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Novel antioxidant production by *Cladophora sp.* and *Spirogyra sp.*

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ABSTRACT

Reactive oxygen species (ROS), such as the superoxide anion, hydroxyl radicals, and hydrogen peroxide, cause lipid oxidation and thereby decrease the nutritional value and appearance of food *Cladophora sp* is one of the largest filamentous green alga commonly available in the ponds of Puri district, Odisha. The antioxidant activities of Cladophora *sp* and *Spirogyra sp.* were measured by free radical scavenging assays. The study aimed at assaying the antioxidant activities both the species. of Puri distict, Odisha. The antioxidant activity of the extracts was investigated including the total phenolic contents). IC^{50} for the DPPH radical scavenging activity was 920 ± 42 µg ml-1. The IC^{50} values for ascorbic acid, quercetin and BHA were 5.05 ± 0.1, 5.28 ± 0.2 and 53.96 ± 3.1 µg ml-1, respectively. Phenol and flavonoid contents of this alga may have led to its good DPPH-scavenging activity. These results indicate that further investigation is needed to identify and purify the responsible antioxidative components. The present study confirms the production of antioxidants from *cladophora sp. And Spirogyra sp.* of Puri District,Odisha state and suggests that two species constitute a potential source for producing antioxidant. Hence *cladophora sp and Spirogyra sp.* can be used as a novel source of natural antioxidant agents for pharmaceutical industries.

Key words: *Cladophora sp., Spirogyra sp.,* irradiance*, antioxidant* activites, flavonoid contents, phenolic contents.

Abbreviations:
BHT: Butylated hydroxytoluene;
DPPH: 2, 2-diphenyl-1-picrylhydrazyl
IC50: Inhibition Concentration
GC-MS: Gas chromatography-mass spectrometry
ROS: Reactive oxygen species;
SOD, superoxide dismutase;
TLC: Thin layer chromatography

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1. INTRODUCTION

Microalgae are able to enhance the nutritional content of conventional food and feed preparation and hence to positively affect humans and animal health due to their original chemical composition, namely high protein content, with balanced amino acids pattern, carotenoids, fatty acids, vitamins, polysaccharides, sterols, phycobilins and other biologically active compounds, more efficiently than traditional crops. Algae are photosynthetic organisms, which possess reproductive simple structures. These organisms constitute a total of 25-30,000 species, with a great diversity of forms and sizes, and that can exist from unicellular microscopic organisms (microalgae) to multicellular of great size (macroalgae). Algae can be a very interesting natural source of new compounds with biological activity that could be used as functional ingredients. Some important algae aspects, such as natural character, easy cultivation, their rapid growing (for many of the species) and the possibility of controlling the production of some bioactive compounds by manipulating the cultivation conditions. In this way, algae can be considered as genuine natural Reactors (Plaza, et al., 2008).Commercial importance, utilization of algae remains below their optimum level in India. Asian countries like Japan, South Korea and China have made algal farming for their nutraceutical and commercial chemicals.

Recently, much research is focused to find naturally occurring antioxidant for use in food or pharmaceutical purposes, as synthetic antioxidants, which are being restricted due to their side effects of carcinogenicity (Zheng and Wang, 2001). Green microalgae are widely used in the life science as the source of compounds with diverse structural forms and biological activities. Marine and fresh water algae have been historically and exceptionally rich source of pharmacologically active metabolites with antineoplastic, antimicrobial and antiviral effects. The aim of this chapter is to review the most important features of microalgae in animal and human nutrition, particularly in the development of novel design-foods rich in carotenoids and polyunsaturated fatty acids with antioxidant effect and other beneficial health properties. Microalgae are an enormous biological resource, representing one of the most promising sources for new products and applications (Pulz and Gross, 2004). They can be used to enhance the nutritional value of food and animal feed, due to their well balanced chemical composition. Moreover, they are cultivated as a source of highly valuable molecules such as polyunsaturated fatty acids, pigments, antioxidants, pharmaceuticals and other biologically active compounds. Moreover the nutritional quality and safety of foods also can easily change due to their toxic compound formation (Yagi 1987; Zainol et al. 2003). These lipid peroxidation by-products, called free radicals, give damage to healthy cells, and thereby create harmful molecules. Free radicals and other reactive oxygen species (ROS) are generated continuously not only via normal physiological processes but also by external stimulations. Normal physiological processes need oxygen in order to carry out their operations as a resultant by-product like ROS are produced within the human body. If these harmful factors accumulate in cell, tissue and other vital organs of the body, then our bodie exposed to dangerous circumstances. Of the external stimulation, ROS can be induced by tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (Robinson et al. 1997; Karawita et al. 2005). ROS including superoxide ($O_2^{\cdot-}$), hydroxyl radical ($HO\cdot$), and hydrogen peroxide (H_2O_2), have the ability to react with a large variety of easily oxidizable cellular components, such as proteins, lipids, nucleic acids, and carbohydrates. Their oxidative damages cause aging and many other diseases, including arthritis, strokes, heart diseases, atherosclerosis, diabetes, cancers and neurodegenerative disorders (Alho and Leinonen 1999; Lemberkovices et al. 2002). Reactive oxygen species (ROS), such as the superoxide anion, hydroxyl radicals, and hydrogen peroxide, cause lipid oxidation and thereby decrease the nutritional value and appearance of food. In addition, ROS are the major cause of qualitative decay of food, which leads to rancidity, toxicity, and destruction of major biochemical components that are important in metabolism (Park et al. 2005).

Antioxidants are important inhibitors against lipid peroxidation, therefore these compounds are utilized to delay or prevent lipid peroxidation in foods and the oxidation of cellular substrates. They exert their effects by scavenging ROS, activating a battery of detoxifying proteins, or by preventing the generation of ROS (Jun et al. 2001). The most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and propyl gallate (PG). However, there has been growing concern over their safety and toxicity. Moreover, some studies (Lu and Foo, 2000) have been reported that there is an inverse relationship between dietary intake of antioxidant-rich foods and the incidence of human diseases. Therefore, development and utilization of more effective antioxidant from natural resources are desired for use in foods or medicinal materials to replace the synthetic antioxidants.

2. MATERIALS AND METHODS

2.1. Chemicals required

Ferrozine, linoleic acid, trichloroacetic acid (TCA), DPPH, potassium ferricyanide and hydrogen peroxide. Gallic acid, quercetin, butylated hydroxyanisole (BHA), ascorbic acid, sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, EDTA, ferric chloride, Muller Hinton Agar, Nutrient broth.

2.2. Collection and preparation of sample

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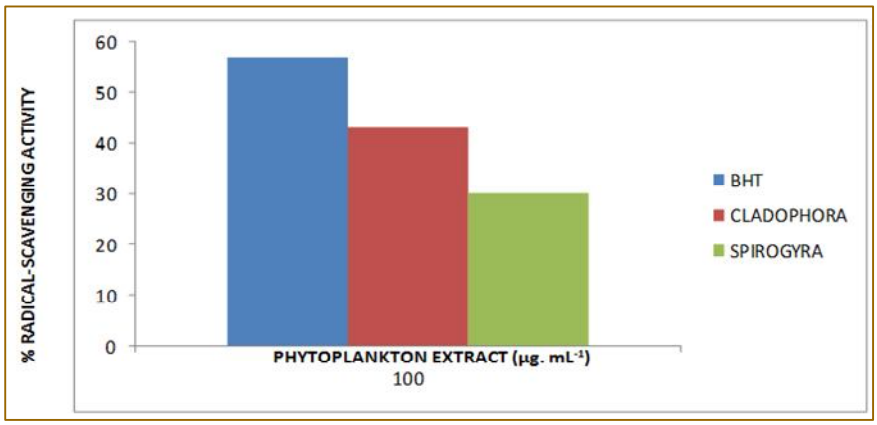

Figure 1

The extracts of *Cladophora* (43.26%) exhibited higher activity than those of and *Spirogyra sp.* (30.17%) in 100 µg of extracts

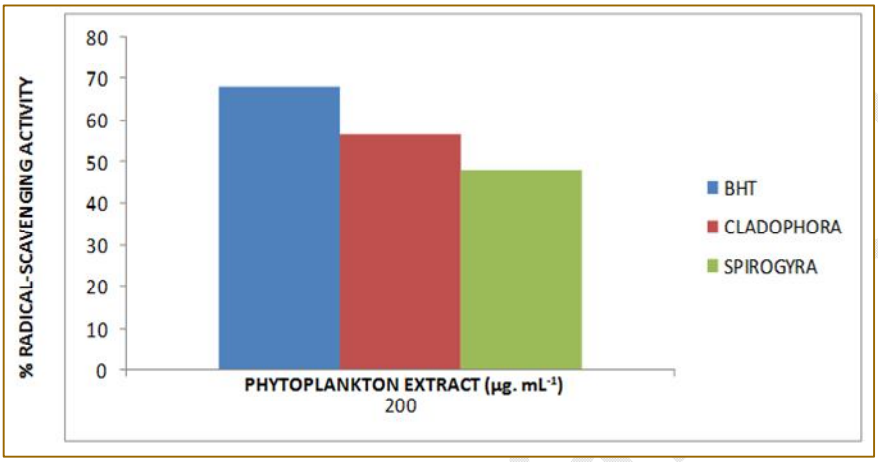

Figure 2

The extracts of *Cladophora* exhibited 57.91% in 200 µg of extracts

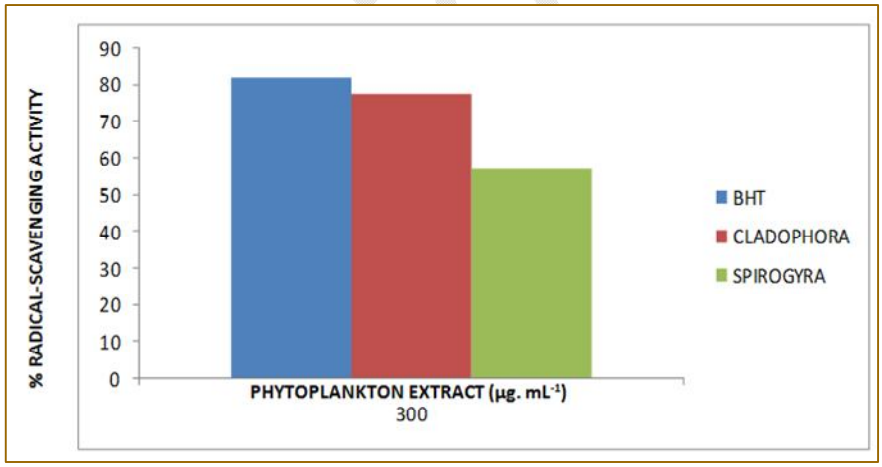

Figure 3

The extracts of *Cladophora* exhibited 78.62 % in 300 µg of extracts

Cladophora sp. and *Spirogyra sp.* were collected manually from the ponds of Puri district. The harvested macroalgae were stored in plastic bags for transportation to the laboratory.

2.3. Collection and preparation of algal extracts

Dried materials were coarsely ground before extraction. 5 g of dried materials were extracted by maceration with 70% ethanol (1 h sonication, filtered; repeated 2 times). The extract was then separated from the sample residue by filtration through Whatman No.1 filter paper. The resultant extracts were concentrated in a rotary evaporator under reduced pressure until a crude solid extract was obtained, which were then freeze-dried for complete solvent which were then freeze-dried for complete solvent removal (0.9 g).

2.4. Determination of total phenolic compounds and flavonoid content

Total phenolic compound content was determined by the Folin- Ciocalteau method (McDonald et al., 2001). Extract (0.5 ml, 1.6 mg ml-1) was mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 min and 2.0 ml of 75 g l-1 sodium carbonate was then added. The absorbance of the reaction was measured spectrophotometrically at 760 nm after 2 h of incubation at room temperature (r.t.). Results were expressed as gallic acid equivalents. Total flavonoids were estimated according to the method of Chang et al. (2002). Briefly, 0.5 ml solution of extract in methanol (1.6 mg ml-1) 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 with a double beam spectrophotometer.

2.5. DPPH radical-scavenging activity

The stable DPPH was used for the determination of free radical scavenging activity of the extract (Koleva et al., 2002). Different concentrations of extract were added at an equal volume, to the methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated three times. Vitamin C, BHA and quercetin were used as standard controls. IC^{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

3. RESULTS AND DISCUSSION

3.1. Determination of total phenolic compounds and flavonoid content

Total phenol compounds were reported as gallic acid equivalents by reference to standard curve (y = 0.0054x + 0.0628, r^2 = 0.987). The total phenolic content was contents 3151 ± 123 mg gallic acid equivalent g-1 of extract. The

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total flavonoid content was 472 ± 45 mg quercetin equivalent g-1 of extract. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources. There are different amounts of phenols and polyphenolic compounds in the phytoplanktons. In this alga, there were high amounts of phenols and polyphenolic compounds. Increasing the levels of flavonoids in the daily diet may decrease the impact or occurrence of certain human diseases because they interact with various biological systems and show anti-inflammatory, hypolipidemic, hypoglycemic and antioxidant.

3.2. DPPH radical-scavenging activity

DPPH is a free radical that accepts an electron or changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Ebrahimzadeh et al., 2010a). IC^{50} for the DPPH radicalscavenging activity was 920 ± 42 µg ml-1. The IC^{50} values for ascorbic acid, quercetin and BHA were 5.05 ± 0.1, 5.28 ± 0.2 and 53.96 ± 3.1 µg ml-1, respectively. Phenol and flavonoid contents of this alga may have led to its good DPPH-scavenging activity. The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables.

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4. CONCLUSIONS

In conclusion, the methanolic and aqueous extracts of the examined *Cladophora sp.*and *Spirogyra sp.* contain antioxidative compounds that can strongly scavenge ROS such as superoxide anion, hydroxyl radical, hydrogen peroxide and DPPH free radical. These results indicate that the methanolic and aqueous extracts of *Cladophora sp* and *Spirogyra sp.* can be potential natural antioxidants in foods and pharmaceutical industries. However, the responsible compounds related to the antioxidant activity of extract *Cladophora sp.* are not yet cleared. Therefore, further studies are required in order to identify the antioxidant compoundof the *C. glomerata* extract. These two species showed good but different levels of antioxidant activities in some models studied. The extracts had high amount of phenols and polyphenolic compounds. Phenols and polyphenolic compounds were in very good amount and higher than those in some plants (Ebrahimzadeh et al., 2010b). DPPH-scavenging activity showed potent activity. Identification of the antioxidant compounds of these extracts will lead to their evaluation in considerable commercial potential in medicine, food production and in the cosmetic industry.

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