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**Research Article**

# *In vitro* antioxidant and spectrophotometric studies of a colonial ascidian *Didemnum psammathodes*

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## Abstract

*Didemnum psammathodes* commonly available in Tuticorin coast was screened for its chemical value. Analysis of phenols and flavonoids were done by Spectrophotometry method. A maximum of 86.13% phenol and 69.07% Flavonoids were observed in *Didemnum Psammathodes*. This study is designed to examine the invitro antioxidant activity of phenolic compounds in the ethanolic extract of *Didemnum psammathodes* by DPPH method. In DPPH system the strongest radical scavenging activity was exhibited by the ethanolic extract ($IC_{50}$ - 49.22) when compared to standard drug ascorbic acid (25.98). An increase in dose has significantly increased the percentage of antioxidant activity. This result free radical induced oxidative reveal that *Didemnum psammathodes* ethanolic extract a promising antioxidant potential against free radical induced oxidative damage. The present observation suggests need for further investigation of *Didemnum Psammathodes* so as to isolate secondary metabolites.

**Keywords:** Phenols, colonial ascidian, *Didemnum Psammathodes*, Flavonoids, antioxidant

## 1. Introduction

Ascidians, commonly called as 'Sea Squirts' are sedentary tunicates. They have an outer protective covering for the body known as test or tunic. The ascidians live exclusively in marine environments, unable to survive in low salinity area. Ascidians feed by filtering organisms and particles from water. Ascidians feed by filtering organisms and particles from water [1]. *Didemnum psammathodes* is a colonial ascidian belonging to the family didemnidae and it is available in all over India. Antioxidants are radical scavengers. The natural antioxidant mechanisms may be insufficient in variety of conditions and hence dietary intake of antioxidant compounds are important [2]. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties [3]. A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity [4]. The scavenging property of *Didemnum psammathodes* may be due to hydroxyl groups existing in the phenols and flavonoids. Hence an attempt was made to study the *invitro* antioxidant activity, phenol and flavonoid of a colonial ascidian, *Didemnum psammathodes*.

## 2. Materials and Methods

### 2.1 Collection of animal material

*Didemnum psammathodes* was collected from Tuticorin coast in the month of May 2013 by SCUBA diving. Epibionts and particles of shell, coral fragments attached to the colony were carefully removed. Identification up to the species level was carried out based on the key to identification of Indian ascidian [5]. A voucher specimen has been submitted in the ascidian collections of the Museum of the Department of Zoology, A. P. C. Mahalaxmi College for Women, Tuticorin – 628002, Tamilnadu, India **Plate 1**.

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**Plate 1: Colony of *Didemnum Psammathodes***

**2.2 Systematic position**

*Didemnum psammathodes* belongs to Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Enterogona, Suborder: Aplousobranchia, Family: Didemnidae, Genus: *Didemnum* and Species: *psammathodes*

**2.3 Preparation of extract**

The specimen was washed several times with sterile sea water. It was dried under shade, homogenized to get a coarse powder which was stored in an air-tight container and used for all further investigations. 0.5 g of the dry powder was ground in a mortar and pestle with ten times volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatant was collected and the extract is used for the estimation of phenols and flavonoids.

**2.4 Chemical analysis**

Phenol was estimated by using Catechol [6]. Flavonoid content was estimated by following [7] method. Elico Sc-177 Scanning mini spectrophotometer was used for the measurement of absorbance.

**2.4.1. DPPH Radical Scavenging Assay**

The antioxidant activity of the animal extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical according to the method described by Brand-Williams *et al*., [8] with slight modifications. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of animal extract solution of varying concentrations (50, 100, 150 and 200 µg/ml). Corresponding blank sample were prepared and L-Ascorbic acid was used as reference standard. Mixture of 1ml methanol and 1ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer (UV-VIS Shimadzu). The inhibition % was calculated using the following formula, Inhibition % = Ac-As/Ac×100

Where Ac is the absorbance of the control

As is the absorbance of the sample

## 3. Results and Discussion

The results of the present study are given in **Figure 2**. Present study indicates *Didemnum psammathodes* contain 69.07% phenols. Phenols are a class of antioxidants which act as free radical terminators [9-11]. The greater amount of phenolic compounds leads to more potent radical scavenging effect. HPTLC studies of *Microcosmus exasperatus* have revealed the presence of phenolic compounds such as gallic acid, ferulic acid, caffeic acid [12]. Present study indicates that *Didemnum psammathodes* contain a high amount 86.13%of flavonoids. Preliminary research indicates that flavonoids may modify allergens, viruses and carcinogens, hence may act as biological "response modifiers". *In vitro* studies show that flavonoids also have anti-allergic, anti-inflammatory, antimicrobial, anticancer, antitumour, antioxidant and anti-diarrheal activities [13-15]. A comparison of result shows *Didemnum psammathodes* has high percentage of flavonoids than the other chemical constituents.

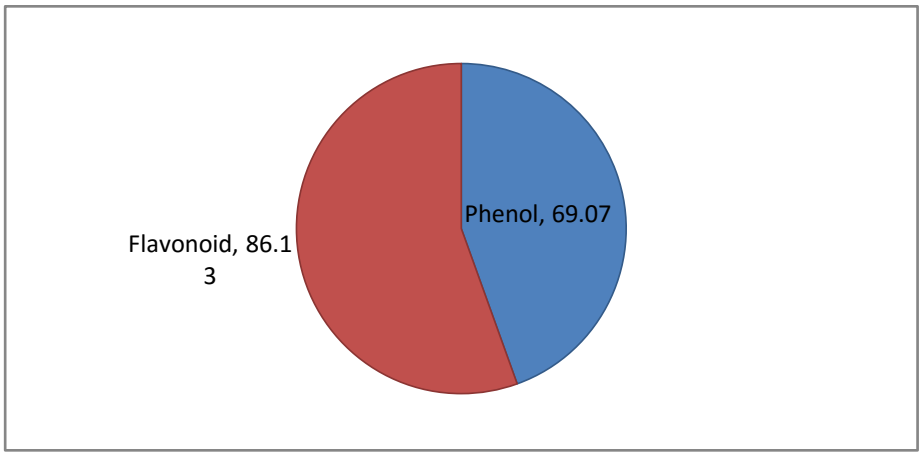


**Figure 2: Percentage of Phenol, Flavonoid**

**Table 1: Antioxidant Studies by DPPH method**

| Con (µg/ml) | % of activity (+ SEM) | |
|---|---|---|
| | DP Extract | Ascorbic acid |
| 50 | 23.64±0.37 | 21.84±0.43 |
| 100 | 38.33±0.28 | 32.92±0.23 |
| 200 | 59.03±0.48 | 51.16±0.65 |
| 400 | 72.88±0.59 | 63.48±0.36 |
| 800 | 83.29±0.21 | 71.42±0.27 |
| 1600 | 92.38±0.56 | 80.34±0.21 |
| IC50 | 49.22 | 25.98 |

*Didemnum psammthodes* has been tested for antioxidant activity previously. The antioxidant activity was assessed by spectrophotometry of the presence of the DPPH radical scavenging activity. When an antioxidant scavenges free radicals by hydrogen donation, the DPPH assay solution becomes lighter in color. Samples with a raw material concentration range of 50.0–1600.0 µg/ml were analyzed. The quality of the antioxidants in the sample was determined by the IC50 values, denoting the concentration of the sample required to scavenge 50% of the DPPH free radicals. The 1/IC50 coefficient was calculated to compare the antioxidant activity of sample. The antioxidant activity of the tested extracts increased with the quantity of raw material in the extract. The antioxidant activity of polyphenols is due mainly to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. A dosage level was increased by higher antioxidant activity when compared to standard drug ascorbic acid. A maximum of antioxidant activity in higher dosage (1600 mg/ml) was observed in DPPH method. The results indicate that the ethanolic extract with their proton donating ability could serve as free radical inhibitors or scavengers acting possibly as primary antioxidants.

## 4. Conclusion

These spectrometric studies suggest the extract of didemnum ascidians presence a lot of chemical compounds and have potency as a drug. Further research is used to investigate and design this sample as drug in future.

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