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Nutritional and pharmacological qualities of selected species of ascidians from Gulf of Mannar, southern coast of peninsular India

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Abstract

Tunicates, a group of marine animals, have been attracted a lot of attention in medical application, food market, water pollution issues and exploited in the food industry. However, limited knowledge of their chemical composition and nutritional profiles prohibited further application. In this study, three common colonial tunicate species, *Polylinum indicum, Polylinum nudum* and *P. saturnium* were subjected to comprehensive composition analysis in terms of moisture, protein, lipids, cellulose, ash, amino acids, fatty acids, non-cellulose carbohydrates and minerals. In addition, antimicrobial activity of the solvent extract of these ascidians was also studied. All the three species had low carbohydrate content, and protein and fat were their main components. Having high quality protein content, these species could be excellent sources of nutritive ingredients. Among amino acids, glutamate, aspartic acid, alanine, leucine and lysine were present at high levels in the ascidians, while the major elements present were sodium, chloride, magnesium and phosphorous. The selected species demonstrated balanced essential to non-essential amino acids ratio. Among polyunsaturated fatty acids, eicosapentaenoic acid and docosahexaenoic acid were found to be prominent. Higher content of vitamin A and vitamin B3 in *Polyclinum saturnium* signified their importance in preventing osteoporosis. The heavy metal contents such as lead, copper, cadmium and nickel were found to present below the recommended level. These species exhibited potent antibacterial activity against several bacterial pathogens. Overall, the results indicate that the chosen species of ascidians can be good sources of high quality protein, lipid, and essential elements. Toxic metals were below threshold limits of recommended standards for human consumption. These species could have been utilized as an alternate source for food supplements.

Keywords: Ascidians, antibacterial activity, food value, nutritional components, *Polyclinum sp.*

Introduction

In current years, the world food catastrophe has become more sober on account of natural disasters such as floods and droughts and the outbreak of the novel coronavirus (2019-nCoV) [Cao et al 2022]. Researchers have looked for alternative protein sources in different regions to

overcome this all-encompassing issue [Liceaga et al 2022]. Marine biological resources, such as fin fishes, shellfishes and seaweed account for abundant food constituents for human beings, and it is decisive to deal with the world food crisis by producing cost-effective and high-quality protein sources. Among these sources, tunicates representing a new type of marine bioresource containing high contents of good-quality protein that can be a complementary food source, thereby helping to ease the food crisis [Lambert et al 2016].

More than 1200 biologically active molecules have been identified from tunicates and tunicate-associated microbial species as signature candidates with potent pharmacological properties [Dou and Dong, 2019], confirming that they are rich in diverse biologically active compounds [Faulkner, 1995]. Screening of bioactive lead molecules from tunicates is mainly used treat various types of cancer and inflammatory diseases, while antimalarial and other drugs are in second and third place [Kaleemullah Khan et al 2021]. Some peptides and alkaloids purified from tunicates show anticancer effects [Jumeri 2011], while sulfated glycosaminoglycan (GAG) can be used for anticoagulation applications [Gandra et al 2000].

Tunicates are a high biomass group of filter-feeding, sedentary animals that have inhabit wide range of marine habitats worldwide. They settle on solid or hard surfaces as well as on soft surfaces submerged into sea water. They are considered as major marine invasive species and contribute significantly to fouling problem in aquaculture and loss of biodiversity. They can compete with shellfish for surface and reduce aquaculture productivity, thus threaten the marine ecosystems and food chains for human beings [Govindharaj et al 2022]. They have become a significant marine fouling organisms due to their higher growth rate, explosion competence, and continued existence abilities compared with sponges, shellfish and seaweeds, [Locke et al 2007].

Control or prevention of their biofouling and invasiveness is more complicated as their reproductive potential and polymorphic nature uphold escalating growth which in turn pave a way for the consequences in the environment. Thus, possible utilization of the tunicates is imperative. Besides, this technique not only helps to restrain the exotic one but also facilitates to conserve the native species. Instead of dumping the tunicates in to the ocean, they show potential to a valuable and abundant marine bioresources, which can be used to produce food, animal feed material, and energy [Zhao et al 2018; Samuelsen et al 2022]. Several edible tunicate species, such as *Ciona intestinalis*, *Halocynthia roretzi*, *H. aurantium*, *Herdmania pallid, Microcosmus hartmeyeri*, *M. sabatieri and M. vulgaris, Pyura pachydermatina, P. chilensis, Styela plicata, S. canopus* and *S. clava* are cultivated and consumed as delicious food in many countries [Lambert et al 2016]. In Asia, Chile, and some Mediterranean countries, several species of edible tunicate are widely available as fresh or dried products in seafood markets. *Pyura chilensis,* locally called piure in Chile, is consumed domestically and exported to other countries, such as Sweden and Japan [Haye and Mu˜noz-Herrera, 2013]. *Halocynthia aurantium* is often called “sea peach” or “ice floe tunicate” (akaboya in Japan) was cultivated in Japan, but production data is lacking [Narita et al 2017]. Another edible tunicate species, *Halocynthia roretzi*, commonly referred to as “sea pineapple”, was first cultivated in Korea in 1982 [Lambert et al 2016]. *Styela plicata* is eaten fresh in Korea and some Mediterranean countries. *Microcosmus hartmeyeri* (harutoboya in Japan) is eaten in Japan. *Microcosmus sabatieri* and *Microcosmus vulgaris* are consumed as popular recipes in France, Italy, and Greece. Historically, the Maoris in New Zealand consumed *Pyura pachydermatina*, which was once a food source for indigenous people living around Botany Bay, Australia [Lambert and Lambert, 1997]. The elemental composition of *Salpa thompsoni* was determined with moisture content of 93.6 % (aggregate form) and 92.3 % (solitary form). Ash content reaches 44 % of the dry weight. Carbon and nitrogen comprise 17–22 % and 3–5% of the dry weight respectively [Dubischar

et al 2006]. It can be concluded that different tunicate species and fractions have different chemical compositions [Wang et al 2014].

In addition to protein and lipids, it can be used as a useful indicator to reflect the environmental pollution for various metals since some tunicate species, such as *Ciona intestinalis*, has a unique capacity to accumulate iron (Fe) from the environment. It also contains many different amounts of trace minerals, such as zinc (Zn), magnesium (Mg), vanadium (V), etc., which are also vital in sustaining the healthy bodies of human beings [Papadopoulou and Kanias, 1977]. The chemical contents of *Halocynthia roretzi* were formerly investigated [Oh et al 1997], Zhao and Li [2016] also determined the prime chemical composition of *H. roretzi*. *Phallusia nigra* accumulate various heavy metals from the sea water and reported as sentinel animal [Abdul Jaffar Ali et al 2015]. Scandinavian startup Pronofa is farming and processing Ciona tunicates, commonly known as sea squirts, in an effort to provide an environmentally friendly alternative to mince produced from conventional terrestrial livestock.(Rob Fletcher, Senior editor, The Fish Site) - 13 May 2024. Although several species of tunicates are consumed as seafood worldwide, very limited studies have focused on the chemical composition and nutritional profile analysis of tunicates of Indian coast. Furthermore, to the best of our knowledge, there is an insufficient data regarding the chemical composition and nutritional profile different species of tunicates. Nutritional and pharmacological status of three colonial ascidians (*Polyclinum nudum, Polyclinum indicum* and *P. saturnium*) collected from the coastal waters of Mandapam, Gulf of Mannar, India, with the aim of exploiting this underutilized resource.

Materials and Methods

During 2021 and 2022, ascidian samples were collected from the pillars of Jetty in the intertidal and subtidal zone of Mandapam coast, Gulf of Mannar, India. The specimens were identified according to Monniot and Monniot [2001]; Cole and Lambert [2009]; and Kott [2002]. Voucher samples have been deposited at the Museum of Biotechnology Department, Islamiah College (Autonomous), Vaniyambadi – 635 752, Tirupattur District, Tamil Nadu, India.

For the analysis of nutritional value of the samples, fresh material was collected and transported to the laboratory, hermetically sealed in plastic bags, and kept in refrigerated containers. Epiphytes and contaminants were removed from the collected material by repeatedly washing in fresh sea water. The cleaned samples were then frozen at -20℃. Drying was performed in a Labconco freeze-dryer, model 18 L (Kansas, KS, USA). Finally, pulverized lyophilized samples were obtained using a mortar and stored in hermetically sealed plastic bags at -20℃. The whole colony was used to avoid leakage of intracellular fluids. Three replicates of ten individuals each were obtained. Each sample was stored at -20°C (for a maximum 7 days) in polyethylene bags.

Analysis of biochemical components

Moisture content was determined by drying 0.1 g of lyophilized sample in an oven at 105°C overnight until a constant weight was obtained. Additionally, ash content was determined by burning the 0.1 g of lyophilized sample in the furnace at 550°C overnight. The crude protein content was measured by the Kjeldahl procedure (nitrogen to protein conversion factor = 6.25). The methods of the Association of Official Analytical Chemists [AOAC, 1995] were used. Protein was measured by a modification of the Lowry procedure [Hartree, 1972] using bovine serum albumin as a standard, lipid content according to Marsh and Weinstein [1966] with a cholesterol standard, and carbohydrate according to the method of Dubois *et al.* [1956] using a glucose standard and preceded by protein precipitation with cold 15% w/v TCA. All biochemical analyses

were run on triplicate subsamples. Results of proteins, glycogens and total lipids were expressed as mg/g of weight wet.

Vitamins

Fat-soluble vitamins A, D, E, K and water-soluble vitamins B1, B2, B6, B12, C were analyzed by HPLC (Merk Hitachi L-74000) as described by Sadasivam and Manickam [1996]. . Folic acid was estimated following the calorimetric procedure of Sethi [1997]. Pyridoxine, pantothenic acid, Vitamin B12 was estimated by the following method recommended in USP NF [2000] Asian Edition.

Amino Acid Profile

Amino acids were estimated as described by Chakraborty and Joseph [2015] and amino acid score of both essential and non essential amino acids were calculated as described by FAO/WHO/UNU, [2007] by the following formula:

Amino acid score = sample amino acid/reference amino acid*100

Fatty acid profile

The fatty acid composition of total lipids was assessed as described by Metcalf *et al*. [1966] and Chakraborty and Paulraj [2009].

Minerals

Mineral contents in the dried portions were determined by atomic absorption spectrometry using an AA-7000 spectrometer (Shimadzu, Japan) with the system of double atomization (flame and electrothermal according to the AOAC method [2000]. Dried ascidian samples (100 mg) were digested with 7 ml HNO3 and 3 ml HCl. After 1 h of open digestion, the samples were digested in a microwave digester (Anton Paar Multiwave Go), and the digested samples were diluted with milliQ water and analyzed for minerals.

Analysis of pharmacological properties

Preparation of crude extracts

Dried materials were soaked separately in 100% A.R grade methanol in the ratio of 1g/20 ml for 10 days at room temperature under constant agitation in Orbital Shaker. The extracts were filtered and then dried overnight at room temperature for complete evaporation of the solvent before subsequent extraction. The dried extracts were re-suspended in Methanol for 24 hrs and the extracts were collected, filtered with Whatman No.1 paper, and concentrated using rotary evaporator (Buchi type). The extract residues were re-suspended in 20 ml of 100% A.R. grade methanol and were transferred to new vials to remove salt precipitates. The methanol soluble extracts were dried and solubilized in deionized water. Different concentrations of extracts were prepared and stored at 0^0C for further use.

Antibacterial assay

Microbial strains

To test the efficiency of the crude extract of the five chosen ascidians, nine bacterial pathogens such as *Clostridium difficile, Bacillus subtilis, Entrococcus faccalis, Acinetobacter iwoffii, Shigella flexneri, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Proteus vulgaris* were chosen. These pure strains were obtained from microbial type culture collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

Antibacterial assay

Different extract were individually tested for their action as antibacterial agents on five bacterial strains using the standard filter disc diffusion method [Laouer *et al.* 2009].

Sterilized discs were soaked with prepared extracts of different concentrations such as 25 mg/ml, 50mg/ml and 100 mg/ml and kept overnight at room temperature. The soaked discs were then aseptically dried to ensure evaporation of the solvents. The specific culture medium recommended by MTCC, Chandigarh (Table .2.1) was used for culturing the specific bacteria. Swabs were prepared from various stock cultures of bacteria and spread over the agar surface. The plates were allowed to dry for 20 min. Dried antimicrobial discs (with impregnated ascidian extracts) and control discs (without extract) were carefully dispensed at uniform distance over the agar surface and were pressured for correct implantation. Incubation period of 48-72 hours at 35^0C was maintained for observation of antibacterial activity of the extracts. The antibacterial activity was evaluated by measuring zones of inhibition of bacterial growth surrounding the extracts. The complete antibacterial analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm radius and the experiment was carried out in triplicates.

Statistical Analysis

All above biochemical analyzes were performed on three samples. Results are expressed as mean (standard deviation) (n=3) and statistically significant differences were reported at $p < 0.05$. Data analysis was performed using SPSS 11.5 software (SPSS Inc., Chicago, IL, USA).

Results

Table 1 shows the percentages of protein, carbohydrate, lipid, ash, and moisture in the colonial ascidians collected in Mandapam coast of Gulf of Mannar, India. Among the three ascidians studied, there is no much difference in the percentage of biochemical compositions present among the species studied ($p = 0.000$). Proteins were the major macronutrient among the constituents investigated in the colonial ascidians, which is followed by lipid and carbohydrate. High protein levels and low lipid contents characterized the overall proximate profile of tunicates studied herein. Highest protein content with 37% was present in *P. saturnium* and *P. indicum*.

The carbohydrate value of *Polyclinum saturnium* in this study is consistent with the result of Tamilselvi (2008) who reported the presence of 6% carbohydrate in the indigenous colonial ascidian *Distaplia nathensis*. Abdul Jaffar Ali (2004) reported comparatively low carbohydrate in *Phallusia nigra* collected from Thoothukudi coast, where as Iyyappan and Ananthan (2016) observed 6.8% carbohydrate in *P. nigra* collected from Palk Bay in South East coast of India. Kumaran and Bragadeeswaran (2014) reported very low levels of carbohydrate in *Eudistoma viride* and *Didemnum psammathodes* collected from Thoothukudi coast. The results obtained from this study showed that the carbohydrate content in present study species of colonial ascidians was higher than other ascidians. Carbohydrates play a negligible role as energy stores in aquatic animals. The present results may also indicate that carbohydrates play a minor role in energy storage in these sedentary and hermaphrodite animals (Love, 1970).

High protein content of 37% was found to present in *P. saturnium* and maximum of 14.5% of lipid and 6.2% of carbohydrate was present in *P. saturnium*. The total lipid amount, also, exhibited no significant differences among the species, with the highest values determined in *P. saturnium* (14.5%), *P. indicum* (14%) and respectively) and the lowest ones in *P. nudum* (12.5%). Based on the reported protein levels, all species can be classified as “high in protein”.

Meenakshi (1997) reported comparatively high protein content than lipid in *Perophora formosana* and *Styela plicata*. Maximum protein of 15.47% was found to present in *Phallusia nigra* (Iyappan and Ananthan [2016], but Abdul Jaffar Ali [2004] observed 22% protein. The colonial ascidians investigated in this study were found to contain rich source of protein, which is essential for

human growth and development. The protein content in ascidians species depends on the species and season. Higher protein values in these species may be due to hermaphroditic gonads and their stable reproductive activity. The fat content of other ascidian species in the literature is presented as follows: *Botrylloides magnicoecum* (14 mg/g) and *Didemnum psammathodes* (1.7 mg/g) (Tamilselvi, 2008), *Phallusia nigra* (3.95%) Iyappan and Ananthan 2017], *Eudistoma viride* (0.23 µgml−1) and *Didemnum psammathodes* (0.32 µgml−1) Kumaran and Bragadeeshwar (2014), *Microcosmus exasperates* (2.64%) Karthikeyan *et al* (2010) and *Polyclinum indicum* (3.1%) and *P. constellatum* (2.8%) Ananthan *et al* [2012].

As expected, the main component of all studied species is the moisture, whose content indicates flesh freshness. The highest values were found in *P. saturnium* (21%). These values did not differ statistically between each other ($p<0.05$), and the lowest was found in *P. nudum* (18%). The highest ash content, which indicates the amount of inorganic compounds in the tissues of tunicates, was found in *P saturnium* followed by *P. indicum* (8%) and *P. nudum* (6%). The differences between values were insignificant, ($p<0.05$).

These species of tunicates represent a good source of protein considering the WHO Daily Value (DV) recommendation of 0.80g/kg/day [WHO, 2007].

Fatty acid composition

Until now, lipids are the primary metabolites from finfish and shellfish that have shown the highest potential for commercial functional foods and additives. Although they are considered the main source of long-chain omega-3 PUFA, some of the tunicate species could be a sustainable, alternative source of these fatty acids. Moreover, lipid classes present in tunicates comprise neutral, polar lipids, sterols and sterol esters, vitamins and carotenoids. Fatty acid composition of the studied species is presented in Table 2.

Thirty fatty acids, including 18:2n-6, 22:6n-3 DHA and 20:5n-3 EPA, that exceeded a minimum of 1% of total fatty acids in the three tunicate samples, were identified (Table 2). Sum of EPA and DHA is very important because they play an important role in the prevention of cardiovascular disorders [Breslow, 2006].

Fatty acid composition differed significantly among the species studied ($p<0.05$), although, saturated fatty acids (SAFAs) were the most predominant fatty acids in all samples, followed by polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs). All the three species have a higher ratio of ΣPUFA / ΣSFA (above 0.45) than the recommended level for a healthy diet [HMSO, 2001].

The content of SAFAs ranged from 35.52% in *P. indicum* to 37.02% in *P. nudum* ($p<0.05$). The main SAFAs were Hexadecanoic acid (palmitic acid) (C16:0, from 9.12 % in *P. nudum* to 10.3 % of total FAs in *P. saturnium*), myristic acid (C14:0, from 2.4% to 4.2% of total FAs in *P. nudum* and *P. saturnium*, respectively), and Octadecanoic acid (stearic acid) (C18:0, from 10.22% to 11.8% of total FAs in *P. saturnium* and *P. nudum*, respectively).

Minerals

Contents of both macro minerals such as Na, Cl, K, P, Ca, I and Mg and microminerals such as Zn, Mn, Fe and Se in fresh soft tissue of the tunicates found in this study are shown in Table 3. Significant differences ($p<0.05$) were found in the distribution of metals among the species analyzed, though the Na, K, Mg, and P were predominant in the tunicates. I and Ca were found in significant quantities in all the species studied. Zn and Fe were found at higher level significantly where as Mn and Se instead, were found generally at lower levels. As regards the minimum and

maximum contents, the highest contents of Cl were found in *P. saturnium* and *P. indicum* (534 ± 5.2 and 520 ± 6.3 mg/100 g, respectively), followed by Mg (498 ± 5.6 mg/g and 478 ± 5.1 mg/g in *P. indicum* and *P. saturnium* respectively), while the lowest levels of K found in *P. nudum* and *P. saturnium* (10.8 ±1.1 and 12.3 ± 1.1mg/100 g, respectively). Ascidians are good sentinel animals of macromineral and trace element availability in the environment [Cravo and Bebianno, 2005]. Calcium and phosphate are vital for sustaining normal physiological status, and imbalances in this regulation lead to cardiovascular disease and chronic kidney disease [Blaine *et al*., 2015].

To the best of our knowledge, no information is available on the nutritional quality for most of the studied species. Thus, we compared our findings with some other bivalve's species of commercial size from different geographical areas.

Vitamins are vital for growth, reproduction, and maintenance of standard healthiness and performance of the organisms. Results of both qualitative and quantitative estimation of vitamins in the whole colony tissues of the three study species is presented in Fig 1. Among them, vitamins A (8.2 mg / 100 g) and B3 (5.1 mg / 100 g) were found in higher concentrations in *Polyclinum saturnium*, and vitamins B2, B1, B5 and B6 were found in tissues in lower concentrations. Water-soluble vitamins are higher than fat-soluble vitamins in the three species. Fat-soluble vitamins play a significant role for the normal growth, metabolism and reproductive cycle of organisms. Vitamin A is very important nutrient involving in cell protection. It can prevent lipids from peroxidation, which exemplifies a number of degenerative diseases.

Accumulation of heavy metals in the experimented ascidian samples varied species to species (Table 5). Mercury was not detected in any of the species analyzed. The disparity in the metal concentration among the species could be attributed to the differences in the uptake of various metals by ascidian species. By virtue of its filter feeding mechanism, the ascidians filter large amounts of water to collect food, accumulate heavy metals from the environment in their bodies. Laws (1981) has observed that ascidians feed on sediments and plankton that cause high concentration of heavy metals. Moreover, the concentration of various metals in phytoplankton is actually close to seawater values and in some cased it is about 3 orders of magnitude higher than seawater values and the concentration in zooplankton is much higher than that of phytoplankton. Of the four metals studied, highest concentration of vanadium was observed. Goldberg *et al*. [1951] reported a greater accumulation of vanadium in ascidians. Miller [1954] noted that members of the order Ascidiidae and families Perophoridae of the order Phlebrobranchia and some species of Aplausobranchia accumulate relatively high concentrations of vanadium. From this study, we concluded that the concentrations of these metals (cadmium, lead, mercury) observed in the study animals were below the Indian Maximum Acceptable Standards [Venugopal and Gopakumar, 2017; Mokhtar *et al* 2009] recommended for fish and seafood products for human consumption. Therefore, it is recommended that research animals be safe for human consumption when maintained in captivity with metal-free water.

Aminoacid profile:

The amino acid compositions of tunicates are given in Table 4. The total amino acid contents in the whole animal body tissues of three colonial ascidians were 5.9, 5.86, and 5.69 mg/100mg respectively. The total amino acid contents were lower than the crude protein contents in the study animals. The proportions of the major groups of essential amino acids—essential (lysine, leucine, arginine, threonine, isoleucine, histidine, phenylalanine, valine, methionine), and non-essential (aspartic acid, tryptophane, alanine, glycine, glutamic acid, serine, proline, tyrosine, and cysteine)—varied among the investigated tunicate species. The contents of conditionally essential and non-essential amino acids were not significantly different ($p>0.05$).

All the three species of ascidians showed a leucine-to-isoleucine ratio (~1.5) as specified by FAO/WHO [FAO/WHO, 1990]. Leucine a branched-chain amino acid, was found to stimulate protein synthesis [Tipton, 2017] and excess leucine influences the metabolic pathways of niacin and tryptophan, leading to the deficiency of niacin [Gopalan and Srikantia, 1960; Raghuramulu *et al.*, 1965]. Rajmohan and Kurup, [1997] reported that cholesteremic properties of the proteins are connected to their amino acids and arginine/lysine ratio.

Amino Acid Scores

Amino acid scores and the first limiting amino acid are established methods of assessing the quality of proteins, which are widely used in studies on the nutritive value of proteins [WHO/FAO/UNU Expert Consultation 2007].

Amino acid scores are summarized in Table 4. Total essential amino acid contents were 288, 286, and 278 mg/g of protein in *P. indicum, P. nudum* and *P. saturnium* respectively. The total essential amino acids content in the studied species was higher than the recommended daily intake (262 mg/g total protein) and varied from 310 mg/g total protein for adductor to 366 mg/g total protein for mantle.

Then, comparing our results with the amino acid composition of the reference protein recommended by FAO/ WHO/UNU, it was found that many main indicators of the amino acids in the parts of the analysed clams were higher than 100.

Antibacterial activity of crude extract of chosen ascidians:

Crude methanol extracts of whole colony of three colonial ascidians such as *Polyclinum indicum, P. nudum* and *P. saturnium* were evaluated for their antibacterial activity against nine bacterial pathogens such as *Clostridium difficile* (CD), *Bacillus subtilis* (BS), *Entrococcus faccalis* (EF), *Acinetobacter iwoffii* (AI), *Shigella flexneri* (SF), *Pseudomonas aeruginosa* (SA), *Escherichia coli* (EC), *Staphylococcus aureus* (SA) and *Proteus vulgaris* (PV). The overall means of the inhibition in diameters feature marked differences between species with different concentrations. Antibacterial activity was measured as the area of no-growth around the paper disc.

Effect of methanol extracts of *Polyclinum nudum*:

This species showed potent antibacterial activity against almost all the bacteria tested as compared to the activity of other two ascidians. The range of influence was between 2 to 6 mm radius and showed maximum of 6 mm radius against CD, EF and EC (Fig 2). Low concentration of the extract also influenced the bacterial growth at broad spectrum.

Effect of methanol extracts of *Polyclinum nudum*:

The zone of inhibition ranged from 0 to 8 ± 0.07 mm radius against the bacterial studies and the zone of inhibition is concentration dependant. The concentrations 50 and 100 mg/ml showed potent activity by exhibiting inhibition zones of 2 to 4 mm and 4 to 8 mm radius respectively (Fig 3).

Maximum activity was shown against AL with inhibitory zone of about 8 ± 0.07 mm radius whereas least activity (4 ± 0.04 mm radius) was shown against *EF, PA* and PV. But no zone of inhibition was observed against BS.

Effect of methanol extracts of *Polyclinum saturnium:*

The methanolic extract of this *P. saturnium* showed comparatively lower activity than shown by *P. nudum*. The range of influence varied widely from 0 to 6 mm radius for the bacterial species tested. Antibacterial effect was high for BS, EC and PV where as no activity was shown against EF, AL and SF (Fig 4).

Application of ANOVA for antibacterial activity between three species and bacterial strains showed significant difference at 5% level ($p<0.005$).

Ascidian extracts inhibited the growth of both gram positive and gram negative bacteria, signifying that these extracts do not selectively inhibit one group of bacteria. Prem Anand and Patterson Edward (2002) reported that the colonial ascidian *Didemnum psammathodes* as a promising source of antibacterial compound and the highest activity was seen against *P. mirabilis, Shigella flexneri* and *S. typhi*. Murugan and Santhana Ramasamy [2003] has reported that for the crude methanol extract of *Didemnum psammathodes*, the average range of inhibition of the bacteria of 7.1 mm. Abdul Jaffar Ali et al [2008] reported the maximum antibacterial activity of the crude methanol extracts of the test and mantle bodies of *Phallusia nigra* against the Gram positive *Staphylococcus aureus*.

The results of the present study indicate that study animals show evidence of significant activity against chosen bacteria. Therefore the current studies depict the existence of effective antimicrobial compounds from study ascidian species of Mandapam coast.

Conclusion

This study presents for the first time a complete nutritional profile with the pharmacologial importance of three colonial ascidians of Gulf of Mannar region. Preliminary toxicological studies suggest that these species are innocuous. With the growth of interest in healthy and alternative food supplements, more people are interested to learn more regarding Indian ascidians. Nevertheless, with proper culturing, monitoring, and preparation of certain tunicate species that are currently an underutilized but highly nutritive food in many parts of the world could be easily cultivated, and the huge numbers of invaders could be harvested and marketed.

Conflict of Interests

The authors have no conflict of interest.

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Table 1. Biochemical compositions in *Polyclinum nudum, P. indicum* and *P. saturnium*

Biochemical compositions (%)	***Polyclinum nudum***	***P. indicum***	***P. saturnium***
Carbohydrate	4.8±0.039	5.7±0.5	6.2±0.59
Protein	30±3.4	37±0.34	37±2.50
Lipid	12.5±1.4	14±0.19	14.5±1.3
Ash	8±0.07	7±0.068	7±0.082
Moisture	56±5.61	54±5.53	52±5.49

Table 2. Fatty acid profile in *Polyclinum nudum, P. indicum* and *P. saturnium*

Saturated fatty acids	**IUPAC no**	***Polyclinum nudum***	***P. indicum***	***P. saturnium***
Lauric acid	12:00	0.36±0.03	0.18±0.02	0.23±0.02
Tridecanoic acid	13:00	0.18±0.02	0.10±0.01	0.16±0.01
Myristic acid	14:00	2.52±0.24	4.2±0.38	2.4±0.26
Pentadecanoic acid	15:00	2.31±0.26	2.24±0.2	2.41±0.23
Hexadecanoic acid	16:00	10.1±1.15	9.12±9.11	10.3±1.15
Heptadecanoic acid	17:00	1.5±0.04	1.1±0.01	1.33±0.01
Octadecanoic acid	18:00	11.8±1.14	11.3±1.11	10.22±1.17
Nonadecyclic acid	19:00	1.65±0.14	1.25±0.2	1.67 ±0.32
Icosanoic acid	20:00	1.5±0.12	1.13±0.01	1.7±0.01
Heneicosanoic acid	21:00	0.16±0.02	0.1±0.02	0.65±0.06
Docosanoic acid	22:00	1.84±1.62	1.8±0.01	1.8±0.02
Tricosanoic acid	23:00	1.2±0.10	1.2±0.01	1.1±0.12
Arachidonic acid	24.00	1.9±0.02	1.8±0.001	1.65±0.05
	ΣSFA*	**37.02±4.6**	**35.52±3.9**	**35.62±4.2**
Monounsaturated fatty acids				
7-Tetradecenoic acid	14:1*n*-7	3.16±0.02	3.21±0.03	3.16±0.03
Cis-6-Pentadecenoic	15:1*n*-6	1.02±0.01	1.1±0.01	1.8**±0.01**
9-Hexadecenoic acid	16:1*n*-7	4.69±0.05	4.12±0.31	4.18±0.05

Cis-7-Heptadecenoic acid	17:1*n*-7	1.4±0.13	1.02±0.00	1.1**±0.01**
cis-Vaccenic acid	18:1*n*-7	1.1±0.01	1.05±0.001	1.07±0.001
11-Octadecenoic acid	18:1*n*-9	14.24±1.03	16.11±1.01	13.61±1.1
Nonadecenoic acid	19:1*n*-8	2.1±0.03	2.9±0.03	1.8**±0.98**
Eicosenoic acid	20:1*n*-9	1.5±0.002	1.2±0.01	1.2±0.01
Erucic acid	22:1*n*-9	2.43±0.03	2.42±0.03	2.3±0.03
Nervonic acid	24:1*n*-9	1.1±0.01	1.9±0.01	1.4±0.01
	ΣMUFA	**32.87±4.1**	**33.37±3.8**	**31.60**±3.2
Polyunsaturated fatty acids				
Linoleic acid	18:2*n*-6	2.33±0.02	2.51±0.02	2.64±0.02
gamma-Linolenic acid	18:3*n*-6	1.24±0.02	1.2±0.01	1.54±0.01
alpha-Linolenic acid	18:3*n*-3	3.1±0.02	2.9±0.02	3.2±0.03
Eicosadienoic acid	20:2*n*-6	2.23±0.03	4.4±0.04	3.9±0.03
Dihomo-γ-linolenic acid	20:3*n*-6	0.99±0.00	1.01±0.00	1.1±0.001
Arachidonic acid	20:4*n*-6	2.91±0.03	2.9±0.02	2.5±0.03
Eicosapentaenoic acid	20:5*n*-3 EPA	8.22±0.54	8.11±0.06	8.32±0.08
Docosapentaenoic acid	22:5*n*-3	1.22±0.01	1.26±0.01	1.22±0.02
Docosahexaenoic acid	22:6*n*-3 DHA	8.23±0.61	8.28±0.07	9.34±0.20
ΣPUFA		**30.47±3.8**	**31.57±3.6**	**32.76±3.1**
Σn-3		**23.75**	**22.55**	**24.08**
Σn-6		**9.7**	**12.02**	**11.68**
Σn-3/Σn-6		**2.45**	**1.88**	**2.06**
DHA + EPA		**17.52**	**19.45**	
ΣPUFA/ΣSFA		**0.87**	**0.97**	

Table 3. Mineral compositions in *Polyclinum nudum, P. indicum* and *P. saturnium*

Macrominerals (mg/g)	***Polyclinum nudum***	***P. indicum***	***P. saturnium***
Sodium	326 ± 29.34	487 ± 45.2	490 ± 45.4
Chloride	430 ± 42.90	520 ± 56.3	534 ± 54.2
Potassium	10.8 ±1.1	15.2 ±2.0	12.3 ± 1.1
Phosphorous	82.32±7.76	80.67±7.80	83.54±8.83
Calcium	13.3 ±1.32	16.2 ± 12.5	15.45± 1.54
Iron	67.2 ± 6.7	89.2 ±8.9	87 ±8.8
Magnesium	412 ±**37.08**	498 ± 45.6	478 ± 45.1
Ca+P	24.1±2.5	31.4±3.8	27.7±2.4
Na/K	30.19±3.5	32.04±3.8	39.84±4.1

Ca/P	0.16±0.02	0.20±0.02	0.18±0.02
Microminerals (mg/kg)			
Zinc	0.96±0.06	0.98±0.01	0.96±0.06
Manganese	0.42±0.03	0.20±0.01	0.29±0.03
Ferrous	1.2±0.20	0.98±0.08	1.3±0.20
Selinium	0.02±0.00	0.03±0.00	0.02±0.00

Table 4. Amino acid score in *Polyclinum nudum, P. indicum* and *P. saturnium*

Ratio	*Polyclinum nudum*	*P. indicum*	*P. saturnium*
ΣAA	5.973	5.86	5.9
ΣEAA/ΣAA	0.489	0.488	0.471
ΣNEAA/ΣAA	0.511	0.512	0.529
Σ EAA/Σ NEAA	0.956	0.953	0.891
Leu/Ileu	3.4	3.5	3.4
Arg/Lys	0.59	0.69	0.55

Table 5. Content of heavy metals in the *Polyclinum nudum, P. indicum* and *P. saturnium*.

(mg/kg)	*Polyclinum nudum*	*P. indicum*	*P. saturnium*	WHO, 1995	FAO(1983)
Cd	0.98±0.10	1.06±0.1.	1.04±0.01	0.05	0.05 – 5.5
Pb	5.12±0.53	5.6±0.45	5.9±0.48	0.2	2.0
Cu	0.34±0.03	0.5±0.04	0.4±0.03	10	30
Ni	2.5±0.19	2.6±0.19	1.4±0.09	1	---
V	56±5.4	67±5.8	68±5.9	---	---
Hg (ppb)	ND	ND	ND	0.001	---

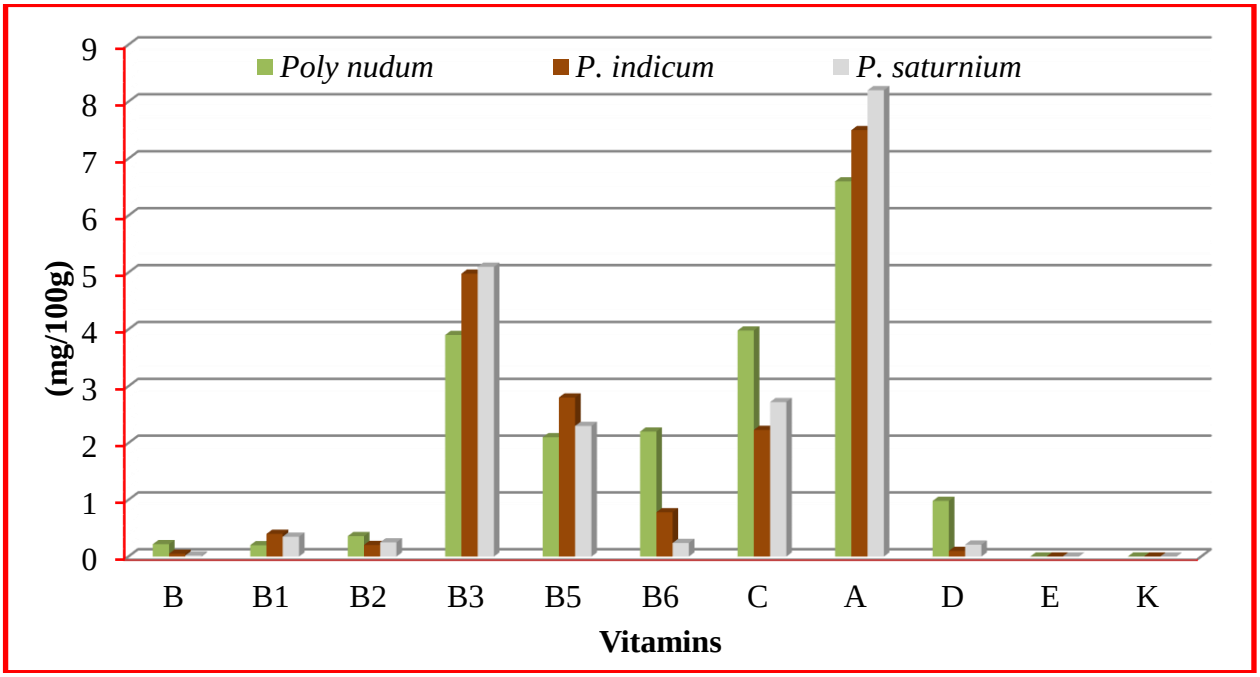

Figure 1. Vitamins estimated in the three colonial ascidians.

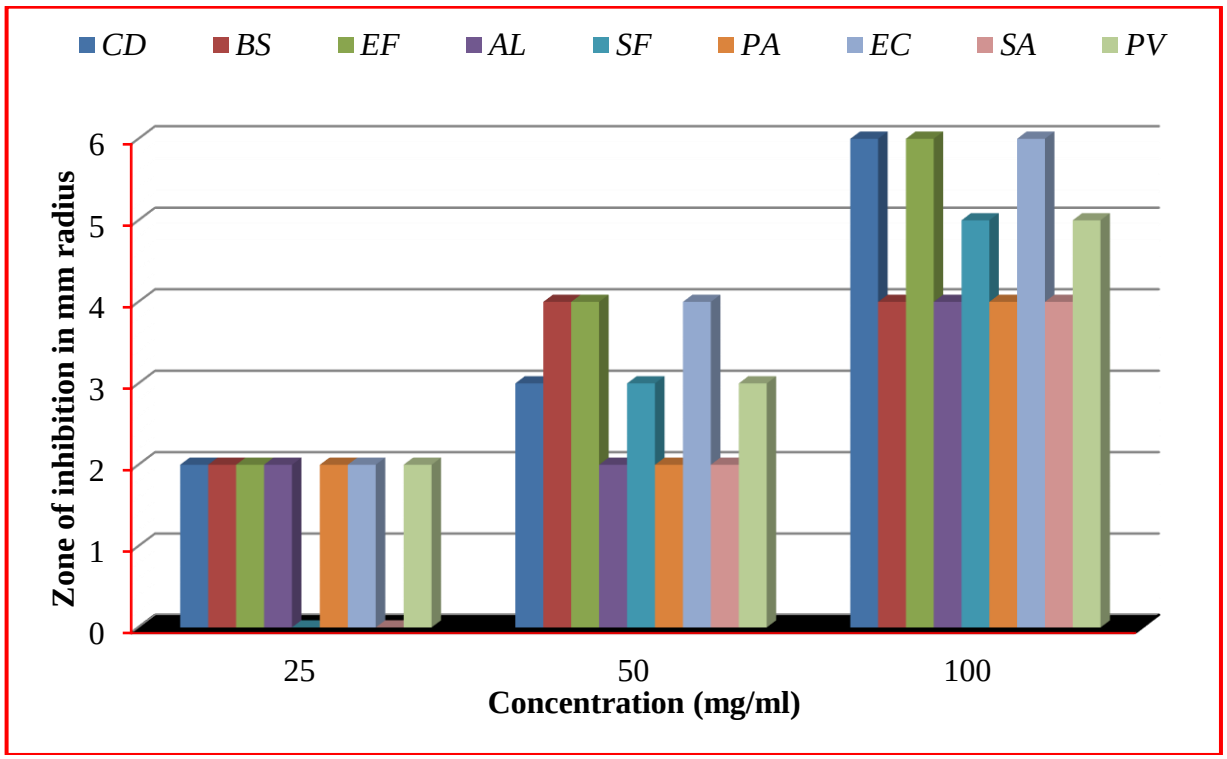

Figure 2. Antibacterial Activity of crude extracts of *Polylinum nudum* against nine bacterial pathogens.

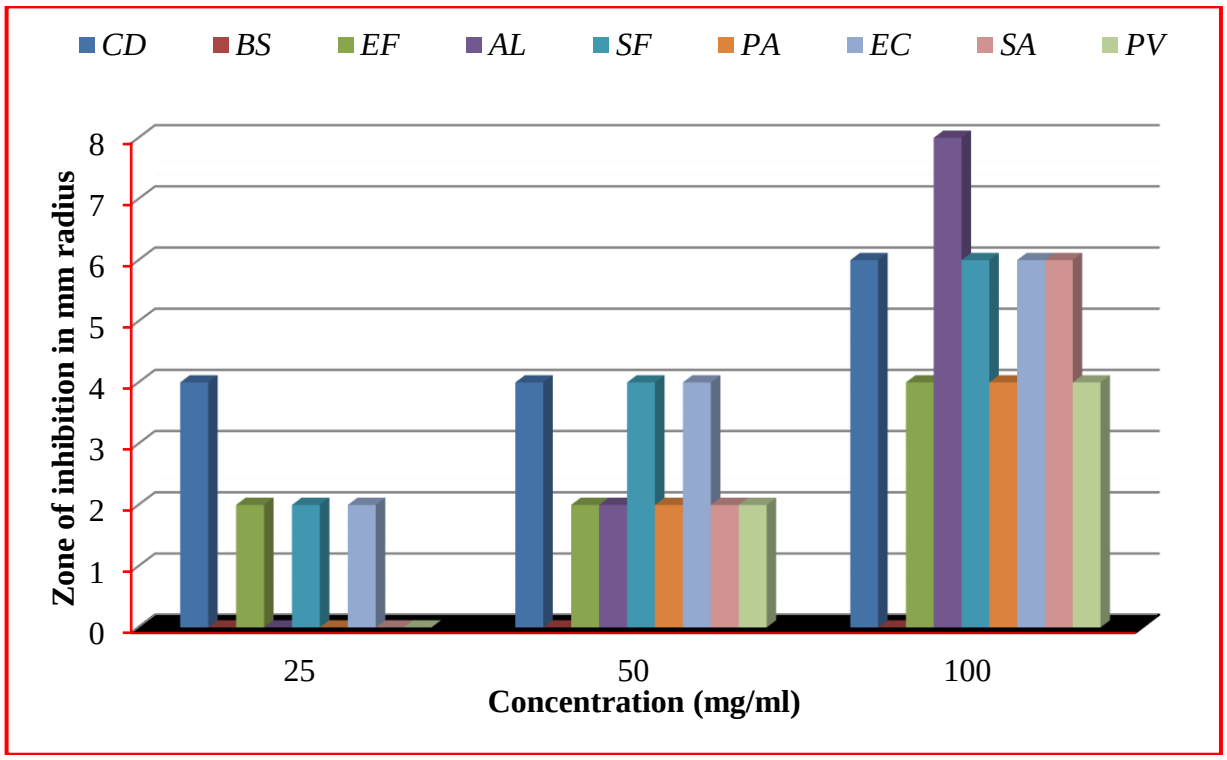

Figure 3. Antibacterial Activity of crude extracts of *Polylinum indicum* against nine bacterial pathogens.

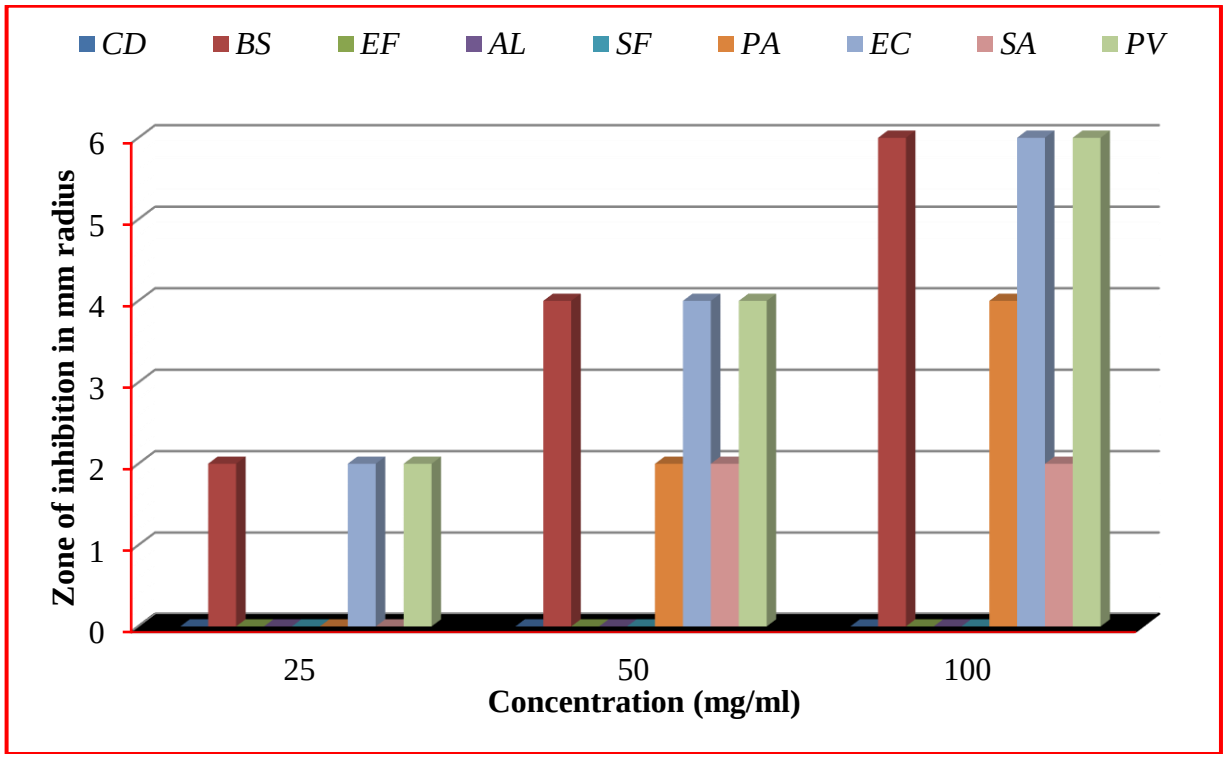

Figure 4. Antibacterial Activity of crude extracts of *Polylinum saturnium* against nine bacterial pathogens