Chemical Composition and Bioactive Properties of Aqueous Extracts From Four Species of Marine Bivalves

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Abstract

This study evaluated the proximate composition, amino acid composition and bioactive properties of four marine bivalves namely *Placuna placenta* (Linnaeus, 1758), *Placuna ephippium* (Philipsson, 1788), *Marcia hiantina* (Lamarck, 1818), and *Anadara inaequivalvis* (Bruguière, 1789). Major proximate components of the bivalve tissues were moisture (74.27 ± 3.20 – 80.18 ± 0.80 %), followed by protein (9.08 ± 0.50–13.54 ± 0.50 %), ash (1.87 ± 0.20–3.57 ± 1.00 %), and lipid (0.60 ± 0.06–1.27 ± 0.30 %). Lysine and threonine were the most abundant essential amino acids (EAAs) in the range of 4–10 % amino acid content, while arginine (7.1–9.8 %) and glutamic acid (4.4–9.1 %) were the most abundant of the non-essential amino acids (NEAAs). *Marcia hiantina* had the highest percentage of total EAAs and NEAAs at 17.3 % and 33.0 %, respectively. Samples were extracted using aqueous extraction, then subjected to biological assays to evaluate bioactivity and extraction in cold compared with hot water. Cold-water extract of *M. hiantina* demonstrated the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric-ion reducing capacity (76.24 ± 7. 15 % and 1.58 ± 0.04 mM Trolox equiv.mL^{-1} sample, respectively) while the cold-water extract of *P. ephippium* exhibited the highest 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity at 93.67 ± 0.29 %. Moreover, hot-water extract of *P. placenta* demonstrated the highest angiotensin I-converting enzyme (ACE) inhibition at 76.01 ± 2.24 %. These results suggest that these four marine bivalve species might possess bioactive peptides eliciting antioxidant and anti-hypertensive activities which can be used as natural supplements and drug alternatives *in lieu* of synthetic medicines.

Keywords: proximate composition, essential amino acids, water extraction, antioxidant activity, ACE inhibitory activity

Introduction

Marine bivalves, which represent the second-largest class in the Phylum Mollusca, are one of the many natural sources found to have an abundance of unique compounds which exhibit functional bioactivities (Shanmugam et al., 2019). Several marine-derived bioactive peptides were proven in the laboratory to have positive biological functions such as anti-inflammatory, anti-cancer, antioxidant, anti-coagulant, and anti-microbial activities (Venugopal and Gopakumar, 2017).

About 22,000 mollusc species are found in the Philippines (Del Norte-Campos et al., 2019), but despite their importance and diversity, Philippine molluscs, like other invertebrates, have received less focus in research and conservation (Ramos et al., 2018). Some of these bivalve molluscs are important commercially, but majority are underutilised, either because they are sold cheaply when they are in abundance or there is an absence of post-harvest processing technique to increase their market value. For instance, *Placuna placenta* (Linnaeus, 1758) and *Placuna ephippium* (Philipsson, 1788) from family Placunidae are more valued for its iridescent shell for decorative purposes rather than its meat. The meat is usually removed from the shell and used as a component for poultry and shrimp feeds. *Marcia hiantina* (Lamarck, 1818) and *Anadara inaequivalvis*

(Bruguière, 1789) are commercially important bivalve species in the Philippines but were not extensively studied. A recent study reported that the protein extracts of these marine bivalves possess antioxidant and antibacterial activities which can be potential sources of bioactive compounds (Salido et al., 2022).

Metabolic processes and environmental exposures induce harmful free radicals and reactive oxygen species (ROS) which can be linked to a variety of health issues, including cancer, cardiovascular disease, diabetes and degenerative disease (Lobo et al., 2014). Antioxidants play a crucial function in neutralising oxidative processes and preventing the adverse effects of free radicals (Flora, 2007). Many low-cost and effective synthetic antioxidants are available in the market but may cause possible risks *in vivo*, limiting their use in food applications (Ito et al., 1985). Thus, the search for natural antioxidants from marine organisms has been gaining momentum in recent years (Anjum et al., 2017).

Hypertension is another major public health issue around the world with associated risks of heart failure and chronic disorders (Lordan et al., 2011). There is an array of synthetic antihypertensive drugs available in the market which include diuretics, Angiotensin II receptor blockers and enzyme inhibitors. While these blood pressure-lowering medications are effective, they come at a high cost to the global healthcare system and have certain negative side effects, shifting the focus on natural food-derived antihypertensive peptides as potential hypertension preventative agents (Wang et al., 2008). Marine organisms have been highlighted as potential sources of angiotensin I-converting enzyme (ACE) inhibitors (Anjum et al., 2017). ACE inhibitors can effectively lower the mean arterial blood pressure, necessary to treat and manage hypertension (Nasution, 2006).

The aim of the present work is to evaluate the chemical composition and biological activities of four species of marine bivalves, *P. placenta, P. ephippium, M. hiantina*, and *A. inaequivalvis*. The proximate composition and essential amino acids were investigated to determine the biochemical indices of these marine bivalves for its optimum utilisation as putative food with bioactive properties.

Materials and Methods

Ethical approval

No live animals were used in the conduct of the study. Bivalve samples and the methods used were approved by the Institutional Animal Care and Use Committee (IACUC) of University of the Philippines Visayas under the project "Biochemical composition and biopotentialities of crude tissue extracts of commercially important marine bivalves in Panay" (Project SP21-17, 26 August 2021).

Collection of samples

Adult bivalve samples namely *P. placenta*, *P. ephippium*, *M. hiantina*, and *A. inaequivalvis* (Fig. 1) were obtained from selected wet markets in Panay Island, Philippines between September to October 2022. Morphological identification up to the species-level was based on a field guide by Laureta (2008). Each bivalve species (~20 kg) was packed in insulated boxes and transported live to the laboratory. Upon arrival, manual separation of the meat from the shell was performed. Samples were then stored frozen at –20 °C.

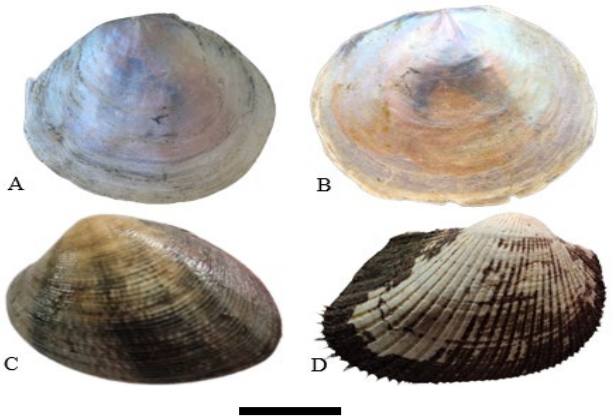

Fig. 1. Bivalve species used in the study. A) *Placuna placenta*; B) *Placuna ephippium*; C) *Marcia hiantina*; and D) *Anadara inaequivalvis*. Scale bar = 10 mm.

Chemical analysis of bivalve tissues

The proximate components of the bivalve samples were evaluated using standard methods (AOAC, 2000). Briefly, the moisture content of the bivalve tissues (2-g sample) was analysed by oven-drying method at 105 °C until constant weight. For crude ash determination, 2-g samples were placed in a porcelain crucible and heated to 600 °C in a temperature-controlled furnace and weighed. The Kjeldahl method (Foss Analytical Höganäs, Sweden) was used to measure the total nitrogen content, and protein content was calculated by multiplying the total nitrogen value by the factor of 6.25. Crude lipid was extracted using a mixture of chloroform-methanol (1:2, v/v) according to Bligh and Dyer (1959). The total lipid content was calculated as a percentage of the dry muscle weight.

The amino acid profile of the bivalve tissues was analysed using ortho-phthalaldehyde in a high-performance liquid chromatography LC-10A/C-R7A amino acid analysis system (Shimadzu, Japan) with 18 amino acid standards following the method by Shimadzu Corporation (Kyoto, Japan). The amino acid composition of the samples (% AA) was expressed as residues per 100 amino acid residues using the equation below:

$$\%\ \mathrm{AA} = \frac{\text{Concentration of an individual amino acid (mM)}}{\text{Concentration of total amino acids tested (mM)}} \times 100$$

Aqueous extraction

Aqueous (hot- and cold-water) extraction was performed using the method of Karaulova et al. (2021) with slight modifications. About 500 g of homogenised bivalve tissues were subjected to boiling for 15 min at 95 °C for hot-water extraction. While for cold-water extraction, the homogenates were macerated for 30 min in cold, distilled water (~4 °C). Subsequently, the extracted tissues were centrifuged at 8000 rpm for 10 min and the supernatant was collected and filtered (Whatman, 0.45 µm PVDF). All collected water-soluble protein extracts were freeze-dried (VaCo5 Zirbus, Germany) to obtain the dry powder and stored at -20 °C for further analysis. The percentage yield of the dried protein extracts was in the range of 8–10 % for both cold- and hot-water extraction.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The scavenging effect on DPPH free radical was determined using the method of Blois (1958) with slight modifications. A 100 µL of sample extract was mixed with DPPH solution (100 µL, 0.1 mM in 96 % ethanol). The mixture was shaken and left in the dark for 30 min. The absorbance of the resulting solution was measured at 515 nm using a UV-visible spectrophotometer (BMG Labtech, Germany). Positive control of ascorbic acid (Sigma Corp., USA) dilutions was prepared in the same manner. The DPPH scavenging activity was determined using this equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \text{ x } 100$$

where "$\text{Abs}_{\text{control}}$" is an absorbance of the reference solution (100 µL of distilled water instead of the test sample), "$\text{Abs}_{\text{sample}}$" is an absorbance of the test solution/positive control.

2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

The ABTS radical scavenging activity was quantified using the method of Re et al. (1999) with slight modifications. A solution containing 7 mM ABTS with 2.45 mM potassium persulfate (final concentration) was left in a dark room for 12–16 h to produce the radical cation ABTS. The radical ABTS solution was diluted in phosphate buffer solution (pH = 7.4) to obtain the working solution and absorbance was read until 734 nm. Trolox was used as positive control and distilled water as negative control. The ABTS scavenging activity was determined by the equation:

$$\text{ABTS radical scavenging activity (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \text{ x } 100$$

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was analysed according to the method of Lee et al. (2021) with slight modifications. One mL reaction solution of each sample, 1 mL of phosphate buffer (0.2 mol.L^{-1}, pH 6.6) and 2 mL of potassium ferricyanide (1 %, w/v) were incubated at 50 °C for 20 min. To terminate the reaction, 1 mL of trichloroacetic acid solution (10 %, w/v) was added. Then, 375 µL distilled water and 75 µL of 0.1 % ferric chloride were added to the solution in a dark room. The absorbance was measured at 700 nm after incubation for 30 min. Trolox was used as positive control and was prepared in a similar manner. The regression value was used to calculate the FRAP value units expressed as mM Trolox equiv.mL^{-1} sample.

Angiotensin I-converting enzyme (ACE) inhibition assay

The inhibitory effect of ACE was evaluated according to the method of Cushman and Cheung (1971) with some modifications (Jimsheena and Gowda, 2009) using hippuril-histidyl-leucine (HHL; Sigma-Aldrich, USA) as the substrate. Briefly, the aliquot was prepared using HHL mixed with 50 mM sodium borate buffer (pH 8.3, containing 0.5 M NaCl). Then, 150 µL of aliquot was mixed with 50 µL each diluted sample and added with 100 µL ACE (Sigma-Aldrich, USA). This was followed by incubation for 30 min at 37 °C. The reaction was then quenched with the addition of 250 µL of 0.05 N HCl. Pyridine (0.4 mL) was added followed by 0.2 mL of benzene sulphonyl chloride (BSC; Sigma-Aldrich, USA) and mixed by inversion for 1 min and cooled on ice. Captopril was used as positive control and the absorbance was measured at 410 nm. The ACE inhibition (%) was calculated using the following formula:

$$\text{ACE inhibitory activity (\%)} = \frac{(\text{A1} - \text{A2})}{(\text{A1} - \text{A3})} \text{ x } 100$$

where "A1" is the absorbance of the positive control, "A2" is the absorbance of the test solution and "A3" is the blank solution (HHL solution only).

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's test were used to analyse the data (mean ± standard deviation). All statistical analyses were performed using R software version 4.1.2 (R Core Team, 2014). *P* values lower than 0.05 were statistically different.

Results

Proximate components of the bivalve tissues

The proximate composition of the four marine bivalves

is shown in Table 1. The major composition of the bivalve tissues was moisture (74.27–80.18 %), followed by protein (9.08–13.54 %), ash (1.87–3.57 %), and lipid (0.60–1.27 %). Significant differences ($P < 0.05$) were detected among the bivalve samples in all the parameters tested. Highest moisture content was observed in *P. ephippium* at 80.18 ± 0.80 %, but this value was not significantly different ($P > 0.05$) from the moisture content of *P. placenta* at 78.65 ± 1.00 %. Protein was found significantly highest ($P < 0.05$) in *A. inaequivalvis* (13.54 ± 0.50 %) and lowest in *P. ephippium* (9.08 ± 0.50 %). Lowest ash content was found in *A. inaequivalvis* (1.87 ± 0.20 %), while *P. placenta* showed the highest lipid content (1.27 ± 0.30 %) among the four bivalve species.

Amino acid composition of the bivalve protein extracts

Table 2 shows the amino acid composition of the bivalve tissues expressed as residues per 100 amino acids. The major amino acid in the bivalve samples was arginine, followed by glutamic acid, glycine and lysine. In general, the two *Placuna* species, *P. placenta* and *P. ephippium*, had almost similar amino acid profiles. *M. hiantina* had the highest percentage of essential amino acids (EAAs) (17.3 %), while *A. inaequivalvis* had the lowest percentage EAAs (10.2 %) among the bivalve species. Lysine and threonine were the most abundant EAAs in all samples, while the rest had less than 3 % amino acid content.

Consistently, *M. hiantina* had the highest percentage of non-essential amino acids (NEAAs) (33.0 %), while *A. inaequivalvis* had the lowest percentage NEAAs (20.5 %). The most abundant NEAAs in the bivalve samples were arginine (7.1 to 9.8 %), glutamic acid (4.4 to 9.1 %), and glycine (2.0 to 5.7 %).

Antioxidant activities of the bivalve samples

The feasibility of hot- and cold-water extraction to recover water-soluble proteins from the bivalve tissues was explored in this study. The shift towards green extraction methods such as aqueous extraction can

Table 1. Proximate composition of the muscle tissue of four marine bivalves.

Bivalves	Parameters (%)			
	Moisture	Ash	Lipid	Protein
Placuna placenta	78.65 ± 1.00^{a}	2.53 ± 0.02^{a}	1.27 ± 0.30^{a}	10.55 ± 0.20^{b}
Placuna ephippium	80.18 ± 0.80^{a}	2.76 ± 0.20^{a}	0.83 ± 0.20^{b}	9.08 ± 0.50^{c}
Marcia hiantina	74.27 ± 3.20^{c}	3.57 ± 1.00^{a}	0.60 ± 0.06^{c}	11.44 ± 0.02^{b}
Anadara inaequivalvis	76.22 ± 0.40^{b}	1.87 ± 0.20^{b}	0.75 ± 0.20^{c}	13.54 ± 0.50^{a}

Values (mean ± standard deviation, n = 3) in the same column followed by different lowercase superscripts are significantly different ($P < 0.05$).

Table 2. Amino acid composition of the bivalve molluscs tissue (% amino acids).

Amino Acid	*Placuna placenta*	*Placuna ephippium*	*Marcia hiantina*	*Anadara inaequivalvis*
Essential amino acids				
Isoleucine	0.5	0.3	0.7	0.2
Leucine	1.5	1.7	2.1	0.8
Histidine	0.1	0.1	0.0	1.5
Lysine	4.5	4.8	5.9	3.6
Methionine	0.8	0.7	1.0	0.5
Phenylalanine	2.0	1.6	2.4	1.2
Threonine	3.6	2.8	4.7	2.2
Tryptophan	0.1	0.1	0.1	0.1
Valine	0.2	0.2	0.4	0.1
Non-essential amino acids				
Aspartic acid	2.3	2.3	3.2	3.8
Serine	0.5	0.4	0.7	0.3
Glutamic acid	7.0	5.7	9.1	4.4
Proline	NDL	NDL	0.4	0.6
Glycine	5.7	5.5	5.4	2.0
Alanine	2.0	1.6	3.7	1.6
Arginine	8.4	9.8	8.8	7.1
Cysteine	NDL	NDL	0.6	0.2
Tyrosine	0.6	0.5	1.1	0.5
Total EAAs	13.3	12.3	17.3	10.2
Total NEAAs	26.5	25.8	33.0	20.5

NDL = Not detectable level; EAAs = essential amino acids; NEAAs = non-essential amino acids.

encourage the synthesis of diverse bioactive compounds from low-grade raw materials with concomitant positive biological activities. The antioxidant activities of the aqueous extracts of the bivalve tissues are shown in Figure 2. For the DPPH radical scavenging activity (Fig. 2A), results show that the cold-water extract of *M. hiantina* gave the highest DPPH radical scavenging activity at 76.24 ± 7. 15 % ($P < 0.05$). This value is not significantly different ($P > 0.05$) from the DPPH radical scavenging activity of ascorbic acid at 81.32 ± 1.55 %. While the hot-water extract of *P. placenta* had the lowest value at 16.09 ± 4.15 % ($P < 0.05$).

Highest ABTS radical scavenging activity was observed in *P. ephippium* for both cold- and hot-water extracts (93.67 ± 0.29 % and 90.84 ± 0.59 %, respectively), but these values were not significantly different ($P > 0.05$) from the values obtained from the hot- and cold-water extracts of *P. placenta* at 90.34 ± 1.04 % (Fig. 2B). The cold-water extract of *M. hiantina* gave the lowest value for the ABTS radical scavenging activity at 85.51 ± 8.23 % ($P < 0.05$).

The antioxidant capacity of the bivalve species was further evaluated with its ability to reduce Fe^{3+} to Fe^{2+}, which can indicate that compounds with higher reducing power possess greater ability to donate hydrogen (Wang et al., 2008). As shown in Figure 2C, significantly high reducing capacity was observed in both *M. hiantina* and *A. inaequivalvis* samples under cold-water extraction at 1.53 ± 0.04 and 1.20 ± 0.15 mM Trolox equiv.mL^{-1} sample, respectively ($P < 0.05$), while lowest FRAP values were obtained in both hot- and cold-water extracts of *P. placenta*. These results agreed with the DPPH radical scavenging activity of *M. hiantina* with the highest activity among the bivalve samples.

ACE inhibitory activity of the bivalve samples

The antihypertensive properties of the four bivalve species were evaluated through its ACE inhibitory activity as shown in Figure 3. Results show that highest ACE inhibitory activity was observed in hot-water extract of *P. placenta* at 76.01 ± 4.24 %, while lowest ACE inhibitory activity was observed in both *Placuna* species at 28.11 ± 11.8 % extracted under cold water. The ACE inhibitory activity of *P. placenta* is not significantly different from those of the positive control, captopril, which is at 91.10 ± 1.50 %.

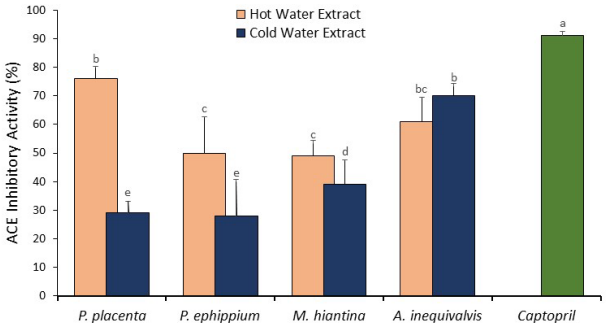

Fig. 3. Angiotensin I-converting enzyme (ACE) activity water-soluble proteins of four bivalve species. Each value represents mean ± SD (n = 3). Values with different letters are significantly different ($P < 0.05$). Positive control is captopril.

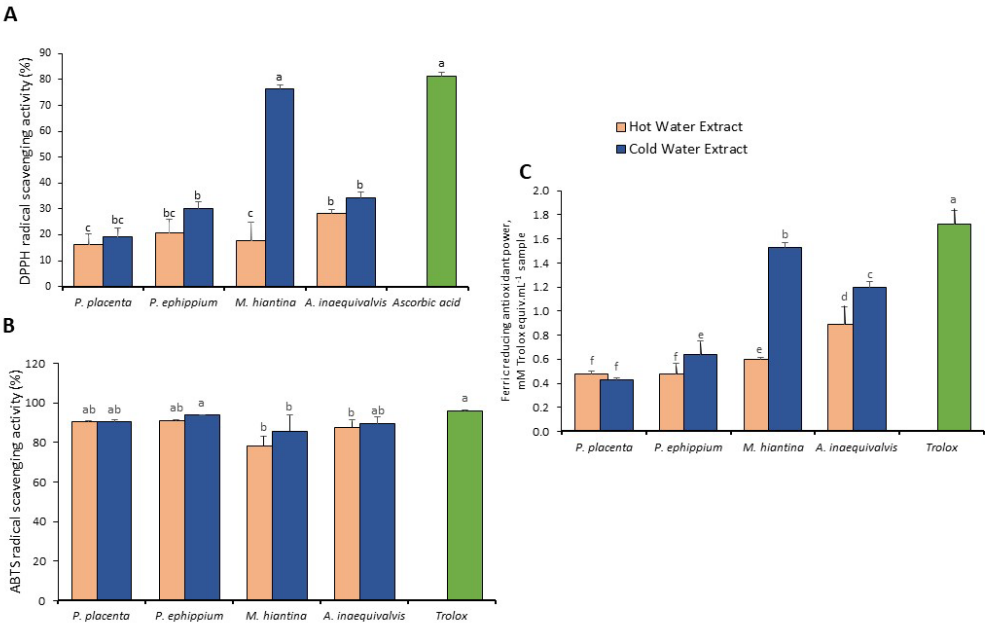

Fig. 2. Antioxidant activities of water-soluble proteins of bivalve tissues. (A) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, (B) 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity, and (C) Ferric reducing antioxidant power (FRAP) of the four bivalve species. Each value represents mean ± SD (n = 3). Values with different letters are significantly different ($P < 0.05$). Ascorbic acid and Trolox were used as positive control.

Discussion

The chemical composition of marine bivalves is hugely influenced by their reproductive cycle, physiological state, and nutritional condition, among other factors (Berthelin et al., 2000). In this study, the bivalves were caught after the spawning season, during the months of September and October. It has been observed that the tissues of some bivalves caught after spawning contain 3 to 4 times less protein, 4 to 5 times less carbohydrates, and 10 to 12% more moisture (Karaulova et al., 2021). The protein contents of the bivalve samples were comparable or slightly higher than the values obtained in other economically important shellfish species (Normah and Noorasma, 2015; Venugopal and Gopakumar, 2017). Generally, the lipid content of the bivalve samples obtained in this study were lower than 3 %, which suggests that these bivalves can be a potential source of low-fat meat (Chen et al., 2012).

Moreover, *M. hiantina* and *A. inaequivalvis* had adequate protein/lipid ratios (approximately 12:1), which, from a nutritional perspective, would mean a good balance of nitrogen and amino acids vital for the human body (FAO/WHO/UNU, 2007). The high ash levels obtained in this study, particularly in *M. hiantina* (3.57 ± 1.00 %), may also indicate important mineral contents suitable for human consumption. Normally, fish and shellfish comprise the majority of naturally occurring elements, with ash contents ranging from 0.4 to 2 % (Biandolino et al., 2020.). Hence, the high levels of ash present in *M. hiantina* indicates that it is rich in minerals such as magnesium, calcium, potassium, and zinc (Ayanda et al., 2019).

The abundance of certain amino acid residues can be correlated to improved antioxidant capacities. For instance, several studies reported that an elevated ratio of hydrophobic amino acids (alanine, leucine, isoleucine, methionine, phenylalanine, and valine) improved antioxidant activities (Chen et al., 1996; Guo et al., 2009). Furthermore, Zainol et al. (2003) also indicated that aromatic amino acids (i.e., tyrosine and phenylalanine) can function as potent electron donors, thereby sequestering the free radicals. A significant amount of hydrophobic amino acids such alanine and leucine (4–5 %), as well as aromatic amino acids, although in low amounts (1–2 %), were present in the bivalve protein extracts used in this study. The presence of hydrophobic amino acids may facilitate peptides to enter target organs through hydrophobic interactions with membrane lipid bilayers and enhance antioxidant activity *in vitro* (Ranathunga et al., 2006). These bivalve species can be a significant source of amino acids needed for optimum health and as functional food with health benefits.

Interestingly, along with the highest percentage of total EAAs among the bivalve samples, *M. hiantina* also showed the highest DPPH radical scavenging activity and highest ferric reducing activity. The significant amounts of lysine and threonine present in *M. hiantina* confirmed its high antioxidant activity. These specific amino acid residues may function as electron donors and interact with free radicals, stabilising them and thus terminating the radical chain reaction (Ranathunga et al., 2006). To date, no studies were conducted on the antioxidant activities of *M. hiantina* but previous literature confirmed that close relative species of *M. hiantina* namely *Meretrix casta* (Gmelin, 1791) and *Meretrix meretrix* (Linnaeus, 1758) were reported to possess high antioxidant activities (Shenai-Tirodkar et al., 2012; Sugesh and Mayavu, 2013). Moreover, the presence of hydrophobic and acidic amino acids in the protein samples can be positively correlated to the enhanced ferric reducing ability (Song et al., 2015).

The ABTS radical scavenging activity of the bivalve samples in this study, which ranged from 86–94 %, was higher as compared to those reported in literature. For instance, the ABTS radical scavenging activity of the mussel, *Perna canaliculus* (Gmelin, 1791), hydrolysed using pepsin and alcalase, showed highest radical scavenging activity at 77 % and 50 % for pepsin and alcalase hydrolysates, respectively (Jayaprakash and Perera, 2020). Furthermore, Yang et al. (2019) assessed the ABTS radical scavenging activity of six antioxidant peptides from blood cockle, *Tegillarca granosa* (Linnaeus, 1758) and observed radical scavenging activity ranging from 45–75 %. ABTS is a reliable method to measure the capacities of antioxidant peptides, and the findings in this study indicated that the aqueous extracts of these bivalves could effectively terminate the chain reaction of ABTS cation radicals, changing them into a colourless form.

Moreover, results also revealed that samples from cold-water extraction exhibited higher antioxidant activity than the samples extracted under hot water. Karaulova et al. (2021) also reported better antioxidant activities from cold-water extracts than hot-water extracts of three bivalve species namely *Anadara broughtoni* (Schrenck, 1867), *Corbicula japonica* (Prime, 1864), and *Spisula sibyllae* (Valenciennes, 1858). One possible cause might be the denaturation of bioactive proteins during heat application. Some proteins get denatured upon heat application at ~60 °C and lose their structure as well as some of its functions. Usually, cold denaturation of proteins can happen at temperatures below 0 °C (Dias et al., 2010). In the present study, cold-water extracts were subjected to 4 °C, so protein denaturation may have been reduced, preserving structural integrity and functionality of the proteins.

ACE activity leads to the conversion of its active vasoconstrictor form, from angiotensin-I to angiotensin-II, and degrades the vasodilator bradykinin resulting in hypertension or increased blood pressure. As a consequence, ACE inhibitors are widely used to prevent the synthesis of angiotensin-II in the treatment of cardiovascular disorders (Je et al., 2009).

Food-derived peptides were shown to exhibit ACE inhibitory activity, are regarded as safe and have milder effects than synthetic drugs (Khan and Kumar, 2019). Based on this, the ACE inhibitory activity of bivalve samples was evaluated. In contrast to the results of the antioxidant activities that cold-water extracts showed higher antioxidant capacities than hot-water extracts, highest ACE inhibitory activity was observed bivalve samples extracted under hot water.

Previous studies confirmed that bivalve species subjected to higher extraction temperatures resulted in better ACE inhibitory activity. Lee et al. (2021) evaluated the crude protein hydrolysates of the pen shell, *Atrina pectinata* (Linnaeus, 1767) extracted using subcritical water hydrolysis obtained at temperatures ranging from 140 to 290 °C for its ACE inhibitory activity and obtained values which ranged from 92.16–96.70 %. Similarly, peptides from the oyster, *Crassostrea gigas* (Thunberg, 1793), was studied for its stability in ACE inhibition and results showed that the extracted nanopeptide (Val-Tyr- Pro-Trp-Thr-Gln-Arg-Phe) was stable at different temperatures (4 °C–100 °C) with ACE inhibition having a close range within 40–50 % (Wang et al., 2008).

Conclusion

Overall, our results indicated that the four marine bivalve species contain balanced nutritional properties, having significant protein content and low in fat. These qualities make them suitable for human consumption and can be employed as dietary products. The study also confirmed the feasibility of aqueous extraction, a less expensive alternative to chemical and enzymatic hydrolysis, to liberate the peptides. Higher antioxidant activities were observed from the cold-water extracts than the hot-water extracts. Consistently, *M. hiantina* demonstrated the highest DPPH radical scavenging activity and ferric-ion reducing capacity (76.24 ± 7. 15 % and 1.58 ± 0.04 mM Trolox equiv.mL^{-1} sample, respectively) which can be correlated to the presence of significant amino acid residues in *M. hiantina* resulting in better antioxidant potential. Furthermore, extracts from the bivalve species also showed promising anti-hypertensive activity measured by ACE inhibition assay. Bioactive peptide identification coupled with *in vivo* experiments to confirm its biopotentialities should be taken into consideration for future studies.

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Author contributions: Rhoda Mae C. Simora: Concept, experimental design, data analysis, drafting and revising of manuscript. Ma. Lorena M. Serisola: Experimentation, data analysis, drafting of manuscript. Nicole Pauline P. Plagata: Experimentation, data analysis. Gleann P. Salido: Experimentation, data analysis. Sharon N. Nuñal: Concept, drafting and revising of manuscript.

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