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Research Article

Analysis of Antimicrobial Activity of Carrageenan Extracted from *Kappaphycus Alvarezii*

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Abstract: The abuse of synthetic antibiotics is one of the leading causes of the emergence of antibiotic-resistant microorganisms that have become the primary health concern. To overcome this resistance and counteract all the side effects of synthetic drugs, attention was diverted towards natural resources for developing antimicrobial substances. This study tests the effect of carrageenan isolated from *Kappaphycus alvarezii* on pathogenic bacteria. Carrageenan is a natural bioactive compound extracted from red algae. Carrageenan was extracted from *K. alvarezii* using hot water extraction. The antimicrobial property of carrageenan was tested using the Minimum Inhibitory Concentration and the bacterial cell growth rate. Moreover, a commercial antibacterial hand wash and anti-acne toner were used as comparative agents. Firstly, a series of concentrations were used, from 25 µg/mL to 10 mg/mL. The results showed no antimicrobial activity of carrageenan extracted from *K. alvarezii* using ethanol toward the strains used in this study, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. However, the carrageenan extracted exhibited a growth-boosting effect over the microorganisms used. Thus, further studies can be carried out using different carrageenan extract methods for *K. alvarezii* to ensure that anti-microbial compounds from *K. alvarezii* can be extracted during the carrageenan extraction.

***Keywords*:** antimicrobial substances, resistant microorganisms, carrageenan, *Kappaphycus alvarezii*, cell growth rate

1. Introduction

All around the world, infectious diseases are the number one cause of mortality and morbidity. There is a remarkably continuous increase in the number of multi-drug resistant microorganisms and a rise of strains with lower susceptibility to antibiotics [1]. This increase has been highly linked to the discriminatory use of broad-spectrum antibiotics, organ transplantation, intravenous catheters and immunosuppressive agents [2-4]. Therefore, scientists are urged to discover new antimicrobial substances from various sources, mainly among natural resources. Synthetic drugs are over-priced and inadequate, often stumble with adulteration and cause undesirable side effects [5-7].

Recently, much attention has been paid to natural resources such as plants and algae for discovering biologically active compounds which exhibit potential antimicrobial effects [8, 9]. Some bioactive compounds from plants such

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as cloves, garlic and cinnamon have shown antimicrobial effects on food-borne microbes [10, 11]. These bioactive compounds are produced in natural products as secondary metabolites [12].

Secondary metabolites are produced within the natural sources of plants and algae, besides the primary compounds produced to ensure their growth and development. Unlike primary metabolites, secondary metabolites do not contribute to the growth and development of plants or algae. They are synthesised randomly and found to have an essential role [12-14]. Some protect the plants and algae from free radicals produced during photosynthesis, some serve as cellular signalling molecules, and others exploit defence mechanisms against herbivores and insects [15]. Because of the increasing demand for screening for new antimicrobial drugs from natural sources, significant interest has been directed toward marine organisms. Seaweeds are a type of marine organism belonging to algae, a huge and diverse group of eukaryotes. These organisms are simple as they do not have distinct roots, leaves or stems. All parts of these organisms have the same observable structure [15].

Seaweeds are a rich source of biologically active and structurally diverse secondary metabolites. Most of the secondary metabolites produced by seaweeds contain bacteriostatic properties that defend against pathogens [16-18]. In recent years, it has been found that phytochemicals extracted from marine algae can be used dependably in the food and pharmaceutical industries as gelling agents. Proteins and polysaccharides in red algae could inhibit the activity of bacteria [19], and high phenol content in red algae protects the algae from severe conditions and other diseases [20-22].

This research used the red algae *Kappaphycus alvarezii* to test its antimicrobial effect on pathogenic bacteria. Seaweeds have been used as fertilisers, food and medicine for centuries. Over the recent years, the demanding use of marine algae has been exceptionally increasing due to the presence of bioactive compounds such as alginate, agar and carrageenan that are nominated to replace the use of synthetic substances that may be unhealthy and pose harm to their users [23]. The main structure of this seaweed polysaccharide is chemically characterised by the repeated units of D-galactose and 3,6-anhydro-L-lgalactose, supplemented with ester sulphate content [20]. In general, seaweeds are categorized into three classes, Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae). *K. alvarezii* belongs to the Rhodophyceae class. Much research has been done on various species of red algae to test their effectiveness in inhibiting the growth of specific disease-causing bacteria, both gram-positive and gram-negative. Some of the findings were extraordinary as they have shown a remarkable effect in inhibiting the bacterial growth for both gram-positive and negative strains [24-27].

As reported by García-Bueno et al. [28], antibacterial is seasoning dependent, where the red seaweed harvesting during winter showed a better antibacterial activity of 16% compared to winter is 5-7%. *Himanthalia elongate* extracted with water: ethyl showed 100% inhibition toward *Salmonella bony* with 10 mg/mL seaweed extract [29]. Studies also showed that the inhibition rate of microbe is affected by the type of seaweed, the solvent used in the extraction, and the extract concentration. The concentration can range from 50 µg/mL to 100 µg/mL [30].

This study aimed to extract carrageenan from *K. alvarezii* and test its antibacterial effect against common pathogens that cause skin infections. Furthermore, it will compare its impact with various commercial solutions that are known to inhibit the growth of selected bacteria. Minimum Inhibitory Concentration (MIC) and cell growth rate methods were used to test for carrageenan's inhibitory effect and compare the solutions.

2. Materials and method

2.1 *Sample extraction*

K. alvarezii was washed to remove all sand and salt residues. Then, the wet weight of fragmented *K. alvarezii* was heated in distilled water at a 1:10 (w/v) and incubated at 80 °C for 2 hours [31]. The mixture was filtered, and the filtrate was collected and left aside to cool for 10 minutes. Cold absolute ethanol was added to the filtrate in a 1:1 ratio (v/v). Precipitation of carrageenan occurred in the form of fibrous coagulum while stirring. Filtration was repeated, and the filtrate was collected and dried at 40 °C for several days until achieving constant weight.

The percentage of extract yield (w/w) was calculated by dividing the amount of collected dry extract (g) by the amount of sample (g) used during the extraction and then multiplied by 100%.

2.2 *Antimicrobial assays*

Four strains of bacteria were used to study the antimicrobial assay of *K. alvarezii*: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The bacterial culture was prepared using the spectrophotometer reading of the turbidity of bacterial cell suspension. All the bacteria used in this study qualified for the suspension culture [32]. A single colony from the nutrient agar culture of each bacterium was inoculated into four tubes, each containing 10 mL nutrient broths. The tubes were incubated in a shaking incubator at 37 °C till the absorbance reading of the culture read 0.3-0.4 at 600 nm.

2.3 *Minimal Inhibitory Concentration (MIC) determination*

MIC was used in this study to determine the lowest concentration of seaweed carrageenan that can prevent the visible growth of a selected microbe. Each plate consists of a set of controls: growth medium only (sterility control), bacteria and growth medium (growth control), distilled water (negative control), three positive controls, which include antibiotics (ampicillin and chloramphenicol), antibacterial hand wash (commercial product), and anti-acne solution (commercial product).

Carrageenan extracted from *K. alvarezii* was dissolved in distilled water to prepare a stock solution of 20 mg/mL. The final carrageenan concentrations used in this study were 25 µg/mL, 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, 300 µg/mL, 600 µg/mL, 800 µg/mL, 1 mg/mL, 1.2 mg/mL, 1.4 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL and 10 mg/mL. *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* were used as the microbes samples. At the end of the preparation, 10% of Resazurin was added to the corresponding wells. The plates were wrapped with aluminium foil to prevent bacteria from dehydrating and were incubated at 37 °C for 24 hours.

2.4 *Bacterial cell growth rate*

All four strains of bacteria were included in this method, *E. coli*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa*. Five agents were used in testing the growth rate of the bacteria, carrageenan extracted from *K. alvarezii*, antibacterial hand wash, anti-acne solution, positive controls ampicillin (100 µg/mL) or chloramphenicol (25 µg/mL) and negative control (distilled water).

As illustrated in Figure 1, each tube contained 5 mL bacterial culture, and the tubes were divided into triplicates. Each triplicate set included a specific agent; the first triplicate held carrageenan solution reaching a final concentration of 10 mg/mL. The second set added 100 µL of the anti-acne solution, while the third set added 100 µL of the antibacterial hand wash solution. The fourth set contained antibiotics as a positive control.

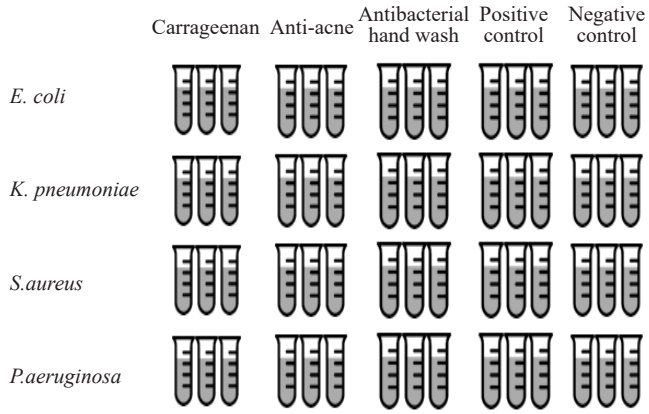

Figure 1. Representation of the 60 tubes used in measuring the bacterial growth rate

The absorbance readings at 600 nm were recorded for all tubes after adding the agents to obtain a zero-hour reading. All the tubes were placed in a shaking incubator for one hour at 120 rpm and 37 °C. After the first hour, all the tubes were removed from the incubator to measure the absorbance reading. Thus the first-hour records were obtained. Then, the tubes were placed in the incubator for another hour; afterwards, the absorbance measurements for all tubes were retaken.

The previous steps were repeated for the third-hour incubation. At the end of this experiment, four records were obtained, control (zero time), after the first hour, second hour and third hour. The plain nutrient broth was used as the blank for this measurement. And a different cuvette was used to measure each triplicate set.

3. Results and discussion

Red seaweed *K. alvarezii* is selected in this project as *K. alvarezii* is a common culturing seaweed in Malaysia. It is famous for its high application in different pharmaceutical, food production and medical [14, 33, 34]. This study extracted carrageenan from the seaweed sample and yielded 13.14 ± 1%. Rupert et al. [14] reviewed that various factors affected the production yield of carrageenan from seaweed [35, 36], such as *K. alvarezii*, from environmental factors to extraction methods. In this experiment, the modified ultrasound-assisted extraction method [37, 38] was applied to harvest the highest percentage of carrageenan [39].

3.1 *Minimum inhibitory concentration*

This study chose concentrations based on various studies; some showed antibacterial effects, and others did not. Sebaaly et al. [40] demonstrated using 325 µg/mL of carrageenan from *Corallina* that showed antibacterial activity against *S. epidermis*. Another study represented 20 mg/mL of carrageenan in preparing carrageenan films [41]. Therefore, a range of low and high concentrations was used in this study to determine the antibacterial activity of carrageenan from *K. alvarezii*.

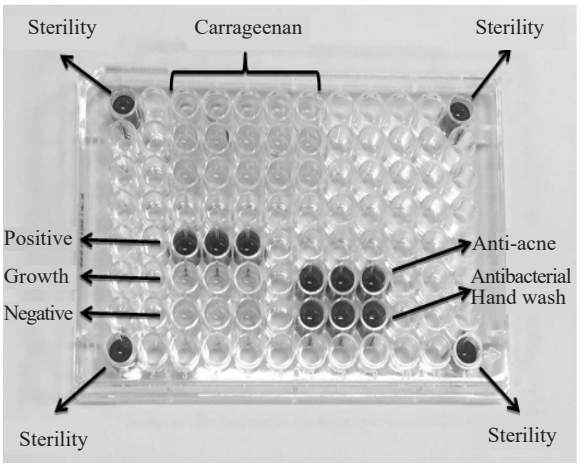

Figure 2. Minimum Inhibitory Concentration (MIC) test for carrageenan. Colour of resazurin changes, which indicates the growth of bacteria

Figure 2 represents sterility control in the corners of the microtiter plate, and the positive control showed no bacteria growth, thus indicating no contaminations reached the plate from outside. As for the negative control, the bacteria grew, and the resazurin colour changed from blue to pink, as expected. Usually, the MIC method is used to evaluate the effectiveness of specific concentrations of an extract and test which lowest concentrations elicit an antibacterial effect. This principle is fundamental in antibiotic development studies because it is difficult for the lowest

concentration that will inhibit bacterial growth, thus preventing the tested bacteria from developing resistance against the antibacterial agent. Even for potential antibiotic development, this is essential. The main advantage of using MIC is the reproducibility, convenience, and economy of reagents and space due to the miniaturisation of this procedure [42]. Moreover, this method can efficiently generate computerised results if an automated plate reader is available. Nevertheless, the main disadvantage of this method is some inflexibility of the drug selection [42].

Based on the findings, all concentrations of carrageenan extracted from *K. alvarezii* show no inhibition toward the bacteria selected in this experiment using the MIC method. These findings were supported by Chua et al. [43], where a crude extract of *K. alvarezii* found an insignificant effect against gram-positive and gram-negative bacteria. On the other hand, Wang et al. [44] reported that Kappa-carrageenan extracted using the enzymatic degradation method shows significant inhibition activities toward selected microbes such as *E. coli* and *S. aureus* in 5 mg/mL. This further proves that the extracted process can be one of the factors affecting the findings in the antimicrobial assay.

3.2 *Bacterial cells growth rate*

This method is used as a confirmative test together with the MIC method. The bacterial growth rate was measured using the absorbance reading of the liquid bacterial culture when mixed and incubated with all five agents, carrageenan, anti-acne, antibacterial hand wash, positive control (antibiotics) and negative control (nutrient broth). According to the results in Figures 3, 4, 5 and 6, carrageenan showed no apparent antibacterial activity toward bacterial growth. The only carrageenan concentration used was 10 mg/mL; as explained previously, this concentration is the highest that could be prepared in liquid form, mixed and used. For this tested carrageenan concentration, no inhibition of bacterial growth was observed. Therefore, the characteristic of *K. alvarezii* in terms of antimicrobial actions still needed to be detected. This emphasises that the tested bacteria have low sensitivity or total lack of sensitivity against *K. alvarezii*.

Few explanations may account for the zero-inhibitory effect of the water extract of *K. alvarezii*. This might be due to the extraction solvent used in this study. It is essential to extract the bioactive compounds with the most suitable solvent as the extracted compound is highly attributed to the type of solvent. Polar solvents will extract polar compounds, while nonpolar solvents isolate nonpolar compounds. Thus the extraction yield and the activity of the extract are highly dependent on the solvent polarity [45]. Franco et al. [46] have documented that mixtures of water and ethanol or methanol consistently achieve a higher and more stable yield. However, ethanol and water extraction are preferable due to their low toxicity and high extraction yield [46]. Another possible reason for the lack of sensitivity is that the outer membrane of bacteria prevents the penetration of the active compounds of *K. alvarezii*. Wherefore, *K. alvarezii* cannot interact with the bacterial cell wall, thus limiting or preventing any possible growth inhibition.

The antimicrobial activity of seaweeds may vary based on the time of harvest, storage conditions and temperatures and extraction methods [47, 48]. Moreover, environmental factors, seasonal changes and stages of development may affect the production and distribution of bioactive constituents in the seaweed. Other factors affecting active phytochemicals capture include the solvents used, the extraction period and the extraction conditions [49].

3.2.1 *Staphylococcus aureus*

Figure 3 represents data on *S. aureus* growth rate using five agents measured over three hours at hourly intervals. The growth rate of this bacterium represents an obvious pattern. Carrageenan extracted from *K. alvarezii* does not show any inhibition pattern. On the contrary, carrageenan caused an increase in bacterial growth over the three hours. However, comparing the growth rate of *S. aureus* with carrageenan against the other tested bacteria shows a higher growth rate. The bacterial cells grew gradually and continuously over three hours. The absorbance readings difference between the first and last record increased by 0.5127 ± 0.1, the highest among all bacteria.

As for the anti-acne and the antibacterial hand wash solutions, both products produce almost similar growth patterns. Both solutions decreased the growth of *S. aureus*. However, their effect is not very remarkable, as the bacterial growth was slightly reduced with a similar effect as a bacteriostatic effect. As the function and the usage of both products are different, thus, the findings for both products in this product display no significant differences. As reported by Mihalache et al. [50], the effect of hand washing in removing the bacteria must couple with rinsing steps. Thus, using hand wash directly in an antimicrobial assay has its limitations. As for the positive control using Chloramphenicol, the growth rate shows a drop in the turbidity measure; Thus, this antibiotic had a bactericidal effect on *S. aureus*. Negative

control shows a predicted growth pattern, where the turbidity measures increased from 0.359 + 0.1 to 1.3287 + 0.1, much higher than carrageenan actions. Both positive and negative controls elicit very predictable actions towards the tested bacterium.

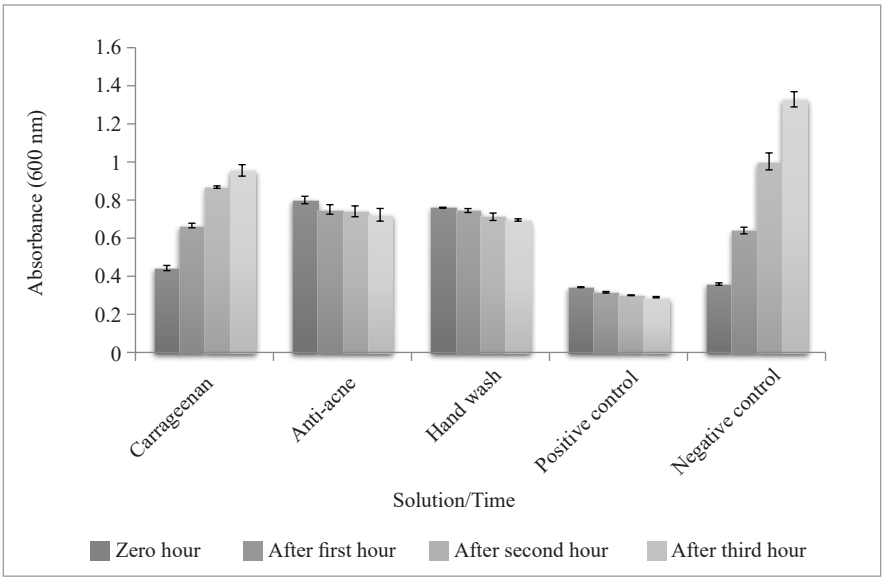

Figure 3. *S. aureus* growth rate using five agents over three hours. Carrageenan with a concentration of 10 mg/mL was tested on *S. aureus* to check its antimicrobial properties by measuring the absorbance reading of the liquid bacterial culture. Anti-acne, antibacterial hand-wash and positive and negative controls were tested on the same bacterium

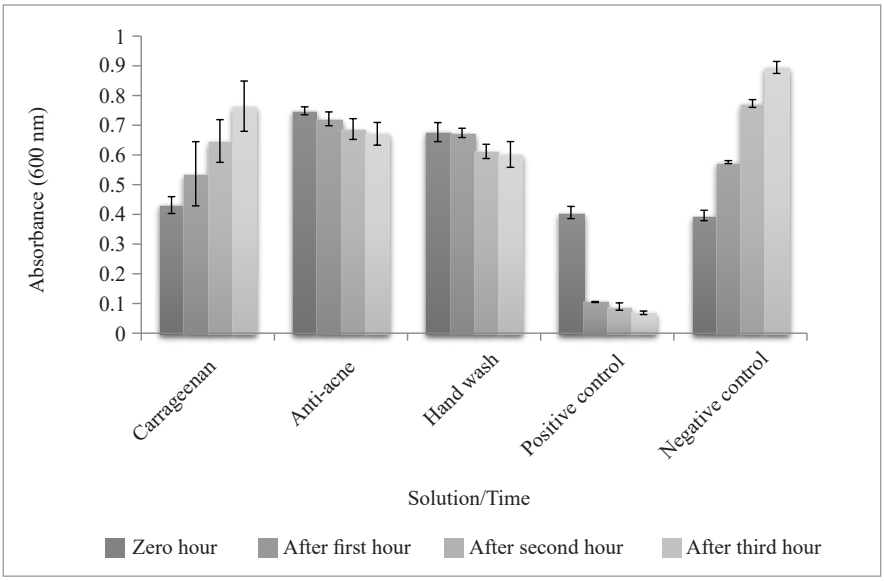

Figure 4. *E. coli* growth rate using five agents over a period of three hours. Carrageenan with a concentration of 10 mg/mL was tested on *E. coli* to check its antimicrobial properties by measuring the absorbance reading of the liquid bacterial culture. Anti-acne, antibacterial hand wash, and positive and negative controls were tested on the same bacterium

3.2.2 *Escherichia coli*

Figure 4 represents a bar chart of the growth rate of *E. coli* using five different solutions, carrageenan, anti-acne,

antibacterial hand wash, and positive and negative controls respective to the represented data. Absorbance was measured over a period of three hours at hourly intervals. Positive control was via using ampicillin of concentration 100 µg/mL. The graph shows the *E. coli* growth rate using the carrageenan sample from *K. alvarezii* shows a remarkable rising trend. Using carrageenan, the zero-hour absorbance reading is 0.4313 ± 0.1; after three hours, the final reading is 0.7643 ± 0.1. Thus, the difference between these two readings represents the growth rate over three hours, which is 0.333 ± 0.1 and considerably high. This means carrageenan does not have any antibacterial effect on *E. coli*.

The anti-acne solution and the antibacterial hand wash proved their antimicrobial properties with *E. coli*, as the graph shows a decrease in absorbance readings over three hours. However, this decrease is slight and gradual; compared to carrageenan, it is clear how the effects elicited were reversed. Moreover, when the result of carrageenan is compared to the positive control, as expected, the positive control (ampicillin) inhibits the growth of *E. coli* with a bactericidal effect.

And the graph shows a dramatic decline in absorbance reading from 0.4063 ± 0.1 to 0.0687 ± 0.1. As for the negative control using nutrient broth, the bacteria grew dramatically gradually, as shown in the graph below. The carrageenan extracted from *K. alvarezii* shows approximately a similar effect on the growth pattern of *E. coli* as the negative control.

3.2.3 *Klebsiella pneumoniae*

Figure 5 illustrates the bar chart of the growth rate of *K. pneumoniae* using the previously mentioned solutions. Absorbance readings were recorded over a period of three hours at hourly intervals. Positive control was via using chloramphenicol of concentration 25 µg/mL. From the graph, the *K. pneumoniae* growth rate using the carrageenan sample from *K. alvarezii* shows a moderate increase.

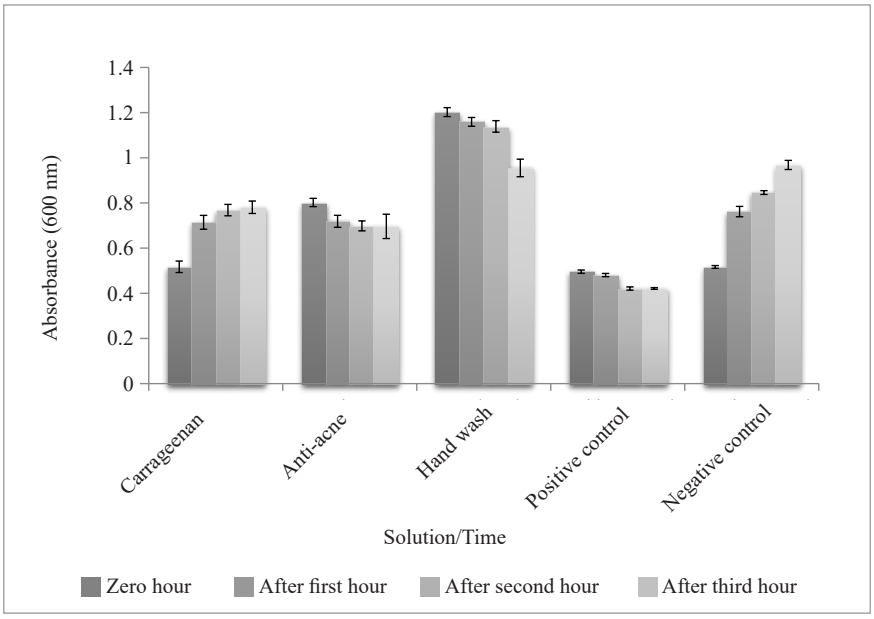

Figure 5. *K. pneumoniae* growth rate using five agents over three hours. Carrageenan with a concentration of 10 mg/mL was tested on *K. pneumoniae* to check its antimicrobial properties by measuring the absorbance reading of the liquid bacterial culture. Anti-acne, antibacterial hand wash and positive and negative controls were tested on the same bacterium

The first absorbance reading (zero hours) was 0.5173 ± 0.1, and after three hours, the final reading was 0.781 ± 0.1; thus, the difference between these two readings is 0.2637 ± 0.1, representing the growth rate over three hours, and it is mildly high. This means carrageenan does not have any antibacterial effect on *K. pneumoniae*. Carrageenan increased the growth of *K. pneumoniae*. However, the increase was not dramatic compared to *E. coli* and *S. aureus*. As Pérez et al.

[51] summarise, seaweed produces metabolites with potent antimicrobial activity. The compounds extracted from the seaweed depend on the type of seaweed, the number of compounds isolated, the solvent used in the extraction and the variety of microbes involved in the experiments. In addition, the methods used in the assessment are factors that should be investigated in determining the antimicrobial effect of seaweed.

Moreover, the effect of the anti-acne solution on the growth of *K. pneumoniae* was notable in decreasing the growth of the bacterial cells. Nevertheless, the antibacterial hand wash showed a more dramatic result in reducing the growth of *K. pneumoniae* over three hours. Regarding the positive control, Chloramphenicol caused a slight effect in killing the bacteria. The results show a slight decrease; thus, it could be bactericidal but requires higher concentrations to have a higher impact. The negative control results illustrate the high growth of *K. pneumoniae*. When comparing the effect of carrageenan and the negative control, it is notable that carrageenan activity is less efficient than the negative control. However, both have a considerable increase in bacterial cells.

3.2.4 *Pseudomonas aeruginosa*

Figure 6 represents a bar chart of the growth rate of *P. aeruginosa* using carrageenan solution, anti-acne, antibacterial hand wash, and positive and negative controls. The data were collected at hourly intervals for a total of three hours. Positive control was done using a chloramphenicol concentration of 25 µg/mL. From the chart below, *P. aeruginosa* growth using a carrageenan sample from *K. alvarezii* shows a mild increase. The first absorbance reading at zero hours was 0.294 ± 0.1, and after three hours, the final reading was 0.513 ± 0.1; thus, the difference between these two readings is 0.219 ± 0.1, representing the growth rate over three hours, and it is considered high.

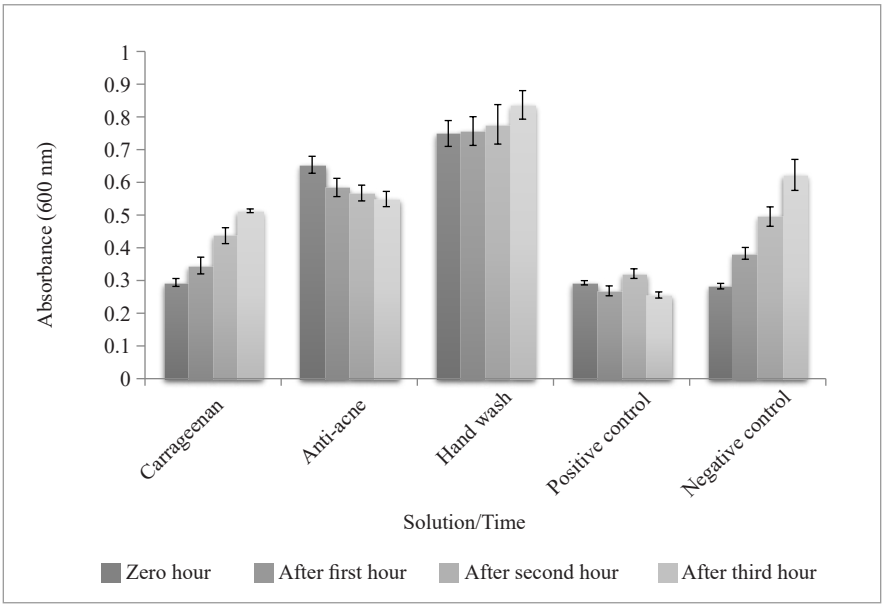

Figure 6. *P. aeruginosa* growth rate using five agents over three hours. Carrageenan with a concentration of 10 mg/mL was tested on *P. aeruginosa* to check its antimicrobial properties by measuring the absorbance reading of the liquid bacterial culture. Anti-acne, antibacterial hand wash and positive and negative controls were tested on the same bacterium

Carrageenan extracted in this experiment has no antibacterial effect on *P. aeruginosa*. Carrageenan increased the growth of *P. aeruginosa*. However, the increase was not remarkable compared to *E. coli* and *S. aureus*.

Furthermore, *P. aeruginosa* showed a moderate decrease in growth using the anti-acne solution. The antimicrobial effect of the anti-acne solution depends on its formulation and treatment time [52]. In this experiment, the maximum treatment duration is only 3 hours compared to experiments conducted by Blaskovich et al. [52], with a minimum of 24 to 72 hours. This demonstrates that to activate the compound in the extracted seaweed, the incubation time for the mixture to take action is a factor that can be investigated. In contrast, the antibacterial hand wash did not affect

its growth as the bacterial growth increased. Thus, this antibacterial hand wash is not effective in eliminating this bacterium. As for the positive and negative controls, the first was done using chloramphenicol. The results show a slight decrease over the first hour. However, an increase in bacterial growth was notable over the second hour of incubation, and a trivial decrease dominated the culture over the last incubation hour. Hence, this antibiotic may not have a substantial impact on *P. aeruginosa*. The negative control illustrates the expected results, as the bacterial growth rate increases gradually over three hours. When comparing the effect of carrageenan extracted from *K. alvarezii* on *P. aeruginosa* and the development of using only nutrient broth, the results are similar and show a very close growth rate.

3.3 *Positive and negative controls*

This study used ampicillin only on *E. coli* as a positive control to inhibit growth. The results showed inhibition in *E. coli* growth with positive control. As for the negative control with standard nutrient broth, *E. coli* growth increased dramatically. Therefore, the results obtained regarding *E. coli* in the present study can be determined reliably as a proper procedure. The good conditions of chemicals and apparatuses used have been performed. The positive control results using chloramphenicol on *K. pneumoniae*, *S. aureus* and *P. aeruginosa* inhibited bacterial growth. This inhibition is clearly illustrated in Figure 3 and Figure 5 for *S. aureus* and *K. pneumoniae*, respectively.

As for *P. aeruginosa*, the action of chloramphenicol in inhibiting growth was somewhat unstable, although the absorbance readings witnessed a reduction, and the MIC testing chloramphenicol showed inhibition of *P. aeruginosa*. As for the negative control using nutrient broth, the bacterial growth increased significantly. The results obtained for all three bacteria can be considered reliable as both positive and negative results were predicted.

The antimicrobial activity of carrageenan extracted from *K. alvarezii* did not cause any inhibition or reduction in bacterial growth compared to the positive controls. The absorbance reading for carrageenan witnessed a continuous increase over the testing period, while the positive control absorbance readings decreased over the same period.

The increasing prevalence of resistance and multi-resistant bacterial strains is alarming and a dreadful threat to the public health system. Although millions of drugs are being synthesised to suppress the activity of bacteria, bacteria have continued to evolve and develop multi-resistance mechanisms against various antimicrobial agents. Therefore, the discovery of new drugs should be considered a priority together with reducing the number of untreatable infections by knowing the reliable means of transmission of diseases. Since bioactive compounds such as lipids, tannins or phenolic compounds have huge potential in pharmacology and other biological actions, they could be a promising antibacterial agent in future studies.

Despite several studies proving the antibacterial effect of carrageenan extracted from various red algae species [51], carrageenan extracted from *K. alvarezii*, and based on this study, is believed to have no antibacterial actions against *E. coli*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa*. However, more research can be done using different solvents and microorganisms. Due to the high gelling property of carrageenan, it is challenging to prepare higher concentrations of carrageenan solution. Therefore, different forms of carrageenan can be used to assess antibacterial properties, such as gel, paste or cream [53]. A different form, such as hydrogel, a natural structure, is a gel form with the disc diffusion assay that can effectively study the antibacterial activities of sticky-type products. Adding different concentrations of carrageenan extract in antibacterial activity will not influence the outcome of the study [32]. These forms will require a completely different methodology to be applied. Thus more studying should be done.

4. Conclusion

In conclusion, the main objectives of this study were to extract carrageenan from *K. alvarezii*, test its antimicrobial activity on a range of bacteria and compare that activity with commercially used antibacterial and anti-acne solutions successfully achieved. Carrageenan extracted from *K. alvarezii* was found to have no antimicrobial effect over *E. coli*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa*. Thus, different extraction methods using other solvents and assessment assays, such as dish diffusion assay, should be applied. In addition, a more green and sustainable extraction method should be developed to effectively extract the compound in seaweed that can be used in the antimicrobial assay.

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Conflict of interest

The authors declare that they have no conflict of interest in the publication.

References

[1] Saha M, Sarkar A. Review on multiple facets of drug resistance: A rising challenge in the 21st century. *Journal of Xenobiotics*. 2021; 11(4): 197-214.
[2] Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*. 2020; 83(3): 770-803.
[3] Vaghasiya Y, Chanda S. Screening methanol and acetone extracts of fourteen Indian medicinal plants for antimicrobial activity. *Turkish Journal of Biology*. 2007; 31(4): 243-248.
[4] Bartoletti M, Giannella M, Tedeschi S, Viale P. Multidrug-resistant bacterial infections in solid organ transplant candidates and recipients. *Infectious disease clinics of North America*. 2018; 32(3): 551-580.
[5] Nisar B, Sultan A, Rubab SL. Comparison of medicinally important natural products versus synthetic drugs-A short commentary. *Natural Products Chemistry & Research*. 2018; 06(02): 1000308.
[6] Rocha T, Amaral JS, Oliveira M. Adulteration of dietary supplements by the illegal addition of synthetic drugs: A review. *Comprehensive Reviews in Food Science and Food Safety*. 2016; 15(1): 43-62.
[7] Leighl NB, Nirmalakumar S, Ezeife DA, Gyawali B. An arm and a leg: The rising cost of cancer drugs and impact on access. *American Society of Clinical Oncology - Educational Book*. 2021; 41: 1-12.
[8] Farahmandfar R, Esmaeilzadeh Kenari R, Asnaashari M, Shahrampour D, Bakhshandeh T. Bioactive compounds, antioxidant and antimicrobial activities of Arum maculatum leaves extracts as affected by various solvents and extraction methods. *Food Science & Nutrition*. 2019; 7(2): 465-475.
[9] Stan D, Enciu AM, Mateescu AL, Ion AC, Brezeanu AC, Stan D, et al. Natural compounds with antimicrobial and antiviral effect and nanocarriers used for their transportation. *Frontiers in Pharmacology*. 2021; 12: 723233.
[10] Somrani M, Ingles MC, Debbabi H, Abidi F, Palop A. Garlic, onion, and cinnamon essential oil anti-biofilms' effect against listeria monocytogenes. *Foods*. 2020; 9(5): 567.
[11] Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li HB. Antibacterial and antifungal activities of spices. *International Journal of Molecular Sciences*. 2017; 18(6): 1283.
[12] Sytar O, Smetanska I. Special issue "Bioactive compounds from natural sources (2020, 2021)". *Molecules*. 2022; 27(6): 1929. Available from: doi: 10.3390/molecules27061929.
[13] Seca AML, Trendafilova A. Secondary metabolites in edible species: Looking beyond nutritional value. *Foods*. 2021; 10(5): 1131.
[14] Rupert R, Rodrigues KF, Thien VY, Yong WTL. Carrageenan from *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae): Metabolism, structure, production, and application. *Frontiers in Plant Science*. 2022; 13: 859635.
[15] Bode-Dalby M. *YOUMARES 9 -The Oceans: Our Research, Our Future*. Springer Open; 2018.
[16] Rosa GP, Tavares WR, Sousa PMC, Pages AK, Seca AML, Pinto D. Seaweed secondary metabolites with beneficial health effects: An overview of successes in in vivo studies and clinical trials. *Marine Drugs*. 2019; 18(1): 8.
[17] Srinivasan R, Kannappan A, Shi C, Lin X. Marine bacterial secondary metabolites: A treasure house for structurally unique and effective antimicrobial compounds. *Marine Drugs*. 2021; 19(10): 1-36.
[18] Farah Nurshahida M, Nazikussabah Z, Subramaniam S, Wan Faizal W, Nurul Aini M. Physicochemical, physical characteristics and antioxidant activities of three edible red seaweeds (*Kappaphycus alvarezii*, *Eucheuma spinosum* and *Eucheuma striatum*) from Sabah, Malaysia. *IOP Conference Series: Materials Science and Engineering*. 2020; 991: 012048.
[19] Chaisuwan W, Phimolsiripol Y, Chaiyaso T, Techapun C, Leksawasdi N, Jantanasakulwong K, et al. The antiviral activity of bacterial, fungal, and algal polysaccharides as bioactive ingredients: Potential uses for enhancing immune systems and preventing viruses. *Frontiers in Nutrition*. 2021; 8: 772033.

[20] Lomartire S, Cotas J, Pacheco D, Marques JC, Pereira L, Goncalves AMM. Environmental impact on seaweed phenolic production and activity: An important step for compound exploitation. *Marine Drugs*. 2021; 19(5): 245.
[21] Generalic Mekinic I, Skroza D, Simat V, Hamed I, Cagalj M, Popovic Perkovic Z. Phenolic content of brown algae (Pheophyceae) species: Extraction, identification, and quantification. *Biomolecules*. 2019; 9(6): 244.
[22] Chojnacka K, Saeid A, Witkowska Z, Tuhy L. Biologically active compounds in seaweed extracts - the prospects for the application. *The Open Conference Proceedings Journal*. 2012; 3(1): 20-28.
[23] Zhang L, Liao W, Huang Y, Wen Y, Chu Y, Zhao C. Global seaweed farming and processing in the past 20 years. *Food Production, Processing and Nutrition*. 2022; 4(1): 23.
[24] Rima M, Chbani A, Roques C, El Garah F. Seaweed extracts as an effective gateway in the search for novel antibiofilm agents against staphylococcus aureus. *Plants (Basel)*. 2022; 11(17): 2285.
[25] Lu WJ, Tsui YC, Chang CJ, Hsu PH, Huang MY, Lai M, et al. Characterization and potentiating effects of the ethanolic extracts of the red seaweed *Gracillaria sp*. on the activity of carbenicillin against vibrios. *ACS Omega*. 2022; 7(50): 46486-46493.
[26] Tang J, Wang W, Chu W. Antimicrobial and anti-quorum sensing activities of phlorotannins from seaweed (Hizikia fusiforme). *Frontiers in Cellular and Infection Microbiology*. 2020; 10: 586750.
[27] Frazzini S, Scaglia E, Dell'Anno M, Reggi S, Panseri S, Giromini C, et al. Antioxidant and antimicrobial activity of algal and cyanobacterial extracts: An in vitro study. *Antioxidants (Basel)*. 2022; 11(5): 992.
[28] García-Bueno N, Decottignies P, Turpin V, Dumay J, Paillard C, Stiger-Pouvreau V, et al. Seasonal antibacterial activity of two red seaweeds, Palmariapalmataand Grateloupia turuturu, on European abalone pathogen Vibrio harveyi. *Aquatic Living Resources*. 2014; 27(2): 83-89.
[29] Cox S, Hamilton Turley G, Rajauria G, Abu-Ghannam N, Jaiswal AK. Antioxidant potential and antimicrobial efficacy of seaweed (Himanthalia elongata) extract in model food systems. *Journal of Applied Phycology*. 2013; 26(4): 1823-1831.
[30] El Shafay SM, Ali SS, El-Sheekh MM. Antimicrobial activity of some seaweeds species from Red sea, against multidrug resistant bacteria. *The Egyptian Journal of Aquatic Research*. 2016; 42(1): 65-74.
[31] Regina Leong ZL, Wan HL, Teo SS. Antimicrobial activity of hydrogel loaded with capsicum annuum, auricularia auricula judae and *Kappaphycus alvarezii*. *Research Journal of Chemistry and Environment*. 2023; 27(4): 1-6.
[32] Zhi Ling RL, Kong LK, Lim LH, Teo SS, Ng HS, Lan JC, et al. Identification of microorganisms from fermented biowaste and the potential for wastewater treatment. *Environmental Research*. 2023; 218: 115013.
[33] Banakar SN, Kumar MKP, Gautam C, Puneeth ME. Foliar application of red seaweed (*Kappaphycus alvarezii*) bioformulations increased the levels of chlorophyll content in rice. *Journal of Pharmacognosy and Phytochemistry*. 2020; 9(1): 408-410.
[34] Bhuyar P, Rahim MH, Sundararaju S, Maiam GP, Govindan N. Antioxidant and antibacterial activity of red seaweed; Kappaphycus arvarezii againt pathogenic bacteria. *Global Journal of Environmental Science and Management*. 2020; 6(1): 47-58.
[35] Jiang F, Liu Y, Xiao Q, Chen F, Weng H, Chen J, et al. Eco-friendly extraction, structure, and gel properties of iota-carrageenan extracted using $Ca(OH)_2$. *Marine Drugs*. 2022; 20(7): 419.
[36] Florez-Fernandez N, Falque E, Dominguez H, Torres MD. Green extraction of carrageenans from mastocarpus stellatus. *Polymers (Basel)*. 2022; 14(3): 554.
[37] Gisbert M, Barcala M, Rosell CM, Sineiro J, Moreira R. Aqueous extracts characteristics obtained by ultrasound-assisted extraction from Ascophyllum nodosum seaweeds: effect of operation conditions. *Journal of Applied Phycology*. 2021; 33(5): 3297-3308.
[38] Ummat V, Tiwari BK, Jaiswal AK, Condon K, Garcia-Vaquero M, O'Doherty J, et al. Optimisation of ultrasound frequency, extraction time and solvent for the recovery of polyphenols, phlorotannins and associated antioxidant activity from brown seaweeds. *Marine Drugs*. 2020; 18(50): 250.
[39] Martin-Del-Campo A, Fermin-Jimenez JA, Fernandez-Escamilla VV, Escalante-Garcia ZY, Macias-Rodriguez ME, Estrada-Giron Y. Improved extraction of carrageenan from red seaweed (Chondracantus canaliculatus) using ultrasound-assisted methods and evaluation of the yield, physicochemical properties and functional groups. *Food Science and Biotechnology*. 2021; 30(7): 901-910.
[40] Sebaaly C, Kassem S, Grishina E, Kanaan H, Sweidan A, Chmit MS, et al. Anticoagulant and antibacterial activities of polysaccharides of red algae *Corallina* collected from Lebanese coast. *Journal of Applied Pharmaceutical Science*. 2014; 4(4): 30-37.
[41] El-Fawal G. Preparation, characterization and antibacterial activity of biodegradable films prepared from carrageenan. *Journal of Food Science and Technology*. 2014; 51(9): 2234-2239.

[42] Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clinical Infectious Diseases*. 2009; 49(11): 1749-1755.
[43] Chuah XQ, Mun W, Teo SS. Comparison study of anti-microbial activity between crude extract of *Kappaphycus alvarezii* and Andrographis paniculata. *Asian Pacific Journal of Tropical Biomedicine*. 2017; 7(8): 729-731.
[44] Wang FF, Yao Z, Wu HG, Zhang SX, Zhu NN, Gai X. Antibacterial activities of kappa-carrageenan oligosaccharides. *Applied Mechanics and Materials*. 2011; 108: 194-199.
[45] Brglez Mojzer E, Knez Hrncic M, Skerget M, Knez Z, Bren U. Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. *Molecules*. 2016; 21(7): 901.
[46] Franco D, Sineiro J, Rubilar M, Sanchez M, Jerez M, Pinelo M, et al. Polyphenols from plant materials: Extraction and antioxidant power. *Electronic Journal of Environmental, Agricultural and Food Chemistry*. 2008; 7(8): 3210-3216.
[47] Cabral EM, Oliveira M, Mondala JRM, Curtin J, Tiwari BK, Garcia-Vaquero M. Antimicrobials from seaweeds for food applications. *Marine Drugs*. 2021; 19(4): 211.
[48] Li Y, Sun S, Pu X, Yang Y, Zhu F, Zhang S, et al. Evaluation of antimicrobial activities of seaweed resources from Zhejiang coast, China. *Sustainability*. 2018; 10(7): 2158.
[49] Cmikova N, Galovicova L, Miskeje M, Borotova P, Kluz M, Kacaniova M. Determination of antioxidant, antimicrobial activity, heavy metals and elements content of seaweed extracts. *Plants (Basel)*. 2022; 11(11): 1493.
[50] Mihalache OA, Borda D, Neagu C, Teixeira P, Langsrud S, Nicolau AI. Efficacy of removing bacteria and organic dirt from hands-A study based on bioluminescence measurements for evaluation of hand hygiene when cooking. *International Journal of Environmental Research and Public Health*. 2021; 18(16): 8828.
[51] Perez MJ, Falque E, Dominguez H. Antimicrobial action of compounds from marine seaweed. *Marine Drugs*. 2016; 14(3): 52.
[52] Blaskovich MAT, Elliott AG, Kavanagh AM, Ramu S, Cooper MA. In vitro antimicrobial activity of acne drugs against skin-associated bacteria. *Scientific Reports*. 2019; 9(1): 14658.
[53] Ruo Xin L, Starlin Chellathurai M, Vijayaraj Kumar P, Ibrahim Khalivulla S, Swee Sen T. Formulation and evaluation of solid lipid nanoparticles containing *Kappaphycus alvarezii* Extract in a cosmetic gel. *Research Journal of Pharmacy and Technology*. 2022; 5812-5818.