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Research Article

Phytochemical Assessment of Enteromorpha

Phytochemical Assessment of Certain Seaweeds of Enteromorpha Species in Coastal Regions of Ramanathapuram, Tamil Nadu, India

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Abstract: Freshly dried seaweeds are being extensively consumed, particularly by peoples living in coastal areas. *Enteromorpha* algae are nutritious and low-calorie food rich in essential Amino acids, Fatty acids, Vitamins, Dietary fiber and Resistant protein of humans. *E. prolifera* has many nutritional compounds and polysaccharides that have attracted extensive interest due to their numerous biological activities. The current studies for uses some Phytochemical components such as Alkaloids, Flavonoids, Phenols, Proteins, free Amino acids, Saponins, Sterols, Terpenoids, Coumarin, Glycosides, Quinones and Tannins are evaluated on the chosen *Enteromorpha intestinalis* species, including *E. flexuosa*, *E. intestinalis* and *E. prolifera*. In addition, Protein, Carbohydrate, Fat and photosynthetic pigments like chlorophyll and carotenoids were also quantitatively determined.

Keywords: *Enteromorpha* species, Phytochemical Assessment, Selected seaweeds.

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1. INTRODUCTION

Algae is known as seaweeds, which are classified permitting to their natural pigments; green, brown, and red seaweeds. Seaweeds are recognized as a source of bioactive compounds.[16] The numbers of metabolites considered by a broad spectrum of biological activities and traditionally used as nutritive specific taste foods in Asia. The freshly dried seaweeds are being extensively consumed, particularly by people living in coastal areas[13]. The invention of valuable chemical compounds; Alginates, Agar, Carrageenan and polysaccharides[11]. These seaweeds are used in food, pharmaceutical and other industries to enhance the nutritional value of food, stabilize emulsions and make products more palatable[19]. They are also used to thicken and stabilize food products, improve the texture of ice cream, and even as a health supplement due to their high concentration of vitamins and minerals[20]. These compounds are known as emulsifiers and they work by binding oil and water molecules together, creating a homogenous mixture. This allows for the uniform dispersion of fat-soluble vitamins and minerals, improving the product's overall nutritional value[21]. They also help to prevent spoilage and extend the shelf life of food products [22]. *Enteromorpha* is nutritious and low-calorie food rich in essential Amino acids, Fatty acids, Vitamins, Dietary fiber and Resistant protein of humans[8]. *Enteromorpha* also contains chlorophyll b and other minerals like Calcium, Magnesium, Iron, etc., Chemical analysis exhibited that *Enteromorpha* species have protein, ash content and total Fatty acids are respectively. They stated that polysaccharides like carbohydrate polymers are used in food, cosmetic and pharmaceutical industries, microbiology, and biotechnology. This type of polysaccharide is a major property of *E. prolifera. E. prolifera* has many nutritional compounds and polysaccharides that have attracted extensive interest due to their numerous biological activities. Polysaccharide compounds use plays a nutritional role as dietary fiber and biological activities like gelling abilities. They stated that *Caulerpa serrulata* and *E. prolifera* were similar percentages of protein which equals that of cereals like oats. *E. intestinalis*- Sulfated polysaccharide reduced tumor mass; increased thymus and spleen mass; increased TNF-α, NO, and reactive oxygen species. *intestinalis* - Methanol extract Antiproliferative[17].

The findings suggest that *Caulerpa serrulata* and *E. prolifera* contain proteins that are comparable in quality to those found in cereals like oats. Furthermore, they demonstrate that the sulfated polysaccharide in *E. intestinalis* has the potential to reduce tumor mass and increase thymus and spleen mass. In contrast, the methanol extract of this species has shown anti-proliferative activity. Moreover, the study highlights the potential therapeutic effects of these seaweeds, suggesting they could be beneficial in the treatment of cancer and other related diseases[23]. However, more research is needed to determine the long-term effects of these seaweeds on cancer and other diseases. Additionally, it is unclear whether the benefits of these seaweeds outweigh the risks. *E. compressa* was observed in Mandapam coast regions Vasanthi and Rajamanikam (2003)[5-17]are reported the differentiation in the chemical constituents of the marine red algae in Tuticorin and Mandapam coast found out the nutritional properties of 20 species of seaweeds from the Gulf of Mannar[2]. The present study carried out the analysis of phytochemical and biochemical pigments such as four marine seaweeds; *E. compressa* (Linnaeus) Nees, *E. flexuosa* (Wulfen) *J. Agardh*, *E. intestinalis* (Linnaeus) Nees and *E. prolifera* (Müller) and *J. Agardh* are belonging to the family Ulvaceae.

2. MATERIALS AND METHODS

2.1. *Sample collection*

Fresh plants of *E. compressa, E. flexuosa, E. intestinalis, and E. prolifera* were collected from the intertidal regions of Ramanathapuram District of 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.[7] The powdered samples were then stored in the refrigerator until use. Plant Authentication is done by P. Sona, Research scholar & Dr. G. Subramanian, Head of the Department of Botany, Arignar Anna Govt. Arts College, Namakkal. The plants were stored with voucher numbers AAGACNTN021 (*Enteromorpha compressa),* AAGACNTN022 (*E. flexuosa),* AAGACNTN023 (*E.intestinalis),* AAGACNTN024 (*E.Prolifera).*

Credit and Source: Google Maps

The Mandapam google satellite map. This place is situated in Ramanathapuram, Tamil Nadu, India, its geographical coordinates are 9° 17' 0" North, 79° 7' 0" East, and its original name are Mandapam.

2.2. Distribution

It can be found in the Bering Sea near Alaska, the Aleutian Islands, Puget Sound, Japan, Korea, Mexico, the Philippines, and Russia. Besides this, it can be found in Israel and European countries such as the Azores, Belgium, Denmark, Ireland, Norway, Poland and the Baltic and Mediterranean Seas. It is also found on the Pacific Ocean's shores, including in New Zealand.

2.3. Description

The fronds have branches are completely tubular, expanding in width to mid-thallus, reaching 15 cm long or more. The cells are irregularly arranged, and the chloroplast is hood-shaped and placed to one side, generally with only one pyrenoid. The species may be 10–30 cm (3.9–11.8 in) long and 6–18 millimeters (0.24–0.71 in) wide. They have rounded tips as well. The algae may be reproductive at all times of the year and has a life cycle with an alternation of generations. The gametophyte and saprophytes are isomorphic, having identical morphology. In some references, the species (*Ulva intestinalis*) is treated as two subspecies: ssp. *intestinalis* (L.) Link and ssp. *Compressa*(L.)[34,35].

2.4. Medicinal properties of Enteromorpha

Enteromorpha is used against goiter and scrofula as an antipyretic to prepare a refreshing liquid and treat sunstroke, bronchitis, cough, and asthma. It is also applied as fish bait. In addition, the dried and crushed fronds can be used as a topping for many foods, in soups, and as a coating. *Enteromorpha* is rich in nutrients with medicinal and health-promoting effects. From a nutritional standpoint, the main properties of sea lettuce are its richness in polysaccharides, protein and Amino acids, Fatty acids, Minerals and Vitamins. Therefore, their nutritional value makes them valuable food supplements[36,37].

2.5. Phytochemical screening

The stored samples were subjected to a qualitative test to examine phytochemical content with the standard procedures[4]. The seaweed powder extracts were tested for Alkaloids, Flavonoids, Glycosides, Phenolic groups, Sugars, Saponins, Steroids, Tannins, Protein, Amino acids and Terpenoids.

2.6. Preparation of extracts[38]

The seaweed specimens were washed thoroughly, placed on blotting paper, and spread out at 37 ±2°C in the shade condition for drying. Next, 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. Finally, 3g powdered samples were packed in the Soxhlet apparatus and extracted with ethanol for 12 hours.

1. **Test for Alkaloids:** 1ml of 1% HCl was added to the 2ml extract in a test tube and treated with a few drops of Mayer's reagent. A creamy white precipitate indicated the presence of alkaloids.
2. **Test for Flavonoids:** Five drops of 1% NH_3 solution were added to 2 ml of extract in a test tube. A yellow coloration was observed for the presence of flavonoids.
3. **Test for 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. Next, 10 ml of Fehling's solution was added and boiled. A brick-red precipitate indicated the presence of glycosides.
4. **Test for Phenolic groups:** To 1ml extract, add 2ml distilled water followed by a few drops of 10% Ferric chloride. Either blue or black color formed showed the presence of phenolic groups.
5. **Test for sugars:** 5-8 drops of 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 color showed the presence of sugar.
6. **Test for Saponins:** About 2 ml of the extract was shaken vigorously with 5 ml distilled water to obtain stable, persistent foam. The formation of emulsion indicated the presence of saponins.
7. **Test for Steroids:** About 0.5 ml of hot acetic anhydride was added with 2 ml 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.
8. **Test for Tannins:** 1 ml of distilled water and 1-2 drops of ferric chloride solution were added in 2 ml extract and observed for brownish green or a blue-black coloration.
9. **Test for Terpenoids:** 2ml of $CHCl_3$ was added in 2ml extract in a test tube. And then, 3 ml of concentrated H2SO4 was carefully added along the test tube wall to form a layer. An interface with a reddish-brown coloration has confirmed the presence of terpenoids.
10. **Test for Coumarin:** For coumarin identification, 1 ml of extract and 1 ml of 10% NaOH were added. The formation of the yellow color indicates the presence of coumarins.
11. **Test for Quinines**: Forquinine identification, 1ml of extract, and 1ml of concentrated sulphuric acid were added. The formation of red color indicated the presence of quinones.
12. **Test for Proteins:** To a 2ml of ethanolic extract, 1ml of 40% NaOH solution was added, and two drops of 1% CuSO4 solution were added. The presence of a peptide linkage of the molecule was indicated by the violet color, which showed the presence of protein.
13. **Test for 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 the purple color formed indicated the presence of amino acids.

2.7. Estimation of total Protein content

The protein was estimated by the Biurette method (Raymont *et al.*, 1964) [12]. To 2 mg of dried algal powdered sample, 2 ml of distilled water followed by 4 ml of burette reagent were added and incubated for 30 minutes at room temperature. Then the mixture was centrifuged for 15 minutes at 4000 rpm. Finally, the supernatant solution was pooled, and the optical density was taken at 540 nm in a spectrophotometer.

2.8. Estimation of total Lipid content

The lipid estimation was carried out using a solvent mixture of chloroform-methanol (2:1). 3 10 mg of dried algal powder sample was taken in a test tube, and 5 ml of chloroform-methanol (2:1) mixture was added. The test tubes with the mixture were closed with aluminum foil and then incubated at 37 ± 2°C for 24 hrs. After the incubation, the sample mixture was filtered using a whatman No.1 filter paper. The filtrate was collected and pooled in a 50 ml pre weighed beaker, which was kept on a hot plate until the solvent evaporated. The residue with the beaker was weighed and calculated to know the total crude lipid of the sample.

2.9. *Estimation of total Sugar content*

The algal sample's total sugar content was estimated using the method described by[14]. A known quantity of the sample was taken in pestle and mortar, added 80% ethanol was, 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. The tube was kept in a boiling water bath for 20 min. After that, it was kept in the darkroom for another 20 minutes and then read in a spectrophotometer at 650 nm.

2.10. *Estimation of total Chlorophyll content*

The amount of chlorophyll present in the freshly collected seaweed was analyzed by the method of Arnon[1]. 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. Next, the pellet was extracted by repeated washing with 80 % acetone until it turned colorless. Then, the extracts were pooled and subjected to determine 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)

2.11. *Estimation of total Carotenoid content*

The same algal chlorophyll extract was used to measure carotenoids. The extract OD was taken at 480 nm[6] Carotenoid (µg/g.fr.wt.) = A.480 + (0.114 X A. 663) – (0.638 X A. 645).

Where A = Absorbance at the respective wavelength

3. RESULTS AND DISCUSSION

3.1. *Qualitative Test of Phytochemicals analysis*

In this study, 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 and Tannin were absent, and the remaining eight phytochemicals were present in the selected four species of *Enteromorpha* (Table-1). The brownest and red algal seaweeds have 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 phytochemicals like Coumarin, Glycosides, Quinone and tannin were absent in all four algal samples (Table-1)

Table 1: Qualitative Phytochemical Screening of Marine Enteromorpha Species

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

Note: +++ (High), ++ (Moderate) - (Absent)

In the above table (Table 1), Alkaloids, Flavonoids, Phenol, Protein and Amino acids, Saponins, Sterols, Sugar and Terpenoids were present in all four species. But Coumarin Glycosides, Quinone, and Tannin were absent in all four species.

3.2. *Estimation of Biochemicals*

Among the four species, alkaloid was present in all the selected four species; *E. compressa*had the maximum Alkaloid of 2.84% than the remaining three species (Table -2) [24]. Flavonoid was also present in all four species of *Enteromorpha,* with a range of 1.33 to 1.63%. It is similar to early reports [25-27]. The phenol compound was present maximally in *E. intestinalis* with 0.66% and a minimum of 0.22% in *E. flexuosa* (Table -2). Biochemical protein, sugar, and the lipid content of the selected four green algal species of *Enteromorpha* were quantitatively estimated (Table -2). The Sugar, Protein and Lipids were maximally 7.94, 6.72, and 2.09 % in *E. prolifera,* respectively. Protein and sugar were minimally 6.78 and 5.77 %, respectively, in *E. compressa,* and lipid was 1.09% in *E. flexuosa*, less than the remaining three species (Table -2; Figures: 1- 4)[28]. This present four species results showed similarity with the early reports[9]. The present study recorded the maximum protein content in the green alga *E. prolifera* and the minimum in the green alga *E. compressa*. Similarly, recorded the highest protein content in brown algae *Tubinaria ornata* from the Gulf of Mannar regions near Rameswaram[2]. These reports support the present study that green algal seaweeds equal brown algal seaweed and red algal seaweeds. The present study showed the lipid content of *Entromorpha* species was significantly equal to green algae *C. adharens* and *U. fasciata.*[10]

Table 2: Protein, Sugar, and lipid contents of marine algal species of enteromorpha

Biochemical (Result in%)	*E.compressa*	*E. flexuosa*	*E.Intestinalis*	*E. prolifera*
Alkaloids	2.84 ±0.02	2.74 ± 0.01	2.64 ±0.05	2.06 ± 0.04
Flavonoids	1.42 ±0.05	1.33 ± 0.04	1.63 ±0.08	1.43 ± 0.05
Lipids	1.30 ±0.02	1.09 ± 0.11	1.42 ±0.31	2.09 ± 0.05
Phenols	0.33 ±0.02	0.22 ± 0.02	0.63 ±0.05	0.52 ± 0.07
Proteins	6.75 ±0.22	7.20 ± 0.04	7.52 ±0.15	7.94 ± 0.02
Sugar	5.74 ±0.01	6.52 ± 0.03	5.85 ±0.23	6.72 ± 0.14

All values are expressed in percentage; ± mean value represents standard deviation.

Alkaloids were found higher in *E. compressa,* Flavonoids higher in *E. Intestinalis,* Lipids higher in *E. prolifera,* Phenols higher in *E. Intestinalis* and Proteins higher in *E. prolifera.*

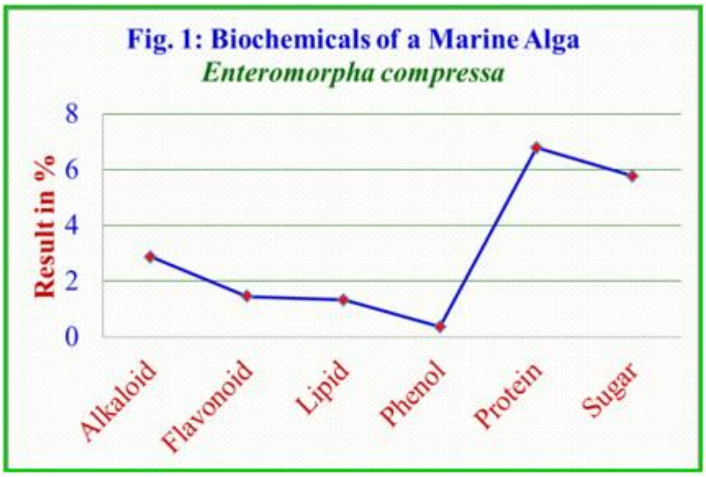

Fig 1: Biochemicals of a Marine Alga *Enteromorpha compressa*

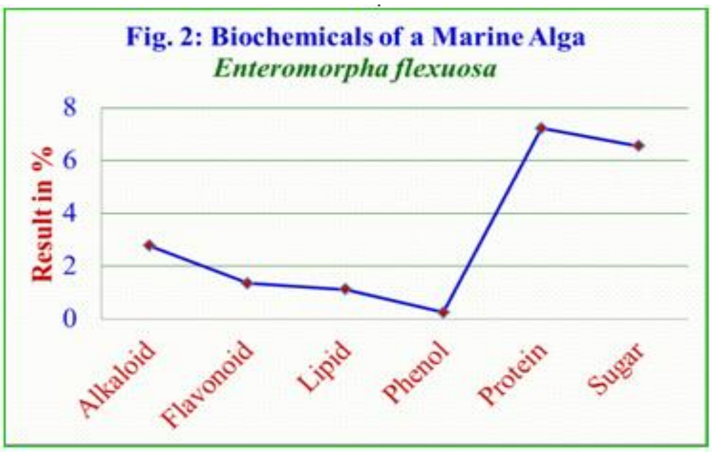

Fig 2: Biochemicals of a Marine Algae *Enteromorpha flexuosa*

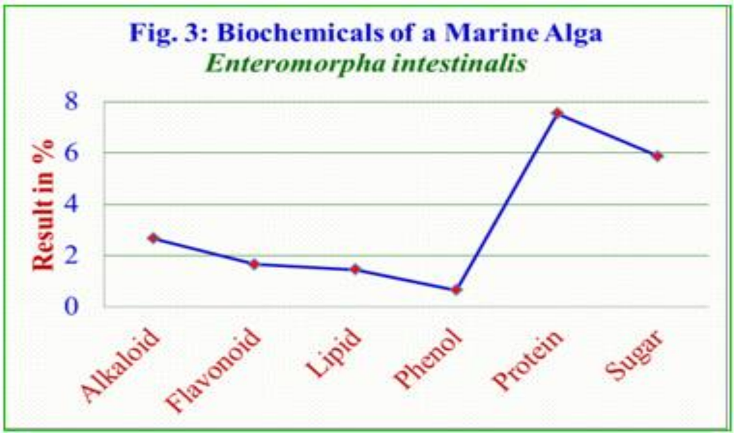

Fig 3: Biochemicals of a Marine Algae *Enteromorpha intestinalis*

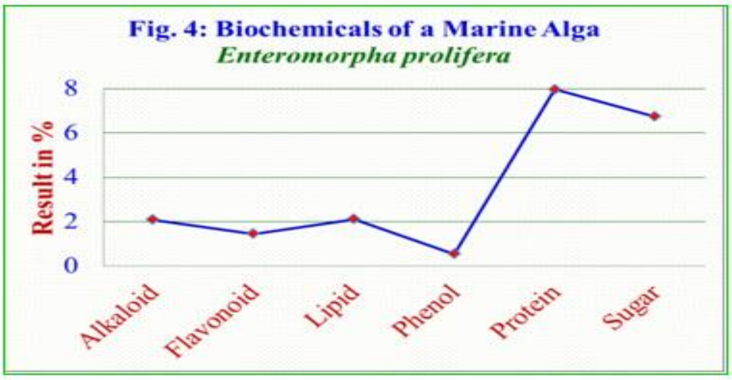

Fig 4: Biochemicals of a Marine Algae *Enteromorpha prolifera*

The sugar, protein, and lipid were maximally 7.94, 6.72, and 2.09 % in *E. prolifera*. Protein and sugar were minimally 6.78, and 5.77 %, respectively, in *E. compressa,* and lipid was 1.09% in *E. flexuosa,* which was less than the remaining three species (Table -2; Figures: 1- 4)

Table.3: pigments of four marine algal species of enteromorpha.

AlgalPigments (mg/g offr.wt.)	*E. Compressa*	*E. flexuosa*	*E.Intestinalis*	*E. prolifera*
Chlorophyll a	0.344 ± 0.031	0.312 ± 0.021	0.391 ± 0.025	0.350 ± 0.052
Chlorophyll b	0.112 ± 0.011	0.124 ± 0.022	0.095 ± 0.023	0.088 ± 0.015
TotalChlorophyll	0.459 ± 0.042	0.439 ± 0.043	0.489 ± 0.048	0.441 ± 0.067
Carotenoids(µg/g.fr.wt)	0.372 ± 0.022	0.383 ± 0.025	0.319 ± 0.011	0.392 ± 0.255

All values are expressed in percentage; ± mean value represents the standard deviation.

The table revealed that algal pigments like chlorophyll 'a', chlorophyll 'b,' total chlorophyll and Carotenoid were present in all four species of Enteromorpha and they were estimated quantitatively with the highest chlorophyll a in *E.*

intestinalis with 0.388±0.022mg/gfr.wt., and the highest chlorophyll b in E. flexuoua with 0.124 mg/g offr. Wt, which is similar to some earlier reports [18,29,30,31], 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.) (Table – 3).The chlorophyll content of green alga *E. intestinalis* was equal to green algae *Caulerpa scalpeliformis* and less than red alga *Acanthafera spicifera*[32,33]. Ten estimated that the maximum carotenoid content in the brown seaweed was higher than the selected species of *Enteromorpha.*

4. CONCLUSION

The phytochemicals, biochemical and photosynthetic pigments were examined with four species of *Entromorpha* which were collected from the intertidal coastal regions of Ramanathapuram District of Tamil Nadu, India. Phytochemicals were screened in all four species. Quantitatively analyzed biochemical was significantly distributed among the species. In addition, the photosynthetic pigments were quantified in all the species. The present study shows that marine macro algae, like *Enteromorpha compressa, E. flexuosa, E. intestinalis, and E. prolifera* are high in nutritive properties. In addition, the green algae *Enteromorpha* species observed a high lipid value. The present findings will be useful for future bio-product productions such as cosmetics, skincare products, industrial food products, and pharmaceutical industries and also very useful to feed cattle and birds.

5. AUTHOR CONTRIBUTION STATEMENT

Sona performed all of the tasks necessary for this study and the publication of this manuscript.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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