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Research Article

FREE RADICAL SCAVENGING POTENTIAL OF EXTRACTS OF *GRACILARIA VERRUCOSA* (L) (HARVEY): AN ECONOMICALLY IMPORTANT SEAWEED FROM CHILIKA LAKE, INDIA

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ABSTRACT

Objective: Marine macroalgae or seaweeds are rich sources of several compounds with biological effects including antioxidant activities. The aim of the study was to evaluate the possible free radical scavenging potential of *Gracilaria verrucosa,* an economically important seaweed from Chilika Lake.

Methods: The algal extracts were obtain with acetone and chloroform and their antioxidant activity was determined by evaluation of total antioxidant capacity by Phosphate Molybdenum method, scavenging of DPPH free radical, total phenol content by Folin–Ciocalteau Phenol reagent (FCP) method and scavenging of Metal chelating activity.

Results: Strong antioxidant capacity of 9.82±1.64 and 12.13±1.63 mg catechol equivalent/gm dry weight (DW) was observed in both acetone and chloroform extracts of *Gracilaria verrucosa* respectively. The total phenol content was found out to be 2.011±0.035 and 1.31±0.028 % catechol equivalent/ gm DW respectively in acetone extract and chloroform extract. The DPPH scavenging potential of the organic extracts was found to be more active in comparison to the standard commercial Butylated hydroxyl toluene (BHT). The Metal chelating activity of the algal extracts was found to be concentration dependent that increases with the increase in concentration of the sample.

Conclusion: From the result, it was concluded that both the extracts are successful in extracting the active antioxidant compounds from the alga species. The biological activities observed in this study conclude that *Gracilaria verrucosa* is a potential source of natural antioxidant and offers opportunities for further research aiming to isolate and identify the specific phenolic compounds responsible for their antioxidant activity.

Keywords: Antioxidant, Chilika Lake, *Gracilaria verrucosa,* Free radicals scavenger, Seaweed.

INTRODUCTION

Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the normal metabolism of oxygen or from exogenous factors and agents. The different forms of reactive oxygen species, includes superoxide anion radicals (O^{2-}), hydroxyl radicals (HO·), hydrogen peroxide (H_2O_2), and singlet oxygen ($1O_2$). [1] Oxidative damage of DNA, protein, lipid, and other molecules caused by ROS is associated with a number of human pathological processes, including atherosclerosis, arthritis, diabetes, chronic inflammation, cataractogenesis, muscular dystrophy, pulmonary dysfunction, tissue damage, and neurological disorders such as Alzheimer's disease, certain types of cancer and aging. [2-4] Recently, various phytochemicals and their effects on health, especially the suppression of active oxygen species by natural antioxidants from tea, spices, herbs and also marine seaweeds have been intensively studied throughout the World. [5]

Marine algae have long been used for food and medicines in many of the Asian countries like, China, Indonesia, Japan and Thailand since ancient times, as they are a rich source of vitamins, minerals, dietary fiber, protein, and various functional polysaccharides. [6] Moreover, seaweeds are also considered to be a rich source of antioxidants such as polyphenols, alkaloids, terpenoids, halogenated compounds etc. [7-8] Chilika lake, which is situated in the southern part of Odisha, India is one of the largest brackish water lake in Asia and is rich in various types of seaweeds and marine macro algae (Chlorophyceae, Rhodophyceae, Cyanophyceae) etc. Its climatic condition is suitable for the growth and development of different type of economic seaweeds from Rhodophyceae family. *Gracilaria verrucosa* **(**L) (Harvey) which also belongs to the family Rhodophyceae is one of the most exploited red seaweed of Chilika, India. *G. verrucosa* which is commonly known for its uses in food and phycocollides has a high growth rate and tolerates high temperatures. The red seaweed, *G. verrucosa* (Hudson) are found growing abundantly in many areas on the Indian coast and has been listed as one of the richest and most promising sources of bioactive primary and secondary metabolites. [9-10] The up-regulation of antioxidant and antioxidant enzymes such as carotenoids, SODs and methods of cellular repair by photo re-activation and nucleotide excision are also strategies of maintaining homeostasis in them. [11] In view of the above factors the present study was conducted to provide a substantiate antioxidant potential of acetone and chloroform extracts of *Gracilaria verrucosa* by conducting different types of antioxidant assays such as total phenol content, total antioxidant capacity, DPPH free radical scavenging assay and metal chelating activity.

MATERIALS AND METHODS

Sample Collection and Preparation of organic algal extract

Samples of *Gracilaria verrucosa* **(**L) (Harvey) were collected from brackish water lagoon of Chilika Lake, Odisha (Kalijai area) which extends between 19º 28' and 19º 54' N Latitude to 85º 05' and 85º 38' E longitude. Collected samples were washed thoroughly with marine water to remove the epiphytes, debris and other marine organisms and were brought to laboratory in plastic bags containing water to prevent evaporation. It was then washed with tap water, dried with tissue paper and kept at -20º C for further analysis. The algal sample was dried in hot air oven at 40 ºC for two days. The extraction was done by Soxhlet extraction techniques. Two different solvents (Chloroform and Acetone) were used successively with gradient polarity. The extracts were evaporated to complete dryness by vacuum distillation and stored in refrigerator for further use. [12]

Evaluation of Total Antioxidant Capacity

The Total Antioxidant Capacity of the algal extracts were determined by the Phosphate Molybdenum method, based on the reduction of Mo (VI) to Mo (V) by antioxidant compound and formation of green phosphate Mo (V) complex with a maximum absorption of 695 nm. [13] Aliquot of 0.1 ml of algal extract (50-100µg/ml) was combined to 2 ml of reagent solution (0.6M H_2SO_4+28mMSodium phosphate + 4 mM Ammonium Molybdate). The tubes were incubated at 90ºC for 90 minutes. It was then cooled to room temperature and the absorbance was measured at 695 nm against blank. The antioxidant capacity was expressed as mg catechol equivalent /gm DW.

Total Phenol Content

Phenolic compounds in the algal extracts were determined with Folin–Ciocalteau Phenol reagent (FCP) method with minor modifications. [14] About 5µl of algal extracts were taken and to it 2ml of 2% Sodium carbonate was added. It was mixed by vortexing vigorously up to 3 min. Then about 0.1ml of 50% FCP was added to it and was kept for 30 min at room temperature in dark. The absorbance of all the extract was measured at 700nm using spectrophotometer (Systronics, 108) using catechol as reference standard. Phenolic content was expressed in catechol equivalent percentage DW.

Scavenging of DPPH free radical

The scavenging effect of acetone and chloroform extracts of the algal sample was determined by standard modified method. [15] Briefly 2.0 ml of DPPH (2,2-diphenylamine -1-picryl hydrazyl) solution was added to the test tube containing 0.1 ml of aliquot of algal extract and standard BHT from 50-100µg/ml concentration. The mixture was vortexed and kept at room temperature for 30 min in dark. The absorbance of the sample solution was then measured at 517 nm by using spectrophotometer (Systronics 108). The IC_{50} value was determined to find out the effective concentration of the extract showing 50 % scavenging. The % scavenging effect of the plant extract against DPPH free radical was calculated using the following equation.

$$\% \text{ Scavenging} = \frac{\mathbf{A_0} - \mathbf{A_0}}{\mathbf{A_0}} \times 100$$

Where, A_0 is the absorbance of control.

A_1 is the absorbance of test sample.

Metal chelating activity

The Metal chelating activity of the algal extracts was estimated by chelation of ferrous ions using standard method. [16] About 0.1 ml of the extract was added to a solution of 0.5 ml ferrous chloride (0.2 mM). Then about 0.2ml of ferrozine (5mM) was added to it and incubated at room temperature for 10 min. The absorbance was measured at 562 nm. BHT was used as a positive control. The IC_{50} value was determined to find out the effective concentration of the extract showing 50 % scavenging. The metal chelating effect of the extracts was calculated from the following equation.

$$\% \text{ Activity} = \frac{A_0 - A_0}{A_0} \times 100$$

Where, A_0 is the absorbance of control.

A_1 is the absorbance of test sample.

Statistical analysis

Mean and standard deviation were determined for each analysis and analyzed using GenStat discovery (edition 3) statistical software package (ANOVA) and the differences between samples were determined by Duncan's Multiple Range test using. Differences were considered statistically significant at a probability level of $p < 0.05$.

RESULTS AND DISCUSSION

The percentage dry weight of the two solvent extracts (Acetone and Chloroform) was calculated by weighing the extracts after complete dryness and the result are shown in Table (1). The percentage dry weight of the solvent extracts was found to be 0.216 % and 0.144 % respectively. Both the extracts showed subsequent total antioxidant capacity of 9.82±1.64 in acetone extract and 12.13±1.63 mg catechol equivalent / gm DW in chloroform extract respectively (Table 1). The strong antioxidant capacity of the acetone and chloroform extracts of *Gracilaria verrucosa* might be attributed to the presence of photochemical such as phenolic compound. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant capacity of many medicinal plants [13]. Total antioxidant capacity in different extracts implies that the extracting solvent used would affect the radical scavenging potency which may be due to the different polarities of each antioxidant compound group present in the algal sample. [17] Phenolic compounds are very important constituents because of their scavenging ability due to their hydroxyl groups. A number of researches have pointed out that seaweed polyphenols are associated with antioxidant activity and play an important role in stabilizing lipid peroxidation. [3] Phenolic acids have repeatedly been implicated as natural antioxidants in fruits, vegetables, and other plants. [5] The total phenol content was measured as 2.011±0.035 % DW in acetone extract and 1.31±0.028 % DW in chloroform extract (Table 1). Phenols are particularly effective antioxidants for polyunsaturated fatty acid and acts as chemoprotective agents, anti-carcinogen, antioxidant, anti-apoptosis and anti-aging. [18] Similar results were also obtained by various researchers, who found a significant correlation between the total phenol content and the antioxidant activity of the same and different macro algae of the same family collected from Egypt and other parts of the World. [19-20]

Table 1: Percentage yield, Phenol content and Total Antioxidant capacity of organic extracts of *Gracilaria verrucosa*

Parameters	Acetone extract	Chloroform extract
Percentage yield (%)	0.216	0.144
Phenol content (% Catechol equivalent per DW)	2.011±0.035	1.31±0.028
Total Antioxidant capacity (mg Catechol equivalent per gram DW)	9.82±1.64	12.13±1.63

All data are expressed in mean ± S.D

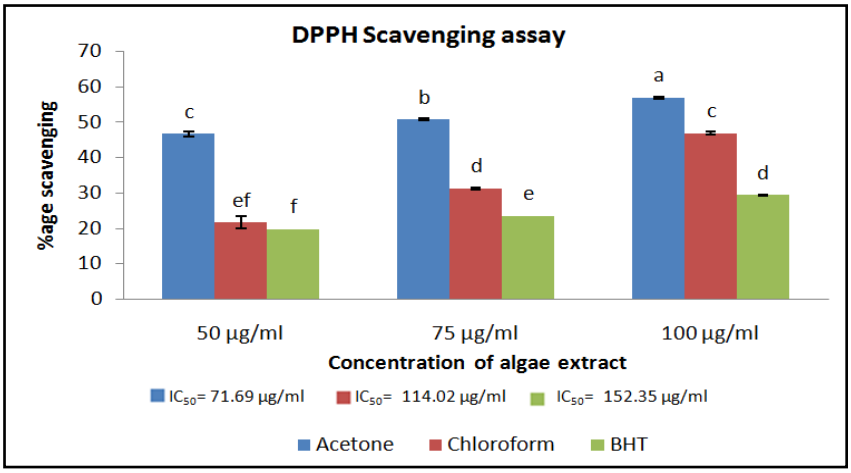

Fig. 1: DPPH free radical scavenging potential of organic extracts of *G. verrucosa*

All data were expressed in mean ± S.D; Difference in superscript letters indicate significance level at *p< 0.05*

Scavenging of DPPH free radical has been widely used to investigate the ability of algal extracts and fractions or compounds that act as free radical scavengers or hydrogen donors. The change in absorbance produced by DPPH was used to evaluate the ability of test compounds to act as free radical scavengers. The results showed that the algal extracts were more active in comparison to standard BHT (Fig 1). Similar results have been reported by various workers on different fractions of *G. verrucosa*. [20] The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability that is often used in many preliminary studies. Hydrogen donor molecules present in the extract that react with the DPPH radical causes a decrease in the intensity of absorption at 517 nm and can help identify extracts with anti-radical compounds. [20] Both the extracts showed significant DPPH radical scavenging activity at $p<0.05$ (LSD 2.08). The IC_{50} values of the acetone extract, chloroform extract and the standard BHT was found out to be 71.69, 114.02 and 152.35 µg/ml respectively. It was conformed that the acetone extract possesses more active DPPH free radical scavenging compounds than the chloroform and standard BHT.

The study on metal chelating activity revealed that the acetone extract has potential chelating activity of 0.08±0.113 %, 5.74±0.240 %, 15.28±0.360 % and chloroform extract showed 0±0, 10.72±0.59 %, 16.97±0.59 % in all the three different concentrations (50 µg/ml, 75 µg/ml, and 100 µg/ml) respectively (Fig 2). The reducing capacity of the solvent extracts may serve as a significant indicator of its potential antioxidant activity by measuring the transformation of Fe^{3+} to Fe^{2+}. [21] Both the extracts showed significant Metal chelating activity at $p<0.05$ (LSD 1.82). The IC_{50} values of the acetone extract, chloroform extract and the standard BHT was found out to be 490.26, 338.51 and 563.93 µg/ml respectively.

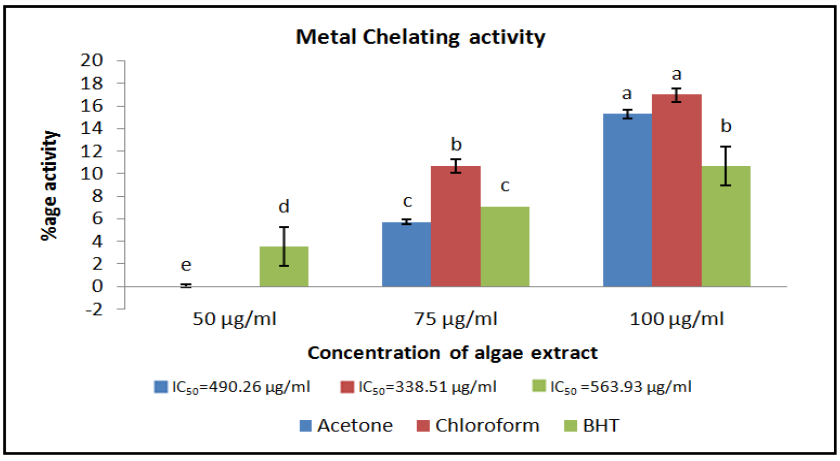

Fig. 2: Metal chelating activity of organic extracts of *G. verrucosa*

All data were expressed in mean ± S.D; Difference in superscript letters indicate significance level at *p< 0.05*

CONCLUSION

The results obtained in the present study clearly demonstrate that the acetone and chloroform extracts of *Gracilaria verrucosa* showed potent antioxidant properties against the different forms of free radicals. It also showed that its antioxidant properties are concentration and time-dependent. The findings of the current work appears to be useful for further research aiming to isolate and identify the specific phenolic compounds responsible for the antioxidant activity of *Gracilaria verrucosa* and exploit the beneficiary properties of the same for its potential use in food processing and pharmaceutical industries.

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