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DETERMINATION OF SELECTED BIOCHEMICAL CONSTITUENTS OF TWO COMMON EDIBLE MOLLUSC SPECIES, *PERNA VIRIDIS* (GREEN MUSSEL) AND *MARCIA RECENS* (HOLTEN, 1802) (YELLOW FOOT CLAM) FROM SELECTED SITES IN THIRUVANANTHAPURAM AND KOLLAM DISTRICTS

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Abstract

Molluscs comprises a diverse array of invertebrate animals found in marine, freshwater, and terrestrial environments worldwide. With over 100,000 described species, mollusks exhibit remarkable morphological diversity, ranging from tiny snails to giant squids. The ecological importance of mollusks cannot be overstated. They play vital roles in ecosystems as herbivores, carnivores, scavengers, and filter feeders, contributing to nutrient cycling and serving as food sources for numerous organisms. Some molluscs, such as oysters and mussels, have significant economic value in aquaculture and fisheries. This study explores the nutritional composition of two common edible mollusk species, *Perna viridis* (green mussel) and *Marcia recens* (yellow foot clam), harvested from the estuarine waters along the southwest coast of India. The investigation focused on determining the protein, carbohydrate, cholesterol, triglyceride, and vitamin C content of these mollusks, providing insights into their potential as seafood sources. Further studies are needed to assess additional health markers and nutritional indices to fully understand the suitability of these mollusks as dietary sources. Overall, this study underscores the nutritional value and potential health implications of consuming *P. viridis* and *M. recens*, highlighting the importance of continued research in this area for fisheries and aquaculture management. Additionally, mollusks are utilized in medical research and environmental monitoring, further highlighting their importance beyond ecological roles. Understanding the diversity and ecological significance of Phylum Mollusca is essential for conservation efforts and the sustainable management of ecosystems.

Keywords Molluscs, mussels, clam, seafood, nutritional value

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INTRODUCTION

Phylum Mollusca is a diverse group of invertebrate animals comprising over 100,000 described species found in marine, freshwater, and terrestrial habitats. They exhibit a remarkable range of morphological diversity, from tiny snails to giant squids. Molluscs typically have a soft body, often enclosed within a calcareous shell, although some species lack shells. Their body plan consists of a head-foot region containing sensory organs and locomotory structures, a visceral mass housing internal organ, and a mantle responsible for shell secretion in shelled molluscs. Molluscs possess a specialized feeding structure called a radula, an open circulatory system, and paired nephridia for excretion. The major classes of mollusks include Gastropoda (snails, slugs), Bivalvia (clams, mussels), Cephalopoda (squids, octopuses), Polyplacophora (chitons), and Scaphopoda (tusk shells). These classes exhibit diverse adaptations for locomotion, feeding, and defense.

Aristotle is the father of the word 'Mollusca'. These soft bodied animals, a large and most important group of invertebrates which occupies all the possible habitats except aerial. According to Darwin Song (2008), molluscs are the largest phylum among the marine water invertebrates and it occupies 23% of total marine living animals. Mollusks play vital ecological roles as herbivores, carnivores, scavengers, and filter feeders, contributing to nutrient cycling and serving as food sources for many organisms. Economically, mollusks such as oysters and mussels are important in aquaculture and fisheries. Additionally, some species, like snails, are used in medical research and environmental monitoring. Overall, Phylum Mollusca represents a fascinating group of animals with widespread distribution and ecological importance.

India boasts of extensive molluscan fishery resources along the long coastline. In the numerous bays, brackish water resources and in the seas around the sub-continent, comprising of marine molluscs belonging to different taxonomic groups, oysters, mussels, window-pan oysters, pearl oysters, chanks, cuttlefish, squid, octopus which have been captured since time immemorial for food, pearls and shells utilized in several ways.

Studies on marine edible molluscs of India date back to the end of the 20th century. According to Yennawar and Tudu, 2014, the molluscs are benthic organisms that live on or in, the bottom of the

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water body with greater than 1.0 mm in size. Its body is made up of head, visceral mass and locomotory or digging foot, epidermis is forming mantle, secrets calcareous spicules or produces one or more shells. Chitinous is ribbon like or radial having small size teeth in the mouth but absent in Bivalves.

Carolus Linnaeus (1758) adopted the name, Mollusca, a term which was in fact proposed by Johnston (1650), but without developing any real concept of the phylum - Mollusca. Cuvier (1795) had shown better understanding of the group, and his concept approximates to modern ideas. In the beginning time, several other groups such as barnacles, brachiopods and other shelled forms animals were classified together with the molluscs.

It is recognized that there are around 85,000 extant species of molluscs. A large number of molluscs also live in terrestrial habitats and also in freshwater bodies like pond, like, river, reservoir, cannel etc. They are very highly diverse group, not only in anatomical structure and in size, but also in habitat and in behaviour. Molluscs species are found 10190 meter deep in the ocean to 5000 meter of elevation. It is a highly diversified group, differs from other groups in size, shape, number as well as its habit and habitat.

Winckworth (1940), estimates 31643 numbers of marine molluscs species, 8765 numbers of freshwater molluscs species and 24503 numbers of terrestrial molluscs species, making a total numbers of 64,911 molluscs species (approx. 65,000). Subba Rao, Dey and Barua (1995) made a conservative estimate 66535 numbers of molluscan species of which the Indian share is 5070 numbers of molluscan species, among them 3400 marine molluscs, 183 fresh water molluscs and 1487 terrestrial molluscs.

Chapman, A. D. (2009), estimates of accepted described living species of molluscs vary from 50,000 to a maximum of 120,000 species. In 1969 David Nicol estimated the probable total numbers of living molluscs at 107,000 of which were about 12,000 freshwater, 35,000 terrestrial and 60000 marine molluscs. The Bivalvia among the marine invertebrates would comprise about 14% of the total and the other five classes is less than 2% of the living molluscs. Haszprunar in 2001 estimated about 93,000 molluscs species which include 23% of all named marine living organisms.

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Molluscs species are second only to arthropods in numbers of all living animal species far behind the arthropods - 1,113,000 but well ahead of chordates - 52,000. About 200,000 living species in total are estimated and 70,000 fossil species, although the total number of molluscs species ever to have existed, whether or not preserved, must be many times greater than the number alive today.

The coastal strip of land where land meets the sea is the home of the richest marine communities. The total number of biomass in a square meter at the low-tide line is at least ten times higher than that of in most abyssal area. Invertebrates are the one of major component of this fauna. They are also the major component in all existing marine habitats. They all together help the entire ecosystem. Measurements of change in marine molluscs communities have for several decades been widely used in identifying and monitoring human impacts on the sea.

Molluscs community analyses have proven to be useful in assessing the environmental impacts of coastal discharges, chemical contamination of the sediments, commercial dredging, sludge dumping, trawling, oil exploration and introduced marine pests. This is largely because benthic organisms are relatively non-mobile and integrate effects of the pollutants over time. Most importantly, however, molluses organisms are comparatively easy to sample identify and count.

The marine molluscs have an important place in an ecosystem for maintaining it. They are the major food source for humans as well as other secondary consumers in the ecosystems which are also important bio-resource like fishes. Molluscs play a major role in ecosystem by filtering phytoplankton and then acting as a good source of food for higher organisms living in upper tropic level such as fishes. They oxygenate the bottom by reworking sediments and play a basic role in breaking down organic materials before bacterial remineralization. They are also used as biological indicators because they can provide information on environmental conditions either due to the sensitivity of single species (indicator species) or because of some general feature that makes them integrate environmental signals for a long period of time. Their role in the ecosystem cannot be over looked as many of them are commercially important species and other are biological indicators as well as important in food web.

Molluscs exhibits of significant diversity in colouration, sculpture and shell shape. The marine molluscs display flamboyance in colour and within and between the species comparison to the

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terrestrial and freshwater molluscs. Diversity is also evident in molluscs in their feeding habits. They are herbivores, carnivores, scavengers and deposit feeders, suspension feeders. Sometime they are considered as parasites and show commensalism.

India supports extensive bivalve fisheries, notably for mussels, oysters, and clams, with an estimated annual production of 84,483 tonnes (CMFRI 2017). Kerala is considered as the 'mussel fishery zone' of India since extensive natural mussel beds are available in the Malabar Coast and exploitation is done from time immemorial. Two species of mussels, green mussel *Perna viridis* and brown mussel *P. indica* are available in Indian coast and are exploited on a commercial basis for edible purpose. *P.viridis* has got a wider distribution along Kerala, Karnataka, Goa, Maharashtra and also in Andamans. *P.indica* has got restricted distribution in south west coast of India from Varkala to Kanyakumari. Clams form a subsistence fishery in Indian coastal waters, lakes, and estuaries, with a potential yield of 113,189 tonnes and the export from India is dominated by the short-neck or yellow-foot clam. A major part of this export is sourced from the Ashtamudi Lake in Kerala state, a designated Ramsar wetland on the southwestern coast of India.

OBJECTIVE OF THE STUDY

The objective of the present study was to determine the protein, carbohydrate, cholesterol, triglyceride and vitamin C composition of two common edible mollusc species, *Perna virdis* (green mussel) and *Marcia recens* (Holten, 1802) (yellow foot clam), available in abundance in the coastal waters of the southwest coast of India.

REVIEW OF LITERATURE

Molluscs are considered as nutritious seafood and culinary delicacies. The phylum Mollusca represents one of the most diverse groups, comprising about 23% of all named marine organisms. Several published information are available on the nutritional profiling of certain marine molluscs (Chiou *et al*., 2001; Salwa *et al*., 2007). They contain relatively low fat and highpolyunsaturated fatty acids (PUFAs), some of which cannot be synthesised by human and must be obtained from their diet (Smoothey, 2013). Evaluation of proximate profiles is often

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essential to guarantee that they meet requirements of commercial specifications and food regulations. Since 1995, edible bivalves like the Indian backwater oyster, *Crassostrea madrasensiss* and the green mussel, *Perna viridis* have been farmed on a commercial scale in the estuaries of Kerala.

Explorative studies on estuarine molluscs of Asthamudi estuary have been initiated by various researchers, though none of the studies was comprehensive (Divakaran *et al*. 1981; Nair *et al*. 1984a,b; Kripa and Mohammedsalih 1999; Jyothilal *et al*. 2015; Ravinesh *et al*. 2020). Most of the studies are based on the commercial bivalves *Marcia recens, M. opima* and *Meretrix casta*. Only very few previous studies have attempted to document the molluscan diversity of Ashtamudi estuary. Recently Arathi *et al*. (2018,2019) recorded the taxonomical status of clam fisheries resources from the Ashtamudi Lake and Biju Kumar *et al*. (2019) recorded the invasion of the charru mussel *Mytella strigata* (Hanley, 1843) from this estuary.

Considering the importance of bivalves as a source of protein rich food for man, there has been considerable work on the biochemical composition of commercially important species from all over the world. From Indian waters, edible bivalves, mussels, clam and pearl oysters were studied for its biochemical composition. Biochemical analysis is the process of partitioning of components in a feed into six categories based on the chemical properties of the components. The six categories are: moisture, ash, crude protein, crude fat, carbohydrate and minerals. Studies on proximate analysis of different faunal components have been carried out by Ackman, 1989; Bruyer *et al*., 1990; Alikunhi, *et al*., 2010; Doty, 1969, 1970; Garcia *et al*., 2006; Hendriks *et al*., 2002; Hua *et al*., 2005; Kristein, 1999; Lapidus *et al*., 1958; Lucking and Marchen, 1996; Nuckles *et al*., 1990, Ehigiator and Oterai, 2012, Fatima, 1996, Nurjahan, Hafiluddin, Nurhayati Tati, Nugraha Rini, 2012; Baby, Hassan, Kabir and Naser, 2010; Osibona, Kusemiju and Akande, 2006, Kumari and Nair, 1989; Nagabhushanan and Talikhedkar, 1977; Sarvaiya, 1989; Bayne, Salkald and Worrall, 1983; Tenjing Sing, Krishnamoorthy and Trippeswamy, 2012; Chattopadhyay, Rathie and Das, 2013; Nair and Rao. 1974; Aziz *et al*., 2013 and other workers.

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MATERIALS AND METHODS

The study was carried out in Ashtamudi estuary (8.8° 57′ N; 76° 34′ to 76.30′–76.40′ E), Kollam district, Kerala, India, and Perumathura coast (8.6252° N, 76.8000° E) during Feb 2022–March 2022. The Ashtamudi Lake is a large, basin-shaped estuary, some 62 km^2 in area and discharging into the Laccadive Sea through a narrow channel less than 300 m wide (Mohamed *et al*., 2013). It is a unique wetland ecosystem, and the name is derived from the planimetric shape with eight branches radiating the central part. Both the study areas provides livelihoods for hundreds of people involved in clam fishing, preparation and packing (CMFRI2019, Appukuttan 1993, 2016, Appukuttan *et al*. 1999, Mohamed *et al*. 2013). The two edible mollusc species, *Marcia recens* (Holten, 1802) (yellow foot clam) [designated as S1] was collected from Ashtamudi and *Perna virdis* (green mussel) (designated as S2] was collected from Perumathura.

Perna viridis **(Asian Green Mussel)**

Marcia recens (Holten, 1802) (**Yellow Foot Clam**)

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All the specimens were collected by handpicking, hand lift netting and grab sampling and estimation of total carbohydrate, protein, cholesterol and ascorbic acid (vitamin C) was done. The procedures are detailed below:

Estimation of total carbohydrate by phenol sulphuric acid method Stock Solution of Extracts:

10 mg of each extract was accurately weighed and made upto 1ml with DMSO.

Materials required

- 5% Phenol
- 96% Sulphuric Acid

Procedure

To 500µL of the sample, 1mL of phenol solution was added. To this solution, 5mL of 96% Sulphuric acid was added and shake well. After 10 minutes of shaking, it was then placed in a water bath at 25-30^{0}C for 20 minutes. After cooling, the absorbance was read at 490nm. The amount of total carbohydrate present in the sample was calculated using the standard graph of glucose.

Protein Estimation : Bradford Method

The protein in solution can be measured quantitatively by different methods. The method described by Bradford uses the capacity of protein to bind a dye, quantitatively. The method is simple, rapid and inexpensive. The assay is based on the ability of proteins to bind Coomassie brilliant blue G250 and form a complex whose extinction coefficient is much greater than the free dye.

Each samples (10µl) were added with 200µl of diluted dye binding solution. One volume of concentrated dye solution was added with four volumes of distilled water for use. It was mixed well and allowed the color to develop for at least 5mins but no longer than 30 minutes. Absorbance was read at 595nm after incubation. A standard graph was plotted and calculated theprotein concentration using the standard curve. Bovine serum albumin was used as the

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standard.

Cholesterol

Concentration of cholesterol was estimated using Coral Clinical systems Cholesterol kit.

To 10µL of each sample, standard and distilled water, 1mL of working reagent was added. It was mixed well and incubated for 5 minutes at 370 C. After incubation, absorbance of samples and standard were read at 505nm against reagent blank.

Triglycerides

Concentration of triglycerides was estimated using ERBA Triglycerides kit, Catalogue No-BLT00059 TG 250)

To 100µL of each sample (Control, Test), standard and distilled water, 1mL of reagent solution was added. It was mixed well and incubated for 10 minutes at 37^0 C. After incubation, absorbance of samples and standard were read at 500nm against reagent blank.

Calculation

Tiglycerides (mg/dL)= ((Absorbance of sample/ Absorbance of Standard) X Concentration of standard)

[Concentration of standard- 200mg/dL]

Estimation of ascorbic acid

Ascorbic acid is first dehydrogenated by bromination. The dehydroascorbic acid is then reacted with 2,3- dinitrophenyl hydrazine to form osazone and dissolved in sulphuric acid to give an orange red colour solution which is measured at 540nm. 0.5g of sample was grinded. 25ml of 4%of oxalic acid was added to the homogenized samples, and centrifuged at 5000rpm for 3minutes. Supernatant was collected. 10ml of the supernatant was transferd into a conical flask and added bromine water dropwise with constant mixing. Makeup to 25ml with 4% oxalic acid.Then Pipette out 0.1ml of brominated sample and makeup the volume 3ml with distilled water. Then added 1ml of DNPH reagent followed by 1-2 drops of thiourea to each tube. Set blank as above with water. Mixed the contents of the tubes thoroughly and kept it 15minutes for incubation at 37ºC of solution. After incubation dissolved the orange- red osazone crystals formed by the addition of 1ml 80% Sulphuric acid. Absorbance measured at540nm.

RESULTS

The total carbohydrate, protein, cholesterol and ascorbic acid (vitamin C) from *Marcia recens*

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(Holten, 1802) (yellow foot clam) [designated as S1] and Perna virdis (green mussel) [designated as S2] are given below.

GLUCOSE- STANDARD GRAPH VALUES

Concentration(µg/mL)	Absorbance at 490nm
200	0.0635
400	0.1008
600	0.1331
800	0.1599
1000	0.1995

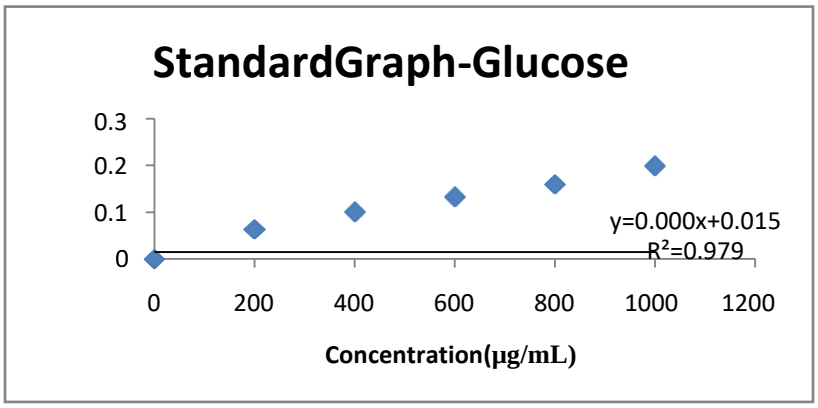

Fig: Standard graph of Glucose for estimation of carbohydrates

Sample code	Absorbance at 490nm	Amount of carbohydrate (mg/mg of sample)
S1	0.0555	0.201
S2	0.05856	0.2163

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PROTEIN

Sample code	Absorbance	Amount of protein(mg)
S1	7.982	13.74
S2	8.595	14.029

CHOLESTEROL

SAMPLE CODE	**ABSORBANCE**	**CONCENTRATION OF CHOLESTEROL(mg/dL)**
S1	0.0602	46.92128
S2	0.098	76.38348

TRIGLYCERIDES

SAMPLE CODE	**ABSORBANCE**	**CONCENTRATION OF TRYGLICERIDE(mg/dL)**
S1	0.1788	179.8793
S2	0.1892	190.3421

ASCORBIC ACID

Concentration(mg/mL)	**Absorbance**
STANDARD: ASCORBIC ACID	
100	0.0669
200	0.1151
400	0.1422
800	0.2414
1000	0.2867

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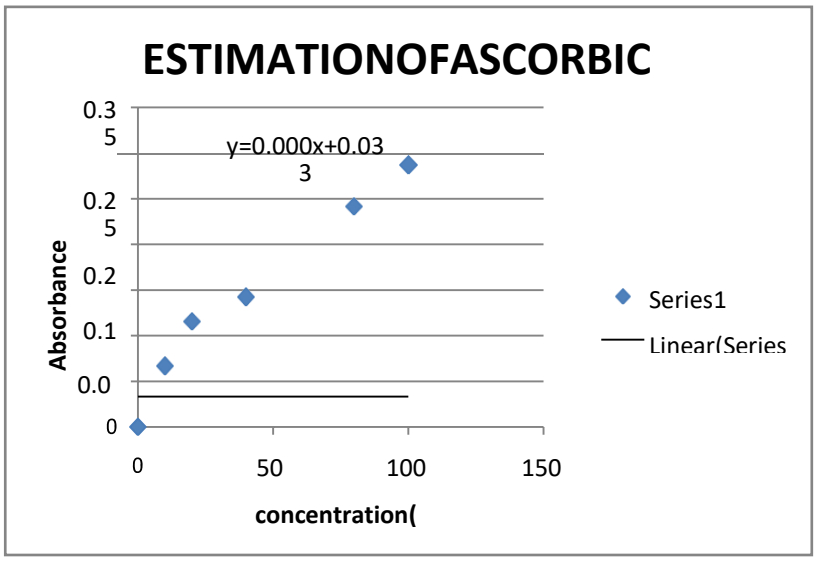
Fig: Standard graph values- Ascorbic acid



Concentration(µg/ml)	Absorbance	Concentration of ascorbic acid (µg/ml)
S1	0.1476	381.66
S2	0.1491	386.66

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DISCUSSION

The present study provided a definite biochemical profile of the edible molluscs *Marcia recens* and *Perna viridis* collected from the estuarine waters of southwest shoreline of India. Molluscs are a source of high-valued nutrients and are effectively accessible and less expensive for poor individuals living in the coastal areas. It is a rich source of minerals, vitamins, long-chain *n*-3 polyunsaturated fatty acids and can conceivably supplant the artificial food supplements available in the market.

The percentage edibility or the condition indices have been utilized to comprehend the biological value of the shellfish used in the present study. The commercial quality and physiological condition of molluscs are tentatively depicted by condition indices. CI is closely identified with the nutrient storage and meat quality. The good CIs, as dictated by different methods, show these shellfish species as potential sources for human consumption.

Marcia recens and *Perna viridis* were found to be rich in protein contents that are fundamental for human proper growth and survival. The proteins, lipids, and minerals are considered as the significant contributors to the nutritional value and organoleptic properties of mollusks (Orban *et al*., 2006). The protein content found in *Perna viridis* was slightly higher when compared to *Marcia recens.* There was no significant difference in carbohydrate values of the two species.

Shellfish meat is rich in key minerals needed for vital enzymes and for different metabolic pools and the present study determined molluscs to be good sources of vitamins. The vitamin C content reported in *Marcia recens* and *Perna viridis* varied from 381.66 to 386.66 µg/mL. This water soluble vitamin is a crucial antioxidative supplement for people, yet an extra outer dietary source is obliged in light of the fact that it is not biosynthesized by the human digestion system (Jeevitha *et al*., 2013). It, furthermore, helps the body assimilate to iron and calcium, assists in wound recovery, and helps cerebrum capacity (Iqbal *et al*., 2004).

P. viridis and *M. recens* demonstrated high cholesterol and triglyceride levels with *P. viridis* (76.38348 mg/dL) showing significantly higher cholesterol content compared to *M. recens*

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(46.92128 mg/dL). However the triglyceride content of *M. recens* (179.8793 mg/dL) was significantly lower than that of *P. viridis* (190.3421 mg/dL). Previous studies showed that bivalve mollusks contain cholesterol within threshold levels. Cholesterol was accounted for as the significant sterol in *M. edulis* and *P. canaliculus* (Murphy *et al*., 2002). Greater lipid content was reported in *P. malabarica* and *V. cyprinoides* (7–8 mg/100 g) when contrasted with the mollusk *M. casta* (5 mg/100 g; Srilatha *et al*., 2013).

Summary & Conclusion

The present study provided understanding into the nutritional composition of two bivalve shellfishes—*P. viridis* and *M. recens*—gathered from the estuarine waters situated on the southwest coast of the Arabian Sea and likewise provided useful information in regard to the importance of these candidate species for fisheries and aquaculture. More noteworthy levels of protein and cholesterol & triglyceride proportion characterized these species.

The present study demonstrated that *P. viridis* and *M. recens* can be utilized as nutritionally rich protein sources of seafood. The carbohydrate and vitamin C contents were also satisfactory. However the levels of cholesterol and triglycerides of both species exceed threshold levelreported so far and could be a matter of concernt. Therefore further studies focusing on the ideal atherogenic index, thrombogenicity index, hypocholesterolemic/hypercholesterolemic ratio, and fatty acid and amino acid based health markers are require to determine the status of these low- esteemed mollusc species as potential health food.

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