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Original Article

Isolation of an Antihypertensive Bioactive Peptide from the Freshwater Mussel *Lamellidens marginalis*

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Abstract

Background: Dietary proteins have long been recognized for their nutritional and functional properties. They are good sources of bioactive peptides with a broad spectrum of pharmacological activities. The freshwater edible mussel *Lamellidens marginalis*, an unconventional low-cost protein source is still underexploited for its health benefit molecules. **Aim:** An attempt has been made to isolate and characterize antihypertensive bioactive peptides from *L. marginalis* using commercially available food grade proteases. **Methods and Materials:** Basic nutrient parameters were studied using different standard procedures. Crude protein was extracted and hydrolysed by commercial enzymes: Alcalase2.4L, Pepsin, and Papain. Low molecular weight peptides (<3KDa) were separated out through ultrafiltration. Antihypertensive activity was analysed using *in vitro* assay and the lowest IC_{50} value was used for mass spectrometry. **Results:** Proximate analyses revealed that mussel meat contains a moderate amount of protein (42.00±0.67%) with several essential amino acids. Two hours of hydrolysis with Alcalase2.4L, Pepsin and Papain were evident maximum and resulted in degree of hydrolysis of 85.45%, 62.30% and 60.35% respectively. Bioactivity of short peptides was measured in terms of percentage of ACE inhibition and IC_{50} values of Alcalase2.4L, Pepsin and Papain were 47.22 µg/ml, 142.79 µg/ml and 502.55 µg/ml respectively. A novel ACE inhibitory peptide having amino acid sequence: isoleucine-glycine-proline-glycine-proline-phenylalanine-serine-arginine (IGPGPFSR; molecular weight 830.953 Dalton) of antihypertensive nature has been identified. **Conclusion:** Present observations suggest that bioactive peptide derived from the freshwater mussel *Lamellidens marginalis*, could serve as a useful supplement in nutraceutical therapy which may help to mitigate hypertension and other cardiovascular ailments the future.

Keywords: Angiotensin-I converting enzyme, Antihypertensive, Bioactive peptide, Functional food, *Lamellidens marginalis*

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INTRODUCTION

Hypertension is the leading cause of cardiovascular disease and premature death worldwide. Globally, an estimated 26% of the world's population has hypertension which is expected to increase to 29% by 2025. This high prevalence of hypertension contributes a tremendous public health burden.[1] The situation in India is more alarming. Approximately 29.8%

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of Indians have hypertension with 33.8% prevalence in urban and 27.6% in rural India.[2] Renin–angiotensin system (RAS) plays a crucial role in cardiovascular control where both the systemic angiotensin-II (Ang-II) and centrally generated Ang-II, signal through complex neural networks and contribute in the development of hypertension. Angiotensin converting enzyme (ACE) and renin are the two crucial components that regulate RAS pathway. Renin catalyses the conversion of angiotensinogen to a decapeptide angiotensin-I which is again converted to an octapeptide angiotensin-II, a potent vasoconstrictor, by ACE, a type-I membrane anchored dipeptidyl carboxypeptidase having Zn^{+2} ion in its active site. ACE also degrades bradykinin, a vasodilator nonapeptide which results in an increase in blood pressure. ACE inhibition is a successful strategy for the treatment of hypertension and congestive heart failure to prevent kidney failure in patients with high blood pressure or diabetes and also to reduce the risk of stroke. Some potent synthetic inhibitors like captopril, lisinopril, enalapril are used successfully against heart failure, hypertension, and diabetic neuropathy.[3] But these inhibitors also exert adverse side effects like dry cough, skin rashes, and allergic reactions. So, the need of the hour is to search for safe and natural alternatives. Food-derived ACE-inhibitory peptides are considered as potent antihypertensive agents. These are preferred over synthetic drugs as usually, they have no adverse side effects and there is virtually no risk of overdosing which is associated with synthetic drugs. Dietary proteins have long been recognized for their nutritional and functional properties. In recent years, a considerable amount of research has focused on the liberation of bioactive peptides (usually with 2-20 amino acid residues) which are encrypted within food proteins, with a view to utilizing such peptides as functional food ingredients aimed at health maintenance. Food derived bioactive peptides have been shown to display a wide range of physiological functions including antihypertensive, antioxidative, opioid agonistic, immunomodulatory, antimicrobial, prebiotic, mineral binding, antithrombotic and hypocholesterolemic effects.[4] Currently, bioactive peptides possessing antihypertensive activities have been studied extensively and peptides from fishes[5] and vegetable proteins[6] are gaining importance for their hypertension regulating properties. Among the unconventional resources of animal proteins, molluscs as a group is regarded as an under-exploited source of health-benefit molecules. The freshwater mussel *Lamellidens marginalis,* found widely distributed in ponds and large bodies of perennial waters in the Indian sub-continent is a well accepted food all over India. The oyster flesh obtained as by-product of pearl fisheries is a cheap protein source. Moreover, ethno-medicinal uses of the flesh of *Lamellidens* among the rural people to treat blood pressure have been documented.[7] This scenario has prompted us to isolate and characterize short peptides prepared from the freshwater edible bivalve *Lamellidens marginalis* against hypertension.

METHODS AND MATERIAL

Materials

The benthic bivalve *Lamellidens marginalis* were collected from the freshwater ponds with the help of local people from the district *South 24Paraganas,* West Bengal, India. Species identification and authentication was done from Zoological Survey of India, Prani Vigyan Bhaban, M-Block, New Alipore, Kolkata-53, [F.No.229–10/98-Mal(Part-1)/6844]. Specimens were brought to the laboratory one week before experimentation. Bivalves of similar size (8cm-10cm long) and average weight (55-70 g with shell) were cleaned by thorough washing with water to remove fouling biomass and algal biomass and allowed to acclimatize in laboratory conditions in a 50. lt glass aquarium. Finally, shells were removed carefully to separate the edible muscle mass. Fleshes were preserved at -20 °C, until analysis.

Commercially available three (3) food grade proteases: Alcalase2.4L (produced by *Bacillus licheniformis*, Subtilisin-A, Sigma Aldrich: P4860, MA, USA), Papain (latex of *Carica papaya*, SR:14049) and Pepsin (from porcine gastric mucosa, Sigma Aldrich: P6887), were used for protein hydrolysis. Angiotensin-I Converting Enzyme (ACE: from rabbit lung; Sigma Aldrich: A6778), Hippuryl-L-histidyl-L-leucine (HHL; Sigma Aldrich: H1635) as the substrate for ACE and Captopril (Sigma Aldrich: C4042) as standard inhibitor of ACE were used for *in vitro* study of antihypertensive activity. All other chemicals and solvents used in this study were of analytical reagent grade.

Methods

Proximate Composition

Ash, carbohydrate, and fibre contents of tissue homogenate were determined by the AOAC (1990) method. Total protein of mussel flesh was determined by Micro-Kjeldahl method.[8] The amount of reducing sugar present in tissue homogenate was analysed by Somogyi method.[9] Tissue Lipid was estimated by the method of Bligh and Dyer[10] with slight modification by Floch *et al.*[11] Amino acid composition of protein was analysed by reverse phase HPLC (Binary Pump-Waters 1525, Waters Dual λ Absorbance Detector 2487) with 150*3.9 mm reverse phase column (Novapack C18, Waters) according to the method of Baker and Han.[12] The whole process was executed using Empower software (v2).

Protein Quality Determination

Protein quality of mussel mass was assessed based on its

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amino acid profile. Essential amino acid index (EAAI) was calculated using amino acid composition of casein as standard. Protein efficiency ratio (PER value) was determined by the method of Alsmeyer *et al.*[13] Biological value was calculated using the FAO pattern of standard protein casein.[14] The quality of protein was determined by calculating the nutritional index (NI). All the above parameters were calculated according to the formulas enlisted below.

$$EAAI = \frac{(lys*thr*val*met*ile*leu*phe*his*tryp)a*}{(Lys*thr*val*met*ile*leu**phe*his*tryp)b*}$$

where, a* represents the content (%) of amino acid is in test sample.

b* represents the content (%) of amino acid is in standard casein.

PER= -0.486 + % Leu - % Tyr

Biological value (BV) = 1.09*EAAI- 11.7

Nutritional index (NI) = (EAAI*% Protein)/100

Isolation and Preparation of Protein Hydrolysates

Preparation of Mussel Protein Isolate

Crude protein isolate was prepared by mixing weighed mussel tissue with phosphate buffer (pH-7.4) at 1:10 w/v, homogenized with a high speed homogenizer (Ultra Turrax T18, IKA®, Werke GmbH&Co.KG, Stufen, Germany) for 30min at 8000 g by keeping the mixture in an ice bath. To remove cell debris, homogenized sample was centrifuged at 5600 g for 30 min. Ammonium sulphate at 50% saturation was slowly added to the supernatant collected, with continuous stirring. Solution was kept in an ice bath for 1h and then centrifuged at 5600 g for 30 min. This time precipitate was collected, dialyzed for 48 h in phosphate buffer (pH-7.4) to to remove the salt and buffer was changed for every 12 h interval. The dialyzed sample was collected and lyophilized to obtain in dried powder form and stored at -20 °C until further analysis.

Preparation of Protein Hydrolysates by Alcalase2.4L

100 ml of deionised water was added to 1.5 g of dried mussel protein isolate, adjusting pH at 8.5 and temperature at 50 °C for 1 h. It was then treated with 0.3% Alcalase2.4L (v/v) with constant stirring to obtain 10 min, 30 min, 60 min and 120 min hydrolysates. Enzyme was rapidly inactivated by heating at 95 °C for 5 min and hydrolysates produced were collected. Particulate materials were removed by centrifugation at 1400 g for 15 min and supernatant was collected and stored at -20 °C until further analysis.

Preparation of Protein Hydrolysates by Pepsin

Mussel Protein Isolate (MPI) was incubated at 50 °C for 1h in shaking condition after being dissolved in 0.1N HCl (1.0% w/v) and pH was adjusted to 2 using 1 N NaOH. 2.1 ml of (0.1%) pepsin solution was added to 40 ml of 1.0% protein solution at 37 °C and hydrolysis was continued for 10 min, 30 min, 60 min, and 120 min with constant shaking. pH of the hydrolysates produced was adjusted to 7.5 (optimum pH: 7.0-8.0) with 1 N NaOH and cooled in ice bath to inactivate the enzyme. It was then centrifuged at 1400 g for 15min and supernatant was collected and stored at -20 °C for further analysis.

Preparation of Protein Hydrolysates by Papain

Mussel Protein Isolate was mixed with distilled water in the ratio of 1:20 (w/v) and pH was adjusted to 10.0 with 1 N NaOH. It was then incubated at 50 °C for 1 h in shaking condition. pH was again adjusted to 8.0 and hydrolyzed with 0.01 g of papain at 37 °C for 10 min, 30 min, 60 min, and 120 min with constant shaking. pH of the hydrolysates obtained was adjusted to 8.0 and enzyme was rapidly inactivated by heating at 95 °C for 5min. Each hydrolysate was then centrifuged and supernatant was collected and stored at -20 °C for further analysis.

Determination of Degree of Hydrolysis

Degree of hydrolysis (DH%) is defined by cleavage percentage of peptide bonds and was determined by the method of Molnar *et al.*[15] 200 µl of mussel protein hydrolysates (MPH_{120}) were mixed with 800 µl of distilled water and 1 ml of ninhydrin, followed by incubation at 100 ºC for 15 min. 5 ml of diluent solution (water:n-propanol=1:1) was added to the mixture and absorbance was measured at 570 nm. Ninhydrin was used to determine the free amino groups. The degree of hydrolysis was calculated from the equation:

$$DH\ \% = h/h_{tot} \times 100$$

[where, h = concentration of peptide bond hydrolysed (meq/gm) and h_{tot} = total amount of the peptide bond (8 amino meq/g)].

Ultrafiltration

Ultrafiltration of 120 min mussel protein hydrolysates (MPH_{120}) was done to separate out low molecular weight peptides (<3KDa) using Vivaspin Unit with 3KDa molecular weight cut-off (MWCO) (VS2091, Sartorius AG, Goettingen, Germany). Membrane fractions from MPH_{120} of all the three enzymes were taken for evaluating ACE-inhibitory activity of the ultrafiltered short peptides.

In vitro ACE-Inhibitory Activity Assay

Angiotensin-converting enzyme by virtue of its action on

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angiotensin-I and bradykinin, plays a crucial role in blood-pressure regulation. In clinical studies the synthetic substrate hippuryl-L-histidyl-L-leucine (HHL) is used to measure serum ACE. The enzyme hydrolyzes the substrate molecule to produce hippurate, which is measured through spectrophotometry at 382 nm. In the present investigation, the ACE inhibitory activity was determined according to the method of Cushman and Cheung *et al.*[16] IC_{50} values of MPH_{120} ultrafiltrates collected from all three enzymes were calculated and the best result was further used for mass spectrometry.

De Novo Sequencing of Peptides by MALDI-TOF/TOF

$AlcH_{120}$ ultrafiltrate was used for amino acid sequencing. The mass analysis of the peptides was performed using a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). 1µl solution of ultrafiltrate was mixed with 1µl of saturated matrix solution containing 4hydroxy-alpha-cyano-cinamic acid (HCCA) in TA (30% acetonitrile + 70% water + 1% tri-fluoroacetic acid) and was spotted on the anchorchip plate. The crystallized peptide spot was analyzed using Ultraflextreme TOF/TOF instrument (Bruker Daltonics, Bremen, Germany). The MS spectra were acquired in reflector mode and MS-MS spectra were acquired in LIFT mode using Flex Control (version 3.4) software. The spectra were acquired in the mass range between 700 and 3500 m/z, with 2000 shots added per sample.

Statistical Analysis

All experiments were conducted in triplicate. Data were analysed for variance between sample groups using oneway ANOVA in Origin Pro-8 (v8.0724) statistical software; MA,USA. Tukey's *T*-test was employed to compare the significant differences between the means at significance limit set at 1% probability.

RESULTS AND DISCUSSION

Proximate Composition

Nutritive value of the fresh water mussel *L. marginalis* has been evaluated. Proximate analyses of dried mussel mass revealed that it contains moderate amount of protein (42±0.67%) and carbohydrate (30.05±0.38%) including reducing sugar (12.65±0.07%) with a very less amount of fat (4.10±0.20%) and fibre (0.10±0.001%). Ash content is about 11.10±0.11%. Differences with previous results may be due to differences in environmental conditions and nature of the diet of the organism, mainly aquatic vegetations.

Amino Acid Composition

Results revealed that oyster protein is composed of good quantity of both the essential (29.95%) and non-essential amino acids (70.05%) which reflect its nutritional value as well. Among the essential amino acids, phenylalanine 4.76±0.02%, isoleucine 4.69±0.03%, lysine 4.88±0.03% are present in moderate amount while the significant quantity of non-essential amino acids including arginine 12.40±0.002%, aspartic acid 13.80±0.001% and glutamic acid 15.60±0.001% have been demonstrated. These amino acids are widely known for their important roles in the maintenance of homeostasis among different physiological functions.

Nutritional Value

Critical analyses with different assessment scores not only indicate the protein quality but also the nutritional value of the freshwater mussel *Lamellidens marginalis* as a supplementary food source. The essential amino acid index (EAAI) of oyster protein is 87.8±0.07%, which is significantly high. Biological value (BV) of the mussel flesh (83.13±0.02%) is well comparable with other animal protein sources like fish (80%) and meat (74%).[17] Protein Efficiency Ratio (PER) is quite within the desirable range (2.52) with nutritional index of 36.87±0.03%.

Degree of Hydrolysis

Figure 1 represents the degrees of hydrolysis of protein isolate from *L. marginalis* which shows gradual increment at a selected time interval. As compared to the whole protein, the concentration of mussel protein isolates increases to 69.9±0.3% after extraction. Mussel protein was hydrolysed by three different proteases: Alcalase2.4L, Pepsin, and Papain at different time intervals (10 min, 30 min, 60 min, and 120 min). MPH_{120} of all the three enzymes produced maximum peptide bond cleavage with 85.45%, 62.30%, and 60.35% degree of hydrolysis respectively. Alcalase2.4L belongs to a family of Serine S8 endoprotease. Its usual preference site for cleavage is the large uncharged residue at the P1 position. On the other hand, Pepsin is mostly an exopeptidase and exhibits preferential cleavage for hydrophobic residues like phenylalanine, tryptophan, proline, or leucine in PI and P1 positions. Papain, a cysteine protease of the peptidase C1 family exhibits broad specificity, cleaving peptide bonds of basic amino acids, leucine or glycine. Papain exhibits a preference for amino acid bearing a large hydrophobic side chain at the P2 position.[18]

ACE Inhibitory Activity of Mussel Protein Hydrolysate

Food derived bioactive peptides are regarded as biological regulators and can perform a variety of functions in the gastrointestinal tract, in intestinal epithelium, and also after systemic absorption. Recently, many biologically active

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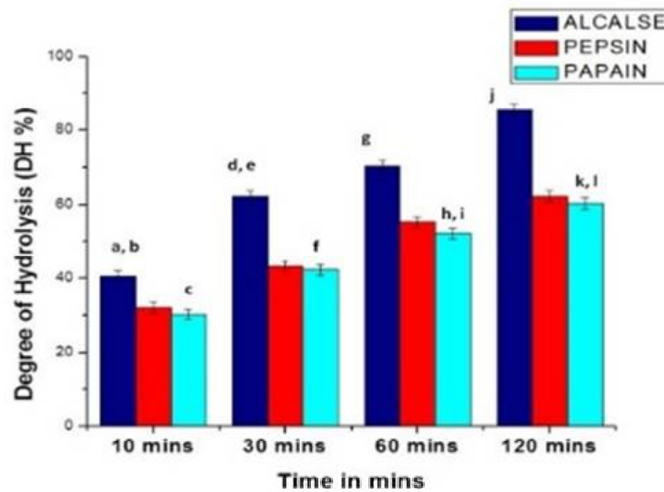
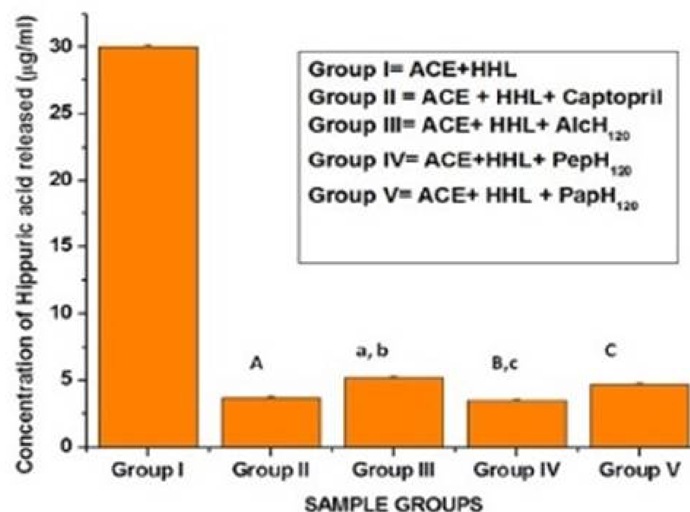
Figure 1: Determination of Degree of Hydrolysis of Protein Hydrolysates



Note: Enzymatic hydrolysis of L *marginalis* protein concentrates with Alcalase2.4L, Pepsin and Papain at different time intervals. All values are expressed as mean±SD for triplicate experiments. All the superscripts here are indicative of statistical differences between hydrolysis at different enzymes and time interval groups at significance level of $P<0.01$. a=$AlcH_{10}$ *vs* $PepH_{10}$; b=$AlcH_{10}$ *vs* $PapH_{10}$, c=$PepH_{10}$ *vs* $PapH_{10}$, d=$AlcH_{30}$ *vs* $PepH_{30}$, e=$AlcH_{30}$ *vs* $PapH_{30}$, f=$PepH_{30}$ *vs* $PapH_{30}$, g=$AlcH_{60}$ *vs* $PepH_{60}$, h=$AlcH_{60}$ *vs* $PapH_{60}$, i=$PepH_{60}$ *vs* $PapH_{60}$, j=$AlcH_{120}$ *vs* $PepH_{120}$, k=$AlcH_{120}$ *vs* $PapH_{120}$, l=$PepH_{120}$ *vs* $PapH_{120}$.

peptides from food proteins have been investigated for their medicinal values. Angiotensin-I-converting-enzyme (ACE) is a zinc metalloproteinase present both in biological fluids and in many tissues. It catalyzes the conversion of angiotensin-I to angiotensin-II, a potent vasoactive peptide that causes blood vessel constriction resulting in increased blood pressure. Thus, ACE inhibitory peptides are the most-searched food derived peptides for their ability to control hypertension.[19] A wealth of literature published during the last few years attest to the potential of the enzymatically hydrolysed food derived peptides having ACE inhibitory/antihypertensive activity. In the present investigation, an attempt has been made to study *in vitro*, ACE inhibitory property of ultrafiltrates (containing <3KDa peptides) of MPH_{120} prepared with Alcalase2.4L, Pepsin and Papain. Captopril, a potent synthetic ACE

Figure 2(a): ACE Inhibitory Activity of Mussel Protein Ultrafiltrates



Note: *In vitro* Angiotensin-I converting enzyme inhibition potential (expressed by means of concentration (µg/ml) of hippuric acid released from ACE substrate (HHL) in presence of standard ACE inhibitor Captopril and *L. marginalis* protein hydrolysates, hydrolysed by Alcalase2.4L, Pepsin and Papain for 120 min, i.e. $AlcH_{120}$, $PepH_{120}$ and $PapH_{120}$ respectively. All values are expressed as Mean±S.D for triplicate experiments. All the superscripts here are indicative of statistical differences between mentioned in the graph. Both the upper-case letters and lower case letters indicate significant differences between the groups at the significance level of 0.05. A=Group II *vs* Group III, B=Group II *vs* Group IV, C=Group II *vs* Group V, a=Group III *vs* Group IV, b= Group III *vs* Group V, c= Group IV *vs* Group V.

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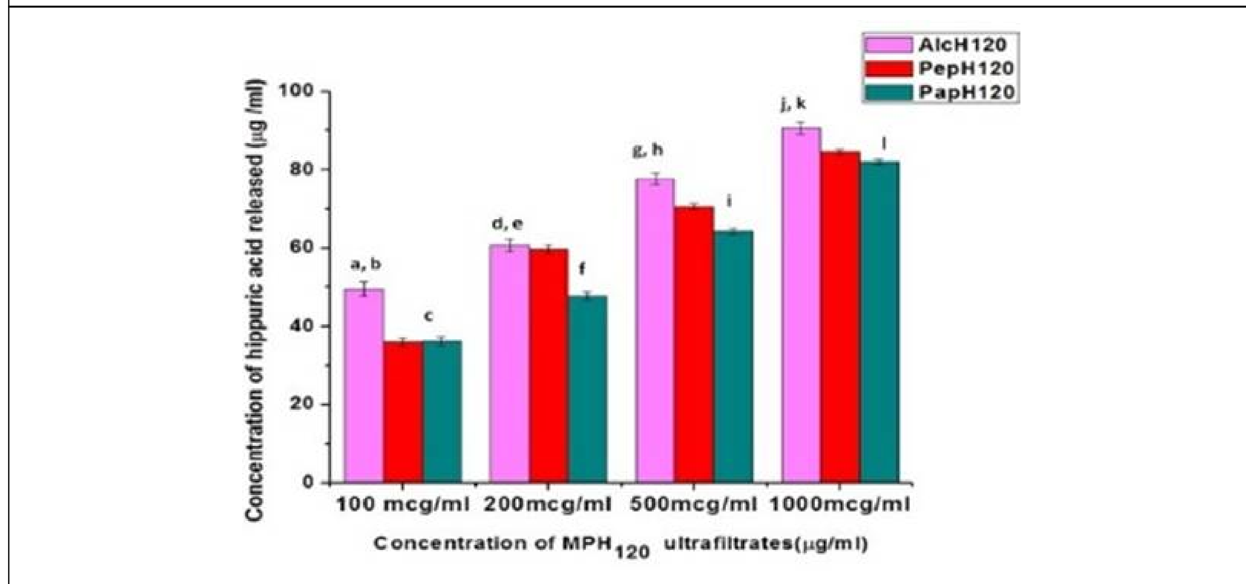
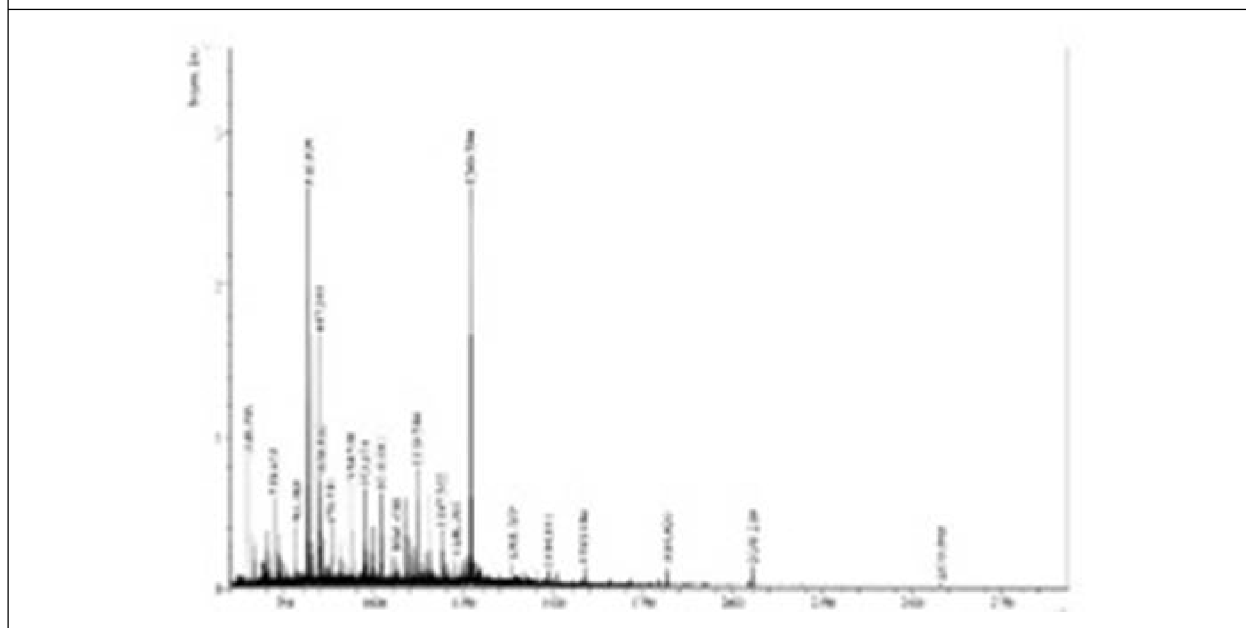
Figure 2(b): Determination of IC_{50} Values of MPH_{120} Ultrafiltrates



Note: ACE inhibitory activity to determine IC_{50} value of different protein hydrolysates of *L. marginalis,* hydrolysed by Alcalase2.4L, Pepsin and Papain for 120 mins studied in different peptide concentrations. All values are expressed as Mean±SD for triplicate experiments. All the superscripts here are indicative of statistical differences between similar concentration of different enzymatic hydrolysates at significance level of $P<0.01$. At 100 mg/ml: a=$AlcH_{120}$ *vs* $PepH_{120}$, b=$AlcH_{120}$ *vs* $PapH_{120}$, c=$PepH_{120}$ *vs* $PapH_{120}$. At 200 mg/ml: d=$AlcH_{120}$ *vs* $PepH_{120}$, e=$AlcH_{120}$ *vs* $PapH_{120}$, f=$PepH_{120}$ *vs* $PapH_{120}$. At 500 mg/ml: g=$AlcH_{120}$ *vs* $PepH_{120}$, h=$PepH_{120}$ *vs* $AlcH_{120}$, i=$PepH_{120}$ *vs* $PapH_{120}$. At 1000 mg/ml: j= $PepH_{120}$ *vs* $AlcH_{120}$, k=$AlcH_{120}$ *vs* $PapH1_{120}$, l=$PapH_{120}$ *vs* $PepH_{120}$.

Figure 3: MS Spectrum of the $AlcH_{120}$ Ultrafiltrate



Note: MALDI-TOF spectrum of the <3kDa permeate of the $AlcH_{120}$ of the protein extracted from *Lamellidens* flesh. The spectrum was internally calibrated using mass peaks of 3 peptide fragments e.g. Bradykinin (1-7), Angiotensin II, Renin substrate, respectively, at m/z 757.39916, 1046.5418 and 1758.9326.

inhibitor has been taken as a standard to compare the potentiality of experimental peptides. Figure 2(a) shows the ACE inhibitory activity of the MPH_{120} ultrafiltrates in comparison to the standard ACE inhibitor. In this study, Group-I indicates the control group (i.e.without any inhibitor of ACE). Group-II shows the inhibitory activity of Captopril as the concentration of released hippuric acid (3.72 µg/ml) is diminished in comparison to Group-I (30.05 µg/ml) due to its competitive inhibitory effect. Group-III which consists of ACE, HHL, and ultrafiltrate of $AlcH_{120}$ also shows a significant reduction in the concentration of released hippuric acid (5.24 µg/ml) which is comparable to the inhibitory activity of Captopril. Similar results have also been obtained in Group-IV (3.52 µg/ml) and Group-V

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Table 1: *de novo* Sequenced Peptides Identified in <3KDa Permeate Obtained from $AlcH_{120}$, Derived from *L. marginalis* Protein with Significant Sequence Matching in Relation to Documented ACE-Inhibitory Peptides

Observed Mass (Dalton)	Obtained Sequence	ACE Inhibitory Peptide	IC_{50} Value	Reference
815.445	MGEPLLR	GEP	3.2 mM	[21]
		LNENLLRFFVAPEPEVFG	280 µM	[22]
830.953	IGPGPFSR	GAHypGPAGPGGIHypGERG	45.6 Âµg/ml	[23]
		GPFPILV	42.4 ÂµM	[22]
		GPP	6.25 µg/ml	[21]
892.435	GPPLYCSR	PPLTQTPV	173.3 µM/l	[24]
973.543	ITGGGVNISR	GVNGEEGVPG	*NR	[23]
1111.524	QSNPPQNGNR	NPPHQIYP	37 µM	[24]
		PQNILP	440 µM	[24]
1137.548	QGGVHYWYK	GVHHA	71.8 ÂµM	[24]

Note: *NR = not reported.

(4.71 µg/ml) containing ACE, HHL, and the ultrafiltrate of $PepH_{120}$, and $PapH_{120}$ respectively. This observation further demonstrates that short peptides from $PepH_{120}$ are more potent ACE inhibitor than the other two MPH_{120} that reduce the release of hippurate and comparable to Captopril.

Moreover, bioactivity is measured in terms of percentage of ACE inhibition, and in this regard, IC_{50} values indicate the peptide concentration required to reduce the ACE activity to 50%. IC_{50} values of MPH_{120} ultrafiltrates of Alcalase2.4L, Pepsin and Papain as shown in Figure 2(b) is 47.22 µg/ml, 142.79 µg/ml, and 502.55 µg/ml respectively. These results clearly demonstrate that <3KDa-ultrafiltrate of $AlcH_{120}$ has the maximum potential to inhibit ACE activity and a lower concentration of peptides (47.22 µg/ml) to reduce ACE activity to 50%, compared to Pepsin and Papain. This observation further confirms that Alcalase2.4L is more potent for the production of short peptides than Pepsin and Papain. Therefore, ultrafiltrate of $AcIH_{120}$ has been selected to perform MALDI-TOF to identify the amino acid sequences of antihypertensive short peptides.

MALDI-TOF Analysis

The mass-spectrum of ultrafiltrate fraction (<3KDa) of $AlcH_{120}$ is presented in Figure 3. From thousands of peptides, six peptides with high intensity and molecular weight ranging from 815-1137Dalton are selected and matched with already registered ACE inhibitory peptide sequences to find out the overlapping sequences among them. Peptides a rich hydrophobic amino acid residues at the C-terminal end and presence of proline residues show significantly high ACE inhibitor activity.[20] Peptides listed in Table 1 show significant sequence matching with that of many well documented ACE inhibitory peptides. A novel ACE inhibitory peptide having amino acid sequence: isoleucine-glycine-proline-glycine-proline-phenylalanine-serine-arginine(IGPGPFSR; molecular weight 830.953 Dalton) of antihypertensive nature has been identified. Purification and further analysis may help to understand the structure-function relationship between ACE and this mussel derived bioactive antihypertensive peptide.

CONCLUSION

Taken together the present observations made in this study propose that this molluscan soft body can be used as an unconventional low cost animal protein source for food security and disease prevention. Present investigation has demonstrated that bioactive peptides derived from the freshwater mussel *Lamellidens marginalis* may serve as a useful supplement for nutraceutical therapy to treat hypertension. Moreover, *Lamellidens* protein hydrolysates can also be a valuable source for industrial applications in developing functional foods. However, the observations made in this investigation are preliminary and therefore should be viewed as a prelude to what future holds.

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