

Figure-4: Estimation of Biochemical Parameters of Nitrate under different time intervals in *G. edulis*

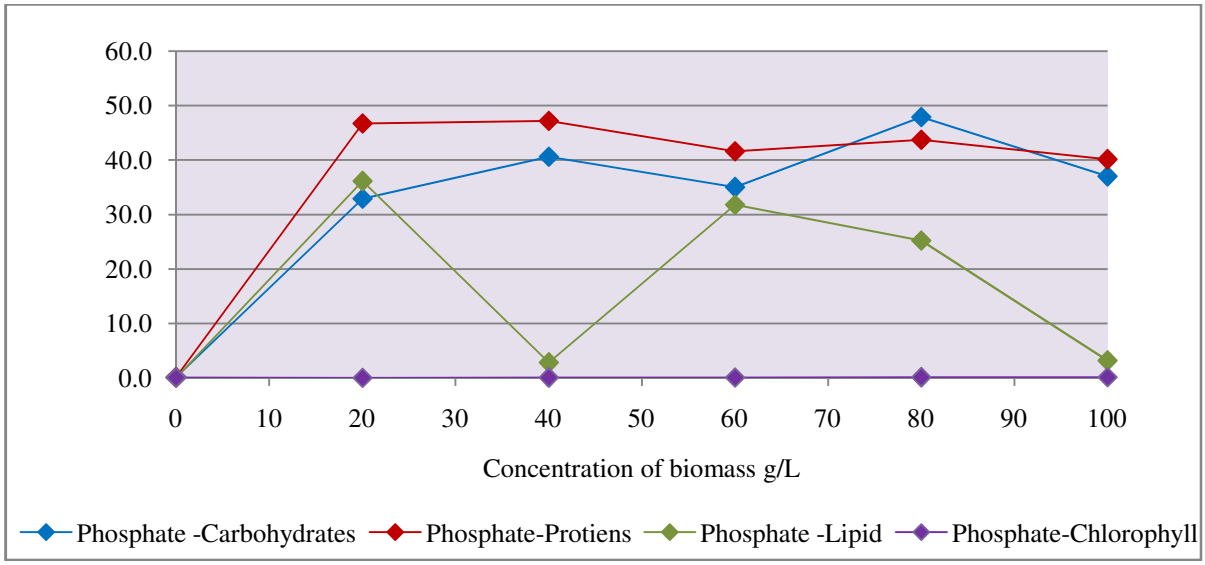

Figure-5: Estimation of Biochemical Parameters of phosphate under different time intervals in *Kappaphycus alvarezii.*

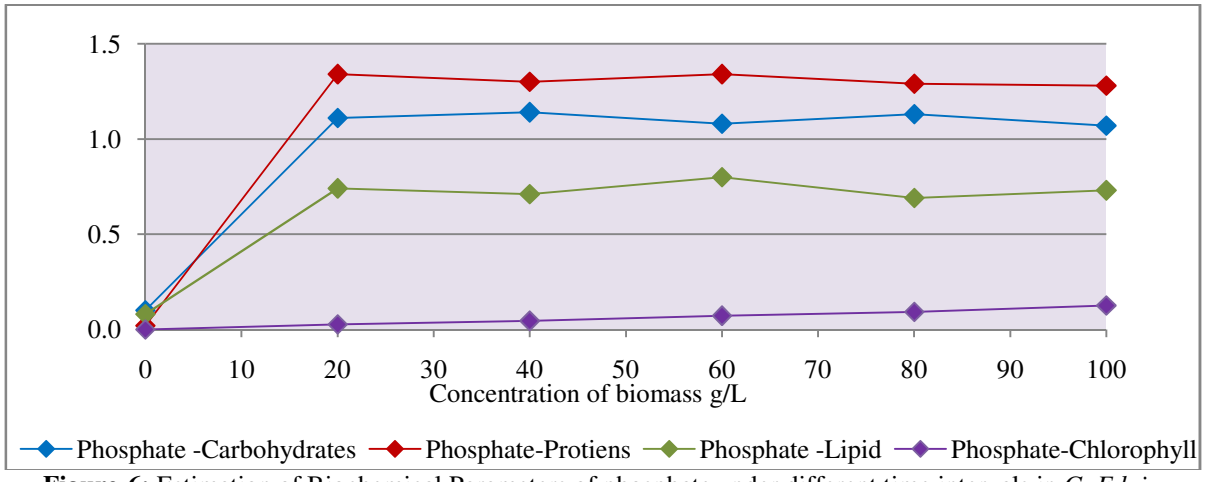

Figure-6: Estimation of Biochemical Parameters of phosphate under different time intervals in *G. Eduis.*

For human and animal consumption certain aminoacids, fatty acids, minerals and vitamins are very essential[17-19]. Proteins, lipids, carbohydrates / polysaccharides, minerals, amino acids and vitamins were found to be in significant amounts[20-27].

The most important component needed for carbohydrate metabolism is respiration. In different seasons the different seaweeds change was noted in carbohydrate content[28,29] analyzed the seaweeds from Maharashtra coast and Kovalam coast showed similar results.

Variable level of carbohydrate were recorded during July in *U. reticulate* (50.24% of DW) according to Sobha V. et al.[30], Norziah M.H[20] recorded the *Gracilariasp* with 48.4% of reported[31] that *Enteromorpha* sp showed 54.71% of dry weight (DW).

The possibility of new developed food are the major use of seaweeds and they are rich in human nutrition. Among Rhodophyceaen members the highest amount was noted in *Acanthophora spicifera* (85.14±0.49mg/g DW) and lowest in *Corynomorpha prismatica* (2.11± 0.77mg/g DW). The observed difference in protein content among species is in corroboration with earlier reports[28]. In *Porphyra tenera* the protein levels were found to be (47% of dry mass) and in *Palmaria palmate* with (35% of dry mass)[32,33] protein levels were found to be greater in these algae. The nature of cellular matrix containing protein can sometimes be entrapped depending on the availability of seaweed. Depending on seasonal variation protein content varies[34].

During oxidation process more amount of energy is produced than other biological compounds. They serves as storage material for living organisms. Marine algae produce a variety of glycerides or lipids having biocidal and antimicrobial properties.

According to Fleurence J.[35] the maximum lipid level was recorded in *Kappaphycus alvarezii* with 34.4mg/g DW[36,37] Crude lipid content in all three-colour forms ranged between 0.66±0.01 to 0.72±0%. Although this content was relatively low to that recorded in other edible red seaweeds like *Ahnfeltiopsis concinna, Asparagopsis taxiformis, Chondrus ocellatus, Eucheuma denticulatum, Gracilaia spp*, *Halymenia formosa, Laurencia spp* and *Porphyra vietnamensis*.

In the present study, *K. alvarezii* carbohydrate content was noted to be highest at 37.1µg/mL at 26.25g/L concentration. The protein content was found to be highest with 20.3µg/mL at 8.75g/L concentration. At 3.5g/L concentration the lipid and chlorophyll content was found to be highest with 10.8µg/L and 0.146µg/mL. In *G. edulis* at 26.25g/L concentration carbohydrate, protein content was found to be high at 37.8µg/mL, 25.5µg/mL and lipid content 19.9µg/mL was highest in 3.5g/L. At 26.25g/L concentration the chlorophyll content was found to be 0.153µg/mL (Table-1).

Table-1: Biochemical estimation of *Kappaphycus alvarezii* and *Gracilaria edulis.*

Biochemical parameter	Concentration of biomass (µg/mL)	Age in days				
Kappaphycus alvarezii		5	10	15	20	25
Chlorophyll a	Control	0.054± 0.021	0.071±0.047	0.088±0.023	0.108±0.035	0.131±0.012
	1.0	0.045±0.056	0.066±0.061	0.082±0.062	0.093±0.058	0.117±0.020
Carotenoids	Control	0.041±0.013	0.058±0.064	0.071±0.025	0.083±0.058	0.111±0.028
	1.0	0.034±0.26	0.047±0.071	0.069±0.046	0.081±0.071	0.104±0.016
Total chlorophyll	Control	0.311±0.061	0.354±0.121	0.377±0.128	0.392±0.134	0.412±0.101
	1.0	0.309±0.034	0.338±0.080	0.368±0.411	0.384±0.037	0.406±0.110
Carbohydrates	Control	0.81±0.022	0.52±0.037	0.65±0.207	0.72±0.016	1.08±0.025
	1.0	37.1±0.006	0.35±0.041	0.61±0.059	1.47±0.012	1.39±0.037
Protein	Control	0.44±0.053	1.90±0.066	0.72±0.023	1.86±0.042	1.32±0.028
	1.0	0.38±0.013	20.3±0.067	0.61±0.008	1.74±0.002	1.18±0.033
Lipids	Control	1.19±0.007	1.15±0.006	1.74±0.061	1.66±0.083	1.28±0.042
	1.0	1.11±0.083	1.02±0.027	10.8±0.89	1.43±0.044	1.11±0.042

Gracilaria edulis						
Chlorophyll a	Control	0.054± 0.021	0.051±0.047	0.057±0.023	0.177±0.035	1.393±0.012
	1.0	0.040±0.056	0.031±0.061	0.034±0.062	1.583±0.058	1.381±0.020
Carotenoids	Control	0.041±0.013	0.058±0.064	0.071±0.025	0.083±0.058	0.111±0.028
	1.0	0.034±0.26	0.047±0.071	0.069±0.046	0.081±0.071	0.104±0.016
Total chlorophyll	Control	0.311±0.061	0.354±0.121	0.377±0.128	0.392±0.134	0.412±0.101
	1.0	0.309±0.034	0.338±0.080	0.368±0.411	0.384±0.037	0.406±0.110
Carbohydrates	Control	0.054± 0.021	0.051±0.047	0.057±0.023	0.177±0.035	1.393±0.012
	1.0	0.040±0.056	0.031±0.061	0.034±0.062	1.583±0.058	1.381±0.020
Protein	Control	0.69±0.053	1.97±0.066	0.72±0.023	1.86±0.042	1.32±0.028
	1.0	0.62±0.013	1.91±0.067	0.64±0.008	1.54±0.002	1.15±0.033
Lipids	Control	1.98±0.007	1.15±0.006	1.74±0.061	1.66±0.083	1.28±0.042
	1.0	1.98±0.083	0.82±0.027	1.73±0.89	1.34±0.044	1.08±0.042

Conclusion

To conclude, this study indicates that seaweeds such as G. edulis and K. alvarezii are promising but acts as a source of nutraceutical values like carbohydrates, proteins and lipids. It can be utilized not only as a source of supplements but also acts as bioactive compounds.

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