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In vitro antioxidant activity of marine red algae *Chondrococcus hornemanni* and *Spyridia fusiformis*

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ABSTRACT

Free radical scavenging activity of the marine red algae Chondrococcus hornemanni and Spyridia fusiformis were performed using the DPPH photometric assay. They are potential as natural sources of antioxidants and have gained recent attention. This study focuses on determining the antioxidant properties of the different solvent extracts, namely chloroform, methanol and aqueous. In general Spyridia fusiformis extract possesses high antioxidant activity followed by Chondrococcus hornemanni which might be helpful in preventing or slowing the progress of various oxidative stress related disorders. The present study suggests that different solvent extracts contain different antioxidant compounds able to scavenge free radicals.

Key words: *Chondrococcus hornemanni* and *Spyridia fusiformis*, antioxidant, total phenolic contents, DPPH.

INTRODUCTION

The oxidative damage caused by reactive oxygen species on lipids, proteins and nucleic acids may trigger various chronic diseases, such as coronary heart disease, atherosclerosis, cancer and ageing [3]. Epidemiological studies have demonstrated an inverse association between intake of fruits and vegetables and mortality from age related diseases, such as coronary heart disease and cancer, which may be attributed to their antioxidant activity [2]. On the other hand, some synthetic antioxidants, such as BHT and BHA, need to be replaced with natural antioxidants, as they were found to be toxic and carcinogenic in animal models [10]. Natural antioxidants such as flavonoids, phenolics, tannins, curcumin and terpenoids are found in various plants [19, 20, 21] which act as reducing agents, hydrogen donators, free radicals scavengers and singlet oxygen quenchers and therefore, as cell saviors [22]. Hence, now a days search for natural antioxidant source is gaining much importance. Thus, it is important to identify new sources of safe and inexpensive antioxidants of natural origin.

Algal biomass and algae-derived compounds have a very wide range of potential applications for human nutrition and health products. Some algae are considered as rich sources of natural antioxidants [5]. Macroalgae have received much attention as potential natural antioxidants [1, 8], and there has been very limited information on antioxidant activity of macroalgae [5]. Present study was aimed to investigate the antioxidant potentials of two distinct marine red algae *Chondrococcus hornemanni* and *Spyridia fusiformis* by using the DPPH photometric assay.

EXPERIMENTAL SECTION

Fresh materials of *Spyridia fusiformis* (Wulfen) and *Chondrococcus hornemanni* (Lyngb) F.Schmitz were collected from intertidal regions of Leepuram, Kanyakumarai, South East Coast of Tamilnadu, India, by hand picking method. The freshly collected samples were thoroughly cleaned using sterilised sea water to remove the sand and salt contents. The sample was also gently brushed with soft brush to remove attached epiphytes, other marine organisms and debris. Dried seaweeds were powdered and soaked in different solvents (chloroform, methanol and water) (1:20, w/v) overnight, filtered and concentrated to crude extract.

2.1 DPPH photometric assay

DPPH scavenging activity was measured by the spectrophotometric method [11]. To a methanolic solution of DPPH (200 µM), 0.05 ml of the test compounds were added at different concentrations (100-900 µg/ml). An equal volume of methanol was added to the control. After 20 min, the decrease in the absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated by using the formula [9]. The experiment was repeated in triplicate.

$$\text{Inhibition (\%)} = \frac{(\text{Control- test})}{\text{Control}} \times 100$$

2.2 Total Phenolic content

The total phenolic concentration was measured using the Folin-Ciocalteau method [12]. In this procedure, 100 µl aliquot of stock sample was mixed with 2.0 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. Then 100 µl of Folin-Ciocalteau's phenol reagent was added. After incubation for 30 min at room temperature in darkness, the absorbance was read at 720 nm using spectrophotometer.

2.3 Total flavonoid content

The total flavonoid content was determined according to the method of [17]. Briefly, a 250 µl of 5% $NaNO_2$ solution was added to 0.5 ml of the stock sample along with 150 µl of 10% $AlCl_3.H_2O$ solution. After 5 min, 0.5 ml of 1M NaOH solution was added and then the total volume was made up of 2.5 ml with ionized distilled water and the absorbance was read 510 nm.

2.4 Statistical Analysis

Data were obtained as the mean and standard deviation (SD) and the IC_{50} values of antioxidant were determined using SPSS version 11.5 for windows.

RESULTS AND DISCUSSION

3.1 DPPH

The marine red algae extracts were tested for their scavenging activity against the stable free radical DPPH by spectrophotometrically. It is visually noticeable as a colour change from purple to yellow. The values in percentage of inhibition of DPPH scavenging activities of the algal extracts were summarized in Tables 1, 2 and Figs. 1a, 1b. *Spyridia fusiformis* revealed the maximum (51.14 ± 0.01) extent of DPPH scavenging activity by the methanolic extract with 100 µg/ml, followed closely by chloroform and then by the aqueous extract. Whereas, the chloroform extract of *Chondrococcus hornemanni* showed the highest radicals scavenging power (64.32 ± 0.05) in the concentration of 100 µg/ml, followed closely by methanol and then by the aqueous extract. Thus, it proved that solvent polarity have great influences in the antioxidant compounds. But these algae show lower scavenging activity in comparison to synthetic antioxidants such as BHT and ascorbic acid, exhibited antioxidative potential in increased concentration.

In general, the scavenging effects on the DPPH radical increased sharply with increasing concentration of all the samples and standards. The results show that these algae had the highest DPPH scavenging activity and this indicates that *Spyridia fusiformis* and *Chondrococcus hornemanni* as a good source of natural antioxidant.

Extracts of red alga from the Rhodomelaceae and Bangiaceae families have been reported to exhibit weak DPPH quenching activity when obtained using water [4, 18]. On the other hand, chloroform, ethyl acetate and acetone extracts from several Rhodomelaceae genera have been reported to exhibit strong DPPH quenching activity *In vitro*

[14]. Thus, the particular solvent used to extract the seaweed material will have a dramatic effect on the chemical species recovered.

3.2 TOTAL PHENOLICS AND FLAVANOID CONTENT

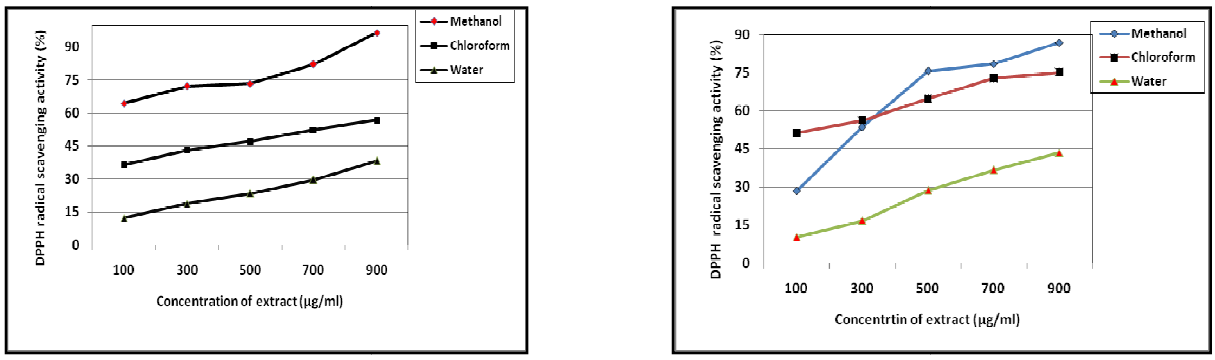
It was also observed that the extract which contained high total phenolic content, showed high antioxidant activity. In the present study total phenolic content was found to be higher in *Chondrococcus hornemanni* (3.14 ± 002 µg/g dry wt than that of *Spyridia fusiformis* (0.59 ± 0.002 µg/g dry wt) of dry material. Similarly, the total flavonoids in the extract were found to be lower in *Spyridia fusiformis* (0.012 ±.0.001 µg/g dry wt) than that of *Chondrococcus hornemanni* (0.013 ± 0.000 µg/g dry wt) of dry material (Table.3). This indicates that phenolic constituents present in these seaweed extracts would be capable of functioning as free radical scavengers. This results show that compounds with the stronger radical scavenging activity in the algae is medium polarity. This trend was observed in both the algae. The scavenging activity of all samples on the DPPH radical was found to be strongly dependent on concentration.

Table.1 Evaluation of the antioxidant effect of different solvents extracted by DPPH radical screening test for *Spyridia fusiformis*

Concentration (µg/ml)	Methanol	Chloroform	Aqueous
100	64.32 ± 0.05	36.35 ± 0.02	12.19 ± 0.02
300	72.15 ± 0.04	43.19 ± 0.02	18.73 ± 0.02
500	73.32 ± 0.03	47.12 ± 0.07	23.36 ± 0.007
700	82.16 ± 0.03	52.28 ± 0.02	29.67 ± 0.01
900	96.44 ± 0.03	56.80 ± 0.007	38.35 ± 0.02
IC_{50}	78	535	1175

Table.2 Evaluation of the antioxidant effect of different solvents extracted by DPPH radical screening test for *Chondrococcus hornemanni*

Concentration (µg/ml)	Methanol	Chloroform	Aqueous
100	28.5 ± 0.04	51.14 ± 0.01	10.21 ± 0.02
300	53.57 ± 0.04	56.19 ± 0.01	16.71 ± 0.007
500	75.57 ± 0.03	64.76 ± 0.01	28.61 ± 0.02
700	78.48 ± 0.007	72.73 ± 0.01	36.68 ± 0.02
900	86.75 ± 0.007	75.03 ± 0.02	43.35 ± 0.02
IC_{50}	280	98	1040



(a) **(b)**

Fig.1 DPPH radical scavenging activity (%) of different solvent extracts from a) *Spyridia fusiformis* b) *Chondrococcus hornemanni*

More recently, Jimenez-Escrig *et al*., [7] determined that the DPPH free radical scavenging by brown and red algal extracts were positively correlated with the total polyphenol content of these extracts. Some authors claim that there is no correlation between the total phenolic content and the radical scavenging capacity [15], so it was very important to examine the correlation between the total phenolic contents and total antioxidant capacity of the studied seaweeds. It is believed that the antioxidant properties of phenolics are a result of their ability to act as reducing agents, hydrogen donors, and free radical quenchers and phenolics can also act as metal chelators which prevent the catalytic function of metal in the process of initiating radicals [13]. The results of the present study revealed that there is a strong correlation between antioxidant activity and phenolic content. It is possible that the antioxidant of both seaweed extract (*Spyridia fusiformis* and *Chondrococcus hornemanni*) can be the result of their high concentration of phenolic compounds.

However, the strength of the existing data is not enough to suggest a reasonable mode of action for antioxidant effects. The data of this study may just enrich the existing comprehensive data of antioxidant activity of algae. Further studies are required to identify the active principles responsible for the significant antioxidant effect.

Table.3 Total phenol and flavonoid content of the experimental algae

S.No	Name of the algae	Major Phyto constituents	
		*Total phenol (µg/g dry wt)	*Flavonoids (µg/g dry wt)
1	*Spyridia fusiformis*	0.59 ± 0.002	0.01 ±0.001
2	*Chondrococcus hornemanni*	3.14 ±0.002	0.01 ± 0.000

*Mean ± SD

CONCLUSION

The present study has shown the antioxidant activity of red marine algae. The antioxidant content of the seaweeds varies significantly with different solvents. Thus the *Spyridia fusiformis* and *Chondrococcus hornemanni* is a potential source for natural antioxidant which will be beneficial as nutraceuticals as well as cosmeceuticals. The findings of the current report are useful for further research aiming at isolating specific phenolic compounds responsible for the antioxidant activity of *Spyridia fusiformis* and *Chondrococcus hornemanni*, thus enabling the seaweeds to produce a high value added product.

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