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Original Article

In vitro antioxidant activity of methanolic extract of green alga *Valoniopsis pachynema*

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ABSTRACT

This study was conducted to investigate the *in-vitro* antioxidant activity of methanolic extract of green alga *Valoniopsis pachynema.* The free radical scavenging activity of the extracts was measured by superoxide; hydroxyl and nitric oxide radicals and the antioxidant potential were compared with commercial antioxidants, such as Butylated Hydroxy Toluene (BHT) and L- ascorbic acid. The antioxidant activity of experimental algae showed a dose dependent increase when compared to commercial antioxidants. The significant free radical scavenging activity of *Valoniopsis pachynema* might be attributed to algal contents of carotenoids, free phenols and fatty acids.

Keywords: *Valoniopsis pachynema*, Superoxide radical, Hydroxyl radical, Nitric oxide radical

INTRODUCTION

Free radicals are responsible for aging and causative agents of various human diseases. Antioxidant compounds play an important role in various fields such as medical field (to treat cancers, cardiovascular disorders, and chronic inflammations), cosmetics (anti- ageing process) and food industries (food preservative) [1].

Seaweeds contain reactive antioxidant molecules, such as ascorbate and glutathione (GSH) when fresh, as well as secondary metabolites including carotenoids (α-and β carotene, fucoxanthin, astaxanthin), mycosporine-like amino acids (mycosporine-glycine), catechins (e.g., catechin, epigallocatechin, gallate, phlorotannins ,eckol and tocopherols (α-,χ-δ-tocopherols) [2] Oxidative stress is a crucial etiological factor to the pathophysiology of variety of degenerative or pathological conditions such as aging, cancer, coronary heart disease, Alzheimer's disease, atherosclerosis and inflammation [3,4].

Multiple mechanisms of enzymatic and non-enzymatic antioxidant systems are present in human body to protect the cellular molecules against reactive oxygen species (ROS) induced damage [5]. The present study was conducted to evaluate the antioxidant potential of methanolic extract of *Valoniopsis pachynema*.

MATERIALS AND METHODS

Collection of algae: The marine green alga was collected from Mandapam coast, Tamilnadu, South India.

P**reparation of Algal extract:** The freshly collected samples were soaked and thoroughly cleaned in sea water to remove the sand and salt contents and shade dried. Dried seaweeds was powdered and soaked in methanol (1:3, w/v) overnight and filtered to collect the methanol fraction. The residue was extracted two more times and the filtrates were combined and concentrated to obtain the crude extract. All the fractions were concentrated by evaporating under vacuum in a rotary evaporator and the dried extract was used for exploring its potential activity.

Invitro Antioxidant Assay: The antioxidant activity of methanolic extract of green alga *Valoniopsis pachynema* was examined. The free radical systems like superoxide, hydroxyl and nitric oxide were used for assessing the free radical scavenging activity of the test extract and the results were compared with the standard antioxidant Butylated hydroxytoluene and L-ascorbic acid.

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Scavenging of superoxide radical: The extent of superoxide generation *in vitro* studied on the basis of inhibition of production of nitro blue tetrazolium formazone by the algae extract measured spectrophotometrically at 560 nm. The 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 without the algal extract, where DMSO (Dimethyl sulfoxide) was added. The initial optical densities of the solutions were recorded at 560 nm and the tubes were illuminated uniformly with the fluorescent lamp for 30 minutes. A 560 was measured again and the difference in O.D was taken as the quantum of superoxide production. The percentage inhibition by the algal sample was calculated by comparing with the O.D of the control tubes. In the present study, the efficiency of the algal extract in inhibiting the *in vitro* generation of superoxide was studied using the methods elaborated by Winterbourn *et al.,* (1975) [6].

Scavenging of hydroxyl radical: Hydroxyl radical scavenging activity was measured according to the method of Kunchadny and Rao (1990) [7], 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 extract in a final volume of 0.1 mL. The reaction mixture was incubated for 1 h at 37ºC. Deoxyribose degradation was measured as thiobarbituric acid reacting substances (TBARS) and the percentage inhibition calculated.

Scavenging of nitric oxide radical: Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction [8, 9, 10]. Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration (100-500 µg/ml) of the methanol extract dissolved in phosphate buffer (0.025 M, pH 7.4) and the tubes were incubated at 25ºC for 5 hr. Control experiments without the test compound, but with equivalent amounts of buffer were conducted in an identical manner. After 5 hrs, 0.5 mL of incubation solution was removed and diluted with 0.5 ml of Griess' reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% napthylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylene diamine was read at 546 nm. The experiment was repeated in triplicate.

The IC_{50} the antioxidant activity in terms of the amount (µg/mL) of the extract necessary for inhibiting 50% of cell growth.

RESULTS AND DISCUSSION

In the present study, the free radical scavenging activities of methanolic extract from the experimental algae *Valoniopsis pachynema* were carried out using superoxide, hydroxyl and nitric oxide radicals.

Superoxide radical scavenging activity: Superoxide anions are the most common free radicals in vivo and are generated in a variety of biological systems and the concentration of superoxide anion increases under conditions of oxidative stress. Hence, an assay was carried out to test whether the methanolic extract of *Valoniopsis pachynema* and scavenge superoxide anions. The results showed that *V. pachynema* at the concentration 500 µg/mL had the higher DPPH scavenging activity of 87.71 ± 0.03% (Table. 1 and Fig. 1). The IC_{50} values of methanolic extract from the experimental algae *V. pachynema* was 98 µg/mL which was higher when compared to the standards BHT (33 µg/mL) and L-ascorbic acid (69.51 µg/mL) (Table.2). 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%). The results of the present investigation are in agreement with those of Le Tutour (1990) [11] who investigated the antioxidant activities of different seaweeds in their studies and reported, *Laminaria digitata* and *Himanthalia elongata* exhibited the most valuable antioxidant activities compared with those of vitamin-E and Butylhydroxy Toluene (BHT). Kuda *et al.,* (2005) [12] reported a good superoxide anion scavenging activity in edible brown seaweed, *Nemacystus decipiens*. Our study also exhibited strong superoxide anion inhibitory effect, in the methanolic extract of *V. pachynema* and it can be used as an application in natural antioxidant source.

Hydroxyl radical scavenging activity: The hydroxyl radical is one of representative reactive oxygen species generated in the body. The hydroxyl radical, OH, is the neutral form of the hydroxide ion (OH). Hydroxyl radicals are highly reactive and consequently short-lived; however, they form an important part of radical chemistry. Hydroxyl radical scavenging activity showed a maximum % of 69.64 ± 0.45 at the concentration 500 µg/mL (Table. 1 and Fig. 1) for *Valoniopsis pachynema*. The IC_{50} values of the methanolic

extract from the experimental algae *V. pachynema* was 394 µg/mL which was higher compared to the standards BHT (55.5 µg/mL) and L-ascorbic acid (81 µg/mL) (Table. 2). The results of the present study indicate that the methanolic extract from the experimental algae has higher and significant effect on scavenging of hydroxyl radical. In this assay, the antioxidant activity was determined based on the ability of the antioxidant components in the samples to inhibit deoxyribose oxidation by reactive OH- generated from Fenton's type of reaction.

Nitric oxide radical scavenging activity: Nitric oxide is generated from amino acid L-arginine by vascular endothelial cells, phagocytes and certain cells in the brain. Scavengers of nitric oxide compete with oxygen and lead to the production of nitric oxide. Nitric oxide radical scavenging activity showed a maximum % of 54.39 ± 0.03 at the concentration 500 µg/mL (Table. 1 and Fig. 1) for *Valoniopsis pachynema*. The IC_{50} values of the methanolic extract from the experimental algae *V. pachynema* was 433 µg/mL which was higher compared to the standards BHT (78.49 µg/mL) and L-ascorbic acid (109.52 µg/mL) (Table. 2). The results of the present study indicate that the methanolic extract from the experimental algae has higher and significant effect on scavenging of nitric oxide radical. The suppression of nitric oxide release may be partially attributed to direct scavenging by the extracts of *V. pachynema*, which decrease the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro* [13]. The results of the present study are similar to Monsuang et al., (2009) [14] who reported that green seaweeds showed significantly higher phenolic content and antioxidant activities than red seaweeds. The results of the study also proves that superoxide radical shows better activity of the methanolic extract of the experimental algae *V. pachynema* when compared to hydroxy radical followed by nitric oxide radical.

Table.1. Effect of methanolic extract of *Valoniopsis pachynema* on superoxide, hydroxyl and nitric oxide radicals

S. No	Concentration (µg/mL)	*Superoxide radical	*Hydroxyl radical	*Nitric oxide radical
1	100	63.5 ± 0.03	22.05 ± 0.02	29.75 ± 0.03
2	200	78.17 ± 0.02	34.12 ± 0.02	39.66 ± 0.04
3	300	82.95 ± 0.04	49.63 ± 0.03	49.36 ± 0.02
4	400	85.21 ± 0.02	57.32 ± 0.04	51.60 ± 0.04
5	500	87.71 ± 0.03	69.64 ± 0.45	54.39 ± 0.03
P-Value		0.000	0.000	0.000
F-Value		1.934555	2.109666	7.561433

* Values are expressed as Mean ± SD of triplicates

Table.2. IC_{50} values of experimental algae *V. pachynema with* standard on free radical scavenging system. (Free radical scavenging assay IC_{50} value (µg/mL))

	Free radical scavenging assay IC_{50} value (µg/mL)			
S.No	**Standard /Experimental algae**	**Superoxide**	**Hydroxyl**	**Nitric oxide**
1	BHT	33	55.5	78.49
2	L-ascorbic acid	69.51	81	109.52
3	*V. pachynema*	98	394	433

Values are expressed as Mean ± SD of triplicates

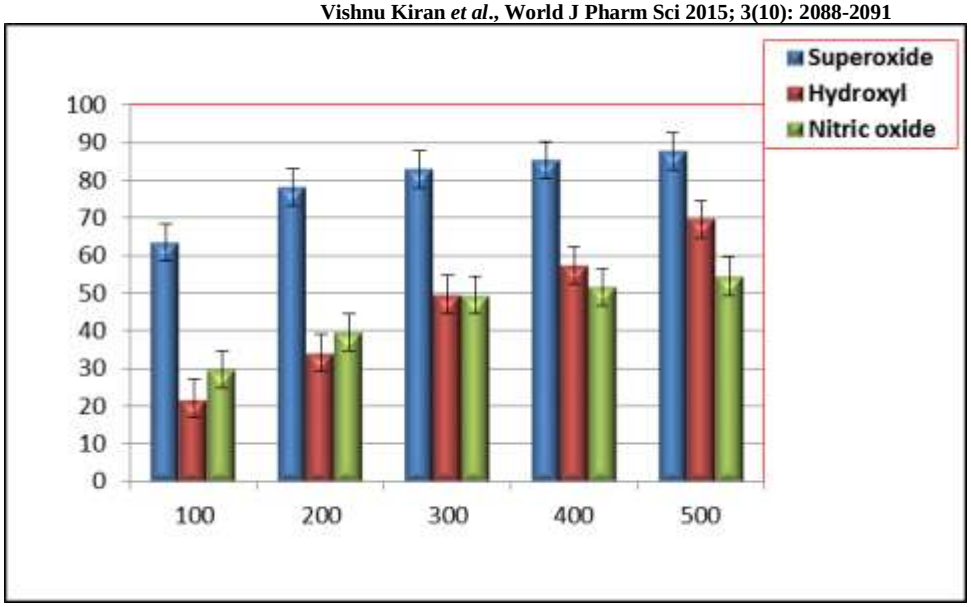

Figure.1. Superoxide, hydroxyl and nitric oxide scavenging activity of methanolic extract from *Valoniopsis pachynema*

CONCLUSION

The results showed that the methanolic extract from the experimental algae *Valoniopsis pachynema* had active radical scavenging activity against superoxide, hydroxyl and nitric oxide radicals. Hence forth the algae *V. pachynema* may be used as good source of natural antioxidants. Furthermore studies are required to isolate and identify the active compounds from this alga and also screen for their invivo studies, mechanism of action.

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