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PARTIAL PURIFICATION AND IDENTIFICATION OF ANTIBACTERIAL PEPTIDES FROM THE ENDOPHYTIC FUNGUS KT31 ISOLATED FROM *Kappaphycus alvarezii*

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Submitted: 17 July 2025/Accepted: 24 September 2025
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How to cite (APA Style 7th): Sofyana, N. T., Mustopa, A. Z., Setyaningsih, I., Tarman, K., & Maulidiani. (2025). Partial purification and identification of antibacterial peptides from the endophytic fungus KT31 isolated from *Kappaphycus alvarezii*. *Jurnal Pengolahan Hasil Perikanan Indonesia,* 28(9), 828-840. http://dx.doi.org/10.17844/vyxqk974

Abstract

Endophytic fungi produce a wide array of secondary metabolites with diverse biological activities, including antibacterial, antifungal, insecticidal, and immunosuppressive effects. The increasing prevalence of infections caused by pathogenic bacteria, such as *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Bacillus pumilus*, and *Staphylococcus aureus*, highlights the urgent need for novel antibacterial agents. This study aimed to determine the optimal concentration of ammonium sulfate for the isolation of endophytic fungus KT31 from *Kappaphycus alvarezii* based on its antibacterial activity. Fungal proteins were extracted using ammonium sulfate precipitation at varying saturation levels and subsequently tested for antibacterial activity against a panel of seven pathogenic bacterial strains. Crude protein extracts demonstrating promising activity were further purified using gel filtration chromatography with Sephadex G-50, followed by molecular weight determination using SDS-PAGE and protein quantification using a Bicinchoninic Acid (BCA) assay. The highest antibacterial activity was observed in the protein fraction precipitated at 80% ammonium sulfate saturation, exhibiting inhibition zones of up to 14 mm against *E. coli* and *B. pumilus*. A notable inhibition zone of 12 mm was observed for the most active chromatographic fraction. SDS-PAGE analysis revealed that the active protein had an estimated molecular weight of 11.27 kDa. These findings suggest that endophytic fungi, particularly the isolate KT31, represent a promising source of novel antibacterial peptides, warranting further investigation for therapeutic applications..

Keywords: antibacterial activity, bioactive peptide, endophytic fungi, protein purification

Purifikasi Parsial dan Identifikasi Peptida Antibakteri dari Kapang Endofit KT31 yang Diisolasi dari Makroalga *Kappaphycus alvarezi*

Abstract

Kapang endofit mampu menghasilkan berbagai metabolit sekunder yang memiliki aktivitas biologis beragam, di antaranya antibakteri, antifungi, insektisida, dan imunosupresif. Peningkatan infeksi akibat

http://dx.doi.org/10.17844/vyxqk974

bakteri patogen misalnya *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Bacillus pumilus*, dan *Staphylococcus aureus* menunjukkan perlunya pencarian agen antibakteri baru. Penelitian ini bertujuan menentukan konsentrasi amonium sulfat terbaik untuk isolasi kapang endofit KT31 yang diisolasi dari *Kappaphycus alvarezii* berdasarkan potensi antibakterinya. Protein kapang diisolasi melalui metode pengendapan amonium sulfat dengan tingkat kejenuhan berbeda, kemudian diuji aktivitas antibakterinya terhadap tujuh isolat bakteri patogen. Ekstrak protein kasar yang menunjukkan aktivitas tertinggi dimurnikan lebih lanjut menggunakan kromatografi gel filtrasi dengan Sephadex G-50. Penentuan bo-bot molekul protein menggunakan metode SDS-PAGE dan kuantifikasi protein menggunakan uji Bicinchoninic Acid (BCA). Aktivitas antibakteri tertinggi diperoleh dari fraksi protein yang dipresipitasi pada kejenuhan amonium sulfat 80%, dengan zona hambat mencapai 14 mm terhadap *E. coli* dan *B. pumilus*. Fraksi terbaik dari hasil kromatografi menunjukkan zona hambat sebesar 12 mm. Analisis SDS-PAGE menunjukkan bahwa protein aktif memiliki estimasi bobot molekul sebesar 11,27 kDa. Hasil ini menunjukkan bahwa kapang endofit KT31 berpotensi sebagai sumber peptida antibakteri baru yang menjanjikan untuk dikembangkan lebih lanjut dalam bidang terapeutik.

Kata kunci: aktivitas antibakteri, kapang endofit, peptida bioaktif, purifikasi protein

INTRODUCTION

Indonesia's marine and coastal ecosystems are significant reservoirs of biodiversity, including microorganisms such as marine-derived endophytic fungi. These organisms are increasingly recognized as prolific producers of structurally diverse secondary metabolites with promising pharmaceutical applications. Endophytic fungi, which asymptomatically colonize plant tissues, are known to synthesize bioactive compounds exhibiting antimicrobial, cytotoxic, antioxidant, and immunomodulatory activities (Tan & Zou, 2001; Huang *et al*., 2007; Kusari *et al*., 2012; Atanasov *et al*., 2021; Isti'anah *et al*., 2024; Budiman *et al*., 2024).

Given the rising global threat of antimicrobial resistance, there is an urgent need to explore novel antimicrobial compounds. Antimicrobial peptides (AMPs) offer promising therapeutic alternatives because of their broad-spectrum activity, low toxicity, and reduced tendency to induce resistance (Mahlapuu *et al*., 2016; Mookherjee *et al*., 2020). While many studies have screened fungal extracts for antimicrobial activity, peptide-based fractions, especially those derived from marine endophytes, remain underexplored.

Marine-derived fungi from Indonesia have been reported to produce metabolites with antibacterial and cytotoxic activities, notably *Nodulisporium* sp. KT29 exhibited strong antibacterial effects against *Vibrio harveyi* and stimulated immune responses in shrimp (Hariati *et al*., 2018; Wahjuningrum *et al*., 2020, 2022), and *Xylaria psidii* KT30 produced extracellular proteins active against gram-positive bacteria and HeLa cells (Inthe *et al*., 2014; Munandar *et al*., 2014; Indarmawan *et al*., 2016). *Mycelium sterilium* KT31 also exhibited antibacterial and cytotoxic potential in preliminary screenings (Tarman *et al*., 2011). However, most previous studies on marine fungi have emphasized non-peptide secondary metabolites, such as polyketides, terpenoids, and alkaloids, or crude extracts without identifying the specific active molecules (Youssef *et al*., 2019). Reports on peptide-derived compounds remain relatively scarce, despite their recognized potential as antibacterial and cytotoxic agents (Huo *et al*., 2022). Given this background, the present study focuses on characterizing bioactive peptides from *Mycelium sterilium* KT31 as a promising source of such activities.

In this study, the marine endophytic fungus *Mycelium sterilium* KT31 was isolated from the red macroalga *K. alvarezii*, a commercially important seaweed widely cultivated in Indonesia. *K. alvarezii* is not only a major source of carrageenan but also harbors diverse microbial communities, including endophytic fungi, that may contribute to the host's defense mechanisms by producing bioactive metabolites. Previous studies have reported that endophytic fungi from *K. alvarezii* produce structurally diverse metabolites with antibacterial and cytotoxic activities, such as dibenzospiroketals from *Aspergillus*

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micronesiensis (Luyen *et al*., 2019). Recently, proteins derived from *K. alvarezii* have been shown to exhibit antimicrobial activity against human pathogens through mechanisms such as membrane perforation and ROS generation (Souza *et al*., 2025). In addition, phytochemical investigations have highlighted *K. alvarezii* as a valuable source of compounds with antimicrobial and biotechnological applications (El-Beltagi *et al*., 2022). Exploring endophytic fungi from this macroalga thus provides dual significance: it enhances our understanding of seaweed–microbe interactions and reveals potential antimicrobial peptides for therapeutic development.

Although fungal-derived antimicrobial peptides (AMPs) have attracted growing scientific interest, their isolation and structural characterization remain technically challenging. The inherent low abundance, structural complexity, and instability of these peptides necessitate laborious fractionation and advanced analytical techniques, such as RP-HPLC, MS/MS, and NMR. For example, peptaibols from *Trichoderma* spp. typically require multi-step chromatographic purification; however, the yields remain low and often insufficient for detailed structural analysis (Szekeres *et al*., 2005). Similarly, a defensin-like peptide isolated from *Aspergillus clavatus* showed potent antibacterial activity, but purification required successive ammonium sulfate precipitation and gel filtration before mass spectrometry confirmation (Ng & Wong, 2013). Furthermore, mechanistic studies are sparse, particularly on AMPs from marine endophytic fungi, despite their potential as novel antimicrobial agents (Ageitos *et al*., 2017). Recent reviews have emphasized marine fungi as a rich yet underexplored source of structurally unique antibacterial compounds, including peptides (Pan *et al*., 2025).

To address this gap, this study focused on the isolation, partial purification, and characterization of peptide fractions from *Mycelium sterilium* KT31. This fungal isolate, obtained from the red macroalga *K. alvarezii*, has previously shown moderate antibacterial and cytotoxic activities in preliminary screenings (Tarman *et al*., 2011), suggesting the presence of bioactive molecules of potential pharmaceutical relevance. The association of KT31 with *K. alvarezii* is also noteworthy, as seaweed–fungal interactions are increasingly recognized as important sources of antimicrobial compounds that may contribute to the host's defense against marine pathogens. By characterizing the peptide fractions of KT31, this study not only explores a unique fungal–algal symbiosis but also contributes to the discovery of novel antimicrobial peptides and supports the development of alternative therapeutics from marine endophytic fungi.

MATERIALS AND METHODS

Fungal Isolate and Culture Conditions

The endophytic fungal isolate *Mycelium sterilium* KT31, isolated from the red macroalga *K. alvarezii,* was obtained from the culture collection of Dr. rer. nat. Kustiariyah Tarman. The fungus was cultivated in Potato Dextrose Broth (PDB; Himedia) supplemented with 0.75 g/L NaCl. A 1% (v/v) inoculum from a 3-day-old preculture was transferred into 300 mL of fresh PDB and incubated for 7 days at room temperature (25 ± 2 °C) on a rotary shaker at 150 rpm. Fungal biomass was harvested by centrifugation at 10,000 rpm for 30 min at 4°C. The supernatant was collected for protein extraction, following the method described by Ilyas *et al.* (2009), while the biomass pellet was discarded, as the focus of this study was on extracellularly secreted metabolites.

Protein Precipitation

The fungal culture supernatant was subjected to ammonium sulfate precipitation at 60%, 70%, 80%, and 90% saturation, following the method described by Onsori *et al.* (2005). The mixtures were incubated overnight at 4°C and centrifuged at 10,000 rpm for 30 min. The resulting pellets were resuspended in 10 mM Tris-HCl buffer (pH 7.4) and dialyzed overnight at 4°C to remove residual ammonium sulfate before being stored at 4°C for further purification.

http://dx.doi.org/10.17844/vyxqk974

Gel Filtration Chromatography

The fungal protein was partially purified using Sephadex G-50 gel filtration chromatography. The column was pre-equilibrated and eluted with 30% ethanol (v/v) in sterile distilled water at a 3:7 ratio. Elution was performed at 4°C, and 1 mL fractions were collected in microtubes (Wachirathiancai *et al*., 2004). All fractions were tested for their antibacterial activity.

Antibacterial Bioassay

The antibacterial activity was assessed using the agar diffusion method. Antibacterial activity was assessed using the agar diffusion method (Aboaba *et al*., 2011). A panel of bacterial strains, including *E. coli*, *S. typhi*, *P. aeruginosa*, *L. monocytogenes*, and *S. aureus,* selected due to their status as well-known human pathogens commonly associated with foodborne illnesses and clinical infections. In addition, *B. subtilis* and *B. pumilus* were included in this assay. Although generally considered non-pathogenic or opportunistic, these *Bacillus* species are frequently employed as model organisms in antibacterial screening studies because of their robust growth, sporulation ability, and relevance in food spoilage and contamination scenarios. All bacterial isolates used in this study were obtained from the bacterial culture collection maintained by the Recombinant Viral Protein Research Group, Genetic Engineering Research Centre, National Research and Innovation Agency (BRIN), Indonesia. Antibacterial assays were performed using chloramphenicol (C) and penicillin (P) as positive controls, and 10 mM Tris-HCl buffer (pH 7.4) as the negative control.

Prior to the assay, the bacteria were cultured in Nutrient Broth (NB) for 16 h at 37°C with agitation. The cell suspensions were adjusted to McFarland standard 2 and homogenized with molten Nutrient Agar (NA). Twenty microliters of each fungal protein fraction was applied to sterile paper disks, which were then placed on the inoculated NA plates. The plates were incubated at 37°C for 20 h, and the inhibition zones were measured.

SDS-PAGE Analysis

The molecular weights of the active protein fractions were determined by SDS-PAGE using an 18% acrylamide separating gel. Electrophoresis was performed at 10 mA for 120 min. The gel was stained using the silver staining method and visualized to estimate protein size based on molecular weight markers (Todorov *et al*., 2007).

Protein Quantification

Protein concentrations were measured using the Bicinchoninic Acid (BCA) assay (Smith *et al*., 1985). Each sample (0.1 mL) was mixed with 2.0 mL of the working reagent and incubated at 37°C for 30 min. Absorbance was measured at 562 nm. Bovine Serum Albumin (BSA) standards ranging from 0.05 to 0.5 mg/mL were used to construct the calibration curve.

RESULTS AND DISCUSSION

Cultivation of Endophytic Fungus KT31 (*M. sterilium*)

The growth characteristics of the KT31 isolate reflected its adaptation to aerated liquid media. During incubation, visible expansion of filamentous biomass occurred in the suspension, forming dispersed white aggregates. This macroscopic morphology is frequently associated with enhanced metabolite secretion in marine-derived endophytic fungi grown under agitation, which supports optimal aeration and nutrient uptake (Bertrand & Munoz, 2019; Pan *et al*., 2024). The harvested culture medium, which showed signs of active fungal metabolism, served as the source of extracellular proteins for subsequent purification (Wang *et al*., 2023). The morphological appearance of KT31 grown on both solid and liquid media is shown in Figure 1 and 2, respectively, illustrating distinct colony characteristics under different culture conditions.

Protein Isolation via Ammonium Sulfate Precipitation

Differential salt precipitation was employed to enrich proteinaceous

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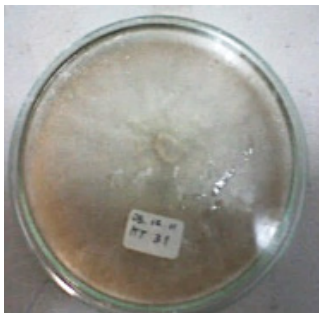

Figure 1 The endophytic fungus *M. sterilium* (KT31) grown on solid medium

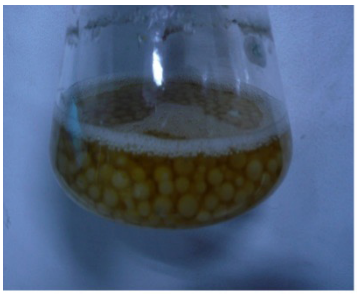

Figure 2 Mycelial morphology of the endophytic fungus KT31 (*M. sterilium*) in liquid medium

compounds with potential bioactivity. This classical method separates proteins based on their solubility profiles at increasing ionic strengths. The 80% ammonium sulfate saturation condition produced a prominent protein pellet, suggesting the effective concentration of soluble components with intermediate hydrophobicity. This fraction was used in downstream bioassays after gentle resuspension in buffered conditions to preserve bioactivity (Wingfield, 2001).

Antibacterial Activity of Precipitated Protein

Bioactivity screening of protein precipitates revealed that the 80% saturated fraction had the most significant antimicrobial effect. Based on the inhibition zone diameters, the crude extract (SP) exhibited weak antibacterial activity, with inhibition zones of 8 mm only against *E. coli* and *L. monocytogenes*. According to standard classifications, inhibition zones less than 10 mm are generally considered low, 10-15 mm moderate, and >15 mm high (Balouiri *et al*., 2016).

In contrast, chromatographic fractions P60% to P90% showed medium antibacterial activity against most tested strains, with zones reaching up to 14 mm in diameter. This increase in inhibition suggests that bioactive compounds with antibacterial properties were more concentrated in the polar fractions following chromatographic separation. Among all the tested samples, this fraction consistently produced the widest inhibition zones, particularly against *E. coli* and *B. pumilus*, with a maximum diameter of 14 mm. These results indicate the presence of potent antibacterial compounds, likely short peptides, that can disrupt bacterial cell integrity.

The antibacterial activity of the KT31 fractions was observed against both Gram-positive (*L. monocytogenes*, *B. pumilus*) and Gram-negative bacteria (*E. coli*, *S. typhi*, *P. aeruginosa*), although inhibition zones were generally larger for Gram-positive strains. This trend is consistent with the mode of action of antimicrobial peptides (AMPs), which preferentially target the exposed peptidoglycan layer of gram-positive bacteria (Wang, 2010). Nevertheless, the detectable inhibition of gram-negative bacteria suggests that KT31 peptides may also interact with the outer membrane, a feature reported for several fungal-derived lipopeptides and peptaibols (Youssef *et al*., 2019; Huo *et al*., 2022).

http://dx.doi.org/10.17844/vyxqk974

These findings align with the known mechanisms of antimicrobial peptides, such as pore formation and membrane destabilization (Bertrand & Munoz, 2019; Chen *et al*., 2024). They not only confirmed the specificity of the bioactive fraction, since no inhibition was observed in the buffer controls, but also highlighted the relatively broad antibacterial spectrum of the KT31-derived peptides, underscoring their potential as promising candidates for novel antimicrobial agents. The lack of activity in the buffer controls also confirmed the specificity of the bioactive fraction. The antibacterial activity of each protein fraction against a panel of pathogenic bacterial strains is presented in Table 1.

Gel Filtration Chromatography and Fraction Bioactivity

Subsequent purification via gel filtration chromatography enabled the further separation of the protein mixture. Chromatographic separation of the crude extract yielded 30 fractions with varying antibacterial activities. Most of them exhibited low inhibition, with zones below 10 mm. However, specific fractions, particularly fractions 11, 12, and 25–30, showed moderate activity (10-12 mm), notably against *E. coli* and *S. typhi*. According to the literature, inhibition zones within this range are considered moderate (Balouiri *et al*., 2016). These findings suggest that the bioactive compounds responsible for antibacterial activity were enriched in select fractions, as these displayed stronger effects than the crude extract, which only weakly inhibited *E. coli* and *L. monocytogenes* (8 mm zones). This underscores the value of chromatographic fractionation in concentrating functional metabolites and enhancing biological efficacy. Among the eluted fractions, only one exhibited

Table 1 Inhibition zone (mm) of endophytic fungus KT31 (*M. sterilium*) against several pathogenic bacteria

Sample	Inhibition zone (mm)						
	S. typhi	*E. coli*	*S. aureus*	*P. aeruginosa*	*B. pumilus*	*B. subtilis*	*Listeria*
SP	-	8	-	-	-	-	8
S60%	-	-	-	-	-	-	-
S70%	-	-	-	-	-	-	-
S80%	-	-	-	-	-	-	-
S90%	-	-	-	-			
P60%	11	12	9	8	13	12	11
P70%	12	13	10	8	13	12	12
P80%	13	14	10	9	14	12	12
P90%	13	14	9	9	12	13	12
C	25	22	25	25	25	25	23
P	10	10	10	9	9	20	9
T	-	-					-

SP : Supernatant after initial filtration
S60%-S90% : Supernatant after ammonium sulfate precipitation at 60-90% saturation
P60%-P90% : Pellet after ammonium sulfate precipitation at 60-90% saturation
C : Chloramphenicol (positive control)
P : Penicillin (positive control)
T : Tris-HCl 10 mM pH 7.4 (negative control)
Low inhibition : <10 mm
Medium inhibition : 10–15 mm
High inhibition : >15 mm

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measurable antibacterial activity, forming a 12 mm inhibition zone against *S. typhi*. Although this was slightly reduced compared to the crude ammonium sulfate pellet, the retained activity confirmed the presence of a functional antibacterial component. This modest reduction may be attributed to sample dilution or partial loss during elution. Nevertheless, the selectivity of Sephadex G-50 for small peptides makes it a suitable medium for initial fractionation (Bertrand & Munoz, 2019; Sarkar & Suthindhiran, 2022).

In terms of bacterial susceptibility, the active fractions showed slightly higher effectiveness against gram-negative bacteria (*E. coli* and *S. typhi*) than gram-positive strains. *P. aeruginosa* remained largely unaffected, possibly because of the intrinsic resistance conferred by its outer membrane and efflux systems (Nikaido, 2003; Li *et al*., 2015). Gram-positive bacteria, including *B. subtilis*, *B. pumilus*, and *L. monocytogenes*, showed limited susceptibility with inhibition zones mostly below 9 mm. These findings indicate that antibacterial compounds may preferentially target components or pathways that are more accessible in gram-negative organisms.

In conclusion, both initial fractionation and subsequent gel filtration chromatography successfully enriched the antibacterial activity of the crude extract. The identification of moderately active fractions, particularly against gram-negative pathogens, highlights the potential of these compounds for further purification and characterization as novel antimicrobial agents. The results of antifungal fraction testing of endophytic fungus KT 31 are shown in Table 2.

Protein Characterization by SDS-PAGE

SDS-PAGE analysis produced distinct protein bands separated according to their molecular weights. The thickness of each band reflects the relative abundance of proteins with similar molecular weights that migrate to the same position on the gel. This observation aligns with the fundamental principle of electrophoresis, where charged molecules migrate under an electric field, and molecules of identical size and charge accumulate at the same or nearby locations.

Molecular weight analysis of the endophytic fungus *M. sterilium* was performed using SDS-PAGE. The resulting gel profile revealed that the target protein band had an estimated molecular weight of 10-15 kDa. The precise molecular weight was determined using a standard protein marker. A single distinct band corresponding to 11.2745 kDa was observed in the SDS-PAGE analysis. This observation indicates that the dominant peptide, likely responsible for the observed bioactivity, was successfully isolated. The size of this compound falls within the typical range for low-molecular-weight antimicrobial peptides derived from endophytic and marine fungi (Youssef *et al*., 2023). The clarity of the band further suggests minimal contamination by other proteins, supporting the effectiveness of our purification workflow. The SDS-PAGE electropherogram is shown in Figure 3.

Protein Quantification Using BCA Assay

The protein concentration of the most active fraction was quantified to evaluate the efficiency of each purification step. Measurements were performed using the Bicinchoninic Acid (BCA) Protein Assay, a widely used colorimetric method based on the biuret reaction. In this assay, peptide bonds reduce Cu^{2+} to Cu^{+} in an alkaline environment, forming a purple complex upon interaction with bicinchoninic acid. The resulting absorbance, measured at 562 nm, directly correlates with the protein content and can detect concentrations within the range of 20-2000 µg/mL (Pierce, 2003).

Quantification was performed using a calibration curve generated from bovine serum albumin (BSA) standards ranging from 0 to 2 mg/mL. A standard curve was used to determine the protein concentrations of samples obtained from the crude extract, ammonium sulfate precipitation (pellet fractions), and final gel filtration fractions. The results of the protein concentration analysis of the endophytic fungus are shown in Table 3.

As shown in Table 3, the total protein content decreased progressively during

http://dx.doi.org/10.17844/vyxqk974

Table 2 Inhibition zone measurements of the endophytic fungal fractions of KT31 against several pathogens

Sample	Inhibition zone (mm)						
	S. typhi	*E. coli*	*S. aureus*	*P. aeruginosa*	*B. pumilus*	*B. subtilis*	*Listeria*
Fraction 1	10	-	7	-	8	-	7
Fraction 2	10	-	7	-	8	-	7
Fraction 3	9	-	7	-	8	-	7
Fraction 4	8	-	-	-	8	-	7
Fraction 5	8	8	-	-	8	-	7
Fraction 6	8	8	-	-	-	-	7
Fraction 7	9	-	-	-	7	-	7
Fraction 8	8	-	7	-	7	-	7
Fraction 9	8	8	7	-	8	-	7
Fraction 10	8	10	-	-	-	-	7
Fraction 11	8	12	-	-	-	-	7
Fraction 12	9	12	-	-	-	-	7
Fraction 13	9	12	-	-	-	8	-
Fraction 14	9	12	-	-	-	8	-
Fraction 15	9	9	-	-	-	9	-
Fraction 16	9	12	-	-	-	9	-
Fraction 17	9		-	-	-	-	-
Fraction 18	9	8	-	-	-	-	-
Fraction 19	9	8	-	-	7	-	-
Fraction 20	9	8	-	7	7	7	-
Fraction 21	9	7	-	7		9	-
Fraction 22	9	-	-	7	7	9	-
Fraction 23	9	-	-	7	7	9	-
Fraction 24	9	-	-	7	7	9	-
Fraction 25	9	12	-	7	-	-	-
Fraction 26	9	12	-	-	-	-	-
Fraction 27	9	12	-	-	-	8	-
Fraction 28	9	12	-	-	-	10	-
Fraction 29	9	12	-	-	-	-	-
Fraction 30	11	12					-

Low inhibition : <10 mm
Medium inhibition : 10–15 mm
High inhibition : >15 mm

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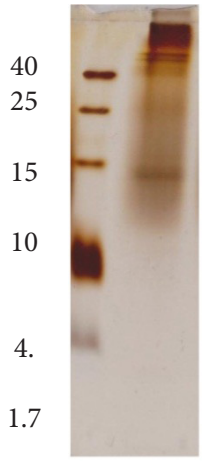

Figure 3 SDS-PAGE electropherogram of ethanol eluate fraction 25

Table 3 Protein concentration of endophytic fungus KT31 (*M. sterilium*)

Stage	Volume (mL)	Protein concentration (mg/mL)	Total protein (mg)
Crude extract	150	0.19	28.5
80% Ammonium sulfate precipitate	1	0.209	0.209
Sephadex G-50 fraction	1	0.1564	0.1564

the purification process. The crude extract contained the highest protein concentration because of the abundance of both target and non-target proteins and other cellular components. In contrast, the pellet fractions obtained after ammonium sulfate precipitation showed a lower protein yield, which can be attributed to the selective precipitation of proteins and potential loss of proteins during filtration. A portion of the protein may have remained in the biomass or supernatant, thus reducing the amount recovered in the final pellet.

The lowest protein concentration was recorded in the final Sephadex G-50 fraction. This was expected because the sample was not pre-concentrated prior to chromatography, and the eluted volume was relatively high compared to the pellet stage. Furthermore, protein loss during gel filtration could also result from nonspecific binding to the matrix or partial degradation. The possibility of autolysis occurring during the purification process cannot be excluded, particularly if temperature fluctuations occurred during the sample handling. These combined factors contributed to the reduced total protein recovery, despite the enrichment of bioactive peptides in the final purified fraction of the protein hydrolysate. Nevertheless, peptide enrichment was evident, as SDS-PAGE revealed a distinct band of approximately 11.27 kDa. This molecular weight falls within the range commonly reported for fungal antimicrobial peptides, including defensin-like proteins and small secreted peptides (Wang, 2010; Niño-Vega *et al*., 2025), and is consistent with previous reports of extracellular proteins from marine fungi exhibiting antibacterial and cytotoxic activities (Munandar *et al*., 2014; Indarmawan *et al*., 2016). Collectively, these observations support the hypothesis that the KT31 fraction contains bioactive peptides with antibacterial potential.

CONCLUSION

The endophytic fungus KT31 (*Mycelium sterilium*) exhibited notable antibacterial activity when cultured in Potato Dextrose Broth (PDB), as demonstrated by an inhibition zone of 14 mm from the ammonium sulfate precipitated pellet and 12 mm from the most active chromatographic fraction. Among the tested conditions, 80% ammonium

sulfate saturation yielded the most effective antibacterial fraction. SDS-PAGE analysis revealed that the purified bioactive protein had a molecular weight of 11.2745 kDa. Future studies are recommended to further purify and characterize the specific peptide(s) responsible for antibacterial activity using high-performance liquid chromatography (HPLC).

ACKNOWLEDGEMENT

The authors thank the National Research and Innovation Agency (BRIN) for providing analytical instruments to support this research under the supervision of Dr. Apon Zaenal Mustopa, M. Si., as the principal investigator.

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