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STUDIES ON PHYTOCHEMICALS OF *ENTEROMORPHA COMPRESSA* (L.) NEES- A GREEN ALGA FROM RAMESHWARAM COASTLINE, TAMIL NADU, INDIA

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ABSTRACT

The study aimed to investigate the biochemicals of *E. compressa* in Rameshwaram Coastline, Tamil Nadu, India. The biochemical composition of total proteins, total carbohydrates, total phenols, total lipids, total chlorophylls, and total carotenoids was analyzed using the standard procedure in fresh algal material. The phytochemical screening of ethanol extract was analyzed using the standard procedure for fourier-transform infrared spectroscopy (FTIR). This study showed that the *E. compressa* contained a high level of carbohydrates (336 mg/g of fw), followed by proteins (204 mg/g of fw.), lipids (17.2 mg/g of fw), phenols (16.3 mg/g of fw.), chlorophylls (3.86 mg/g of fw) and low level of carotenoids (2.12 mg/g of fw). The presence of functional groups such as amides, phosphorus compound, alcohols, phenols, and halogen compounds was confirmed by FTIR. The biochemical study of *E. compressa* showed the presence of various phytochemicals. The studied green seaweed possesses several bioactive compounds and used as medicine and food.

KEYWORDS: Phytochemical, FTIR, Green Alga, *Enteromorpha compressa*, Rameshwaram.

INTRODUCTION

Seaweeds are known as marine macroalgae in habitats brackish water environment. Seaweeds are found in the intertidal and subtidal regions up to where photosynthetic light of 0.01% prevails and also in the coastal region between high tide and low tide. As the first organism in the marine food chain, seaweeds provide nutrients and energy for all other living organisms.[1,2] Seaweeds provide shelter and habitat for many coastal animals. Seaweeds are also traditionally consumed in a different part of the world. Recently human consumption of green algae (Chlorophyceae), brown algae (Phaeophyceae) and red algae (Rhodophyceae) is high in Asia, mainly in Japan, China, and Korea. Those countries, seaweeds are often utilized or taken as marine vegetables. Japanese are the main consumers with an average of 1.6 kg (dry weight) per year per capita.[3] The seaweed could be taken by humans as food and are resources of useful industrial products namely phycocolloids, carrageenan, alginates, and agar. Algal phycocolloids are being used in the food industry as a thickening and emulsifying agents. A few numbers of the algae are being used to prepare soil conditioner for horticulture. The remaining algae use include medicine, animal feed, cosmetics and fish bait.[4] Many research studies have been conducted to investigate the phytochemicals present in seaweeds especially carbohydrates, proteins, lipids, phenols.[5,6] The chemical composition of tropical seaweeds was estimated seasonally and reported that the protein content of green seaweeds was greater than the brown and red seaweeds. Furthermore protein content of seaweeds also found higher concentration in green seaweed compared with some higher plants. Lipid extracts of some edible seaweed showed antioxidant activity and synergistic effect with the tocopherol.[7] Seaweeds are also used as manure for agricultural and horticultural crops due to the presence of minerals, trace elements and plant growth regulators which occur in water-soluble form and enhances the disease resistance in field crops.[8-9] Hence, the present work was aimed to screen and evaluate the biochemical of *E. compressa* in Rameswaram coastline, Tamil Nadu, India.

MATERIALS AND METHODS

Collection of Sample

Enteromorpha compressa is green algal seaweed shows much attention in the recent years as it has the potential to supplement native vegetation. *E. compressa* was collected from Rameswaram coastline in the southeast coast of Tamil Nadu, India during January 2016. Samples were rinsed with seawater to remove debris and epiphytes. The entire epiphytes

were removed using a soft brush. In the laboratory, the seaweeds are once again washed in freshwater and stored in the refrigerator for further analysis.

Phytochemical of *Enteromorpha compressa*

Estimation of Biochemicals

The total carbohydrates[10], Proteins[11], lipids[12], phenols[13], chlorophylls[14] and carotenoids[15] were estimated by the standard method in fresh plant sample.

Phytochemical analysis

The different extracts were tested for alkaloids, anthraquinones, catechin, flavonoids, glycosides, phenolic groups, reducing sugars, saponins, steroids, tannins, and terpenoids. Phytochemical screening of the extracts was carried out according to the standard methods.[16]

Preparation of extracts

For the preparation of different extracts, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature 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, acetone, benzene, chloroform, petroleum ether and water for 12 hours separately.

1. Test for 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.

2. Test for Anthraquinones: About 2 ml extract was mixed with benzene and 1ml of 10% ammonia solution was added. The presence of a pink, red or violet color indicated the anthraquinones.

3. Test for Catechin: About 2 ml extract was mixed with enriching reagent and a few drops of Conc. HCl. Formation of pink colour showed the presence of catechin.

4. Test for 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.

5. 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. 10ml of Fehling's solution was added and boiled. A brick-red precipitate indicated the presence of glycosides.

6. Test for Phenolic groups: To 1ml extract, add 2ml distilled water followed by a few drops of 10% ferric chloride. The formation of blue or black colour indicates the presence of phenolic groups.

7. Test for Reducing 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 red-brick precipitate showed the presence of reducing sugars.

8. Test for 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.

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

10. Test for 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.

11. Test for 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.

FTIR analysis

Fourier-transform infrared spectroscopy analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups of phytochemicals. The peak values of the FTIR were recorded. Each analysis was repeated thrice and confirmed the spectrum.

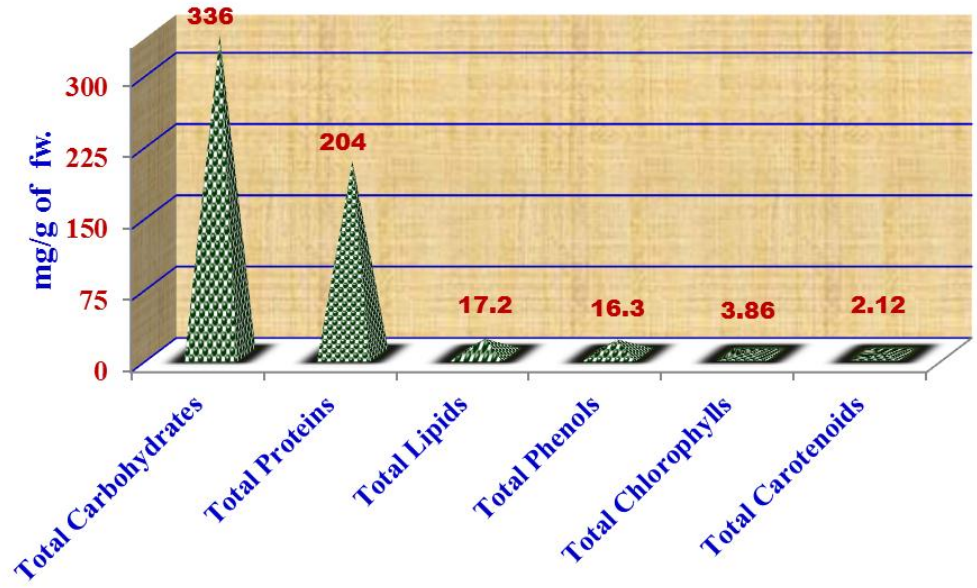
RESULT AND DISCUSSION

The amount of total carbohydrate, total protein, total lipid, total phenols, total chlorophylls and total carotenoids of *E. compressa* were presented in the Fig.1. The concentration of

carbohydrates was found to be 336 mg/g in fresh weight. In the present study, protein content showed a remarkable amount of 204mg/g. The total lipids and phenols contents in *E. compressa* were found relatively low compared to total carbohydrates and total proteins. The seaweed contains 17.2mg/g of lipids and 16.3mg/g of phenols (Fig. 1).

The total chlorophylls and total carotenoids were observed 3.86mg/g and 2.12mg/g respectively. Carbohydrate is one of the important components for metabolism and it supplies the energy needed for respiration and other most important processes. Proteins have essential roles in all the biological processes. The protein functional activities can be described by enzymatic catalysis, transport and storage, mechanical sustentation, growth, and cellular differentiation control. In general, the lipids are also rich in - C = O - bonds, are providing much more energy in the metabolic process of oxidation than other biological constituents. They are forming a readily available storage material for living organisms. Of course, seaweeds exhibit low lipid contents.[17]

Fig. 1: Concentration of Biochemicals of a Marine Green Alga *E. compressa*.



In seaweeds, the lipids are ubiquitously distributed, especially in several resistance stages. Total chlorophyll content was the summative value of the chlorophyll ‘a' and chlorophyll ‘b'. Therefore it showed that similar trend and concentration gradient like the constituting two above mentioned parameters. Total chlorophyll content was also recorded the highest in green algae when compared to red algae. Thus carotenoid concentrations were found to be

varied in different algal groups and collaborate with the earlier report. In general, the phycobilin compound was observed to be more in red algae than in green algae and brown algal seaweeds. Most of the red seaweeds consist of a higher amount of red pigment phycoerythrin in addition to chlorophyll contents.[18]

Phytochemical analysis

In the phytochemical analysis of *Enteromorpha compressa* eleven different types of secondary metabolites (alkaloids, anthraquinones, catechins, flavonoids, glycosides, phenolic groups, reducing sugars, saponins, steroids, tannins, and terpenoids) were tested in six different extracts of *E. compressa*. The positive results showed the presence of alkaloids, anthraquinones, catechin, flavonoids, glycosides, phenolic groups, reducing sugars, saponins, steroids, tannins, and terpenoids. Phenolic groups showed the maximum presence, being found in five different extracts and saponin in five extracts followed by alkaloids, catechin, found in four extracts and glycosides and tannins found in only five extracts respectively. Among the six different extracts, the benzene and ethanol extracts showed the presence of the maximum number of compounds. Next to benzene, and ethanol and chloroform extracts showed the presence of seven compounds and the aqueous extract showed the presence of six compounds and the acetone extract showed five compounds (Table. 1).

Table 1: Phytochemical analysis of crude extracts of *Enteromorpha compressa* (L) Nees.

S.No.	Phytochemicals	Solvents					
		Aqueous	Ethanol	Acetone	Benzene	Chloroform	Petroleum Ether
1	Alkaloids	-	+	-	+	+	+
2	Anthraquinones	-	+	-	+	+	-
3	Catechin	+	-	+	+	-	+
4	Flavonoids	-	+	-	+	+	-
5	Glycosides	+	+	+	+	+	-
6	Phenolic groups	-	+	+	+	+	+
7	Reducing sugars	+	-	-	+	-	-
8	Saponins	+	+	+	+	-	+
9	Sterols &Steroids	-	-	-	-	+	-
10	Tannins	+	+	-	+	+	+
11	Terpenoids	+	-	+	-	-	+
Note: (+) indicates the present. (–) symbol indicates not detected							

FTIR Analysis

The fourier-transform infrared spectroscopy analysis spectrum was taken to identify the functional group of the bioactive components based on the peak value in the region of infrared radiation. The ethanol extract of *E. compressa* showed characteristic absorption

bands at 3386 cm-1 and 1055 cm-1 (C - O) for a hydroxyl (-OH) group 2925 cm-1, 2859 cm-1 (for CH stretching), 1380 cm-1 (for C-H bending), 1700 cm-1 for carbonyl group (C= O) and at 1613 cm-1 for C= C group. The fresh crude ethanol extract of *E. compressa* was passed into the fourier-transform infrared spectroscopy and the functional groups of the components were separated based on its peak ratio. It was confirmed the presence of functional groups such as amides, a phosphorus compound, alcohols, phenols, and halogen compounds, etc.

Seaweeds are rich in polysaccharides, minerals and certain vitamins[19], but also contain bioactive substances like proteins, lipids, and polyphenols with antibacterial, antiviral and antifungal properties as well as many others.[20] Therefore seaweeds give great potential as a supplement in functional food or for the extraction of compounds. Physiologically active substances in seaweeds affect the maintenance of human homeostasis directly.[21] The marine algal seaweeds are low in calories from a nutritional perspective. The lipid content was low and even though the carbohydrate content was high, most of this is dietary fibers and not taken up by the human body. Naturally, seaweeds are having halogenated compounds[22] and the compounds are being dispersed in several various classes of metabolites, such as indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons.[23] They possess biological activities of pharmacological interest, such as antibacterial[24] and anti-tumoural.[25] The most specific and notable producers of halogenated compounds are seaweeds in the marine environment.[26] The compounds are predominantly derivate of sesquiterpenes diterpenes, triterpenes, acetogenins, fatty acids, and brominated indoles. In addition to antimicrobial and cytotoxic properties also play multifunctional ecological roles such as acting as a feeding defendant.[27, 28] Seaweed extracts with different solvents such as water, ethanol, ethyl acetate, petroleum ether, and chloroform have shown antibacterial, anti-inflammatory and anti-pathogenic effects.[29-31]

CONCLUSION

The biochemical of the present study suggest that *E. compressa* have considerable carbohydrates, proteins, lipids, phenols, chlorophylls and carotenoids for the use of food and pharmaceutical industry as a source in reparation of nutrient supplements, medicine, and fine chemical synthesis. The carbohydrates content was higher however, phenol values were lower. It was found that *E. compressa* was appeared to be interesting potential sources of plant food proteins owing to their high carbohydrate level. Besides, eleven different types of

secondary metabolites (alkaloids, anthraquinones, catechins, flavonoids, and glycosides, phenolic groups, reducing sugars, saponins, steroids, tannins, and terpenoids) were also observed. The presence of functional groups was found in *E. compressa* such as amides, a phosphorus compound, alcohols, phenols and halogen compounds with the help of FT-IR.

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