

Thoothukudi Coast and Antioxidant Activity of Ascidian

Dr.K. S. Manjunatha

Department of Pharmaceutical Chemistry, Kuvempu University, Post Graduate Centre, Kadur-577548, Karnataka, India.

Ascidians are a rich source of bioactive secondary metabolites. *Phallusianigra* is a simple ascidian belonging to the family Ascidiidae found in plenty throughout the year along the Thoothukudi coast of India. Antioxidant activity was performed by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method for different extracts of *Phallusianigra* which showed that the alcoholic extract of the animal on higher concentration possesses better antioxidant potential when compared to that of the standard ascorbic acid. They exhibited strong antioxidant DPPH radical scavenging activity with absorbance of 0.1984 and 0.0553 for ascorbic acid and ethanolic extract respectively. The strongest antioxidant activity of ethanolic extract may be due to the presence of flavonoids and phenols.

Keywords: *Phallusianigra*, DPPH, Flavonoids..

1. Introduction

The ocean is considered to be an untapped source for many things including potential drugs. Ascidians are marine sedentary organisms. In some countries, mainly those of the Far East and certain parts of the Mediterranean, ascidians are eaten by man and are sufficiently important to merit an entry in the F.A.O. yearbook of Fishery statistics [1]. *Microcosmussulcatus*, *Styelaplicata* and *Polyarpapomaria* are taken as food in the Mediterranean [2]. *Halocynthia roretzi* in Japan, is even cultured in the North of Honshu [3] for human consumption and *Pyurachilensis* is popular in South America [4] as a food source. Margalino and Destefano found that the flesh of *Microcosmussulcatus* is almost as digestible as whole egg and the protein content is higher [5]. Such is their abundance in some localities that ascidians have been considered as a possible source of cellulose, vanadium, protein and other chemicals [6,7]. Though the nutritive value of many natural resources of these seas has gained much attention, the importance of ascidians as food or drug has been totally neglected in our country. *Phallusianigra* is a simple ascidian belonging to the family Ascidiidae occurring as the major component of fouling community on the hulls of ships, piers, pilings, harbour installations and materials used for aquaculture operations in the Tuticorin Port Area. Previous studies show that the animal possesses antipyretic [8], analgesic anaesthetic [9], anti-inflammatory [10], wound healing [11] and antimicrobial activities [12,13]. No reports are available on the antioxidant activity of different extracts of the simple ascidian *Phallusianigra*. Hence, the present study focuses on the chemical investigation of antioxidant activity of the different extract of *Phallusianigra* by DPPH method.

The DPPH assay method is based on the reduction of DPPH, a stable free radical [14]. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical scavenging antioxidant) and is reduced to the DPPH-H form as consequence the absorbance is decreased from the DPPH [15]. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured [16]. More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug [17].

2. Materials and methods

2.1 Collection and identification

Phallusianigra (Fig. 1) was collected from Green Gate area ($8^{\circ}48'N$ and $78^{\circ}11'E$) of Thoothukudi Port, Tamil Nadu by SCUBA diving and identified using key to identification of Indian ascidians [18]. A voucher specimen (AS2083) was deposited in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin 628002, Tamil Nadu, India.

**Fig.1:Phallusianigra Sav.****2.2 Preparation of extract**

The whole animal was dried in shade and homogenized to get a coarse powder. The powder was successively extracted with various solvents such as petroleum ether (40° - 60° C), benzene, chloroform, ethanol, methanol and water.

2.3 DPPH Radical Scavenging Assay

The antioxidant activity of the animal extract was measured on the basis of the scavenging activity of the diphenyl-2-picrylhydrazyl (DPPH) free radical according to the method described by Brand-Williamset al [19] with slight modifications. 1 ml of 0.1 mM DPPH solution in methanol was mixed with 1 ml of animal extract solution of varying concentrations (50, 100, 150 and 200 μ g/ml). Corresponding blank sample were prepared and Ascorbic acid was used as reference standard. Mixture of 1 ml methanol and 1 ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-Visspectrophotometer (UV-VIS Shimadzu). The inhibition % was calculated using the following formula, Inhibition% = $A_{\text{c}} - A_{\text{s}} / A_{\text{c}} \times 100$

Where A_{c} is the absorbance of the control

A_{s} is the absorbance of the sample [20]

3. Results and Discussion

The results of antioxidant activity of the different extracts at varying concentrations are represented in the Table 1. The ethanol extract of the animal showed the significant antioxidant potential when compared to that of the standard ascorbic acid by DPPH scavenging assay method.

Table 1: Absorbance of different extract of *Phallusianigra* at varying concentration

Concentration (μ g/ml)	Petroleumether	Benzene	Chloroform	Ethanol	Methanol	Water			Standard Ascorbic acid
500.4944	0.52550	0.21240	0.19980	0.11390	0.11220	0.3126			
1000.48760	0.48790	0.21180	0.09210	0.10590	0.10800	0.2989			
1500.39220	0.42250	0.19890	0.07260	0.09760	0.07670	0.2126			
2000.23450	0.41000	0.09760	0.05530	0.06450	0.06210	0.1984			

Absorbance of control at 517 nm 0.3846

Radical scavenging method for different extracts of *Phallusianigra* showed that the chloroform, ethanol, methanol and aqueous extract of the animal at higher concentration possess better antioxidant potential when compared to that of the standard ascorbic acid. They exhibited strong antioxidant DPPH radical scavenging activity with absorbance of 0.1984 and 0.0553 for ascorbic acid and ethanol extract respectively. Generally, the antioxidant properties of these extracts were found to be concentration dependent. Based on the results obtained, highly significant antioxidant potential was observed in the ethanol, methanol and aqueous extracts which are more polar in DPPH assay [21]. A preliminary chemical screening of the ethanol extract of *Phallusianigra* showed the presence

of alkaloids, terpenoids, flavonoids, glycosides, phenolic compounds and tannins [22]. The strongest antioxidant activity of ethanol extract may be due to the presence of any of these chemical constituents.

4. Conclusion

The results of the present study suggest that the alcoholic and aqueous extract of *Phallusianigra* illustrates highly significant antidiabetic activity, which may be due to the presence of flavonoids and phenols.

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