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Antioxidative potentials of four green filamentous algae of Indian Sunderbans

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Abstract

In search of potent natural antioxidants from green algal community of Indian Sundarbans, the largest Mangrove forest of the World (22º22'81"N, 88º47'91"E; 22º08'38"N, 88º42'24"E),four filamentous Chlorophycean members viz, *Cladophora glomerata, Chaetomorpha area, Rhizoclonium crassipellitum* and *Pithophora cleveana* were tested for their antioxidant potentials. Antioxidant properties were estimated in terms of polyphenol contents using three different solvent systems (water, ethanol and methanol). Methanol was found to be the best solvent system for antioxidative properties. High phenolic (2.48-9.8 mg GAE / g DW), flavonoids (7.21-24.6 mg/ g DW) and carotenoid (1.72-9.9mg/ g DW) contents, and free radical scavenging activities were determined from methanolic extracts of all four algae. Out of all four chlorophycean members *Rhizoclonium crassipellitum* is proved to be the most antioxidant rich strain showing maximum content of polyphenol (Total phenol content - 9.8 mg GAE/g dry tissue and total flavonoid content - 24.6 mg /g dry tissue respectively), total carotenoid content (9.95 mg/g DW), DPPH radical scavenging activity (IC_{50} - 7.759mg/mL), ferrous ion chelating activity (IC_{50} -7.08mg/mL) and superoxide radical scavenging activity (IC_{50} - 9.37 mg/mL).

Keywords: Green Algae, Antioxidant Property.

Introduction

Since the ancient times chlorophycean members have found their usage as nutritional supplements or food sources in Asiatic countries. Some of the most biotechnologically important green algal genera being commonly used in human food additives are *Chlorella vulgaris*, *Haematococcus pluvialis*, *Dunaliella salina* etc. According to Hills and Nakamura (1976), *Chlorella sp* has several by-products that have found profound usage in fruit and vegetable preservations. Furthermore, addition of microalgae to pasta, snack foods or drinks as nutritional supplements or natural food colorants has been reported by Becker (2004). *Chlorella* also finds wide usage in animal feed for cats, dogs, aquarium fish, ornamental birds, horses, poultry, cows and breeding bulls (Spolaore *et al.* 2006). Works of several authors have shown the use of different algae as feed ingredients such as *Porphyridium, Isochrysis, Pavlova, Chaetoceros, Gracilaria, Palmaria and Arthrospira* as efficient color elicitors and growth enhancers in cichlid fish, rainbow trout, fish larvae, bivalve mollusks, black tiger prawns and several gastropods (O'Connor et al. 1997; Kop & Durmaz 2008). Khatoon *et al.* (2010) used fresh algal biomass of *Nostoc ellipsosporum* and *Navicula minima* in formulation of algae based value added feed (VAF). Oxidative stress in the body of living organisms leads to the production of several reactive oxygen and reactive nitrogen species abbreviated as ROS and RNS respectively. These bring about changes in the normal cellular redox status, immune function and intracellular functioning of a particular organism leading to the development of various types of diseases such as cancer, cardiovascular disorder, atherosclerosis, diabetes mellitus, Alzheimer's disease etc. Therefore, a true and proper antioxidant rich diet is required which must have high reducing and scavenging activities. Different micro organisms like bacteria, fungi and algae are being used as alternative sources to functional food or feed. Macro- or microalgae can be considered as hubs of several bioactive compounds and are both nutritive as well as protective in nature (Kumar & Ganessan 2007). The bioactive compounds of algae comprise mostly of secondary metabolites like phenols, flavones, flavonols, pigments (b-carotene), sulphated polysaccharides and vitamins, which can be used effectively in the feed, pharmaceuticals as well as nutraceutical industries (Borowitzka & Borowitzka 1988; Koivikko *et al.* 2008).

Till date, majority of the works have been done on antioxidant properties of seaweeds or marine algae. These works have suggested that seaweeds have a high content of natural antioxidants which have allowed them to be used in

varieties of folk medicines since ages (Yoshie *et al.* 2002; Santoso *et al.* 2004). Brown and red algal genera such as *Sargassum swartzii*, *Sargassum kjellmanianum*, *Cystoseira myrica*, and *Colpomenia sinuosa* have high contents of phenols, flavonoids and phlorotannins (Nagayama *et al.*1989; Mayer & Lehmann 2000; Sadati *et al*. 2011) which impart them with antioxidative , anticarcinogenic as well as anti ageing efficiency .

Pigments also play major roles in antioxidant property of algae. Several pigments like α – carotene, ß- carotene, lutein, canthaxanthin and astaxanthin make the algal biomass active against different diseases. Some authors such as Yamamoto *et al*. (1986) and Okai *et al*. (1996a) have reported that many algae have anticancerous activities due to rich pigment content. Astaxanthin also plays a very important role as free radical scavenger especially in *Haematococcus pluvialis* and in *Dunaliella bardawil* (Kobayashi *et al*. 1992; Shaish *et al*.1993). Another important component of algal bioactive compounds are algal polysaccharides, which have both antioxidant properties as well as anticancer activity (Okai *et al*. 1993 ; Okai & Higashi 1994). Antioxidative effect has been reported to be high with a sulfoglyco lipid fraction isolated from *Porphyridum creuntum* (Berge *et al*. 2002).Green algal taxa, *Ulva fasciata* and *Ulva lactuca* commonly used in soups and salads, also have been reported to contain high antioxidant and antibacterial properties.

The algal flora of Sundarbans, the largest tiger inhabiting, less explored mangrove forest of South East Asia was investigated by the present group(Satpati *et al*. 2013). Since the green algal genera have been less exploited, thereby in our present investigation, four dominant Chlorophycean members of Indian Sundarbans viz; *Cladophora glomerata, Chaetomorpha area ,Rhizoclonium crassipellitum and Pithophora cleveana* were selected for antioxidant property study. To test the best activity of the collected biomass three different solvents (water, ethanol and methanol) were used as extracting media for polyphenolic compounds. Apart from those carotenoids, radical scavenging activities and total antioxidant capacity of the collected algae were also analyzed.

Material Methods

Four different chlorophycean members viz., *Cladophora glomerata* (Linnaeus)Kützing , *Chaetomorpha area* (Dillwyn) Kützing *, Rhizoclonium crassipellitum* West&G.S.West and *Pithophora cleveana* Wittrock were collected from south-eastern part of Indian Sundarbans (22º22'81"N, 88 º 47'91"E; 22 º 08'38"N, 88 º 42'24"E). The collected samples were initially washed under running tap water. They were exposed to 0.5% povidone iodine solution overnight and were further subjected to thorough washing under running tap water. A small portion of biomass was taken for preparation of different algal extracts, for evaluating their antioxidant potentials (IC_{50}) in relation to phenols, flavonoids, carotenoids and radical scavenging activities.

a. Total Phenol Estimation

Total phenolic content of the algal biomass was determined by Folin–Ciocalteau method (Singleton & Rossi 1965). An aliquot of 6.0 mL of double-distilled water was taken, to which 0.1 mL of algal extract and 0.5 mL of Folin–Ciocalteau reagent was added. Furthermore 1.5 mL of Na_2CO_3 was added and the volume was made up to 10.0 mL with distilled water. After incubation for 30 min at 25ºC, the absorbance was measured at 760 nm and was expressed as gallic acid equivalents.

b. Total Flavonoid Estimation

Total flavonoid content of the algae was determined by colorimetric method (Zhishen1999). Briefly, an aliquot of 0.5 mL of sample extract was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm. The results were expressed as quercetin equivalents.

c. Total Carotenoid Estimation

A spectrophotometric analysis was done for quantitative estimation of carotenoid of the algal biomass using the standard protocols (Sachindra & Mahendrakar 2005).

d. DPPH Scavenging Assay

The free radical scavenging activity of the algae was estimated using the protocol of Blois (1958). Here, 2.0 mL of methanolic extract was mixed with 2.0 mL (0.16 mM) methanol DPPH solution. The mixture was vortexed for few minutes and incubated in dark for 30 minutes. The absorbance of the above mixture was measured at 517nm. Sample blank and control was prepared. The scavenging effect was calculated according to the above methodology.

e. Ferrous Ion Chelating Assay

Iron chelating ability is another promising test for determining the antioxidant ability of a particular sample. Here, the chelating ability of methanolic extract of the algae was estimated using the standard protocol of Decker & Welch (1990). Briefly, 1mL of sample was added to 2mM $FeCl_2$ (0.5mL) and 5mM ferrozine (0.2mL), the mixture was shaken vigorously and incubated at room temperature for 10 minutes. The absorbance of the mixture was then measured at 562nm. The percentage inhibition of ferrozine –Fe2+ complex formation by the extract was calculated using the respective formula:

% Inhibition + $(1- A_{1sample} / A_{0control)} \times 100$ [A_0 – Absorbance of the control

A_1 – Absorbance of the sample extract. The control contained all the above reagents except the algal extract.]

f. Superoxide Radical Scavenging Assay

Non enzymatic superoxide radical scavenging assay was performed using the methodology described by Robak *et al*. (1988). Here 1mL of sample was added to a reaction mixture of 0.2mL of PMS, 0.2mL of (0.5 mM) NBT, 1 mL of NADH, 1 mL of (0.1 M) potassium buffer (pH 7.2) and then the mixture was warmed at 32°C for 3 to 5 minutes. The reading of the mixture was taken at 560 nm.

The scavenging effect (%) was calculated according to the formula –

$$(1- \text{Abs of Sample}_{560nm} / \text{Abs of Control}_{560nm}) \times 100$$

g. Total Antioxidant Capacity

Total antioxidant capacity of crude methanolic extract was determined according to the method of Prieto *et al*. (1999). Briefly, an aliquot of 0.3 mL of sample was mixed with 3.0 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95° C for 90 minutes in the water bath. Absorbance of all the sample mixtures was measured at 695 nm and the total antioxidant capacity was expressed as the number of equivalents of ascorbic acid in milligram mg/per gram of extract.

h. Statistical analysis

All biochemical parameters of the four algal strains were analysed by ANOVA. The significance of difference between the means was determined by Duncan's multiple range test ($P < 0.05$) using SPSS for windows (10.0) (Duncan 1995).

Results

a.Solvent extraction of algal polyphenols

In the first phase of experimentation, the polyphenolic contents of selected algae were estimated using three solvent systems (ethanol, water and methanol) (Fig. 1a, b). Both total phenol content (TPC) and total flavonoid content (TFC) were found to be maximum in methanolic extracts of all four algae. Therefore the rest of the experiments were carried out using the methanolic extracts of algae.

b.Total Polyphenol Content

The polyphenolic contents of selected algae are represented in terms of total phenolic content (TPC) and total flavonoid content (TFC) (Figure 1a, b). Both TPC and TFC were significantly higher in *Rhizoclonium* ($P<0.05$) (9.8 mg GAE/g dry tissue and 24.6 mg QE /g dry tissue respectively) than *Pithophora* , *Cladophora* and *Chaetomorpha* respectively as depicted in figure 1a and b.

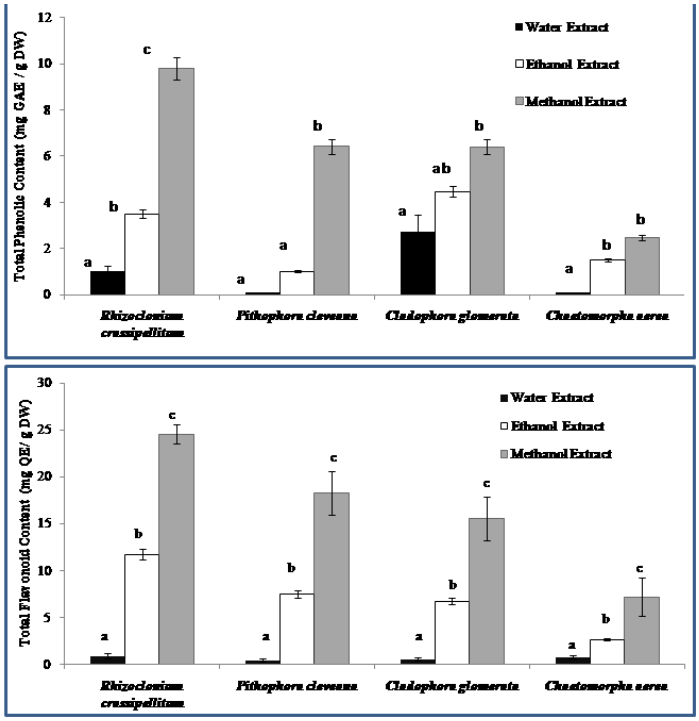

Figure1. Graphical Representation of the total polyphenolic contents of selected algae. a. Total Phenol Content. b. Total Flavonoid Content.

c.Total Carotenoid Content

Spectrophotometric analysis of methanolic extracts of the studied taxa showed total carotenoid content (TCC) in the range 1.3-10.4 mg / g DW (Figure 2). Significantly high carotenoid contents wereobserved in *Rhizoclonium* and *Chaetomorpha* (P<0.05) followed by *Cladophora* and *Pithophora* (9.95 mg/g DW, 8.605 mg/g DW, 5.548 mg/g DW and 1.72 mg/g DW respectively) .

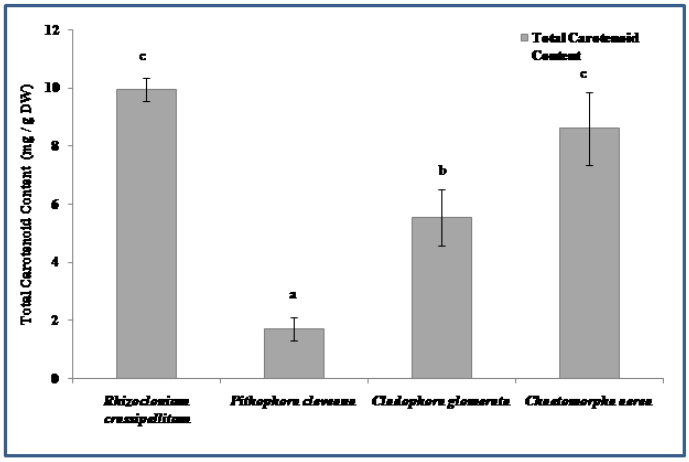

Figure2. Graphical representation of the total carotenoid content of selected algae.

d.Radical Scavenging Activity

To evaluate the radical scavenging ability of the selected strain three basic tests were performed, which were DPPH radical scavenging activity, ferrous ion chelating activity and superoxide radical scavenging activity (Figure 3a, b, c).

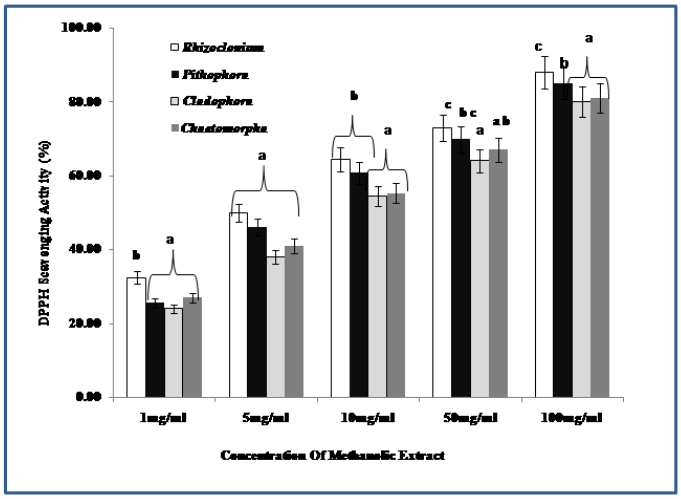

Figure3a. Graphical representation of the DPPH radical scavenging activity (%) of selected algae at different extract concentrations.

Reduction of 1,1- diphenyl 1,2 picrylhydrazyl (DPPH*), a stable radical by antioxidant to a yellow colored diphenylpicrylhydrazine is one of the most convenient and easy method for analyzing the free radical scavenging activity. Best radical scavenging activity was observed in methanolic extract of *Rhizoclonium* (IC_{50} -7.759mg/mL) followed by *Pithophora* (IC_{50} – 8.235 mg/mL), *Cladophora* (IC_{50} -9.17mg/mL) and *Chaetomorpha* (IC_{50} - 9.04 mg/mL) (Figure 3a). The differences in the DPPH radical scavenging activity amongst the selected genera were found to be significant according to Duncan's multiple range test ($P < 0.05$).

The oxy-radical formation is prevented by metal ion chelating activity of an antioxidant molecule as it reduces the concentration of the catalyzing transition metals in lipid protein bilayer thereby stabilizing the oxidized form of metal ion. Maximum ferrous ion chelating activity was shown by *Rhizoclonium* (IC_{50} – 7.08mg/mL) followed by *Pithophora* (IC_{50} – 7.32 mg/mL), *Cladophora* (IC_{50} – 32.19 mg/mL) and *Chaetomorpha* (IC_{50} – 9.01mg/mL) (Figure 3b)..

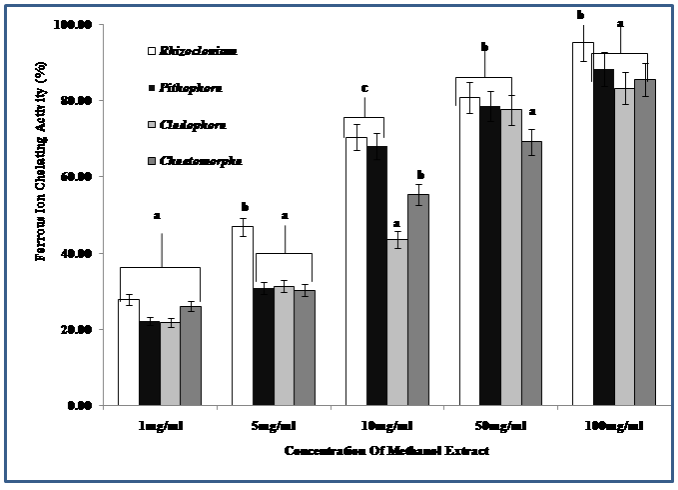

Figure3b. Graphical representation of the Ferrous Ion Chelating Activity (%) of selected algae at different extract concentrations.

Superoxide anion radical is converted to hydrogen peroxide by superoxide dismutase. Though superoxide radical itself is a relatively weak oxidant, it decomposes to form stronger, reactive oxidative species, such as singlet oxygen and hydroxyl radicals, which initiate peroxidation of lipids. In case of Superoxide radical scavenging activity, *Rhizoclonium* showed significantly more activity (IC_{50} - 9.37 mg/mL) ($P<0.05$) followed by *Pithophora* (IC_{50} – 49.38 mg/mL), *Cladophora* (IC_{50} – 50 mg/mL) and *Chaetomorpha* (IC_{50} – 45.24 mg/mL) (Figure 3c).

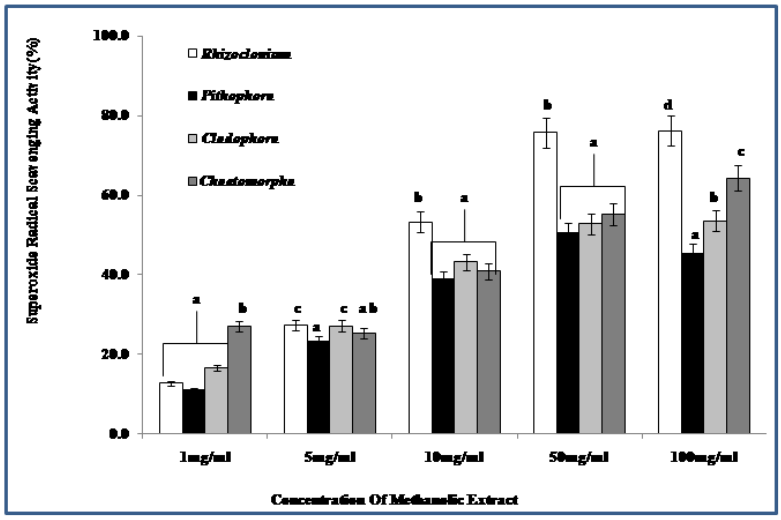

Figure3c. Graphical representation of the Superoxide Radical Scavenging Activity (%) of selected algae at different extract concentrations.

e.Total Antioxidant Capacity

The total antioxidant capacity (TAC) of the crude methanol extract has been represented in the (figure 4). Maximum TAC was noted in case of *Rhizoclonium* (598µg AAE/gm dry mass), followed by *Pithophora* (545 µg AAE/gm dry mass), *Cladophora*. (317µg AAE/gm dry mass) and *Chaetomorpha* (243µg AAE/gm dry mass) at 100mg/mL concentration. Significant differences in the TAC level were observed amongst the selected genera according to Duncan's multiple range test ($P < 0.05$).

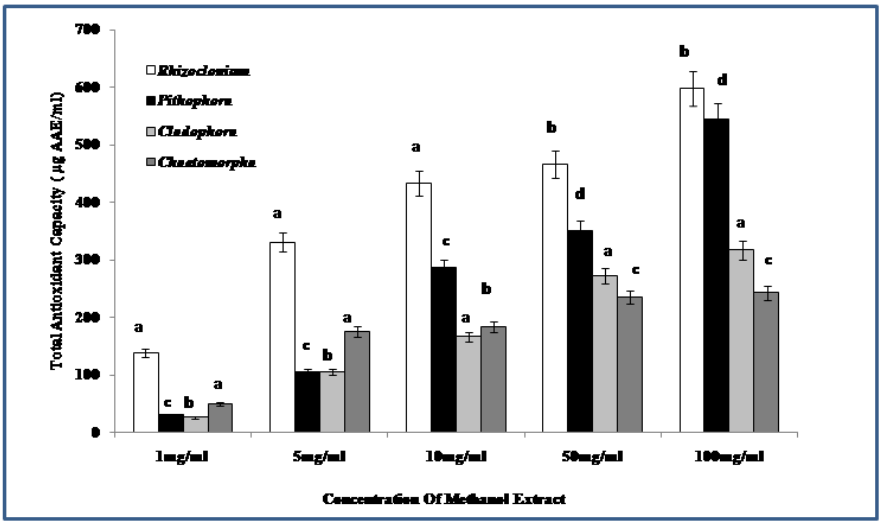

Figure 4. Graphical representation of the Total Antioxidant Capacity of selected algae at different extract concentrations.

Discussion

Seaweeds along with several microalgae are good source of bioactive compounds. All these compounds have remarkable antioxidative properties and are effective against several diseases (Newman *et al*. 2003). Works of Kuda *et al*.(2005) detected antioxidant properties in dried 'kayamo-nori' a brown alga, *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae).Among the seaweeds, *Rhodomela confervoides* and *Symphyocladia latiuscula* were compared to the well-known antioxidant butylated hydroxytoluene for their high activity (Vadlapuri 2012). One of the key bioactive compounds actively participating in the antioxidative system of algae is polyphenols (Reddy *et al*. 2003).

In the present investigation the selected green algal genera were found to be rich sources of phenol, flavonoids and carotenoids. Significantly high phenol contents were observed in methanolic extracts of all four types of algae. Similar observation was studied by Waterman & Mole (1994) and Koivikko *et al*. (2005) who reported methanol, ethanol and acetone as effective extractants of phenolic compounds. Amongst all the genera *Rhizoclonium carssipellitum* proved itself to be the richest source of phenolics. Athukorala *et al*. (2003) reported *Gratelopia filicina* (red algae) remarkable source of phenolics. The flavonoid content of all four algae was found to be significantly higher. Markham *et al*. (1969) showed the presence of good content of flavonoids in *Nitella hookeri*. Chen *et al*. (2003) and Moffitt *et al*. (2007) also reported the presence of flavonoids in the cyanobacteria *Anabaena variabilis* , *Nostoc puncti-forme* and in green microalga *Chlorella pyrenoidosa.*

Furthermore, in our experimentation, maximum radical scavenging activity was found to be present in case of algae having maximum phenol contents. Our observation is in accordance to the observation of Athurokula *et al*. (2003) who also showed maximum radical scavenging activity by *Grateloupia filicina*. Similarly, works of Chandini *et al*. (2008) also portrayed significant amount of radical scavenging activity in *Sargassum marginatum, Padina and Turbinaria*. It has also been observed that reactive oxygen species scavenging activity of our selected algae was directly related to their total phenolic contents.

Other important bioactive compounds in algae are the carotenoids. These tetra-terpenoid C- 40 units. The present algae used in the experiment also proved to be good sources of carotenoids. In our study, *Rhizoclonium* showed maximum carotenoid content followed by *Chaetomorpha* and *Cladophora*. According to Del Campo *et al*. (2000) chlorophycean microalgae accumulate carotenoids with different biological activities. Thus, all four studied green algal taxa tested viz., *Cladophora glomerata, Chaetomorpha area, Rhizoclonium crassipellitum, Pithophora cleveana* were found to be potent source of antioxidants, among all *Rhizoclonium* showed best results.

Conclusion

From the present investigation it was observed that the chlorophycean members selected from Sunderbans are rich in bioactive compounds. They can thus efficiently combat different degenerative diseases and hence can be effectively included in various pharmaceutical applications. Being rich in carotenoid contents these algal genera can also be effectively used in different fish feeding programmes.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflict of interest in the present study.

References

Athukorala ,Y., Lee, K.W., Song, C., Ahn, C.B., Shin, T.S., Cha, Y.J., Shahidi and F., Jeon, Y.J. 2003. Potential antioxidant activity of marine red alga *Grateloupia filicina* extracts. J. Food. Lipids. 10:251–265.

Berge, J. P., Debiton, E., Dumay, J., Durand, P. and Barthomeuf, C. 2002. In vitro anti-inflammatory and anti-proliferative activity of sulfolipids from the red alga *Porphyridium cruentum*. J. Agricult. and Food. Chem. 50: 6227-6232.

Becker, W. 2004. Microalgae in human and animal nutrition. In A. Richmond (Ed) Handbook of Microalgal Culture, Blackwell, Oxford,. 312-351.

Borowitzka, M. A. and Borowitzka, L.J. 1988. Micro-algal biotechnology. Cambridge, UK: Cambridge University Press. 96–100.

Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical, Nature. 181: 1199-1200.

Chandini, S. K., Ganesan, P. and Bhaska, N. 2008. In vitro antioxidant activities of three selected brown seaweeds of India. Food. Chem.107: 707–713.

Chen, K., Feng, H., Zhang, M. and Wang, X. 2003. Nitric oxide alleviates oxidative damage in the green alga *Chlorella pyrenoidosa* caused by UV-B radiation. Folia. Microbiol. (Praha). 48: 389–393.

Decker, E.A. and Welch, B. 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. J. Agri. Chem. 38: 674–677.

Del- Campo, J.A., Moreno, J., Rodríguez, H., Vargas, M.A., Rivas, J. and Guerrero, M.G. 2000. Carotenoid content of chlorophycean microalgae: factors determining lutein accumulation in *Muriellopsis sp.* (Chlorophyta). J. Biotechnol. 7: 76(1):51-9.

Duncan, D. B. 1995. Multiple range and multiple F tests. Biometrics. 11:1-42.

Hills, C. and Nakamura, H. 1978. Food from sunlight. World Hunger Research Publ., Boulder Creek, CA.
Kobayashi, M., Kakizono, T., Yamaguchi, K., Nishio, N. and Nagai, S. 1992. Growth and astaxanthin formation of *Haematococcus pluvialis* in heterotrophic and mixotrophic conditions. J. Fer-ment. Bioeng.74: 17-20.

Koivikko, R., Loponen, J., Honkanen,T. and Jormalainen, V. 2005. Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga *Fucus vesiculosus*, with implications on their ecological functions. J.Chem.Ecol. 31:195-212.

Koivikko, R. 2008.Brown algal phlorotannins improving and applying chemical methods. Turun Yliopisto, Turku.

Kuda, T., Tsunekawa, M., Hishi, T. and Araki, Y. 2005. Antioxidant properties of dried 'kayamo-nori', a brown alga *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae). Food. Chem. 89: 617–622.

Kumar, K.S., Ganessan. K. and Rao, P.V.S. 2007. Antioxidant potential of solvent extract of *Kappaphycus alverezii* (Doty). Doty – edible seaweed. Food. Chem. 107: 289 – 295.

Kop, A. and Durmaz,Y. 2008. The effect of synthetic and natural pigments on the colour of the cichlids (*Cichlasoma severum sp*.,Heckel 1840). Aquac.Intro.16:117–122.

O'Connor, W.A. and Heasman, M.P.1997. Diet and feeding regimens for larval doughboy scallops, *Mimachlamys asperrima*. Aquac. 158:289-303.

Khatoon, N., Sengupta, P., Homechaudhuri, S.and Pal, R. 2010 a. Evaluation of algae based feed in Goldfish (*Carassius auratus*) nutrition. Proceed. Zoo.Soc. 63 (2): 109–114.

Markham, K.R. and Porter, L.J. 1969. Flavonoids in the green algae (chlorophyta). In Plant Flavonoids in Biology and Medicine; Cody,V., Middleton, E., Harborne, J. B., Eds.; Alan R. Liss: New York. 15-24.

Mayer, A.M.S and Lehmann, V.K.B. 2000. Marine pharmacology in 1998: Marine compounds with antibacterial, anticoagulant, anti-inflammatory, anthelmintic, antiplatelet, antiprotozoan, and antiviral activities; with actions on the cardiovascular, endocrine, immune, and nervous systems; and other miscellaneous mechanisms of action. The Pharmaco.42: 62-69.

Moffitt, M. C., Louie, G.V., Bowman, M.E., Pence, J., Noel, J. P. and Moore, B.S. 2007. Discovery of two cyanobacterial phenylalanine ammonia lyases: kinetic and structural characterization. Biochem.46 : 1004–1012.

Nagayama, Y., Takahashi, M., Fukuyama, Y. and Kinzyo, Z. 1989. An antiplasmin inhibitor, eckol, isolated from the brown alga *Ecklonia kurome* OKAMURA. Agric. and Bio. Chem. 63: 3025–3030.

Newman, D.J., Cragg, G. M. and Snader, K.M.2003.Natural products as source of new drugs over the period 1981-2002. J. Natural. Products. 66: 1022- 1037.

Okai, Y. and Higashi-Okai, K.1994. Identification of antimutagenic activities in the extracts of an edible brown alga, Hijikia fusiforme (Hijiki)by umu gene expression system in *Salmonella typhimurium* (TA 1535/pSK 1002). J. Sci. of Food. and Agric. 66:103–109.

Okai, Y., Higashi-Okai, K. and Nakamura, S.C.1993. Identification of heterogeneous antimutagenic activities in the extract of edible brown seaweeds, *Laminaria japonica* (Makonbu) and *Undaria pinnatifda* (Wakame) by umu gene expression system in *Salmonella typhimurium* . Mut.Research. 302: 63–70.

Okai, Y., Higashi-Okai, K., Yano, Y. and Otani, S. 1996a. Identification of antimutagenic substances in an extract of edible red alga, *Porphyra tenera* (Asakusa-nori). Cancer. Letters .100: 235–240.

Prieto, P., Pineda, M. and Aguilar,M.1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269; 337–341.

Reddy,L., Odhav, B. and Bhoola, K. D. 2003. Natural products for cancer prevention: a global perspective. Pharma and Therap. 99: 1–13.

Robak, J. and Gryglewski, R.J. 1988. Flavonoids are scavengers of superoxide anions. Biochem. Pharmacol. 37:837–841.

Sachindra, N. M. and Mahendrakar, N.S. 2005. Process optimization for extraction of carotenoids from shrimp waste with vegetable oils. Biores. Technol. 96:1195-1200.

Sadati, N., Khanavi, M., Mahrokh, A., Nabavi, S.M.B., Sohrabipour,J., Hadjiakhoondi, A. 2011.Comparison of Antioxidant Activity and Total Phenolic Antioxidant activities of marine algae 203 Contents of some Persian Gulf Marine Algae. J. Med. Plants. 10 (37): 73-79.

Santoso, J., Yoshie-Stark,Y. and Suzuki,T.2004. Anti-oxidant activity of methanol extracts from indonesian seaweeds in an oil emulsion model. Fisheries.Sci.70:183-188.
Satpati, G. G., Barman, N. and Pal, R. 2013. A study on green algal flora of Indian Sundarbans mangrove forest with special reference to morphotaxonomy. Journ. Algal. Biomass. Utln. 4 (1): 26–41.

Shaish, A., Avron, M., Pick,U. and Ben-Amotz, A.1993. Are active oxygen species involved in induction of b-carotene in *Dunaliella bardawil*? Planta. 190: 363-368.

Singleton, V.L. and Rossi, J.A., Jr 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungtic acid reagent. Amer. Journ. Enol. and Vitic. 16:144-158.

Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, A. 2006. Commercial applications of microalgae. Journ. Biosci. Bioeng. 101: 87-96.

Vadlapudi, V. 2012. Antioxidant activities of marine algae: A review. Medicinal Plants as Antioxidant Agents: Understanding Their Mechanism of Action and Therapeutic Efficacy. 189-203.

Waterman,P.G. and Mole, S.1994. Analysis of Phenolic Plant Metabolites. Blackwell Scientific Publications: Oxford, UK. 85-87.

Yamamoto, I., Maruyama, H., Takahashi, M. and Komiyama, K. 1986. The effect of dietary or intraperitoneally injected seaweed preparations on the growth of sarcoma-180 cells subcutaneously implanted into mice. Cancer. Letters. 30: 125–131.

Yoshie, Y., Wang, W., Hsieh, Y.P. and Suzuki, T. 2002. Compositional difference of phenolic compounds between two seaweeds, *Halimeda spp.* Journal Tokyo University of Fisheries. 88: 21–24.

Zhishen, J., Mengcheng, T. and Jianming, W. 1999. Food. Chem. 64:555-559.