Investigating the Synergistic Antibacterial Effects of *Ulva lactuca* and *Fucus vesiculosus* Extracts on the Growth of *Staphylococcus epidermidis*

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This study investigates the antibacterial properties of extracts from marine macroalgae, *Ulva lactuca* (green algae) and *Fucus vesiculosus* (brown algae), individually and in combination, with a focus on combating antibiotic-resistant pathogens. The escalating challenge of antibiotic resistance requires the exploration of alternative antimicrobial agents derived from natural sources. Utilizing the agar well diffusion method, the study evaluates the inhibitory effects of the algae extracts on *Staphylococcus epidermidis*. Chloramphenicol serves as the positive control, while 5% dimethyl sulfoxide acts as the negative control. The combination of *Ulva lactuca* and *Fucus vesiculosus* extracts did not exhibit a significantly greater antibacterial effect than *Ulva lactuca* alone. Chloramphenicol demonstrated strong antibacterial activity, validating the experimental setup. *Ulva lactuca* displayed significantly higher antibacterial efficacy than *Fucus vesiculosus*, challenging the initial hypothesis. The observed relationships between *Ulva lactuca*, *Fucus vesiculosus*, and their combination suggest complex interactions among bioactive compounds. After analyzing the data through the use of a one-way ANOVA and a Tuckey's HSD test, it was determined that while *Ulva lactuca* alone outperformed the combination of both extracts, the combination showed a slight advantage over *Fucus vesiculosus* alone, hinting at potential synergistic effects. Acknowledging limitations, including equipment constraints and time considerations, emphasizes the need for future studies to address these factors for more robust findings. Despite unexpected outcomes, this research contributes to understanding the intricate dynamics of algae extracts and opens avenues for further exploration in the quest for effective antimicrobial strategies against antibiotic resistance.

Introduction

In recent years, there has been a notable rise in infectious diseases and an overuse of antibacterial agents composed of chemical combinations (Suganya et al., 2020). This has resulted in a higher occurrence of microorganisms developing antibiotic resistance. Therefore, it has become imperative to investigate fresh antimicrobial substances derived from natural sources, including plants and other biological alternatives. As stated by Suganya et al. (2020), affiliated with the Department of Pediatric and Preventive Dentistry, Indira Gandhi Institute of Dental Sciences, Puducherry, India, macroalgae and seaweeds from marine environments offer a compelling alternative due to their abundant availability and capacity to produce bioactive secondary compounds. The marine environment is a valuable source of unique bioactive compounds. Marine organisms, including bacteria, protozoans, plants, and animals, display numerous beneficial biological properties that hold promise for applications in promoting human health (Suganya et al., 2020). Seaweeds, in particular, are essential marine resources known for their potential to produce antibacterial substances (Saritha et al., 2013). They contain antimicrobial compounds, making them a source of bioactive metabolites. They also contain numerous inorganic and organic substances that are useful and beneficial to human health. For example, the secondary or non-primary metabolites that are produced by the seaweeds, have been applied across various industries, from food and fragrances to pigments, insecticides, and medicinal applications (Saritha et al., 2013). Selected seaweeds also have shown promise in combating gram-positive bacteria. Additionally, marine algae are increasingly recognized by the biotechnology industry due to their discovery of numerous bioactive compounds (Saritha et al., 2013).

Staphylococcus epidermidis is a gram-positive and coagulase-negative strain of cocci bacteria that forms clusters (Lee et al., 2023). They are the most prevalent species of coagulase-negative Staphylococci to inhabit human skin and mucosa and are usually considered harmless. Specifically, they constitute ~90% of the Staphylococci recovered from the anterior nares when *S. aureus* is absent (Siciliano et al., 2023; Lee et al., 2023). However, *Staphylococcus epidermidis* can become a troublesome pathogen under specific conditions. In cases where the host's defenses are compromised, such as with the presence of foreign bodies, neutropenia, or following surgeries, it can cause infections, often associated with medical devices like catheters (Siciliano et al., 2023). Furthermore, these infections are prevalent contributors to nosocomial infections, which are healthcare-associated infections acquired while receiving medical care and were not present at the time of admission (Lee et al., 2023; Sikora & Zahra, 2023). In the United States, these infections often lead to severe complications while treating patients (Lee et al., 2023). Specifically, *Staphylococcus epidermidis* causes approximately 20% of all orthopedic device-related infections and 50% in late developing infections (Brescó et al., 2017). The ability of *Staphylococcus epidermidis* to form biofilms on these devices enhances its persistence and pathogenicity (Brescó et al., 2017). Furthermore, it has developed a significant level of antibiotic resistance, making treatment of this bacteria challenging (Siciliano et al., 2023).

Seaweeds fall into the category of marine macroalgae, with additional categorization into brown algae (Phaeophyta), red algae (Rhodophyta), and green algae (Chlorophyta). *Ulva lactuca* (sea lettuce), for example, is classified as a green alga and is implicated in the widespread occurrence of green tides or blooms, which are a direct outcome of human activities. Additionally, the decomposition of these algal blooms results in the release of dangerous vapors (Dominguez et al., 2019). However, *Ulva lactuca* can also be very beneficial due to its numerous bioactive compounds. It has also been recorded to possess antimicrobial properties against significant pathogens like *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus* (MRSA) (Suganya et al., 2020). Furthermore, *Ulva lactuca* contains various secondary metabolites, including alkaloids, triterpenoids, steroids, saponins, phenolic compounds, and flavonoids (Ardita at al., 2021). These compounds have the potential to enhance the healing of hospital-acquired wound infections due to their antibacterial, anti-inflammatory, antioxidant, and anticoagulant properties. Additionally, Ulva contains ulvans, which are sulfated heteropolysaccharides that contribute to the cell wall's strength and flexibility, protecting it from drying out during tides (Dominguez et al., 2019). This polysaccharide exhibits a range of beneficial properties, including antiviral, antitumor, anticoagulant, lipid-lowering, hepatoprotective, immunostimulating, antidepressant, and anxiolytic activities (Dominguez et al., 2019). Hence, there is growing demand for their application in the pharmaceutical and food industries. With its multiple active components, *U. lactuca* extract is believed to be effective against bacteria that are resistant to multiple treatments. Its diverse array of active components allows the extract from *U. lactuca* to address multi-resistant bacteria by employing various antimicrobial mechanisms to overcome the bacterial resistance barriers (Ardita at al., 2021).

Fucus vesiculosus, commonly known as bladderwrack, falls into the category of brown algae and can be found to grow in the Baltic Sea, Atlantic Ocean, and North Pacific Ocean (U.S. National Library of Medicine, 2021). Furthermore, it is one of the most prominent brown algae of the genus Fucus (Obluchinskaya et al., 2022). Aside from its use as a food ingredient, *F. vesiculosus* is applied in cosmetics, biofertilizers, animal feed, and the pharmaceutical industry (Obluchinskaya et al., 2022). *F. vesiculosus* is a valuable source of health-enhancing substances, including fucoidans, polyphenols, fucoxanthin, and essential minerals. Fucoidans are sulphated polysaccharide unique to brown algae (Saeed et al., 2021); they belong to the Fucan family and exhibit a wide range of pharmacological effects, such as antioxidative, anti-obesity, antidiabetic, anti-aging, antimicrobial, antitumor, anticoagulant, and anti-inflammatory properties (Obluchinskaya et al., 2022). Furthermore, phlorotannins, a distinct group of polyphenolic compounds also exclusive to brown algae, demonstrate significant biological activities, including antioxidant, antibacterial, and antidiabetic effects (Obluchinskaya et al., 2022).

In light of the escalating challenges posed by antibiotic-resistant pathogens and the need for novel antimicrobial agents, this study aims to explore the potential of marine macroalgae, specifically *Ulva lactuca* and *Fucus vesiculosus*, as sources of natural compounds with antibacterial properties. These algae hold promise due to their diverse bioactive metabolites, including fucoidans, polyphenols, and ulvans, which have demonstrated various health-enhancing and antimicrobial characteristics. By investigating the antibacterial efficacy of these algae against pathogens like *Staphylococcus epidermidis*, the research seeks to contribute to the development of alternative antimicrobial strategies with broader applications. This research addresses the pressing issue of antibiotic resistance while utilizing sustainable resources from the marine environment, offering potential solutions to enhance human health and healthcare practices. In a time of increasing health concerns and a need for effective antibacterial agents, this study is positioned to bridge a gap in the field, offering a promising and eco-friendly path to combat infections and nosocomial complications.

Literature Review

Evaluation of Antibacterial Properties

Currently, when evaluating the antibacterial properties of selected compounds, the two most commonly used methods are agar disk diffusion and agar well diffusion. The agar disk-diffusion test, established in 1940, is the widely recognized method employed in numerous clinical microbiology labs for routine assessments of antimicrobial susceptibility. This method is recognized by Balouiri et al. (2016), who are affiliated with the Laboratory of Microbial Biotechnology at the University Sidi Mohamed Ben Abdellah in Fez, Morocco. For this method, the standardized microorganism inocula are applied to agar plates, followed by the placement of small filter paper discs (approximately 6 mm in diameter) containing the desired concentration of the test compound on the agar surface. The Petri dishes are then incubated under suitable conditions. Typically, the antimicrobial agent diffuses into the agar, inhibiting the germination and growth of the test microorganism, and the diameters of the growth inhibition zones are measured.

Although agar well diffusion is similar to agar disk diffusion, agar well diffusion serves a more specific purpose and is commonly used to assess the antimicrobial activity of plant or microbial extracts (Balouiri et al., 2016). The one major difference between these two methods is that disk diffusion uses a filter paper disc, while well diffusion uses wells that are punched into the agar. Furthermore, as illuminated by a study conducted by Valgas et al., in 2007, the agar well diffusion method demonstrated heightened sensitivity as compared to the agar disk diffusion method, particularly when loaded with natural products. Its heightened sensitivity makes it advantageous to the ongoing investigation into the synergistic antibacterial properties of *U. lactuca* and *F. vesiculosus* extracts, by enabling a more discerning detection of potential antibacterial interactions.

Moreover, it is worth noting that the most commonly used methods for the detection of antimicrobial activity of natural products fall into three distinct groups: bioautographic, diffusion, and dilution methods (Valgas et al., 2007). Among these, bioautographic and diffusion methods are recognized as qualitative techniques, primarily serving to indicate the presence or absence of substances with antimicrobial activity (Valgas et al., 2007). In contrast, dilution methods are classified as quantitative assays, enabling the determination of the minimal inhibitory concentration (Valgas et al., 2007). This categorization highlights the diversity of screening techniques available, each offering a specific perspective on the antimicrobial properties of natural products. The choice of method depends on the research objectives and the nature of the substances being investigated. Since the goal of this research is to investigate the antibacterial properties of algae extracts, agar well diffusion stands out as the most suitable approach.

Bioactive Compounds in Algae

Perez et al. (2016), affiliated with the Department of Functional Biology and Health Sciences at the University of Vigo, state that seaweeds produce metabolites as protective mechanisms in response to the necessity of coping with environmental stressors and challenges. These metabolites possess antiviral, antiprotozoal, antifungal, and antibacterial properties. Marine organisms, which are always adapting to competitive and hostile environments, produce complex secondary compounds as responses to ecological pressures. Some of these compounds, including antimicrobials, inhibit the growth of competing microorganisms, defend against grazers, and prevent the attachment of epiphytes (Perez et al., 2016). Specifically, the substances isolated from green, brown and red algae that show potent antimicrobial activity belong to polysaccharides, fatty acids, phlorotannins, peptides, pigments, lectins, alkaloids, terpenoids, and halogenated compounds (Perez et al., 2016; Ardita et al., 2021; Silva et al., 2020). Polysaccharides, which play a structural role in the algal cell wall, can be neutral or acidic, linear or branched. Green, red, and brown algae are all defined by the sulfated polysaccharides that they are composed of. These polysaccharides are primarily characterized by the presence of sulfate groups and consist of repeating units of rhamnose, xylose, and uronic acids (Silva et al., 2020). Although, the antibacterial properties of polysaccharides depend on molecular weight, charge density, and sulfated content, several studies have shown that sulfated polysaccharides extracted from different seaweed are able to inhibit the growth of pathogenic bacteria (Silva et al., 2020). Pigments, which make up the color of the algae, have shown to have antibacterial properties against gram positive bacteria, like *S. agalactiae*, *S. aureus*, and *S. epidermidis* (Silva et al., 2020).

Role of Antibacterial Agents in Healthcare

According to the European Centre for Disease Prevention and Control, antimicrobial resistance leads to more than 25,000 fatalities annually in Europe (Shannon & Abu-Ghannam, 2016). As microorganisms continue to evolve new tactics to escape the effects of antibiotics, the emergence of multiple drug-resistant bacterial strains has become a pressing concern. In light of the escalating resistance of pathogens to antibiotics, there is a growing public health imperative to investigate and create cost-effective and efficient natural antimicrobial agents with improved potential, reduced side effects compared to antibiotics, enhanced bioavailability, and minimal toxicity (Perez et al., 2016).The large-scale cultivation of macroalgae holds significant promise as a valuable source of potential compounds that could be harnessed for the development of innovative drugs capable of managing emerging diseases or strains of pathogenic microorganisms that have developed resistance to conventional treatments (Perez et al., 2016).

Early studies screened 151 British marine algae species for antibiotic production, revealing significant antibacterial activity in various species (Parsaeimehr & Lutzu, 2016). In a separate study, 82 marine macroalgae from various classes were screened for antimicrobial activity, and 84% of the algae from this taxon showed antimicrobial activity (Parsaeimehr & Lutzu, 2016). Similar results were found in a study of algae extracts from the North Aegean Sea, where various algal classes demonstrated antimicrobial activity against diverse bacteria (Parsaeimehr & Lutzu, 2016). These findings highlight algae's potential as a source of valuable antimicrobial compounds.

Establishing a Gap

Numerous articles delve into the individual antibacterial properties of *Ulva lactuca* and *Fucus vesiculosus* extracts. Yet, a substantial gap exists in the comprehension of how combining these extracts might influence their antibacterial efficacy. This research gap holds particular significance in the present era, where escalating antibiotic resistance presents a pressing global health challenge. Exploring unconventional sources of antibacterial agents is, therefore, of paramount importance, and algae, renowned for their diverse bioactive compounds, may offer a promising solution. Furthermore, delving into potential synergies among these algae extracts could pave the way for the creation of more potent and adaptable antibacterial treatments, potentially mitigating the threat of bacterial resistance. The practical implications of this research are substantial; it could lead to the development of a potent and versatile antibacterial agent with applications in healthcare, potentially bolstering infection prevention and treatment capabilities. Moreover, integrating algae-based antibacterials into disinfectants could result in more efficient and environmentally friendly disinfection solutions. Hence, this study revolves around assessing how the combination of *Ulva lactuca* and *Fucus vesiculosus* extracts impact the growth of *Staphylococcus epidermidis*.

Methods

Similar to the methods outlined by EL-Sayed, El-Sheekh, and Makhlof (2023), the current study implemented comparable procedures for algae extraction. Specifically, *Ulva lactuca* and *Fucus vesiculosus* algae powders were soaked in 99% methanol for 72 hours. However, a difference from the previously mentioned methodology occurred in this study: the algae powders were soaked in methanol at a 1:25 (weight/volume), as opposed to the 1:10 ratio utilized in the referenced research study. For the first hour, each extract was continuously stirred at room temperature using a magnetic stirrer. Additionally, each of the beakers were labeled respectively and covered with parafilm to prevent contaminants from being introduced into the extracts, as shown in Figure 1. Later, the extracts were filtered through vacuum filtration, by using buchner funnels, filter paper, and a side-arm flask, which can be seen in Figure 2. Gradually, small quantities of the extracts were incrementally introduced into the buchner funnel until complete filtration was achieved. Following the methodology outlined in EL-Sayed's, El-Sheekh's, and Makhlof's 2023 study, the filtrates were concentrated at 50 °C. However, due to the unavailability of a rotary evaporator, the concentration was achieved by evaporation under low heat (~50 °C) in a drying oven for approximately 24 hours or until minimal liquid was left. Subsequently, the concentration process was finalized using a water bath at ~50 °C for ~3 hours, as displayed in Figure 3.

This method yielded approximately 1 gram of crude extract from each alga. In accordance with the methods described by Miss, Hoare, and Hughes (2019), the crude extracts were then stored in a freezer (-20 °C) until further use to reduce the rate of degradation of the extracts. Continuing with the procedures, the final step involved suspending the weighted crude extracts in 20 mL of 5% dimethyl sulfoxide. This suspension resulted in a crude extract with a concentration of 50 mg/mL. The positive control variable used for the experiment was chloramphenicol and the negative control was 5% dimethyl sulfoxide. Notably, all procedures, excluding concentration in the oven and vacuum filtration, were conducted under a fume hood, in order to minimize exposure to the methanolic fumes.

Agar well diffusion was used to assess the antibacterial properties of these extracts, a commonly utilized method for testing the antibacterial properties of natural plant extracts, as demonstrated in studies conducted by Manivannan & Subramanian (2023) and by Miss, Hoare, and Hughes (2019). To begin, 30 Mueller Hinton agar plates were prepared. Following the guidelines from Carolina Biological, 750 mL of Mueller Hinton agar was prepared by mixing 28.5 grams of Mueller Hinton agar powder with 750 mL of distilled water, then autoclaved. The agar was then poured into 30 Petri dishes measuring 90 mm and allowed to cool at room temperature. Afterward, the dishes were stored in a refrigerator, while upside down, until they were needed for experimentation (11 days). The agar plates were then inoculated with standardized inocula of the *Staphylococcus epidermidis*. Following this, 5 holes, each with a diameter of 6 mm, were punched aseptically with a sterile 6 mm cork borer. Each well was labeled on the underside of the petri dishes according to the substance being placed in the well. Finally, 20 µL of each algae extract, and the controls were introduced into each well. The agar plates were then incubated at 37 °C for 24 hours, and the zone of inhibition for each sample was measured, using the metric side of a ruler, to determine the antibacterial properties of the extracts, as shown in Figure 4. These methods were well-suited for investigating the influence of combining *Ulva lactuca* and *Fucus vesiculosus* extracts on *Staphylococcus epidermidis* growth in a controlled setting, aligning with the research question. A One-Way ANOVA and Tukey's HSD test were used to determine the significance of the data collected. The experimental design diagram is shown in Figure 5.

Results

The results of the antibacterial assay provided insights into the effectiveness of the tested substances. Chloramphenicol, functioning as the positive control, showed a significant mean zone of inhibition of 25.41 mm, indicating strong antibacterial activity. In contrast, the negative control (5% DMSO) showed no observable inhibition, serving as a baseline for comparison. The *Ulva lactuca* extract demonstrated a mean zone of inhibition of 7.12 mm, and the *Fucus vesiculosus* extract displayed a mean zone of inhibition of 5.48 mm. The combination of the *Ulva lactuca* extract and the *Fucus vesiculosus* extract resulted in a mean zone of inhibition of 5.67 mm. The data is presented below in Table 1, providing a condensed version of the raw data outlined in Appendix A.

A one-way ANOVA was utilized in order to understand the significance of the differences among treatment groups. The one-way ANOVA revealed a highly significant overall effect ($F = 2653.19$, $p < 0.001$, $\alpha = 0.05$), indicating substantial variations in antibacterial efficacy, and the null hypothesis was rejected. A post-hoc Tukey's HSD test allowed for pairwise comparisons, providing a Tukey's HSD value of 0.73, helping to identify specific differences among treatment groups, and whether these differences were significant.

Chloramphenicol showed a significant mean difference of 25.414 mm compared to the negative control (5% DMSO). When comparing the *Ulva lactuca* to *Fucus vesiculosus*, the mean difference was 1.638 mm, meaning that the *Ulva lactuca* extract had significantly greater antibacterial efficacy as compared to that of *Fucus vesiculosus*, as the mean difference (1.638 mm) is greater than Tuckey's HSD value of 0.73. When comparing the *Ulva lactuca* extract to the combination of *Ulva lactuca* and *Fucus vesiculosus*, the mean difference was 1.449 mm, meaning *Ulva lactuca* alone displayed significantly greater antibacterial properties than the combination of *Ulva lactuca* and *Fucus vesiculosus*. The data can be

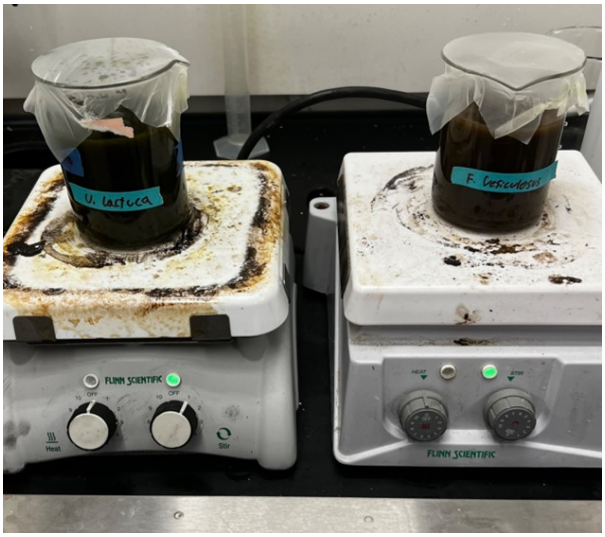

Figure 1. *Magnetic Stirring of Algae Extracts.* The figure displays the beakers containing the algae extracts, each labeled with the respective algae used. Positioned on magnetic stirrer hot plates with the heat turned off, the beaker on the left holds *Ulva lactuca*, while the one on the right contains *Fucus vesiculosus*. Notably, both beakers are securely covered with parafilm.

Figure 2. *Vacuum Filtration of Algae Extracts.* This figure displays the vacuum filtration setup, used for separating the extracts from the algae powder. Suspended by a ring stand, the setup features a side-arm flask on the bottom, with a Buchner funnel securely attached. Notably, the yellow tubing connects to a dual hose inlet adapter, establishing a vacuum effect by linking to a sink (not shown). Positioned in the bottom right corner is the beaker containing the algae extract

Figure 3. *Evaporation Using a Water Bath.* This figure displays the water bath setup used, in order to evaporate the remaining liquid in the extracts. Notably, the extract-filled beakers are positioned within a larger beaker filled with water, resting on top of hot plates. Additionally, each beaker is equipped with a thermometer immersed in the water, ensuring continuous monitoring of water temperature during the evaporation process.

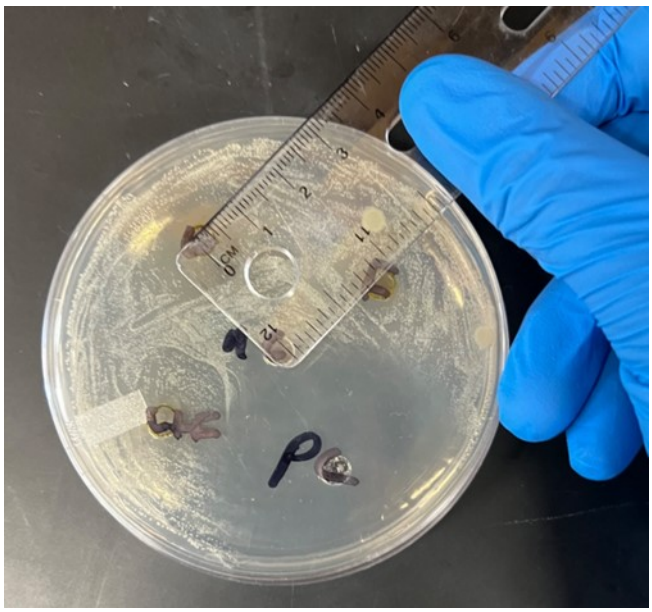

Figure 4. *Measuring Zone of Inhibition.* The figure illustrates the data collection process, wherein the inhibition zones of each experimental group were measured. The groups are indicated as follows: "U" (Top left) for *Ulva lactuca*, "F" (Top right) for *Fucus vesiculosus*, "U+F" (Bottom left) for the combination of *Ulva lactuca* and *Fucus vesiculosus* extracts, "NC" (Center) for the negative control, and "PC" for the positive control. The zones of inhibition were assessed by utilizing the metric side of the ruler to measure the distance from the center of the well to the edge of the inhibition zone.

Figure 5. *Experimental Design Diagram*

Title of the Experiment

The effect of combining *Ulva lactuca* and *Fucus vesiculosus* extracts on growth of *Staphylococcus epidermidis* in a controlled setting.

Hypothesis

It is hypothesized that the combination of the *Ulva lactuca* and *Fucus vesiculosus* extracts would result in greater antibacterial effects on the *Staphylococcus epidermidis*, as opposed to using the extracts individually. This is based on the unique properties of each algae species. Specifically, *Fucus vesiculosus* contains fucoidans known for their bacteriostatic effects on the studied bacteria. These fucoidans prevent the bacteria from receiving sufficient nutrition, leading to the inhibition of their growth. When combined with the extract from *Ulva lactuca*, which contains its own set of bioactive compounds, it's anticipated that the different modes of action of these compounds could work synergistically, potentially intensifying the overall antibacterial impact.

Independent Variable

Levels of Independent Variable	*Ulva lactuca* extract (20 µL)	*Fucus vesiculosus* extract (20 µL)	Combination of *Ulva lactuca* and *Fucus vesiculosus* (20 µL)	Chloramphenicol (Positive control) (20 µL)	5% Dimethyl sulfoxide (Negative control) (20 µL)
Number of Repeated Trials	30	30	30	30	30

Dependent Variable

Zone of inhibition (mm)

Control Group

Chloramphenicol is the positive control.

5% dimethyl sulfoxide is the negative control.

Constants

The volume of substances added to each well. The solvent (5% dimethyl sulfoxide) used for each substance. Source from which each substance is obtained. Type of agar that is used. Size of each petri dish.

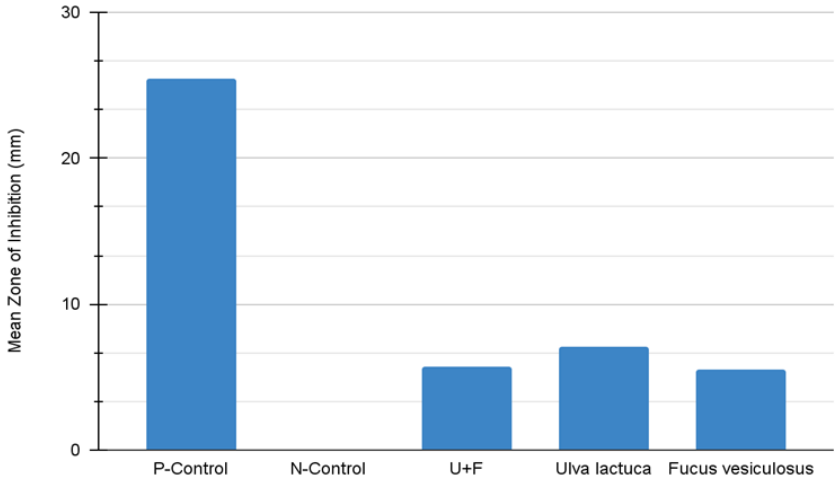

Figure 6. *Mean Zone of Inhibition.* This figure shows the mean zone of inhibition of each group. It can be seen that the positive control group had a mean zone of inhibition of 25.41 mm, and the negative control had a mean zone of inhibition of 0 mm. As shown in the graph U+F stands for the combination of *Ulva lactuca* and *Fucus vesiculosus* extracts, which had a mean zone of inhibition of 5.67 mm. The *Ulva lactuca* extract by itself had a mean zone of inhibition of 7.12 mm. The *Fucus vesiculosus* extract by itself had a mean zone of inhibition of 5.48 mm.

Table 1. *Mean Zones of Inhibition*

Groups	*Count*	*Mean*	*Variance*
P-Control (mm)	30	25.414	1.466
N-Control (mm)	30	0	0
U+F (mm)	30	5.672	1.487
U (mm)	30	7.121	1.226
F (mm)	30	5.483	0.973

Table 1 is a condensed version of the raw data that presents the mean zone of inhibition for each independent variable and control. Notably, the "P-Control" (positive control) exhibits a mean zone of inhibition of 25.41 mm, while the "N-Control" (negative control) shows no inhibition with a mean of 0 mm. The combination of both extracts, represented as "U+F," displays a mean zone of inhibition of 5.672 mm. Additionally, "U" (*Ulva lactuca* extract) has a mean zone of inhibition of 7.121 mm, and "F" (*Fucus vesiculosus* extract) registers a mean zone of inhibition of 5.483 mm. The variance represents the variation in zones of inhibition within each group, with 30 trials conducted for each group.

Table 2. *One-Way ANOVA Summary Table*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10934.300	4	2733.575	2653.194	< 0.001	2.436
Within Groups	144.241	140	1.030			

Table 2 displays the sum of squares for both between and within the treatment groups, the degrees of freedom for between and within groups, the mean square for between and within groups, the F-ratio, P-value, and the critical F-value.

seen in Appendix B. This suggested the potentially heightened antibacterial efficacy of the *Ulva lactuca* extract alone, which can be seen in Figure 6 Similarly, the mean difference between *Fucus vesiculosus* and the combination of extracts was 0.189 mm, suggesting a slight advantage of the combination over *Fucus vesiculosus* alone. However, it was important to highlight that the observed mean difference of 0.189 mm did not reach statistical significance, as it was below Tukey's HSD value of 0.73 mm. Consequently, the observed difference, although indicating a slight favorability towards the combination, was not statistically significant enough to draw definitive conclusions regarding the superiority of the combination over *Fucus vesiculosus* alone.

Discussion and Conclusion

The investigation into *Ulva lactuca* and *Fucus vesiculosus* extracts, both individually and in combination, aimed to contribute insights to the development of alternative antimicrobial strategies. The results presented unexpected findings that challenged the initial hypothesis, stating that the combination of the *Ulva lactuca* and *Fucus vesiculosus* extracts would result in greater antibacterial effects on the *Staphylococcus epidermidis*, as opposed to using the extracts individually, and added complexity to the understanding of interactions between these algae extracts.

The unexpected finding, shown in Figure 6, that the combination of *Ulva lactuca* and *Fucus vesiculosus* did not exhibit a significantly greater mean zone of inhibition than *Ulva lactuca* alone challenges the initial hypothesis of a synergistic effect. This suggests that the combination may not amplify antibacterial efficacy as anticipated, calling for a reassessment of the presumed synergies. Notably, the significant differences observed among treatment groups, particularly the higher inhibition seen with *Ulva lactuca* compared to the combination, underscore the complex nature of the interactions between different algae extracts. While the results align with studies highlighting the antibacterial properties of *Ulva lactuca* and *Fucus vesiculosus* individually (Dominguez & Loret, 2019; Obluchinskaya et al., 2022), the lack of a synergistic effect in the combination contrasts with some literature suggesting potential synergies (EL-Sayed et al., 2023). These inconsistencies emphasize the need for a detailed understanding of algae extract interactions that may be influenced by specific bioactive compounds.

Delving into the relationships between *Ulva lactuca* and the combination of extracts, the mean difference of 1.449 mm, which is greater than Tuckey's HSD value of 0.73, suggests that *Ulva lactuca* alone may possess a stronger inhibitory effect than the combination of both extracts. This could be as a result of specific bioactive compounds in *Ulva lactuca* that might interact differently when combined with *Fucus vesiculosus*, potentially diminishing their individual effects. In contrast, when comparing *Fucus vesiculosus* with the combination of both extracts, the mean difference of 0.189 mm, which is smaller than Tuckey's HSD value of 0.73, indicates a slight advantage of the combination over *Fucus vesiculosus* alone. While this difference is statistically insignificant, it calls for exploration into potential synergistic effects between the two extracts. The combination might be capitalizing on the individual strengths of each algae species, to a degree, resulting in a cumulative antibacterial impact.

The observation of the combination of both extracts being less effective than *Ulva lactuca* alone but more effective than *Fucus vesiculosus* alone could result from the interactions of compounds within the extracts. *Ulva lactuca* may contain dominant antibacterial agents that, when combined with *Fucus vesiculosus*, experience some form of interference or alteration, affecting their potency. On the other hand, the combination might still outperform *Fucus vesiculosus* on its own, due to the additive or synergistic effects that enhance antibacterial activity.

Acknowledging limitations is crucial for a comprehensive understanding of the study's results. The unavailability of specific laboratory equipment, notably rotary evaporators, and time constraints are recognized as significant constraints. These limitations may have influenced the extraction process, potentially impacting the overall quality of the algae extracts. It is important to note that the absence of certain equipment might

have introduced variations in the concentration and composition of the extracts, introducing a degree of uncertainty into the results. Despite these challenges, the observed differences in antibacterial efficacy among the treatment groups remain significant, providing valuable insights.

To pave the way for future investigations, it is recommended that future studies address these limitations by ensuring access to necessary laboratory equipment and allowing sufficient time for the extraction processes. Moreover, exploring alternative combinations or concentrations of algae extracts could offer a more detailed understanding of their antibacterial potential. This approach aims to enhance the applicability of findings in subsequent studies, contributing to the continuous advancement of knowledge in this research domain.

In conclusion, the study contributes to the understanding of the antibacterial properties of *Ulva lactuca* and *Fucus vesiculosus* extracts. Despite the outcome regarding the combination, the research advances knowledge of algae extracts' antibacterial efficacy and sets the stage for further investigations. This emphasizes the need for detailed approaches in exploring potential synergies within natural compounds, ultimately contributing to efforts in finding natural alternatives to combat bacterial resistance.1

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Appendix A

Table A1. *Full Data Table for the Zone of Inhibition of Each Experimental Group; Raw Data*

P-Control	N-Control	U+F (mm)	U (mm)	F (mm)	Trial #
25	N/A	3	9	5	1
24	N/A	5	5	6	2
25	N/A	5	6	4	3
27	N/A	5	9	3.5	4
26	N/A	5	7	4	5
25	N/A	7	6	6	6
26	N/A	4	8	5.5	7
25	N/A	4.5	6	4.5	8
26	N/A	5.5	7.5	6.5	9
24	N/A	5	8	4.5	10
25	N/A	4	7	5	11
26	N/A	6	6	5.5	12
25	N/A	7.5	7.5	5	13
27	N/A	6	6.5	5.5	14
27	N/A	6	6.5	7	15
24	N/A	5.5	8	6	16
26	N/A	7.5	9	7	17
25	N/A	4.5	7	5	18
27	N/A	7	7	5	19
25	N/A	7	8	6.5	20
26	N/A	6.5	6	7	21
27	N/A	6.5	7	6.5	22
24	N/A	8	6.5	4.5	23
26	N/A	6.5	9	6	24
25	N/A	4	8.5	7	25
24	N/A	5	6	6	26
27	N/A	6.5	7	4.5	27
22	N/A	6	6.5	5.5	28
26	N/A	5.5	6	5	29
25	N/A	5.5	6.5	5.5	30

Appendix B

Table B1. *Tuckey's HSD and Mean Differences of Inhibition Zones*

Tuckey's HSD		
HSD=q*√(MS/nk)		**HSD= 0.72756068**
q=3.86	MS(within)=1.03	nk=29

Groups	Mean differences	Statistical Significance
PC - NC	25.414	Yes
PC - U+F	19.742	Yes
PC - U	18.202	Yes
PC - F	19.931	Yes
U+F - NC	5.576	Yes
U - NC	7.121	Yes
F - NC	5.483	Yes
U - F	1.638	Yes
U - U+F	1.449	Yes
U+F - F	0.189	No