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COMPARATIVE ANALYSIS OF FLAVONOID AND TANNIN EXTRACTION FROM ULVA LACTUCA USING MACERATION AND ULTRASONIC-ASSISTED (UAE) TECHNIQUES FOR ANTIBACTERIAL ACTIVITY

Analisis Komparatif Ekstraksi Flavonoid dan Tanin Dari *Ulva Lactuca* Menggunakan Teknik Maserasi dan Ultrasonic-Assisted Untuk Aktivitas Antibakteri

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ABSTRACT

This research involved *Streptococcus agalactiae* and *Streptococcus iniae* bacteria and can lead to widespread mortality in gourami fish. The use of conventional antibiotics in the management of these diseases has led to resistance problems and environmental pollution. *Ulva lactuca* algae are known to contain bioactive compounds such as triterpenoids, flavonoids, and saponins that have antibacterial potential against Streptococcus. This study aims to evaluate the potential of *Ulva lactuca* algae extract as an alternative treatment for *streptococcosis* in Gourami (*Osphronemus gouramy*). In vitro antibacterial activity test through inhibition zone test using disc diffusion method with treatment in the form of negative control (P-) or without treatment, positive control (P+) with amoxicillin antibiotic administration, *Ulva lactuca* extract administration. The inhibition zone test was conducted by testing *Ulva lactuca* extract at concentrations of 0.005%, 0.01%, 0.015%, 0.02%, 0.025%, 5%, 10%, 15%, 20%, 40%, 60%, 80%, and 100%. The content test of algae extracts by maceration method showed flavonoid content of 94.746 µg/g and tannin content of 10.845 µg/g. The content test of algae extracts with *Ultrasonic-Assisted Extraction* (UAE) method showed flavonoid content of 59.919 µg/g and tannin content of 8.3025 µg/g. The results showed that *Ulva lactuca* extracts at all concentrations have not shown the ability to inhibit bacteria. This can be caused by the cell wall of Streptococcus sp bacteria, which is gram positive, is too thick and rigid so it is necessary to optimize the *Ulva lactuca* extraction process and its concentration to increase the effectiveness of *Streptococcus* sp. inhibition.

Keywords: Gourami, Maceration, *Streptococcus* sp., *Ultrasonic-Assisted Extraction*, *Ulva lactuca*.

ABSTRAK

Penelitian ini melibatkan bakteri *Streptococcus agalactiae* dan *Streptococcus iniae* yang dapat menyebabkan kematian yang meluas pada ikan gurami. Penggunaan antibiotik konvensional dalam penanganan penyakit ini telah menimbulkan masalah resistensi dan pencemaran lingkungan. Alga *Ulva lactuca* diketahui mengandung senyawa bioaktif seperti triterpenoid, flavonoid, dan saponin yang memiliki potensi antibakteri terhadap *Streptococcus sp*. Penelitian ini bertujuan untuk mengevaluasi potensi ekstrak alga *Ulva lactuca* sebagai alternatif pengobatan streptokokus pada ikan gurami (*Osphronemus gouramy*). Uji aktivitas antibakteri secara in vitro melalui uji zona hambat menggunakan metode difusi cakram dengan perlakuan berupa kontrol negatif (P-) atau tanpa perlakuan, kontrol positif (P+) dengan pemberian antibiotik amoksisilin, pemberian ekstrak *Ulva lactuca*. Uji zona hambat dilakukan dengan menguji ekstrak *Ulva lactuca* pada konsentrasi 0,005%, 0,01%, 0,015%, 0,02%, 0,025%, 5%, 10%, 15%, 20%, 40%, 60%, 80%, dan 100%. Uji kandungan ekstrak alga dengan metode maserasi menunjukkan kandungan flavonoid sebesar 94,746 µg/g dan kandungan tanin sebesar 10,845 µg/g. Uji kandungan ekstrak alga dengan metode *Ultrasonic-Assisted Extraction* (UAE) menunjukkan kadar flavonoid sebesar 59,919 µg/g dan kadar tanin sebesar 8,3025 µg/g. Hasil penelitian menunjukkan bahwa ekstrak *Ulva lactuca* pada semua konsentrasi belum menunjukkan kemampuan untuk menghambat bakteri. Hal ini dapat disebabkan karena dinding sel bakteri *Streptococcus sp* yang merupakan bakteri gram positif terlalu tebal dan kaku sehingga perlu dilakukan optimasi proses ekstraksi *Ulva lactuca* dan konsentrasinya untuk meningkatkan efektifitas penghambatan bakteri *Streptococcus* sp.

Kata Kunci: Gurami, Maserasi, *Streptococcus* sp., *Ultrasonic-Assisted Extraction, Ulva lactuca*

INTRODUCTION

Gourami fish (*Osphronemus gouramy*) has an important role in the fishing industry as a source of animal protein and is one of the main commodities in the freshwater fisheries sector. Gourami fish are widely cultivated by the community, given the high market demand for this type of fish (Kristina, 2015). Gourami fish is native to Indonesian waters with high economic value and is widely consumed by the community. Fisheries production in the gourami commodity in Indonesia reached 176,113.78 tons in 2021 (Sadya, 2022). This fish species occupies the main position along with several other fish species, such as carp, tilapia, catfish, and catfish. However, gourami fish farming is not free from problems.

An obstacle in gourami farming is that it is susceptible to various diseases, including bacterial, parasitic, and fungal infections. Diseases that often attack gourami are diseases caused by pathogenic bacteria that cause 50-100% mass mortality (Yanti & Prayitno, 2015). The phenomenon of fish mortality has an impact on the decline in aquaculture production resulting in large losses. One of the diseases that cause mass mortality in fish farming is streptococcosis. This disease is caused by bacteria from the genus *Streptococcus*, especially the species *Streptococcus iniae* and *Streptococcus agalactiae*. These bacteria are gram-positive pathogens that can attack various types of freshwater fish including gourami.

Mass infection with *Streptococcus agalactiae* bacteria is acute and can cause very high mortality rates in farmed fish (Wulandari et al., 2018). Streptococcosis disease can cause death between 30-50% of the fish population within a week. Handling of fish infected with streptococcosis is generally done by giving antibiotics to fish. However, intensive use of antibiotics or chemicals can cause various problems such as bioaccumulation, pollution, pathogen resistance, and suppression of the fish immune system (Manoppo & Kolopita, 2017). This indirectly impacts the environment and decreases the effectiveness of synthetic

antibiotics, so it is necessary to find alternative treatments that are more environmentally friendly. One source of natural antibiotics is algae.

Indonesian waters are rich in biological resources such as marine algae that can be utilized as an alternative to overcome these problems. The type of seaweed or green algae that is often found and widespread is *Ulva sp*. or also known as sea lettuce. *Ulva lactuca* is a natural resource that has potential as an antibacterial because it contains bioactive compounds such as triterpenoids, flavonoids, and saponins (Liswandari, 2018). The use of natural materials such as *Ulva lactuca* as antibiotics can be a solution to reduce negative impacts on the environment and health. This alga has the capacity to reproduce sexually and through thallus fragmentation, giving it the ability to multiply rapidly (Dominguez & Loret, 2019). The content in this alga can be utilized by extraction to obtain secondary metabolites.

Streptococcosis disease which is massive and causes huge losses requires effective treatment, one of which is by using *Ulva lactuca*. Information on the effectiveness of the algae has not been widely researched to solve the disease problem. Therefore, this research is needed to determine the effectiveness of *Ulva lactuca* macroalgae extract against streptococcosis disease caused by *Streptococcus agalactiae* bacteria in gourami.

RESEARCH METHODS

Time and Place

This research was conducted over a 4-month period in April – July 2024 at the Fish Cultivation Laboratory of the Fish Disease Division and the Fisheries and Marine Resources Exploration Laboratory, Faculty of Fisheries and Marine Science, Brawijaya University.

Tools and Materials

The tools used are rotary evaporator, analytical balance, blender, mortar pestle, glass jar, filter paper, autoclave, incubator, petri dish, test tube, beaker glass, erlenmeyer, ose needle, paper disc, bunsen, aquarium, aluminum foil, ruler, microscope, mask, and latex gloves. The materials used were *Ulva lactuca* algae, *Streptococcus agalactiae* bacterial isolate, gourami fish, TSA (Tryptone Soya Agar), TSB (Tryptone Soya Broth), Blood Agar, amoxicillin, 90% ethanol, fish feed, spirtus, 70% alcohol, and Na-phis.

Research Design

In vitro antibacterial activity test, independent variables are 7 groups consisting of negative control (P-) or without treatment, positive control (P+) by giving amoxicillin antibiotics, giving *Ulva lactuca* extracts with concentrations of 50 ppm (P1), 100 ppm (P2), 150 ppm (P3), 200 ppm (P4), and 250 ppm (P5). The dependent variable is the diameter of the inhibition zone. In vivo antibacterial activity test on gourami, the independent variable is 5 groups consisting of negative control (P-) or without treatment, positive control (P+) by giving amoxicillin antibiotic, giving *Ulva lactuca* extract with 3 best concentrations of inhibition zone test results. The dependent variable is the survival rate and recovery rate of the fish.

Research Procedures

1. **Extraction of *Ulva lactuca***

Ulva lactuca macroalgae were cleaned and dried, then pulverized using a blender and mortar pestle. Once smooth, the algae were macerated by soaking in 90% ethanol in a ratio of 1:10 for 3 days in a glass container wrapped in black plastic to avoid evaporation. After that, the algae maceration was filtered and concentrated using a vacuum rotary evaporator at 55°C to obtain a thick extract. Then the thick extract was diluted with distilled water to obtain concentrations of 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm (Metungun et al., 2023; Sari, 2016).

2. ***Streptococcus sp.* Bacterial Culture**

Streptococcus sp. bacteria were inoculated on TSA (Trypticase Soy Agar) media with an ose needle, then streaked and stored upside down for 24 hours. One colony was separated, dipped in liquid TSB (Trypticase Soy Broth) media, and incubated for 24 hours and homogenized (Yanti & Prayitno, 2015).

3. **Antibacterial Activity Test of *Ulva lactuca* Extract (ZI Test)**

Antibacterial activity testing using the disc diffusion method was carried out by preparing a bacterial suspension that had previously been diluted to a concentration of 10-7 bacterial cells/ml, then spread on agar media, and placed paper discs that had been soaked in the test extract solution on the media. After incubation for 24 hours at 30°C, a clear zone around the disc was observed to evaluate antibacterial activity, by measuring the diameter of the clear zone (Amalia & Sari, 2017; Attamimi, 2022; Keintjem et al., 2019; Liswandari, 2018).

4. **Gourami Fish Acclimatization (*Osphronemus gouramy*)**

The test fish used were 15 cm gourami in healthy condition. Acclimatization was carried out first on the test fish in a tub for 7 days. After acclimatization, the fish are fed for 24 hours (Pribadi et al., 2021). Gourami Fish Infection with *Streptococcus sp.* bacteria. Gourami fish are infected with *Streptococcus agalactiae* bacteria through immersion for 60 minutes and given a small incision on the body to ensure infection then, returned to the maintenance container (Rahmi et al., 2021).

5. **Application of *Ulva lactuca* Extract to Gourami Fish**

Gourami fish that have been infected with streptococcosis disease are applied through immersion for 1 hour then, the fish are returned to the maintenance container. During the maintenance of fish, water conditions are maintained by changing water and flushing every day and feeding twice a day (Rahmi et al., 2021).

RESULTS

1. ***Ulva lactuca* Macroalgae Extract**

Extraction of *Ulva lactuca* was carried out with two different extraction methods, namely maceration method and *Ultrasonic-Assisted Extraction* (UAE) method. Extraction of *Ulva lactuca* by maceration method was carried out by macroalgae samples macerated with 90% ethanol using a ratio of 1:10 for 72 hours and stirred periodically. The maceration results from *Ulva lactuca* macroalgae samples were filtered and then concentrated using a rotary evaporator at 55°C until they became thick extracts. *Ulva lactuca* macroalgae extraction is not only done using a rotary evaporator, but also using a vacuum evaporator. This tool will extract *Ulva lactuca* macroalgae in powder form.

Figure 1. *Ulva lactuca* extract

Ulva lactuca extraction using *Ultrasonic-Assisted Extraction* (UAE) method is done by extracting algae powder with UAE at 40°C for 15 minutes with the ratio of algae and ethanol

solvent 1:10. Then filtering is done using Whatman filter paper to produce a liquid extract, then evaporated for 4.5 hours at 50°C to get a thick extract.

2. Zone of Inhibition Test

In vitro antibacterial activity test through inhibition zone test using disc diffusion method with treatment in the form of negative control (P-) or without treatment, positive control (P+) by giving amoxicillin antibiotic, giving *Ulva lactuca* extract with concentration of 50 ppm (P1), 100 ppm (P2), 150 ppm (P3), 200 ppm (P4), and 250 ppm (P5). The test results showed that the *Ulva lactuca* extract tested did not show a clear zone. In the treatment of *Ulva lactuca* extract with concentrations of 5% (P1), 10% (P2), 15% (P3), and 20% (P4) also did not show a clear zone. This is not in accordance with the research of Maray et al. (2023) which showed the ability to inhibit Streptococcus agaloctiae ATCC13813 by *Ulva lactuca* extract with a diameter of 10.3 mm. Based on the criteria for antimicrobial strength according to (Keintjem et al., 2019), namely the diameter of the inhibition zone ≤ 5 mm (weak category), the diameter of the inhibition zone 5-10 mm (medium category), the diameter of the inhibition zone 10-20 mm (strong category), and the diameter of the inhibition zone ≥20 mm (very strong category). The experiment was conducted again by testing *Ulva lactuca* extract at concentrations of 5%, 10%, 15%, 20%, 40%, 60%, 80%, and 100%. The experiment was conducted using the UAE extraction method to optimize the maceration extraction method.

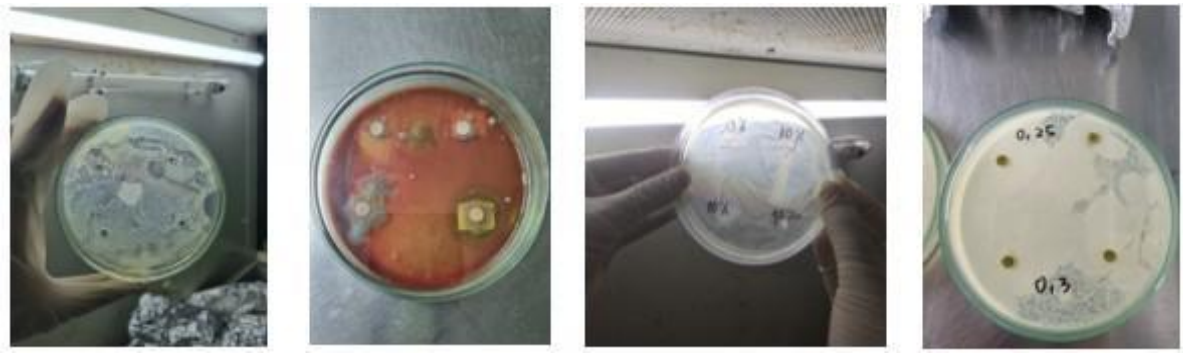

Figure 2. Zone of Inhibition Test of *Streptococcus agalactiae* with *Ulva lactuca* Extract

However, the experimental results still did not show a clear zone. There are several factors that can affect these results. Different results can occur due to the extraction process that has not been optimized. In addition, based on the research of (Sari, 2016), flavonoids which are antibacterial compounds in ulva, can only inhibit the growth of gram-negative bacteria. This is due to differences in cell wall structure and thickness with gram-positive bacteria. *Streptococcus agalactiae* is a gram-positive bacterium that has a thicker and stiffer cell wall than gram-negative bacteria (Maray et al., 2023).

Table 1. Diameter of Inhibition Zone Test

Test	Diameter of Inhibition Zone Test														
	K - (aquades)	K+ (Amoxicillin)	0,005%	0,01%	0,015%	0,02%	0,025%	5 %	10 %	15 %	20 %	40 %	60 %	80 %	100%
1	0	22	0	0	0	0	0	-	-	-	-	-	-	-	-
2	0	21	0	0	0	0	0	-	-	-	-	-	-	-	-
3	0	21	-	-	-	-	-	0	0	0	0	-	-	-	-
4	0	23	-	-	-	-	-	0	0	0	0	0	0	0	0

3. Characterization Using FTIR

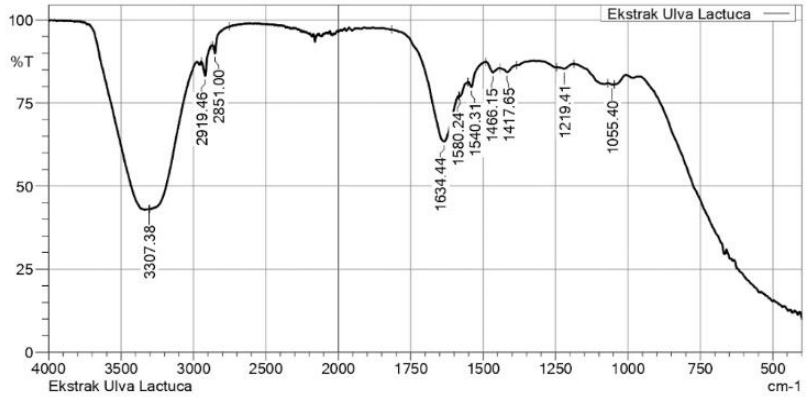

Figure 3. The FTIR Spectrum of *Ulva lactuca*

The *Fourier Transform Infrared* (FTIR) give the information in the IR spectrum to be analyzed is presented and can be observed in the table below.

Table 2. IR Analysis of *Ulva lactuca* Extract

Wavenumber (cm^{-1})	**Vibrational Bonds**
3307.38	O-H, broad stretching
2919.46	C-H sp3, stretching
2851.00	C-H sp3, stretching
1634.44	C=O, stretching
1466.15	C-H, bending
1417.65	C-H, bending
1219.41	C-C, stretching
1055.40	C-O, stretching

4. Flavonoid Test and Tannin Test

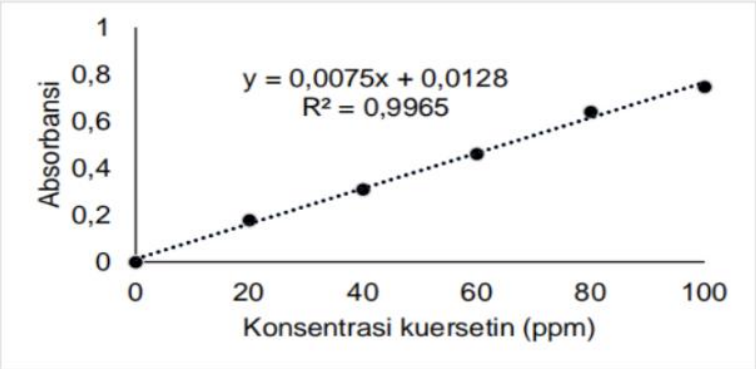

Figure 4. Standard Curve of *Ulva lactuca* Extract in Flavonoid Test

Flavonoid test was conducted on *Ulva lactuca* extracts made using UAE and maceration methods to obtain a standard curve at a certain absorbance.

Table 3. Flavonoid content of *Ulva lactuca* extract samples

Sample	Mass of The Sample (g)	Absorbance Value (Y)	C1 Value (X)	Flavonoid Concentration (µg/g)
A	2	0,912	0,3321	59,919
B	2	1,434	0,4388	94,746

In the extract sample made using the UAE method (sample A), the flavonoid content was 59.919 µg/g while in the extract sample made using the maceration method (sample B), the flavonoid content was 94.746 µg/g. The flavonoid content of sample B extracted by maceration method was higher than that of sample A extracted using *Ultrasonic-Assisted Extraction* (UAE) method. This indicates that the maceration method is more effective in extracting flavonoid compounds from *Ulva lactuca* compared to the UAE method.

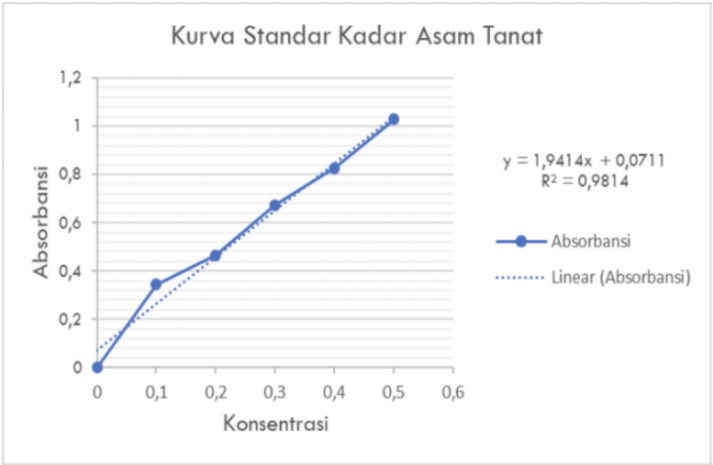

Figure 5 Standard curve of *Ulva lactuca* extract in tannin test

In addition, the tannic acid content test or tannin test was carried out on *Ulva lactuca* extracts made by UAE and maceration methods so that a standard curve of tannic acid content at a certain absorbance was obtained.

Table 4. Tannin content of *Ulva lactuca* extract samples

Sample	Mass of The Sample (g)	Absorbance Value (Y)	C1 Value (X)	Tanin Concentration (µg/g)
A	2	0,716	0,3321	8,3025
B	2	0,923	0,4388	10,845

In the extract sample made using the UAE method (sample A), the tannin content was 8.3025 µg/g while in the extract sample made using the maceration method (sample B), the tannin content was 10.845 µg/g. Just like flavonoids, the tannin content in sample B extracted by maceration method was also higher compared to sample A using UAE method. This indicates that maceration method is also more effective in extracting tannin compounds from *Ulva lactuca* compared to UAE method.

Extraction of *Ulva lactuca* by maceration method showed higher results in flavonoids and tannins content compared to the *Ultrasonic-Assisted Extraction* (UAE) method. One of the main factors is the much longer extraction duration in the maceration method, i.e. 72 hours, compared to UAE which only lasts for 15 minutes. The longer extraction time provides a greater opportunity for the solvent (90% ethanol) to thoroughly extract the bioactive compounds from the algae matrix. In addition, the maceration process involves periodic stirring, which ensures better contact between the sample and the solvent, so that compounds such as flavonoids and tannins can be dissolved more effectively.

In addition to duration, the stability of bioactive compounds during the extraction process is also an important consideration. Maceration methods, which are performed without the use of high energy or drastic temperature changes, tend to better maintain the stability of active compounds such as flavonoids and tannins. In contrast, although UAE utilizes ultrasonic technology that can accelerate the extraction process, the ultrasonic waves can potentially cause disturbances to the stability of the compounds through the formation of free radicals or localized temperature increases. This, together with the short extraction duration, may cause the content of flavonoids and tannins extracted via UAE to be lower compared to maceration.

DISCUSSION

The extraction results for *Ulva lactuca* show notable differences in the effectiveness of the maceration method compared to the *Ultrasonic-Assisted Extraction* (UAE) method. The maceration process, which involves soaking the algae in ethanol for 72 hours, produced significantly higher levels of flavonoids (94.746 µg/g) and tannins (10.845 µg/g). In comparison, the UAE method, which takes just 15 minutes, yielded lower concentrations of flavonoids (59.919 µg/g) and tannins (8.3025 µg/g). This difference is likely due to the longer extraction time and the gentler conditions of maceration, which allow the solvent to interact more thoroughly with the algae and better preserve sensitive compounds. On the other hand, while the UAE method is much faster, it uses ultrasonic waves that can generate localized heating and free radicals, which might degrade some of the bioactive compounds. Additionally, the short duration of the UAE process limits the time for the solvent to fully penetrate the algae matrix. Unlike maceration, UAE does not involve stirring, which could further reduce its efficiency in extracting compounds (Rujiyanti et al., 2020).

The results of the zone of inhibition test revealed that *Ulva lactuca* extracts did not show significant antibacterial activity against *Streptococcus agalactiae*, as no clear inhibition zones were observed at any concentration. This lack of antibacterial effect can be attributed to several factors. First, *Streptococcus agalactiae* is a gram-positive bacterium with a thick, rigid cell wall that acts as a barrier to the penetration of antibacterial compounds. Flavonoids and tannins, the primary bioactive compounds in *Ulva lactuca*, are generally more effective against gram-negative bacteria, which have thinner cell walls. Additionally, the concentration of active

compounds in the extract may not have been high enough to produce a visible effect, suggesting that higher concentrations of *Ulva lactuca* extract or more potent solvents might be required to achieve inhibition. The extraction methods used in this study may also not have fully optimized the release of these bioactive compounds, and further improvements in the extraction process could enhance their antibacterial potential. Lastly, the antibacterial efficacy could depend on the synergistic effects between different compounds, and the lack of synergy in this extract might have reduced its overall effectiveness. While these results suggest that *Ulva lactuca* extracts may not be effective against *Streptococcus agalactiae*, further optimization of the extraction process, testing higher concentrations, and exploring its effects on gram-negative bacteria could yield more promising results.

In the tests, the negative control (aquades) showed no inhibition, with a diameter of 0 mm, while the positive control (amoxicillin) exhibited significant antibacterial activity, with inhibition zones ranging from 21 to 23 mm, demonstrating its effectiveness. For the *Ulva lactuca* extracts, none of the concentrations, including 0.005%, 0.01%, 0.015%, 0.02%, and higher concentrations up to 100%, produced any measurable inhibition zones. The results consistently showed 0 mm inhibition, indicating that the *Ulva lactuca* extracts did not inhibit the growth of *Streptococcus agalactiae* at the tested concentrations. This suggests that the bioactive compounds in the extracts, despite being present in measurable amounts, were not effective in this test. The lack of inhibition at all tested concentrations may be attributed to factors such as the thick cell wall of the gram-positive bacteria, which hinders the penetration of the compounds, or the insufficient concentration of active compounds in the extracts.

The FTIR spectrum analysis of *Ulva lactuca* extracts reveals the presence of functional groups that are indicative of bioactive compounds. Key peaks identified in the spectrum include a broad band at 3307.38 cm^{-1}, corresponding to O-H stretching vibrations, which are characteristic of hydroxyl groups commonly found in phenolic compounds such as flavonoids and tannins. These groups are known to play a role in antioxidant and antibacterial activities. Another significant peak appears at 1634.44 cm^{-1}, associated with C=O stretching vibrations, which are indicative of carbonyl groups. These groups are often present in flavonoid structures, contributing to their biological activity. Additionally, peaks at 2919.46 cm^{-1} and 2851.00 cm^{-1} correspond to C-H stretching vibrations, suggesting the presence of aliphatic compounds. The C-O stretching vibration at 1055.40 cm^{-1} further supports the existence of alcohols or esters, which are integral components of tannins and other bioactive substances. The spectrum also highlights peaks at 1466.15 cm^{-1} and 1417.65 cm^{-1}, associated with C-H bending, which may indicate the presence of saturated hydrocarbons. The FTIR data align with the chemical properties of flavonoids and tannins identified in the extracts. These functional groups validate the potential of *Ulva lactuca* extracts as sources of antibacterial and antioxidant compounds. However, the effectiveness of these compounds depends on their interaction with bacterial cell walls, as observed in subsequent antibacterial tests. This analysis underscores the importance of characterizing bioactive compounds in the extract and optimizing their extraction for potential applications in antimicrobial treatments.

The flavonoid and tannin content of *Ulva lactuca* extract, as measured in this study, revealed significant differences between the two extraction methods used—maceration and *Ultrasonic-Assisted Extraction* (UAE). The maceration method yielded higher concentrations of flavonoids (94.746 µg/g) and tannins (10.845 µg/g) compared to the UAE method, which resulted in 59.919 µg/g flavonoids and 8.3025 µg/g tannins. These findings highlight the influence of extraction techniques on the yield of bioactive compounds. The higher concentrations obtained from the maceration method can be attributed to several factors, primarily the longer extraction time (72 hours) and the gentler conditions under which the process is carried out. The prolonged contact between the solvent (ethanol) and the algae allows for more thorough extraction of flavonoids and tannins, which are known to be sensitive to

temperature and mechanical forces. On the other hand, the UAE method, which uses ultrasonic waves to facilitate extraction, produced lower concentrations of both flavonoids and tannins. The shorter extraction time (15 minutes) likely limited the opportunity for the solvent to fully penetrate the algae matrix, resulting in a lower yield of bioactive compounds. Furthermore, the ultrasonic waves, while aiding in the extraction process, can generate localized heating and free radicals, which might destabilize sensitive compounds like flavonoids and tannins, reducing their overall yield. Flavonoids and tannins are well-known for their antioxidant and antimicrobial properties, and the higher concentrations obtained from maceration suggest that this method is more effective for extracting these beneficial compounds from *Ulva lactuca* (Arifin & Ibrahim, 2018). However, the UAE method offers advantages in terms of time and energy efficiency. With further optimization of the UAE method, such as adjusting extraction parameters like temperature, solvent type, and duration, it may be possible to increase the yield of flavonoids and tannins while preserving their bioactivity.

CONCLUSION

From the results of our research, it can be concluded that *Ulva lactuca* extract is less than optimal in inhibiting the growth of *Streptococcus agalactiae* bacteria. This can be seen from the experiments that have been carried out, showing no clear zone formed in the inhibition zone test. This can be caused by the cell wall of *Streptococcus agalactiae* bacteria, which is gram-positive, too thick and rigid. However, from the results of flavonoid and tannin content tests using FTIR, *Ulva lactuca* has high flavonoid and tannin content, which is 59.919 µg/g and 94.746 µg/g for flavonoid compounds and 8.3025 µg/g and 10.845 µg/g for tannin compounds.

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