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Biochemical Composition and Nutritional Value of Marine Tunicates *Styela plicata* and *Styela canopus* from Mandapam, South-East Coast of India

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Abstract: In certain countries, few species of ascidians have been recognized as delicious food for human consumption. In India, a significant level of information is not available related to nutritional composition of ascidians. This study involved the biochemical and nutritional analysis of two solitary ascidian species such as *Styela plicata* and *S. canopus* collected from Mandapam coast, Tamil Nadu, Southeast coast of India. Both the species had low carbohydrate content, and protein and fat were their main components. Having high quality protein content, both the species could be excellent sources of nutritive ingredients. Among amino acids, glutamate, aspartic acid, alanine, leucine and lysine were present at high levels in both bivalve 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 B_3 in *S. plicata* signified their importance in preventing osteoporosis. The heavy metal contents such as lead, copper, cadmium and nickel were found to be present below the recommended level. 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.

Keywords: Ascidians, Food value, Nutritional components, *Styela plicata, Styela canopus*

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Introduction

Ascidians are one of the most diverse marine benthic communities. Ascidians are an important component of tropical marine ecosystems due to their sedentary nature, abundance, dual morphology (solitary and gregarious), potential for invasiveness, and diversity. They are energy sources for many valuable biomolecules, including proteins, amino acids, lipids, and secondary metabolites (Rinehart, 2000; Haefner, 2003; Jain *et al*., 2008; Qing Hou *et al*., 2021). It is prey for many organisms, used as nutritional food and medicine, as an indicator to evaluate water quality, and to warn of the invasive status of alien species and its changes (Abdul Jaffar Ali, 2004; Tamilselvi, 2008).

Some species of ascidians are eaten in many parts of the world, including Japan, Korea, Chile, and Europe (Lambert, 2005). It is a popular food around the world, including South America, the Mediterranean, Korea, Japan, Chile (Mikami *et al* 2010), and the Antarctic Peninsula (Koplowitz *et al*., 2009). Most ascidian species are inedible, but some solitary ascidians of the families Steylidae and Pyridae are collected from the wild or farmed. Ascidians are highly nutritious (Lee *et al*., 1995; Odate and Pavlik, 2007; Meenakshi, 2009; Ananthan *et al*., 2012; Choi *et al*., 2014; Roger-Busatto and Ujevic, 2014). The nutritional value of a large number of solitary and social ascidians has been analyzed, suggesting that they may be useful high-protein, low-calorie seafood (Lee *et al*., 1995; Choi *et al*., 2006; Meenakshi, 2009; Tamilselvi *et al*., 2010; Kang *et al*., 2011; Ananthan *et al*., 2012). Due to low awareness, ascidians are not popular as an alternative food source for animals and humans in India.

In India, Meenakshi (2009) reported that several species of ascidians contain high levels of carbohydrates, proteins, and lipids. Radha Lakshmi *et al.* (2017) observed that black molly fed a diet supplemented with *Polyclinum nudum* and *Didemnum psammathodes* had high growth rate and fecundity. .

Knowledge about the biochemical composition of edible organisms is very important because nutritional value is reflected in the biochemical composition (Nagabhushnam and Manet, 1978). The increasing demand for protein-rich and high-lipid products has led to the need to find alternative sources in regions such as India where ascidians are not traditionally eaten (Meenakshi, 2009). *H. pallida* is rich in proteins, lipids and carbohydrates and can be processed into food, at least in the form of spicule-dissolved pickles, in India and other areas where the invader is very common (Tamilselvi *et al*., 2010). In an increasingly interconnected world, alien ascidians have transcended their original borders and colonized new areas so successfully that they are considered invasive. In this way, ascidians can be said to be a rich biological resource from a nutritional and functional perspective. The nutritional value of ascidians can supplement the nutrients needed by humans and animals. Active utilization of exotic ascidians will not only reduce commercial and economic losses caused by ascidians but also help reduce the impact of these species.

This study analyzed the biochemical and nutritional status of two solitary ascidians (*Styela plicata* and *S. canopus*) collected from the coastal waters of Mandapam, Gulf of Mannar, India, with the aim of exploiting this underutilized resource.

Materials and Methods

Specimen Collection and Identification

Chosen ascidians were sampled by scuba diving from a pier post on the coast of Mandapam, India (9°28′86″N, 79°13′29″E) at depths of 1–3 m between April 2020 and May 2022. After the specimens were collected, samples were thoroughly washed with treated seawater to remove sand, debris, and epibionts before being transported to the laboratory. The specimens were identified according to Monniot and Monniot (2001); Kott (2002) and Cole and Lambert (2009). Voucher samples have been deposited at the Museum of Biotechnology Department, Islamiah College (Autonomous), Vaniyambadi – 635 752, Tirupattur District, Tamil Nadu, India.

Sample preparation

Ascidian tissues were shade dried for few days. The dried tissues were made into powder for biochemical analysis.

Proximate Composition Analysis

The amount of protein, carbohydrate and lipid was estimated by the method of Bradford *et al*. (1976),

Table 1: Biochemical compositions in *Styela plicata* and *S. canopus*

Biochemical compositions (%)	***Syela plicata***	***S. canopus***
Carbohydrate	6.7	6.3
Protein	51	48
Lipid	15.3	13
Ash	10	12
Moisture	32	31

Dubois *et al*. (1956) and Folch *et al*. (1957), respectively. Moisture content was determined by drying the samples in an oven at 105°C until a constant weight was obtained (AOAC, 1990). Total ash content was determined by incineration of samples in a furnace at 550°C for 8-12 h (AOAC, 1990).

Vitamins

Fat-soluble vitamins A, D, E, K and water-soluble vitamins B_1, B_2, B_6, B_{12}, 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 B_{12} 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

Minerals were estimated by inductively coupled plasma mass spectrometry (iCAPTM Q, Thermo Fisher). 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.

Statistical Analysis

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

Results and Discussion

The proximate compositions of *Styela plicata* and *S. canopus*, such as protein, carbohydrates, and fat content, were estimated in per cent, and the proximate composition results represented in the Table 1 which showed that proteins were dominant, followed by carbohydrates and fats.

While the average percentage of total carbohydrates in *S. plicata* was 6.7±0.6, it was lower in *S. canopus* (6.3±0.59). The carbohydrate value of *S. plicata* in this study is consistent with the result of Tamilselvi (2007) in the colonial ascidian *Distaplia nathensis*. While Abdul Jaafar Ali (2004) reported low carbohydrate value in *Phallusia nigra*, 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*. The results obtained from this study showed that the carbohydrate content in *S. canopus* and *S. plicata* was higher than other ascidians.

Total carbohydrate content was reported to be higher in *S. plicata*. Ascidians have been reported to store large amounts of carbohydrates during the growing season, presumably as a source of energy during active growth, and use them throughout the year. Love (1970) reported that carbohydrates play a minor 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. It is generally believed that carbohydrates are an immediate energy product and therefore are not stored for a long time.

Protein contains the maximum amount of all biochemical components. The average percentage of protein contained in *S. plicata* is 51, but in the case of *S. canopus* the percentage is low (48%). Ascidian is reported to contain high amounts of protein (40-70%). Maximum protein of 15.47% is estimated to be present in *P. nigra* (Iyappan and Ananthan (2016), but Abdul Jaffar Ali (2004) observed 22% protein. Meenakshi (1997) also reported in common and colonial ascidians that the protein percentage was found to be higher than lipid and estimated the maximum protein concentration of about 61.75% and 66.25% in *Perophora formosana* and *S. plicata* respectively. The higher amount of insoluble protein in *Cnemidocarpa verrucosa* reflects the contribution of insoluble protein to structural materials, including connective tissue. Madin *et al*. (1981) found that the main contribution came from protein, equivalent to 26.6% of the dry weight of Atlantic salp. The ascidians analyzed in this study were found to be a rich source of protein, which is essential for human growth and development. Previous reports showed comparable protein content in *P. nigra, E. viride* and *D. psammathodes*. The protein content in ascidians species depends on the species and season. The higher protein values in both species may be due to hermaphroditic gonads and their constant reproductive activity.

Lipids were higher than carbohydrates, but lower than proteins. The average percentage of lipids present in *S. plicata* is 15, whereas the percentage in *S. canopus* is lower to 13. 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), *P. nigra* (3.95%) Iyappan and Ananthan (2017), *Eudistoma viride* (0.23 μgml^{-1}) and *D. 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). Several reports on proximate composition have shown that the nutritional value of sea squirts is equal to or greater than that of other marine benthic communities such as sponges (Mc Clintock *et al.*, 2004).

The amount of minerals present in body tissues is shown in Table 2. A total of 7 macrominerals and 5 microelements were detected. Among macrominerals, calcium (305 mg/g) and chloride (8 mg/g) were observed at increasingly lower levels in *S. plicata*, while comparatively lower levels of these macro-minerals were observed in *S. canopus*.

The trace minerals such as zinc, iron, manganese and copper were also detected but selenium was negligible in both the species tested. Mineral content shows some differences between species. Ascidians are good bioindicators of macromineral and trace element availability in the environment (Cravo and Bebianno, 2005). Calcium and phosphate are important for maintaining normal physiological status, and imbalances in this regulation lead to cardiovascular disease and chronic kidney disease (Blaine *et al*., 2015). K content in experimental animals was found to exceed 15 mg/kg dry weight, which is important for enzyme systems controlling various electrochemical and catalytic functions (Suhail, 2010). The balanced ratio of sodium to potassium

Table 2: Mineral compositions in *Styela plicata* and *S. canopus*

Macrominerals (mg/g)	***Syela plicata***	***S. canopus***
Sodium	14.5 ± 1.5	12.6 ± 1.3
Chloride	8 ± 0.9	5 ± 0.5
Potassium	56 ± 5.2	45 ± 4.8
Phosphorous	97.76±0.97	90.43±0.85
Calcium	305 ± 0.32	298 ± 24
Iron	81.8 ±0.9	78.2 ± 0.8
Magnesium	65 ± 5.2	45 ± 4.4
Ca+P (2:1)	402.76	388.43
Na/K	0.259	0.28
Ca/P	3.12	3.30
Microminerals (mg/kg)		
Zinc	0.69±0.087	0.59±0.06
Manganese	0.4±0.04	0.32±0.03
Copper	0.35±0.03	0.26±0.01
Ferrous	0.45±0.05	0.4±0.04
Selinium	0.03±0.00	0.03±0.00

(<1.0) in ascidians has been shown to be safe for people with heart disease. The ascidian species considered in this work had a high Ca+P content (>95 mg/kg-1:2:1 Ca:P). The balanced ratio of sodium to potassium (<1.0) in ascidians has been shown to be safe for people with heart disease. Selenium is important for the normal functioning of endocrine tissues and is a constituent of amino acids such as selenocysteine and selenomethionine, which have antioxidant functions (Venugopal and Gopakumar, 2017), and its content is significantly higher ($p < 0.05$) in *S. plicata* and *S. canopus* than other ascidian species.

Amino acid profile, both essential and nonessential, is depicted in the Figures 1 and 2, respectively. Balanced amounts of essential and non-essential amino acids (0.12-0.72 mg/100 mg) have shown that these acids are a good source of protein. The most abundant essential amino acid in *S. plicata* is leucine (0.72±0.006 mg/100 mg), followed by lysine (0.62±0.05 mg/ 100 mg) and arginine (0.44± 0.005 mg/ 100 mg). The same trend was also observed in *S. canopus*.

Amino acid scores of ascidian species in terms of essential and non-essential amino acids are shown in Table 3. Ascidians showed a leucine-to-isoleucine ratio (~1.5) as specified by FAO/WHO (FAO/WHO, 1990). Leucine is a branched-chain amino acid that has been reported to stimulate protein synthesis (Tipton, 2017). Excess leucine affects the metabolic pathways of niacin and tryptophan, causing niacin deficiency (Gopalan and Srikantia, 1960; Raghuramulu *et al*., 1965). Cholesteremic properties of proteins are related to their amino acid content and arginine/lysine ratio (Rajmohan and Kurup, 1997). The arginine/lysine ratio in ascidians was ~2.3, indicating their importance in maintaining a good cholesterolemic index. Aromatic amino acids are precursors to the synthesis of catecholamines and thyroid hormones (Logan and Rice, 1987), found to be significantly present in *S. plicata* ($p<0.05$). This duly demonstrated that ascidian protein could effectively supplement the limiting amino acids in the daily diet. Amino acid scores were found to be significantly higher than in the study species than the other ascidian species ($p<0.05$).

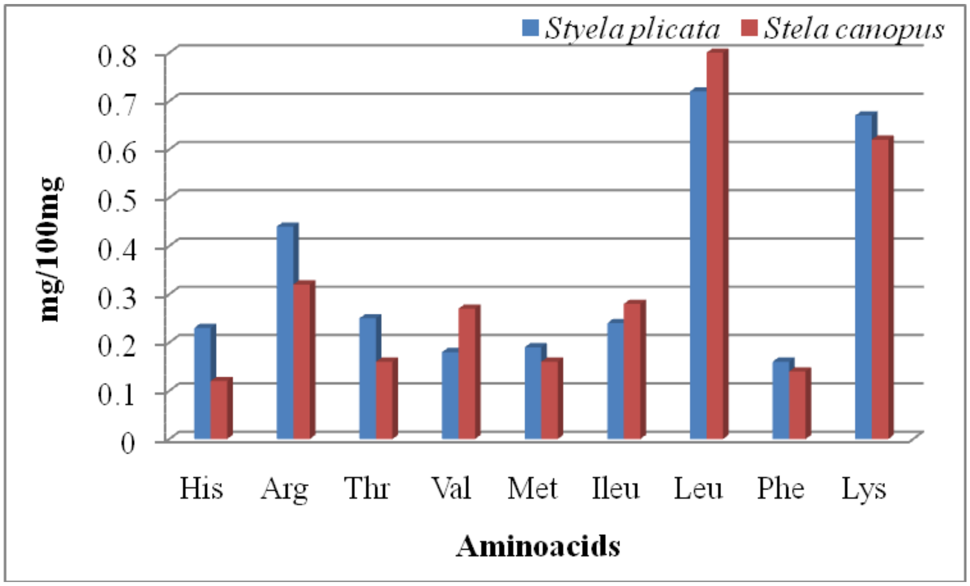

Fig. 1: Essential amino acid profile in *Styela plicata* and *S. canopus.*

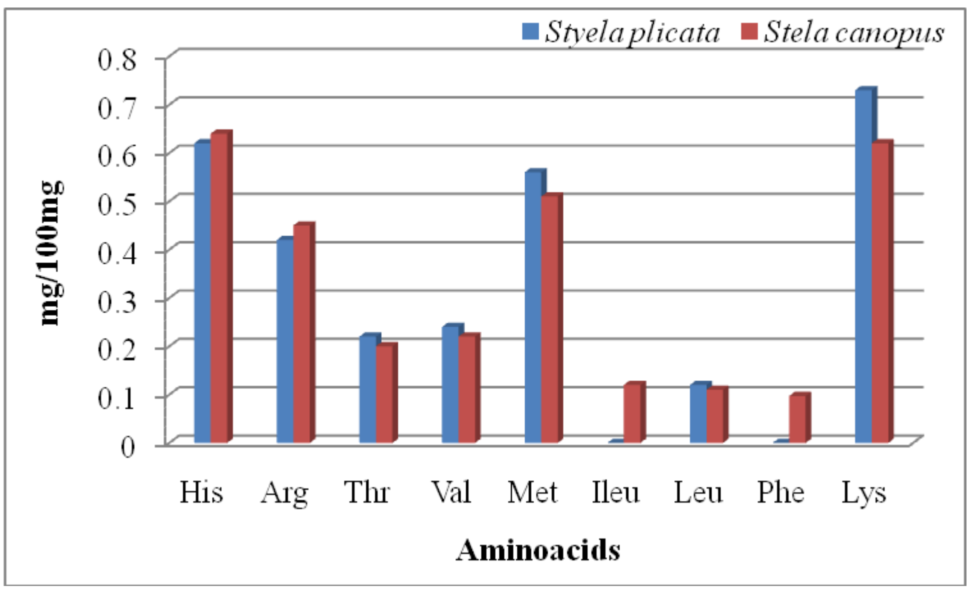

Fig. 2: Non-essential amino acid profile in *Styela plicata* and *S. canopus.*

Table 3: Aamino acid score in *Styela plicata* and *S. canopus*

Ratio	*Styela plicata*	*S. canopus*
ΣAA	5.99	5.837
ΣEAA/ΣAA	0.514	0.492
ΣNEAA/ΣAA	0.486	0.508
Σ EAA/Σ NEAA	1.058	0.967
Leu/Ileu	3	2.9
Arg/Lys	0.66	0.52

Table 4; Fatty acid profile in *Styela plicata* and *S. canopus*

Saturated fatty acids	IUPAC no	*Styela plicata*	*Styela canopus*
Lauric acid	12:00	0.28±0.02	0.32±0.03
Tridecanoic acid	13:00	0.38±0.02	0.30±0.02
Myristic acid	14:00	4.3±0.04	4.2±0.04
Pentadecanoic acid	15:00	2.1±0.06	2.8±0.02
Hexadecanoic acid	16:00	9.1±0.15	8.8±0.77
Heptadecanoic acid	17:00	1.8±0.04	1.2±0.01
Octadecanoic acid	18:00	8.8±0.14	8.2±0.13
Nonadecyclic acid	19:00	3.65±0.4	3.25±0.2
Icosanoic acid	20:00	1.8±0.02	1.4±0.01
Heneicosanoic acid	21:00	0.16±0.02	0.1±0.02
Docosanoic acid	22:00	2.1±0.02	2.9±0.02
Tricosanoic acid	23:00	1.8±0.04	1.4±0.01
Arachidonic acid	24.00	1.98±0.02	1.75±0.15
	ΣSFA*	**38.25±4.2**	**36.62±3.2**
Monounsaturated fatty acids			
7-Tetradecenoic acid	14:1*n*-7	3.3±0.03	3.2±0.02
Cis-6-Pentadecenoic	15:1*n*-6	1.02±0.01	1.1±0.01
9-Hexadecenoic acid	16:1*n*-7	4.92±0.04	4.3±0.05
Cis-7- Heptadecenoic acid	17:1*n*-7	1.4±0.03	1.02±0.00
cis-Vaccenic acid	18:1*n*-7	1.03±0.001	1.2±0.001
11-Octadecenoic acid	18:1*n*-9	8.43±0.02	8.24±0.03
Nonadecenoic acid	19:1*n*-8	2.1±0.03	2.9±0.03
Eicosenoic acid	20:1*n*-9	1.9±0.002	1.8±0.002
Erucic acid	22:1*n*-9	2.45±0.03	2.58±0.03
Nervonic acid	24:1*n*-9	1.8±0.01	1.9±0.01
	ΣMUFA	**28.35±4.1**	**28.35±3.8**
Polyunsaturated fatty acids			
Linoleic acid	18:2*n*-6	2.43±0.02	2.33±0.02
gamma-Linolenic acid	18:3*n*-6	1.4±0.01	1.24±0.01
alpha-Linolenic acid	18:3*n*-3	3.3±0.03	3.1±0.03
Eicosadienoic acid	20:2*n*-6	3.32±0.03	3.23±0.03
Dihomo-γ-linolenic acid	20:3*n*-6	1.3±0.01	1.99±0.01
Arachidonic acid	20:4*n*-6	3.1±0.03	2.91±0.03
Eicosapentaenoic acid	20:5*n*-3 EPA	9.4±0.02	11.22±0.02
Docosapentaenoic acid	22:5*n*-3	1.62±0.01	1.22±0.01
Docosahexaenoic acid	22:6*n*-3 DHA	8.12±0.3	8.23±0.3
ΣPUFA		**33.39±4.1**	**35.47±3.7**
Σn-3		**22.44**	**23.77**
Σn-6		**11.5**	**11.7**
Σn-3/Σn-6		**1.95**	**2.03**
DHA + EPA		**17.52**	**19.45**
ΣPUFA/ΣSFA		**0.87**	**0.97**

Fatty acids are important substances required by the body for various metabolic functions. The details of fatty acid profile is presented in the Table 4, and the total amount of saturated fatty acids (SFA) is 38.25% in *S. plicata* and 36.62% in *S. canopus*; Among them, palmitic acid stands out (9.1% and 8.8%, respectively), followed by octadecanoic acid (8.8 and 8.2%). More PUFA was registered in SC (35.47%), and the sum of EPA and DHA in *S. plicata* and *S. canopus* was 17.5% and 19.45%, respectivelywhich is very important because they play an important role in the prevention of cardiovascular disorders (Breslow, 2006). The n-3/n-6 ratio is a useful biomedical marker; Increasing this score is critical for preventing coronary heart disease and reducing cancer risk (Chakraborty *et al*., 2014), whereas *S. canopus* shows a higher n-3/n-6 PUFA (2.03). Both species have a higher ratio of ΣPUFA / ΣSFA (above 0.45) than the recommended value for a

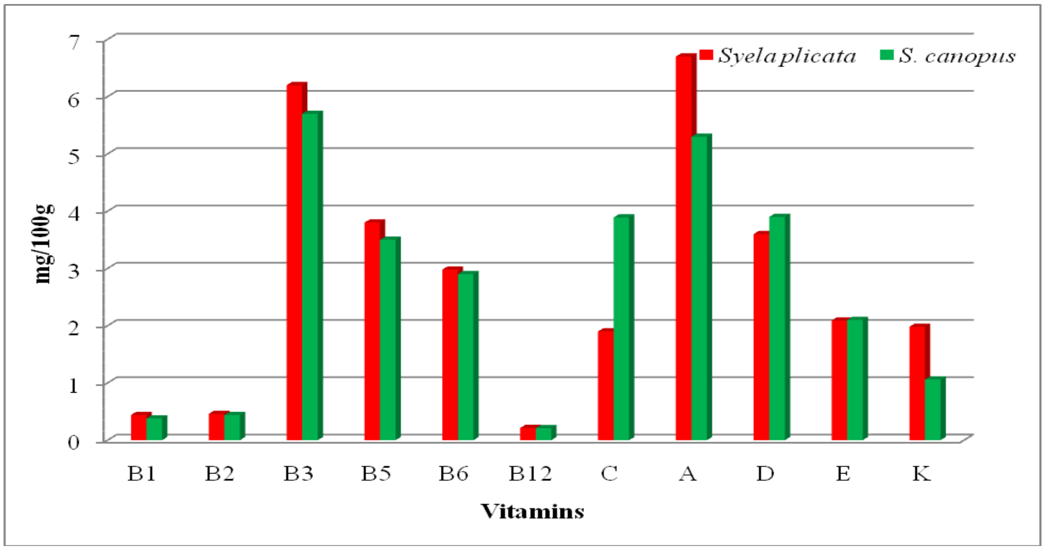

Fig. 3: Vitamins in *Styela plicata* and *S. canopus.*

Table 5: Content of heavy metals in the *S. canopus* and *S. plicata* specimens

(mg/kg)	*Syela canopus*	*S. plicata*	WHO, 1995	FAO(1983)
Cd	0.81	0.69	0.05	0.05 – 5.5
Pb	0.49	0.41	0.2	2.0
Cu	1.45	1.28	10	30
Ni	2	2.2	1	---
V	29.2	34.1	---	---
Hg (ppb)	ND	ND	0.001	---

healthy diet (HMSO, 2001).

Vitamins are organic chemical compounds that are essential for growth, reproduction, and maintenance of normal health and functioning of the organism. Detailed information about the vitamins found in the tissues of the organism is presented in Figure 3. Among them, vitamins A (6.7 mg / 100 g) and B_3 (6.2 mg / 100 g) were found at higher concentrations, and vitamins B_2, B_1 and B_{12} were found in tissues in lower concentrations (0.466, 0, 44 and 0,213 mg / 100 g in *S. plicata* and 0,44, 0,38 and 0,21 mg/100g in *S. canopus*), respectively. Water-soluble vitamins are higher than fat-soluble vitamins in both species. Water soluble vitamins were slightly higher (17.02 mg/100g) in *S. canopus* than *S. plicata* (15.99mg/100g) whereas fat soluble vitamins were higher in *S. plicata* (14.37 mg/100g) than *S. canopus* (12.36 mg/100g).

The composition and concentration of heavy metals in the analyzed ascidian samples varied (Table 5). Heavy metal concentrations in *S. plicata* and *S. canopus* were: 0.81 ppm and 0.69 ppm for cadmium, 1.45 ppm and 1.28 ppm for copper, 0.49 ppm and 0.41 ppm for lead, 2.0 ppm and 2.2 ppm for nickel, and 29.2 ppm and 34.1 ppm for V, respectively. Mercury was not detected in any species analyzed. The variations in the metal concentration between the species may be due to differences in the uptake of various metals by ascidian species. Because ascidians are sedentary and filter large amounts of water to collect food, they can accumulate heavy metals from the environment in their bodies. Furthermore, as pointed out by Laws (1981), ascidians feed on sediments and plankton that accumulate heavy metals in large quantities, and metal concentrations in phytoplankton are actually close to seawater values and are reported to be about 3 orders of magnitude higher than seawater values and the concentration of zooplankton is much higher than that of phytoplankton. Of the four metals studied, vanadium had the highest accumulation. This observation is also consistent with that of Goldberg *et al*. (1951) who also observed a greater accumulation of vanadium in ascidians. Miller (1954) also 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 (Mokhtar *et al.*, 2009; Venugopal and Gopakumar, 2017) 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.

Conclusion

Many scientific studies have demonstrated a direct cause-effect relationship between the consumption of marine based food and a reduction in the risk of cardiovascular diseases of social importance. This is due to the need to increase the intake of n-3 PUFAs, essential elements, fat-soluble vitamins, sterols and antioxidants. Studies on nutritional compositions of commercial seafood products in India are limited. This may be due to a lack of knowledge about the benefits of these nutrients. The nutritional values of sea squirts have not been highlighted yet, so consumption of these nutrient-rich sea squirts has not attracted attention. The presented preliminary results on the nutritional composition of colonial ascidians described them as valuable and promising resources. Its high content of proteins, essential fatty acids and other nutritional compounds highlights its promising potential for the development of health-enhancing products.

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