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Research Article

FREE RADICAL SCREENING ACTIVITY OF MARINE SPONGE *AURORA GLOBOSTELLATA*

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ABSTRACT

Objective: Marine sponges are sessile organisms which produce chemicals to prevent themselves from the predators; these chemicals are the secondary metabolites. These metabolites are said to contain various biological activities. A research has been made for analyzing the antioxidant activity of crude extracts of sponges using hexane, ethyl acetate, ethanol, water as solvents and compared using gallic acid as standard antioxidant.

Methods: The free radical scavenging activity was analyzed using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical.

Results: The significant radical scavenging activity shown by hexane extract with inhibitory concentration IC_{50} as 30.62 µg/ml which is nearby the activity of gallic acid with IC_{50} 18.93 µg/ml. The method is based on the spectrophotometric measurement of the DPPH concentration change resulting from the reaction with an antioxidant.

Conclusion: Therefore, based on the analysis the hexane extract of the sponge has shown to have more antioxidants may be due to the presence of phenols and flavonoids. The research can be further done by the isolation of the antioxidant compound through chromatographic techniques, which could come up with the isolation of novel compound for various therapeutics.

Keywords: 1,1-diphenyl-2-picryl-hydrazyl, Chemo preventive, Absorbance, Cytotoxic, Antioxidant, *Aurora globostellata.*

INTRODUCTION

Marine environment is said to contain a wide array of organic and inorganic substances which are helpful for various therapeutic uses for human health. There are various marine organisms which provide an arsenal of a wide array of medicinal compounds, where sponges are said to be fat-free, low-calorie, and richest source of minerals [1]. As they are deep inside the sea, they are said to be free from pollutants and radiations. The research area of marine biotechnology is aggressively focused toward discoveries of novel compounds, which are obtained from marine organisms by their production of secondary metabolites. These metabolites have high medicinal values which are used as antioxidant, antiviral, anti-inflammatory, anticancer, antiproliferative, and antitumor activities [2]. This may be due to the presence of phenolic compounds, organic acids, and sulfated polysaccharides [3].

Marine sponges (phylum *Porifera*) that have existed for 700-800 million years and hence primitives of the animals. They are the most widespread species with approximately 15,000 species in marine environment [4-6]. Since the sponges contain active compounds in them, they play a major role in constructing the coral reefs. The bioactive compounds present in marine sponges are higher than those in plants. Still it is not understood how, when, where these compounds are produced [7]. Hence, the antioxidants present in marine sponges can be analyzed through the study and on further research the compounds can be isolated through wet lab techniques.

Antioxidants

All living organisms utilize oxygen for metabolism and dietary nutrients for energy production to survive. Hence, oxygen plays an important role in mediating chemical reactions which metabolize fats, proteins, and carbohydrates to produce energy. At the same time, it is a highly reactive atom capable of becoming part of potentially damaging molecules called as free radicals. These free radicals attack the healthy cells of the body leading to severe disorders. The compounds that have the ability to donate electrons and stabilize free radicals are said to possess antioxidant properties. Since free radicals contain unpaired electron, they are unstable and capture electrons from others to neutralize themselves which initially stabilizes the free radical but generates another, which is a chain reaction where more than thousands of free radicals can be formed within few seconds. These free radicals formation can be controlled by compounds called the antioxidants. When the antioxidants are deficient, the damage caused by free radicals is accumulated. These antioxidants are naturally occurring and also man-made which helps in prevention of cell damage. The marine sources are said to be rich in antioxidants which are primarily focused in the research paper.

METHODS

Sponge collection and identification

The marine sponge species were collected from Rameswaram coast, and cleaned with distilled water, frozen immediately until extraction. A small piece of sponge sample was cut and sent for identification at Zoological Survey of India [5]. It was then deposited under National Zoological Collections of MBRC/ZSI. The sponge was identified using spicules by nitric acid digestion method following Identification keys (Hooper, Thomas).

Extraction

The freeze-dried sponges were taken washed with distilled water and crushed using solvents such as hexane, ethyl acetate, ethanol, and water. Based on increasing order of polarities these solvents were selected. These extracts are further used for the antioxidant assay.

Phytochemical analysis

The qualitative analysis was carried out using methods described by Harborne (1998). The extracted Sponges were analyzed for the presence of various biochemical constituents as described by Chairman *et al.*, 2012 [6]. The phytochemical analysis is performed for finding the presence of bioactive constituents such as flavonoids, terpenoids, alkaloids, reducing sugar, phenolic compounds, saponin, tannin, aromatic acid, and xanthoprotein.

1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity

The hydrogen atom donating ability of the marine sponge extracts using different solvents was determined by the decolorization of the methanol solution of DPPH [7]. DPPH in the solution reacts with the antioxidant compound in the sample, which can donate hydrogen and reduce the DPPH. The color change from deep violet to light yellow was visualized, and the absorbance read at 517 nm on a UV-visible spectrophotometer.

Dissolving 29 mg of DPPH in 100 ml of methanol and kept at overnight incubation in the freezer. DPPH was diluted with methanol, and the absorbance was read at 517 nm to obtain O.D to 1. The diluted solution was then used for the further procedure.

The percentage of inhibition was calculated by the following:

% antioxidant activity=$[A_c - A_s A_c] \times 100$,

where A_c=Absorbance of control and A_s=Absorbance of the sample.

RESULTS

The importance of marine organisms as the source of secondary metabolites led to the determination of radical scavenging activity of marine sponge *Aurora globostellata*.

The marine sponges collected are extracted with different solvents based on polarity. Using DPPH assay, the extract with high antioxidant activity is analyzed by comparing with gallic acid as standard. The sponge extracts exhibited dose-dependent scavenging activity in various strengths. The phytochemical analysis was performed for finding the presence of bioactive constituents such as flavonoids, terpenoids, alkaloids, reducing sugar, phenolic compounds, saponin, tannin, aromatic acid, and xanthoproteins; the hexane extract was found to contain flavonoids, terpenoids, phenolic compounds, and aromatic acid.

DPPH is a stable free radical (deep purple color) owing its delocalization of spare electrons which prevents its dimerization and side reactions, as that happens with most of the free radicals [8]. DPPH radical scavenging activity was expressed as percentage inhibition that is inhibitory concentration $(IC)_{50}$ values were determined and compared with reference standard antioxidant gallic acid. The results of the hexane extract were found to having highest radical scavenging activity with IC_{50} value as 30.62 µg/ml, followed by water with 43.61 µg/ml, ethyl acetate with 48.17 µg/ml, and ethanol with 57.85 µg/ml. The standard gallic acid was found to have IC_{50} as 18.3 µg/ml as given in Table 1. The antioxidant activity of the hexane extract is nearby the activity of standard. The presence of antioxidants can be due to the presence of greater amounts of phenolic and flavonoid contents in the sponges [9]. The difference in DPPH radical scavenging activity of the extracts could

Table 1: Free radical scavenging activity of the marine sponge extracts

Serial number	Concentration of gallic acid (µl)	Volume of diluted DPPH	Incubate the tubes under dark condition for 15 minutes	Absorbance at 517 nm	Percentage of inhibition	IC_{50}
DPPH activity of standard - gallic acid						
1	10	3		0.647	36±1.2	18.93±1.33
2	20	3		0.484	51.6±1.0	
3	30	3		0.374	65.3±1.7	
4	40	3		0.13	87±1.5	
5	50	3		0.05	95±2.0	
DPPH activity of sponge *Aurora globostellata* - hexane						
1	10	3		0.850	15±0.7	30.62±1.0
2	20	3		0.672	32.8±1.0	
3	30	3		0.472	52.8±1.4	
4	40	3		0.242	75.8±1.8	
5	50	3		0.200	80.0±2.1	
6	60	3		0.195	80.5±2.4	
DPPH activity of sponge *Aurora globostellata* - ethyl acetate						
1	10	3		0.868	13.5±1.6	48.17±1.8
2	20	3		0.786	21.5±0.7	
3	30	3		0.678	32.2±0.9	
4	40	3		0.587	41.3±1.3	
5	50	3		0.489	51.1±1.2	
6	60	3		0.398	60.2±1.7	
DPPH activity of sponge *Aurora globostellata* - ethanol						
1	10	3		0.865	13.5±0.4	57.85±2.5
2	20	3		0.775	22.5±0.9	
3	30	3		0.789	25.9±1.1	
4	40	3		0.683	31.1±1.7	
5	50	3		0.556	44.4±2.2	
6	60	3		0.455	54.5±2.6	
DPPH activity of sponge *Aurora globostellata* - water						
1	10	3		0.871	12.9±1.2	43.61±1.8
2	20	3		0.770	23±1.1	
3	30	3		0.668	33.2±1.6	
4	40	3		0.534	46.6±0.8	
5	50	3		0.425	57.5±1.7	
6	60	3		0.315	68.5±1.9	

*Values are mean±SD, n=3; and values with different letters shows significant difference at $p<0.05$ level. DPPH: 1,1-diphenyl-2-picryl-hydrazyl, SD: Standard deviation, IC: Inhibitory concentration [20]

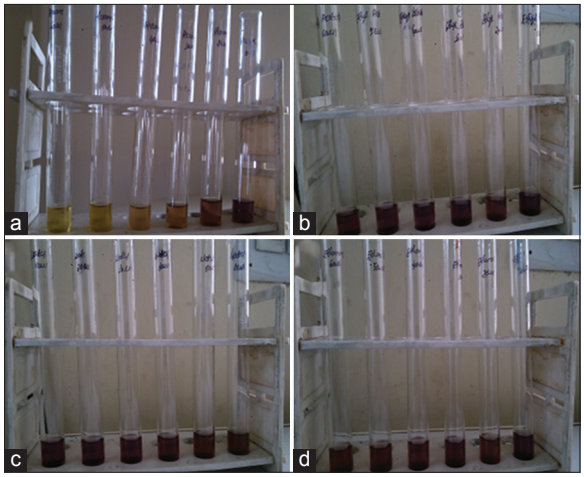

Fig. 1: Observance of decolorisation from purple color to yellow color. Extracts of marine sponges. (a) Hexane, (b) Ethyl acetate, (c) Water, (d) Ethanol

be due to the concentration of phenolic and flavonoid compounds [10].

The hexane extract was able to reduce the stable radical DPPH to the yellow color to give significant inhibitory percent, but the free radical scavenging activity of other solvents was poor. The absorption strength is decreased at 517 nm resulting in decolorization from the purple color to yellow color as shown in Fig. 1. This assay is preferably used widely to investigate the free radical scavenging ability of pure compounds.

DISCUSSION

There have been a growing number of biological activities indicating potential antioxidant, anti-inflammatory, antibacterial, antifungal, and cytotoxic activity of extracts from marine sponges [11-14], among all these antioxidants play a major role in treating various disorders. The effect of antioxidants on DPPH radicals is thought to be due to their hydrogen donating ability [15-18]. The reasons for focusing on radical scavenging activities are due to the vital role of free radicals in different diseases mainly different cancer types. The antioxidants act by inhibiting the lipid peroxidation mechanism. This method is preferable because of relatively short time taken for analysis. The IC_{50} of hexane extract was significantly higher than that of the other fractions with the order Hexane>water>ethyl acetate>ethanol. Although the hexane extract was not highly active than the standard, the IC_{50} was nearby the standard. As a result, the hexane extract of sponge *A. globostellata* was found to have high antioxidants, which can be used as a lead source for isolating novel metabolites as chemoprotective agents for various cancers. Similarly, the active fractions of the marine sponge *Haliclona exigua* were found to contain antioxidant compounds confirmed through gas chromatography-mass spectrometry analysis and also the main constituents in the fractions was found to be antibacterial, antioxidant, anti-inflammatory, and anticancer compounds [19].

CONCLUSION

The hexane extract of marine sponge *A. globostellata* was found to possess bioactive properties such as flavonoids, terpenoids, phenolic compounds, and aromatic acids. This extract exhibited a good antioxidant activity when compared to other extracts. Further investigations are being carried out to analyze the anticancer activity of sponges through cell line assays and also in the identification of the antioxidant compounds by characterizing them.

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