



ESTIMATION OF VITAMIN COMPONENTS IN SELECTED GREEN ALGAL SEA WEEDS COLLECTED FROM GULF OF MANNAR ISLANDS, TAMIL NADU STATE SOUTH INDIA

Lalitha.N and Dhandapani.R*

PG and Research Department of Botany, Government Arts College, Ariyalur-621 713, Tamil Nadu, India

*Corresponding Author Email: paniroever2007@rediffmail.com

ABSTRACT

Vitamins are more important in nutrition aspects, now a day's sea weeds are providing nutrients in various ways. In Bay of Bengal region, grown number of sea weeds and algal species 18 different seaweeds collected from the study area and analyzed the vitamin contents such as A, C, E and B complex vitamins such as B1, B2, B3, B5, B6, B7, B9 and B12 by standard methods. The results are discussed with the literature.

KEY WORDS

Vitamins, Sea weeds, Algal species, Nutrition

INTRODUCTION

Seaweeds grow primarily in the intertidal zone, and their ceaseless need to protect themselves against oxidative stress from UV radiation, desiccation and extreme temperature fluctuation at low tide explain the abundance of powerful anti-oxidants such as carotenoids, phlorotannins, ascorbic acid (vitamin C), tocopherol (vitamin E), poly phenols, chlorophyll derivatives and mycosporine like amino acids found in them. In comparison with terrestrial plants, seaweeds are particularly rich in iodine, which is indispensable to the functioning of the thyroid and of the nervous organization. They are rich in vitamins, minerals, protein, polyunsaturated fatty acid and dietary fibers [1,2] and numerous clinical studies have shown the health benefits of seaweed consumption [3]. Nowadays, seaweed consumption is increasing due to their natural constitution. Still, they contain 80–90 % water and their dry weight basis contains 50 % carbohydrates, 1–3 % lipids, and 7–38 % minerals. Their protein contents are highly variable (10–47 %) with high ratios of essential amino acids [4]. Because of their low-fat abundance and the presence of protein and carbohydrate substances,

they can contribute few calories to the diet. However, the chemical composition and the abundance of carbohydrates vary among seaweed species. Seaweeds are a rich source of minerals, especially macro and micronutrients necessary for human nutrition; however, the nutritional properties of seaweeds are usually determined from their biochemical composition alone viz., proteins, carbohydrates, vitamins, amino acids, etc., [5,6]. The mineral fraction of some seaweed even accounts for up to 40% of dry matter [7], however, in some cases the mineral content of the seaweeds is recorded even higher than that of land plants and animals' products [8]. Seaweeds are known to be of low-calorie content, rich in polysaccharides, minerals, vitamins, proteins, steroids and dietary fibers [9] making them increasingly sought for commercial purposes. According to [10] 100 g seaweed contains more than the daily requirement of vitamin A, B and B12 and two third of vitamin C. *Enteromorpha* contains vitamins C and β -carotene, which among the nutrients protect cells against powerful oxidizing agents [11]. The present investigation is to analyze vitamins in sea weeds.

MATERIALS AND METHODS

Study area

The Gulf of Mannar, the first Marine Biosphere Reserve (GOMMBR) in the South and South East Asia, running down south from Rameswaram to Kanyakumari in Tamil Nadu, India is situated between Longitudes 78008 E to 79030 E and along Latitudes from 8035 N to 9025 N. This Marine Biosphere Reserve encompasses a chain of 21 islands [12].

Collection of seaweeds

The green algal seaweeds were collected at a depth of 1-2 m from the coastal area of the Gulf of Mannar islands, in between Rameswaram to Kanyakumari, Tamilnadu, and South East Coast of India. Algae were collected during the low tide period in the early morning time, cleaned and removed the epiphytes and necrotic parts on them by using seawater and tape water and then rinsed with sterile water to remove any associated debris. The cleaned fresh material was air-dried [13]. The samples were identified and further confirmed as the marine green algae in chlorophyceae by using authentic floras and books [14-17]. They are namely *Caulerpa chemnitzia* (Esper) J.V.Lamououx, *Caulerpa peldala* J.V.Lamouroux, *Caulerpa racemosa* (Forsskål) J.Agardh, *Caulerpa scalpelliformis* (R.Brown ex Turner) C.Agardh, *Caulerpa serrulata* (Forsskål) J.Agardh, *Caulerpa sertularioides* (S.G.Gmelin) M.A.Howe, *Caulerpa taxifolia* (M.Vahl) C.Agardh, *Caulerpa veravalensis* Thivy & V.D.Chauhan, *Chaetomorpha aerea* (Dillwyn) Kützing, *Chaetomorpha antennina* (Boryde Saint-Vincent) Kützing, *Codium adhaerens* C.Agardh, *Codium tomentosum* Stackhouse, *Enteromorpha compressa* (Linnaeus) Nees, *Enteromorpha intestinalis* (Linnaeus) Nees, *Halimeda macroloba* Decaisne, *Halimeda tuna* (J.Ellis & Solander) J.V.Lamououx, *Ulva lactuca* Linnaeus, and *Ulva reticulata* Forsskål. Vitamins have diverse biochemical functions, including function as hormones, antioxidants and mediators of cell signaling and regulators of cell and tissue growth and differentiation. A simple, sensitive, highly accurate UV-visual spectrophotometric method for determination of water-soluble vitamin in pharmaceutical capsule and tablet dosage form was developed and validated. The validation is the collection and evaluation of data. In the present study, the UV-visible spectrometric method was calibrated and validated for the estimation of water-soluble vitamins (vitamin B and C). The results showed that the present

method has wide applicability for routine quality control and it was well established by the assay of vitamins (Vitamin – A (Parrish *et al*, 1985), vitamin B1, vitamin B2, vitamin B3, vitamin B5, vitamin B6, vitamin B7, vitamin B9, vitamin B12 and vitamin C) in pharmaceutical preparations [18]. Vitamin B complex and vitamin C were procured from Syncom Healthcare Limited, Dehradun. All the chemicals were of analytical reagent grade of Merck (Germany) unless otherwise specified. The standard procedures were followed for the different vitamins in the seaweed samples [19-21].

Vitamin – A.

The reagent for vitamin-A was prepared by mixing 25 g of antimony trichloride in 100 mL of chloroform contained 1 % v/v acetic anhydride. Dried samples were dissolved in 1 mL of chloroform each. The solutions were kept in the cuvettes of the spectrophotometer. Two mL of the reagent was added for each sample and absorbancy was read within 15 seconds at 620 nm.

For estimating the amount of Vitamin-A in samples, it is essential to find out the interference in absorbancy caused by β -carotene in the samples at this wavelength. Separate standard graphs using the above procedure had to be prepared for vitamin-A and β -carotene 86 using different concentrations. The amount of β -carotene present in each sample was estimated previously had to be taken into account to find out the absorbance of β -carotene at 620 nm and the values of absorbancy thus obtained in the present standard graph of β -carotene was deduced from the total absorbancy of the samples estimated in the present procedure. The corrected values of absorbancy were used for estimating the amount of vitamin-A in the samples by using the standard graph of vitamin-A [22].

VitaminC (Ascorbic acid)

Ascorbic acid of the extracts was determined using ascorbic acid as standard, with some modifications. The samples (1 g) and 4 ml oxalic acid (1%) were mixed, homogenized for 1 min, and filtered. Polyvinylpyrrolidone (PVPP) (100 g) was added to 2.5 ml of the filtered sample, to remove phenols, and 2-3 drops of H₂SO₄ (25%) were added, to reduce the pH to below 1. Absorbance of the mixture was determined at 254 nm. Results were expressed as mg ascorbic acid (AA) 100 g⁻¹ fresh weight (fw) [23].

Vitamin E

0.1 ml hexanic extract of algae was mixed with 1 ml phosphomolybdenum reagent solution and incubated

at 37°C for 90 min with vigorous shaking. The absorbance was measured at 695 nm. Vitamin E content was expressed as -tocopherol equivalents per gram of extract [24].

Vitamin B Groups

Diluent Preparation

Double distilled water was used as the diluent while 3 % dipotassium phosphate solution was used to folic acid because it is insoluble in water. Vitamin B1, B2, B3, B5, B6, B7, B9, B12 and vitamin C stock solutions.

Accurately weighed amounts, 50 mg of thiamine hydrochloride (vitamin B1), 60 mg of riboflavin (vitamin B2), 50 mg of nicotinamide (vitamin B3), 50 mg of pantothenic acid (vitamin B5), 50 mg of pyridoxine hydrochloride (vitamin B6), 50 mg of biotin (vitamin B7), 40 mg of folic acid (vitamin B9), 25 mg of cyanocobalamin (vitamin B12) and 50 mg of ascorbic acid (vitamin C), were taken into 100 ml volumetric flask separately and 50 ml of diluent was added and sonicated to dissolve. The volume was made up to the mark with diluent. The working standard solutions of vitamins contained 500 µg/ml of thiamine hydrochloride (vitamin B1), 600 µg/ml of riboflavin (vitamin B2), 500 µg/ml of nicotinamide (vitamin B3), 500 µg/ml of pantothenic acid (vitamin B5), 500 µg/ml of pyridoxine hydrochloride (vitamin B6), 500 µg/ml of biotin (vitamin B7), 400 µg/ml of folic acid (vitamin B9), 250 µg/ml of cyanocobalamin (vitamin B12) and 500 µg/ml of ascorbic acid (vitamin C). The solutions were then filtered through Whatman filter paper no1.

Standard preparation

Stock solution

Two ml of thiamine hydrochloride (vitamin B1), 2 ml of riboflavin (vitamin B2), 2 ml of nicotinamide (vitamin B3), 2 ml pantothenic acid (vitamin B5), 2 ml of pyridoxine hydrochloride (vitamin B6), 1 ml of biotin (vitamin B7), 10 of folic acid (vitamin B9) and 10 of ascorbic acid (vitamin C) were transferred to a 100 volumetric flask and the volume was made up with diluent and mixed well. In case of vitamin B12, 1 ml was transferred to 250 ml volumetric flask and further diluted 2 ml into 50 ml volumetric flask. This final solution contain 10, 12, 10, 10, 10, 5, 40, 0.04 and 50 µg/ml of vitamin B1, vitamin B2, vitamin B3, vitamin B5, vitamin B6, vitamin B7, vitamin B9, vitamin B12 and vitamin C respectively.

Sample preparation

The average weight of 20 capsule and tablets were determined and crushed to a fine powder. An amount equivalent to average weight of sample i.e. 1.0 mg of vitamin B1, 1.5 mg of vitamin B2, 10 mg of vitamin B3, 5 mg of vitamin B5, 1.0 mg of vitamin B6 and 50 mg vitamin C, were taken into 100 ml volumetric flask and 50 ml of diluent was added and sonicated to dissolve. The volume was made up to the mark with diluent. The solutions were then filtered through whatman filter paper 1. Above sample solution, 5 ml of vitamin B3, 10 of vitamin B5 and 5 ml of vitamin C were taken into 50 ml volumetric flask separately and 20 ml of diluent was added to dissolve. The volume was made up to the mark with diluent while 20 ml of vitamin B2 was taken into 25 ml volumetric flask. Vitamin B9, equivalent to 1.0 mg was taken into 25 ml volumetric flask and diluent (3 % dipotassium phosphate solution) was added and sonicated to dissolve. The volume was 89 made up to the mark with diluent. This final solution contain 10, 12, 10, 10, 10 and 50 µg/ml of vitamins B1, vitamin B2, vitamin B3, vitamin B5, vitamin B6 and vitamin C respectively.

Procedure for Water Soluble Vitamins

Vitamin B1 (Thiamine hydrochloride)

Five ml of the standard and sample was taken in marked test tubes. In each test tube, 5 ml NH₄OH (0.1M) and 0.5 ml 4-Amino phenol solution added and mixed well, then kept for 5 minute added 10 ml chloroform and separate of chloroform layer. The absorbance recorded chloroform layer at 430 nm against blank.

Vitamin B2 (Riboflavin)

Five ml of the standard and sample solution was taken in marked test tubes. In each test tube, 2 ml hydrochloric acid (1 M), 2 ml glacial acetic acid, 2 ml hydrogen peroxide, 2 ml potassium permagnate (15% w/v) and 2 ml phosphate buffer (pH 6.8) added and mixed well and absorbance recorded at 444 nm against blank.

Vitamin B3 (Nicotinamide)

Two ml of the standard, sample and blank solution was taken in marked test tubes. In each test tube, 5 ml sulphanic buffer (pH 4.5), 5 ml water and 2 ml cyanogen bromide solution (10% w/v) added and mixed well and absorbance recorded at 450 nm against blank and recorded an interval of 2 minutes.

Vitamin B5 (Pantothenic acid)

Hydrolysis of standard and sample 5 ml of standard and sample solution was taken into 50 ml volumetric flask. In each volumetric flask, 2 ml hydrochloric acid (1 M) added and mixed well, then heat 5 hours at $690 \text{ C} \pm 10 \text{ C}$ for affecting the hydrolysis and cool at room temperature. After that 2 ml of hydroxylamine reagent (7.5% in 0.1M sodium hydroxide), 5 ml sodium hydroxide (1M) and kept for 5 minutes now adjust pH 2.7 ± 0.1 with 1 M hydrochloric acid and make up the volume with water. Procedure 5 ml of the standard and sample hydrolysis solution was taken in marked test tubes. In each test tube, 1 ml of 1% ferric chloride solution (in water) added and mixed well remove the air bubbles and absorbance recorded at 500 nm against blank.

Vitamin B6 (Pyridoxine hydrochloride)

Two ml of the standard and sample solution was taken in marked test tubes. In each test tube, one ml of ammonium buffer (in water), 1 ml of 20% sodium acetate (in water), 1ml of 5% boric acid (in water) and 1 ml dye (2,6- di-chloroquinine chorimide) solution added and mixed well. It was absorbance recorded at 650 nm against blank.

Vitamin B7 (Biotin)

Weigh accurately equivalent to 500 mcg of vitamin B7 of sample was taken into 100 ml volumetric flask and 10 ml of dimethyl sulfoxide was added to dissolve. Heat the flask on water bath at 600 to 700 C for 5 minutes. The volume was made up to the mark with dilute water. Filter and absorbance recorded at 294 nm against blank of sample as well as standard.

Vitamin B9 (Folic Acid)

Two ml of the standard and sample solution was taken in marked test tubes. In each test tube, 2 ml of 0.02 % potassium permanganate solution, 2 ml 2 % sodium nitrate solution, 2 ml 4 M hydrochloric acid solution, 1 ml 5 % ammonium sulphamate solution and 1 ml dye solution (0.1 % N, N diethyl aniline dye solution in iso propyl alcohol) added and mixed well, then kept for 15 minutes at room temperature. It was absorbance recorded at 535 nm against blank.

Vitamin B12 (Cyanocobalamin)

Weigh accurately equivalent to 1 mcg of vitamin B12 of sample was taken into 25 ml volumetric flask and 10 ml of water was added to dissolve. 1.25 gm of dibasic sodium phosphate, 1.1 gm of anhydrous citric acid and 1.0 gm of sodium metabisulphate was added. The volume was made up to the mark with water. The solution was autoclaved at 1210 C for 10 minutes. Filter and absorbance recorded at 530 nm against blank of sample as well as standard.

STATISTICAL ANALYSIS

The Content of vitamins in respective methods were expressed by mean \pm SD, with the help of Graph Pad Prism (Version 7).

RESULTS AND DISCUSSION

Totally 18 seaweeds were collected from the east coast of India, especially in Mannar Biosphere Reserve, during the month of January 2014 and estimated vitamins such as vitamin A, C, E, B1,B2,B3,B5,B6,B9 and B12 (Table 1 and 2) measurable difference in nutritional composition were apparent among 18 species studied. Vitamin A, C and E recorded in higher level among 18 green algal seaweeds such as *Caulerpa chemnitzia*, *C. racemosa*, *C. serrulata*, *C. taxifolia*, *Cheatomorpha area*, *condium adhaerens*, *Enteromorpha compressa*, *Halimeda Macroloba*, *Ulva lactuca*. (Fig 1-3). Seaweeds are rich sources of vitamin C, vitamin B-complex, e.g. folic acid and vitamin A precursors, such as β - carotene [25-27]. Vitamin C is an antioxidant, protecting hydrogen/electron carriers within the cellular phone and maintains suitable redox levels for enzyme systems. Ascorbate is also implied in the biosynthesis of hormones and deoxyribonucleic acid. The algae belonging to Chlorophyceae and Phaeophyceae has higher annual mean contents of the vitamin C than Rhodophyceae [28]. Seasonal variations in ascorbic acid concentrations are apparently dependent on hydrographic parameters and solar radiation and growth intensity of plants [29]. Vitamin E is a generic term applied to tocopherols and tocotrienols, which show similar nutritional properties to α -tocopherol. Higher levels of α -tocopherol in commercially important brown alga *Himanthalia elongata*. [30,31] quantified α -tocopherol level in *Cystoseira sp.* and *Ulva sp.*

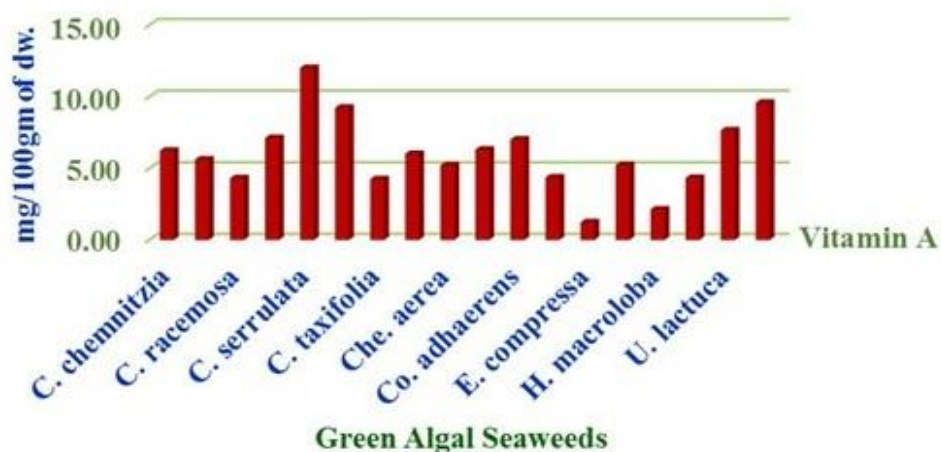


Figure.1. Estimation of Vitamin A Content in different algal seaweeds (mg/100gm dr.wt.)

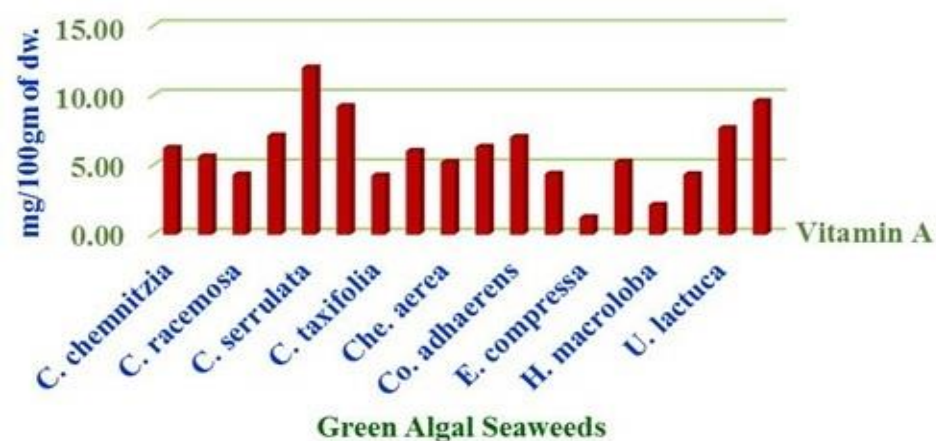


Figure.2. Estimation of Vitamin C content in different algal seaweeds (mg/100gm dr.wt.)

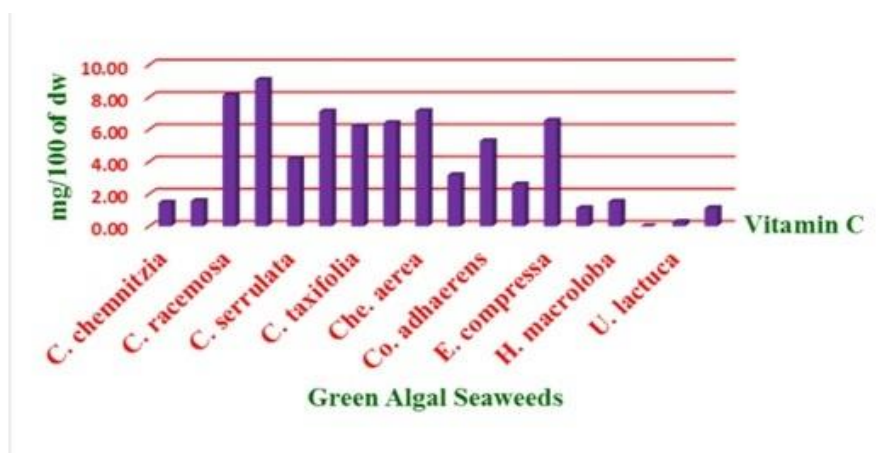


Figure. 3. Estimation of Vitamin E content in different algal seaweeds (mg/100gm dr.wt.)

Table 1. Quantity of Vitamin A, Vitamin C and Vitamin E of green algal seaweeds collected from Gulf of Mannar Islands

S.No.	Seaweeds	Vitamin A	Vitamin C	Vitamin E
		mg/100gm of dw.		
1	<i>Caulerpa chemnitzia</i>	6.23 ± 0.03	1.48 ±0.06	1.05 ±0.06
2	<i>Caulerpa peldala</i>	5.62 ± 0.01	1.57 ±0.02	1.04±0.08
3	<i>Caulerpa racemosa</i>	4.31 ± 0.04	8.14 ±0.05	1.21±0.05
4	<i>Caulerpa scalpelliformis</i>	7.12 ± 0.02	9.08 ±0.03	nd
5	<i>Caulerpa serrulata</i>	12.01 ± 0.02	4.21 ±0.04	1.02±0.01
6	<i>Caulerpa sertularioides</i>	9.23 ± 0.01	7.13 ±0.01	1.21±0.02
7	<i>Caulerpa taxifolia</i>	4.24 ± 0.01	6.22 ±0.12	0.35±0.03
8	<i>Caulerpa veravalensis</i>	6.12 ± 0.06	6.41 ±0.14	0.88± 0.04
9	<i>Cheatomorpha aerea</i>	5.21 ± 0.05	7.16 ±0.01	0.62±0.01
10	<i>Cheatomorpha antennina</i>	6.31 ± 0.01	3.18 ±0.04	0.85±0.04
11	<i>Codium adhaerens</i>	7.01 ± 0.05	5.29 ±0.02	1.05±0.01
12	<i>Codium tomentosum</i>	4.361 ± 0.01	2.6 ± 0.06	2.07±0.03
13	<i>Enteromorpha compressa</i>	1.234 ± 0.02	6.56 ±0.01	1.87±0.02
14	<i>Enteromorpha intestinalis</i>	5.231 ± 0.01	1.13 ±0.06	0.86±0.05
15	<i>Halimeda macroloba</i>	2.13 ± 0.02	1.53 ±0.05	1.84±0.01
16	<i>Halimeda tuna</i>	4.332 ± 0.05	nd	0.64±0.03
17	<i>Ulva lactuca</i>	7.654 ± 0.06	0.27 ±0.04	0.33±0.04
18	<i>Ulva reticulata</i>	9.584 ± 0.01	1.13 ±0.12	0.86±0.01

Note: mg/100gm of dry weight and ± Standard Deviation (n = 4). n.d., not detected

Table 2: Quantity of Vitamin B1, B2, B3, B5, B6, B7, B9, and B12 of Green Algal seaweeds

S.No	Seaweeds	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin B5	Vitamin B6	Vitamin B7	Vitamin B9	Vitamin B12
		mg/100gm of dw.							µ/ gm of dw
1	<i>Caulerpa chemnitzia</i>	0.44 ± 0.03	0.33 ± 0.02	0.73 ± 0.02	0.33±0.03	0.12 ± 0.03	0.05±0.01	1.05 ±0.01	0.35 ±0.02
2	<i>Caulerpa peldala</i>	0.13 ± 0.02	nd	0.09 ± 0.01	0.34± 0.06	0.11 ± 0.01	nd	1.04±0.02	0.42 ±0.03
3	<i>Caulerpa racemosa</i>	0.14 ± 0.01	0.29 ± 0.02	0.29 ± 0.05	0.64±0.01	0.12 ± 0.02	0.23±0.05	1.21±0.15	0.32 ±0.01
4	<i>Caulerpa scalpelliformis</i>	0.12 ± 0.04	0.49 ± 0.02	0.23 ± 0.06	0.12±0.07	0.11 ± 0.01	0.33±0.01	0.63±0.21	0.11 ±0.01
5	<i>Caulerpa serrulata</i>	nd	0.11 ± 0.01	0.16 ± 0.03	0.69±0.06	nd	0.02±0.02	1.02±0.01	nd
6	<i>Caulerpa sertularioides</i>	0.11 ± 0.01	0.31 ± 0.02	0.19 ± 0.06	0.05± 0.03	0.22 ± 0.06	0.31±0.01	1.21±0.14	0.45 ±0.03
7	<i>Caulerpa taxifolia</i>	0.14 ± 0.02	0.46 ± 0.06	nd	0.64±0.01	0.11 ± 0.01	0.15±0.03	0.35±0.12	0.31 ±0.03
8	<i>Caulerpa veravalensis</i>	0.22 ± 0.06	0.45 ± 0.01	0.39 ± 0.02	0.31±0.02	0.19 ± 0.06	0.88±0.04	0.88±0.02	0.47 ±0.04
9	<i>Cheatomorpha aerea</i>	0.15 ± 0.04	nd	0.07 ± 0.01	nd	nd	0.62±0.05	nd	0.17 ±0.01
10	<i>Cheatomorpha antennina</i>	0.37 ± 0.01	0.32 ± 0.11	0.26 ± 0.05	0.5 ±0.06	nd	0.85±0.02	0.85±0.03	0.07 ±0.02
11	<i>Codium adhaerens</i>	0.15 ± 1.02	0.66 ± 0.03	0.64 ± 1.02	0.7 ±0.04	0.32 ± 0.04	0.05±0.06	1.05±0.02	0.09 ±0.03
12	<i>Codium tomentosum</i>	0.23 ± 1.02	0.11 ± 0.04	0.81 ± 1.04	0.12±0.05	0.12 ± 0.01	nd	2.07±0.01	nd
13	<i>Enteromorpha compressa</i>	0.14 ± 1.07	0.54 ± 0.06	0.12 ± 0.02	nd	0.22 ± 0.04	0.83±0.04	1.87±0.01	0.12 ±0.06
14	<i>Enteromorpha intestinalis</i>	0.22 ± 0.02	nd	0.41 ± 0.03	0.33±0.01	0.41 ± 0.02	0.26±0.04	0.86±0.06	0.14 ±0.05
15	<i>Halimeda macroloba</i>	0.16 ± 0.06	0.62 ± 0.03	nd	0.16±0.03	nd	0.84±0.01	1.84±0.01	nd
16	<i>Halimeda tuna</i>	0.35 ± 0.01	0.23 ± 0.04	0.31 ± 0.01	0.04±0.02	0.06 ± 0.05	0.63±0.12	0.64±0.03	0.27 ±0.01
17	<i>Ulva lactuca</i>	0.71 ± 0.02	0.43 ± 0.05	0.21 ± 0.05	0.03±0.01	nd	0.32±0.03	0.33±0.01	0.13 ±0.12
18	<i>Ulva reticulata</i>	0.64 ± 0.03	0.62 ± 0.03	nd	0.14±0.04	nd	0.26±0.14	0.86±0.04	0.08 ±0.23

CONCLUSION

Marine algae are emerging as a good source for bioactive substances in pharmaceutical industry and also as a source of food and other health aspects. The vital nutrient components such as vitamin A (beta carotene, retinol) vitamin B1 vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxin), vitamin B7 (biotin), folic acid (B9), vitamin B12 (cobalamin), vitamin C (ascorbic acid) and vitamin E were qualitatively and quantitatively measured with different algal species.

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***Corresponding Author:**

Dhandapani.R*

Email: paniroever2007@rediffmail.com