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**Research Article**

# *In vitro* antioxidant studies of a colonial ascidian *Ecteinascidia venui* Meenakshi, 2000

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## Abstract

Oxidative stress has been implicated with the pathology of many diseases such as inflammatory conditions, cancer, diabetes and aging. Free radicals contribute to more than one hundred disorders in humans. Therefore the need for the search of antioxidants from natural origin has been greatly felt in the recent years. Keeping this view on, an attempt was made to evaluate free radical scavenging activities of various concentrations of different extracts of *Ecteinascidia venui* by methods like DPPH, Hydroxyl, superoxide, ABTS and reducing power using *in vitro* models. Petroleum ether extract of *Ecteinascidia venui* at 800 µg/ml showed better scavenging activity in DPPH (114.21%). The methanol extract (129.14, 120.71%) exhibited highest hydroxyl and ABTS cation radical scavenging activity. Ethanol (129.17%) extract noted strong Superoxide dismutase assay. Like the antioxidant activity, reducing power of the extracts increase with increase in concentration. Ascorbic acid and trolox were used as standard and the results were compared. This study indicates the significant potential of *Ecteinascidia venui* and suggests its safe use as a therapeutic antioxidant.
**Keywords:** Colonial ascidian, *Ecteinascidia venui*, antioxidant.

## 1. Introduction

Antioxidants are useful in retarding oxidative deterioration of food materials especially those with high lipid content and have the capacity to protect living cells from oxidative damage that occur due to formation of free radicals and reactive oxygen species during most of the metabolic activities [1]. Oxidative damage of cellular constituents in the human body results in cell injury and death associated with pathogenesis of various chronic diseases like carcinomas, coronary heart disease and many other health problems related to aging [2]. This has lead to an increase in the interest of natural substances exhibiting antimicrobial and antioxidant properties that are supplied to human and animal as food components or as specific pharmaceutics [3]. Among these, living organisms are the primary sources of naturally occurring antioxidants for humans. It has been well known that the essential oils and plant extracts have antimicrobial and antioxidant effects. Antioxidants play a significant role in the prevention of diseases and do have a capacity to reduce oxidative stress by chelating trace elements or scavenging free radicals and protecting antioxidant defenses [4]. Researchers have shown more interest to isolate antioxidant rich compounds from the natural sources like marine organisms, plants etc., In this context the recent focus has been on the marine sedentary organisms called ascidians. *Ecteinascidia venui* Meenakshi, 2000 is a colonial ascidian occurring in the Tuticorin Port Area [5]. Various species of ascidians from Indian water has been proved to exhibit potent pharmacological activities [6-37]. Review of literature reveals that only chemical investigation of the ethanolic extract of *Ecteinascidia venui* has been carried out so far [38-40]. In the present study, an attempt has been made to investigate the *in vitro* antioxidant properties of the different solvent extracts of *Ecteinascidia venui* using DPPH, Hydroxyl, Superoxide, ABTS scavenging assays and reducing power.

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## 2. Materials and methods

### 2.1Collection of animal material

*Ecteinascidia venui* 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 ascidians [41]. A voucher specimen AS 2247 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.

### 2.2 Systematic position

*Ecteinascidia venui* belongs to Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Enterogona, Suborder: Phlebobranchia, Family: Perophoridae, Genus: *Ecteinascidia* and Species: *venui*

### 2.3 Antioxidant studies

#### 2.3.1 DPPH radical scavenging activity

DPPH radical scavenging activity of antioxidant component is used to study free radical. Blois method [42] is based on the reduction of DPPH in methanol solution in presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H. 0.1 mM solution of DPPH in methanol was prepared. 1 ml of this solution was added to 3 ml of various solvent extracts at different concentration (50, 100, 200, 400 & 800 µg/ml). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity.

#### 2.3.2 Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al*., (1987) [43]. Stock solutions of EDTA (1mM), $FeCl_3$ (10 mM), Ascorbic acid (1mM), $H_2O_2$ (10mM) and Deoxyribose (10 mM), were prepared in distilled de ionized water. The assay was performed by adding 0.1ml EDTA, 0.01ml of $FeCl_3$, 0.1ml $H_2O_2$, 0.36 ml of deoxyribose, 1.0 ml of solvent extracts at different concentration (50, 100, 200, 400 & 800 µg/ml), 0.33 ml of phosphate buffer (50 mM, pH 7.9) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 °C for 1h. 1 ml portion of the incubated mixture was mixed with 1ml of 10% TCA and 1ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation.

#### 2.3.3 Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Srinivasan *et al*., (2007) [44]. The superoxide anion radicals were generated in 3 ml of Tris-HCl buffer (16 mM, pH 8.0), containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution,1 ml extract of different concentration (50, 100, 200,400 & 800 µg/ml), and 0.5 ml Tris-HCl buffer (16 mM, pH 8.0). The reaction was started by adding 0.5 ml PMS solution (0.12 mM) to the mixture, incubated at 25°C for 5 min and the absorbance was measured at 560 nm against a blank, ascorbic acid.

#### 2.3.4 ABTS+ cation radical scavenging activity

ABTS assay was based on the slightly modified method of Huang *et al*. (2011) [45]. ABTS radical cation (ABTS+) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium per sulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS+ solution was diluted with ethanol and after addition of 100 µl of sample or trolox standard to 3.9 ml of diluted ABTS+ solution, absorbance was measured at 734 nm by UV-VIS spectrophotometer exactly after 6 min. The results are expressed as trolox equivalent antioxidant capacity (TEAC).

#### 2.3.5 Reducing power

The reducing power of the extract was determined by the method of Kumar and Hemalatha (2011) [46]. 1ml of solution containing 50, 100, 200, 400 & 800 µg/ml of extract was mixed with sodium phosphate buffer 5 ml (0.2 M, pH 6.6) and potassium ferricyanide 5 ml (1%). The mixture was incubated at 50°C for 20 min. Then 5 ml of 10% trichloroaceticacid was added and centrifuged at 980 g (10 min at 5°C) in a refrigerator centrifuge. The upper layer of the solution (5 ml) was diluted with 5.0 ml of distilled water and ferric chloride and absorbance read at 700 nm.

**2.4 Statistical analysis**

The capability to scavenge free radical was calculated by using the following formula. Scavenging effect (% inhibition) = {($A_0$–$A_1$)/$A_0$) X 100} Where, $A_0$ is the absorbance of the control reaction, and $A_1$ is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged. Analyses were performed in triplicates. The data were statistically evaluated using analysis of variance (ANOVA) with SPSS 15.0. Duncan's multiple range test was carried out in order to test any significant differences between the solvents used and the treatment methods.

## 3. Results and discussion

*Ecteinascidia venui* extracts exhibited potent *in vitro* antioxidant activity in DPPH, hydroxyl, superoxide, ABTS radical scavenging assays and reducing power in comparison to the known antioxidants such as ascorbic acid and trolox.

**3.1 DPPH radical scavenging activity**

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Ecteinascidia venui* is presented in figure 1. The radical scavenging effect was found to increase with increasing concentrations. *Ecteinascidia venui* extracts and standard showed maximum percentage inhibition in the following order - petroleum ether (114.21), methanol (99.12), benzene (98.24), ethyl acetate (91.71), ethanol (91.37) and standard (79.15) with $IC_{50}$ values of 52.92, 46.24, 45.26, 40.74, 41.37 and 23.46 µg/ml respectively. Petroleum ether extract of *Ecteinascidia venui* at 800 µg/ml showed significant scavenging activity compared to the standard ascorbic acid. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [47]. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The removal of free radicals may serve as a possible preventative intervention for the diseases [48].

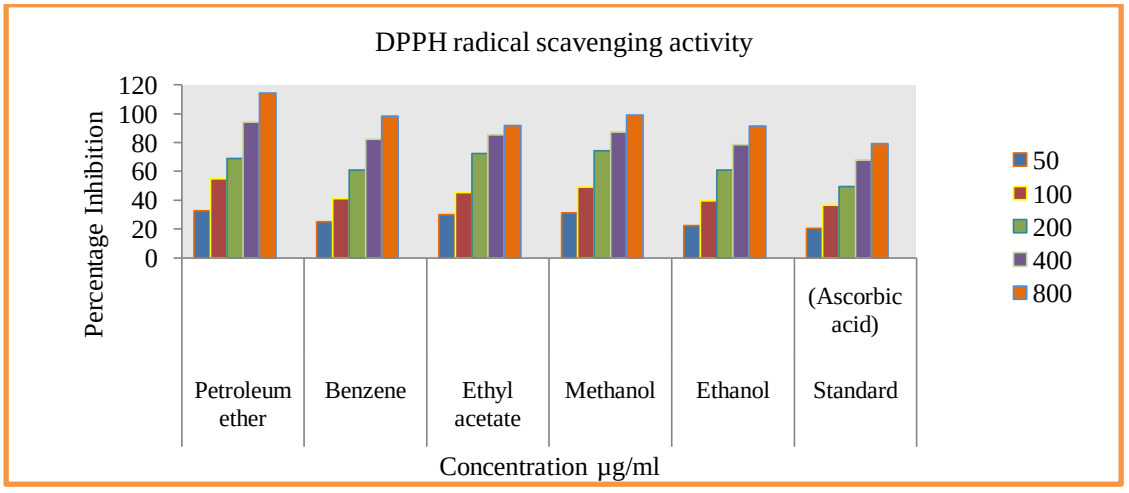


**Figure 1: DPPH radical scavenging activity of different extracts of *Ecteinascidia venui***

**3.2 Hydroxyl radical scavenging activity**

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Ecteinascidia venui* is given in figure 2. The hydroxyl radical scavenging effect was found to increase with increasing concentrations. *Ecteinascidia venui* extracts and standard showed maximum percentage inhibition in the following order - methanol (129.14), ethanol (112.14), ethyl acetate (103.37), benzene (102.44), petroleum ether (89.41) and standard (102.57), with $IC_{50}$ values of 48.44, 41.44, 39.78, 36.21, 31.71 and 35.48 respectively. The methanol extract exhibited high antioxidant activity. Hydroxyl radicals are known to be the most reactive of all the reduced forms of dioxygen which initiate cell damage *in vitro* [49]. Effect of the above extract on hydroxyl radicals generated by $Fe^{2+}$ ions was measured by determining the degree of deoxyribose degradation, an indicator of TBA-MDA adducts formation. Hydroxyl scavenging activity was higher in methanol followed by ethanol compared to standard ascorbic acid.

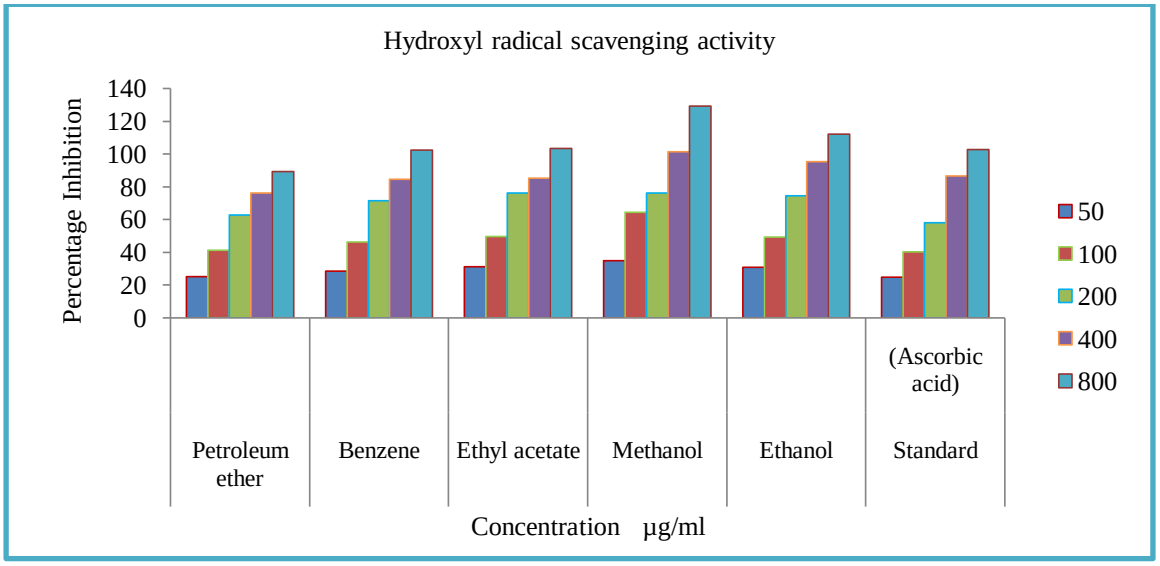


**Figure 2: Hydroxyl radical scavenging activity of different extracts of *Ecteinascidia venui***

### 3.3 Superoxide radical scavenging activity

Different solvent extracts of *Ecteinascidia venui* were subjected to superoxide radical scavenging assay and the results are noted in figure 3. The effect was found to increase with increasing concentrations. *Ecteinascidia venui* extracts and standard showed maximum percentage inhibition in the following order - ethanol (129.17), methanol (118.11), ethyl acetate (108.11), petroleum ether (99.81), benzene (99.26) and standard (101.21) with $IC_{50}$ values of 42.82, 40.75, 36.67, 31.84, 30.84 and 32.81 respectively. The ethanol extract exhibited high antioxidant activity. Superoxide anions are the most common free radicals *in vivo* and are generated in a variety of biological systems, either by auto-oxidation processes or by enzymes. The concentration of superoxide anions increases under conditions of oxidative stress and related situations [50]. Moreover, superoxide anions produce other kinds of cell damaging free radicals and oxidizing agents [51]. The results indicate that *Ecteinascidia venui* is an effective scavenger of superoxide anions and this may be due to the presence of multiple antioxidants with relatively high superoxide scavenging activity.

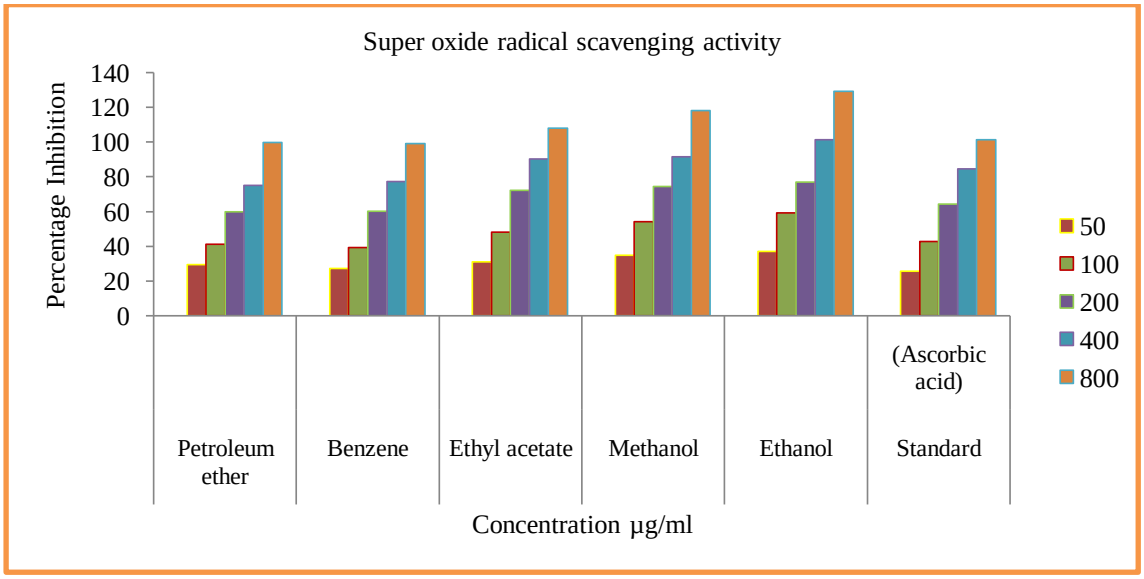


**Figure 3: Superoxide dismutase radical scavenging activity of different extracts of *Ecteinascidia venui***

### 3.4 ABTS+ cation radical scavenging activity

ABTS+ cation radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Ecteinascidia venui* is shown in figure 4. The cation radical scavenging effect was found to increase with

increasing concentrations. *Ecteinascidia venui* extracts and standard showed maximum percentage inhibition in the following order - methanol (120.71), ethyl acetate (101.21), ethanol (99.41), petroleum ether (99.12), benzene (89.41) and standard (108.41) with $IC_{50}$ values of 44.26, 35.54, 34.07, 35.66, 30.85 and 39.65 (µg/ml) respectively. The methanol extract exhibited high antioxidant activity. The ABTS+ scavenging assay, which employs a specific absorbance (734 nm) at a wavelength remote from the visible region and requires a short reaction time, can be used as an index that reflects the antioxidant activity of the test samples [52]. The antioxidant activities against ABTS or DPPH were correlated with the concentration, chemical structures, and polymerization degrees of organ antioxidants [53].

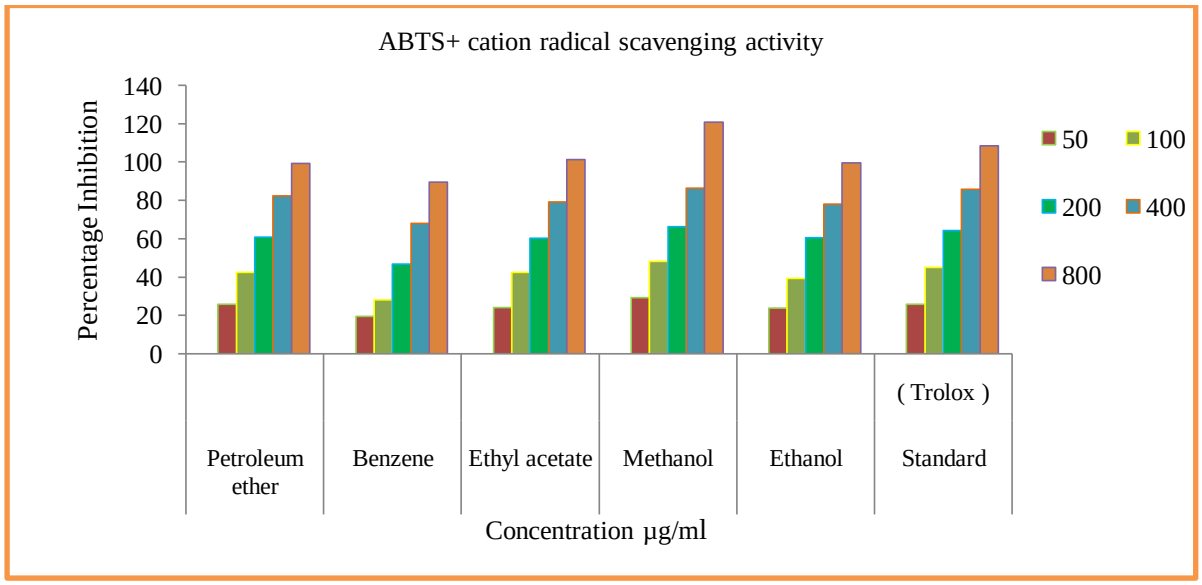


**Figure 4: ABTS+ cation radical scavenging activity of different extracts of *Ecteinascidia venui***

### 3.5 Reducing power

Reducing ability of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Ecteinascidia venui* is compiled in figure 5. The radical scavenging effect was found to increase with increasing concentrations. A higher absorbance indicated a higher reducing power. Among the solvent tested, ethanol and methanol extracts exhibited higher reducing ability. So, comparison with ascorbic acid or trolox, it is clear that *Ecteinascidia venui* extracts possess potential antioxidant activity.

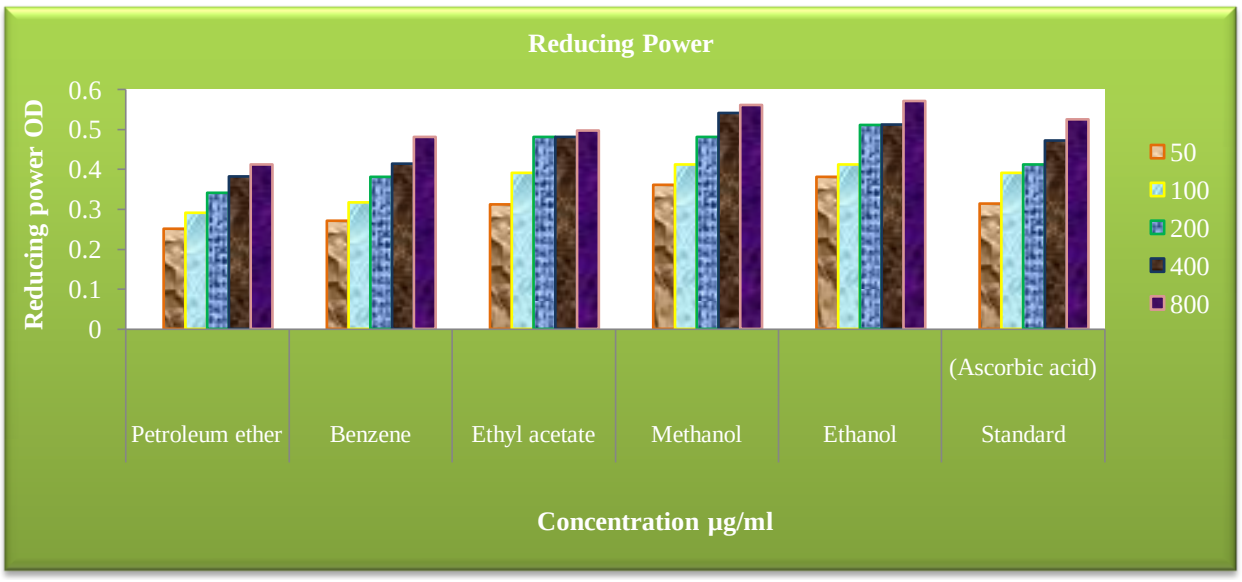


**Figure 5: Reducing power of different extracts of *Ecteinascidia venui***

**Table 1: $IC_{50}$ values of different extracts of colonial ascidian *Ecteinascidia venui***

| Solvent | Radical scavenging activity - $IC_{50}$ (µg/ml) | | | |
|---|---|---|---|---|
| | DPPH | Hydroxyl | Superoxide | ABTS+ cation |
| **Petroleum ether** | 52.92 | 31.71 | 31.84 | 35.66 |
| **Benzene** | 45.26 | 36.21 | 30.84 | 30.85 |
| **Ethyl acetate** | 40.74 | 39.78 | 36.67 | 35.54 |
| **Methanol** | 46.24 | 48.44 | 40.75 | 44.26 |
| **Ethanol** | 41.37 | 41.44 | 42.82 | 34.07 |
| **Ascorbic acid** | 23.46 | 35.48 | 32.81 | - |
| **Trolox** | - | - | - | 39.65 |

Many epidemiological studies show that phenolic compounds have beneficial effects on human health due to their antioxidant activity. Since a good correlation between antioxidant activities and reducing power has been proved, it has been used extensively to measure the total antioxidants in the sample. Various mechanisms, including reducing capacity, prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging have been claimed to explain the antioxidant activities. The reducing capacity of compound may serve as a significant indicator of its potential antioxidant activity [54].

## 4. Conclusion

In conclusion, the present study clearly indicates that the different extracts of *Ecteinascidia venui* show strong antioxidant activity when compared with standard ascorbic acid or trolox. The extracts were found to contain noticeable amount of total phenol and flavonoids which might play a major role in antioxidant defence. Furthermore, it adds to our understanding of the antioxidant activities of ascidians inorder to employ them in improving the health status, preventing chronic diseases and cancer.

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## References

[1] Fatouma AL, Prosper ,E François E, Nabil M, Adwa A, Samatar Djama, Louis-Clément O, Ismael B, Mamoudou D.Antimicrobial and antioxidant activities of essential oil and methanol extract of *Jasminum sambac* from Djibouti. *African Journal of Plant Science.*2010; 4(3):038-043.

[2] Nantitanon W, Chowwanapoonpohn S, Okonogi, S. Antioxidant and antimicrobial activities of *Hypitis suaveolens* essential oil. *Scientia Pharmaceutica.* 2007; 75: 35- 46.

[3] Azuma Y, Nakawo KM, Jiankany H, Watanabe O. Metting induced relocation of ions in glacier and in a seasonal snow-pack. *IAHS Publications*. 1995; 223:287-287.

[4] Banerjee A, Dasgupta N, De B. *In vitro* study of antioxidant activity of *Sygyium cumini* fruit. *Food Chemistry*. 2005; 90:727-733.

[5] Meenakshi VK, Studies on a few aspects of ascidians- Taxonomy, Biofouling, Bio indicators and Economic Importance, Final Technical Report submitted to the University Grants Commission, Hyderabad, 1996, 1-30.

[6] Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Antipyretic and Analgesic activity of *Phallusia nigra* Savigny, 1816. *Annals of Biological Research*. 2011; 2(4):192-196.

[7] Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Chemical Investigation of the Simple ascidian *Phallusia nigra* Savigny, 1816 of Tuticorin coast by GC-MS. *International Journal of Pharma and Bio Sciences*. 2011; 2(4):382-387.

[8] Meenakshi VK, Paripooranaselvi M, Gomathy S, Chamundeswari KP. Antiproliferative activity of *Phallusia nigra* Savigny, 1816 against Dalton's Lymphoma Ascites. *International Journal of Chemical and Pharmaceutical Sciences*. 2012; 3(2):70-75.

[9] Meenakshi VK, Paripooranaselvi M, Senthamarai S, Gomathy S, Chamundeswari, KP. Antitumor and immunomodulatory activity of *Phallusia nigra* Savigny, 1816 against Ehrlich ascites carcinoma. *Research Journal of Pharmaceutical Sciences*.2012; 1(2):7-12.

[10] Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Antimicrobial activity of the methanolic extract of *Phallusia nigra* Savigny. *Journal of Natural Product and Plant Resources*. 2012; 2(5):579-583.

[11] Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Anaesthetic activity of *Phallusia nigra* Savigny. *Annals of Biological Research*.2012; 3(4):1863-1865.

[12] Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Wound healing activity of the methanolic extract of *Phallusia nigra* Savigny. *International Journal of Chemical and Pharmaceutical Sciences*. 2012; 3(3): 45-51.

[13] Meenakshi VK, Senthamarai S, Paripooranaselvi M, Gomathy S, Shanmuga Priya D. Chamundeswari KP. Antibacterial activity of simple ascidian *Ascidia sydneiensis* (Family: Ascidiidae) against human pathogens. *Journal of Microbiology and Biotechnology Research*. 2012; 2(6): 894-899.

[14] Meenakshi VK, Senthamarai S, Paripooranaselvi M, Gomathy S. Sankaravadivu S. Chamundeswari KP. *In vitro* and *in vivo* antitumor and immunomodulatory studies of *Microcosmus exasperatus* against DLA bearing mice. *European Journal of Applied Engineering and Scientific Research*. 2013; 2(3):18-25.

[15] Gopalakrishnan S, Meenakshi VK, Shanmugapriya D. Anti-Inflammatory activity of Simple Ascidian, *Phallusia nigra* Savigny. *International Journal of Pharmaceutical sciences Review and Research.* 2013; 22(2):162-167.

[16] Meenakshi VK, Paripooranaselvi M, Senthamarai S, Gomathy S, Chamundeswari, KP. Immunomodulatory activity of ethanol extracts of *Phallusia nigra* Savigny, 1816 against Dalton's lymphoma ascites. *European Journal of Applied Engineering and Scientific Research*. 2013; 2(1):20-24.

[17] Meenakshi VK, Paripooranaselvi M, Sankaravadivu S, Gomathy S, Chamundeeswari KP. Immunomodulatory activity of *Phalusia nigra* Savigny, 1816 against S-180. *International Journal of Current Microbiology and Applied Sciences*.2013; 2(8):286-295.

[18] Meenakshi VK, Paripooranaselvi M, Senthamarai S, Gomathy S, Sankaravadivu S Chamundeeswari KP. Antitumor effect of ethanolic extract of *Phallusia nigra* Savigny, 1816 on S-180 tumor bearing mice. *International Journal of Pharmacological Screening Method*. 2014; 4(1):20-25.

[19] Meenakshi VK, Paripooranaselvi M, Gomathy S, Senthamarai S, Chamundeeswari, KP, Sankaravadivu S. Immunostimulating activities of *Phallusia nigra* Savigny, 1816 on sarcoma-180 tumor-bearing mice. *International Journal of Medicinal Chemistry and Analysis*. 2014; 4(2):62-69.

[20] Meenakshi VK, Delighta Mano Joyce MI, Paripooranaselvi M, Gomathy S. CNS depressant activity of the simple ascidian *Microcosmus exasperatus* Heller, 1878. *International Journal of Current Microbiology and Applied Sciences.* 2013; 2(10):16-25.

[21] Meenakshi VK, Gomathy S, Senthamarai S, Paripooranaselvi M, Chamundeswari, KP. Hepatoprotective activity of the ethanol extract of simple ascidian, *Microcosmus exasperatus* Heller, 1878. *European Journal of Zoological Research.* 2013; 2(4):32-38.

[22] Meenakshi VK, Delighta Mano Joyce MI, Paripooranaselvi M, Gomathy S, Chamundeswari KP. Protective Effect of *Microcosmus exasperatus* against isoproterenol induced myocardial ischemia- A biochemical and histopathological approach. *International Journal of Pure and Applied Bioscience*. 2014; 2(1):62-70.

[23] Meenakshi VK, Delighta Mano Joyce MI, Paripooranaselvi M, Gomathy S. Antihyperlipidemic Activity of *Microcosmus exasperatus* Heller, 1878. *Journal of Chemical, Biological and Physical Sciences.* 2014; 4(3):1379-1387.

[24] Kohila Subathra Christy H, Jothibai Margret R, Meenakshi VK. Antipyretic and analgesic activity of *Phallusia arabica* Savigny, 1816. *International Journal of Medicinal Chemistry and Analysis*. 2014; 4(3):162-165.

[25] Kohila Subathra Christy H, Jothibai Margret R, Meenakshi VK. Antiinflammatory activity of the simple ascidian of *Phallusia arabica* Savigny, 1816. *International Journal of Biological and Pharmaceutical Research.* 2014; 5(7):553-558.

[26] Kohila Subathra Christy H, Jothibai Margret R, Meenakshi, VK. Chemical screening and anaesthetic activity of *Phallusia arabica* Savigny, 1816. *International Research Journal of Pharmaceutical and Applied Sciences*. 2014; 4(1):24-28.

[27] Meenakshi VK, Gomathy S, Senthamarai S, Paripooranaselvi M, Chamundeswari, KP. Antifertility activity of simple ascidian, *Microcosmus exasperatus* Heller, 1878. *International Journal of Pharmaceutical Sciences Review and Research*. 2014; 24(1): 230-236

[28] Delighta Mano Joyce MI, Meenakshi VK, Paripooranaselvi M, Gomathy S. Anaesthetic, analgesic and antipyretic activities of *Microcosmus exasperatus* Heller, 1878. *World Journal of Pharmaceutical Research*. 2015; 4(7):1770-1779.

[29] Delighta Mano Joyce MI, Meenakshi VK, Paripooranaselvi M, Gomathy, S. Evaluation of anti–inflammatory activity of *Microcosmus exasperatus*. *European Journal of Pharmaceutical and Medical Research*. 2015; 2(4):682-692.

[30] Delighta Mano Joyce MI, Meenakshi VK, Paripooranaselvi M, Gomathy S. Wound Healing Activity of the ethanolic extract of *Microcosmus exasperatus* Heller, 1878. *Journal of Environmental and Applied Bioresearch*. 2015; 3(4):226-229.

[31] Kohila Subathra Christy H, Jothibai Margret R, Meenakshi VK. Evaluation of wound healing activity of *Phallusia arabica*. *World Journal of Pharamaceutical Research*. 2015; 4(3):162-165.

[32] Paripooranaselvi M, Meenakshi VK, Gomathy, S. Inhibition of HLCA-549 cell proliferation and survival by ethanolic extract of *Phallusia nigra* Savigny, 1816. *European Journal of Biomedical and Pharmaceutical Sciences*. 2015; 2(2):216-230.

[33] Paripooranaselvi M, Meenakshi VK. Ethanolic extract of *Phallusia nigra* Savigny, 1816 induced immunomodulations in HLCA-549 bearing mice. *World Journal of Pharmaceutical Research.* 2015; 4(11):1168-1181.

[34] Shanmugapriya D, Kohila Subathra Christy H, Sankaravadivu S. Antimicrobial activity of simple ascidian *Phallusia nigra*. *World Journal of Pharmaceutical research*. 2015; 4(9): 822-827.

[35] Shanmugapriya D, Kohila Subathra Christy H, Sankaravadivu S, Stella Packiam. Antidiabetic activity of the ethanolic extract of a simple ascidian *Phallusia nigra* World *Journal of Pharmaceutical research*. 2015; 4(11):1557-1563.

[36] Shanmugapriya D, Kohila Subathra Christy H, Sankaravadivu S, Stella Packiam. Antioxidant activity of the simple ascidian *Phallusia nigra* of Thoothukudi coast. *International Journal of Pharmaceutical Chemistry*. 2015; 410-412.

[37] Shanmugapriya D, Kohila Subathra Christy H, Sankaravadivu S. Hepatoprotective activity of ethanol extracts of *Phallusia nigra* against $CCl_4$ induced hepatotoxicity in rats. *World Journal of Pharmaceutical research*. 2015; 5(1):648-655

[38] Sankaravadivu S, Jothibai Margret R, Meenakshi VK. Infrared and gas chromatogram-mass spectral studies of the ethanolic extract of *Ecteinascidia venui* Meenakshi, 2000. *International Journal of Chemical Pharmaceutical Sciences*. 2013; 4(2):84-89.

[39] Sankaravadivu S, Jothibai Margret R, Meenakshi VK. Spectrophotometric studies of a colonial ascidian *Ecteinascidia venui* Meenakshi, 2000. *International Journal of Pharmacy and Biological Sciences*. 2013; 3(4):159-163.

[40] Sankaravadivu S, Jothibai Margret R, Meenakshi VK. Preliminary Screening and IR Spectral studies of a colonial ascidian *Ecteinascidia venui* Meenakshi, 2000. *Journal of Chemical, Biological and Physical sciences*. 2015; 5(4):4205-4210.

[41] Meenakshi, Biology of a few chosen ascidian, Ph.D thesis, M.S. University, Tirunelveli, 157-173, 1997.

[42] Blois MS. Antioxidant determination by the use of a stable free radical. Nature.A. 1958; 26:1199-1200.

[43] Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals. *Analytical Biochemistry.* 1987; 65:215-219.

[44] Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B. Antioxidant activity of *Caesalpinia digyna* root. *Journal of Ethnopharmacology.* 2007; 113:284-291.

[45] Huang MH, Huang SS, Wang BS, Sheu MJ, Hou WC. Antioxidant and anti-inflammatory properties of *Cardiospermum halicacabum* and its reference compounds *ex vivo and in vivo*. *Journal of Ethnopharmacology*.2011; 133:743-750.

[46] Kumar RS, Hemalatha S. *In vitro* antioxidant activity of alcoholic leaf extract and subfractions of *Alangium lamarckii Thwaites*. *Journal of Chemical and Pharmaceutical Research.* 2011; 3:259-267.

[47] Koleva II, Van Beek TA, Linseen JPH, de groot A, Evstatieva L. Screening of plant extracts for antioxidant activity a cimparatvr study on three testing methods. *Phytochemical Analysis*. 2002; 13:8-17.

[48] Gyamfi MA, Yonamine M, Anja Y. Free-radical scavenging action of medicinal herbs from Ghana Thonningia sanguine on experimentally induced liver injuries. *General Pharmacology*.1999; 32:661-667.

[49] Rollet-Labelle E, MJ Grange, C Elbim C Marquetty MA, Gougrot-Pacidalo, C Pasquier. Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis. *Free radical Biology and Medicine*. 1998; 24:563-572.

[50] Lee, JC HR Kim, J Kim, YS Jang. Antioxidant activity of ethanol extract of the stem of *opuntia ficus-indica var. saboten*. *Journal of Agricultural and Food Chemist.* 2002; 50:6490-6496.

[51] Hu C, DD Kitts.Studies on the antioxidant activity of *Echinacea* root extract. *Journal of Agricultural and Food Chemist*. 2000; 48:1466-1472.

[52] Wu LC, Hsu HW, Chen YC, Chiu CC, Lin YI, Ho JA. Antioxidant and antiproliferative activities of red pitaya. *Food Chemistry*. 2006; 95:319-327.

[53] Oszmianski J, Wojdylo A, Lamer-Zarawska E, Swiader K. Antioxidant tannins from Rosaceae plant roots. *Food Chemistry*. 2007; 100:579-583.

[54] Hsu B, Coupar IM, Ng K. Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. *Food Chemistry*. 2006; 98:317-328.