PANAINOOL

Transdisciplinary Insights in Life Sciences

ISSN: 2584-2161(Online)
Journal Homepage: www.panainool.com

RESEARCH ARTICLE

Antioxidant and antimitotic activity of two colonial ascidians from Mandapam Coast, Gulf of mannar Biosphere, India.

Ramaswamy Tamil Selvi[1], Vadivel Balamurugan[1*], & H. Abdul Jaffar Ali[2]

*[*1]PG and Research Department of Biotechnology, Sri Vinayaga College of Arts and Science, Ulundurpet, Tamil Nadu, India.*
[2]Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi, Tamil Nadu, India.

ARTICLE HISTROY

Received 21 November 2023
Revised 22 November 2023
Accepted 27 November 2023

Keywords

Antioxidants
Antimitotic
Eudistoma microlarvum
Eudistoma laysani

ABSTRACT

Coastal environments have always been targeted for research as they are provided with rich biodiversity. Ascidians (Phylum: Chordata, Class: Ascidiacea), or sea squirts, are the most important and most diverse class of the sub-phylum Tunicata (also referred to as Urochordata). They comprise approximately 3000 described species found in marine habitats, from shallow water to deep sea. There are no freshwater species, and most cannot tolerate salinities below 20%. The most commonly available colonial species are *Eudistoma laysani* and *Eudistoma microlarvaum* on the Mandapam coast, Gulf of Mannar. We were chosen to evaluate the antioxidant and antimitotic activities. The crude methanol extract of *E. microlarvum* showed good antioxidant and antimitotic activity, which are the main contributing factors for anticancer potential. This study sheds light on the potential use of *E. microlarvum* in the treatment of cancer.

✉ Balamurugan
balakal78@gmail.com

Introduction

Recent shifts in the global environment and work lifestyles have given rise to a multitude of severe illnesses, with cancer emerging as the second most prevalent cause of mortality on a global scale (Sung et al., 2021). Cancer starts when cells multiply too quickly and without control. This makes a mass of undifferentiated cells that turn into cancerous cells and show a wide range of symptoms. Despite the advancements made in the control of cancer through interventions like surgery, radiation therapy, and chemotherapy, these treatment modalities come with drawbacks (Debela et al., 2021).

Marine-derived items utilized in cancer treatments have the potential to mitigate harmful side effects as compared to herbal medications (YUN et al., 2019; Zuo & Kwok, 2021). Significant advancements have been achieved in the field of cancer treatment, with a notable contribution stemming from the utilization of pharmaceuticals produced from plants (Dehelean et al., 2021). The

oceans serve as vast reservoirs of non-conventional resources consisting of marine organisms. These organisms not only supply sustenance for the continuously growing global population but also serve as valuable sources for colors, scents, pesticides, and life-saving pharmaceuticals (Landrigan et al., 2020).

Scientific research has consistently directed its attention towards coastal habitats due to their abundant biodiversity (Herbert-Read et al., 2022). According to studies by Macedo et al., (2021) and Vladkova et al., (2022) marine organisms are well-known as valuable sources of natural antioxidants. In recent years, there has been a growing interest in the investigation of antioxidant activity within the realm of marine invertebrates, due to their significant biological potential (Romano et al., 2022).

Tunicates are known to possess a variety of phytochemical that has potent antioxidant properties. Cell division in the meristematic tissues of the growing root of an onion exhibits similarities to the division of tumors in humans (Carletti et al., 2022). Therefore, it is possible to utilize these meristematic cells for the first drug screening aimed at evaluating their potential anticancer properties (Rolnik et al., 2021). There may be concerns about applying what we know about plant tissue to animals and people, but it is important to note that plant cells are much more resistant to colchicine, which is a powerful anti-cancer drug that works by stopping the formation of microtubules. Hence, there exists a potential for chemical substances to influence the chromosomes of plants and animals (Parthasarathy et al., 2021; Williams & Omoh, 1996). Because of this, it is thought that if tunicate extract can kill *A. cepa* root cells, it might also be able to stop animal and human cells from dividing (Aşkin Celik & Aslantürk, 2010).

Ascidians, commonly known as tunicates, are a prominent class within the sub-phylum Tunicata (or Urochordata) of the phylum Chordata, exhibiting significant diversity and size. Ascidians exhibit a sessile lifestyle throughout their adult lives, subsequent to a short-lived larval phase (Shenkar & Swalla, 2011). The initial comprehensive elucidation of ascidians was provided by Schlosser in 1756, in a correspondence titled "Description of a colonial ascidian, *Botryllus schlosseri*, collected in the vicinity of the British Islands" (Reem et al., 2021). The term "tunicate" is derived from the presence of a polysaccharide-based tunic that surrounds the organism and serves as a pliable skeletal structure. The taxonomic group under consideration encompasses an estimated total of 3000 documented species, which exhibit a wide distribution across diverse marine environments ranging from shallow coastal regions to the depths of the ocean (Abdul Jaffar Ali, 2009; Tamilselvi et al., 2011). According to recent phylogenomic investigations, it has been proposed that tunicates are the closest relatives to vertebrates (DeBiasse et al., 2020; Delsuc et al., 2006). However, this assertion contradicts the findings derived from rRNA and mitochondrial data (Swalla & Smith, 2008). They play a significant role in enhancing marine biodiversity. In this historical context, the present study aimed to assess the antioxidant and antimitotic properties of methanolic extracts derived from two commonly found colonial ascidians, namely *Eudistoma laysani* and *Eudistoma microlarvum*.

Materials and Methods

Study area

The Mandapam seaside area (9°16'16.6"N 79°08'04.0"E) was selected as the location for the current study (Fig 1). The aforementioned coastal area is situated inside the Gulf of Mannar Biosphere in India (Fig 2). Various collection procedures, including scraping, peeling, and dislodging, were utilized to gather ascidians from the submerged surfaces of pillars in the Jetty region under investigation. This was accomplished by employing tools like a sharp knife and scalpel (Fig 3).

In this study, two colonial ascidians, namely *Eudistoma laysani* and *E. microlarvum* (Fig 4), were obtained from the Mandapam coast, located on the

southeast coast of India. These ascidians are abundantly available in the area.

Fig 1. Map shows the study location of Gulf of Mannar

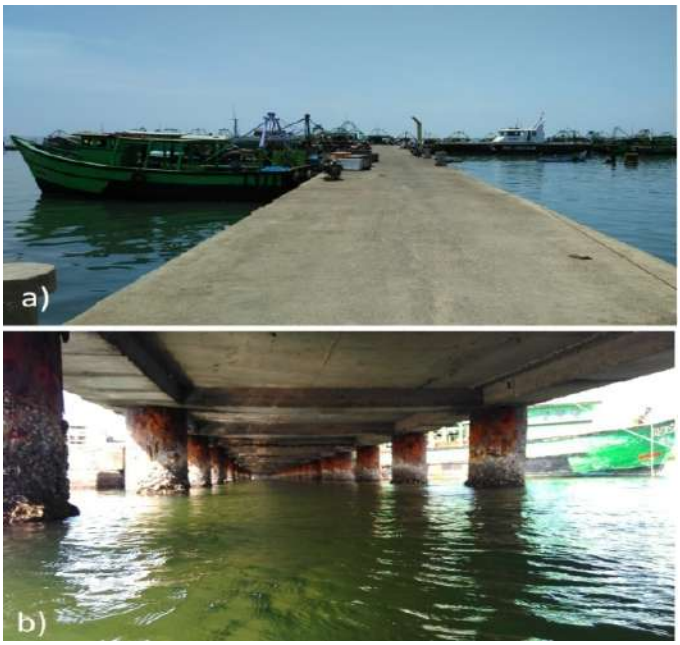

Fig 2. Photo images shown the ascidians sampling sites - (a) Mandapam Jetty and (b) Pillars of the Jetty

Fig 3. Methods of collection - (a)Dislodging the whole colony, (b) Scrapping and peeling of colony

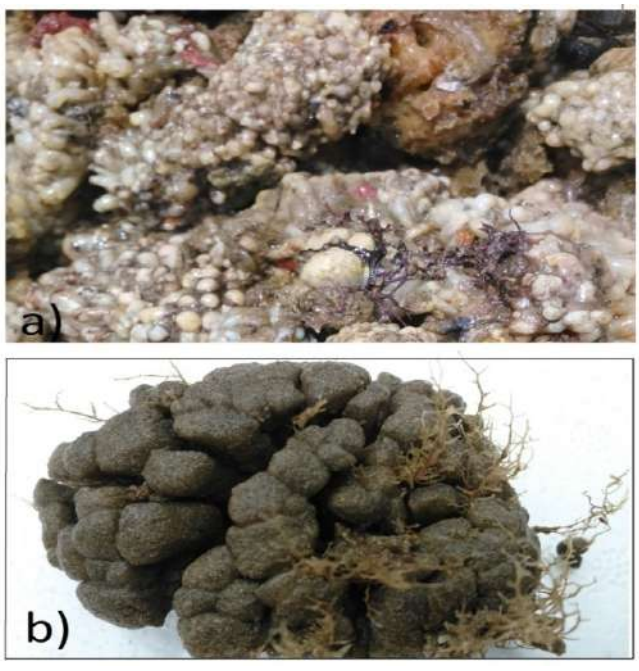

Fig 4. Collected samples - (a) A bunch of Colonial ascidian *Eudistoma laysani*; (b) A bunch of Colonial ascidian *Eudistoma microlarvum*

Process of animal material preparation

The entire population of the studied animals was utilized for the current investigation. The recently acquired specimens were individually cleansed and rinsed using uncontaminated seawater in order to eliminate any contaminants as well as any accompanying flora and fauna (Bayne, 1996). The specimens were subjected to a drying process in a shaded environment at ambient temperatures. The dried samples underwent grinding and sieving procedures to eliminate shell particles and were subsequently utilized for the production of crude methanol extracts (Deli et al., 2019).

Preparation of crude extracts

The dried materials were individually immersed in methanol of 100% A.R. grade at a ratio of 1g / 20 ml for duration of 10 days. This process took place at room temperature and involved continuous agitation using an orbital shaker. The extracts underwent filtration and were subjected to overnight drying at room temperature to ensure complete solvent evaporation prior to further extraction. The dried extracts were reconstituted in methanol for duration of 24 hours. Subsequently, the resulting extracts were gathered, subjected to filtration using Whatman No. 1 paper, and concentrated through the employment of a rotary evaporator of Buchi type. The remaining leftovers from the extract were suspended in 20 ml of methanol of analytical reagent quality and subsequently transferred to new vials in order to eliminate any salt precipitates. The extracts that were soluble in methanol were subjected to drying and subsequently dissolved in deionized water. Various quantities of extracts were made and stored at a temperature of 0°C for future utilization.

Antioxidant activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method

The assessment of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The stable free radical known as DPPH exhibits its highest absorption peak at a wavelength of 515 nm. The absorption of this substance is diminished when it is subjected to reduction by an antioxidant or a free radical species (Szerlauth et al., 2019). The utilization of the DPPH method enables a direct examination of the extract or antioxidant's capacity to contribute hydrogen and/or electrons in order to neutralize the DPPH radical. The presence of antioxidants in the solution leads to the suppression of the radical, resulting in a noticeable alteration in color from a dark purple hue to a pale yellow shade. Additionally, the absorbance at a wavelength of 515 nm exhibits a drop. The assessment of antioxidant activity in the tested compounds is based on the reduction in absorbance seen over a specific reaction time, with ascorbic acid serving as the standard. The methanolic extracts of two ascidians were individually combined with 95% methanol to create the stock solution at a concentration of 10 mg/10 ml. The test samples were generated by diluting a stock solution with methanol to achieve concentrations of 5 µg/ml, 10 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, and 125 µg/ml. The diluted test samples, measuring 1 ml in volume, were introduced into a solution of DPPH in methanol with a concentration of 0.004% and a volume of 3 ml. The mixture was incubated in darkness for a duration of 30 minutes to allow for the occurrence of the reaction. The measurement of absorbance was conducted at a wavelength of 515nm using a colorimeter. The standard utilized in this study was ascorbic acid. Combining 1 mL of methanol with 0.004% of 1 mL of DPPH solution created a blank solution. The measurement of optical density was recorded, and the percentage inhibition of DPPH activity can be calculated using the following formula as

% inhibition of DPPH activity = (Abs control - Abs sample) / Abs control ×100.

Antimitotic activity

Antimitotic activity refers to the ability of a substance or compound to inhibit or disrupt the process of mitosis, which is checked the antimitotic activity by looking at what happened to the root meristematic cells of *Allium cepa*. *Allium*

cepa bulbs were germinated in tap water for duration of 48 hours at an ambient temperature. The bulb that exhibited standardized root development was utilized for the purposes of experimentation.

Treatment

Various concentrations, specifically 1, 3, and 5 mg/mL, of two extracts derived from ascidians were generated. The present work employed various solutions to investigate the antimitotic impact through a bioassay approach, utilizing the root tips of Allium cepa. The sprouting roots were subjected to varying concentrations of ascidian extracts for a duration of approximately three hours. At the same time, distilled water was used as a control solution and methotrexate at a concentration of 0.1 mg/ml was used as a standard solution (Fig 6(a) & (b)). Following exposure, the juvenile roots were carefully removed and made ready for examination for mitosis.

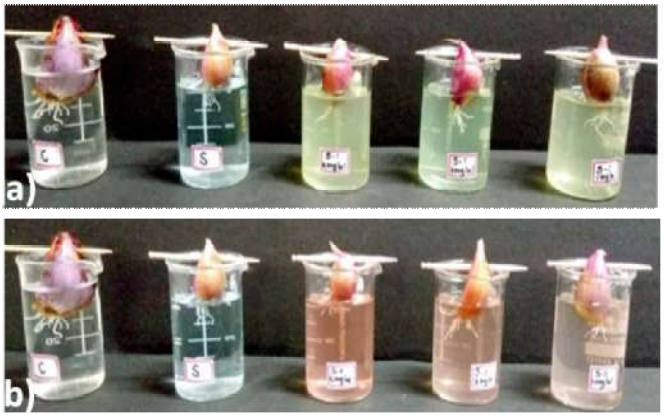

Fig 6. Onion roots exposed to Control, Standard and three different concentrations of extract of (a) *E. Laysani* and (b)*E. microlarvum*

Procedures for making squash

A root tip was surgically removed, incubated with 1N hydrochloric acid, rinsed, and stained with aceto-orcein. The tip was then manipulated into smaller fragments, pulverized, and covered with a cover slip. The stain was removed to prevent loss of cellular material. The slide was then used for observation, and the cellular material was carefully dispersed using a cover slip. The calculation of the mitotic index was performed utilizing the subsequent formula:

Mitotic index = Dividing cells / Total cells ×100

Statistical Analysis

The findings are reported as means ± standard deviation (SD) and are subjected to statistical analysis using analysis of variance (ANOVA).

Results

Antioxidant activity

The antioxidant activities of the crude extract from two species of ascidians are presented in Table 1. The DPPH free radical scavenging test was used to measure the antioxidant activity of crude extracts from two different ascidians. Concentrations used ranged from 5 to 125µg/ml. Ascorbic acid was utilized as the standard for comparison. The inhibitory activity exhibited a positive correlation with increasing concentration, reaching a maximum value of 75.42%. This observation suggests the presence of a potent antioxidant.

Table 1. Antioxidant activity of crude methanol extracts of chosen colonial ascidians.

Concentrations (µg/ml)	*E. laysani*	*E. microlarvum*	Ascorbic acid
5	24.02±2.4	18.14±1.63	44 ±3.9
10	36.35±3.27	29.12±3.2	55 ±4.9
25	48.19±5.3	39.25±4.71	66±7.2
50	61.22±5.5	51.23±6.1	78±8.5
100	68.27±6.1	61.22±5.5	81±8.1
125	75.42±8.29	69.25±6.2	86±8.6
IC_{50}	**46**	**27**	**15**

To test the antioxidant abilities of two types of ascidian, their IC_{50} values were found at five different concentrations as 5µg/ml, 25µg/ml, 50µg/ml, 100µg/ml, and 125µg/ml. The findings indicate that *Eudistoma laysani* has the highest level of free radical scavenging activity compared to the other specimens.

Plotting the percentage inhibition against the quantities of the methanol extract for both the

samples and the standard yielded the IC_{50} value. This analysis was performed using MS Excel, as shown in Fig 7a-c. The inhibition percentage of the *E. laysani* extract varied between 24.023% and 75.42%, with an IC_{50} value of 46 µg. With a value of 27µg, the IC_{50} value for the extract of *E. microlarvum* was found to be between 18.14 and 69.25 %.

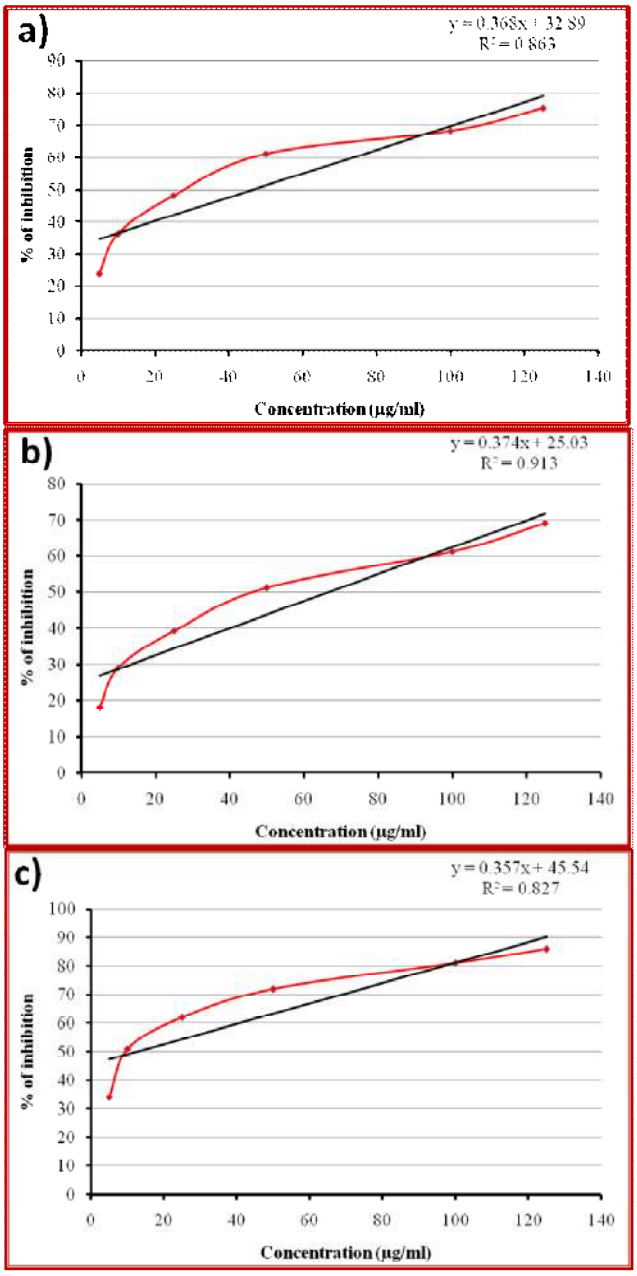

Fig 7. Graphical representation of % inhibition vs concentrations of methanol extract of a) *E. Laysani*, b) *E. microlarvum* and Ascorbic acid (r^2 value of IC_{50}).

As shown in Table 1, the scavenging capacities of all samples exhibited a correlation with their respective concentrations. Both of the two samples exhibited a clear scavenging action on the DPPH radical. The DPPH radical scavenging effect of the extract of *E. microlarvum* was found to be significantly superior to that of E. laysani ($P < 0.05$). The IC_{50} value, which represents the concentration of the extract that resulted in 50% scavenging activity of DPPH, was determined.

Antimitotic activity

The antimitotic actions exhibited by the whole extracts were found to be similar to those of the standard. Table 2 presents the activity observed for three distinct concentrations of the entire extract and standard. The cellular divisions were distinguished, and the quantities of cells in each stage of cell division, namely prophase, metaphase, anaphase, or telophase, were documented.

The experiment revealed that the extract exhibited a reduction in the mitotic activity of *A. cepa* root tips, which was dependent on the dosage administered. In each instance, the phases were distinguished, and it was seen that the quantity of non-dividing cells had an upward trend with a corresponding rise in the concentration of the extract.

In the control group, the proportion of cells that were not undergoing division was found to be 16%, while the mitotic index was determined to be 84.4. Upon exposure to a standard solution with a concentration of 1 mg/ml, the proportion of actively proliferating cells in the root tips exhibited a quick decline, reaching a value of 15%. When different amounts of methanolic extracts from two species of ascidian were put on the root tips, they showed different levels of antimitotic activity.

In each of the conducted studies, prophase exhibited a prevailing presence, subsequently followed by metaphase and anaphase. Telophase was only found in the cells subjected to the control water, while a small percentage (1-2%) of telophase was observed in the treated cells. Upon treatment of the cells with *E. laysani* extracts, there

Table 2. Antimitotic activity of crude methanol extracts of chosen colonial ascidians.

Sample	Dose (mg/ml)	3hrs						
		TC	Dividing cells				TDC	MI
			P	M	A	T		
Control	0	256±21.0	112±11.1	60±6.2	32±3.2	12±1.1	216±2.8	84.4±8.0
Standard (Methotrexate)	1	230±18.7	13±3.5	12±1.0	7±0.6	4±0.4	36±3.4	15.6±1.7
E. laysani	1	219±21.8	68±6.7	42±5.9	29±2.6	19±1.8	148±16.5	67.5±6.3
	3	221±22.3	55±5.81	36±3.7	20±2.1	10±1.4	121±11.5	54.7±5.0
	5	220±21.8	47±4.4	34±3.3	15±1.8	7±0.3	103±10.3	46.8±4.0
E. microlarvum	1	207±18.3	44±4.5	27±2.4	22±2.5	8±0.9	101±12.7	48.8±4.8
	3	215±24.8	48±4.1	25±2.7	22±2.1	9±0.9	104±10.4	48.3±4.0
	5	220±25.2	33±3.1	18±1.8	15±1.6	3±0.2	69±6.7	31.3±3.6

was an observed rise in the proportion of cells that were not undergoing division. Specifically, at dosages of 1, 3, and 5 mg/ml, the percentages of non-dividing cells were found to be 32%, 45%, and 53%, respectively. The mitotic index exhibited a sudden reduction to 46.8 at a dosage of 5 mg/ml. The extract of *E. microlarvum* exhibited notable and statistically significant activity when tested at a concentration of 5 mg/ml. The observed percentages of non-dividing cells ranged from 51% at a concentration of 1 mg/ml, to 52% at a dose of 3 mg/ml, and finally to 69% at a concentration of 5 mg/ml.

The results of a one-way analysis of variance (ANOVA) indicated a statistically significant effect of therapy on mitotic activity, with a p-value ≤ 0.005.

The concentration of the extracts exhibited an inverse relationship with the quantity of cells entering prophase. The progression of cell division phases is inversely correlated with the concentration of the aqueous extract, as prophase is not observed in the cells.

Discussion

In recent times, there has been a growing corpus of studies focused on antioxidants, with a specific emphasis on their potential to counteract the perceived harmful impacts of free radicals within the human body, as well as their ability to impede the degradation of lipids and other constituents found in food (Sharifi-Rad et al., 2020). A multitude of methodologies and adaptations have been suggested in order to assess antioxidant activity and elucidate the mechanisms by which antioxidants operate (Munteanu & Apetrei, 2021). One of the currently prevalent approaches involves the utilization of the stable free radical known as diphenylpicrylhydrazyl (DPPH) (Ionita, 2021). A singular antioxidant molecule has the capacity to engage in a reaction with only one individual free radical (Pizzino et al., 2017). Hence, it is imperative to continuously replace endogenous antioxidant reserves, either through endogenous processes or by means of supplementation. Numerous natural and synthetic substances have been extensively studied over the course of several decades to assess their effectiveness in mitigating oxidative stress (Heo & Jeon, 2009; Lobo et al., 2010). Consumers exhibit a preference for naturally derived antioxidants owing to apprehensions about the potential toxicity and carcinogenicity associated with synthetic antioxidants.

According to Soares et al., (1997), it was hypothesized that the ability of antioxidants to scavenge DPPH radicals is attributed to their capacity to donate hydrogen. In recent times, there has been an increased focus on the evaluation of natural biomaterials for screening purposes in various clinical scenarios (Gulcin & Alwasel, 2023).

This shift in attention might be attributed to the limitations imposed on the utilization of synthetic antioxidants, which are known to possess carcinogenic properties (Kim et al., 2002).

The current study involved the assessment of the crude methanolic extract derived from two colonial ascidians. The evaluation was conducted by measuring the free radical-scavenging activity using the DPPH method. The obtained results were then compared to those of ascorbic acid. According to the basic idea behind this method, a solution containing methanolic DPPH (2,2-diphenyl-1-picrylhydrazyl) is reduced when an antioxidant that gives off hydrogen is present. This decrease happens because of the reaction that creates the non-radical form DPPH (Li et al., 2007; Yang et al., 2022), showed that the extract could effectively lower the stable radical DPPH, leading to the formation of diphenylpicrylhydrazine, which is clearly visible as yellow.

Figure 5.1 shows how the total antioxidant capacity of the studied ascidians changes with concentration. The total antioxidant capacity is given in terms of ascorbic acid equivalents in µg/mL of extract. Among the two species, *E. microlarvum* demonstrated noteworthy antioxidant activity. According to Manggau et al., (2022), three main crude polysaccharides from *Sargassum pallidum*, a type of brown seaweed, were tested against DPPH radicals and found to have less than 20% scavenging power at a concentration of 3.8 mg/mL. All of the findings demonstrated significant DPPH radical scavenging capabilities, suggesting their potential as main antioxidants. Minglv Sun et al. did a study in 2011 that found the EC_{50} values of oligosaccharide from three different parts of the simple ascidian Styela clava to be 1.35, 0.77, and 0.90 mg/mL, when tested against DPPH radicals. Furthermore, the research indicated that VOS exhibited the most potent antioxidant properties among the three samples. According to (Jana et al., 2011), the methanolic extract from *Bursa spinosa* showed strong antioxidant activity. This might be because it contains phenolic compounds. The gastropod *Pleuroploca trapezium*'s methanolic extract, which had an IC_{50} value of 4021 g/ml, had significant scavenging activity against the DPPH radical.

The current study shows that the methanolic extract from *E. microlarvum* has a good amount of antioxidant activity. Consequently, this particular species holds potential for further exploration and utilization following additional evaluation. The antioxidant activity of the methanolic extract of Babylonia zeylanica was shown to be potent, with a value of 78.6±0.40 at a concentration of 10 mg/ml, as determined by the conducted study.

The surplus of free radicals present in the body undergoes the oxidation of low-density lipoproteins (LDL), rendering them potentially harmful. The process of aging has been associated with various severe pathologies, such as cerebral stroke, diabetes mellitus, rheumatoid arthritis, Parkinson's disease, Alzheimer's disease, and cancer. It is believed that the presence of too many free radicals accelerates these diseases. From a physiological standpoint, the presence of oxygenated free radicals is considered crucial among radical species. Reactive oxygen species (ROS) encompass a variety of highly oxidizing species, which can be classified as either radical or non-radical in nature.

The numerous health benefits that naturally derived (exogenous) antioxidants with low molecular weight provide are primarily to blame for the growing interest in antioxidants. Using this method is meant to stop diseases before they happen. These diseases are linked to oxidative stress, which happens when free radicals damage important parts of cells like lipids and nucleic acids.

The mitotic index is a measure of the frequency of cell division and is considered a significant indicator for evaluating the rate of root growth and assessing the cytotoxicity of various treatments. The findings of the current investigation indicate that, among the four ascidians examined, the methanolic extract derived from *E. microlarvum* exhibited noteworthy anti-mitotic efficacy. The methanolic extracts exhibited a decline in mitotic

index (MI) values, as shown in Table 2, which was found to be dependent on the concentration. According to the findings of Fernandes et al., (2007) and Newman & Cragg, (2020), alterations in the MI, whether they increase or decrease, can be indicative of the cytotoxic effects exerted by the drugs under investigation.

Conclusion

The methanolic extract of *E. microlarvum* has shown efficacy in inhibiting cell divisions. It is plausible that the component within this extract exerts its effects on human cell lines by mimicking the mechanisms observed in onion cells, hence diminishing cell viability. In light of this, the administration of the extract in humans has the potential to hinder cell proliferation through direct interaction with DNA, resulting in DNA fragmentation. Additionally, it may impede the folic acid pathway or interact with cell receptors and enzymes, triggering signals for cell apoptosis, as documented by Patil & Bhat, (1992).

The antioxidant capacity of the extract has been determined in a previous experiment conducted in the current study. The provided extract exhibited notable antioxidant activity, which may potentially contribute to its anticancer properties. This work elucidates the possible application of *E. microlarvum* in the treatment of cancer.

Acknowledgement

The authors' thankful to the Sri Vinayaga College of Arts and Science, Tamil Nadu, India for providing necessary facilities for carried out of this work.

References

Abdul Jaffar Ali H, Tamilselvi, M. and S. V. (2009). Distribution of Alien and Cryptogenic Ascidians along the Southern Coasts of Indian Peninsula. *World Journal of Fish and Marine Sciences*, *1*(4), 305–312.

Aşkin Celik, T., & Aslantürk, O. S. (2010). Evaluation of cytotoxicity and genotoxicity of Inula viscosa leaf extracts with Allium test. *Journal of Biomedicine & Biotechnology*, *2010*, 189252. https://doi.org/10.1155/2010/189252

Bayne, K. (1996). Revised Guide for the Care and Use of Laboratory Animals available. American Physiological Society. In *The Physiologist* (Vol. 39, Issue 4).

Carletti, A., Cardoso, C., Lobo-Arteaga, J., Sales, S., Juliao, D., Ferreira, I., Chainho, P., Dionísio, M. A., Gaudêncio, M. J., Afonso, C., Lourenço, H., Cancela, M. L., Bandarra, N. M., & Gavaia, P. J. (2022). Antioxidant and Anti-inflammatory Extracts From Sea Cucumbers and Tunicates Induce a Pro-osteogenic Effect in Zebrafish Larvae. *Frontiers in Nutrition*, *9*(May), 1–13. https://doi.org/10.3389/fnut.2022.888360

Debela, D. T., Muzazu, S. G., Heraro, K. D., Ndalama, M. T., Mesele, B. W., Haile, D. C., Kitui, S. K., & Manyazewal, T. (2021). New approaches and procedures for cancer treatment: Current perspectives. *SAGE Open Medicine*, *9*, 20503121211034370. https://doi.org/10.1177/20503121211034366

DeBiasse, M. B., Colgan, W. N., Harris, L., Davidson, B., & Ryan, J. F. (2020). Inferring Tunicate Relationships and the Evolution of the Tunicate Hox Cluster with the Genome of Corella inflata. *Genome Biology and Evolution*, *12*(6), 948–964. https://doi.org/10.1093/gbe/evaa060

Dehelean, C. A., Marcovici, I., Soica, C., Mioc, M., Coricovac, D., Iurciuc, S., Cretu, O. M., & Pinzaru, I. (2021). Plant-Derived Anticancer Compounds as New Perspectives in Drug Discovery and Alternative Therapy. *Molecules*, *26*(4). https://doi.org/10.3390/molecules26041109

Deli, M., Ndjantou, E. B., Ngatchic Metsagang, J. T., Petit, J., Njintang Yanou, N., & Scher, J. (2019). Successive grinding and sieving as a new tool to fractionate polyphenols and antioxidants of plants powders: Application to Boscia senegalensis seeds, Dichrostachys glomerata fruits, and Hibiscus sabdariffa calyx powders. *Food Science & Nutrition*, *7*(5), 1795–1806. https://doi.org/10.1002/fsn3.1022

Delsuc, F., Brinkmann, H., Chourrout, D., & Philippe, H. (2006). Tunicates and not cephalochordates are the closest living relatives of vertebrates. *Nature*, *439*(7079), 965–968.

https://doi.org/10.1038/nature04336

Fernandes, T. C. C., Mazzeo, D. E. C., & Marin-Morales, M. A. (2007). Mechanism of micronuclei formation in polyploidizated cells of Allium cepa exposed to trifluralin herbicide. *Pesticide Biochemistry and Physiology*, *88*(3), 252–259. https://doi.org/https://doi.org/10.1016/j.pestbp.2006.12.003

Gulcin, İ., & Alwasel, S. H. (2023). DPPH Radical Scavenging Assay. *Processes*, *11*(8). https://doi.org/10.3390/pr11082248

Heo, S.-J., & Jeon, Y.-J. (2009). Protective effect of fucoxanthin isolated from Sargassum siliquastrum on UV-B induced cell damage. *Journal of Photochemistry and Photobiology. B, Biology*, *95*(2), 101–107. https://doi.org/10.1016/j.jphotobiol.2008.11.011

Herbert-Read, J. E., Thornton, A., Amon, D. J., Birchenough, S. N. R., Côté, I. M., Dias, M. P., Godley, B. J., Keith, S. A., McKinley, E., Peck, L. S., Calado, R., Defeo, O., Degraer, S., Johnston, E. L., Kaartokallio, H., Macreadie, P. I., Metaxas, A., Muthumbi, A. W. N., Obura, D. O., … Sutherland, W. J. (2022). A global horizon scan of issues impacting marine and coastal biodiversity conservation. *Nature Ecology & Evolution*, *6*(9), 1262–1270. https://doi.org/10.1038/s41559-022-01812-0

Ionita, P. (2021). The Chemistry of DPPH· Free Radical and Congeners. *International Journal of Molecular Sciences*, *22*(4). https://doi.org/10.3390/ijms22041545

Jana, K., Chatterjee, K., Ali, K. M., Ghosh, A., Bera, T. K., & Ghosh, D. (2011). Antioxidant potential of hydro-methanolic extract of seed of Caesalpinia bonduc: An in vitro study. *Journal of Advanced Pharmaceutical Technology & Research*, *2*(4), 260–265. https://doi.org/10.4103/2231-4040.90884

Kim, I. G., Jung, I. L., Oh, T. J., Kim, K. C., & Shim, H. W. (2002). Polysaccharide-enriched fraction isolated from Duchesnea chrysantha protects against oxidative damage. *Biotechnology Letters*, *24*(16), 1299–1305. https://doi.org/10.1023/A:1019812202099

Landrigan, P. J., Stegeman, J. J., Fleming, L. E., Allemand, D., Anderson, D. M., Backer, L. C., Brucker-Davis, F., Chevalier, N., Corra, L., Czerucka, D., Bottein, M.-Y. D., Demeneix, B., Depledge, M., Deheyn, D. D., Dorman, C. J., Fénichel, P., Fisher, S., Gaill, F., Galgani, F., … Rampal, P. (2020). Human Health and Ocean Pollution. *Annals of Global Health*, *86*(1), 151. https://doi.org/10.5334/aogh.2831

Li, X. M., Li, X. L., & Zhou, A. G. (2007). Evaluation of antioxidant activity of the polysaccharides extracted from Lycium barbarum fruits in vitro. *European Polymer Journal*, *43*(2), 488–497. https://doi.org/https://doi.org/10.1016/j.eurpolymj.2006.10.025

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, *4*(8), 118–126. https://doi.org/10.4103/0973-7847.70902

Macedo, M. W. F. S., Cunha, N. B. da, Carneiro, J. A., Costa, R. A. da, Alencar, S. A. de, Cardoso, M. H., Franco, O. L., & Dias, S. C. (2021). Marine Organisms as a Rich Source of Biologically Active Peptides. *Frontiers in Marine Science*, *8*(July), 1–23. https://doi.org/10.3389/fmars.2021.667764

Manggau, M., Kasim, S., Fitri, N., Aulia, N. S., Agustiani, A. N., Raihan, M., & Nurdin, W. B. (2022). Antioxidant, anti-inflammatory and anticoagulant activities of sulfate polysaccharide isolate from brown alga Sargassum policystum. *IOP Conference Series: Earth and Environmental Science*, *967*(1), 12029. https://doi.org/10.1088/1755-1315/967/1/012029

Munteanu, I. G., & Apetrei, C. (2021). Analytical Methods Used in Determining Antioxidant Activity: A Review. *International Journal of Molecular Sciences*, *22*(7). https://doi.org/10.3390/ijms22073380

Newman, D. J., & Cragg, G. M. (2020). Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *Journal of Natural Products*, *83*(3), 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285

Parthasarathy, A., Borrego, E. J., Savka, M. A., Dobson, R. C. J., & Hudson, A. O. (2021). Amino acid–derived defense metabolites from plants: A potential source to facilitate novel antimicrobial development. *Journal of Biological Chemistry*, *296*, 100438.

https://doi.org/10.1016/j.jbc.2021.100438

Patil, B. C., & Bhat, G. I. (1992). A Comparative Study of MH and EMS in the Induction of Chromosomal Aberrations on Lateral Root Meristem in Clitoria ternatea L. *Cytologia*, *57*(2), 259–264. https://doi.org/10.1508/cytologia.57.259

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*, *2017*, 8416763. https://doi.org/10.1155/2017/8416763

Reem, E., Douek, J., & Rinkevich, B. (2021). A critical deliberation of the 'species complex' status of the globally spread colonial ascidian Botryllus schlosseri. *Journal of the Marine Biological Association of the United Kingdom*, *101*(7), 1047–1060. https://doi.org/10.1017/S0025315422000029

Rolnik, A., Soluch, A., Kowalska, I., & Olas, B. (2021). Antioxidant and hemostatic properties of preparations from Asteraceae family and their chemical composition - Comparative studies. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *142*, 111982. https://doi.org/10.1016/j.biopha.2021.111982

Romano, G., Almeida, M., Varela Coelho, A., Cutignano, A., Gonçalves, L. G., Hansen, E., Khnykin, D., Mass, T., Ramšak, A., Rocha, M. S., Silva, T. H., Sugni, M., Ballarin, L., & Genevière, A.-M. (2022). Biomaterials and Bioactive Natural Products from Marine Invertebrates: From Basic Research to Innovative Applications. *Marine Drugs*, *20*(4). https://doi.org/10.3390/md20040219

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. El, Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., … Sharifi-Rad, J. (2020). Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Frontiers in Physiology*, *11*(July), 1–21. https://doi.org/10.3389/fphys.2020.00694

Shenkar, N., & Swalla, B. J. (2011). Global diversity of Ascidiacea. *PloS One*, *6*(6), e20657. https://doi.org/10.1371/journal.pone.0020657

Soares, J. R., Dinis, T. C., Cunha, A. P., & Almeida, L. M. (1997). Antioxidant activities of some extracts of Thymus zygis. *Free Radical Research*, *26*(5), 469–478. https://doi.org/10.3109/10715769709084484

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

Swalla, B. J., & Smith, A. B. (2008). Deciphering deuterostome phylogeny: molecular, morphological and palaeontological perspectives. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *363*(1496), 1557–1568. https://doi.org/10.1098/rstb.2007.2246

Szerlauth, A., Muráth, S., Viski, S., & Szilagyi, I. (2019). Radical scavenging activity of plant extracts from improved processing. *Heliyon*, *5*(11), e02763. https://doi.org/10.1016/j.heliyon.2019.e02763

Tamilselvi, M., Sivakumar, V., Ali, H. A. J., & Thilaga, R. D. (2011). Distribution of Alien Tunicates (Ascidians) in Tuticorin Coast , India. *World Journal of Zoology*, *6*(August 2007), 164–172.

Vladkova, T., Georgieva, N., Staneva, A., & Gospodinova, D. (2022). Recent Progress in Antioxidant Active Substances from Marine Biota. *Antioxidants (Basel, Switzerland)*, *11*(3). https://doi.org/10.3390/antiox11030439

Williams, G. O., & Omoh, L. E. (1996). Mitotic effects of the aqueous leaf extract of Cymbopogon citratus in Allium cepa root tips. *Cytobios*, *87*(350), 161–168.

Yang, T., Hu, Y., Yan, Y., Zhou, W., Chen, G., Zeng, X., & Cao, Y. (2022). Characterization and Evaluation of Antioxidant and Anti-Inflammatory Activities of Flavonoids from the Fruits of Lycium barbarum. *Foods (Basel, Switzerland)*, *11*(3). https://doi.org/10.3390/foods11030306

YUN, C. W. O. N., KIM, H. J. O. O., & LEE, S. H. U. N. (2019). Therapeutic Application of Diverse

Marine-derived Natural Products in Cancer Therapy. *Anticancer Research*, *39*(10), 5261–5284. https://doi.org/10.21873/anticanres.13721

Zuo, W., & Kwok, H. F. (2021). Development of Marine-Derived Compounds for Cancer Therapy. *Marine Drugs*, *19*(6). https://doi.org/10.3390/md19060342