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Research Article

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Phytochemical analysis of green algae *Chlorococcum* sp., *Stigeoclonium* sp. and *Enteromorpha* sp

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ABSTRACT

The aim of the present paper is to evaluate the photochemicals, lipids and proteins of green algae Chlorococcum sp. Stigeoclonium sp. and Enteromorpha sp. The green algae Chlorococcum sp. Stigeoclonium sp. and Enteromorpha sp. showed the phytochemical constituents like Flavonoids and phenols compounds. The presence of a variety of chemical constituents, such as phenols, flavonoids were analyzed in three green algae by TLC. In the present investigation Enteromorpha sp. showed the highest amount of Flavonoids and phenol was found to be highest in Stigeoclonium sp. The quantitative estimation in above three species showed the presence of highest amount of lipids in Stigeoclonium sp and highest amount of proteins was estimated in Chlorococcum sp.

Key words: Flavonoids, Phenols *Chlorococcum* sp. *Stigeoclonium* sp. and *Enteromorpha* sp

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INTRODUCTION

Algae are being extensively used for deriving components that are widely used in pharmaceutical industries, medical industries and food industries. The bioactive components derived from algae are used for various purposes. Microalgae can be utilized in the production of nutritional supplements, antioxidants, cosmetics, natural dyes and poly unsaturated fatty acids (3). Brown, red and green algae are rich in molecules with antiviral, antioxidant, antifungal and antimicrobial activities because of its great nutritional value, *S. platensis* is being used since olden times as a resource of food. It is prospered in nutrients like minerals, protein, carbohydrates, vitamins and (γ)-linolenic acid. Algae contain variety of components like secondary metabolites including phenols and flavonoids. Phenols, sometimes called phenolics, are one of the main secondary metabolites present in the plant kingdom.)

Flavonoids are largest group of polyphenolic compounds and are known to contain a broad spectrum of chemicals and biological activities including antioxidant and free radical scavenging properties. They are remarkable group of plant metabolites. Flavonoids are perhaps best known to enhance the effects of ascorbic acid.(13) these are widely distributed in plant fulfilling many functions. Researchers have become interested in flavonoids and other phenol for their medicinal properties, especially their potential role in the prevention of cancer and heart diseases (4). Fresh water green alga *Chlorococcum humicola* is found to be a rich source of flavonoids, carotenoids, polyphenols, fatty acids etc. It has a wide variety of benefits used in medical and food industry because of its possible role in prevention of diseases like cancer(1). It is a potential source of pharmaceuticals and neutraceuticals. For many generations, marine algae *Enteromorpha sp.* has been extensively used as food for humans and animals and as organic fertilizers. It is found that the alga *Enteromorpha* sp. also contains bioactive components like flavonoids and polyphenols with potent antioxidant activity(12). Algae have the potential to generate significant quantities of commercially viable biofuel. Growing algae for biofuel can be one of the sustainable options of harvesting solar

energy optimally. *Chlorococcum* sp. has been studied for the production of biofuel and was found to contain high amounts of lipids (8). The aim of this work is to detect an existence of bioactive components and to quantify them in *Chlorococcum* sp., *Stigeoclonium* sp. and in *Enteromorpha* sp. and to check their industrial applications. It also involves the study of *Chlorococcum*, a freshwater unicellular alga, *Stigeoclonium* a filamentous chlorophycean alga and marine alga *Enteromorpha* for their other industrial and environmental applications.

EXPERIMENTAL SECTION

Collection of algae: In this investigation two fresh water algae *Chlorococcum* sp. and *Stigeoclonium* sp. as well as a marine algal species *Enteromorpha* were collected to carry out numerous tests. *Chlorococcum* sp. and *Stigeoclonium* sp. were obtained from Ballaleshwar Lake, Panvel, Navi Mumbai. All three algal sp. were grown in controlled conditions in suitable medium and subjected to various analysis. *Enteromorpha* sp. marine algae was collected from Marine Drive, Mumbai. It was used as fresh and dried samples for different analysis.

Culturing of algae:
Chlorococcum sp. and *Stigeoclonium* sp. were isolated using serial dilution and streaking techniques and made axenic using BG-11 broth. Basal BG-11 medium was selected for culturing of algae for being suitable. The pH of medium was maintained between7.1 to 7.3 after autoclaving. The cultures were grown for around 20 days. The culture flasks were kept in sunlight. The profusely grown algae were sub-cultured at regular intervals and used for experimental procedures.

Preliminary Tests for detection of components: Preparation of algal extract for preliminary tests , 200mg of dried algal powder was crushed in 10ml of methanol using a mortar and pestle and it was filtered through muslin cloth. The filtrate was collected in a clean test tube. This served as the sample for detection of various components in algae. (Extracts of all the three algae *Chlorococcum* sp. *Stigeoclonium* sp. and *Enteromorpha* sp. were prepared using the above mentioned procedure.)

Separation of components by Thin layer chromatography: Thin layer chromatography technique was used for the separation of active components Flavonoid and Phenol of *Chlorococcum* sp. *Stigeoclonium* sp. and *Enteromorpha* sp. Various solvent combinations gave separated phytoconstituents which were observed under U.V. light.

Quantitative Estimation of Total Phenol and Flavonoids : The amount of total phenol in *Chlorococcum* sp. *Stigeoclonium* sp. and *Enteromorpha sp.* was determined spectrophotometrically using Folins- Ciocalteu reagent and total Flavonoids by aluminium chloride colorimetric assay, total lipid content of *Chlorococcum* sp. *Stigeoclonium* sp. *Enteromorpha sp.* was estimated according to Bligh and Dyer's method and proteins by Lawry's methods .

RESULTS AND DISCUSSION

Chemical analysis carried out for detection of Flavonoids and phenols from three algae *Chlorococcum* sp. *Stigeoclonium* sp. and *Enteromorpha* sp. preliminary qualitative tests clearly revealed the presence of Flavonoids and Phenols compounds. The test algae were proved to be a good source of bioactive components i.e. Flavonoids and Phenol . The algal extracts of *Chlorococcum* sp. *Stigeoclonium* sp. and *Entermorpha* sp. were tested for the presence of Flavonoids and Phenols using thin layer chromatography technique (Table 1). In TLC specific solvent system were used to separate compounds Fig 1 shows the TLC profile of the phytocompounds present in *Chlorococcum* sp. *Stigeoclonium* sp. and *Entermorpha* sp. Bioactive components reported like phenolics, alkaloids , Flavonoids and tannins in marine algae *Gracillaria corticata* and *Spirulina platensis*.(7). In the present investigation *Enteromorpha* sp. showed the highest amount of Flavonoids. It was found to be 11.4 mg/g of tissue. It was comparatively low in *Stigeoclonium* sp. and *Chlorococcum* sp. In *Chlorococcum* sp. and *Stigeoclonium* sp. The amount of flavonoids was found to be 10.57 mg/g and 8.02 mg/g. (Table 2) similar results reported from brown algae and green algae (14) .In current study the total phenolics estimation was carried out in *Chlorococcum* sp. *Stigeoclonium* sp. and *Enteromorpha* sp. the total phenolic content was found to be highest in *Stigeoclonium* sp. which was 22.64 mg/g tissue. It was comparatively low in *Chlorococcum* sp and *Enteromorpha* sp. (Table 3).Some researchers have investigated the amount of phenol in *Ulva* to be ranging from 5.08±0.65 to 1.258±0.126 in different species(10, 11) The quantitative estimation in above three species showed the presence of highest amount

of lipids in *Stigeoclonium* sp. It showed 34.23% of lipids while *Chlorococcum* sp. showed presence of 27.84% of lipids. *Enteromorpha* contained the lowest amount of lipids which was 4.55%.The proteins were quantitatively estimated in three algae by Lowry's method. The highest amount of proteins was estimated in *Chlorococcum* sp.(Table 4) . The lowest amount of proteins was found in *Enteromorpha* sp. Chlorococcum sp contained 22.38 mg of proteins per gram. Stigeoclonium sp. had 13.33 mg/g proteins and Enteromorpha sp showed 10.56 mg proteins per gm. (16)

Table. (1) TLC profile of Flavonoids in *Chlorococcum, Stigeoclonium sp.* and *Enteromorpha sp*

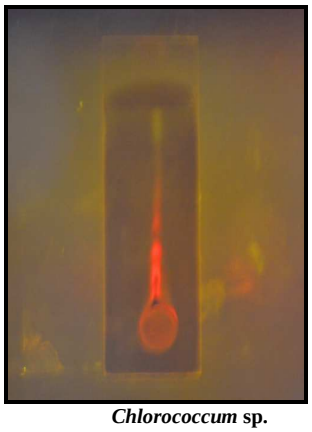
Name of algae	R.F values of flavonoids
Chlorococcum sp.	0.41±0.02,0.63±0.01, 0.67±0.01, 0.94±0.02
Stigeoclonium sp.	0.14 ± 0.01 0.33± 0.02
Enteromorpha sp	0.20±0.03, 0.32±0.02.

Table 2 amounts of Flavonoids and Phenols in *Chlorococcum* sp.*Stigeoclonium* sp. and *Enteromorpha* sp.

Name of algae	Amount of flavonoids	Amount of total phenolics
Chlorococcum sp.	10.57±0.2 mg/g	8.584±0.2mg/g
Stigeoclonium sp.	8.02±0.1 mg/g.	22.64±0.3mg/g
Enteromorpha sp.	11.41±0.1 mg/g	19.644±0.07mg/g

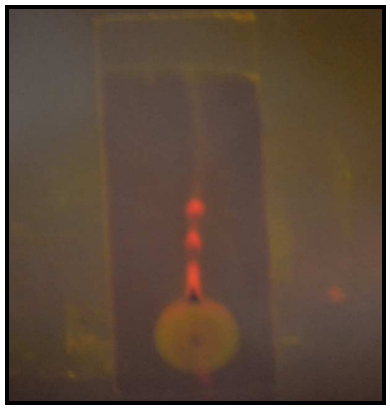
Table 3 TLC profile of total Phenol of *Chlorococcum sp.* , *Stigeoclonium sp.* and *Enteromorpha sp.*

Name of the algae	R.F. values of total phenolics
Chlorococcum	0.33± 0.01, 0.56±0.01, 0.62±0.02.
Stigeoclonium	0.42±0.01, 0.48±0.03, 0.56±0.02, 0.69±0.02 , 0.97±0.03
Enteromorpha	0.54±0.01, 0.60±0.03, 0.77±0.02



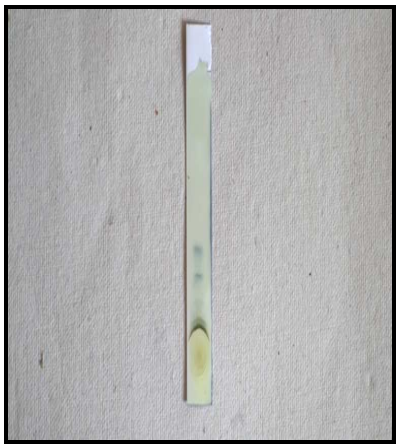
***Chlorococcum* sp.**

***Stigeoclonium* sp.**

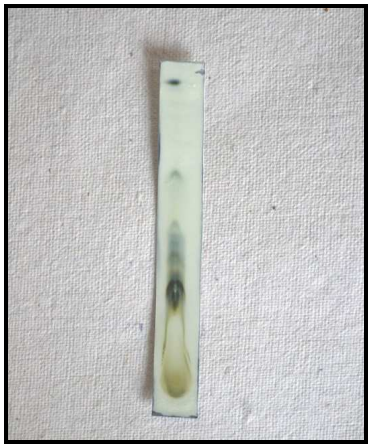


***Enteromorpha* sp**

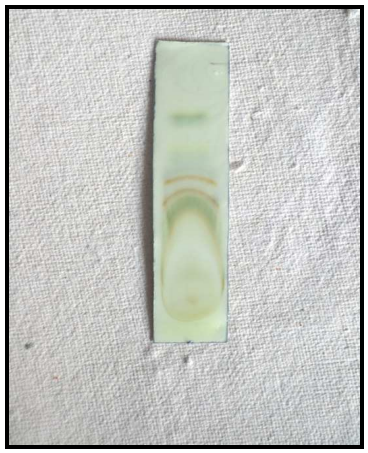
Fig : 1 TLC Profile of the Flavonoids in *Chlorococcumsp.* , *Stigeoclonium sp.* and *Enteromorpha* sp



Chlorococcum sp.



***Stigeoclonium* sp.**



***Enteromorpha* sp**

Fig : 2 TLC Profile of the Phenols in *Chlorococcumsp.* , *Stigeoclonium sp.* and *Enteromorpha* sp

Table 4 The amount of proteins and lipid in *Chlorococcum sp.* , *Stigeoclonium sp.* and *Enteromorpha* sp

Name of alage	Amount of proteins	Amount of lipids(%)
Chlorococcum sp.	22.38±0.3mg/g	27.84%
Stigeoclonium sp.	13.33±03mg/g	34.23%
Enteromorpha sp.	10.56±0.1mg/g	4.55%

CONCLUSION

The results of this phytochemical investigation suggest that algae *Chlorococcum* sp., *Stigeoclonium* sp. and *Enteromorpha* sp contains important phytochemicals like phenols, and flavonoids which may contribute to its biological activities. The present study reveals algae extract might be utilized as a natural source of antioxidant, supplement in food and pharmaceutical industry.

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