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Original Article

POTENTIALITY OF PROTEIN HYDROLYSATE FROM *ANADARA GRANOSA* AS NUTRACEUTICAL AGENT: ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

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ABSTRACT

Objective: This study aims to extract protein hydrolysate from *Anadara granosa* and assess its impact on protein solubility, antioxidant, and antibacterial activities.

Methods: Several methods were used, including the isolation of enzyme bromelain, protein extraction from *A. granosa*, and the breakdown of protein using trypsin and bromelain. Together with its protein solubility, antioxidant activity (IC50) against free radicals (DPPH), the protein hydrolysate's antibacterial activity (MIC and inhibition zone) against *Staphylococcus aureus* and *Escherichia coli* was evaluated.

Results: With a molecular weight of 10 kDa and an IC50 of 83.81 mg/ml, the trypsin protein hydrolysate fraction showed remarkable antioxidant activity after 5 h of incubation. At a dosage of just 0.25 mg/ml, the *A. granosa* protein and its corresponding hydrolysate had inhibitory zones against *S. aureus* and *E. coli* that were comparable to those observed in samples treated with amoxicillin. Using trypsin as an enzyme for 3 or 5 h produced the strongest hydrolyzed product. The trypsin hydrolysate was better than the bromelain hydrolysate because of its antioxidant and antibacterial activities.

Conclusion: Based on the results, antioxidant and antibacterial activities, and protein solubility were influenced by enzymatic hydrolysis.

Keywords: Enzymatic hydrolysis, Blood clams, DPPH-scavenging, Inhibition zone

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INTRODUCTION

There is great potential for cultivating a wide variety of shellfish, including *Anadara granosa*, in the waters surrounding Indonesia. *A. granosa* also provides a substantial and nutrient-dense source of protein, ash, protein, lipids, minerals, and vitamins, as well as complete and balanced essential amino acid profiles. The species can be processed into fisheries products or turned into protein hydrolysate because of its 19.48% protein content [1]. Bioactive compounds from food, such as protein, have a significant impact on human body function [2].

Numerous techniques, including heat, enzymatic proteolysis, acid hydrolysis, and alkaline hydrolysis, can be used to create protein hydrolysate [3]. The advantages of enzymatic proteolysis over chemical methods have been shown in several reports. This process allows free amino acids to exist since the hydrolysate contains peptides with a lower molecular weight. Furthermore, the superior emulsion quality enables the high water solubility and quick body absorption of the products [4]. Several aquatic creatures have produced similar products, such as snakehead fish for the anti-lipidemic effects [5], catfish and sea cucumbers as sources of collagen [6, 7], and octopus-hydrolyzed protein for energy drinks for athletes [8, 9].

The bioactive components of *A. granosa*'s protein possess antibacterial and antioxidant activities, as reported in previous studies [10, 11], The protein hydrolysate of this species is poorly understood, despite the existing literature. In line with several studies, hydrolysis is often carried out through enzymatic or fermentation processes [5, 12–14]. The proteolytic enzymes that are often used during hydrolysis include trypsin, pepsin, alcalase®, bromelain, and papain [14–18]. Previous studies have shown that bromelain can easily be extracted from pineapple [19, 20], has the ability to enhance protein bioactivity [17, 21]. Given that numerous studies have documented the use of bromelain for protein hydrolysis, tryptic hydrolysate was also tested to maximize antioxidant and antibacterial activities. The production of hydrolysate is expected to increase the soluble protein levels, as well as antioxidant and antibacterial activities of *A. granosa* because it increases the solubility and absorption of short-chain peptides and free amino acids. This allowed the product to be used as a nutraceutical agent (in the form of food supplements) to increase body immunity. Therefore, this study aimed to increase the potential of *A. granosa* protein as an antioxidant and antibacterial agent using enzymatic hydrolysis. The product obtained can be consumed as a dietary supplement to improve the body's natural defenses.

MATERIALS AND METHODS

Material and equipment

The materials used were *A. granosa*, pineapple fruit, *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). Analytical-grade chemicals, such as ethanol, methanol, sodium chloride, sodium carbonate, calcium chloride, ammonium sulfate, 2-mercaptoethanol, tris(hydroxymethyl) aminomethane, trichloroacetic acid (TCA), Triton X-100, Folin-Ciocalteu, Lowry A, Lowry B, and l-tyrosine were purchased from Merck. The other reagents, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, phosphate-buffered saline (PBS), and bovine serum albumin (BSA) were obtained from Sigma Aldrich. In addition, nutrient agar, nutrient broth, and Mueller-Hinton agar were purchased from Himedia. A UV-Vis spectrophotometer (Shimadzu, UV-1800), centrifuge (Hitachi, CF16RXII), Amicon centrifugal filter 10 kDa (Merck), and antibacterial activity test equipment were also used in the procedures.

Preparation of *Anadara granosa* crude protein

The sample was cleaned, separated from the shell, weighed to 500 g, and processed using a blender. In addition, protein was isolated using buffer (Tris-HCl 0,1 M pH 8,3; NaCl 2 M; $CaCl_2$ 0,01 M, 2-mercaptoethanol 1%, Triton X-100 0.5%), and the mixture was then chilled overnight in a refrigerator at 4 °C for a night before filtered through a Buchner funnel. The filtrate was defrosted 2-3 times before centrifuging at 6000 rpm and 4 °C for 30 min, and the

supernatant was collected in a cup. The obtained protein isolate was tested for its activity and protein content [22].

Enzyme preparation

Bromelain was extracted by crushing young skinless pineapples using a food processor. The resulting juice was filtered through Whatman No. 41 filter paper. Acetone was added at a ratio of 1:1 ($^{v}/_{v}$), and the mixture was maintained at 4 °C overnight. For 15 min, the solution was centrifuged at 4 °C for 6000 g. The precipitate was then combined at a 1:1 (w/v) ratio with PBS (pH 7.4). Enzyme activity was determined using the Bergmeyer method, which was based on the ability of proteases to hydrolyze substrates into tyrosine. Furthermore, tyrosine with Folin-Ciocalteu's reagent formed a complex compound, and the absorbance was examined at a wavelength of 578.0 nm. The unit of enzyme activity was the quantity of enzyme that, under test conditions, could create one mol of tyrosine every minute [20, 23, 24].

Protein hydrolysate preparation

The 2 types of enzymes used in the hydrolysis process were crude bromelain extract and trypsin, with the conditions for bromelain hydrolysis, pH 7, temperature 45 ℃, and 10% bromelain concentration. Furthermore, the pH for trypsin hydrolysis was 8, the temperature was 37 ℃ and the enzyme concentration was 1%. Both experiments used a substrate enzyme ratio of 1:6 with duration variations of 1(H1), 3(H2), and 5(H3) hours [23, 25]. The hydrolysate of protein was then filtered using an *Amicon* centrifugal filter at 10 kDa to obtain a fraction (F1-3) with a lower molecular weight [26].

Soluble protein content

The Lowry method was used to determine the soluble protein content, and BSA was used as a standard. A total of 4 ml of the sample solution and 5.5 ml Lowry B reagent were put in a beaker. After homogenization, the mixture was incubated at room temperature for 10–15 min. In this study, 0.5 ml Lowry A reagent was added to the mixture, which was then incubated for 30 min at room temperature. The absorbance was then examined at a wavelength of 740.6 nm [27, 28].

The DPPH scavenging activity

The DPPH radical-scavenging activity of the isolated protein, hydrolysate, and hydrolysate fraction of 10 kDa was investigated, as previously described by Brand-Williams in 1995. Aliquots of the samples at 5 different concentrations were placed in a volumetric flask with 1 ml of freshly prepared 200 µg/ml DPPH in methanol. After 35 min of incubation, scavenging activity was measured spectrophotometrically by measuring the decrease in absorbance at 517 nm, and the blank value was determined using distilled water. The results were expressed as inhibition capacity (%) = $\frac{AO-A}{AO}100$, where *A* and *A0* were the absorbance of the test and blank solution, respectively [28–30].

The antibacterial sensitivity test

The antimicrobial activity of the protein isolate, hydrolysate, and hydrolysate fraction of 10 kDa was determined using the agar disk diffusion method (Kirby-Bauer Test). A Mueller Hinton Agar (MHA) plate was seeded equally with 100 µl of bacterial culture that was cultivated overnight and adjusted to a McFarland Standard of 0.5. Subsequently, wells with a diameter of 5 mm were made on solid media using sterile drill discs [31, 32]. Tests were run on each sample using 100 µl of each sample after dilution with 50 percent ($^{v}/_{v}$) DMSO solvent added to each well. Another well was used as the control and received the same amount of DMSO as the solvent. The antibacterial activity was assessed by measuring the width of the inhibition zone after the agar plates were kept at 37 °C for 24 h [31–33].

The minimum inhibition concentration test

The term "minimum inhibitory concentration" (MIC) referred to the highest dilution or lowest concentration of an antimicrobial agent necessary to prevent or inhibit bacterial growth, and these tests were performed using the microdilution method. For the MIC, many concentrations were tested, including 100%, 75%, 50%, 25%, 12.5%, 6.25%, and 3.125% ($^{v}/_{v}$). MHA agar plates were uniformly seeded with 100 µl of bacterial culture that was cultivated overnight and corrected to a McFarland Standard of 0.5. A sterile drill disc was used to drill the well into a solid medium with a diameter of 5 mm. Furthermore, 100 µl** of each concentration was added to each well. The control wells received the same amount of DMSO as the solvent, and the agar plates were inspected after a 24-hour incubation period at 37 °C. The lowest concentration for any organism that did not show growth was recorded as the MIC of that organism [32, 34].

Statistical analysis

Each experiment was performed 3 times using a purposive sampling design, and the data were analyzed using analysis of variance (ANOVA). SPSS online software was used for the Shapiro-Wilk test to compare means.

RESULTS AND DISCUSSION

Enzymatic activity

The enzyme was isolated from pineapple juice which was precipitated using acetone with ratio 1:1 ($^{v}/_{v}$). This was determined using the Bergmeyer method for the enzymatic activities [20, 23, 24]. The extraction process was intended to separate crude enzymes from pineapple tissue. Additionally, it should be emphasized that to obtain enzymes with excellent activity, the sources must meet the necessary criteria. The temperature was maintained at 10 °C, and it was necessary to add a buffer solution with a pH of 7.5 during the extraction process. However, the enzyme was separated from pineapple tissue by centrifugation, and crude bromelain was obtained by drying the supernatant [19].

The enzymatic activity of bromelain was assessed by using a casein substrate, and the absorbance was measured at a wavelength of 290 nm using a Shimadzu UV-visible spectrophotometer. The wavelength of 290 nm was used because in determining the maximum wavelength of tyrosine, it was found that the maximum wavelength was 290 nm. The enzyme activity obtained was 0.0213 U/ml for crude bromelain extract and 0.35574 U/ml for commercial trypsin. However, this result was lower than the result obtained by Kahiro *et al.* [32]. Bromelain isolated from the crown of pineapple using the standard method had a yield value of 80-100 U/ml, and many factors could lead to this. The primary cause was the use of various isolation methods. Bromelain was a mixture of thiol endopeptidases and other substances because this enzyme was still a combination of multiple enzymes and could contain chemicals that were not enzymes, and crude extracts normally have poor activity [19, 20, 35].

Soluble protein content

Fig. 1 showed the soluble protein concentrations of the isolate, hydrolysate, and protein hydrolysate fraction of *A. granosa*. H0 was the soluble protein concentration of the protein isolate 32.4769±0.0115 mg/ml). Additionally, hydrolyzing procedures enhanced protein solubility.

As shown in fig. 1, there was an increase in soluble protein concentration due to enzymatic hydrolysis. In additional, hydrolysis with trypsin increased the solubility of *A. granosa* protein with increasing hydrolysis time, and the concentration of dissolved protein was greater when compared to hydrolysis with bromelain. In addition, the results of fractionation using 10 kDa filtration. The highest concentration of soluble protein was indicated by the 5-hour trypsin hydrolysate fraction of 10 kDa, which was 41.0114±0.0757 mg/ml.

Protein solubility was the factor that affected the overall utility of a protein in food systems more than any other functional characteristic. The equilibrium between hydrophobic and ionic interactions determined a protein's solubility. Hydrophobic interactions promoted protein-protein interactions and decreased solubility, while ionic interactions stimulated protein-water interactions and increased solubility. When the pH approached the isoelectric point, the hydrolysate had higher protein solubility than native protein [36]. In 1951, Lowry *et al.* described the reaction mechanism underlying the Lowry assay, stating that the reaction between a protein in a buffered alkaline copper solution and the Folin-Ciocalteu reagent, which comprised phosphotungstic and phosphomolybdic acids, generated cuprous ions and reduced the

Folin-Ciocalteu reagent, thereby yielding a discernible blue hue [27]. Everette *et al.* proposed that Folin-Ciocalteu reagent reduction and the oxidation of aromatic residues, primarily tryptophan and tyrosine, were both necessary for the reaction to occur. Additionally, studies had demonstrated that cysteine was also reactive to the Folin-Ciocalteu assay and that cysteine residues in protein also contributed to the absorbance observed in the Lowry assay [37, 38]. In this study, the longer the hydrolysis period, the more protein could be dissolved because the peptide bond was broken inside or in the middle, producing more short-chain peptide bonds.

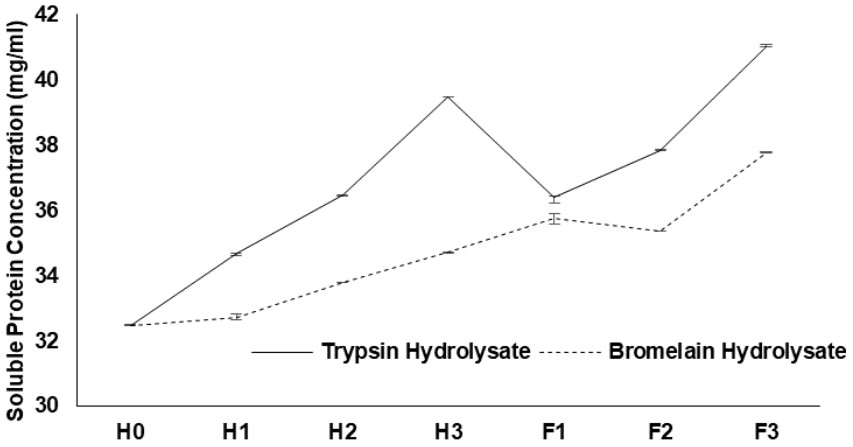

Fig. 1: The soluble protein concentration of the isolate (H0), protein hydrolysate (H1-3), and protein hydrolysate fraction (F1-3) of *Anadara granosa,* data were presented as mean±SD (n=3)

Antioxidant activity

Determining the antioxidant activity of foods as well as the capacity of components to capture free radical compounds or hydrogen donors, was a common application of the DPPH method. This was an uncomplicated, rapid, and cost-effective approach to evaluate the radical-scavenging capabilities of multiple compounds. The DPPH technique applies to both solid and liquid samples and did not need a special antioxidant component. Therefore, this was more often used than other methods [30, 39]. DPPH free radicals were detected at a maximum absorbance of 517 nm in ethanol and methanol. However, the effect of antioxidant inhibition was due to their ability to donate hydrogen. The absorbance value decreased when DPPH-free radicals came in contact with proton donor substances, such as antioxidants, which captured the radicals. The absorbance value obtained could be used to calculate the percentage of DPPH free radical inhibition, and the greater the percentage of inhibition, the higher the antioxidant activity [30]. The IC_{50} values of the protein hydrolysate of *A. granosa* were shown in fig. 2. In addition, the lowest IC_{50} was indicated by the 10 kDa fraction of trypsin hydrolysate for 5 h, which was 83.81 µg/ml and was classified as a strong antioxidant. All of the fraction of 10 kDa of the protein hydrolysate showed strong antioxidant activity. However, for both protein hydrolysate, trypsin and bromelain showed moderate antioxidant activity, except for the 5 h trypsin hydrolysate (strong activity). The IC_{50} of the protein hydrolysate was higher than that of ascorbic acid (4.16 81 g/ml), indicating that the antioxidant activity was not as strong as that of ascorbic acid.

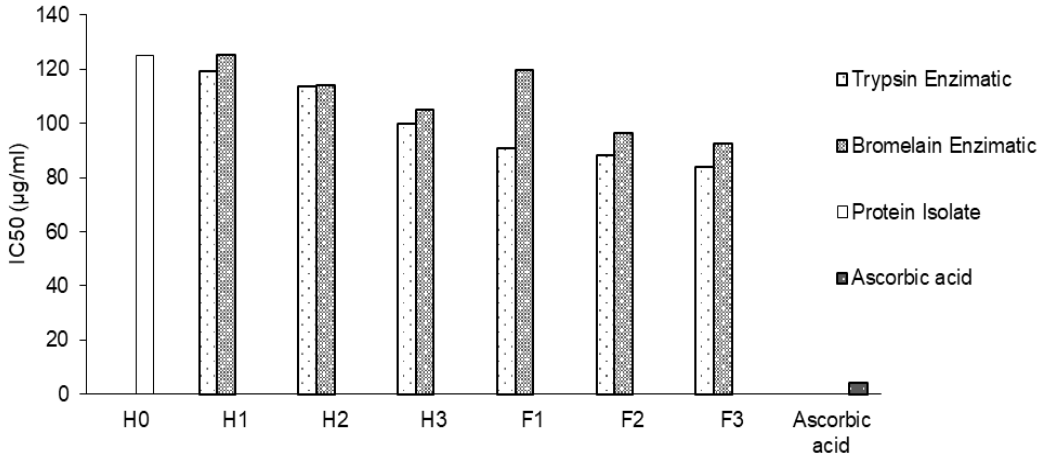

Fig. 2: The IC_{50} of isolate (H0), hydrolysate (H1-3), protein hydrolysate fraction (F1-3), and control (ascorbic acid). Data were presented as mean of triplicate

The structure of the substrate molecule could influence the antioxidant activity [39]. Protein exhibited antioxidant activity owing to their amino acid residues. Branched-chain amino acids (Val, Leu, and Ile), basic amino acids (His, Arg, and Lys), hydrophobic amino acids (Leu, Gly, and Val), and aromatic residues (Phe) were all known to have substantial antioxidant activity in peptides [40–43]. The proposed reaction of amino acids with DPPH was shown in fig. 3.

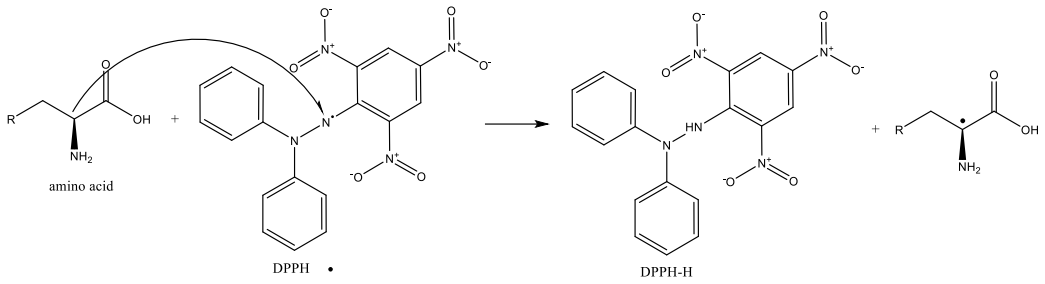

Fig. 3: The proposed reaction mechanism of antioxidant activity of amino acid neutralized the free radical DPPH

Antioxidants generally inhibited the initiation or spread of radical stated in various ways. Depending on their work, antioxidants could be categorized as either primary or secondary. The chain-breaking antioxidants, such as phenolic compounds with one or more hydroxyl groups, could provide a radical with a hydrogen atom and a single electron, neutralizing it (-OH). Although the term "radical scavenging" did not accurately describe the action, it was well known. Substances that blocked or postponed lead chains were classified as secondary or preventative antioxidants. These secondary antioxidants could act in several ways, such as chelating transition metals, scavenging oxygen, and quenching singlet oxygen [43, 44].

Antibacterial sensitivity

Fig. 4 and 5 showed the inhibition zones of the isolate, hydrolysate, and fraction protein hydrolysate of *A. granosa* towards *E. coli* and *S. aureus*. There was a difference between the average diameter of the inhibition zone of each hydrolysis method and the 10 kDa hydrolysate fraction. The highest inhibition zone diameter was indicated by the 10 kDa hydrolysate fraction for 5 h (F3) against *S. aureus* bacteria with an inhibition zone diameter of 14.40±0.20 mm. This fig. was also higher than that of the positive control amoxicillin at a concentration of 0.25 mg/ml. Additionally, trypsin hydrolysate was more effective than crude bromelain hydrolysate and had an inhibition zone larger than that of the positive control (sig.<0.05, 95% confidence interval). Protein hydrolysate with trypsin and crude bromelain was more active against *S. aureus* (gram-positive) than against *E. coli* (gram-negative), as well as the 10 kDa fraction. These data were analyzed using SPSS two-way ANOVA with a significance 0.003 (trypsin hydrolysate *E. coli* vs. *S. aureus*) and 0.011 (crude bromelain hydrolysate of *E. coli* vs. *S. aureus*), with a 95% confidence interval. In addition, it was possible to draw the conclusion that the protein present in *A. granosa* was hydrolyzed utilizing a range of enzymes, affecting the growth inhibition zone of *E. coli* and *S. aureus*, and then fractionated depending on its molecular weight.

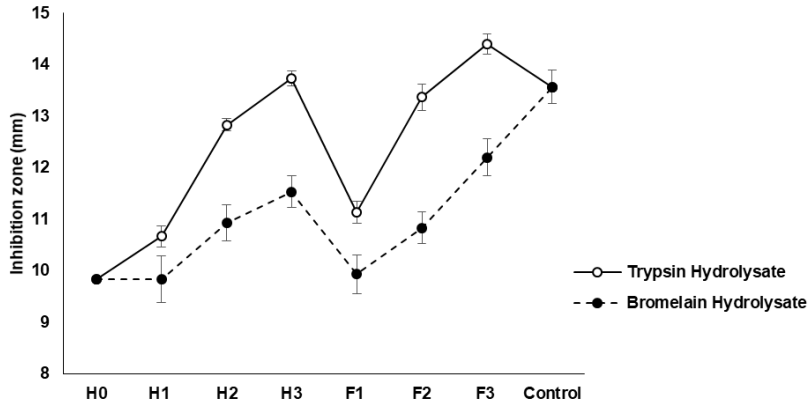

Fig. 4: The inhibition zone of the protein isolate (H0), hydrolysate (H1-3), and fraction of hydrolysate (F1-3) protein of *Anadara granosa* against *Staphylococcus aureus,* using amoxicillin as the positive control. Data were presented as mean±SD (n=3)

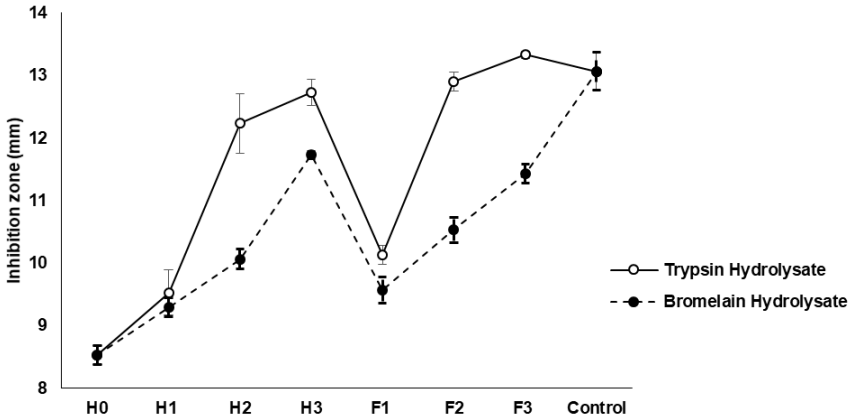

Fig. 5: The inhibition zone of the protein isolate (H0), hydrolysate (H1-3), and fraction of hydrolysate (F1-3) protein of *Anadara granosa* against *Escherichia coli,* using amoxicillin as the positive control. Data were presented as mean±SD (n=3)

The isolate, hydrolysate, and hydrolysate fractions were more active against g-positive bacteria than g-negative bacteria. The outer cell envelope of Gram-negative bacteria was an asymmetric, highly hydrophobic bilayer composed of lipopolysaccharides and phospholipids with a negative charge. This electrostatic charge could impart impermeability to antimicrobial treatments by serving as a protective barrier against hydrophobic compounds. Porins, which were pore-forming protein that served as transport channels, were also present in this bilayer [45], allowing tiny hydrophilic molecules (<600 Da) to enter passively. Additionally, size exclusion impaired the permeability of hydrophilic substances [46]. The structural properties of the peptides, such as the overall density of cationic charges, length of the peptide sequence, quantity of hydrogen bond donors, and 3-D conformation, could also affect how antimicrobial peptides interact with inner and outer membranes [47]. The positively charged and water-repellent characteristics of peptides, which could induce cell rupture by interacting with the lipopolysaccharides of the bilayer, generally influenced the antibacterial action of hydrolysate extracts [48, 49].

Minimum inhibition concentration

As shown in table 1, the MIC of trypsin hydrolysate was lower than that of crude bromelain hydrolysate. This indicated that the trypsin hydrolysate was more active than the crude bromelain hydrolysate but not as active as amoxicillin. The 10 kDa fraction of trypsin hydrolysate for 5 h showed the highest inhibition zone compared to the other samples and was more active against *S. aureus* than against *E. coli*. The results showed that the MIC of the protein hydrolysate was 25% ($^{v}/_{v}$). When converted to soluble protein concentration, it equaled 10.25 mg/ml of protein in the 10 kDa fraction and was hydrolyzed by trypsin for 5 h, while amoxicillin 0.25 mg/ml gave 13.07±0.31 mm against *E. coli* and 13.57±0.32 mm against *S. aureus*. Yang *et al.* (2017) reported the minimum inhibitory concentration (MIC) for amoxicillin against *E. coli* was 32 μg/ml, while for *S. aureus it was* 0.125 μg/ml [50].

Table 2: The MIC of isolate and protein hydrolysate of *Anadara granosa*

Concentration % (v/v)	Inhibition zone against *Escherichia coli (mm)*±SD (*n = 3*)												
	H 0	Trypsin						Bromelain					
		H1	H2	H3	F1	F2	F3	H1	H2	H3	F1	F2	F3
100	8.53 ±0.15	9.53 ±0.15	12.23 ±0.15	12.73 ±0.06	10.13 ±0.21	12.90 ±0.20	13.33±0.15	9.30 ±0.36	10.07±0.47	11.73 ±0.21	9.57 ±0.15	10.53 ±0.15	11.43 ±0.06
75	7.47 ±0.23	8,10 ±0.20	10.50 ±0.20	10.67 ±0.15	8.17 ±0.21	10.47 ±0.23	11.13±0.32	7.57 ±0.42	7.90 ±0.20	9.20 ±0.26	7.60 ±0.44	9.00 ±0.17	9.27 ±0.15
50	6.73 ±0.15	6.50 ±0.26	8.67 ±0.21	9.00 ±0.20	6.60 ±0.26	8.20 ±0.26	9.13 ±0.25	6.70 ±0.20	6.63 ±0.25	7.10 ±0.20	6.60 ±0.17	7.07 ±0.15	7.10 ±0.20
25	-	-	6.87 ±0.25	7.33 ±0.15	-	7.23 ±0.21	7.57 ±0.31	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-	-	-	-
3.125	-	-	-	-	-	-	-	-	-	-	-	-	-
Control (-)	-	-	-	-	-	-	-	-	-	-	-	-	-
Concentration % (v/v)	Inhibition zone against *Staphylococcus aureus (mm)*±SD (*n = 3*)												
	H0	Trypsin						Bromelain					
		H1	H2	H3	F1	F2	F3	H1	H2	H3	F1	F2	F3
100	9.83 ±0.06	10.67 ±0.21	12.83 ±0.12	13.73±0.15	11.13 ±0.21	13.37 ±0.25	14.40 ±0.20	9.83 ±0.45	10.93 ±0.35	11.53 ±0.31	9.93 ±0.38	10.83 ±0.31	12.20 ±0.36
75	8.17 ±0.21	8.82 ±0.25	11.37 ±0.15	11.37±0.38	9.27 ±0.21	11.73 ±0.21	12.43 ±0.35	7.37 ±0.25	8.90 ±0.46	9.13 ±0.35	7.57 ±0.29	8.40 ±0.50	10.07 ±0.15
50	6.77 ±0.15	7.63 ±0.31	9.40 ±0.44	9.07 ±0.47	7.67 ±0.21	9.63 ±0.23	9.67 ±0.21	6.43 ±0.40	7.13 ±0.25	7.10 ±0.26	6.33 ±0.15	6.80 ±0.26	7.50 ±0.46
25	-	-	7.57 ±0.42	7.37 ±0.38	-	7.57 ±0.06	7.67 ±0.21	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-	-	-	-
3.125	-	-	-	-	-	-	-	-	-	-	-	-	-
Control (-)	-	-	-	-	-	-	-	-	-	-	-	-	-

In this study, the 10 kDa hydrolysate fraction was found to possess greater activity than both its hydrolysate parent and isolated protein counterparts. This finding confirms previous findings where isolated proteins with higher molecular weight demonstrated lower antibacterial activity. A 30 kDa hydrolysate fraction showed a greater inhibitory zone against *S. aureus* and *E. coli* bacteria as reported by Borrajo *et al.* (2020) [48], thus providing evidence of its antioxidant and antibacterial activities [47, 48]. To accurately ascertain its antioxidant/antibacterial activities, an analysis of its molecular weight was essential [48].

CONCLUSION

In conclusion, the pineapple-extracted enzyme had lower activity than trypsin enzyme purchased from the market. *A. granosa's* protein solubility increased after hydrolysis. Protein hydrolysate and isolates showed moderate antioxidant activities. The fraction 10 kDa had strong antioxidant activity. The isolate, hydrolysate, and hydrolysate fractions of 10 kDa *A. granosa's* protein had growth-inhibiting effects on *S. aureus* and *E. coli,* which was comparable to 0.25 mg/ml amoxicillin. The MIC of protein hydrolysate and 10 kDa hydrolysate fraction on *S. aureus* and *E. coli* was at a concentration of 50% using both hydrolysis methods, bromelain, and trypsin. Based on the results, the best activity was observed for the hydrolysate and the 10 kDa hydrolysate fraction with trypsin for 3 and 5 h, with a minimum inhibition concentration of 25% ($^{v}/_{v}$). Trypsin hydrolysate was more active than bromelain hydrolysate as an antioxidant and antibacterial agent.

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AUTHORS CONTRIBUTIONS

Y. M. P., S. Y., and L. L. participated in the research design; Y. M. P., S. Y., L. T., and V. W. conducted the research; Y. M. P. and L. L. wrote the manuscript.

CONFLICT OF INTERESTS

Declared none

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