Biochemical Composition and Preliminary Qualitative Analysis of Marine Clam *Gafrarium divaricatum* (Gmelin) From Mumbai, West Coast of India

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ABSTRACT :

The present work investigates the potential use of the whole tissue of clam *Gafrarium divaricatum (Gmelin)* as a food source in a coastal region of India. In order to evaluate its food value, a detailed biochemical analysis of the whole tissue has been carried out. The results of biochemical analysis show very high protein content (26.32%), Carbohydrate (11.23%) and lipid (1.29%). The protein consists of 10 essential and 9 non essential amino acids which are as follows (Lysine 14.36%, Histidine 9.02, Methionine 8.92 %) and (Alanine 5.94%, Aspartic acid 4.98, Asparagine 3.79, Tyrosine 3.52 and Proline 3.21%) are the predominant essential amino and non essential amino acids. *G. divaricatum* consists of 6 different fatty acids out of which 2 are saturated fatty acids (SFA), one monounsaturated fatty acids (MUFA) and three polyunsaturated fatty acids (PUFA). SFA, MUFA and PUFA content was 27.18, 11.02, 12.47, 17.96, 11.38 and 11.38% respectively in fat (1.29 gm/100gm). In addition it contains Vitamins such as Vitamin A (112.3 IU), Vitamin C (24.11 mg/g), D (13.96 IU), B_{12} (1.98µg/g.), E (1.14 mg/g), K (0.59 mg/g) and B_6 (0.31mg/g). Mineral composition of whole tissue showed presence of Calcium 312.74 mg/g, Sodium 89.93mg/g, Magnesium 61.11 mg/g, Potassium 21.38 mg/g as major and Copper 1.43, iron 1.37 and zinc 0.38 mg/g as minor quantity. The test for secondary metabolites shows the presence of alkaloids, phenolics, terpenes, carotenoids and steroids. This shows that the clam species is a good alternative food source to fish and can be very well exploited after its toxicity evaluation. The presence of secondary metabolites will have some medicinal values like hepatoprotective activity and anti oxidant activity which is currently under investigation.

Keywords: Antioxidant activity, Biochemical analysis, Gafrarium divaricatum, Hepatoprotective activity and Secondary metabolites.

INTRODUCTION:

The biochemical analysis is also known as percentage composition of some fundamental elements like water, protein, lipids, carbohydrate and minerals for human diet [Ramakrishnan and Venkat rao, 1995]. The high protein content foods availability is the biggest problem in some developing countries. The knowledge about nutrition of edible living organisms is tremendously significant since the nutritive value is reflected in its biochemical analysis [Nagabhushanam and Mane, 1978]. The edible species of Marine bivalve mollusks are tasteful and it will get more importance next to fish and prawn. Marine mollusks are economically important species and it is easy to cultivate in coastal region. The marine mollusks are having leading components of bivalve fishery in aquaculture coastal area [Jones and Alagarswami, 1973] and it forms an important source of nutrition for coastal folks [Verlecar et al., 2006 and Parulekar et al., 1984]. It is not only exploited as a food source, it has enormous contents of natural bioactive compounds which are being identified, isolated and characterized with vast scope for treatment of human ailments [Fenical, 1993]. Now a day, many researchers have focused their research on mollusk to isolate secondary metabolites, although some tiny part of secondary metabolites only investigated from marine mollusk.

Some marine gastropods and bivalves have got an immense inquisitiveness to produce bio-natural products, yielding a variety of active compounds and several drugs leads are presently in clinical trial [Cimino and Gavagnin, 2006].

Based on the mollusks evolutionary records and one kind of life style of the species, recurrent for minor classes of Mollusks species is unfounded due to the lack of knowledge about novel pathways for secondary metabolism in this family. Much more arguments are enduring about the secondary metabolites of the phylum Mollusks because of lack of clarity of natural active secondary metabolites production is everywhere contained by the phylum Mollusks. Hence, in future the drug discovery fields will get excellent scope on this phylum and it is good to explore novel bioactive natural products with newer mode of action in the phylum of mollusks. [Sreejamole and Radhakrishnan, 2010]

The present work investigates the Biochemical analysis and qualitative preliminary evaluation of the major secondary metabolites present in the whole tissue of the clam *Gafrarium divaricatum* in Methanol and n-hexane extracts.

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MATERIALS AND METHODS

Collection of sample:

Investigated species were collected during low tide period from Nariman Point area of Mumbai coast and the collection of species are immediately transferred to laboratory in live condition. Specimens were then washed with distilled water to remove associated debris for methanol extract, the species of *G. divaricatum* 200 g. were dissected and the whole body tissues were blot dried with tissue paper to remove extraneous water content of the tissues. The whole body tissues (10% w/v) were homogenized and extracted in methanol (90% v/v) by agitation in rotary shaker for 24 hours. The step wise methanol extraction procedure included repeated extractions at every 6 hours intervals. Initially, the whole extract contents were centrifuged (8000 × g for 10 minutes at 4 °C) and supernatant was collected in separate vial. The tissue pellet obtained in consequent steps was further treated similarly with methanol to achieve maximum extraction and recovery of the bioactive compounds. All the fractions were finally pooled together, filtered through Whatman filter paper No.1 and concentrated through Rota evaporator and stored in deep freezer for further analysis.

The n-hexane extract, the 200 gram of clam species were homogenized and extracted with n-hexane and then agitated for 15 minutes by using a magnetic agitator. The extract was filtered through filter paper made up of cellulose under vacuum, the residue was repeatedly extracted and final extracts were made up to 3 mL and stored in deep freezer at -20 °C for further analysis [César J Lodeiros et al., 2001].

Estimation of Protein:

The Folin-Ciocalteu Phenol method of Lowry et al., was used for the estimation of total protein content in the catfish [Lowry et al., 1951].

Estimation of Carbohydrate:

The total carbohydrate level was estimated by phenol- sulphuric acid method of Dubois et al., 1956.

Estimation of Lipid:

The total lipid content was estimated gravimetrically by following Folch et al., [Folch et al., 1957].

Estimation of amino acids:

The experimental samples were finely grounded for estimating the amino acids in the HPLC (Merck Hitachi L-7400) following the method of Baker et al., 1994.

Fatty acid analysis:

For fatty acid analysis, the samples were homogenized with chloroform: methanol (2:1 v/v) mixture and they were extracted using the method of Bligh et al., 1959. After the fat extraction, they were esterifies with 1% H_2So_4 and fatty acid methyl esters were prepared by following the procedure of AOAC (1995). The identification and quantification of fatty acids were done using Gas Chromatography (Hewlett Packard 5890 model).

Estimation of vitamins:

The fat soluble vitamins A, D, E and K and the water soluble vitamins B1, B6, B12 and C were analyzed in the HPLC (Merk Hitachi L-74000) following the method described by Sadasivam and Manickam (1996). The folic acid was estimated by following the calorimetric procedure of Sethi (1997). The pyridoxine, panthothenic acids were estimated by following the methods suggested in USP NF 2000 Asian edition [Sethi, 1997].

Estimation of minerals:

The minerals were estimated by following the method of Guzman and Jimenez [Guzman and Jimenez, 1992].

PRELIMINARY IDENTIFICATION OF THE CHEMICAL COMPONENTS TEST

The chemical elements of alkaloids, flavonoids, phenolics, saponins and sterols are present in the three extracts of *P. malabarica* were carried out using various general detection reagents as described by [Cannell, 1998].

Thin layer chromatography (TLC) of the extracts:

The Concentrated extracts dissolved in suitable solvents and each extracts were spotted on readymade TLC plate (10 x 10cm) and allowed to develop with individual solvent systems for each extracts. A few drops of ammonium solution were added to the solvent system for methanol and aqueous extracts for better resolution. The developed TLC plates were visualized under UV lamp fixed in UV chamber; afterward they were exposed to iodine vapor to visualize the components which were UV invisible. The solvent systems used for each extracts were given below.

Methanol extract:

Methanol: Dichloromethane: chloroform (30:35:35)

Acetone & n-Hexane (1:3) extract:

Hexane: ethyl acetate (70:30)

Spray reagent detection method on TLC:

The developed TLC plates were visualized under UV light after spraying with various spray reagents to find out the presence of secondary metabolites like alkaloids, phenolics, steroids and terpenes according to standard protocols illustrated by [Cannell, 1998].

RESULT

Proximate composition:

In this present study the protein, carbohydrate and lipid content of *G. divaricatum* was investigated and the marine clam was found to contain 26.32%, 11.23% and 1.29% respectively. As per the result the protein is the predominant source in this clam. Table 1 shows the proximate composition of *G. divaricatum.*

S.No:	Proximate composition %	
1	Protein	26.32
2	Carbohydrate	11.23
3	Lipid	1.29
4	Ash	5.56
5	Moisture	6.11

Table 1: Proximate composition of *Gafrarium divaricatum.*

Essential and non essential amino acid:

In this study totally 10 essential and 9 non essential amino acids are discovered in the investigated sample. Lysine 14.36%, Histidine 9.02, Methionine 8.92 % are the predominant essential amino acid and Alanine 5.94%, Aspartic acid 4.98, Asparagine 3.79, Tyrosine 3.52 and Proline 3.21% are the predominant non essential amino acids. Ta-

ble. 2 & 3 shows the essential and nonessential amino acid level.

S.No	Essential Amino Acids %	
1	Threonine	1.41
2	Arginine	0.16
3	Histidine	9.02
4	Valine	7.26
5	Methionine	8.92
6	IsoLeucine	5.53
7	Phenylanine	2.31
8	Leucine	6.54
9	Lysine	14.36
10	Tryptophan	3.89

Table 2: Essential amino acid of *Gafrarium divaricatum.*

S.No	Non-essential Amino Acids %	
1	Aspartic Acid	4.98
2	Glutamic Acid	1.47
3	Asparagine	3.79
4	Serine	0.13
5	Glycine	2.18
6	Alanine	5.94
7	Cysteine	1.02
8	Tyrosine	3.52
9	Proline	3.21

Table 3: Non-essential amino acid of *Gafrarium divaricatum*

Fatty acid profile:

In *G. divaricatum* totally 6 different fatty acids were established in whole body tissue; they are 2 saturated fatty acids (SFA), one monounsaturated fatty acids (MUFA) and three polyunsaturated fatty acids (PUFA). Among the SFAs Palmitic (C16:0) was the major acid. The percentage availability of SFA, MUFA and PUFA content was 27.18, 11.02, 12.47, 17.96, 11.38 and 11.38% in *G. divaricatum* (Table. 4).

S.No	Fatty Acids profile %	
1	Palmitic Acid	27.18
2	Stearic Acid 18:0	11.02
3	Oleic Acid 18:1	12.47
4	Linolenic Acids	17.96
5	Alpha Linolenic Acid	11.38
6	Moroctic Acid 18:4	8.11

Table 3: Fatty acid profile of *Gafrarium divaricatum.*

Vitamins and Minarals:

The Vitamin compositions of studied clam's level are presented in Table 5. In this study it is observed to have 7 Vitamins and in these vitamin A 112.3 IU is observed as a highest level in the whole body tissue followed by Vitamin C, D, B_{12}, E, K and B_6 are 24.11 mg/g, 13.96 IU, 1.98µg/g., 1.14, 0.59 and 0.31 respectively.

Minerals are present in very good level in *G. divaricatum.* In this Calcium 312.74 mg/g, Sodium 89.93mg/g, Magnesium 61.11 mg/g, Potassium 21.38 mg/g is the predominant minerals on the investigated species. Copper 1.43, iron 1.37 and zinc 0.38 mg/g are observed in minor quantity. The list of minerals which are present in the body tissue is given in Table. 6.

S.No	Vitamins	Mg/g
1	Vitamin-A	112.3 IU
2	Vitamin-D	13.96 IU
3	Vitamin-E	1.14
4	Vitamin-B6	0.31
5	Vitamin-B12	1.98 µg/g
6	Vitamin-C	24.11
7	Vitamin-K	0.59

Table 4: Vitamins of *G. divaricatum.*

Qualitative analysis:

The occurrence and nonappearance of biochemical elements are found out by preliminary qualitative chemical analysis. The results are shown in Table 5.

The different kind of biochemical elements of clam species are identified by Thin Layer Chromatography (TLC) system. The methanol extracted species of *G. divaricatum* show different colored spots on TLC using the solvent system of n-hexane: ethyl acetate (7:3). Under UV observation of developed TLC plate, 254 nm and 365 nm developed TLC plate showed complete different color spots like pink, yellow, brown, violet and some other colors spots too. The Rf values on the TLC are shown in Table 6. In n-hexane and methanol extracts the numbers of spots shown are seven and five respectively. The colorful spots are observed only under the UV radiation. The florescent spot under UV are due to presence of carotenoids (astaxanthin and its methyl esters) Through the Spray reagent detection method the developed TLC plates of the two solvent extracts showed the presence of some organic compounds like alkaloids, phenolics, terpenes and steroids (Table 7).

S.No	TEST	Methanol	Acetone & n-hexane
1	**Alkaloids**		
	Mayer's test	-V	+V
	Dragendorff's reagent	-V	-V
	Wagner's reagent	-V	+V
2	**Flavonoids**		
	Shinoda's test	-V	-V
3	**Poly phenols**	+V	+V
4	**Sterols**		
	Liebermann-Buchard test	+V	+V
	Salkowski reaction	+V	+V
5	**Saponins**	+V	-V

Table 5: Components of *G. divaricatum* extracts identified by general detection reagents.

n-hexane	Methanol
A1- 0.08	M1- 5.6
A2- 0.15	M2- 5.7
A3- 0.22	M3- 4.3
A4- 0.24	M4- 4.5
A5- 0.37	-
A6- 0.68	-
A7- 0.81	-

Table 6: Rf value of different extract of *G. divaricatum.*

S.No.	Identified compounds of the three extracts by Rf value		
	Compounds	Acetone & n-hexane	Methanol
1	Alkaloids	A1, A2 & A3	-
2	Phenolics	A7	-
3	Terpenes	A4 & A7	M3& M4
4	Steroids	-	M1 & M2

Table 7: Compounds detected in the extracts *G. divaricatum* using different spray reagents on TLC.

DISCUSSION

Marine foods are the very essential food source for many folks due to their great nutrition contents and economically cheap. Marine natural products have drawn the attention of researchers in recent years due to their pharmacological value. Protein is essential for the sustenance of life and exists in largest quantity of all nutrients as a component of the human body [Okuzumi and Fujii, 2000]. In this current study *G. divaricatum* having good content of protein, carbohydrate, lipid, ash and moisture content like 26.32%, 11.23%, 1.29%, 5.56% and 6.11% respectively. Our present research revealed that the protein content is more prominent when compared to the carbohydrate and lipids contents on this sample. Therefore, the results suggest that the *G. divaricatum* can be considered as another potential food source for of providing cheapest animal protein. Much more work is needed to carry out, on research some other marine mollusks. Some researchers have reported 11.9 % of protein has been observed in surf clam *Mactra violacea* [Laxmilatha, 2009]. Arularasan, 2009 has studied the protein content on male and female species of an edible sea snail of *Strombus canarium* that has protein content in the range from 47.86 to 70.18% taken from Gulf of Mannar. Shanmugam et al., (2001) have mentioned the protein level of *Bursa spinosa* which varied from 18.71 to 29.81% at Parangipettai coast. 23.51% of protein was observed by Periyasamy et al., 2014 on Marine Bivalve *Donax incarnatus* at Cuddalore coast. Carbohydrates are a group of organic compounds that includes sugars, starches and fiber, which is a major source of energy for animals. In our examined Clam species the carbohydrate level is around 11.23%. Manikandarajan et al., and Eswar et al., have analyzed the carbohydrate level at Parangipettai coast on Catfish, *Arius maculatus*, *Plotosus lineatus* and puffer fish of *Lagocephalus inermis* and *Lagocephalus lunaris.* These show carbohydrate level varying from 2.15 gm to 1.98 gm head and body part of the cat fish, 1.87 % and 1.96 % at whole body tissue of Puffer fish. Carbohydrate levels of 0.84 % to 3.04% are noticed on *Pythia plicata* by Shanmugam (1987). When compared with fish, the molluscan species are very cheap and economically valued.

The lipids are highly well-organized source of energy, in that they contain more than twice the energy of carbohydrate and proteins. 1.29% of lipid content is noticed on an investigated clam sample. In male and female species of *Rapana rapiformis* the lipid content is 0.85-2.12% and 0.95-2.96% respectively [Rajkumar, 1995]. In *Babylonia zeylanica* and *Pleuroploca trapezium* species 10.38% and 1.97% of highest lipid content were noticed by Nirmal (1995).

Protein values are obviously reflected in the essential amino acids concentrations. Present study found total of 19 essential and non essential amino acids. This study reveals that the clam meats are higher in essential amino acids (59.4%) than that of (26.24%) of nonessential amino acids. The result reveals that the meat *G. divaricatum* is a potential source of food due to an elevated level of quality protein, as well as balanced essential amino acids. Ajaya Bhaskar, Arularasan et al., and Babu et al., have observed the amino acid concentrations on different marine mollusks in South East Coast of India. They observed that the total amino acid level in *Perna viridis* is 95.76%, *Crassostrea madrasensis* is 98.4% and in *Meretrix casta* is 65.17%. *Strombus canarium* is 80.97% of essential and 15.07% of non essential amino acids, *Bursa spinosa* it is 50.01% of essential amino acids and 46.79% of nonessential amino acids respectively. This study clearly demonstrates that these marine mollusks might be a good potential source of amino acid for all sections of people who suffer from malnutrition.

Periyasamy (2014) has reported 28.13 % of palmitic acid and 18.74 % of Linolenic acid on *D. incarnatus* from Cuddalore Southeast coast of India. Shanmugam, in (2007) has reported the *Donax cuneatus* to contain good quantity of saturated, mono and polyunsaturated fatty acids in the range of 35.28%, 12.71%, 11.72% respectively. This study suggested that the marine animals are richest source of PUFA.

Mollusk are containing a variety of minerals, vitamins, essential and non essential amino acids and high quality protein [King et al., 1990; Leu et al., 1981; Connor and Lin, 1982; Skonberg and Perkins, 2002; USDA, 2003; Okuzumi and Fujii, 2000 and Periyasamy et al., 2014]. Pigott and Tucker stated that the flesh of fish and shell fish are important sources of vitamin A. In these studies, Clam body tissue showed the major source of vitamin A and vitamin C which constituted 105.6 and 23.84 mg/g. Ajayabhaskar (2002) has studied the vitamin level on three different mollusk species green mussels *P. viridis*, true oyster of *C. madrasensis* and yellow clam species of *M. casta.* These species contain a significant level of Vitamins like Vitamin B1 (0.11), B2 (031) and B6 (0.31). Shellfish covered in the present study, showed complete range of vitamins as required for good health.

Minerals also constitute important components of hormones, enzymes and enzyme activators in human nutrition [Khan, 1992]. Mineral components such as sodium, potassium, magnesium, calcium, iron, phosphorus and sulphur are important for human nutrition [Erkan and Ozden, 2007]. Srilatha et al., (2013) have estimated the Vitamins content on Clam *M. casta* from station one Parangipettai and second station of Cuddalore coastal area. She has found the level of the vitamin A (14.40IU, 8.200IU), vitamin D (200IU, 150IU), vitamin E (1.18 mg/g., 1.06 mg/g) and Vitamin K (0.62 mg/g, 0.18 mg/g) and from the Station I and II. Ozden and Erkan have studied the vitamin B mainly in muscle of gonad and eggs, while B2 was detected in the digestive gland, gonad and eggs. The main source of

vitamin B1 (0.11), B2 (0.31) and B6 (0.31) were identified on *P. viridis*, *C. madrasensis* and *M. casta* by Ajaya Bhaskar. Preliminary chemical evaluation of the Methanol and n-hexane extracts of clam *G. divaricatum* has demonstrated the presence of secondary metabolites like alkaloids, poly phenols, sterols and saponins. Sreejamole & Radhakrishnan, (2010) and Eswar et al., (2015) have estimated the preliminary qualitative biochemical estimation of the different extracts from Green mussel *Perna viridis* and Clam *Paphia malabarica* species. They have also visualized the different colored zones on developed TLC plate under UV light which showed similarity in Rf values to the separated bands for extract of *P. viridis* and *P. malabarica*.

In this present investigation, *G. divaricatum* extracts have shown various group of secondary metabolites like alkaloids, polyphenols, terpenes, steroids and saponins on TLC and detected by spray reagent detection method. Under UV light the developed TLC plate showed completely different color spots with different Rf values on n-hexane extract and while in the methanol extract some spots have shown fluorescence and some have not. It might be due to the fact that some analysts have not observing the spots on visible or UV radiation. In general, the chromatographic zones usually appear in dark background only. The whole chromatogram of n-hexane extract illustrated fluorescence on UV light, that reflected individual zones of different colors like blue, green, purple and white. These colors indicate the presence of alkaloids, flavanoids and saponins.

In normal light absorbance, some separated chromatographic zones appear as colorless. Some components of methanol extracts that are not visible in the normal light were detected under UV and exposing to iodine vapors.

In the present study the *G. divaricatum* extracts has shown flavonoids in methanol extract. Flavonoids and polyphenolic are a good source of potent of antioxidant agents, may also be a potential therapeutic agents against a wide range of diseases, disorders and it may have potential to decrease a variety of tumors and preventing menopausal symptoms in human diet. Polyphenols with its phenol rings can act as electron scavenger to reduce free radical activity [Cushnie and Lamb, 2005; DeSousa et al., 2007; Chakraborty et al., 2013 and Meenakshi et al., 2009].

The n-hexane extracts of clam showed the occurrence of alkaloids and the alkaloids are isolated judiciously from some marine mollusks organisms where aliphatic nitrogen holding elements are quiet uncommon [Cannell, 1998]. The physical, chemical and biological characteristics of phenolic compounds are used as drugs in many diseases and disorders [Aliyu, et al., 2009]. In the clam *G. divaricatum* extract showed the positive results on steroids and it is important element to the human diet because it has connection with sex hormone [Okwu, 2001]. Saponins are also found in clam extracts and Chinese traditional medicine. These are considered as key ingredients and it is increasingly becoming clear why these clams show natural medicinal activities. Saponins are identified as an inhibitor of anti-inflammatory activity. The physical, chemical and biological characteristics of saponins have uses in drug productions. These saponins show at higher concentrations antimicrobial, anti inflammatory, anti-feedent and hemolytic effects [George et al., 2002; Mandal et al., 2005 and Xu et al., 1996].

Conclusion:

The clam *Gafrarium divaricatum* has been found to be a rich source of protein. It also contains carbohydrates, lipid, Minerals and Vitamins. The presence of varies secondary metabolites such as alkaloids, phenolics, terpenes, carotenoids and steroids shows a tremendous potential for its medicinal value. Currently its therapeutics potential for hepatoprotective activity and anti oxidant activities is under study in vivo animal models.

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