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A COMPARATIVE ANALYSIS OF THE ANTIOXIDANT POTENTIAL OF *CHAETOMORPHA ANTENNINA* AND *CERATOPHYLLUM SUBMERSUM*

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ABSTRACT

Marine environment is an exceptional reservoir of biologically active natural products. The common major components like proteins, carbohydrates, steroids, glycosides can be extracted using polar solvents such as methanol. In most of the developing countries, kelp forest resources like seaweeds which are potentially strong bioactive component are used to fulfill the requirements as nutritious food, biofertilizers, biofuels and pharmaceuticals to cure different diseases. Antioxidants are increasingly being recommended because they act directly on oxidative processes and may prevent diseases and problems associated with disease control and aging. Therefore, there is a constant search for antioxidants from natural sources for the isolation of antioxidant bio molecules. Seaweeds are an excellent source of natural antioxidant bioactive compounds which can be used as dietary supplements or as antioxidants in controlling bacterial infection in aquaculture farms as well as in the food industry. A great deal of effort has therefore focused on using available techniques to identify natural antioxidants from seaweeds. The present study is a comparative analysis of the antioxidant potential of Chaetomorpha antennina and Ceratophyllum submersum.

KEYWORDS: *Marine Environment, Natural Product, Chaetomorpha antennina, Ceratophyllum submersum and Antioxidant Potential.*

INTRODUCTION

Pharmaceutically valuable products from microalgae and its industrial commercialization is a gateway to a multibillion dollar industry. Microalgae are autotrophic and ubiquitous in nature. They represent a major untapped resource of genetic potential for valuable bioactive agents (**Seedevi *et. al.*, 2013)**. This proven ability of microalgae to produce these compounds places these micro organisms in biotechnological spotlight for applications and commercialization as in the pharmaceuticals. Metabolites from seaweeds have been shown to possess anti-aging, photo-protective, moisturing and whitening properties (**Sahaa *et. al.*, 2008)**. Compared to the terrestrial plants and animal-based foods, seaweed is rich in health-promoting molecules and materials such as dietary fibre, omega-3 fatty acids, essential amino acids and vitamins A, B, C and E which is essential for cosmeceutical product development. In addition, marine algae are considered as sea vegetables not only for consumption, but also as an alternative medicine since ancient times for skin- related diseases. In other words, the marine environment is many folds richer in its biodiversity, thereby making marine organisms and metabolites unique. Metabolites from seaweed have potential antioxidant, anti-inflammatory, antidiabetic, antitumor, antihypertensive and anti-allergic properties, as well as their role in hyaluronidase enzyme inhibition, neuroprotection and bone-related diseases (**Rashed. K, Medda. R** and **Pintus F, (2016).**

TYPES OF SEAWEEDS

Seaweeds are classified into different groups according to their pigments e.g., green algae, red algae and brown algae.

GREEN ALGAE

The "Green algae" is the most diverse group of algae, it belong to the class Chlorophyceae with more than 7000 species growing in a variety of habitats. The green algae is a 'paraphyletic' group because it excludes the plantae (**Aseer Manilal *et. al.*, 2012)**. Like the plants, the green algae contain two forms of chlorophyll, which they use to capture light energy to fuel the manufacture of sugars, but unlike plants they are primarily

aquatic. They are found both in fresh and salt water environment and some even live on land in very wet soils. Some of the most well-known are sea lettuce. Green algae are an important food source. They also contain beta carotene, which is used as food coloring and also for cancer prevention.

RED ALGAE

Red algae or Rhodophyta is one of the oldest groups of eukaryotic algae. Red algae are red in color because of the presence of the pigment phycoerythrin; this pigment reflects red light and absorbs blue light. Because blue light penetrates water to a greater depth than light of longer wavelengths, these pigments allow red algae to photosynthesize and live at greater depths. It has high level of protein and vitamin. Red algae are also commercially important. Carrageenan is a gel used to stabilize man made products such as ice creams, paste, facial creams etc. (**Subathraa K** and **TV Poonguzhali, 2013**).

BROWN ALGAE

Brown algae is the largest and most complex type of algae. Brown algae contain chlorophyll a and c and a pigment called fucoxanthin, which gives the color. Fucoxanthin is not found in other algae or plants. Brown algae include a number of edible seaweeds. All brown algae contain alginic acid (alginate) in their cell walls, which is extracted commercially and used as an industrial thickening agent in food and for other uses. The polysaccharide is a major component of brown algae and is not found in land plants. Alginic acid can also be used in aquaculture (**Premalatha *et.al.*, 2011)**.

CLASSIFICATION - GREEN ALGAE

Binomial name : *Chaetomorpha antennina* (Kutzing), 1847
Kingdom : Plantae
Division : Chlorophyta
Class : Ulvophyceae
Order : Cladophorales
Family : Cladophoraceae
Genus : Chaetomorpha
Species : antennina

Chaetomorpha contains vitamin C and A. Some species are edible such as *C.crassa*, *C.linum* and *C.brachygona. C.crassa* is consumed as salad or dessert in far eastern countries due to its character of gelatinization (**Soad M. Mohy El-Din *et al.*, 2015)**. *Chaetomorpha* has possible applications in medicine, dietary supplements, cosmetics and food industries (**Chang, V.S.** and **Teo, S.S, 2016).** The aim of the present study is to identify the effects of *Chaetomorpha antennina* and its polysaccharides as a potential antioxidant agent. The extract would be characterized using FTIR and its Antioxidant activity using Phosphomolybdenum method.

AQUATIC WEEDS

Binomial name : *Ceratophyllum submersum*
Kingdom : Plantae
Clade : Angiosperms
Order : Ceratophyllales
Family : Ceratophyllaceae
Genus : ceratophyllum
Species : submersum

Many disorders in human organism such as atherosclerosis, arthritis, Alzheimer disease, Cancer etc.**,** may be the result of increased concentrations of free radicals in an organism (**Meenakshi Bhattachaerjee, 2016).** Reactive oxygen species (ROS) and nitrogen (RNS) species are the most frequent pro-oxidants, either originating from normal metabolism or induced by UV radiation and different pollutants (**Maria Filomena *et.al.*, 2015)**. Harmful effects of disturbed antioxidant-prooxidant balance can be largely prevented by the intake of antioxidant substances. Antioxidants have already been found in seaweeds (**Bharathiraja *et.al.*,**

2015). Due to their natural origin, the antioxidants obtained from seaweeds are of greater benefit in comparison to synthetic ones (**M. S. Leelavathi** and **Prasad M. P, 2015).** The use of natural antioxidants from seaweeds does not induce side effects, while synthetic antioxidants are found to have genotoxic effect (**Mitali Priyadharshini Pati *et. al.*, 2016)**. The aim of the present study is to study the comparative analysis of the antioxidant property of *Chaetomorpha antennina* and *Ceratophyllum submersum.*

MATERIALS AND METHODOLOGY

COLLECTION, PROCESSING AND EXTRACTION OF SEAWEEDS

Chaetomorpha antennina and *Ceratophylum submersum* were collected from a pond in Ajax (Thiruvottiyur) in Chennai. The samples were manually collected; epiphytes and debris were removed by washing in running tap water and washed again with distilled water. The samples were then allowed to shade dry for 7 days at room temperature and were made into a fine powder using an electric blender. The materials required for the extraction of Seaweeds are Aquatic plants, *Chaetomorpha antennina* and *Ceratophyllum submersum*, Solvent (Methanol) 500ml and Conical flask (500 ml). 10gms of the dried Green algae and aquatic plant were extracted separately in 100ml of Methanol (1: 10 ratio) for 3 days in a separate conical flask. The solvent were filtered using a muslin cloth or filter paper. The filtrates were stored in screw capped container for further analysis.

FREE RADICAL SCAVENGING ACTIVITY

DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY (**Phosphomolybdenum method by Pireto *et al.*)**

The materials required for Free Radical Scavenging Activity are 28mM Sodium phosphate, 4mM Ammonium molybdate, 0.6M Sulphuric acid, Sample extracts and Polysaccharides. 1ml of the extract was mixed with 1ml of the standard reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695nm against a reagent blank. The Total Antioxidant Capacity was expressed as milligram of Ascorbic Acid Equivalence (AAE) per gram of extract.

DETERMINATION OF REDUCING POWER ASSAY (Oyaizu *et al.)*

A reductant is not necessarily an antioxidant but an antioxidant is commonly a reductant. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. The materials required for Determination of Reducing Power Assay are 1% Potassium ferricyanide, 0.2M Sodium phosphate buffer, 10% Trichloroacetic acid, 0.1% Ferric chloride, Samples extract and Polysaccharides. The reaction mixture contained 1ml of various concentrations of extracts (2-10 mg/ml), 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 0.2 M sodium phosphate buffer. The mixture was incubated at 50°C for 30 minutes and the reaction was terminated by the addition of 2.5 ml of 10%TCA, followed by centrifugation at 3000rpm for 10 minutes. 2.5 ml of the upper layer was mixed with 2.5 ml of the deionized water and 0.5 ml of the 0.1% Ferric chloride. The absorbance was measured at 700 nm against blank. The reducing power ability of the sample is determined by the increase in absorbance of sample. BHT was used as standard for comparison.

HYDROGEN PEROXIDE SCAVENGING ASSAY (**Ruch *et.al***)

Hydrogen peroxide is a weak oxidizing agent and can inactive a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H2O2 can probably react with Fe2+ and possibly Cu2+ ions to form hydroxyl radical and this ,may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The materials required for Hydrogen Peroxide Scavenging Assay are 43mMHydrogen peroxide, 1M Phosphate buffer, Ascorbic acid, Weed extracts and Polysaccharides. A solution of hydrogen peroxide(43mM) is prepared in phosphate Buffer (1M pH 7.4).

Different concentration of sample was added to a hydrogen peroxide solution(0.6 ml , 43 mM). Absorbance of hydrogen peroxide at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. The free radical scavenging activity was determined using the Formula

Percentage scavenging (H2 O2) = ((Ao-A1) / Ao) x 100

RESULTS AND DISCUSSION

COLLECTION OF SAMPLES

Chaetomorpha antennina and *Ceratophyllum submersum* were collected from shores of Royapuram beach. The seaweeds (*Chaetomorpha antennina* and *Ceratophyllum submersum)* were washed with water, the calcareous, stones and epiphytes were removed by manual setting and intensively washed with tap water and then again with distilled water. The aquatic weeds were shade dried for 7-10 days. The bioactive compounds from seaweeds were obtained by using the solvent extraction. Methanol was taken in a conical flask and the weeds were weighed using an electronic weighing balance and added to the solvents in the ratio of 1:10 and left for 2-3 days and the extract was filtered using a muslin cloth.

EXTRACTION OF CRUDE POLYSACCHARIDES

Extraction resulted by yielding 0.5g of green solid crude polysaccharides from 10g of *Chaetomorpha antennina* and 0.4g of brownish green crude polysaccharides from 10g of *Ceratophyllum submersum***.**

FREE RADICAL SCAVENGING ACTIVITY

DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY

The total antioxidant activity of the extract and the polysaccharides were measured spectrophotometrically through Phosphomolybdenum method. The higher the absorbance, the stronger is the antioxidant activity. The total antioxidant activity was found to be higher in *Chaetomorpha* extract than *Ceratophyllum*. The total antioxidant capacity is expressed as milligram of Ascorbic Acid Equivalence (AEE) per gram of extract **(Tables 1 & 2)**.

S.NO	SAMPLE	O.D. (695nm)
1	Ascorbic acid (Standard)	0.894
2	*Chaetomorpha* extract	0.857
3	*Chaetomorpha* polysaccharides	0.789

Table 1 : Total Antioxidant Activity of *Chaetomorpha antennina*

S.NO	SAMPLE	O.D (695nm)
1	Ascorbic acid (Standard)	0.894
2	*Ceratophyllum* extract	0.736
3	*Ceratophyllum* polysaccharide	0.650

Table 2 : Total Antioxidant Activity of *Ceratophyllum submersum*

DETERMINATION OF REDUCING POWER

In reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be monitored by measuring the formation of Prussian blue at 700nm. A higher absorbance indicated the higher reducing power. The reducing power capacity of *Chaetomorpha* extract was higher than *Ceratophyllum submersum* (**Tables 3 & 4).**

Table 3 : Reducing Power Capacity of *Chaetomorpha antennina*

S.NO	**SAMPLE**	**O.D (700nm)**
S.NO	**SAMPLE**	**O.D (700nm)**
1	*Chaetomorpha* extract	0.841
1	*Ceratophyllum* extract	0.754
2	*Chaetomorpha* polysaccharide	0.780
2	*Ceratophyllum* polysaccharide	0.650

Table 4: Reducing Power Capacity of *Ceratophyllum submersum*

HYDROGEN PEROXIDE SCAVENGING ACTIVITY

The reactive oxygen species (ROS) such as superoxide anion (O_2^-), Hydrogen peroxide, hydroxyl radical, single oxygen and peroxynitrite are known to cause oxidative damage, contributing to the development of chronic diseases such as cancer, heart disease and cerebrovascular disease (**Tables 5 & 6**). The highest scavenging activity was found in *Chaetomorpha* polysaccahrides.

S.NO	**SAMPLE**	**O.D (230nm)**	**% SCAVENGED (H_2O_2)**
1	Ascorbic acid (Standard)	0.993	-
2	*Chaetomorpha* extract	0.873	12.08%
3	*Chaetomorpha* polysaccharide	0.813	18.1%

Table 5 : Hydrogen scavenging activity of *Chaetomorpha antennina*

S.NO	**SAMPLE**	**O.D (230nm)**	**% SCAVENGED (H_2O_2)**
1	Ascorbic acid (Standard)	0.993	-
2	*Ceratophyllum* extract	0.710	28.4%
3	*Ceratophyllum* polysaccharide	0.970	2.3%

Table 6 : Hydrogen Scavenging Activity of *Ceratophyllum submersum*

CONCLUSION

Natural antioxidants are an interesting alternative in view of their variety of structures and chemical interactions, as well as the numerous biological activities they can perform. Intensive research activities are currently being carried out on plant antioxidants to meet this challenge. Indeed, several studies have reported that polyphenols, such as flavonoids, hydroxycinnamic acids and proanthocyanidins, act as powerful antioxidants. The flavonols (e.g., quercetin) and hydroxyicinnamic acids (e.g., caffeic and ferulic acids) were determined to be more potent antioxidants than ascorbic acid. Phenolic antioxidants have been recognized as an important class of food ingredients and are currently added to various food products in order to provide additional health benefits. Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention .There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities may be more effective over the long term. The present study highlights the Antioxidant Potential of *Chaetomorpha antennina* to be more significant than that of *Ceratophyllum submersum.* Thus Antioxidants are of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery.

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