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Nutrient composition of marine benthic algae found in the Gulf of Kutch coastline, Gujarat, India

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Abstract

Fifteen species of marine benthic algae that belong to the phylum Rhodophyta, Phaeophyta and chlorophyta were collected in Sikka and Vadinar, Gulf of Kutch, India and investigated concerning their biochemical (total carbohydrate, soluble proteins, total lipid, reducing sugar, amino acid and vitamin C) and pigment composition (chlorophyll a, b and carotenoids). The total carbohydrate content (% dry weight) ranged from 8.6 ± 0.7% to 42.4 ± 0.7%; soluble proteins from 4.3 ± 0.4% to 32.4 ± 2.5%, lipid content from 0.9 ± 0.3% to 5.2 ± 0.4%, reducing sugar 1.9 ± 0.3 to 8.6 ± 0.1 mgg^{-1}, amino acid 26.5 ± 1.9 to 152.3 ± 4.0 mgg^{-1}, Vitamin C from 0.2 ± 0.03 to 0.4 ± 0.02 mgg^{-1}. Chlorophyll a content ranged between 0.62 ± 0.03 to 2.5 mgg^{-1}, chlorophyll b from 0.24 ± 0.03 to 1.43 ± 0.12 mgg^{-1} and carotenoids from 15.0 ± 0.35 to 25.2 ± 1.42 μgg^{-1}. The biochemical and micronutrient values of the studied algae showed their potential of being source of ingredients with high nutritional values and utilization for food and pharmaceutical industries.

Key words: Marine benthic algae, biochemical composition, pigments

Introduction

There are about 8,000 species of marine macro algae along the world's coast line and they may extend as deep as 270m (Luning, 1990). A total of 25 species of green sea weeds, 90 species of brown and 350 species of red sea weeds are found in the world sea area that are commercially important because of their protein, amino acids and mineral contents (Santhanam et al, 1980). These marine algae have evolved unique and highly specialized biochemical pathways to adapt to their seawater medium and survival pressures which gave rise to unparallel variety of biochemical composition in marine algae. For human benefit these nutritional biochemical constituents have been used for centuries (Dawczynski et al., 2007). Many algae species have been used in the industry principally for the extraction of phycocolloids (algin, carrageenan, and agar) and as a source of pharmaceutical substances. They are being used as herbal medicine, fertilizer, fungicides, and herbicides and for direct use in human nutrition too (Cardozo et al., 2007). Certain edible seaweeds contain significant quantities of protein, lipids, minerals, vitamins (Norziah et al, 2002) and 20 – 50% minerals in their dry weight (Kazutosi, 2002). Biochemical compositions of marine algae in Indian coast have been studied considerably (Subba Rao et al. 2007; Chakraborty & Santra, 2008). The potential areas in India for luxuriant growth of seaweeds are south Tamil Nadu coast, Gujarat coast, Lakshadweep and Andaman Nicobar Islands. The total standing crop of seaweeds from Intertidal and shallow waters of all maritime states and Lakshadweep Islands was estimated as 91339 tons (wet WI.) (Kaliaperumal and Kalimuthu 1997). Gujarat has the longest

available seacoast in India and the substratum is rocky in many parts of Gujarat, which provides the suitable environment for algal growth. It fosters about 300 species of algae. Intertidal survey of marine algal resources along the Gujarat coast of India revealed great diversity and the algal biomass along this coastline contributes substantially to the total standing crop of the Indian coast. The Gulf of Kutch in this zone is a luxuriant ecosystem with abundance of hundreds of marine organisms including micro and macro algae. The region was notified as a Marine National Park and Sanctuary (MNPS) in 1982 for its rich biodiversity. Since 1991, coral reefs and mangroves in the region have additionally been accorded the highest degree of protection under the 1991 Coastal Regulation Zone (CRZ) Notification. Some work on biochemical composition of marine algae from Gulf of Kutch had been done in the past but most of them were focussed to the effect of pollution on their biochemical composition, where limited parameters were studied, since the main thrust

was on pollution aspects (Jadeja and Tewari, 2011). The present study was attempted to characterize the predominant marine benthic algal species found in the Sikka and Vadinar areas of the Gulf of Kutch in terms of biochemical composition. These two sites were chosen owing to their general conditions for algal proliferation i.e. nutrient enrichment and limited focus on such type of previous studies.

Materials and Methods

Study site

The sampling sites selected, Sikka and vadinar are neighbouring sites that undergo similar oceanographic conditions in Gulf of Kutch. Vadinar is situated at $22^0$28'N and $69^0$43'E. Sikka is located in $22^0$26'N and $69^0$49' E. Organic input in this region arises from direct defecation process of the population residing along the coastal habitat and drainage of sewage. This is also a factor for consideration for the present study area because it supports the growth and proliferation of some selected macro algal species in the habitat.

Seaweed sampling, Identification and preparation

Algae were handpicked from substratum (mud and concrete surfaces) of the intertidal zone and placed into food-grade plastic bags for transfer to the laboratory in insulated (cool) containers. Specimens were chosen because of their different morphological and physiological characteristics and also for their widespread occurrence in Sikka and Vadinar coastal waters. Immediately after reaching the laboratory (within 4 hrs) the samples (0.5 – 1 kg) were washed twice with local seawater from the sampling site followed by single distilled water to remove any adhering impurities, sand debris and epiphytes and examined morphologically and microscopically with reference to literatures on marine benthic seaweeds (The Diversity and Distribution of Seaweeds of Gujarat Coast (Developments in Applied Phycology) by Bhavnath Jha 2009).

Fresh samples were utilized for chlorophyll and carotenoid estimation immediately and the rest of the biomass preserved for the other biochemical analysis at 4^0C.

Biochemical analysis

The total carbohydrate content of the samples were determined by the method of Hedge & Hofreiter (1962), popularly known as Anthrone method. In this method the carbohydrate content was measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis, and estimating the resultant monosaccharides.

The "Lowry method" (Lowry et al., 1951) was used for protein determination with Folin-Ciocalteau reagent. Extractant was 0.2M Phosphate buffer (pH – 7.0).

The method developed by Bligh and Dyer (1959) was followed for total lipid determination of the benthic algal species after extraction with chloroform: methanol: water (2:1:0.8 v/v/v). Determination of the total lipid was done by gravimetric method and expressed in terms of percentage of dry weight basis.

The total free amino acid content of the algal species was determined according to the method of Lee and Takahasi (1966) with slight modification.

The reducing sugar content of the samples was determined by di nitro salicylic acid method as developed by Miller (1972).

Ascorbic acid, the water-soluble and heat- labile vitamin had been analyzed by the method of Sadashivam and Balasubramanian (1987).

Amount of the ascorbic acid (mg/100gm) sample was calculated in this way

$$x = \frac{0.5\text{mg X V2 X 100 ml X 100}}{\text{V1 ml X 5 ml X Wt. of the sample}}$$

Where V1 is the titrant volume of the dye Dichlorophenol-indophenol for the standard (100mg ascorbic acid in 100ml of 4% oxalic acid) and V2 is the titrant volume of the dye for the sample.

Chlorophyll was estimated spectrophotometrically according to the method of Arnon (1949) by extracting in 80% acetone. Calculation of Chlorophyll 'a', 'b' and Total Chlorophyll was done as follows:

Chl a: [12.7 (D 663) – 2.69 (D 645)] X V/1000 X 1/W
Chl b : [12.7 (D 663) – 2.69 (D 645)] X V/1000 X 1/W
Where D = Optical density
V = Final volume of 80% acetone –chlorophyll extract
W = Fresh weight of the algae (in gm)

Carotenoids were isolated and estimated by the method of Davies (1965) with a little modification. Algal extraction was carried out in in 80% alkaline acetone combined with an equal volume of cyclohexane.

Results

Table 1 shows the proximate biochemical constituents of the algae. The carbohydrate concentrations of the algae varied from 8.6 ± 0.7 to 42.4 ± 0.7% dw. The maximum concentration was recorded from *Gracilaria verrucosa* (42.4 ± 0.7%) followed by *Acanthophora delilei* (36.4 ± 0.6%), *Ulva lactuca* (36.0 ± 1.2 %), *Hypnea musciformis* (34.9 ± 1.7%), *Enteromorpha compressa* (32.7 ± 1.7%) and *Enteromorpha linza* (30.9 ± 2.0%) among the significant ones. The minimum carbohydrate content

was observed in *Caulerpa scalpeliformis* (8.6 ± 0.7%) followed by *Caulerpa racemosa* (9.4 ± 0.7%), *Fucus* (17.7 ± 0.7%) and *Padina gymnospora* (18.3 ± 1.3%). The rest of the algae showed values within this range. Significant variation among the different groups was not visible.

In the present study protein content exhibited notable variation with the highest value of 32.4 ± 2.5% in *Caulerpa scalpeliformis*, followed by 24.8 ± 0.4 % in *Caulerpa racemosa*, 24.8 ± 0.4% in *Lola capillaries*, while the lowest values of 4.3 ± 0.4% in *Fucus*, 5.5 ± 0.7% in *Acanthophora specifera*, 5.9 ± 0.5% in *Hypnea* were observed.

The total lipid content in the algae was within the range of 0.9 ± 0.3 to 5.2 ± 0.4%. Highest lipid content was observed in *Ulva lactuca* (5.2 ± 0.4%), followed by 4.4 ± 0.4% in *Caulerpa racemosa* and 3.6 % in *Caulerpa scalpeliformis* and *Lola capillaris*, all belonging to chlorophyceae. The lowest lipid contents were found in *Sargassum polycustum* (0.9 ± 0.3%) followed by 1.8 ± 0.4% in *Fucus*, 1.9 ± 0.1% in *Hypnea musciformis* and *P. gymnospora*, all belonging to Phaeophyceae. Red algae showed moderate lipid concentration values.

Reducing sugar contents in the present investigation ranged between 1.9 ± 0.3 and 9.2 ± 0.4 mgg^{-1}. *L. capillaris* showed high content of 9.2 ± 0.4 mgg^{-1}, followed by 8.6 ± 0.1 mgg^{-1} in *P. gymnospora*, 7.5 ± 0.3 mgg^{-1} in *C. scalpeliformis.* Lowest value was recorded in *U. lactuca* (1.9 ± 0.3 mgg^{-1}) followed by 2.5 ± 0.3 mgg^{-1} in *D. bartayresiana*, 3.0 ± 0.4 mgg^{-1} in *C. racemosa.*

Total free amino acid content was recorded within the range of 26.5±1.9 to 152.3±4.0 mgg^{-1}. The maximum total free amino acid was observed in the species *H. musciformis* followed by 121.7± 4.1 mgg^{-1} in *S. polycustum*, 107.6 ± 2.5 mgg^{-1} in *G. verrucosa*. Most of the green algae showed lower values, while the minimum value was observed in *D. bartayresisna* (26.5 ± 1.9 mgg^{-1}), followed by *A. delilei* (42.2 ± 0.8 mgg^{-1}) and *Fucus* (45.0 ± 1.2 mgg^{-1}).

Individual variation of Vitamin C content within the species was very meager. Range of Vitamin C was from 0.2 ± 0.03 to 0.4 ± 0.02 mgg^{-1}. So group wise variation was not found in our observations of Vitamin C content in the algae.

The data on Chlorophyll a, b and carotenoids are given in Table2. The overall pigment data observation revealed higher concentrations of chlorophyll in green algae. Chlorophyll a ranged from 0.62 ± 0.03 to 2.50 ± 0.13 mgg^{-1}. Highest concentration of chlorophyll a was observed in *C. racemosa* (2.50 ± 0.13 mgg^{-1}) followed by *E. compressa* (2.24 ± 0.02 mgg^{-1}) and *U. lactuca* (2.01± 0.10 mgg^{-1}). Lowest chlorophyll a was observed in red algae *A. specifera* (0.62± 0.13 mgg^{-1}) followed by *G. verrucosa* (0.73 ± 0.04 mgg^{-1}) and *A. delelei* (0.77 ± 0.09 mgg^{-1}). Brown alga *D. bartayresiana* exhibited considerable quantity of Chlorophyll a (1.78 ± 0.03 mgg^{-1}). Similarly Chlorophyll b was also observed maximum in the green algae *U. lactuca* (1.43 ± 0.12 mgg^{-1}) followed by *E. compressa* (1.17± 0.07 mgg^{-1}) and *E. linza* (1.07± 0.08 mgg^{-1}). Lowest concentration of Chlorophyll b was recorded in *G. verrucosa* and *D. dictyota* (0.24± 0.03 mgg^{-1}) followed by *H.musciformis* and *A. delelei* (0.26 ± 0.02 mgg^{-1}).

Carotenoids were however recorded higher in the red and brown algae except green alga *U. lactuca*. Maximum carotene content was read in *D. bartayresiana* (25.20 ± 1.42 µgg^{-1}), 21.70 ± 1.04 µgg^{-1} in *Fucus* and 21.30 ± 1.04 µgg^{-1} in *U. lactuca*. Minimum carotenoids were recorded in green algae *E.linza* (13.47 ± 5.62 µgg^{-1}), *C. scalpeliformis* (15.00 ± 0.35 µgg^{-1}) and *G. verrrucosa* (15.67± 0.90 µgg^{-1}).

Table 1. Biochemical composition of the studied algae species (Data ± SE, n=3)

Species	Carbohydrate (% dry wt)	Protein (% dry wt)	Lipid (% dry wt)	Reducing sugar (mg/g)	Total amino acids(mg/g)	Vitamin C(mg/g)
Acathophora specifera	29.8 ± 1.2	5.4 ± 0.7	2.3 ± 0.6	7.2 ± 0.4	56.4 ± 4.8	0.3 ± 0.08
Acanthophora delilei Lamour	36.4 ± 0.6	4.6 ± 0.4	2.0 ± 0.4	7.2 ± 0.3	42.2 ± 0.8	0.2 ± 0.03
Gracilaria verrucosa	42.4 ± 0.7	6.0 ± 0.1	2.6 ± 0.6	6.4 ± 0.3	107.6 ± 2.5	0.3 ± 0.03
Hypnea sp.	34.9 ± 1.7	5.9 ± 0.5	1.9 ± 0.1	3.6 ± 0.3	152.3 ± 4.0	0.4 ± 0.02
Caulerpa	8.6 ± 0.7	32.4 ±	3.6 ±	7.5 ± 0.3	87.8 ± 1.7	0.3 ±

scalpeliformis		2.5	0.5			0.02
Enteromorpha compressa	32.7 ± 1.7	10.2 ± 0.8	3.5 ± 0.3	7.1 ± 0.9	77.2 ± 1.1	0.4 ± 0.04
Ulva lactuca Linn.	36.0 ± 1.2	17.6 ± 0.9	5.2 ± 0.4	1.9 ± 0.3	64.6 ± 4.3	0.4 ± 0.01
lola capillaries	28.8 ± 0.4	24.8 ± 3.1	3.6 ± 0.5	9.2 ± 0.4	78.3 ± 8.1	0.4 ± 0.04
Caulerpa racemosa	9.4 ± 0.7	24.8 ± 0.4	4.4 ± 0.4	3.0 ± 0.4	96.5 ± 3.9	0.3 ± 0.03
Enteromorpha linza	30.9 ± 2.0	15.3 ± 0.8	3.2 ± 0.4	5.0 ± 0.2	83.6 ± 2.5	0.3 ± 0.04
Padina gymnospora	18.3 ± 1.3	13.0 ± 1.4	1.9 ± 0.1	8.63 ± 0.1	76.6 ± 7.4	0.3 ± 0.05
Dictyota bartayresiana	22.1 ± 0.3	5.7 ± 0.5	2.0 ± 1.2	2.5 ± 0.3	26.5 ± 1.9	0.3 ± 0.05
Fucus spp.	17.7 ± 0.7	4.3 ± 0.4	1.8 ± 0.4	4.3 ± 1.2	45.0 ± 1.2	0.4 ± 0.01
Sargassum polycustum	24.1 ± 0.7	15.7 ± 0.6	0.9 ± 0.3	3.9 ± 0.3	121.7 ± 4.1	0.4 ± 0.02
Dictyota dichotoma	25.1 ± 0.5	7.6 ± 0.5	2.8 ± 0.1	4.03 ± 0.2	46.9 ± 1.0	0.3 ± 0.03

Table 2. Pigment composition of the studied algae species (Data ± SE, n=3)

Algae Species	**Chlorophyll a (mg/g)**	**Chlrophyll b (mg/g)**	**Carotenoids (µg/g)**
Acathophora specifera	0.62 ± 0.03	0.36 ± 0.05	18.17 ± 0.58
Acanthophora delilei Lamour	0.77 ± 0.09	0.26 ± 0.02	19.3± 0.75
Gracilaria verrucosa	0.73 ± 0.04	0.24 ± 0.03	15.67 ± 0.90
Hypnea musciformis	0.91 ± 0.05	0.26 ± 0.03	20.47 ± 1.23
Enteromorpha linza	1.75 ± 0.07	1.07 ± 0.08	13.47 ± 5.62
Ulva lactuca Linn.	2.01 ± 0.10	1.43 ± 0.12	21.10 ± 0.78
lola capillaries	1.54 ± 0.08	1.14 ± 0.08	17.43 ± 1.26
Caulerpa racemosa	2.50 ± 0.13	0.73 ± 0.11	17.07 ± 1.03
Enteromorpha compressa	2.24 ± 0.02	1.17 ± 0.07	17.23 ± 0.51
Caulerpa scalpeliformis	1.88 ± 0.10	0.84 ± 0.05	15.00 ± 0.35
Padina gymnospora	1.29 ± 0.05	0.39 ± 0.07	20.17 ± 1.50
Dictyota bartayresiana	1.78 ± 0.03	0.56 ± 0.05	25.20 ± 1.42
Fucus spp.	1.58 ± 0.09	0.80 ± 0.04	21.70 ± 1.04
Sargassum polycustum	0.86 ± 0.09	0.31 ± 0.03	21.30 ± 1.04
Dictyota dichotoma	1.38 ± 0.07	0.24 ± 0.05	20.43 ± 0.60

Discussion

Considerable difference in biochemical parameters in the algae were evident apparently but there was no significant variation in various biochemical parameters of different species as shown by one way ANOVA at 0.05 level of significance (F=0.251, Fcrit=1.82). variation in pigments are also non significant among the different species (F=.027,

Fcrit=2.04) as shown by one way ANOVA at 0.05 level of significance.

Carbohydrates in algae are of immense importance since they are stored in different forms like laminarin in brown algae, as paramylon in euglenophyta and leucosin in golden brown algae as food reserves and energy. The carbohydrate content in the studied algal species showed considerable individual variation, which might be due to different season and growth stage (Fleurence 1999). Concentrations were within the ranges of green algae (8.60 – 35.97%), red algae (29.87 – 42.40%) and brown algae (17.70 – 25.13%) of dry weight.

The protein contents are comparable with that of high-protein plant foods such as soybean. Values were similar to previous studies on some species in different biotopes of the world (Matanjun et al. 2009; Chakraborty and Santra, 2008) and higher than algal carbohydrate concentrations recorded by Manivannan et al. (2008).

Generally algal protein is called complete protein with all the essential amino acids-unlike most plant foods. Marine algae are often termed as superfood due to high concentration of protein in them. Our study also revealed considerably high quantities of protein in the studied algal species from Gulf of Kutch. Range of protein concentration in the algae was from 4.33% in *Fucus* to 32.40% in *C. scalpeliformis*. Marked Individual protein content difference was also evident from the data. Our data were very similar to protein content of seaweeds from mandapam studied by Manivannan (2008). Dave and Parekh (1997) also studied protein values of eight genera of green algae of Saurashtra coast, which share the same oceanographic conditions like our investigation site. However some of the algae like *caulerpa* and *Sargassum* exhibited higher concentrations than same species reported by Matanjun et al (2009).

Lipids have been recognized as essential components in human and animal nutrition and are used as feed additives in aquaculture. Data presented in the literature show that the lipid content in marine algae are less than 4% (McDermid & Stuercke, 2003). In contrast, our results showed that *U. lactuca* has about 5.2% and *C. racemosa* has 4.43% of lipid, which is comparable with higher lipids values for *Caulerpa* species collected from the Hawaiian coast (McDermid and Stuercke, 2003) and *Caulerpa* from Veraval coast of Gujarat (Kumari et al. 2011). There was significantly lower percentage of lipid in the other algal species (0.93- 3.6%). Species wise variation of the lipid content is attributed to the state of nutrition cells.

Sugars that contain aldehyde groups that are oxidised to carboxylic acids are classified as reducing sugars. Examples are glucose, fructose, glyceraldehydes, lactose, arabinose and maltose, except for sucrose. Data on reducing sugar content of algae is scanty. However the author have estimated this parameter in previous study on Sunderban mangroves (Chakraborty and Santra, 2008) and found that the reducing sugar content in the algal species are normally higher in the Kutch ecosystem. The range of reducing sugar content was between 1.93 and 18.10 mgg^{-1}.

Various studies on the relationship between the nutritive value of marine macro algae and culture organisms have demonstrated that the content of essential amino acids is the principal factor in their dietary value (Watanabe *et al*., 1983; Tibaldi and Lanari, 1991).The total amino acid content in the present study ranged from 26.50 to 152.33 mgg^{-1}. High total amino acid content in *in H. Musciformis, S. Polycustum* and *G. Verrucosa* was comparable with previous studies of Kumar and Kaladharan (2007).

Generally it is believed that algae with brighter thalli are found to be rich in vitamin-C (Sarojini and Sarma, 1999). However in the present study Vitamin C was found to be significantly low in the studied algae. The range of Vitamin C content was from 0.26 to 0.41 mgg^{-1} exhibiting no significant species variation (as seen by one way ANOVA). This is contrasting to earlier observations in the algae of south east coast of India (Anantharaman et al. 2011).

The data of chlorophyll a, b and carotenoids are presented in Table2. All chlorophylls serve as the primary means algae use to intercept light in order to fuel photosynthesis. Carotenoids are the pigments involved in the initial absorption of water, which rules as an environmental factor of growth and development. Chlorophyll a content varied from 0.62 to 0.91 mgg^{-1} in red algae, 0.86 to 1.88 mgg^{-1} in brown algae and 1.54 to 2.50 mgg^{-1} in green algae. Chlorophyll b varied from 0.24 to 0.36 mgg^{-1} in red algae, 0.24 to 0.80 mgg^{-1} in brown algae and 0.73 to 1.43 mgg^{-1} in green algae. Carotenoids varied from 15.67 to 20.47μgg^{-1} in red algae, 20.17 to 25.20 μgg^{-1} in brown algae and 13.47 to 21.10 μgg^{-1} in green algae. The data on pigment concentrations showed higher concentrations than previous studies by Jayashankar and Ramalingam (1993). The data shows apparently a clear variation among groups though one way ANOVA does not support the assumption with F value 0.01 and F_{crit} value of 5.143 suggesting non significant species variation at 0.05 level of significance. However green algae showed higher variations of chlorophyll a and b while brown algae

exhibited higher carotenoid concentrations compared to the other groups.

Conclusion

Thus results of the present study suggests that the marine benthic macro algae found in the selected areas of Gulf of Kutch are rich in nutrient and pigments composition though individual species wise variation was not significant at 0.05 level of significance. Considerable concentrations of carbohydrate, proteins, amino acids, chlorophyll a, b and carotenoids were recorded, though lipid was present in limited quantity and thus can be a potential health food in human diets and may be used to the food industry as a source of ingredients with high nutritional value. Micronutrients are also present in significant quantities, which hint that the species can be used for biomonitoring studies for pollution as well.

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