

**INTERNATIONAL JOURNAL OF ADVANCES IN
PHARMACY, BIOLOGY AND CHEMISTRY****Research Article****Biochemical Composition of Marine Bivalve *Donax
incarnatus* (Gmelin, 1791) from Cuddalore
Southeast coast of India****N. Periyasamy*, S. Murugan, P. Bharadhirajan.**

C A S in Marine Biology, Faculty of Marine Sciences

Annamalai University, Parangipettai, Tamilnadu, India – 608 502.

Abstract

The marine molluscs have been given more importance, because they have both ecological and economically importance to mankind. Bivalve molluscs comprise major marine fishery resources; they have rich in biochemical compounds. The present study deals with biochemical composition of common beach clam, *D. incarnatus*. The proximate composition of protein, carbohydrate, lipid, amino acids and fatty acids were studied. The results of proximate composition in *D. incarnatus* showed the percentage of protein was high 23.51%, followed by the carbohydrate 10.23% and lipid 1.34 %. The total essential amino acids were found to be as 58.21 % and non-essential amino acids were 35.4 % in body tissue. Among the EAA and NEAA Lysine & Glutamate were found high as 13.28 % and 9.03 % on dry matter basis in bivalve tissue. In the analysis, the fatty acid profile by gas chromatography revealed the presence of higher amount of SFA (palmitic acid 28.13 %) and PUFA (Linolenic acid 18.74 %). The details of the vitamins detected in *D. incarnatus* tissue. Among them, vitamin A and C were found in higher levels (105.6 mg/g & 23.84 mg/g). In the present study, totally 5 macro minerals and 2 trace minerals were reported. The *D. incarnatus* body tissue contributed maximum calcium (315.2 mg/g) and sodium (91.69 mg/g) of minerals. The result shows that marine mollusc (*D. incarnatus*) tissue is a valuable food recipe for human consumption, due to its high quality protein and well-balanced amino acids fatty acids, vitamins and minerals.

Key words: Mollusc, *D. incarnatus*, Proximate composition, Amino acids, Fatty acids, Vitamins and Minerals**INTRODUCTION**

The knowledge on biochemical composition of any edible organisms is extremely important since the nutritive value is reflected in its biochemical contents¹. A new species should be recommended for human consumption only after assessing the nutritive value of the species with regards to its nutritional qualities². The demand for protein rich food is increasing, especially in developing countries, stimulating the exploration of unexploited or non-traditional resources. Marine molluscs are commercially valuable species and easy to cultivate in coastal areas. Marine molluscs are important for marine ecology and human diet, since it is an important source of nutrients. Consumption of

marine molluscs provides an inexpensive source of protein with a high biological value, essential minerals and vitamins. Additionally, the molluscs muscle contains little saturated fat and significant amount of Vitamin C. Molluscs is also a good source of minerals such as calcium, potassium, zinc, iron, phosphorus and copper².

Protein is the essential substance of life and accordingly exists in the largest quantity of all nutrients as a component of the living beings. The ratio of carbohydrate was less when compare to the other nutrients such as proteins and lipids in animal tissues, especially in aquatic animals³. Lipids are the major sources of metabolic energy and essential

materials for the formation of cell and tissue membranes⁴. Shellfish proteins are rich in essential amino acids (EAA). They are required for the maintenance of growth, reproduction and synthesis of vitamins. Aquatic animal fats are good sources of essential fatty acids that are not synthesized in the human body. Fatty acids have a very distinctive character compared to fatty acids from other sources. They consist not only essential fatty acids, but also a significant source of omega-3 fatty acids- especially eicosapentaenoic acid (EPA, C20:5n3) and docosahexanoic acid (DHA, C22:6n3). These fatty acids play a vital role in human nutrition, disease prevention and health promotion⁵. Vitamins are organic compounds representing a minor fraction in the day today life. Fish is a good source of vitamins A, B and C. Vitamin content may be considerably influenced by methods of handling, storage and preparation of sea food⁶. Seafood includes the following important macro minerals: Ca, P, Mg and the electrolytes (Na and K). Trace minerals present in marine organisms are Mn, Fe, Co, Cu, Zn, Ni, Mo and Cr (essential), Al, Ti, V and Ag (non-essential) and Pb and Cd (toxic). Marine organisms are good sources of I, Ca and P which ranged from 70 to 80% in the shellfishes.

As the world population is growing, the per capita consumption of seafood is also increasing rapidly. Because of health consciousness, the modern day man is interested in taking seafood more in view of its nutritional superiority than all other sources of food accessible to him. There remains no considerable study on *Donax* sp with regard to their nutritive value. Though bivalve muscles are being consumed in coastal people and islands, in India there is no evidence to support the *D. incarnatus* as edible. Hence, the present work was planned to study the proximate composition of *D. incarnatus* through estimating their major biochemical components such as total protein, carbohydrate and lipid content in the whole body tissue apart from the amino acids, fatty acids profile, vitamins and minerals content *D. incarnates*.

MATERIALS AND METHODS

The marine bivalve *D. incarnatus* were collected from the Cuddalore (Lat, 11^o 45' 0N; Long, 79^o 45' 0E) Southeast coast of India. *D. incarnatus* is intertidal marine sandy beach habited. They were brought to the laboratory, the animal shell was removed and tissue were dried at 50 °C in an oven and used for biochemical analysis. The proximate compositions of the experimental samples were determined by using standard methods; viz., protein⁷, carbohydrate⁸ and lipid⁹. The experimental

molluscs samples were dried at 55°C for 24 hours in an oven and the dried samples were finely ground for estimating the amino acids in the HPLC (Merck Hitachi L-7400) following the method of¹⁰. For fatty acid analysis, the samples (body tissue) were homogenized with chloroform: methanol (2:1 v/v) mixture and the samples were extracted using the method of¹¹. After the fat was extracted, it was esterified with 1% H₂SO₄ and fatty acid methyl esters were prepared by following the procedure of¹². Identification and quantification of fatty acids were done using Gas chromatography (Hewlett Packard 5890 model). The vitamins were estimated the fat soluble vitamins A, D, E and K and the water soluble vitamins B₁, B₂, B₆, B₁₂ and C were analysed in the HPLC (Merck Hitachi L-74000) following the method described by¹³. The folic acid was estimated by following the calorimetric procedure of¹⁴. The pyridoxine, panthothenic acid and vitamin B₁₂ were estimated by following methods suggested in USP NF 2000 Asian edition. The minerals were estimated soft tissue *D. incarnatus* by following the method¹⁵.

RESULTS

The proximate composition such as protein, carbohydrate and lipid contents of *D. incarnatus* tissue are presented. The results of the present study revealed that the protein composition were high 23.51% followed by carbohydrate 10.13% and lipid 1.34%. The percentage compositions of essential and non-essential amino acids are presented in tissue (Table. 1). The total essential amino acids were found to be as 58.21 % and non-essential amino acids were 35.4 %. Among the essential amino acids Lysine were found high as 13.28 % on dry matter basis in bivalve tissue. The non-essential amino acids Glutamate were found maximum as 9.03 % on dry matter basis.

In *D. incarnatus* 7 different fatty acids were found in body tissue; they are three saturated fatty acids (SFA), one monounsaturated fatty acids (MUFA) and three polyunsaturated fatty acids (PUFA). Among the SFAs palmitic (C16:0) were the major acids. The percentage availability of SFA, MUFA and PUFA content was 44.2, 12.34 & 37.84% in *D. incarnatus* (Table. 2).

The details of the vitamins detected in body tissue are presented in Table.3. Among them, vitamin A (105.6 mg/g) and C (23.84 mg/g) were found in higher levels, whereas vitamin K and B₆ were noticed as lower levels in tissue (0.61 mg/g and 0.33 mg/g).

The quantity of minerals presented in the body tissue (Table 4). Totally, 5 macro minerals and 2

trace minerals were detected. Among the macro minerals, calcium (315.2 mg/g) and copper (1.45 mg/g) were observed at higher and lower levels in *D. incarnatus*, whereas other macro-minerals sodium and magnesium were in negligible level. The trace minerals such as iron (1.41 mg/g) and zinc (0.34 mg/g) were also detected.

DISCUSSION

Foods from the sea have for hundreds of years been a source of high quality protein. In the five basic groups seafood belongs to the same category as meat, poultry, eggs, dried beans and peas-all major sources of protein. Protein is essential for the sustenance of life and exists in largest quantity of all nutrients as a component of the human body¹⁶. The present investigation revealed that the maximum level of protein content in *D. incarnatus* body tissue was 26.93%.³ assessed the percentage of protein which was ranged from 19.25 to 27.9% in the Mesogastropod, *Bursa spinosa*. The protein content was varied from 29.81% to 18.71% reported by^{17, 18} contributed the percentage of protein ranged from 47.86% to 70.18% males and from 49.64% to 72.21% in females *Strombus canarium* gastropoda. Carbohydrates are a group of organic compounds including sugars, starches and fiber, which is a major source of energy for animals. In the present study the percentage of carbohydrates in the body tissue 10.13%.¹⁹ estimated maximum levels (5.31%) of carbohydrate in *L. quadricentus* and (4.69%) in *N. pyramidalis*.²⁰ reported the carbohydrate in *Pythia plicata* values from 0.84 % to 3.04%. In *D. cuneatus*, generally the carbohydrate content was found to be high when compared to other molluscs. The lipids are highly efficient as source of energy, in that they contain more than twice the energy of carbohydrate and proteins. In the present study lipid content of tissue were 1.34 & 2.11%.²¹ reported on lipid content in *Rapana rapiformis* range from 0.85-2.12% in male and 0.95-2.96% in female.²² found the highest level of lipid 10.38% in *Babylonia zeylanica* and 1.97% in *Pleuroploca trapezium* respectively. This clearly indicated the potential source for the proximate composition for human consumption. The determined in this research showed that marine bivalve *D. incarnatus* tissue is value food due to high quality protein.

Biological value of protein is obviously reflected upon its essential amino acids concentration. In the present study body tissue showed the essential amino acids, Lysine (13.28%) was maximum and the minimum Arginine (0.14%) and nonessential amino acids, Glutamate (9.03%) was maximum and

minimum as serine (0.12%), both essential and nonessential amino acids concerned (93.61%). The total amino acids composition in molluscan, *Perna viridis* 95.76%, *C. madrassensis* 98.4% and *Meretrix casta* 65.17% was reported by^{2, 18} found the percentage of essential amino acids was more (80.97%) than those non essential amino acids (15.07%) from the gastropod *S. canarium*.²³ analyzed the amino acids content of *Nertia crepidularia*, total of six amino acids recorded four essential amino acids (68.5%) and two non-essential amino acids (31.01%) respectively. In the present study, molluscs have noticed high value of EAA leucine in tissue. The molluscs have a balanced distribution of all essential amino acids required for an adult per day. This study clearly demonstrates that these marine molluscs can be well used as the potential source of amino acid by all sections of people to do way with malnutrition. In *D. cuneatus*, 7 different fatty acids were found in tissue; they are three saturated fatty acids (SFA), one monounsaturated fatty acids (MUFA) and three polyunsaturated fatty acids (PUFA). Among the SFAs palmitic acid (C16:0) were the major acids. The percentage availability of SFA, MUFA and PUFA content was 44.2, 12.34 & 37.84% in *D. incarnatus*. The second type of fat is MUFA. In the present study, minimum amount Oleic acid (12.34) was found in tissue.²⁴ reported that the presence of higher amount of saturated fatty acids (35.28%) than mono (12.71%) and polyunsaturated (11.72%) fatty acids wedge clam *Donax cuneatus*. Gastropods have been found to contain 18: 1 major fatty acid²⁵. Oleic acid (18:1) contributed more than 10% in *Chlamys tehuecha*^{26, 27} suggested tract the marine animals are richest source of PUFA. In the present study, body tissue showed the dominance of poly unsaturated fatty acid, Linolenic acid (18.74%). In the present study, body tissue showed the high value of saturated fatty acids, Palmitic acid (16:0) which were found to be 28.13%. The *D. incarnatus* tissue could be a better alternative source (against tubercle bacilli and bactericidal effect), since it contains palmitic acids. The results of the present study provide not only the information about the fatty acid composition but also recommended the consumption of this wedge clam *D. incarnatus* since they are rich in Palmitic acid and Linolenic acid. It could also be added that the consumption of marine bivalves is a nutritional assurance to millions of malnourished hungry people. The malnutrition problem in our country can be overcome by effective utilization of nutrient rich molluscan seafood.

Vitamins are organic chemical compounds essential for promoting growth, reproduction and maintenance

of normal body health and function. ⁶ stated that the flesh of fish and shell fish flesh in nut considered to be important sources of vitamin A while high of at fishes and shell fish (e.g. eel, mackerel and bivalve) contain moderate amounts. In the present study, body tissue showed the dominance of vitamin A and vitamin C which constituted 105.6 and 23.84 mg/g. ² estimated the vitamin level from three species of bivalve namely *P. viridis*, *C. madrasensis* and *M. casta* and found vitamin B1, B2 and B6 as 0.11, 0.31 & 0.31 respectively. Shellfish covered in the present study showed complete vitamin profile as the levels required from good health. Minerals are the chemical elements, which are involved in the building of organisms and are necessary for its proper functioning. Totally, 5 macro minerals and 2 trace minerals were detected. Among the macro minerals, calcium (315.2mg/g) and copper (1.45 mg/g) were observed at higher and lower levels in body tissue, whereas other macro-minerals sodium and magnesium were in negligible level. The trace minerals such as iron (1.41 mg/g) and zinc (0.34 mg/g) were also detected. ²⁸ reported more than 40% of soluble copper and zinc in the oyster *Ostrea edulis*. ²⁹ localized Zn and Fe in lysosomes of a number of cell types in *Mytilus edulis*. ³⁰ explained the importance of Ca, Mg, and K in the human nutrition. ³¹ reported that sea foods in general are excellent sources of I, Ca, P, Na, Fe, Zn and oysters are good sources of Fe and Cu. Minerals in different species of pearl shells from South China Sea were rich, particularly in Ca, P and Zn contents ³². Shellfish can absorb minerals directly from the aquatic environment through gills and body

surface. In the present study tissue showed higher level of calcium and good source of minerals.

The role of shellfishes in human health has been well understood due to role of nutrients present in shellfishes. In general, seafood is one of the most nutritionally balanced foods. The seafood diet helps to control weight and goes a long way towards preventing heart diseases. Studies on amino and fatty acid composition of commercial seafood in India are limited. This might be due to lack of awareness on benefits of these nutrients particularly from shellfish tissue. The nutritional values of bivalve are not brought to the limelight so far, so consumption of these nutrient rich bivalves has not attracted attention. The results of the present study provide information about the amino acids and fatty acid composition, but also suggest the consumption of this bivalve tissue. It is rich in amino acids (Lysine), fatty acids (palmitic acid, linolenic acid). Further, the presence of amino acids (Lysine) and fatty acids (16:1 and 18:3) in *D. incarnatus* tissue adds more value through the possibility. From the above observation it is clear that the body tissue of *D. incarnatus* with rich nutritive value can be used for alternate source as an regular sea food which supplies nutrients for the growing children, pregnant women and people suffering from malnutrition.

ACKNOWLEDGEMENT

Authors are thankful to UGC major research project (File No.41-164/2012, SR) for giving facilities, financial and encouragement during the study period.

Table 1. Essential and non essential amino acids of *D. incarnatus*

EAA	% of amino acids	NEAA	% of amino acids
Phenylalanine	2.26	Glycine	2.12
Lysine	13.28	Serine	0.12
Histidine	8.92	Glutamic acid	1.35
Methionine	9.04	Cystine	0.63
Arginine	0.14	Glutamate	9.03
Leucine	6.62	Alanine	6.04
Threonine	1.36	Proline	3.32
Isolucine	5.63	Aspartate	4.36
Valine	7.32	Tyrosin	3.28
Tryptophan	3.64	Aspartic acid	5.15

Table 2. Fatty acid profile of *D. incarnatus*

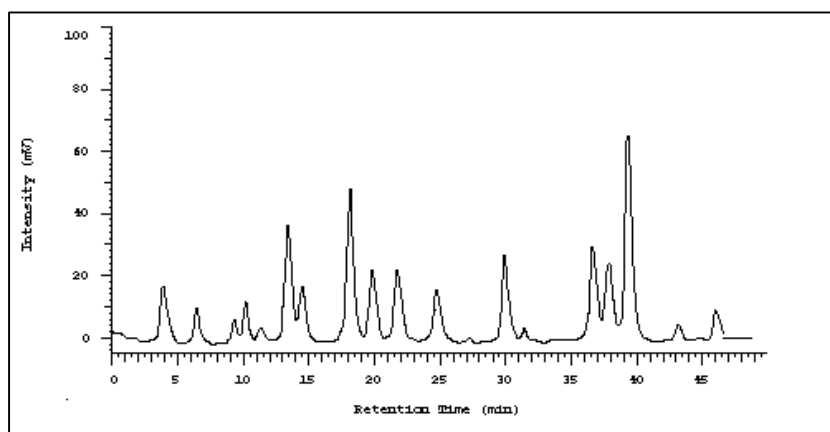
S. No	Fatty acids	Carbon atom (n)	% of fatty acids
Saturated Fatty Acids			
1	Palmitic acid	C16:0	28.13
2	Margaric acid	C17:0	5.73
3	Stearic acid	C18:0	10.34
Total			44.2
Mono Unsaturated Fatty Acids			
4	Oleic acid	C18:1	12.34
Total			12.34
Poly Unsaturated Fatty Acids			
5	Linolenic acid	C18:3	18.74
6	Alpha Linolenic acid	C18:3	11.27
7	Stearidonic or Moroctic acid	C18:4	7.83
Total			37.84

Table 3. Vitamin content of *D. incarnatus* (mg/g)

S. No	Vitamins	Body tissue
1	Retinol (A)	105.6
2	Calciferol (D)	14.2
3	Tocopherol (E)	1.27
4	Vitamin (K)	0.61
5	Pyridoxin (B6)	0.33
6	Cobalamin (B12)	2.08
7	Vitamin (C)	23.84

Table 4. Minerals content of *D. incarnatus* (mg/g of the sample)

S. No	Minerals	mg/g
	Macro	
1	Calcium	315.2
2	Sodium	91.69
3	Potassium	20.36
4	Copper	1.45
5	Magnesium	60.54
Trace		
6	Iron	1.41
7	Zinc	0.34

**Fig. 1. Standard graph for amino acids**

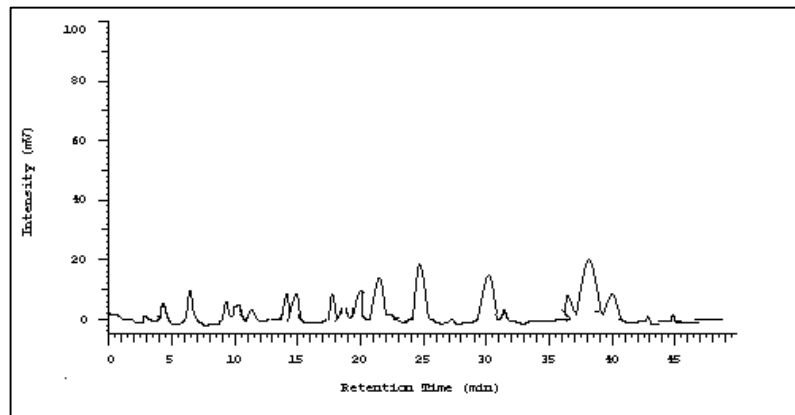


Fig. 2. Estimation of amino acids from *D. incarnates*

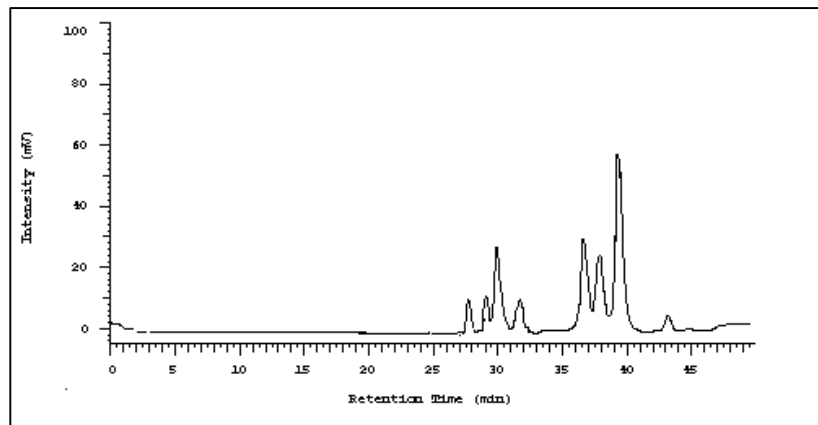


Fig. 3. Standard graph for fatty acids

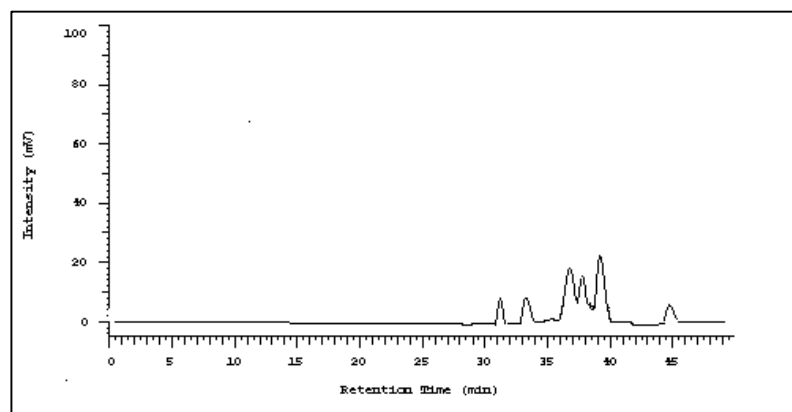


Fig. 4. Estimation of fatty acids from *D. incarnatus*

REFERENCES

1. Nagabhushanam R and Mane VH. Seasonal variation in the biochemical composition of *Perna viridis* at Ratnagiri on the West Coast of India. *Hydrobiologia*, 1978; 57(3): 69-72.
2. Ajayabhaskar D. Nutritional evaluation of molluscan sea food. Ph. D., Thesis, Annamalai University India, 2002; pp 129.
3. Babu A, Kesavan K, Annaduri D and Rajagopal S. *Bursa spinosa*-A mesogastropod fit for human consumption. *Advance Journal of Food Science and Technology*, 2010; 2(1):79-83.
4. Sargent JR. Origins and function of egg lipids nutritional implications. In; Bromage. N. R. R. J. Roberts. (Eds), *Broodstock Management and Egg and Larval Activity*. Black Well Sciences, 1995; 353-372.
5. Frenoux JMR, Prost Belleville ED and Prost JL. A polyunsaturated fatty acid diet lowers blood pressure and improves antioxidant status in spontaneously hypertensive rats. *J Nutr*, 2001; 131: 39-45.
6. Pigott GM and Tucker BW. *Seafood: Effects of Technology on nutrition*, Marcel Dekker, Inc., New York and Basel, 1990; pp 362.
7. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the tolin phenol reagent. *J Biol Chem*, 1951; 193: 265-273.
8. Dubois M, Giller KA, Hamilton JK, Roberts RA and Smith F. Colorimetric method for determination of sugars and related substances. *Analyt Chem*, 1956; 28: 350-356.
9. Floch J, Lees M and Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem*, 1956; 226: 497-509.
10. Baker DH and Han Y. Ideal amino acid profile for broiler chicks during first three weeks post hatching. *Poult Sci*, 1994; 73: 1441-1447.
11. Bligh EG and WJ Dyer. A rapid method for total lipid extraction and purification. *Can J Biochem Physiol*, 1959; 37: 911-917.
12. AOAC. *Official Methods of Analysis*, 16th Ed., Association of Official Analytical Chemists, Washington, DC, 1995.
13. Sadasivam S and Manickam A. *Biochemical Methods*. 2nd Edition, New Age International (p) limited, Publishers, 1996; 179-186.
14. Senthil PD. *Quantitative Analysis of Drugs in Pharmacological Formulations* 3rd edition, 1997; 589-590.
15. Guzman HM and Jimenez CE. Concentration of coral reefs by heavy metals along the Caribbean coast of central Africa (costarica and panama) *Mar Poll Bull*, 1992; 24(11): 554-561.
16. Okuzumi M and Fujii T. *Nutritional and Functional Properties of Squid and Cuttle Fish 35th Anniversary of Commemorative Publication*, 2000; pp 223.
17. Shanmugam A, Purusothaman A, Sambashivam S, Vijayalakshmi S, and Balasubramanian T. *Cephalopods of Parangipettai coast, East coast of India*. CAS in marine biology, Annamalai University India, 2001; pp 45.
18. Arularasan S. Studies on eco-biology of the dog conch *Strombus canarium* (Linnaeus, 1758) (Gastropoda: Prosobranchia: Strombidae) from Gulf of Mannar Marine Biosphere Reserve, Southeast Coast of India. Ph. D. Thesis, Annamalai University, Parangipettai India, 2009; pp 180.
19. Thivakaran GH). Studies on the Littorids *Littorina fuadricentua* (Philippi) and *Nodilittorina pyramidalis* away and *Granimard* (1983), *Gastropoda*. Pros Branlria; *Cittornidae* from the Tranqubar rocky shore south coast of India. Ph. D., Thesis Annamalai University, India, 1988; pp 179.
20. Shanmugam A. Studies of *Pythia plicata* (Gray). (Gastropoda; Pulmonata: Elobiidae) from the Pitchavaram mangroves. Ph.D. Thesis. Annamalai University India, 1987; pp 127.
21. Rajkumar T. Studies on *Rapana rapiformis* Born (Mollusca: Gastropoda: Muricidae: Rapaninae) from the Parangipettai coastal waters, India. Ph. D., Thesis, Annamalai University, India, 1995; pp 185.
22. Nirmal A. Biochemical studies on prosobranchian gastropods *Babylonia zeylonica* (Neogastropods: Buccinidae: Fascioliariidae). M. Sc., Dissertation, Annamalai University, India, 1995; pp 30.
23. Palpandi C. Studies on mollusca, biology, biochemistry and heavy metal accumulation in *Nerita* (*Dostia*) *crepidularia* Lamarck, 1822 from mangroves of Vellar estuary South east Coast of India. Ph. D., Thesis, Annamalai University India, 2010; pp 318.
24. Shanmugam A, Palpandi C and Sambasivam S. Some valuable fatty acids exposed from wedge clam *Donax cuneatus* (Lin), *African Journal of Biochemistry Research*, 2007; 1 (2); 014-018.
25. Johns RB, Nichols PD, Perry GJ. Fatty acid components of nine species of molluscs of the littoral zone from Australian waters. *Comp. Biochem. Physiol*, 1980; 62B: 207-214.
26. Pollero RJ, Maria Re, Brenner R. Seasonal

-
- changes of the lipids of the molluscs *Chlamys* *tehuelpa*. Catedra de Bioquímica, Instituto de Fisiología, Facultad de ciencias medicas, Universidad Nacional de La plata, Argentina and centro Nacional Patagonica Madryn, Argentina. *Comp Biochem Physiol*, 1979; 64A: 257– 263.
27. Nair PGV and Mathew S. Biochemical composition of fish and shellfish. CIFT Technology Advisory Series, 2000; pp 14.
28. Coombs TL. The nature of zinc and copper complexes in the oyster *Ostrea edulis*. *Marine biology*, 1974; 28: 1-10.
29. Lowe DE and Moore MN. The cytochemical distributions of zinc (ZnII) and iron (FeII) in the common mussel *Mytilus edulis* and their relationship with lysosomes. *J Mar Biol Ass U.K*, 1979; 59: pp 581.
30. Rajagopal S, Lyla PS and Ajmalkhan S. Seafood the nutritional insurance. *Seafood Exp J*, 1993; 45-46.
31. Devadas R (1994). Foods of aquatic origin in human nutrition and their importance in the Indian context. Symposium on Nutrients and Bioactive Substances in Aquatic Organisms. Cochin, India, 1994; 16, 177-194.
32. Laihao L, Shigians D, Peigily C, Xiangiang W, Yanyan and Maojiar W. Evaluation on nutrition components of pearl shell meat. *J Fish China*, 1999; 23(4): 392-397.