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The Potential of Red Algae Methanol Extract (*Kappaphycus alvarezii*) as an Antibacterial Agent Toward *Pseudomonas aeruginosa* and *Enterococcus faecalis* Bacteria

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Abstract

Urinary tract infection (UTI) is a disease caused by both Gram-negative and Gram-positive bacteria. Red algae (*Kappaphycus alvarezii)* contain bioactive compounds derived from secondary metabolites with antibacterial properties. This study investigates the potential of *Kappaphycus alvarezii* methanol extract to inhibit the growth of *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 using the microdilution broth method, determining Minimum Inhibitory Concentration (MIC) values ranging from a concentration of 500 µg/mL to 1.9531 µg/mL. To elucidate the inhibition mechanism, nucleic acid, protein, and Ca^{2+} ion leakage assay were performed via spectrophotometry at MIC 1, MIC 2, and MIC 3 concentrations. Morphological changes in the bacterial cell membrane were further examined using Scanning Electron Microscopy (SEM) at MIC 2. The results demonstrated that, the methanol extract of *Kappaphycus alvarezii* exhibited significant antibacterial activity against *Enterococcus faecalis* bacteria with an MIC value of 1.9531 µg/mL, but showed no significant inhibitory effect on *Pseudomonas aeruginosa*. The extract inducted membrane leakage, as indicated by a higher leakage value compared to negative controls and commercial antibiotics. In addition, the methanol extract of *Kappaphycus alvarezii* caused alterations in bacterial cells. These findings highlight the potential of *Kappaphycus alvarezii* methanol extract as a promising antibacterial agent.

Keywords: Antibacterial; *Enterococcus faecalis*; Urinary Tract Infection; *Kappaphycus alvarezii*; *Pseudomonas aeruginosa*.

Abbreviations: 2,3,5-Triphenyltetrazolium Chloride (TTC), American Type Culture Collection (ATCC), Clinical and Laboratory Standards Institute (CLSI), *Enterococcus faecalis* (*E. faecalis*), *Kappaphycus* alvarezii (*K. alvarezii*), Minimum Inhibitory Concentration (MIC), Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), Nutrient Broth (NB), Phosphate-Buffered Saline (PBS), *Pseudomonas aeruginosa* (*P. aeruginosa*), World Health Organization (WHO), Scanning Electron Microscope (SEM).

INTRODUCTION

Infection is a serious health problem, and its incidence continues to rise in various parts of the developing world. These increasing infections can be caused by various microorganisms, such as bacteria, fungi, or parasites. According to the *Global Research on Antimicrobial Resistance* in 2022, 13.7 million cases of disease were caused by infections, with 7.7 million cases being caused by various types of pathogenic bacteria (Ikuta et al., 2022). One of the most common infections, which is increasing annually, is urinary tract infections (UTIs), which are ranked second among infectious diseases (Jannah et al., 2022).

In fact, urinary tract infections (UTIs) can be caused by *Pseudomonas aeruginosa* (*P. aeruginosa*) or *Enterococcus faecalis* (*E. faecalis*). According to the 2021 *Global Burden of Disease* data, the increase in urinary tract infections was estimated at 66.45% over the past 30 years (He et al., 2025). Furthermore, the increase in cases in Indonesia was estimated at 90-100 cases per 100.000 people, or 180.000 cases per year (Yashir, M., & Apriani, 2019). However, the prevention of urinary tract infections nowadays is still limited to the administration of synthetic antibiotics, which can lead to resistance if used inappropriately. In 2024, the World Health Organization (WHO) successfully categorized the *P. aeruginosa* bacteria as a high-priority antibiotic resistance group (Sari et al., 2022). Furthermore, the *E. faecalis* bacteria were also included in the high-priority antibiotic resistance group (Mancuso et al., 2021).

Currently, many studies have been conducted to develop natural antibiotics derived from natural ingredients, including red algae (*Kappaphycus alvarezii)*. *Kappaphycus alvarezii* is a red algae from the sea that is still being developed as an anticancer therapeutic drug and

antibiotic. *K. avarezii* contains several bioactive compounds resulting from secondary metabolites that have the potential to act as antibacterial, anticancer, anti-inflammatory, and other biological activities (Sari et al., 2022). A study by Marhaeny et al. (2024) showed that *K. alvarezii* contains alkaloids, flavonoids, steroids, and tannins with potential antibacterial, anti-inflammatory, or anticancer agents. In addition, *K. alvarezii* also produces carrageenan which can act as an antibacterial (Alizadeh et al., 2023). Tiarani et al. (2025) stated that *K. alvarezii* has the potential to inhibit the growth of *P. aeruginosa* bacteria. Similarly, Naqvi et al. (2022) found that the methanol extract of red algae can inhibit the growth of *E. faecalis* bacteria.

Although *K. alvarezii* has the potential antibacterial activity, a molecular study into the inhibitory mechanisms of active compounds against bacteria is still rare. Therefore, this study explored the potential of *K. alvarezii* methanol extract as a natural antibacterial agent in inhibiting the growth of bacteria classified as resistant to synthetic antibiotics. Furthermore, further testing was conducted to determine the extract's ability to induce membrane leakage and changes in bacterial cell morphology.

MATERIALS AND METHODS

Materials

The materials used in this study consisted of methanol extract of *K. alvarezii, E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, Mueller Hinton Agar (MHA) media, Mueller Hinton Broth (MHB) media, Nutrient Broth (NB) media, 0.9% physiological NaCl, sterile distilled water, phosphate buffer pH 7.0, 1× PBS pH 7.4, 1% DMSO, chloramphenicol, 2.5% glutaraldehyde, ethanol, and 2,3,5-Triphenyltetrazolium Chloride (TTC) reagent.

Procedures

Preparation of Test Bacterial Suspension

One loop of *E. faecalis* and *P. aeruginosa* bacteria, rejuvenated for 24 hours, was taken. The sample was then inoculated into 9 ml of 0.9% physiological NaCl until the optical density (OD) reached the McFarland standard of 0.5. Once the turbidity met the standard, the bacteria were inoculated into nutrient broth or Mueller-Hinton broth.

Preparation of Test Solution

The test solution was prepared by weighing 10 mg of *K. alvarezii* methanol extract, dissolving it in 5 ml of 1% DMSO and vortexing to obtain a 2,000 µg/mL stock solution. The 2,000 µg/mL stock solution was diluted to produce the desired final extract concentration using the formula $V1 \times M1 = V2 \times M2$.

Preparation of Control Solution

A positive control solution was prepared by weighing 10 mg of chloramphenicol powder and then dissolving it in 10 ml of sterile distilled water and homogenizing with a vortex as a positive control stock solution of 1,000 µg/mL. The negative control solution used 1% DMSO without other ingredients.

Preparation of 2,3,5-Triphenyltetrazolium Chloride (TTC) Reagent

This reagent was prepared to visualize the MIC results obtained in a 96-well plate. This reagent was prepared by weighing 10 mg of TTC powder, dissolving it in 10 ml of phosphate buffer at pH 7.0, and vortexing to obtain a 0.1% TTC stock solution. The TTC solution was filtered using a 0.22 µm syringe filter. The TTC reagent was then diluted with phosphate buffer from 0.1% to 0.01% using the formula $V1 \times M1 = V2 \times M2$.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC value was determined using the microdilution broth method in accordance with the Clinical and Laboratory Standards Institute (CLSI) 2024 standards (Morrissey & Patel, 2025). The bacterial suspension of *E. faecalis* and *P. aeruginosa*, incubated for 18-24 hours was diluted using 9 ml of 0.9% physiological NaCl until the turbidity matched the McFarland 0.5 standard. After that, the bacterial suspension was put into Mueller-Hinton broth. In a 96-well plate, 100 µL of Mueller Hinton broth was added, then a 2.000 µg/mL extract stock solution was added to the first well and dilution was carried out starting from an extract concentration of 500 µg/mL to 1.9531 µg/mL. In addition, in other wells, a positive control in the form of chloramphenicol and a negative control of 1% DMSO were added. After all were successfully added, the prepared bacteria were inoculated into each well until the final volume reached 200 µL, then the microplate was incubated in a 37°C incubator for 24 hours. After 24 hours, the microplate was added with 20 µL of 2,3,5-Triphenyltetrazolium Chloride (TTC) reagent and incubated again at 37°C for 1-4 hours. The color change was observed to be pink or red.

Nucleic Acid and Protein Leakage Testing

The nucleic acid leakage test was performed by taking 10 ml of the prepared bacterial suspension and placing it in a centrifuge tube. The test was then centrifuged at 3500 rpm for 20 minutes. The resulting bacterial pellet was washed twice with 1× PBS pH 7.4. After washing, 1× PBS pH 7.4 and extracts with concentrations of MIC 1, MIC 2, MIC 3, and the positive control were added to the centrifuge tube containing the pellet, reaching a final volume of 10 ml. Afterward, the centrifuge tube was incubated on a shaker at 150 rpm for 18-24 hours. The bacterial pellets, which were successfully incubated for

18-24 hours, were centrifuged again at 3500 rpm for 20 minutes. The resulting supernatant was measured for nucleic acid leakage at 280 nm and protein leakage at 260 nm using a UV-Vis spectrophotometer.

Ca^{2+} Metal Ion Leakage Test

The nucleic acid leakage test was performed by taking 10 ml of the prepared bacterial suspension and placing it in a centrifuge tube, then centrifuging it at 3500 rpm for 20 minutes. The resulting bacterial pellet was washed twice with 1× PBS pH 7.4. After washing, 1× PBS pH 7.4 and extracts with MIC 1, MIC 2, MIC 3, and positive control were added to the centrifuge tube containing the pellet until a final volume of 10 ml was reached. The centrifuge tube was then incubated on a shaker at 150 rpm for 18-24 hours. The bacterial pellets that had been incubated for 18-24 hours were then centrifuged again at 3500 rpm for 20 minutes, and the resulting supernatant was measured using an Anatomic Absorption Spectrophotometer (AAS).

Observation of Bacterial Morphological Changes Using a Scanning Electron Microscope (SEM)

SEM testing was performed by adding an extract at a concentration of MIC 2 to a suspension of *E. faecalis* ATCC 29212 bacteria, then incubating it on a shaker at 150 rpm for 9-12 hours. Afterward, the pellet was centrifuged at 10,000 rpm and washed with 1× PBS pH 7.4. The washed pellet was fixed in 2.5% glutaraldehyde for 12 hours at 4°C. Afterward, another wash was performed using 1× PBS pH 7.4 and continued with dehydration using ethanol in stages (50%, 60%, 70%, 80%, 90%, and 100% for 10 minutes each). The successfully fixed pellets were then viewed under an electron microscope.

Data analysis

The data obtained from the research results were analyzed using the SPSS 27 statistical application. Statistical tests began with normality and homogeneity tests with a 95% confidence level ($\alpha = 0.05$). If the normality test value $\alpha > 0.05$ and the homogeneity test value $\alpha > 0.05$, a One-Way ANOVA test was performed to assess the potential for an increase in contamination between treatments. However, if the data were not normally distributed or homogeneous, a Kruskal-Wallis test was performed followed by a Mann-Whitney test.

RESULTS AND DISCUSSION

Results

Determination of the Minimum Inhibitory Concentration (MIC) Value

The antibacterial activity of *K. alvarezii* methanol extract against *E. faecalis* and *P. aeruginosa* bacteria was tested by qualitatively determining the MIC value in a 96-well plate. Test results were obtained by visualizing the color of the TTC reagent added to each well. A positive result for bacterial growth was indicated by a pink to red color change in each well where the TTC reagent was added, while a negative result, indicating the absence of bacterial growth, was indicated by a clear color in each well (De Souza et al., 2024). The results of the study indicated that the methanol extract of *K. alvarezii* had the potential to inhibit the growth of *E. faecalis* bacteria with an MIC value of 1.9531 µg/mL. However, for *P. aeruginosa*, the methanol extract did not have the potential to inhibit the growth of these bacteria, as the addition of the TTC reagent resulted in a pink color. The results of determining the MIC value can be seen in Table 1 below.

Table 1. The Results of Minimum Inhibitory Concentration (MIC).

Treatment	Test	Bacteria	
		P. aeruginosa	***E. faecalis***
positive control	1	-	-
	2	-	-
Negative control	1	+	+
	2	+	+
500 µg/mL	1	+	-
	2	+	-
250 µg/mL	1	+	-
	2	+	-
125 µg/mL	1	+	-
	2	+	-
62.5 µg/mL	1	+	-
	2	+	-
31.25 µg/mL	1	+	-
	2	+	-
15.625 µg/mL	1	+	-
	2	+	-
7.1825 µg/mL	1	+	-
	2	+	-
3.9062 µg/mL	1	+	-
	2	+	-
1.9531 µg/mL	1	+	-
	2	+	-

Nucleic Acid, Protein, and Ca^{2+} Metal Ion Leakage Testing

Based on the MIC values obtained, the inhibitory mechanism of *K. alvarezii* methanol extract toward E. *faecalis* and *P. aeruginosa* bacteria through membrane leakage was investigated. The nucleic acid, protein, and Ca^{2+} metal ion leakage tests were conducted with the five treatments: a positive control, a negative control, and various extract concentrations ranging from 1.9531 µg/mL, 3.9062 µg/mL, and 500 µg/mL. The results of these leakage tests will be statistically tested using *One-Way* ANOVA to determine significant differences in leakage between treatment groups compared to the negative control. The results of nucleic acid, protein, and Ca^{2+} metal ion leakage are shown in Figure 1 below.

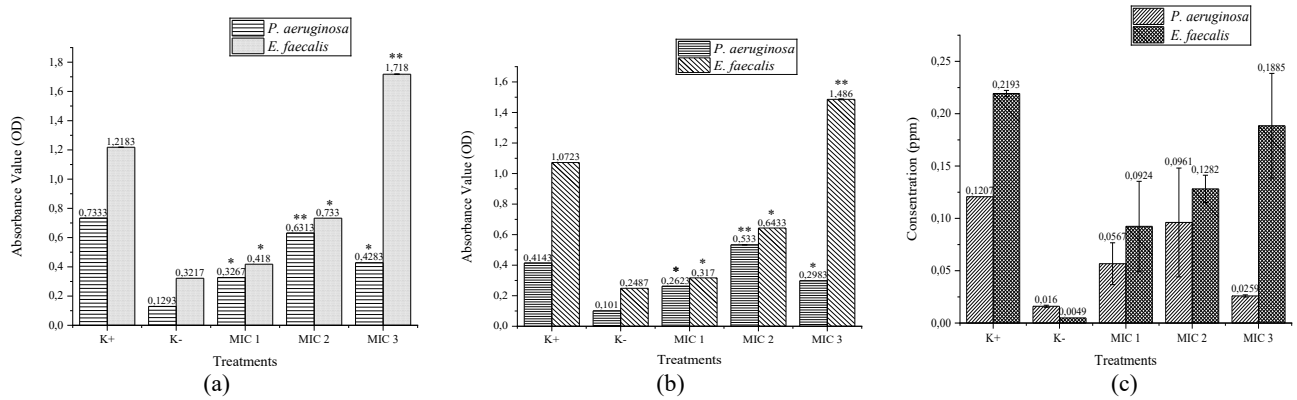

Figure 1. The Results of Nucleic Acid, Protein, and Ca^{2+} Metal Ion Leakage Tests.
Description: (a) Nucleic acid leakage; (b) Protein leakage; (c) Ca^{2+} metal ion leakage; *: significant increase in leakage compared to the negative control; **: significant increase in leakage compared to the negative control and potential to cause leakage compared to commercial antibiotics (chloramphenicol); K+: Positive control (chloramphenicol); K-: Negative control (without additional substances); MIC 1: 1.9531 µg/mL; MIC 2: 3.9062 µg/mL; MIC 3: 500 µg/mL.

The data show that the addition of extracts with various concentrations can cause bacterial cell leakage toward *E. faecalis* and *P. aeruginosa* as evidenced by the higher leakage values of nucleic acids, proteins, and Ca^{2+} metal ions compared to negative controls. In *E. faecalis* bacteria, the leakage value is directly proportional to the increase in the concentration of the added extract. However, in *P. aeruginosa* there is a decrease in the leakage value at a MIC concentration of 3. In addition, in *E. faecalis*, a concentration of 500 µg/mL has the potential to cause leakage compared to the positive control, while in *P. aeruginosa* a concentration of 3.9062 µg/mL also has the potential to cause leakage. Statistical analysis was carried out using nonparametric One-Way ANOVA and continued with the Mann-Whitney test to confirm a significant increase in leakage and the potential to cause leakage in the treatment ($p < 0.05$).

Observation of Bacterial Morphological Changes Using a Scanning Electron Microscope (SEM)

To demonstrate the inhibitory mechanism and membrane leakage, this study conducted SEM observations to detect morphological changes in the bacteria. If the extract was proven to inhibit bacterial growth and cause membrane damage, the SEM results demonstrated damage to bacterial morphology and changes in shape, either cell lysis or deformation. Figure 2 below shows the results of SEM testing on *E. faecalis* bacteria with the addition of the extract at a concentration of 3.9062 µg/mL.

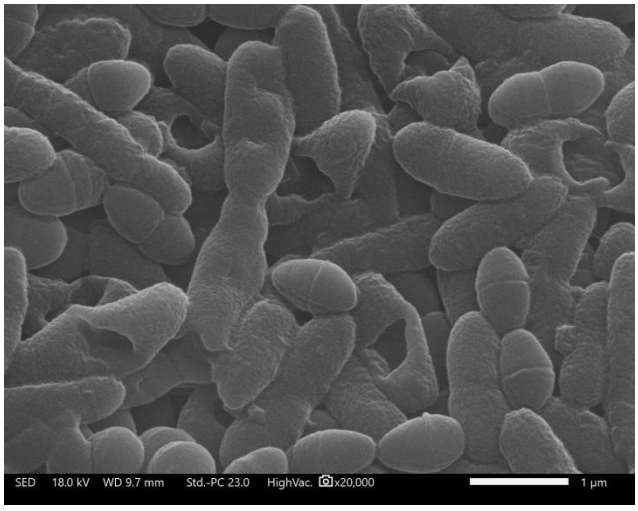

Figure 2. The SEM test results of *E. faecalis* bacteria with the addition of extract concentration 3.9062 µg/mL (Magnification 20,000×).

Discussion

This study demonstrated that the methanol extract of *K. alvaerezii* exhibited quite variable antibacterial activity. However, the extract had greater potential to inhibit the growth of *E. faecalis* bacteria than *P. aeruginosa* bacteria. MIC results indicated that the lowest extract concentration had the least inhibitory effect on the growth of infection-causing bacteria. For *P. aeruginosa* bacteria, the methanol extract showed no significant inhibition of bacterial growth. This was evidenced by the fact that the highest concentration of the extract still allowed *P. aeruginosa* to grow.

This difference in results may be due to differences in the structure of the cell wall layers in these bacteria. *P. aeruginosa* was generally difficult to inhibit with antibacterial compounds because it was a Gram-negative bacterium with a membrane layer consisting of three layers, which had been shown to exhibit resistance to antibacterial compounds. This is consistent with research

conducted by Mawengkang et al. (2022), which found that Gram-negative bacteria remain difficult to control because they have developed resistance to various commercial antibiotics. The difficulty in controlling antibiotic resistance in Gram-negative bacteria was due to the presence of a strong layer that is difficult for antibacterial compounds to penetrate. Gram-negative bacteria have three cell wall layers: an inner membrane, a thin layer of peptidoglycan, and an outer membrane composed of strong lipopolysaccharides that are difficult for antibacterial compounds to penetrate (Soleimani et al., 2021). *E. faecalis* was easily inhibited by the methanol extract of *K. alvarezii* because this bacterium was classified as Gram-positive, with a cell wall layer that consisted of only a single layer surrounded by peptidoglycan. According to Rizi (2022), Gram-positive bacteria are generally more sensitive to antibacterial compounds because they lack an outer membrane, which can act as a barrier to the entry of these compounds. This can also allow small antibacterial compound molecules to easily penetrate the bacterial cell membrane and inhibit bacterial growth (Abdullahi et al., 2022).

The inhibition of *E. faecalis* bacterial growth by *K. alvarezii* methanol extract was also due to the antibacterial activity mechanisms of several bioactive compounds it contained, such as alkaloids, flavonoids, saponins, and tannins. This is consistent with research conducted by Marhaeny et al. (2024), which found that bioactive compounds, such as alkaloids, flavonoids, saponins, and tannins, exhibit antibacterial activity through several inhibitory mechanisms. Flavonoids inhibit bacterial growth by disrupting DNA gyrase and ATPase activity in bacteria (Leswara et al., 2024). Tannins inhibit ribosome transcription and translation (Leswara et al., 2024). Saponins interact with lipids, triggering cell rupture, which facilitates antibacterial compounds' inhibition of bacterial cell metabolism (Mewengkang et al., 2022). Furthermore, alkaloids target DNA chains, causing damage and destruction of bacterial cells (Pratiwi et al., 2021). In this study, the extracts that were able to inhibit bacterial growth were indicated by no color change after the addition of TTC reagent. Meanwhile, the extracts that were not able to inhibit bacterial growth were indicated by a color change from pink to red after the addition of TTC reagent. The presence of a color change after being dropped with TTC reagent indicates the presence of bacterial metabolic activity detected by the TTC reagent, resulting in a redox reaction (de Souza et al., 2024). This reaction can occur when tetrazolium salts penetrate the bacterial cell membrane and then activate the oxidoreductase and dehydrogenase enzymes, resulting in the transfer of hydrogen and electrons through the dehydrogenase reaction, resulting in the formation of a red formazan color (Braissant et al., 2020).

The antibacterial effectiveness of *K. alvarezii* methanol extract not only inhibits bacterial growth but also triggers cell leakage by disrupting cell permeability, allowing several important intracellular components of bacterial cells, such as metal ions, ATP, nucleic acids, and proteins, to diffuse out (Iseppi et al., 2020). To determine the presence of cell leakage, nucleic acid, protein, and Ca^{2+} metal ion leakage tests were conducted to indicate the presence of cell leakage in the bacterial membrane due to exposure to antibacterial compounds through the addition of *K. alvarezii* methanol extract. The results of the study showed that there was cell leakage in both *E. faecalis* and *P. aeruginosa* bacteria, resulting in measurable leakage values for nucleic acids, proteins, and Ca^{2+} metal ions. This leakage is caused by the mechanism of bioactive compounds resulting from secondary metabolites in disrupting bacterial cell permeability. Based on the LC-HRMS results conducted by Asthisa et al. (2021), *K. alvarezii* contains phenolic compounds in the form of alkaloids, flavonoids, saponins, and tannins, which have potential antibacterial properties. The presence of phenolic compounds in the extract will cause interactions between the compounds and the phospholipid components found in the bacterial cell membrane, disrupting the permeability of the bacterial cell membrane and triggering bacterial cell damage. This is in line with research conducted by Rizky & Sogandi (2018), which found that phenolic compounds can cause cell leakage because they contain OH-, which is toxic to bacteria. Therefore, if there is an interaction between phenolic compounds and bacterial cells, it will facilitate organic acids to enter the cytoplasmic membrane and cause bacterial cell leakage.

In fact, alkaloid compounds can play a role in disrupting the components contained in peptidoglycan, causing the peptidoglycan to be incompletely arranged and causing bacterial cell damage (Lestari et al., 2024). Tannins target the polypeptide wall in the cell wall, disrupting the cell wall formation process in bacteria (Khairunnisa *et al*., 2025). Saponins increase the permeability of the bacterial cell membrane by reducing the surface tension of the cell wall. This ruptures the cell wall, allowing important intracellular components to diffuse out (Mewengkang et al., 2022). Furthermore, the flavonoids in *K. alvarezii* inhibit peptidoglycan formation in bacteria, making them susceptible to cell lysis due to the absence of peptidoglycan, which acts as a barrier to the entry of antibacterial compounds (Hamzah et al., 2023).

The results showed that in *E. faecalis*, the leakage values of all three were directly proportional to the concentration of the added extract. This is consistent with the research by Auza et al. (2025), which showed that increasing the concentration of the added extract led to increased leakage values in tests for nucleic acids, proteins, and Ca^{2+} metal ions. However, this was not the case for *P. aeruginosa* bacteria, as it actually decreased at the highest concentration. This may be due to the "eagle effect." The eagle effect is a paradoxical response in antibacterial testing whereby higher concentrations of antibacterial compounds actually decrease the rate of

bacterial cell death (Jarrad et al., 2018). Generally, bacteria experiencing this effect activate the SOS gene, which inhibits cell division and produces protective enzymes that inhibit antibiotic uptake within the bacterial cells (Lai et al., 2023).

Based on the permeability tests conducted, all extract concentrations applied in each treatment were found to induce permeability. This was indicated by the permeability values for each treatment, which were higher than those of the negative control. However, the optimal concentrations capable of inducing permeability of nucleic acids, proteins, and Ca2+ metal ions were 3.9062 µg/mL for *P. aeruginosa* and 500 µg/mL for E. faecalis. Furthermore, the extract at these concentrations showed promising potential, as the resulting leakage values were equivalent to or greater than those of the commercial antibiotic chloramphenicol. These findings indicate that *K. alvarezii* extract has potential as a candidate for a novel antibiotic. To demonstrate the inhibition of bacterial growth and cell leakage due to the addition of *K. alvarezii* methanol extract, the observations were performed by using an electron microscope to detect membrane damage and deformation of the bacterial cells. SEM observations showed that the bacterial cells experienced an increase in cell size and an increase or swelling. This enlargement or swelling is caused by the antibacterial compounds in the extract entering the cells, leading to their accumulation (Panjaitan, 2020).

SEM observations also revealed damage to bacterial cells in the form of bacterial cell lysis. This bacterial cell lysis is caused by the extract containing saponins, which can reduce surface tension on the cell wall and lead to bacterial cell rupture (Mewengkang et al., 2022). Other damage that occurred was the formation of holes in the surface of the bacterial cell wall. The presence of holes indicates that the antibacterial compounds contained in the extract are capable of causing damage to the cell wall and leading to hole formation (Panjaitan, 2020).

These research results align with the research conducted by Srikong et al. (2017), which showed that bioactive compounds, such as phenolic compounds and their derivatives, found in algae, cause morphological changes in bacterial cells. Furthermore, these bioactive compounds can also damage the membrane and cell wall of bacteria, either through gaps or lysis, which can lead to cell death.

Therefore, these findings align with previous similar research on the potential of red algae extract as a candidate for a natural antibacterial therapeutic agent that can suppress increasing resistance to synthetic antibiotics. Furthermore, the methanol extract of *K. alvarezii* used in this study had the potential to inhibit bacterial growth, particularly against Gram-positive bacteria, and could cause cell leakage in bacteria. In the future, further exploration of the *K. alvarezii's* potential as a natural antibacterial therapeutic agent should be conducted by purifying the bioactive compounds contained in the extract to better understand the compounds with the most potential for antibacterial activity. Furthermore, in vivo evaluation and safety testing are also necessary.

CONCLUSIONS

The methanol extract of *K. alvarezii* demonstrated its potential to inhibit bacterial growth, particularly against Gram-positive bacteria such as *E. faecalis* ATCC 29212, with an MIC of 1.9531 µg/mL. However, no significant inhibitory effect was observed against Gram-negative bacteria such as *P. aeruginosa.* Nucleic acid, protein, and Ca^{2+} metal ion leakage tests showed that *K. alvarezii* methanol extract induced leakage in both *E. faecalis* and *P. aeruginosa* bacteria, with leakage values for each treatment being higher than those of the negative control. Furthermore, *K. alvarezii* methanol extract induced morphological changes in bacteria, including damage and deformation of bacterial cell shape. This study demonstrated that *K. alvarezii* methanol extract had the potential as a natural antibacterial agent, but further in vitro and in vivo research is needed.

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