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Original Article

Antioxidant Activity of Six Marine Sponges Collected from the Persian Gulf

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Abstract

Compounds especially from natural sources are capable of protecting against reactive oxygen species (ROS) mediated damage. Therefore, there is a growing interest in novel substances exhibiting antioxidant properties. Several marine environments can provide a rich source of novel biologically active compounds. The aim of this paper is to evaluate *in vitro* antioxidant properties of six sponge species collected from Kish Island in the Persian Gulf. We evaluated the effects of different concentrations of the dichloromethane and methanolic extracts of six sponges on scavenging DPPH and OH free radicals. The activities of these extracts were compared with those of commercial antioxidants such as gallic acid. The maximum level of DPPH radical scavenging (0.234± 0.033 mg/ml) was observed for the methanolic extract of *Pseudosaberites clavatus* in the reaction mixture. Also, most of sponge extracts exhibited medium to high hydroxyl radical scavenging activity. The results of this study suggest that marine sponges of the Persian Gulf are promising sources of antioxidants.

Keywords: Antioxidant activity; Marine sponges; Persian Gulf; Reactive free radical.
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1. Introduction

Even today, natural products have a major role for innovative drugs. Research on natural products has shifted toward marine environments as a rich source of biologically active agents for clinical applications. Sponges, a group of marine invertebrate, multicellular and immobile, are a point of interest for marine natural product researchers as a source of novel bioactive products [1]. There have been a growing number of investigations indicating potential antioxidant, antiinflammatory, antibacterial, antifungal and cytotoxic activity of extracts from marine sponges [2-9]. Among these bioactive compounds, antioxidants are of special interest due to the role of free radicals in many ailments including cancer, aging and atherosclerosis [10-13]. The Persian Gulf is an unexplored and presumably rich source of marine natural products. There are few reports on the bioactivity of the Persian Gulf sponges, most notably a report on *in*

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vitro antimicrobial activity of sponges collected from Nay Band Bay, Iran [14, 15]. As a part of our screening studies in order to find promising marine natural products from the Persian Gulf region, herein, we present our initial findings on the antioxidant activity of dichloromethane and methanolic extracts of six sponges: *Fascaplysinopsis reticulata, Callyspongia siphonella, Niphates furcata, Callyspongia sp., Callyspongia clavata* and *Pseudosaberites clavatus* collected from coral Island of Kish (26.32 ºN, 53.58 ºE) in the Persian Gulf. A survey of literature showed that there are reports on anti-malarial and anti- cancer activity of natural products isolated from *Callyspongia siphonella* as well as reversal alkaloids from *Fascaplysinopsis reticulata*. We have not been able to find any report on the natural products of the other four sponges [16-20].

2. Materials and methods

2.1. Chemicals and reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl radical) and gallic acid were purchased from Sigma Chemical Company. All other reagents were obtained from Merck Chemical Company.

2.2. Sponge collection

Six species of marine sponges were collected from the north coast of the Persian Gulf, near Kish Island (26.32 ºN, 53.58 ºE). The samples were cleaned and washed with distilled water and immediately frozen and maintained at -20 °C prior to extraction.

2.3. Identification of sponges

Five of the samples were identified by Michelle Kelly, National Centre for Aquatic Biodiversity and Biosecurity, National Institute of Water and Atmospheric Research Ltd, Auckland, New Zealand. *Callyspongia sp.* was identified by Rob W.M. Van Soest, Institute for Systematic and Ecology (Zoological Museum), University of Amsterdam, Amsterdam, the Netherlands. A voucher specimen was deposited at Table 1 for each sponge.

2.4. Extraction

The freeze-dried sponges were ground to a fine powder. One gram of the powdered sponge sample was then extracted successively with dichloromethane (20 ml) followed by methanol (20 ml) at room temperature overnight on a shaker. The residue was dried and extracted again using the above-mentioned procedure, twice. The combined dichloromethane extract (60 ml total) and methanolic extract (60 ml total) were filtered through filter paper and concentrated in vacuum. Dried residue was stored at -20°C and used in antioxidant assays.

2.5. Bio Autography

Extracts were diluted with methanol and applied on a sheet of TLC and then developed with *n*-hexane:ethyl acetate (10:3). Active bands could be visualized as yellow spots against purple background by spraying DPPH solution in methanol.

2.6. Hydroxyl Radical Scavenging Assay

The colorimetric 2-deoxyribose oxidative degradation method was used as the reference method for the determination of OH radical scavenging activity of extracts. The reacting mixture for the deoxyribose assay [21, 22] made up to the final volume of 1 ml contained the following reagents: 200 µl pH 7.4 phosphate buffer (100 mM) solution, 200 µl deoxyribose (15 mM), 200 µl $FeCl_3$ (50 mM), 100 µl ascorbic acid (100 mM) (ascorbic acid and $FeCl_3$ premixed immediately before its addition to the reaction mixture), 100 µl EDTA (100 mM), 100 µl H2O2 (10 mM) and 100 µl of the dichloromethane or methanol extracts of six sponges with the concentration of 0.5, 0.25, 0.125 and 0.062 mg/ml. Reaction mixtures were incubated at 37 °C for 1 h. At

Table 1. Sample collection specificities.

Voucher number	Depth	Place	Time of collection	Name of sponge
NIWAKD 6674	<5m	Kish Island, Persian Gulf, Iran	10-08-2010	Callyspongia clavata
NIWAKD 6676	<5m	Kish Island, Persian Gulf, Iran	10-08-2010	*Pseudosaberites clavatus*
22172	5-10m	Kish Island, Persian Gulf, Iran	10-08-2010	*Callyspongia sp.*
NIWAKD 6667	<5m	Kish Island, Persian Gulf, Iran	13-06-2010	*Niphates furcata*
NIWAKD 6669	<5m	Kish Island, Persian Gulf, Iran	13-05-2010	Callyspongia siphonella
NIWAKD 6666	<5m	Kish Island, Persian Gulf, Iran	13-06-2010	*Fascaplysinopsis reticulata*

the end of the incubation period, deoxyribose degradation was measured by the thiobarbituric acid (TBA) method [23]. Thus, 1 ml of TBA (1% w/v) and 1 ml of trichloroacetic acid (TCA) (2.8% w/v) were added to the mixture and heated in a boiling water bath for 20 min to develop the pink colored malondialdehyde–thiobarbituric acid adduct, and the absorbance of the resulting solution (2.0 ml) was measured at 532 nm. The reaction mixture not containing the test sample was used as control. The inhibition ratio of the extract (%) was calculated using the following formula: inhibition ratio (%) = $100\times[A_0\text{-}A/A_0]$ where A_0 and A are the absorbances of the system in the absence and presence of scavenger, respectively. The assay was repeated three times for each concentration.

Table 2 depicts OH radical scavenging effect of the two solvent extracts of six sponge species.

2.6. DPPH radical scavenging assay

Free radical scavenging activities of the dichloromethane and methanol extracts of the sponges along with the reference standards such as gallic acid were determined by the DPPH free radical method [24]. The absorbance was monitored at 490 nm at different concentrations of each sample extract dissolved in 100 µl methanol (1.5 to 3200 µg /ml) and mixed with 100 µl of the methanolic DPPH solution (50 µM), after standing at room temperature for 30 min. The percentage of scavenging has been

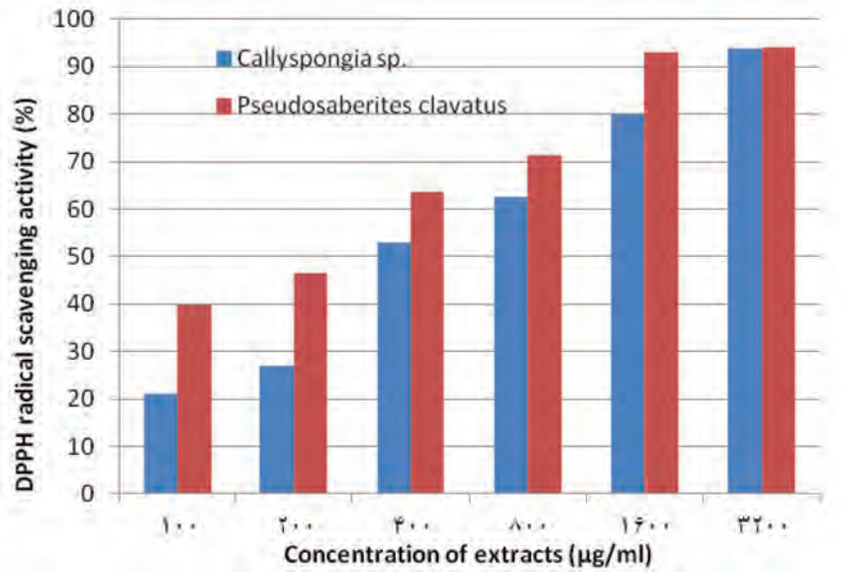

Figure 1. DPPH free radical scavenging activity of methanol extract of *Pseudosaberites clavatus* and *Callyspongia sp.* Values are means of triplicate determinations (n=3)±standard deviation.

Table 2. Hydroxyl radical scavenging activities of sponge extracts

	Percent of OH radical Inhibition in CH2Cl2 extract*			
Name of sponge	0.5**	0.25	0.125	0.062
Fascaplysinopsis reticulata	71.2±1.69	82.0±1.09	82.7±2.14	81.8±0.59
Niphates furcata	82.9±0.98	80.9±0.82	79.7±2.01	74.6±0.88
Callyspongia siphonella	71.4±1.40	72.3±1.54	69.3±1.93	72.1±1.34
Callyspongia sp.	9.1±5.8	9.8±1.19	10.8±3.61	-2.2±1.68
Callyspongia clavata	22.4±1.46	46.3±2.77	46.6±3.86	47.6±4.22
Pseudosaberites clavatus	20.3±3.55	14.9±3.00	39.0±2.70	43.5±9.63
	Percent of OH radical Inhibition in MeOH extract*			
Name of sponge or standard	0.5**	0.25	0.125	0.062
Fascaplysinopsis reticulata	75.0±3.25	75.7±5.79	72.5±2.95	72.9±0.99
Niphates furcata	71.6±2.08	75.4±3.30	75.1±4.28	80.5±3.09
Callyspongia siphonella	48.4±9.55	46.6±1.91	40.3±2.25	35.3±4.81
Callyspongia sp.	33.5±4.89	22.0±2.53	17.0±3.93	-2.7±1.07
Callyspongia clavata	66.2±1.99	68.4±2.71	70.4±1.15	73.2±0.87
Pseudosaberites clavatus	81.4±2.56	73.6±2.85	51.7±1.93	23.7±13.88
Gallic acid	73.6±0.73	65.4±0.82	60.7±2.07	72.4±0.45
BHT	57.7±0.10	65.5±1.19	63.2±1.59	62.6±1.22
Quercetin	53.2±3.49	49.0±1.83	55.1±3.05	51.5±2.26

*Values are mean of three independent determinations (n=3)±SD, standard deviation.
**Concentrations in mg of dried sponge extract or standard in 1 ml solvent.

calculated as the ratio of the absorption of the sample solution mixed with DPPH solution (OD_T) relative to the control (DPPH solution without the extract OD_C). The experiments were carried out in quadruplicate and average values were taken. Radical scavenging activity was calculated by using the following equation: DPPH radical scavenging activity (%) = $100-[[(OD_T-OD_B)/OD_C]\times100]$ where OD_B represents the absorbance of the sample extract solution alone. Anti-radical activity was defined as IC_{50} (the concentration of the extract generating 50% of radical scavenging). Gallic acid methanolic solution was used as the reference antioxidant. Table 3 depicts DPPH radical scavenging effects of two solvent extracts of the six sponge species.

2.7. Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) using Instat Version 3.10 software. *P* values>0.05 were regarded as non-significant and *P* values<0.001 as very significant.

3. Results and discussion

3.1. Hydroxyl radical scavenging assay

One of the reactive free radicals formed in biological systems is hydroxyl radical, known as a highly damaging species. This radical has the capacity to cause numerous cellular disorders such as carcinogenesis and cytotoxicity [25, 26]. Phytochemicals present in sponges can act as antioxidants and prevent disorders due to oxidative damage [27]. Results for OH radical scavenging activity of the two solvent extracts of six sponge species along with the reference standards such as gallic acid, quercetin and butylated hydroxyl toluene (BHT) are shown in Table 2. In the present study, dichloromethane extracts of *Callyspongia siphonella, Niphates furcata* and *Fascaplysinopsis reticulata* showed highest level of hydroxyl radical scavenging activity in comparison with the same dose of their methanolic extracts. On the other hand, methanolic extracts of *Callyspongia* sp., *Callyspongia clavata* and *Pseudosaberites clavatus* were more active than their corresponding dichloromethane extracts at the same concentration. The order of hydroxyl

Table 3. DPPH radical scavenging activities of sponge extracts.

Name of sponge or standard	IC_{50} CH2Cl2 extract±SD*	IC_{50} MeOH extract±SD
Fascaplysinopsis reticulata	0.605**±0.010	0.328±0.011
Callyspongia siphonella	≥1	≥1
Niphates furcata	≥1	≥1
Callyspongia sp.	≥1	0.530±0.040
Callyspongia clavata	≥1	≥1
Pseudosaberites clavatus	≥1	0.234±0.033
Gallic acid	-	0.610×10^{-3}±0.090

*Values are mean of three independent determinations (n=3)±SD, standard deviation.
**Concentrations in mg of dried sponge extract in 1 ml methanol.

radical scavenging among three tested standards was gallic acid>BHT>quercetin. There is no significant difference between gallic acid with BHT and BHT with quercetin (p>0.05), but there is a significant but not very high difference between gallic acid and quercetin (p<0.01).

In comparison with gallic acid, both extracts of *Niphates furcata* and *Fascaplysinopsis reticulata* showed superior OH radical scavenging activity (p<0.01), while dichloromethane extract of *Callyspongia siphonella*, methanolic extract of *Callyspongia clavata* and *Pseudosaberites clavatus* were found to be as effective as gallic acid (p>0.05). The order of hydroxyl radical scavenging activity at the same concentration for methanolic extracts was found to be *Fascaplysinopsis reticulata, Pseudosaberites clavatus, Niphates furcata* as effective as *Callyspongia clavata* (p>0.05) and *Callyspongia siphonella* to be more effective than *Callyspongia sp.* (p<0.001), and for dichloromethane extracts: *Fascaplysinopsis reticulata*, *Callyspongia siphonella* were as effective as *Niphates furcata* (p>0.05) and *Callyspongia clavata* was more effective than *Pseudosaberites clavatus* (p<0.001). There was no significant difference between *Fascaplysinopsis reticulata* and *Niphates furcata* activities in both extracts (p>0.05).

3.2. DPPH radical scavenging assay

DPPH radical is a stable free radical having an absorption maximum at 515 nm. In the presence of the compounds capable of donating H-atom or an electron there is a decrease in the absorbance at 515 nm which means a stable diamagnetic molecule has formed [28, 29]. The free radical scavenging activity of the dichloromethane and methanolic extracts of six different sponge species along with the reference standard gallic acid were determined by the DPPH radical method [24], and the results expressed as IC_{50} values are shown in Table 3. Percentage of DPPH radical scavenging activities of all the extracts were dose-dependent (Figure 1). There was not a very significant difference between methanolic extract of *Fascaplysinopsis reticulata* and *Pseudosaberites clavatus* (p<0.05) in DPPH radical scavenging activity test. Dichloromethane extract of *Fascaplysinopsis reticulata* and methanolic extract of *Callyspongia sp.* also showed good DPPH radical scavenging activities. None of the extracts was better than gallic acid in scavenging DPPH radical.

4. Conclusion

In this study, we meant to disclose our findings on antioxidant activities of several marine sponges from the Persian Gulf. Among these sponges, dichloromethane and methanolic extracts of *Niphates furcata* and *Fascaplysinopsis reticulata* exhibited higher OH radical scavenging activity compared to standard gallic acid. However, in DPPH radical scavenging assay, none of the extracts showed impressive activity compared to standard gallic acid. These results indicate

the promising antioxidant potential of some the Persian Gulf sponges.

Acknowledgments

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