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Research Article

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Evaluation of antioxidant property of methanolic extract of red algae *Chondrococcus hornemannii* and *Spyridia fusiformis*

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ABSTRACT

The aim of the present study was to evaluate the antioxidant activity of marine red algae Chondrococcus hornemannii and Spyridia fusiformis. The methanolic extract of the algae C. hornemannii and S. fusiformis screened for antioxidant activities against superoxide and ABTS free radicals and were compared to that of standard BHT and α-tocopherol. The phenolic related compounds are mainly responsible for the higher rate of antioxidant activity. These results indicated that both seaweeds could be potential sources of development novel antioxidant compounds.

Key words: *Chondrococcus hornemannii, Spyridia fusiformis,* antioxidant, Super oxide, ABTS.

INTRODUCTION

Seaweeds are multicellular macroalgae used as a potential renewable resource in the field of medical and commercial environment [1], many of which have commercial applications in pharmaceutical, medical, cosmetic, nutraceutical, food and agricultural industries. The seaweed contains numerous pharmacologically important bioactive constituents such as flavonoids, carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals. Nowadays seaweeds are used as dietary food supplements in daily life and it regulates the human health [2].

Algae from the three groupings traditionally known as Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae) produce compounds with varying bioactivities that might have pharmaceutical applications [3]. Accordingly, interest in the search for natural antioxidants from algae has been increasing in recent years. Natural antioxidants, found in many algae, are important bioactive compounds that play an important role against various diseases and ageing processes [4], through the protection of cells from oxidative damage. Research into the natural products chemistry and chemical defenses of algae over the past 40 years has resulted in the isolation of over 15,000 novel compounds, many of which have been shown to have bioactive properties [1, 5-10]. Therefore, the studies on natural antioxidant are attracted by investigators and consumers for use in foods or medicinal materials to replace synthetic antioxidant.

Antioxidant activity has been reported in numerous genera of marine algae, including *Ahnfeltiopsis, Colpomenia, Gracilaria, Halymenia, Hydroclathrus, Laurencia, Padina, Polysiphonia* and *Turbinaria* [11]. The detected

antioxidant compounds in algae from these genera and others have potential anti-aging, dietary, anti-inflammatory, antibacterial, antifungal, cytotoxic, anti-malarial, anti-proliferative, and anticancer properties [1, 4].

However, uses of the synthetic antioxidants such as butylated hydroxyanisol, butylated hydroxytoluene (BHT) have been suspected to be a possible cause of liver damage and carcinogenesis [12, 13]. Consequently, nowadays most of the literatures are more publishing on finding alternative antioxidants from natural origin. According to that novel finding of marine seaweed is a valuable antioxidant source, it consists of high levels of antioxidant compounds [14, 15, 16]. Among the red algae, the *C. hornemannii* and *S. fusiformis* is known to produce the largest number and diversity of secondary metabolites, ultimately making it the world's most chemically complex seaweed. Based on that seaweeds and their extracts are beneficial to health and some even have been reported to retain biological activity of potential medicinal value. Hence, the present study investigated to evaluate the antioxidant activity of methanolic extract of marine red algae *C. hornemannii* and *S. fusiformis.*

EXPERIMENTAL SECTION

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

2.1 Scavenging of superoxide radical

In the present study, the efficiency of the algal extracts in inhibiting the generation of superoxide radical was studied using the methods elaborated by Winterbourn *et al*., [17]. Assay tubes contained 0.2 ml of the extract (corresponding to 20 mg extract) with 0.2 ml EDTA, 0.1 ml nitro blue tetrazolium, 0.05 ml riboflavin and 2.64 ml phosphate buffer. The control tubes were set up with DMSO (Dimethyl sulfoxide) solution instead of the algal extracts. The initial optical densities of the solutions were recorded at 560 nm and the tubes were illuminated uniformly with the fluorescent lamp for 30 mins. A_{560} was measured again and the difference in O.D was taken as the quantum of superoxide production. The percentage of inhibition by the algal samples was calculated by comparing with O.D of the control tubes.

2.2 ABTS radical cation decolorisation assay

In this improved version of $ABTS^{\cdot}$, a free radical is generated by persulfate oxidation of 2, 2-azinobis (3-ethylbenzoline-6-sulfonic acid) - ($ABTS^{2-}$). ABTS radical cation was produced by reacting ABTS solution (7 mM) with 2.45 mM Ammonium PerSulphate and the mixture was allowed to stand in the dark at room temperature for 12-16 hrs before use. For the study, different concentrations (100-500 µg/ml) of methanolic extract (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with ethanol to make 1ml. The absorbance was read at 745 nm and the percentage inhibition was calculated.

2.3 Total Phenol content

The total phenolic concentration was measured using the Folin-Ciocalteau method [18]. In this procedure, 100 µl aliquot of stock sample were 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.

2.4 Total flavonoid content

The total flavonoid content was determined according to the method of [19]. 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.

2.5 Statistical Analysis

Data were obtained as the mean and standard deviation (SD) and the IC_{50} values of antioxidant were determined using SPSS version 17.0 for windows.

RESULTS AND DISCUSSION

3.1 Superoxide anion scavenging activity

Superoxides are produced from molecular oxygen due to oxidative enzymes of the body as well as via non enzymatic reaction such as autooxidation by catecholamines [20]. The scavenging activity towards the superoxide radical (O_2^-) is measured in terms of inhibition of the generation of O_2^-. The probable mechanism of scavenging the superoxide anions may be due to the inhibition effect of the extract towards generation of superoxides in the *in vitro* reaction mixture. Superoxide and hydroxyl radicals are the two most effective representative free radicals. In cellular oxidation reactions, superoxide radical is normally formed first and its effects can be magnified because it produces other kinds of cell damaging free radicals and oxidizing agents [21]. Superoxide scavenging activity of *S.fusiformis* exhibited a maximum of s62.50 ± 0.04% and *C. hornemannii* shows 52.38 ± 0.04%, which is higher than the standard BHT and L-ascorbic acid whose scavenging effect was 61.48 ± 0.01 and 58.01 ± 0.04% respectively (Table.1). The IC_{50} value of methanolic extracts of *C.hornemannii* was 80 µg/ml and *S.fusiformis* was 96 µg/ml and standard BHT was 32 and L-ascorbic acid was 68.51 respectively.

The methanolic extracts of *Grateloupia lanceolata*, *Ahnfeltiopsis flabelliformis*, *Martensia denticulata*, *Bonnemaisonia hamifera*, *Carpopeltis affinis* and *Prionitis cornea* are found to have relatively higher superoxide anion scavenging activities (over 83%). Nagai and Yukimoto [22] recorded a significant superoxide anion scavenging activity in a beverage made from *S.fusiformis* and Kuda *et al*., [23] reported a good superoxide anion scavenging activity in edible brown seaweed, *Nemacystus decipiens*. In agreement with these observations, the present study also exhibited strong superoxide anion inhibitory effect of *C. hornemannii* and *S. fusiformis* and they can be used as an application in antioxidant source.

3.2 ABTS radical scavenging activity

ABTS assay is a simple indirect method for determining the activity of natural antioxidants. In the absence of phenolics, ABTS radical is rather stable, but it reacts energetically with an H-atom donor, such as phenolics, been converted into a non-colored form of ABTS [24]. The ABTS radical cation-scavenging assay performed showed that the antioxidant activity increases with an increase in the concentration. Total antioxidant capacity of the algal extracts was evaluated by its ability to scavenge $ABTS^+$ radical cation. *C.hornemannii* shows 37.10 ± 0.03% and *S.fusiformis* shows 46.27 ± 0.04% (Table.1), of inhibition at 100 µg/ml concentration and these are significantly lower than that of the standard BHT (98.99 ± 0.02%) and L-ascorbic acid (98.85 ± 0.03%). The IC_{50} value of methanolic extract was 290 µg/ml for *S. fusiformis* and 476 µg/ml for *C.hornemannii* was higher than that of standard BHT (32.5 µg/ml) and L-ascorbic acid (45.1 µg/ml).

The results indicated that methanolic extract has a significant effect on scavenging free radicals. The active substances in the alcoholic extract of *Turbinaria conoides* behave as primary and secondary antioxidants [25]. ABTS radical scavenging activity of four red seaweeds was reported by Sachindra *et al*., [26]. However, the limitations of ABTS assay, such as the capability of a sample to react with ABTS radical rather than to inhibit the oxidative process and the slow reaction of many phenolics [27] necessitate compatible evaluation of antioxidant activity using other assays as well bring in this gap. The antioxidant activity of the extracts is strongly dependent on the types of solvent used due to compounds with different polarity exhibiting different rates of antioxidant potential [28]. The methanolic extract of *C.hornemannii* was consistent with broad antioxidant activities via both single electron transfer and hydrogen atom transfer system [29].

3.3. Phytochemicals

More recently, [30] extracts were positively correlated with the total polyphenol content of these extracts. There are few reports stating that no correlation between the total phenolic content and the radical scavenging capacity [33], so it was very important to examine the correlation between the total phenolic contents so, it was very important to examine the correlation between the contents of total phenolic compounds and related antioxidant efficiency. Many researches stated that phenolic compounds are one of the most effective antioxidants in brown algae [25, 31]. In our study, the methanolic extract of *C.hornemannii* and *S. fusiformis* (Table.2) had significantly higher phenol content. It is possible that the antioxidant of both seaweed extracts (*C.hornemannii* and *S. fusiformis*) can be the result of their high concentration of phenolic compounds (Table.2). It is possible that the antioxidant of both seaweed extracts (*C.hornemannii* and *S. fusiformis*) can be the result of their high concentration of phenolic compounds.

Table.1 Effect of methanolic extract of *C.hornemannii* and *S. fusiformis* on different antioxidant models

S.No	Concentration (µg/ml)	Free radical scavenging activity (inhibition %)			
		C.hornemannii		*S.fusiformis*	
		Superoxide radical	ABTS	Superoxide radical	ABTS
1	100	52.38 ± 0.04	37.10 ± 0.03	62.50 ± 0.03	46.27 ± 0.04
2	200	57.14 ± 0.08	43.40 ± 0.04	76.17 ± 0.02	46.84 ± 0.04
3	300	61.90 ± 0.08	52.43 ± 0.04	80.95 ± 0.04	47.13 ± 0.06
4	400	63.10 ± 0.09	58.30 ± 0.02	83.21 ± 0.02	48.42 ± 0.04
5	500	66.66 ± 0.04	58.59 ± 0.03	85.71 ± 0.03	48.56 ± 0.03
IC_{50}	BHT	32	32.5		
	L-ascorbic acid	68.51	45.1		
	C. hornemannii	80	476		
	S. fusiformis	96	290		

Values are expressed as Mean ±SEM, n=3

Table.2 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	*Chondrococcus hornemannii*	3.14 ±0.002	0.01 ± 0.000
2	*Spyridia fusiformis*	0.59 ± 0.002	0.01 ±0. 001

**Values are expressed Mean ±SD*

CONCLUSION

In the present study the methanol extracts of marine red algae *C.hornemannii* and *S. fusiformis* at varying concentrations were shown as a potential reducing agent, superoxide radical and ABTS radical scavengers. The methanolic extracts showed *C.hornemannii* and *S. fusiformis* showed significant antioxidant activity and the efficacy was comparable with commercial antioxidants. From the present study, it can be concluded that the methanolic extract of seaweeds can be used as easily accessible, source of natural antioxidants and as a possible food supplement or in the pharmaceutical industry. The results shown here indicate that red algae *C.hornemannii* and *S. fusiformis* extracts can be a good source of natural antioxidant. However, the responsible compounds related to the antioxidant activity of the algal extract are not yet cleared. Further investigation is needed to isolate and identify the specific class of compound that is responsible for the antioxidant activity.

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