Biochemical Composition Of The Marine Ascidian Eudistoma Viridae From Thoothukudi Coast.

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ABSTRACT
An investigation on the Ascidian fauna in Tuticorin coast, India was carried out to update the biochemical value of colonial ascidians Eudistoma viridae. Ascidians are marine filter-feeders commonly investigated for marine natural products development, such as anti-cancer and anti-malarial drugs. Biochemical study was carried out to evaluate the protein, carbohydrate, lipid, aminoacids, and fatty acids content of ascidian in tissue a dry matter (DM) basis. It could be inferred from this study that Eudistoma viride has 4.3% of protein, 3.2% of carbohydrates and 2.3% of lipid. amino acid content in Eudistoma viridae was 46.3%. Among them, the essential amino acids (EAA) 23.8% and non-essential amino acids (NEAA) 22.5% were observed. The highest concentration is formed by lysine (3.3%) for essential and aspartic acid (4.1%) for non -essential amino acids. Valine (1.1%) and serine (0.9%) shows the lowest concentration of essential and non -essential amino acids respectively. There are seven individual fatty acids were identified in the present study. Among them the poly unsaturated ,fatty acids were the dominant fatty acids. The mono unsaturated fatty acid (MUFA) and saturated fatty acid were the second most common fatty acids. The polyunsaturated fatty acid (12.0%), mono unsaturated fatty acid (7.7%) and saturated fatty acid (5.2%) contributed to the total amount of fatty acids. These results indicated that the biochemical composition and subsequently the nutritional value of these ascidians are not only genetically determined but also influenced by its maturity stage and type of ingested food.

Keywords: Eudistoma viridae, carbohydrate, protein, lipid, amino acid and fatty acid.

INTRODUCTION

Thoothukudi is situated in Gulf of Mannar which is one of the marine hotspot areas in India. This coastal area is provided with natural substrates (small stones, embedded rocks, coral pieces, molluscan shells, etc) and artificial substrates (jetty, cement blocks, hull of boats and other fishing harbour installations) which are ideal for settlement of ascidians. Ascidians are signature species for novel compounds with various activities such as anti-tumour(Menna, 2009), anti-inflammatory (Chan.,et.al., 2011) and anti-leukemic (Kobayashi, 1988). Ascidians are consumed by marine animals as food in many parts of the world (Lambert, 2005). Some ascidians could also be used as potential indicators of coastal water quality (Carballo, 2002). Though they are filter feeders, they can be used for monitoring of heavy metals (Philip, 2002). In light of the above, biochemical investigation was carried out to explore the ascidians fauna in Thoothukudi coast situated in Gulf of Mannar, India.

Marine ascidians have been the focus of intensive chemical investigation in recent years and they are very rich sources for unique and biologically active secondary metabolites, which serve as chemical defence. Faulkner (2000) Carbohydrates, proteins and lipids are directly involved in energy production, growth and development. These are widely distributed in nature, occurring in one form to another in virtually all organisms having a key role in metabolic processes such as respiration and nutrient assimilation.

The ratio of carbohydrate was less when compared to the other nutrients such as proteins and lipids in animal tissues (Rinehart, 2000). Carbohydrates constitute only a minor percentage of total biochemical composition. Protein is essential for the sustenance of life and accordingly exists in the largest quantity of all nutrients as a component of body tissues (Menna, 2009,). Protein is essential for normal function, growth and maintenance of body tissues. Amino acids are serve as body builders, and role played by amino acids in isosmotic intracellular regulation has been illustrated in several investigators (Kott, 2001). The analysis of fatty acids have become increasingly important, because they consist not only essential fatty acids, but also a significant source of omega-3 fatty acids.

Therefore, in the present study, an attempt has been made evaluate the proximate composition of basic biochemical constituents such as protein, carbohydrate, lipid, amino acid and fatty acid profiles of the Ascidian Eudistoma viridae from Thoothukudi coastal area.

MATERIAL AND METHODS

Samples of Eudistoma viridae were collected during the low tide from the inter tidal area of Tuticorin coastal area of Gulf of Mannar and brought to the laboratory for further study. The Ascidians were kept in a glass trough in tap water for 24 hours, for emptying and cleaning the gut. The entire body tissue was dried at 50°C (constant temperature) for 24 hours in the hot air oven. Then the dried tissues was powdered and the required quantity of powder was taken for the estimation of total carbohydrate, protein, lipid, amino acids and fatty acids. The estimation of total carbohydrate content followed by the procedure of Anthrone method (Roe, 1955). Lowry et al. (1951) method was followed for quantification of total proteins. The procedure suggested by Bragdon (1951) was adopted to determine total lipids. The chloroform – methanol extraction procedure was used for extracting lipid from the various body parts. The lipid content was estimated gravimetrically by following Folch et al., (1959) method. Amino acids were evaluated by following the standard procedure adopted by (Baker and Han, 1994) in HPLC– Lachrom E merck in SPD-10A VP Detector. Fatty acids were analyzed by the standard procedure followed by Kashiwagi et al., (1997).

RESULTS

The biochemical composition (g/100g) such as carbohydrate, protein, lipid, amino acids and fatty acids of Eudistoma viridae are evaluated. The of the present study revealed that the protein contents (4.3 g/100g) were high in the Ascdian, followed by carbohydrate (3.2g/100g) and lipid 2.3g/100g) respectively. The Ascidian collected from Thoothukudi coast was recorded as proximate composition viz., low amount of lipid 2.3 g/100g , carbohydrates, 3.2.8g/100g and 4.3 g/100g of protein . Of the three components, protein was found to be maximum followed by mand carbohydrate and lipid.

Biochemical composition of Eudistoma viridae

The present study shows that the biochemical composition of the colonial ascidian, Eudistoma viridae in the dry weight basis and biochemical constituents like, protein ,carbohydrate and lipids were 4.3%, 3.2% and 2.3% respectively (Table.1). Amino acid analysis is used to determine the amino acid composition or content of protein and peptides. Total amino acid content of Eudistoma viridae was 46.3%. Among them, the essential amino acids (EAA) was found to be 23.8%, non-essential amino acid (NEAA) was 22.5%. The results of the present study revealed that lysine, arginine, histidine, methionine, phenylalanine, tryptophan contributed as major essential amino acids. Aspartic acid, glutamic acid, cysteine, proline, glutamine are contributed as major non-essential amino acids. Essential amino acids such as valine, isoleucine, and leucine showed the lowest concentration of 1.1% and lysine (3.3%). The minimum and the highest concentration formed maximum amount of non essential amino acids was recorded as serine (0.9%) and aspartic acid (4.1%) (Table.2).

Table 1. Biochemical composition of Eudistoma virida

Protein	4.3%
Carbohydrates	3.2%
Lipid	2.3%

Table 2. Amino acid composition of Eudistoma viridae

Essential aaminoacids	**Percentage of EAA in Eudistoma viridae**
Threonine	2.2%
Arginine	3.0%
Histidine	3.0%
Valine	1.1%
Methionine	3.0%
Isoleucine	1.1%
Phenylalanine	3.0%
Leucine	1.1%
Lysine	3.3%
Trptophen	3.0%
Total	**23.8%**
Non Essential aminoacids	**Percentage of NEAA in Eudistoma viridae**
Aspartic acid	4.1%
Glutamic acid	4.0%
Asparagine	1.0%
Serine	0.9%
Glutamine	3.0%
Glycine	1.1%
Alanine	1.1%

Cysteine	3.3%
Tyrosine	1.0%
Proline	3.0%
Total	**22.5%**

Table 3. Composition of fatty acids in Eudistoma viridae

Saturated fatty acid	**Position of the Carbon atom**	**Concentration of saturated fatty acids in percentage**
Palmitic acid	C6 : 0	1.1%
Stearic acid	C17 : 0	Nil
Margaric acid	C18 : 0	4.1%
Total		**5.2%**
Mano unsaturated fatty acids (MUFA)		
Oleic acid	C18 : 1	7.7%
Poly unsaturated fatty acids (PUFA)		
Linolenic acid	C18 : 2 ω - 6	8.1%
Alpha linolenic acid	C18 : 3 ω - 3	3.9%
Morotic acid	C 18 : 4	In traces
Total		**12.0%**

The fatty acid composition of Eudistoma viridae was analyzed, and the result showed seven fatty acids. Among them, three were saturated fatty acids, one was monounsaturated fatty acid and three were polyunsaturated fatty acids. In the present study, the poly unsaturated fatty acids (12.0%) were found to be dominant fatty acid and most of which were C16:2 (8.1%) and C18:3 (3.9%). Monounsaturated fatty acid (MUFA) of oleic acid occupies second position, contributed 7.7% of total fatty acids represented by C18:1. At the same time Omega - 6 and Omega -3 fatty acids were accounted for 8.1% and 3.9% of total PUFA.

The saturated fatty acid (SFA) occupying third position and contributed 5.2% of total fatty acids. The analysis of fatty acid composition of the Eudistoma viridae showed quantitative differences in the percentage of individual acids (Table.3). A total of (MUFA) mono unsaturated fatty acids recorded in the body tissues of Eudistoma viridae were 7.7%. A high content of polyunsaturated fatty acids were found in the body tissues of Eudistoma viridae (12.0%).

DISCUSSION

Biochemical studies are much important from the nutritional point of view. The biochemical constituent in animals are to vary with season, size of the animal, stage of maturity, temperature and availability of food etc. Biochemical components such as protein carbohydrates and lipids are essential for the body growth and maintenance. Protein is essential for the sustenance of life and exists in largest quantity of all nutrients as component of the human body (Okuzumi and Fujii., 2000). In the Eudistoma viridae protein was found to be the major constituent (4.3%). High levels of protein occur in body components of the Antartic solitary ascidian Cnemidocarpa verrucosa and reflect, the contribution of insoluble protein to the structural materials including connective tissue (Mc Clintock et al 1999.) Madin et al., (1981) analyzed the biochemical components of North Atlantic salp (Tunicata: Thaliacea) species and found proteins to be the major contributor which correspond to 26.6% of the dry weight. The higher protein concentration measured by Madin et al., (1981) is likely to have resulted from lower lipid content in salps. Ali (2008) reported the higher amount of protein in the body tissues of Phallusia nigra.

The present study investigation revealed that the maximum level of protein content in Eudistoma viridae is 4.3%, whereas Ananthan et al., 2012 reported a minimum protein content of 2.2% in Polyclinum indicum and maximum amount of 8.29% from Microcosmus exasperatus from Palk bay. As protein was determined by subtraction, it is also possible some fraction of this insoluble material is attributable to a protein polysaccharide complex of fibers (tunicin) known to occur in various concentrations in the tunic of ascidians (Kott, 1989). The result of the present study agrees well with the findings of Ali (2008), Karthikeyan (2011), and Ananthan et al.,(2012).

In the present observation carbohydrate content was slightly higher than the lipid and lower than that of protein. The result of the present study agrees well with the findings of Ananthan et al., 2012. Carbohydrates in fishery products contain no dietary fibre but only glucides, the majority of also contain traces which consist of glycogen. They glucose, fructose, sucrose and other mono and disaccharides (Okuzumi et al., 2000). Lichun-yan et al., (2007) analysed and evaluated nutritional of composition of ascidian Halocynthia roretzi and analysed 9.19% of carbohydrate in edible parts of the ascidian. He recorded higher concentration of protein (39.60%) and lower concentration of fat (6.53%). The present study is in agreement with the earlier reports (Karthikeyan et al., 2010).Tamil selvi et al.,(2010) carbohydrates constitute only a minor percentage of the total biochemical composition.

In Eudistoma viridae the lipid content was very low (2.3%) as compared to protein (4.3%) and carbohydrate (3.2%). The lower value of lipids in ascidian was reported by **Park et al., (1992).** The present study is in agreement with the earlier

reports of Karthikeyan et al., (2011) and Ananthan et al., (2012) in Microcosmus sexasperatus and respectively. Determining the composition of lipids can be mportant in determining nutritional condition because cell membranes are made of polar lipids but energy is stored as neutral lipids, mostly triglycerides. In general, lipid content has been very low in the ascidians as compared to protein and carbohydrate (Karthikeyan et al.,2011). The lower value of lipids in ascidians have been already reported by Park et al., (1991). In general, ascidians do not feed when they are in spawning season. Their nutrient requirement is met by body reserves and as a result, the level of lipids falls steadily as spawning progress. When lipid is drawn from muscle reserves, it is replaced by water so, that the total mass of the flesh remains much same (Mc Clintock et al., 2004). The water content of muscle obviously rises and this makes the flesh weak. This is the reason for the poor eating equality of spawning ascidians. This tissue energy levels are similar to measurements for the tropical colonialascidian, Cystodytes lobatus and nine species of ascidians from Canadian Artic. This study makes it crystal clear that the biochemical compositions which also can be interpreted as the nutritional representation of ascidians.

Biological value of protein is obviously reflected upon its essential amino acids concentration. Totally, 20 amino acids were estimated in the body tissue of Eudistoma viridae. Total essential concentration of Eudistoma viridae was 23.8% and the total concentration of non-essential amino acid estimated in Eudistoma viridae was 22.5% and they were comparable with the reports by Park et al.. (1991). Li Chun-yan et al., (2007) reported that 51.40% of essential amino acids and 29.5% of NEAA in Halocynthia roretzi. In the present study also EAA content was higher (23.8%) than NEAA (22.5%). Karthikeyan et al., (2011) recorded higher concentration of EAA (27.22%) than the NEAA(3.99%) in Microcosmus exasperatus. The results of the present work is very similar to the above findings. Marine species are excellent sources of fatty acids. In the body tissues of Eudistoma viridae, seven fatty acids are analysed. Among them, three are saturated fatty acids (stearic acid >palmitic acid > margaric acid), one mono unsaturated fatty acid (oleic acid) and three poly unsaturated fatty acids (linolenic acid > Alpha linolenic acid >morotic acid) were recorded. Totally, maximum value of polyunsaturated fatty acid PUFA (12%) and minimum value of saturated fatty acid (5.2%) was recorded in Eudistoma viridae.

The of the present work very similar to the findings of Nanton and Castell (1999) who found significantly higher content of PUFA in harpacticoid copepods. Karthikeyan et al., (2011) analyzed the saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) in Microcosmus exasperatus, and their contents ranged from 0.131% to 1.612%, 1.304% to 1.546% and 1.021 to 1.732% of total fatty acids respectively. An increasing demand for good quality animal protein for the exploding population has led to effective and increasing exploitation of the aquatic resources. The present investigation revealed that there is maximum level of protein contents in tissue collected is (4.3g/100g).

Among the non-essential amino acids aspartic acid was low (0.035%) in the body tissues and proline was high (0.525%). In general, the essential amino acids are necessary for the animals because the animals are unable to synthesize it and should be consumed always through external source in the form of diets. In the present study, it has been recorded high value of EAA Valine, Phenyl alanine and Threonine in the body tissues of Ascidian. This study clearly demonstrates that these Ascidian can be well used as the potential source of amino acids. In Eudistoma viridae, totally seven fatty acids were found. They are three saturated fatty acids (SFA), one mono unsaturated fatty acid (MUFA) and three polyunsaturated fatty acids (PUFA). PUFA was observed in highquantity (12.0%). MUFA was high (7.7%) followed by the present study, body tissue showed the domin PUFA. The variations observed in the present study might be due to the seasonal conditions at the time of study conducted

CONCLUSION

From the present study it was observed that marine Ascidian Eudistoma viridae can be regarded as a good source of protein, carbohydrate, lipid, aminoacids and fattyacids and can be recommended as an ideal food item and can also be employed as a supplement of protein and other nutritive matter so as to balance human nutrition to prevent nutritional deficiencies in the future.

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