International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 20-11-2020; Accepted: 06-12-2020: Published: 15-12-2020
www.botanyjournals.com
Volume 5; Issue 6; 2020; Page No. 612-616

Detection and estimation of phytochemical of *Enteromorpha* species collected from coastal areas of Ramanathapuram district, Tamil Nadu, India

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Abstract
The present study on the phytochemical constituents like alkaloids, flavonoids, phenols, proteins and free amino acids, saponins, sterols, terpenoids, coumarin, glycosides, quinones, and tannin were qualitatively investigated in the selected species of *Enteromorpha*. The biochemical composition of protein, carbohydrate, lipid, and photosynthetic pigments like chlorophyll and carotenoid were quantitatively estimated. The phytochemical, data reveals there is no significant variation among species. Coumarin, glycosides, quinine and tannins were negative in all *Enteromorpha* species and others are positive. The quantitative investigated results of phytochemicals reveals less significantly varies among in the all four selected species of *Enteromorpha.* All values are expressed in percentage; ± mean value represents standard deviation. The maximum alkaloid percentage was 2.87 in *E. compressa and maximum flavanoid* is 1.66% in *E. Intestinalis. E. prolifera found to have maximum sugar, lipid and protein content (6.75, 2.12 and 7.97%).* The phenol level (0.66%) is higher in *E. Intestinalis. E. flexuosa* comparatively moderate in alakloids, sugar and protein and least among flavanoids, lipids and phenols than other species. All the four species have more than 0.4 mg/g of total chlorophyll and 0.3 mg/g of carotenoids. The maximum total chlorophyll content was 0.492 mg/g in *E. Intestinalis* and maximum carotenoid is 0.395 mg/g in *E. prolifera*.

Keywords: carotenoids, chlorophyll, flavanoids, seaweed, marine

Introduction

Research findings reveal that incorporation of seaweed meal in bioorganic fertilizer has resulted in improved growth and yield performance, better nutrient efficiency, and plant growth promotion properties due to rich source of bioactive compounds (Faulkner, 2001) [5]. The FAO (2014) [4] reported that 38% of seaweeds in the 2012 used in foods and beverages. They can produce many numbers of metabolites which are characterized by a broad spectrum of biological activities. They have traditionally been used as nutritive and characteristic taste foods in Asia, and the freshly dried seaweeds are being extensively consumed, particularly by people living in coastal areas. They are also used for the production of valuable chemical compounds; alginates, agar, carrageenan, and polysaccharides. Edible Chlorophyta species have 16-22.1% of protein, 12.4-18.7% of ash, and 43.4-60.2% of carbohydrate as a percentage of dry matter. Common edible seaweeds *Enteromorpha* species are often growing on the rocks of intertidal zones, on the gravel of mud beaches, and widely distributed in the oceans of the world (Yimin Qin, 2018) [15]. Wang *et al.*, (2013) [14] reported that *Enteromorpha* algae as a nutritious and low-calorie food with rich in essential amino acids, fatty acids, vitamins, dietary fiber, and resistant protein of humans. *Enteromorpha* also contains chlorophyll b and different kinds of minerals like calcium, magnesium, iron, etc. Chemical analysis exhibited that *Enteromorpha* species has 9-14% protein, 32-36% ash content, and also n-3 fatty acids and n-6 fatty acids are 10.4 and 10.9g/100 g of total fatty acid respectively (Aguilera-Morales *et al*., 2005) [1]. Chao Zhao *et al*., (2016) [2] reported that the polysaccharide like carbohydrate polymers are used food, cosmetic and pharmaceutical industries to microbiology and biotechnology. This type of polysaccharide is a major property of *E. prolifera*. The species of *E. prolifera* has many nutritional compounds and polysaccharides which are having attracted extensive interest due to their multiple biological activities. The uses of polysaccharide compounds play a nutritional role as dietary fiber and also biological activities like gelling abilities (Chao *et al*., 2016) [2]. *Caulerpa serrulata* and *E. prolifera* were similar percentages (12-15%) of protein which equals that of cereals like oats (13.3%) (Morales de Leon *et al*., 2000) [9]. *E. intestinalis* - Sulfated polysaccharide reduced tumour mass; increased thymus and spleen mass; increased TNF-α, NO, and reactive oxygen species. Jiao *et al*. (2009) [6]. *E. intestinalis* - Methanol extract Antiproliferative (Paul and Kundu, 2013) [11]. *E. compressa* was observed in Mandapam coast (Kaliaperumal *et al*., 2002) [7]. Vasanthi and Rajamanikam (2003) [13] reported that the differentiation in the chemical constituents of the marine red alga in Tuticorin and Mandapam coast. Dinesh *et al*., (2007) [3] found out the nutritive properties of 20 species of seaweeds from the Gulf of Mannar. The present study was carried out the analysis of phytochemical, biochemical and pigments with four marine seaweeds; *E. compressa* (Linnaeus) Nees, *E. flexuosa* (Wulfen) J. Agardh, *E. intestinalis* (Linnaeus) Nees and *E. prolifera* (Müller) J. Agardh., are belonging to the family Ulvaceae.

Materials and Methods

Sample collection

Fresh plants of *E. compressa*, *E. flexuosa*, *E. intestinalis*, and *E. Prolifera* were collected from the intertidal regions of Ramanathapuram district, Tamil Nadu, India and then they were brought to the laboratory. The algae were washed thoroughly with tap water to remove extraneous materials. The well-washed samples were dried in the oven at 37 °C, till constant weight. The dried samples were individually ground in an electric mixer (Lima-Filho *et al*., 2002) [8]. The powdered samples were then stored in the refrigerator until use.

Phytochemical screening

The stored samples were subjected to a qualitative test for the investigation of phytochemical content with the standard procedures. The seaweed powder extracts were tested for alkaloids, flavonoids, glycosides, phenolic groups, sugars, saponins, steroids, tannins, protein, and amino acids and terpenoids.

Preparation of extracts

The seaweed specimens were washed thoroughly, placed on blotting paper, and spread out at 37 ±2 °C in the shade condition for drying. The shade dried samples were grounded to a fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 3g powdered samples were packed in Soxhlet apparatus and extracted with ethanol for 12 hours.

Qualitative phytochemistry (Trease and Evans, 2002) [12]

Detection of Alkaloids: 1ml of 1% HCl was added to the 2ml of extract in a test tube and was treated with a few drops of Mayer's reagent. A creamy white precipitate indicated the presence of alkaloids.

Detection of Flavonoids: Five drops of 1% NH_3 solution was added to 2 ml of extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

Detection of Glycosides: 2ml of 50% H_2SO_4 was added to the 2ml of extract in a boiling tube. The mixture was heated in a boiling water bath for 5 min. 10ml of Fehling's solution was added and boiled. A brick-red precipitate indicated the presence of glycosides.

Detection of Phenolic groups: To 1ml extract, add 2ml distilled water followed by a few drops of 10% Ferric chloride. The formation of either blue or black colour showed the presence of phenolic groups.

Detection of sugars: 5-8 drops Fehling's solution was added to 2ml extract. The mixture was heated in a boiling water bath for 5 min. A precipitate formed with either red or brick colour showed the presence of sugar.

Detection of Saponins: About 2 ml of the extract was shaken vigorously with 5ml distilled water to obtain stable persistent foam. The formation of emulsion indicated the presence of saponins.

Detection of Steroids: About 0.5 ml of hot acetic anhydride was added with 2ml of ethanol extract. The mixture was treated with Libermann reagent. The appearance of a ring of blue-green showed the presence of sterol and steroids.

Detection of Tannins: 1ml of distilled water and 1-2 drops of ferric chloride solution was added in 2 ml extract, and observed for brownish green or a blue-black coloration.

Detection of Terpenoids: 2ml of $CHCl_3$ was added in 2ml extract in a test tube. And then, 3 ml concentrated H_2SO_4 was carefully added along the wall of the test tube to form a layer. An interface with a reddish-brown coloration has confirmed the presence of terpenoids.

Detection of Coumarin: For coumarin identification, 1 ml of extract, 1 ml of 10% NaOH was added. The formation of the yellow colour indicates the presence of coumarins.

Detection of Quinines: For quinine identification, 1 ml of extract, 1 ml of concentrated sulphuric acid was added. The formation of red color indicated the presence of quinones.

Detection of Proteins: To a 2ml of ethanolic extract, 1ml of 40% NaOH solution was added and the added 2 drops of 1% CuSO4 solution. The presence of a peptide linkage of the molecule was indicated by the violet color which showed the presence of protein.

Detection of Amino Acids: To 2 ml of ethanolic extract, 2 ml of Ninhydrin reagent was added and laid in a water bath for about 20 minutes. The visual aspect of purple color formed indicated the presence of amino acids.

Estimation of Protein

The protein was estimated by Biurette method. To 2 mg of dried algal powdered sample, 2ml of distilled water followed by 4 ml of biurette reagent were added and incubated for 30 minutes at room temperature. Then the mixture was centrifuged for 15 minutes at 4000 rpm. The supernatant solution was pooled and the optical density was taken at 540 nm in a spectrophotometer.

Estimation of Lipid

The lipid estimation was carried out by using a solvent mixture of the chloroform-methanol (2:1). About 10 mg of dried algal powder sample was taken in a test tube and added 5 ml of chloroform-methanol (2:1) mixture. The test tubes with the mixture were closed with aluminum foil and then incubated at 37 ± 2 °C in 24 hrs. After the incubation, the sample mixture was filtered by using a Whatman No.1 filter paper. The filtrate was collected and pooled in a 50 ml preweighed beaker, which was kept on a hot plate until the solvent evaporated. The residue with beaker was weighed and calculated to know the total crude lipid of the sample.

Estimation of Sugar

The total sugar content of the algal sample was estimated by using the method described. A known quantity of the sample was taken in pestle and mortar, added 80% ethanol in it and then ground well and centrifuged at 4000 rpm. About 5 ml of the supernatant was taken in a test tube; 5 ml of anthrone reagent was added in it. The tube was kept in a boiling water bath for 20 min. After that, it was kept in a dark room for another 20 minutes, and then it was read in a spectrophotometer at 650 nm.

Estimation of Chlorophyll

The amount of chlorophyll present in the freshly collected seaweed was analyzed by Uv spectroscopic method. About 500 mg of a fresh sample of seaweed was kept in a pestle and mortar with an adequate amount of 80% acetone and then it was ground well. The homogenate liquid was centrifuged at 3000 rpm for 10 minutes and the supernatant was stored. The pellet also was extracted by repeated washing with 80% acetone till it turned into colourless. The extracts were pooled and subjected to determining the chlorophyll content. The extract absorbance was observed at 645 nm and 663 nm in a spectrophotometer. The chlorophyll content was calculated by using the following formula

$$\text{Chlorophyll 'a' (mg/g. fr.wt.)} = \frac{12.7 \times A663 - 2.69 \times A645}{a \times 1000 \times W} \times V$$

$$\text{Chlorophyll 'b' (mg/g. fr.wt.)} = \frac{22.9 \times A645 - 4.68 \times A663}{a \times 1000 \times W} \times V$$

$$\text{Total Chlorophyll (mg/g. fr.wt.)} = \frac{20.2 \times A645 + 8.02 \times A663}{a \times 1000 \times W} \times V$$

Where A = Absorbance at respective wavelength = Volume of extract (ml), W = Fresh weight of the sample (g)

Estimation of Carotenoid

The same algal chlorophyll extract was used to measure carotenoid. The extract OD was taken at 480 nm. Carotenoid: µg/g.fr.wt. = A.480 + (0.114 X A. 663) – (0.638 X A. 645).

Where A = Absorbance at the respective wavelength

Results and Discussion

Phytochemicals analysis

The present study exploited qualitative and quantitative significance among the the four collected seaweed comes under Enteromorpha spp. Totally 12 important phytochemicals namely alkaloids, glycosides, coumarins, flavonoids, phenols, protein and amino acids, quinones, saponins, sterols, sugar, tannin, and terpenoids were qualitatively tested in the selected four species of a genus *Enteromorpha* (Table -1). Among the 12 phytochemicals, coumarin, glycosides, quinone, tannin were absent, the remaining 8 phytochemicals were detected in the selected all the four species of *Enteromorpha*. The most of brown and red algal seaweeds are having agar, alginic acid, laminarin, fucoidan, galactans, carrageenan, xylan, and mannans naturally, but in the green algal seaweeds mostly the presence of alkaloids, flavonoids, phenol, protein and amino acid, sterols, sugar, and terpenoids. Some of the phytochemicals like coumarin, glycosides, quinone, and tannin were absent in all the four algal samples. Alkaloid was present in all the selected 4 species, among the 4 species; *E. compressa* had the maximum of alkaloid 2.87% than the remaining three species (Table -2). Flavonoid was also present in all the four species of *Enteromorpha* with a range of 1.36 to 1.66%. The phenol compound was present maximally in *E. intestinalis* with 0.66% and a minimum of 0.25% in *E. flexuosa* (table -2). Biochemical of protein, sugar, the lipid content of the selected four green algal species of *Enteromorpha* were quantitatively estimated. The sugar, protein, and lipid were maximally 7.97, 6.75, and 2.12% in *E. prolifera* respectively. Protein and sugar were minimally 6.78 and 5.77% respectively in *E. compressa* and lipid was 1.12% in *E. flexuosa* which was less than the remaining three species (Figures: 1). This present four species results showed the similarity with the early reports. In the present study, the maximum protein content was recorded in the green alga *E. prolifera*, and the minimum in the green alga *E. compressa*. Similarly, Dinesh *et al*., (2007) [3] recorded the highest protein content in brown alga *Tubinaria ornata* from the Gulf of Mannar region near to Rameswaram. These reports are supportive of the present study that the green algal seaweeds which were equal to brown algal seaweed than red algal seaweeds. The present study showed the lipid content of *Entromorpha* species was significantly equal to green algae *C. adharens* and *U. fasciata* (Muthuraman and Ranganathan, 2004) [10].

Photosynthetic pigments

The table 3 revealed the algal pigments like chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll and carotenoid were present in all the four species of *Enteromorpha* and they were estimated quantitatively. *E. Compressa* showed 0.347≥0.115≥0.462 mg/g of Chlorophyll a≥ Chlorophyll a≥ Total Chlorophyll≥ and 0.375 µg/g of Carotenoids. The content of Chlorophyll a, Chlorophyll b and Total Chlorophyll in *E. flexuosa is* 0.315≥0.127≥0.442 mg/g and Carotenoids is 0.386 µg/g. Similarly chlorophyll (mg/g) and carotenoid (µg/g) values among *E. Intestinalis* 0.394≥0.098≥0.492≥0.322 and 0.353≥0.091≥0.444≥0.395 for *E. prolifera.* the highest chlorophyll a in *E. intestinalis* with 0.394 ± 0.025 mg/g fr.wt, and the highest chlorophyll b in *E. flexuosa* with 0.127 mg/g of fr.wt. The species *E. intestinalis* had the highest total chlorophyll (0.492mg/g of fr.wt.) and *E. prolifera* had the maximum of carotenoid (0.395 µg/g of fr. wt.) The chlorophyll content of green alga *E. intestinalis* was equal to green alga *Caulerpa scalpeliformis*, and less than to red alga *Acanthafera spicifera* (Muthuraman and Ranganathan, 2004) [10]. Muthuraman and Ranganathan (2004) [10] estimated that the maximum carotenoid content in the brown seaweed which was higher than the selected species of *Enteromorpha.*

Conclusion

The phytochemical, biochemical, and photosynthetic pigments were analyzed with four species of *Entromorpha* which were collected from the intertidal coastal regions of Ramanathapuram district, Tamil Nadu, India. From the present study, it is evident that marine macroalgae like *Enteromorpha compressa, E. flexuosa, E. intestinalis, E. prolifera,* are high in nutritive properties. The present findings will be useful for future bioproduct productions such as cosmetics, skincare products, food industrial products, and pharmaceutical industrial and also very useful to feed for cattle and birds.

Table 1: Qualitatively phytochemical screening of marine Enteromorpha species

Phytochemicals	*E. compressa*	*E. flexuosa*	*E. intestinalis*	*E. prolifea*
Alkaloids	+	+	+	+
Coumarin	-	-	-	-
Flavanoids	+	+	+	+
Glycosides	-	-	-	-
Phenol	+	+	+	+
Protein and Amino acid	+	+	+	+
Quinone	-	-	-	-
Saponin	+	+	+	+
Sterols	+	+	+	+
Sugar	+	+	+	+
Tannin	-	-	-	-
Terpenoids	+	+	+	+

Note: + (present), - (absent)

Table 2: Quantification of biomlecules from Enteromorpha species

Biochemical (Result in %)	*E. compressa*	*E. flexuosa*	*E. Intestinalis*	*E. prolifera*
Alkaloids	2.87 ± 0.02	2.77 ± 0.01	2.67 ± 0.05	2.09 ± 0.04
Flavonoids	1.45 ± 0.05	1.36 ± 0.04	1.66 ± 0.08	1.46 ± 0.05
Lipids	1.33 ± 0.02	1.12 ± 0.11	1.45 ± 0.31	2.12 ± 0.05
Phenols	0.36 ± 0.02	0.25 ± 0.02	0.66 ± 0.05	0.55 ± 0.07
Proteins	6.78 ± 0.22	7.23 ± 0.04	7.55 ± 0.15	7.97 ± 0.02
Sugar	5.77 ± 0.01	6.55 ± 0.03	5.88 ± 0.23	6.75 ± 0.14

Table 3: Estimation of pigments of Enteromorpha spp extract.

Algal Pigments (mg/g of fr.wt.)	*E. Compressa*	*E. flexuosa*	*E. intestinalis*	*E. prolifera*
Chlorophyll a	0.347 ± 0.031	0.315 ± 0.021	0.394 ± 0.025	0.353 ± 0.052
Chlorophyll b	0.115 ± 0.011	0.127 ± 0.022	0.098 ± 0.023	0.091 ± 0.015
Total Chlorophyll	0.462 ± 0.042	0.442 ± 0.043	0.492 ± 0.048	0.444 ± 0.067
Carotenoids (µg/g.fr.wt)	0.375 ± 0.022	0.386 ± 0.025	0.322 ± 0.011	0.395 ± 0.255

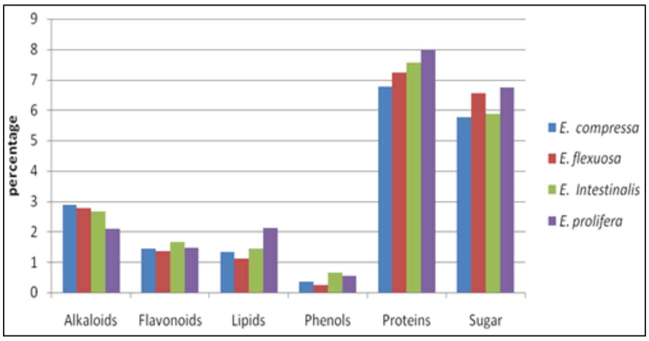

Fig 1: Percentage of major biocomponents among *Enteromorpha* spp

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