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Preliminary investigations on antimicrobial, antioxidant and nutritional properties of freshwater snail *Brotia costula* (Rafinesque, 1833)

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ABSTRACT

The present study aimed to evaluate the bioactive properties of freshwater snail *Brotia costula* (Rafinesque, 1833) meat along with its nutritional and functional properties. The soft tissue of *B. costula* had excellent nutritional properties as reflected in the high protein (13.83%), carbohydrate (10.70%) and polyunsaturated fatty acid (PUFA) content (37.7% of total fatty acid) of the meat. Among protein fractions, the major fraction was myofibrillar (51.40%) followed by sarcoplasmic (23.86%), stroma (17.89%) and alkali-soluble protein fractions (11.67%), whilst high non-protein nitrogen (27.23%) was also observed. To assess the bioactive properties, snail meat was extracted in three different solvents *viz.* 100% ethanol (l00EE), 50% ethanol (50EE) and 100% water (100WE). Among the three solvent extracts, 100% ethanol extract (100EE) showed excellent antioxidative properties as confirmed by four different antioxidant assays. The FTIR spectral analysis revealed strong absorbance for functional groups (phenol and carbonyl) in 100 EE tissue extract. The 100 EE showed maximum inhibition against spoilage bacteria (*Pseudomonas putida*, 21.3 mm) than pathogenic bacteria (*Escherichia coli*, 11.6 mm). Overall, it can be concluded that *B. costula* may be used as a good nutritional source and a potent natural antioxidant and antimicrobial agent in food and feed preparations.

Keyword: Antioxidant activity, Antibacterial activity, Cholesterol, Freshwater snail, FTIR

Introduction

Molluscs are considered a delicacy and fondly consumed around the world. In the phylum Mollusca, gastropods are essential classes containing snails having varying shapes and sizes. The distribution of snails is so diverse that they are found in freshwater, marine water and land. Freshwater molluscs are sufficiently available in and around rivers/reservoirs banks, pond and paddy fields, thus consumed mostly by tribal and economically poor populations worldwide. These people often consume them to satisfy their hunger and health benefits (Mahata, 2002). Snail contains a substantial quantity of protein and low fat with a good amount of polyunsaturated fatty acids (PUFAs), mainly essential fatty acids, which are primarily not synthesised by humans and should be obtained through diet. In some African countries, snails are marketed as "Congo meat" and popularly regarded as traditional food. In those countries, snails are locally available and offer a supplemental diet to boost the nutrition for the poor African population. Cagiltay *et al*. (2011) revealed that about 100 g of snail meat could meet 30% of the daily essential amino acid requirements of a person weighing 75 kg. Another study on snails ushered that it contains more PUFA than saturated fatty acids (SFA) and minerals, indicating its superiority to other conventional meats (Obande *et al*., 2013).

In addition to the nutritional importance of molluscs, snails are also believed to be rich in bioactive compounds that have valuable pharmaceutical (anti-tumour) and biomedical applications (antimicrobial, anti-inflammatory and antioxidant properties) (Nagash *et al*., 2010; Cheung *et al*., 2015). Pangestuti and Kim (2017) reported that these bioactivities exerted by the mollusc meat are frequently correlated with peptides, sterols, terpenes, polypropionates, nitrogen compounds, derivatives of fatty acids, mixtures and alkaloids.

In Bangladesh, some workers investigated the role of snails as supplementary feed for prawns. Many molluscs, such as green mussels (Shanmugam *et al*., 2020) and terrestrial snails (Ulagesan *et al*., 2018), have been studied for their nutritional and functional significance in addition to their bioactive compounds. However, studies on the freshwater molluscs or snails are too little and mostly restricted to nutritional composition. The indigenous people of landlocked North-east India are fond of eating small molluscs, especially snails caught chiefly from rivers or small reservoirs. The present investigation determined the antibacterial, antioxidant and nutritional

properties of the freshwater snail *Brotia costula* meat. Snail species of *Brotia* (family Pachichylidae), occurring in freshwater habitats, was selected for this study as it is fondly consumed among tribes due to its wide availability in the local markets.

Materials and methods

Sample collection

Live snails *B. costula* (Fig. 1) were procured from the tribal people, who harvest freshwater snails in the morning hours and sell on the same day in the local markets of Lembucherra, Tripura, India. The snails were collected in August-October 2020. The live snails were transported to the laboratory in polythene bags within half an hour. Snails were subjected to the depuration process upon arrival in the laboratory, performed in a plastic tub of 50 l capacity filled with freshwater for up to 48 h. Water exchange was done at an interval of 8 h to remove the adhered materials and to clean the gastrointestinal tract.

Morphometric characteristics

The morphometric characteristics of *B. costula* used for the study are given in Table 1. The average weight, length and width were 16.72±5.09 g, 6.7±0.52 cm and 1.67±0.31 cm (n=20), respectively. The total length was taken by measuring the distance between the anterior and the posterior region. Then the entire width was measured from the dorsal side to the ventral side of the animal. The soft tissues withdrawn from the shell were weighed separately to determine the total weight of the animal.

Analysis of proximate and physico-chemical composition

The proximate composition (moisture, protein, carbohydrate, fat, ash) of *B. costula* tissue was performed following the methods described in AOAC (2016).

Analysis of fatty acids

The fatty acid composition was determined in the extracted oil samples following Bligh and Dyer (1959) method. The AOAC method was followed to esterify the lipid extract. The esterified sample was kept in an amber vial to minimise oxidation during analysis and placed in a refrigerator until use. Gas chromatography-mass spectrometry (GC-MS) was performed on Shimadzu Qp2010 quadrupole Gas Chromatography-Mass Spectrometer (GC-MS) instrument equipped with a carbowax (30 m × 0.25 mm ID; 0.25 im film thickness) capillary column (Cromlab S.A). One millilitre of the sample was injected into the GC-MS for analysis of fatty acids. Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. The peak area was quantified and expressed as a percentage of total fatty acids.

Table 1. Morphometric yield, bio-chemical and functional properties of *B. costula*)

Parameters	Means±SD
Meat yield (%)	36.82±4.72
Moisture (g 100 g meat^{-1})	71.20±0.62
Protein (g 100 g meat^{-1})	13.83±0.91
Sarcoplasmic protein (% of total proteins)	23.86±0.61
Myofibrillar protein (% of total proteins)	51.40±1.56
Stroma protein (% of total proteins)	17.89±1.43
Alkali soluble protein (% of total proteins)	11.67±1.56
Non-protein nitrogen (% of total proteins)	27.23±0.61
Carbohydrate (g 100 g meat^{-1})	10.70±0.04
Fat (g 100 g meat^{-1})	1.26±0.58
Ash (g 100 g meat^{-1})	5.17±0.56
Cholesterol (mg 100 g fat^{-1})	81.76±1.55
pH	8.93±0.15
Solubility (%)	74.63±0.91
Water holding capacity (%)	40.45±1.35
Emulsion capacity (ml oil mg protein^{-1})	0.88±0.71
Foaming capacity	22.53±0.9

Values are expressed as means±SD, n = 3.

Determination of cholesterol content

The cholesterol content of fat extracted from *B. costula* tissue was determined by the method described by Zlatkis *et al*. (1953). By comparing the absorbance (at 560 nm using UV spectrophotometer) with the standard curve, the amount of cholesterol was calculated and expressed as mg 100 g^{-1} of lipid.

Fig. 1. Freshwater snail *B. costula*

Characterisation of snail extracts by FTIR spectroscopy

The lyophilised crude tissue extracts prepared in different solvents were subjected to Fourier transform infrared spectroscopy (FTIR) analysis to identify and characterise a functional group. The infrared spectrum of an organic compound provides a unique fingerprint, which is readily distinguished from the absorption patterns of all other compounds. The IR spectra of the samples were recorded with a 3000 Hyperion Microscope with Vertex 80 FTIR System. One part of the crude extract was mixed with 99 parts of dried potassium bromide and it was scanned between 900-4000 cm^{-1} (wave number) at a speed of 65 spectra s^{-1} and with a programmed slit opening and air as reference.

Functional properties analysis

Protein solubility

The protein solubility of the snail samples was determined by the method of Benjakul and Bauer (2000). and the protein content was estimated by the Biuret method (Robinson and Hogden, 1940).

Water holding capacity (WHC)

The water holding capacity (WHC) was determined by the method outlined by Barrera *et al*. (2002). Three replicates were performed and the average was shown as the value of WHC and was expressed as percentage.

Emulsifying and foaming capacity

The emulsifying capacity (EC) of muscle proteins of snail was assessed by a method described by Swift *et al*. (1961). Emulsion capacity was expressed as ml of oil per mg protein. The average of three replicates was reported as emulsion capacity values. Foaming capacity (FC) of snail muscle proteins were tested according to the method tailored by Don *et al*. (1991) and was expressed as percentage.

Preparation of crude extract of B. costula in different solvents

Snail shells were opened and appropriately washed with distilled water. For crude extraction, 30 g of wet tissue was macerated with 60 ml (1:2) solvent systems: 100% water, 100% ethanol and 50% ethanol individually. The homogenised mixture was incubated for 48 h at 4°C and subsequently centrifuged at 10,000 rpm for 10 min. The supernatants from each extract were collected and pellets were subjected to re-extraction with 30 ml of each solvent (1:1) and centrifuged. The supernatant was pooled and concentrated by a rotary evaporator with reduced pressure, freeze-dried and stored at -20°C until use.

Total phenol content

The total phenolic content of tissue extracts prepared in different solvents was determined using Folin-Ciocalteu reagent according to the method described by Singleton and Rossi (1965) using gallic acid as standard.

Antimicrobial activity

Snail tissue extracts were tested for inhibition of bacterial and fungal growth against spoilage bacteria *viz*. *Bacillus subtilis, Pseudomonas putida, Bacillus megaterium* and *Bacillus stratosphericus* as well as pathogenic bacteria *viz. Escherichia coli, Vibrio parahaemolyticus* and *Aeromonas* sp. and the fungus, *Aspergillus niger*. The microbial assay was performed by the agar well diffusion method (Bayer *et al*., 1966). The plates were incubated at 37°C for 24 h for bacteria and at 37ºC for 48 h for fungi. The zone of inhibition around the well was measured. The assays were carried out in triplicates, and the values were expressed as Mean±standard deviation.

Antioxidant assays

The antioxidant potential of different solvent extracts of snail was evaluated through 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Dhanabalan *et al*., 2017); 2, 2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) ABTS assay (Arnao *et al*., 2001), hydrogen peroxide (H_2O_2) assay (Ruch *et al*., 1989) and ferric ion reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996).

Statistical analysis

All data were analysed and interpreted using one-way ANOVA with Statistical Package for Social Sciences (SPSS, version 16.0 for windows). The significance of differences was defined at $p<0.05$. Tukey multiple comparison tests were used for *post hoc* analysis ($p<0.05$).

Results and discussion

Proximate composition, fatty acid profile, protein fractions and cholesterol content of B. costula

Proximate composition of B. costula

The moisture content of *B. costula* was 71.20% (Table 1) higher than the values reported for some other freshwater mollusc species *viz. Brotia bengalensis* (65.80%), *B. dissimilis* (67.73%) (Debnath *et al*., 2016) and marine molluscs such as *Lunella torquata* (68.50%) (Ab Lah *et al*., 2017) and *Rapana venosa* (70.75%) (Luo *et al*., 2017), while the value was found to be lower compared to another freshwater snail *Phaeocystis globosa* (73.80%) and land snails such as *Achatina fulica* (90.27%), *Limcolaria* sp. (89.98%) and *Helix pomatia* (75.41%) (Adegoke *et al*., 2010). The difference in the

moisture content of mollusc species varies due to season, environment, sex and age (Baby *et al*., 2010).

In the present study, the recorded amount of protein in the snail species was 13.83% (Table 1). The value obtained was comparable with other freshwater snail species such as *B. dissimilis* (11.18%), *P. globosa* (15.59%) (Debnath *et al*., 2016) and *Abacetus convexiusculus* (12.92%) (Baby *et al*., 2010). But, the content in *B. costula* was noticeably less in comparison to land snails such as *Achatina marginata* (19.53%), *Limicolaria* sp. (18.66%) and *Achatina achatina (*19.27%) (Fagbuaro *et al*., 2006). Luo *et al*. (2017) assessed the protein content of marine snail (*R. venosa*) (19.15%), which was higher than the experimental species. Ab Lah *et al*. (2017) postulated that predatory gastropod whelks contain higher protein than reported for herbivores. The difference in protein content of snails from different habitats may be due to differences in diet, environment and region.

In the present investigation, lipid content was 1.26% as depicted in Table 1. Generally in snails, the amount of fat in fresh specimens seldomly exceed 2%. The lipid content of snails is lower than other animals and extensively used against hypertension and other related ailments in humans. The fat content in other freshwater snails was near 1% (Debnath *et al*., 2016). However, the value for land snails (*A. marginata*, *A. achatina* and *Limicolaria* sp*., H. pomatia, H. nemoralis*) (Fagbuaro *et al*., 2006) and many marine snails (*L. torquata, T. militaris* 5.6% and *L. undulata* 5.2%) (Ab Lah *et al*., 2017) was higher (5-8.5%) than that registered for the experimental species (*B. costula*) (1-2%). This variation in fat content in snails could also be related to food availability and environment.

Molluscs contain a good quantity of carbohydrates compared to finfishes. In the present study, the content of carbohydrates in *B. costula* was found to be 10.70% (Table 1). Debnath *et al*. (2016) reported almost similar values in other freshwater snail species (*B. bengalensis,* 11.97%; *B. dissimilis,* 11.46%). However, the values reported for *P. globosa* (5.62%) were considerably lesser than *B. costula*. In land snails, the reported amounts of carbohydrates (*A. marginata*, *A. achatina* and *Limicolaria* sp.) were less than 1% (Fagbuaro *et al*., 2006). Similarly, the carbohydrate content reported in marine snails (*L. torquata*, *L. undulata, T. militaris, L. quadricentus* and *N. pyramidalis*) is also around 3-5% (Ab Lah *et al*., 2017). This indicates that the carbohydrate content in freshwater snails is relatively higher than the land and marine snails. Higher carbohydrate levels could be due to over-activeness of the snails for reproduction with the onset of monsoon (Debnath *et al*., 2016).

Snails are reported to contain good quality minerals. Ash content provides a measure of the total amount of minerals within samples. Various minerals, often in tiny amounts, are essential for human health. In the present study, the ash content observed was 5.17% (Table 1).

Fatty acid profile of B. costula

The fatty acid profile of *B. costula* observed in the present study is given in Table 2. The major portion of the fatty acids in *B. costula* comprised polyunsaturated fatty acids (PUFA) (37.7% total fatty acids) followed by saturated fatty acids (SFA) (28.22% of total fatty acids) and monounsaturated fatty acids (MUFA) (25.33%). The value obtained for PUFA in *B. costula* was higher, whereas SFA content was lower than reported for freshwater mussel (*L. marginalis*) (Haldar *et al*., 2014). These MUFAs and PUFAs have been considered as good fats which should help to improve HDL levels in the blood and could be helpful in the maintenance of normal blood cholesterol

Table 2. Fatty acid profile of fat extracted from *B. costula* meat

Fatty acids	Percentage
Saturated fatty acids (SFA)	
C12:0	0.26±0.007
C13:0	1.57±0.098
C14:0	1.78±0.035
C15:0	1.13±0.063
C16:0	13.42±1.25
C17:0	2.41±0.098
C18:0	5.37±0.52
C19:0	0.9±0.028
Total saturated fatty acids	26.84
Monounsaturated fatty acids (MUFA)	
C16:1, n-7	2.59±0.67
C17:1	0.4±0.03
C18:1, n-9	16.97±0.62
C20:1	3.78±0.77
Total monounsaturated fatty acids	23.74
Polyunsaturated fatty acids (PUFA)	
C18:2, n-6	11.23±0.042
C18:3, n-3	7.06±1.90
C20:3, n-3	0.92±0.021
C20:3, n-6	1.69±0.05
C20:4, n-6 (AA)	10.33±0.15
C20:5, n-3 (EPA)	1.31±0.23
C22:4, n-6	2.95±0.16
C22:6, n-3 (DHA)	0.42±0.035
Total polyunsaturated fatty acids	35.91
Total n-3 polyunsaturated fatty acids	9.71
Total n-6 polyunsaturated fatty acids	26.2
Ratio n-3/n-6	0.37

Values are expressed as means±SD, n = 3.

levels (Karnjanapratum *et al*., 2013). The results suggests that consumption of this freshwater snail is good from the nutrition point of view and for people suffering from cardiac diseases.

Among PUFA, the content of linoleic acid (C18:2 n-6) (11.26%) was higher in comparison to arachidonic acid (C18:4 n-6) (10.44%) and linolenic acid (C18:3 n-3) in the fat extracted from *B. costula* was 8.41%. Almost similar findings were observed for marine snails (*P. trapezium*) (Prem Anand *et al*., 2010). Linoleic and linolenic acids content were comparable with our experimental freshwater snail species. However, the values obtained for EPA and DHA were insignificant in *B. costula*, compared to freshwater mussel (DHA, 31.6 and EPA, 8.3%) (Mahanty *et al*., 2015). The dietary intake of food with a high ratio of n-3/n-6 is nutritionally beneficial for humans. FAO has recommended that the proportion of n-3/n-6 in the diet be higher than 0.2 (FAO/WHO, 2003) and similarly, the UK Department of Health recommended that the n-3/n-6 ratio should be higher than 0.25 (Hmso, 1994). This ratio for *B. costula* was 0.42, that was well above the recommended value. Among SFA, palmitic acid (C16:0; 14.31% of total fatty acids) was the major saturated fatty acid while oleic acid (C18:1; 17.41% of total fatty acids) was the main MUFA observed in *B. costula*.

Cholesterol content in B. costula

Cholesterol is a very important biomolecule for life. However, consuming higher amount of cholesterol than the daily requirement may pose severe threat to human health. American heart association proposed dietary cholesterol consumption to 300 mg day^{-1} for healthy individuals. In the present study, the amount of cholesterol was found to be 81.76 mg 100 g^{-1} (Table 1) that was lower than the value reported for average cholesterol concentration in Captain Cook marine snail (132 mg 100 g^{-1}) (Mason *et al*., 2014). Zhu *et al*. (1994) reported that cholesterol was the principal sterol in molluscs, usually accounting for 85% or more of the sterols measured. However, molluscs are considered a high cholesterol food but consist only about one-quarter of the cholesterol concentration of a whole egg. Similarly, Zhu *et al*. (1994) reported a higher cholesterol value in different land snails such as *Helix sportella* and *V. columbiana* (86.6-118.4 mg 100 g^{-1}). Moreover, the content was found to be lesser in *B. costula* compared to raw meat of other shellfishes such as cuttlefish 130 to 162; squid 188 to 198; Antarctic krill 33.7 to 103; shrimp 118 to 169; crab 54 to 67; lobster 220; red shrimp 57.8 to 60.8; *Macrobrachium rosenbergii* 139 and oyster160 mg 100 g^{-1}, from Indian markets (Rosa and Nunes, 2004; Turan *et al*., 2011).

Proteins fractions profile of B. costula

The percentage of different protein fractions in freshwater snails is depicted in Table 1. Among protein fractions, the major fraction was myofibrillar protein (51.40%) followed by sarcoplasmic protein (23.86%), stroma protein (17.89%) and alkali-soluble fractions (11.67%). In comparison to finfish, shrimp and squid (Gopakumar, 2002), the myofibrillar fraction was slightly less while stroma and alkali-soluble fractions were higher in *B. costula*. The higher amount of stroma (25-31%) and sarcoplasmic proteins (28-33%) were also documented in clam species (Tabakaeva *et al*., 2018) in comparison to *B. costula*. However, the quantity of myofibrillar protein fractions in clam (*A. broughtoni* and *M. chinensis*) (Karnjanapratum *et al*., 2013) were lesser in comparison to values obtained for *B. costula* in the present study.

FTIR analysis of freshwater snail extracts

In the present investigation, the FTIR spectra of the lyophilised samples of *B. costula* extracted in different solvents are shown in Table 3 and Fig. 2. The results indicated that 100% ethanolic lyophilised extract of *B. costula* showed more functional groups than other solvent extracts. Among three different solvents, the 100% ethanol extract lyophilised sample showed the highest (9) significant peaks 3391, 2942, 1667, 1532, 1415, 1150, 1021, 760 and 582 cm^{-1} than other solvent tissue extracts (Fig. 2). In 100% ethanolic extract, the peak 3391 cm^{-1} indicated broad, strong NH stretching and OH stretching.

Table 3. FTIR analysis of tissue extracts of *B. costula* in different solvents

Band position (cm^{-1})	Tissue extracts in different solvents		
	100% ethanol	50% ethanol	100% water
3400-3200	Strong, broad OH and NH stretching	Nil	Nil
2942	Asymmetrical stretching of CH_2	Asymmetrical stretching of CH_2	Asymmetrical stretching of CH_2
1667	C=O bonded to N-H stretching	C=O bonded to N-H stretching	Nil
1532	NH bend coupled with CN stretch	Nil	Nil
1415	COH stretch	Nil	Nil
1150-1021	C-O (single bond stretch)	C-O (single bond stretch)	C-O (single bond stretch)
850-760	C-O-S stretching	Nil	Nil
760-582	Skeletal stretch	Skeletal stretch	Nil

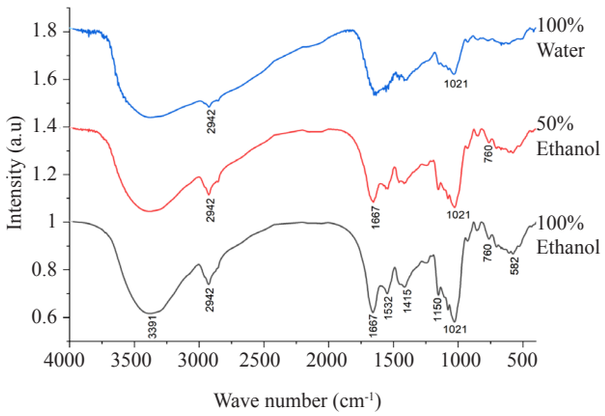

Fig. 2. FTIR spectra of lyophilised tissue extract of *B. costula* in different solvents

The wave number 2942 cm^{-1} showed distinctive asymmetrical stretching of CH_2. Similarly, the wave number 1532 cm^{-1} indicated the bending of NH coupled with CN stretch. Amide III functional group showed a peak at 1415 cm^{-1}, a complex system mainly associated with CH_2 residual groups from glycine and proline. A band at 1021 cm^{-1} represented the acetylamino group, indicating the asymmetric in-phase ring stretching mode and 582 cm^{-1} OH showed plane bending. The wave number of 1150 cm^{-1} revealed CO stretching with single bonds. Likewise, in freshwater snail (*Pila virens*), 7 peaks with frequencies ranging from 403.12 to 3994.58 cm^{-1} and different functional groups were reported by Gayathri *et al*. (2017). Similarly, in the case of marine snail *H. davidis*, peaks ranging from 665.44 to 3996.51 cm^{-1} have been reported (Giftson and Patterson, 2016). The FTIR spectra of the lyophilised sample of *B. costula* peak values revealed that bioactive compound (such as phenol or alcohol, carbonyl) signals at different ranges indicating medicinal value due to high quality of antimicrobial compounds. Similarly, the IR spectrums of *Perna viridis* tissue extract showed different peaks that were said to be responsible for the chemical properties. The present findings were comparable with the findings of Salas and Chakraborty (2020) in which methanolic extract of a marine gastropod showed broad absorption bands in the region of 3300-3500 cm^{-1} which might characterise O-H stretching vibrations of phenols or alcohols and the N-H stretching vibration corresponding to amide and amine groups.

Functional properties of B. costula

The functionality of the protein indicates its usefulness in food formulations. Solubility, water holding capacity, emulsion capacity and foaming ability are the essential functional properties of the protein irrespective of the source of the proteins. Solubility is one of the important functionalities that bear other functional properties such as viscosity, gelling ability and emulsion capacity. In the present study, the solubility of the protein from the soft tissue of *B. costula* was 74.63% (Table 1). The observed value was higher than the value reported for freshwater mollusc *T. fuscatus* var *radula* by Ogungbenle and Omowole (2012), who opined that the solubility of freshwater mollusc protein depends on the pH of the dissolving medium and the value was highest around pH 2.

In food formulation, water-protein interaction is one of the critical criteria for better protein functionality. Water holding capacity is the ability of the protein to imbibe water and retain it against gravitational force within a protein matrix. In the present study, the water holding capacity of snail species was 40.45% (Table 1) and this value was lesser than that of value reported for squid (58%) (Mehta and Nayak, 2017). The lower water holding capacity of snails might be related to higher hydrophobic amino acid residues in the meat composition. The higher hydrophobic amino acids residues tend to bind less water, thus yielding lower WHC.

Emulsion and foaming capacity are surface active properties of the proteins, hence fondly correlated with surface hydrophobicity. The emulsion capacity (EC) can be defined as the ability of the protein to emulsify oil in a specified condition. The myofibrillar fractions of the protein are responsible for the emulsifying power of the meat that reduces the surface tension between water and oil interface in the food formulations. In the present investigation, the emulsifying capacity recorded was 0.88 ml mg^{-1} of protein (Table 1). The value is higher than that of the value reported for green mussel (0.76-0.81 ml mg^{-1}) (Binsi *et al*., 2007) but lower than Indian squid (2.23 ml mg^{-1}) (Mehta *et al*., 2017). The difference in emulsifying properties of proteins from different sources is due to their conformation and solubility in a given solvent. However, the proteins from molluscs having better emulsion capacity may be used as cheap emulsifying agents in various food formulations.

An increase in concentration enhances higher protein-protein interaction, increasing viscosity and facilitating the formation of cohesive protein film at the interface, yielding foam formation. In the present study, the foaming capacity of snail was 22.53% (Table 1). The foaming values were comparable with green mussel (*Perna canaliculus*) powder extract (27.27%) and lower than sea snail *H. trunculus* (75%) (Vijaykrishnaraj *et al*., 2015). To assess foam stability, the foam volume was measured after 5, 15 and 30 min and found to be 93.89, 88.54 and 81.71%, respectively of the original value. This suggested that snail proteins had excellent foaming stability and can

be used as an additive in the food items as bulking agents and bulk stabilisers.

Total phenol content of B. costula extracts

In the present investigation, the tissue extracts were prepared in three different solvents *viz.* 100% ethanol (100EE), 100% water (100WE) and 50% ethanol (50EE) to find a better solvent candidate for tissue extraction to achieve the maximum activity. Among these three solvents, 100EE (18.77 mg GAE g^{-1}) was shown to have maximum phenolic content followed by 50EE (11.63 mg GAE g^{-1}) and 100WE (6.90 mg GAE g^{-1}) (Table 4). In the present study, the total phenolic content value was comparable with marine mollusc species such as *Amphioctopus marginatus* (27.51 GAE g^{-1}), *Sepiella inermis* (24.33 GAE g^{-1}), *Crassostrea madrasensis* (=*Magallana bilineata* (19.16 GAE g^{-1}) and *Uroteuthis duvaucelii* (10.08 GAE g^{-1}) (Krishnan and Chakraborty, 2019) whereas, in marine snails (*Babylonia spirata* and *Chicoreus ramosus*) the content was higher than the value observed in the present investigation (Salasa and Chakraborty, 2020). Phenolic compounds are well characterised for their antibacterial and antioxidative properties. However, natural sources of these compounds are not so available cheaply. The FTIR spectra supported the findings well, where the presence of broad, strong NH stretching and OH stretching or phenol was quite clear at 3391 cm^{-1} (Fig. 2) in all three extracts. Phenolic compounds can scavenge free radicals, inhibit lipid peroxidation and chelate ferrous ions in the biological system (Espinosa *et al*., 2015) and are effective deactivators of electronically excited sensitiser molecules concerned with producing radicals and singlet oxygen.

Antibacterial activity of B. costula extracts

Just like total phenolic assessment, the tissue extracts were prepared in three different solvents. After extraction, all three extracts were lyophilised. Further, the lyophilised extracts prepared from freshwater snails were screened against bacteria (three human pathogenic bacteria and four spoilage bacteria) and fungal species to test their antimicrobial efficacy. Among three different solvents, 100EE showed maximum activity against spoilage bacteria followed by pathogenic bacteria, whereas fungal strain *A. niger* was observed to be resistant against all three other solvent tissue extracts (Fig. 3). The results of FTIR analysis showed maximum functional groups, mainly phenolic or OH group in the region of 3300-3500 cm^{-1} (3391 cm^{-1} precisely) in 100EE followed by 50EE (Fig. 2).

Among spoilage bacteria, the maximum inhibition zone was formed against *P. putida* (21.3 mm), followed by *B. stratosphericus* (13 mm) (Fig. 3). In the case of pathogenic bacteria, the maximum antibacterial activity was found against *Aeromonas* sp. (12 mm) followed by *E. coli* (11.6 mm) in the extract prepared in 100% ethanol as solvent (Fig. 3). Almost similar trends were obtained for extract prepared in 50% ethanol but with lesser inhibition which indicated that this freshwater snail might be a potential source of natural antimicrobial molecules. FTIR analysis (Fig. 2) and phenolic content (Table 4) of 100EE confirmed the strong presence of alcohol or phenolic compound in *B. costula* meat, showing antimicrobial activity against microorganisms. So, anti-spoilage bearing compounds may be prepared from ethanolic extract of *B. costula* for future storage studies.

Furthermore, the *post hoc* test (Tukeys test) also showed that 100EE was more effective against the spoilage microorganism than 50EE and 100WE. The test also established that extract prepared in 100EE had antimicrobial potential against *P. putida* compared to other spoilage and pathogenic bacteria. Almost similar

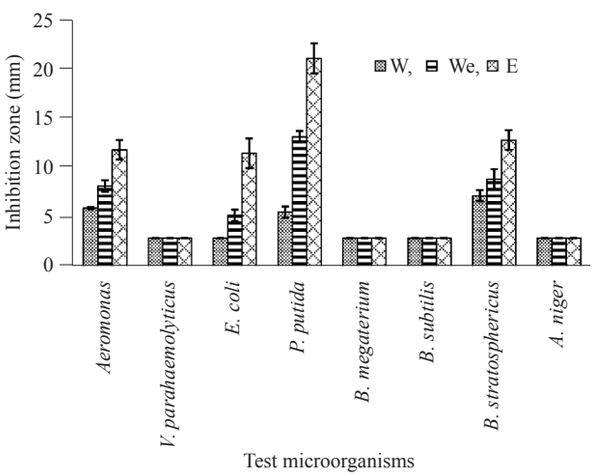

Fig. 3. Antibacterial activity of tissue extracts of *B. costula* in different solvents against different test microorganisms (w-Water extract, We-Water ethanol and E-100 % ethanol)

Table 4. Total phenolic content and antioxidant activities of tissue extracts prepared from *B. costula* in different solvents

Snail extracts	Total phenolic content (mgGAE g^{-1})	DPPH assay (%)	ABTS assay (%)	H_2O_2 assay (%)	FRAP assay (µMol $FeSO_4$ mg^{-1})
100% water extract	6.90±0.86[a]	24.37±0.34[a]	25.82±1.11[a]	20.83±0.78[a]	3554.68±41.56[a]
50% ethanol extract	11.63±1.56[b]	34.06±1.87[b]	38.90±1.52[b]	28.78±0.22[b]	5620.93±55.91[b]
100% ethanol extract	18.77±1.12[c]	54.14±1.11[c]	52.86±1.11[c]	47.11±1.57[c]	8259.39±46.86[c]

Values means±SD, n=3. Mean values bearing different superscripts (a, b, c) in a column are significantly different ($p<0.05$)

antimicrobial activities against spoilage bacteria were reported in various freshwater snail species with different natural antimicrobial substances in snails (Altaf *et al.*, 2018). However, some novel and uncharacterised mechanisms of action that might ultimately benefit the ongoing global search for clinically useful antimicrobial agents need to be explored to explain the characteristics of antimicrobial activity of *B. costula*.

Antioxidant activity of B. costula tissue extracts

Considering the utilisation of freshwater snail meat as a functional food, various antioxidative assays *viz.* DPPH, ABTS, H_2O_2 radical scavenging activity and FRAP assay were performed to understand its potential as a natural antioxidant. Antioxidant molecules play an important role in nutritional preservation and preventing colour and flavour deterioration in food manufacturing. In the present investigation, the values obtained for all four antioxidants assays (DPPH, ABTS, H_2O_2 radical scavenging activity and FRAP) were higher in the case of lyophilised extract prepared from *B. costula* in 100% ethanol followed by 50% ethanol and 100% water.

In the present study, the DPPH activities of tissue extract of *B. costula* tissue, prepared in different solvents *viz.* 100EE, 50EE and 100WE were 54.14, 34.06 and 24.37% respectively (Table 4). This indicated that DDPH activity in 100EE had better radical scavenging than the other two solvent extracts. The value reported for two marine gastropods *viz. B. spinosa* (Subhapradha *et al*., 2013) and *P. trapezium* (Prem Anand *et al*., 2010) were lower than the value reported in the present investigation. Likewise, the DPPH radical scavenging activity of the different solvent extracts from *B. costula* was comparable with the organic extracts of the squid tissues (*U. duvaucelii*, 58%) and *Donax cuneatus*, 32%) (Nazeer and Naqash, 2013) but higher than marine bivalves *Meretrix meretrix* extracts (34.56%) (Sugesh *et al*., 2019). However, DPPH activities were reported for methanolic tissue extracts of another freshwater snail (74.83%) (Gayathri *et al*., 2017) and marine water mussels (79.86%) (Shanmugam *et al*., 2020) were slightly higher than the value obtained in our study. This difference in the DPPH value may be due to the solvent, as different solvents have different extraction capacities of the antioxidative compounds, as observed in the FTIR graph (Fig. 2). Like DPPH activity, the 100EE prepared from *B. costula* had higher ABTS activity (52.86%) followed by 50EE (38.90%) and 100WE extracts (25.82%). The value reported for terrestrial snail (Ulagesan *et al*., 2018) was higher (70%) than that documented in the present study.

The trends for the H_2O_2 activity also were similar to DPPH and ABTS activity. The activities of extracts prepared from *B. costula* in 100EE, 50EE and 100WE were 47.11, 28.78 and 20.83%, respectively (Table 4). However, the hydrogen peroxide scavenging activity was higher (88.12%) in crude tissue extract of *P. viridis* (Madhu *et al*., 2014) compared to the values obtained in our investigation. Hydrogen peroxide is an oxidising agent, which when enter the cell, can probably react with Fe^{2+} and possibly Cu^{2+} to form hydroxyl radicals, leading to toxic effects. Hence, the present study concluded that 100% ethanolic extract of *B. costula* might possess higher antioxidants which could probably be used as a natural, accessible source for treating human diseases.

The ferrous ion ($Fe^{2+)}$ is a pro-oxidant that interacts with hydrogen peroxide (Fenton reaction) to produce reactive oxygen species (ROS) and the hydroxyl (OH) free radical, which may initiate or accelerate lipid oxidation. The complex formation of the ferrous ion is disrupted when chelating agents are present, resulting in decreased colour. As for reducing power, the presence of antioxidants causes reduction of the ferric cyanide complex to its ferrous form. In the present investigation, the FRAP activity of extracts prepared from *B. costula* in 100% ethanol, 50% ethanol and 100% water were 8259.39, 5620.93 and 3554.68 µMol $FeSO_4$ mg^{-1} respectively (Table 4).

To access a better assay of antioxidant property, the Karl person correlation coefficient was computed between the total phenolic content and different antioxidant assays (DPPH, FRAP, ABTS, H_2O_2 radical scavenging activity). The highest correlation coefficient was calculated between the total phenolic content and FRAP assay ($R^2 = 0.9978$), followed by DPPH scavenging assay ($R^2 = 0.9933$), ABTS activity ($R^2 = 0.9904$) and with H_2O_2 activity was the lowest ($R^2 = 0.9885$). Therefore, the best method for determining the antioxidant capacity in *B. costula* extracts was the FRAP method, followed by the DPPH method.

Freshwater snail meat is an excellent nutritional source as it contains considerable amount of unsaturated fats, low cholesterol and good protein. Further, snail meat was shown to have good functionality, especially emulsion and foaming capacities. The study also suggested that 100% of ethanol may be recommended as a solvent to achieve maximum bioactivity. FTIR spectral analysis also deduced the presence of a strong phenol group at 3400-3200 cm^{-1} in lyophilised extracts of *B. costula* prepared in 100% ethanol. The study concluded that the lyophilised extract of *B. costula* might be used as a natural antioxidant and antibacterial additive to reduce the artificial antioxidants and preservatives in the food and feed industry.

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