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Research Article

Evaluation of antioxidant activity of methanol extracts of red algae *Chondrococcus hornemannii* and *Spyridia fusiformis*

Murugesan S* and Bhuvaneswari S

Unit of Algal Biotechnology and Bionano Technology, PG and Research, Department of Botany, Pachaiyappa's College, Chennai-600 030 India

***Correspondence Info:**
Murugesan S
Unit of Algal Biotechnology and Bionano Technology, PG and Research, Department of Botany, Pachaiyappa's College,
Chennai-600 030 India
E-mail: smurugesan5@gmail.com

Abstract

The antioxidant activity of methanol extracts of the red seaweed, *Chondrococcus hornemannii* and *Spyridia fusiformis* was analyzed through two different radicals such as nitricoxide and hydroxyl radicals. The extract from *S. fusiformis* had the highest antioxidant potential, which was also found to be equivalent to the antioxidant activities of some commercial antioxidants (BHT and L-ascorbic acid). The antioxidant assay was performed at the concentration ranging from 100 – 500 µL. The present study confirms that *C. hornemannii* and *S. fusiformis* received special attention and used as a source of natural antioxidant.

Keywords:
Antioxidant activity,
Red algae, Nitric oxide, Hydroxyl radicals.

1. Introduction

Seaweeds are potential renewable resources in the marine environment. Marine organisms are a rich source of structurally novel and biologically active metabolites. 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 [1-3]. In light of the broad spectrum of their reported biological activities, algae have been suggested as a promising source of bioactive substances that might have pharmaceutical applications [4].

Accordingly, interest in the search for natural antioxidants from algae has been increasing in recent years. The overall aim of this type of research is discovery of compounds and/or extracts that can counteract free radical-induced and other oxidative stress processes, and in so doing decrease the incidence of human diseases directly related to these processes [5]. Natural antioxidants from algae are known to play an important role against various diseases and aging processes [6].

In light of the potential commercial uses of algal antioxidant compounds in the medicine, food, pharmaceutical, and cosmetic industries [6], we saw the need to ascertain whether algae could be a natural source of such compounds. Therefore, the present study was conducted to evaluate the antioxidant activity of extracts from marine red algae *Chondrococcus hornemannii* and *Spyridia fusiformis* using nitricoxide radicals and hydroxyl radicals assay.

2. Materials and methods

2.1 Collection and extraction of marine algae

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, were identified by standard manual [7]. The freshly collected samples were thoroughly cleaned using sterilized sea water to remove the sand and salt contents. Dried seaweeds were powdered and soaked in methanol overnight, filtered and concentrated to crude extract. The crude extract powder was stored at room temperature.

2.2 Scavenging of nitric oxide radical

Nitric oxide generated from sodium nitroprusside was measured by the Griess reaction [8]. Sodium nitroprusside (5 mM) in standard phosphate buffer solution was incubated with different concentration (100-500 µg/mL) of the methanol extract of the experimental algae was dissolved in phosphate buffer (0.025 M, pH 7.4) and the solutions were incubated at 25^{o}C for 5 hrs. After 5 hrs, 0.5 mL of the incubation solution was removed and diluted with 0.5 mL of Griess' reagent (1% Sulphanilamide, 2% orthophosphoric acid and 0.1% naphthalene diaminedihydrochloride). The absorbance of the chromospheres formed during the diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthalene diamine was read at 546 nm.

2.3 Scavenging of hydroxyl radicals

Hydroxyl radical scavenging activity was measured using the method of Kunchandy and Rao [9] by studying the competition between deoxyribose and test extract for hydroxyl radical generated by Fenton's reaction. The reaction mixture contained deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbate (0.1 mM), $KH_2PO_4^-$ KOH buffer (20 mM, pH 7.4) and various concentrations of the sample extracts in a final volume of 0.1 mL. The reaction mixture was incubated for 1 hr at 37^{o}C. Deoxyribose degradation was measured as thiobarbituric acid reacting substances (TBARS) and the percentage of inhibition was calculated.

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 17.0 for windows.

3. Results

3.1 Nitric oxide Radical scavenging activity

Suppression of NO^- release may be attributed to a direct NO scavenging effect as the seaweed extracts decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro* as shown in Table.1. The results showed that *S. fusiformis* and *C. hornemannii* had scavenging activity of 29.72 ± 0.03 and 12.07 ± 0.02% (Table.1) respectively, and these values are comparably lower than that of the standard BHT (52.68 ± 0.03%) and L-ascorbic acid (41.62 ± 0.05%). The IC_{50} values of the nitric oxide radical assay were compared to the standard antioxidants BHT (77.49 µg/mL) and L-ascorbic acid (108.52 µg/mL). The IC_{50} values of methanol extracts of red algae *C. hornemannii* was 480 µg/mL and *S. fusiformis* was 430 µg/mL. It was also found that the IC_{50} value of the algal extracts were higher than that of L-ascorbic acid and BHT (Table.1).

3.2 Hydroxyl Radical scavenging activity

The scavenging effect of OH was investigated using the Fenton reaction and the results were shown as an inhibition rate in Table.1. *S. fusiformis* exhibited the lower inhibition of about 20.05 ± 0.02% and *C. hornemannii* shows 12.62 ± 0.03% of inhibition against hydroxyl radicals and this is higher than the standard BHT as well as the L-ascorbic acid, which exhibited 61.42 ± 0.01 and 56.28 ± 0.02% of inhibition, respectively. In the present study, it was observed that the methanol extract of *C. hornemannii* showed IC_{50} values of 355 µg/mL whereas *S. fusiformis* at 392 µg/mL. However, IC_{50} of BHT and ascorbic acid was found to be only 54.50 µg/mL and 80 µg/mL respectively (Table.1).

Table.1 Effect of methanol 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*	
		Nitricoxide radical	Hydroxyl radical	Nitricoxide radical	Hydroxyl radical
1	100	12.07 ± 0.02	12.62 ± 0.03	29.72 ± 0.03	20.05 ± 0.02
2	200	21.98 ± 0.05	26.43 ± 0.04	39.62 ± 0.04	32.12 ± 0.02
3	300	29.72 ± 0.07	39.55 ± 0.03	47.36 ± 0.02	48.63 ± 0.03
4	400	31.57 ± 0.08	51.03 ± 0.03	48.60 ± 0.04	56.32 ± 0.04
5	500	39.01 ± 0.07	66.91 ± 0.04	51.39 ± 0.03	69.62 ± 0.45
IC_{50}	BHT	77.49	54.5		
	L-ascorbic acid	108.52	80		
		480	355	430	392

Values are expressed as Mean ± SEM, n=3

4. Discussion

4.1 Nitric oxide free radical scavenging activity

Nitricoxide is generated when sodium nitroprusside reacts with oxygen to form nitrite. Seaweed compounds inhibit nitrite formation by competing with oxygen to react with nitric oxide directly. Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes [10]. Excess concentration of NO is associated with several diseases [11]. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxinitrite anions, which acts as free radicals [12]. The results of the present study suggest that *C. hornemannii* and *S. fusiformis* might be potent and novel therapeutic agents for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO and its oxidation product, peroxynitrite.

4.2 Hydroxyl radical scavenging activity

The hydroxyl radical is the most reactive free radical and can be formed from superoxide anion and hydrogen peroxide. The free radical has an extremely short half-life but is capable of causing great damage in living organisms [13-14]. It is now widely held that the mutagenic capacity of oxygen is due to the direct interaction of hydroxyl radicals, which are produced through the interaction of hydrogen peroxide and superoxide with transition metals [15]. The hydroxyl radical is a major reactive oxygen species causing lipid peroxidation as it can abstract a hydrogen atom from phospholipids membranes [16]. The methanol extract showed a similar effect compared to the effect exhibited by phenolic compounds in the studies carried out by Shon *et al* [17] suggesting that they can be used to minimize the adverse effects from the hydroxyl radical. Hydroxyl radicals are known to be capable of abstracting hydrogen atoms from membranes and they bring about peroxidic reactions of lipids [18]. Therefore, the strong hydroxyl radical scavenging activity in aqueous extract of red algae indicates that some aqueous extract could be used as an application in antioxidant source

Nahas *et al* [19] reported the high antioxidant activity of extracts from brown seaweeds, comparable to that of commercial antioxidants. However, in the present study their antioxidant activity (*C.hornemannii* and *S.fusiformis*) was lower than that of the commercial antioxidants (L-ascorbic acid and BHT). The present study suggested that both the seaweed extracts possess high antioxidant activity that might be helpful in preventing or slowing the progress of various oxidative stress related disorders.

The results of the antioxidant assays indicated that the methanol extracts of *C.hornemannii* and *S. fusiformis* are the best source of antioxidant compounds. It has been reported that sun drying and subsequent storage of algae will considerably decrease the levels of these labile antioxidants such as L-ascorbate and BHT [20]. A requirement for endogenous antioxidant capacity in algae is implicit, due to the fact that algae, as intertidal organisms, require protection against UV irradiation [21].

5. Conclusion

The methanol extract of *C.hornemannii* and *S. fusiformis* showed high antioxidant activities. The finding of this work are useful for further research to identify, isolate and characterize the specific compound which is responsible for the highest antioxidant activity and which may promote their use as natural sources of antioxidants.

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