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Studies on Antioxidant Present In Two Ascidians

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Abstract

Eudistoma viride and Phalluisa nigra are the colonial and simple ascidians belonging to the family ascididae and polycitoridae available all over India. This study is designed to examine the *invitro* antioxidant activity of phenolic compounds in the ethanolic extract of ascidians by DPPH method with all extracts. In DPPH system the strongest radical scavenging activity was exhibited by the different extracts of Eudistoma and Phallusia when compared to standard ascorbic acid. An increase in dose has significantly increased the absorbance of antioxidant activity. These results reveal the ethanolic extract of the two ascidians display a promising antioxidant potential against free radical induced oxidative damage.

Keywords: Colonial ascidian*, Eudistoma viride, Phallusia nigra,* antioxidant activity.

Introduction

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, causes the deterioration of food products during processing and storage [1]. Ascidians commonly called “Sea Squirts” are filter feeding organisms. They have a lot of chemical constituents in simple and colonial ascidians. *Eudistoma viride* and *Phallusia nigra* are colonial and simple ascidians belonging to the family polycitoridae, ascididae and it is available in all over India. Though considerable attention has been directed towards the identification of plants with antioxidant activity, no comparative results were reported in antioxidant studies using ascidians. The aim of the study is to prepare antioxidant rich fractions from Eudsitoma and Phallusia and to evaluate their antioxidant activity by DPPH method.

Materials and Methods

Collection of animal material:

Eudistoma viride and Phallusia nigra were 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 **Figure 1& 2**.

Figures 1 & 2: Simple ascidian- *Phallusia nigra* and 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*

Phallusia nigra belongs to Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Enterogona, Suborder: Phlebobranchia, Family: Ascididae, Genus: *Phallusia* and Species: *nigra*

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

Chemical analysis

Phenol was estimated by using catechol [3]. Flavonoid content was estimated by following [4] method. 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 samples were prepared

and L-ascorbic acid was used as a 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 **2**. Present study indicates *Eudistoma viride* and *Phallusia nigra* contain 10.7 g/100g, 11.2 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* and *Phallusia nigra* contain a high amount (40 g/100g, 50 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 *Phallusia nigra* has high percentage of flavonoids than the other chemical constituents.

Table 1: Absorbance of different extracts 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

Table 2: Absorbance of different extracts of *Phallusia nigra* at varying concentrations

Concentration ($\mu g/ml$)	Petroleum ether	Benzene	Chloroform	Ethanol	Methanol	Water	Standard ascorbic acid
50	0.4944	0.5255	0.2124	0.1998	0.1139	0.1122	0.3126
100	0.4876	0.4879	0.2118	0.0921	0.1059	0.1080	0.2989
150	0.3922	0.4225	0.1989	0.0726	0.0976	0.0767	0.2116
200	0.2345	0.4100	0.0976	0.0553	0.0645	0.0621	0.1984

Radical scavenging method for different extracts of *Eudistoma viride* and *Phallusia nigra* showed that the chloroform, ethanol, methanol and aqueous extracts of the animal on higher concentrations possess better antioxidant potential when compared to that of the standard ascorbic acid. They exhibited strong antioxidant DPPH radical scavenging activity than ascorbic acid and ethanolic extract. Generally, the antioxidant properties of these extracts were found to be concentration dependent.

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* and *Phallusia nigra* 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. It can be concluded that ascidians have a lot of antioxidant activity due to their presence of phenols and flavonoids respectively.

Conclusion

These spectrometric studies suggest the chemical compounds in the extracts of *Eudistoma* and *Phallusia leading to high radical scavenging activity.* High radical scavenging was observed in *Eudistoma viride* and *Phallusia nigra.* The findings of the present study support the view that ascidians are promising sources of potential antioxidants and may be efficient as preventing agents in some diseases. Further research is on to investigate and design this sample as drug in future.

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References

1. Williams. Pregnancy planning and antepartum management, Text book of Obstetrics. 2006; 22nd Edition, 226.
2. Meenakshi, V.K., Ph.D thesis, Manonmaniam Sundaranar University, Tirunelveli, (1997).
3. Khatiwora, E, Adsul BV, Kulkami, MM, Deshpande, NR, Kashalkar, RV. Spectroscopic determination of total phenols and flavonoids contents of *Ipomoea carnea*. International Journal of Chem Tech Research. 2010; 2(3): 1698-1701.

4. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. *Journal of Food Drug Analysis.* 2002*;* 10: 178-182.
5. Brand-williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity .Lebensmittel Wissenschaft and Technologies. 1995; 28(1): 25-30.
6. Ebrahimzadeh MA, Hosseinimehr SJ, Hamidinia A, Jafari M. Antioxidant and free radical scavenging activity of *Feijoa sallowiana* fruits peel and leaves.Pharmacology online. 2008; 1: 7-14.
7. Meenakshi, VK, Gomathy, S, Senthamarai, S, Paripooranaselvi, M, Chamundeswari, KP. Analysis of vitamins by HPLC and phenols, flavonoids by HPTLC *Microcosmus exasperatus.* European Journal of Zoological Research. 2012; *1($)*: 105-110.
8. Cushnie TPT, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. International Journal of Antimicrobial Agents. *2011;* 38(2): 99-107.
9. De Sousa, R.R., Queiroz, K.C., Souza, A.C., Gurgueira, S.A., Augusto, A.C., Miranda, M.A., Peppelenbosch, M.P., Ferreira, C.V., Aoyama, C.H., Phosphoproteins levels, MAPK activities and NF kappa B expression are affected by fisetin. Journal of Enzyme Inhibiting Medical Chemistry. 2007; 22(4): 439-444.
10. Gryglewski KJ, Korbut, J. On the mechanism of antithrombotic action of flavonoids. Biochemical Pharmacology. 1987; 36: 317-321.
11. Schuier, M, Sies, H, Illek, B, Fischer, H. Cocoa-related flavonoids inhibit CFTR-mediated chloride transport across T84 human colon epithelia. Journal of Nutrition, 2005; 135(10): 2320–2325.
12. Spencer P, Jeremy, E. Flavonoids: modulators of brain function? British *Journal of Nutrition*, 99*:* ES60–77.
13. Zakaria ZA, Rofiee MS, The LK, Salleh MZ, Sulaiman MR,Somchit MN. Bauhinia purpurea leaves extracts exhibited in vitro antiproliferative and antioxidant activities. *African journal of Biotechnology* 2011; 10(1): 65-74.