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Nutritional significance of the Sea Slug *Kalinga ornata* from southeast coastal waters of India

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Abstract
The present study was carried out to investigate the nutritional potential of sea slug *K. ornate* from Indian coastal waters. The collected sea slug *K. ornate* samples were tested for biochemical assays (carbohydrate, protein, lipid, amino acids and fatty acids). All these assays were followed by standard methods. In bio chemical analysis; The protein content in *K. ornate* tissue was found to be 12.1 $mgmL^{-1}$, the total carbohydrate content was found to be 3.8 $mgmL^{-1}$, total lipid content was observed to be 3.63 $mgmL^{-1}$, Total fatty acids shows 15.23% of total saturated fatty acids (Σ of SFAs), 25.15% of total monounsaturated fatty acids (Σ of MUSFAs) and 54.24% poly-unsaturated fatty acids (Σ of PUFAs) shows high amount of palmitic acid (C16:0) 6.67%, oleic acid (C18:1) 25.15% and alpha linolenic acid (C18:3) 48.16%. Amino acids such as leucine, lysine, phenylalanine, glycine and proline were observed in high amount. Minerals such as sodium and potassium shows high amount in sea slug *K. ornata*. From the above observation it is clear that the body tissue of *K. ornata* has rich nutritive value and it can be used for alternative source for food.
Keywords: sea slug, *K. ornate*, nutritional potential, PUFAs, minerals

INTRODUCTION

Nutrition is the study of food, constitutes macronutrients and micronutrients in relation to the living organisms. Macronutrients stimulate calories from, carbohydrates, proteins and fats as they serve to maintain our body weight. Essential micronutrients such as vitamins, minerals, certain amino acids and fatty acids play an important role in a healthy life. Proteins are required for the growth and repair of the body muscles, cells and tissues. They defend the body from infection and diseases are obtained from meat, dairy products, egg and fishes. However, as they also contain definite amounts of fat, they should be consumed in smaller quantities than other types of food.

Shellfish are exoskeleton bearing aquatic invertebrates used as food. They can be divided into 2 groups namely; molluscs and crustaceans. Marine molluscs include clams, mussels, oysters, winkles and scallops. Some crustaceans commonly consumed are shrimps, prawns, lobsters, crayfish and crabs are valuable nutritional source. Such nutrients play a vital role in growth, development, maintenance of normal body function of physical activity and health (Nagabhushanam and Mane, 1978).

Molluscs constitute the second largest invertebrates and most successful phyla next to insects (Bouchet, 1991). Molluscs are commercially valuable species and are easy to cultivate in coastal areas. Molluscs are an important source of nutrients in the human diet, which provides an inexpensive health care application. Additionally, molluscs muscle contains saturated fatty acid, vitamin C and minerals such as calcium, potassium, zinc, iron, phosphorus and copper (Ajayabhaskar, 2002).

Shellfish such as mussel and clam contains approximately 20 to 28% calories from fat. It also provides dietary protein with essential amino acids and consumed under in a low-fat diet (King *et al*., 1990). The concentration and functional forms of the minerals need to be maintained within a narrow range for ordinary metabolic activities in cells and tissues. The principal functions of essential minerals include skeletal structure, maintenance of colloidal systems and regulation of acid base equilibrium. Minerals constitutes essential components of hormones and enzymes in human nutrition. Mineral deficiencies can cause biochemical, structural and functional pathologies depend on the degree of mineral deprivation.

Sea food contains the following important minerals such as calcium, phosphorus, magnesium and the electrolytes (sodium and potassium). Heavy metals are environmentally ubiquitous, readily dissolved in water and taken up by aquatic organisms (Alam *et al*., 2002). Trace minerals present in marine organisms are Mn, Fe, Co, Cu, Zn, Ni, Mo and Cr as essential minerals, whereas Al, Ti, V and Ag as non-essential and some of the toxic metals are encountered such as Pb and Cd.

Marine organisms are good sources of I, Ca and P which ranged from 70 to 80% in shellfishes. In the present study, this chapter deals with biochemical assays like estimation of carbohydrate, protein, lipid, amino acids and minerals have been planned to evaluate the nutritional significance of sea slug *K. ornata*.

MATERIALS AND METHODS

The collected sea slug *K. ornata* was thoroughly washed with sea water for removal of sand, mud and overgrowing organisms at the site of collection, then immediately the sample was stored in an insulated box containing ice cubes and transported to the laboratory. The collected specimens were identified by the standard literature of taxonomic guide by Clark and Rowe, (1971). Sea slug tissues (5 gm) were dried and powderized for biochemical analysis.

Estimation of protein

Protein concentration was estimated by the method Bradford (1976) Bovine serum albumin (2 $mgmL^{-1}$) was used as reference standard at concentration 20, 40, 60, 80 and 100 µL. The assay relies on the binding of dye coomassie blue G25 to the protein molecule measured calorimetrically at 595 nm.

Estimation of carbohydrate

Total carbohydrate was estimated by phenol-sulphuric acid method of Dubois *et al*. (1956). Glucose (1 μgmL^{-1}) was used as a reference standard concentration (20, 40, 60, 80 and 100 $\mu g\mu L^{-1}$). The absorbance was measured at 490 nm in a spectrometer (HITACHI-220S UV).

Total and free amino acids estimation

Total free amino acids were determined by the method described by Hamilton and Van slyke, (1943). 10% pyridine (1 mL) was used as a reference standard. The absorbance was measured at 570 nm in spectrophotometer (HITACHI-220S UV).

Amino acid

The samples for the analyses of free amino acids were prepared by the method of Huesgen (1998). The amino acids were determined by an amino acid analyser (Shimadzu- High performance liquid chromatography, company HITACHI, Detector-SPD 10 AVP, LP pump LC-10AT VP).

Estimation of lipid

The amount of lipid was determined by the method described by Folch *et al*. (1957). 1 gm dry sample was dried to constant weight in a drying oven (60°C, 24 hrs). Dried samples were homogenized with chloroform: methanol mixture (2:1 v/v), mixed in a vortex mix in 2800 × g and filtered. The extract was shaken and equilibrated with 0.25 volume of a saline The extracted lipids were concentrated by a rotary evaporator. Extracted lipids were weighed in vials using a microelectronic balance (±0.001 mg) in order to calculate the total lipid content.

Fatty acid estimation

In fatty acid analyses, following pre-process were followed such as saponification, methylation, extraction, basic wash and FAMEs were separated by GC, finally fatty acids were analysed by gas chromatography according to the method of Lepage and Roy, (1986). The fatty acid samples were analysed by gas phase chromatography (network gas chromatograph model 6890N, Agilent technologies, USA). Samples were injected by split injector, split the ratio 100:1, used column was ultra 2 capillary column, Sherlock version 4.5 with EUKARY database was utilised.

Estimation of minerals and metals

The metals were analysed by Tuzen *et al*., (2009), the tissue was washed with double distilled water and was dried in hot air oven at 60°C for 24 hrs. Dried samples were homogenised in a blender and 1gm of homogenate was digested by microwave digestion system (maximum pressure 800 psi, maximum temperature 220°C). After digestion, residues diluted to 50 mL with ionized water. The metal analysis of samples was carried out by using inductively coupled plasma atomic emission spectroscopy. The metal contents were expressed as $mgkg^{-1}$ dry weight, (dw).

RESULTS

The amount of protein content in *K. ornata* was found to be 12.1 $mgmL^{-1}$in dry weight (Fig. 1). The total carbohydrate estimated by phenol sulphuric acid method using glucose as standard and the total carbohydrate content was found to be 3.8 $mgmL^{-1}$. The total free amino acid content in *K. ornata* tissues was found 4.56 $mgmL^{-1}$. In sea slug 18 essential and non-essential amino acids were analysed (Table 1). From Following amino acids such as leucine, lysine, Asparagine, glutamic acid and tryptophan were recorded in very trace amount phenylalanine, glycine and proline were observed in high amount from the sea slug. The estimation proves that amino acids are rich in sea slug *K. ornata.* The total lipid content in *K. ornata* tissue was recorded to be 3.63 $mgmL^{-1}$ (Fig.1). The metal concentration in tissue of *K. ornate* was given in the table 2. Sodium and potassium was recorded in enormous amount 1.088 and 0.317 in the tissue**.** In fatty acid estimation *K. ornata* shows 15.23% of total saturated fatty acids (Σ of SFAs), 25.15% of total mono unsaturated fatty acids (Σ of MUSFAs) and 54.24% polyunsaturated fatty

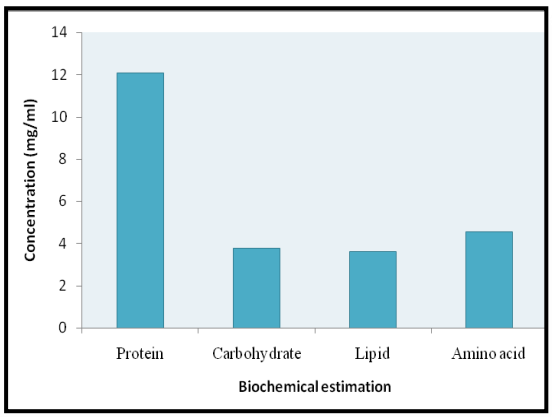

Fig. 1. Biochemical composition of sea slug *K. ornata*

Table 1. Amino acid content of sea slug *K. ornata*

S. No.	Amino Acids	Percentage
1	Aspartic acid	2.183
2	Glutamic acid	0.708
3	Asparagine	0.538
4	Serine	0.903
5	Glycine	7.998
6	Threonine	4.243
7	Arginine	4.506
8	Alanine	3.787
9	Cystine	0.399
10	Tyrosine	4.375
11	Histidine	0.699
12	valine	6.565
13	Isoleucine	3.998
14	phenyl alanine	9.898
15	Leucine	44.677
16	Lysine	38.060
17	Proline	7.344
18	Tryptophan	0.853

acids (Σ of PUFAs). Saturated fatty acids (Σ of SFAs), mono unsaturated fatty acids (Σ of MUSFAs) and polyunsaturated fatty acids (Σ of PUFAs) shows high amount of palmitic acid (C16:0) 6.67mg/g, oleic acid (C18:1) 25.15mg/g and alpha linolenic acid (C18:3) 48.16% respectively (Table 3).

DISCUSSION

Foods from the sea have for hundreds of years given rise to high quality protein. In five basic groups, sea foods are the major source of protein like that of meat, poultry, eggs, dried beans and peas. Protein is critical to the substance of life and exists in largest largest quantity of all nutrients as a component of the human body (Guzman and Jimenez, 1992). Biochemical studies are of great importance from the nutritional point of view. The biochemical constituents in animals are known to vary with season, size of the animal, stage of maturity

Table 2. Minerals content of sea slug *K. ornate*

S.No.	Minerals	*K. ornata* (mgkg^{-1})
1	Copper	0.0048
2	Zinc	0.0027
3	Iron	0.001
4	Sodium	1.088
5	Potassium	0.317
6	Calcium	0.032
7	Magnesium	0.066
8	Phosphorous	< 0.1

temperature and availability of food. The amount of carbohydrates, proteins, lipids, such as phospholipids (PLs), sterols, fatty acids and minerals were higher in their body and higher concentration of free amino acids has also been found in the body tissue of the study animal. In addition to the protein, carbohydrates, amino acids and fatty acids were also estimated to understand the nutritive value (Nurjanah *et al*., 2012).

In the present investigation sea slug *K. ornata* was found to reveal high amount of proteins, carbohydrates, lipids, amino acids, fatty acids content which shows high nutrient value and thus surpasses many marine food source in terms of value added marine food.

The protein and carbohydrate were 12.1 mg/ml and 3.8 mg/ml respectively in sea slug tissues (Periyasamy *et al*., 2014), the maximum levels of protein content in *D. incarnatus* body tissues were 26.93%. Babu *et al*. (2010) assessed the percentage of protein which was ranged from 19.25 to 27.9% in the meso gastropod, *Bursa spinosa*. Premanand *et al*. (2010) reported the higher percentage of protein (10.348%) and carbohydrates (4.307%) content in *Pleuroploca trapezium*.

Thivakaran (1988) estimated the maximum levels (5.31%) of carbohydrates in *I. quadricentus* and 4.96% in *N. pyramidalis*. The total lipid content of this sea slug shows 2.81 mg/ml and it is very low as compared to protein and carbohydrate. The lower value of lipid in molluscs was reported by Premanand *et al*. (2010). The low level of lipid (1.3%) content in *D. incarnatus* was reported earlier (Periyasamy *et al*., 2014). Biological value of protein is obviously reflected upon its essential amino acids concentration. In the present study, body tissue showed the maximum percentage of essential amino acid leucine (44.67%) and minimum cystine (0.399%) and non-essential amino acids, glycine (7.99%) was maximum and glutamate (0.70%) as minimum. Total amino acid composition of molluscan species such as *Perna viridis*, *Crassostrea madrasensis* and *Meretrix casta*

Were 95.76, 98.4 and 65.17% respectively (Ajayabhaskar, 2002). In the present study, molluscs have noticed extreme value of EAA leucine in its tissue. The molluscs have a balanced distribution of all essential amino acid required for an adult per day. This study clearly demonstrates that these marine molluscs can be well used as the potential source of amino acids.

In the present study, *K. ornata* shows 15.23% of total saturated fatty acids (Σ of SFAs), 25.15% total mono unsaturated fatty acids (Σ of MUSFAs) and 54.24% polyunsaturated fatty acids (Σ of PUFAs). In saturated fatty acids (Σ OF SFAs), palmitic acid (C16:0) shows high amount of 6.67 mg/g, in monounsaturated fatty acids (Σ OF MUSFAs) oleic acid shows (C18:1) 25.15 mg/g, in polyunsaturated fatty acids (Σ OF PUFAs) alpha linolenic acid (C18:3) shows high amount of 48.16% and 6.08% of linoleic acid (C18:2).

Unsaturated fatty acid content was higher than saturated fatty acid in tissues of *K. ornata*. All tissues of elongatulus were found to have similar amount of unsaturated fatty acids (Ekin and Bashan, 2010). Similar results are also found by several researchers (Person and Sereflisan, 2010; Shanmugam *et al*., 2007; Murphy *et al*., 2003). Alpha linolenic acid was dominant in meat tissue, presented 54.24% of poly unsaturated fatty acid, 25.15% of mono unsaturated fatty acid and 15.23% of saturated fatty acid. Linolenic acid is an important component of cell membrane has a beneficial effect in health and controls chronic diseases (Simopuolos, 1999).

Minerals are chemical elements, which are involved in the building of organisms and are required for its proper functioning totally 5macro minerals and 2 trace minerals were detected. Among the macro minerals such as sodium (1.088 mg/g) and potassium (0.317 mg/g) were observed at higher and lower levels in body tissue respectively; where as other macro minerals calcium (0.032 mg/g), magnesium (0.066 mg/g) and copper (0.0048 mg/g) were in negligible level. The trace minerals such as iron (0.001 mg/g) and zinc (0.0027 mg/g) were also detected (Devadas, 1994) reported that sea food in general are excellent sources of I, Ca, P, Na, Fe and Zn. Molluscs are good source of Mg and Cu. Shellfish can absorb minerals directly from the aquatic environment through gills and body surface. From the above observation it is clear that the body tissue of *K. ornata* has rich nutritive value and it can be used for alternative source for food.

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REFERENCES

1. Nagabhushanam, R. and V.H. Mane, 1978. Seasonal variation in the biochemical composition of *Perna viridis* at Ratnagirii on the west coast of India. *J. Hydrobiologia*, 57(3): 69-72.
2. Ajayabhaskar, D., 2002. Nutritional evaluation of molluscan sea food. *Ph.D., Thesis, Annamalai University, India*, 129 pp.
3. King, I., M.T. Childs, C. Dorsett, J.G. Ostrander and E.R. Monsen, 1990. Shellfish: Proximate composition, minerals, fatty acids, and sterols. *Journal of the American Dietetic Association,* 90: 677-685.
4. Alam, M.G.M., A. Tanaka, G. Allinson, L.J.B. Laurenson, F. Stagnitti and E.T. Snow, 2002. A comparison of trace element concentrations in cultured and wild carp (*Cyprinus carpio*) of lake Kasumigaura, Japan. *Ecotoxicology and Environmental Safety*, 53: 348-354.
5. Clark, A. M and F. E. W. Rowe, (1971). Monograph of shallow water Indo west pacific echinoderms. British Museum (National History) Publication 690: 1-238.
6. Bradford, M.M., 1976. A rapid and sensitive method for the quantisation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem*., 72: 248-254.
7. Dubois, M., K.A. Giles, J.K. Hamilton, P.A. Rebors, F. Smith, 1956. Calorimetric method for determination of sugars and related substances. *Anal. Chem*., 28: 350-356.
8. Hamilton, P.B. and D.D. Van Slyke, 1943. Amino acid determination with ninhydrin. *J. Biol. Chemist.*, 150: 231-233.
9. Huesgen, A.G., 1998. Sensitive and reliable amino acid analysis in protein hydrolysates using the HP1100 SERIES HPLC. *Hewlett Packard technical note,* 12: 5966-6110.
10. Folch J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*., 226: 497-509.
11. Lepage, G. and C.C. Roy, 1986. Direct transesterification of all classes of lipids in a one step reaction. *J. lipid res.*, 27: 114-120.

12. Premanand, T., C. Chellaram, S. Kumaran and C. Felicia Shanthini, 2010. Biochemical composition and antioxidant activity of *Pleuroploca trapezium* meat. *J. Chem. Pharm. Res.,* 2(4): 526-535.
13. Thivakaran, G.A., 1988. Studies on the Littorinids *Littorina quadricentus* (Philippi) and *Nodilittoria pyramidalis* (Quoy and Gaimard, 1833) (Gastropoda: Prosbranchia: Littorinidae) from the Tranquebar rocky shore, Southeast Coast of India. *Ph.D., Thesis, Annamalai University, India,* 179 pp.
14. Ajayabhaskar, D., 2002. Nutritional evaluation of molluscan sea food. *Ph.D., Thesis, Annamalai University, India,* 129 pp.
15. Ekin, I. and M. Bashan, 2010. Fatty Acid Composition of Selected Tissues of *Unio elongatulus* (Bourguignat, 1860) (Mollusca: Bivalvia) collected from Tigris river, Turkey. *Turkish Journal of Fisheries and Aquatic Science,* 10: 445-451.
16. Person, B. and H. Sereflisan, 2010. The proximate composition and fatty acid profiles of edible parts of two freshwater mussels. *Turkish Journal of Fisheries and Aquatic Sciences,* 10: 71-74.
17. Shanmugam, A., C. Palpandi and S. Sambavisam, 2007. Some valuable fatty acids exposed from wedge clam *Donax cuneatus* (Linnaeus). *African Journal of Biochemistry Research,* 1(2): 014-018.
18. Murphy, K.J., N.J. Mann and AJ. Sinclair, 2003. Fatty acid and sterol composition of frozen and freeze-dried New Zealand Green Lipped Mussel (*Perna canaliculus*) from three sites in New Zealand. *Asia Pacific J. Clin. Nutr.,* 12(1): 50-6.
19. Simopuolos, A.P., 1999. Essential fatty acids in health and chronic disease. *American Journal of Clinical Nutrition,* 70(3): 560-569.
20. Devadas, R.P., 1994. Foods of aquatic origin in human nutrition and their importance in the Indian context. In: Nutrients and bioactive substances in aquatic organisms. *Society of Fisheries Technologists, Cochin, India.* 299 pp.
21. Tuzen, M., I. Karaman, D. Citak and M. Soylak, 2009. Mercury (II) and methyl mercury determinations in water and fish samples by using solid phase extraction and cold vapour atomic absorption spectrometry combination. *Food and Chemical Toxicology,* 47: 1648-1652.
22. Guzman, H.M. and C.E. Jimenez, 1992. Concentration of coral reefs by heavy metals along the Caribbean coast of central Africa (Costarica and Panama). *Mar. Poll. Bull.,* 24(11): 554-561.
23. Nugraha, 2012. Nutritional and antioxidant properties of sea slug (*Discodoris sp.*) from Pamekasan Indonesia Sea water. *European journal of scientific research,* 79(1): 40-47.
24. Periyasamy, N., S. Murugan and P. Bharadhirajan, 2014. Biochemical composition of marine bivalve *Donex incarnates* (Gmelin, 1791) from Cuddalore south east coast of India. *International Journal of Advances in Pharmacy, Biology and Chemistry*, 3(3): 575-582.
25. Babu, A., K. Kesavan, D. Annadurai and S. Rajagopal, 2010. *Bursa spinosa* mesogastropod fit for human consumption. *Adv. J. Fd. Sci. Tech*., 2: 79-83.