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ANTIOXIDANT ACTIVITY OF A COLONIAL ASCIDIAN *EUDISTOMA VIRIDE* USING DPPH METHOD

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ABSTRACT

Eudistoma viride is a colonial ascidian belonging to the family polycitoridae and it is available in all over India. This study is designed to examine the *invitro* antioxidant activity of *Eudistoma viride* by DPPH method with different extracts. In DPPH system, the strongest radical scavenging activity was exhibited by the ethanolic extract of *Eudistoma* when compared to standard drug ascorbic acid. An increase in dose have significantly increased the absorbance of antioxidant activity. This result reveal that *Eudistoma viride* of ethanolic extract a promising antioxidant potential against free radical induced oxidative damage.

KEYWORDS: Colonial ascidian, *Eudistoma viride*, antioxidant activity.

INTRODUCTION

Since ancient times, the medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities. Antioxidants are the compounds that when added to food products, especially to lipids containing foods, can increase the life by retarding the process of lipid peroxidation, with is one of the major reasons for deterioration of food products during processing and storage.[1]

Ascidians commonly called as "Sea Squirts" and filter feeding organisms. In most of the countries ascidians were taken as food. *Eudistoma viride* is a colonial ascidian belonging to the family polycitoridae and it is available in all over India. In recent years considerable attention has been directed towards the identification of ascidians with antioxidant activity. The aim of the study were to prepare antioxidant rich fractions from *Eudsitoma* of different extracts and to evaluate their antioxidant activity using DPPH method.

MATERIALS AND METHODS

Collection of animal material

Eudistoma viride 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.[2] 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**.

Plate 1: Colony of *Eudistoma viride*

Systematic position

Eudistoma viride belongs to Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Enterogona, Suborder: Aplousobranchia, Family: Polycitroidae, Genus: *Eudistoma a*nd Species: *viride.*

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. The powder was successively extracted with various solvents such as petroleum ether (40^0-60^0 C), benzene, chloroform, ethanol, methanol and water for DPPH method. 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.

Chemical analysis

Phenol was estimated by using Catechol.[3] Flavonoid content was estimated by following[4] standard methods. Elico Sc-177 Scanning mini spectrophotometer was used for the measurement of absorbance.

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*.,[5] 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

RESULTS AND DISCUSSION

The results of the present study are given in Table 1 & Figure 1. Present study indicates *Eudistoma viride* contain 107 g/100g phenols. Phenols are a class of antioxidants which act as free radical terminators.[6-8] 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.[9] Present study indicates that *Eudistoma viride* contain a high amount 40 g/100g 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.[10-12] A comparison of result shows *Eudistoma viride* has high percentage of flavonoids than the other chemical constituents.

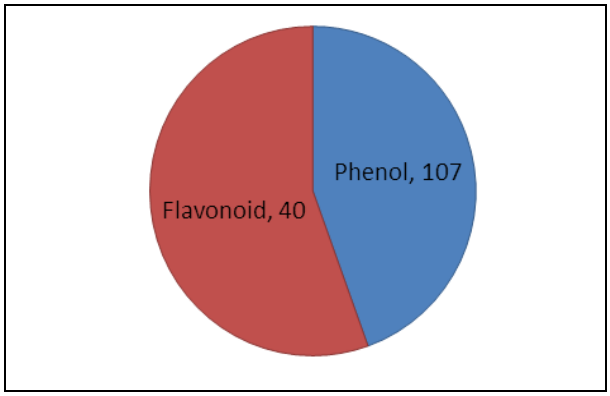

Figure 1: Total Phenol and Flavonoid

Table 1: Absorbance of different extract of *Eudistoma viride* at varying concentrations

Concentration ($\mu g/ml$)	Petroleum ether	Benzene	Chloroform	Ethanol	Methanol	Water	Standard ascorbic acid
50	0.5955	0.5255	0.2825	0.2292	0.1089	0.1282	0.3116
100	0.5206	0.4267	0.2722	0.1971	0.1002	0.1050	0.1919
150	0.5222	0.4025	0.2029	0.1726	0.0926	0.0807	0.1116
200	0.4955	0.3902	0.1923	0.1605	0.0755	0.0582	0.1913

Absorbance of control at 517 nm 0.3846

Radical scavenging method for different extracts of *Eudistoma viride* showed that the chloroform, ethanol, methanol and aqueous extract of the animal on 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 ascorbic acid and ethanolic 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.[13] A preliminary chemical screening of the ethanolic extract of *Eudistoma viride* showed the presence of flavonoids and phenolic compounds. The strongest antioxidant activity of the above extracts may be due to the presence of any of these chemical constituents.

CONCLUSION

These spectrometric studies suggest the extract of *Eudistoma* presence a lot of chemical compounds. High radical scavenging was observed in *Eudistoma viride.* The findings of the present study support the view of ascidians are promising sources of potential antioxidants and may be efficient in preventing agents in some other diseases. Further research is used to investigate and design this sample as drug in future.

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