



## Bioactive Compounds and Antibacterial Activity of Red Macroalgae (*Kappaphycus alvarezii*) from Aru Islands, Indonesia

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### ABSTRACT

The red macroalga *Kappaphycus alvarezii* is a valuable marine resource widely cultivated in Indonesian waters. Beyond its economic importance as a source of carrageenan, this species contains a variety of bioactive secondary metabolites that can serve as natural antibacterial agents for fishery product preservation. This study aims to evaluate the antibacterial activity and phytochemical constituents of *K. alvarezii* collected from the waters of Aru Islands, Indonesia, using methanol and chloroform as extraction solvents. Extraction yields of methanol and chloroform extracts were 9.4% and 8.7%, respectively. Both extracts exhibited antibacterial activity against *Staphylococcus aureus*, with the methanol extract showing a higher inhibition zone ( $10.10 \pm 0.71$  mm) than the chloroform extract ( $8.25 \pm 0.28$  mm). However, neither extract was effective against *Escherichia coli*. Phytochemical analysis revealed the presence of alkaloids, flavonoids, and terpenoids in both extracts, while saponins were detected only in the methanol extract. These findings suggest that the methanol extract of *K. alvarezii* possesses higher antibacterial potency and could be developed as a natural preservative to enhance the shelf life and safety of fishery products. Further studies involving fractionation and GC–MS or LC–MS profiling are recommended to identify the active compounds responsible for the antibacterial effect.

### INTRODUCTION

Indonesia is a maritime country, with 70% of its territory consisting of ocean. One of the most potential biological resources from Indonesian marine waters is macroalgae, which comprises various types. Marine macroalgae, commonly known as seaweed, is a type of marine biodiversity with significant potential to provide value in the

pharmaceutical industry (**Biris-Dorhoi et al., 2020; Costa et al., 2023**). Unfortunately, the lack of research on the potential benefits of marine macroalgae for coastal communities has prevented marine macroalgae from being used to their fullest potential. Studies reveal that marine algae are widely used in a variety of industries, including medicines, cosmeceuticals, and nutraceuticals (**Carpena et al., 2023; Vijayaram et al., 2024**). However, the application of macroalgae in the pharmaceutical industry is still quite limited, despite the enormous potential of macroalgae in Indonesia, particularly in Maluku as an archipelagic nation, to be utilized as pharmaceutical raw materials.

The potential of macroalgae on Maluku Islands, Indonesia, has been investigated by a number of studies, but only in relation to Seram Island, namely in the seas of Kotania Bay, West Seram (**Arfah & Patty, 2014**), and East Seram (**Rugebregt et al., 2021**). However, the study focused more on the description of biodiversity and conservation efforts. Research focusing on the potential of macroalgae as antibacterial and antioxidant is quite limited, especially from the Aru Islands. In fact, macroalgae, particularly red algae show a lot of potential for development as antibacterials (**Teo et al., 2021**).

*Kappaphycus alvarezii* is a species of red algae (Rhodophyta) which was formerly known as *Eucheuma cottonii* (**Jalal, 2023**). The *K. alvarezii* extract is reported to contain primary and secondary metabolites. Primary metabolites, including vitamins, minerals, fiber, alginate, carrageenan, and agar, are frequently utilized as ingredients in cosmetic products for skin care. In addition to its primary content which has economic value, the content of secondary metabolites from *K. alvarezii* has the potential to be a producer of various bioactive metabolites with very broad activities as antibacterials (**Silva et al., 2020; Arias et al., 2023; Vijayaram et al., 2024**), antioxidants, antivirals, antifungals and cytostatics (**Rajapaksha et al., 2024**). **Pushparaj et al. (2014)** conducted research on a *K. alvarezii* extract from Tamil Nadu, India, using a variety of solvents, including ethanol, methanol, ethyl acetate, acetone, and chloroform. The results revealed that the methanol extract had the highest antibacterial activity against *B. subtilis*, measuring 13mm, while the lowest activity was observed against *E. coli*, measuring 3mm.

Our previous research revealed that n-hexane extract of *K. alvarezii* (*E. cottonii*) had weak antibacterial activity against *S. aureus* ( $2.1 \pm 0.14$ ), and *E. coli* ( $0.45 \pm 0.03$ ) (**Yunita et al., 2024**). Because n-hexane is a non-polar solvent that extracts molecules in a limited and selective manner, it is likely that some secondary metabolites were not fully extracted, resulting in weak antibacterial activity (**Malekzadeh et al., 2016; Das et al., 2023**). This serves as the basis for our current research to explore the antibacterial potential of *K. alvarezii* using different solvents than those previously employed.

Another previous study also reported that the ethanol and n-hexane extracts of *K. alvarezii* has several bioactive contents, such as phenol and flavonoids, which act as antibacterial and antioxidants by disrupting the function of the plasma membrane, eventually damaging the cell walls of pathogenic bacteria (**Das et al., 2023; Yunita et al., 2024**). Recent research also identified several bioactive proteins from *K. alvarezii*

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with antifungal potential (Souza *et al.*, 2025), further emphasizing the pharmacological relevance of this species. Exploration of secondary metabolites and antibacterial properties of *Kappaphycus alvarezii* using different extraction solvents, such as chloroform and methanol, needs to be carried out to profile the potential of secondary metabolites from the marine resources of the Aru Islands, Indonesia. Therefore, investigating the antibacterial and phytochemical properties of *K. alvarezii* is essential to support its valorization as a sustainable and locally available source of natural preservatives for fishery product processing in coastal communities. Accordingly, this study aims to examine and compare the antibacterial activity of the red macroalga *K. alvarezii* from the waters of the Aru Islands using two different extraction solvents.

### MATERIALS AND METHODS

#### Experimental design

The research used true experimental laboratory approach with a post-test only controlled group design. This research was conducted in July-September 2023. The waters around Jabulenga Village, Aru Islands, Maluku, with coordinates of 5°46'52.82"S - 134°21'8.69"E (Fig. 1), were sampled with purposive sampling method, while laboratory tests were carried out at the Basic Biology Laboratory of the Faculty of Teacher Training and Education, Biology, Pattimura University, Ambon. Determination of *K. alvarezii* was carried out at the Biology Department, Faculty of Mathematics and Science, Universitas Pattimura.

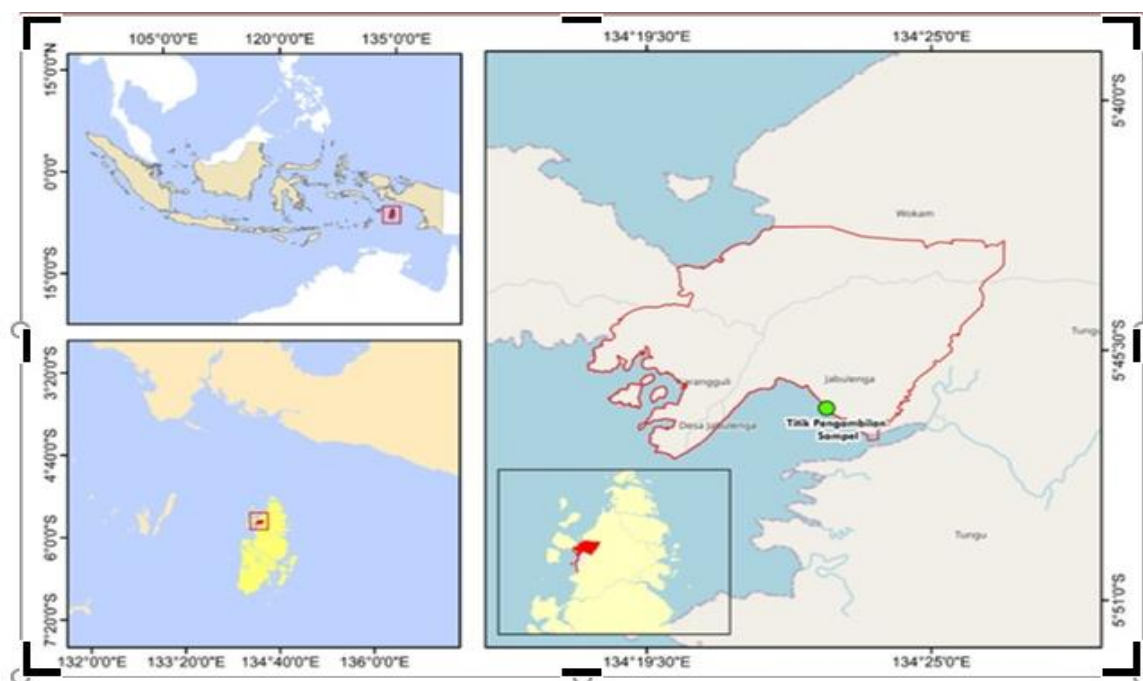


Fig. 1. Sampling site of *K. alvarezii*

### Sample preparation

Zip-loc bags were used to collect red algae (*K. alvarezii*) collected from the waters of Jabulenga Village, Aru Islands, Indonesia. The process of wet sorting involved thoroughly washing the red algae under running water after removing any dirt that might adhere to it. The red algae were air dried and kept out of the sun's direct rays to preserve their chemical composition for  $\pm 7$  days. The dried *K. alvarezii* was cut and processed into a coarse powder by blending it. This powder is called dry simplicial powder (Yunita et al., 2024).

### Preparation of extract

50g of simplicial powder of *K. alvarezii* was inserted in an erlenmeyer. Maceration was performed with a ratio of 1:3 using two different solvents, namely methanol and chloroform, and the extraction was done for 2x24 hours. Soaking was intended to draw out organic compounds contained in the simplicial powder. The solution was then filtered using filter paper and concentrated with an evaporator until a thick and concentrated extract was generated. These two extracts were then diluted and serial concentrations of 10%, 30%, 50%, 80%, 100% were performed. While yield percentage of *K. alvarezii* extract was calculated by the following formula (Dhanani et al., 2017):

$$\% \text{Yield extract} = \text{Concentrated extract (g)} \times 100 / \text{Simplicia weight (g)}$$

### Re-culturing pathogenic bacteria

*Staphylococcus aureus* and *Escherichia coli* were the pathogenic microorganisms used in the current study. The pathogenic bacterial cultures were obtained from the Maluku Province Health and Equipment Calibration Laboratory. Both pathogenic bacteria were re-cultured in Nutrient Agar media and incubated at room temperature for 24 hours.

### Antibacterial testing

The Kirby-Bauer disk diffusion method was used to investigate the antibacterial activity of methanol extract of *K. alvarezii*, chloroform extract of *K. alvarezii*, and positive and negative controls. Ampicillin was used for the positive control and sterile distilled water was used for the negative control. Using sterile cotton buds, pathogenic bacteria were swabbed in Mueller Hinton Agar (MHA; Merck) to conduct antibacterial testing. Placed atop the pathogenic bacterial culture, sterilized 6mm paper disks previously soaked in 200 $\mu$ l of each concentration of ethanol and chloroform extract of *K. alvarezii*. All treatments were repeated three times. For one to three days, the petri dish was kept at 37°C for 3 days in an incubator covered with plastic wrap. The antibacterial zone was assessed as the clear zone that developed around the paper disc with the following formula (Davis & Stout, 1971):

$$\frac{(VD - PD) + (HD - PD)}{2}$$

Where, VD: Vertical zone diameter, HD: Horizontal zone diameter, PD: Paper disk diameter.

The inhibitory zone results were categorized according to size into four categories: weak (<5 mm), moderate (6-10 mm), strong (11-20 mm), and extremely strong (>20 mm) (Davis & Stout 1971).

### Phytochemical testing

The presence of secondary metabolites, such as tannins, terpenoids, alkaloids, phenolics, flavonoids, and saponin, in the crude extract of *K. alvarezii* was determined by qualitative standard screening using phytochemical tests. The qualitative findings were expressed using the phytochemical's presence/positive reaction (+) and absence/negative reaction (-) (Yunita *et al.*, 2023).

### Data analysis

Data were descriptively evaluated and tabulated and provided as Figs. and Tables. The experimental data were provided as mean  $\pm$  SEM from three replicates. The difference in standard deviation between extract concentrations was computed. Data were analyzed using Kruskal-Wallis, and differences between samples were examined using the Dunn's test. A *P*-value of <0.05 is considered statistically significant. All statistical analyses were carried out using Microsoft Excel 2024.

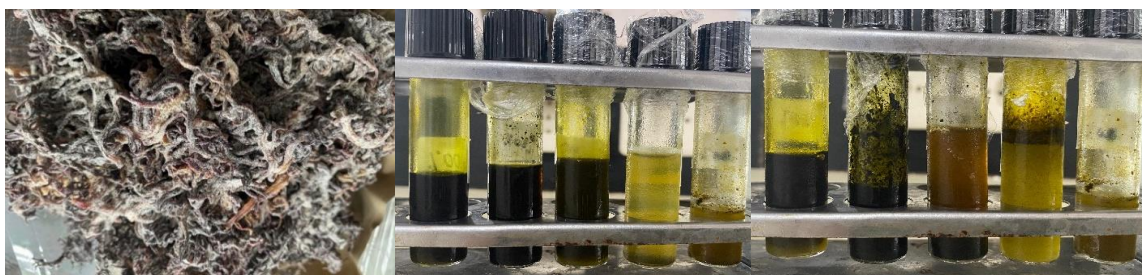
## RESULTS

Air dried-red macroalgae *K. alvarezii* were extracted by the maceration procedure using methanol and chloroform as the solvents. However, there was no noticeable variation in color. A comparison of the two extracts of *K. alvarezii* is presented in Fig. (2). Meanwhile, the extraction yields from the two solvents are shown in Table (1).

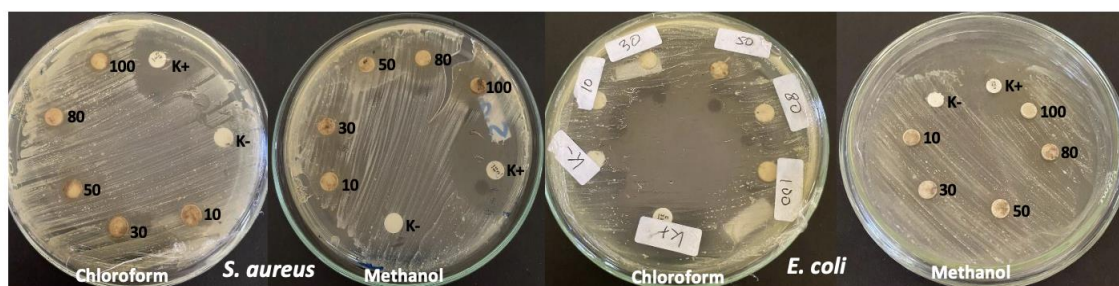
**Table 1.** Yield percentage of methanol and chloroform extract of *K. alvarezii*

| Simplicia weight | Solvent    | Concentrated extract (gr) | Yield percentage (%) |
|------------------|------------|---------------------------|----------------------|
| 750              | Methanol   | 70.5                      | 9.4                  |
| 750              | Chloroform | 65                        | 8.7                  |

The antibacterial test was conducted *in vitro* on Mueller Hinton Agar (MHA) medium using the Kirby-Bauer disk diffusion method. Ampicillin was used as the positive control, and sterile aqua dest was used as the negative control. The visualization of the antibacterial test is presented in Fig. (3).



**Fig. 2.** air-dried *K. alvarezii*; (left); Methanol extract of *K. alvarezii* (middle); Chloroform extract of *K. alvarezii* (right)



**Fig. 3.** Visualization of the antibacterial activity of chloroform and methanol extracts of *K. alvarezii* against *S. aureus* and *E. coli* on MHA media

In this current study, solvents used to extract macroalgae *K. alvarezii* were methanol and chloroform with 5 different concentrations ranging from 10, 30, 50, 80, to 100%. The results revealed that both the methanol and chloroform extracts of *K. alvarezii* exhibited inhibitory efficacy in the antibacterial test, particularly on the growth of *S. aureus*, with relatively similar sizes, as shown in Table (2). However, neither of the two extracts examined in this study was able to inhibit *E. coli* from growing.

Table (2) demonstrates that methanol extract of *K. alvarezii* was able to inhibit the growth of *S. aureus* at a concentration of 50% ( $2.00 \pm 0.00$  mm) with a weak category to 100% ( $10.10 \pm 0.71$  mm) with a moderate category, while chloroform extract of *K. alvarezii* was able to inhibit the growth of *S. aureus* at all concentrations with inhibition zones ranging from  $1.50 \pm 0.71$ , with a weak category, to  $8.25 \pm 0.28$ , with a moderate category. Meanwhile, both extracts of *K. alvarezii* couldn't be able to inhibit the growth of *E. coli* in all concentration tested.

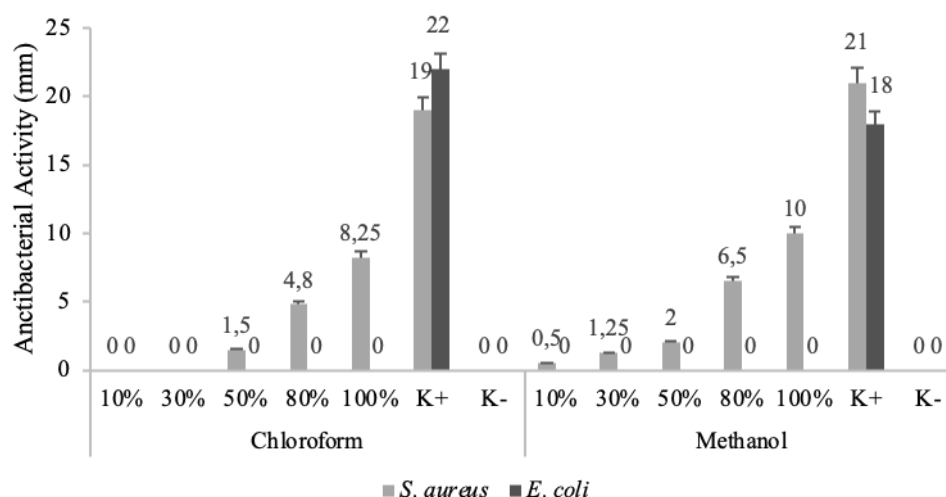
Nevertheless, the antibacterial activity shown by ampicillin as a positive control was still much greater than the two extracts tested in this study. Ampicillin as positive control showed strong inhibition against pathogenic *S. aureus* and *E. coli* with the inhibitory zone of  $19.00 \pm 0.00$  and  $21.00 \pm 0.71$  mm, respectively. A comparison of antibacterial activity between ampicillin and the two extracts can be seen in Fig. (4).

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**Table 2.** Antibacterial activity of chloroform and methanol extracts of *K. alvarezii* against of *S. aureus* and *E. coli*

| Concentration (%) | Solvent    | Antibacterial activity (Mean $\pm$ SD mm) |             |                  |             |
|-------------------|------------|-------------------------------------------|-------------|------------------|-------------|
|                   |            | <i>S. aureus</i>                          | Category    | <i>E. coli</i>   | Category    |
| 10                | Methanol   | 0.00 $\pm$ 0.00 <sup>a</sup>              | None        | 0.00 $\pm$ 0.00  | None        |
| 30                |            | 0.00 $\pm$ 0.00 <sup>a</sup>              | None        | 0.00 $\pm$ 0.00  | None        |
| 50                |            | 2.00 $\pm$ 0.00 <sup>c</sup>              | Weak        | 0.00 $\pm$ 0.00  | None        |
| 80                |            | 6.50 $\pm$ 0.35 <sup>c</sup>              | Weak        | 0.00 $\pm$ 0.00  | None        |
| 100               |            | 10.10 $\pm$ 0.71 <sup>d</sup>             | Moderate    | 0.00 $\pm$ 0.00  | None        |
| K+                |            | 21.00 $\pm$ 0.71                          | Strong      | 18.00 $\pm$ 0.18 | Very Strong |
| K-                | Chloroform | 0.00 $\pm$ 0.00                           | None        | 0.00 $\pm$ 0.00  | None        |
| 10                |            | 0.50 $\pm$ 0.00 <sup>a</sup>              | Weak        | 0.00 $\pm$ 0.00  | None        |
| 30                |            | 1.25 $\pm$ 0.18 <sup>b</sup>              | Weak        | 0.00 $\pm$ 0.00  | None        |
| 50                |            | 1.50 $\pm$ 0.70 <sup>b,c</sup>            | Weak        | 0.00 $\pm$ 0.00  | None        |
| 80                |            | 4.80 $\pm$ 0.28 <sup>d</sup>              | Weak        | 0.00 $\pm$ 0.00  | None        |
| 100               |            | 8.25 $\pm$ 0.28 <sup>e</sup>              | Moderate    | 0.00 $\pm$ 0.00  | None        |
| K+                |            | 19.00 $\pm$ 0.00                          | Very Strong | 22.00 $\pm$ 0.35 | Very Strong |
| K-                |            | 0.00 $\pm$ 0.00                           | None        | 0.00 $\pm$ 0.00  | None        |

Description: Different superscript letters (a, b, c, d, e) in the treatment group column indicate statistically significant differences ( $P < 0.05$ ).



**Fig. 4.** Comparison of antibacterial activity of methanol and chloroform extracts of *K. alvarezii* on the growth of *S. aureus* and *E. coli* compared to positive and negative controls

Phytochemical analysis was performed to determine the active metabolites that are present in both extracts and have the potential to function as antibacterials. The results of the phytochemical test were then compared between the two extracts to see the content of active compounds contained qualitatively. The results showed that both extracts contained alkaloids, flavonoids, and terpenoids. Nevertheless, the methanol extract also

showed a positive reaction to saponin which was not shown by the chloroform extract (Table 3).

**Table 3.** Phytochemical test results of methanol and chloroform extracts of *K. alvarezii*

| No | Parameter tested | Chloroform Extract |        | Methanol Extract |        |
|----|------------------|--------------------|--------|------------------|--------|
|    |                  | Indicator          | Result | Indicator        | Result |
| 1  | Alkaloids        | Yellow             | +      | Yellow           | +      |
| 2  | Flavonoids       | Greenish Yellow    | +      | Greenish Yellow  | +      |
| 3  | Terpenoids       | Red Ring           | +      | Red Ring         | +      |
| 4  | Steroids         | Yellow Ring        | -      | Yellow Ring      | -      |
| 5  | Phenolic         | Blue               | -      | Blue             | -      |
| 6  | Saponins         | Foam               | -      | Foam             | +      |
| 7  | Tannins          | Orange             | -      | Orange           | -      |

## DISCUSSION

The extraction process's treatment is more effective when a higher yield is obtained without affecting other qualities. According to the findings of this study, it is reasonable to assume that methanol extract of *K. alvarezii* contains more bioactive components than the chloroform extract. This acknowledges with the findings revealed by **Dhanani *et al.* (2017)**, which showed that a high yield value suggested a high concentration of bioactive components. A number of variables might affect the yield results, such as the type of solvent used, the temperature and time of the extraction process, the maceration process, the extraction method used (**Zlotek *et al.*, 2016**).

Extraction by maceration is one of the main methods used to extract natural materials using universal solvents, such as ethanol and methanol, and chloroform. The type of solvent used, the solvent to simplicial ratio, the extraction temperature, and the extraction duration are some of the critical variables affecting the antibacterial activity of *K. alvarezii* extract (**Rajaram *et al.*, 2021**; **Das *et al.*, 2023**).

Our previous research showed that *K. alvarezii* extract using n-hexane as a solvent showed much smaller results, where n-hexane extract could only inhibit the growth of *S. aureus* ranging from  $0.27 \pm 0.03$ - $2.1 \pm 0.14$  at a concentration of 50- 100%, and  $0.17 \pm 0.05$  -  $0.45 \pm 0.03$  against *E. coli* at a concentration of 80- 100% (**Yunita *et al.*, 2024**). Variations in its size can be explained by changes in the pace at which the antibacterial material diffuses into the agar medium and the concentration of bioactive antibacterial compounds within the inhibition zone. Other factors that are reported to influence the establishment of the inhibitory zone include the sensitivity of antibacterial growth, the interaction between the active component and the medium, and the temperature of the incubation period (**Silva *et al.*, 2020**).

Consistent with the findings of this investigation, **Hamid *et al.* (2024)** also reported that the methanol extract of *K. alvarezii* from Sabah waters, Malaysia had slightly higher

antibacterial activity ( $4.63 \pm 0.03$ ) than the chloroform extract ( $3.10 \pm 0.01$ ). In another study, the methanol extract of *K. alvarezii* using the disc diffusion method also showed a fairly large inhibition zone of  $10.6 \pm 0.2$  mm against *S. aureus* and  $8.2 \pm 0.1$  mm against *E. coli* (Rajapaksha *et al.*, 2024). Nevertheless, the antibacterial activity shown by ampicillin as a positive control was still much greater than the two extracts tested in this study. A comparison of antibacterial activity between ampicillin and the two extracts can be seen in Fig. (3).

Ampicillin as positive control showed strong inhibition against pathogenic *S. aureus* and *E. coli* with the inhibitory zone of  $19.00 \pm 0.00$  and  $21.00 \pm 0.71$  mm, respectively. Ampicillin works in two stages. In the initial step, the drug attaches itself to membrane-bound penicillin-binding proteins (PBPs), which are main receptors. These proteins are essential for the morphogenetic development of the cell wall peptidoglycan in relation to the cell cycle. As such, PBPs' function is promptly stopped upon deactivation by bound antimicrobials. The physiological effects of this receptor-ligand interaction are the focus of the second stage. The final phases of peptidoglycan production in the cell wall are mediated by PBPs. Given the importance of peptidoglycan in preserving the integrity of the cell wall, any disruption can result in lysis and ultimately lead to the death of the cell, particularly in the hypotonic environment in which it inhabits (Guz *et al.*, 2023; Jiang *et al.*, 2023).

The antibacterial activity of *K. alvarezii* extract is related to the presence of bioactive compounds, such as alkaloid, saponin, flavonoid, terpenoid, anthraquinone glycosides, steroids, and essential oils, which are known to disrupt bacterial cell membranes, disrupt enzymatic processes, and inhibit nucleic acid synthesis (Cotas *et al.*, 2020; Martić *et al.*, 2023). Phytochemical analysis was performed to determine the active metabolites that are present in both extracts and have the potential to function as antibacterials. The results of the phytochemical test were then compared between the two extracts to see the content of active compounds contained qualitatively. The results showed that both extracts contained alkaloids, flavonoids, and terpenoids.

Methanol is reportedly a superior solvent for the reliable extraction of antimicrobial compounds from medicinal plants, according to reports in the literature. There are two possible explanations for this. Firstly, the presence of methanol may enhance the physiologically active components' nature and potential, such as terpenoids, alkaloids, steroids, flavonoids, and essential oils. Second, a higher number or quantity of active ingredients with antibacterial activity might have been created due to methanol's increased extraction capacity (Pushparaj *et al.*, 2014; Rajaram *et al.*, 2021). This is also in accordance with the results obtained by Pushparaj *et al.* (2014) who found that methanol extract had the highest activity in inhibiting the growth of human pathogens compared to ethanol, chloroform, ethyl acetate, and acetone extracts. Meanwhile, the phytochemical content of *K. alvarezii* found protein ranging from  $9.5 \pm 0.96\%$  -  $16.26 \pm 2.21\%$ , lipid  $0.57 \pm 0.53$ – $1.38 \pm 0.44$ , carbohydrate  $17.10 \pm 0.28$ – $26.62 \pm 1.37\%$ . Its

protein content can even reach 47% which includes polysaccharides with antioxidant and antimicrobial properties (**Rajaram et al., 2021**).

The antibacterial activity observed in this study highlights the potential of *Kappaphycus alvarezii* as a valuable marine resource beyond its conventional use in carrageenan production. The bioactive compounds identified—particularly alkaloids, flavonoids, terpenoids, and saponins—are known to possess broad antimicrobial functions that can inhibit the growth of spoilage and pathogenic bacteria. Such properties are of particular relevance to the fishery processing sector, where the use of natural antimicrobial agents is increasingly emphasized to ensure product safety and extend shelf life (**Silva et al., 2020; Fernandes et al., 2023**).

Recent studies have demonstrated that extracts from marine macroalgae can effectively suppress foodborne bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, and *Pseudomonas* spp., which are commonly associated with the deterioration of seafood products (**Cotas et al., 2020; Martić et al., 2023**). Macroalgae-derived extracts, rich in phenolic and flavonoid compounds, have been recognized as promising alternatives to synthetic preservatives, aligning with global trends toward clean-label and eco-friendly food production (**Biris-Dorhoi et al., 2020; Arias et al., 2023**).

The valorization of *K. alvarezii* as a natural preservative source could thus provide added economic value for coastal communities, particularly in regions such as the Aru Islands, where seaweed cultivation is abundant. Its antibacterial and antioxidant potential offers opportunities for application in developing sustainable fishery products with extended shelf life and improved microbial safety (**Fernandes et al., 2023; Vijayaram et al., 2024**). Further applied research is recommended to evaluate its effectiveness in actual seafood matrices and under different storage conditions, supporting the integration of *K. alvarezii* bioactives into value-added fishery processing.

Nonetheless, this study proposed the promising potential of methanol and chloroform extracts of *K. alvarezii* as antibacterials against *S. aureus* as a Gram-positive bacterium. However, this study has limitations due to the inability to inhibit *E. coli* as a representative of Gram-negative pathogenic bacterium. The reason for the lack of antibacterial activity against *E. coli* could be because the solvent applied to extract the active compounds from *K. alvarezii* did not perform to its full potential. Qualitative phytochemical tests also limit information on the levels of active compounds. Further investigation on GCMS or LCMS analysis needs to be carried out to obtain complete quantitative information on active compounds so that the right solvent for extracting *K. alvarezii* can also be evaluated.

## CONCLUSION

The study concludes that both methanol and chloroform extracts of *K. alvarezii* are able to inhibit the growth of *S. aureus*, however, methanol extract can inhibit the growth

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of *S. aureus* in a wider range of concentrations compared to methanol extracts. Nevertheless, both extracts are unable to inhibit the growth of *E. coli* at all concentrations tested. As both extracts contained alkaloids, flavonoids, and terpenoids, the specific compounds responsible for antibacterial activity could not be determined. Future studies should perform quantitative identification of active compounds.

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