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Biochemical composition and antioxidant activity of *Pleuroploca trapezium* meat

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ABSTRACT

The Horse conch, Pleuroploca trapezium, is a large carnivorous gastropod, which is available in all seasons along the southeast coast of India. Their beautiful shell and operculum are being used for ornamental and medicinal purpose. A small section of the fisher folk consumes the flesh of this gastropod but the majority of them are not aware of the real value and delicacy. The present research highlights the nutritive value of the meat of P.trapezium such as its protein, low lipid, vitamins, minerals and trace metal contents and Polyunsaturated Fatty Acids (PUFAs) like, Linoleic, Linolenic, Eicosapentaenoic acid (EPA) Arachidonic and Eicosatrienoic acids. The meat also has good antioxidant activity, hence justifying the need to popularize this meat as important seafood. The information of the nutritional quality of this meat should be transferred to the local fishermen in order to give them an idea of the value of this resource so that effective actions can be taken for the proper utilization of this gastropod resource.

Key words: *Pleuroploca trapezium*, biochemical analysis, PUFA, antioxidant activity

INTRODUCTION

Nutritionists have known for years that seafood is a source of top-quality protein. Seafood can make a significant contribution to the nutrient needs of all consumers, especially growing children and the elderly. Nutrition is the net effect of the process by which an organism ingests and uses food for growth and maintenance of the body. Foods are composed of specific nutrients: Proteins, fats, carbohydrates, vitamins and minerals. An added advantage of seafood is that its protein is highly digestible. The protein in seafood is more readily broken down and absorbed than the protein in red meats and poultry. This advantage makes seafood an excellent food choice for people of all ages. Seafood consumption can help maintain a balanced nutrient intake compatible with a low-fat diet. The total amount of fat in seafood is very low in most varieties and the fat is rich in PUFA. On a unit calorie basis, seafood can provide a broad range of nutrients. Most nutrition researchers now say that eating seafood once or twice a week may be beneficial in preventing coronary heart disease. The high content of PUFA in seafood lowers serum cholesterol levels and cholesterol levels are not significant in most seafood products. In general, seafood is one of the most nutritionally balanced foods.

Many studies on bioactive compounds from molluscs exhibiting antitumour, antibacterial and antiviral activities have been reported worldwide [1], [2], [3]. Reactive oxygen species and free radicals are formed in the body as a consequence of normal metabolic reactions, exposure to ionizing radiation and by the influence of many xenobiotics. Reactive oxygen species are an important part of the defense mechanisms against infection, but excessive generation of free oxygen radicals may damage tissue [4]. Free radicals are known to be generated through biological and environmental interactions [5] and they have been implicated in more than one hundred disease conditions in humans, including cardiovascular, brain and ocular dysfunctions, arthritis, ischemia and reperfusion carcinogenesis, and AIDS [6]. Antioxidants are chemical substances which can scavenge free radicals and are implicated in the prevention of heart diseases, cancer, ageing etc. Gastropods have not been utilized in identifying and extending the natural antioxidant present in them and screened for their antioxidant potential. Hence attempt has been made to study the natural antioxidant present in the gastropod.

Over the last few years, utilization of fish and sea foods for human consumption has increased worldwide, primarily due to growing concern for healthy nutrition [7]. For better utilization and processing of new resources analysis for proximate chemical composition and nutritional components becomes a prerequisite, especially in case of new varieties of sea food hitherto not analyzed. An understanding of the composition is vital to evaluate each species of fish in terms of quality. The present study was carried out to understand the nutritional quality of the underutilized gastropod *P. trapezium*, thus to highlight its significance as a new seafood resource for consumption.

MATERIALS AND METHODS

Study Area: The Molluscan samples were collected from the local shell dealer in live conditions from Tuticorin area (Lat 8º45 and Long 78º13’E) of southeast coast of India (Fig. 1).

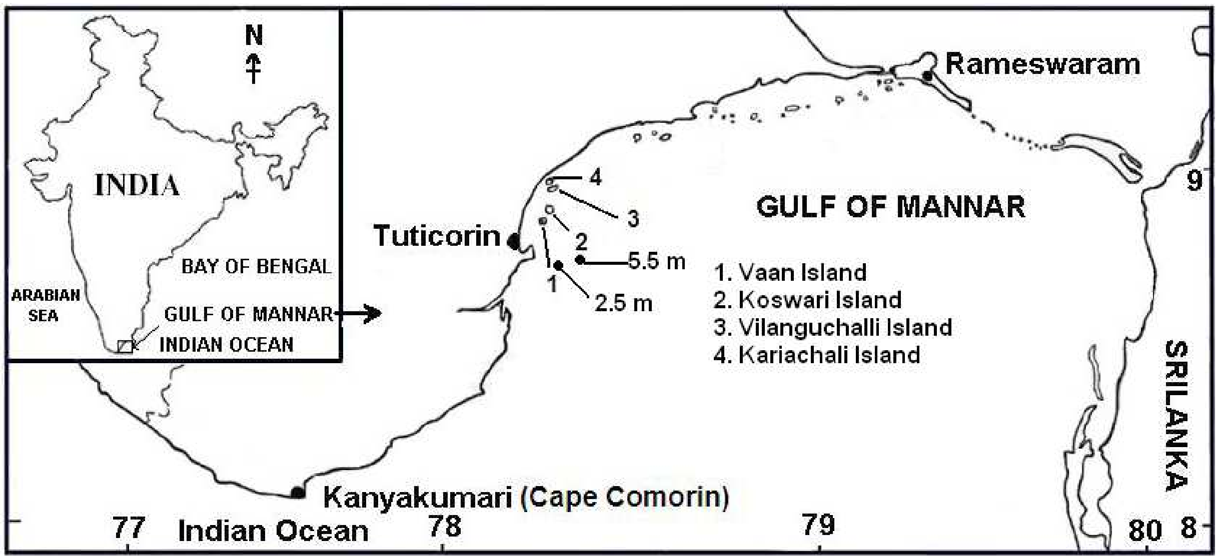

Fig.1

Biochemical analysis: The foot and adductor muscle were removed by breaking the shell and then was washed and dried in a hot air oven at 50 to 55°C. The well dried meat was powdered and it was used for the biochemical analysis. The carbohydrate, protein and lipid contents of the *Pleuroploca* meat powder was estimated by the method of Dubois *et al*. (1956), Raymont *et al*. (1964) and Bligh and Dyer (1959) respectively [8], [9],10]. The vitamin, mineral and heavy metal content of *Pleuroploca trapezium* meat were analyzed at A TO Z Pharmaceuticals (P) Ltd., Chennai. Dried and finely powdered samples were used for the analysis**.**

Fresh raw *Pleuroploca* meat sample of 100 g was extracted for total lipid content by following the method of Bligh and Dyer (1959) [10]. The lipid was extracted using the solvents such as methanol and chloroform. A higher concentration of methanol at the start of the extraction ensures good separation ability and the presence of chloroform ensures good solvation ability. The fatty acids present in *Pleuroploca* meat lipids were converted to Fatty Acid Methyl Ester (FAME) using the BF_3 - Methanol method described in Association of Official Analyticl Chemist (AOAC, 1990) [11]. 250 mg of lipid was taken in a 50 ml round bottom flask to which 4 ml of 0.5M alcoholic NaOH was added and refluxed for 5-10 minutes. 5 ml of BF_3 methanol was added to the mixture and refluxed for 1 minute. The contents were transferred to a stoppered test tube and 15 ml of saturated NaCl solution was added. The FAME was extracted twice with 5 ml of hexane. The upper hexane layer was collected into a screw cap test tube and dried under the steam of nitrogen to get a final concentration of 100 mg / ml hexane. Fatty acid composition was determined by gas chromatography. The quantification was done by the method described by Candela *et al*. (1996) [12]. The fatty acid Methyl Esters (FAME) prepared by BF3 – methanol was quantitatively separated on a Perkin-Elmer Autosystem X L gas chromatograph fitted with a Flame Ionization Detector (FID). A fused silica capillary column (PE-225) (0.25mm ID x 30 m length) was used. The operating conditions were injector temperature 250^o C, detector temperature 300^o C; and the oven temperature was increased from 70^o C to 80^o C at the rate of 3^o C / min and then to 220^o C at the rate of 10^o C / min. The carrier gas used was

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nitrogen at 20 psi pressure. Peaks were identified by comparison of their retention times with those of standard mixtures (Sigma Chemical Co., St. Louis, USA, 99 % purity specified for GC).

Antioxidant Activity: The foot and adductor muscle of *Pleuroploca trapezium* were cut into small pieces and airdried. It was then extracted with methanol by cold steeping and filtered. The filtrate was evaporated and the methanolic extract of the meat was used for determining the antioxidant activity. The analysis was done at Inspec Laboratories Pvt. Ltd., Chennai by DPPH (1, 1 Dipheny1 2-Picryl hydrazyl) free radical scavenging method. An accurately weighed quantity (1.0135 gm) of the methanolic extract was dissolved and made up to 50 ml with 80 % Methanol (2% w/v) and kept as a stock solution. This was further diluted with 80 % methanol to obtain different concentrations (Test extract). The test was carried out by adding 0.5 ml of 0.022 % DPPH free radical solution (SIGMA-Aldrich) to the extract. The resultant solution was incubated at room temperature for 30 minutes and absorbances were measured at 517 nm. Control absorbance was also taken by using DPPH solution. Ascorbic acid was used as standard The percentage activity was calculated by using the following formula:

$$\% \text{ Antioxidant activity} = \frac{\text{Control (ac)} - \text{Test (at)}}{\text{Control (ac)}} \times 100$$

Where ac = Absorbance of control
And at = Absorbance of Test
IC_{50} = (50 % Inhibition concentration) value was obtained by linear regression method using percentage activity concentrations.

RESULTS AND DISCUSSION

In the present study, the percentage composition of protein, carbohydrates and fat of the gastropod *Pleuroploca trapezium* meat are in comparison with the values given for molluscs in general by Mukundan (1968) [13]. He reported that molluscs have approximately 8-10% of proteins (by weight), 4-5% of carbohydrates, 2-3% of minerals with but 1-2% of fat (Table1). The edible part of *P.trapezium* meat was found to be low in fat with 1.74%. Ackman (2000) [14] reported that oysters, mussels, clams etc. are lean, lipid ranging from 1-3% and in crustaceans the fat content ranges from 0.5-1.5 %. Seafood is generally low in fat, and are not considered as a significant source of fat-soluble vitamins, A, .D, E and K, but the water soluble vitamins like Niacin, B_{12}, B_6 and thiamine are found in fair amounts with little Vitamin C [15]. In *P.trapezium* meat also the vitamins were present as in other seafoods (Table 1). Seafood has also been reported as an excellent source of minerals and fishes are one of the most important sources of calcium. Other minerals in seafood include Zinc (oysters and crustaceans), iron (oysters, bluefish and shrimp), copper (oysters, crabs and lobsters), potassium (mussels, scallops and clams), and iodine, phosphorus, and selenium (all seafood in general) (15). Devadas (1994) [16] reported that fish and other seafoods are rich in calcium, phosphorus, sodium, iron and zinc. The sodium content in seafood is generally low but in *P.trapezium* high amount (120 mg/100g) was observed as in the case of other molluscs (1968) [13]. The important nutritional elements like Calcium, Phosphorus, Magnesium, Potassium and Sodium are present in *P.trapezium* meat in appreciable quantities to provide the nutritional requirement of these minerals in the diet of human population (Table 1).

Table 1

Parameters	**Quantity**
Protein %	10.348
Carbohydrate %	4.307
Lipid %	1.74
Vitamins:	trace amount
Vitamin B_1	trace amount
Vitamin B_2	1.01mg
Vitamin B_6	1.01mg
Vitamin B_{12}	0.2509mcg
Vitamin C	0.189mg
Niacinamide	0.0125mg
Minerals:	
Calcium	8.09mg
Magnesium	3.11mg
Sodium	120mg
Potassium	78.5mg
Phosphorus	2.98mg
Trace Metals:	
Iron	0.025mg
Zinc	0.008mg
Copper	Absent
Lead	Absent

Trace metals like Iron and zinc were present in *P.trapezium* meat as 0.025 and 0.008 mg/100g respectively, whereas copper and lead were undetectable (Table 1). It has already been reported that among the heavy metals iron, copper, zinc, cobalt, manganese, magnesium, molybdenum, selenium, nickel, chromium and tin are biologically essential for the correct functioning of biochemical processes [17]. Karppahen and Stabel (1976) [18] reported 0.07 to 2.26 ppm and 0.10 to 17.0 ppm copper in fresh fish and fish products respectively. Zinc is an important element performing a variety of functions in the body, as it is a cofactor for a number of enzymes. The recommended daily dietary requirement of Zinc for an adult according to NRC, USA is 15mg. Higher levels of Zn were observed in squid (24.25 ppm) and cuttlefish (24.02 ppm) [19] followed by fishes (5-10 ppm) [20]. Metals such as lead, cadmium, mercury and arsenic are considered as non-essential and are not required for sustenance of life. These metals are particularly toxic in high concentration and usually inhibit enzymatic activities. These metals were not encountered in the present study, and thus the meat of *Pleuroploca trapezium* is safe for human consumption.

The capacity of marine animals especially the bivalve mollusks to accumulate potentially toxic trace metals in their tissues, far in excess of ambient level is well known [21], [22], [23]. In contrast, the carnivorous gastropods have been found to contain lesser amounts of trace metals in their tissues than in the animals they were feeding. Nott and Nicolaidou (1989) [24] reported that in marine gastropods, high concentrations of heavy metals occur in the digestive gland where these metals are accumulated within intracellular mineralized granules as phosphates and within lysosomal residual bodies in association with sulphur in a form that renders them unavailable to the general metabolic processes of the animal and also to a carnivore that eats the tissue. Thus, uptake by the first animal results in bioamplification but transfer to the second animal results in bioreduction. Lack of biomagnification in carnivore gastropod along a food chain has also been reported by Young (1977) [25] and Ireland and Wootton (1977) [26] . In the present study also, the trace metals were found in less quantities in the foot and adductor muscles, and this may be attributed to the lack of biomagnification in the carnivorous gastropod *Pleuroploca trapezium.*

Fish and shellfish provide an almost unlimited variety of fatty acids with beneficial role in human health. There are no risks attached to these fatty acids and generally, except for deep fried portions, they are relatively low in fat compared to farm meats. The analysis of fatty acid profile of *Pleuroploca trapezium* meat revealed the presence of eicosapentaenoic acid (EPA), which is an omega – 3 polyunsaturated fatty acid (Fig. 2 and Table 2).

Fig. 2

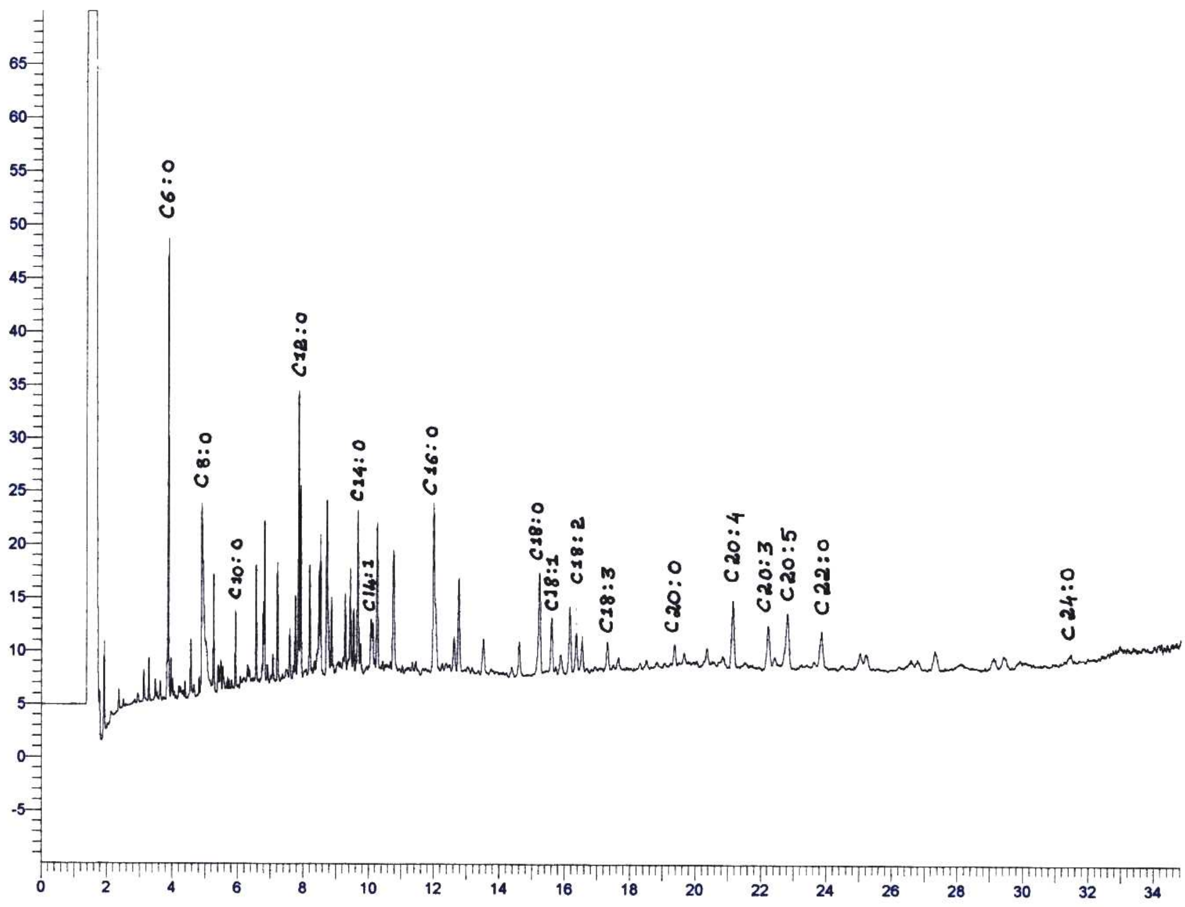

Table 2

Fatty acid		Composition (mg/100 mg of lipid)
Saturated Fatty Acids:		
C6:0	Caproic acid	0.022
C8:0	Caprolyic acid	0.166
C10:0	Capric acid	0.050
C12:0	Lauric acid	0.093
C14:0	Myristic acid	0.600
C16:0	Palmitic acid	0.088
C18:0	Stearic acid	0.050
C20:0	Arachidic acid	trace
C22:0	Behenic acid	trace
C24:0	Lignoceric acid	trace
Mono Unsaturated Fatty Acids (MUFA):		
C14:1	Myristoleic acid	0.035
C18:1	Oleic acid	0.0028
Poly Unsaturated Fatty Acids (PUFA):		
C18:2	Linoleic acid	0.640
C18:3	Linolenic acid	0.008
C20:3	Eicosatrienoic acid	trace
C20:4	Arachidonic acid	0.004
C20:5	Eicosapentaenoic acid (EPA)	trace

The essential fatty acids such as the polyunsaturated Linoleic and the Linolenic acids were also present. The C_{20} and C_{22} marine polyunsaturated fatty acids, often referred to as simply "omega-3" fatty acids, participate in complex body metabolic processes [27], [28]. Recent epidemiological studies have suggested that the consumption of oceanic fish containing omega – 3 polyunsaturated fatty acids may offer considerable protection against cardiovascular disease. Dietary omega-3 fatty acids have also been found to alter favourably plasma lipid levels including triglyceride lowering, reducing blood pressure in individuals with mild hypertension, as well as inhibiting atherosclerosis (hardening of arteries) in various animal trials. Recent evidence also indicates that omega 3 fatty acids may offer potential benefit in various inflammatory disorders including rheumatoid arthritis, psoriasis of the skin etc. The presence of abundant levels of omega-3 fatty acids in seafood may offer potential marketing strategies for encouraging the consumption of seafood based on the health effects of the unique fatty acid components [29] reported crab as good source of EPA. Linolenic acid itself seldom exceeds 1% of the fatty acids of marine animal lipids. Linolenic acid is also beneficial [30]

Table 3

Sample	Concentration (micro g/ml)	Absorbance		Anti Oxidant Activity %	Ic_{50} (micro g/ml)
		Control	Sample		
Meat Extract	810	0.437	0.392	10.297	4021
	1620	0.437	0321	26.545	
	2430	0.437	0.286	34.554	
	3240	0.437	0.251	42.563	
	4050	0.437	0.217	50.340	
Ascorbic acid					0.6422

The other major series of Polyunsaturated Fatty Acids, called omega-6 series, are chiefly represented in marine lipids by the parent 18:2n6 (linoleic acid) and its successor 20:4n6 (arachidonic acid) and these two seldom totally more than 2% of total fatty acids. Linoleic acid also exhibits some potential to lower total blood cholesterol levels. These PUFAS are complemented by palmitic acid (16:0) with rather less myristic acid (14:0) and relatively little stearic acid (18:0). The relative proportions in fish lipids of 20:1 and 22:1 are variable since these are of exogenous origin [31]. In the present study also the fatty acids such as linoleic acid, arachidonic acid, palmitic acid, myristic acid and stearic acid were present in the fatty acid profile of *Pleuroploca trapezium*. Rebhung, (1997) [32] reported that palmitic acid, stearic acid, oleic acid, linolenic acid, arachidonic acid, docosatetraenoic acid, docosapentaenoic acid, docosahexaenoic acid and eicosapentaenoic acid as major fatty acids in the gastropod, *Trochus niloticus*.

Antioxidants are chemical substances that, when present at low concentrations, compared to those of the oxidisable substrates considerably delay or inhibit oxidation of the substrate. A free radical may be an atom or a molecule with one or more unpaired electrons. The free radicals are capable of independent existence and can cause oxidative tissue damage [33]. Free radicals are produced *in vivo* from various biochemical reactions and also from the respiratory chain as a result of occasional leakage [34]. Increased production of reactive oxygen species or a decreased efficiency of an antioxidant system appears to be a major contributing factor to a number of degenerative processes such as cancer, coronary heart diseases, cataract, arthritis, ageing, and AIDS [35]. This implies that the formation of free radicals largely through the oxidative damage leading to tissue injury is responsible for most if not all human diseases. Thus antioxidants which can scavenge free radicals have an important role in biological system and their use is implicated in the prevention of cancer, heart diseases, aging etc. Antioxidants act at several different stages in an oxidative sequence. They act as (a) scavengers, imitating free radicals such as hydroxyl, aloxyl and paroxyl species, (b) breaking the chain of initiated sequence, (c) quenching or scavenging singlet oxygen. The gastropod *P.trapezium* meat is having natural antioxidant potential as its methanolic extract was found to exhibit a good scavenger of DPPH radical with an IC_{50} value of 4021 micro gram/ml (Table 3).

The present study highlights the nutritive value of the meat of *P.trapezium* such as its protein content, low lipid content, presence of vitamins, minerals and trace metals, PUFAs such as Linoleic, Linolenic, EPA, Arachidonic and Eicosatrienoic acids. The meat also has good antioxidant activity, hence justifying the need to popularize this meat as important seafood. The information of the nutritional quality of this meat should be transferred to the local fishermen in order to give them an idea of the value of this resource so that effective actions can be taken for the proper utilization of this gastropod resource.

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