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IN VITRO ANTIOXIDANT STUDIES ON COLONIAL ASCIDIANS *EUDISTOMA VIRIDE* AND *DIDEMNUM PSAMMATHODES*

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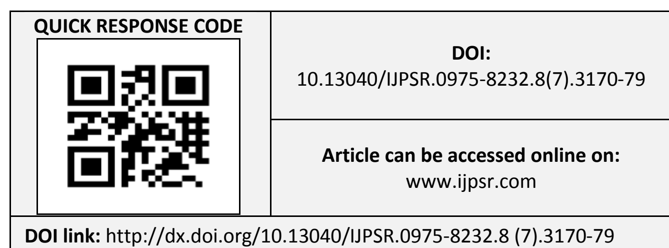
ABSTRACT: Ascidians are rich source of novel bioactive agents as potential source of therapeutic drugs. Evaluation of antioxidant potential of ascidians, *Eudistoma viride* and *Didemnum psammathodes* extracts was undertaken. In DPPH radical scavenging assay *E. viride* II showed 34% to 93% scavenging activity and *D. psammathodes* II showed 45% to 96% scavenging activity in different ascidian concentrations. In reducing power assay the ascidian fraction *E. viride* II and *D. psammathodes* II showed 2.3 and 2.23 reducing power in 100µgmL^{-1}. In Hydroxyl (OH) radicals scavenging activities the ascidian *E. viride* II and *D. psammathodes* II showed high radical scavenging activity 96% and 98% at 100 µgmL^{-1} concentration. In H_2O_2 scavenging assay the ascidians fractions *E. viride* II and *D. psammathodes* II showed 78% and 89% of at 100µgmL^{-1} concentration. In nitric oxide radical scavenging activity, the ascidians fractions *E. viride* II showed 64% at 100µgmL^{-1} and *D. psammathodes* II showed 72% at 100µgmL^{-1} concentration. The finding throws light on the higher efficiency of scavenging activity of natural products improved to synthetic drugs. These results indicate that ascidian represent a promising biological resource for derivation of new compounds with antioxidant potential which was far reaching implication in biomedical research and therapeutics.

INTRODUCTION: Antioxidants are defined as any substances that, when present at low concentrations compared with those oxidizable substrates, significantly delay or prevent oxidation of that substrate. Antioxidants inhibit the formation of free radical species, convert existing free radicals into less harmful molecules and prevent injurious chain reactions.



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Oxidation is an unavoidable reaction in all living organisms. Hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide radicals ($O^{2\bullet-}$) and hydroxyl radicals ($OH^\bullet$), which are collectively known as reactive oxygen species (ROS) are derived from the metabolism of oxygen in an aerobic system [1]. Free radicals and other ROS are normally formed during the oxidative metabolic process. Highly reactive free radicals, which are formed by exogenous chemicals, stress or in the food system, are capable of oxidizing bio-molecules, cause destructive and irreversible damage to the components of a cell [2]. Moreover, ROS are predominant cause of qualitative decay of foods, leading to rancidity, toxicity and destruction

of biomolecules important in physiologic metabolism. DNA, cell membranes, proteins and other cellular constituents are target site of the degradation processes and consequently induce different kinds of serious inflammatory diseases in human such as atherosclerosis, rheumatoid arthritis, muscular dystrophy, cataracts, neurological disorders, cancers and invariably aging process [3].

Cancer is a major cause of mortality worldwide and cancer incidened rapidly increase from year to year [4]. As ROS are involved in cancer development, compounds with high ROS reduction ability are likely to prevent cancer incidence and morbidity. Due to safety concerns being identified in the use of synthetic antioxidants, considerable interest has arisen in research for the alternative sources of natural antioxidants [5]. The use of natural antioxidants has the advantage that the consumer, considered to be safe because of no chemical contamination, readily accepts them and no safety tests are required by the legislation if the food component is generally recognized as safe (GRAS). Therefore, many investigations have been initiated to develop new, safe and natural sources of antioxidants and cancer chemo-preventive agents.

It was suggested recently that generation of free radicals play a major role in the progression of a wide range of pathological disturbances such as brain dysfunction and oxidative stress. Free radicals, together with other derivatives of oxygen are inevitable by- products of biological redox reactions. Several synthetic antioxidants such as tertbutyl atedhydroxyquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxyl-toluene (BHT) are commercially available and widely used. However, these antioxidants have been restricted for use in foods as they are suspected to be carcinogenic [6].

For this reason, governmental authorities and consumers are concerned about the safety of the food and also about the potential harmful effects of synthetic additives on health. Recently there has been a growing interest in the search for natural antioxidants. Much attention has been focused on the use of marine natural antioxidants because of the increasing research on marine natural products [7]. Studies have shown that marine organisms like tunic and/or sea squirts produce several kinds of antioxidant substances such as xantholphyll, β-carotene, astaxanthin, and lycopene compounds [8-10]. Previously few works have been carried out on anti-oxidant activities of crude, fractions and oligosaccharides from different parts of ascidian *Styela clava* in china but in India until now no work has been carried on anti-oxidant activities. In the present study, the anti-oxidant properties from biofoulant ascidians *E. viride* and *D. psammathodes* from Tuticorin coastal waters were evaluated.

MATERIALS AND METHODS:

Specimen Collection and Identification: Ascidians were collected as common and persistent biofoulants from the rocks of Hare Island Tuticorin Coast (Lat. 8º 46’ 20. 72" N and Long. 78º 11’ 57. 91" E), India (**Fig. 1**) by SCUBA diving at the depth ranging from 1 to 3 m between September, 2014. **Fig. 2** shows the colonies of *E. viride* and *D. psammathodes*. The samples were thoroughly washed with treated sea water and removed sand, mutt and overgrowing organisms at the site collection and transported to laboratory and specimens were identified by the standard literature of Monniot and Monniot [11]; Cole and Lambert [12]; Kott [13]. A Voucher specimen No: AS 2234 and AS 2233 has been deposited in the Museum (National Collections of Ascidians) of the Department of Zoology, A.P.C. Mahalaxmi College for women, Tuticorin – 628002.

Extraction: The freshly collected ascidians *Eudistoma viride* (wet wt. 1.43 Kg) and *Didemnum psammathodes* (wet wt. 1.665 Kg) were soaked in methanol (0.5 L×3) dichloromethane: methanol (1:1, 0.5 L×3) at room temperature for 3 days. This extraction process was repeated up to ascidian materials turned colourless. The combined extract was filtered through Whatman®No.1 filter paper and concentrated by rotary evaporator (VC100A Lark Rotavapor® at 35 ºC) with reduced pressure to give a dark green and dark brown gummy mass. The resultant residues were separately extracted with ethyl acetate (0.5L). The collected ethyl acetate extracts were concentrated and used for further purification process.

Sample Elution: The concentrated ascidian ethyl acetate extracts were subjected to gel filtration Sephadex LH-20 (column size 1.5 cm × 22 cm),

ethyl acetate: hexane (3:7 v/v), followed by silica gel column chromatography eluting with ethyl acetate: hexane (3:7 v/v). The eluting samples were fractionated every 5 mL into a test tube and monitored by thin layer chromatography (TLC) to identify the same compound. The collected fractions were concentrated by rotary evaporator (VC100A Lark Rotavapor® at 35 ºC) with reduced pressure.

Thin Layer Chromatography (TLC): Samples were analysed by TLC coupled to chemical tests for identification of different secondary metabolites according to MINSAP [14]. For analytical TLC, aluminium sheets (10 x 4 cm) coated with silica gel 60 F_{254}, were used. The chromatography was run in a chamber with ethyl acetate: hexane: water (3:7:1 v/v) as medium as the mobile phase under UV light at 254 nm and charred with sulphuric acid regent and heated.

Antioxidant Activity Determination (*In-vitro* assay):

DPPH Scavenging Assay: The scavenging effects of samples for DPPH radical were determined according to reference of Sun *et al.,* [15]. The ascidian crude *E. viride* and *D. psammathodes* extracts and fractions were taken in different concentrations (20, 40, 60, 80 and 100 μgmL^{-1}) and mixed with DMSO. The absorbance of the resulting solution was measured at 517nm. The change in absorbance with respect to the control (containing DPPH only without sample, expressed as 100% free radicals) is calculated as percentage scavenging. Ascorbic acid (20, 40, 60, 80 and 100 μgmL^{-1}) was used as standard antioxidant.

Reducing Power Assay: The reducing powers of the ascidian crude and fractions were determined according to reference of Oyaizu [16]. The ascidian crude *E. viride* and *D. psammathodes* extracts and fractions were taken in different concentrations (20, 40, 60, 80 and 100 µg/mL). Ascorbic acid (20, 40, 60, 80 and 100 μgmL^{-1}) was used as standard antioxidant. Higher absorbance of the reaction mixture indicated greater reducing power.

Hydroxyl Radical Assay: Hydroxyl (OH) radical assay was performed according to the modified method of Halliwell *et al.,* [17]. Ascorbic acid (20, 40, 60, 80 and 100 μgmL^{-1}) was used as standard antioxidant. The absorbance of the mixture was measured at 532nm. DMSO with sample was used as a blank. Hydroxyl (OH) radicals scavenging activity was evaluated as the inhibition rate of α-deoxyribose oxidation by hydroxyl radical.

Hydrogen peroxide (H_2O_2) Scavenging Activity: H_2O_2 scavenging activity was determined according to the method of Muller [18]. DMSO with sample was used as a blank. α -tocopherol (20, 40, 60, 80 and 100 μgmL^{-1}) was used as standard antioxidant. After incubation the absorbance of the mixture was measured at 405 nm.

Nitric Oxide Radical Scavenging Activity: Nitric oxide radical scavenging activity was determined according to the method reported by Marcocci *et al.,* [19]. The nitric oxide radicals scavenging activity was calculated. Ascorbic acid (20, 40, 60, 80 and 100 μgmL^{-1}) was used as standard antioxidant. The absorbance at 540nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated according to the following equation:

Each experiment was performed 3 times, and data were expressed as the mean± standard deviation (SD). One way analysis of variance (ANOVA) and Duncan's new multiple range test were used to determine the differences among these means at $p < 0.05$.

Fourier Transform Infrared Spectroscopy (FT-IR): The collected fractions of ascidian were dried well. The dried power sample approximately 5 mg was mixed with 1000mg of dried KBr was subjected to a pressure of 5×10^6 pa and made into clear pellet of 3 mm diameter and 1 mm thickness. Absorbance spectra were recorded using Nicolet Avatar-360 FTIR Spectrometer equipped with a KBr beam splitter and an air - cooled DTGS detector (Department of Chemistry, Annamalai University). The absorption of light intensity of the peak was calculated using the base line method. The frequencies for all sharp bands were accurate to $0.01cm^{-1}$.

RESULTS AND DISCUSSION: In the present investigation, ethyl acetate extracts of ascidian *E. viride* and *D. psammathodes* were concentrated under reduced pressure to give a dark green and

brownish gummy mass of 19.11 gm and 18.73 gm (in wet weight).

FIG. 1: THE COLONIAL ASCIDIAN COLLECTION SITE

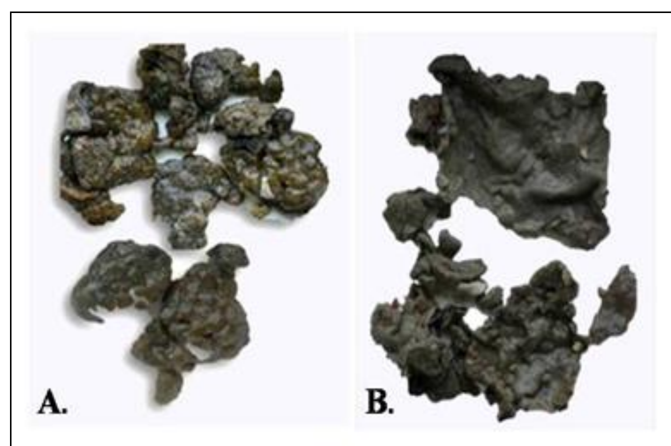

FIG. 2: COLLECTED COLONIAL ASCIDIAN (A) *E. VIRIDE* AND (B) *D. PSAMMATHODES*

In TLC the eluted fractions were tested for identify the similar compound fractions. The chromatography was run in a chamber with ethyl acetate: hexane: water (3:7:1 v/v) as medium as the mobile phase. The **Fig. 3** and **Fig. 4** showed TLC plate of the ascidian *E. viride* and *D. psammathodes* crude and fractions. In this investigation, the TLC plate of the ascidian *E. viride* showed intensive spot (R*f* value= 8.6 and 5.3 cm). Mobile phase move upto 9 cm. The TLC plate of the *D. psammathodes* showed intensive spot (R*f* value = 7.6 and 4.9 cm). Mobile phase moved upto 8.5 cm.

Many antioxidants work by scavenging free radicals. Owing to different antioxidant components having different scavenging activities against various reactive oxygen species such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen (^-O) or peroxyl radical (^-OH), many methods for measuring these properties *in vivo* and *in vitro* have been developed. The most common method involves the determination of the disappearance of free radicals using a spectro-photometer, such as 2,2-azinobis (3-ethylbenzo-thiazoline-6- sucfonic) acid radical ($ABTS^{\bullet+}$) and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) [20].

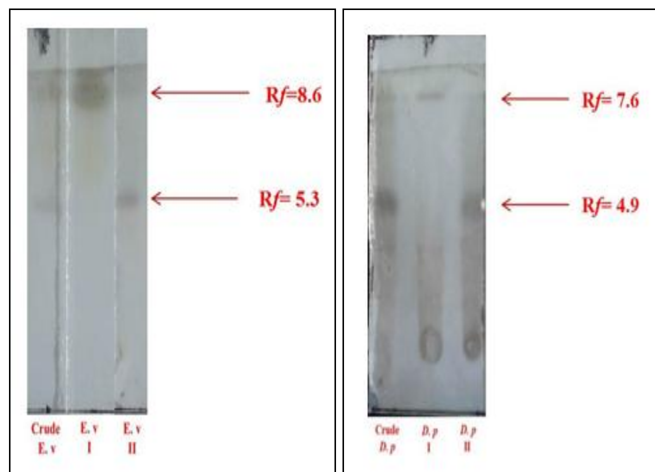

FIG. 3: TLC PLATES OF THE ASCIDIAN (A) *E. VIRIDE* (B) D. PSAMMATHODES CRUDE AND FRACTIONS

DPPH is a free radical compound and has been widely used to test the free radical scavenging abilities of various samples [21]. The DPPH free radicals scavenging method is a colorimetric assay and can be used to evaluate within a short time [22]. DPPH is a stable nitrogen-centered free radical, the colour of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction are considered as antioxidants and radical scavengers [23]. The result of the DPPH radical scavenging activity analyses are presented in **Fig. 4**. In this assay fractions *E. viride* II and *D. psammathodes* II showed power full scavenging activity. *E. viride* II showed 34% to 93% scavenging activity and *D. psammathodes* II showed 45% to 96% scavenging activity in different ascidian concentrations (**Fig. 4**).

Table 1 showed the one way ANOVA of DPPH scavenging activity of ascidian in this the values were significant at 0.05% level and *p* values were lower than 0.05% level ($p<0.05$). Nacional *et al.,* [24] observed obvious scavenging effect on DPPH radical. EC_{50} of tunicae, viscera and whole body extract of DPPH radicals were shown as 1.35, 0.77

and 0.90 $mgmL^{-1}$ and At 1 $mgmL^{-1}$, the scavenging ability were 37.29%, 65.42% and 55.94% respectively. Lee *et al.,* [25] studied that the DPPH radical scavenging activity for the fleshy part of *S. clava* was higher than that of tunic part and water extracted showed the highest value (53.0% at10 $mgmL^{-1}$). Previous studies have suggested that the DPPH radical scavenging capacities of extracts are largely affected by the presence and position of the phenolic hydroxyl group. The anti-radical activity of the phenolic compound is, in turn, dependent on its molecular configurations, *i.e.* the availability of phenolic hydrogens as well as the potential for stabilization of the resulting phenoxyl radicals formed by hydrogen donation [26].

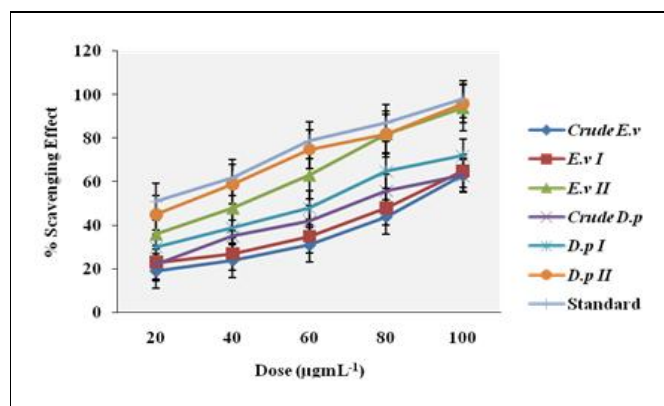

FIG. 4: DPPH SCAVENGING ACTIVITY OF ASCIDIAN *E. VIRIDE* AND *D. PSAMMATHODES*
Each value is the Mean ± SD of triplicate measurements; Values with different symbols indicate significant differences ($p<0.05$).

TABLE 1: ONE WAY ANOVA OF DPPH SCAVENGING ACTIVITY OF ASCIDIAN *E. VIRIDE* AND D. PSAMMATHODES EXTRACTS

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5024.567	5	1004.913	2.814095	0.038788*	2.620654
Within Groups	8570.4	24	357.1			
Total	13594.97	29				

*Significant at 0.05% level ($p< 0.05$)

In the reducing power assay, the presence of antioxidants in the samples would result in reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. The concentration dependency of antioxidant activity was investigated as a function of reducing power (**Fig. 5**), as this gives a general view of reductones present in the sample. The reducing power increased with increasing concentration in all tested samples. In this assay the ascidian fraction *E. viride* II and *D. psammathodes* II showed 2.3 and 2.23 reducing power in 100μgmL^{-1}.

Table 2 showed the one way ANOVA of reducing power of ascidian. In this the values were significant at 0.05% level and *p* values were low than 0.05% level ($p < 0.05$). The concentration dependency of antioxidant activity was investigated as a function of reducing power, as this gives a cue of reductones present in the sample. The present results are supported by previous studies. The carotenoids of *S. clava* were found to have strong reducing power, with an OD of 1.025 when a concentration of 120μgmL^{-1} was evaluated [24]. Ganesan *et al.,* [27] reported that the reducing power of MeOH extracts from some red seaweeds was low at the mg level, as indicated by an optical density (OD) of <0.2 [28], who examined MeOH extracts of red seaweed, *P. morrowii*, also reported this trend. Sun *et al.,* [29] depicted the reducing power of tunicae, viscera and whole body extract. Among the three samples, viscera extract showed the highest reducing power revealing. The presence of reductones in the crude extract and fractions as suggested by Duh [30].

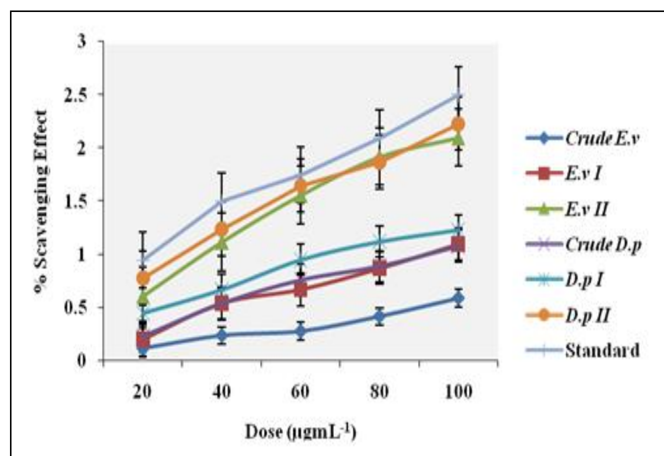

FIG. 5: REDUCING POWER ACTIVITY OF ASCIDIAN *E. VIRIDE* AND *D. PSAMMATHODES*
Each value is the Mean ± SD of triplicate measurements; Values with different symbols indicate significant differences ($p<0.05$).

TABLE 2: ONE WAY ANOVA OF REDUCING POWER ACTIVITY OF ASCIDIAN *E. VIRIDE* AND D. PSAMMATHODES EXTRACTS

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5.759097	5	1.151819	6.628477	0.000524*	2.620654
Within Groups	4.17044	24	0.173768			
Total	9.929537	29				

*Significant at 0.05% level ($p < 0.05$)

The hydroxyl radical, generated in the system by the fenton reaction, is known to be a highly potent oxidant, which can react with all biomacro-molecules functioning in living cells [31]. Among reactive oxygen species, hydroxyl radicals are the most reactive and often induce severe oxidative damage to important biomolecules such as proteins, DNA, PUFA and nucleic acids, causing aging, cancer and several other diseases [32]. Earlier researchers have suggested that two mechanisms might be responsible for the hydroxyl radical scavenging ability of sulfated saccharides. One suppresses the generation of hydroxyl radical and the other scavenges hydroxyl radicals generated [33]. In hydroxyl radical assay the ascidian crude and fractions showed high hydroxyl radical scavenging activities (**Fig. 6**). The scavenging activities were oriented with increasing ascidian concentration. In this the ascidian *E. v* II and *D. p* II showed high radical scavenging activity 96% and 98% at 100µgmL^{-1} concentration. **Table 3** showed the one way ANOVA of hydroxyl radical activity of ascidian, in this the values were significant at 0.05% level and *p* values were higher than 0.05% level ($p>0.05$). Previous reports also proved this result, as all crude oligosaccharides were found to exhibit the ability to scavenge hydroxyl radicals in a concentration-dependent manner [29].

Scavenging effect of viscera extract on hydroxyl radicals was the highest among three samples ($P < 0.05$). The EC_{50} of tunicae extract, of viscera extract and whole body extract against hydroxyl radical was 6.32, 3.45 and 5.55mgmL^{-1}, at 5mgmL^{-1} respectively. The scavenging effect in percentage of tunicae extract, viscera extract and whole body extract was 39.67%, 72.51% and 45.04%, respectively. Scavenging effect of oligosaccharides extracted from different parts of *Styela clava* was higher than the fractions [29]. Previous studies reported that the sulphate content and molecular weight may affect the antioxidant activities [34 - 35].

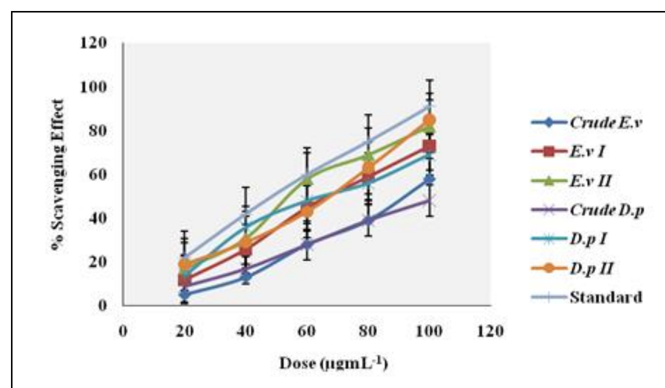

FIG. 6: HYDROXYL RADICAL ACTIVITIES OF ASCIDIAN *E. VIRIDE* AND *D. PSAMMATHODES*
Each value is the Mean ± SD of triplicate measurements; Values with different symbols indicate significant differences ($p > 0.05$).

TABLE 3: ONE WAY ANOVA OF HYDROXYL RADICAL ACTIVITIES OF ASCIDIAN *E. VIRIDE* AND D. PSAMMATHODES EXTRACTS

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2978.3	5	595.66	0.968605	0.456591*	2.620654
Within Groups	14759.2	24	614.9667			
Total	17737.5	29				

*Significant at 0.05% level ($p>0.05$)

The measurement of H_2O_2 scavenging activity is also a useful method determining the ability of antioxidants to decrease the level of peroxidants such as H_2O_2 [36]. H_2O_2 is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol ($^-$SH) groups. Hydrogen peroxide can cross cell membrane (bio membrane) rapidly. Once inside the cell, H_2O_2 can probably react with Fe^{+2} and possibly Cu^{+2} to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. Results obtained in the present study indicate that ascidian extracts showed remarkable radical scavenging activity. Measurement of H_2O_2

scavenging activity is known to be one of the most useful methods for determining the ability of an antioxidant to decrease the levels of pro-oxidants such as H_2O_2. In H_2O_2 scavenging assay of the ascidian both crude and fractions showed significant scavenging activities. The ascidians fractions *E. viride* II and *D. psammathodes* II showed 78% and 89% of H_2O_2 scavenging activity at 100 µgmL^{-1} concentration (**Fig. 7**).

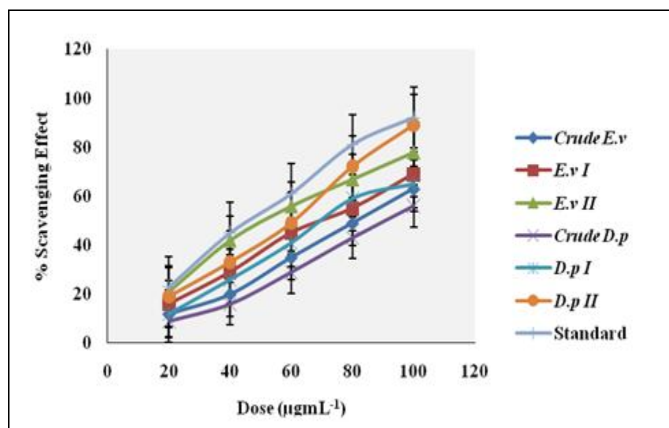

FIG. 7: HYDROGEN PEROXIDE (H_2O_2) SCAVENGING ACTIVITIES OF ASCIDIAN *E. VIRIDE* AND D. PSAMMATHODES EXTRACTS
Each value is the Mean ± SD of triplicate measurements; Values with different symbols indicate significant differences (p>0.05).

The **Table 4** showed the one way ANOVA of hydrogen peroxide scavenging activity of ascidian. In this the values were significant at 0.05% level and p values were higher than 0.05% level ($p > 0.05$).There are few reports in hydrogen peroxide scavenging activity on ascidian extracts. Jumeri and Kim [37] observed AH had higher H_2O_2 scavenging activity than either TH or PH, with IC_{50} values of 904.7, 940.0 and 2241.5 µgmL^{-1} for AH, TH, and PH at concentration of 1000 µgmL^{-1}.

However, the IC_{50} values of H_2O_2 scavenging activity of the hydrolysates were higher than those of ascorbic acid (IC_{50}, 48.0µgmL^{-1}) and α-tocopherol (IC_{50}, 49.9µgmL^{-1}). IC_{50} for scavenging of H_2O_2 were 169 ± 7.25 for *B. hyrcana* leaves, 175 ± 6.95 for *C. speciosum*, 640 ± 11.67µgmL^{-1} for *V. odorata* leaves and 663 ± 9.38µgmL^{-1} for *H. officinalis* aerial parts. Though hydrogen peroxide itself is not very reactive, it can cause cytotoxicity by giving rise to hydroxyl radicals inside the cells. Alter the structure and function of cellular components and consequent to cell damage [38]. The above few finding also throw light on the higher efficiency of scavenging activity of natural products improved to synthetic drugs.

TABLE 4: ONE WAY ANOVA OF HYDROXYL RADICAL ACTIVITIES OF ASCIDIAN *E. VIRIDE* AND *D. PSAMMATHODES*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1971.5	5	394.3	0.780277	0.573721*	2.620654
Within Groups	12128	24	505.3333			
Total	14099.5	29				

*Significant at 0.05% level (p > 0.05).

Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of cellular damage. The scavenging activities were oriented with increasing ascidian concentration. The ascidians fractions *E. viride* II showed 64% at 100 µgmL^{-1} and *D. psammathodes* II showed 72% at 100 µgmL^{-1} (**Fig. 8**).

The **Table 5** showed the one way ANOVA nitric oxide radical scavenging activity of ascidian. In this the values were significant at 0.05% level and p values were low than 0.05% level ($p < 0.05$). The action was concentration dependent. The values were comparable to that of the standard *i.e* glacial acetic acid.

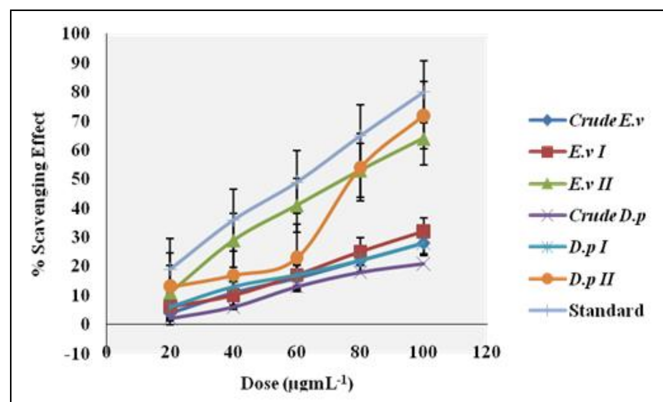

FIG. 8: NITRIC OXIDE RADICAL SCAVENGING ACTIVITIES OF ASCIDIAN *E. VIRIDE* AND D. PSAMMATHODES EXTRACTS
Each value is the Mean ± SD of triplicate measurements; Values with different symbols indicate significant differences (p<0.05).

The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine is used as the marker for NO scavenging activity [39]. The chromophore formation was not complete in the presence of different test extracts, which scavenges the NO thus formed from the sodium nitroprusside and hence the absorbance decreases as the concentration of the extracts increases.

TABLE 5: ONE WAY ANOVA OF NITRIC OXIDE RADICAL SCAVENGING ACTIVITIES OF ASCIDIAN *E. VIRIDE* AND *D. PSAMMATHODES*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3325.867	5	665.1733	2.788207	0.040121	2.620654
Within Groups	5725.6	24	238.5667			
Total	9051.467	29				

*Significant at 0.05% level ($p < 0.05$).

In this study a preliminary attempt has been made to assay the potent biomedical compounds from the ascidian by FTIR analysis and peaks were observed and assigned as amide groups. The FT-IR analysis (**Fig. 9**) shows the following functional groups present in the ascidian *E. viride* fraction II (*E. viride* II).

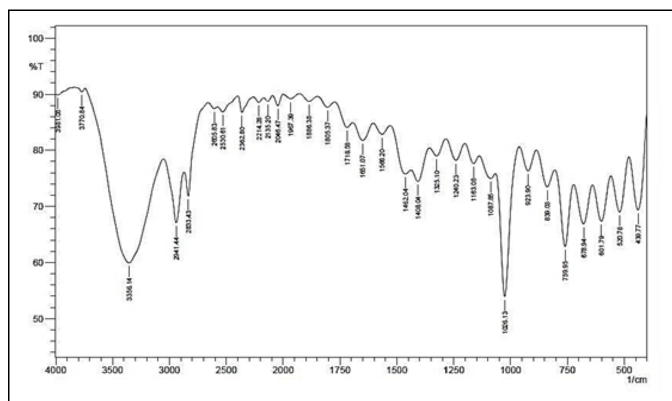

FIG. 9: FT-IR ANALYSIS OF ASCIDIAN *E. VIRIDE* FRACTION II (*E. V* II)

Wave number 3335.14 cm^{-1} (stretch) represents the presence of N–H stretching groups (doublet for primary amines; singlet for secondary amines). Wave number 2941.44 and 2833.43 cm^{-1} (stretch) represents aliphatic structure like aldehydes (H–C=O: C–H stretch Asymmetric and Symmetric). Wave number 2214.28 and 2135.20 cm^{-1} (weak, stretch) represent the present of Alkynes (C≡C) group. Wave number 1718.58, 1651.07 cm^{-1} represent the C=O (stretch) that shows the occurrence of aldehydes, ketones, carboxylic acids and esters groups. Wave number 1566.20 cm^{-1} shows the occurrence of 1° amines (N–H bend). Wave number 1462.04 cm^{-1} represent the (C-C=C) Asymmetric Stretch or (C–H) bend like alkanes. 1325.10 cm^{-1} wave number represent the nitro groups (N=O Bend) and C–H rock alkanes. 1163.08, 1087.85, 1240.23 and 1026.13 cm^{-1} wave number represents the C–O stretch such like alcohols, ethers, carboxylic acids and esters. Wave number 923.90 cm^{-1} represents O–H bend carboxylic acids. 839.03, 759.95 and 678.94 cm^{-1} (stretch) shows the presence of =C–H bends alkenes.

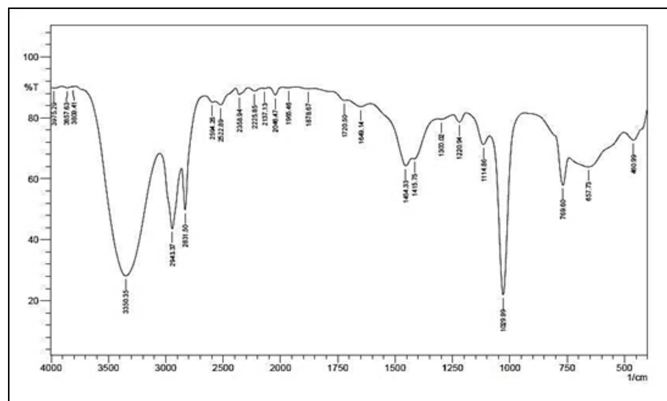

FIG. 10: FT-IR ANALYSIS OF ASCIDIAN D. PSAMMATHODES FRACTION II (*D. P* II)

The FT-IR analysis (Fig. 10) shows the functional groups present in ascidian *D. psammathodes* fraction II (*D. p* II). Wave number 3350.35 cm^{-1} (stretch) represents N–H stretching (doublet for primary amines; singlet for secondary amines). Wave number 2943.37 and 2831.50 cm^{-1} (stretch) represents aliphatic structure like aldehydes (H–C=O: C–H stretch Asymmetric & Symmetric), 2225.85 and 2137.13 cm^{-1} (stretch) shows the presence of C≡C Alkynesor C≡N stretch nitriles functional groups in this fraction. Wave number 1965.46 cm^{-1} shows the occurrence of phenyl ring substitution overtones. Wave number 1454.33 and 1415.75 cm^{-1} shows the presence of C–C stretch (in–ring) aromatics and C–H bend alkane's functional groups, Wave number 1220. 94, 1114.86 and 1029.99 cm^{-1} express the presence of the C–O

(stretch) such like alcohols, ethers, carboxylic acids, esters and C–N stretch aliphatic amines groups in this fraction. The wave number 769.60 cm^{-1} shows the presence of C-H Alkenes (s) bend and Phenyl Ring Substitution Bands in the fraction.

CONCLUSION: In this study the results revealed that fraction II (*D. psammathodes* II) has the highest antioxidant and free radicals scavenging activities. These findings are in agreement with observations from other studies on ascidians and support the fact that functional properties of antioxidative compounds are highly influenced by parameters such as molecular mass, concentration, chemical interactions and ligand binding etc. The results of the present study suggest that ascidian extract could be a potential therapeutic agent in oxidative stress-induced diseases and can be used as an accessible source of natural antioxidants.

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