Int J Adv Life Sci Res. Volume 9(2) 100-109
https://doi.org/10.31632/ijalsr.2026.v09i02.009

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

Journal homepage http://ijalsr.org

Original Article

Assessment of Antioxidant and Antibacterial Activities of Pokea Clam (*Batissa violacea celebensis*) Protein Hydrolysates: An *in vitro* Approach

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Abstract

The pokea clam (*Batissa violacea celebencis* Martens, 1897), a freshwater bivalve of the Corbiculidae family, is indigenous to the Pohara River in Konawe, Southeast Sulawesi. Recognised as an endemic species, it has been traditionally utilised by local populations for the treatment of ailments such as jaundice, malaria, asthma, high blood pressure, and fever. Marine-derived bioactive peptides, especially from bivalves, can be extracted via enzymatic hydrolysis, producing protein hydrolysates known for their potential in functional food applications. These hydrolysates possess notable health-promoting properties, including antioxidant and antibacterial effects, which may offer advantages over synthetic alternatives. In this study, the antioxidant capacity of pokea clam protein hydrolysates was analysed using DPPH and ABTS methods, resulting in IC_{50} values of 52.304 mg/mL and 81.268 mg/mL, respectively. Antibacterial testing via the agar diffusion technique demonstrated strong inhibitory effects against *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*, with respective inhibition zone diameters of 28 mm, 23 mm, and 18.5 mm. These findings underscore the potential of pokea clam protein hydrolysates as therapeutic agents for oxidative stress and bacterial infections.

***Keywords**: Antibacterial; Antioxidant; Batissa violacea; Pokea Clam*

Introduction

Indonesia ranks among the world's richest regions in biodiversity, providing abundant sources of natural antioxidants. One of these is the pokea clam (*Batissa violacea celebensis* Martens, 1897), a mollusc species endemic to Indonesian waters. Research has indicated that, like certain plants and animals, clams are capable of producing peptide hydrolysates with significant antioxidant potential (Sheih *et al*., 2009). Phytochemical investigations of pokea clam extracts—using solvents such as ethanol, methanol, ethyl acetate, and n-hexane—have identified multiple secondary metabolites with bioactive properties, including flavonoids, alkaloids, terpenoids, steroids, and saponins (Rasyid *et al*., 2022). Compounds with antioxidant activity often feature hydroxyl groups bound to aromatic rings and include diverse classes such as phenolics, flavonoids, alkaloids, and tannins (Deswati *et al*., 2024). Oxidative stress occurs when the production of reactive oxygen species (ROS)—such as hydroxyl radicals, superoxide anions, and lipid peroxides—surpasses the body's capacity to neutralise them.

Received on :8th September 2025; Revised version received on :23rd February 2026; Accepted: 24th February 2026

Although ROS are involved in normal metabolic activities, their overproduction can cause damage to cells and contribute to the development of serious health conditions (Li *et al*., 2022; Rey *et al*., 2023). Based on the results of research conducted by Sernita *et al*. (2016), it was found that pokea shell extract can inhibit the growth of *Staphylococcus aureus* bacteria at concentrations of 40 to 100%. Research on the specific antibacterial antioxidants of pokea shell protein hydrolysate has not yet been conducted. The uniqueness of this study lies in its focus for the first time on empirically testing the antioxidant and antibacterial activity of the protein hydrolysate of the pokéa shellfish, so that it is not only limited to the identification of potential. The results of this study are expected to make a significant contribution to the development of pokea shellfish as a source of natural bioactive compounds for applications in the food and health industries, as well as increase the added value of local commodities in Southeast Sulawesi.

To assess the antioxidant performance of compounds, researchers often rely on several assays. One widely used method is the ABTS assay, which measures a substance's ability to neutralise free radicals. The ABTS radical is generated by reacting ABTS diammonium salt with potassium persulfate in ethanol, and the reduction in absorbance is monitored at 753 nm using a spectrophotometer (Dawidowicz & Olszowy, 2013; Dong *et al*., 2015; Ilyasov *et al*., 2020). Another established method is the DPPH assay, where antioxidant molecules interact with DPPH radicals, leading to their neutralisation through electron or hydrogen atom donation, ultimately converting DPPH to its non-radical form (Kedare & Singh, 2011; Baliyan *et al*., 2022). Protein hydrolysates obtained from clams have been found to include important secondary metabolites like alkaloids and saponins, which enhance their antioxidant and antimicrobial potential. These hydrolysates are produced by breaking down proteins using enzymatic, acidic, or alkaline hydrolysis, resulting in bioactive peptides and amino acids. Given their functional properties, they are extensively utilised in both the food and pharmaceutical industries as an effective way to boost the availability of amino acid–rich protein sources.

Bioactive compounds with antioxidant and antibacterial properties are essential for maintaining human health and ensuring food safety. These agents are often incorporated into dietary supplements to enhance immune function and reduce the risk of diseases linked to oxidative stress, such as cancer, diabetes, cardiovascular disorders, and premature ageing. In food preservation, they help prevent lipid oxidation and inhibit microbial growth that leads to spoilage (Najafian & Babji, 2012; Intarasirisawat *et al*., 2014). Protein hydrolysates with antioxidant potential offer a natural substitute for synthetic antioxidants like Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), and Propyl Gallate (PG), which are associated with potential toxicity and genotoxic risks (Luo *et al*., 2013). During normal metabolic activity, the body generates Reactive Oxygen Species (ROS) and free radicals, which can damage healthy cells and contribute to disease development (Rasyid *et al*., 2022; Hidayati *et al*., 2019). Although both natural and synthetic antioxidants can neutralise these harmful molecules, the latter are often linked to adverse effects (Floegel *et al*., 2011). Naturally derived antioxidants are therefore considered safer and more sustainable, particularly when integrated with advanced technologies such as nanobiotechnology, which enhance their antimicrobial applications (Thaipong *et al*., 2006). Exploring new therapeutic agents from natural sources remains crucial. The pokea clam, a native mollusc found in Indonesian waters, has demonstrated antioxidant activity in solvent extracts (methanol, ethanol, ethyl acetate, and n-hexane), yet its protein hydrolysates have not been previously studied. Thus, this research aims to investigate the antioxidant and antibacterial potential of enzymatically derived protein hydrolysates from pokea clams.

Material and Methods

Preparation of protein hydrolysates from the pokea clam (Batissa violacea celebensis Martens, 1897*)*

The production of protein hydrolysate from kerangpokea was based on a modification of the fish protein hydrolysate production method developed by Liceaga-Gesualdo and Li-Chan (1999). The hydrolysis process began with the soaking of 60 grams of dried striped mussels for 4-6 hours. After chopping, the small pieces of mussel meat were weighed, and 100 g (wet weight) was obtained.

Then, it was homogenised with water in a ratio of 1:4 (1 part mussel meat (wet weight) mixed with 4 parts water) for 2 minutes. The mixture is stirred, and the pH value is adjusted to pH 7 at a temperature of 55°C to produce optimal enzyme activity. It is then hydrolysed by adding the papain enzyme at various concentrations. Enzyme activity is stopped by increasing the stirring temperature to 85°C for 20 minutes. The samples taken are filtered with filter paper. The liquid phase is collected and precipitated, then dried with a vacuum rotary evaporator at a temperature of 80-90°C for 45 minutes to obtain the hydrolysate product in powder form.

The reagents and chemicals used in this study included distilled water, 1 M hydrochloric acid, bovine serum albumin (BSA, Sigma-Aldrich), DPPH (1,1-diphenyl-2-picrylhydrazyl, PT. Smartlab Indonesia), papain enzyme (Sigma-Aldrich), ethanol (analytical grade, PT. Smartlab Indonesia), and casein hydrolysate (Sigma-Aldrich). Additional materials included Lowry A reagent (a 1:1 mixture of Folin-Ciocalteu reagent and distilled water) and Lowry B reagent (comprising 2% Na_2CO_3, 0.1 N NaOH, 1% $CuSO_4 \cdot 5H_2O$, 1 M sodium hydroxide, 10% sodium hydroxide, and 2% sodium potassium tartrate), as well as analytical-grade methanol and vitamin C/ascorbic acid.

Antioxidant Activity Using the DPPH Assay

A 100 ppm DPPH solution was prepared by dissolving 10 mg of DPPH in 96% ethanol (100 mL). A matching stock solution of pokea clam hydrolysate (100 ppm) was made in methanol and serially diluted to 10–50 ppm. The optimal absorbance wavelength was determined by scanning a mixture of 1 mL ethanol and 3 mL DPPH between 450 and 600 nm using a UV-Vis spectrophotometer. Ascorbic acid (5 mg/50 mL methanol) served as a standard and was similarly diluted. Each sample (1 mL) was mixed with 2 mL DPPH, incubated at 37°C, and measured at the selected wavelength. A decrease in absorbance, coupled with a colour shift from violet to yellow, indicated free radical scavenging activity (Liu *et al*., 2015).

Antioxidant Activity Using the ABTS Assay

To generate the ABTS radical cation, 1.5 mL of 0.0406 g/10 mL ABTS and 0.007 g/10 mL potassium persulfate solutions were mixed and incubated in the dark for 12 hours. A working solution was prepared by diluting 1 mL of this mixture to 50 mL with methanol. Vitamin C standards (10–50 ppm) were prepared in methanol. For the assay, 0.15 mL of each standard or sample was mixed with 2.85 mL of the ABTS reagent, incubated at room temperature (30 minutes, dark), and measured at 750 nm. Antioxidant capacity was expressed as IC_{50} values, determined via linear regression: y = bx + a, where y = 50% inhibition (Rohmah, 2022; El-Guour *et al*., 2023).

Antibacterial Assay

Mueller Hinton Agar (MHA) was used to culture *E. coli*, *S. aureus*, and *S. typhi*. Test solutions were prepared by dissolving 1 g of hydrolysate in 4 mL of sterile water. Ampicillin (10 µg), tetracycline (30 µg), and chloramphenicol (250 µg) served as positive controls for *E. coli*, *S. aureus*, and *Salmonella*, respectively, while sterile water was the negative control. The disc diffusion method was applied using 15 mL MHA per Petri dish. Bacteria were spread evenly, and discs impregnated with test solutions were applied. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured to assess antibacterial activity (Nursyam, 2017).

Results

Based on the results of a preliminary study, it is known that the process of hydrolysis of pokea clam proteins using the papain enzyme at 6% on the substrate for a hydrolysis time of 48 h produced the best protein hydrolysates. The pokea clam protein hydrolysates produced from the preliminary study were then used in this study. These pokea clam protein hydrolysates took the form of a paste. To examine the possibility of using these protein hydrolysates as an ingredient in food supplements or nutraceutical products, analyses were carried out, which included an analysis of antioxidant activity. Data on antioxidant activity testing using the DPPH and ABTS methods on protein hydrolysates from pokea clams using a vitamin C comparator solution are presented in Table 1.

Table 1: *Evaluation of Antioxidant Activity at Different Concentrations with DPPH And ABTS Assays, Ascorbic Acid (Standard), and Protein Hydrolisates of Pokea Clams (DPPH And ABTS % Inhibition)*

Concentration (mg/ml)	Protein Hydrolisates DPPH and ABTS % Inhibition	Standard DPPH and ABTS % Inhibition
10	28.62 ± 32.47	54.22 ± 0.75
20	44.78 ± 20.83	58.62 ± 2.09
30	50.36 ± 19.67	61.16 ± 2.32
40	52.82 ± 20.70	67.14 ± 2.92
50	56.29 ± 16.91	69.81 ± 3.33

Table 2: *Antioxidant Activity at Different Concentrations with DPPH And ABTS Assays, Ascorbic Acid (Standard), And Protein Hydrolisates of Pokea Clams (DPPH And ABTS IC_{50})*

Concentration (mg/ml)	Protein Hydrolisates DPPH (IC_{50})	Standard DPPH (IC_{50})	Protein Hydrolisates ABTS (IC_{50})	Standard ABTS (IC_{50})
10	52.304	0.481	81.268	4.761
20				
30				
40				
50				

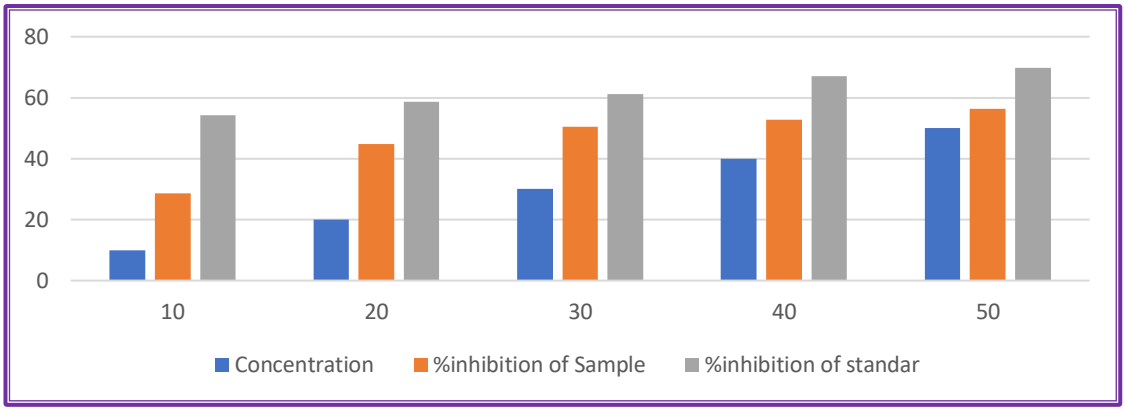

Figure 1: *DPPH and ABTS Free Radical Scavenging Activity, % Inhibition for Standard Drug, and % Inhibition for Pokea Clam Protein Hydrolysates Tested at Various Concentrations*

Antibacterial Activity Test of Pokea Clam Protein Hydrolysates

The disc diffusion method was used to assess antibacterial activity, as it is widely favoured for its straightforward and practical application. The effectiveness of the antibacterial agent is determined by measuring the diameter of the clear zone that forms around the paper disc, which reflects the degree of bacterial growth inhibition.

Table 3: *Antimicrobial Activity of Pokea Clam Protein Hydrolysates*

Microbes	Zone of Inhibition (diameter in mm)	Category
S. aureus	27	Sensitive
(Control: Tetracycline)	28	Sensitive
E. coli	23	Sensitive
(Control: Ampicillin)	18.5	Sensitive
S. typhi	27	Sensitive
(Control: Chloramphenicol)	23	Sensitive
Negative Control (Sterile Aquadest)	-	-

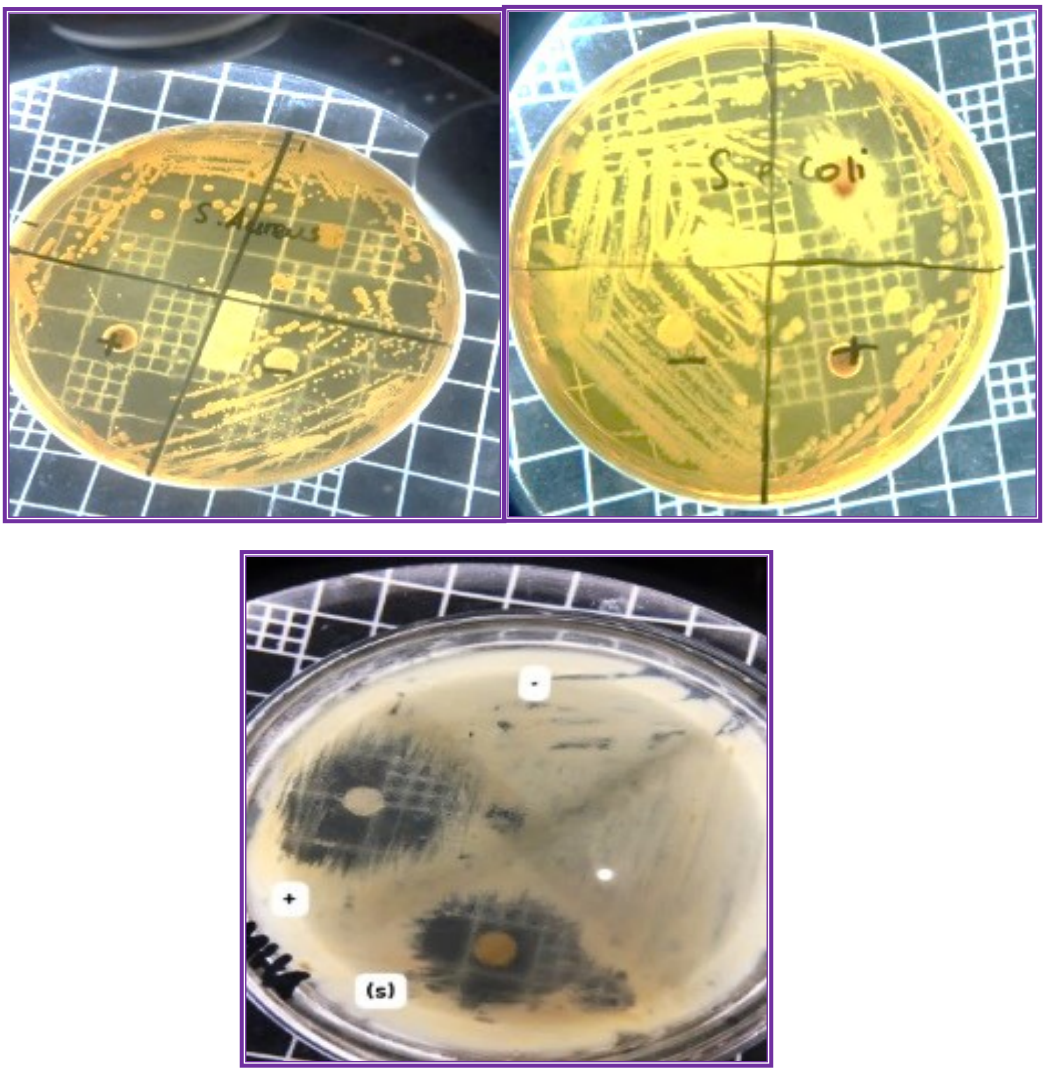

Figure 2: *Results of the Inhibition Zone Test of Pokea Clam Protein Hydrolysates Against S. Aureus, E. Coli, And Salmonella Typhi*

Discussion

Protein hydrolysates are extensively applied in food and pharmaceutical industries for their functional and bioactive properties. In food systems, they enhance emulsifying, foaming, and gelling capacities, supporting processing efficiency and product stability (Tang *et al*., 2023). Hydrolysates from sources like corn gluten and soy also improve emulsion and foam stability for diverse formulations (Mirzaee *et al*., 2024). Beyond functionality, their antioxidant, antihypertensive, and antidiabetic effects make them valuable in functional foods and nutraceuticals (Mirzaee *et al*., 2024; Liceaga & Hall, 2015). Fish protein hydrolysates, for instance, are known for antimicrobial benefits and are used as nutritional supplements (Das *et al*., 2021). In pharmaceuticals, their biocompatibility and biodegradability support applications in controlled drug delivery (Falsafi *et al*., 2024), while lipid-based encapsulation systems improve their oral bioavailability and stability (Falsafi *et al*., 2024). Thus, producing protein hydrolysates is not only a means to enhance amino acid-rich protein availability but also a gateway to developing bioactive compounds. This study aims to evaluate the antioxidant and antibacterial activities of *pokea* clam (*Batissa violacea celebensis*) protein hydrolysates obtained via enzymatic hydrolysis. A lower IC50 value indicates stronger antioxidant potential. Antioxidant strength is categorised based on IC50 values: very strong when <50 ppm, strong between 50 and 100 ppm, moderate within 101–250 ppm, weak if 251–500 ppm, and considered inactive when exceeding 500 ppm (Zarai *et al*., 2013). Vitamin C demonstrates a low IC50 value because it is a natural antioxidant

in its pure form, which contributes to its high efficacy in neutralising DPPH free radicals. This compound contains free hydroxyl groups that act as radical scavengers (Faisal &Handayani, 2019; Rahaman *et al*., 2023).

The antioxidant capacity of pokea clam protein hydrolysates was analysed using DPPH and ABTS methods, resulting in IC_{50} values of 52.304 mg/mL and 81.268 mg/mL, respectively. Antioxidant results show being in the strong category. The antioxidant potential of pokea clam protein hydrolysates is largely attributed to their diverse secondary metabolites, particularly alkaloids. While flavonoids are commonly recognised for their antioxidant activity, their role may not be solely decisive, as other compounds such as phenolics, alkaloids, and saponins also contribute significantly (Zhou *et al*., 2012; Hasanuddin *et al*., 2023). Alkaloids exhibit strong antioxidant properties due to the presence of nitrogen atoms with lone electron pairs that can neutralise free radicals (Faisal &Handayani, 2019; Rahaman *et al*., 2023). The efficacy of these metabolites is determined by their molecular structure, including the type and number of hydroxyl groups, which influence their reactivity toward ROS and RNS (Odeleye *et al*., 2016). Specifically, hydroxyl groups positioned on aromatic rings enhance the compounds' ability to stabilise radicals through electron or hydrogen donation (Fauziah *et al*., 2021). Additionally, Odeleye *et al*. (2016) reported that bioactive components in New Zealand surf clams demonstrate similar antioxidant capabilities, suggesting their potential in functional food and health-promoting applications.

The ABTS method has advantages, including the determination of a wide variety of antioxidant compounds (phenols, amino acids, vitamin C, and vitamin E) in food components and convenience operationally. According to Kurniasari *et al*. (2022), the ABTS method has the advantages of being able to interact with antioxidants quickly, being applicable at various pH levels, and being soluble in both water and organic solvents. However, this method is weak in a few respects, including that it uses the kinetic assay; the reactions involved in the ABTS assay are uncertain because they may occur with oxidising agents, enzymes, and radical cations, possibly resulting in excessive results; and it lacks biological relevance due to the use of ABTS cations, which are not found in food or biological systems. According to Al-Hmoud *et al*. (2014), although the ABTS method has advantages, including a higher degree of sensitivity and applicability at various pH levels, which leads it to be considered better than the DPPH method, the fact that it is expensive is a disadvantage. Compared to the ABTS method, the DPPH method tends to be more sensitive at acidic pH. DPPH is able to reflect the body's defence system against free radicals (Karadag *et al*., 2009).

The results demonstrate that pokea clam protein hydrolysates possess notably strong antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*. This effect is largely linked to the presence of alkaloid compounds in the hydrolysates. Alkaloids are known to act as antibacterial agents by compromising the structural integrity of peptidoglycans in bacterial cell walls, destabilising membranes, and ultimately causing cell lysis. In addition, these compounds disrupt bacterial protein synthesis, thereby interfering with critical metabolic functions. Prior studies have shown that alkaloids are effective against both Gram-positive and Gram-negative bacteria (Chandran *et al*., 2009). These findings align with previous reports where pokea clam extracts were able to inhibit the growth of *S. aureus* at concentrations between 40% and 100%. Similar results were also obtained in research involving razor clams, whose ethyl acetate extracts produced inhibition zones of 32.10 ± 0.17 mm for *S. aureus* and 32.06 ± 1.07 mm for *E. coli*. In the case of *S. typhi*, pokea clam hydrolysates showed strong inhibitory activity, comparable to that of standard antibiotics. These outcomes are consistent with ethnomedicinal practices in Pohara Village, where pokea clams have traditionally been used to treat liver-related diseases, including typhoid fever.

The antibacterial efficacy of *pokea* clam protein hydrolysates is likely associated with the remarkable ecological adaptability of bivalves. Living in dynamic and often contaminated aquatic environments, bivalves such as *Batissa violacea celebensis* are continuously exposed to a range of stressors, including pathogenic microorganisms, antibiotics, and heavy metals such as Zn, Cd, Ni, and Pb (De Souza *et al*., 2024; Hamed *et al*., 2024). These pollutants can adversely affect the physiological functions of bivalves, disrupt their internal microbial communities, and exert selective pressure that

may enhance the production of bioactive defence compounds. For instance, certain species like *Perna perna* and *Crassostrea rhizophorae* exhibit species-specific capacities to accumulate and tolerate metal contaminants, reflecting adaptive physiological strategies shaped by their habitat conditions (De Souza *et al*., 2024). In polluted waters, the accumulation of both antibiotic residues and antibiotic-resistant bacteria (e.g., *Aeromonas* spp., *Vibrio* spp.) poses not only ecological risks to bivalves but also potential public health concerns through human consumption (Baralla *et al*., 2021; Albini *et al*., 2022). This contamination contributes to the development and spread of antibiotic resistance, further pressuring bivalves to evolve innate immune strategies, including the synthesis of antimicrobial peptides (AMPs) and other bioactive metabolites with broad-spectrum antibacterial activity. According to Rodrigues *et al*. (2025), these AMPs serve as a crucial first line of defence, especially under fluctuating environmental conditions. Moreover, heavy metals may disrupt the bivalve microbiome and select for metal-resistant and antibiotic-resistant bacterial strains, such as *Vibrio* spp., which could trigger increased host-pathogen interactions and further stimulate the production of antimicrobial compounds as a compensatory response (Pavón *et al*., 2022). Despite their relatively simple immune systems, bivalves have thus become an important source of bioactive molecules with pharmaceutical and nutraceutical potential. The presence of alkaloids, peptides, and AMPs in *pokea* clam protein hydrolysates likely reflects these evolutionary and ecological pressures, supporting their observed inhibitory effects against both Gram-positive and Gram-negative bacteria in vitro.

Despite possessing relatively simple anatomical structures—lacking specialised immune organs—the bivalves are capable of mounting effective defence responses through the secretion of antimicrobial peptides (AMPs) and other secondary metabolites. These AMPs, as noted by Rodrigues *et al*. (2025), exhibit broad-spectrum antibacterial activity, enabling bivalves to survive in fluctuating marine conditions where pathogenic load can vary drastically. The ability of these peptides to disrupt bacterial membranes and interfere with essential cellular pathways allows them to act rapidly and effectively against both Gram-positive and Gram-negative bacteria. In the case of pokea clam protein hydrolysates, their inhibitory action against *Staphylococcus aureus*, Escherichia coli, and Salmonella typhi is likely mediated by these bioactive compounds. Upon interaction with bacterial cells, such compounds are believed to penetrate the cell wall and cytoplasmic membrane, causing structural destabilisation. Once internalised, they interfere with key metabolic pathways, including protein biosynthesis, purine metabolism, and nucleic acid replication, leading to cellular dysfunction and eventual bacterial death. This mode of action not only supports their potential use in food preservation or therapeutic applications but also underscores the importance of marine bivalves as a promising source of natural antimicrobial agents in the search for alternatives to conventional antibiotics.

Limitations

This study was limited to in vitro assays for antioxidant and antibacterial activities. It did not include IC_{50} quantification, compound isolation, or in vivo validations.

Future Research Scope

Future research should explore bioactive compound identification and molecular mechanisms. In vivo studies and product development trials are needed for clinical and commercial relevance.

Conclusion

The DPPH and ABTS assays demonstrated that pokea clam protein hydrolysates possess strong antioxidant activity, with minimal variation between the two methods, suggesting their interchangeable use in evaluating antioxidant potential. Additionally, the hydrolysates exhibited notable antibacterial effects against pathogenic bacteria. These findings indicate that pokea clam protein hydrolysates hold promise as a natural source of antioxidant and antimicrobial agents, supporting their potential application as functional food ingredients.

Conflict of Interest

No competing interests have been reported by the authors.

Acknowledgement

The authors acknowledge the Directorate General of Higher Education, Research, and Technology of the Republic of Indonesia for funding support in 2024 and thank the Institute for Research and Community Service (LPPM) of Mandala Waluya University for institutional facilitation.

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