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Qualitative and quantitative biochemical analysis of some green marine algae from Veraval coast, Gujarat

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ABSTRACT

Edible green algal species are the most widely distributed and diverse macroalgae in the world and are considered as an important source of bioactive molecules which serve as multi-product sources for nutritional and pharmaceutical applications. The present investigations were carried out concerning biochemical constituents from three species of green marine algae *Ulva conglobata*, *Caulerpa racemosa*, and *Bryopsis plumosa* collected from the coast of Veraval Chowpati, Gujarat India. The biochemical composition was analysed using *uv* - spectrophotometer to evaluate its food value and find out variations in composition during the investigation period. The reducing sugar, proline, and starch contents were high in *Bryopsis plumosa* followed by *Caule rpa racemosa* and *Ulva conglobata*. Proline content is higher than the total amino acids in three algal species. The protein contents following by Lowry method *Caulerpa racemosa* have a high amount with 1667.32 ± 18.42 (µgg-1 dry wt.) followed by *Bryopsis plumosa* 1394.98 ± 18.78 (µgg-1 dry wt.) and *Ulva conglobata* 292.72 ± 17.85 (µgg-1 dry wt.). The protein content was recorded maximum in *Bryopsis plusmosa* and *Caulerpa racemosa* than in the *Ulva conglobata*. All the assays carried out in the present work showed that all selected green algae are good sources of biochemical. According to the biochemical composition values of the studied algae, they have the potential to be a source of constituents with high nutritional value and use in the food, dietary, and pharmaceutical industries.

Keywords: Biochemical composition, marine algae, protein content.

INTRODUCTION

Marine algae are the primary producers of the aquatic food chains which are generally visible to the naked eye. Coastal algae, such as seaweeds, are resources too valuable as a source of biochemical compounds for human consumption and the environment in many countries (Chapman 1980; Rao *et al*., 1986). Vinuganesh *et al*., 2022 reported that Seaweeds are significant marine ecosystem components that contribute to global primary production and serve as a breeding and feeding ground for many marine life forms. Based on their nutrient content, pigmentation, morphology, anatomy and chemical composition, Seaweeds can be classified into three groups like as green algae (Chlorophyata), brown algae (Phaeophyta) and red algae (Rhodophyta) (Dawczynski *et al*., 2007). According to Razak *et al*. (2020), Based on their pigmentation, 12,272 different species of algae are categorised as belonging to the Phaeophyta (brown algae), Rhodophyta (red algae), and Chlorophyta (green algae). Seaweed may be raised in tight spaces using aquulation systems or straight in the ocean. Macroalgae are multicellular photosynthetic organisms which belong to the lower plants, they have leaf-like thallus structures as their main structure instead of roots, stems and leaves distinctly marked (Lobban *et al*., 1985). Chlorophyta is the division of green algae, with the same ratio of chlorophyll a and chlorophyll b as higher plants (Lobban and Wynne, 1981; Bold and Wynne, 1985). Kaimala *et al.* (2015) observed that green seaweeds normally are existent in the intertidal zone between the high and low water marks as well as in shallow water where sunlight is abundant. Khokher and Joshi (2016) reported that green seaweeds belong to Chlorophyceae which are usually found closer to shores for they can tolerate more sunlight and have chlorophyll a and chlorophyll b as main photosynthetic pigments, hence the term chlorophyceae.

Seaweeds are one of the most important and renewable marine living resources as the food, fodder, fertilizer and a source of medicinal drugs since ancient time but, today seaweeds are the raw material for industrial production of agar, algin and carrageenan as well as they continue to be widely consumed as food in Asian countries (Mishra *et al*., 1993). According to Dhargalkar and Verlecar (2009) some edible macro algae were commonly used as additives to make snacks and first food ingredients, so gradually seaweeds have been

taken as food by peoples every day. They are the source for the production of phytochemials and phycocolloids which are use as gelling, stabilising and thickening agents in food, pharmaceuticals, dairy, textile, paper, paint and varnish in industry (Kaliaperumal, 2003). The source of the pharmaceutical from the algae was being used as herbal medicine, fungicides, and herbicides and for direct use in human nutrition too (Cardozo *et al*., 2007).

Several edible green seaweed species contain significant quantities of proteins, lipids, vitamins, polysaccharides, essential amino acids, minerals, and have a low-fat content (Norziah and Ching, 2002; Kotiya *et al*., 2011). Fleurence (1999) reported that seaweeds that belong to the Chlorophyta also contain a considerable amount of protein (10 - 47% DW, dry weight basis) which remains a potential source of nutrition for humans and animals. According to Hayes (2015), and Roohinejad *et al*. (2017) Seaweeds are rich in source of antioxidants, dietary fiber, essential amino acids, minerals, vitamins, phytochemicals, and bioactive compounds present in seaweeds which have health-promoting properties. It is believed that Seaweeds are considered as an important rich source of bioactive molecules as well as enzymes and biochemical constituents (Mumtaj, 2015). Chakraborty and Bhattacharya (2012) have shown that the Gulf of Kutch coastline might be a potential source of primary metabolites composition. These properties include anti-diabetic activity, anti-oxidant, anti-inflammatory, dyslipidemia, bone-health, heart-health and mental-health benefits. A species variety of seaweed that is fit for human consumption includes contains considerable quantities of protein, lipids, minerals, vitamins (Norziah and Ching, 2002), and in fact 20 – 50 % of its dry weight is made up of minerals (Kazutosi, 2002). *U. lactuca* bas been examined by Ortiz *et al*. (2006) who found that it has a high protein content comparable to the traditional high protein plant sources extending its direct use in human nutrition or for the development of stabilized meals for animal nutrition. Kaliaperumal (2003) studied the seaweeds which have formed rich sources of protein in *Ulva rigida*, *U. fasciata*, *U. stenophylla*, *Caulerpa scalpelliformis*, *Cladophora monumentalis*, *Bryopsis spp.*, *Porphyra virtnamensis*, *Centocerous clavulatum* and *Acanthophora spicifiera*; this algal protein has broadly aminoacids counting iodine.

According to research, seaweed has 16-30% of protein on a dry weight basis which amount is higher than other food materials such as cereals, eggs and fish. However, only a few studies have been undertaken on the quality and quantity of biochemical composition of green seaweed. In light of this, the current study examines these characteristics in relation to three species of very useful marine green macroalgae *Ulva conglobata*, *Caulerpa racemosa* and *Bryopsis plumosa* for their high quantity by analysing biochemical quantity.

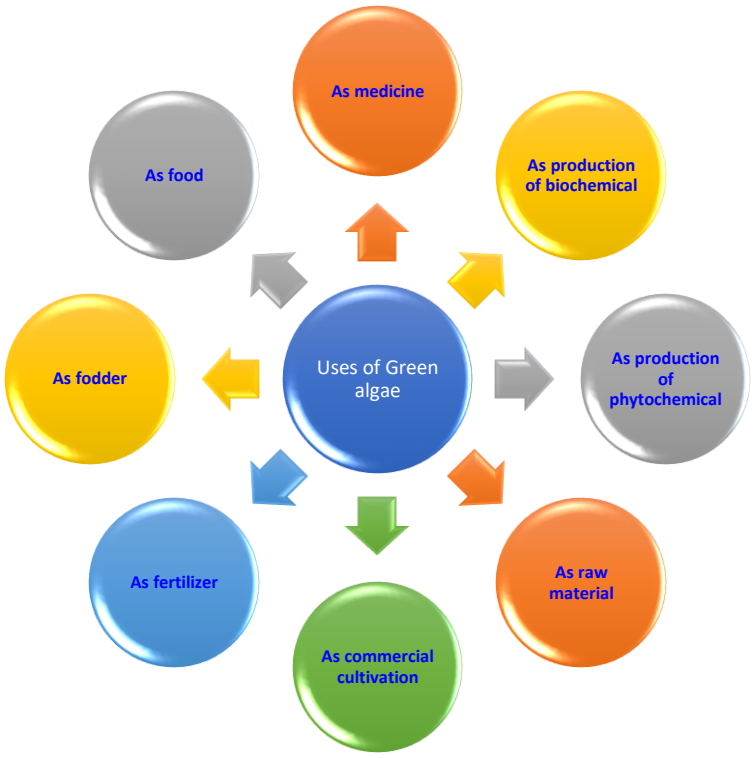


Fig.1. Uses of Green Algae

MATERIAL AND METHODS

Study site:

The sampling sites selected, Veraval chowpati is neighbouring sites large coastal industrial center. Veraval chowpati is situated at (Lat: 20^{o} 54' 35.04" N and Long: 70^{o} 21' 8.56" E) Veraval - Gir Somnath district of Gujarat states of India. The substratum of this coast is mainly rocky also with few sandy patches which significantly supports for too much algal diversity (Fig. 2).

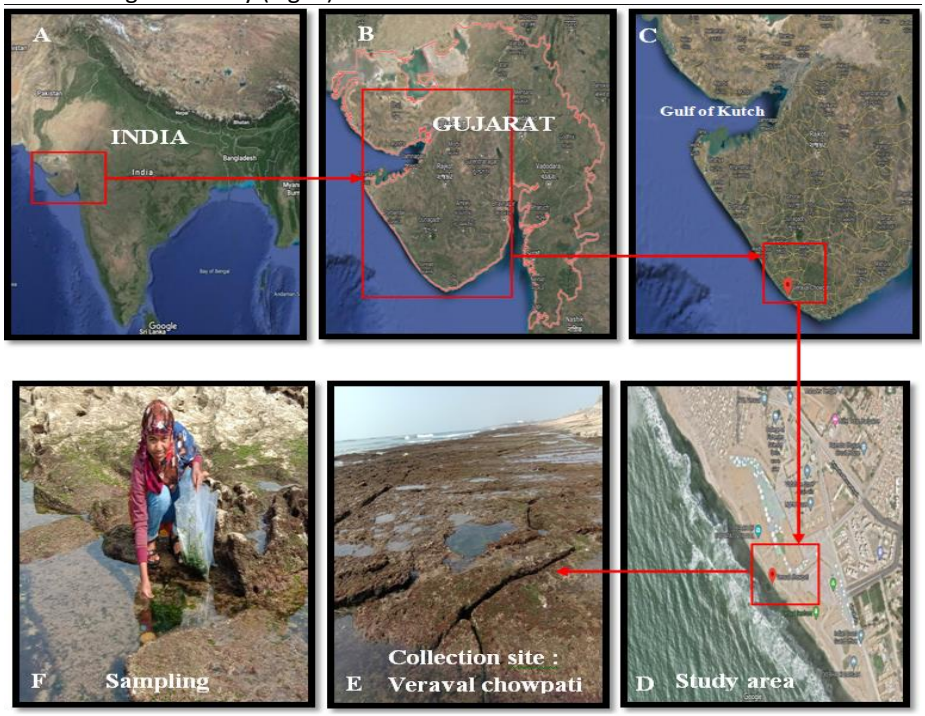


Fig. 2. Study area; (A) Gujarat coast, (B) Gulf of Kutch in Gujarat, (C) Veraval coast in Gulf of Kutch, (D) Study area: Veraval chowpati, (E) Collection site: Veraval chowpati, (F) Sampling

Collection of seaweed and identification:

During the survey different species of algae were collected from Veraval chowpati during month of December 2019 for study. Collected algae/seaweeds were washed with surrounded sea water to remove adhered sands and take in food-grade polythene bags individually by hand picking and transported in wet acclimatize to laboratory. The materials were cleaned exhaustively to remove any adhering impurities, sand debris and epiphytes by repeated washing in water and again washed in distilled water to remove all the salt on the surface. Some of fresh algal sample was stored in deionised water in fridge and other washed out materials were drying in sun light and make in to dry powdered form and finally stored in fridge for analysis. The eight species of green seaweeds were collected and identified on the basis of morphological criteria like size, shape, colour etc. with reference to literatures on marine benthic seaweeds (Jha *et al*., 2009). Among them three species were selected for further studies of biochemical constituents for their high quantity.

Preparation of sample:

Qualitative analysis was done by shake extraction method by Gadhavi *et al*. (2019) using water extract, methanol extract and extract (80% ethanolic extract for sugar and amino acid, 0.1 N NaOH extract for protein and 52 % perchloric acid extract for starch). For quantitative analysis 1 gm of algae powder crushed into 80 % ethanol (10ml) and homogenized using a pinch of sand and centrifuged residue was again extracted with 10 ml of ethanol twice. The supernatant was taken and total volume was made 20 ml extract which used for estimation of reducing sugar and amino acids. The pellet after removing the supernatant was dissolved in 10 ml of 0.1 N NaOH for 12 hour after that shaking it properly it was centrifuged at 4000 - 5000 rpm for 20 minutes. The supernatant was taken for estimation of protein. For the estimation of starch, extraction method was done according to McCready *et al.* (1950) with slight modifications. 1 gm of each sample was soaked in conical flasks containing 10 ml of water as a solvent and put on the shaker for 24 hours at 55 ^{o}C. After then heat in boiling water bath for 30 minutes and centrifuged at 1000 - 2000 rpm for 30 minutes. The extracts were filtered through filter paper and the supernatants used for estimation of starch.

Qualitative analysis

Qualitative analysis was conducted using ten tests described by Phukan and Shil (2017) and Chop et al. (1993).

1) Benedict's test:

1 ml of Benedict's reagent was added in 0.5 ml extract. The mixture is heated on a boiling water bath for 5 min. A characteristic coloured (green, yellow or red) ppt indicates the presences of reducing sugar.

2) Nin-hydrin test:

2 ml of Extract were heated with few drops of 2% nin-hydrin solution in boiling water bath for 5 min. A characteristic purple or bluish (total amino acids), brown, yellow (proline) colour indicates the presence of amino acids.

3) Biuret test:

1 ml extract was treated with two drop of 2% copper sulphate solution. In this mixture add 1 ml of ethanol (95%) is added, followed by an excess of potassium hydroxide palate. The pink or violet colour in the ethanolic layer indicates the presence of proteins.

4) Dye binding test:

In 1 ml extract, add 5 ml Bradford reagent (diluted dye solution). Mix the contents of the test tube by vortexing / shaking the tubes and take in the incubation (allow the colour to develop for at least 5 min but more than 30 min) for 5 – 30 min. The red dye turns blue when it binds with protein.

5) Heller's test (Albumin protein)

In 1 ml extract, add concentrate HNO_3 from the side of test tube. A white ring formed at junction which indicates the presence of proteins

6) Xanthohydrin test:

1 ml extract was heated with concentration HNO_3 in boiling water bath. Cool it. After then add 40% NaOH drops in that mixture. The orange colour indicates the presence of protein.

7) PPT of proteins by salt Heavy metals ($AgNO_3$):

2 ml of extract treated with a few drops of $AgNO_3$. A white precipitate formation or any other changes in mixture represents the proteins.

8) Turbimetric test:

1 ml of extract was mixed with an equal volume of the 50% TCA solution in a test tube. Turbidity is developed immediately at room temperature, which indicates the present of the proteins.

9) Coagulation test:

An Extract was mixed with equal volume of water and when boiled then it leads to coagulation. It represents the presents of albumin, globulin proteins.

10) Iodometric test:

In 0.5 ml extract mixed with o.5 ml water add 1 ml citrate buffer and boil in water bath than after add 1 ml I_2KI solution. After 10 minutes incubation purple or bluish colour indicates the presence of starch.

Quantitative analysis

Reducing sugar Estimation: The total reducing sugar was estimated using the DNS method of Miller (1959).

Total amino acid Estimation: The estimation of total amino acid was done by the nin–hydrin method of Lee and Takahasi (1966).

Proline Estimation: The proline content was estimated using the nin–hydrin method of Bates *et al*. (1973).

Protein Estimation: The protein was estimated by following the lowry method of Lowry *et al*. (1951).

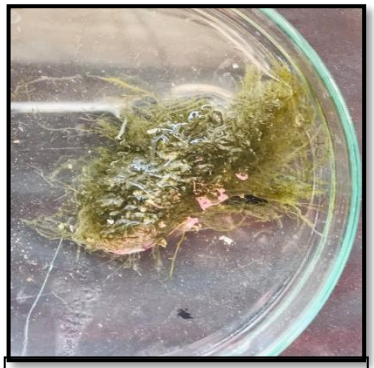
Starch Estimation: The starch content was estimated by iodometric method following to Gilber and Spragg (1964).

RESULTS

Collection and Identification of the Samples:

The survey reported eight species of green marine algae; they were identified as *Ulva conglobata* Kjellman, *Bryopsis plumosa* (Hudson) C. Agardh, *Caulerpa racemosa* (Forsskal) J. Agardh, *Caulerpa scalpelliformis* (Brown ex Turner) C. Agardh var. *denticulata* Borgesen, *Caulerpa sertularioides* (S. Gmelin) Howe f. *brevipes* (J. Agardh) Svedelius, *Caulerpa veravalensis* (Thivy and Chauhan), *Chaetomorpha spiralis* Okamura, *Chamaedoris auriculata* Borgesen and prepared a checklist of algae with morphological criteria using the literature Jha *et al*. (2009).

Ulva conglobata **Kjellman**



Bryopsis plumosa **(Hudson) C. Agardh**

Caulerpa racemosa **(Forsskal) J. Agardh**

Caulerpa scalpelliformis **(Brown ex Turner) C. Agardh var. denticulate Borgesen**

Caulerpa sertularioides **(S. Gmelin) Howe f. brevipes (J. Agarth) Svedelius**

Caulerpa veravalensis **(Thivy and Chauhan)**

Chaetomorpha spiralis **Okamura**

Chamaedoris auriculata **Borgesen**

PLATE - 1. Green algal species recorded at Veraval Chowpati, Gujarat

Table 1. Checklist of Green algal species recorded at Veraval Chowpati, Gujarat

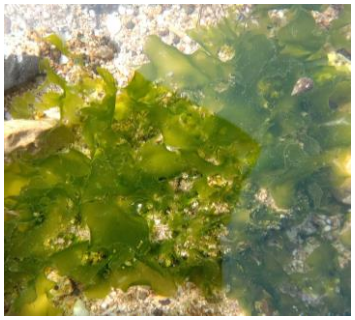
SR. NO.	SPECIES	FAMILY	USES
1	*Ulva conglobata* Kjellman	Ulvaceae	Used as food and animal feed in china as "Oyster vegetable". It's also used for Pharmaceutical substances.
2	*Bryopsis plumose* (Hudson) C. Agardh	Bryopsidaceae	It is useful in Food, animal feed and agricultural. It is explored sources of compounds for Pharmaceutical and nutraceutical industry.
3	*Caulerpa racemosa* (Forsskal) J. Agardh	Caulerpaceae	Used as fish feed and as a medicine for humans to treat high blood pressure and rheumatism.
4	*Caulerpa scalpelliformis* (Brown ex Turner) C. Agardh var. *denticulata* Borgesen		Used as against culex pipiens, food and animal feed.
5	*Caulerpa sertularioides* (S. Gmelin) Howe f. *brevipes* (J. Agardh) Svedelius		It is used to treat high blood pressure and goiter and also used for antibacterial, antifungal and antitumor.
6	*Caulerpa veravalensis* (Thivy & Chauhan)		That is widely used as a decorative plant in aquaria.
7	*Chaetomorpha spiralis* Okamura	Cladophoraceae	It is used as salad (food) and used for making the thick mat in fishponds.
8	*Chamaedoris auriculata* Borgesen	Siphonocladaceae	Used as medicinal and neutraceutical.

***Ulva conglobata* Kjellman:** The complete, undivided, leafy and spherical lobes/mass of the algal thallus was yellow to bright green in color. It was sub-cartilaginous at the base and membranous at the top portions. (Plate II)

***Caulerpa racemosa* (Forsskal) J. Agardh**: The color of the alagal thallus was pale green, it is ramiform and creeping type, growing as patches and it is coenocytes with prostrate rhizomes and erect assimilators; assimilates often much crowded on the rhizomes, simple or sparingly forked, number of side-shoots covered with clavately to spherical branchlets; stalks of the branchlets short. Most commonly associated with dead corals. (Plate III)

***Bryopsis plumosa* (Hudson) C. Agardh:** Algal thallus was light to olive green in color, growing as tufts or patches, coenocytic, with erect fronds that were naked below. Thallus has a soft silky texture with ramiform and feathery, regularly plumose above with basal rhizomatous portions; branching pinnate and bi-pinnate with long branches below and short ones above giving arrowhead appearance. (Plate IV)

Plate II



Ulva conglobata

Plate III

Caulerpa racemosa

Plate IV

Bryopsis plumosa

Qualitative biochemical analysis:

The results of the ten tests for the qualitative biochemicals are depicted in Table 1. *Ulva conglobata* and *Bryopsis plumosa* were observed to reflect the maximum positive result in extract and *Caulerpa racemosa* was observed with equal positive results in methanol extract and extract. Thus, the majority of the solvents gave positive results and confirmed the presence of primary metabolites in the three algal species.

Table 2. Detection of biochemical in three species of green algae

Sr. no.	Name of the Test	*Ulva conglobata*			*Caulerpa racemosa*			*Bryopsis plumosa*		
		WE	ME	E	WE	ME	E	WE	ME	E
1	Benedict's test	+	−	+	+	−	+	−	−	+
2	Nin-hydrin test	+	+	+	+	+	+	+	+	+
3	Biuret test	+	+	+	+	+	+	+	+	+
4	Dye binding test	+	+	+	+	+	+	+	+	+
5	Hellers test	−	+	+	+	+	+	−	+	+
6	Xanthohydrin test	−	−	−	−	+	+	−	−	+
7	By Heavy metals ($AgNO_3$)	+	+	+	−	+	+	−	+	+
8	By acid reagent	−	−	+	−	+	−	+	+	+
9	Coagulation test	+	+	+	+	+	+	+	+	+
10	Iodometric test	+	−	−	+	−	−	+	−	−

WE = Water extract
ME = Methanol extract
E = Extract

+ Positive
− Negative

Quantitative biochemical analysis:

Table 3 shows the proximate biochemical constituents of green algae.

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

Species	Reducing sugar (µg/g)	Total amino acid (µg/g)	Proline (µg/g)	Protein (µg/g)	Starch (µg/g)
Ulva conglobata	435.34 ± 9.74	1016.38 ± 13.59	1140.48 ± 24.99	292.72 ± 17.85	166.68 ± 6.38
Caulerpa racemosa	585.71 ± 10.03	1000.05 ± 13.42	1911.81 ± 24.91	1667.32 ± 18.42	494.63 ± 6.40
Bryopsis plumosa	663.95 ± 10.20	805.79 ± 14.17	2012.13 ± 24.31	1394.98 ± 18.78	511.21 ± 6.67

Total Reducing Sugar:

In the present study the calibration curve of the sugar obtained by the series of different concentration of reducing sugar (glucose) and standard curve equation was y = 0.0006x, R^2 = 0.9949 (Fig. 3) where y is absorbance at 540 nm and X is concentration of sugar (µgg^{-1}). The reducing sugar content was recorded maximum amount in *Bryopsis plumosa* 663.95 ± 10.20 µgg^{-1} dry wt. followed by *Caulerpa racemosa* 585.71 ± 10.03 µgg^{-1} dry wt. and *Ulva conglobata* with 435.34 ± 9.74 µgg^{-1} dry wt. (Fig. 4).

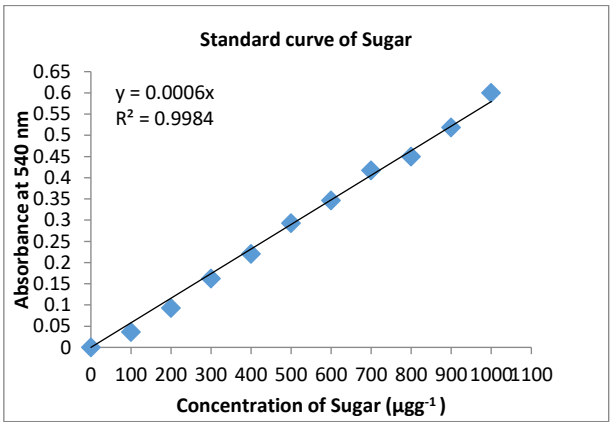


Fig. 3. Calibration curve of Reducing Sugar

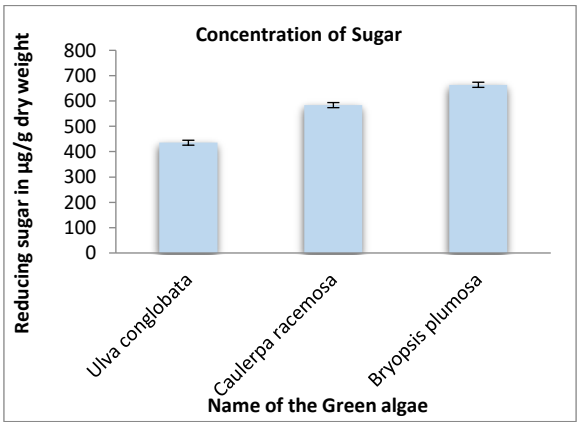


Fig. 4. Reducing sugar content in green seaweeds

Total Starch:

The calibration curve of the starch obtained by the series of different concentration of starch and standard curve equation was y = 0.001x, R^2 = 0.9955 (Fig 5) where y is absorbance at 600 nm and x is concentration of starch (µgg^{-1}). Starch content was ranged from 166.68 ± 6.38 µgg^{-1} dry wt. to 511.21 ± 6.67 µgg^{-1} dry wt., in that the highest starch content was recorded in *Bryopsis plumosa* 511.21 ± 6.67 µgg^{-1} dry wt. followed by *Caulerpa racemosa* 494.63 ± 6.40 µgg^{-1} dry wt. and *Ulva conglobata* 166.68 ± 6.38 µgg^{-1} dry wt. (Fig. 6)

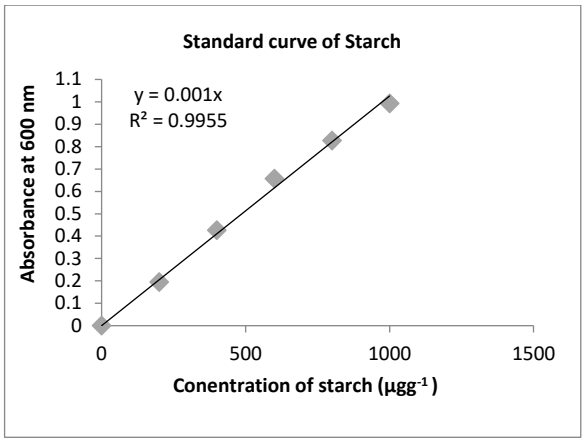


Fig. 5. Calibration curve of total Starch

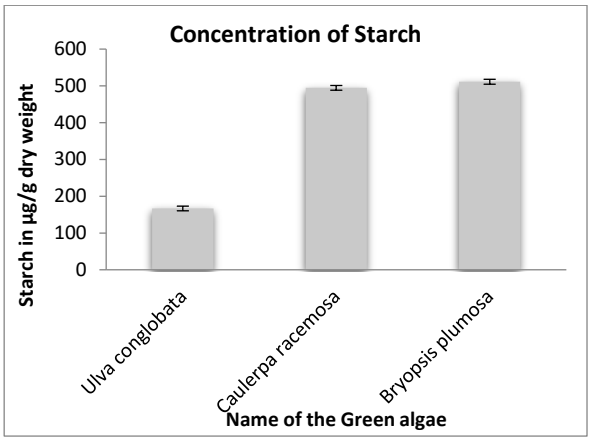


Fig. 6. Starch content in green seaweeds

Total amino acids:

The calibration curve of the total amino acid obtained by the series of different concentration of glutamic acid and standard curve equation was y = 0.0006x, R^2 = 0.9974 (Fig. 7) where y is absorbance at 540 nm and X is concentration of total amino acid (µgg^{-1}).Total amino acid content was recorded within the range of 805.79 ± 14.17 µgg^{-1} dry wt. to 1016.38 ± 13.59 µgg^{-1} dry wt. The maximum total amino acid content was observed in the species *Ulva conglobata* 1016.38 ± 13.59 µgg^{-1} dry wt. followed by 1000.05 ± 13.42 µgg^{-1} dry wt. in *Caulerpa racemosa* and 805.79 ± 14.17 µgg^{-1} dry wt. in *Bryopsis plumosa*(Fig. 9).

Total Proline:

The calibration curve of the proline obtained by the series of different concentration of proline and standard curve equation was y = 0.0009x, R^2 = 0.9924 (Fig. 8) where y is absorbance at 420 nm and X is concentration of proline (µgg^{-1}). Proline content in the present investigation ranged between 1140.48 ± 24.99 µgg^{-1} dry wt. to 2012.13 ± 24.31 µgg^{-1} dry wt. *Bryopsis plumosa* showed high content with 2012.13 ± 24.31 µgg^{-1} dry wt. followed by 1911.81 ± 24.91 µgg^{-1} dry wt. in *Caulerpa racemosa* and 1140.48 ± 24.99 µgg^{-1} dry wt. in *Ulva conglobata.* The overall amino acid data observation of green algae revealed higher concentration of proline than total amino acid. (Fig. 9).

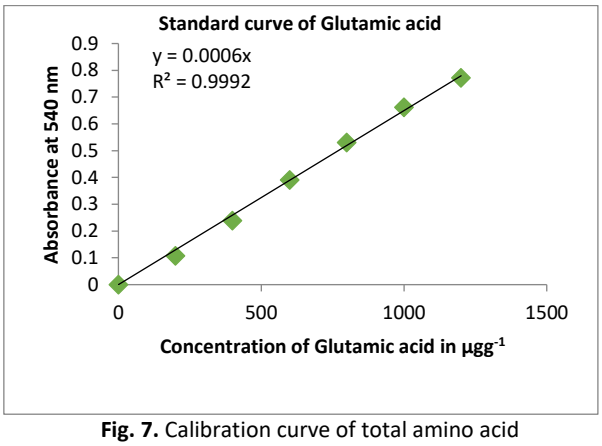


Fig. 7. Calibration curve of total amino acid

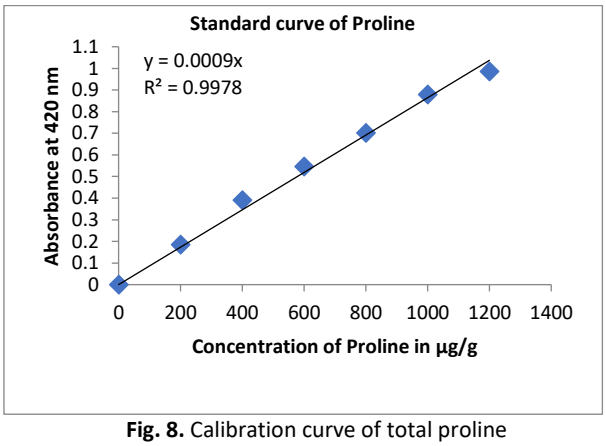


Fig. 8. Calibration curve of total proline

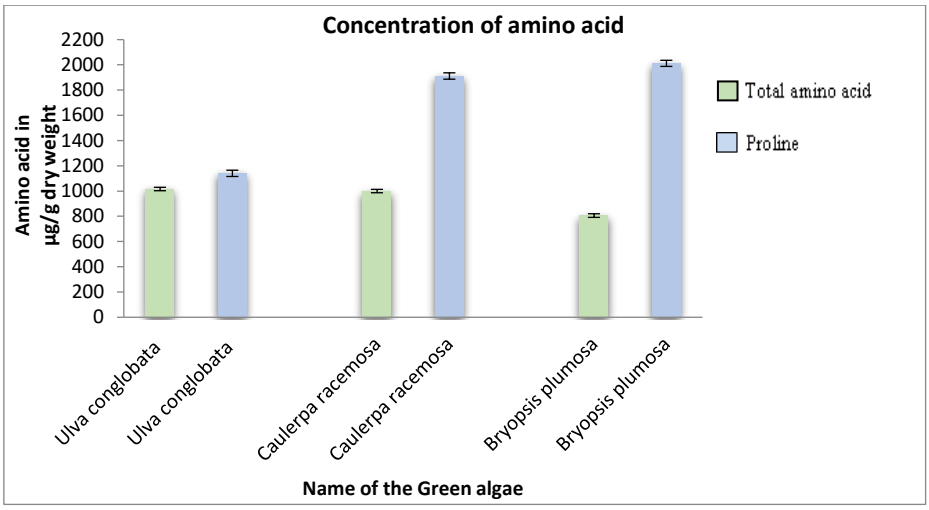


Fig. 9. Total amino acid and proline content in green seaweeds

Total Protein:

The calibration curve of the protein by Lowry method obtained by the series of different concentration of protein (Bovine serum albumin)and standard curve equation was y = 0.0001x, R^2 = 0.9957 (Fig. 10) where y is absorbance at 750 nm and x is concentration of protein (µgg^{-1}). Protein content in the present investigation ranged between 292.72 ± 17.85 µgg^{-1} dry wt. to 1667.32 ± 18.42 µgg^{-1} dry wt. The maximum amount of protein was observed in *Caulerpa racemosa* 1667.32 ± 18.42 µgg^{-1} dry wt. followed by *Bryopsis plumosa* 1394.98 ± 18.78 µgg^{-1} dry wt. and *Ulva conglobata* 292.72 ± 17.85 µgg^{-1} dry wt. Among the three species samples protein content was observed maximum in *Caulerpa racemosa* and low amount in *Ulva conglobata*. *Caulerpa racemosa* and *Bryopsis plumosa* gave the similar type results than the *Ulva conglobata*. (Fig. 11)

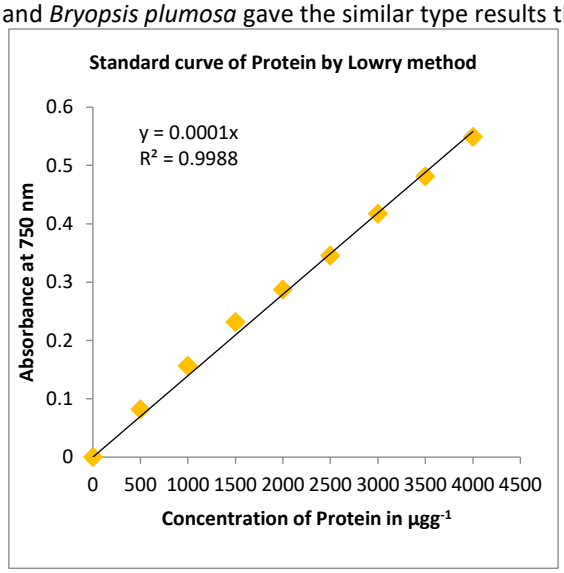


Fig. 10. Calibration curve of Protein by Lowry method

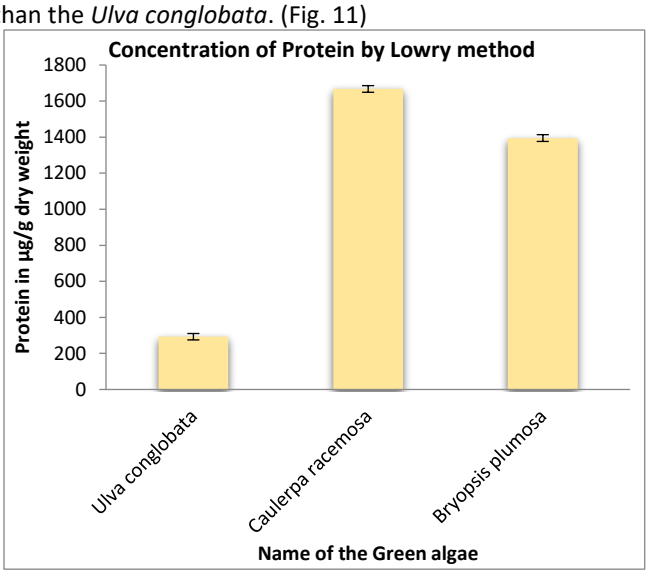


Fig. 11. Protein contents in green seaweeds by Lowry method

DISCUSSION

Substantial differences in biochemical parameters in the green algae were conspicuous apparently but there was no remarkable variation in various biochemical parameters of varied species. Sugar is one of the simple forms of carbohydrates, which are an important source of energy. The values of reducing sugar content in the studied algal species were compatible with earlier studies on other marine algae, among them *Ulva lactuca Linn*. with 1.9 ± 0.3 (mgg^{-1}) and *Caulerpa racemosa* with 3.0 ± 0.4 (mgg^{-1}) (Chakraborty and Bhattacharya, 2012) and higher in Ulva species by Chakraborty and Santra 2008. The total amino acid contents of the algae in the present study are comparable to other marine seaweeds like as *Caulerpa peldata* with 3.9 ± 1.3 (mgg^{-1}) and *Bropsis sp.* with 4.30 ± 0.1 (mgg^{-1}) of the Mandabam Sea coast (Mumtaj, 2015).

The data on starch content showed a higher concentration than the previous study in some algal species; among them recorded the high content result in green algae *Chaetomorpha antennia* with 405 ± 0.01

(µgg^{-1}) followed by *Ulva conglobata* and *Caulerpa racemosa* from Olaikuda and Vadakkadu, Rameshwaram (Roy and Anantharaman, 2017).

Total amino acid values were low compared to previous reports of the nutrition composition of marine algae from the Gulf of Kutch (Chakraborty and Bhattacharya, 2012). The result indicated that a substantial amount of the total amino acids were responsible for the special flavor and taste of the seaweeds (Wong and Cheung, 2000). According to present research work and previous research work total amino acid content also varied in *Ulva conglobata* with *Ulva lactuca Linn.* and *Bryopsis plumosa* with *Bropsis sp.* it conclude that content of total amino acid have variation in species to species.

The value of the proline content in the present study showed trivial individual differences between the three species and the content was high than the total amino acid. The values of proline content in studied algal species were compatible with previous studies in some algal species; among them recorded the result *Ulva lactuca Linn*. with 4.54 ± 0.22 (mgg^{-1}). on biochemical composition from Sundarban (Chakraborty and Santra, 2008). . Based upon the study, all the species have high proline content than the total amino acids. This are the marine algae so proline content, salinity increase, may be algae use the proline amino acid and make the defensive protein so therefore it is different and proline content is high than the total amino acid.

The protein contents were high quality because of the substantial total amino acid content and this essential amino acid profile was close to those of egg and soya protein (Valerie *et al*., 1999). Values were similar to previous studies on biochemical composition from Sundarban (Chakraborty and Santra 2008) and similar content in different marine seaweed species; among them recorded the result *Caulerpa peldata* with 5.71 ± 2.6 (mgg^{-1}) and *Bropsis sp.* with 6.44 ± 0.1 (mgg^{-1}) reported by Mumtaj 2015, and also higher protein content was observed by Manivannan *et al*., 2009). Algal protein also known as complete protein including all the essential amino acids and high concentrations of protein in marine algae is termed a superfood (Chakraborty and Bhattacharya 2012). During the present studies observed highest protein composition in *Caulerpa racemosa and Bryopsis plumosa* which is strongly supports the view for great potential as a good source of protein. Their protein contents differ according to the species to species.

CONCLUSION

Review work on green algal species reported that they produce metabolites and minerals like as protein, polysaccharides, lipid, carbohydrate, fibers, ash, minerals, potash, iodine, nitrogen, mannitol etc. which possesses basic nutrients with main biochemical composition. Biochemical analysis of the marine algae will be a good to reveal its metabolite constituents like sugar, amino acids, protein and starch which resultant as major ingredients of food, medicine, nutritional etc. The overall result of this present study suggests that the green algae which are found lavishly in the selected area of the Veraval coast of Gujarat are rich in primary metabolites composition through individual species wise variation. Substantial concentrations of reducing sugar, total amino acid, proline, protein and starch content were recorded, though protein content was in high quantity thus can be explored for food ingredients with high nutritional values, potential healthy food in human diets, feed and fertilizer purpose and it can also have great implications in medicine, nutraceutical and pharmaceutical industries as a source of basic materials in the preparation of nutrient supplement products.

REFERENCES

Bates, L. S., Waldeen, R. P. & Teare I. D. (1973). Rapid determination of free proline for water stress studies. *Plant soil,* (39), 205-207.

Bold, H. C. & Wynne, M. J. (1985). Introduction to the algae structure and reproduction, second edition, Prentice – Hall Inc., *Englewood Clifts, NJ,* (07632), 1-33.

Cardozo, K. H. M., Guaratini, T., Barros, M. P., Falcao, V. R., Tonon, A. P., Lopes, N. P., Campos, S., Torres, M. A., Sauza, A. O., Colepicolo, P. & Pinto, E. (2007). Metabolites from algae with economical impact. *Comparative Biochemical Physiology.* (146), 60-78.

Chakraborty, S. & Bhattacharya, T. (2012). Nutrient composition of marine benthic algae found in the Gulf of Kutch coastline, Gujarat, India. *Journal of Algal Biomass Utilization* 3 (1), 32-38.

Chakraborty, S. & Santra, S. C. (2008). Biochemical composition of eight benthic algae collected from Sunderban. *Indian Jjournal of Marine Sciences* 37 (3), 329-332.

Chapman, D. J. (1980). Seaweed and their uses. 2nd edition. Chapman & Hall, New York: 334.

Chop, W. S., Chung, K. J., Chang, M. S., Chun, L. K., Lee, H. W. & Hong, S. Y. (1993). A Turbimetric Determination of Protein by Trichloroacetic acid. *Archives of Pharmacal Research* 16 (1), 57-61.

Dawczynski, C., Schibert, R. & Jahreis, G. (2007). Amino acids, fatty acids and dietary fibre in edible seaweed products. *Food Chemistry* 103, 891-899.

Dhargalkar, V. K. & Verlecar, X. N. (2009). Southern Ocean seaweeds: a resource for exploration in food and drugs. *Aquaculture* 287, 229-242.

Fleurence, J. (1999). Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Sciences and Technology* 10, 25–28.

Gadhvi, K., Depani, P., Karangiya R. & Vyas, S. (2019). Phytochemical Analysis of Algal Species found in Sutrapada Coastal Region Of Gujarat. Junagadh, India. *European Journal of Medicinal Plants* 29(1), 1-9.

Gilbert, G. A. & Spragg S. P. (1964). Iodometric determination of amylose. *In R. L. Whistler Methods in carbohydrate chemistry* (4), 168–169.

Hayes, (2015). Seaweeds: a nutraceutical and health food. In: Tiwari BK, Troy DJ (eds) Seaweed sustainability: food and non-food applications. Academic Press, London, 365–387.

Jha, B., Reddy, C. R.,Thakur, M. C. & Umamaheswara, R. M. (2009). Seaweeds of India. The Diversity and Distribution of Seaweeds of Gujarat Coast, Springer, New York: 1-59.

Kaliaperumal, N. (2003). Products from seaweeds. Regional Center of Central Marine Fisheries Research Institute Marine Fisheries – 623 520, Ramnad District, Tamilnadu. *SDMRI Research Publication no.* 3(2), 33 – 42.

Kasimala, M. B., Mebrahtu, L., Magoha, P. P. & Asgedom, G. (2015). A Review On Biochemical Composition And Nutritional Aspects Of Seaweeds. *Carib. Journal of Science and Technology* (3), 789-797.

Kazutosi, (2002). Seaweeds Kaiso: Bountiful harvest from the seas. Sustenance for health and wellbeing Japan seaweed Association. Kochi.

Khokher, S. & Joshi, N. H. (2016). Diversity of marine macro algae of Okha cost, Gulf of Kutch, Gujarat. *International Journal of Global Science Research ISSN: 2348-8344* 3 (5), 333-339.

Kotiya, A. S., Gunalan, B., Parmar, H. V., Jaikumar, M, Dave, T., Solanki, J. B. & Makwana, N. P. (2011). Growth comparison of the seaweed *Kappaphycus alvarezii* different coastal areas of Gujarat coat, India. *Advances in Applied Science Research* 2(3), 99-106.

Lee Y. & Takahasi T. (1966). An improved colorimetric determination of amino acids with the use of ninhydrin. *Analytical Biochemistry* 14, 71 – 77.

Lobban, C. S. & Wynne, M. J. (1981). The biology of seaweeds. First's ed. Blackwell Scientific Publications.

Lobban, C. S., Harrioson, P. J. & Duncan, M. J. (1985). The Physiological Ecology of Seaweed. Cambridge University Press. Cambridge.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurements with the folin phenol reagent. *Journal of Biological Chemistry* 193, 265-275.

Manivannam, K., Thirumaran, G., Karthikai, D. G., Anantharaman, P. & Balasubramanium, T. (2009). Proximate composition of different group of seaweeds from Vedalai Coastal Waters (Gulf of Mannar): southeast coast of India. *Middle East Journal of Scientific research* 4(2), 72 – 77.

McCready, R. M., Guggolz, J., Silviera, V. & Owens, H. S. (1950). Determination of Starch and Amylose in Vegetables. *Analytical Chemistry* 22(9), 1156 – 1158.

Miller, G. L. (1959). Use of Dinitrosalicyclic acid reagent for determination of reducing sugar. *Annals of Chemistry* 31, 426 – 428.

Mishra, V. K., Temelli, F., Ooraikul, S. F. & Craigie, J. S. (1993). Lipids of the red alga *Palmaria palmata*. *Botanica Marina 36 (2)*, 169-174.

Mumtaj, S. (2015). Study on the Biochemical Characterization Of Marine Seaweeds of Mandabam sea Coast. *Internatinal Journal Curr. Microbiology in Appied Science* 4 (10), 273-281.

Norziah, M. H. & Ching, Ch. (2002). Nutritional composition of edible seaweeds *Gracilaria changgi*. *Food Chemistry 68*, 69 -76.

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., Navarrete, E., Osorio, A. & Rios, A., (2006). Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. *Food Chemistry* 99, 98–104.

Phukan, R. & Shil, D. (2017). Preliminary phytochemical screening and *In-vitro* anticonvulsant activity of methanolic extract of leaves of *Cinnamomum tamala* (Buchham) T. Nees and Eberm (Family- Lauraceae), *Indian Journal of Pharmacognosy* 4(10), 345-351.

Rao, P. P., Rao, P. S. & Karmarkar, S. M. (1986). Biological investigations on genus *Sargassum* I. Antifungal activity of fraction of different species of *Sargassum*. *Phycos.,* 25, 6-11.

Raymont, J. E. G., Austin, J. & Lineford, E. (1964). Biochemical studies on zooplankton. The biochemical composition of *Neomycis integar. Journal of Marine Science* 28, 354 – 363.

Razak, S. A., Aroyehun, Q., B., Palaniveloo, K. , Nagappan, T., Rahmah, N. S., Jin, G. W., Chellappan, D. K., Chellian, J., & Kunnath, A. P., (2020). Bioprospecting Cultivated Tropical Green Algae, *Caulerpa*

racemosa (Forsskal) J. Agardh: A Perspective on Nutritional Properties, Antioxidative Capacity and Anti-Diabetic Potential. *Foods* 9, 1313.

Roohinejad, S., Koubaa, M., Barba, F.J., Saljoughian, S., Amid, M., & Greiner, R. (2017). Application of seaweeds to develop new food products with enhanced shelf-life, quality and health-related beneficial properties. *Food Research International* 99: 1066–1083.

Roy, S. & Anantharaman, P. (2017). Biochemical Compositions of Seaweeds Collected from Olaikuda and Vadakkadu, Rameshwaram, Southeast Coast of India. *Journal Marine Science Research Development* 7(7), 240.

Valerie, A., Irmouli, G., Fleurence, J., Lamghari, R., Lucon, M., Rouxel, C., Barbaroux, O., Bronowicki, J. P., Villaume, C. & Gueant, J. L. (1999). Nutritional value of proteins from edible seaweed Palmaria palmata (Dulse). *Journal of Nutritional Biochemistry* 10, 353-359.

Vinuganesh, A., Kumar, A., Prakash, S., Alotaibi, M. O., Saleh, A. M., Mohammed, A. E., Beemster, G. T., AbdElgawad, H. (2022). Influence of seawater acidification on biochemical composition and oxidative status of green algae *Ulva compressa*. *Science of The Total Environment* 806 (1).

Wong, K. H., Peter, C. K., & Cheung, (2000). Nutritional evaluation of some subtropical red and green seaweed: part 1-proximate composition, amino acid profiles and some physico-chemical properties. *Food chemistry* 71(4), 475-482.